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Risks for human health related to the presence of plant lectins in food

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

The European Commission asked the European Food Safety Authority (EFSA) to assess the risk related to the presence of plant lectins in food. Based on the available evidence, the CONTAM Panel considered only phytohaemagglutinin (PHA), a legume lectin from beans (*Phaseolus* sp.), for the risk characterisation. Effects of PHA in the small intestine were considered as the critical effect in subacute studies in rats. A lower confidence limit of the benchmark dose $(BMDL)_{10}$ of 22.9 mg/kg body weight (bw) per day for an increase in small intestine dry weight was selected as the most appropriate reference point for the risk characterisation. The establishment of a health-based guidance value for PHA was not considered appropriate due to the limitations and uncertainties in the current toxicological evidence, and the margin of exposure (MOE) approach was used for the risk characterisation. The Panel considered that acute exposure resulting in MOEs above 100 is not expected to raise a health concern. As no occurrence data were submitted to EFSA, data for PHA presence in food were identified by a literature search. An arbitrary acute exposure scenario, where only 50% of the lectins are inactivated due to insufficient cooking of food containing lectins (e.g. beans), would result in MOEs below 100. The Panel, accounting for the uncertainties affecting the exposure and hazard assessments, concluded with at least 95% probability that such a dietary exposure would raise health concerns. The Panel also noted that exposure to completely deactivated lectins in food prepared following adequate food processing practices (e.g. soaking and boiling) would not raise health concerns. No risk characterisation could be performed for other lectins due to the lack of relevant toxicological data and/or in some cases lack of occurrence data.

KEYWORDS

antinutritional compounds, food, food processing, gastrointestinal toxicity, human exposure, phytohaemagglutinin, plant lectins, risk assessment

*Deceased, active until February 2025.

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SUMMARY

The European Commission asked the European Food Safety Authority (EFSA) to assess the risk related to the presence of plant lectins in food.

Lectins are carbohydrate-binding proteins naturally occurring in many plants, including legumes, grains and vegetables in variable concentrations. Plant lectins are also called phytohaemagglutinins. Improperly prepared food containing plant lectins, especially legumes, can cause adverse effects in humans.

Plant lectins are a diverse group of proteins, lacking catalytic activity and are characterised by their structural variability and specific reversible binding to sugars. Based on structural and phylogenetic relationships, up to 13 groups of plant lectins have been identified, of which five were considered relevant in this Opinion for their potential risk for human health; these include (1) legume lectins, (2) *Galanthus nivalis* agglutinin (GNA)-related lectins, (3) jacalin-related lectins, (4) hevein-like domain-containing lectins and (5) type 2 ribosome-inactivating proteins (RIPs-2) which contain a lectin chain. The biological activity and potential health impacts of dietary lectins primarily depend on the presence and characteristics of their carbohydrate-binding sites, which vary in structure, shape, size and specificity across lectin groups but share common functional characteristics.

To obtain highly purified lectins, while maintaining their carbohydrate-binding activity, requires multiple steps and a combination of different techniques. This process starts with preparing an aqueous extract from the plant using buffered solutions, followed by various chromatographic techniques. Among these, affinity chromatography is considered the most efficient for the purification of lectins, leveraging the high specificity of lectins to bind certain carbohydrates. The efficacy of the various purification steps is typically monitored by SDS-PAGE or haemagglutination assay. No certified reference materials are available.

A number of different techniques are applied for the determination of lectins in biological matrices and foods. Detection and quantification of lectins is usually performed using the highly sensitive and specific enzyme-linked immunosorbent assays (ELISA). The results from ELISA depend on the type and specificity of the antibodies used (polyclonal or monoclonal), which determines whether they bind exclusively to the active lectins or also to inactive forms and/or lectins bound to glycoproteins. Mass spectrometry-based techniques, capable of detecting both active and denatured lectins, are also used for the identification and quantification of lectins in food or food products. Other methods less commonly used are surface plasmon resonance (SPR) and biosensors. To determine lectin activity, the haemagglutination assay is one of the most commonly used tests; however, a number of factors (e.g. the type of red blood cells used, the trypsin treatment of red blood cells and the purity of the lectins) affect the sensitivity and specificity of this assay and limit the robustness of the results. Overall, it is challenging to compare results obtained from different techniques for estimating the lectin concentration or evaluating the lectin activity.

Hazard identification and characterisation

Based on few toxicokinetic studies available in rodents, the majority of ingested lectins [up to 90% of phytohaemagglutinin (PHA) and over 75% of concanavalin A (Con A)] pass unchanged (remain structurally intact and retain their biological activity) through the gastrointestinal tract. Absorption is low with minor amounts of lectin (up to 4% of PHA and 3% of tomato lectin) being absorbed and reaching the major organs through the systemic circulation in rodents. There is no evidence of degradation in rodents, as approximately 90% of lectins in faeces were functionally active.

Limited evidence on toxicokinetics of active lectins [peanut agglutinin (PNA) and wheat germ agglutinin (WGA)] in humans showed rapid detection in the systemic circulation after consumption of peanuts or wheat germ, suggesting absorption occurs in the upper gastrointestinal tract, probably in the stomach. Moreover, the detection of lectins in faeces using haemagglutination assays implied that unabsorbed material retains its biological activity. Significant IgG and IgM responses to lectins or to proteins ingested at the same time as the lectin may indicate alterations in the gut barrier, eventually leading to an increased translocation of dietary antigens.

A limited number of acute toxicological studies were retrieved in experimental animals. Con A affected some enzymatic activities in the small intestine of rats at 250 mg/kg bw and reduced feed intake at 3600 mg/kg bw. No acute or long-term toxicity studies were identified for PHA or soybean agglutinin (SBA).

The main targets in repeated dose subacute toxicity studies in rodents exposed to lectins by gavage or via diet were the gastrointestinal tract, pancreas and immune system.

All evaluated lectins showed a similar general toxicity pattern, e.g. many of the investigated lectins reduced feed intake (PHA, SBA) and body weight (PHA, SBA, WGA). Comparisons with ad libitum and pair-fed controls indicated that the reduced body weight may be due to reduced feed uptake and/or lowered digestibility and retention of nitrogen by the body, which may contribute to antinutritive effects. PHA reduced the body weight of rats at doses above 7 mg/kg bw per day. Further common findings in the pancreas were elevated weight (PHA, SBA, PNA, WGA), hypertrophy and vacuolation as shown by histopathology and altered enzyme activities (SBA, PSA). The most sensitive endpoints were increased weight of the pancreas (PNA, 0.1 mg/kg bw per day; SBA, 44 mg/kg bw per day) and hypertrophy of the exocrine pancreas (PHA 32.5 mg/kg bw per day).

Regarding gastrointestinal toxicity, lectins bind to the brush border membrane of the small intestine via many and varied carbohydrate-binding sites. The effects elicited by the various lectins depend on the specificity of the binding to the carbohydrate sites. In general, the most potent lectins caused damage to the villi, microvilli and crypts (PHA at 3 mg/kg bw

per day; Con A at 8 mg/kg bw per day). Increases in small intestine weight (PHA at 32.5 mg/kg bw per day) and length (SBA at 112 mg/kg bw per day; PHA at 120 mg/kg bw per day) were commonly observed as were changes in the activities of the brush border enzymes such as alkaline phosphatase (ALP) (PHA at 3 mg/kg bw per day) and sucrase (Con A at 600 mg/kg bw per day). In addition, PHA at doses from 20 mg/kg bw per day led to a higher bacterial number in the small intestine and changes in the microbial composition and diversity, which are associated with alterations in the mucus layer and reduced nutrient absorption. The majority of these effects on the gut are reversible on return to a normal diet without lectin.

Lectins (PNA, PHA, ASAI/II, WGA) administered orally in rodents may disturb the immune system in the gut (PHA from 0.5 mg/kg bw once weekly for 7 weeks and SBA from 60 mg/kg bw per day for 20 days), leading to an antibody response against the lectin and potentially against other dietary proteins, which instead of inducing oral tolerance increases the risk of triggering an allergic response.

PHA administered in rats (PND14) altered small intestine morphology at 2 mg/kg bw per day and affected body weight gain, liver and pancreas weights, maturation of the gastrointestinal (GI) tract and the immune system at 50 mg/kg bw per day. For SBA and other lectins, no developmental studies were identified.

The evidence for genotoxicity was weak as non-standard tests and study designs were used. However, since lectins are proteins, no genotoxic properties are expected via a direct DNA interaction. There is no evidence of carcinogenicity from the one study retrieved which primarily investigated intestinal carcinogenesis.

The available human data only pertained to the association between postulated acute exposure to (non-purified) lectins and gastrointestinal effects, metabolic effects and food allergy in a limited number of small studies. No quantitative analysis was performed, relevant control groups were not used, confounders were not taken into consideration, lectin identification was done occasionally and only qualitatively, and the presumed lectin activity was assessed via haemagglutination assay. Consumption of partially processed red and white kidney beans can cause GI symptoms, namely nausea, vomiting, abdominal pain and diarrhoea. Red and white kidney beans have been associated with acute allergic reactions which might be attributed to the lectin activity. The available evidence on lectin-related allergenicity consists of studies assessing skin prick test (SPT) reactions and IgE antibody assays (either lectin- or plant-specific) and has certain limitations (including variable documentation on allergy history and infrequent distinction between specific and non-specific lectin binding). The CONTAM Panel considered that lectins from edible plants might induce sensitisation and/or that allergic reactions may occur in subjects allergic to other lectins present in edible plants (due to potential cross-reactivity).

Regarding the mode of action, the activity of legume lectins and possible deleterious effects on human health depends on four key features. Firstly, the structure and the carbohydrate-binding activity of lectins and the specificity of the binding to specific carbohydrates (simple sugars or complex glycans). Secondly, the number of carbohydrate-binding sites, e.g. tetrameric being more active than dimeric. Thirdly, the overall lectin content in the seeds and fourthly, the structural arrangement of sub-units and their interaction with glycans. Lectins exert their effects on the gut through specific binding to the carbohydrate moieties on the epithelial cells and altering the function of the microvilli, villi and crypt cells. It is possible that by binding to receptors in the gut, and perhaps mimicking binding of natural ligands, lectins modulate intracellular/intercellular signalling pathways that affect viability, proliferation and differentiation of epithelial cells.

Lectins bind to the carbohydrate moieties present on the surface of many but not all microorganisms, and this likely influences the proportion of ingested lectin available to bind to the gut epithelial cells. Conversely, the changes induced by ingested lectins on the gut epithelium may influence the gut microbiota composition. As the plant lectins hold the capacity to adversely alter the gut barrier and the microbiota, plant lectins have been implicated in the possible induction of autoimmune disorders and allergic reactions. Lectin may affect or disrupt the interplay between the gut barrier and the microbiota by disturbing mucus production or epithelial cell differentiation.

Lectins may induce adverse immunotoxicological reactions, with the type of immune response depending on the animal species, the underlying immune polarisation (i.e. whether the immune system is skewed towards a Th1, Th2 or other type of immune response), and the amount of lectin that reaches the circulation in the animals. The latter may largely depend on the properties of the specific lectin and the amount ingested.

Lectins may cause the release of cholecystokinin (CCK) from enteroendocrine cells in the intestinal tract into the bloodstream, which stimulates the exocrine pancreas to produce digestive juice and to grow.

The available epidemiological data on GI and metabolic effects are limited both in terms of volume and validity and cannot be used further for risk characterisation. Therefore, the CONTAM Panel relied on data from subacute studies in experimental animals to identify reference points for the human risk characterisation.

Acknowledging the lack of data from lectins other than PHA for the identification of critical effects and dose–response analysis, the Panel evaluated the impact on the risk assessment by restricting the risk characterisation to PHA alone. The Panel considered small intestine weight, large intestine weight and pancreas weight in rodents as the critical effects for acute PHA toxicity, using a benchmark response (BMR) of 10% based on the standard deviation of the control groups, the intercorrelation of the effects and on consideration of the biological relevance of the endpoints (e.g. reversibility of the effects). The Panel noted that PHA is considered the most toxic among the lectins from edible plants because of its specificity to bind complex glycans containing a bisecting N-acetylglucosamine (GlcNAc) and of its high affinity to human enterocytes. Benchmark dose (BMD) modelling was performed according to the 2022 EFSA Guidance on the use of the BMD approach in risk assessment. A $BMDL_{10}$ of 22.9 mg/kg bw per day for increase in small intestine dry weight was selected as the most appropriate reference point for the risk characterisation.

Occurrence and dietary exposure assessment for the European population

As no occurrence data on lectins were available in the EFSA Data Warehouse, occurrence data in food were identified by a literature search. Lectins are present in seeds and especially in legume seeds in few mg/g dry weight, a low quantity compared to seed storage proteins. The results reported in the literature are estimated using different methodologies (e.g. ELISA, LC–MS, haemagglutination assay, based on purification yields) and the reliability of the results depends largely on the method used.

Due to the limitations in the toxicological evidence, the Panel considered only PHA for risk characterisation, and thus, dietary exposure assessment was conducted only for PHA. Based on the outcome of the toxicological assessment, acute dietary exposure to PHA was estimated.

The CONTAM Panel selected a study reporting a concentration of 24.9 mg/g of PHA in raw kidney beans as representative of high PHA levels for the acute exposure assessment. This study was selected because the ELISA method quantified only biologically active lectins. The value of 24.9 mg/g of PHA in raw kidney beans was also used as a characteristic high concentration of PHA for all the different types of beans that belong to *Phaseolus* sp. (e.g. runner beans, French beans, etc.).

Acute dietary exposures to PHA were estimated based on consumption data from the EFSA Comprehensive European Food Consumption Database and expressed as mean exposures and high exposures, the latter based on the highest reliable percentiles. Acute dietary exposure was estimated separately for each food item rather than for the total diet and was based solely on consuming days.

Two exposure scenarios were considered. A scenario representing adequate processing practices applied to beans from *Phaseolus* sp. before consumption (e.g. after soaking and boiling). In this scenario, it is assumed that most of the initial lectin activity is eliminated and thus leads to a safe consumption of beans, so no dietary exposure was estimated. The second exposure scenario represented a situation where deviations from adequate processing practices occur, resulting in relatively high levels of active lectins remaining in the processed commodities. To assess this scenario, an arbitrary value of 50% was selected, assuming that only half of the active lectins originally present in the raw primary commodities are inactivated in the processed commodities because of insufficient treatment. Under this exposure scenario, the highest mean exposure estimates would range between 23.5 mg/kg bw per day and 35.0 mg/kg bw per day following the consumption of 'Borlotti or other common beans (dry)' and Lima beans (dry), respectively. For the highest reliable percentile, the exposure estimates would be between 58.9 mg/kg bw per day (97.5th percentile) and 75.8 mg/kg bw per day (99th percentile) via the consumption of 'Borlotti or other common beans (dry)' and 'Beans and vegetables meal', respectively. The highest reliable percentile would be in the population group 'Other children'.

Regarding food processing, thermal and non-thermal treatments are applied to reduce the impact of lectins on human health. However, a sufficiently long cooking time is needed because lectins may be partly resistant to heat inactivation. The effect of soaking on the reduction in lectin content seems to depend on the type of seeds and the duration of water inhibition. Soaking (6–12 h) combined with heat treatment (≥ 100°C, > 30 min) are highly effective conditions for deactivating lectins from plant sources (e.g. PHA present in kidney beans), which usually results in a 90%–100% reduction of the lectin activity. However, since the effect of the soaking time can vary depending on the kidney bean varieties, it is advisable to continue soaking until the beans have softened before cooking. Other cooking processes, home-based (e.g. steaming, microwaving and roasting) or industrial (extrusion and autoclaving), yield varying degrees of reduction in lectin activity.

Considerations for the Risk Characterisation

Due to the limitations and uncertainties in the current evidence, the establishment of a health-based guidance value for PHA was considered not appropriate. Instead, the margin of exposure (MOE) approach was used for the risk characterisation.

The CONTAM Panel considered that MOEs > 100 do not raise a health concern. This MOE would cover variability with respect to kinetic and dynamic differences between animal species and humans and within the human population.

Comparison of the PHA acute exposure estimates for the arbitrary exposure dietary scenario with only 50% deactivation of PHA and the $BMDL_{10}$ of 22.9 mg/kg bw per day resulted in MOEs of about 0.3 at the highest reliable percentile exposure. Since this MOE is below the value considered safe for human health, 50% deactivation of the initial active PHA in foods (as selected in the arbitrary exposure scenario) would raise health concerns. The Panel also noted that exposure to completely deactivated lectins in food prepared following adequate food processing practices would not raise health concerns.

No conclusions could be made for other lectins due to the lack of relevant toxicological data and/or, in some cases, lack of occurrence data.

Uncertainty Analysis

The uncertainty analysis performed resulted in the conclusion that it is extremely likely (with at least 95% certainty) that PHA poses a health concern under the scenario of 50% deactivation. The experts agreed that this conclusion holds for all age groups.

Recommendations

The CONTAM Panel provided recommendations to fill the gaps for a robust risk assessment of lectins.

The development and validation of analytical methods is essential to quantify active and non-active lectins in different food commodities, using certified reference materials. The collection of occurrence data for different lectins using appropriate and comparable analytical methods, considering active and non-active lectins and food processing conditions, will allow a more robust exposure assessment.

Appropriate studies in humans and rodents are needed to address ADME, immunotoxicity and gastrointestinal endpoints, using primarily purified PHA.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background

Lectins are ubiquitous proteins characterised through their ability to bind different types of carbohydrates. Lectins of the plant kingdom, sometimes called phytohaemagglutinins, are naturally occurring proteins that are found in many plants. Some foods that contain higher amounts of lectins include beans, peanuts, lentils, tomatoes, potatoes, eggplant, fruits, wheat and other grains. It is well known that functional lectins from insufficiently prepared legumes can cause adverse human health effects. It is appropriate that EFSA assesses the risk related to the presence of plant lectins in food.

1.1.2 | Terms of reference

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002, the Commission asks EFSA for an assessment of the risk related to the presence of plant lectins in food.

1.2 | Interpretation of the Terms of Reference

The CONTAM Panel considered that the Scientific Opinion should cover lectins from edible plants. Lectins from other non-plant sources, i.e. algal, fungal, bacterial and animal lectins were considered out of scope for this assessment. In addition, closely structurally related molecules such as glucosidases with haemagglutinating activity, lectin-like proteins, type I ribosome-inactivating proteins and lectin binders were considered out of scope for this assessment. Lectins as pharmaceutical formulations along with lectins in feed were also considered out of scope.

The CONTAM Panel decided that this Opinion should address the following points:

- Identify as the scope of the Opinion the types of lectins in edible plants, including herbs used as food, with a special focus on plants that in the edible parts contain higher amounts of lectins;
- Evaluate the analytical methods to identify, quantify and characterise the activity of plant lectins and limitations thereof;
- Evaluate the toxicity of lectins from edible plants and identify toxicity patterns, including antinutritional effects, if any;
- Identify a reference point from toxicological studies and derive a HBGV when applicable;
- Evaluate the occurrence profiles of lectins (i.e. composition and levels) in edible plants from literature data;
- Assess the impact of cooking and processing of food on the activity of plant lectins in food;
- Estimate the dietary exposure of the EU population to plant lectins present in food considering different food preparation scenarios;
- Assess the human health risks for the European population, including specific groups of the population if relevant.

1.3 | Supporting information for the assessment

1.3.1 | Chemistry and physicochemical properties

Plant lectins comprise a vast group of ubiquitous proteins characterised by a large diversity of structural organisation, with various sugar-binding specificities. By definition, plant lectins do not hold any catalytic activity. Defined as proteins involved in the specific and reversible binding to simple and complex carbohydrates, they are widely distributed within the plant kingdom in all plant parts.

1.3.1.1 | *Structural organisation of lectins*

Basically, most plant lectins result from the non-covalent association of monomers with either identical (hololectins) or different (heterolectins) structural domains to form homo- or hetero-dimers or homo- or hetero-tetramers (Van Damme et al., 1998). Depending on the structure of the monomers, up to 13 groups of structurally and phylogenetically related plant lectins have been identified, with varying carbohydrate-binding specificities (Tsaneva & Van Damme, 2020). Only five of these groups are of relevance with respect to their potential risk for human health and will be initially considered in this Opinion for further assessment; these include (1) legume lectins, (2) *Galanthus nivalis* agglutinin (GNA)-related lectins, (3) jacalin-related lectins, (4) hevein-like domain-containing lectins and (5) type 2 ribosome-inactivating proteins (RIPs-2) which contain a lectin chain.

– **Legume lectins**, which consist of dimers like *Lens culinaris* agglutinin (LCA) from lentil (*Vicia lens*, syn. *Lens culinaris*) (Loris et al., 1994), *Pisum sativum* agglutinin (PSA) from pea (*Pisum sativum*) (Prasthofer et al., 1989) and favin from

broad bean (*Vicia faba*) (Reeke Jr. & Becker, 1986) or tetramers like phytohaemagglutinin-L (PHA-L) (Hamelryck, Dao-Thi, et al., 1996) and phytohaemagglutinin-E (PHA-E) (Nagae et al., 2016) from kidney bean (*Phaseolus vulgaris*), concanavalin A (Con A) from jack bean (*Canavalia ensiformis*), soybean agglutinin (SBA) from soybean (*Glycine max*) (Olsen et al., 1997) and peanut agglutinin (PNA) from peanut (*Arachis hypogaea*) (Banerjee et al., 1996). All these lectins are built from the non-covalent association of a monomeric structural scaffold known as the jelly roll structure, which in fact corresponds to a β-sandwich structure made of two β-sheets connected by loops. Each monomer contains a carbohydrate-binding site (CBS) made of few hydrophilic amino acids belonging to the loops connecting both β-sheets, stabilised by two Ca^{2+} and Mn^{2+} ions liganded to some amino acids which participate in the CBS (Table 1A,B). The dimeric lectins result from the symmetrical non-covalent association of two monomers made of a heavy/long (α) and a light/short (β) chain, to form a long β-sandwich $\alpha_2\beta_2$ with two CBS at its extremities (Table 1C). The tetrameric lectins consist of the non-covalent association of four single-chain (α) monomers, each monomer containing a CBS (Table 1D). Tetrameric lectins (α_4) differ by the mode of arrangement of their monomers and display various carbohydrate-binding specificities (Brinda et al., 2005).

- ***Galanthus nivalis* agglutinin (GNA)-related lectins**, which owe their name to the snowdrop (*Galanthus* spp.) tetrameric lectin GNA (Hester et al., 1995) (Table 1G), are mainly represented in this Opinion by the dimers like *Allium sativum* agglutinin (ASA) from garlic (*Allium sativum*) (Chandra et al., 1997; Van Damme et al., 1992), *Allium cepa* agglutinin (ACA) from onion (*A. cepa*) (Van Damme et al., 1998) or *Allium porrum* agglutinin (APA) from leek (*A. porrum*) (Table 1F) (Van Damme et al., 1998) resulting from the arrangement of two monomeric structures with a β-prism II[1] fold. Each β-prism II scaffold, made of three bundles of β-strands linked by loops, contains three carbohydrate-binding sites with a strict high-mannose-binding specificity (Table 1E) (Barre et al., 2001). Accordingly, dimeric lectins and tetrameric lectins of this group consist of hexavalent (6 CBS) and dodecavalent (12 CBS) lectins, respectively. All the so-called monocot bulb lectins belong to the GNA-related lectins group (Barre et al., 2019).
- **Jacalin-related lectins** (JRL) constitute a group of seed lectins occurring in fruits from the Moraceae, including jacalin from jackfruit (*Artocarpus heterophyllus*) (Sankaranayanan et al., 1996), artocarpin from chempedak (*A. integer*) (Pratap et al., 2002) and *Maclura pomifera* agglutinin (MPA) from Osage orange (*Maclura pomifera*) (Huang et al., 2010). They present a tetrameric organisation (Table 1K) of a structure exhibiting a β-prism I[1] fold (Table 1H,I). The lectins differ by the cleavage (two-chain monomer of jacalin) or not (single chain monomer of MPA) and their carbohydrate-binding specificities, Gal/GalNAc-binding specificity for jacalin and MPA versus Mannose-binding specificity for artocarpin. In addition, jacalin exhibits some promiscuity of its monosaccharide-binding specificity since it also accommodates mannose and other monosaccharides (Bourne et al., 2002; Jeyaprakash et al., 2005). Other JRL with a mannose-binding specificity possess a dimeric organisation like BanLec (Table 1J), the banana (*Musa acuminata*) lectin (Meagher et al., 2005), which contains two CBSs in each monomer.
- **Hevein-like domain-containing lectins** are represented by the pokeweed (*Phytolacca americana*) mitogen PWM (Table 1M) (Börjeson et al., 1966) and wheat germ agglutinin (WGA) from wheat (*Triticum aestivum*) (Wright, 1990) and both consist of hevein-like domains. Chitinases of classes I and IV, built from a hevein-like domain linked by an extended loop to a second domain with chitinase activity (Table 1O), also belong to the hevein-like lectins group. Hevein-like domains consist of a polypeptide of 43 amino acid residues exhibiting a β-α-α-β fold and possessing a strong affinity for chito-oligosaccharides (Andersen et al., 1993) (Table 1L). In the dimeric WGA, two monomers built from four hevein-like domains associate to create a multivalent chitooligosaccharide-binding structure (Table 1N).
- **Type 2 ribosome-inactivating proteins (RIP-2)** like ricin from castor bean (non-edible) (*Ricinus communis*) are considered in the Opinion because they are implicated in cases of intentional or accidental ingestion. They are lectins resulting from the covalent association by a single disulfide bridge of two distinct chains: a β-chain, which corresponds to a lectin made of two domains with a β-trefoil fold (Table 1P,Q), and an α-chain containing eight α-helices and a left-handed twist of six β-strands responsible for the toxic RNA *N*-glycosidase activity (Rutenber et al., 1991) (Table 1R). Usually, the lectin chain of RIP-2 contains two carbohydrate-binding sites which may recognise sialylated or non-sialylated Gal/GalNAc[2]-containing complex glycans (Van Damme et al., 1998). The jequirity bean (*Abrus precatorius*) contains another toxic type 2 RIP, abrin, also built from an α and β chain linked by a disulfide bond (Bagaria et al., 2006). In addition to the type 2 RIPs ricin and abrin, castor bean and jequirity bean also contain less toxic lectins, *Ricinus communis* agglutinin (RCA) and *Abrus precatorius* agglutinin (APA), which consist of dimeric type 2 RIPs (ab2) with slightly different amino acid sequences (Olsnes et al., 1974).
- **Other groups of lectins** built from the association of different structural scaffolds have been identified, but as they are not present in foods, they are considered out of scope of this Scientific Opinion.

1.3.1.2 | *Lectin stability*

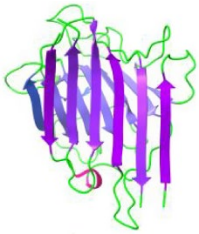
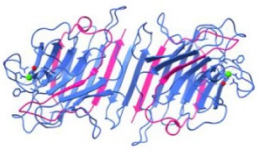
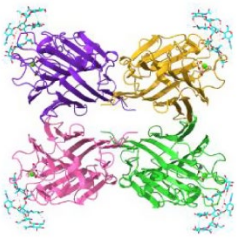
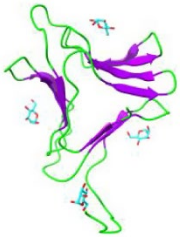
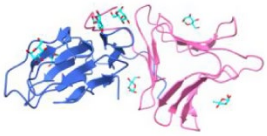
The structural scaffolds that build lectins consist of β-sheets organised in β-sandwich or β-prism structures. These structural β-sheet arrangements observed in monomers display a high degree of stability (Molla & Mandal, 2013), and both the dimeric and tetrameric arrangement of monomers increase the stability of the quaternary structure by creating a high

[1]The β-prism I and β-prism II refer to two different modes of organisation of the β-strands forming the β-prism structure: in β-prism I, the β-strands are oriented parallel to the axis of the prism (elongated prisms of jacalin) whereas in β-prism II, the β-strands are oriented perpendicular to the axis of the prism (flattened prisms of GNA).

[2]Galactose and N-acetylgalactosamine.

number of ionic interactions between the monomers (Sinha et al., 2005). Ionic interactions are the most predominant in the association of dimers or tetramers, but also other hydrophilic interactions, hydrophobic bonds and aromatic stackings are involved. Moreover, the strength of the interactions occurring at the dimeric and tetrameric interface may affect the stabilisation of the lectin structure as in the case of GNA-related lectins (Bachhawat et al., 2001). The stability also depends on the mode of arrangement in tetramers; for example, SBA, which slightly differs from Con A by the arrangement of monomers, exhibits a higher conformational stability than Con A (Sinha & Surolia, 2005). In addition, regarding the effect of sugar moiety on lectin stability, the sugar moiety of SBA enhances the stability of the tetrameric structure, compared to Con A which is not glycosylated (Halder et al., 2016; Nagai & Yamaguchi, 1993). The stabilisation of dimeric and tetrameric GNA-related lectins mainly depends on the strength of interactions occurring at the dimeric and tetrameric interface (Bachhawat et al., 2001).

TABLE 1 Description of the structural scaffolds found in the main groups of food/diet lectins, and their oligomerisation in more complex structures, mostly dimeric or tetrameric. A few structures of non-food lectins (GNA, pokeweed mitogen, ricin) are included to reflect the structural complexity of lectins.

	Lectin structure	PBD code	Lectins	Plant	Description
Legume lectins (b-sandwich structure)					
A		1LEN	LCA monomer	*Vicia lens*	Front view of the lentil lectin monomer which consists of a β-sandwich made of two β-sheets linked by loops (coloured violet and pale violet).
B		1LEN	LCA monomer	*Vicia lens*	Lateral view of the lentil monomer showing the two β-sheets forming the β-sandwich structure (coloured violet and pale violet), the green and red dots correspond to the Ca^{2+} and Mn^{2+} ions which stabilise the CBS of the lectin they indicate the localisation of the CBS at the surface of LCA).
C		1LEN	LCA dimer	*Vicia lens*	Dimer built from two symmetrically disposed monomers, each monomer consists of a light chain (coloured pink) and a heavy chain (coloured blue).
D		5AVA	PHA-E tetramer	*Phaseolus vulgaris*	Homotetramer built from four monomers associated in two dimers. Monomers are coloured yellow, green, pink and violet. The lectin is complexed to bisected N-glycans (coloured cyan) which occupy the four CBS.
GNA-like lectins (β-prism-II structure)					
E		1MSA	GNA monomer	*Galanthus nivalis*	Monomer of GNA with a β-prism II fold, containing three carbohydrate-binding sites CBSs occupied by a mannose residue (coloured cyan).
F		1BWU	ASA dimer	*Allium sativum*	Dimeric organisation of ASA, monomers (coloured pink and blue). Each monomer contains three CBS occupied by mannose (coloured cyan).

(Continues)

TABLE 1 (Continued)

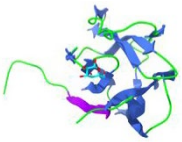
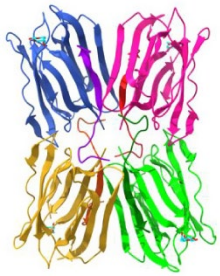
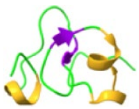
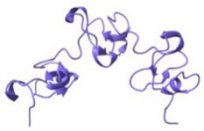
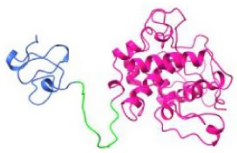
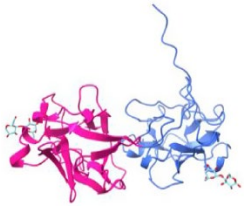
	Lectin structure	PBD code	Lectins	Plant	Description
G		1MSA	GNA tetramer	*Galanthus nivalis*	Tetrameric organisation of GNA, monomers (coloured blue, yellow, green and pink). The 12 (4 × 3) CBS are occupied by a mannose residue (coloured cyan).
Jacalin-related lectins (β-prism-I structure)					
H		1UGW	Jacalin monomer	*Artocarpus integrifolia*	Monomer of jacalin made of three bundles of β-strands associated in a β-prism I fold, lateral view. It contains a single CBS at the top of the monomer occupied by a galactose residue (coloured cyan).
I		1UGW	Jacalin monomer	*Artocarpus integrifolia*	Upper view of the jacalin monomer showing the organisation of the three bundles of β-strands in a β-prism-I structure.
J		3MIU (J)	BanLec	*Musa acuminata*	Dimeric organisation of the BanLec, made of two monomers with a β-prism-I structure (coloured violet and pink), in complex with a pentamannoside (coloured cyan).
K		1UGW	Jacalin tetramer	*Artocarpus integrifolia*	Tetrameric organisation of jacalin, composed of 4 monomers coloured pink, green, yellow and violet, each monomer containing a single CBS.
Hevein-like lectins (chitinases I and IV)					
L		1WKX	Hevein	*Hevea brasiliensis*	Monomer of 43 amino acids with a β-α-α-β fold, with allergenic properties (Hev b 6.02 allergen).
M		1ULK	PWM	*Phytolacca americana*	Organisation of the pokeweed mitogen in three hevein-like domains.
N		2UVO	WGA	*Triticum aestivum*	Dimeric organisation of WGA, made of two tetramers with 4 hevein-like domains.
O		Modelled with alphaFold	Cas s 5 allergen from chestnut	*Castanea sativa*	Allergen (Class I chitinase) made of a N-terminal hevein-like domain (coloured blue), linked by a loop (coloured green) to an active C-terminal domain with chitinase activity (coloured pink).

TABLE 1 (Continued)

Lectin structure	PBD code	Lectins	Plant	Description
Type II RIPs (β-chain)				
P 	Modelled with alphaFold	Ricin β-chain	*Ricinus communis*	Upper view of a β-trefoil domain containing a single CBS, showing the organisation in three bundles of β-strands.
Q 	3RTJ	Ricin β-chain	*Ricinus communis*	β-chain built from two β-trefoil domains, each containing a CBS complexed to lactose [Gal(β1,4)Glc)] (coloured cyan).
R 	3RTJ (R)	Ricin	*Ricinus communis*	Type 2 RIP made of a a-chain (coloured violet) covalently linked by a disulfide bridge (coloured green) to the β -chain (coloured pink) containing two CBS complexed to lactose (coloured cyan).

Abbreviations: CBS, carbohydrate-binding site; PDB, Protein Data Bank unique accession or identification code; RIP, ribosome-inactivating protein.

1.3.1.3 | *Lectin biosynthesis*

Lectin biosynthesis starts with monomers that are synthesised in the endoplasmic reticulum, eventually glycosylated in the Golgi apparatus, and finally accumulate in the vacuoles where they associate to form dimeric or tetrameric structures. Pulse-chase experiments with ^{14}C-labelled amino acids and subcellular fractionations indicated that PSA, the pea (*Pisum sativum*) two-chain lectin, is first synthesised as a precursor pro-lectin of 23 kDa in the endoplasmic reticulum, which further migrates in the protein bodies where it is processed to its mature form made of non-covalently bound light (6 kDa) α-chain and heavy (17 kDa) β-chains (Higgins et al., 1983).

The association of monomers into tetramers has been studied in detail for soybean lectin, SBA, which is in fact a dimer of dimers (Figure 1). The monomers are dimerised via interfaces between polar residues (canonical interfaces) that are different from those ensuring tetramerisation of the dimers (non-canonical interfaces) (Figure 1) (Sinha & Surolia, 2005). Ionic interactions predominantly ensure the stability of all these oligomeric structures. The monomers of mannose-specific dimeric lectins from the Fabeae tribe, e.g. LCA or PSA, associate via canonical interfaces to form the 12-stranded contiguous sheet described as the canonical mode of legume lectin dimerisation (Chatterjee & Mandal, 2005).

Many lectins, and especially legume lectins, are synthesised and processed in the vacuoles of seed cells, which become progressively dehydrated during the seed maturation process to give the protein bodies in mature seeds (Higgins et al., 1983). The flattened shape of the lectin dimers and tetramers, by enabling easy stacking of lectins in protein bodies, will favour their accumulation during the seed ripening process. The localisation of lectins in the protein bodies of mature seeds, and especially legume seeds, has been demonstrated by immuno-histochemical techniques using anti-lectin antibodies as primary antibody and a secondary antibody either peroxidase-labelled (Van Driessche et al., 1981) or coupled to gold particles (Boisseau et al., 1985; Díaz et al., 1990; Greenwood et al., 1984; Herman & Shannon, 1984; Manen & Pusztai, 1982).

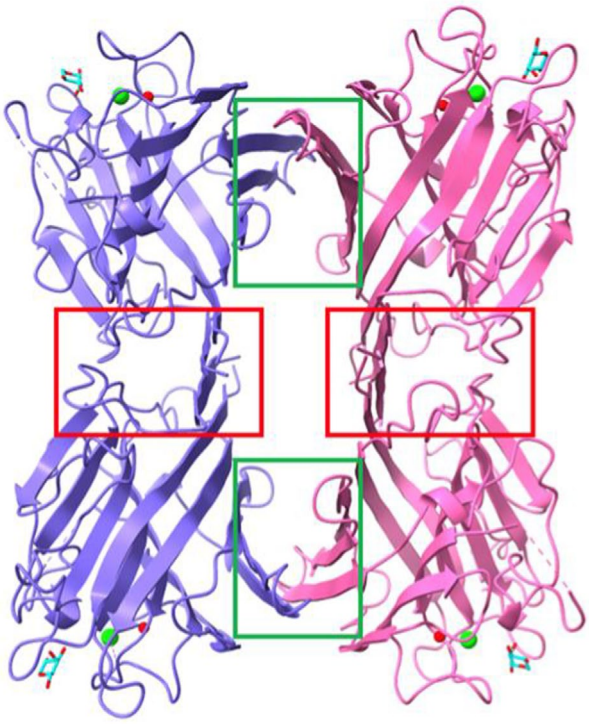


FIGURE 1 Tetrameric organisation of SBA (PDB code 1SBE): Two dimers (violet and pink), each resulting from the side-by-side association ('canonical interfaces' – red frames) of the six-stranded back faces of two β-sandwich monomers to form a 12-stranded β-sheet dimer, associate face-to-face via the outermost strands of their six-stranded back faces ('non-canonical interfaces' – green frames) to form a tetramer, which is in fact a dimer of dimers. The CBS are occupied by galactose residues (cyan). The Ca^{2+} (green dot) and Mn^{2+} (red dot) ions stabilising the CBSs are represented.

1.3.1.4 | *Carbohydrate-binding site (CBS) and carbohydrate-binding specificity of lectins*

Regardless of their structural organisation, the specific recognition of either N- or O-glycans by their CBS will play an important role in the activity and possible deleterious effects of dietary lectins on human health. The activity of lectins depends on the occurrence of CBS in the different structural scaffolds building the dimeric and tetrameric lectins from the different groups of lectins. The association of monomers into dimers or tetramers preserves the accessibility of the CBS(s) carried by each monomer. Although the overall structure, shape, size and specificity of the CBSs differ from one lectin group to another, they nevertheless share similar characteristics such as the ability to bind simple and complex glycans and to agglutinate red blood cells.

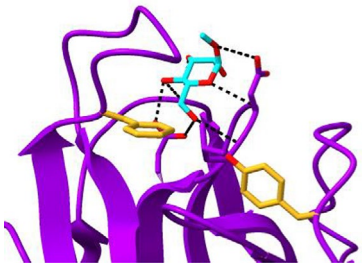
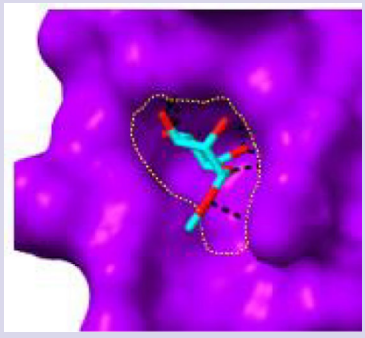
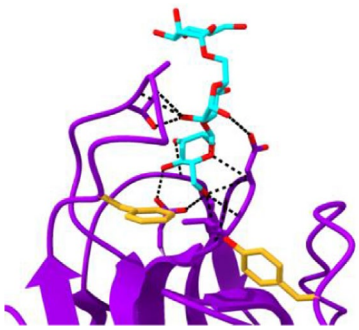
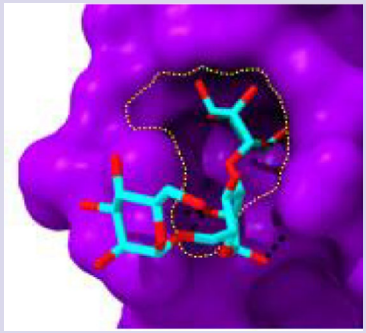
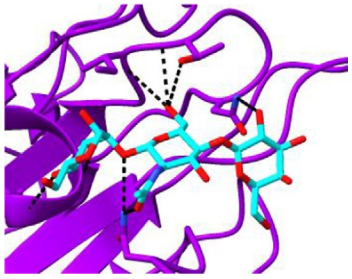
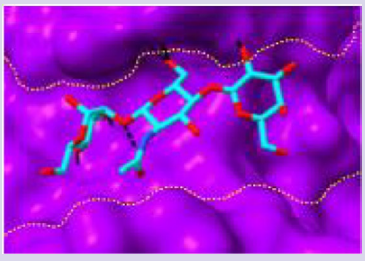
Usually, the CBS corresponds to an area of variable shape and size on the surface of the lectin monomer, which often possesses a shallow cavity in the central region capable of recognising and binding a monosaccharide. The shallow cavity corresponds to the monosaccharide-binding site of the lectin, responsible for the broad sugar-binding specificity, e.g. mannose-specific or galactose-specific lectins. A network of hydrogen bonds created between a few hydrophilic and often charged amino acids forming the CBS and the hydroxyl groups of the pyranose ring of the monosaccharides anchor the sugar into the site. A stacking interaction between the pyranose ring of the sugar and aromatic residue(s) (phenylalanine, tyrosine, tryptophan) located in the vicinity of the monosaccharide-binding pocket often completes the binding (Bourne et al., 1990; Naismith et al., 1994; Pratap et al., 2002; Ravishankar et al., 1999) (Table 2A,B).

The overall CBS recognises and binds complex glycan chains and corresponds to the extended binding site of the lectins, responsible for their fine sugar-binding specificity, e.g. the recognition of complex type N-glycans or high-mannose type glycans by the lectins. The shape and size of the CBS explain the extreme diversity of glycan structures that can be specifically recognised by lectins from the different groups. The interaction of complex glycan chains with the extended CBS involves a greater number of hydrogen bonds and stacking interactions (Bourne et al., 1992; Jeyaprakash et al., 2004; Rani et al., 1999) (Table 2C,D).

As a notable exception, PHA from kidney bean differs from most other lectins in that it is not readily inhibited by monosaccharides, e.g. Gal and GalNAc (Kornfeld & Kornfeld, 1970). A biantennary galactosylated N-glycan with bisecting GlcNac (see Appendix A) was identified by Cummings and Kornfeld (1982) as the best inhibitor for the PHA-E erythroagglutinating activity (Table 2E,F).

The affinity of lectins for simple sugars, e.g. for mannose or galactose, is far weaker compared to the affinity measured for complex type or high-mannose type N-glycans (Debray et al., 1981; Kornfeld & Ferris, 1975). Depending on the fine specificity of their CBS, lectins from the different groups, and especially legume lectins specific for complex and high-mannose type N-glycans present in many foods and food products, will play a prominent role in the recognition and binding to the different glycans decorating the cells from the digestive tract. In addition, the number of CBS may play a role in the functionality of lectins. When comparing bivalent lectins (2 CBS) to tetravalent lectins (4 CBS), the ability of tetrameric lectins to bind to glycan chains is often greater than that of dimeric lectins because of an enhanced avidity.

TABLE 2 Carbohydrate-binding sites of lectins.

	Lectin structure	PBD code	Lectin	Plant	Description
A		1J4U	Artocarpin	*Artocarpus heterophyllus*	Cartoon showing the network of hydrogen bonds (black dashes) anchoring Man (cyan) to the amino acids forming the mono-saccharide-binding site/pocket of artocarpin. Residues Y62 and F86 (orange) interact with Man via a stacking interaction.
B		1J4U	Artocarpin	*Artocarpus heterophyllus*	Molecular surface of artocarpin showing Man anchored by hydrogen bonds (black dashes) to the shallow monosaccharide-binding site/pocket (yellow dots).
C		1VBO	Artocarpin	*Artocarpus heterophyllus*	Cartoon showing the interaction of trimannoside Man3 with the CBS of artocarpin via a network of hydrogen bonds (black dashes) and stacking interactions with Y62 and F86 (orange) with two out of the three Man residues of Man_3.
D		1VBO	Artocarpin	*Artocarpus heterophyllus*	Molecular surface of artocarpin showing that the monosaccharide-binding site/pocket (yellow dots) participates in the binding of a Man residue of the trimannoside Man_3.
E		3WCS	PHA-E	*Phaseolus vulgaris*	Cartoon showing the interaction via a network of hydrogen bonds (black dashes) of the trisaccharide Galβ1-4GlcNAcβ1-2Man (cyan) from a biantennary galactosylated N-glycan with bisecting GlcNac with the CBS of PHA-E from kidney bean.
F		3WCS	PHA-E	*Phaseolus vulgaris*	Molecular surface of PHA-E showing the anchorage of the trisaccharide Galβ1-4GlcNAcβ1-2Man (cyan) via a network of hydrogen bonds (black dashes) to the extended CBS (yellow dots) of PHA-E.

Abbreviations: CBS, carbohydrate-binding site, Man, mannose; PDB, Protein Data Bank unique accession or identification code.

1.3.1.5 | *Closely structurally related molecules*

Kidney beans also contain closely structurally related molecules like the α-amylase-inhibitor a-AI1 (Bompard-Gilles et al., 1996) and arcelin, an insecticidal protein which only occurs in pinto bean, a cultivated variety of kidney bean (Hamelryck, Poortmans, et al., 1996; Mourey et al., 1998). Both these PHA-related proteins exhibit a three-dimensional structure similar to that of PHA-E and PHA-L but differ by the lack of one (arcelin) or two (aAI1) loops in the vicinity of the carbohydrate-binding site that abolishes their sugar-binding properties (Rougé et al., 1993). However, the α-amylase inhibitory activity is not restricted to PHA-related proteins since a dimeric fetuin-specific lectin with an unusual β-propeller structure isolated from chickpea seeds (*Cicer arietinum*) (Kumar et al., 2015) presents an inhibitory activity towards various α-amylases (Wang, Chen, et al., 2017).

1.3.2 | Sampling and methods of analysis

1.3.2.1 | *Methods for isolation and purification of plant lectins*

Although a number of purified plant lectins are nowadays available from commercial sources, in many studies, especially those requiring larger amounts of lectins, the lectins have been isolated in-house from the specific plant material and purified using methodologies that have proven effective for the specific classes of lectins. In this section, an overview is presented of the most commonly applied techniques for the preparative isolation and purification of lectins from plant matrices. To obtain highly purified lectins, while maintaining the quaternary structure and thus carbohydrate-binding activity of the lectins, in many cases multiple steps and a combination of different techniques are required (Nascimento et al., 2012; Pohleven et al., 2012). This text is provided as supporting information to the evaluation of the purity of lectins in the animal toxicity studies.

Extraction of plant matrices

The first step in the isolation of lectins is the preparation of an aqueous extract from the plant material. Typically, this involves the extraction of freshly ground material with buffered aqueous solutions (typically PBS or Tris buffers). In the process, other biomolecules as well as many plant metabolites are coextracted, and these need to be removed in subsequent purification steps, which may vary across lectin.

Affinity chromatography

Since the discovery by Agrawal and Goldstein (1967) of the retention of Con A by a Sephadex column and its subsequent elution, in a pure form, by a glucose solution, affinity chromatography has become a cornerstone in the purification of lectins. Affinity chromatography makes use of the high specificity of lectins to bind to certain carbohydrates. A wide array of carbohydrate affinity columns is nowadays available that contain specific mono, di- or polysaccharide ligands immobilised to a solid support made of polymers (e.g. agarose, Sepharose, dextran, chitin) (Calderón de la Barca et al., 1991; Jiang et al., 2019; Rameshwaram & Nadimpalli, 2008). Typical ligands include mannose, galactose, lactose, glucose, rhamnose. There are also protocols available for custom preparation of columns with specific immobilised ligands. Columns containing more complex ligands such as the glycoproteins (asialo)fetuin, mucin and ovalbumin are also used (Pohleven et al., 2012; Rizzi et al., 2003). The binding of lectins to simple carbohydrates is relatively weak and the lectins can be released by adding a solution of the corresponding carbohydrate in excess. When complex ligands are used, this results in much stronger affinity with the lectin, and then, more stringent conditions such as high or low pH or high molarity salt buffers are required to release the lectins (Pohleven et al., 2012). Limitations of affinity chromatography are sensitivity to impurities present in the extract to be applied, and the inability to separate lectins with similar specificity (including isoforms). A second affinity column with a different carbohydrate specificity can be used (Pohleven et al., 2012). In Appendix A, some examples are provided of lectin – affinity ligand combinations that have been described in the literature.

Ion exchange chromatography (IEC)

In IEC, the resin contains chemically bonded ionisable functional groups that are either positively (for anion exchange) or negatively (for cation exchange) charged. The method makes use of the difference in charge properties and ion exchange capacity of proteins. The net charge of the proteins (including lectins) is a function of the pH and elution can be achieved by changing the pH of the column or by increasing the ionic strength by adding a salt solution (Desmiaty et al., 2024; Naik et al., 2017).

Gel filtration

In gel filtration (or size exclusion chromatography), proteins are primarily separated on the basis of their molecular size. Larger proteins will experience less retention on the column and are eluted earlier. Typically, columns based on dextran

(Sephadex), agarose (Sepharose) or polyacrylamine (Bio-Gel) are used with different pore sizes (Nascimento et al., 2012). Gel filtration can also be used for desalting of lectin fractions.

Dialysis

Dialysis is used to remove small molecules (metabolites), carbohydrates and salts that may interfere with the haemagglutination assay and/or spectroscopic analysis. Typically, dialysis membranes with cut-offs from 2 kDa to 20 kDa are used.

The efficacy of the various purification steps is typically monitored with the haemagglutination assay and is expressed as a purification factor compared to the crude extract (Campos et al., 1997; Naik et al., 2017). The presence of other proteins in the final lectin product can be checked with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). In PAGE, proteins are separated based on their net molecular charge under an electric field. However, when the proteins have been denatured with SDS, separation is primarily based on the difference in molecular weight (Hearn et al., 1982; Naeem et al., 2006). In this way, SDS-PAGE can also be used to estimate the molecular weight of novel lectins.

1.3.2.2 | *Analytical methods for detection and quantification of plant lectins*

Some methods described above for the preparative purification of lectins are less suited for the efficient quantification and high-throughput analysis of lectins in biological matrices and foods. For the determination of plant lectins in biological matrices, a number of different techniques are used. The Panel notes that no certified reference materials are available.

Enzyme-linked immunosorbent assay (ELISA)

In the enzyme-linked immunosorbent assay (ELISA), lectin-specific monoclonal or polyclonal antibodies are used to capture and/or detect the lectin. The results from ELISA depend on the antibodies used (polyclonal or monoclonal) and specificity; this determines whether the antibodies bind to the active lectins exclusively and/or inactive lectins and/or glycoprotein bound lectin. In particular, sandwich type ELISA methods show very high sensitivity, enabling their use for the detection of intact lectins such as WGA in biological matrices including serum (LOQ: 10 ng/mL) (Güll et al., 2007). A commercial sandwich ELISA for SBA reported an LOQ of 31.25 pg/mL in 100-fold diluted soybean extract (Breeze et al., 2015).

Boniglia et al. (2003) described an ELISA method in which PHA from kidney bean protein was selectively trapped by thyroglobulin coated on microtitre plates. Detection was performed with rabbit anti-PHA IgG. Thermally denatured PHA did not elicit an ELISA response, showing that the test was specific for PHA in its active form. The reported sensitivity was 30 µg/g and a good correlation with the haemagglutination assay was obtained (Boniglia et al., 2003). In a modified version of this ELISA, fetuin coated on the microtitre plates was used to bind PHA present in raw and processed kidney beans (Nciri et al., 2015).

Vincenzi et al. (2002) developed ELISAs for WGA, PNA and Con A using specific glycoproteins coated on microtitre plates to selectively bind the lectins (asialofetuin for PNA and Con A and ovalbumin for WGA). Lectin-specific antibodies were used to detect and quantify the respective lectins. The reported sensitivity was 30 ng/mL for WGA and 80 ng/mL for PNA and Con A in solution. When applied to extracts of wheat germ and roasted peanuts, a good correlation was observed with results obtained with an agglutination assay (Vincenzi et al., 2002). The same approach was used for SBA (asialofetuin was selected to bind the lectin), and then, ELISA was used for the quantification of SBA in soybean sprouts as well as some soy-containing food products (Rizzi et al., 2003). Interestingly, the use of glycoproteins for capturing lectins means that only active lectins are quantified because they have functional CBS capable of interacting with the corresponding glycoproteins, while denatured lectins would not be detected.

A competitive ELISA for the quantification of *Lens culinaris* agglutinin (LCA) in lentils was developed by Cuadrado et al. (2002). Application in the natural fermentation of lentils showed a strong decrease in LCA content in line with the results of a haemagglutination assay run in parallel.

Enzyme-linked lectin assay (ELLA)

The enzyme-linked lectin assay (ELLA) is similar to ELISA, with the difference being that polyacrylamide-linked carbohydrates are used instead of antibodies for the specific binding and detection of the lectin. Breeze et al. (2015) developed and validated an ELLA method for SBA using α-GalNAc-PAA as SBA-specific binding carbohydrate. The ELLA assay was 10,000-fold less sensitive (0.375 µg/mL) compared to a commercially available ELISA assay for SBA (31.25 pg/mL), but the assay was shown fit for purpose for the quantification of SBA in a set of commercial soybean varieties available at the US market.

Liquid chromatography coupled with mass spectrometry (LC-MS)

Mass spectrometry-based techniques are not commonly used in the quantification of lectins in food or food products. This is linked to the high molecular weight of the lectins, which can exceed the limited mass range of most LC-MS instruments. However, under acidic conditions, lectins, like other proteins, become multicharged, which reduces their mass-to-charge (m/z) ratio, allowing their apparent mass to fit within the range of the LC-MS detector. By deconvolution of the mass signals, the molecular weight of the lectins can be calculated (Anta et al., 2010; Castro-Rubio et al., 2007). It is noted that LC-MS quantifies both active and denatured lectins.

Capillary electrophoresis-mass spectrometry (CE-MS) has been successfully applied for the analysis of *Urtica dioica* agglutinin (UDA) isolectins from root samples of stinging nettle, after affinity chromatographic purification on a tailored Sepharose-4B column. LODs or LOQs were not specified but can be estimated from the presented data to be in the order of 30 µg/g (Ganzera et al., 2005). Con A was measured in spiked bean extracts using CE-MS after aptamer-affinity extraction and SPE concentration. A limit of detection (LOD) of 2.5 µg/g was reported (Vergara-Barberán, Simó-Alfonso, et al., 2023).

In matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS), the molecular weight of the lectin does not limit its detectability. One potential application to food analysis was published recently by Vergara-Barberán, Catalá-Icardo, et al. (2023) and described the analysis of Con A in a number of spiked extracts of different bean species using MALDI-TOF-MS in combination with aptamer-based selective extraction. The LOD and LOQ of the method were 5 and 15 µg/g in bean matrix, respectively.

Instead of analysing the intact lectin, LC-MS/MS can be used for the analysis of peptide tryptic digests of the lectin. Quantification can be achieved by using synthetic isotopically labelled marker peptides. This approach has been successfully used for the analysis of allergenic (non-lectin) proteins in soybeans (Hill et al., 2017). Wen et al. (2021) applied the approach for the quantification of SBA in a collection of soybeans. After defatting, aqueous extraction and tryptic digestion of the isolated protein residue, nano-LC-Q-Exactive-MS was used. An LOD of 35.5 µg/g in soybean was reported. Recently, Li et al. (2024) reported a quantitative UHPLC-MS/MS method for PHA-L and PHA-E in common beans based on tryptic digestion and isotope dilution mass spectrometry (IDMS) of two signature and one common peptide. The authors reported LODs and LOQs in the range of 2.3–12.3 ng/g and 7.7–40.8 ng/g, respectively, which were considerably lower than other reported values.

Surface Plasmon Resonance (SPR)

In surface plasmon resonance (SPR), the binding of lectins to a sugar ligand immobilised on a biochip is measured with a laser. Because lectins have different affinities and specificities to ligands, an array of sugar-coated biochips can be used to differentiate between lectins and obtain a (semi-)quantitative concentration. For Con A, an LOD of 0.5 µg/mL solution was obtained and a reported limit of determination (considered by the CONTAM Panel corresponding to the limit of quantification) of 10 µg/mL. No experiments with legume extracts were carried out; thus, it is not known how the method performs with real samples (Vornholt et al., 2007). Methods deploying SPR for the detection in biological samples of the lectins, ricin and abrin, have been described with detection limits as low as 0.6 ng/mL (Luo et al., 2022; Stern et al., 2016).

Biosensors

Biosensors are electrochemical devices that function as an electrochemiluminescence platform for the detection of specific macromolecules including lectins. When properly designed and optimised, biosensors can detect specific lectins at very low concentrations (pg/mL amounts or less) (Sha et al., 2019; Wang, Zhong, et al., 2017). An advantage is that the method is relatively fast and can generate quantitative results (Fan et al., 2016). A rapidly increasing number of specifically designed electrodes has been described in recent years (Li et al., 2023). In most of these publications, the focus is on Con A and the area of application is in clinical applications, such as the detection of Con A in biological samples including serum. An application for the detection of SBA spiked in serum has been described as well (Liu et al., 2019).

Applications of biosensors in food analysis have not been described thus far.

1.3.2.3 | *Methods for determination of activity of plant lectins*

Haemagglutination assay

The haemagglutination assay is one of the most commonly used tests for confirming lectin activity. It is a semi-quantitative test performed in a standard 96-well plate, in which a lectin solution is diluted in sequential steps of 2, starting with the pure lectin. A suspension of red blood cells is added to each well, and after shaking of the plate to promote mixing, it is left to stand at room temperature and then placed in the cold (4°C) before reading the results. Positive haemagglutination results in a pinkish disc occupying all or part of the surface of the well. The absence of haemagglutination is indicated by a red dot in the centre of the well, which corresponds to non-agglutinated red blood cells settling at the bottom of the well. The last well-showing positive haemagglutination is used to calculate the titre, which corresponds to the inverse of the last dilution yielding a positive haemagglutination result or the haemagglutinating units (HU) if the lectin/protein concentration of the starting solution is known (e.g. 300 HU/mg protein or dw seed) (Adamcová et al., 2021; Grant et al., 1983). Since changes in both the ionic strength and pH can interfere with the oligomeric state of tetrameric proteins, the buffer solution used for haemagglutination assays should be carefully selected. As a rule, haemagglutination assays, whatever the type of RBCs used, must be performed with a phosphate-buffered saline solution (150 mM) with a pH in the range between pH 7.2 and pH 7.5 or in other buffered tris (TBS) saline solutions commonly used for the storage of the assayed lectin. This is to avoid any unwanted change in the oligomeric state of the lectin and an abnormal binding capacity of the RBCs (RBCs crenellated in hypertonic buffer, RBCs either swollen or lysed in buffer that is too hypotonic) used in the haemagglutination test. Moreover, a low ionic strength can cause some haemolysis of RBCs which completely masks haemagglutination.

The sensitivity and specificity of the haemagglutination assay depends on a number of factors such as the type of red blood cells used [human (type A, B and O; note that different type of human erythrocytes may show different activity) or

animal (e.g. rat, rabbit)] and whether the red blood cells have been trypsin treated (TT) or not (NTT). Trypsin treatment may make the cells more susceptible to the agglutination potency of specific lectins, although this is not a general rule for all lectins. The degree of purity of the lectins is also very important: non-purified extracts of plant organs (seeds, roots, stems, leaves, etc.) are often rich in compounds that are capable of interfering with haemagglutination (polyphenolic compounds, tannins, heterosides, etc.) or contain haemolytic compounds (saponins). For all these reasons, it is often difficult to compare results obtained for the same lectin in different laboratories (Adamcová et al., 2021). The agglutination assay can be automated by using a microplate reader (Gulzar et al., 2023).

The higher number of CBSs together with a more versatile distance between two adjacent CBSs observed in tetrameric lectins, compared to dimeric lectins, should explain both the higher affinity and avidity of tetrameric lectins for cell surface glycans (for further information, see Section 1.3.1.4).

Modifications of the classical haemagglutination assay include the latex agglutination assay, in which latex beads coated with glycoproteins containing specific carbohydrate functional groups (e.g. ovalbumin, fetuin, mucin, horseradish peroxidase) are used to bind the lectin (Kaul et al., 1991).

A brush border lectin agglutination assay (BBLAA) has been developed which measures the capability of lectins to agglutinate the intestinal brush border membrane, which is the target membrane of the intestinal epithelium (Irish et al., 1999).

1.3.3 | Sources

Of particular relevance to the present risk assessment are three different situations. First, the lectins that remain after processing of plants with high lectin content, mainly legumes. Second, the lower amounts of lectins active when consumed in raw fruits and vegetables. Third, the lectins present in low concentration in food commonly consumed in high amounts (e.g. wheat-based food). In the section, the baseline (before processing) lectin content of these food categories will be discussed.

As discussed above, plant seeds can be a major source of lectins, e.g. PHA from kidney bean (*Phaseolus vulgaris*), SBA from soybean (*Glycine max*) or PNA from peanut (*Arachis hypogaea*) (Liener, 1986). However, pronounced differences can occur in the lectin content of legume seeds as shown from a survey of the relative concentration of lectins measured by semi-quantitative haemagglutination of erythrocytes of various origins, in legume seeds consumed in the UK (Grant et al., 1983). The highest lectin concentrations in unprocessed legume seeds were measured in runner bean (*P. coccineus*), red and white kidney bean (*P. vulgaris*) and tepary bean (*P. acutifolius*), while other beans like lima bean (*P. lunatus*), mung bean (*Vigna radiata*; syn *P. aureus*) and adzuki bean (*Vigna angularis*; syn *P. angularis*) or pinto bean (*P. vulgaris*) contained lower lectin amounts. Low amounts of lectins are contained in other legumes, as well, such as lentil (*Vicia lens*), pea (*Pisum sativum*), winged bean (*Psophocarpus tetragonolobus*), chickpea (*Cicer arietinum*), black-eyed pea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*), broad bean (*Vicia faba*) and soybean (*Glycine max*).

Besides seeds, many other plant organs like the edible bulbs from monocot plants and the edible fruits from many plants also contain appreciable amounts of lectins and should be considered as relevant sources of dietary lectins. Typical examples are lectins from the bulbs of monocot plants, like ASA from garlic (*Allium sativum*), ACA from onion (*A. cepa*) or APA from leek (*A. porrum*) (Van Damme et al., 1998). Lectin chitinases of classes I and IV are present in a variety of fruits like chitinase I from kiwifruit (*Actinidia chinensis* var. *deliciosa*; syn. *Actinidia deliciosa*) (Díaz-Perales et al., 1998) or chitinase I from tomato (*Solanum lycopersicum*) (Díaz-Perales et al., 1999), which contain an active hevein-like domain. In addition, some fruits from the Moraceae, such as jackfruit (*Artocarpus heterophyllus*), Cempedak (*Artocarpus integer*) or osage orange (*Maclura pomifera*), contain highly reactive jacalin-related lectins in their seeds but not in their flesh parts (Van Damme et al., 1998). The level of lectin contamination in food preparations containing plants where lectins are present in the seeds but not in the flesh is unknown.

Finally, non-edible plants and/or plant parts can be the source of lectins in the following situations: (a) accidental intake, (b) intentional intake with the purpose of self-harm, (c) intentional intake as herb infusions. RIP-2s such as ricin from castor bean (*Ricinus communis*) (Van Damme et al., 1997) is an example of such uses (Stirpe, 2004).

Lists of food plants and plant products containing appreciable amounts of potentially toxic lectins have been published. From these lists and other semi-quantitative data retrieved from different studies (Grant et al., 1983; Grant et al., 1995; Hossaini, 1968; Krüpe, 1956; Nachbar & Oppenheim, 1980; Thompson, 2019), Table 3 lists the most relevant edible plants and plant organs that may pose a potential risk to human health.

As lectin concentrations are affected by the ripening status, the Panel notes that changes in environmental conditions may affect the concentration of lectins in plants (Tsaneva & Van Damme, 2020; Zandalinas et al., 2021).

TABLE 3 Structural organisation, localisation in plants and specificity of lectins occurring in foods and food products.

Lectin domain structure	Domain association	Plant species (accepted plant species and synonyms as reported in the original article)	Plant family	Lectin name	Localisation in plant	Mono-saccharide-binding specificity	Glycan-binding specificity	Reference
β-sandwich (legume lectins)	Dimeric (a_2b_2)	Lentil (*Vicia lens;* syn. *Lens culinaris*)	Fabaceae	LCA	Seed	Man	Complex + high mannose glycans	Debray et al. (1981), Liener (1986), Loris et al. (1994)
		Pea (*Pisum sativum*)	Fabaceae	PSA	Seed	Man	Complex + high mannose glycans	Debray et al. (1981), Einspahr et al. (1986), Liener (1986)
		Grass pea/White vetch (*Lathyrus sativus*)	Fabaceae	LSL	Seed	Man	Complex + high mannose glycans	Debray and Rougé (1984), Liener (1986), Sletten and Kolberg (1983)
		Faba bean/broad bean (*Vicia faba*)	Fabaceae	Favin	Seed	Man	Complex + high mannose glycans	Debray et al. (1981), Liener (1986), Reeke Jr. and Becker (1986)
		Chickpea (*Cicer arietinum*)	Fabaceae	CAL	Seed	GalNAc	Fetuin	Gautam et al. (2018)
	Tetrameric (a_4)	Kidney bean (*Phaseolus vulgaris*) Runner bean (*P. coccineus*) Tepary bean (*P. acutifolius*) Azuki bean (*Vigna angularis*; syn. *P. angularis*) Mung bean (*Vigna radiata*; syn. *P. aureus*)	Fabaceae	PHA	Seed	Gal/GalNAc*	Complex type glycans	Grant et al. (1983), Hamelryck, Dao-Thi, et al. (1996), Krüpe (1956), Liener (1986), Suseelan et al. (1997), Valadez-Vega et al. (2011), Yamashita et al. (1983)
		Lima bean (*P. lunatus*)	Fabaceae	LBA	Seed	Gal/GalNAc*	Complex type glycans	Sparvoli et al. (2001)
		Soybean (*Glycine max*)	Fabaceae	SBA	Seed	Gal/GalNAc	Group A substance	Debray et al. (1981), Hossaini (1968), Liener (1986), Nachbar and Oppenheim (1980), Olsen et al. (1997), Pereira et al. (1974)
		Peanut (*Arachis hypogaea*)	Fabaceae	PNA	Seed	Gal/GalNAc/T	Asialo-glycophorin and asialo-fetuin, galactosides, O-glycans	Banerjee et al. (1996), Lotan et al. (1975), Liener (1986), Nachbar and Oppenheim (1980), Ravishankar et al. (1999)
		Jack bean (*Canavalia ensiformis*)	Fabaceae	Con A	Seed	Man/Glc	Complex + high mannose glycans	Debray et al. (1981), Kornfeld and Ferris (1975), Naismith et al. (1994)
		Horse gram (*Macrotyloma uniflorum; syn. Dolichos biflorus*)	Fabaceae	DBA	Seed	Gal/GalNAc	Complex glycans	Etzler et al. (1981), Hamelryck et al. (1999), Hossaini (1968), Liener (1986)

TABLE 3 (Continued)

Lectin domain structure	Domain association	Plant species (accepted plant species and synonyms as reported in the original article)	Plant family	Lectin name	Localisation in plant	Mono-saccharide-binding specificity	Glycan-binding specificity	Reference
β-prism II (GNA-like lectins)	Dimeric	Garlic (*Allium sativum*)	Amaryllidaceae	ASA	Bulb	Man	High-mannose glycans	Chandra et al. (1999), Nachbar and Oppenheim (1980)
		Leek (*Allium porrum*)	Amaryllidaceae	APA	Bulb	Man	High-mannose glycans	Liener (1986), Van Damme et al. (1993)
		Onion (*Allium cepa*)	Amaryllidaceae	ACA	Bulb	Man	High-mannose glycans	Van Damme et al. (1993)
		Shallot (*Allium ascalonicum*)	Amaryllidaceae	AAA	Bulb	Man	High-mannose glycans	Van Damme et al. (1993)
		Banana (*Musa acuminata*)	Musaceae	BanLec	Fruit	Man	High-mannose glycans	Liener (1986), Meagher et al. (2005), Winter et al. (2005)
β-prism I (Jacalin-related lectins)	Tetrameric (a_4)	Jackfruit (*Artocarpus heterophyllus;* syn. *Artocarpus integrifolius var. heterophyllus*) Chempedak (*Artocarpus integer*)	Moraceae	Jacalin	Seed	Gal/GalNAc/T	Complex glycans and O-glycans	Sankaranayanan et al. (1996)
		Jackfruit (*Artocarpus heterophyllus;* syn. *Artocarpus integrifolius var. heterophyllus*)	Moraceae	Artocarpin	Seed	Man	Complex + high mannose glycans	Jeyaprakash et al. (2004), Barre et al. (2004), Debray et al. (2009), Pratap et al. (2002)
		Osage orange (*Maclura pomifera*)	Moraceae	MPA	Seed	Gal/GalNAc/T/ Forssman disaccharide	Complex glycans and O-glycans, group A + H substance	Huang et al. (2010), Lee et al. (1998)
Hevein-like	Hevein-like domain + Chitinase domain	Avocado (*Persea americana*)	Lauraceae	Chitinase I	Fruit	GlcNAc, SIA	(Sialo) chitooligosaccharides	Díaz-Perales et al. (1998), Díaz-Perales et al. (1999)
		Custard apple (*Annona cherimola*)	Annonaceae	Chitinase I	Fruit	GlcNAc, SIA	(Sialo) chitooligosaccharides	Gamboa et al. (2005)
		Banana (*Musa acuminata*)	Musaceae	Chitinase I	Fruit	GalNAc, SIA	(Sialo) chitooligosaccharides	Sánchez-Monge et al. (1999)
		Chestnut (*Castanea sativa*)	Fagaceae	Chitinase	Fruit/Seed	GlcNAc, SIA	(Sialo) chitooligosaccharides	Díaz-Perales et al. (1998), Díaz-Perales et al. (1999)
		Eggplant (*Solanum melongena*)	Solanaceae	Chitinase	Fruit	GlcNAc, SIA	(Sialo) chitooligosaccharides	Gamboa et al. (2005)
		Kiwifruit (*Actinidia chinensis* var. *deliciosa;* syn. *A. deliciosa*)	Actinidiaceae	Chitinases I/IV	Fruit	GlcNAc, SIA	(Sialo) chitooligosaccharides	Díaz-Perales et al. (1999)

(Continues)

TABLE 3 (Continued)

Lectin domain structure	Domain association	Plant species (accepted plant species and synonyms as reported in the original article)	Plant family	Lectin name	Localisation in plant	Mono-saccharide-binding specificity	Glycan-binding specificity	Reference
		Mango (*Mangifera indica*)	Anacardiaceae	Chitinase IV	Fruit	GlcNAc, SIA	(Sialo) chitooligosaccharides	Díaz-Perales et al. (1999)
		Maize (*Zea mays*)	Poaceae	Chitinase IV	Fruit/seed	GlcNAc, SIA	(Sialo) chitooligosaccharides	Volpicella et al. (2014)
		Papaya (*Carica papaya*)	Caricaceae	Chitinase I	Fruit	GlcNAc, SIA	(Sialo) chitooligosaccharides	Díaz-Perales et al. (1999)
		Passion fruit (*Passiflora edulis*)	Passifloraceae	Chitinase I	Fruit	GlcNAc, SIA	(Sialo) chitooligosaccharides	Díaz-Perales et al. (1999)
		Tomato (*Solanum lycopersicum*)	Solanaceae	Chitinase I	Fruit	GlcNAc, SIA	(Sialo) chitooligosaccharides	Díaz-Perales et al. (1999), Hossaini (1968), Peumans et al. (2003)
		Potato (*Solanum tuberosum*)	Solanaceae	STA	Tuber	GlcNAc, SIA	(Sialo) chitooligosaccharides	Liener (1986), Van Damme et al. (2004)
		Grape (*Vitis vinifera*)	Vitaceae	Chitinase IV	Fruit	GlcNAc, SIA	(Sialo) chitooligosaccharides	Robinson et al. (1997)
		Wheat (*Triticum aestivum*)	Poaceae	WGA	Fruit/seed	GlcNAc, SIA	(Sialo) chitooligosaccharides	Debray et al. (1981), Volpicella et al. (2014), Wright (1990)
		Rice (*Oryza sativa*)	Poaceae	Rice lectin	Seed	GlcNAc	(Sialo) chitooligosaccharides	Liener (1986), Poola et al. (1986), Tabary et al. (1987)
		Buckwheat (*Fagopyrum esculentum*)	Polygonaceae	Antimicrobial peptides: Fa-AMP1 Fa-AMP2	Seed	GlcNAc***	Chitooligosaccharides***	Fujimura et al. (2003)
		Turnip (*Brassica rapa*)	Brassicaceae	Endochitinase-like Bra n 2 allergen	Root	GlcNAc***	Chitooligosaccharides ***	Hänninen et al. (1999)
β-trefoil (β-chain)	Monomer	Castor bean (*Ricinus communis*)**	Euphorbiaceae	RCA Ricin β-chain	Seed	Gal/GalNAc/SIA	(Sialo)complex glycans	Debray et al. (1981), Rutenber et al. (1991)
		Dwarf elder (*Sambucus ebulus*)	Adoxaceae	Ebulin β-chain	Leaf, rhizome	Gal/GalNAc/SIA	(Sialo)complex glycans	Pascal et al. (2001)
		Elderberry (*Sambucus nigra*)	Adoxaceae	SNA-I β-chain	Fruit	Gal/GalNAc/SIA	(Sialo)complex glycans	Peumans et al. (1998)

Abbreviations: Gal, Galactose; GalNAc, N-acetylgalactosamine; Man, Mannose; SIA, sialic acid; T, T-antigen (Thomsen-Friedenreich antigen Gal1, 3GalNAc).

*Usually, monosaccharides (Gal/GalNAc) do not react or react very weakly with lectins of *Phaseolus* and *Vigna*. They readily interact with Gal/GalNAc-containing complex glycans of the N-acetyllactosaminic type. **Non-edible plant. ***Binding specificity not confirmed.

1.3.4 | Previous risk assessments

No quantitative risk assessment has been carried out by any national or international food safety authority; information regarding the occurrence of active lectins and their consumption is considered largely unknown.

The German Federal Institute for Risk Assessment (BfR) published a statement in January 2024 on the health risks related to lectins in plant-based food (BfR, 2024). Following a request by the Federal Ministry of Food and Agriculture (BMEL), BfR was requested to comment on the topic of lectins in food, including the occurrence of lectins in food, the effects on human health and measures to reduce their content in food. In addition, BfR was requested to provide an opinion on the need to carry out regulatory follow-up actions at EU level, including for example, a request to the European Food Safety Authority (EFSA) to assess the risks to human health associated with the presence of lectins in food or a request to the European Reference Laboratory (EURL) for mycotoxins and plant toxins to work on analytical aspects for the determination/quantification of lectins in food. In this statement, it is noted that the consumption of lectins from plant-based food can cause adverse health effects in humans, particularly in children, including nausea, abdominal pain and diarrhoea. Since lectins are deactivated by heat treatment, the severity of the adverse effects is associated with the type and/or conditions of food preparation. Another point raised in the statement was the allergenic potential of plant lectins and the need to investigate further the underlying mechanisms of action.

1.3.5 | Legislation - recommendations from member states

No maximum levels/activity limits for lectins in food are set in the EU and no specific regulatory provisions are applicable for lectins in legumes or other food products.

A preparation of kidney bean lectins (*Phaseolus vulgaris*) has been authorised in the EU as a feed additive for suckling piglets (content 220–660 HAU per animal/day).[3]

Few member states have published preparation recommendations for consumers to avoid acute health effects from legume lectins or lectins in other edible plants:

- The Swedish Food Agency in its risk management report published in 2017[4] recommends soaking and boiling of raw dried beans and peas before eating them, where boiling should be in fresh water. Primarily, the Swedish Food Agency advises to follow the instruction on the product label and in the absence of information on the food product label regarding preparation of raw dried beans and peas, recommends soaking for at least 12 h, rinsing and boiling for at least 30 min. It is highlighted that microwave heat treatment is not sufficient to destroy lectins. For fresh beans and peas, it is suggested to heat, for example, by boiling before consumption. It is also highlighted that red kidney beans have high lectin content and require thorough preparation.
- The Finnish Food Authority, in 2022,[5] published an article in their website on instructions for use of dried beans. The Finnish Food Authority recommends soaking of all types of beans overnight (for a minimum of 12 h), rinsing and boiling for at least 30 min or longer if necessary. Despite the recommendation to treat all dried beans in the same way, a list of recommended cooking times for different varieties of beans is also available.
- The German Federal Institute for Risk Assessment (BfR) in its statement published in January 2024 on the health risks associated with lectins in plant-based food (BfR, 2024) provides preparation recommendations for the consumption of plants containing lectins. In particular, BfR recommends for dried leguminous vegetables that they should be soaked in water for at least 5 h; then, the water should be discarded, and consequently, the pulses should be boiled in fresh water for 30 min. For other edible plants, such as sugar snaps, tomatoes, bananas and peas which contain low concentration of lectins, this preparation recommendation does not apply, and according to the Deutsche Gesellschaft für Ernährung (DGE), they can also be consumed raw.
- The Food Safety Authority of Ireland (FSAI) published an article in July 2024[6] which provides precautionary advice on uncooked or raw bean consumption. In particular, FSAI recommends dried beans to be soaked for at least 12 h, to discard the water used for soaking and to boil the beans in 'fresh' water for at least 30 min. For fresh beans, the recommendation is thorough rinsing in fresh water and cooking/boiling for 10 min before consumption. FSAI also notes that canned beans or beans in jars should be prepared and consumed following the manufacturer's instructions.
- The Austrian Agency for Food and Health safety (AGES) published in August 2024[7] an article on lectins recommending few practices to reduce the lectin content in food, i.e. overnight soaking; cooking in water, which was highlighted as a more effective method than soaking in water alone to reduce the lectins, as they are sensitive to heat; fermenting; sprouting; removal of the outer husk from cereals or pulses; use of tinned pulses, which are already pre-cooked and avoid the use of a slow cooker for pulses.

[3]Commission Implementing Regulation (EU) 2016/1833 of 17 October 2016 concerning the authorisation of a preparation of kidney bean lectins (*Phaseolus vulgaris* lectins) as a feed additive for suckling piglets (holder of authorisation Biolek Sp. z o.o.). OJ L 280, 18.10.2016, p. 19–21.

[4]https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2017/riskhanteringsrapport-lektiner-i-baljvaxter-livsmedelsverkets-rapportserie-nr-14-del-1-2017.pdf.

[5]https://www.ruokavirasto.fi/en/foodstuffs/food-sector/food-information/labelling/instructions-for-use-and-warning/beans.

[6]https://www.fsai.ie/news-and-alerts/latest-news/fsai-provides-precautionary-advice-on-uncooked-or.

[7]https://www.ages.at/en/human/nutrition-food/residues-contaminants-from-a-to-z/lectins.

2 | DATA AND METHODOLOGIES

The current risk assessment on plant lectins in food was developed applying a structured methodological approach, by developing an initial protocol to plan the methodologies to cover each step of the scientific assessment, adapted to the mandate requirements (EFSA Scientific Committee, 2023), and by thoroughly documenting the process (Annex A).

The draft Scientific Opinion underwent a public consultation from 24 July 2025 until 18 September 2025. The comments received were taken into account when finalising the Scientific Opinion and are presented and addressed in Annex F.

2.1 | Evidence collection and appraisal

An extensive literature search was conducted to retrieve information in systematic reviews and peer-reviewed original studies according to the EFSA Guidance (EFSA, 2010) relevant to the following seven areas: 1: Chemistry and physicochemical properties, 2: Analytical methods, 3: Occurrence in food and exposure 4: Data on toxicokinetics in experimental animals and in humans studies 5: Data on toxicity in experimental animals and 5: Data on observations in humans (including epidemiological studies, case reports, biomarkers of exposure) and 7: Data on mode of action. Details on the controlled vocabulary and free text search strings are available in Annex B. The search was performed in Web of Science and PubMed databases until March 2024. An additional literature search was performed in the same databases using the same controlled vocabulary to identify studies on toxicokinetics, on toxicity in experimental animals and observations in humans published from February 2024 to June 2025.

The selection of the scientific papers for inclusion or exclusion followed a systematic review methodology and was based on consideration of the extent to which the study was relevant to the assessment (inclusion/exclusion criteria are detailed in Section 3.1.3 of the protocol in Annex A), irrespective of the results. Limitations in the information used are documented in this Scientific Opinion.

For studies in experimental animals that were directly relevant to the risk assessment question, a tailored OHAT Risk of Bias Tool (as included in the NTP-OHAT Approach for Systematic Review; Rooney et al., 2014) was used as a critical appraisal tool, as detailed in Annex C. Included studies that were not directly relevant to the risk assessment question, but contributed to the risk assessment as supportive evidence, were described narratively.

2.2 | Supporting information for the assessment

Information on previous assessments and legislation was gathered from previous assessments by national and international bodies by checking their official websites and from EU legislative documents. The information was summarised in a narrative way based on expert knowledge and judgement.

2.3 | Hazard identification and characterisation

Information relevant for the sections under hazard identification and characterisation was identified after a literature search and study appraisal which were conducted as described in *Section 1.1 Evidence collection and appraisal*.

Benchmark dose (BMD) analyses were carried out according to the most recent EFSA Scientific Committee Guidance on BMD modelling at the time of this assessment (EFSA Scientific Committee, 2022) and the Bayesian BMD Modelling web-app (https://zenodo.org/record/7334435#.Y5osYXbMLD4) available at the EFSA R4EU platform (https://r4eu.efsa.europa.eu/). This tool, developed and maintained by EFSA, is freely available and extensively documented (Hasselt University, 2022; Verlinden et al., 2024).

2.4 | Occurrence data

As no occurrence data on lectins were available in the EFSA Data Warehouse by the end of 2024, scientific literature was assessed to identify data that would allow estimating acute dietary exposure to selected lectins. Section 2.1 details the methodology followed to select the scientific literature. The attention on the occurrence of lectins was put on PHA, the lectins present in *Phaseolus* sp. (e.g. kidney beans, lima beans, etc.).

2.5 | Food consumption data

The EFSA Comprehensive European Food Consumption Database (EFSA Comprehensive Database) provides a compilation of existing national information on food consumption at the individual level and was first built in 2010 (EFSA, 2011a; Huybrechts et al., 2011; Merten et al., 2011). Details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011a). The latest version of the Comprehensive Database, updated in December 2024, contains results from a total of 58 different dietary surveys carried out in 24 different Member States covering 98,014 individuals.

Within the dietary studies, subjects are classified in different age classes as follows:

Infants[8]:	> 12 weeks to < 12 months old
Toddlers:	≥ 12 months to < 36 months old
Other children:	≥ 36 months to < 10 years old
Adolescents:	≥ 10 years to < 18 years old
Adults:	≥ 18 years to < 65 years old
Elderly:	≥ 65 years to < 75 years old
Very elderly:	≥75 years old

Twelve surveys provided information on specific population groups: 'Pregnant women' (7),[9] 'Lactating women' (2)[10] and 'Vegetarians' (3).[11]

The food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. Consumption data were collected using single or repeated 24- or 48-h dietary recalls or dietary records covering from 3 to 7 days per subject. Because of the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.

When for one country and age class two different dietary surveys were available, only the most recent one was used. Not all countries provided consumption information for all age groups, and in some cases, the same country provided more than one consumption survey.

As the dietary exposure assessment refers to acute exposure, dietary surveys with only one day per subject were also considered (EFSA, 2011b). In Annex E (Table E1), the dietary surveys and the number of subjects available for the acute exposure assessment are described.

2.6 | Food classification

Consumption and occurrence data were codified according to the FoodEx2 classification system (EFSA, 2011a, 2011c). Since 2018, all consumption records in the Comprehensive Database as well as all occurrence data submitted to EFSA have been codified according to the FoodEx2 classification system (EFSA, 2015). The FoodEx2 classification system consists of a large number of standardised basic food items aggregated into broader food categories in a hierarchical parent–child relationship. Additional descriptors, called facets, are used to provide additional information about the codified foods (e.g. information on food processing and packaging material).

2.7 | Dietary exposure assessment

The CONTAM Panel considered it appropriate to estimate acute dietary exposure to PHA only (see Section 3.1).

For estimating acute dietary exposure to PHA, food consumption and body weight data at the individual level were retrieved from the EFSA Comprehensive Database. Occurrence data and consumption data were linked at the relevant FoodEx2 level.

In the absence of occurrence data reported to EFSA, the starting point was the concentrations reported in the scientific literature for PHA (beans from *Phaseolus* sp.) in raw primary commodities. When needed, for the exposure estimations under a particular scenario, the concentrations were adjusted to account for the effects of food processing on the active lectins. Reverse yield factors and recipes were considered, as described in the EFSA raw primary commodity model (EFSA, 2019), to derive the final concentration of the lectins in the consumed commodities.

Acute dietary exposures to PHA were calculated by combining the derived lectin concentrations with the total consumption of each food in 1 day at individual level in each dietary survey and age class. Individual acute exposures per day and body weight were obtained for all individuals. The following formula describes the calculations made:

$$\bar{e}_i = \frac{x_f \cdot c_{f,d,i}}{\mathrm{bw}_i}$$

[8]According to the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants under 16 weeks of age, the exposure assessment for these infants should be carried out separately from that for older infants, following the procedure described in the guidance (EFSA Scientific Committee, 2017). Based on this guidance, infants under 16 weeks of age should be excluded from the dietary exposure estimation of the infants age group. However, for the exposure assessment of lectins, due to uncertainty in the reported individual ages of infants in the Comprehensive Database, the cut-off age was based on a validated existing age group in this database corresponding to 12 weeks of age. Thus, food consumption data of infants between 12 and 16 weeks of age were also included in the exposure assessment. As the number of children within this age range in the database is limited, it is not expected that this will have affected the exposure estimate of lectins for infants of 16 weeks up to 12 months of age.

[9]Austria: ≥ 19 years to ≤ 48 years old; Cyprus: ≥ 17 years to ≤ 43 years old; Latvia: ≥ 15 years to ≤45 years old; Romania: ≥ 19 years to ≤ 49 years old; Spain: ≥ 21 years to ≤ 46 years old; Poland: 20 years old to 42 years old; Portugal: 17 years old to 46 years old.

[10]Greece: ≥ 28 years to ≤ 39 years old, Estonia: 18 years old to 45 years old.

[11]Romania: ≥ 12 years to ≤ 74 years old, Poland: 6 years old to 9 years old, Poland: 18 years old to 50 years old.

where

$\bar{e}_i$	is the acute exposure of individual i through the consumption of food group *f*
x_f	is the reported concentration derived for food group *f*
$c_{f,d,i}$	is the consumed amount of food group *f* by individual *i* in 1 day *d*
bw_i	is the individual body weight of individual *i*

Acute dietary exposure was estimated individually for each food, not for the whole diet, and considering consuming days only. The individual exposure estimates across dietary surveys were grouped by population group to obtain more robust data and a larger sample size, allowing the estimation of higher reliable percentiles representing high exposure. For the highest reliable percentile, the minimum total number of consuming days to consider them reliable was $n = 5$ for P50, $n = 11$ for P75, $n = 29$ for P90, $n = 59$ for P95, $n = 119$ for P97.5 and $n = 299$ for P99 (EFSA, 2011b).

Two exposure scenarios were considered. The first scenario represents adequate processing practices applied to beans from *Phaseolus* sp. before being consumed (e.g. soaking and boiling). In this scenario, the assumption is that most of the initial lectin activity present in the raw primary commodity is eliminated. No exposure estimations are, however, provided for this scenario because it is assumed that the adequate processing leads to a safe consumption of beans. The evidence of the efficacy of processing to inactivate lectins is shown in the scientific references provided in Section 3.2.4.1. However, literature also describes that modifications of the processing (e.g. reduction of cooking time and/or soaking time and pressure, etc.) might lead to higher levels of active lectins remaining in the processed commodities (Del Valle et al., 1983; Leontowicz, Leontowicz, Kostyra, et al., 2001; Venter & Thiel, 1995). The second exposure scenario aims to represent a situation where deviations from adequate processing practices occur, resulting in relatively high levels of active lectins remaining in the processed commodities. To assess this scenario, an arbitrary value of 50% was selected, representing that only half of the active lectins originally present in the raw primary commodities are inactivated in the processed commodities as a result of insufficient treatment as described in the literature (see Table 13 and Appendix D).

2.8 | Risk characterisation

The general principles of the risk characterisation for chemicals in food were applied as described by the WHO/IPCS (2009) along with the different EFSA guidance documents relevant to this step of the risk assessment (see Annex A).

3 | ASSESSMENT

3.1 | Hazard identification and characterisation

3.1.1 | Toxicokinetics

3.1.1.1 | *Toxicokinetic studies in experimental animals*

There were few studies on toxicokinetics for plant lectins in animal models and these are described below.

Absorption and distribution

Kilpatrick et al. (1985) assessed the digestion and absorption of tomato lectin in Lister rats. Rats ($n = 4$) were fed fresh tomato juice (diet constituted by 5% of tomato protein) for 10 days. Using immunodiffusion with lectin-specific antibodies, active tomato lectin was found in the faeces. In a subsequent experiment, Lister rats (weighing approximately 100 g) were fed I^{125}-labelled tomato lectin diluted into fresh tomato juice added 50 mg/mL of BSA (to stabilise the lectin) to a final concentration of 1 μCi/mL of which 1 mL was administered by gavage. Immediately after administration, the content of I^{125}-labelled tomato lectin was highest in the stomach contents and epithelium (about 95% of the ingested lectin). After 1.5 and 3 h, 50%–60% of the ingested tomato lectin was in the small intestine content and epithelium, and after 3 h ca. 25% of the ingested tomato lectin was in the colon contents and epithelium. The binding of lectin to the epithelial cells in the duodenum was confirmed by histochemical staining with peroxidase-labelled antibodies against tomato lectin. In terms of absorption, in samples collected from the internal organs and serum only about 3% of the radioactivity administered as I^{125}-labelled tomato lectin could be found after 3 h, mostly in serum and liver. In summary, the experiments showed that most tomato lectin is not absorbed and mainly passes unchanged through the gastrointestinal tract as shown by immunodiffusion and by the amount of I^{125} recovered in faeces.

[^{3}H]-labelled Kintoki bean (*Phaseolus vulgaris*) lectin was administered to 4 weeks old fasted ddY mice (male) by gavage (1 mL containing 10 mg [^{3}H]-lectin, 2×10^6 cpm, 25,200 HA units). Radioactivity in various organs, blood, urine and faeces was counted after 0.5, 2, 5 and 24 h (Hara et al., 1984). At 30 min, approximately 66% of the total radioactivity bound to the epithelium. This proportion had decreased to 18% at 24 h. Between 65% and 70% of the [^{3}H]-lectin found in the gut

was recovered as active lectin. The total radioactivity collected from kidneys, liver, spleen, pancreas, brain, lung, heart and thymus was approximately 3%. Radioactivity in the gut corresponding to active lectin molecules was measured by haemagglutinating activity. The total recovery of the administered lectin (HA-activity 25,200 units) was between 59% and 45% between 0.5 and 24 h. This confirms that lectins bind to the gut epithelium, but there is little evidence of absorption and distribution to other organs.

Metabolism and excretion

Several studies indicate that lectins pass through the gut unchanged indicating little or no metabolism (Kilpatrick et al., 1985; Pusztai et al., 1990; Rubio et al., 2006).

In rats, the proportion of the fed PHA that remained unchanged through the digestive tract in an immunoreactive and functionally active form was found to be 90% of the administered amount (Pusztai et al., 1990); for further results, see Section 3.1.4.2.

Rubio et al. (2006) investigated the amount of PHA excreted unchanged and functionally active via the digestive tract in pigs ($n = 3$, bw 100 ± 2 kg) cannulated in the distal part of the ileum and fed an amount of PHA of 5.96 g/kg feed. The pigs were fed the kidney bean-containing feed for 3 days, and after the third day, 81% of the PHA was recovered in the small intestine content.

Nakata and Kimura (1985) performed a series of feeding studies in male Wistar rats (weighing 100 g on average) with Con A (further results, see Sections 3.1.3, 3.1.4.2). Rats were fed Con A (2%) for 24 h and then a normal diet. Approximately 63% of the Con A was detected in the faeces after 72 h. In another experiment, rats were administered 50 mg Con A in 5 g feed (equivalent to 500 mg/kg bw), fasted for 24 h and given access to standard feed ad libitum for the rest of the experiment. All faeces were collected in 24 h intervals up to 120 h. The amount of the unchanged Con A found in the faeces collected at 24, 48, 72 and 96 h was 26.2, 42.8, 10.9 and 8.7% of the administered dose, respectively, and no Con A was detected in faeces collected in the 96–120 h period. The data showed that a large proportion (> 89%) of ingested Con A may pass unchanged through the digestive tract.

When PHA (a mixture of E3L or L4; mixture composition not reported) was labelled with I^{125} and administered orally to rats, Pusztai et al. (1989) found that most labelled PHA was associated with the epithelial tissue and the content in the small intestine was highest after 1 h. The blood content of I^{125}-labelled PHA was estimated to be up to 0.8% (after 3 h) of the amount found in the small intestine, and low levels (less than that found in blood) were detected in collected organs (liver, kidney, pancreas, spleen).

In summary, based on the limited number of toxicokinetic studies in animals, the major proportion of ingested lectins (up to 90% of PHA and over 75% of Con A) pass unchanged (remain structurally intact and retain their biological activity) through the gastrointestinal tract, with several lectins binding to the epithelium of the small intestine. Minor but variable proportions of lectins (up to 4% of PHA and 3% of tomato lectin) are absorbed and reach the major organs through the systemic circulation.

3.1.1.2 | *Toxicokinetic studies in humans*

Wang et al. (1998) assessed in healthy UK volunteers the absorption of active PNA after consumption of either raw peanuts ($n = 2$; 200 g; local market) or roasted salted peanuts ($n = 5$; 200 g) in the morning 2–3 h after a light breakfast. Blood samples were taken at 0.5, 1, 1.5, 2, 4, 24, 48 and 72 h after peanut ingestion. PNA up to 5 µg/mL was detected in serum by immunoblot 1–4 h after ingestion in both volunteers after raw peanuts' ingestion and in four of the five volunteers after roasted peanuts' ingestion; the peak concentrations were observed between 1 and 1.5 h. PNA was not detected after 4 h in most of the cases. Haemagglutinating activity test (neuraminidase-treated human red blood cells) was also positive in the study participants 1–4 h after peanut ingestion.

Brady et al. (1978) assessed whether WGA can be absorbed by the human small intestine unchanged. Three healthy volunteers ingested 100 g of unprocessed wheat germ or 100–300 g of processed wheat germ daily for 4 days with faecal collections being obtained on days 3 and 4. Faecal extract, obtained 48 h after the consumption of a diet containing 100 g of unprocessed wheat germ daily, revealed by means of affinity chromatography a single protein band with the same mobility as standard WGA indicating that WGA recovered from the faeces was intact and biologically active (haemagglutination assay ranging from 0 to +4). WGA could not be detected in the faeces when 100 g of processed wheat germ daily was ingested. Small amounts of WGA were detected in faecal samples after 48 h of a diet containing 150 g of processed wheat germ daily. WGA was consistently detected in faecal samples after 48 h when 250 g or more of processed wheat germ was ingested daily. One subject with an ileostomy, but otherwise normal small intestine, ingested 100 g of processed wheat germ over 24 h. Ileostomy effluent was collected over the 24-h period. WGA active in haemagglutination assay was present in the ileostomy effluent collected over this period.

A number of studies that assessed the lectin-binding profile of immunoglobulins other than IgE are available and described in detail in Section 3.1.5.2.2. Significant response levels of IgG and IgM to oral antigens may indicate alterations in or breakdown of the epithelial barrier in the gut.

Based on the above, active PNA and WGA can be rapidly detected in the systemic circulation after eating peanuts or wheat germ suggesting uptake that occurs at an early part in the gastrointestinal tract, probably in the stomach. Moreover, the detection of lectin with a haemagglutination assay in faeces implies that unabsorbed material retains biological activity.

3.1.2 | Toxicity in experimental animals

Further to the eligibility criteria for study selection mentioned in the Protocol, Annex A, **inclusion criteria**, were studies where the administered lectins were derived from edible and unprocessed plant parts. Studies using preparations in which the lectin was present in a purified form and lectins purchased from commercial sources were also considered. Studies were selected if the concentration and the extraction and purification steps were clearly reported. Of these, only studies with lectins of high purity were further included (see Section 1.3.2.1 Methods for isolation and purification of plant lectins).

Exclusion criteria encompassed studies where lectins derived from non-edible plant parts and studies either lacking sufficient information on the purification steps, composition and/or lectin concentration of the used preparations.

In the text in Sections 3.1.3 Acute toxicity studies and 3.1.4 Repeated dose toxicity studies, statistically significant changes are reported unless it is stated differently.

3.1.3 | Acute toxicity studies

Kuku et al. (2012) studied the acute toxicity of TCL lectins purified by affinity chromatography on Lactose-Sepharose, from African walnut (*Plukenetia conophora*, syn *Tetracarpidium conophorum*, Euphorbiaceae). Male Swiss albino mice received a single dose of 0, 500, 750, 1000, 1500, 2000 or 2500 mg/kg bw by gavage ($n = 6$ per group) and were observed for a follow-up period of 14 days. Mortality, body and organ weights and histopathological changes of liver, kidney, lungs, pancreas, spleen, heart and brain were recorded. There were no alterations except reduced body weights (approximately –20%, estimated by the CONTAM Panel from data in Figure 3 of the publication) at doses of 750 mg/kg bw and above on day 15 post-treatment (food intake not reported). However, the CONTAM Panel notes that this protein, TLC lectin, has not been structurally characterised.

Nakata and Kimura (1985) studied the impact of Con A (commercial source, ≥ 90% purity) on the intestinal enzyme activities in young male Wistar rats (~100 g bw); for further results, see Sections 3.1.1.1 and 3.1.4.2. Following a 48-h period of reduced feed intake, controls received 5 g of the basal diet supplemented with 25 mg of casein, while a second group was fed 5 g of the basal diet containing 25 mg of Con A (250 mg/kg bw). Animals were sacrificed at 8, 16 and 24 h after feeding ($n = 4$ per group and time point). In the jejunum of the controls, the activities of sucrase, alkaline phosphatase and leucine aminopeptidase showed a tendency to increase during the observation period, while the activities remained unchanged or were even depressed by Con A, leading to significant differences from respective levels in control animals for all three enzymes.

A study of Larue-Achagiotis et al. (1992) reported that on the first experimental day male Wistar rats ($n = 4$) consumed considerably less food when receiving purified Con A (purified by affinity chromatography on Sephadex G 100) at 3% via diet (equivalent to 30,000 mg/kg feed and approximately 3600 mg/kg bw). There was a follow-up period of 5 days, but no further data are given.

To summarise, TCL lectins from African walnut, administered by gavage at 750–2500 mg/kg bw, reduced the body weight of mice as the only effect. In rats, Con A affected some enzymatic activities (sucrase, alkaline phosphatase and leucine aminopeptidase) in the small intestine at 250 mg/kg bw and reduced feed intake at 3600 mg/kg bw. No acute toxicity studies were identified for PHA or SBA.

3.1.4 | Repeated dose toxicity studies

3.1.4.1 | *General toxicity (bw, nutrition, liver, pancreas, kidney)*

Seven studies were identified on the effects of PHA on general toxicity (Table 4).

Possible antinutritive effects of **PHA** were investigated repeatedly. Two studies on ad libitum fed rats reported unchanged feed intake and body weight gain when PHA was applied via gavage every third day or three times per week at 50 mg/kg (Alatorre-Cruz et al., 2018; for further results, see Sections 3.1.4.2 and 3.1.4.3; Pita-Lopez et al., 2020, for further results, see Section 3.1.4.2). Two further studies comprised ad libitum fed controls and additional controls, being pair-fed with the lectin treated group (Rossi et al., 1984; for further results, see Section 3.1.4.2; Rouanet et al., 1985; for further results, see Section 3.1.4.2). In these studies, rats on the PHA diet showed a significantly lower food or nitrogen intake and body weight than that of the ad libitum fed controls but similar to that of respective pair-fed controls. To be specific, the body weight was reduced by 20% when compared to ad libitum fed untreated animals. The data indicate that the reduced growth of rats given PHA at 300 mg/kg bw per day for 17 days or at 437.5 mg/kg bw per day for 5 days was due to reduced N digestibility or uptake of food, respectively.

Three studies used pair-fed controls only (Banwell et al., 1993; for further results, see Section 3.1.4.2; Bardocz et al., 1995 and 1996; for further results, see Section 3.1.4.2). In these studies, body weight or body weight gain of rats remained unaffected after 7 days of PHA at 120 mg/kg bw per day or was reduced in conventional rats after 5 days of PHA at 120 mg/kg bw per day (Banwell et al., 1993) and after 10 days at 7 mg/kg bw per day (Bardocz et al., 1996). Reduced dry weight of the gastrocnemius muscle (– 2% at 7 mg/kg bw per day and –9.4% at 14 mg/kg bw per day) and of total muscle dry weight (–4%) and of total body lipid weight (–6.7%) both at 38 mg/kg bw per day, were reported as well (statistical significance

not given) (Bardocz et al., 1996). These effects became increasingly pronounced at higher dose levels; however, the difference between the proportional loss of body and muscle weights was not significant ($p > 0.05$). Banwell et al. (1993) noted a reduced dry and wet weight as well as N- and lipid-content of the faeces after 1 week of PHA treatment at 120 mg/kg bw per day.

The relative liver weight remained unchanged by PHA given at a dose of 120 mg/kg bw per day for 7 days (Banwell et al., 1993) or applied every third day at 50 mg/kg (Alatorre-Cruz et al., 2018). An increased relative liver weight, however, was reported in rats treated for 10 days with a considerably higher dose, i.e. 525 mg/kg bw per day.

In a dose–response study, PHA was administered at 0–525 mg/kg bw per day for 10 days. The dry weight of the pancreas was increased in a dose-dependent manner starting at 32.5 mg/kg bw per day and reaching a plateau at around 262 mg/kg bw per day; statistical analysis is not clearly reported. This increase in dry weight may be due to hypertrophy of the exocrine part of the gland, as reported by Bardocz et al. (1995). Alatorre-Cruz et al. (2018) focused on alterations of the pancreas histopathology; the organ showed hypertrophy and trabecular widening after the 6-week administration period of 50 mg/kg bw every third day; after the 2-week recovery period, atrophy was observed, as indicated by a decrease in the mean pancreatic area of acini being lower than that of the untreated controls. The authors suggested that the regression of the tissue may be a compensatory process.

To summarise (Table 4), PHA reduced the body weight of rats due to lowered food intake and/or digestibility of nitrogen at 7 mg/kg bw per day. Hypertrophy of the exocrine pancreas in rats was reported from 32.5 mg/kg bw per day.

TABLE 4 In vivo studies on the effects of phytohaemagglutinin (PHA) in rats.

Lectin tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	LOAEL/NOAEL (mg/kg bw per day)*	Findings	Reference**
PHA (lectin rich fraction obtained by gel filtration on Sephadex G-75|purity not reported)	Rats, SD, sex NR ($n = 12$/gr) Non-pair-fed controls	0 or 50 mg/kg bw 3 times per week	Gavage	6 weeks	50 mg/kg bw 3 times per week (NOAEL – only one dose tested)	↔ bw and feed consumption	Pita-López et al. (2020)
PHA (purified by dialysis and gel filtration on Sephadex G-75|purity not reported)	Rat, SD, sex NR ($n = \sim 12$/gr) Non-pair-fed controls	0 or 50 mg/kg bw every third day	Gavage	43 days, recovery for 14 days until day 57	50 mg/kg bw every third day (LOAEL – only one dose tested)	↔ bw and feed consumption ↓ insulin (day 22), albumin (day 43, day 57), glucose (day 43) and urea (day 57) in serum; ↔total serum protein, a-amylase, creatine and GPT in serum ↔ rel weight of liver, spleen, kidney, pancreas, heart and thymus *Pancreas histopathology:* hypertrophy, vacuolation trabecular widening (day 43) and recovery with atrophy (day 57)	Alatorre-Cruz et al. (2018)
PHA (purified by affinity chromatography on Fetuin-Sepharose-4B|purity not reported)	Rat, Hooded-Lister, sex NR ($n = 5$/gr) Pair-fed Controls	0, 0.6, 1.3, 2.6, 5.2, 10.5, 15, 21, 30 or 42 mg/rat per day; equivalent to 0, 7.2, 15.6, 31.2, 62.4, 126, 180, 252, 360 or 504 mg/kg bw per day (82–84 g bw[b])	Diet	10 days	7.2 (LOAEL)	↓ Dose-dependently for dry bw and dry weight of M. gastrocnemius	Bardocz et al. (1996)
PHA (purified by affinity chromatography on Fetuin-Sepharose-4B|purity not reported)	Rat, Hooded-Lister, sex NR ($n = 5$/gr) Pair-fed Controls	0, 3.5, 7, 14, 21, 30 or 42 mg/rat per day; equivalent to 0, 42, 84, 168, 252, 360 or 504 mg/kg bw per day (82–84 g bw[b])	Diet	10 days	42 (LOAEL)	↓ Dose-dependently for dry bw and dry weight of M. gastrocnemius, total muscle dry weight and total lipid weight	

(Continues)

TABLE 4 (Continued)

Lectin tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	LOAEL/NOAEL (mg/kg bw per day)*	Findings	Reference**
PHA (purified by affinity chromatography on Fetuin-Sepharose-4B|purity not reported)	Rat, Hooded-Lister, sex NR ($n=5$/gr) pair-fed Controls	0, 0.65, 1.3, 2.6, 5.2, 10.5, 15, 21, 30 or 42 mg/rat per day; equivalent to 0, 8.1, 16.3, 32.5, 65, 131.3, 187.5, 262.5, 375, 525 mg/kg bw per day (80 g bw[b])	Diet +1 gavage at day of sacrifice (half dose 2 h before sacrifice, not accounted in the dose)	10 days	32.5 (Reference point)	↑ Dose-dependently for relative dry weight of pancreas[c]	Bardocz et al. (1995)
PHA (purified by affinity chromatography on Fetuin-Sepharose-4B|purity not reported)	Rat, Hooded-Lister, sex NR ($n=5$/gr) Pair-fed controls	0 or 42 mg/rat per day; equivalent to 0, 525 mg/kg bw per day (80 g bw[b])	Diet +1 gavage of (half dose 2 h before sacrifice, not accounted in the dose)	10 days	525 (Reference point L- only one dose tested)	↓ Dry body weight ↑ Relative dry weight of pancreas and liver	
PHA (purified by affinity chromatography on thyroglobulin-Sepharose 4B column|purity not reported)	Rat, SD kept germ-free, m ($n=6$/gr) Pair-fed Controls	0 or 22.2 mg per rat per day; equivalent to 0 or 120 mg/kg bw per day (185 g bw[b])	Diet	7 days	120 (LOAEL-only one dose tested)	↑ Dry and wet weight and content of nitrogen and fat of faeces; ↔ bw, kidney weight and liver wet weight; ↓ Spleen weight	Banwell et al. (1993)***
PHA (purified by affinity chromatography on thyroglobulin-Sepharose 4B column|purity not reported)	Rat, SD kept germ-free, m ($n=6$/gr) Pair-fed Controls	0 or 22.2 mg per rat per day; equivalent to 0 or 120 mg/kg bw per day (185 g bw[b])	Diet	5 days	120 (LOAEL-only one dose tested)	↓Body weight gain	
PHA (purified by affinity chromatography on thyroglobulin-Sepharose CL-4B|purity not reported)	Rat, SD, m ($n=6$/gr) Non-pair-fed and pair-fed controls	0 or 0.25% of dry matter of diet (equal to 0 or 2500 mg/kg feed); equivalent to 0 or 300 mg/kg bw per day[a]	Diet	17 days	300 (LOAEL – only one dose tested)	Compared to pair-fed controls: ↓ bw, nitrogen digestibility and uptake	Rouanet et al. (1985)
PHA (purified by affinity chromatography on thyroglobulin-Sepharose 4B column|purity not reported)	Rat, Wistar, m ($n=4$/gr) Non-pair-fed and pair-fed controls	0 or 1% in diet (equal to 0 or 10,000 mg/kg feed); equivalent to 0 or 437.5 mg/kg bw per day (96 g bw[b] consumption of 4.2 g/day)	Diet	5 days	437.5 (LOAEL-only one dose tested)	Compared to non-pair-fed control: ↓ Food consumption and bw gain; Compared to pair-fed control: ↔ bw gain and food consumption	Rossi et al. (1984)

Abbreviations: bw, body weight; GPT, glutamate pyruvate transaminase; gr, group; LOAEL, lowest observed adverse effect level; M., muscle; m, male; *n*, sample size; NOAEL, no observed adverse effect level; NR, not reported; rel, relative; SD, Sprague Dawley.

*NOAEL and LOAEL extracted from the publication. **Studies are ordered by chronological order, starting with the most recent. ***The authors reported reduction of PHA haemagglutinating activity of 5% after irradiation.

[a]Applying EFSA default values (EFSA Scientific Committee, 2012).

[b]Weights as reported at the beginning of the study.

[c]Weight of pancreas reported for seven doses instead of 10 tested.

Table 5 provides information on the effects of different lectins on general toxicity. Studies in the text below are presented according to the lectin studied.

Several studies reported significantly reduced body weight or body weight gain in **SBA**-treated animals when compared to pair-fed controls (Zang, Li, Piao, & Tang, 2006 and 2006b; Tang et al., 2006; Li, Li, & Qiao, 2003; Pusztai, Ewen, et al., 1993; for further information, see Sections 3.1.4.2 and 3.1.4.3). This effect became evident at 44 mg/kg bw per day and above (Zang, Li, Piao, & Tang, 2006 and 2006b). Li et al. (2003 and 2003b) conducted two dose–response studies with SBA, administering 10–187 mg/kg bw per day via the diet to SD rats for up to 20 days. There was significantly reduced gastrointestinal N-digestibility and significantly increased loss of nitrogen via faeces and urine, starting at 10 mg/kg bw per day (for further information, see Section 3.1.4.2). The body weights and the feed conversion rates were reduced at the highest dose of 240 mg/kg bw per day. However, in two studies of Czerwinski et al. (2005, 2006), the feed intake, the body weight gain and the feed efficiency ratio remained unchanged by SBA, given at 111 mg/kg bw per day for up to 31 days; the studies comprised non-pair-fed controls. The authors reported on significantly elevated N-excretion via the urine but unchanged N-digestibility and N-uptake by the gastrointestinal tract; the overall N-retention by the body was reduced.

In several dose–response studies with SBA in SD rats (doses ranging between 10 and 187 mg/kg bw per day) by Zang et al. (2006 and 2006b) and by Li et al. (2003 and 2003b), the relative pancreas weight was increased significantly at doses of 44 mg/kg bw per day and above. At 44 mg/kg bw per day and higher doses, there were significantly elevated plasma levels of cholecystokinin, a hormone inducing hypertrophy and hyperplasia of rodent pancreas (for more details, see Section 3.1.6.1) (Zang, Li, Piao, & Tang, 2006 and Li 2003b). This was associated with induced pancreatic activities of trypsin, chymotrypsin and amylase at 44 mg/kg bw per day (Zang 2006b). However, in Wistar rats treated with SBA at 111 mg/kg bw per day up to 31 days, the relative weight, the trypsin activity of the pancreas tissue and enzymatic activities and other components of the pancreatic juice remained unaltered (Czerwinski et al., 2005; Czerwinski et al., 2006). Lowered serum insulin and unchanged blood glucose levels were reported at 44 mg/kg per day and above (Czerwinski et al., 2005; Czerwinski et al., 2006; Li, Li, & Qiao, 2003; Zang, Li, Piao, & Tang, 2006).

SBA lowered the weight of the spleen and kidney in one report at 88 mg/kg bw per day (Zang, Li, Piao, & Tang, 2006) or left it unaltered in another study at up to 187 mg/kg bw per day (Li, Li, and Qiao, 2003). Elevated weight of the small intestine and caecum (44 mg/kg bw per day) and unchanged weight of the liver, heart, lung, colon, stomach and thymus were reported by Zang, Li, Piao, and Tang (2006) and Li, Li, and Qiao (2003).

To summarise, lowered body weight gains at unaltered food intake and a reduced uptake and/or retention of nitrogen by the body started at doses of SBA of 44 mg and 12 mg/kg bw per day, respectively. At 44 mg/kg bw per day and above, plasma cholecystokinin concentrations and relative pancreas weights were elevated; plasma insulin was lowered at unchanged blood glucose concentrations. Altered pancreatic enzyme activities were also reported.

Kelsall et al. (2002) administered **PNA** at 0 or 0.1 mg/kg bw per day for 24 weeks via the diet to male rats, which served as controls in a study that primarily investigated intestinal carcinogenesis (for details, see Section 3.1.4.7). These animals also received 16 weekly subcutaneous applications of DMH (1,2-dimethylhydrazine). A slight but significantly increased weight of the colon, relative pancreas weight (+18%) and weight of pancreatic acini (+24%) and septal tissue (+39%) was the outcome.

PSA was administered as extract at 0 or 111 mg/kg bw per day to rats for 14 days (Czerwinski et al., 2006). This regimen elevated the feed intake and body weight gain and reduced blood glucose levels and the trypsin activity in the pancreatic tissue. The feed efficiency ratio and the amylase and trypsin activities in the pancreatic juice remained unchanged.

Kayashima et al. (2005) kept rats on a cholesterol-enriched diet and treated the animals with **Con A** at 0 or 662 mg/kg bw per day for 12 days. Body weight and feed intake remained unchanged when compared to non-pair-fed controls. The faecal excretion of cholesterol and coprostanol was increased by Con A, associated with reduced serum total cholesterol and hepatic cholesterol concentrations and an elevated ratio of serum HDL cholesterol to total cholesterol. The authors concluded that intestinal absorption and digestibility of cholesterol were lowered by Con A. The serum level of HDL, triglycerides, phospholipids, total protein and glucose remained unaffected.

CAL was studied at doses of 0, 50, 100 or 200 mg/kg bw per day administered by gavage to rats for 22 days (Gautam et al., 2018). Reduced serum triglycerides and creatinine levels were observed in all tested doses; the highest dose induced atrophy of hepatocytes. The body weights remained unchanged.

WGA, a lectin from wheat (*Triticum aestivum*), was administered to male Swiss mice at 0 or 167 mg/kg bw per day for up to 3 weeks, which lowered serum glucose and the glycogen content in muscles and liver (Zaremba et al., 1991). When tested in male Lister Hooded rats at 0 or 525 mg/kg bw per day via the diet for 9–10 days, the gain of dry and wet body weights was reduced, combined with a lowered digestibility of nitrogen and nitrogen balance, and elevated loss of nitrogen via faeces (Pusztai, Ewen, et al., 1993).

Lectins purified from seeds of ***Moringa oleifera*** were given by gavage at 0 or 5 mg/kg bw per day to male C57BL mice for a period of 21 days (Yurre et al., 2020). There were no alterations in body weights, blood glucose levels, glucose tolerance or insulin resistance. The relative heart weights and several cardiac functions were unchanged as well.

Rubi et al. (1991) treated male rats for 10 days with **favin** (purified from fava beans) at 0 or 645 mg/kg bw per day via diet. The controls received either a diet with lectin-depleted bean protein or a diet based on lactalbumin. All animals received the same amount of proteins in feed supplemented with amino acids. The authors stated that animals were fed ad libitum but indicated that feed intake was identical for all treatment groups. Compared to the lactalbumin control, the favin-treated animals exhibited no significant effect on dry weights and lipid/nitrogen content of the body as well as on weight and water/nitrogen/lipid content of faeces, nitrogen concentration of the urine and digestibility of nitrogen, lipids and dry matter. The net protein utilisation was reduced. Compared to the controls on a lectin-depleted bean protein diet, favin elevated the dry weights and lipid/nitrogen content of the body, reduced weight and nitrogen content of faeces and nitrogen and urea concentration of the urine and elevated the net protein utilisation; the digestibility of nitrogen, lipids and dry matter was unaltered. It is likely that the lectin-depleted protein fraction of the beans contained components complicating the interpretation of the data. Compared to the lactalbumin control, favin exerted a relatively small effect on the nitrogen utilisation.

To conclude, across all evaluated lectins, a similar toxicity pattern was observed, e.g. many of the lectins investigated reduced feed intake (PHA, SBA) and body weights (PHA, SBA, WGA). Comparisons with ad libitum and pair-feeding controls indicated that the reduced body weights may be due to reduced feed uptake and/or lowered digestibility and retention of nitrogen by the body. Further common findings were elevated weight of the pancreas (PHA, SBA, PNA, WGA), pancreas hypertrophy and vacuolation as shown by histopathology (see details in Section 3.1.4.2) and altered pancreatic enzyme activities (SBA, PSA). The most sensitive endpoints were increased weight of pancreas (PNA at 0.1 mg/kg bw per day; SBA 44 mg/kg bw per day) or nitrogen loss via faeces (SBA at 10 mg/kg bw per day) (Table 5).

TABLE 5 In vivo studies on the effects of legume lectins, except PHA, in rats and mice.

Lectin or item tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	LOAEL/NOAEL (mg/kg bw per day) *	Findings	Reference**
CAL (purified by ion-exchange chromatography on DEAE-cellulose|purity not reported)	Rat, Wistar, m ($n=6$/gr) Non-pair fed controls	0, 50, 100 or 200 mg/kg bw diluted in distilled water	Gavage	22 days	50 (LOAEL – only one dose tested)	↔ rel change in bw, ↓ serum triglyceride (50 mg/kg bw and above) and serum creatinine (50 and 200 mg/kg bw); ↔ total protein, albumin, uric acid and cholesterol in serum *Histopathology:* Atrophy of hepatocytes (200 mg/kg bw)	Gautam et al. (2018)
Con A (commercial source|purity not reported)	Rat, Wistar, m ($n=7$/gr) Fed cholesterol-enriched diet Non-pair fed controls	0 or 0.34% in diet (equal to 0 or 3400 mg/kg feed); equivalent to 0 or 662 mg/kg bw per day (79g bw [b] consumption of 15.4 g/day)	Diet	12 days exposure +12 days observation	662 (LOAEL – only one dose tested)	↔ food intake and bw gain Serum: ↓ conc. of total cholesterol; ↔ conc. of HDL, triglycerides, phospholipids, total protein, glucose; ↑ratio of HDL/total cholesterol; Liver: ↓ conc. of cholesterol; ↔ rel weight; conc. of triglycerides and phospholipids; Faeces: ↑ excretion of cholesterol+coprostanol; ↔ dry weight; excretion of acid sterols; ↓ absorption and digestibility of cholesterol	Kayashima et al. (2005)
Favin lectin (purified by affinity chromatography on Sephadex G-100 |purity not reported)	Rat, Hooded-Lister, m ($n=4$) (lactalbumin controls, $n=8$) unclear whether pair-fed	0 (lactalbumin controls) or 0.7% in the diet (equal to 7000 mg/kg feed); equivalent to 645 mg/kg bw per day (85 g bw[b] consumption of 7.83 g/day)	Diet	10 days	645 (LOAEL – only one dose tested)	↔ food intake; Body: ↔ dry weight and N–/lipid conc.; Urine: ↔ conc. of N; ↑ conc. of urea; Faeces: ↔ dry weight and N–/water–/lipid-conc.; Digestibility: ↔ for N, lipids and dry matter; ↓ protein utilisation	Rubi et al. (1991)
Moringa oleifera seeds (lectins purified by dialysis and affinity chromatography on chitin|purity not reported)	Mice, C57Bl, m ($n=11$–14/gr) Non-pair fed controls	0 or 5 mg/kg bw per day	Gavage	21 days	5 (NOAEL-only one dose tested)	↔ blood glucose levels, glucose tolerance or insulin resistance; ↔ bw; relative heart weight; heart weight/tibia length; ↔ cardiac electric activity, left ventricular functions, cardiac mitochondrial function	Yurre et al. (2020)
PNA (commercial source|purity not reported)	Rat, Wistar, m ($n=9$/gr) Non-pair-fed controls	0 or 2 mg/kg diet; equivalent to 0 or 0.1 mg/kg bw per day[a]	Diet all rats received 16 weekly sc injections of solvent	Approx 24 weeks	0.1 (LOAEL- only one dose tested)	↔ body weight ↑ weight of colon, rel pancreas weight and of pancreatic acini and septal tissue	Kelsall et al. (2002)
PSA (purified by affinity chromatography on Con A-Sepharose 4B|purity not reported)	Rat, Wistar, m ($n=10$/gr) Non-pair-fed controls	0 or 15 mg/rat per day; equivalent to 0 or 111 mg/kg bw per day (135 g bw[b])	Diet	14 days	111 (LOAEL – only one dose tested)	↑ feed intake and bw gain ↔feed efficiency ratio Plasma: ↓ glucose; ↔ protein conc. and amylase activity Pancreas: ↔ rel weight and protein content; ↓ trypsin activity; In pancreatic juice: ↔ amylase and trypsin activity, conc. of protein, Ca, P, Na, K, Cl	Czerwinski et al. (2006)

TABLE 5 (Continued)

Lectin or item tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	LOAEL/NOAEL (mg/kg bw per day) *	Findings	Reference**
SBA (purified by affinity chromatography on epoxy-activated N-acetyl-D-galactosamine-sepharose-6B|purity not reported)	Rat, SD, m + f (n = 6/sex/gr) Pair-fed controls	0, 3.5, 7.0, 10.5 or 14.0 mg/rat per day; equivalent to 0, 44, 88, 131 or 175 mg/kg bw per day (80 g bw [b])	Gavage	10 days	44 (LOAEL)	↓ bw gain and weight of carcass (44 mg/kg bw); ↓ weight of kidney and spleen (88 mg/kg bw); ↑ weight of pancreas (44 mg/kg bw), small intestine and caecum (44 mg/kg bw) ↔ weight of liver, heart, lung, colon, stomach Plasma: ↑ urea (88 mg/kg bw) and AST (44 mg/kg bw); ↓ blood insulin level (44 mg/kg bw); ↔ glucose, cholesterol, triglyceride, HDL, ALT	Zang, Li, Piao, and Tang (2006)
SBA (purified by affinity chromatography on epoxy-activated N-acetyl-D-galactosamine-sepharose-6B|purity not reported)	Rat, SD, m (n = 6/gr) Pair-fed controls	0, 3.5, 7.0, 10.5 or 14.0 mg/rat per day; equivalent to 0, 44, 88, 131 or 175 mg/kg bw per day (80 g bw[b])	Gavage	14 days	44 (LOAEL)	↓ bw gain (44 mg/kg bw); ↑ serum CCK (44 mg mg/kg bw) and intestinal CCK expression Pancreas: ↑ rel dry weight (44 mg/kg bw); ↑ RNA, DNA and protein content, activity of (chymo)trypsin and amylase (44 mg/kg bw)	Zang, Li, Wang, et al. (2006)
SBA (purified by affinity chromatography on epoxy-activated N-acetyl-D-galactosamine-sepharose-6B|> 95% purity)	Rat, SD, m (n = 6/gr) Pair-fed controls	0, 0.05, 0.10, 0.15 or 0.20% soybean agglutinin in diet (equal to 0, 500, 1000, 1500 or 2000 mg/kg feed); equivalent to 0, 60, 120, 180 or 240 mg/kg bw per day[a])	Diet	20 days	120 (NOAEL) 180 (LOAEL)	↓ bw (180 mg); ↑ rel weight of small intestine (240 mg), ↔ weight of spleen and thymus; For immunological parameters see section 3.1.4.3	Tang et al. (2006)
SBA (purified by affinity chromatography using Con A-Sepharose 4B|purity not reported)	Rat, Wistar, m (n = 10/gr) Non-pair-fed controls	0 or 15 mg/rat per day; equivalent to 0 or 111 mg/kg bw per day (135 g bw[b])	Diet	14 days	111 (LOAEL – only one dose tested)	↔ feed intake, bw gain and feed efficiency ratio Plasma: ↑ protein conc.; ↔ glucose conc. and amylase activity Pancreas: ↔ rel weight and protein content; ↓ trypsin activity; In pancreatic juice: ↔ amylase and trypsin activity, conc. of protein, Ca, P, Na, K, Cl	Czerwinski et al. (2006)
SBA (purified by affinity chromatography on Con A-Sepharose 4B|purity not reported)	Rat, Wistar, m (n = 10/gr) Non-pair-fed controls	0 or 15 mg/rat per day; equivalent to 0 or 111 mg/kg bw per day (135 g bw[b])	Diet	31 days	111 (LOAEL – only one dose tested)	↔ feed intake, bw gain and feed efficiency ratio Plasma: ↔ protein and glucose conc. and amylase activity Pancreas: ↔ rel weight, protein content and trypsin activity; Determined at day 5: ↔N-intake, N in faeces, dry matter intake and protein digestibility; ↑ N-excretion in urine ↓ N-retention	Czerwinski et al. (2005)
SBA (purified by affinity chromatography on epoxy-activated N-acetyl-D-galactosamine-sepharose-6B| highly purified[c])	Rat, SD, m (n = 6/gr) Pair-fed controls	0, 0.1, 0.2 or 0.4 g/kg diet; equivalent to 0, 10, 20 or 40 mg/kg bw per day (70 g bw[b] consumption of 7 g/day)	Diet	10 days	10 (LOAEL)	↓ N-digestibility (10 mg/kg bw), N-retention (20 mg/kg bw), N-balance (20 mg/kg bw) ↑ N in faeces (20 mg/kg bw) and urine (40 mg/kg bw); ↑ rel pancreas weight (40 mg/kg bw) ↔ N-intake, faeces weight, urine production, rel dry weight of pancreas: protein, RNA and DNA content per g dry pancreas	Li, Li, Qiao, Zhu, and Huang (2003)

TABLE 5 (Continued)

Lectin or item tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	LOAEL/NOAEL (mg/kg bw per day) *	Findings	Reference**
SBA (purified by affinity chromatography on epoxy-activated N-acetyl-D-galactosamine-sepharose-6B|purity not reported)	Rat, SD, m ($n=6$/gr) Pair-fed controls	0, 0.4, 0.6 or 0.8 g/kg diet; equivalent to 0, 33, 49 or 66 mg/kg bw per day (85 g bw [b] consumption of 7 g/day)	Diet	10 days	33 (LOAEL)	↓ N-digestibility (33 mg/kg bw), N-retention (33mg/kg bw), N-balance (33 mg/kg bw) ↑ N in faeces (33 mg/kg bw) and urine (33 mg/kg bw); ↔ bw gain, feed conversion ratio, N-intake, faeces weight, urine production	Li, Li, and Qiao (2003)
SBA (purified by affinity chromatography on epoxy-activated N-acetyl-D-galactosamine-sepharose-6B|purity not reported)	Rat, SD, m ($n=6$/gr) Pair-fed controls	0, 0.4, 0.8, 1.2 or 2.0 g/kg diet; equivalent to 0, 37, 75, 112 or 187 mg/kg bw per day (75 g bw[b] consumption of 7 g/day)	Diet	20 days	75 (NOAEL) 112 (LOAEL)	↓ plasma insulin (112 mg/kg bw); bw gain (187 mg/kg bw), weight of dry carcass (187 mg/kg bw) and feed conversion ratio (187 mg/kg bw); ↑ plasma CCK (187 mg/kg bw), rel dry weight of pancreas (187 mg/kg bw); RNA and DNA content/g dry pancreas weight (187 mg/kg bw) ↔ rel dry weight of liver, kidney and spleen; protein/g dry pancreas weight	
WGA (partially purified by dialysis|purity not reported)	Rats, Lister Hooded, m ($n=4$/gr) Pair-fed controls	7 g/kg diet; equivalent to 525 mg/kg bw per day (80 g bw[b] consumption of 6 g/day)	Diet	9–10 days	525 (LOAEL – only one dose tested)	↓ bw gain, dry bw, N-balance, N-digestibility; urine volume; thymus weight ↑ faeces weight and N excretion in faeces ↔ N-intake and N-retention; Urine: ↔ urea, ammonia, creatinine, free alpha-amino-N; Serum: ↔ 3-OH-butyrate	Pusztai, Ewen, et al. (1993)
WGA (Purified WGA from commercial source|highly purified[c])	Mice, Swiss, m ($n=10$/gr) Non-pair-fed controls	0 or 167 mg/kg per day	Gavage	7, 14 or 21 days	167 (LOAEL-only one dose tested)	↓serum glucose (14 day), glycogen content in muscle (14 day) and liver (14 and 21 day)	Zaremba et al. (1991)

Abbreviations: AST, aspartate aminotransferase; bw, body weight; m, male; gr, group; CAL, Cicer arietinum lectin; CCK, cholecystokinin; Con A, Concanavalin A; f, female; HDL, high density lipoprotein; Immunol, immunological; LOAEL, lowest observed adverse effect level; N, nitrogen; *n*, sample size; NOAEL, no observed adverse effect level; PNA, Peanut agglutinin; PSA, Pisum sativum; rel, relative; SBA, Soybean agglutinin; sc, subcutaneous; SD, Sprague Dawley; WGA, Wheat germ agglutinin.

*NOAEL and LOAEL extracted from the publication. **Studies are ordered by lectin name and then by chronological order, starting with the most recent one.

[a]Applying EFSA default values (EFSA Scientific Committee, 2012).

[b]Weights as reported at the beginning of the study.

[c]Determined by SDS-PAGE.

3.1.4.2 | *Gastrointestinal toxicity*

Table 6 provides information on the effects of different lectins on gastrointestinal toxicity. Studies in the text below are presented according to the lectin studied.

From the early 1980s, it was clear that several lectins adversely affected the structure and form of the small intestine. Multiple studies including light and electron microscopy, immunohistochemistry and histology have observed similar findings with adverse effects particularly to the villi (see Table 6).

Later studies by Gupta and Sandhu (1997, 1998) confirmed that histological changes to the brush border membrane (BBM) were also caused by ASA110 (lectin isolated from *Allium sativum*) at 160 mg/kg bw per day when administered for 7 days.

ASA_{110}

In two studies by Gupta and Sandhu (1997, 1998), the effect of ASA_{110} from *Allium sativum* by gavage in male Swiss albino rats was examined. The exposures were 160 mg/kg per day for 7 days. The first study showed decreased body weight gain (89.5% vs. 91% in control and treated respectively) and a decreased food intake especially after day 4. Immunohistochemistry showed binding of ASA_{110} to cells of lamina propria of the jejunum and to the BBM and basal region (Brunner's gland) of duodenum. ASA_{110} was not altered in its passage through the gut (Gupta & Sandhu, 1997). Same exposure of Swiss albino rats to ASA_{110} (160 mg/kg per day for 7 days) also resulted in some functional disturbance in the enzyme activity in the intestine

and histological changes in the brush border membranes. ASA_{110} decreased ALP and total ATPase activity and increased acid phosphatase activity. Increases in sucrase and maltase were not significant. The total protein was increased, but there were no changes in nucleic acid content. Micrographs of the brush border membranes showed thinning and sloughing of villi, and increased numbers of goblet cells and vacuolisation (Gupta & Sandhu, 1998).

In summary, ASA110 bound to the cells of lamina propria of the jejunum and passed through the gut unaltered. Histological changes were observed to the BBM together with changes in BBM enzymes.

Con A

In a study on male SD rats, Fitzgerald et al. (2001) examined the effect of Con A via gavage on epithelial proliferation in the intestine. Rats were fed total parenteral nutrition via the right external jugular vein for 3 days while Con A was infused into the stomach (25 mg/rat and bw: 200–250 g equivalent to 100–125 mg/kg bw per day) for 4 days. Con A did not affect body weight gain, length of colon or weight of stomach, small intestine, caecum or colon. Nor did it affect crypt branching. Samples were collected along the entire length of both the small intestine and colon, specifically from areas located at 10%, 50% and 90% of their respective lengths. Con A did increase proliferation in the proximal small intestine (50%) and a smaller, but significant, increase in proliferation in the mid and distal small intestine. In the colon, Con A increased proliferation only in the proximal region. There was an increase in the number of metaphases per crypt in only the 10% areas of small intestine and colon. In the 50% and 90% areas, there were no changes in the number of metaphases per crypt. In a series of seven experiments using male Wistar rats, Nakata and Kimura (1985) showed that Con A at 1000 mg/kg bw (100 mg/rat, rats of 100 g bw) was essentially unchanged during passage through the digestive tract and 90% was recovered in the faeces after 72 h (for further information, see Sections 3.1.1.1 and 3.1.3). By following the fate of the Con A for up to 72 h the authors suggested Con A bound to the luminal surfaces of the small intestine and affected the BBM. The content of nitrogen found in the faeces was approximately double that found in the faeces of rats on the basal diet when Con A was administered for 4 days at 600 mg/kg bw per day (0.5% in diet). Decrease in enzyme activities localised to the BBM (sucrase, ALP and leucine aminopeptidase) occurred after exposure to Con A.

Zárate and Chaia (2012) examined the effects of Con A with or without *Proprioni bacterium acidipropionici* on the small intestine of mice. Male BALB/C mice (24–26 g) fed daily 8 mg/kg bw per day Con A for 21 days showed decreased body weight gain and food intake. There was an increase in stomach weight but no change to the small intestine, liver or spleen. Con A exposure increased epithelial cell proliferation and shortening and shedding of microvilli (for further details, see Section 3.1.4.2.1).

In summary, Con A passed through the digestive tract almost unchanged. Con A binds to the BBM, resulting in decreases in BBM enzymes. There were inconsistencies in the effect of Con A on organ weights. Con A did increase proliferation in specific areas of the small intestine and colon. Changes were seen in the structure of the small intestine with shortening and loss of microvilli.

PHA

In Hooded Lister rats fed PHA or WGA for 10 days (42 mg lectin per day, equivalent to 525 mg/kg bw per day), the lectins altered the jejunal crypts, which were elongated with shorter villus length. Semi-quantitative immunohistochemical staining showed decreases in HSP72 and HSP90 (Ovelgönne et al., 2000). For further information, see Section 3.1.6.1.

In a study comparing germ-free and conventional Sprague Dawley rats pair-fed PHA at 1% or 10,000 mg/kg feed (equivalent to 1200 mg/kg bw per day) of dietary protein for 7 days, Larson et al. (1989) found little difference in the histology of the epithelia of the jejunum or ileum. There were increases in sphingomyelin and non-acid glycolipid excretion in faeces from both conventional and germ-free rats to approximately the same extent. Germ-free rats did excrete more gangliosides, particularly N-acetyneuraminosyl-lactosylceramide, which is restricted to the duodenum, upper jejunum and large intestine. The authors concluded that the effects of PHA were due to binding of the lectin to enterocyte brush border membrane and were not associated with the microflora of the rats.

Banwell et al. (1984) fed male Sprague Dawley rat diets of crude raw kidney beans (RKB) or purified PHA (1% or 10,000 mg/kg feed, equivalent to 1200 mg/kg bw per day) for 7 days. The rats fed RKB had a lower body weight gain compared to control animals, while those fed PHA lost body weight. Both treatments resulted in diarrhoea with increased faecal wet and dry weights. There was also an increase in total nitrogen excretion. There was lower nitrogen excretion in urine but greater excretion in faeces. These effects were reversible when animals reverted to a normal diet. Sections of jejunum, ileum and cecum were obtained from experimental rats and were stained by indirect immunofluorescence with anti-PHA-L, normal rabbit serum or anticasein antisera. Fasting the animals for up to 24 h resulted in loss of staining in caecum and ileum. The staining of sections of control small intestine layered in vitro showed staining for both PHA L and PHA E in brush border and goblet cells. Sections layered with casein showed no staining in PHA and RKB treated rats. Staining was restricted to the brush border with a segmented pattern of staining in animals treated for 4 days and a continuous 'band like' pattern of staining in animals fed for longer times (10 days). In general, the staining was associated with the proximal small intestine but some staining was visible in the caecum. It was noted that lectin staining to the brush border of the jejunum was also seen in germ-free rats exposed to PHA. Light microscopy indicated no changes to the jejunum or ileum. Transition electron microscopy (TEM) also showed no effects on microvilli, terminal web, microfilaments or cell organelles. The authors concluded that PHA adherence to the intestinal mucosal surface was an important cause of nutritional and malabsorptive changes seen.

Bardocz et al. (1995) examined the effect of purified PHA on the gastrointestinal tract in 30-day-old Hooded Lister rats (Rowett strain) in a series of experiments (for further results, see Section 3.1.4.1). The first experiment compared rats fed a lactalbumin diet (LA) with those fed PHA 525 mg/kg bw per day. The dry weight/dry body weight of stomach, jejunum, SI, caecum, pancreas, liver and colon all increased over 10 days. In terms of wet weight, all organs except liver and stomach also showed increases. Protein, RNA and DNA content increased in stomach, jejunum and small intestine. RNA increased in all tissues while DNA did not change in colon, caecum and pancreas. The second experiment measured the effect of 375 or 525 mg/kg bw per day over time (day 0, 1, 3, 5, 7, 10). Dry weight of small intestine, pancreas and large intestine increased with time. Increased length of the small intestine was seen from day 3, with the higher dose having a greater effect. The third experiment measured a dose response (0, 8.1, 16.3, 32.5, 65, 131.3, 187.5, 262.5, 375, 525 mg/kg bw per day) for 10 days. The small intestine, large intestine and pancreas showed an increase in dry weight with dose. Small intestine increased from 32.5 mg/kg bw per day, pancreas dry weight from 32.5 mg/kg bw per day (see Section 3.1.4.1), and the large intestine from 131.3 mg/kg bw per day and above. Binding of PHA in the stomach was to the surface epithelium and parietal region. Slower emptying of the stomach with PHA was observed. In the small intestine, binding was to the BBM and changes in the length and thickness of the small intestine were noted. The effect on length was dose dependent. In the large intestine, binding was to the surface and increased weight; protein and RNA were observed with a NOAEL of 65 mg/kg bw per day. The final experiment measured the effect of LA diet for 6 days, PHA diet for 3 days followed by LA for 3 days or LA for 3 days followed by PHA for 3 days to investigate reversibility. Lectin-induced changes were fully or partially reversible within 3 days.

Lafont et al. (1988) exposed male Sprague Dawley rats (80–90 g) to PHA in the basal diet (0, 3, 30, 48, 96, 144, 192, 300 mg/kg bw per day) for 9 days. Food intake was not affected until 48 mg/kg bw per day PHA, administered via the diet. It then decreased as a linear function of the log of PHA concentration. The calculated average daily weight gain was linear. Light microscopy showed no differences in the morphology of the small intestine. Electron microscopy showed that, at the lowest dose (3 mg/kg bw per day PHA), the microvilli were shortened and irregular. The intestinal injuries were dose dependent. There was increased endocytic activity in most of the duodenal enterocytes. These effects were increased and magnified at the higher doses and at the highest dose cell death was apparent. ALP was decreased at all doses of PHA. In a control group where food intake was restricted to approximately the same level as the top dose of PHA, there was no damage observed in the duodenum suggesting that the effects were due to PHA and not to decreased food intake. A compensatory hyperplasia measured by increased [3H] thymidine into DNA was seen with all doses of PHA.

Banwell et al. (1993) carried out a number of studies in germ-free and conventional Sprague Dawley rats fed PHA (22.2 mg PHA/rat per day, equivalent to 120 mg/kg bw per day) for 3–10 days. In the germ-free rats, the small intestine length, wet and dry weight was increased significantly, but there was no change in body weight gain. The spleen weight decreased, but no change was observed in the weights of the liver, kidney or colon. The mean wet weight of mucosal scrapings of both the proximal and distal small intestine were significantly increased. Protein, DNA and faecal output weight, fat and nitrogen were increased while sucrase activity decreased. PHA antibody staining was observed on the brush border membrane. Crypt depth and mitotic index was increased in the jejunum, but only mitotic index was increased in the ileum. DNA content and [^{3}H] thymidine uptake were also increased in the jejunum indicating increased rate of epithelial cell renewal. Increases were seen in the jejunum in the number of goblet cells per crypt, intraepithelial and lamina propria cells. Similar, but smaller, changes were seen in the ileum. Increases in crypt cell DNA content and [^{3}H]thymidine uptake indicated epithelial cell hyperplasia. In contrast, in conventional rats, body weight gain was decreased. The length and weight of the small intestine were greater than control, but once the animals were returned to a normal diet, the small intestine weight decreased. In a separate study using partially purified PHA at 2.5, 5.0 and 7.5% (equal to 25,000, 50,000 and 75,000 mg/kg feed) for 5 days, the increase in weight of the small intestine was shown to be dose dependent, but the increases in DNA content and hexose were not. In the final study where rats were exposed to PHA with and without antibiotics (metronidazole and kanamycin both at 0.5 mg/mL), the trophic response was seen in both treatments, but the effect was greater with no antibiotics present. For further results, see Section 3.1.4.1.

In a study in Wistar rats (96 g) fed PHA (1% in the diet or 10,000 mg/kg feed, equivalent to 437.5 mg/kg bw per day) for 5 days, Rossi et al. (1984) observed ultrastructural changes in the jejunum in the animals fed PHA (see below, for further results, see Section 3.1.4.1). The growth rate of the rats was lower in the PHA-fed animals (1.0 g per day per rat) vs. control rats (2.7 g per day per rat). Food intake was also decreased. The absorptive cells of the jejunum in the PHA-treated rats showed abnormal villi. The villi were shorter and thicker and were irregular and sparse compared to those in the control animals. The terminal web below the brush border was disorganised, and many cells contained cytolysosomes (enlarged lysosomes). The rats that were pair-fed with the PHA-treated animals showed no changes in the ultrastructure of the jejunum indicating that the changes were due to the presence of the lectin.

Rouanet et al. (1985) investigated the effects of PHA on growing rats. Male Sprague Dawley rats (82 +/– 1 g) were fed PHA (0.25% or 2500 mg/kg dry matter in feed equivalent to 300 mg/kg bw per day) for 17 days. Growth of the PHA- and pair-fed group (no PHA) was decreased compared to controls suggesting the growth retardation was due to decreased food intake. Protein digestibility was decreased in the PHA-fed animals. Several duodenal and jejunal enzyme activities were decreased in the treated rats (enterokinase, AP, LNA, sucrase and GGT). Mucosal protein content was not changed in the duodenum but was increased in the jejunum with PHA. In the duodenum, the length of the villi was decreased and the crypt length increased. In the jejunum, the length of the villi did not change, but the length of the crypts increased. For further results, see Section 3.1.4.1.

Bardocz et al. (1996) carried out five studies on the effect of PHA on growth, body composition and plasma insulin in Hooded male Lister rats (82–84 g). In the first experiment, rats were given a range of doses of PHA from 0 to 42 mg/rat per

day (Experiment 1a 0, 2.6, 5.2, 10.5, 21, 42 mg/rat per day; Experiment 1b 0, 0.6, 1.3, 15, 30 mg/rat per day). In both experiments, the dry body weight and dry weight of gastrocnemius muscle were decreased. In addition, there was loss of lipid weight with increasing PHA dose. For further results, see Section 3.1.4.1.

In summary, there is evidence that PHA induces a trophic response in the gut. PHA binding to the BBM is observed and this binding is important in the changes observed. There are differences in the magnitude of the effects seen in germ-free versus conventional rats with germ-free animals showing greater effects. The most commonly observed changes were in body weight gain and changes in the histology of the small intestine (LOAEL 3 mg/kg bw per day). Light microscopy generally showed little change, but electron microscopy found damage to the microvilli, which were shorter and irregular. Crypt depth and mitotic index were also increased and the terminal web disorganised. BBM enzymes such as ALP, where measured, were decreased by PHA.

Tepary Bean Lectin (*Phaseolus acutifolious*)

In a study by Alatorre-Cruz et al. (2018), rats were treated with TBLF (50 mg/kg bw) via gavage administration every 3 days for 6 weeks with a 2-week recovery period with no lectin. In rapidly growing 5-week-old Sprague Dawley rats, it was shown that Tepary bean lectin fraction (TBLF) did not affect the rat development, with no differences seen in body weight gain, food intake or rat length. However, rats in the final phase of growth (15 weeks old) showed a decrease of 10% in body weight gain. After 6 weeks, the weight of the small intestine was increased by 14% without any change in length. The weight of the colon decreased by 20%. Spleen weight was not affected (for further information, see Sections 3.1.4.1 and 3.1.4.3). Histopathology showed intestinal structural changes, including thinning of the intestinal tissue in the duodenum and activation of the immune system in the Peyer's patches. The ileum showed villi with broadening of the base and narrowing at the top of the villi. Crypt depth and villus height were decreased, and villus length decreased on day 43. There were few changes in the biochemical parameters, but changes in the immune system and pancreas were observed (Section 3.1.4.3).

Pita-López et al. (2020) showed that the effects of Tepary bean lectin fraction (TBLF) on the intestines of the rat were temporary, and recovery occurred after 6 weeks (for further results, see Sections 3.1.4.1 and 3.1.4.2.1). Five-week-old Sprague Dawley rats were given TBLF (50 mg/kg bw 3 times per week) for 6 weeks by gavage. This was followed by 6 weeks on a normal diet. In these young rats, TBLF had no effect on body weight gain. Mucus production was increased by 62% in the treated rats, which may be an indication of inflammation. There was no change in the weight or length of the SI, although macroscopically there was intestinal thinning. In the duodenum, villi decreased in height but increased in width, with Lieberkuhn crypt atrophy and decreased crypt depth. In the jejunum and ileum, there was an increase only in the width of the villi and decreased crypt depth. The authors indicated the damage to the intestine was moderate. Some damage was observed in the colon to the prismatic epithelium and a change to the microbiota profile in the treated rats. There was also an increase in membrane permeability. These adverse effects were reversed after 6 weeks of normal diet, with only the crypt depth of the duodenum being partially recovered.

TBLF produced histological observable damage to the duodenum and ileum in terms of decreased crypt depth and shortened villus height. The changes were almost all reversible after 2–6 weeks on a normal diet.

Kintoki Bean Lectin

Hara et al. (1983) examined the oral toxicity of KBL in rats and mice. Male mice (ddY strain) fed KBL by gavage at 20, 40 and 60 mg/mouse (mice of 20 g bw, equivalent to 1000, 2000 and 3000 mg/kg bw per day) for 6 days also lost body weight, with one-third and two-thirds of the 2000 and 3000 mg/kg bw per day dose dying within a few days. Male Wistar rats fed KBL at 0.4% or 4000 mg/kg in diet (equivalent to 480 mg/kg bw per day) for 5 days showed significantly decreased absorption of protein, carbohydrate and lipid as well as loss of body weight. Histological analyses showed disruption to the epithelial cells of the villi and shortening of the villi of the small intestine in the presence of KBL. In both rats and mice, no effects were observed on liver, pancreas, spleen and kidney. The authors concluded that the growth retardation due to KBL is the result of damage to the intestinal cells and decreased nutrient absorption.

In summary, Kintoki bean lectin was damaging to rats and mice from 1000 mg/kg bw per day. Histology showed disruption and damage to the intestinal cells and decreased nutrient absorption.

Winged Bean Lectin

Higuchi et al. (1984) showed that feeding winged bean lectin to rats decreased growth and damaged the villi of the small intestine. Male Wistar albino rats were fed isolated WBL at 0, 0.6, 1.2, 3.5, 5.0, 10% or 6000, 12,000, 35,000, 50,000 and 100,000 mg/kg in feed (equivalent to 0, 720, 1440, 4200, 6000 and 12,000 mg/kg bw per day) for 7 days. Body weight, liver and spleen weight and food intake decreased with increasing % of lectin with significant decrease starting from 1440 mg/kg bw per day. Significant mortality occurred above 6000 mg/kg bw per day. Rats fed 10% WBL or 100,000 mg/kg in feed (equivalent to 12,000 mg/kg bw per day) in the basal diet for 5 days showed decreased intestinal enzyme activities (sucrase, maltase, ALP, gamma GT, leucine aminopeptidase). Light microscopy showed decreased height and abnormal forms of the villi of the small intestine.

In summary, Winged bean lectin decreased body weight and food intake of rats from 1440 mg/kg bw per day. Damage to the villi of the small intestine was also observed.

Peanut Lectin

Henney et al. (1990) showed that peanut lectin induced a mild hyperplasia in weanling male Sprague Dawley rat small intestines but only at 0.2% or 2000 mg/kg diet (equivalent to 120 mg/kg bw per day) in the diet for 23 days. Other doses 0.004 and 0.04% or 40 and 400 mg/kg diet (equivalent to 1.2 and 12 mg/kg bw per day) showed no effect on the parameters measured in the study (mucosal weight, protein and DNA content and brush border enzymes, maltase, g-glutamyltranspeptidase and ALP). There were no effects on the histology of the small intestine. At the highest dose, 120 mg/kg bw per day increases in mucosal weight, protein and DNA content, and GGT activity was noted but without altered villus morphology.

In summary, peanut lectin showed no effect on the histology of the gut, but at the highest dose (120 mg/kg bw per day), there was a mild hyperplasia of the small intestine.

SBA (Soybean Agglutinin)

In a study in weaned piglets, Zhao et al. (2011) found that 0.1% or 1000 mg/kg diet (56 mg/kg bw per day) and greater SBA in the diet increased intestinal permeability to D-lactic acid and ileal mucosa diamine oxidase. 0.05% or 500 mg/kg diet (28 mg/kg bw per day) had no effect on permeability. Higher doses (> 0.1% or 1000 mg/kg diet) also decreased the expression of tight junction proteins, occludin and ZO-1. No morphological changes in the intestine were observed.

Tang et al. (2006) examined the effects of soybean agglutinin (SBA) on growth and immune function in male Sprague Dawley rats. Rats were fed SBA in the diet for 20 days (0, 0.05, 0.10, 0.15, 0.20% or 0, 500, 1000, 1500 and 2000 mg/kg feed, equivalent to 0, 60, 120, 180 or 240 mg/kg bw per day). Body weight gain in the rats decreased linearly with increasing concentration of SBA and was significant at 180 mg/kg bw per day and above. The relative weight of the small intestine also increased in a dose dependent manner and was significant at the top dose (240 mg/kg bw per day), which by the CONTAM Panel was considered to be due to the reduction in body weight gain. There was a significant decrease in lymphocyte proliferation in spleen, lymph nodes and blood; however, there was no change in the weight of spleen or thymus. For further information, see Sections 3.1.4.1 and 3.1.4.3.

Li, Li, and Qiao (2003) undertook two studies to examine the effects of SBA on nitrogen metabolism of intestine and pancreas in rats. In the first experiment, male Sprague Dawley rats (85 g) were fed SBA (0, 0.4, 0.6, 0.8 mg/g equivalent to 0, 33, 49 or 66 mg/kg bw per day) in diet for 20 days. No change was seen in the weight, food or nitrogen intake. Faecal nitrogen loss increased, and nitrogen digestibility decreased with increasing concentration of SBA and was significant at all doses. In the second experiment, male Sprague Dawley rats (75 g) were fed SBA (0, 0.4, 0.8, 1.2, 2.0 mg/g equivalent to 0, 37, 75, 112 or 187 mg/kg bw per day) for 20 days. Below 187 mg/kg bw per day, no significant changes were seen. At the top dose, a decrease in body weight gain and feed intake and an increase in cholecystokinin were noted. Insulin was decreased at the top two doses. Decreases in the length of jejunal villi and increases in crypt depth and number of crypt cells were seen at the top dose. Increased protein and RNA content as well as increased putrescine, spermidine and spermine were also seen at the top dose. No change in the brush border epithelium and no diarrhoea were observed in the study. Dry weights of liver, kidney and spleen were not changed. For further information, see Section 3.1.4.1.

In summary, effects of SBA varied with test species. Piglets showed increased intestinal permeability but no histological changes. In turkey poults, doses below 125 mg/kg bw per day showed no antinutritive effects and little effect on gut architecture. In rats, decreased body weight gain was significant at and above 180 mg/kg bw per day. Similarly, SBA increased nitrogen loss and decreased nitrogen digestibility but without affecting the BB epithelium or intestine at 240 mg/kg bw per day. Effects were noted on jejunal villi and crypts.

Other lectins

In addition to the studies on individual lectins described above, there were a number of studies testing several lectins. These are described below.

In a study by Pusztai et al. (1990), rats (80–90 g; no strain given) were exposed to 470 mg/kg bw per day of plant lectins (PHA, SBL or SBA, SNA I, SNA II, VFL) for 10 days (sub-acute). All lectins except *Vicia faba* lectin (VFL) caused a decrease in body weight gain over the 10 days. In addition to intestinal growth, there was accumulation of polyamines, known growth promoters. The dry weight of the jejunum increased with PHA, SBL, SNA I and II, indicating hyperplastic growth. There was an increase in crypt length and increased number of enterocytes (hyperplasia) with all lectins. Binding patterns were different, with SNA I showing strong binding initially but little on day 10. PHA, SBL and SNA II underwent endocytosis. VFL was not toxic, was not bound, was not endocytosed and did not promote growth (Pusztai et al., 1990). For further results, see Section 3.1.1.1.

Pusztai, Ewen, et al. (1993) investigated the antinutritive effects of WGA and another N-acetylglucosamine-specific lectin from nettle rhizomes (UDA) (for further information, see Sections 3.1.4.1 and 3.1.4.3). Male hooded Lister rats (80 g) were given 7000 mg/kg purified individual lectins (equivalent to 525 mg/kg bw per day) in the diet for 10 days. Both lectins decreased the body weight gain in the rats, with WGA being the most effective. WGA-treated rats showed greater faecal excretion, lost more total nitrogen, showed an apparent decrease in N digestibility and lost greater relative lipid content than control rats. UDA had no effect on body N or lipid. WGA increased the weight of the SI after 10 days being 45% heavier than controls. Growth of the SI was also noted with enlarged crypts and higher numbers of crypts in the jejunum in the presence of WGA. Protein, RNA, DNA and polyamine content of the SI were also increased, but other parts of the gut showed

no change. UDA showed similar changes, but these were modest and did not reach statistical significance. WGA enlarged the pancreas (18%) and decreased the thymus weight (> 20%). UDA was stated not to affect the thymus and other tissues, but the results were not shown. Monospecific antibody staining found extensive binding of WGA to the brush border epithelium and some staining of basolateral membrane and walls of venules and lacteals. No endocytosis of UDA was found.

Overall summary of the gastrointestinal toxicity

In summary, lectins pass through the gut relatively unchanged and binding to the brush border membrane is observed and needed for their effects. Lectins have been found to affect the small intestine to a greater or lesser extent depending on the lectin used. In general, the most potent lectins cause damage to the villi and microvilli and crypts (PHA at 3 mg/kg bw per day; Con A at 8 mg/kg bw per day). Body weight gain and food intake are often decreased, and the reduced body weight gain is often attributed to the decreased food intake rather than a toxicity per se. The CONTAM Panel also notes that reduced body weight gain may also be attributed to increased excretion of faecal nitrogen i.e. nitrogen not absorbed in the gut due to BBM damage and altered gut permeability. Increases in weight (PHA at 32.5 mg/kg bw per day) and length (SBA at 112 mg/kg bw per day; PHA at 120 mg/kg bw per day) of the small intestine are commonly observed as are changes in the activities of the brush border enzymes such as ALP (PHA at 3 mg/kg bw per day) and sucrase (Con A at 600 mg/kg bw per day). The majority of the effects on the gut are reversible on return to a normal diet minus lectin.

TABLE 6 In vivo studies on the gastrointestinal effects of lectins in animals.

Lectin tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	LOAEL/NOAEL (mg/kg bw per day)*	Findings	Reference**
ASA_{110} (purified by affinity chromatography on asialofetuin-linked amino activated silica affinity column|high purity[c])	Rats, Swiss albino, m (*n* = 6/gr)	160 mg/kg bw per day	Gavage	7 days	160 (LOAEL – only one dose tested)	↓ in ALP (~30% of control); ↑ in acid phosphatase (~25% of control); ↓ in total ATPase (~45% of control); thinning and sloughing of villi and ↑ number of goblet cells and vacuolisation in brush border membranes; ↔ in DNA or RNA; ↑ in total protein content (~35% of control)	Gupta and Sandhu (1998)
ASA_{110} (purified by affinity chromatography on asialofetuin-linked amino activated silica affinity column|purity not reported)	Rats, Swiss albino, m (*n* = 6/gr)	160 mg/kg bw per day (twice a day, 80 mg/kg bw per dose)	Gavage	7 days	160 (LOAEL – only one dose tested)	↓ in bw gain (~ 7%); ↓ food intake on day 8 (~60% of control); ASA binds to cells of lamina propria of jejunum and toe BBM of duodenum and basal region (Brunner's gland)	Gupta and Sandhu (1997)
Con A (commercial source|purity not reported)	Rats, SD, m (*n* = 6/gr)	25 mg/rat; equivalent to 100–125 mg/kg bw per day (200–250 g bw[b])	Gavage	4 days	100–125 (LOAEL – only one dose tested)	↑ metaphases per crypt in 10% small intestine and 10% colon ↔on bw gain; ↔ on length of colon; ↔ on weight of stomach, small intestine, caecum, colon cf. TPN; ↔ on crypt branching	Fitzgerald et al. (2001)
Con A (commercial source|purity not reported)	Rats, Wistar, m (*n* = 4/gr)	0.5% in diet (equal to 5000 mg/kg feed); equivalent to 600 mg/kg bw per day (100 g bw[b])	Diet	4 days	600 (LOAEL – only one dose tested)	↔ Con A in GIT; ↓ sucrase, ALP and leucine aminopeptidase ↑ N in the faeces	Nakata and Kimura (1985)
Con A (commercial source|purity not reported)	Mice, BALB/C, m (*n* = 15/gr)	8 mg/kg bw per day	Diet	21 days	8 (LOAEL – only one dose tested)	↓ bw and food intake, ↑ stomach weight; ↑ epithelial cell proliferation; shortening and shedding of microvilli; ↑ populations of enterobacteria and enterococci; ↓ disaccharidase activity	Zárate and Chaia (2012)
Kintoki bean lectin (purified by double gel filtration on Sepharose 4B column|purity not reported)	Mice, ddY, m (*n* = 3/gr)	20, 40, 60 mg; equivalent to 1000, 2000 and 3000 mg/kg bw per day (20 g bw [b])	Gavage	7 days	1000 (LOAEL)	↓ bw in dose dependent manner, damaged villi and cells of small intestine, ↔ on liver, spleen, kidney and pancreas	Hara et al. (1983)

(Continues)

TABLE 6 (Continued)

Lectin tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	LOAEL/NOAEL (mg/kg bw per day)*	Findings	Reference**
Kintoki bean lectin (purified by double gel filtration on Sepharose 4B column|purity not reported)	Rats, Wistar, m ($n=6$/gr)	0.4% (equal to 4000 mg/kg feed); equivalent to 480 mg/kg bw per day[a]	Diet	5 days	480 (LOAEL – only one dose tested)	↓ bw ↓ absorption of protein, carbohydrate and lipid, histological damage to villi ↔ on liver, spleen, kidney and pancreas	
PHA (purified by dialysis and gel filtration on Sephadex G-75|purity not reported)	Rat, SD, sex NR ($n=$~12/gr)	0 or 50 mg/kg bw every third day	Gavage	43 days, recovery for 14 days until day 57	50 mg/kg bw every third day (LOAEL- only one dose tested)	↓ insulin (day 22), albumin (day 43, day 57), glucose (day 43) and urea (day 57) in serum; ↔ rat development; ↑ in SI weight 14%; ↓ in colon weight 20%	Alatorre-Cruz et al. (2018)
PHA (purified by affinity chromatography on thyroglobulin-Sepharose 4B column|purity not reported)	Rat, SD kept germ-free, m ($n=6$/gr)	0 or 22.2 mg per rat per day; equivalent to 0 or 120 mg/kg bw per day (185 g bw[b])	Diet	7 days	120 (LOAEL – only one dose tested)	↔ bw or liver, colon kidney weight;↓ spleen weight; ↔ plasma or tissue content of gastrin, enteroglucagon or peptide YY *Histopathology:* ↑crypt depth and mitotic index in Jejunum; ileum ↑mitotic index; PHA staining on BBM	Banwell et al. (1993)***
	Adult male SD rats ($n=4$-6/gr)	0 or 22.2 mg per rat per day; equivalent to 0 or 120 mg/kg bw per day (185 g bw [b])	Diet	5–9 days	120 (LOAEL – only one dose tested)	↑ wet weight and length of small intestine	
PHA (purified by affinity chromatography on Fetuin-Sepharose-4B|purity not reported)	Rat, Hooded-Lister, sex NR, ($n=5$/gr)	0, 0.6, 1.3, 2.6, 5.2, 10.5, 15, 21, 30 or 42 mg/rat per day; equivalent to 0, 7.2, 15.6, 31.2, 62.4, 126, 180, 252, 360 or 504 mg/kg bw per day (82–84 g bw[b])	Diet	10 days	7.2 (LOAEL)	↓ dose-dependently for dry body weight and dry weight of M. gastrocnemius (the difference between the proportional loss of body and muscle weights was not significant ($p>0.05$)	Bardocz et al. (1996)
		0, 3.5, 7, 14, 21, 30 or 42 mg/rat per day; equivalent to 0, 42, 84, 168, 252, 360 or 504 mg/kg bw per day (82–84 g bw [b])	Diet	10 days	42 (LOAEL)	↓ dose-dependently for dry bw and dry weight of M. gastrocnemius, total muscle dry weight and total lipid weight	
PHA (purified by affinity chromatography on Fetuin-Sepharose-4B|purity not reported)	Rat, Hooded-Lister, sex NR, ($n=5$/gr)	0, 0.65, 1.3, 2.6, 5.2, 10.5, 15, 21, 30 or 42 mg/rat per day; equivalent to 0, 8.1, 16.3, 32.5, 65, 131.3, 187.5, 262.5, 375, 525 mg/kg bw per day (80 g bw[b])	Diet +1 gavage at day of sacrifice (half dose 2 h before sacrifice, not accounted in the dose) Diet	10 days	32.5 (Reference point)	↑ in small intestine weight (2.6 mg) ↑ in large intestine weight (10.5 mg)	Bardocz et al. (1995)[d]
		0, or 42 mg/rat per day; equivalent to 0 or 525 mg/kg bw per day (80 g bw[b])		3 days	525 mg/kg bw per day	Binding to surface epithelium and parietal region of stomach (42 mg); binding to BBM in SI, more pronounced in jejunum than ileum (42 mg); large intestine: binding to surface of colon and caecum (42 mg); effects fully or partially reversible	

TABLE 6 (Continued)

Lectin tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	LOAEL/NOAEL (mg/kg bw per day)*	Findings	Reference**
PHA (purified by affinity chromatography on Fetuin-Sepharose-4B-CL|purity not reported)	Rats, SD, m ($n=6$/gr)	0, 0.0025, 0.025, 0.04, 0.08, 0.12, 0.16, 0.25% (equal to 0, 25, 250, 400, 800, 1200, 1600, 2500 mg/kg feed); equivalent to 0, 3, 30, 48, 96, 144, 192, 300 mg/kg bw per day[a]	Diet	9 days	3 (LOAEL) 48 (NOAEL) 96 (LOAEL)	↓ ALP duodenal; ↑ compensatory hyperplasia; *Electron microscopy* Damage to microvilli in duodenum ↓ bw and food intake (at 96 mg/kg bw per day)	Lafont et al. (1988)
PHA (purified by method of Felsted et al. (1975)|purity not reported)	Rat, SD, germ free or conventional adult, m ($n=6$/gr)	1% (equal to 10,000 mg/kg feed); equivalent to 1200 mg/kg bw per day[a]	Diet	7 days	1200 (LOAEL – only one dose tested)	↑ faecal excretion of sphingomyelin and non-acid glycosphingolipids; ↔between GF and conventional rats; GF rats gained weight; conventional rats no change in bw; no change in histology (not shown)	Larson et al. (1989)
PHA (purified by affinity chromatography on fetuin-Sepharose-4B|purity not reported)	Rats, Hooded Lister, m ($n=2$/gr) pair fed control	42 mg/day equivalent to 525 mg/kg bw per day (80 g bw[b])	Diet	10 days	525 (LOAEL – only one dose tested)	Altered crypts and villi of jejunum; ↓ HSP72 and HSP90	Ovelgönne et al. (2000)
PHA (lectin rich fraction obtained by gel filtration on Sephadex G-75|purity not reported)	Rats, SD, sex NR ($n=12$/gr)	0 or 50 mg/kg bw, 3 times per week	Gavage	6 weeks	50 mg/kg bw 3 times per week (NOAEL – only one dose tested)	↔ bw and feed consumption ↑ mucus production and permeability; *Histology:* ↑ villus width in duodenum, jejunum, ileum; ↓ villus height in duodenum; ↓crypt depth and crypt atrophy in duodenum, jejunum, ileum; Colon damage to prismatic epithelium and altered microbiota; Effects reversible after 6 weeks	Pita-López et al. (2020)
PHA (purified by affinity chromatography on thyroglobulin-Sepharose4B column|purity not reported)	Rat, Wistar, m($n=4$/gr)	0 or 1% in diet (equal to 0 or 10,000 mg/kg diet); equivalent to 0 or 437.5 mg/kg bw per day (96 g bw[b] consumption of 4.2 g/day)	Diet	5 days	437.5 (LOAEL – only one dose tested)	↓ bw gain and food consumption *Histology:* Jejunal villi shorter and thicker, sparse and irregular; cells contained large cytolysosomes	Rossi et al. (1984)
PHA (purified by affinity chromatography on thyroglobulin-Sepharose CL-4B|purity not reported)	Rat, SD, m ($n=6$/gr)	0 or 0.25% of dry matter of diet (equal to 0 or 2500 mg/kg dm of diet); equivalent to 0 or 300 mg/kg bw per day[a]	Diet	17 days	300 (LOAEL – only one dose tested)	↓ bw, nitrogen digestibility and uptake; ↓ duodenal and jejunal enzymes	Rouanet et al. (1985)
PNL or PNA (purified by affinity chromatography on cyanogen bromide activated Sepharose 4B and N-E-aminocaproyl-β-D-galactopyranosylamine|high purity[c])	Rats, SD weanling, m (*n*: NR)	0, 0.004, 0.04, 0.2% (equal to 0, 40, 400, 2000 mg/kg feed); equivalent to 0, 1.2, 12, 120 mg/kg bw per day[a]	Diet	23 days	12 (NOAEL) 120 (LOAEL)	↑ mucosal weight, protein and DNA content and GGT activity; No morphological changes	Henney et al. (1990)

(Continues)

TABLE 6 (Continued)

Lectin tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	LOAEL/NOAEL (mg/kg bw per day)*	Findings	Reference**
SBA (purified by affinity chromatography on epoxy-activated N-acetyl-D-galactosamine-sepharose-6B|purity not reported)	Rat, SD, m ($n=6$/gr)	0, 0.4, 0.6 or 0.8 g/kg diet; equivalent to 0, 33, 49 or 66 mg/kg bw per day (85 g bw[b] consumption of 7 g/day)	Diet	10 days	33 (LOAEL)	↑ faecal output, N loss and urine N excretion(33 mg/kg bw); ↓ N-retention and N-digestibility (33 mg/kg bw)	Li, Li, and Qiao (2003)
		0, 0.4, 0.8, 1.2, 2.0 g/kg diet; Equivalent to 0, 37, 75, 112 or 187 mg/kg bw per day (75 g bw [b] consumption of 7 g/day)	Diet	20 days	112 (NOAEL) 187 (LOAEL)	↓ bw gain, food intake (187 mg/kg bw); ↑ CCK, protein, RNA and polyamine content (187 mg/kg bw); ↔ dry weight liver, kidney, spleen; *Histology:* ↓ length of jejunum; ↑ crypt depth and number of crypt cells	
SBA (purified by affinity chromatography on epoxy-activated N-acetyl-D-galactosamine-sepharose-6B|> 95%)	Rat, SD, m ($n=6$/gr)	0, 0.05, 0.10, 0.15 or 0.20% soybean agglutinin in diet (equal to 0, 500, 1000, 1500 or 2000 mg/kg SBA in diet); equivalent to 0, 60, 120, 180 or 240 mg/kg bw per day[a]	Diet	20 days	120 (NOAEL) 180 (LOAEL)	↓ bw (180 mg/kg bw); ↑ weight of small intestine (240 mg/kg bw), ↑ lymphocyte proliferation; ↓ cytokines and antibodies in plasma	Tang et al. (2006)
SBA (purification method not reported|≥ 95% purity)	Weaned piglets, cross bred barrows, NR ($n=5$/gr)	0, 0.05, 0.10, 0.15, 0.20% (equal to 500, 1000, 1500, 2000 mg/kg feed); equivalent to 0, 28, 56, 84, 112 mg/kg bw per day (7.07 bw[b] and 0.35 kg/day feed intake NRC (2012))	Diet	7 days	28 (NOAEL) 56 (LOAEL)	↑ permeability; ↓tight junction protein expression	Zhao et al. (2011)
WGA (purified by affinity chromatography on N-acetylglucosamine|purity not reported)	Rats, Hooded Lister, m ($n=2$/gr); pair fed controls	42 mg/day; equivalent to 525 mg/kg bw per day (80 g bw[b])	Diet	10 days	525 (LOAEL – only one dose tested)	Altered crypts and villi of jejunum; ↓ HSP72 and HSP90	Ovelgönne et al. (2000)
WGA (partially purified by dialysis|purity not reported)	Rats, Lister Hooded, m ($n=4$/gr)	7000 mg/kg diet; equivalent to 525 mg/kg bw per day (80 g bw[b] consumption of 6 g/day)	Diet	9–10 days	525 (LOAEL- only one dose tested)	↓ bw gain, dry bw, N-balance, N-digestibility; ↑ N excretion in faeces; ↑ weight of SI; Jejunum – crypts enlarged, more crypt cells; ↑ Protein, RNA, DNA and polyamine contents of SI; Binding to basolateral membrane and endocytosis	Pusztai, Ewen, et al. (1993)
Winged Bean Lectin (purified by dialysis against distilled water|30% purity)	Rats, Wistar albino, m ($n=6$/gr)	0, 0.6, 1.2, 3.5, 5.0, 10.0% (equal to 0, 6000, 12,000, 35,000, 50,000, 100,000 mg/kg feed); equivalent to 0, 720, 1440, 4200, 6000 and 12,000 mg/kg bw per day[a]	Diet	7 days	720 (NOAEL) 1440 (LOAEL)	↓ bw at 1440 mg/kg bw per day and above. Mortality at 6000 mg/kg bw per day and above	Higuchi et al. (1984)

TABLE 6 (Continued)

Lectin tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	LOAEL/NOAEL (mg/kg bw per day)*	Findings	Reference**
Winged Bean Lectin (purified by dialysis against distilled water|30% purity)	Rats, Wistar albino, m ($n=6$/gr)	10% (equal to 10,000 mg/kg feed); equivalent to 12,000 mg/kg bw per day[a]	Diet	5 days	12,000 (LOAEL – only one dose tested)	↓ intestinal enzymes; abnormal villi	
UDA (purified by affinity chromatography on N-acetylglucosamine and chitin-agarose columns, respectively|purities not reported)	Rats, hooded Lister (Rowett), m ($n=4$/gr)	42 mg/rat equivalent to 525 mg/kg bw per day (80 g bw[b])	Diet	10 days	525 (LOAEL – only one dose tested)	↓ bw gain; UDA ↔ body N or lipid; ↑ weight of SI; jejunum enlarged crypts and higher number of crypts Protein, RNA, DNA and Polyamine content increased in SI Other parts of gut not affected; Antibody staining in brush border epithelium; UDA no endocytosis	Pusztai, Ewen, et al. (1993)
Other Lectins							
PHA, SBL or SBA, SNA I and SNA II, VFL (purified by affinity chromatography on fetuin-sepharose-4B; cross-linked guar gum; unknown and Sephadex G-100 columns, respectively|purities not reported)	Rats, no strain reported, sex NR ($n=4$/gr)	0.7% in diet (equal to 7000 mg/kg feed; equivalent to 470 mg/kg bw per day (85 g bw[b] consumption of 5.7 g/day)	Diet	10 days	470 (LOAEL – only one dose tested)	↓ bw gain (except VFL); ↑ jejunum dry weight (except VFL); ↑ crypt length & number of enterocytes	Pusztai et al. (1990)

Abbreviations: ALP, alkaline phosphatase; ASA, *Allium sativum* agglutinin; BBM, brush border membrane; bw, body weight; CCK, cholecystokinin; cf., crypt fission; Con A, Concanavalin A; dm, dry matter; DNA, deoxyribonucleic acid; DSA, *Datura stramonium* agglutinin; GF, germ free; GGT, γ-Glutamyltranspeptidase; GIT, gastrointestinal tract; gr, group; HSP, heat shock protein; LOAEL, lowest observed adverse effect; m, male; M., muscle; N, nitrogen; *n*, sample size; NOAEL, no observed adverse effect; NR, not reported; NRC, National Research Council; PHA, phytohaemagglutinin; PNL, peanut lectin; PNA, peanut agglutinin; rel, relative; RNA, ribonucleic acid; SBA, soybean agglutinin; SBL, soybean lectin; SD, Sprague Dawley; SI, small intestine; TPN, total parenteral nutrition; UDA, *Urtica dioica* agglutinin; VFL, *Vicia faba* lectin; WGA, wheat germ agglutinin.

*NOAEL and LOAEL extracted from the publication. **Studies are ordered by lectin name and then by chronological order, starting with the most recent one. ***The authors reported a reduction of PHA haemagglutinating activity of 5% after irradiation.

[a]Applying EFSA default values (EFSA Scientific Committee, 2012).

[b]Weights as reported at the beginning of the study.

[c]Determined by qualitative methods (e.g. SDS-PAGE, native-PAGE).

[d]Weight of small intestine reported for 11 doses instead of 10 reported as tested.

3.1.4.2.1 | *Gut microbiome*

Feeding PHA (0.5% or 5000 mg/kg feed equivalent to 316 mg/kg bw per day) to weanling rats for 21 days (Banwell et al., 1983) showed a many fold higher number of microorganisms in the jejunum and ileum of the PHA-fed rats compared to the control group, indicative of bacterial overgrowth. In conventional, but not in germ-free rats, feeding RKB containing the same amount of PHA-affected nutrient absorption revealing that the change in gut microbiota played a key role for the reduced nutrient absorption induced by PHA.

Pusztai, Grant, et al. (1993) administered doses in the range of 20–100 mg PHA/day (21, 30, 42, 54, 60, 69, 72, 78, 81 and 99 mg/day[12]) equivalent to 262.5, 375, 525, 675, 750, 862.5, 900, 975, 1012.5 and 1237.5 mg/kg bw per day by gavage to 30-day-old Hooded-Lister (80 ± 1 g) rats for 3 days with or without a 3-day subsequent wash-out period, or gave 10^8–10^9 *E. coli*/mL in the drinking water for 6 days, prior to sacrifice and examination of the small intestines. The administration of *E. coli* bacteria did not significantly increase the number of coliform bacteria in homogenised wet sections of jejunum. In PHA-administered rats, the number of coliform and lactobacilli was at least two- to threefold higher compared to the same sections of jejunum from control rats administered water or *E. coli*. Especially *E. coli* with capability to bind mannose residues showed a dose-dependent increase both in washed and unwashed tissues indicating a strong adherence to the glycocalyx in the PHA treated animals. In PHA treated rats sacrificed after a 3-day wash-out period, the bacterial count had almost reverted to the background level, thus showing the reversible nature of the effect of PHA on the bacterial count. The authors reported that when Con A or WGA were administered instead of PHA, WGA induced a similar but weaker effect

[12]Doses estimated from article graphs, in the article reported as a range of 20–100 mg lectin per rat per day.

on bacterial growth while Con A had no effect. These data showed that some lectins may affect the growth condition of bacteria in the gut and further change the glycosylation pattern of the mucosa.

In a study where Sprague Dawley rats (50–70 g) were fed 0.5% PHA or 5000 mg/kg feed (corresponding to 316 mg/kg bw per day) for 7 days and then sacrificed, the gut barrier in the jejunum and ileum was studied by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) as regards morphology and occurrence of bacteria. In contrast to the control animals showing an almost complete coverage of the epithelial cells with secreted mucus, the epithelium of the PHA-fed rats displayed discontinuities in the mucosal layer (Banwell et al., 1985). In the PHA-fed animals, the lack of mucus led to a massive increase of bacteria close to and on the epithelial surface. A fluorescent photomicrograph of a cross section of the jejunal lumen stained with fluorescently labelled anti-PHA antibodies displayed PHA as a continuous layer on the epithelial surface.

Rats, 14 days old, were orally administered PHA (100 mg/kg bw; $n = 10$) once by gavage or water ($n = 10$) (Marungruang et al., 2018). At 17 days of age, all rats were administered human serum albumin (HSA, 1250 mg/kg bw) by gavage which was used as a marker for gut intestinal barrier permeability, and 3 h later, the animals were sacrificed and blood samples and the content of cecum collected. As the gut barrier is immature at birth and mature gradually during the first 4 weeks, control water-fed rats were also sacrificed at day 28, 3 h after an oral administration of HSA. Compared to the control group administered water, the PHA-administered rats showed a reduced gastrointestinal uptake of HSA (> 90% reduction) comparable to the HSA uptake in 28-day-old rats. The gut microbiota composition in the cecum of rats treated with PHA was altered compared to control rats. The phylae Proteobacteria and Firmicutes were decreased when treated with PHA ($p < 0.0001$ and $p < 0.001$, respectively) and the phylum Bacteroidetes was increased ($p < 0.0001$). Hence, 3 days after administration of a single dose of PHA to suckling rats, the gut barrier is less permeable than in the corresponding control rats, and the composition of the gut microbiota has changed. For further results, see Section 3.1.4.5.

Zárate and Chaia (2012) examined the effects of Con A with or without *Propionibacterium acidipropionici* on the small intestine of mice. Male BALB/C mice (24–26 g) were fed daily 8 mg/kg bw of Con A for 21 days. In terms of bacteria adhering to the intestinal mucosa, Con A increased enterobacteria and enterococcal populations but had no effect on lactobacilli, bifidobacteria or propionibacteria. Con A also decreased the specific activity of disaccharidases, sucrases, lactases, trehalases and maltases. Treatment of mice with *Proprionibacterium acidipropionici* had little effect alone but when given together with Con A it prevented all of the Con A-induced changes observed.

Pita-López et al. (2020) examined the effect of feeding the tepary bean (*Phaseolus acutifolius*) lectin fraction to 5 weeks old Sprague Dawley rats for 6 weeks (gavage, 3 times 50 mg/kg bw each week, approximately 20 mg/kg bw per day) on the microbial diversity of faeces. The microbial diversity was analysed by bacterial DNA electrophoretic profile in samples taken at weeks 0, 2, 4 and 6 and showed a gradual decrease in diversity in the tepary bean lectin fed rats during the 6 weeks of feeding. The reduction in faecal diversity was paralleled with reduced intestinal integrity and increased jejunal mucus content.

In summary, orally administered lectin (PHA) in doses from 20 mg/kg bw may lead to a higher bacterial number in the small intestine and changes in the microbial composition and diversity, and associated with alterations in the mucus layer and reduced nutrient absorption. These effects seem reversible and disappear a few days after the end of lectin feeding.

3.1.4.3 | *Immunotoxicity*

Immunotoxicology describes (unintended) modulation of the immune system following exposure to the lectins. Hence, effects on size or weight of lymph organs, increased production and release of leucocytes into the circulation, abrogation of oral tolerance against dietary proteins and increased activation of lymphocytes may be consequences of lectins that are administered orally and may eventually lead to allergies, autoimmune diseases or other adverse immune-related diseases.

Effects on lymph nodes, spleen, thymus and blood

Increased size or weight of lymph nodes may be a sign of increased immune activity due to either strongly immune stimulating compounds or increased translocation of microbes and dietary compounds from the gut. Increased activity upon mitogen treatment may indicate a higher number of cells that proliferate in response to the mitogen.

Feeding PHA-E (100 mg/kg bw per day) to Wistar rats (male) for 90 days led to increased weight of the mesenteric lymph nodes while feeding 30 mg/kg bw per day for the same period did not show this effect (Kroghsbo et al., 2008) (Table 7). For further results, see Section 3.1.4.4.

Sprague–Dawley rats, 14 days old, were administered a single dose of PHA via gavage at 0, 5, 10, 50 or 250 mg/kg bw and then sacrificed after 12 or 72 h. The weight of the spleen was decreased only after 12 h by the highest dose (250 mg/kg bw), and the weight of the thymus decreased 72 h after the highest dose (Prykhod'ko et al., 2009). This was accompanied by an increased number of T cells ($CD3^+$ cells) at the two highest doses and an increased number of B cells ($CD19^+$ cells) at the highest dose in the small intestine (Table 8). For further results, see Section 3.1.4.5.

A low dose of lectin from black turtle beans (*P. vulgaris* L, purity > 94%) was administered to BalbC mice by gavage on days 1, 4, 7, 14, 21 and 28 (200 µL emulsified lectin solution containing 5 µg lectin (equivalent to 0.2 mg/kg bw per dose) in a PBS and CFA emulsion) as sensitisation.[13] A control group received only PBS and CFA emulsion. All groups received 200 µL

[13]Sensitisation: the induction of the process in which the immune system first recognises and responds to an antigen.

of emulsified lectin solution containing 15 mg of lectin (600 mg/kg bw) 1 week after the last sensitisation and were sacrificed after 1 h. In the mice receiving the repeated low dose of lectin, the spleen index (mg spleen per g bw) showed a fourfold increase compared to the control group (He et al., 2021) (Table 7). For further results, see Section 3.1.4.4.

Sprague Dawley rats, 5 weeks old ($n = 62$) were fed lectin from tepary bean every third day for 43 days, at a dose of 50 mg/kg bw per day, and groups were sacrificed 9, 22, 43 days, respectively, or 2 weeks after cessation of lectin administration. A control group was administered saline after the same schedule. The rats did not reveal any alterations in the weight of the spleen or thymus compared to the control group (Alatorre-Cruz et al., 2018) (Table 4). For further results, see Sections 3.1.4.1 and 3.1.4.2.

Male Sprague Dawley rats ($n = 6$) were fed with purified soybean agglutinin (SBA) at the doses of 0, 60, 120, 180 or 240 mg/kg bw per day for 20 days and then sacrificed. Spleen and thymus were weighed, and lymphocyte proliferation after stimulation of spleen cells and cells from mesenteric lymph nodes with concanavalin A (a T-cell mitogen) was assessed. There were no differences in the weight of spleen or thymus (Tang et al., 2006). Con A stimulation of cells isolated from both spleen and mesenteric lymph nodes from rats fed 120 mg/kg bw per day or higher of SBA showed a dose-dependent lower proliferation. In parallel, the production of interleukin (IL)-2 and interferon (IFN)-gamma showed a dose-dependent decrease from a dose of 180 mg/kg bw. Likewise, the concentration of IL-2 and IFN-gamma and of IgG in plasma decreased with increasing SBA dose from an SBA concentration of 180 mg/kg bw. Together, this indicates that SBA in the feed affected the number and type of T cells in the gut-associated lymph nodes and in circulation, as well as the circulating IgG (Table 7).

When Sprague Dawley rats, 26 days old (78–80 g), were fed soybean lectin (SBA) in doses of 0, 44, 88, 131 or 175 mg/kg bw per day for 10 days, the weight of the spleen decreased with increasing doses of SBA, giving a relative decrease in dry weight of 29.8% with the highest dose compared to the control group (Zang, Li, Piao, & Tang, 2006) (Table 5). For further results, see Section 3.1.4.1.

When 19-day-old weaning male hooded Lister (Rowett, 80 ± 1 gram) were fed 7% WGA or UDA corresponding to 70,000 mg/kg in the feed equivalent to 525 mg/kg bw per day for 10 days, the weight of the thymus was reduced by over 20% in the WGA-fed rats, while in the UDA-fed rats, there was no thymic weight reduction. In both WGA and UDA-fed animals, the wet weight of the small intestine was increased (45% for WGA and 15% for UDA) while the weight of no other tissue was affected including the dry weight of the different part of the gut (Pusztai, Ewen, et al., 1993) (Section 3.1.4.1).

Vaz et al. (2012) immunised 5-week-old Swiss albino mice with WGA on day 0, 15 and 30 (250 mg/kg per injection (0.5 mL)) ($n = 5$) and 3 days before starting the oral treatment, with the same dose intraperitoneally. The WGA immunised mice were then orally administered WGA (27 mg WGA/kg bw per day) or saline for 7 days. Then, the mice were sacrificed by decapitation, and histopathological evaluation of jejunum of the animals was performed, and cell composition as well as specific cytokines (IL-4, IL-5) and chemokines (eotaxin, Rantes (CCL5)) in blood were measured. The histological examination showed that only the jejunum from WGA-fed mice showed moderate lymphocytic infiltrate filling the stroma of villi and a submucosa with numerous eosinophils compared to non-immunised animals treated with saline. Animals challenged with native WGA showed a significant ($p < 0.05$) increase of eotaxin, IL-4 and IL-5 when compared to the saline fed control group.

In summary, orally administered lectins (PHA, SBA, WGA) showed varying and even opposite effects on the size of the lymph organs, such as the spleen and the thymus, which may be due to the highly dynamic nature of these organs and differences in experimental parameters. Two studies (PHA or WGA) assessing the lymphocyte numbers in the small intestine found increased lymphocytes indicating recruitment of lymphocytes to the gut (for further details, see Section 3.1.4.5).

Abrogation of oral tolerance against another dietary protein

One study investigated the ability of lectins to abrogate the induction of oral tolerance by assessing the effect of co-feeding a dietary antigen with lectin. Feeding mice red kidney bean extract containing 1 mg/mL PHA together with the hen's egg white protein ovomucoid (2 mg/mL) equivalent to 250 mg PHA/kg bw and 500 mg ovomucoid/kg bw in the drinking water for three consecutive days abrogated the induction of oral tolerance towards ovomucoid. A subsequent feeding experiment with the same amount of pure PHA (PHA-P, 5 mg/day equivalent to 250 mg/kg bw), together with ovomucoid likewise resulted in abrogation of oral tolerance against ovomucoid. The antibody response against PHA in the extract feeding experiment was also assessed and showed an increase in PHA antibodies after red kidney bean extract feeding. When PHA-P was administered iv or ip, it did not affect induction of tolerance against fed ovomucoid indicating the importance of the oral route for the effect of the lectin. The effect of oral administration of extracts of soy, peanut and pea (corresponding to 5, 3.5 and 2.5 mg/day equivalent to 100, 140 and 200 mg/kg bw per day of the respective lectin) was tested. Of these, only the soy extract abrogated the oral tolerance induction to ovomucoid. Of note, the lectin doses administered in the peanut and pea extracts were lower, which may be the reason for the lack of effect (Kjær & Frøkiaer, 2002).

Feeding ovalbumin (OVA) immunised rats WGA (27 mg/kg bw per day) for five consecutive days followed by an oral OVA challenge (100 µg) gave rise to an IgE response/titre against OVA comparable to OVA immunised and challenged but non-WGA-fed rats (Watzl et al., 2001). Only the WGA-fed rats showed an increased serum level of mast cell protease II indicative of mast cell degranulation at the day of oral OVA challenge. Hence, WGA may lead to higher OVA absorption in the gut resulting in stronger mast cell stimulation. For further results, see Section 3.1.4.4.

Hence, feeding lectins (PHA, WGA) for 3–5 days adversely affects the capability to establish oral tolerance against co-administered proteins.

3.1.4.4 | *Allergenicity*

The typical immunological response to a protein antigen administered orally is the induction of oral tolerance, characterised by a specific downregulation of an antibody response against the antigen (Pabst & Mowat, 2012). Oral tolerance may be abrogated if adverse conditions in the gut, such as disturbance in epithelial renewal or in mucus production, occur. Hence, an antibody response against a fed antigen often indicates disruptions in the immunological homeostasis of the gut mucosa. Various plant lectins have been demonstrated to react with IgE from allergic human individuals and to promote mast cell and basophil degranulation in vitro in the presence of serum from atopic individuals (see Section 3.1.3.3). Animal testing has been used to investigate if lectins, either orally administrated or through injection (sc or ip), give rise to a specific antibody response towards the administered lectin or a co-administered allergen/antigen (Table 7).

Eight- to 10-week-old male Brown Norway rats (6 per group) were daily administered peanut agglutinin (PNA, 99% pure) or OVA (purity 98%) by gavage (1 mg in 1 mL of water equivalent to 10 mg/kg bw) for 42 days or water (control group). The rats were bled weekly, and serum was tested for PNA or OVA-specific IgG and IgE by ELISA (Dearman et al., 2001). PNA induced high IgG titres in all animals peaking from day 21, while OVA induced lower titres and were slower in reaching the peak response (28 days). IgE antibodies in serum against the administered protein measured by the passive cutaneous anaphylaxis (PCA) test in naïve rats did not reveal a positive reaction in any of the rats. In the same publication, groups ($n = 6$) of young adult (8–12 weeks old) female BALB/c mice were administered PNA or OVA by gavage (0.25 mg/day in 1 mL water equivalent to 10 mg/kg bw per day). The mice were sacrificed after 28 or 42 days, and serum was tested for specific IgG by ELISA and IgE by PCA test in naïve mice. PNA-specific IgG in mice administered PNA was high both at day 28 and day 42, while OVA-specific IgG in OVA administered mice was lower and dropped from day 28 to day 42, and not all mice showed a response (Dearman et al., 2001). All mice in the PNA administered group showed positive PCA tests, both at day 28 and 42, while in the OVA administered group 5/6 and 4/6 on day 28 and 42, respectively, showed a positive PCA test. A positive PCA test is apparently not depending on the lectin feeding but on the animal species or strain.

Feeding rats with PHA-E (100 mg/kg bw per day) for 90 days induced increased weight of the mesenteric lymph nodes and increased total serum IgA but did not affect the antibody titre against PHA-E (Kroghsbo et al., 2008). When rats were fed 30 mg/kg bw, no significant effects were seen. When rats were fed lower doses of PHA-E (4.5–72 mg/kg bw per day) for 28 days, the PHA-E-specific IgG1 in blood increased at all doses fed and the PHA-E-specific IgA at doses from 18 mg/kg (Kroghsbo et al., 2008). IgE levels were not measured, but in another study where PHA (L and E) was administered by gavage to Sprague–Dawley male rats for 10 days (50 mg/kg bw per day) or administered alternately by gavage and subcutaneously did not lead to increased total IgE in blood, which was the only isotype of antibodies measured (Haas et al., 2001, for further results, see Section 3.1.6). From these data, the fed amount, the length of the feeding period or both seems to play a key role for the consequence of feeding PHA. Moreover, the type of animal and in particular the specific strain used may affect the specific outcome of the feeding trial. For further details, see Section 3.1.4.3.

The lectin from black turtle beans (BTB) shows high homology with PHA (He et al., 2015). When mice were administered 5 mg BTB lectin (equivalent to 278 mg/kg bw) in a complete *Freund's* adjuvant/PBS emulsion by oral gavage six times during a 4-week period and then challenged orally with 15 mg (equivalent to 833 mg/kg bw) lectin, there was an increase in specific IgE and IgG1 antibody levels as well as an increase in the serum level of histamine and mast cell protease 1 indicating an allergic reaction in the lectin-fed mice upon challenge (He et al., 2021). For further results, see Section 3.1.4.3. Of note, the mice administered native BTB lectin weighed less than the control groups. Histopathologic examination at the end of the study clearly showed that the intestines of the mice fed the native lectin, but not the intestines of control mice, had typical lectin-induced disruptions (with small intestine villi atrophy, brush border membranes detachment and even partial necrosis of intestinal cells). Whether there is a relation between the increased lectin-specific antibody levels and other allergic manifestations on the one hand and the reduced weight and intestinal disruption on the other hand is not clear.

The dietary garlic lectins ASA-I and ASA-II administered orally to BALB/c mice were tested for their immunogenicity potential, compared to PHA (Clement & Venkatesh, 2010). Like PHA, both ASA-I and ASA-II lectins enhanced IgG production (3-fold increase) and displayed a twofold increased phagocytic activity towards rat peritoneal cells. For further results, see Appendix C.

Feeding OVA immunised (10 µg OVA ip) adult Brown Norway male rats WGA by gavage (27 mg/kg bw per day) for five consecutive days followed by an oral OVA challenge (100 µg equivalent to 1 mg/kg bw) gave rise to an IgE response against OVA comparable to OVA immunised and challenged non-WGA fed rats (Watzl et al., 2001). Only the WGA fed rats showed an increased serum level of mast cell protease II indicative of mast cell degranulation at the day of oral OVA challenge. Hence, WGA may lead to higher OVA absorption in the gut resulting in stronger mast cell stimulation. For further results, see Section 3.1.4.4 and Table 7.

The albumin fraction of jackfruit (*Artocarpus heterophyllus*), known to contain a lectin called FIISP (MW of 43 kD). Only when the albumin fraction (1 mg/day, equivalent to 3–4 mg/kg bw per day) from jackfruit containing the lectin was fed to mice for 8 days before an immunisation ip with the albumin fraction, an FIISP-specific IgE response was raised in the immunised mice (Restum-Miguel & Prouvost-Danon, 1985) indicating that the oral route is important for the induction of the IgE response. For further results, see Appendix C.

Lavelle et al. (2000) administered different lectins (5 mg/kg bw of PHA, WGA, tomato lectin (LEA)) or OVA (5 mg/kg bw) by gavage three times (on days 1, 14 and 35), and lectin-specific antibodies in blood, saliva and by mucosal wash were measured 2, 5 and 7 weeks after administration of the first dose. The specific antibody response upon the oral administration was compared to the response to the egg-white protein OVA. No increase in IgA from mucosal samples in mice orally administered lectin was found, but in contrast to OVA, administration of the lectins PHA, WGA and UEA-I induced specific IgG antibody and serum IgA responses upon administration of the lectin.

Taken together, the animal feeding studies support that lectin (PNA, PHA, ASAI/II, WGA) administered orally may disturb the immune system in the gut, leading to an antibody response against the lectin and potentially against other dietary proteins instead of inducing oral tolerance. This disruption increases the risk of triggering an allergic response. Based on the data identified in the literature as provided above, due to high variation in administration schemes (i.e. amount of lectin, frequency and length of administration, antibody classes measured, animal species), no quantitative information can be directly associated with the antibody response.

TABLE 7 Animal feeding experiments with lectins or extract measuring antibody responses.

Lectin tested (purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Antibodies measured	Findings	Reference*
ASAI/II or PHA (purified by anion-exchange chromatography on Q-Sepharose|purity not reported)	Mice, BALB/c, sex NR (n = 5–8/gr)	0.5–5 mg/kg bw ASAI or II, once weekly for 7 weeks	Gavage	Anti -lectin IgG in blood	ASAI or II or PHA Specific IgG high at day 12, 35 and 55. ASAI and PHA higher than ASAII. All higher titres than anti-ovalbumin	Clement and Venkatesh (2010)
Black turtle bean lectin (BTB-lectin) (PHA-type), (extracted and purified by reverse micellar extraction|94% purity)	Mice, BalbC, f (n = 8/gr)	5 mg; equivalent to 278 mg/kg bw once weekly by gavage for 5 weeks in emulsion. Challenge by 15 mg lectin; equivalent to 833 mg/kg bw (18 g bw[b])	Gavage	IgE, IgG1 (BTB-specific)	Reduced bw, increased splenic index, increased anaphylactic symptoms, increased IgE and IgG levels	He et al. (2021)
FIISP Albumin extract of Jackfruit (fraction with high haemagglutinating activity purified by ion-exchange chromatography on SP-Sephadex C-50|purity not reported)	Mice, DBA/2, f (n = 5–8/gr)	1 mg/day; equivalent to 3–4 mg/kg bw per day for 8 days (250–300 g bw[b])	Gavage	IgE total and specific increased	Unheated extract of Jackfruit	Restum-Miguel and Prouvost-Danon (1985)
PHA (commercial source|purity not reported)	Mice, BALB/c, f (n = 4–6/gr)	5 mg/day; equivalent to 250 mg/kg bw per day for 3 days[a]	in drinking water	PHA and ovomucoid specific Ig	Specific PHA Ig in PHA fed mice, abrogation of oral tolerance to in PHA fed mice	Kjær and Frøkiær (2002)
PHA (purified by affinity chromatography on Ovomucoid-Sepharose 4B|purity not reported)	Rats, Sprague–Dawley, m (n = 4/gr)	6 mg per day; equivalent to 50 mg/kg bw per day for 10 days (120 g bw[b])	Gavage	IgE no effect	Non-specific	Haas et al. (2001)
PHA (purified by affinity chromatography on Fetuin-Sepharose-4B|purity not reported), **WGA, UEA, LEA** (commercial source|purity not reported)	Mice, BALB/c, f (n = 10/gr)	5 mg/kg bw on day 1, 14 and 35 of lectin or OVA	Gavage	Specific IgG and IgA in blood, specific IgA in saliva	Higher specific IgG and IgA in mice administered PHA, WGA or UEA than with OVA	Lavelle et al. (2000)
PHA-E (Recombinant PHA-E expressed in *E. coli*|purity not reported)	Rats, Wistar, f/m (n = 5-16/sex/gr)	30 or 100 mg/kg bw per day for 90 days or 4.5–72 mg/kg bw per day for 28 days	Diet	Total IgA, IgG, IgM and PHA-E specific IgM, IgG and IgM	100 mg/kg bw for 90 days increased the total IgA level Feeding 4.5–72 mg/kg bw for 28 increased PHA-E specific IgG1 and IgA but not total antibody levels	Kroghsbo et al. (2008)

(Continues)

TABLE 7 (Continued)

Lectin tested (purity)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Antibodies measured	Findings	Reference*
PNA (commercial source|99% purity)	Rats, Brown Norway, m ($n=6$/gr)	10 mg/kg bw per day for 42 days	Gavage	Specific IgG and IgE against PNA compared to specific ab against OVA after OVA administration	Stronger IgG response against PNA than OVA No IgE response	Dearman et al. (2001)
	Mice, BALB/c, f ($n=6$/gr)	10 mg/kg bw per day for 28 or 42 days	Gavage		Stronger IgG and IgE responses against PNA than OVA	
SBA (purified by affinity chromatography on epoxy-activated N-acetyl-D-galactosamine-sepharose-6B|> 95%)	Rats, Sprague–Dawley, m ($n=6$/gr)	0, 0.05, 0.10, 0.15 or 0.20% (equal to 0, 500, 1000, 1500 or 2000 mg/kg in feed); equivalent to 0, 60, 120, 180 or 240 mg/kg bw per day for 20 days[a]	Diet	IgA, IgG, IgM	Non-specific	Tang et al. (2006)
WGA (commercial source|purity not reported)	Rats Brown-Norway adult, m ($n=6$–8) OVA specific	10 mg per day for 5 days; equivalent to 27 mg/kg bw per day; 100 µg OVA oral challenge	Gavage	OVA-specific IgE Mast cell protease Ifn-g	No effect of oral WGA on anti-OVA IgE Higher Mast cell protease in WGA fed rats	Watzl et al. (2001)

Abbreviations: ab, Antibody; ASA, *Allium sativum* agglutinin; BTB, black turtle bean; bw, body weight; Con A, Concanavalin A; f, female; FIISP, jackfruit lectin; gr, group; IgE, Immunoglobulin E; IgG, Immunoglobulin G; IgM, Immunoglobulin M; Ifn-g, Interferon gamma; LEA, *Lycospersicum esculentum* agglutinin; m, male; *n*, sample size; OVA, ovoalbumin; PHA, phytohaemagglutinin; PNA, peanut agglutinin; UEA, *Ulex europaeus* agglutinin; WGA, wheat germ agglutinin.
*Studies are ordered by lectin name and then by chronological order, starting with the most recent one.

[a]Applying EFSA default values (EFSA Scientific Committee, 2012).
[b]Weights as reported at the beginning of the study.

3.1.4.5 | *Developmental and reproductive toxicity*

No studies on the effects of lectins from edible plants on reproduction and prenatal development were retrieved from the literature.

With regard to the postnatal development, there are a few studies on PHA only. Multiple effects of PHA were explored in a dose–response study on PND14 rats treated via a single gavage of PHA at 0, 2, 10, 50 or 250 mg/kg bw (Prykhod'ko et al., 2009). The relative weights of liver and pancreas were increased at 50 and 250 mg/kg bw, respectively; the relative weight of the thymus was reduced at 250 mg/kg bw, while the body weight remained unchanged at all doses (absolute weight changes not reported). The study focused specifically on the maturation of the small intestine. At 50 and 250 mg/kg bw, there was shortening of villi and reduced activities of disaccharidases, followed by precocious maturation at the 72 h time point, as indicated by increased activities of maltase and sucrose and lowered activity of lactase. Exposure to the two highest doses elevated the number of mucosal CD19+ (B lymphocytes) and CD3+ cells (T lymphocytes) in the small intes-tine, which occurred in untreated weaned rats later in development, i.e. at the ages of 21–28 days. The authors concluded that the immune system may be involved in the precocious maturation of the gastrointestinal tract (for further results, see Section 3.1.4.3). The same group studied the impact of a single dose of 100 mg/kg bw in rats at PND 14 (Marungruang et al., 2018). On PND17, the PHA-treated animals showed an altered composition of the gut microbiota and less intestinal permeability than the controls (for further results, see Section 3.1.4.2). One study on piglets was conducted between PND9 and 11 (Prykhod'ko et al., 2010). PHA was administered in purified form at a single dose of 0 or 50 mg/kg bw with a subse-quent observation period of 7 days. The authors reported no effects on plasma proteins, WBC, IgG, acute phase proteins, CRP, insulin, cholecystokinin (CKK) and GLP2 levels in blood (Prykhod'ko et al., 2010).

Linderoth et al. (2005) treated suckling SD rats at PND10 or PND14 via single gavage of PHA at 0 or 50 mg/kg bw. One or 3 days after this treatment, findings included increased width/depth of crypts, reduced length of villi and enhanced growth of the proximal and distal small intestine with significantly increased numbers of PCNA+ cells and of cells per crypt. In the PHA-treated animals, the intestinal disaccharidases showed an adult-l ike pattern, as indicated by reduced lactase but increased maltase and sucrase activities.

Linderoth et al. published two further studies on suckling SD rats at PND14, receiving PHA at 0 or 50 mg/kg bw either once or three times per gavage (Linderoth, Prykhod'ko, Pierzynowski, & Westrom, 2006 and 2006b; for further results, see Section 3.1.4.2). Enteric exposure to PHA led to growth and precocious maturation of the gastrointestinal tract. In the first 24 h, PHA bound to the gut mucosal lining and was accompanied by villus shortening and decreased disaccharidase activity. From day 1 to 3, PHA binding was decreased and PHA was taken up by enterocytes. Increased crypt cell proliferation, gut

growth, sucrase and maltase activities occurred. Growth of the pancreas and increased digestive enzymes were also observed. Morphologically, from 24 to 72 h, PHA increased intestinal crypt size (width and depth) in the small intestine with villus length in the proximal small intestine increased at 72 h. In parallel study arms, the suckling rats received at PND10 or PND14 one additional gavage of BSA and bovine IgGs 1–27 h after the last PHA treatment; the absorption of these macromolecules was determined via blood levels of BSA and IgGs, which were reduced significantly by PHA in both age groups and under any experimental condition (Linderoth et al., 2005; Linderoth, Prykhod'ko, Pierzynowski, & Westrom, 2006, 2006b).

PHA reduced body weights but significantly increased the relative weights of the liver, stomach and pancreas and the pancreatic protein and trypsin and amylase activities (Linderoth et al., 2005; Linderoth, Prykhod'ko, Pierzynowski, & Westrom, 2006, 2006b).

Based on the adult-like disaccharidase patterns, the reduced absorption of macromolecules and the altered morphology of the small intestine, the authors concluded that PHA induced precocious functional maturation of the gastrointestinal tract in suckling rats.

To summarise, adverse effects of PHA, i.e. an altered small intestine morphology, were seen at 2 mg/kg bw, given once to rats on PND14. Body weight gain, liver, pancreas and pancreatic enzyme activities, the maturation of the GI tract and the immune system were affected at 50 mg/kg bw per day. In piglets of PND9–11, one study did not find any adverse PHA effect when applied at 50 mg/kg per day for 31–35 days (Table 8).

TABLE 8 Developmental and reproductive toxicity studies on the effects of phytohaemagglutinin (PHA) in rats and piglets.

Lectin tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose	Route of administration	Duration of exposure	Observation period	LOAEL/ NOAEL (mg/ kg bw per day)*	Findings	Reference**
PHA (purified by affinity chromatography on Ovomucoid-Sepharose|purity not reported)	Piglet, PND9/10, sex NR (n = 5–6/gr) α-lactalbumin gavage as control	0 or 50 mg/ kg bw	Gavage	Once	Up to 7 days post treatment	50 (NOAEL – only one dose tested)	In blood: ↔ insulin, cholecystokinin, GLP2, plasma proteins, WBC, IgG, acute phase proteins, CRP	Prykhod'ko et al. (2010)
PHA (purified by affinity chromatography on Ovomucoid-Sepharose-4B|purity not reported)	Rat, SD PND14, sex NR (n = 5-8/gr) α-lactalbumin gavage as control	0, 2, 10, 50 and 250 mg/kg bw	Gavage	Once	12 or 72 h	2 (LOAEL)	↔ bw and rel weight of stomach; ↑ rel weights of pancreas (50 mg/kg bw, 72 h) and liver (250 mg/kg bw, 12 h and 72 h); ↓ rel weight of thymus and spleen (250 mg/kg bw, 12 h and 72 h) Small intestine: ↑ length of small intestine (250 mg/kg bw at 72 h) ↑ rel weight of proximal tract (50 mg/kg bw, 72 h); ↔ rel weight of distal part; height of villi: ↓ in proximal and distal part (50 mg/kg bw, 12 h) and ↑ (2 mg/kg bw, 72 h); ↑ crypt depth in proximal part (2 mg/kg bw at 12 h; 50 mg/kg bw at 72 h); ↑ crypt depth in distal part (2 mg/kg bw at 12 h, 250 mg/kg bw at 72 h); ↓ lactase activity in prox and distal part (50 mg/kg bw, 12 h; 250 mg/kg bw, 72 h); ↓ maltase activity in prox and distal part (50 mg/ kg bw, 12 h); ↑ maltase in prox (250 mg/kg bw, 72 h) and distal part (50 mg/ kg bw, 72 h); ↑ sucrase activity in prox (50 mg/ kg bw, 72 h) and distal part (250 mg/kg bw, 72 h); ↑ infiltration with cd19+ cells in prox and distal part (50 mg/kg bw, 12 h) and in distal part (250 mg /kg bw at 72 h); ↑ infiltration with cd3+ cells in prox and distal part (50 mg/kg bw, 12 and 72 h)	Prykhod'ko et al. (2009)

(Continues)

TABLE 8 (Continued)

Lectin tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose	Route of administration	Duration of exposure	Observation period	LOAEL/ NOAEL (mg/ kg bw per day)*	Findings	Reference**
PHA (purified by affinity chromatography on Fetuin-Sepharose-4B|purity not reported)	Rat, SD PND14, sex NR ($n = 10$/gr)	0 or 50 mg/ kg bw per day	Gavage	3 days	1 day after end of treatment	50 (LOAEL-only one dose tested)	↔ bw and rel weight of liver and spleen, ↓ rel weight of thymus, ↑ rel weight of stomach, prox and distal small intestine, and of pancreas ↑ pancreatic protein and trypsin activity and ↔ amylase activity; ↑ rel length of prox and distal small intestine ↓ lactase activity and ↑ activity of sucrase and maltase in prox small intestine ↑ corticosterone levels in plasma ↓ uptake of bovine IgG and bovine serum albumin by GI tract when applied 24 h after treatment;	Linderoth, Prykhod'ko, Pierzynowski, and Westrom (2006)
PHA (purified by affinity chromatography on Fetuin-Sepharose-4B|purity not reported)	Rat, SD PND14, sex NR ($n = 134$)	0 or 50 mg/ kg bw	Gavage	Once	1, 3, 6, 12, 24, 48 and 72 h after treatment	50 (LOAEL-only one dose tested)	↔ bw Small intestine: ↑ rel weight of distal tract (24–72 h); ↑ rel weight of prox tract (48 h); ↑ rel length of the whole tract (48 and 72 h); length of villi in prox part: ↓ (6 and 24 h) and ↑ (72 h); crypt depth/width in prox and distal part: ↑ (24 and 48 h); ↑ PCNA + cells in prox and distal part (24 h); ↓ lactase activity (12–48 h); ↑ maltase and sucrase activity (48 and 72 h); ↓ uptake of bovine IgG and bovine serum albumin by GI tract when applied 1–72 hrs after treatment; Liver and pancreas: ↑ rel weight of liver and pancreas (48 and 72 h) and of stomach (48 h); ↑ activity of trypsin (48 and 72 h) and amylase (72 h)	Linderoth, Prykhod'ko, Ahrén, et al. (2006)
PHA (purified by affinity chromatography using Fetuin-Sepharose-4B|purity not reported)	Rat, SD PND10, sex NR ($n = 7$-20/gr)	0 or 50 mg/ kg bw per day	Gavage	3 days	1 day or 3 day after end of treatment	50 (LOAEL – only one dose tested)	↓ bw (day 1) ↑ rel weight of liver (day 1), stomach (day 1), and of pancreas (day 1 and day 3); ↑ pancreatic protein (day 1 and day 3) and trypsin activity (day 1 and day 3); Proximal small intestine: ↑ rel weight (day 1 and day 3), diameter (day 1), cells per crypt (day 1), PCNA+ cells (day 1) Distal small intestine: ↑ rel weight (day 1 and day 3), diameter (day 1), cells per crypt (day 1) ↓ uptake of bovine IgG and bovine serum albumin by GI tract;	Linderoth et al. (2005)

TABLE 8 (Continued)

Lectin tested (purification technique|purity)	Study design (species, strain, sex, group size)	Dose	Route of administration	Duration of exposure	Observation period	LOAEL/ NOAEL (mg/ kg bw per day)*	Findings	Reference**
	Rat, SD PND14, sex NR (n = 7-20/gr)	0 or 50 mg/ kg bw per day	Gavage	3 days	1 day or 3 day after end of treatment	50 (LOAEL – only one dose tested)	↓ bw (day 1 and day 3) ↑ rel weight of liver (day 1), stomach (day 1 and day 3) and of pancreas (day 1 and day 3); ↑ pancreatic protein (day 1 and day 3) and trypsin activity (day 1 and day 3); Proximal small intestine: ↑ rel weight (day 1 and day 3), diameter (day 1 and day 3), cells per crypt (day 1) ↓ lactase activity (day 1); ↑ activity of sucrase and maltase (day 1) Distal small intestine: ↑ rel weight (day 1 and day 3), diameter (day 1), cells per crypt (day 1), ↓ % of supranuclear vacuole containing cells (day 1 and day 3) ↓ lactase activity (day 1); ↑ activity of sucrase and maltase (day 1) ↓ uptake of bovine IgG and bovine serum albumin by GI tract	

Abbreviations: bw, body weight; CRP, C-reactive protein; GI, gastrointestinal; GLP-2, glucagon like peptide-2; gr, group; IgG, Immunoglobulin G; LOAEL, lowest observed adverse effect level; n, sample size; NOAEL, no observed adverse effect level; PCNA, proliferating cell nuclear antigen; PND, postnatal day; prox, proximal; rel, relative; SD, Sprague Dawley; WBC, white blood cells.

*NOAEL and LOAEL extracted from the publication. **Studies are ordered by chronological order, starting with the most recent one.

3.1.4.6 | *Genotoxicity*

Three studies were identified that reported investigating genotoxicity. However, of these, only one used a standard genotoxicity test system (Rolim et al., 2011).

Rolim et al. (2011) assessed the potential mutagenicity of an extract of *M. oleifera* seed powder and the water-soluble *M. oleifera* lectin (WSMoL) purified via chitin chromatography. Mutagenicity was evaluated using both a cell-free plasmid DNA assay (i.e. DNA breaks were assessed in the pBC plasmid carrying the chloramphenicol-resistance gene followed by gel electrophoresis) and *Salmonella* Typhimurium (Ames and Kado tests) assays using TA97, TA98, TA100 and TA102 strains, in the presence or absence of hepatic S9. The lectin, WSMoL, was not mutagenic in any of these assays.

Faheina-Martins et al. (2011) assessed the potential genotoxicity of lectins from *Canavalia brasiliensis* (Con Br) and *Cratylia floribunda* (CFL) and commercially sourced *Canavalia ensiformis* (Con A) lectin in the human breast cancer cell line MCF-7. DNA breaks were evaluated by the alkaline comet assay after a 24-h exposure to the lectins. At all tested concentrations, significant apoptosis, some necrosis and DNA breaks were observed by visual assessment. Therefore, it is difficult to determine the exact origin of the DNA breaks.

De Oliveira Dos Santos et al. (2022) used a *Drosophila melanogaster* model to assess the genotoxicity of different concentrations of native Con A (4.4, 17.5 and 70 μg/mL). All concentrations of native Con A induced an increase in DNA breaks (> 50% increase in damage index and damage frequency) in *Drosophila* homogenates on day 7. However, this change coincided with overall toxicity (decreased cell viability and increased markers of oxidative stress), making it difficult to determine the specific origin cause of the DNA breaks.

In summary, there is limited evidence that Con A and related lectins cause DNA breaks in the systems tested. There is no clear evidence supporting mutagenicity or genotoxicity. The Panel notes that, since lectins are proteins, no genotoxic properties are expected via a direct DNA interaction.

3.1.4.7 | *Carcinogenicity*

Only one study investigating intestinal carcinogenesis was retrieved. This study was not a standard carcinogenicity study but was a 24-week low dose (40 mg/rat/day) study using peanut lectin (PNA) either alone or in combination and with or without dimethylhydrazine, a potent colon carcinogen commonly used as an initiator of carcinogenesis. The number of animals per group was low for a carcinogenicity study (n = 9). No significant carcinogenesis of the colon was noted, but a small increase in the weight of the colon of lectin-fed animals was noted. The pancreas weight increased, but there was no evidence of tumour formation (Kelsall et al., 2002).

3.1.5 | Observations in humans

All the relevant studies assessed acute toxicity.

3.1.5.1 | *Gastrointestinal, metabolic and antinutritional effects*

Seven publications were identified on gastrointestinal and antinutritional effects related to lectins, where the lectins under study were of edible plant origin and were identified and/or quantified. None of these studies provided quantitative data on the lectins under study. Two additional articles related to human studies where lectins were of edible food origin; the relevant lectins were not identified but were indirectly associated with the food consumed. Six additional studies were identified that pertained to lectins coming from herbal preparations of non-edible plant parts, and 22 pertained to lectins from non-edible plants (nine on accidental poisoning, 13 on intentional self-harm intake) (Tables B1–B6, Appendix B).

In the text below, information is provided on clinical data related to lectins from edible plants. The information related to accidental or intentional intake of lectins from non-edible plants is provided in Appendix B.

3.1.5.1.1 | *Interventional studies*

Three small intervention studies were identified. Lectin HA was evaluated in two studies. Lectin identification was performed in two studies, but quantitative lectin characterisation was not reported in any study. The endpoints under study were assessed in one study each and pertained to gastrointestinal symptoms, glycaemic response and rectal epithelial proliferation.

Petry et al. (2016) reported on the safety of low-phytic acid[14] bean varieties assessed as regards their efficacy to enhance iron bioavailability in a randomised crossover trial in Rwanda ($n = 25$). Each bean meal was served for five consecutive days, two times per day, in the morning after an overnight fast between 07.00 and 09.00 and for lunch between 11.00 and 13.00 from Monday to Friday. SDS-PAGE showed that the cooked low-phytic acid beans contained PHA-L but no PHA-E. No quantitative information was provided. The consumption of low-phytic acid beans was associated with gastrointestinal side effects in most of the study participants (nausea, vomiting, abdominal pain, abdominal distention, diarrhoea); the gastrointestinal effects occurred during the first 48 h of consumption and then subsided or resolved. Four subjects with vomiting were referred for medical assessment and recovered completely within 3 days. No side effects were reported in the other study arms (high-iron biofortified beans; a commonly consumed control bean).

Rea et al. (1985) investigated whether lectin concentrations of 16 foods and total lectin intakes in test meals were related to glycaemic response (glycaemic index based on the area under the glucose response curve; AUC) in normal or diabetic individuals. Six legumes and 10 non-leguminous foods were cooked in a conventional manner, were aliquoted into meal portions containing 50 g of estimated available carbohydrate and were fed as breakfast test meals ($n_{min} = 10$). Finger prick blood samples were analysed for glucose, and the AUC for a particular food was reported; the AUCs were calculated geometrically for each subject, and statistically significant differences were assessed. Moreover, aliquots of the tested food samples were freeze-dried, ground and analysed for lectin HA; further lectin characterisation was not reported. Lectin HA in the foods varied widely from < 1.3 (lower limit of detection) to 360 HU/mg. Lectin activity (expressed as HU/mg sample or HU/g available carbohydrate) was inversely associated with the glycaemic indices for normal and diabetic individuals. In a subsequent trial, dry red beans obtained from a local supermarket were pressure cooked (15 psi, 121°C) for either 30 min (1300 mL water, 1 tsp salt) or 60 min (1600 mL water, 1 tsp salt) and were fed in a random order as breakfast test meals (with 600 mL tea or coffee over approximately 10 min) after an overnight fast and using white bread as control ($n = 7$). Dry red beans produced statistically significant lower glycaemic responses than white bread; the glycaemic response to beans cooked for 30 min was statistically significantly lower than that to beans cooked for 60 min.

Ryder et al. (1998) assessed whether peanut ingestion can alter rectal epithelial proliferation. Study participants ($n = 36$) with normal colonic mucosa consumed 100 g of peanuts per day for 5 days; exposure to lectins was assessed via PNA lectin histochemistry (for detection of TF antigen); immunohistochemistry was used for detection of PNA bound to the colonic epithelium; faecal lectin activity was measured by haemagglutination assay against neuraminidase-treated human group O red blood cells; and the rectal mitotic index was also assessed. Ten patients (28%) had epithelial positivity for PNA binding sites (defined as 5% of cells showing positivity) in the colonic mucosa before ingestion of peanuts. PNA was detected in 24 of 36 patients (67%) after the period of peanut ingestion, eight of whom tested positive for PNA receptor. Faecal HA increased overall from a mean of 84 (± 22) to 355 (± 102) HU/mg faeces after peanut supplementation of the diet; of note, there was no observed change in six patients without further data on study compliance. The proliferative response correlated with the faecal HA.

3.1.5.1.2 | *Case reports and case series*

Four studies reported on case reports or case series related to ingestion of red ($n = 3$) or white ($n = 1$) kidney beans in France, the UK and Japan. In all three studies related to red kidney beans, GI symptoms were reported, and lectin activity was assessed via haemagglutination assay. No further lectin characterisation was reported.

[14]Phytic acid prevents the absorption of iron, zinc and calcium and may promote mineral deficiencies.

Red kidney beans

Noah et al. (1980) reported seven incidents of gastroenteritis in the UK (n_{cases} = 43) during 1978–1979 that were associated with eating red kidney beans. All consumed food was processed (soaked and/or cooked). The incubation period ranged from 1 to 3 h. Nausea, vomiting, diarrhoea and abdominal pain were the associated clinical symptoms. No death was reported, and two subjects required hospital admission. In all outbreaks, microbiological pathogens were excluded as the source of the outbreak. For one of the outbreaks (n_{cases} = 15), an archived sample of beans was examined (measured on a saline extract of the beans by reaction with rabbit blood erythrocytes). The HA of the sample was 19,000 HAU/g dry weight.

An updated study by the same group (Rodhouse et al., 1990) reported 50 relevant incidents of red bean consumption (n > 200) from 1976 to 1989, with food analysis available for 31 incidents via haemagglutination assay. After careful consideration of the clinical symptoms (nausea, vomiting and diarrhoea) and haemagglutination assay results, only 32 incidents were deemed compatible with the diagnosis of red kidney bean poisoning. Of these, nine incidents were corroborated by high haemagglutinating activity (> 3200 HAU/g), measured on bean extracts by reaction with rabbit blood erythrocytes and, in eight cases, some soaking and/or cooking was reported.

Watier-Grillot et al. (2023) reported on an incident of acute gastroenteritis among customers of a dining facility at a military base in Brittany, France, in 2018, related to chilli con carne served at lunch. Of the 1700 exposed individuals, 200 developed GI symptoms acutely after exposure (median incubation period: 3.3 h). A high haemagglutinating activity using rabbit blood erythrocytes, estimated at 66,667 HAU/g dry weight sample, was found in the raw red kidney beans used in the chilli dishes. Residual haemagglutinating activity above the method detection limit of 400 HAU/g was measured in the chilli con carne sample. The haemagglutinating activity of the beans alone was estimated to be 2000 HAU/g (dry weight), based on the relative proportion to the other ingredients.

White kidney beans

Ogawa and Date (2014) reported on a major incident in Japan where a TV programme advocated a weight loss intervention including the addition of poorly processed white kidney bean powder in cooked rice. More than 1000 people reported gastrointestinal symptoms including vomiting and/or diarrhoea; 467 people needed medical consultation and 122 patients were admitted to the hospital. Two samples provided by viewers of the TV programme who followed the proposed intervention were further tested and the PHA activity was measured by a haemagglutination test using guinea pig and human erythrocytes. No quantitative results on the measured HA were reported, but the authors claimed that the main cause of the symptoms was high PHA activity remaining due to insufficient heating to inactivate the lectin in the beans, especially Shirohana beans (*P. coccineus*), which contain lectins with high activity.

Summary

The available epidemiological evidence on the association between acute exposure to lectins and gastrointestinal and metabolic endpoints is characterised by a limited number of small studies. The observational evidence does not include analytical studies with relevant control groups. Presumed lectin activity was assessed via haemagglutination assay in most of the studies. No further information was provided in any of the studies on the possibility of the observations being attributed to other effects (e.g. disaccharidase activity). Based on the above, the available epidemiological data on GI and metabolic effects cannot be used for risk characterisation.

3.1.5.2 | *Allergy and allergenicity*

3.1.5.2.1 | *Allergic reactions*

Plant lectins have already been identified as allergens by the WHO/IUIS Allergen Nomenclature Sub-Committee (https://allergen.org), including, but not limited to, Tri a 18 (wheat germ agglutinin WGA) and a few chitinases with a hevein domain from banana (Mus a 2), mango (Man i 1), turnip (Bra r 2), chestnut (Cas s 5), corn (Zea m 8), buckwheat (Fag e 4) and avocado (Per a 1) (Table C.2). Evidence from case reports of allergic reactions and allergenicity studies has implicated additional members from different groups of lectins as relevant allergens (Barre et al., 2020). Overall, chitinases of classes I and IV, which contain a hevein-like domain, and legume lectins are the biggest contributors to food allergies (for further information, see Section 3.1.5.2.2).

Of note, other proteins than lectins have also been identified as the major allergens in most of the commonly eaten foods. For example, the major food allergens in soy are reported to be the soybean seed 34-kDa oil-body-associated protein (Gly m Bd 30K/P34), Gly m Bd 28K/P28 and Gly m 5, all belonging to the vicilin storage proteins in soy (Wiederstein et al., 2023). For peanuts, Ara h 2 and Ara h 6, both storage proteins, are reported as the major allergens (Zhuang & Dreskin, 2013), and for wheat, proteins in the gluten fractions are regarded as major food allergens (Tatham & Shewry, 2008).

In this section, the human studies reporting on allergic reactions directly or indirectly related to lectins are described.

González-de-Olano et al. (2018) described the case of an infant presenting with allergy after exposure to garlic lectin. A 9-month-old female infant experienced itchy erythema affecting the ear, trunk and groin, along with cough (normal pulmonary auscultation) within minutes after the accidental intake of a homemade garlic sauce. She was still breastfeeding

(positive maternal history for exposure to garlic sauce) and she had never eaten this food before. The patient and her parents did not have atopic dermatitis or any additional atopic background. The garlic extract was analysed using SDS-PAGE, which revealed protein bands ranging from 55 kDa to 4 kDa. Immunoblotting assays with the patients' serum were performed under reducing and non-reducing conditions (with and without 2-mercaptoethanol, respectively) and revealed IgE reactivity with an apparent molecular weight of approximately 8.5 kDa and 9 kDa under non-reducing conditions, and a faint band of approximately 9 kDa under reducing conditions.

Rougé et al. (2011) reported a case identifying phaseolin and PHA as putative allergens in France (for further information, see Table C.2). The case report was related to the ingestion of cooked kidney beans by a 23-year-old female with no history of atopy and a resulting anaphylactic shock (incubation time, 30'). Of note, the patient had experienced less severe systemic reactions always related to kidney bean ingestion in the past. Skin prick test was positive for crushed cooked kidney bean and skin prick tests for peanut and soybean were negative. Allergens related to kidney beans were sought and five main IgE-reacting protein fractions were identified. Western blot was performed in the presence of the PHA inhibitory sugars (fetuin, galactose) to prevent nonspecific interaction of the lectin with CCD of both bean and serum glycoproteins.

Matsui et al. (2023) reported on a case report involving a 7-year-old girl with Down syndrome in Japan with a history of suspected episodes of beta-lactam allergy who presented with severe allergic symptoms, including multiple hives, conjunctival hyperaemia, difficulty breathing, vomiting and swollen face, after consumption of white bean soup (potential sensitisation reported 1 year before). The total IgE was low (29 IU/mL) and a white bean-specific IgE was detected (13.4 kUA/L). The skin prick test using boiled white bean was positive and the oral food challenge with 2 g of boiled white bean was also indicative of white bean allergy. To further investigate cross-reactivity, skin prick tests to red kidney beans, azuki beans, runner beans and white beans were performed which all tested positive; serum levels of pea-, soybean- and peanut-specific IgE were 1.38, 1.1 and 0.44 kUA/L, respectively. Western blotting and mass spectrometric analysis were performed and, among other proteins, phytohaemagglutinin (lectin_legB domain-containing protein) and the mannose-binding Flt3 receptor-interacting lectin (FRIL, Q9M7M4) (as a fragment) were identified.

Laiseca García et al. (2023) reported on two paediatric cases of allergy to white kidney bean in Spain with cross-reactivity to red kidney bean. Confirmation was done through skin prick testing and IgE measurements. In both cases, SDS-PAGE immunoblotting detected two main protein fractions with molecular masses of 48–50 kDa and 28–31 kDa, which could correspond to phaseolin and PHA, respectively. However, in the absence of mass spectrometry identification, it is not possible to conclude on the equivalence of the 28–31 kDa protein band to PHA.

Summary

The available evidence on the association between acute lectin exposure and allergic reactions pertains to five cases (among which four were children) of allergic reactions that were thoroughly investigated. Based on the above, the CONTAM Panel concludes that red and white kidney beans can be associated with acute allergic reactions which might be attributed to the lectin activity.

3.1.5.2.2 | *Allergenicity studies*

The specific reaction that is induced against an allergen can be classified into four main hypersensitivity reaction types: three antibody-mediated (type I–III) and one cell- mediated (type IV). Lectins can be related to all four types of hypersensitivity reactions.

IgE-mediated food allergy (Type I)

IgE-mediated allergy is the most common form of hypersensitivity reaction and by far the most predominant type in food allergy, and it emerges immediately upon the encounter, e.g. oral intake of the allergen. In this section, the assessed evidence is organised based on the diagnostic tools used (i.e. skin prick testing, lectin-specific IgE antibody detection, food-specific IgE antibody detection).

Lectin-specific skin prick testing and IgE studies

Diagnosis of allergy in humans is first and foremost done by a skin-prick test. The suspected allergen is placed on the skin of the allergic person who will react by developing a wheal and flare reaction [a raised bump (wheal) surrounded by a red area (flare)] in the affected skin if there are IgE antibodies present against the allergen that elicit mast cell degranulation after the allergen application. Of note, the precise causal relationship between specific allergens and atopy and allergy may not be identifiable even after a positive skin prick test due to the presence of other allergens that could play a more major role.

Conversely, the only existing evidence for lectins evoking a specific allergic response in humans is the presence of lectin-specific IgE antibodies in serum from humans suffering from food allergy. However, IgE antibodies are glycoproteins; thus, in any test of IgE reactions against plant lectins, it must be tested whether the antibodies bind specifically to lectins or whether it is the lectin that non-specifically binds to the carbohydrate moieties present on the antibodies. Independently of whether the IgE antibodies bind to the lectin, or the lectin binds to the sugar moieties on the IgE, the binding may cause degranulation of mast cells and basophils and thus, the release of histamine and other bioactive substances. This dual-action

possibility is also a problem when assessing results by the basophil activation test (BAT) where IgE on basophils and mast cells cross-linked by lectin may cause degranulation in both cases (Pramod et al., 2007, Krithika et al., 2018) for further results, see Appendix C. A simple way of distinguishing a specific lectin–IgE interaction from a non-specific interaction is to first add a sugar that inhibits the carbohydrate-binding activity of the lectin to the medium, e.g. Man for a Man-specific lectin, which will allow to differentiate a specific interaction with IgE (uninhibited in this case) from a non-specific IgE interaction induced by sugars (inhibited in this case). This simple test should also prevent a lectin from interacting non-specifically with IgE antibodies directed against (an)other unrelated protein(s), to give a false-positive reaction in the degranulation test. Regarding the basophil activation test, it is important to keep in mind that only sera from allergic individuals hold appreciable levels of IgE and it is only IgE antibodies in the blood that bind to the receptors on the basophils and mast cells. Sera from healthy non-allergic individuals will not cause degranulation and are thus not suitable as controls when investigating whether serum from an allergic individual after addition of a specific allergen (lectin) causes degranulation.

In the following section, knowledge regarding the binding of IgE from allergic/atopic patients to lectins from edible plants is summarised. The Panel acknowledges that, if IgE from an allergic individual binds to a lectin, it signifies that the lectin has either given rise to the production of IgE against the allergen, or that the lectin cross-reacts with other allergens, or that the lectin binds to the N-glycans of the IgE molecules. The Panel also acknowledges that, even if IgE binds specifically to a lectin in vitro, or a lectin binds to the N-glycans of IgE in vitro, it does not necessarily imply that the lectin upon ingestion induces an allergic reaction. To do so, this requires that the lectin is absorbed from the gastrointestinal tract and may further depend on the state of the lectin (native/denatured). Regarding the possibility of an absorbed lectin giving rise to an allergic reaction through the binding to the N-glycans of IgE, this further requires that the lectin retains its activity by retaining its native structure and that the lectin does not bind to N-glycans on cells and other proteins it may encounter before it reaches the IgE bound on mast cells.

The Panel identified two studies where the skin prick test studies were lectin specific and six studies where lectin-specific IgE was assessed. Kasera et al. (2013) assessed the allergenicity of the major allergen of kidney bean (*Phaseolus vulgaris*). The purified protein appeared as a single band at 31 kDa on SDS-PAGE; it showed haemagglutination to human RBCs, and mass spectrometric analysis identified it as PHA. Patients with a history of kidney bean allergy ($n = 25$) were skin prick tested (SPT) with crude kidney bean extract and all of them tested positive. A subgroup was also tested with the purified protein (1 mg/mL) ($n = 14$) and 78% of the participants tested positive. Specific IgE was estimated in sera ($n = 25$) by ELISA and 22 individuals (88%) showed positive IgE values against the purified protein. Periodate oxidation did not reduce the IgE binding of 31 kDa protein as observed on immunoblot thus supporting specific binding. The 31 kDa protein remained stable for 60 min on incubation with pepsin. Cross-reactivity was observed with peanut and black gram.

Pramod et al. (2006) screened 48 atopic and 20 non-atopic individuals by skin prick test (SPT) with DBA (100 mg/mL), the galactose-specific lectin from horse gram (*Dolichos biflorus*) seeds (for further information, see Table C.2). No information on allergic reactions related to horse gram consumption was provided for any of the study participants. Moreover, DBA-specific IgE was detected by dot-blot and ELISA in the individuals with atopy. Histamine release (HR) assay was carried out using leucocytes from non-atopic and atopic subjects and rat peritoneal exudate cells. Regarding SPT, 10 subjects positive for DBA were detected, whereas none of the 20 non-atopic subjects tested were SPT-positive. Among the 10 SPT-positive participants, eight and two were reported as barely and moderately positive, respectively, by means of the wheal/flare diameter. These two individuals had also threefold higher DBA-specific Ig-E titres compared to the remaining 8 and had also a positive correlation to basophil histamine release.

Shibasaki et al. (1992) evaluated the IgE-binding capacity and histamine-releasing response of 12 lectins of different carbohydrate-binding specificities including Con A and 7 lectins from edible food (LCA from lentil, PSA from pea, SBA from soybean, PNA from peanut, PHA-E and PHA-L from kidney bean, WGA from wheat). IgE from allergic patients (children, asthma, high sensitivity to house dust mites, $n = 10$) displayed a high affinity for Con A, LCA, PSA and PHA-L, a lower affinity for WGA, and did not bind to PHA-L, SBA and PNA. No information on food allergy was provided for any of the study participants. The binding of IgE to the lectins was prevented by incubating the lectins with the competitive monosaccharides (Man, Gal/GalNAc, GlcNAc) or glycopeptides (for PHA-E, PHA-L), prior to adding the IgE. Only two lectins, Lotus A and UEA-I, were not competitively inhibited by the lectin-specific sugars or glycopeptide. In addition, Con A, LCA, PSA and WGA induced a significant release of histamine from leucocytes of allergic patients, while no histamine release was measured after stimulation by the lectins of leucocytes from non-allergic subjects; however, non-allergic subjects do not produce much IgE which may explain the lack of histamine release. The histamine-releasing response was also inhibited by the competitive monosaccharides of Con A (Glc), LCA and PSA (Man) and WGA (GlcNAc). These results suggest that lectins with different carbohydrate-binding specificities interact with the N-glycan moiety of IgE hereby inducing a non-specific histamine release from the stimulated leucocytes.

Gruber et al. (2005) evaluated the effect of the non-enzymatic browning reactions on the immunological activity of peanut proteins, including the PNA. The proteins were thermally treated in the absence and presence of carbohydrates and carbohydrate degradation products. The IgE-binding activities of the thermally treated proteins were then compared to those measured for the native, non-reacted counterparts. Single sera samples of individuals allergic to peanuts ($n = 7$), individuals sensitive to sweet cherry ($n = 5$) and non-allergenic controls ($n = 2$) were tested via ELISA for IgE binding. Six of seven patients allergic to peanuts showed an increased level of IgE binding, whereas all the remaining participants did not show any binding to the agglutinin, suggesting that non-specific interaction of PNA with the N-glycan moiety of IgE is unlikely.

Vojdani (2015) measured IgG and IgE antibodies against peanut proteins, agglutinins and oleosins in the general population ($n = 288$). No information on food allergy was provided for any of the study participants. At a cut-off of 0.45 optical

density (OD) for peanut agglutinin, 49 of the specimens (17%) showed an elevation in IgG antibodies. At a cut-off of 0.46 OD, 15 of the tested specimens (5%) had very significant elevations of IgE antibodies against peanut agglutinins, and an additional 18 (6%) showed moderate elevations. Epitope similarity existed between peanut proteins, peanut agglutinin and peanut oleosin to some extent regarding the production of IgG antibodies and, to a greater extent, of IgE antibodies.

Vojdani et al. (2020) evaluated the presence of anti-lectin IgG, IgA, IgM and IgE antibodies [wheat germ agglutinin (WGA), peanut agglutinin (PNA), soybean agglutinin (SBA), phytohaemagglutinin (PHA-E + L), lentil lectin, pea lectin] in healthy donors ($n = 500$). No information on food allergy was provided for any of the study participants. The percentage elevation of IgE antibodies was 15% for WGA, 12% for PNA, 14% for SBA, 8% for PHA, 12% for lentil lectin and 12% for pea lectin. Comparing the elevations among the six lectins, the highest to lowest for IgE were WGA, SBA, PNA, pea lectin, lentil lectin and PHA.

Food-specific IgE studies

The studies described in this section report on IgE assays specific for food or food extracts.

Kasera et al. (2011) characterised allergens of kidney bean among the Indian population ($n = 355$) and established its cross reactivity with other legumes. Inhibition assays revealed extensive cross reactivity among kidney bean, peanut, black gram and pigeon pea. Patients with a history of legume allergy were tested by SPT with 10 legumes. Identification of major allergens of kidney bean was carried out by mass spectrometry. Specific IgE (sIgE) and total IgE were estimated in sera by enzyme-linked immunosorbent assay. Kidney bean exhibited sensitisation in 78 (22%) patients. Sera from 30 kidney bean sensitive individuals exhibited basophil histamine release (16%–54%) which significantly correlated with their SPT ($r = 0.83$, $p < 0.0001$) and sIgE ($r = 0.99$, $p < 0.0001$). An LC-MS/MS analysis identified four allergens of kidney bean showing significant matches with known proteins, namely, lectin (phytohaemagglutinin, PHA-E), phaseolin, alpha-amylase inhibitor precursor and group 3 late embryogenesis abundant protein.

Mateo-Morejón et al. (2017) assessed the properties of peanut allergens in allergic patients ($n = 316$) and in adult non-allergic volunteers ($n = 303$) in Cuba. SPT was performed using two glycerinated allergenic extracts, prepared from raw or roasted peanuts. The prevalence of sensitisation to raw peanut was 4.6% in the general adult population, 18.6% in the adult food-allergic patients and 25.8% in food allergic children. History of allergic reactions to peanut was captured in the SPT-positive participants. The IgE-binding profile of 26 selected SPT-positive patients was further analysed by immunoblotting by which the major allergens Ara h 2 and Ara h 6 were identified as the IgE-binding targets, while PNA was identified in the IgG4 binding profile along with an Ara h 3 fragment.

Lectin-specific non-IgE antibody studies

A number of studies assessed the binding profile of immunoglobulins other than IgE to lectins. The interpretation of the findings of these studies remains uncertain. However, a strong long-lasting specific antibody response towards a food protein (including plant lectins) may indicate a disturbed immune system with a breakdown of tolerance. This may in some individuals lead to allergy towards the plant lectin and towards other dietary proteins. Furthermore, a breakdown of oral tolerance might also increase the risk for developing autoimmunity. IgA is a normal but often transient response to orally presented antigens. This reflects the healthy reaction towards any antigens (proteins, microorganisms) that succeed in traversing the mucosal barrier and mounting a local mucosal immune response. As long as it is confined to the mucosa, this is transient and is not expected to lead to allergy or autoimmunity.

In type II and III, antibodies of the IgG subclasses are produced against the foreign antigen, either because the antigen has bound to free molecules (Type II) or molecules bound to surface molecules on the host cells (Type III). This process introduces new antigens into circulation, triggering the immune system to produce IgG antibodies against them. Significant response levels of IgG and IgM to oral antigens may indicate alterations in or breakdown of the epithelial barrier in the gut and lectins entering into the circulation, conditions that have been associated with autoimmunity. Additionally, IgG4 response could be used as a marker of acquired tolerance often resulting in a less severe allergic disease.

Fälth-Magnusson and Magnusson (1995) compared the serum antibody levels IgA, IgG and IgM to WGA and to gliadin in children under investigation for coeliac disease (CD), as compared to reference children. The levels of IgA and IgG to WGA as well as gliadin were significantly higher in coeliac children on a gluten-containing diet compared to children on a gluten-free diet and reference children.

As discussed above, Vojdani et al. (2020) evaluated the presence of anti-lectin IgG, IgA, IgM and IgE antibodies [wheat germ agglutinin (WGA), peanut agglutinin (PNA), soybean agglutinin (SBA), phytohaemagglutinin (PHA-E + L), lentil lectin, pea lectin] in healthy donors ($n = 500$). The percentage elevation of antibodies against different lectins ranged from 12% to 16% (IgG), 9.7%–14.7% (IgA), 12%–18% (IgM) and 7.8%–14.6% (IgE). Comparing the elevations for each isotype antibody among the six lectins, the highest to lowest for IgG, respectively, were pea lectin, WGA, PHA, lentil lectin, PNA and SBA. The highest to lowest for IgA, respectively, were SBA, PNA, pea lectin, PHA, lentil lectin and WGA. The highest to lowest for IgM, respectively, were pea lectin, WGA, lentil lectin, SBA and PHA equally, and PNA. The highest to lowest for IgE, respectively, were WGA, SBA, PNA, pea lectin, lentil lectin and PHA. Serial dilutions and inhibition study confirmed that these reactions were specific.

As discussed above, Mateo-Morejón et al. (2017) assessed the properties of peanut allergens in allergic patients ($n = 316$) and in adult non-allergic volunteers ($n = 303$) in Cuba. The IgG4-binding profile of 26 selected SPT-positive patients was further analysed by immunoblotting and PNA was identified along with an Ara h 3 fragment.

On a different note, Tchernychev and Wilchek (1996) purified antibodies to three structurally related legume lectins (Erythrina corallodendron lectin (ECorL), peanut agglutinin (PNA) and soybean agglutinin (SBA)) and to one cereal lectin (wheat germ agglutinin (WGA)) by affinity chromatography from human sera and examined their binding specificity. Both the anti-SBA and anti-ECorL antibodies bound to all three denatured legume lectins. Similarly, the affinity-purified anti-WGA antibodies bound to denatured WGA. Thus, the purified anti-lectin antibodies recognised both active and denatured forms of the lectins. The presence of non-fractionated human IgG or affinity purified anti-SBA, -ECorL or-WGA antibodies (40 µg/mL) did not affect the lectin-mediated agglutination of sialidase-treated human erythrocytes.

The latex-fruit syndrome

Latex-fruit syndrome, also known as latex-food syndrome, is characterised by a variety of clinical manifestations, ranging from simple oral reactions (the so-called Oral Allergy Syndrome OAS, which consists of allergic manifestations confined to mouth and throat) to severe systemic or anaphylactic reactions, which are quite common with avocado, banana, chestnut and kiwi, the foods most frequently implicated in latex-fruit syndrome (Blanco et al., 1994; for further results, see Appendix C). Up to 50% of latex-allergic individuals may develop allergy to various fruits, but it remains unclear whether rubber tree latex sensitisation always precedes allergic reaction towards heveins from fruits or not (Radauer et al., 2011). However, in most cases of latex-fruit syndrome, the IgE response to hevein-like domain is elicited by hevein (Hev b 6.02) as sensitising allergen (Wagner & Breiteneder, 2002). Among other chitin/chitobiose-binding lectins, the reaction towards the quite heat-resistant WGA lectin is frequent in wheat allergic patients (50%–70%), and wheat dependent, exercise-induced anaphylaxis (WDEIA) (> 80%) (Dramburg et al., 2023; for further results, see Appendix C). Interestingly, chitinases responsible for the latex-fruit syndrome were identified as ethylene-induced proteins (defence protein) and heat-sensitive proteins (inactivated by cooking) (Sánchez-Monge et al., 2000).

Summary

The available evidence on lectin-related allergenicity consists of studies assessing SPT reactions and IgE assays (either lectin- or plant-specific). The medical history of food allergy or plant-specific allergy was variably reported for the study participants. Discerning specific vs. non-specific lectin binding by adding a sugar that inhibits the carbohydrate-binding activity of the lectin to the medium was rarely done. Positive SPT was reported in varying frequency and the IgE-binding profile was linked to at least eight plant lectins.

3.1.6 | Mode of action (MOA)

In this section, the Panel considers the totality of the MOA evidence associated with the toxicity of lectins, and focuses primarily on PHA and SBA. While many suggestions have been proposed, the major MOA remains unclear.

3.1.6.1 | *Mode of action – Gastrointestinal toxicity*

There is robust evidence that lectins such as PHA and SBA can affect the length and weight of the small intestine. The MOA is via binding to gastrointestinal epithelial cells causing both structural and functional damage to the intestine. Structural injury is mainly observed in the villi, microvilli and crypts, compromising gut function. The effects observed include reduced amino acid absorption, altered nitrogen excretion, impaired digestive efficiency and decreased body weight gain. In addition, the binding of lectin may disrupt the mucosal integrity.

The activity and possible deleterious effects on human health of legume lectins appear to depend on four key features namely:

- Structure and the carbohydrate-binding activity, the specificity of the binding to specific carbohydrates (simple sugars or complex glycans). PHA, for example, has affinity only for complex glycans, whereas SBA binds simple sugars.
- the number of the carbohydrate-binding sites (CBS), e.g. tetrameric being more active than dimeric
- structural arrangement of sub-units and their interaction with glycans (see Section 1.3.1.4 and Appendix A)
- the overall lectin content in the seeds

More specifically, in the gastrointestinal tract, lectins selectively bind to the surface of the small intestine and the parietal cells of the stomach, the brush border epithelium of the small intestine and to the surface membrane of the cecum and colon. This binding of lectins to complex carbohydrates on the surface of cells is likely to influence the effects of natural ligands and may produce a wide range of local and systemic effects. PHA, for example, produces a time-and-dose dependent hyperplasia of the small intestine by increasing the number of crypt cells, an effect that is greater in the jejunum than ileum. In epithelial cells, PHA increased DNA, RNA and polyamine content and increased protein synthesis, but the

mechanisms underlying these effects are not clear (Banwell et al., 1993; Bardocz et al., 1995). In addition, upon binding to the gut cell-surface carbohydrates, lectins are readily internalised into the cells and can activate a number of intracellular signalling pathways. Finally, the internalised lectins can reach the bloodstream and affect other types of cells and organs, such as the pancreas (see Section 3.1.6.3). Lectins can also interact with gut microbiota, influencing microbial growth and composition (see Section 3.1.6.1.1).

The glycosylation pattern on enterocytes can vary according to their differentiation state, which changes as the cells move up towards the tip of the villi. This process has important toxicological implications, as the glycosylation may determine if and where an ingested lectin binds to the epithelial surface and thus which effects it exerts. A study of the glycosylation pattern of CaCo-2 cells in different differentiation states revealed that in less differentiated cells mannose was found to be the dominating sugar moiety while in fully differentiated cells a decrease in high mannose type glycans and a concurrent increase in fucosylated and sialylated complex/hybrid type glycans was seen (Park et al., 2015). This shift in surface glycan composition alters the susceptibility of enterocytes to different lectins. Lectins that preferentially bind mannose residues are more likely to affect immature enterocytes near the crypts, potentially disrupting cell proliferation and differentiation. Conversely, lectins targeting complex glycans may target mature cells at the villus tip, thereby influencing gut homeostasis, nutrient absorption and mucosal integrity.

A study of jejunal biopsies (small intestine) from healthy children and children with villous atrophy due to active coeliac disease showed that the binding capacity of lectins holding different CBS differed markedly between the two groups (Pittschieler et al., 1994). Feeding 2-week-old rats PHA via gavage at 50 mg/kg bw per day for 3 days altered the protein/glycoprotein pattern in the small intestine as seen by changes in the expression of brush-border membrane vesicle proteins, in particular Na^+/H^+ exchangers (Kruszewska et al., 2003), indicating that at least some plant lectins by binding may alter the differentiation of epithelial cells.

The CBS of food lectins may determine where on the villi they bind and with which strength and frequency. This was demonstrated by Boldt and Banwell (1985) who incubated I^{125}-labelled E4 or L4 PHA isomers with microvillous membrane vesicles. Membrane binding was saturable and reversible and was shown to be to the carbohydrate moiety using oligosaccharide inhibitors which abolished binding. Both isomers bound to the vesicles but E4 was estimated to have three to four times more sites to bind compared to L4. Conversely, L4 bound with an order of magnitude higher affinity to the vesicle. The authors suggested this indicates that PHA E4 and L4 each have their own set of protein targets on the epithelial cells. A similar study using intestinal explants from the small intestines of young pigs, 7 weeks old, also found differences in the distribution of bound E4 and L4 isoforms of PHA (Kik et al., 1991). Furthermore, the villi were shorter and irregularly positioned, enterocyte heights were higher and the number of tied off clusters of small vesicles was higher than in controls. The study further demonstrated that E4 but not L4 resulted in reduced sucrase-isomaltase activity. This demonstrates variation in the lectins' capacity to bind to and affect the cells of the intestines.

When human and rat intestinal epithelial cell lines (Intestine-407) were stimulated with 0.1 µg/mL PHA, the proliferation of the cells increased as measured by [^{3}H]-thymidine-incorporation (Otte et al., 2001). In contrast, when the porcine intestinal cell line IPEG-J2 was cultured with 0, 0.5 or 2 mg/mL SBA, a dose-dependent increase in cell apoptosis, the proportion of cells in the G0/G1 phase, and decreased expression of cyclin D1 and BCL-2 was found, indicative of cell growth and differentiation arrest (Pan et al., 2021). Considering the vast difference in the concentration of lectin and that distinct lectins were added to the cells in the two experiments, the concentration of lectin as well as the specificity of the lectin binding is likely to impact the eventual effect. Thus, ingested lectins may change the glycosylation pattern of the epithelial cells and their lectin-binding properties.

In electrochemical studies in excised strips of rabbit ileum in vitro, Dobbins et al. (1986) suggested that PHA may deregulate intestinal fluid and electrolyte transport leading to diarrhoea and malabsorption. PHA (1 mg/mL) added to the mucosal solution, but not to the serosal solution, increased the short-circuit current and decreased Na^+ and Cl^- absorption and tissue conductance but had no effect on the second messengers, cAMP and cGMP. No effects were seen on glucose or amino acid absorption.

Santiago et al. (1993) suggested that lectins may negatively affect intestinal absorption and transport of glucose. Using a rat everted intestinal sac model from the first third of the intestine, pretreatment with PHA (50–200 mg/mL) decreased the absorption and transport of glucose from the luminal to serosal side, but there was no effect on glucose metabolism. The authors concluded that these data do not support a major role for changes in absorption and transport as part of the toxicity of PHA.

Various studies on loops of intestine and explants found that while the structure of the intestine was affected, PHA had no effect on either calcium or sucrose uptake (Ayyagari et al., 1993) and that sucrase-isomaltase activity was decreased with PHA E4 but not with PHA L4 (Kik et al., 1991).

Using an in vitro model of uptake into the rabbit jejunal mucosa, Alvarez and Torres-Pinedo (1982) found that SBA and saponins both increased the uptake of [^{125}I] glycinin, a major storage protein of soybean, and that the effect of both together produced a synergistic effect on glycinin uptake. The authors postulated separate membrane binding sites to account for the co-operative effect of both together.

In differentiated CaCo-2 cells, HSP70 and HSP90 were decreased by exposure to PHA, while WGA decreased HSP70 only (Ovelgönne et al., 2000). For further results, see Section 3.1.4.2.

In summary, lectins produce their effects on the gut through specific binding to the carbohydrate moieties on the epithelial cells and altering the structure and function of the microvilli, villi and crypt cells. It is possible that by binding

to receptors in the gut, lectins can act as agonists or antagonists of intracellular/intercellular signalling pathways such as those described above that affect viability, proliferation and differentiation.

3.1.6.1.1 | *Effects on the microbiota in the small intestine*

Mucus production by goblet cells is vital for maintaining intestinal health as it forms a protective physical barrier that prevents microorganisms from reaching and crossing the surface of the epithelium. The number of goblet cells and their secretion of mucin are affected by a complex interplay of factors, including neural mechanisms, immune cells, diet and the gut microbiome (Yang & Yu, 2021). Lectins may affect the generation of goblet cells and mucus production, e.g. by affecting the microbiota composition and by halting mucus secretion. Miyake et al. (2007) demonstrated that WGA but not SBA frustrated the exocytosis of mucus from the goblet cells.

Direct evidence regarding the effects of PHA on the microbiota was reported. In rats fed PHA, the epithelial cells showed a diminished mucus coverage compared to control rats. The lack of mucus covering the cells led to a strong increase of bacteria at the epithelial surface (Banwell et al., 1985). In a further study (Pusztai, Grant, et al., 1993), rats fed PHA had an increased number of coliform bacteria and lactobacilli compared to α-lactalbumin-fed rats. In particular, the PHA-induced increase in mannose-binding bacteria indicates that changes in the composition of the microbiota, the mucus production and/or the differentiation of the epithelial cells and their expression of carbohydrates may influence the bacterial environment.

Additional evidence of lectin effects on the gut microbiota comes from a study where mice were gavaged with Con A (8 mg/kg bw) daily for up to 3 weeks. These mice showed reduced weight gain and increased proportions of enterococcus and enterobacteria (Zárate & Chaia, 2012). In another group, mice fed with the same Con A treatment were concomitantly administered *Propionebacteria acidipropionici* CRL1198 in the drinking water (5×10^8 CFU/mL), which reversed the effects of feeding Con A (Zárate & Chaia, 2012). This indicates that some bacteria may bind lectins, thereby reducing their binding to the gut epithelial cells, highlighting how specific probiotics may help reduce the harmful effect of dietary lectins.

Thus, lectins may bind to the carbohydrate moieties present on the surface of many but not all microorganisms, and this is likely to influence how much of the ingested lectin that binds to the gut epithelial cells. Conversely, the changes the ingested lectin may confer on gut epithelium may influence the gut microbiota composition.

Together, the microbiota and the gut barrier are tightly related, and the changes induced by ingested plant lectin may affect the composition and functionality of both.

3.1.6.2 | *Autoimmunity and allergenicity*

A disturbed and compromised gut barrier as well as dysbiosis are regarded as risk factors for autoimmune diseases and allergies (Akdis, 2021). As plant lectins hold the capacity to adversely alter the gut barrier and the microbiota, plant lectins have been suggested to cause induction of autoimmune disorders and allergies. The apparent capacity of some lectins to abrogate oral tolerance to co-administered antigens and to compromise the epithelial barrier supports that some plant lectins through their action in the small intestine may lead to the development of autoimmunity or allergy in susceptible individuals (see Section 3.1.4.3). Regarding both humans and animals, induction of a strong antibody response towards food proteins requires a breakdown of the mucosal tolerance that under healthy conditions ensures immune homeostasis. Lectin-specific IgE antibodies have been identified in human allergic individuals (see Section 3.1.4.4), and animal studies have demonstrated that orally administered lectin may cause induction of an allergic response towards the lectin and co-administered antigens. Whether an IgE response is induced seems, however, to depend on the specific animal species and strains used (see Section 3.1.4.3), which may imply that only human individuals prone to induce allergic responses will induce an IgE response against lectins.

The carbohydrate-binding property of lectins may further enhance immune response. As an example, the mitogenic activity of lectins on cells directly involved in various immune (Th cells) responses including allergic (Th2 cells) responses could alter the immune response towards foreign antigens. In this respect, most lectins activate different subsets of T lymphocytes (PHA, SBA, PNA), and more rarely B lymphocytes (Carvalho et al., 2018). The hevein-like domain-containing lectin UDA from stinging nettle (*Urtica dioica*) differs from many other lectins by its capacity to activate both $CD4^+$ and $CD8^+$ T lymphocytes (Galelli & Truffa-Bachi, 1993). It is, however, important to keep in mind that various physiological conditions contribute to whether this eventually leads to an IgE-mediated reaction. Hence, lectins may enhance an ongoing specific immune response. Allergenicity related MoA considerations are available in Appendix C.

A common feature in autoimmune diseases is the involvement of tissue-specific T-lymphocytes and antibodies (Seiringer et al., 2022) that cause immune reactions towards the body's own tissue. Loss of oral tolerance may induce T cells and immunoglobulins with reactivity against food antigens and commensal bacteria. This increases the risk of introducing T cells and antibodies cross-reacting with self-molecules, the so-called antigenic mimicry (Garabatos & Santamaria, 2022). To maintain tolerance against environmental antigens, the integrity of the gut barrier is a prerequisite as an intact gut barrier is essential for preventing the microbiota and diet from triggering adverse adaptive immune responses. In healthy individuals, the majority of the gut microbiota is prevented from entering the bloodstream by the epithelial barrier mechanisms and the few microorganisms that get through this barrier are trapped e.g. by the mesenteric lymph nodes and the liver, providing 'firewalls' for the systemic dissemination of the microbiota (Balmer et al., 2014; Spadoni et al., 2015). The intestinal barrier may be compromised by dysbiosis (i.e. disruption of the gut microbiome), the overgrowth of specific bacteria

species (Manfredo-Vieira et al., 2018; McHugh, 2018), or other factors that compromise the integrity of the epithelial barrier, such as disturbance of the continuous formation and renewal of epithelial cells, and inhibition of mucus secretion. Such events weakening the barrier may cause the translocation of high loads of microorganisms into the circulation (Manfredo-Vieira et al., 2018). Dietary lectins may directly affect the composition of the microbiota or compromise the gut barrier by disturbing the differentiation and mucus production by the gut epithelial cells. It should be noted, however, that a direct cause relationship between lectins and autoimmunity remains to be demonstrated.

Another putative way by which lectins may induce the production of self-reactive lymphocytes is if lectins absorbed into circulation bind to cell surface glycoproteins or extracellular matrix glycoproteins. Hereby they may act as new antigens, triggering the production of T cells and B cells with specificity towards the lectin but also towards the glycoproteins that are targeted by the lectin. This may eventually lead to autoimmunity in some individuals. For further results, see Section 3.1.5.2.2.

In summary, lectins may, by binding to the gut epithelium, disturb the gut–microbiota balance, which in turn may lead to loss of oral tolerance, eventually increasing the risk of development of allergy or autoimmunity.

3.1.6.3 | *Mode of action – General toxicity and pancreas toxicity*

In several reports, lectins tended to decrease the body weight of rats despite unaltered feed intake, which may indicate antinutritional effects of these compounds (for details, see Section 3.1.4.1). Partially purified (no affinity chromatography) lectins may also contain residual (chymo)trypsin inhibitors or alpha-amylase inhibitors, which could impair the digestion of proteins and carbohydrates. However, alpha-amylase inhibitors, the predominant Kunitz inhibitor of trypsin and the less abundant Bowman–Birk inhibitor of (chymo)trypsin have no carbohydrate-binding activities and therefore do not bind to immobilised fetuin or thyroglobulin used in the affinity chromatography process to purify lectins. Therefore, possible antinutritive effects of highly purified lectins, as evaluated in the present Opinion, are likely due to direct effects on the absorption of nutrients by the gastrointestinal mucosa (for details, see Section 3.1.6.1).

Many studies, however, reported that the food intake was reduced when animals were treated with lectins purified by affinity chromatography (for details, see Section 3.1.4.1). This effect on food intake could be due to an increased serum level of cholecystokinin (CCK), as often observed in lectin-treated animals.

Lectins are known to bind to the surface of the small intestine. Among other modes of action, lectins interact with inclusion cells (I cells), a subtype of enteroendocrine cells which release CCK in response to ingested long-chain fatty acids or amino acids. Jordinson et al. (1997) showed that soybean lectin (SBL) or SBA, PNL or PNA and WGL or WGA, infused into the duodenum of rats, cause releases of CCK to the blood stream elevating dose-dependently the plasma levels. In enriched cultures of I cells, the lectin-induced secretion was stimulated through calcium flux via L-type calcium-sensing receptors (Jordinson et al., 1998). CCK may interact with orexin neurons in the hypothalamus and the nucleus tractus solitarius to regulate appetite (Tsujino et al., 2005). The brain itself is a source of CCK locally produced in hypothalamic dopamine neurons (Rehfeld et al., 1984) since the CCK circulating in the bloodstream cannot cross the blood–brain barrier. However, Gibbs et al. (1973) described that exogenous CCK inhibits food intake in rats. The exogenous hormone may induce satiety via afferent vagal fibres reaching the hypothalamus, the nucleus tractus solitarius and area postrema (Smith et al., 1981). In addition to effects in the central nervous system, CCK stimulates the secretion of pancreas juice including digestive enzymes, the contraction of the bile bladder, the small intestine and of the colon, causes relaxation of the musculus sphincter oddi and interferes with the activity of gastrin. These complex actions of CCK together may mediate the effects of lectins on the food intake and body weight of the experimental animals.

CCK has been shown to regulate the growth of the pancreas in rats, mice and guinea pigs (Smith & Solomon, 2013). CCK-treated rodents exhibit increased pancreatic weight due to hypertrophy and hyperplasia of the exocrine part, as shown by an elevated ^{14}C-thymidine uptake into replicating DNA of the acinus cells and an overall increased DNA content of the organ (Mainz et al., 1973).

In mice harbouring deficient CCK-A receptors, however, pancreatic enzyme secretion and growth were impaired, and the pancreatic DNA content was reduced when compared to wild-type mice (Takiguchi et al., 2002). These studies suggest that an intact CCK peptide-CCK receptor axis is crucial for CCK to induce proliferation of acinar cells in rodent pancreas. Thus, it appears very likely that the elevated weight and DNA content of the pancreas of lectin-treated animals is due to a lectin-induced elevation of plasma CCK levels.

In rodent models, exogenous CCK accelerated tumour formation in the pancreas by shortening the latency period and/or elevating the cancer incidence using doses that also caused hypertrophy and hyperplasia of the pancreas. This tumour promoting effect was shown for N-nitroso-bis-2-oxopropylamine-induced ductal carcinomas in hamsters (Howatson & Carter, 1985) and in a Kras transgenic mouse model (Carrière et al., 2009). Although lectins enhance CCK levels and induce pancreatic hypertrophy and hyperplasia in rodents, the few studies that are available on this topic to date have not demonstrated a tumour-promoting effect of lectins.

Purhonen et al. (2008) studied the effect of intraduodenal administration of PHA on plasma CCK levels and gallbladder contraction in five healthy volunteers. They received an intraduodenal saline infusion for 30 min, followed by PHA or heat-inactivated PHA applied at increasing doses of 150 µg, 1.5 mg and 15 mg for 30 min each. At all doses of PHA or heat-inactivated PHA, the plasma CCK levels remained unaffected. However, at the lowest dose of PHA, contraction of the gallbladder occurred and was increased dose-dependently. Blocking the muscarinic or the CCK-A receptors completely abolished the PHA-induced contraction. Heat-inactivated PHA exerted no effect.

To summarise, lectins may cause the release of CCK from enteroendocrine I cells in the intestinal tract to the blood. As a result, the gallbladder contracts and the exocrine pancreas is stimulated to produce digestive juice. In rodents, CCK is causally involved in the induction of growth in the exocrine part of the pancreas. Although this information is reported in the Opinion, the relevance to humans remains unknown.

3.1.6.4 | *Mode of action – Immunotoxicity*

Relatively few studies have investigated the immune toxicity of lectins or elucidated the mechanisms underlying such toxicity. Many of the studies only report lymphoid organ weights, which may be very dynamic, depending on the specific time point examined (see Section 3.1.4.3).

If the gut barrier is compromised upon lectin intake, induction of tolerance is disturbed leading to increased translocation of antigens and microorganisms. The presence of immunoglobulins in serum specific for food antigens, including lectins, indicates that these antigens have been absorbed into the circulation in small but sufficient amounts to trigger a specific immune response. Lectins may hold specific capacity to evoke such response as their putative binding to cell surfaces makes them more immunogenic. Apart from lectin-specific IgE in circulation, other subclasses of antibodies, in particular IgG, have been found in sera from humans and animals. The pattern of antibody production is likely influenced by individual factors, such as existing allergies or an allergy prone (atopic) immune system (i.e. atopic predisposition).

Some studies have also measured IgA levels (Kroghsbo et al., 2008; Lavelle et al., 2000). IgA antibodies indicate an immune response initiated in mucosa-associated lymphoid tissues, such as Peyer's patches lining the small intestines and typically function to confine the bacteria within the intestinal lumen (Pabst & Slack, 2020). Lectins may also induce an IgA antibody response here, e.g. if bound to the surface of bacteria or host cells.

Depending on the subclasses of antibodies that are produced, subsequent exposures to lectin may lead to different hypersensitivity reactions such as an allergic reaction (an IgE mediated type 1 hypersensitivity reaction, or an IgG/IgM mediated type 2 or 3 reaction). The latter may give rise to local inflammatory reactions due to e.g. formation of immune complexes or complement activation. Whether the amounts of lectin that have been found to be present in the body are sufficient to give rise to such symptoms remains, however, to be explored. Most examples of this kind of reaction are known from treatment with non-human monoclonal antibodies or penicillin (Goedvolk et al., 2003; Wang et al., 2008).

The ingested lectins may cause proliferation of lymphocytes provided that enough of the ingested lectin reaches the circulation. A significant proportion of the lectin absorbed may be drained from the gut tissue into local lymph nodes (mesenteric lymph nodes) or filtered and trapped in the spleen. Upon binding to surface proteins on lymphocytes, lectins may lead to their proliferation, acting as mitogens. Lymphocytes, including T cells, readily proliferate when stimulated by antigen presenting cells. However, some plant lectins, including PHA and SBA, also hold the capacity to stimulate proliferation in T cells (Chan et al., 2011; Schechter et al., 1976). The lectins can bind to and crosslink T-cell receptors through binding to the carbohydrate moieties, which will induce a signalling cascade that activates the transcription factor nuclear factor of activated T cells (NFAT). Activation of NFAT induces interleukin (IL)-2, which upon binding to the IL-2 receptor on the T cells induces their proliferation (Carvalho et al., 2018). The activation may depend on an accessory signal, e.g. IL-6 produced by the monocytes, which are abundant in blood and in recent years also acknowledged to be abundant in lymph nodes (Jakubzick et al., 2013: Desch et al., 2016), facilitating the upregulation of the IL-2 receptor (Baroja et al., 1988). Mitogenicity has been widely studied in vitro, and lectins have been used experimentally to induce T-cell proliferation (Carvalho et al., 2018). When lectins are administered orally or via gavage to experimental animals, there are a few reported examples of an increase in the weight or size of T-cell-rich lymph organs, such as the thymus (Padiyappa et al., 2022), mesenteric lymph nodes (Kroghsbo et al., 2008) or spleen (He et al., 2021; Padiyappa et al., 2022). However, other studies found no effect on lymph organ weight (see, e.g Tang et al., 2006). The discrepancies in these findings may reflect that the absorbed quantity of lectin in many cases is too low to induce sufficient cell proliferation to cause significant changes in the weight of the lymph organs; however, no measures of the lectin concentration in circulation in these studies were given.

Overall, the type of immune response seen may depend on the animal species and the amount of lectin that reaches the circulation in the animals. The latter may largely depend on the properties of the specific lectin and the amount ingested.

3.1.7 | Considerations of critical effects and dose–response analysis

3.1.7.1 | *Considerations of critical effects*

Although evidence on all lectins from edible plants is included in the present Opinion, critical effects and dose–response analysis could only be considered for PHA and SBA due to limitations in available data. The Panel notes, however, that the availability and quality of toxicological data is limited also for these lectins.

The available human data only pertained to the association between postulated acute exposure to lectins (non-purified lectins were assessed) and gastrointestinal effects, metabolic effects, and food allergy in a limited number of small studies. No quantitative analysis was performed, relevant control groups were not used, confounders were not taken into consideration, lectin identification was done occasionally and only qualitatively and the presumed lectin activity was assessed via haemagglutination assay. Overall, for gastrointestinal effects, the cumulative evidence in case studies supports effect in humans and, for food allergy, red and white kidney beans can be associated with acute allergic reactions which might be

attributed to the lectin activity. Given that GI effects dominate the limited evidence stemming from human data and that, in some case reports, the HA of the implicated food items was measured, the Panel considered the potential of human data to be used for the risk characterisation and a LOAEL to be identified. However, the Panel noted the following limitations of such an approach: (a) not all reported cases could be linked to lectin consumption, (b) HA assay is a semi-quantitative method thus precluding precise estimates of the lectin content based on the HA titres, (c) there is no indication of a correlation between HA titres and GI effects, and (d) other confounding or mediating factors such as gut microbiota impact, presence of other agents that could bind lectins in the gut. Based on all the limitations indicated above, the Panel decided that the available human data could not be used further in the current risk assessment.

PHA

No acute toxicity data were identified for PHA in the experimental animals.

The most common finding in repeated dose toxicity studies was changes in the gut morphology (see Section 3.1.4.2); PHA was observed to cause damage to the villi, microvilli and crypts. Increases in the weight of the small intestine were commonly observed and were considered the critical effects. Changes to the activities of the brush border enzymes (e.g. ALP) were also noted. Gut microbiota changes (see Section 3.1.4.2.1) were also observed as well as hypertrophy of the exocrine pancreas (see Section 3.1.4.1) and effects on gut maturation (see Section 3.1.4.5).

The Panel noted that gut microbiota changes were not considered further as it was unclear whether these changes were adverse and they were not consistently quantified; therefore, the observed effects cannot be evaluated. Body weight gain and food intake were often decreased, and body weight loss was often attributed to the decreased food intake, reduced nutrient absorption, and possible increased N loss affecting also nutrient absorption (see Section 3.1.4.1). Effects on the liver, spleen, kidney and thymus were not considered further (see Sections 3.1.4.1 and 3.1.4.3), since the adverse effects related to the administration of PHA were observed only at doses higher than those required to elicit effects in the small intestine. Body, muscle and lipid weight as well as nitrogen digestibility were not considered further due to potential confounding related to reduced feed intake. Animal studies support that PHA administered orally induces an antibody response against the lectin and potentially against other proteins present in the diet, rather than promoting oral tolerance, thereby increasing the risk of allergic responses (see Section 3.1.4.4). However, due to high variation in the administration schemes (i.e. amount of lectin, frequency and length of administration, antibody classes measured, animal species), these data cannot be considered further in the current risk assessment.

Based on the above, the CONTAM Panel considered increases in small intestine weight, large intestine weight and pancreas weight in rodents as the critical effects for acute PHA toxicity. Of these critical effects, the observations in the gastrointestinal tract in experimental animals were considered relevant to the adverse effects observed in humans; however, the Panel noted that no human studies with purified lectins were retrieved. Moreover, the Panel considered the increase in small intestine weight as a relevant endpoint for establishing a reference point in human hazard characterisation. This effect reflected the binding of lectins to the intestinal BBM leading to mucosal changes, hyperplasia or inflammation, all potentially mediated by microbiota–lectin interactions. Therefore, the Panel considered the increase in small intestine weight as a relevant endpoint to establish a reference point for human hazard characterisation and identified the Bardocz et al. (1995) rat study as the critical study. Effects on the dry weight of the pancreas, small intestine and large intestine were observed at doses above 32.5 mg/kg bw per day, accompanied by histopathological changes in the gastrointestinal tract. The limitations of this study are detailed in the UA table (Appendix E).

SBA

No acute toxicity data were identified for SBA in experimental animals.

The identified toxicological studies with SBA investigated the subacute effects in gastrointestinal toxicity, general toxicity (organ weights) and histopathology, and immunotoxicity. For GI effects, the effects of SBA varied with test species. Piglets showed increased intestinal permeability but no histological changes (Zhao et al., 2011). The observed increase in the relative small intestinal weight in rats was attributed to the decrease in body weight and was not considered as a critical effect; in addition, potential binding of SBA to sugar content in the diet cannot be excluded due to the specificity of SBA to Gal/GalNAc containing glycoproteins present in the diet (Tang et al., 2006). Plasma cholecystokinin concentrations and relative pancreas weights were elevated; the plasma insulin levels were lowered at unchanged blood glucose concentrations (see Section 3.1.4.1). Altered pancreatic enzyme activities were reported as well. However, due to the unknown relevance of these pancreas-related endpoints for humans, these endpoints were not considered further. SBA lowered the weight of the spleen and kidney in one report (Zang, Li, Piao, & Tang, 2006) or left it unaltered in another study (Li, Li, & Qiao, 2003). Body weight gain and food intake were often decreased, and the body weight loss was often attributed to the decreased food intake (see Section 3.1.4.1). Body, muscle and lipid weight as well as nitrogen digestibility were also not considered further due to potential confounding related to reduced feed intake (see Section 3.1.4.1). Additionally, the Panel noted that, while PHA exhibits specific binding to complex carbohydrates, SBA binds to simple carbohydrates (see Appendix A) frequently occurring in the feed. Thus, when animals consume SBA mixed with feed, it is likely that the SBA binds to these carbohydrates in the feed, e.g. Tang et al. (2006). Based on the above, the CONTAM Panel considered that the evaluated effects related to SBA could not be considered further.

General considerations:

Acknowledging the lack of data from lectins other than PHA to be considered for the identification of critical effects and dose–response analysis, the Panel evaluated the impact on the risk assessment of restricting the risk characterisation to PHA alone. The Panel noted that PHA is considered the most toxic among the lectins of edible plants because of the specificity of PHA to bind to complex carbohydrates and of its high affinity to human enterocytes (Pusztai et al., 1990). More specifically, PHA differs from other edible lectins by its very specific affinity for complex glycans with a bisecting GlcNAc residue linked to the mannose residue from the trimannoside core (see Annex A). Due to this particular carbohydrate-binding specificity, PHA is less able than other edible lectins to interact with different sugar compounds occurring in the diet. This results in a higher amount of free PHA which, in turn, could be available for interacting with the glycans of enterocytes upon feeding. Conversely, most other lectins, which display a more versatile affinity for various glycans present in the diet, are less available for binding to the gut surface. Thus, both the lectin affinity for particular bisected complex glycan structures together with the lack of affinity for other simple or complex sugar compounds present in the diet would explain the different activity of PHA towards the small intestine compared with other edible lectins such as SBA. As a consequence of these particular properties, consuming active PHA in undercooked beans would account for some deleterious effects, unlike other food lectins with different specificities. Based on the above, the Panel considered that PHA-related effects are more sensitive compared to the effects of other lectins.

3.1.7.2 | *Dose–response analysis*

The CONTAM Panel performed benchmark dose (BMD) modelling according to the 2022 EFSA Guidance on the use of the BMD approach in risk assessment (EFSA Scientific Committee, 2022, see Section 2.3). The results of the BMD modelling for the critical study in rats exposed in diet (Bardocz et al., 1995) are summarised in Table 9. Details of the BMD analyses, including the individual reports of the modelling, are shown in Annex D.

For continuous data, the 2022 EFSA guidance recommends estimating the 90% credible interval by using a BMR that takes into account biological relevance (EFSA Scientific Committee, 2022). For all the endpoints (i.e. pancreas, small intestine and large intestine weight in rats), the CONTAM Panel applied the same BMR of 10% based on the standard deviation of the control groups (range 4%–9%), the intercorrelation of the effects and considerations on the biological relevance of the endpoints (e.g. reversibility of the effects).

The CONTAM Panel selected the $BMDL_{10}$ of 22.9 mg/kg bw per day for increase in small intestine dry weight as the reference point for the risk characterisation.

TABLE 9 Benchmark dose (BMD) modelling for the critical study of PHA (for details of the BMD analyses, see Annex D).

			mg/kg bw (per day)		
Reference	**Endpoint**	**BMR**	**BMDL**	**BMD**	**BMDU**
Bardocz et al. (1995)	Increase in pancreas dry weight	10%	34.0	76.7	145.1
	Increase in small intestine dry weight	10%	22.9	47.4	82.6
	Increase in large intestine dry weight	10%	75.2	123.0	393.8

Abbreviations: BMD, benchmark dose; BMDL, benchmark dose lower confidence limit; BMDU, benchmark dose upper confidence limit; BMR, benchmark response; LOAEL, lowest observed adverse effect level.

3.1.8 | Approach for risk characterisation

The CONTAM Panel concluded that, due to the limitations and uncertainties in the current evidence, the establishment of a HBGV for PHA was not appropriate. Instead, the margin of exposure (MOE) approach was used for the risk characterisation.

According to the EFSA Scientific Committee Guidance on selected default values (EFSA Scientific Committee, 2012), an MOE of 100, covering variability with respect to kinetic and dynamic differences between animal species and humans (factor $4 \times 2.5 = 10$) and within the human population (factor $3.2 \times 3.2 = 10$) is considered sufficient to cover the human health effects, provided that additional information would not have resulted in a lower RP.

Due to the lack of data on chronic toxicity in humans and animals, the indications of acute effects in humans and the availability of data only on subacute effects in rats, the CONTAM Panel focussed its assessment on acute health concerns. The Panel also considered that, despite the lack of long-term toxicity studies (reproductive toxicity, immunotoxicity) and carcinogenicity studies, no additional factor needs to be applied as lectins are mainly excreted in their active form (> 90%, see Section 3.1.1) and extrapolation of results from sub-acute studies to assess acute health effects is considered to adequately address acute health concerns.

3.2 | Occurrence data

3.2.1 | Occurrence data on food submitted to EFSA

As already commented in Section 2.4, no occurrence data on lectins were available in the EFSA Data Warehouse. As an alternative, occurrence data of lectins in food were identified by a literature search and study appraisal which were conducted as described in Section 2.1.

3.2.2 | Previously reported occurrence data in the open literature

Usually, the lectin content of seeds and especially of legume seeds is in the order of a few mg/g dry weight. Moreover, it is often difficult to compare results because they depend on the techniques used for estimating the lectin concentration (quantitative radial immunodiffusion or ELISA using either primary or secondary antibodies) or evaluating the lectin activity (results from semi-quantitative haemagglutination of untreated or trypsin-treated red blood cells of different origins, expressed as haemagglutination titre[15] or units HU/g dw or fw). In addition, estimations of the lectin content based on purification yields constitute a serious limitation in evaluating the lectin content and should be treated with caution as they may not represent the entire amount of lectins in the edible plant analysed (Tables 10 and 11).

TABLE 10 Lectin content (SBA) in soybeans reported in the literature.

Legume seed	Lectin	Mean lectin concentration [ranges]	Haemagglutinating activity [ranges]	Analytical technique	Reference*
Concentration of lectins measured by analytical method					
Soybean	SBA	1.82 mg/g – 12.32 mg/g [4–9 mg/g (70% of 320 samples)] fw		LC–MS/MS	Wen et al. (2021)
Soybean	SBA	[2.03–2.92 mg/g (54 samples)] dw		ELLA (Enzyme linked lectin assay)	Breeze et al. (2015)
Soybean	SBA	1.93 mg/g (3 samples) fw		ELISA	Causse et al. (1986)
Soybean	SBA	3.40 mg/g (3 samples) dw		Radial immunodiffusion	Causse and Rougé (1983)
Haemagglutinating activity					
Soybean	NR		3328 ± 706 HU/g	Haemagglutination assay (rabbit erythrocytes, NTT)	Adamcová et al. (2021)
Soybean (conventional)	SBA		3.51 ± 0.27 [0.17–8.63] HU/mg fw	Haemagglutination assay (details not reported)	Zhou et al. (2011)
Korean Large Black Soybean	KSL		4096 titre/mg	Haemagglutination assay using rabbit erythrocytes	Fang et al. (2010)
Soybean	NR		12,800 HA/g soybean flour	Haemagglutination assay (Human O erythrocytes)	Chen et al. (1977)
Lectin isolation					
Soybean	SBA	3.6 mg/g fw		Extraction + affinity chromatography	Calderón de la Barca et al. (1991)
Soybean	SBA	2.8 mg/g**		Protein concentration was determined by spectrophotometry using Folin phenol reagent as reported by Lowry et al. (1951)	Calderón de la Barca et al. (1991)
Soybean	SBA	2–9.8 mg/g fw		Liquid spectrometry and protein measurement by spectrophotometry using Folin phenol reagent as reported by Lowry et al. (1951)	Pull et al. (1978)

[15]Defined as the inverse of the greatest dilution of lectin sample giving visible haemagglutination.

TABLE 10 (Continued)

Legume seed	Lectin	Mean lectin concentration [ranges]	Haemagglutinating activity [ranges]	Analytical technique	Reference*
Soybean	SBA	2.56 mg/g**		Protein was determined photometrically	Allen and Neuberger (1975)
Reviews					
Soybean	NR	< 1 mg/g		NR	Pryme and Aarra (2021) (review)
Soybean	NR	0.2–3 mg/g		NR	Nasi et al. (2009) (review)
Soybean	NR	< 2 mg/g		NR	Peumans and Van Damme (1998) (review)
Soybean	NR	0.2–2 mg/g		NR	Peumans and Van Damme (1996) (review)

Abbreviations: dw, dry weight; ELISA, enzyme-linked immunosorbent assay; fw, fresh weight; HA, haemagglutinating activity; HU, haemagglutinating units; KSL, Korean soybean lectin; LC-MS/MS, liquid chromatography tandem mass spectrometry; NR, not reported; NTT, not trypsin treated; SBA, soybean agglutinin.

*Studies are ordered by the most reliable analytical technique and chronological order. **Figures calculated from concentrations in soybean meal (factor of 1.25 applied).

TABLE 11 Lectin content (PHA) in different *Phaseolus* beans reported in the literature.

Legume seed	Lectin	Mean lectin concentration [ranges]	Haemagglutinating activity [ranges]	Analytical technique	Reference*
Common beans					
Concentration of lectins measured by analytical method					
Common bean	PHA	41.69–51.96 µg/g (PHA-L: 23.67–43.85 µg/g PHA-E: 8.07–18.82 µg/g) (18 samples) powder ww		LC-MS/MS of signature peptides in tryptic digest with isotope dilution MS	Li et al. (2024)
Kidney bean	PHA	Common bean: 24.9 mg/g (1 sample) powder ww Lectin-null beans: 0.003 mg/g powder ww		ELISA	Boniglia et al. (2008)
Haemagglutinating activity					
Kidney bean	NR		13,214 ± 2828 HU/g	Haemagglutination assay (rabbit erythrocytes, NTT)	Adamcová et al. (2021)
Kidney bean	PHA	0.8 mg/g fw[a] in whole pods 25.6 mg/g fw[b] in whole pods 51.2 mg/g fw[c] in whole pods 12.8 mg/g fw[a] seeds 102.4 mg/g fw[b] seeds 102.4 mg/g fw[c] seeds		Haemagglutination assay (rabbit erythrocytes, NTT)	Sun et al. (2019)
Kidney bean	Kidney bean lectin		1.90 + −0.05 HU/mg	Haemagglutination assay (rabbit erythrocytes trypsin treated)	Qayyum et al. (2012)
Brown kidney bean	NR	4.28 mg/g	32,649 HU/mg	Affinity chromatography; Gel filtration Haemagglutination assay (rabbit erythrocytes)	Chan et al. (2012)

(Continues)

TABLE 11 (Continued)

Legume seed	Lectin	Mean lectin concentration [ranges]	Haemagglutinating activity [ranges]	Analytical technique	Reference*
Phaseolus vulgaris seeds	NR		1:128 titre	Haemagglutination assay (Human A and B erythrocytes)	Wang and Liu (1975)
Lectin isolation					
Kidney bean, Different Cultivars	NR	114–195 mg/g dw (globulin protein fraction) 30–46 mg/g dw (albumin protein fraction)	3–600 µg/mL** 3–150 µg/mL**	Extraction of proteins and precipitation of albumins and globulins; Haemagglutination assay (rabbit erythrocytes)	Pusztai et al. (1979)
Reviews					
Kidney bean	NR	< 10 mg/g		NR	Pryme and Aarra (2021), Peumans and Van Damme (1998) (reviews)
Kidney bean	NR	1–10 mg/g		NR	Nasi et al. (2009), Peumans and Van Damme (1996) (reviews)
Pinto beans					
Haemagglutinating activity					
Pinto Bean	*Phaseolus vulgaris* lectin		Human O erythrocytes: 128 titre (HU) ww Human B erythrocytes: > 256 titre (HU) ww Human A erythrocytes: > 256 titre (HU) ww	Haemagglutination assay	Menéndez-Rey et al. (2021)
Pinto bean	NR		13,563 ± 2875 HU/g	Haemagglutination assay (rabbit erythrocytes, NTT)	Adamcová et al. (2021)
Pinto bean	NR		12,800 HU/g beanflour	Haemagglutination assay (Human O erythrocytes)	Chen et al. (1977)
Runner beans					
Haemagglutinating activity					
Runner bean			3328 ± 706 HU/g	Haemagglutination assay (rabbit erythrocytes, NTT)	Adamcová et al. (2021)
Runner bean	*Phaseolus coccineus* lectin		Human A erythrocytes: 2048 titre Goat erythrocytes: 2 titre Pig erythrocytes: 2 titre Sheep erythrocytes: 8 titre Mouse erythrocytes: 16 titre	Haemagglutination assay	Feria et al. (1996)
Runner bean	*Phaseolus coccineus* lectin	Human A erythrocytes: 0.465 mg/g Human B erythrocytes: 0.465 mg/g Human O erythrocytes: 0.465 mg/g	Human A erythrocytes 3.9 µg/mL** Human B erythrocytes: 3.9 µg/mL** Human O erythrocytes: 3.9 µg/mL**	Haemagglutination assay (Human A erythrocytes)	Ochoa and Kristiansen (1982)

TABLE 11 (Continued)

Legume seed	Lectin	Mean lectin concentration [ranges]	Haemagglutinating activity [ranges]	Analytical technique	Reference*
Reviews					
Runner bean	NR	1–10 mg/g		NR	Nasi et al. (2009), Peumans and Van Damme (1996) (reviews)
Lima beans					
Haemagglutinating activity					
Lima bean (red)	Red lima bean lectin	0.75 + –0.28 mg/g dw		Haemagglutination assay (rat and rabbit erythrocytes NTT, rat trypsin treated erythrocytes)	Oboh et al. (1998)
Lima bean (white)	White lima beans lectin	27.49 + –7.09 mg/g dw			
Other type of beans (reviews)					
Tepary bean	NR	1–10 mg/g		NR	Nasi et al. (2009), Peumans and Van Damme (1996) (reviews)
Black bean	NR		26,429 ± 5603 HU/g	Haemagglutination assay (rabbit erythrocytes, NTT)	Adamcová et al. (2021)
Borlotti bean			13,312 ± 2822 HU/g		
Green bean, haricot vert			6656 ± 1411 HU/g		
Rashti bean			13,312 ± 2822 HU/g		
White bean			13,263 ± 2812 HU/g		

Abbreviations: dw, dry weight; ELISA, enzyme-linked immunosorbent assay; fw, fresh weight; HU, haemagglutinating activity; LC–MS/MS, liquid chromatography tandem mass spectrometry; NR, not reported; NT, neuraminidase treated; NTT, non-trypsin treated; PHA, phytohaemagglutinin; TT, trypsin treated; ww, whole weight.

[a]Cultivar Yongshengxianfeng, [b]: cultivar Cuiyun 2, [c]: cultivar Zihuayoudou.

*Studies are ordered by the most reliable analytical technique and chronological order. **Minimum concentration that gave haemagglutination.

Compared to other seed soluble proteins and especially seed storage proteins, lectins represent only a small fraction of seed proteins, e.g. 2.0%–2.5% in lentil (2.1 mg/g fw) (Howard et al., 1972; Rougé, 1974a; Rougé, 1974b), pea (2.0 mg/g fw) (Rougé, 1975) and soybean (1.96 mg/g fw) (Causse et al., 1986).

All the values reported in Tables 10 and 11 were calculated from unprocessed seeds (expressed in fresh or dried weight) and are expected to be different from the lectin concentrations in the corresponding food processed commodities (with different water content, reduction in lectin content after cooking, etc.).

3.2.3 | Selected occurrence data from the scientific literature for the dietary exposure assessment

As mentioned in Section 2.4, the lack of occurrence data in the EFSA Data Warehouse required the use of occurrence data from the scientific literature to conduct the dietary exposure assessment. The CONTAM Panel noted that, due to the limitations in toxicological data (see Section 3.1.7.1), SBA was not considered further for risk characterisation and an exposure assessment was not performed. The selection of data for PHA is described below.

3.2.3.1 | *Selected data from the scientific literature on Phytohaemagglutinin (PHA)*

Among the different scientific papers assessed, most of the studies assessed haemagglutinating activity and only two studies measured the concentration of lectins (Table 11). The CONTAM Panel used the study from Boniglia et al. (2008) to select the concentration of PHA to be used for the exposure estimations. Boniglia and coworkers identified using ELISA the presence of 24.9 mg/g of PHA in one sample of a raw commercial Italian variety of kidney beans. It is assumed that pods are not included, since the concentration of lectins in pods is reported separately (0.36 mg/g). This study was selected because the ELISA methodology applied in this work quantifies only active lectins (Boniglia et al., 2003). Additionally, these results are not dependent on many factors as occurs with the haemagglutination assay (see Section 1.3.2.3), one of the most commonly used tests for confirming lectin activity.

Typically, for deterministic estimates of acute dietary exposure, an estimate of the top end of the range of the concentration data distribution is required. The available data on PHA are difficult to compare as they are reported with different methods, in many cases using the haemagglutination assay or without details on how they were analysed. Still, the

value reported by Boniglia and coworkers (24.9 mg/g) is considered to represent a relatively high concentration of PHA in *Phaseolus* sp. as compared to other reliable studies (Li et al., 2024, see Table 11) that reported much lower values of PHA in common beans analysed by LC-MS/MS.

The value of 24.9 mg/g of PHA in raw kidney beans was also used as a characteristic high concentration of PHA for all the different types of beans that belong to *Phaseolus* sp. (e.g. runner beans, French beans, etc.). To derive the PHA concentration in French beans and immature beans, a 90% moisture content was assumed as compared to the 12% in mature seeds, based on the study by Sinkovič et al. (2024) in French beans. A concentration of 2.8 mg/g of active lectins was assigned to immature seeds, regardless of whether they refer to seeds with or without pods.

Starting from the selected value of 24.9 mg/g in the raw beans, different PHA concentrations were derived for composite foods containing *Phaseolus* sp. beans as an ingredient (e.g. canned or jarred common beans, beans and vegetables meal, legume (beans) soup). The concentrations of active PHA in the composite foods were derived using reverse yield factors and recipes as described in the EFSA's Raw Primary Commodity model (EFSA, 2019).

Table 12 provides an overview of the levels of active PHA in different *Phaseolus* sp. beans and bean-containing products, considering the initial concentrations of active lectins in the raw beans and composite foods. The last column shows the concentrations of active PHA assuming that 50% of the lectin activity remains in the foods as consumed after processing (see Section 2.7).

TABLE 12 Concentration of active PHA in selected beans (*Phaseolus* sp.) and bean-containing products with no processing applied, and under an arbitrary exposure scenario assuming that 50% of the lectin activity remains in the foods as consumed after processing.

	FoodEx name	Initial concentration of active PHA[a] (mg/g)	Concentration of active PHA under 50% scenario[b] (mg/g)
Phaseolus coccineous	Runner beans (dry)	24.9	12.5
	Runner beans (without pods)[c]	2.8	1.4
	Runner beans (with pods)[c]	2.8	1.4
Phaseolus vulgaris	French beans (with pods)[c]	2.8	1.4
	French beans canned	2.8	1.4
Phaseolus lunatus	Lima beans (dry)	24.9	12.5
	Lima beans (with pods)[c]	2.8	1.4
	Lima beans (without pods)[c]	2.8	1.4
Phaseolus vulgaris	Kidney bean (dry seeds)	24.9	12.5
	Kidney bean (fresh seeds)[c]	2.8	1.4
Phaseolus vulgaris	Common bean sprouts[c]	2.8	1.4
	Flageolets (dry seeds)	24.9	12.5
	Flageolet (fresh seeds)[c]	2.8	1.4
	Borlotti or other common beans (dry)	24.9	12.5
	Field bean (fresh seeds)[c]	2.8	1.4
	Navy bean (fresh seeds)[c]	2.8	1.4
	Field beans (dry seeds)	24.9	1.4
	Navy beans (dry seeds)	24.9	12.5
	Borlotti or other common beans (without pods)[d]	2.8	1.4
Composite foods	Canned or jarred common beans	24.9	12.5
	Beans and vegetables meal	12.5	6.3
	Legume (beans) soup	11.2	5.6
	Prepared legume (beans) salad	6.2	3.1
	Legume (beans) soup, dry	112	56.0

[a]Active PHA present in the raw mature beans (24.9 mg/g) and immature beans (2.8 mg/g). The concentrations of active PHA in the composite foods were derived using reverse yield factors and recipes as described in the EFSA raw primary commodity model (EFSA, 2019).

[b]Scenario where 50% of the active PHA present in the raw primary commodity remains in the processed commodities as consumed. See the section on dietary exposure for more details on the exposure scenario.

[c]Immature seeds.

3.2.4 | Food processing and storage

3.2.4.1 | *Effects of food processing*

Different processing techniques including thermal treatments such as cooking, cooking extrusion, autoclaving, microwaving, and non-thermal treatments including soaking, germination, fermentation, dehulling, extraction, high-pressure,

irradiation, enzymatic treatment by foreign proteases have been applied to foods and food products to reduce the impact of lectins on human health. However, lectins may be resistant to heat inactivation that occurs during the cooking process of edible seeds, which necessitates a sufficiently long cooking time. Details on the studies retrieved on the effect of food processing techniques to the concentration or haemagglutinating activity of lectins are available in Appendix D (Tables D.1 and D.2), and information is summarised in Table 13.

TABLE 13 Summary table of the efficacy of different processing methods on lectin inactivation.

Material (isolated lectin/plants/food preparation)	Method	Conditions	Impact on lectin concentration or activity	Supporting references
SBA from soybean, DLL-II lectin from *Dolichos lablab* var. *lignosus* seeds Amaranth grains, mung bean, pigeon pea, chilean bean (except black variety), chickpea	Germination	Time: ≥ 72 h (except for Paucar-Menacho et al. (2010): 42 h and Vishweshwaraiah et al. (2018): 48 h)	≥ 50% reduction (measured by haemagglutination assay, spectrometry, ELISA and RT-qPCR)	Anaemene and Fadupin (2022), Valadez-Vega, Lugo-Magaña, Figueroa-Hernández, et al. (2022), Poblete et al. (2020), Vishweshwaraiah et al. (2018), Paucar-Menacho et al. (2010), Mubarak (2005), El-Adawy (2002)
PHA from kidney bean *Vigna unguiculata* var. *unguiculata*		Time: 96 h	< 30% reduction (measured by haemagglutination assay and ELISA)	Kalpanadevi and Mohan (2013), Shimelis and Rakshit (2007)
Black chilean bean, pea seeds, faba bean, kidney bean, *Dolichos biflorus* seeds		Time: ≥ 72 h (except Orúe et al. (1998): 24 h)	< 10% reduction (measured by haemagglutination assay and radioimmunoassay)	Poblete et al. (2020), Alonso, Aguirre, and Marzo (2000), Alonso et al. (1998), Orúe et al. (1998), Talbot and Etzler (1978)
LCA from lentils PHA from white bean		Time: ≥ 24 h	No significant change in lectin activity (measured by ELISA)	Nciri et al. (2015), Cuadrado et al. (2000)
Soybean	Germination + Boiling	Germination: 3 days Boiling: 100°C, 5 min	100% decrease (measured by haemagglutination)	Felipe et al. (2006)
PHA from kidney bean Pigeon pea, peas (whole and split), lentils (whole and split), beans, chickpea, soybean, velvet bean, *Dolichos lablab var. vulgaris* seeds, *Vigna unguiculata* subsp. *unguiculata*, navy bean, red kidney bean	Soaking	Time: 3–18 h	< 15% decrease (measured by haemagglutination and ELISA)	Shi et al. (2018), Kalpanadevi and Mohan (2013), Shimelis and Rakshit (2007), Onwuka (2006), Vijayakumari et al. (1996), Vijayakumari et al. (1995), Dhurandhar and Chang (1990)
Lupin seeds, *Sesbania aculeata* seeds, pea seeds, faba bean, kidney bean, green and white faba beans		Time: 12–96 h	No change in lectin activity (measured by haemagglutination)	Sahni et al. (2021), Luo and Xie (2013), Embaby (2010), Alonso, Aguirre, and Marzo (2000), Alonso et al. (1998)
PHA from white bean		Time: 12 h	No significant change increase (measured by ELISA)	Nciri et al. (2015)
Mung bean, pigeon pea		Time: 6–18 h	50% decrease (measured by haemagglutination)	Onwuka (2006), Mubarak (2005)
Lima bean		Time: 3–9 h	100% decrease (measured by haemagglutination)	Adeparusi (2001)

TABLE 13 (Continued)

Material (isolated lectin/plants/food preparation)	Method	Conditions		Impact on lectin concentration or activity	Supporting references
Velvet bean, *Dolichos lablab* var. *vulgaris* seeds, *Vigna unguiculata* subsp. *unguiculata*	Soaking with bicarbonate	Time: 1.5–12 h		≤ 23% decrease (measured by haemagglutination)	Kalpanadevi and Mohan (2013), Vijayakumari et al. (1996), Vijayakumari et al. (1995)
PHA from black bean Soybean	Malting	6 h steeping; 24 h and 48 h of germination at 20°C and kilning at 60°C		≤ 76% decrease (measured by haemagglutination and ELISA)	Trugo et al. (1999), Muzquiz et al. (1998)
Dark red kidney beans, soybeans, mung beans, large white kidney bean, brown haricot bean, small white canning bean, red speckled sugar bean, red kidney bean, beans, peas (whole and split), lentils (whole and split), chickpea, dry bean, whole black turtle soup bean, navy bean, red kidney bean, white kidney bean, faba bean	Soaking (≥ 4 h) + Cooking	80–90°C	≥ 60 min	100% decrease (measured by haemagglutination, ELISA and affinity chromatography)	Felipe et al. (2006), Venter and Thiel (1995), Dhurandhar and Chang (1990), Coffey et al. (1985)
		90–100°C	≥ 60 min (except Leontowicz et al. (1998): 20 min)		Shi et al. (2018), Mubarak (2005), El-Adawy (2002), Coffey et al. (1985), Grant et al. (1982)
			30–45 min		Nciri et al. (2015), Felipe et al. (2006), Leontowicz et al. (1998), Dhurandhar and Chang (1990), Coffey et al. (1985), Grant et al. (1982), Antunes and Sgarbieri (1980)
Dosa containing *Lathyrus sativus*			≤ 30 min		Armour et al. (1998), Venter and Thiel (1995), Bonorden and Swanson (1992), Dhurandhar and Chang (1990), Ayyagari et al. (1989), Grant et al. (1982)
		60°C	120 min		Felipe et al. (2006)
PHA from kidney bean Black bean Dosa containing *Lathyrus sativus*		90–100°C (time: NR)			Morales-de León et al. (2007), Shimelis and Rakshit (2007), Ayyagari et al. (1989)
Pigeon pea, *Vigna unguiculata* subsp. *unguiculata*		90–100°C	≥ 30 min	≤ 90% decrease (measured by haemagglutination and ELISA)	Kalpanadevi and Mohan (2013), Onwuka (2006)
Soybean		100°C	20 min	75% decrease (measured by haemagglutination)	Leontowicz et al. (1998)
Large white kidney bean, red speckled sugar bean, red kidney bean		85°C	60 min	≤ 50% decrease (measured by haemagglutination)	Venter and Thiel (1995)

(Continues)

TABLE 13 (Continued)

Material (isolated lectin/plants/food preparation)	Method	Conditions		Impact on lectin concentration or activity	Supporting references
WGA from pasta and wholemeal pasta, PHA from corn–bread–sardine meal, bean meal, sorghum–bean–sardine meal, rice–bean–sardine meal Small white canning bean, black bean, soybean, white faba bean Dhal containing *Lathyrus sativus*	Cooking/Boiling	100°C	< 40 min	≥ 90% decrease (measured by haemagglutination and ELISA)	Luo and Xie (2013), Mosha et al. (2005), Matucci et al. (2004), Rodríguez-Bürger et al. (1998), Venter and Thiel (1995), Ayyagari et al. (1989), Del Valle et al. (1983), Hussein et al. (1980)
Large white kidney bean, red kidney bean, red speckled sugar bean, brown haricot, bambara groundnut seeds, sweet lupin seeds, pigeon pea			40–60 min		Embaby (2010), Omoikhoje et al. (2009), Onwuka (2006), Venter and Thiel (1995)
Velvet bean, ayocote bean, black bean, white kidney bean, other beans			> 60 min		Vijayakumari et al. (1996), Almeida et al. (1991), Grant et al. (1982), Sotelo-López et al. (1978)
PHA from white bean and kidney bean		97°C	35 min		Nciri et al. (2015), Shimelis and Rakshit (2007)
Isolated lectins from black bean, isolated lectin from Zihua snap bean, isolate lectins from black turtle bean		80–90°C	≥ 5 min		Valadez-Vega, Lugo-Magaña, Betanzos-Cabrera, and Villagómez-Ibarra (2022), Jiang et al. (2019), He et al. (2014)
Soybean, isolate lectins from black turtle bean		60–70°C	45 min	≥ 75% decrease (measured by haemagglutination and affinity chromatography)	He et al. (2014), Hussein et al. (1980)
Whole black turtle soup bean		97.8°C	40 min		Bonorden and Swanson (1992)
PHA from white bean Bitter lupin seeds, *Vigna unguiculata* subsp. *unguiculata*, green faba bean		100°C	30–40 min	≤ 85% decrease (measured by haemagglutination)	Nciri et al. (2015), Kalpanadevi and Mohan (2013), Luo and Xie (2013), Embaby (2010)
Cowpea, broad beans, lentils, dry peas, jackfruit seed meal, winged bean seeds			≥ 80 min	< 50% decrease	Igene et al. (2006), Onwuka (2006), Ravindran et al. (1996), Sotelo-López et al. (1978)
Green and white faba bean	Cooking + Soaking (+ Dehulling)	100°C	30 min	100% decrease (measured by haemagglutination)	Luo and Xie (2013)
Functional lectins from soybean, carbohydrate-binding lectins from soybean Defatted meal from peanut and soybean seeds	Steaming	≥ 100°C	≥ 5 min	100% decrease (measured by haemagglutination and immunoassay)	Fasina et al. (2003), Qin et al. (1996), Ahmed (1986)
PHA from navy and pinto beans		82°C	–	≤ 80% decrease (measured by ELISA)	Kelkar et al. (2012)

TABLE 13 (Continued)

Material (isolated lectin/plants/food preparation)	Method	Conditions		Impact on lectin concentration or activity	Supporting references
Ojo de cabra, flor de mayo, black and white beans	Microwave	2450 MHz		≤ 50% decrease (measured by haemagglutination)	Hernández-Infante et al. (1998)
Bitter and sweet lupin seeds		6 min		≥ 75% decrease (measured by haemagglutination)	Embaby (2010)
Sesbania aculeata seeds		3 min		100% decrease (measured by haemagglutination)	Sahni et al. (2021)
Mung beans, soybean, chickpea, velvet beans	Soaking (12 h) + Microwave	15 min		≥ 94% decrease (measured by haemagglutination)	Gurumoorthi et al. (2013), Mubarak (2005), El-Adawy (2002)
Soybean	Soaking (6 h) + Microwave	4 min		50% decrease (measured by haemagglutination)	Felipe et al. (2006)
Ojo de cabra, flor de mayo, black and white beans	Soaking (–) + Microwave	2450 MHz		≤ 50% decrease (measured by haemagglutination)	Hernández-Infante et al. (1998)
Green and white faba beans	Microwave (+Soaking + Dehulling)	6 min		No change in lectin activity (measured by haemagglutination)	Luo and Xie (2013)
Tree bean, lima bean, *Sesbania aculeata* Defatted meal from peanut and soybean seeds, papad roasted containing *Lathyrus sativus*	Roasting	≥ 120°C	≥ 20 min	≥ 90% decrease (measured by haemagglutination)	Sahni et al. (2021), Sathya and Siddhuraju (2015), Adeparusi (2001), Ayyagari et al. (1989), Ahmed (1986)
Amaranth grains Popped seeds of *Lathyrus sativus*		200°C	90 s		Valadez-Vega, Lugo-Magaña, Figueroa-Hernández, et al. (2022), Ayyagari et al. (1989)
Soybean and yambean flours		100°C	≥ 60 min	100% decrease (measured by haemagglutination)	Ojimelukwe et al. (1995b)
Beans (variety Ojo de cabra)		135°C	15 min	84% decrease (measured by haemagglutination)	Almeida et al. (1991)
Vigna unguiculata		120°C	15 min	75% decrease (measured by haemagglutination)	Umapathy et al. (1998)
Corn–soybean tortilla		200°C	30 min	50% decrease (measured by haemagglutination)	Del Valle et al. (1983)
Chapathi containing *Lathyrus sativus*		NR	NR	0% decrease (measured by haemagglutination)	Ayyagari et al. (1989)
Cowpea (*Vigna unguiculata*)	Soaking* (+ Dehulling) + Roasting *For 16 h in sodium bisulphite solution	119°C	30 min	100% decrease (measured by haemagglutination)	Olivera-Castillo et al. (2011)
Kidney bean (whole pod)	Stir-fry	18 min		100% decrease (measured by haemagglutination)	Sun et al. (2019)
Muruku containing *Lathyrus sativus*	Deep-frying	NR			Ayyagari et al. (1989)

(Continues)

TABLE 13 (Continued)

Material (isolated lectin/plants/food preparation)	Method	Conditions		Impact on lectin concentration or activity	Supporting references
PHA contained in rice, bean and whole carob fruit blends, LCA from lentils and lentil flour Pea, kidney bean, faba bean, *Sesbania aculeata* Protein extract from faba bean, lentil flour, bean flours	Extrusion	Temperature: ≥125°C		≥ 90% reduction in lectin content (measured by ELISA or haemagglutination assay)	Sahni et al. (2021), Ciudad-Mulero et al. (2020), Arribas et al. (2019a), Morales et al. (2015), Marzo et al. (2011), Marzo et al. (2002), Alonso et al. (2001), Leontowicz, Leontowicz, Kostyra, et al. (2001), Leontowicz, Leontowicz, Biernat, et al. (2001), Alonso, Aguirre, and Marzo (2000), Martín-Cabrejas et al. (1999), Orúe et al. (1998)
PHA from navy and pinto beans		Temperature: 85°C		78% reduction (measured by ELISA)	Kelkar et al. (2012)
Rice, pea and whole carob fruit blends, spring pea (*Pisum sativum* var. *fidelia*), soybean		Temperature: ≥ 125°C		75% reduction (measure by haemagglutination assay)	Arribas et al. (2019b), Leontowicz, Leontowicz, Kostyra, et al. (2001)
Transgenic soybean meal	Extrusion + Cooking	Moisture content: 39%	Temperature: 165°C	84% reduction (measured by qPCR)	Tian et al. (2014)
			Temperature: 135°C	≥ 50% decrease (measured by qPCR)	
		Moisture content: ≥ 32%	Temperature: 150°C		
White bean, bitter lupin seed, lima bean, kidney bean, chilean bean, alfalfa seeds, *Dolichos lablab* var. *vulgaris*, navy bean, green faba bean Soybean flour, *Jatropha curcas* meal, jackfruit seed meal	Autoclaving	121°C	5–20 min	100% decrease (measured by haemagglutination and ELISA)	Sahni et al. (2020), Luo and Xie (2013), Embaby (2010), Adeparusi (2001), Ravindran et al. (1996), Dhurandhar and Chang (1990)
White bean, sweet lupin seed, kidney bean, chilean bean, white faba bean Soybean flour			20–30 min	≥ 90% decrease (measured by haemagglutination)	Poblete et al. (2020), Nciri et al. (2015), Luo and Xie (2013), Embaby (2010), Machado et al. (2008), Shimelis and Rakshit (2007)
Dolichos lablab var. *vulgaris* seeds *Jatropha curcas* meal			> 30 min		Makkar and Becker (1999) Vijayakumari et al. (1995)
Velvet bean, cucurbit seeds Soybean flour			30–60 min		Vijayakumari et al. (1996) Friedman et al. (1991), Kanwar et al. (1991)
Vigna unguiculata subsp. *unguiculata*		103.4 kPa	30 min	100% decrease (measured by haemagglutination)	Kalpanadevi and Mohan (2013)
Winged beans		121°C	30 min	75% decrease (measured by haemagglutination)	Igene et al. (2006)

TABLE 13 (Continued)

Material (isolated lectin/plants/food preparation)	Method	Conditions		Impact on lectin concentration or activity	Supporting references
Mung bean, tree bean, kidney bean, soybean, chickpea, dry bean, common bean, green and white faba bean Kichdi containing *Lathyrus sativus*	Soaking (≥ 6 h) + Autoclaving	121°C	5–35 min	100% decrease (measured by haemagglutination and ELISA)	Sathya and Siddhuraju (2015), Luo and Xie (2013), Shimelis and Rakshit (2007), Felipe et al. (2006), Mubarak (2005), El-Adawy (2002), Carbonaro et al. (2000), Ayyagari et al. (1989), Antunes and Sgarbieri (1980)
Faba bean		120°C	20 min	88% decrease (measured by haemagglutination)	Carbonaro et al., 2000
Green and white faba bean	Autoclaving + Soaking (+ Dehulling)	121°C	20 min	100% decrease (measured by haemagglutination)	Luo and Xie (2013)
Bean	Canning	≥ 115°C	≥ 40 min	100% decrease (measured by ELISA)	Pedrosa et al. (2015), Olmedilla-Alonso et al. (2013)
Tree bean, lentils	Anaerobic fermentation	72 h		≥ 90% decrease (measured by haemagglutination and ELISA)	Sathya and Siddhuraju (2015), Cuadrado et al. (2002)
Sourdough from whole wheat flour	Aerobic fermentation	72 h		70% decrease (measured by spectrometry)	Tovar and Gänzle (2021)
Pigeon pea		24 h		50% decrease (measured by ELISA)	Anaemene and Fadupin (2022)
Vigna racemosa seeds	Open fermentation	48 h		20% decrease (method: NR)	Difo et al. (2015)
Vigna racemosa seeds, pea flour	Controlled fermentation			100% decrease (method: NR)	
				No change in lectin content (measured by ELISA)	Barkholt et al. (1998)
Black beans	Cooking + Fermentation	Cooking (100°C): 22 min Fermentation: 15 min		100% decrease (measured by haemagglutination)	Rodríguez-Bürger et al. (1998)
Dokla containing *Lathyrus sativus*	Pressure cooking+ Fermentation+ Steaming	NR		100% decrease (measured by haemagglutination)	Ayyagari et al. (1989)
Bitter and sweet lupin seeds, mung beans	Dehulling	NR		≤ 43% decrease (measured by haemagglutination)	Embaby (2010), Mubarak (2005)
Pea seeds, faba bean (green and white), kidney bean				No change in lectin activity (measured by haemagglutination)	Luo and Xie (2013), Alonso, Aguirre, and Marzo (2000), Alonso et al. (1998)
What germ agglutinin	Gamma irradiation	10 kGy		100% decrease (measured by haemagglutination)	Vaz et al. (2012)
Vigna unguiculata subsp. unguiculata				50% decrease (measured by haemagglutination)	Tresina and Mohan (2011)
Jatropha curcas meal	Hydrolysis	Cellulase (5 mg/g) + pectinase 910 mg/g)		No change in lectin activity (measured by haemagglutination)	Xiao et al. (2011)

(Continues)

TABLE 13 (Continued)

Material (isolated lectin/plants/food preparation)	Method	Conditions	Impact on lectin concentration or activity	Supporting references
Red kidney bean	Ultrahigh pressure	450 MPa	100% decrease (measured by haemagglutination)	Lu et al. (2015)
SBA from soybean		550 MPa	63% decrease (measured by ELISA)	Han et al. (2023)
Red kidney bean	High hydrostatic pressure	450 MPa	75% decrease (measured by haemagglutination)	Liu et al. (2013)
High molecular weight α-Galactosidase-haemagglutinin, isolated lectins from black bean Surco variety	pH	3.5–6.5	100% decrease (measured by haemagglutination)	Valadez-Vega, Lugo-Magaña, Betanzos-Cabrera, and Villagómez-Ibarra (2022), Jiang et al. (2019), del Campillo et al. (1981)
Isolated lectins from black bean Surco variety, isolated lectin from Zihua snap pea		9.5–12.0		Valadez-Vega, Lugo-Magaña, Betanzos-Cabrera, and Villagómez-Ibarra (2022), Jiang et al. (2019)
Isolated lectin from black turtle bean, isolated ASA II lectin from garlic		1.0–4.0	≥ 50% decrease (measured by haemagglutination)	Zhao et al. (2019) Clement and Venkatesh (2010)
Isolated ASA II lectin from garlic		10.0–12.0		Clement and Venkatesh (2010)
Isolated lectin from Zihua snap pea		11.0	40% decrease (measured by haemagglutination)	Jiang et al. (2019)
Isolated lectin from Zihua snap pea		2.0–5.0	≤ 30% decrease (measured by haemagglutination)	Jiang et al. (2019)
Isolated lectin from Zihua snap pea, isolated ASA II lectin from garlic		6.0–10.0	≤ 10% decrease (measured by haemagglutination)	Jiang et al. (2019), Clement and Venkatesh (2010)

Abbreviations: ASA, *Allium sativum* agglutinin; DLL-II, *Dolichos lablab* var. *lignosus* lectin; ELISA, enzyme-linked immunosorbent assay; kGy, kilogray; LCA, *Lens culinaris* agglutinin; MHz, megahertz; MPa, megapascal NR, not reported; PHA, phytohaemagglutinin; qPCR, quantitative polymerase chain reaction; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; SBA, soybean agglutinin; WGA, wheat germ agglutinin.

*Studies are ordered by processing method (physical treatments, thermal treatments, mechanical treatments, other) and then by alphabetical order.

3.2.4.1.1 | *Effect of non-thermal treatments*

Soaking, germination and malting

The effects of soaking are highly dependent on the structure, thickness, and composition of the seed coat. The water enters into the seed and triggers different processes such as the hydration of the cells and the activation of different enzymes but also provokes the leakage of small molecules and macromolecules out of the seed. In addition, the soaking time in tap water will influence the leakage of solutes and the degree of softening of the seeds prior to cooking.

Soaking of dry seeds in tap water is a well-known process to reduce their content of antinutrients, especially small antinutrient molecules like phytic acid, tannins and polyphenols, due to a leakage of solutes from the seeds as a result of a major influx of water. However, the effects of soaking on macromolecules and in particular on lectins and α-amylase or trypsin inhibitors from legume seeds are limited. In this respect, soaking of different legume seeds, in distilled water (1:5 seed:water ratio) for 4 h at room temperature, resulted in a decrease in the haemagglutination units (HU/mg dry matter) ranging from 0.6% for soybean to 4.3% for lentil (Table 14) (Shi et al., 2018).

TABLE 14 Effects of various processing treatments on the active lectin content of legume seeds. Haemagglutination assay performed in rabbit erythrocytes. Data are expressed as haemagglutination units/mg dry matter (HU/mg), decrease expressed as percentage (– %). Adapted from Shi et al. (2018)).

Seed	Lectin	Raw/untreated	Soaked*	Cooked**
Pea (*Pisum sativum*)	PSA	5.7	5.5 (– 3.4%)	0.17 (– 97.0%)
Lentil (*Vicia lens*), yellow	LCA	11.1	10.6 (– 4.3%)	0.33 (– 97.0%)
Lentil (*Vicia lens*), green	LCA	11.0	10.8 (– 2.0%)	0.33 (– 97.0%)
Faba bean (*Vicia faba*)	Favin	5.52	5.49 (– 0.6%)	0.10 (– 95.5%)
Chickpea (*Cicer arietinum*)	CAL	2.74	2.70 (– 1.7%)	0.17 (– 93.7%)
Kidney bean (*Phaseolus vulgaris*), red	PHA	88.5	86.5 (– 2.4%)	0.17 (– 99.8%)
Pinto bean (*Phaseolus vulgaris*)	PHA	88.6	87.3 (– 1.4%)	0.17 (– 99.8%)
Navy bean (*Phaseolus vulgaris*)	PHA	88.4	86.8 (– 1.9%)	0.17 (– 99.8%)
Turtle bean (*Phaseolus vulgaris*), black	PHA	87.7	87.0 (– 0.7%)	0.17 (– 99.8%)
Soybean (*Glycine max*)	SBA	692.8	687.5 (– 0.8%)	2.70 (– 99.6%)

*In distilled water (1:5 seed:water ratio) for 4 h at room temperature. **Cooked at 95°C in water (1:5 seed:water ratio) for 1 h.

Soaking of dry mung bean (*Vigna radiata*) seeds in tap water during 12 h reduced the haemagglutinating activity (1360 HU/g dw) by 50%, compared to raw seeds (2670 HU/g dw) (Mubarak, 2005). Conversely, soaking in water (pH 6.9) for 12 h of kidney bean (Tunisian Twila, Beldia and Coco varieties) seeds resulted in an unexpected slight increase (1.95% for Twila, 8.0% for Coco and 10.76% for Beldia) of the PHA content (measured in semi-quantitative haemagglutination of human group O RBCs and expressed as g/kg). It is unclear whether the increase of the PHA activity is related to the release of sugar bound lectins, a better availability due to a better solubilisation of lectins or it is due to the uncertainty in the analytical method used. However, a cooking time of 35 min resulted in the complete elimination of the lectin activity in the previously soaked kidney bean varieties (Nciri et al., 2015).

During the soaking period, germination has begun and is accompanied by an intense proteolytic activity due to the activation of proteases, which provide the young plant with the amino acids it needs for its own protein syntheses. This proteolysis mainly concerns the seed storage proteins that have been deposited in the protein bodies during the seed maturation process. Lectins, which have been accumulated in the cotyledons of lentil and pea seeds during the seed ripening process, will be hydrolysed at the same rate as seed storage proteins to finally disappear approximately 3 weeks after the onset of germination (Howard et al., 1972; Rougé, 1974a; Rougé, 1974b; Rougé, 1975). Lectins present in the embryo axis disappear at a faster rate since no lectin is detected in the young plant approximately 1 week after the onset of germination (Rougé, 1974a; Rougé, 1974b; Rougé, 1975) (Figure 2).

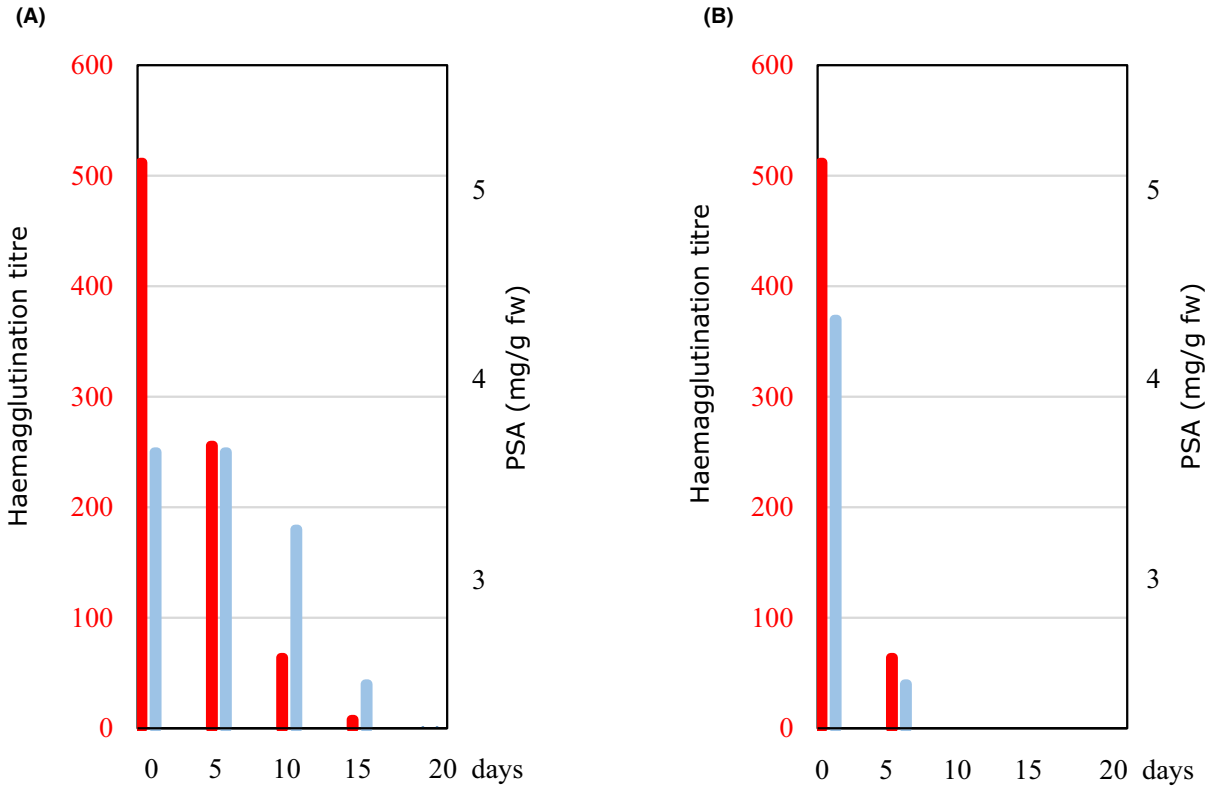


FIGURE 2 (A) Haemagglutinating activity (expressed as haemagglutination titre, red colour) and lectin content (expressed as mg/g fresh weight, light blue colour) of pea cotyledons during the initial stages of germination. (B) Haemagglutinating activity (expressed as haemagglutination titre, red colour) and lectin content (expressed as mg/g fresh weight, light blue colour) of pea embryo axis/young plant during the initial stages of germination. Adapted from Rougé (1975).

A very similar decrease in the lectin content was reported in seedlings of *Phaseolus vulgaris* (Mialonier et al., 1973) and *Dolichos biflorus* in 5 days (Talbot & Etzler, 1978). During germination, the lectin content of cotyledons and young plants of two varieties of peanut (*Arachis hypogaea*), estimated by haemagglutination, decreased gradually to finally disappear 3 weeks after the onset of germination (Pueppke, 1979). More recently, using a combination of native-PAGE, haemagglutination, immunodetection and real-time qPCR, for monitoring the evolution of DLL-II, the galactose-specific lectin from *Dolichos lablab*, Vishweshwaraiah et al. (2018) confirmed the rapid decline in the haemagglutinating activity during the initial stages of germination (3 days). During the post-germination period, only a negligible haemagglutinating activity could be monitored in the young plant. Germination of mung bean seeds (12 h soaking and 3 days germination in dark) reduced the haemagglutinating activity by 79% (560 HU/g dw), compared to dry raw seeds (2670 HU/g dw) (Mubarak, 2005).

The effect of germination upon the haemagglutinating activity and lectin (LCA) content of lentil (*Vicia lens*), under different conditions of watering (daily (wd) or alternate (wa) days) and light (dark (d) or 6 h day^{-1} light (l)), was investigated after 3 days and 6 days of treatment, respectively (Cuadrado et al., 2000). Three days and 6 days after the onset of germination, a decrease in the haemagglutinating activity was observed whatever the watering and light treatment applied to seedlings, except for the 3d (wd,l) samples which received daily watering in the dark which displayed an increase of the haemagglutinating activity (Table 15). Similarly, a slight decrease or no change of the LCA content was measured by competitive ELISA in the cotyledons of the 3d (wd,d) and 3d (wa,d), and the 3d (wd,l) and 3d (wa,l) samples, respectively. Conversely, an increase of the LCA content was observed in all the 6 days samples, whatever the watering and light conditions applied to the seedlings. These results suggest that the watering and light conditions applied to the germinating seedling have little effect on the evolution of haemagglutinating activity but readily affect the evolution of the lectin content, which exhibits an unexpected increase in the samples collected 6 days after the onset of germination. However, the lack of correlation between the evolution of the haemagglutinating activity and the lectin content, combined with the differences in sensitivity and specificity of the haemagglutination and ELISA techniques used, render these results difficult to interpret. It is important to note that the ELISA technique depends on the antibody used and may or may not detect the proportion of lectin bound to glucoproteins.

TABLE 15 Effect of germination upon haemagglutinating activity (HU) and LCA content (ELISA) of lentil seeds under different time (3 (3d) or 6 (6d) days) watering regime (daily (wd) or on alternate days (wa)) and light (dark (d) or 6h day^{-1} light (l)) conditions. The coloured arrows indicate increasing tendency (green), decreasing tendency (red) or equivalence (black), by comparison with the control (non-germinated seeds). Adapted from Cuadrado et al. (2000).

Germination conditions	Haemagglutinating activity (HU or lectin equivalent g/kg dw)	LCA content (mg/g dw)
Control	250 ± 80	0.85 ± 0.09
3d (wd,d)	210 ± 20 ↘	0.74 ± 0.09 ↘
3d (wd,l)	380 ± 41 ↗	0.87 ± 0.18 →
3d (wa,d)	150 ± 12 ↘	0.75 ± 0.06 ↘
3d (wa,l)	120 ± 10 ↘	0.87 ± 0.15 →
6d (wd,d)	170 ± 13 ↘	1.15 ± 0.12 ↗
6d (wd,l)	170 ± 36 ↘	1.54 ± 0.18 ↗
6d (wa,d)	170 ± 24 ↘	1.17 ± 0.09 ↗
6d (wa,l)	125 ± 3 ↘	1.01 ± 0.009 ↗

A study on the impact of a 1- and 2-day malting process (6 h steeping; 24 and 48 h of germination at 20°C and kilning at 60°C) on the lectin content in black beans (*Phaseolus vulgaris*) and soybean (*Glycine max*) revealed an important decrease of the lectin content (measured by ELISA) and activity (haemagglutinating activity expressed as HU (mg/g dw) (Table 16) (Muzquiz et al., 1998).

TABLE 16 Impact of malting on the haemagglutinating activity and lectin content (mean ± standard error) of black beans and soybeans. Adapted from Muzquiz et al. (1998).

Seed	Hemagglutinating activity HU (g/kg)	PHA content (mg/g dw)
Black bean		
Raw	5.13 ± 0.00	0.166 ± 0.4
1 day malting	1.92 ± 0.64 (−63%)	0.091 ± 0.1 (−46%)
2 day malting	1.28 ± 0.00 (−75%)	0.039 ± 0.1 (−76%)
Soybean		
Raw	2.56 ± 0.00	Not analysed
1 day malting	1.60 ± 0.96 (−37%)	Not analysed
2 day malting	0.80 ± 0.48 (−69%)	Not analysed

Since the lectin content quickly diminishes in germinating legume seedlings, germination appears as an interesting and easy process for reducing the concentration of lectins in legume seeds. However, its effectiveness in significantly reducing lectin content requires sufficient time to reach the early stages of germination. In addition, some studies reported no impact of germination on the lectin content (Alonso, Aguirre, & Marzo, 2000; Alonso et al., 1998)

Fermentation

The release of a complex set of hydrolytic enzymes, especially proteolytic enzymes, by the bacterial and fungal microorganisms currently used in fermentation processes makes fermentation an effective process for reducing the concentration of lectins in legume seeds and derived products. Natural fermentation of finely ground lentil seed flour by its own surface microorganisms (*Bacillus* was reported as the predominant microorganism and lactic bacteria, *Lactobacillus* and *Pediococcus*, were observed at 24 h of the natural fermentation) resulted in an important decrease in the lectin content (measured by ELISA), up to 98% decrease after 72 and 96 h of fermentation (Cuadrado et al., 2002). However, studying the effects of fermentation (for 48 h) by lactic acid bacteria (*Pediococcus pentosaceus*, *Lactococcus raffinolactis*, *Lactobacillus plantarum*) or fungi (*Rhizopus microsporus*, *Geotrichum candidum*) on the potential allergenicity of peas, Barkholt et al. (1998) noted no reduction in the lectin content as measured by ELISA.

Enzymatic treatment

Thermomechanical (milling and extrusion at high temperature and high pressure for a short time, conditions not reported) and enzyme-facilitated processing of soybean meal (treatment with an unspecified mixture of enzymes – most probably proteases) enhanced the in vitro kinetics of protein digestion and the protein and amino acid digestibility in weaned pigs

and reduced the lectin (SBA) content by 85.5% (29 mg/kg), compared to the untreated soybean meal (339.4 mg/kg) (Nu et al., 2020).

Dehulling

Dehulling of mung bean (*Vigna radiata*) seeds resulted in a decrease of 22.5% of the haemagglutinating activity (1800 HU/g dw), compared to raw bean seeds (2670 HU/g dw) (Mubarak, 2005).

pH treatment

Acidic treatment at pH 1.5 used to improve the emulsifying properties (Jiang et al., 2009), and in combination with heating at moderate temperature (50–60°C), the gelling ability of soy protein isolates (Liu et al., 2015), readily affects the allergenicity of soy proteins, most probably by promoting structural modifications as shown for different allergens (Lin et al., 2015; Rahaman et al., 2016). Similarly, the acidic treatment at low pH (1.0–3.5) of PHA (94% purity) isolated from black turtle bean (*Phaseolus vulgaris*) resulted in the monomerisation and progressive unfolding of the lectin. This led to a noticeable decrease in its potential allergenicity as evaluated with a direct ELISA, in vitro digestibility by pepsin and trypsin, and haemagglutinating activity (50% decrease at pH 1.0 and 1.5) (Zhao et al., 2019). All these modifications suggest conformational changes in the surface IgE-binding epitopes and the CBS of PHA. Unfolding and refolding of PHA-L, the leucoagglutinin from kidney bean, studied at acidic pH (2.5), shows that a dimeric intermediate occurs during the refolding. In addition, the refolding kinetics was dependent on the protein concentration, which provides another evidence for an intermediate state during the refolding process (Biswas & Kayastha, 2004). Basic treatment up to pH 9 also induces subunit dissociation and unfolding of the tetrameric jacalin lectin as evidenced by circular dichroism (CD) and fluorescence studies (Banerjee et al., 2024). Upon alkaline treatment, the haemagglutinating activity of PHA is slightly decreased (−40% at pH 11) (Jiang et al., 2019). Similarly, the stability of garlic lectin ASA-II is also impacted at alkaline pH since a decrease in the haemagglutinating activity of 60% occurs at pH 10 as well as pH 12 (Clement & Venkatesh, 2010). By contrast, CD spectra showed that both the secondary and tertiary structure of the phloem exudate lectin of cucumber (*Cucumis sativus*) remained nearly unaltered in the pH range 3–10 (Nareddy & Swamy, 2018).

High pressure

The effect of ultra-high pressure (UHP) from 350 MPa up to 550 MPa on the inactivation of SBA in soybean seeds was measured by the ability of SBA to interact with specific antibodies in a sandwich ELISA technique. It was shown that a pressure of 500 MPa for 15 min is required to obtain 60% inactivation of the lectin. The inactivation corresponds to conformational changes in the secondary structure of SBA that have been detected by various techniques including UV spectroscopy, fluorescence spectra and circular dichroism. After the treatment at 500 MPa, the secondary structure of SBA was strongly altered, with an increase in beta-sheets and random coil structures. At the same time, the cytotoxicity in mice of the UHP-treated SBA was reduced (Han et al., 2023).

At a pressure of 600 MPa and temperatures of 50, 60 and 70°C applied for 10 min, about 50% (50°C), 75% (60°C) and 95% (70°C) of PHA from *Phaseolus vulgaris* was inactivated as measured by haemagglutination of human red blood cells (RBCs). Horse gram (*Dolichos biflorus*) lectin (DBA) was inactivated by 75% after 10 min at 600 MPa and 40°C (Butz et al., 1996).

Irradiation

Gamma irradiation of WGA, using doses of 1.0, 10.0 and 25.0 kGy at a rate of 8.25 kGy/h, applied to reduce the allergenicity of the WGA, resulted in the dissociation of the dimeric structure and major structural changes in the lectin monomer. At high doses of irradiation, CD spectra revealed an important effect on disulfide bridges and the production of partially unfolded monomers. At the dose of 25.0 kGy, large amorphous aggregates are produced, whose insolubility is checked by a decrease of intrinsic fluorescence and a high binding of bis-ANS (4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid), used as a probe for hydrophobicity. The haemagglutinating activity of WGA was eliminated at irradiation doses of 10.0 and 25.0 kGy (Vaz et al., 2012). Very similar results were obtained with Con A, submitted to different doses (1.0, 10.0 and 25.0 kGy) of γ-irradiation. The inactivation of Con A corresponds to a fragmentation of the lectin monomer, which increases in a dose–response dependent manner. At irradiation doses of 10.0 and 25.0 kGy, the fragments collapse in particularly hydrophobic large amorphous aggregates and the haemagglutinating activity is eliminated (Vaz et al., 2013). More recently, the impact of different doses (2.5, 5, 10 and 15 kGy) on the bioactive principles of two coastal sand dune legumes, *Canavalia cathartica* (non-edible) *and C. maritima*, was investigated by Supriya and Sridhar (2019). Upon irradiation no alteration occurred in the haemagglutinating activity of the lectin towards A, B and O type RBCs, while the activity towards AB type RBCs decreased at 2.5 kGy and further decreased at higher 10 and 15 kGy doses (Supriya & Sridhar, 2019).

3.2.4.1.2 | *Effect of thermal treatments*

Although most lectins in edible plants, and legume seeds in particular, are made up of tightly associated monomers which seem to be rather resistant to thermal denaturation, these are in fact heat-sensitive proteins that offer only limited

resistance to sufficiently intense and prolonged heating. Thermal stability of PHA from black turtle bean (*Phaseolus vulgaris*) was investigated by fluorescence assays (based on the natural fluorescence of a lectin) and hemagglutinating activity at moderate temperatures in the range between 70°C and 90°C during treatment times from 0 to 30 min (He et al., 2014). Thermal treatment significantly affected the stability of the lectin and was associated with conformational changes (unfolding), which reduce the haemagglutinating activity of the lectin, especially at the higher temperature (90°C). A short-term treatment (5 min) at a higher temperature (90°C) is efficient at eliminating the PHA activity.

Cooking

The term 'cooking', commonly used to describe the heat treatment of food, is rather non-specific because if the conditions are not specified, it may refer to different domestic cooking processes such as boiling, frying, braising, etc. or cooking at industrial level, extrusion cooking or autoclaving.

Domestic cooking

Boiling

Boiling of seeds from different lectin-containing legumes usually results in a sharp decline in the haemagglutination titres (Table 17), with the exception of soybean, whose lectin SBA seems to be more resistant to heat inactivation, as after 20 min of boiling 25% of the haemagglutinating activity still remained (Leontowicz et al., 1998).

TABLE 17 Evolution of haemagglutination titres obtained with different red blood cell groups of raw and boiled seeds from faba bean, pea and soybean (the haemagglutination titres correspond to the inverse of the maximum active dilutions). Adapted from Leontowicz et al. (1998)).

	Haemagglutination of blood groups			
Seed	**O**	**A**	**B**	**AB**
Faba bean				
Raw	100	100	50	200
Boiled 10 min	50	50	0	50
Boiled 20 min	0	0	0	0
Pea				
Raw	100	100	100	200
Boiled 10 min	50	50	50	50
Boiled 20 min	0	0	0	0
Soybean				
Raw	800	800	400	1600
Boiled 10 min	400	600	200	800
Boiled 20 min	200	200	100	400

The haemagglutinating activity of raw cowpea (*Vigna unguiculata*) heated at 120°C for 15 min declined from 25.6 units (unheated) to 6.4 units (heated) (Umapathy et al., 1998). Upon cooking in water at 95°C for 1 h, the haemagglutinating activity (expressed as HU/mg dw) of most Canadian pulses and soybean was drastically reduced by 93.77% for chickpea (*Cicer arietinum*) up to 99.61% for soybean (*Glycine max*) and 99.81% for *Phaseolus vulgaris* beans (red kidney bean, pinto bean, navy bean and black bean) (Shi et al., 2018) (Table 14). Different thermal treatments of mung bean (*Vigna radiata*) seeds, including boiling in tap water for 90 min at 100°C, autoclaving at 120°C under 15 MPa for 35 min and microwave cooking in tap water for 15 min, completely eliminated their haemagglutinating activity (Mubarak, 2005).

Roasting

Using haemagglutination of neuraminidase-treated group A RBCs to quantify the lectin content, the effects of dry and moist roasting on the lectin activity of Florunner peanut and Bragg soybean were investigated (Ahmed, 1986). Both dry (air heated at 177°C) and moist (steam at 121°C) heating of peanut and soybean defatted meal resulted in the complete inactivation of the lectins provided that a heating time of 30 min is respected (Table 18). In this respect, moist heating is more efficient than dry heating to inactivate the peanut and soybean lectins.

TABLE 18 Active lectin content (expressed as mg/g defatted meal, mean values ± standard error from 3 replications) of dry (air heated at 177°C) and moist (steam at 121°C) heated peanut and soybean seeds. Adapted from (Ahmed, 1986).

Heat treatment	Peanut	Soybean
Untreated	144.7 ± 2.13	112.1 ± 2.77
15 min dry heated	120.7 ± 0.71	43.5 ± 2.38
15 min moist heated	9.2 ± 0.60	0.6 ± 0.05
20 min dry heated	108.6 ± 1.09	0.0
20 min moist heated	0.4 ± 0.02	0.0
30 min dry heated	0.0	0.0
30 min moist heated	0.0	0.0

Frying and braising

The time-dependent effects of stir-frying and braising on the PHA content of freshly harvested kidney beans (*Phaseolus vulgaris*, Zihuayoudou strain) were investigated by Sun et al. (2019), using a semi-quantitative haemagglutination method or rabbit RBC (detection limit of 0.2 mg/g). Both cooking methods resulted in a decrease in the lectin content; the temperatures used for stir-frying and braising were not reported in the study (Table 19).

TABLE 19 PHA content of stir-fried and braised Zihuayoudou kidney beans.

Cooking method	Treatment time (min)	PHA content (mg/g)	Cooking method	Treatment time (min)	PHA content (mg/g)
Stir-frying	0	51.2	**Braising**	0	51.2
	3	12.8		5	0.4
	6	3.1		10	ND
	9	1.6		15	ND
	12	0.8		20	ND
	15	0.2		25	ND
	18	ND		30	ND

Abbreviations: ND, PHA content lower than the detection limit (< 0.2 mg/g).

Microwaving

The microwaving treatment applied to various lectin-containing seeds usually resulted in the decrease or complete disappearance of the lectin activity measured with semi-quantitative haemagglutination techniques of human or animal red blood cells (RBCs), except for PHA from different varieties of kidney bean (*Phaseolus vulgaris*) which remain active after the microwaving treatment of the seeds, whereas a conventional cooking method (boiling for 210–270 min) resulted in inactivation of lectins (Hernández-Infante et al., 1998) (Table 20).

TABLE 20 Effects of microwave treatment of food and non-food lectin-containing seeds on the lectin activity measured by semi-quantitative haemagglutination of human or animal RBCs.

Food seeds	Soaking pre-treatment	Microwaving treatment	Haemagglutinating activity of raw foods (maximum dilution causing haemagglutination	Haemagglutinating activity of treated foods (maximum dilution causing haemagglutination)	Reference
Faba (*Vicia faba*) Pea (*Pisum sativum*) Lentil (*Vicia lens*) Chickpea (*Cicer arietinum*) Soybean (*Glycine max*) Kidney bean (*Phaseolus vulgaris*)	Soaking in tap water for a time necessary to acquire a moisture content of 24%–25% (wet seeds)	Microwave oven generating 0.56 KW power at 2450 MHz for the time necessary to release a cooked smell without any toasted grains	0 3 2 3 5 11–12*	0 0 0 0 0 7–8*	Hernández-Infante et al. (1998)

TABLE 20 (Continued)

Food seeds	Soaking pre-treatment	Microwaving treatment	Haemagglutinating activity of raw foods (maximum dilution causing haemagglutination	Haemagglutinating activity of treated foods (maximum dilution causing haemagglutination)	Reference
Chickpea (*Cicer arietinum*)	Soaking for 12 h in distilled water (1:10 ratio) at ~25°C	Seeds soaked in tap water (1:10 ratio) were cooked in microwave oven at 2450 MHz until softening of about 50% of the seeds	6.22 ± 0.22 HU/mg dw of seed	0.00 ± 0.00 HU/mg dw of seed	El-Adawy (2002)
Mung bean (*Vigna radiata*)	Soaking for 12 h in distilled water (1:10 ratio) at ~25°C	Seeds soaked in tap water (1:10 ratio) were cooked in microwave oven at 2450 MHz until softening of about 50% of the seeds	2670 HU/g dw of seed	0.00 HU/g dw of seed	Mubarak (2005)
Bitter lupin (*Lupinus albus, var termis*) Sweet lupin (*Lupinus albus*)	Dry seeds	Seeds soaked in water (1:10 ratio) were cooked in microwave oven for 6 min	16 HU (1 g seed flour/25 mL PBS pH 7.2) 32 HU (1 g seed flour/25 mL PBS pH 7.2)	4 HU (1 g seed flour/25 mL PBS pH 7.2) 4 HU (1 g seed flour/25 mL PBS pH 7.2)	Embaby (2010)

Abbreviations: dw, dry weight; HU, haemagglutinating activity; KW, kilowatts; MHz, megahertz; PBS, phosphate-buffered saline; RBC, red blood cells.
*Different values depend on the variety of kidney bean used.

Industrial cooking

Extrusion

The combination of high temperature (up to 200°C) and high pressure (up to 20 MPa) in the currently used twin-screw extruders creates favourable conditions to mitigate the lectin components of legume-derived food products and other antinutritional factors, i.e. trypsin inhibitors (TI), phytic acid and polyphenolic compounds (Pasqualone et al., 2020). Complete elimination of the lectin activity (PHA), measured by a competitive indirect ELISA assay, was reported in extruded products (125°C) prepared from rice, bean and whole carob fruit (Arribas et al., 2019a), while the same group measured the haemagglutinating activity in products prepared from rice, pea and carob fruit, using trypsin-treated rat blood cells and reported a reduction in activity between 50% and 90% (Arribas et al., 2019b). Extrusion cooking (140°C) of lentil (*Vicia lens*) flour resulted in a reduction higher than 90% in lectin content and activity, as measured with a competitive indirect ELISA assay and a haemagglutination assay, respectively (Ciudad-Mulero et al., 2020). Extrusion cooking of pea (*Pisum sativum*) and kidney bean (*Phaseolus vulgaris*) seed meals provoked a significant reduction in lectin activity, as measured with a haemagglutination assay using trypsin-treated rabbit blood cells, from 8 HU per 100 mg and 1024 HU per 100 mg in raw pea and kidney bean, respectively, to non-detectable in both extruded pea and kidney bean (Alonso et al., 2001). Extrusion at 140°C and humidity 25% was effective in reducing the haemagglutinating activity of both raw and germinated pea (*Pisum sativum*) by 98% ($p < 0.05$) (Orúe et al., 1998). Low-temperature (85°C) extrusion and steam cooking (82°C) reduced the lectin concentration (PHA) in kidney bean (*Phaseolus vulgaris*) flour by 85%–95% compared to raw bean flour, as measured by ELISA (Kelkar et al., 2012).

Autoclaving

Application of different heat treatments including cooking at 100°C, microwaving, and autoclaving of green and white faba bean (*Vicia faba*) seeds resulted in the decrease or disappearance of the lectin activity (measured as the titre) in both seed samples (Luo & Xie, 2013) (Table 21).

TABLE 21 Effects of different heat treatments on the lectin activity (expressed as titre) of green and white faba beans. Adapted from Luo and Xie (2013).

	Green faba bean		White faba bean	
Heat treatment	Lectin (HU*)	Reduction (%)	Lectin (HU*)	Reduction (%)
Untreated	28		56	
Microwaving (6 min)	7	75	7	87.5
Cooking 100°C (30 min)	4	85.7	0	100
Autoclaving 121°C (20 min)	0	100	0	100

*Haemagglutinating activity (HU) was measured by a serial dilution using a 3% suspension of trypsin-treated rabbit erythrocytes; one unit of HA was defined as the reciprocal of the highest dilution giving positive agglutination.

Summary

A substantial body of evidence suggests that heat treatment is a highly effective method for deactivating lectins from plant sources, with numerous studies demonstrating that exposure to elevated temperatures can significantly reduce or eliminate lectin activity. However, depending on the plants, the heating process and its duration, and the previous soaking of seeds in water, some discrepancies in the results have been noticed:

1. The effect of soaking on the reduction in lectin content depends on the type of seeds and the duration of water imbibition. Limited soaking for 3–18 h generally reduces lectin content by around 10%–20%, with a few exceptions: for lupin seeds (no reduction after 96 h soaking), mung bean and pigeon pea (50% reduction after 18 h soaking) and lima bean (100% reduction after 9 h soaking). Soaking (≥4 h) coupled to cooking is a more efficient way for deactivating lectins, which usually results in a 90%–100% decrease in the lectin activity, except for a few kidney bean varieties (≤50% decrease).
2. The processes for cooking, at home or industrial, influence the decrease in lectin activity: steaming, microwaving and roasting, with or without prior soaking, considerably reduce the lectin activity (≥75% and up to 100%), with a few exceptions (50% reduction by microwaving for soybean). Extrusion and autoclaving at 121°C for ≥30 min, with or without soaking, reduce the lectin activity by ≥75% and up to 90%–100%.
3. Other industrial processes used for reducing the lectin activity of edible seeds, such as canning, fermentation with various microorganisms, gamma-irradiation, hydrolysis and ultra high-pressure, have different effects on the decrease of lectin activity, ranging from 20% up to 100% decrease depending on the plants.

3.2.5 | Effects of food storage on the hazard identification and characterisation

Storage conditions, especially for legume seeds, can have a major influence on their taste, palatability, nutritional and possibly toxic properties. In particular, the phenomenon known as 'hard-to-cook' (HTC) defect in kidney beans (*Phaseolus vulgaris*) has been thoroughly investigated (Reyes-Moreno & Paredes-López, 1993). This phenomenon, which occurs under improper storage conditions of high temperature and humidity, diminishes the beans' taste and nutritional qualities and requires a longer cooking time to acquire the softening necessary for their consumption. Although the molecular mechanism of the HTC process is still poorly understood, it probably results from an alteration in the structure and solubility of pectin from the cell walls (Chigwedere et al., 2018; Chigwedere et al., 2019; Njoroge et al., 2014; Yi et al., 2016). According to the now prevailing pectin–cation–phytate theory proposed to explain the development of hardening in kidney beans, the hydrolysis of phytates by phytases would release Ca^{2+} ions which migrate to the middle lamella of cotyledonary cells to transform soluble pectins into insoluble (Chen et al., 2023; Perera et al., 2023; Wainaina et al., 2022; Zhu et al., 2023). Inappropriate storage, leading to HTC, increases the persistence of PHA and represents a food hazard if the beans are not cooked for a prolonged time. The haemagglutinating activities (HU/kg seed meal) and PHA content (g PHA/kg seed meal) of improperly stored (HTC) samples from different cultivars of kidney bean (*Phaseolus vulgaris*) were found slightly increased compared to freshly harvested (FH) samples (Table 22) (Martín-Cabrejas & Esteban, 1995).

TABLE 22 Influence of improper storage (HTC) on haemagglutinating activity (rat erythrocytes) and PHA content of different cultivars of kidney bean (*Phaseolus vulgaris*) harvested 5 years earlier. Adapted from Martín-Cabrejas and Esteban (1995)).

Cultivar	Cooking time (min)	Haemagglutinating activity (HU*/kg seed meal)	Lectin content (ELISA**) (g PHA/kg seed meal)
Fresh Mwitemania	15	9.69	5.9 ± 0.1
HTC Mwitemania	75	9.69	7.4 ± 0.2
Fresh Canadian Wonder	25	9.69	6.5 ± 0.3
HTC Canadian Wonder	> 300	19.38	35.0 ± 1.4
Fresh Mwezi Moja	10	9.69	6.8 ± 0.5
HTC Mwezi Moja	120	9.69	11.6 ± 0.3
Fresh Rose Coco	20	9.69	3.5 ± 0.3
HTC Rose Coco	135	9.69	12.4 ± 0.6
Fresh Red Haricot	20	4.87	3.1 ± 0.5
HTC Red Haricot	75	9.69	10.2 ± 0.7

*One HU defined as the amount of material which caused agglutination of 50% of erythrocytes. **rabbit anti-PHA IgG antibodies and goat anti-rabbit IgG conjugate.

3.2.5.1 | *Occurrence of natural PHA-free cultivars among wild or cultivated bean varieties*

Kidney/common bean (*Phaseolus vulgaris*) cultivars are extremely numerous and some of them are apparently lectin-free. Brücher (1968) observed no haemagglutinating activity in 10% of the South American bean cultivars tested, and Brown et al. (1982) could detect seven cultivars with no haemagglutinating activity against RBCs out of the 107 South African bean cultivars tested. Depending on the cultivars, the apparently lectin-free cultivars predominantly contain either PHA-related α-amylase inhibitors (Moreno & Chrispeels, 1989; Peddio et al., 2023; Pusztai et al., 1981) or different variants of arcelin (Cardona et al., 1990; John & Long, 1990; Lioi et al., 2003; Osborn et al., 1988), an insecticidal protein towards bruchid pests (Cardona et al., 1989). This extreme diversity observed in the distribution of PHA and PHA-related proteins among diverse varieties of *P. vulgaris* and closely related beans such as tepary beans (*P. acutifolius*), lima beans (*P. lunatus*), runner bean (*P. coccineus*), comes from the fact that the synthesis and accumulation of lectins (PHA-E, PHA-L), arcelins and α-amylase inhibitors (α-AI) is under the control of a multigene family known as the APA (A for arcelin, P for phytohaemagglutinin and A for α-amylase inhibitor) cluster (Mirkov et al., 1994). Depending on the varieties of beans, very high variations occur in the levels of the different proteins encoded by the APA genes.

At the molecular level, both lectins PHA-E and PHA-L consist of homo-tetramers whereas arcelins and α-AIs consist of homodimers (Figure 3).

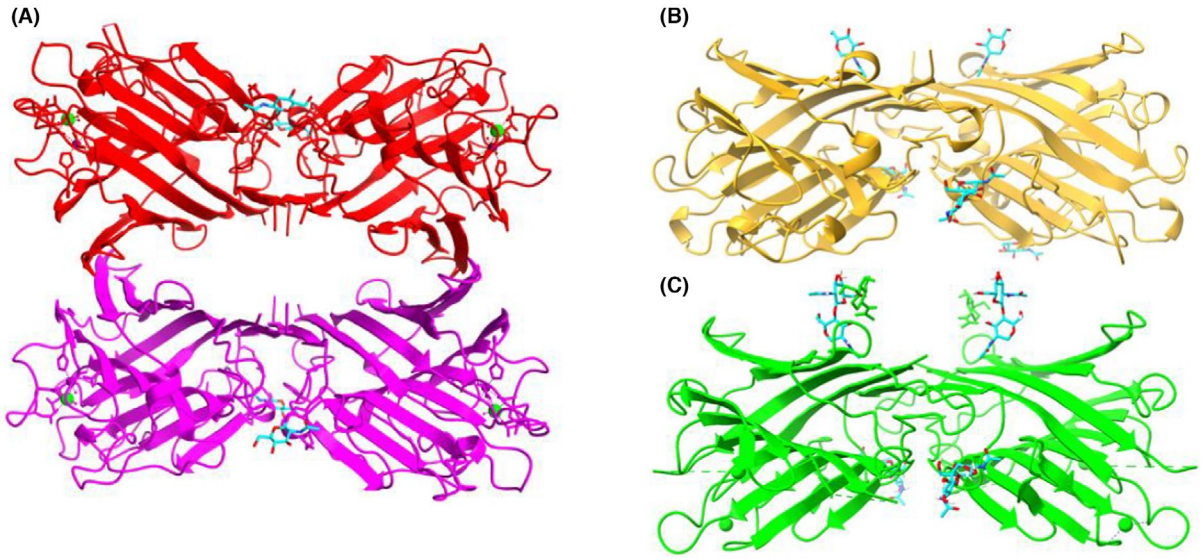


FIGURE 3 (A) Three-dimensional structure of the homo-tetramer PHA-E (PDB code 5AVA), resulting from the non-covalent association of two dimers, coloured red and pink, respectively. Each monomer, made of the non-covalent association of two monomers, contains a CBS. The Ca^{2+} and Mn^{2+} ions are represented by green and purple balls, respectively. The *N*-glycans linked to the dimers are represented in sticks coloured cyan. (B, C) Three-dimensional homo-dimeric structures of arcelin-1 (B) and α-AI-1 (C), showing the non-covalent association of both monomers in each dimer. *N*-glycans linked to both dimers are coloured cyan.

The homodimeric α-AI-1molecule interacts with porcine or insect α-amylase in such a way that its regions corresponding to the deleted and no more active CBS lodge into the V-shaped active site region of the enzyme and totally block the α-amylase activity (Figure 4). As a result of this steric hindrance, the amino acid residues responsible for the hydrolytic cleavage of starch become completely blocked and the access of starch to the active site of α-amylase is no more possible.

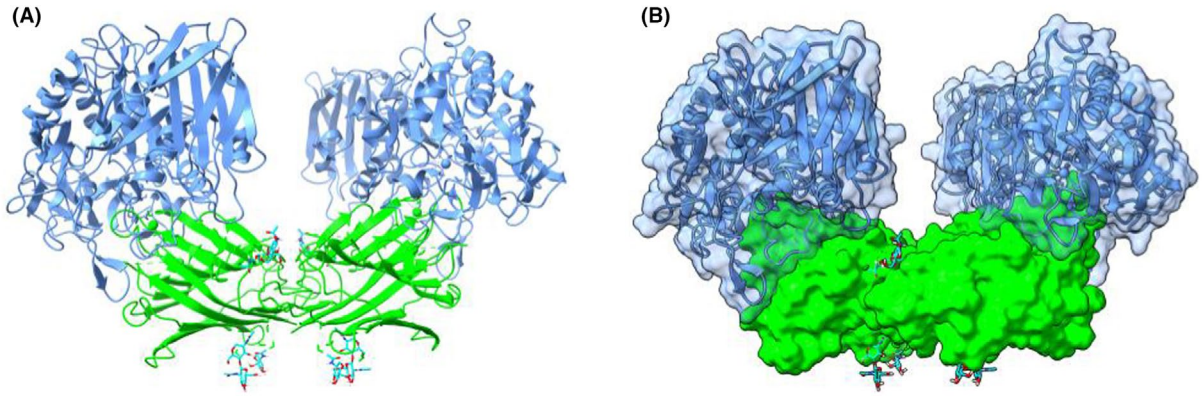


FIGURE 4 Three-dimensional structure of α-AI-1 in complex with two molecules of bovine a-amylase (PDB code 1DHK) (A) and molecular surface of the complex shown in transparency (B). The a-AI-1 and both α-amylase proteins are coloured green and blue, respectively. Note that the a-amylase inhibitor drives like a wedge into the α-amylase region corresponding to the active-site-containing channel of the enzyme, blocking α-amylase hydrolytic activity.

Because of their inhibitory activities on human α-amylases, their presence in appreciable quantities in the seeds of many bean varieties and despite their low resistance to digestive proteases and heat denaturation (Le Berre-Anton et al., 1998), the regular consumption of improperly cooked α-AI-containing beans could have some antinutritional effects, e.g. by lowering blood glucose. In this respect, in toxicity/antinutritional studies performed with crude bean extracts, both the activity of α-amylase- and trypsin-inhibitors should be considered if they have not been eliminated before performing the toxicity assays.

3.3 | Acute dietary exposure assessment for humans

Table 23 shows the acute dietary exposure estimations for PHA via the consumption of individual food commodities in different population groups (consuming days only, see Section 2.7. for details). The food commodities listed in the table are those whose consumption would lead to the highest acute dietary exposures, expressed as mean exposures and high exposures, the latter based on the highest reliable percentiles. Detailed exposure estimates across different population groups via the consumption of different beans (*Phaseolus* sp.) and bean-containing products are shown in Annex E.

In an arbitrary dietary exposure scenario assuming that 50% of the lectin activity remains in the processed commodities, the highest mean exposure estimates would range between 23.5 mg/kg bw per day and 35.0 mg/kg bw per day following the consumption of 'Borlotti or other common beans (dry)' and Lima beans (dry), respectively. For the highest reliable percentile, the exposure estimates would be between 58.9 mg/kg bw per day (97.5th percentile) and 75.8 mg/kg bw per day (99th percentile) via the consumption of 'Borlotti or other common beans (dry)' and 'Beans and vegetables meal', respectively. The highest reliable percentile exposure estimate would be in the population group 'Other children' (see Annex E, Table E3).

TABLE 23 Acute dietary exposure to PHA (mean exposure and high exposure, consuming days only) following the consumption of different beans (*Phaseolus* sp.) and beans containing commodities, under an arbitrary scenario, assuming that 50% of lectin activity remains in the processed commodities[a].

Acute dietary exposure to PHA (mg/kg bw per day)					
	Highest mean	**Population group**		**Highest reliable percentile[b]**	**Population group**
Lima beans (dry)	35.0 ($n = 15$)	Other children	Beans and vegetables meal	75.8 (P99, $n = 431$)	Other children
Borlotti or other common beans (dry)	30.1 ($n = 45$)	Infants	Borlotti or other common beans (dry)	71.4 (P97.5, $n = 145$)	Toddlers
Beans and vegetables meal	27.9 ($n = 431$)	Other children	Canned or jarred common beans	63.9 (P97.5, $n = 144$)	Toddlers
Navy beans (dry seeds)	25.9 ($n = 74$)	Toddlers	Navy beans (dry seeds)	61.5 (P95, $n = 74$)	Toddlers
Borlotti or other common beans (dry)	23.5 ($n = 145$)	Toddlers	Borlotti or other common beans (dry)	58.9 (P97.5, $n = 296$)	Other children

Abbreviations: bw, body weight; *n*, number of consuming days; P, percentile.

[a]Dietary exposure scenario as described in section 2.7.

[b]The highest percentile that can be reliably estimated when applying the following minimum sample size for each percentile ($\alpha = 0.05$): 5 consuming days for the P50, 11 for the P75, 29 for the P90, 59 for the P95, 119 for the P97.5, and 299 for the P99.

3.4 | Considerations for the risk characterisation

The acute dietary exposure estimates for PHA (see Section 3.3), in the arbitrary scenario considering only 50% deactivation of PHA, were compared with the $BMDL_{10}$ of 22.9 mg/kg bw per day, established as a reference point for PHA based on the increase in small intestine weight in rats (see Section 3.1.7.2). This resulted in an MOE of about 0.3 at the highest reliable percentile exposure (75.8 mg/kg bw per day via the consumption of 'Beans and vegetables meal').

The CONTAM Panel noted that the MOE is below 100, considered to cover the human health effects (see Section 3.1.8) and, therefore, deactivation of 50% of the active PHA initially present in the foods (as selected in the arbitrary exposure scenario) would raise health concerns. The CONTAM Panel also noted that PHA exposure from food commodities processed in accordance with adequate food processing practices (see Section 3.2.4) that would result in complete deactivation of lectins does not raise health concerns.

No conclusions could be made for other lectins due to the lack of toxicological data and, in some cases, lack of adequate occurrence data.

3.5 | Uncertainty analysis

The purpose of the uncertainty analysis is to identify and quantify the specific uncertainties in the risk assessment and combine them to assess the overall certainty of the final conclusion, as recommended in EFSA's guidance on uncertainty analysis (EFSA Scientific Committee, 2018a). The risk assessment is limited to the only group of lectins with sufficient evidence, namely: PHA. The uncertainty assessment was done separately for the hazard assessment: ensuring that the selected reference point (RP) for PHA – the lowest BMDL from the critical study and critical endpoint – is protective, and for the exposure assessment: estimating the likelihood that the different exposure scenarios are below the RP. The latter part includes the risk characterisation. The uncertainty assessment is done for acute exposure of European high consumers, namely the 99th percentile.

In a first step, sources of uncertainties related to PHA hazard identification and characterisation, as well as to the exposure assessment to PHA, were listed and discussed (Appendix E). The focus was on uncertainties specific to this assessment, excluding standard uncertainties. Standard uncertainties, such as those covered by extrapolation factors and food consumption data from national surveys, were considered using standard approaches (EFSA, 2011a, 2019). The impact of all non-standard uncertainties was evaluated to identify those most influencing the outcomes of hazard identification and characterisation and exposure estimations.

The sources of uncertainty were further discussed individually. Additional evidence was reviewed to quantify the possible impact on the reference point used in the current assessment. Semi-formal structured methods of Expert Knowledge Elicitation (semi-formal EKE, Annex B.8 of EFSA Scientific Committee (2018b)) using behavioural aggregation were applied to characterise uncertainty of the Reference Point, a high exposure for PHA and overall uncertainty in the MOE.

The EKE were performed with the quartile method for individual judgements and the probability method for consensus judgements. The approximate probability method was used for overall uncertainty elicitation (EFSA Scientific Committee, 2018b).

The potential impact of the main uncertainties affecting the exposure assessments was explored on one scenario: Exposure to lectins in all consumed food under a scenario of 50% deactivation from processing by the consumer or industrial processes. The scenario was considered as conditional factor and thus uncertainties related to the degree of deactivation were not taken into account.

Uncertainty analysis objectives

Assessments must indicate the identified sources of uncertainty and characterise their overall impact on the conclusions. It is recommended to quantify the overall uncertainty of conclusions using probabilities to avoid the ambiguity inherent to qualitative approaches. However, if legislation or risk managers require conclusions to be presented without probabilities, this is acceptable provided that the associated probabilities are defined elsewhere (EFSA Scientific Committee, 2018b). The present uncertainty analysis was conducted with the objective of addressing the assessment question on the risks for human health related to the presence of PHA in food. An MOE approach, calculated as the ratio of the reference point (BMDL) and a high exposure to the population, was applied for the risk characterisation. The risk is characterised by comparing the MOE against a product of uncertainty factors to ensure a minimal distance. The uncertainties pertaining to each of these two components (hazard identification and characterisation as well as exposure assessment) were identified and quantified separately and combined for the overall uncertainty assessment. The uncertainty analysis, focussed on the hazard identification, acute P99 exposure for all age groups. The uncertainty analysis was conducted following the guidance of the EFSA Scientific Committee (2018a). The combined impact of uncertainties on the principal conclusions in each part of the assessment was quantified using % probabilities. These are reported below as % certainty for the more probable outcome for the conclusion, following EFSA's guidance on communication of uncertainty (EFSA, 2019).

Hazard identification and characterisation

Possible limitations of the hazard assessment were systematically screened to identify those uncertainties, which may have a high impact on the Reference Point. The following uncertainties were considered of higher priority regarding the estimation of the Reference Point for PHA. The magnitude and direction of the impact of these uncertainties on the Reference Point were evaluated by a structured expert elicitation (see Appendix E for full identification of sources of uncertainty):

- Limitations in the design of the direct measurements of the effect in experimental animals. **Moderate impact:** the use of histological images to prove the increase in the weight is linked to damage. Limitations in the method from poor image quality or possibility for quantitative analysis of histological images. Increase in intestine weight is measured directly.
- Uncertainty due to studies carried out only in one sex or certain age groups, duration of studies, sample size and biomarkers. **Low to moderate impact:** sex was not specified. The impact of the sex on the critical effect is low with an unclear direction. A moderate impact of age, due to changes in gut maturation, where older animals may need more time to recover from any effect. Study carried out on 30-day-old animals. Relatively short duration of treatment (10 days, sub-acute) and small number of animals per group (5).
- Limited information on other effects that could be considered as critical. **Moderate impact:** the critical study focusing on small intestine, pancreas and nitrogen balance; data on other organs (e.g. liver, kidney) or other metabolic parameters not provided.
- Uncertainties on the MoA of the substance in animals and uncertainties in the strength, consistency and specificity of the association of the key events and the critical effect in humans. **Moderate impact:** the suggested MOA (i.e. lectins binding to animal gut produce pleiotropic effects).
- Uncertainty on the human relevance of the MOA identified in experimental animals. **Moderate impact:** lectins may affect the gut in humans, but there are no studies in humans with pure lectins, only with food containing lectins (e.g. beans). The MOA may be similar in animals and humans.
- Uncertainty on the biological relevance of the adversity of the selected BMR. **Moderate impact:** unclear whether small intestine weight changes are directly relevant for humans. Sufficient information to justify the selection of the BMR.
- Uncertainty regarding the dose response analysis, e.g. trend occurrence, large data variation, possible covariates, lack of raw data points. **Moderate impact:** response is not normalised to body weight, which makes the BMDL less conservative.

After considering the impact of non-standard sources of uncertainty on the Reference Point for PHA, it was concluded that with a 50% probability the Reference Point is between 15 and 25 mg/kg bw per day, and a 90% probability that it is between 10 and 35 mg/kg bw per day, and a 50% probability that it is above 20 mg/kg bw per day via expert knowledge elicitation on the question: 'If all non-standard sources of uncertainty were resolved, what would be the value of the reference point for the acute hazard assessment?' (see Annex F for details and reasonings).

Dietary exposure assessment

The discussions on uncertainty concluded that the main uncertainties identified during the EKE analysis refer to (see Appendix E for full identification of sources of uncertainty):

- Selection/generation of suitable occurrence values for exposure estimation from the literature. **High impact:** extrapolation made from just one type of *Phaseolus* species (and only one sample) to all *Phaseolus* species. 10-fold higher concentration as compared to other results reported in the literature (Table 11) which is considered an adequate value to estimate acute exposure. Limited occurrence data produced with reliable methods, collected from few countries and from few years. No data on processed commodities; recipes and factors used from the RPC model to derive the amounts of lectins in processed commodities. The results in the study selected (in which ELISA method was used to quantify PHA in one sample of kidney beans from Italy) are consistent with other studies and reviews.
- Uncertainty due to lack of validated methods to measure active lectins, including certified reference materials and proficiency tests. **Moderate impact:** PHA consists of a mixture of 5 isomers (E4, E3L, E2L2, EL3 and L4 for PHA) and the proportions of which may vary depending on the bean varieties. Under these conditions, it is difficult to establish certified reference materials of PHA. In fact, the best references for pure lectins, e.g. PHA (PHA-L4 or PHA-E4), would be crystallised lectin samples.
- Uncertainty in consumption data. **Low to moderate impact:** the duration of the dietary surveys (2–3 days duration) might have an impact on capturing food commodities that are not typically consumed, leading to only few consumers available (e.g. specific types of beans from *Phaseolus* sp.).

The uncertainty assessment considered only the median of the high exposure estimates by consuming days of all European surveys in the EFSA Comprehensive database (instead of all surveys separately) to assess the European exposure and related uncertainties. The high exposure estimates were selected from the dietary surveys in the population group with the highest acute exposure, i.e. 'Other children'.

Acute dietary exposures to PHA were estimated based on consumption data from the EFSA Comprehensive European Food Consumption Database and expressed as mean exposures and high exposures, the latter based on the highest

reliable percentiles. Acute dietary exposure was estimated individually for each food, not for the whole diet, and considering consuming days only. The highest exposed population group was 'Other children' following the consumption of 'Beans and vegetables meal' (99th percentile). The uncertainty analysis was performed for this group of population, and the level of protection for all age groups was considered when characterising the overall uncertainty.

After considering the impact of non-standard sources of uncertainty on a high exposure to PHA via food following the arbitrary scenario with only a 50% lectin deactivation, the experts provided a 50% probability that a high exposure P99 to PHA for the population group 'Other children' is between 7 and 14 mg/kg bw per day, a 90% probability that it is between 4 and 20 mg/kg bw per day, and a 50% probability that it is below 10 mg/kg bw per day using an expert knowledge elicitation on the question: 'Assuming the ideal situation: (i) that a representative diet study for daily food consumption of European other children is given; (ii) that all concentrations of lectins are measured with sufficient quality for quantification of the contamination of the (eaten) products before processing at the consumer. What would be the 99th percentile (i.e. the exposure exceeded by 1 person out of 100) of the daily intake of lectins of European 'Other children', when their food has been processed by the consumer (leading to a 50 % lectin deactivation)?'

Together with the most important uncertainties identified and categorised for the occurrence data by the informal EKE (Appendix E), the CONTAM Panel also noted the uncertainties and limitations related to the use of the EFSA Comprehensive Database. The main uncertainties have been described by EFSA (2011a) and generally relate to the use of different dietary survey methodologies, standard portion sizes, representativeness of samples included in surveys.

The uncertainties affecting the food consumption data were not specific for the PHA exposure assessment. They are 'standard uncertainties' across all Opinions and were considered to have low priority. It is generally accepted that the estimates from the EFSA Comprehensive Database are generally considered to be fit for purpose, provided there are no non-standard uncertainties, as it is the case for PHA.

Overall output of the Uncertainty analysis

Uncertainty in the MOE was characterised for the lectin and the highest exposed age group taking into account the deactivation scenario of 50% and the Reference Point for the acute hazard assessment to lectins via food.

MOE (age group) = Reference Point/P99 Exposure age group.

An MOE less than 1 implies that the Reference Point is exceeded by the highest exposure. The outcome of interest is that the MOE is less than the factor for considering standard sources of uncertainty, which in this case is $10 \times 10 = 100$. The outcome of interest is then:

There is a health concern when 'MOE < 100'. The characterisation of overall uncertainty aims to answer the question: how certain are the experts that there is a health concern?

In this EKE, the experts considered any additional sources of uncertainty on the probability of this outcome.

- Selection of critical studies and endpoints. **High impact:** the RP might be different if uncertainties affecting non-critical studies and endpoints or uncertainties due to gaps in the toxicological evidence were resolved, e.g. by obtaining more or better data, including acute toxicity studies. One or several studies reporting effects at lower doses than the critical effect which were not selected for setting the critical RP due to insufficient degree of purification, study design unclear, combination of routes of application, and limited relevance. There is uncertainty about the reference point due to lack of studies on carcinogenicity, immunotoxicity (including allergy), reproduction and neurotoxicity.
- Lack of human data. **High impact:** lack of appropriate human data which could be used for the risk assessment, e.g. with purified lectins, quantified lectins consumed, endpoints from the GI system.

The impacts of several non-standard sources of uncertainty are expressed by probability distributions for the Reference Point and P99 Exposed age group. These distributions were combined into an uncertainty about the MOE from which one can calculate the probability that the MOE is too small (i.e. less than the factor for standard sources of uncertainty) given all sources of uncertainty considered so far.

Infants under 16 weeks of age are not expected to be exposed to food containing lectins, and therefore the WG agreed that the MOE for the highest exposed age group (other children) is protective for all age groups.

Taking into account the impact of all non-standard sources of uncertainty, the experts were at least 95% certain, i.e. that it is extremely likely that there is a health concern with PHA under the scenario of 50% deactivation using an expert knowledge elicitation on the question: 'If all non-standard sources of uncertainty were resolved, what is your probability that the MOE is below the factor 100?'

The experts agreed that the conclusion holds for all age groups, noting that infants under 16 weeks of age are not expected to be exposed to food containing lectins.

4 | CONCLUSIONS

4.1 | Hazard identification and characterisation

4.1.1 | Toxicokinetics

Rodents

- Based on the few toxicokinetic studies available in rodents, the majority of ingested lectins (up to 90% of PHA and over 75% of Con A) pass unchanged (remain structurally intact and retain their biological activity) through the gastrointestinal (GI) tract.
- Minor amounts of lectin (up to 4% of PHA and 3% of tomato lectin) are absorbed and reach the major organs through the systemic circulation in rodents.
- There is no evidence of degradation in rodents, as approximately 90% of lectins in faeces were functionally active.

Human Observations

- Active PNA and WGA are rapidly detected in the systemic circulation after eating peanuts or wheat germ, suggesting absorption occurs in the upper GI tract, probably in the stomach. Moreover, the detection of lectins in faeces using haemagglutination assays implies that unabsorbed material retains its biological activity.
- Significant IgG and IgM responses to lectins or to proteins ingested at the same time as the lectin may indicate alterations in the gut barrier, eventually leading to an increased translocation of dietary antigens.

4.1.2 | Toxicity in experimental animals

- No acute toxicity studies were identified for PHA or SBA.
- No long-term toxicity studies were identified for PHA or SBA.

Gastrointestinal toxicity:

- Lectins bind to the brush border membrane (BBM) of the small intestine via many and varied carbohydrate binding sites. The effects elicited by the various lectins depend on the specificity of the binding to the carbohydrate sites.
- BBM enzymes such as ALP and sucrase are often decreased by lectins.
- Lectins cause an increase in the weight and often the length of the small intestine. Lectins also cause histological damage to the small intestine, mainly to the villi and crypts. Both of these changes affect the structure and function of the gut.
- The effects on the gut result in decreased body weight gain and reduced food intake, accompanied by altered nitrogen absorption, which may contribute to antinutritive effects.
- Lectins may increase bacterial load in the small intestine and alter microbial composition and diversity, which is associated with alterations in the mucus layer and reduced nutrient absorption.
- The majority of the effects on the gut are reversible upon returning to a normal diet without lectin.

Immunotoxicity:

- Lectins may disturb the gut mucosal immune system and thereby abrogating oral tolerance against to antigens.

Developmental toxicity

- In rats (PND14), PHA affected body weight gain, liver and pancreas weights, small intestine morphology and maturation of the GI tract and the immune system. For SBA and other lectins, no studies were identified.

Genotoxicity

- The evidence for genotoxicity is weak as non-standard tests and study designs were used; however, since lectins are proteins, no genotoxic properties are expected via a direct DNA interaction.

Carcinogenicity

- There is no evidence of carcinogenicity from the one study retrieved which primarily investigated intestinal carcinogenesis.

4.1.3 | Observations in humans

- Consumption of partially processed red and white kidney beans can cause GI symptoms, namely, nausea, vomiting, abdominal pain and diarrhoea.
- Lectin identification was done occasionally and only qualitatively.
- Consumption of red and white kidney beans is associated with acute allergic reactions which might be attributed to the lectin activity.
- The available evidence on lectin-related allergenicity consists of studies assessing skin prick test reactions and IgE antibody assays (either lectin- or plant-specific).
- The available evidence on lectin-related allergenicity has certain limitations, including variable documentation on allergy history and infrequent distinction between specific and non-specific lectin binding.
- The CONTAM Panel considered that lectins from edible plants might induce sensitisation and/or that allergic reactions may occur in subjects allergic to other lectins present in edible plants (due to potential cross-reactivity).

4.1.4 | Mode of action

- Lectins produce their effects on the gut through specific binding to the carbohydrate moieties on the epithelial cells and altering the structure and function of the microvilli, villi and crypt cells. It is possible that by binding to receptors in the gut, and perhaps mimicking binding of natural ligands, lectins modulate intracellular/intercellular signalling pathways that affect viability, proliferation and differentiation of epithelial cells.
- Lectins bind to the carbohydrate moieties present on the surface of many but not all microorganisms and this likely influences the proportion of ingested lectin available to bind to the gut epithelial cells. Conversely, the changes induced by ingested lectins on the gut epithelium may influence the gut microbiota composition.
- As the plant lectins hold the capacity to adversely alter the gut barrier and the microbiota, plant lectins have been implicated in the possible induction of autoimmune disorders and allergic reactions.
- Lectin may affect or disrupt the interplay between the gut barrier and the microbiota by disturbing mucus production or epithelial cell differentiation.
- Lectins may induce adverse immunotoxicological reactions, with the type of immune response depending on the animal species, the underlying immune polarisation (i.e. whether the immune system is skewed towards a Th1, Th2 or other type of immune response), and the amount of lectin that reaches the circulation in the animals. The latter may largely depend on the properties of the specific lectin and the amount ingested.
- Lectins may cause the release of cholecystokinin (CCK) from enteroendocrine cells in the intestinal tract into the bloodstream, which stimulates the exocrine pancreas to produce digestive juice and to grow

4.2 | Critical effects and derivation of BMD

- The Panel considered only PHA for the derivation of critical effects based on the availability both of occurrence data (albeit limited) and of relevant endpoints.
- The available epidemiological data on GI and metabolic effects are limited both in terms of volume and validity and cannot be used further for risk characterisation.
- Small intestine weight, large intestine weight and pancreas weight in rodents were selected as the critical effects for acute PHA toxicity.
- The Panel used a BMR of 10% based on increase in organ weights (pancreas and small or large intestine weight).
- PHA is considered the most toxic among the lectins of edible plants because of the specificity of PHA to bind to complex carbohydrates and of its high affinity to human enterocytes.

4.3 | Occurrence and exposure for the European population

4.3.1 | Analytical methods, food processing and occurrence in food

- To isolate and purify lectins, the first step is the preparation of a buffered saline aqueous extract from the plant material.
- As a second step, affinity chromatography is considered highly specific for purification of lectins due to their specificity for carbohydrates.
- Quantification of lectins is usually performed using the highly sensitive and specific sandwich-type ELISA methods, enabling the detection of active lectins in biological matrices including serum.
- The increasing use of quantitative mass spectrometry-based techniques allows a precise identification and quantification of lectins in food or food products. It is noted that LC-MS quantifies both active and denatured lectins.

- To determine lectin activity, the haemagglutination assay is one of the most commonly used tests for confirming lectin activity, despite its well-known limitations.
- It is difficult to compare results obtained by different techniques for estimating the lectin concentration or evaluating the lectin activity.
- Soaking (6–12 h) combined with heat treatment (≥ 100°C, > 30 min) are highly effective conditions for deactivating lectins from plant sources (e.g. PHA present in kidney beans). However, since the effect of the soaking time can vary depending on the kidney bean varieties, it is advisable to continue soaking until the beans have softened before cooking.
- No occurrence data on lectins were available in the EFSA Data Warehouse. As an alternative, occurrence data of lectins in food were identified in the literature to perform dietary exposure to PHA.

4.3.2 | Exposure assessment

- Assuming an arbitrary dietary exposure scenario where 50% of PHA activity remains in different processed commodities (beans from *Phaseolus* sp. and bean-containing products), the mean acute dietary exposure to PHA could be as high as 35.0 mg/kg bw per day following the consumption of Lima beans (dry). In high consumers, the acute dietary exposure to PHA could reach 75.8 mg/kg bw per day via the consumption of 'Beans and vegetable meals' (99th percentile exposure).

4.4 | Considerations for the risk characterisation

- Among all lectins in edible plants evaluated, the amount of occurrence and toxicological data allowed risk characterisation only for PHA.
- Due to limited hazard data (occurrence and toxicological) for PHA, the derivation of the HBGV was not considered appropriate; instead, the MOE approach was followed to assess possible health effects in humans.
- The Panel considered an MOE of 100 as appropriate to address health concerns (covering kinetic and dynamic differences between animal species and humans and within human population).
- An arbitrary dietary exposure scenario was considered, where 50% of the lectins are inactivated as a result of insufficient cooking of food containing lectins (e.g. beans)
- Comparison of the reference point and exposure estimates for PHA resulted in MOEs of about 0.3 at the highest reliable percentile exposure.
- The Panel noted that, under the arbitrary dietary exposure scenario where only 50% of the lectins are inactivated, this MOE would lead to adverse health effects in humans.
- The Panel also noted that exposure to completely deactivated lectins in food prepared following adequate food processing practices would not raise health concerns.

5 | RECOMMENDATIONS

Occurrence

- To develop and validate analytical methods that allow the simultaneous quantification of active and non-active lectins in different food commodities.
- To make available certified reference materials for the analysis of different lectins.
- To collect occurrence data for different lectins (primarily for PHA, SBA) present in raw primary commodities and processed food (where the processing conditions are clearly described). The occurrence data should be produced using appropriate and comparable analytical methods that distinguish between active and non-active lectins.
- To conduct dedicated studies to better understand the effect of food processing on the deactivation of lectins, analysing the lectins with validated analytical methods that allow the quantification of active and non-active lectins simultaneously.

Hazard

- Appropriate toxicokinetic studies in rodents and in humans (primarily with PHA), including all ADME parameters and using validated analytical methods to measure active lectins.
- Long-term low-dose toxicity studies in rodents (primarily with purified PHA, in the active form), focusing on immunotoxicity and gastrointestinal endpoints, including effects on the microbiome.
- More information is needed on the mode of action of lectins (primarily PHA) to inform the association of the specific structures and carbohydrate-binding sites with the effects of lectins in the GI system (including the microbiome), using new and sensitive techniques applied ex vivo and in vivo.
- Human studies are needed with adequate characterisation of the type and amount of lectins of high purity ingested, and appropriate consideration of confounding factors, e.g. trypsin inhibitors, adequate characterisation of the relevant

endpoints pertaining to both acute and chronic toxicity, in order to directly associate lectin effects with human adverse effects in the immune and gastrointestinal systems, including the microbiome.

ABBREVIATIONS

LECTINS		
Abbreviation	**Complete name**	**Plant species**
AAA	*Allium ascalonicum* agglutinin	shallot (*Allium ascalonicum*)
ACA	*Allium cepa* agglutinin	onion (*Allium cepa*)
APA	*Allium porrum* agglutinin	leek (*Allium porrum*)
APA	*Abrus precatorius* agglutinin	jequirity bean (*Abrus precatorius*)
ASA	*Allium sativum* agglutinin	garlic (*Allium sativum*)
BanLec	Banana lectin	banana (*Musa acuminata*)
CAL	*Cicer arietinum* lectin	chickpea (*Cicer arietinum*)
Con A	Concanavalin A	jack bean (*Canavalia ensiformis*)
DBA	*Dolichos biflorus* agglutinin	horse gram (*Macrotyloma uniflorum;* syn. *Dolichos biflorus*)
DLL-II	D-galactose specific lectin	field bean (*Dolichos lablab*)
DSA	*Datura stramonium* agglutinin	thorn apple (*Datura stramonium*)
$F_{II}SP$	Purified lectins from seeds of Jack fruit	jack fruit (*Artocarpus integrifolia*)
GNA	*Galanthus nivalis* agglutinin	snowdrop (*Galanthus nivalis*)
JRL	Jacalin-related lectins	seed lectins of *Moraceae* fruits.
LBA	Lima bean agglutinin	lima bean (*Phaseolus lunatus*)
LCA	*Lens culinaris* agglutinin	lentil (*Vicia lens syn. Lens culinaris*)
LSL	*Lathyrus sativus* lectin	grass pea (*Lathyrus sativus lectin*)
MPA	*Maclura pomifera* agglutinin	osage orange (*Maclura pomifera*)
PHA	Phytohaemagglutinin	kidney bean (*Phaseolus vulgaris*)
PHA-E	Phytohaemagglutinin-E	kidney bean (*Phaseolus vulgaris*)
PHA-L	Phytohaemagglutinin-L	kidney bean (*Phaseolus vulgaris*)
PNA/PNL	Peanut agglutinin	peanut (*Arachis hypogaea*)
PSA	*Pisum sativum* agglutinin	pea (*Pisum sativum)*
PWM	Pokeweed mitogen	pokeweed (*Phytolacca americana*)
RCA	*Ricinus communis* agglutinin	castor bean (*Ricinus communis*)
SBA/SBL	Soybean agglutinin/lectin	soybean (*Glycine max*)
SNA	*Sambucus nigra* agglutinin	elderberry (*Sambucus nigra*)
STA	*Solanum tuberosum* agglutinin	potato (*Solanum tuberosum*)
UDA	*Urtica dioica* agglutinin	stinging nettle (*Urtica dioica*)
UEA	*Ulex europaeus* agglutinin	gorse (*Ulex europaeus*)
VFL/Favin	*Vicia faba* lectin/Fava bean lectin	broad bean (*Vicia faba*)
WGA/WGL	Wheat germ agglutinin/lectin	wheat (*Triticum aestivum*)

AGES	Austrian Agency for Food and Health safety
ALP	alkaline phosphatase
ARfD	acute reference dose
AST	aspartate aminotransferase
AUC	area under curve
BAT	basophil activation test
BBM	brush border membrane
BfR	German Federal Institute for Risk Assessment
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDU	benchmark dose upper confidence limit
BMR	benchmark response
bw	body weight
CBS	carbohydrate binding site

CCK	cholecystokinin
CD	celiac disease
CNS	central nervous system
CRP	C-reactive protein
d	day
DNA	deoxyribonucleic acid
dw	dry weight
ELISA:	enzyme-linked immunosorbent assay
EURL	European Reference Laboratory
f	female
fw	fresh weight
GalNAc	*n*-acetyl galactosamine
GI	gastrointestinal
GLP2	glucagon-like peptide-2
HA	haemagglutinating activity
HAU	haemagglutinating activity units
HBGV	health based guidance value
HTC	hard-to-cook
HU	haemagglutination units
IgE	immunoglobulin e
IgG	immunoglobulin g
IgM	immunoglobulin m
IL	interleukin
ip	intraperitoneal
iv	intravenous
kDa	kilodalton
kGy	kilogray
LC–MS	liquid chromatography mass spectrometry
LOAEL:	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantification
m	male
Man	mannose
MHz	megahertz
MPa	megapascal
n	sample size
n.d.	not detected
NFAT	nuclear factor of activated t cells
NOAEL	no observed adverse effect level
NR	not reported
NTT	not trypsin-treated
OAS	oral allergy syndrome
P95	95th percentile
P99	99th percentile
PCNA	proliferating cell nuclear antigen
PND	postnatal day
prox	proximal
qPCR	quantitative polymerase chain reaction
RBC	red blood cells
rel	relative
RIP	ribosome-inactivating protein
RNA	ribonucleic acid
RT	room temperature
RT-qPCR:	real-time quantitative polymerase chain reaction
sc	subcutaneous
SD	Sprague Dawley
SDS-PAGE:	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE	standard error
SI	small intestine
SIA	sialic acid
sp.	species
SPT	skin prick test

TI	trypsin inhibitor
TT	trypsin-treated
UHP	ultra-high pressure
WBC	white blood cells
WHO/IUIS	World Health Organization and International Union of Immunological Societies

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REFERENCES

Adamcová, A., Laursen, K. H., & Ballin, N. Z. (2021). Lectin activity in commonly consumed plant-based foods: Calling for method harmonization and risk assessment. *Food*, *10*(11), 2765. https://doi.org/10.3390/foods10112796

Adeparusi, E. O. (2001). Effect of processing on the nutrients and anti-nutrients of lima bean (*Phaseolus lunatus* L.) flour. *Food/Nahrung*, *45*(2), 94–96. https://doi.org/10.1002/1521-3803(20010401)45:2<94::AID-FOOD94>3.0.CO;2-E

Agrawal, B. B. L., & Goldstein, I. J. (1967). Protein-carbohydrate interaction. VI. Isolation of concanavalin A by specific adsorption on cross-linked dextran gel. *Biochimica et Biophysica Acta*, *147*, 262–271. https://doi.org/10.1016/0005-2795(67)90404-7

Aguilera, Y., Díaz, M. F., Jiménez, T., Benítez, V., Herrera, T., Cuadrado, C., Martín-Pedrosa, M., & Martín-Cabrejas, M. A. (2013). Changes in nonnutritional factors and antioxidant activity during germination of nonconventional legumes. *Journal of Agricultural and Food Chemistry*, *61*(34), 8120–8125. https://doi.org/10.1021/jf4022652

Ahmed, E. M. (1986). Lectin quantitation in peanut and soybean seeds. *Peanut Science*, *13*(1), 4–7. https://doi.org/10.3146/i0095-3679-13-1-2

Akdis, C. A. (2021). Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions? *Nature Reviews Immunology*, *21*, 739–751. https://doi.org/10.1038/s41577-021-00538-7

Alatorre-Cruz, J. M., Pita-López, W., López-Reyes, R. G., Ferriz-Martínez, R. A., Cervantes-Jiménez, R., De Jesús Guerrero Carrillo, M., PJA, V., López-Herrera, G., Rodríguez-Méndez, A. J., Zamora-Arroyo, A., Gutiérrez-Sánchez, H., de Souza, T. R., Blanco-Labra, A., & García-Gasca, T. (2018). Effects of intragastrically-administered Tepary bean lectins on digestive and immune organs: Preclinical evaluation. *Toxicology Reports*, *5*, 56–64. https://doi.org/10.1016/j.toxrep.2017.12.008

Al-Eissa, Y. A., Bahakim, H., Mahdi, A. W., Saddique, A. A., & Amoh, K. N. (1989). Accidental Castor oil seed poisoning in Saudi children. *Annals of Saudi Medicine*, *9*(6), 609–611. https://doi.org/10.5144/0256-4947.1989.609

Alhamdani, M., Brown, B., & Narula, P. (2015). Abrin poisoning in an 18-month-old child. *The American Journal of Case Reports*, *16*, 146–148. https://doi.org/10.12659/AJCR.892917

Allen, A. K., & Neuberger, A. (1975). A simple method for the preparation of an affinity absorbent for soybean agglutinin using galactosamine and CH-Sepharose. *FEBS Letters*, *50*(3), 362–364. https://doi.org/10.1016/0014-5793(75)80528-x

Allen, H. J., & Johnson, E. A. (1977). A simple procedure for the isolation of L-fucose-binding lectins from *Ulex europaeus* and *Lotus tetragonolobus*. *Carbohydrate Research*, *58*(2), 253–265. https://doi.org/10.1016/s0008-6215(00)84352-9

Almeida, N. G., Delabarca, A. M. C., & Valencia, M. E. (1991). Effect of different heat treatments on the antinutritional activity of *Phaseolus vulgaris* (variety Ojo de Cabra) lectin. *Journal of Agricultural and Food Chemistry*, *39*(9), 1627–1630. https://doi.org/10.1021/jf00009a018

Alonso, R., Aguirre, A., & Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chemistry*, *68*(2), 159–165. https://doi.org/10.1016/S0308-8146(99)00169-7

Alonso, R., Grant, G., Dewey, P., & Marzo, F. (2000). Nutritional assessment in vitro and in vivo of raw and extruded peas (*Pisum sativum* L.). *Journal of Agricultural and Food Chemistry*, *48*(6), 2286–2290. https://doi.org/10.1021/jf000095o

Alonso, R., Orúe, E., & Marzo, F. (1998). Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. *Food Chemistry*, *63*(4), 505–512. https://doi.org/10.1016/S0308-8146(98)00037-5

Alonso, R., Rubio, L. A., Muzquiz, M., & Marzo, F. (2001). The effect of extrusion cooking on mineral bioavailability in pea and kidney bean seed meals. *Animal Feed Science and Technology*, *94*(1–2), 1–13. https://doi.org/10.1016/S0377-8401(01)00302-9

Al-Tamimi, F. A., & Hegazi, A. E. (2008). A case of castor bean poisoning. *Sultan Qaboos University Medical Journal*, *8*(1), 83–87. https://doi.org/10.18295/2075-0528.2699

Alvarez, J. R., & Torres-Pinedo, R. (1982). Interactions of soybean lectin, soyasaponins, and glycinin with rabbit jejunal mucosa in vitro. *Pediatric Research*, *16*(9), 728–731. https://doi.org/10.1203/00006450-198209000-00005

Anaemene, D., & Fadupin, G. T. (2022). Anti-nutrient reduction and nutrient retention capacity of fermentation, germination and combined germination-fermentation in legume processing. *Applied Food Research*, *2*(1), 100059. https://doi.org/10.1016/j.afres.2022.100059
Anandan, S., Kumar, G. K. A., Ghosh, J., & Ramachandra, K. S. (2005). Effect of different physical and chemical treatments on detoxification of ricin in castor cake. *Animal Feed Science and Technology*, *120*(1–2), 159–168. https://doi.org/10.1016/j.anifeedsci.2004.10.002
Andersen, N. H., Cao, B., Rodríguez-Romero, A., & Arreguin, B. (1993). Hevein: NMR assignment and assessment of solution-state folding for the agglutinin-toxin motif. *Biochemistry*, *32*(6), 1407–1422. https://doi.org/10.1021/bi00057a004
Añibarro, B., Fontela, J. L., & De La Hoz, F. (1997). Occupational asthma induced by garlic dust. *Journal of Allergy and Clinical Immunology*, *100*(6 Pt 1), 734–738. https://doi.org/10.1016/s0091-6749(97)70266-5
Anta, L., Luisa Marina, M., & García, M. C. (2010). Simultaneous and rapid determination of the anticarcinogenic proteins Bowman-Birk inhibitor and lectin in soybean crops by perfusion RP-HPLC. *Journal of Chromatography A*, *1217*(45), 7138–7143. https://doi.org/10.1016/j.chroma.2010.09.026
Antunes, P. L., & Sgarbieri, V. C. (1980). Effect of heat treatment on the toxicity and nutritive value of dry bean (*Phaseolus vulgaris* var. Rosinha G2) proteins. *Journal of Agricultural and Food Chemistry*, *28*(5), 935–938. https://doi.org/10.1021/jf60231a004
Armour, J. C., Perera, R. L. C., Buchan, W. C., & Grant, G. (1998). Protease inhibitors and lectins in soya beans and effects of aqueous heat-treatment. *Journal of the Science of Food and Agriculture*, *78*(2), 225–231. https://doi.org/10.1002/(SICI)1097-0010(199810)78:2<225::AID-JSFA109>3.0.CO;2-1
Arribas, C., Cabellos, B., Cuadrado, C., Guillamón, E., & Pedrosa, M. M. (2019a). Bioactive compounds, antioxidant activity, and sensory analysis of rice-based extruded snack-like fortified with bean and carob fruit flours. *Food*, *8*(9), 381. https://doi.org/10.3390/foods8090381
Arribas, C., Cabellos, B., Cuadrado, C., Guillamón, E., & Pedrosa, M. M. (2019b). The effect of extrusion on the bioactive compounds and antioxidant capacity of novel gluten-free expanded products based on carob fruit, pea and rice blends. *Innovative Food Science & Emerging Technologies*, *52*, 100–107. https://doi.org/10.1016/j.ifset.2018.12.003
Arribas, C., Cabellos, B., Cuadrado, C., Guillamón, E., & Pedrosa, M. M. (2020). Cooking effect on the bioactive compounds, texture, and color properties of cold-extruded Rice/bean-based pasta supplemented with whole carob fruit. *Food*, *9*(4), 415. https://doi.org/10.3390/foods9040415
Asero, R., Mistrello, G., Roncarolo, D., Antoniotti, P. L., & Falagiani, P. (1998). A case of garlic allergy. *Journal of Allergy and Clinical Immunology, 101*(3), 427–428. https://doi.org/10.1016/s0091-6749(98)70261-1
Assiri, A. S. (2012). Ricin poisoning causing death after ingestion of herbal medicine. *Annals of Saudi Medicine*, *32*(3), 315–317. https://doi.org/10.5144/0256-4947.2012.315
Ayyagari, R., Raghunath, M., & Rao, B. S. (1993). Early effects and the possible mechanism of the effect of concanavalin A (Con A) and *Phaseolus vulgaris* lectin (PHA-P) on intestinal absorption of calcium and sucrose. *Plant Foods for Human Nutrition*, *43*(1), 63–70. https://doi.org/10.1007/bf01088097
Ayyagari, R., Rao, B. S. N., & Roy, D. N. (1989). Lectins, trypsin inhibitors, BOAA and tannins in legumes and cereals and the effects of processing. *Food Chemistry*, *34*(3), 229–238. https://doi.org/10.1016/0308-8146(89)90143-X
Bach, M. K., & Brashler, J. R. (1975). Inhibition of IgE and compound 48/80-induced histamine release by lectins. *Immunology*, *29*(2), 371–386.
Bachhawat, K., Kapoor, M., Dam, T. K., & Surolia, A. (2001). The reversible two-state unfolding of monocot mannose-binding lectin from garlic bulbs reveals the dominant role of the dimeric interface in its stabilization. *Biochemistry*, *40*(24), 7291–7300. https://doi.org/10.1021/bi0027783
Baenziger, J., & Kornfeld, S. (1974). Structure of the carbohydrate units of IgE immunoglobulin. II. Sequence of the sialic acid-containing glycopeptides. *Journal of Biological Chemistry*, *249*(6), 1897–1903. https://doi.org/10.1016/S0021-9258(19)42870-6
Bagaria, A., Surendranath, K., Ramagopal, A. U. A., Ramakumar, S., & Karande, A. A. (2006). Structure-function analysis and insights into the reduced toxicity of *Abrus precatorius* agglutinin I in relation to abrin. *Journal of Biological Chemistry*, *281*(45), 34465–34474. https://doi.org/10.1074/jbc.M601777200
Balmer, M. L., Slack, E., de Gottardi, A., Lawson, M. A., Hapfelmeier, S., Miele, L., Grieco, A., Van Vlierberghe, H., Fahrner, R., Patuto, N., Bernsmeier, C., Ronchi, F., Wyss, M., Stroka, D., Dickgreber, N., Heim, M. H., McCoy, K. D., & Macpherson, A. J. (2014). The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota. *Science Translational Medicine*, *6*(237), 237ra66. https://doi.org/10.1126/scitranslmed.3008618
Banerjee, B., Wang, X., Kelly, K. J., Fink, J. N., Sussman, G. L., & Kurup, V. P. (1997). IgE from latex-allergic patients binds to cloned and expressed B cell epitopes of prohevein. *The Journal of Immunology*, *159*(11), 5724–5732. https://doi.org/10.4049/jimmunol.159.11.5724
Banerjee, R., Das, K., Ravishankar, R., Suguna, K., Surolia, A., & Vijayan, M. (1996). Conformation, protein-carbohydrate interactions of a novel subunit association in the refined structure of peanut lectin-lactose complex. *Journal of Molecular Biology*, *259*(2), 281–296. https://doi.org/10.1006/jmbi.1996.0319
Banerjee, S., Naresh, M., & Swamy, M. J. (2024). Effect of temperature and pH on the structure and stability of tumor-specific lectin jacalin and insights into the location of its tryptophan residues: CD, DSC and fluorescence studies. *International Journal of Biological Macromolecules*, *260*(Part 2), 129451. https://doi.org/10.1016/j.ijbiomac.2024.129451
Banwell, J. G., Abramowsky, C. R., Weber, F., Howard, R., & Boldt, D. H. (1984). Phytohemagglutinin-induced diarrheal disease. *Digestive Diseases and Sciences*, *29*(10), 921–929. https://doi.org/10.1007/BF01312481
Banwell, J. G., Boldt, D. H., Meyers, J., & Weber, F. L., Jr. (1983). Phytohemagglutinin derived from red kidney bean (*Phaseolus vulgaris*): A cause for intestinal malabsorption associated with bacterial overgrowth in the rat. *Gastroenterology*, *84*(3), 506–515. https://doi.org/10.1016/0016-5085(83)90074-4
Banwell, J. G., Howard, R., Cooper, D., & Costerton, J. W. (1985). Intestinal microbial flora after feeding phytohemagglutinin lectins (*Phaseolus vulgaris*) to rats. *Applied and Environmental Microbiology*, *50*(1), 68–80. https://doi.org/10.1128/aem.50.1.68-80.1985
Banwell, J. G., Howard, R., Kabir, I., Adrian, T. E., Diamond, R. H., & Abramowsky, C. (1993). Small intestinal growth caused by feeding red kidney bean phytohemagglutinin lectin to rats. *Gastroenterology*, *104*(6), 1669–1677. https://doi.org/10.1016/0016-5085(93)90644-r
Barbosa-Lorenzi, V. C., Cecilio, N. T., de Almeida Buranello, P. A., Pranchevicius, M. C., Goldman, M. H., Pereira-da-Silva, G., Roque-Barreira, M. C., Jamur, M. C., & Oliver, C. (2016). Recombinant ArtinM activates mast cells. *BMC Immunology*, *17*(1), 22. https://doi.org/10.1186/s12865-016-0161-0
Bardocz, S., Grant, G., Ewen, S. W. B., Duguid, T. J., Brown, D. S., Englyst, K., & Pusztai, A. (1995). Reversible effect of phytohemagglutinin on the growth and metabolism of rat gastrointestinal tract. *Gut*, *37*(3), 353–360. https://doi.org/10.1136/gut.37.3.353
Bardocz, S., Grant, G., & Pusztai, A. (1996). The effect of phytohaemagglutinin at different dietary concentrations on the growth, body composition and plasma insulin of the rat. *British Journal of Nutrition*, *76*(4), 613–626. https://doi.org/10.1079/bjn19960067
Barkholt, V., Jergensen, P. B., Sørensen, D., Bahrenscheer, J., Haikara, A., Lemola, E., Laitila, A., & Frøkiær, H. (1998). Protein modification by fermentation: Effect of fermentation on the potential allergenicity of pea. *Allergy*, *53*(46 Suppl), 106–108. https://doi.org/10.1111/j.1398-9995.1998.tb04976.x
Barnes, D. J., Baldwin, B. S., & Braasch, D. A. (2009). Degradation of ricin in castor seed meal by temperature and chemical treatment. *Industrial Crops and Products*, *29*(2–3), 509–515. https://doi.org/10.1016/j.indcrop.2008.09.006
Baroja, M. L., Ceuppens, J. L., Van Damme, J., & Billiau, A. (1988). Cooperation between an anti-T cell (anti-CD28) monoclonal antibody and monocyte-produced IL-6 in the induction of T cell responsiveness to IL-2. *The Journal of Immunology*, *141*(5), 1502–1507. https://doi.org/10.4049/jimmunol.141.5.1502
Barre, A., Benoist, H., & Rougé, P. (2023). An overview of fruit allergens: Structural, functional, phylogenetical, and clinical aspects. *Allergie*, *3*(3), 134–176. https://doi.org/10.3390/allergies3030010
Barre, A., Bourne, Y., Van Damme, E. J., Peumans, W. J., & Rougé, P. (2001). Mannose-binding plant lectins: Different structural scaffolds for a common sugar-recognition process. *Biochimie*, *83*(7), 645–651. https://doi.org/10.1016/s0300-9084(01)01315-3

Barre, A., Bourne, Y., Van Damme, E. J. M., & Rougé, P. (2019). Overview of the structure-function relationships of mannose-specific lectins from plants, algae and fungi. *International Journal of Molecular Sciences*, *20*(2), 254. https://doi.org/10.3390/ijms20020254

Barre, A., Peumans, W. J., Rossignol, M., Borderies, G., Culerrier, R., Van Damme, E. J. M., & Rougé, P. (2004). Artocarpin is a polyspecific jacalin-related lectin with a monosaccharide preference for mannose. *Biochimie*, *86*(9–10), 685–691. https://doi.org/10.1016/j.biochi.2004.09.001

Barre, A., Van Damme, E. J. M., Benoist, H., & Rougé, P. (2020). Are lectins relevant allergens in plant food allergy? *Food*, *9*(12), 1724. https://doi.org/10.3390/foods9121724

Benamor, M., Gharbi, E., Bouzid, S., Chakroun-Walha, O., & Rekik, N. (2020). Ricin poisoning after oral ingestion of castor beans: A case report and literature review. *African Journal of Emergency Medicine: Revue Africaine de la Medecine D'urgence*, *10*(4), 274–276. https://doi.org/10.1016/j.afjem.2020.06.002

BfR (German Federal Institute for Risk Assessment). (2024). Lektine in pflanzenbasierten Lebensmitteln: Gibt es ein gesundheitliches Risiko? BfR-Stellungnahme, 03/2024. https://doi.org/10.17590/20240121-185904-0

Bispham, W. N. (1903). Report of cases of poisoning by fruit of *Ricinus communis*. *The American Journal of the Medical Sciences*, *126*(2), 319.

Biswas, S., & Kayastha, A. M. (2004). Unfolding and refolding of leucoagglutinin (PHA-L), an oligomeric lectin from kidney beans (*Phaseolus vulgaris*). *Biochimica et Biophysica Acta*, *1674*, 40–49. https://doi.org/10.1016/j.bbagen.2004.04.017

Blanco, C., Carrillo, T., Castillo, R., Quiralte, J., & Cuevas, M. (1994). Latex allergy: Clinical features and cross-reactivity with fruits. *Annals of Allergy*, *73*(4), 309–314.

Blanco, C., Díaz-Perales, A., Collada, C., Sánchez-Monge, R., Aragoncillo, C., Castillo, R., Ortega, N., Alvarez, M., Carrillo, T., & Salcedo, G. (1999). Class I chitinases as potential panallergens involved in the latex-fruit syndrome. *Journal of Allergy and Clinical Immunology*, *103*(3 Pt. 1), 507–513. https://doi.org/10.1016/s0091-6749(99)70478-1

Boisseau, C., Moisand, A., Père, D., & Rougé, P. (1985). Immunocytochemical localization of the *Lathyrus ochrus* (L.) DC seed lectin in seeds and seedlings. *Plant Science*, *42*(1), 25–34. https://doi.org/10.1016/0168-9452(85)90024-X

Bojar, D., Meche, L., Meng, G., Eng, W., Smith, D. F., Cummings, R. D., & Mahal, L. K. (2022). A useful guide to lectin binding: Machine-learning directed annotation of 57 unique lectin specificities. *ACS Chemical Biology Journal*, *17*(11), 2993–3012. https://doi.org/10.1021/acschembio.1c00689

Boldt, D. H., & Banwell, J. G. (1985). Binding of isolectins from red kidney bean (*Phaseolus vulgaris*) to purified rat brush-border membranes. *Biochimica et Biophysica Acta*, *843*(3), 230–237. https://doi.org/10.1016/0304-4165(85)90143-6

Bompard-Gilles, C., Rousseau, P., Rougé, P., & Payan, F. (1996). Substrate mimicry in the active center of a mammalian a-amylase: Structural analysis of an enzyme-inhibitor complex. *Structure*, *4*(12), 1441–1452. https://doi.org/10.1016/S0969-2126(96)00151-7

Boniglia, C., Carratù, B., Di Stefano, S., Giammarioli, S., Mosca, M., & Sanzini, E. (2008). Lectins, trypsin and α-amylase inhibitors in dietary supplements containing *Phaseolus vulgaris*. *European Food Research and Technology*, *227*(3), 689–693. https://doi.org/10.1007/s00217-007-0773-y

Boniglia, C., Fedele, E., & Sanzini, E. (2003). Measurement by ELISA of active lectin in dietary supplements containing kidney bean protein. *Journal of Food Science*, *68*(4), 1283–1286. https://doi.org/10.1111/j.1365-2621.2003.tb09640.x

Bonorden, W. R., & Swanson, B. G. (1992). Thermal stability of black turtle soup bean (*Phaseolus vulgaris*) lectins. *Journal of the Science of Food and Agriculture*, *59*(2), 245–250. https://doi.org/10.1002/jsfa.2740590216

Börjeson, J., Reisfield, R., Chessin, L. N., Welsh, P. D., & Douglas, S. D. (1966). Studies on human peripheral lymphocytes in vitro. I. Biological and physiochemical properties of the pokeweed mitogen. *Journal of Experimental Medicine*, *124*(5), 859–872. https://doi.org/10.1084/jem.124.5.859

Bouchard, P., Moroux, Y., Tixier, R., Privat, J. P., & Monsigny, M. (1976). An improved method for purification of wheat germ agglutinin (lectin) by affinity chromatography. *Biochimie*, *58*(10), 1247–1253. https://doi.org/10.1016/s0300-9084(76)80124-1

Bourne, Y., Houlès Astoul, C., Zamboni, V., Peumans, W. J., Menu-Bouaouiche, L., Van Damme, E. J. M., Barre, A., & Rougé, P. (2002). Structural basis for the unusual carbohydrate-binding specificity of jacalin towards galactose and mannose. *Biochemical Journal*, *364*(Pt. 1), 173–180. https://doi.org/10.1042/bj3640173

Bourne, Y., Nesa, M. P., Rougé, P., Mazurier, J., Legrand, D., Spik, G., Montreuil, J., & Cambillau, C. (1992). Crystallization and preliminary X-ray diffraction study of *Lathyrus ochrus* isolectin II complexed to the human lactotransferrin N2 fragment. *Journal of Molecular Biology*, *227*(3), 938–941. https://doi.org/10.1016/0022-2836(92)90233-a

Bourne, Y., Roussel, A., Frey, M., Rougé, P., Fontecilla-Camps, J. C., & Cambillau, C. (1990). Three-dimensional structures of complexes of *Lathyrus ochrus* isolectin I with glucose and mannose: Fine specificity of the monosaccharide-binding site. *Proteins*, *8*(4), 365–376. https://doi.org/10.1002/prot.340080410

Brady, P. G., Vannier, A. M., & Banwell, J. G. (1978). Identification of the dietary lectin, wheat germ agglutinin, in human intestinal contents. *Gastroenterology*, *75*(2), 236–239. https://doi.org/10.1016/0016-5085(78)90409-2

Breeze, M. L., Leyva-Guerrero, E., Yeaman, G. R., Dudin, Y., Akel, R., Brune, P., Claussen, F., Dharmasri, C., Golbach, J., Guo, R., Maxwell, C., Privalle, L., Rogers, H., Liu, K., Shan, G. M., Yarnall, M., Thiede, D., & Gillikin, N. (2015). Validation of a method for quantitation of soybean lectin in commercial varieties. *Journal of the American Oil Chemists Society*, *92*(8), 1085–1092. https://doi.org/10.1007/s11746-015-2679-3

Brinda, K. V., Surolia, A., & Wishveshwara, S. (2005). Insights into the quaternary association of proteins through structure graphs: A case study of lectins. *Biochemical Journal*, *391*(Pt. 1), 1–15. https://doi.org/10.1042/BJ20050434

Brown, J. W. S., Osborn, T. C., Bliss, F. A., & Hall, T. C. (1982). Bean lectins I: Relationships between agglutinating activity and electrophoretic variation in the lectin-containing G2/albumin seed proteins of French bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics*, *62*(3), 263–271. https://doi.org/10.1007/BF00276249

Brücher, O. (1968). Absence of phytohemagglutinin in wild and cultivated beans from South America. *American Society for Horticultural Science, Proceedings of the Tropical Region*, *12*, 68–85.

Bublin, M., Mari, A., Ebner, C., Knulst, A., Scheiner, O., Hoffmann-Sommergruber, K., Breiteneder, H., & Radauer, C. (2004). IgE sensitization profiles toward green and gold kiwifruits differ among patients allergic to kiwifruit from 3 European countries. *Journal of Allergy and Clinical Immunology*, *114*(5), 1169–1175. https://doi.org/10.1016/j.jaci.2004.07.016

Burks, A. W., Cockrell, G., Connaughton, C., Guin, J., Allen, W., & Helm, R. M. (1994). Identification of peanut agglutinin and soybean trypsin inhibitor as minor legume allergens. *International Archives of Allergy and Immunology*, *105*(2), 143–149. https://doi.org/10.1159/000236816

Butz, P., Fister, H., & Tauscher, B. (1996). Inactivation of lectins by ultrahigh pressure. In Bardocz S and Pusztai A (eds.). Effects of antinutrients on the nutritional value of legume diets. COST 98. *European Communities*, *2*, 66–68.

Cabanillas, B., Rodríguez, J., Blanca, N., Aranzazu Jiménez, M., & Crespo, J. F. (2009). Clinically relevant cross-reactivity between latex and passion fruit. *Annals of Allergy, Asthma & Immunology*, *103*(5), 449. https://doi.org/10.1016/S1081-1206(10)60370-5

Calderón de la Barca, A. M., Vázquez-Moreno, L., & Robles-Burgueñà, M. R. (1991). Active soybean lectin in foods: Isolation and quantitation. *Food Chemistry*, *39*(3), 321–327. https://doi.org/10.1016/0308-8146(91)90149-I

Campos, J. E., Martinez-Gallardo, N., Mendiola-Olaya, E., & Blanco-Labra, A. (1997). Purification and partial characterization of a proteinase inhibitor from tepary bean (*Phaseolus acutifolius*) seeds. *Journal of Food Biochemistry*, *21*(4), 203–218. https://doi.org/10.1111/j.1745-4514.1997.tb00215.x

Carbonaro, M., Grant, G., Cappelloni, M., & Pusztai, A. (2000). Perspectives into factors limiting in vivo digestion of legume proteins: Antinutritional compounds or storage proteins? *Journal of Agricultural and Food Chemistry*, *48*(3), 742–749. https://doi.org/10.1021/jf991005m

Cardona, C., Kornegay, J., Posso, C. E., Morales, F., & Ramirez, H. (1990). Comparative value of four arcelin variants in the development of dry bean lines resistant to the Mexican bean weevil. *Entomologia Experimentalis et Applicata*, *56*(2), 197–206. https://doi.org/10.1111/j.1570-7458.1990.tb01397.x

Cardona, C., Posso, C. E., Kornegay, J., Valor, J., & Serrano, M. (1989). Antibiosis effects of wild dry bean accessions on the Mexican bean weevil and the bean weevil (*Coleoptera*: *Brichidae*). *Journal of Economic Entomology*, *82*(1), 310–315. https://doi.org/10.1093/jee/82.1.310

Carlini, C. R., & Udedibie, A. B. (1997). Comparative effects of processing methods on hemagglutinating and antitryptic activities of *Canavalia ensiformis* and *Canavalia braziliensis* seeds. *Journal of Agricultural and Food Chemistry*, *45*(11), 4372–4377. https://doi.org/10.1021/jf970466+

Carrière, C., Young, A. L., Gunn, J. R., Longnecker, D. S., & Korc, M. (2009). Acute pancreatitis markedly accelerates pancreatic cancer progression in mice expressing oncogenic Kras. *Biochemical and Biophysical Research Communications*, *382*(3), 561–565. https://doi.org/10.1016/j.bbrc.2009.03.068

Carvalho, E. V. M. M., Oliveira, W. F., Coelho, L. C. B. B., & Correia, M. T. S. (2018). Lectins as mitosis stimulating factors: Briefly reviewed. *Life Sciences*, *207*, 152–157. https://doi.org/10.1016/j.lfs.2018.06.003

Castro-Rubio, F., Marina, M. L., & García, M. C. (2007). Perfusion reversed-phase high-performance liquid chromatography/mass spectrometry analysis of intact soybean proteins for the characterization of soybean cultivars. *Journal of Chromatography A*, *1170*(1–2), 34–43. https://doi.org/10.1016/j.chroma.2007.09.035

Causse, H., Lemoine, A., & Bougé, P. (1986). Quantification of soybean seed lectin in soybean tissues during the life-cycle of the plant, by an enzyme linked immunosorbent assay (ELISA). In T. Bog-Hansen & E. Driessche (Eds.), *Proceedings of IUB symposium No. 144, the seventh international lectin meeting Bruxelles* (Vol. *5*, pp. 667–676). https://doi.org/10.1515/9783112315958-082

Causse, H., & Rougé, P. (1983). Lectin release from imbibed soybean seed and its possible function. In T. C. Bog-Hansen & G. A. Spengler (Eds.), *Proceedings of the Fifth Lectin Meeting Bern, May 31–June 5, 1982* (Vol. *3*, pp. 559–572). De Gruyter. https://doi.org/10.1515/9783112315941-059

Challoner, K. R., & McCarron, M. M. (1990). Castor bean intoxication. *Annals of Emergency Medicine*, *19*(10), 1177–1183. https://doi.org/10.1016/s0196-0644(05)81525-2

Chan, Y. S., Wong, J. H., Fang, E. F., Pan, W., & Ng, T. B. (2012). Isolation of a glucosamine binding leguminous lectin with mitogenic activity towards splenocytes and anti-proliferative activity towards tumor cells. *PLoS One*, *7*(6), e38961. https://doi.org/10.1371/journal.pone.0038961

Chan, Y. S., Wong, J. H., & Ng, T. B. (2011). A glucuronic acid binding leguminous lectin with mitogenic activity toward mouse splenocytes. *Protein & Peptide Letters*, *18*(2), 194–202. https://doi.org/10.2174/092986611794475110

Chandra, N. R., Dam, T. K., Surolia, A., & Vijayan, M. (1997). Crystallization and preliminary crystallographic studies on the mannose-specific lectin from garlic. *Acta Crystallografica Section D*, *53*(Pt. 6), 787–788. https://doi.org/10.1107/S0907444997007385

Chandra, N. R., Ramachandraiah, G., Bachhawat, K., Dam, T. K., Surolia, A., & Vijayan, M. (1999). Crystal structure of a dimeric mannose-specific agglutinin from garlic: Quaternary association and carbohydrate specificity. *Journal of Molecular Biology*, *285*(3), 1157–1168. https://doi.org/10.1006/jmbi.1998.2353

Chatterjee, A., & Mandal, D. K. (2005). Quaternary association and reactivation of dimeric concanavalin A. *International Journal of Biological Macromolecules*, *35*(1–2), 103–109. https://doi.org/10.1016/j.ijbiomac.2005.01.005

Chen, D., Bernaerts, T., Debon, S., Oduah, C. O., Zhu, L., Wallecan, J., Hendrickx, M., & Kyomugasho, C. (2023). Novel insights into the role of the pectin-cation phytate mechanism in ageing induced cooking texture changes of red haricot beans through a texture-based classification and in situ cell wall associated mineral quantification. *Food Research International*, *163*, 112216. https://doi.org/10.1016/j.foodres.2022.112216

Chen, L. H., Thacker, R. R., & Pan, S. H. (1977). Effect of germination on hemagglutinating activity of pea and bean-seeds. *Journal of Food Science*, *42*(6), 1666–1668. https://doi.org/10.1111/j.1365-2621.1977.tb08451.x

Chen, Y. T., Hsu, L. H., Huang, I. P., Tsai, T. C., Lee, G. C., & Shaw, J. F. (2007). Gene cloning and characterization of a novel recombinant antifungal chitinase from papaya (*Carica papaya*). *Journal of Agricultural and Food Chemistry*, *55*(3), 714–722. https://doi.org/10.1021/jf062453e

Chen, Z., Posch, A., Cremer, R., Raulf-Heimsoth, M., & Baur, X. (1998). Identification of hevein (Heb b 6.02) in *Hevea* latex as a major cross-reacting allergen with avocado fruit in patients with latex allergy. *The Journal of Allergy and Clinical Immunology*, *102*(3), 476–481. https://doi.org/10.1016/s0091-6749(98)70138-1

Chen, Z., Posch, A., Lohaus, C., Raulf-Heimsoth, M., Meyer, H. E., & Baur, X. (1997). Isolation and identification of hevein as a major IgE-binding polypeptide in *Hevea* latex. *The Journal of Allergy and Clinical Immunology*, *99*(3), 402–409. https://doi.org/10.1016/s0091-6749(97)70059-9

Chigwedere, C. M., Nkonkola, C. M., Rai, S., Kyomugasho, C., Jamsazzadeh Kermani, Z., Pallares Pallares, A., Van Loey, A. M., Grauwet, T., & Hendrickx, M. E. (2019). Cotyledon pectin molecular interconversions explain pectin solubilization during cooking of common beans (*Phaseolus vulgaris*). *Food Research International*, *116*, 462–470. https://doi.org/10.1016/j.foodres.2018.08.062

Chigwedere, C. M., Olaoye, T. F., Kyomugasho, C., Jamsazzadeh Kermani, Z., Pallares, A., Van Loey, A. M., Grauwet, T., & Hendrickx, M. E. (2018). Mechanistic insight into softening of Canadian wonder common beans (*Phaseolus vulgaris*) during cooking. *Food Research International*, *106*, 522–531. https://doi.org/10.1016/j.foodres.2018.01.016

Ciudad-Mulero, M., Fernández-Ruiz, V., Cuadrado, C., Arribas, C., Pedrosa, M. M., Berrios, J. D. J., Pan, J., & Morales, P. (2020). Novel gluten-free formulations from lentil flours and nutritional yeast: Evaluation of extrusion effect on phytochemicals and non-nutritional factors. *Food Chemistry*, *315*, 126175. https://doi.org/10.1016/j.foodchem.2020.126175

Clement, F., Pramod, S. N., & Venkatesh, Y. P. (2010). Identity of the immunomodulatory proteins from garlic (Allium sativum) with the major garlic lectins or agglutinins. *International Immunopharmacology*, *10*(3), 316–324. https://doi.org/10.1016/j.intimp.2009.12.002

Clement, F., & Venkatesh, Y. P. (2010). Dietary garlic (*Allium sativum*) lectins, ASA I and ASA II, are highly stable and immunogenic. *International Immunopharmacology*, *10*(10), 1161–1169. https://doi.org/10.1016/j.intimp.2010.06.022

Coattrenec, Y., Jaques, D., Jandus, P., Harr, T., & Spoerl, D. (2017). Anaphylactic shock following castor bean contact: A case report. *Allergy, Asthma and Clinical Immunology*, *13*, 50. https://doi.org/10.1186/s13223-017-0221-x

Coffey, D. G., Uebersax, M. A., Hosfield, G. L., & Brunner, J. R. (1985). Evaluation of the haemagglutination activity of low-temperature cooked kidney beans. *Journal of Food Science*, *50*(1), 1750–3841. https://doi.org/10.1111/j.1365-2621.1985.tb13281.x

Cuadrado, C., Burbano, C., Gelencsér, E., Pedrosa, M. M., Ayet, G., Muzquiz, M., Pusztai, A., & Hajós, G. (2000). Influence of germination on lectin in *Lens culinaris* seeds. *Acta Alimentaria*, *29*(3), 231–240. https://doi.org/10.1556/aalim.29.2000.3.3

Cuadrado, C., Hajós, G., Burbano, C., Pedrosa, M. M., Ayet, G., Muzquiz, M., Pusztai, A., & Gelencser, E. (2002). Effect of natural fermentation on the lectin of lentils measured by immunological methods. *Food and Agriculture Immunology*, *14*(1), 41–49. https://doi.org/10.1080/09540100220137655

Cummings, R. D., & Kornfeld, S. (1982). Characterization of the structural determinants required for the high affinity interaction of asparagine-linked oligosaccharides with immobilized *Phaseolus vulgaris* leukoagglutinating and erythroagglutinating lectins. *Journal of Biological Chemistry*, *257*(19), 11230–11234. https://doi.org/10.1016/S0021-9258(18)33746-3

Czerwinski, J., Leontowicz, H., Leontowicz, M., & Gralak, M. A. (2005). Response of rats to a moderate intake of soyabean lectin. *Journal of Animal and Feed Sciences*, *14*(Suppl. 1), 537–540. https://doi.org/10.22358/jafs/70731/2005

Czerwinski, J., Leontowicz, H., Leontowicz, M., & Krzeminski, R. (2006). The influence of lectins isolated from pea and soyabean seeds on performance and pancreatic enzyme activity in rats. *Journal of Animal and Feed Sciences*, *15*(4), 651–659. https://doi.org/10.22358/jafs/66934/2006

Datta, P. K., Basu, P. S., & Datta, T. K. (1984). Isolation and characterization of *Vicia faba* lectin affinity purified on chitin column. *Preparative Biochemistry & Biotechnology*, *14*(4), 373–387. https://doi.org/10.1080/10826068408070642

de Haan, P., Reidinga-Saenen, L. M., & Korporaal-Heijman, J. A. (2016). Intoxication by ingestion of castor beans: Case report. *Netherlands Journal of Critical Care*, *24*(2), 20–22.

De Oliveira Dos Santos, A. M., Duarte, A. E., Costa, A. R., da Silva, A. A., Rohde, C., Silva, D. G., de Amorim, E. M., da Cruz Santos, M. H., Pereira, M. G., Deprá, M., de Santana, S. L., da Silva Valente, V. L., & Teixeira, C. S. (2022). *Canavalia ensiformis* lectin induced oxidative stress mediate both toxicity and genotoxicity in *Drosophila melanogaster*. *International Journal of Biological Macromolecules*, *222*(Pt B), 2823–2832. https://doi.org/10.1016/j.ijbiomac.2022.10.061

Dearman, R. J., Caddick, H., Stone, S., Basketter, D. A., & Kimber, I. (2001). Characterization of antibody responses induced in rodents by exposure to food proteins: Influence of route of exposure. *Toxicology*, *167*(3), 217–231. https://doi.org/10.1016/s0300-483x(01)00462-0

Debray, H., Coddeville, B., Bomfim, L. R., & Ramos, M. V. (2009). A simple micro-method for determining precise oligosaccharidic specificity of mannose-binding lectins. *Glycobiology*, *19*(12), 1417–1426. https://doi.org/10.1093/glycob/cwp091

Debray, H., Decout, D., Strecker, G., Spik, G., & Montreuil, J. (1981). Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosylproteins. *European Journal of Biochemistry*, *117*(1), 41–55. https://doi.org/10.1111/j.1432-1033.1981.tb06300.x

Debray, H., & Rougé, P. (1984). The fine sugar specificity of the *Lathyrus ochrus* seed lectin and isolectins. *FEBS Letters*, *176*(1), 120–124. https://doi.org/10.1016/0014-5793(84)80924-2

del Campillo, E., Shannon, L. M., & Hankins, C. N. (1981). Molecular properties of the enzymic phytohemagglutinin of mung bean. *The Journal of Biological Chemistry*, *256*(14), 7177–7180. https://doi.org/10.1016/S0021-9258(19)68944-1

Del Valle, F. R., Pico, M. L., Camacho, J. L., & Bourges, H. (1983). Effect of processing parameters on trypsin inhibitor and lectin contents of tortillas from whole raw corn-soybean mixtures. *Journal of Food Science*, *48*(1), 246–249. https://doi.org/10.1111/j.1365-2621.1983.tb14835.x

Desch, A. N., Gibbings, S. L., Goyal, R., Kolde, R., Bednarek, J., Bruno, T., Slansky, J. E., Jacobelli, J., Mason, R., Ito, Y., Messier, E., Randolph, G. J., Prabagar, M., Atif, S. M., Segura, E., Xavier, R. J., Bratton, D. L., Janssen, W. J., Henson, P. M., & Jakubzick, C. V. (2016). Flow Cytometric analysis of mononuclear phagocytes in nondiseased human lung and lung-draining lymph nodes. *American Journal of Respiratory and Critical Care Medicine*, *193*(6), 614–626. https://doi.org/10.1164/rccm.201507-1376OC

Desmiaty, Y., Chaidir, C., Utami, A. V., Okta, F. N., & Nugroho, G. A. (2024). Optimization strategy for purification and isolation of active lectin from *Colocasia esculenta* L. Schott Rhizome. *Journal of Applied Biology and Biotechnology*, *12*(3), 223–228. https://doi.org/10.7324/JABB.2024.173070

Dhurandhar, N. V., & Chang, K. C. (1990). Effect of cooking on firmness, trypsin inhibitors, lectins and Cystine/cysteine content of navy and red kidney beans (*Phaseolus vulgaris*). *Journal of Food Science*, *55*(2), 470–474. https://doi.org/10.1111/j.1365-2621.1990.tb06789.x

Díaz, C. L., Hosselet, M., Logman, G. J., van Driessche, E., Lugtenberg, B. J., & Kijne, J. W. (1990). Distribution of glucose/mannose-specific isolectins in pea (*Pisum sativum* L.) seedlings. *Planta*, *181*(4), 451–461. https://doi.org/10.1007/BF00192997

Díaz-Perales, A., Collada, C., Blanco, C., Sánchez-Monge, R., Carrillo, T., Aragoncillo, C., & Salcedo, G. (1998). Class I chitinases with hevein-like domain, but not class II enzymes, are relevant chestnut and avocado allergens. *The Journal of Allergy and Clinical Immunology*, *102*(1), 127–133. https://doi.org/10.1016/s0091-6749(98)70063-6

Díaz-Perales, A., Collada, C., Blanco, C., Sánchez-Monge, R., Carrillo, T., Aragoncillo, C., & Salcedo, G. (1999). Cross-reactions in the latex-fruit syndrome: A relevant role of chitinases but not of complex asparagine-linked glycans. *The Journal of Allergy and Clinical Immunology*, *104*(3 Pt. 1), 681–687. https://doi.org/10.1016/s0091-6749(99)70342-8

Difo, V. H., Onyike, E., Ameh, D. A., Njoku, G. C., & Ndidi, U. S. (2015). Changes in nutrient and antinutrient composition of *Vigna racemosa* flour in open and controlled fermentation. *Journal of Food Science and Technology*, *52*(9), 6043–6048. https://doi.org/10.1007/s13197-014-1637-7

Dobbins, J. W., Laurenson, J. P., Gorelick, F. S., & Banwell, J. G. (1986). Phytohemagglutinin from red kidney bean (*Phaseolus vulgaris*) inhibits sodium and chloride absorption in the rabbit ileum. *Gastroenterology*, *90*(6), 1907–1913. https://doi.org/10.1016/0016-5085(86)90260-x

Dramburg, S., Hilger, C., Santos, A. F., de Las, V. L., Aalberse, R. C., Acevedo, N., … Hoffmann-Sommergruber, K. (2023). EAACI molecular Allergology User's guide 2.0. Pediatric allergy and immunology: Official publication of the European society of. *Pediatric Allergy and Immunology*, *34*(Suppl 28), e13854. https://doi.org/10.1111/pai.13854

Dvořák, R., Pechová, A., Pavlata, L., Filípek, J., Dostálová, J., Réblová, Z., … Poul, J. (2005). Reduction in the content of antinutritional substances in pea seeds (*Pisum sativum* L.) by different treatments. *Czech Journal of Animal Science*, *50*(11), 519–527. https://doi.org/10.17221/4257-CJAS

EFSA (European Food Safety Authority). (2010). Application of systematic review methodology to food and feed safety assessments to support decision making. *EFSA Journal*, *8*(6), 1637. https://doi.org/10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority). (2011a). Report on the development of a food classification and description system for exposure assessment and guidance on its implementation and use. *EFSA Journal*, *9*(12), 2489. https://doi.org/10.2903/j.efsa.2011.2489

EFSA (European Food Safety Authority). (2011b). Use of the EFSA comprehensive European food consumption database in exposure assessment. *EFSA Journal*, *9*(3), 2097. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority). (2011c). The food classification and description system FoodEx 2 (draft-revision 1). *EFSA Supporting Publications*, *8*(12), EN-215. https://doi.org/10.2903/sp.efsa.2011.EN-215

EFSA (European Food Safety Authority). (2015). The food classification and description system FoodEx2 (revision 2). *EFSA Supporting Publications*, *12*(5), EN-804. https://doi.org/10.2903/sp.efsa.2015.EN-804

European Food Safety Authority, Dujardin, B., & Kirwan, L. (2019). Annex A to the technical report on the raw primary commodity (RPC) model - Input data [Data set]. *Zenodo*. https://doi.org/10.5281/zenodo.2537955

EFSA (European Food Safety Authority), Hart, A., Maxim, L., Siegrist, M., Von Goetz, N., da Cruz, C., Merten, C., Mosbach-Schulz, O., Lahaniatis, M., Smith, A., & Hardy, A. (2019). Guidance on communication of uncertainty in scientific assessments. *EFSA Journal*, *17*(1), 5520. https://doi.org/10.2903/j.efsa.2019.5520

EFSA Scientific Committee. (2012). Guidance on selected default values to be used by the EFSA scientific committee, scientific panels and units in the absence of actual measured data. *EFSA Journal*, *10*(3), 2579. https://doi.org/10.2903/j.efsa.2012.2579

EFSA Scientific Committee, Hardy, A., Benford, D., Halldorsson, T., Jeger, M. J., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J. R., Silano, V., Solecki, R., Turck, D., Bresson, J.-L., Dusemund, B., Gundert-Remy, U., … Mortensen, A. (2017). Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. *EFSA Journal*, *15*(5), 4849. https://doi.org/10.2903/j.efsa.2017.4849

EFSA Scientific Committee, Benford, D., Halldorsson, T., Jeger, M. J., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J. R., Silano, V., Solecki, R., Turck, D., Younes, M., Craig, P., Hart, A., Von Goetz, N., … Hardy, A. (2018a). Guidance on uncertainty analysis in scientific assessments. *EFSA Journal*, *16*(1), 5123. https://doi.org/10.2903/j.efsa.2018.5123

EFSA Scientific Committee, Benford, D., Halldorsson, T., Jeger, M. J., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J. R., Silano, V., Solecki, R., Turck, D., Younes, M., Craig, P., Hart, A., Von Goetz, N., … Hardy, A. (2018b). Scientific Opinion on the principles and methods behind EFSA's guidance on uncertainty analysis in scientific assessment. *EFSA Journal*, *16*(1), 5122. https://doi.org/10.2903/j.efsa.2018.5122

EFSA Scientific Committee, More, S. J., Bampidis, V., Benford, D., Bragard, C., Halldorsson, T. I., Hernández-Jerez, A. F., Bennekou, S. H., Koutsoumanis, K., Lambré, C., Machera, K., Mennes, W., Mullins, E., Nielsen, S. S., Schrenk, D., Turck, D., Younes, M., Aerts, M., Edler, L., … Schlatter, J. (2022). Guidance on the use of the benchmark dose approach in risk assessment. *EFSA Journal*, *20*(10), 7584. https://doi.org/10.2903/j.efsa.2017.4849

EFSA Scientific Committee, More, S., Bampidis, V., Benford, D., Bragard, C., Hernandez-Jerey, A., Bennekou, S. H., Koutsoumanis, K. P., Lambré, C., Machera, K., Mullins, E., Nielsen, S. S., Schlatter, J., Schrenk, D., Turck, D., Younes, M., Kraft, A., Naegeli, H., Tsaioun, K., … Halldorsson, T. I. (2023). Guidance on protocol development for EFSA generic scientific assessments. *EFSA Journal*, *21*(10), 8312. https://doi.org/10.2903/j.efsa.2023.8312

Einspahr, H., Parks, E. H., Suguna, K., Subramanian, E., & Suddath, F. L. (1986). The crystal structure of pea lectin at 3.0-Å resolution. *Journal of Biological Chemistry*, *261*(35), 16518–16527. https://doi.org/10.1016/S0021-9258(18)66597-4

El Mauhoub, M., Khalifa, M. M., Jaswal, O. B., & Garrah, M. S. (1983). "Ricin syndrome". A possible new teratogenic syndrome associated with ingestion of castor oil seed in early pregnancy: A case report. *Annals of Tropical Paediatrics*, *3*(2), 57–61. https://doi.org/10.1080/02724936.1983.11748269

El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. *Plant Foods for Human Nutrition*, *57*, 83–97. https://doi.org/10.1023/A:1013189620528

Embaby, H. E. (2010). Effect of soaking, dehulling, and cooking methods on certain antinutrients and in vitro protein digestibility of bitter and sweet lupin seeds. *Food Science and Bioechnology*, *19*, 1055–1062. https://doi.org/10.1007/s10068-010-0148-1

Entlicher, G., Kostir, J. V., & Kocourek, J. (1970). Studies on phytohemagglutinins. III. Isolation and characterization of hemagglutinins from the pea (*Pisum sativum* L.). *Biochimica et Biophysica Acta*, *221*(2), 272–281. https://doi.org/10.1016/0005-2795(70)90267-9

Etzler, M. E., Gupta, S., & Borrebaeck, C. (1981). Carbohydrate binding properties of the *Dolichos biflorus* lectin and its subunits. *Journal of Biological Chemistry*, *256*(5), 2367–2370. https://doi.org/10.1016/S0021-9258(19)69788-7

Faheina-Martins, G. V., da Silveira, A. L., Ramos, M. V., Marques-Santos, L. F., & Araujo, D. A. (2011). Influence of fetal bovine serum on cytotoxic and genotoxic effects of lectins in MCF-7 cells. *Journal of Biochemical and Molecular Toxicology*, *25*(5), 290–296. https://doi.org/10.1002/jbt.20388

Fälth-Magnusson, K., & Magnusson, K. E. (1995). Elevated levels of serum antibodies to the lectin wheat germ agglutinin in celiac children lend support to the gluten-lectin theory of celiac disease. *Pediatric Allergy and Immunology*, *6*(2), 98–102. https://doi.org/10.1111/j.1399-3038.1995.tb00267.x

Fan, Y., Tan, X. R., Ou, X., Lu, Q. Y., Chen, S. H., & Wei, S. P. (2016). A novel "on-off" electrochemiluminescence sensor for the detection of concanavalin A based on Ag-doped g-C_3N_4. *Electrochimica Acta*, *202*, 90–99. https://doi.org/10.1016/j.electacta.2016.04.013

Fang, E. F., Wong, J. H., Lin, P., & Ng, T. B. (2010). Biochemical and functional properties of a lectin purified from Korean large black soybeans – A cultivar of glycine max. *Protein and Peptide Letters*, *17*(6), 690–698. https://doi.org/10.2174/092986610791190309

Fasina, Y. O., Classen, H. L., Garlich, J. D., Swaisgood, H. E., & Clare, D. A. (2003). Investigating the possibility of monitoring lectin levels in commercial soybean meals intended for poultry feeding using steam-heated soybean meal as a model. *Poultry Science*, *82*(4), 648–656. https://doi.org/10.1093/ps/82.4.648

Felipe, P., Sok, D. E., Heo, O. S., Kim, H. C., Yoon, W. K., Kim, H. M., & Kim, M. R. (2006). Effect of cultivar and processing on the hemagglutinin activity of soybean. *Journal of Food Science and Technology*, *15*(1), 91–95.

Felsted, R. L., Leavitt, R. D., & Bachur, N. R. (1975). Purification of the phytohemagglutinin family of proteins from red kidney beans (*Phaseolus vulgaris*) by affinity chromatography. *Biochimica et Biophysica Acta, Protein Structure*, *450*(1), 72–81. https://doi.org/10.1016/0005-2795(75)90316-5

Feria, M., Pérez-Santiago, A., Cuevas, D., Martínez, M., & Córdoba, F. (1996). Purification and partial characterization of a new anti-A1 lectin of *Phaseolus coccineus* collected in Oaxaca. *Mexico. Preparative Biochemistry & Biotechnology*, *26*(1), 31–46. https://doi.org/10.1080/10826069608000048

Fitzgerald, A. J., Jordinson, M., Rhodes, J. M., Singh, R., Calam, J., & Goodlad, R. A. (2001). Comparison of the effects of concanavalin-A and epidermal growth factor on epithelial cell proliferation in the rat intestine. *Alimentary Pharmacology & Therapeutics*, *15*(7), 1077–1084. https://doi.org/10.1046/j.1365-2036.2001.01025.x

Förster-Waldl, E., Marchetti, M., Schöll, I., Focke, M., Radauer, C., Kinacyan, T., Nentwich, I., Jäger, S., Schmid, E. R., Boltz-Nitulescu, G., Scheiner, O., & Jensen-Jarolim, E. (2003). Type I allergy to elderberry (*Sambucus nigra*) is elicited by a 33.2 kDa allergen with significant homology to ribosomal inactivating proteins. *Clinical & Experimental Allergy*, *33*(12), 1703–1710. https://doi.org/10.1111/j.1365-2222.2003.01811.x

Friedman, M., Brandon, D. L., Bates, A. H., & Hymowitz, T. (1991). Comparison of a commercial soybean cultivar and an isoline lacking the Kunitz trypsin inhibitor: Composition, nutritional value, and effects of heating. *Journal of Agricultural and Food Chemistry*, *39*(2), 327–335. https://doi.org/10.1021/jf00002a022

Fujimura, M., Minami, Y., Watanabe, K., & Tadera, K. (2003). Purification, characterization, and sequencing of a novel type of antimicrobial peptides, Fa-AMP1 and Fa-AMP2, from seeds of buckwheat (*Fagopyrum esculentum* Moench.). *Bioscience, Biotechnology, and Biochemistry*, *67*(8), 1636–1642. https://doi.org/10.1271/bbb.67.1636

Galelli, A., & Truffa-Bachi, P. (1993). *Urtica dioica* agglutinin. A superantigenic lectin from stinging nettle rhizome. *Journal of Immunology*, *151*(4), 1821–1831. https://doi.org/10.4049/jimmunol.151.4.1821

Gamboa, P. M., Sánchez-Monge, R., Díaz-Perales, A., Salcedo, G., Ansótegui, J., & Sanz, M. L. (2005). Latex-vegetable syndrome due to custard apple and aubergine: New variations of the hevein symphony. *Journal of Investigational Allergology and Clinical Immunology*, *15*, 308–311.

Ganzera, M., Piereder, D., Sturm, S., Erdelmeier, C., & Stuppner, H. (2005). *Urtica dioica* agglutinin: Separation, identification, and quantitation of individual isolectins by capillary electrophoresis and capillary electrophoresis-mass spectrometry. *Electrophoresis*, *26*(9), 1724–1731. https://doi.org/10.1002/elps.200410369

Garabatos, N., & Santamaria, P. (2022). Gut microbial antigenic mimicry in autoimmunity. *Frontiers in Immunology*, *13*, 873607. https://doi.org/10.3389/fimmu.2022.873607

Gautam, A. K., Srivastava, N., Nagar, D. P., & Bhagyawant, S. S. (2018). Biochemical and functional properties of a lectin purified from the seeds of *Cicer arietinum* L. 3. *Biotech*, *8*(6), 272. https://doi.org/10.1007/s13205-018-1272-5

Geethanandan, K., Joseph, A. J., Sadasivan, C., & Haridas, M. (2013). Carbohydrate binding and unfolding of *Spatholobus parviflorus* lectin: Fluorescence and circular dichroism spectroscopic study. *Applied Biochemistry and Biotechnology*, *171*(1), 80–92. https://doi.org/10.1007/s12010-013-0340-x

Geiselhart, S., Nagl, C., Dubiela, P., Pedersen, A. C., Bublin, M., Radauer, C., Bindslev-Jensen, C., Hoffmann-Sommergruber, K., & Mortz, C. G. (2018). Concomitant sensitization to legumin, fag e 2 and fag e 5 predicts buckwheat allergy. *Clinical & Experimental Allergy*, *48*(2), 217–224. https://doi.org/10.1111/cea.13068

Gibbs, J., Young, R. C., & Smith, G. P. (1973). Cholecystokinin elicits satiety in rats with open gastric fistulas. *Nature*, *245*(5424), 323–325. https://doi.org/10.1038/245323a0

Goedvolk, C. A., von Rosenstiel, I. A., & Bos, A. P. (2003). Immune complex associated complications in the subacute phase of meningococcal disease: Incidence and literature review. *Archives of Disease in Childhood*, *88*(10), 927–930. https://doi.org/10.1136/adc.88.10.927

González-de-Olano, D., Barra-Castro, A., Riaño-Avanzini, T., Pastor-Vargas, C., & Bartolomé, B. (2018). Allergy to a garlic lectin in an infant after the first intake: Difficulties identifying the route of sensitization. *Journal of Investigational Allergology and Clinical Immunology*, *28*(4), 253–254. https://doi.org/10.18176/jiaci.0245

Grant, G., More, L. J., McKenzie, N. H., Dorward, P. M., Buchan, W. C., Telek, L., & Pusztai, A. (1995). Nutritional and haemagglutination properties of several tropical seeds. *Journal of Agricultural Science*, *124*(3), 437–445. https://doi.org/10.1017/S0021859600073391

Grant, G., More, L. J., McKenzie, N. H., & Pusztai, A. (1982). The effect of heating on the haemagglutinating activity and nutritional properties of bean (*Phaseolus vulgaris*) seeds. *Journal of the Science of Food and Agriculture*, *33*(12), 1324–1326. https://doi.org/10.1002/jsfa.2740331220

Grant, G., More, L. J., McKenzie, N. H., Stewart, J. C., & Pusztai, A. (1983). A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK. *British Journal of Nutrition*, *50*(2), 207–214. https://doi.org/10.1079/BJN19830090

Greenwood, J. S., Keller, G. A., & Chrispeels, M. (1984). Localization of phytohemagglutinin in the embryonic axis of *Phaseolus vulgaris* with ultra-thin cryosections embedded in plastic after indirect immunolabeling. *Planta*, *162*, 548–555. https://doi.org/10.1007/BF00399921

Gruber, P., Becker, W. M., & Hofmann, T. (2005). Influence of the Maillard reaction on the allergenicity of rAra h 2, a recombinant major allergen from peanut (*Arachis hypogaea*), its major epitopes, and peanut agglutinin. *Journal of Agricultural and Food Chemistry*, *53*(6), 2289–2296. https://doi.org/10.1021/jf048398w

Güll, I., Wirth, M., & Gabor, F. (2007). Development of a sensitive and reliable ELISA for quantification of wheat germ agglutinin. *Journal of Immunological Methods*, *318*(1–2), 20–29. https://doi.org/10.1016/j.jim.2006.07.025

Gulzar, H., Pervez, S., Nawaz, M. A., Ullah, R., Jan, T., Kan, F. A., Jan, A., Zahoor, M., Ali, E. A., & Umar, M. N. (2023). An automated reading of semi-quantitative hemagglutination results in microplates: Micro-assay for plant lectins. *Open Chemistry*, *21*(1), 20220342. https://doi.org/10.1515/chem-2022-0342

Gupta, A., & Sandhu, R. S. (1997). *In vivo* binding of mannose specific lectin from garlic to intestinal epithelium. *Nutrition Research*, *17*(4), 703–711. https://doi.org/10.1016/S0271-5317(97)00040-7

Gupta, A., & Sandhu, R. S. (1998). Effect of garlic agglutinin and garlic extracts on the rat jejunum. *Nutrition Research*, *18*(5), 841–850. https://doi.org/10.1016/S0271-5317(98)00069-4

Gurumoorthi, P., Janardhanan, K., & Kalavathy, G. (2013). Improving nutritional value of velvet bean, *Mucuna pruriens* (L.) DC. var. *utilis* (Wall.ex.Wight) L.H. Baily, an under-utilized pulse, using microwave technology. *Indian Journal of Traditional Knowledge*, *12*(4), 677–681.

Haas, H., Falcone, F. H., Schramm, G., Haisch, K., Gibbs, B. F., Klaucke, J., Pöpelmann, M., Becker, W. M., Gabius, H. J., & Schlaak, M. (1999). Dietary lectins can induce in vitro release of IL-4 and IL-13 from human basophils. *European Journal of Immunology*, *29*, 918–927. https://doi.org/10.1002/(SICI)1521-4141(199903)29:03<918::AID-IMMU918>3.0.CO;2-T

Haas, H., Herzig, K. H., André, S., Galle, J., Gronow, A., & Gabius, H. J. (2001). Low-dose intragastric administration of *Phaseolus vulgaris* agglutinin (PHA) does not induce immunoglobulin E (IgE) production in Sprague-Dawley rats. *Glycoconjugate Journal*, *18*, 273–275. https://doi.org/10.1023/A:1013740413012

Halder, S., Surolia, A., & Mukhopadhyay, C. (2016). Dynamics simulations of soybean agglutinin (SBA) dimer reveals the impact of glycosylation on its enhanced structural stability. *Carbohydrate Research*, *428*, 8–17. https://doi.org/10.1016/j.carres.2016.04.009

Hamelin, E. I., Johnson, R. C., Osterloh, J. D., Howard, D. J., & Thomas, J. D. (2012). Evaluation of ricinine, a ricin biomarker, from a non-lethal castor bean ingestion. *Journal of Analytical Toxicology*, *36*(9), 660–662. https://doi.org/10.1093/jat/bks077

Hamelryck, T. W., Dao-Thi, M. H., Poortmans, F., Chrispeels, M. J., Wyns, L., & Loris, R. (1996). The crystallographic structure of phytohemagglutinin-L. *Journal of Biological Chemistry*, *271*(34), 20479–20485. https://doi.org/10.1074/jbc.271.34.20479

Hamelryck, T. W., Loris, R., Bouckaert, J., Dao-Thi, M. H., Strecker, G., Imberty, A., Fernandez, E., Wyns, L., & Etzler, M. E. (1999). Carbohydrate-binding, quaternary structure and a novel hydrophobic binding site in two legume lectin oligomers from *Dolichos biflorus*. *Journal of Molecular Biology*, *286*(4), 1161–1177. https://doi.org/10.1006/jmbi.1998.2534

Hamelryck, T. W., Poortmans, F., Goossens, A., Angenon, G., Van Montagu, M., Wyns, L., & Loris, R. (1996). Crystal structure of arcelin-5, a lectin-like defence protein from *Phaseolus vulgaris*. *Journal of Biological Chemistry*, *271*(51), 32796–32802. https://doi.org/10.1074/jbc.271.51.32796

Han, X., Sun, Y., Huangfu, B., He, X., & Huang, K. (2023). Ultra-high-pressure passivation of soybean agglutinin and safety evaluations. *Food Chemistry: X*, *18*, 100726. https://doi.org/10.1016/j.fochx.2023.100726

Hänninen, A. R., Mikkola, J. H., Kalkkinen, N., Turjanmaa, K., Ylitalo, L., Reunala, T., & Palosuo, T. (1999). Increased allergen production in turnip (*Brassica rapa*) by treatment activating defence mechanisms. *The Journal of Allergy and Clinical Immunology*, *104*(1), 194–201. https://doi.org/10.1016/S0091-6749(99)70135-1

Hara, T., Mukunoki, Y., Tsukamoto, I., Miyoshi, M., & Hasegawa, K. (1984). Susceptibility of kintoki bean lectin to digestive enzmes *in vitro* and its behaviour in the digestive organs of mouse *in vivo*. *Journal of Nutritional Science and Vitaminology*, *30*(4), 381–394. https://doi.org/10.3177/jnsv.30.381

Hara, T., Tsukamoto, I., & Miyoshi, M. (1983). Oral toxicity of Kintoki bean (*Phaseolus vulgaris*) lectin. *Journal of Nutritional Science and Vitaminology*, *29*(5), 589–599. https://doi.org/10.3177/jnsv.29.589

Hasselt University. (2022). EFSA platform for Bayesian benchmark dose analysis. *EFSA Supporting Publications*, *19*(12), EN-7740.

He, S., Elfalleh, W., Li, J., Zhao, J., He, Q., & Sun, H. (2018). In vivo anaphylactic assessment of low-dose lectin from the black turtle soup bean (*Phaseolus vulgaris*). *Current Topics in Nutraceutical Research*, *16*(1), 105–112.

He, S., Shi, J., Li, X., Ma, Y., & Xue, S. J. (2015). Identification of a lectin protein from black turtle bean (*Phaseolus vulgaris*) using LC-MS/MS and PCR method. *LWT- Food Science and Technology*, *60*(2), 1074–1079. https://doi.org/10.1016/j.lwt.2014.10.015

He, S., Shi, J., Ma, Y., Xue, S. J., Zhang, H., & Zhao, S. (2014). Kinetics for the thermal stability of lectin from black turtle bean. *Journal of Food Engineering*, *142*, 132–137. https://doi.org/10.1016/j.jfoodeng.2014.06.008

He, S., Zhao, J., Elfalleh, W., Jemaà, M., Sun, H., Sun, X., Tang, M., He, Q., Wu, Z., & Lang, F. (2018). In silico identification and in vitro analysis of B- and T-cell epitopes of the black turtle bean (*Phaseolus vulgaris* L.) lectin. *Cellular Physiology and Biochemistry*, *49*(4), 1600–1614. https://doi.org/10.1159/000493496

He, S., Zhao, J., Zhang, Y., Zhu, Y., Li, X., Cao, X., Ye, Y., Li, J., & Sun, H. (2021). Effects of low-pH treatment on the Allergenicity reduction of black turtle bean (*Phaseolus vulgaris* L.) lectin and its mechanism. *Journal of Agricultural and Food Chemistry*, *69*(4), 1379–1390. https://doi.org/10.1021/acs.jafc.0c06524

He, X., Lu, S., Cheng, L. W., Rasooly, R., & Carter, J. M. (2008). Effect of food matrices on the biological activity of ricin. *Journal of Food Protection*, *71*(10), 2053–2058. https://doi.org/10.4315/0362-028X-71.10.2053

Hearn, M. T., Smith, P. K., & Mallia, A. K. (1982). Isolation of the *Maclura pomifera* hemagglutinin on a deoxymelibiotol affinity support and preliminary characterization by buffer electrofocusing and high-performance liquid chromatography. *Bioscience Reports*, *2*(4), 247–255. https://doi.org/10.1007/BF01136724

Helm, R. M., & Froese, A. (1981). Binding of the receptors for IgE by various lectins. *International Archives of Allergy and Immunology*, *65*(1), 81–84. https://doi.org/10.1159/000232741

Henney, L., Ahmed, E. M., George, D. E., Kao, K. J., & Sitren, H. S. (1990). Tolerance to Long-term feeding of isolated Peanut lectin in the rat: Evidence for a trophic effect on the small intestines. *Journal of Nutritional Science and Vitaminology*, *36*(6), 599–607. https://doi.org/10.3177/jnsv.36.599

Herman, E. M., & Shannon, L. M. (1984). Immunocytochemical localization of concanavalin A in developing jack-bean cotyledons. *Planta*, *161*, 97–104. https://doi.org/10.1007/BF00395468

Hernández-Infante, M., Sousa, V., Montalvo, I., & Tena, E. (1998). Impact of microwave heating on hemagglutinins, trypsin inhibitors and protein quality of selected legume seeds. *Plant Foods for Human Nutrition*, *52*, 199–208. https://doi.org/10.1023/A:1008033610737

Hester, G., Kaku, H., Goldstein, I. J., & Wright, C. S. (1995). Structure of mannose-specific snowdrop (*Galanthus nivalis*) lectin is representative of a new plant lectin family. *Nature Structural & Molecular Biology*, *2*(6), 472–479. https://doi.org/10.1038/nsb0695-472

Higgins, T. J., Chrispeels, M. J., Chandler, P. M., & Spencer, D. (1983). Intracellular sites of synthesis and processing of lectin in developing pea cotyledons. *Journal of Biological Chemistry*, *258*(15), 9550–9552. https://doi.org/10.1016/s0021-9258(17)44702-8

Higuchi, M., Tsuchiya, I., & Iwai, K. (1984). Growth-inhibition and small intestinal lesions in rats after feeding with isolated winged bean lectin. *Agricultural and Biological Chemistry*, *48*(3), 695–701. https://doi.org/10.1271/bbb1961.48.695

Hill, R. C., Oman, T. J., Wang, X., Shan, G., Schafer, B., Herman, R. A., Tobias, R., Shippar, J., Malayappan, B., Sheng, L., Xu, A., & Bradshaw, J. (2017). Development, validation, and Interlaboratory evaluation of a quantitative multiplexing method to assess levels of ten endogenous allergens in soybean seed and its application to field trials spanning three growing seasons. *Journal of Agricultural and Food Chemistry*, *65*(27), 5531–5544. https://doi.org/10.1021/acs.jafc.7b01018

Horowitz, B. Z., Castelli, R., Hughes, A., Hendrickson, R. G., Johnson, R. C., & Thomas, J. D. (2020). Massive fatal overdose of abrin with progressive encephalopathy. *Clinical Toxicology (Philadelphia, Pa.)*, *58*(5), 417–420. https://doi.org/10.1080/15563650.2019.1655150

Hossaini, A. A. (1968). Hemolytic and hemagglutinating activities of 222 plants. *Vox Sanguinis*, *15*(6), 410–417. https://doi.org/10.1159/000467098

Hosseini, N., Hosseini, L., & Noorafshan, A. (2017). Management of Castor Bean Seed-Associated Intraventricular Hemorrhage with fresh frozen plasma: A case report. *Iranian Red Crescent Medical Journal (IRCMJ)*, *19*(1), 1–4. https://doi.org/10.5812/ircmj.27834

Howard, I. K., & Sage, H. J. (1969). Isolation and characterization of a phytohemagglutinin from the lentil. *Biochemistry*, *8*(6), 2436–2441. https://doi.org/10.1021/bi00834a028

Howard, I. K., Sage, H. J., & Horton, C. B. (1972). Studies on the appearance and location of hemagglutinins from a common lentil during the life cycle of the plant. *Archives of Biochemistry and Biophysics*, *149*(1), 323–326. https://doi.org/10.1016/0003-9861(72)90328-1

Howatson, A. G., & Carter, D. C. (1985). Pancreatic carcinogenesis-enhancement by cholecystokinin in the hamster-nitrosamine model. *British Journal of Cancer*, *51*, 107–114. https://doi.org/10.1038/bjc.1985.15

Huang, J., Xu, Z., Wang, D., Ogata, C. M., Palczewski, K., Lee, X., & Young, N. M. (2010). Characterization of the secondary binding sites of *Maclura pomifera* agglutinin by glycan array and crystallographic analyses. *Glycobiology*, *20*(12), 1643–1653. https://doi.org/10.1093/glycob/cwq118

Huang, J., Zhang, W., Li, X., Feng, S., Ye, G., Wei, H., & Gong, X. (2017). Acute abrin poisoning treated with continuous renal replacement therapy and hemoperfusion successfully: A case report. *Medicine*, *96*(27), e7423. https://doi.org/10.1097/MD.0000000000007423

Hussein, L., El-Saied, H. M., & Abu-Salem, F. (1980). Some factors affecting the haemagglutinin of soybean. *Zeitschrift für Ernährungswissenschaft*, *19*(4), 233–237. https://doi.org/10.1007/BF02023781

Huybrechts, I., Sioen, I., Boon, P. E., Ruprich, J., Lafay, L., Turrini, A., Amiano, P., Hirvonen, T., De Neve, M., Arcella, D., Moschandreas, J., Westerlund, A., Ribas-Barba, L., Hilbig, A., Papoutsou, S., Christensen, T., Oltarzewski, M., Virtanen, S., Rehurkova, I., … Van Klaveren, J. (2011). Dietary exposure assessments for children in Europe (the EXPOCHI project): Rationale, methods and design. *Archives of Public Health*, *69*(1), 4. https://doi.org/10.1186/0778-7367-69-4

Igene, F. U., Oboh, S. O., & Aletor, V. A. (2006). Nutrient and anti-nutrient components of raw and processed winged bean seeds (*Psophocarpus tetragonolobus*). *The Indian Journal of Animal Sciences*, *76*(6), 476–479.

Ingle, V. N., Kale, V. G., & Talwalkar, Y. B. (1966). Accidental poisoning in children with particular reference to castor beans. *The Indian Journal of Pediatrics*, *33*, 237–240. https://doi.org/10.1007/BF02749307

Irish, G. G., Maenz, D. D., & Classen, H. L. (1999). A new assay for functional lectins: The brush border lectin agglutination assay (BBLAA). *Animal Feed Science and Technology*, *76*(3–4), 321–333. https://doi.org/10.1016/S0377-8401(98)00214-4

Jackson, L. S., Tolleson, W. H., & Chirtel, S. J. (2006). Thermal inactivation of ricin using infant formula as a food matrix. *Journal of Agricultural and Food Chemistry*, *54*(19), 7300–7304. https://doi.org/10.1021/jf061199n

Jackson, L. S., Triplett, O. A., & Tolleson, W. H. (2015). Influence of yogurt fermentation and refrigerated storage on the stability of protein toxin contaminants. *Food and Chemical Toxicology*, *80*, 101–107. https://doi.org/10.1016/j.fct.2015.03.003

Jackson, L. S., Zhang, Z., & Tolleson, W. H. (2010). Thermal stability of ricin in orange and apple juices. *Journal of Food Science*, *75*(4), T65–T71. https://doi.org/10.1111/j.1750-3841.2010.01570.x

Jakubzick, C., Gautier, E. L., Gibbings, S. L., Sojka, D. K., Schlitzer, A., Johnson, T. E., Ivanov, S., Duan, Q., Bala, S., Condon, T., van Rooijen, N., Grainger, J. R., Belkaid, Y., Ma'ayan, A., Riches, D. W., Yokoyama, W. M., Ginhoux, F., Henson, P. M., & Randolph, G. J. (2013). Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity*, *39*(3), 599–610. https://doi.org/10.1016/j.immuni.2013.08.007

Jang, D. H., Hoffman, R. S., & Nelson, L. S. (2010). Attempted suicide, by mail order: *Abrus precatorius*. *Journal of Medical Toxicology*, *6*(4), 427–430. https://doi.org/10.1007/s13181-010-0099-1

Jeyaprakash, A. A., Jayashree, G., Mahanta, S. K., Swaminathan, C. P., Sekar, K., Surolia, A., & Vijayan, M. (2005). Structural basis for the energetics of jacalin-sugar interactions: Promiscuity versus specificity. *Journal of Molecular Biology*, *347*(1), 181–188. https://doi.org/10.1016/j.jmb.2005.01.015

Jeyaprakash, A. A., Srivastav, A., Surolia, A., & Vijayan, M. (2004). Structural basis for the carbohydrate specificities of artocarpin: Variation in the length of a loop as a strategy for generating ligand specificity. *Journal of Molecular Biology*, *338*(4), 757–770. https://doi.org/10.1016/j.jmb.2004.03.040

Jiang, B., Wang, X., Wang, L., Lv, X., Li, D., Liu, C., & Feng, Z. (2019). Two-step isolation, purification, and characterization of lectin from Zihua snap bean (*Phaseolus vulgaris*) seeds. *Polymers*, *11*(5), 785. https://doi.org/10.3390/polym11050785

Jiang, J., Chen, J., & Xiong, Y. L. (2009). Structural and emulsifying properties of soy protein isolate subjected to acid and alkaline pH-shifting processes. *Journal of Agricultural and Food Chemistry*, *57*(16), 7576–7583. https://doi.org/10.1021/jf901585n

John, M. E., & Long, C. M. (1990). Sequence analysis of arcelin-2, a lectin-like plant protein. *Gene*, *86*(2), 171–176. https://doi.org/10.1016/0378-1119(90)90276-W

Jordinson, M., Beales, I. L. P., & Calam, J. (1998). Soybean agglutinin stimulated cholecystokinin release from cultures rabbit Jejunal cells requires calcium influx via L-type calcium channels. *Peptides*, *19*(9), 1541–1547. https://doi.org/10.1016/S0196-9781(98)00088-6

Jordinson, M., Playford, R. J., & Calam, J. (1997). Effects of a panel of dietary lectins on cholecystokinin release in rats. *American Journal of Physiology*, *273*(4), G946–G950. https://doi.org/10.1152/ajpgi.1997.273.4.G946

Kalpanadevi, V., & Mohan, V. R. (2013). Effect of processing on antinutrients and in vitro protein digestibility of the underutilized legume, *Vigna unguiculata* (L.) Walp subsp. *unguiculata*. *LWT- Food Science and Technology*, *51*(2), 455–461. https://doi.org/10.1016/j.lwt.2012.09.030

Kanwar, J. R., Sharma, P. B., & Kanwar, S. S. (1991). Biochemical and nutritional characteristics of non-conventional protein sources. *Journal of the Science of Food and Agriculture*, *55*(1), 141–151. https://doi.org/10.1002/jsfa.2740550113

Kasera, R., Singh, A. B., Lavasa, S., Nagendra, K., & Arora, N. (2013). Purification and immunobiochemical characterization of a 31 kDa cross-reactive allergen from *Phaseolus vulgaris* (kidney bean). *PLoS One*, *8*(5), e63063. https://doi.org/10.1371/journal.pone.0063063

Kasera, R., Singh, B. P., Lavasa, S., Prasad, K. N., Sahoo, R. C., & Singh, A. B. (2011). Kidney bean: A major sensitizer among legumes in asthma and rhinitis patients from India. *PLoS One*, *6*(11), e27193. https://doi.org/10.1371/journal.pone.0027193

Kaul, R., Read, J., & Mattiasson, B. (1991). Screening for plant-lectins by latex agglutination tests. *Phytochemistry*, *30*(12), 4005–4009. https://doi.org/10.1016/0031-9422(91)83453-R

Kayashima, T., Okazaki, Y., Katayama, T., & Hori, K. (2005). Dietary lectin lowers serum cholesterol and raises fecal neutral sterols in cholesterol-fed rats. *Journal of Nutritional Science and Vitaminology*, *51*(5), 343–348. https://doi.org/10.3177/jnsv.51.343

Kelkar, S., Siddiq, M., Harte, J. B., Dolan, K. D., Nyombaire, G., & Suniaga, H. (2012). Use of low-temperature extrusion for reducing phytohemagglutinin activity (PHA) and oligosaccharides in beans (*Phaseolus vulgaris* L.) cv. Navy and pinto. *Food Chemistry*, *133*(4), 1636–1639. https://doi.org/10.1016/j.foodchem.2012.02.044

Kelsall, A., FitzGerald, A. J., Howard, C. V., Evans, R. C., Singh, R., Rhodes, J. M., & Goodlad, R. A. (2002). Dietary lectins can stimulate pancreatic growth in the rat. *International Journal of Experimental Pathology*, *83*(4), 203–208. https://doi.org/10.1046/j.1365-2613.2002.00230.x

Kik, M. J., Koninkx, J. F., van den Muysenberg, A., & Hendriksen, F. (1991). Pathological effects of *Phaseolus vulgaris* isolectins on pig jejunal mucosa in organ culture. *Gut*, *32*(8), 886–892. https://doi.org/10.1136/gut.32.8.886

Kilpatrick, D. C., Pusztai, A., Grant, G., Graham, C., & Ewen, S. W. (1985). Tomato lectin resists digestion in the mammalian alimentary canal and binds to intestinal villi without deleterious effects. *FEBS Letters*, *185*(2), 299–305. https://doi.org/10.1016/0014-5793(85)80927-3

Kjær, T. M., & Frøkiaer, H. (2002). Modulation of ovomucoid-specific oral tolerance in mice fed plant extracts containing lectins. *British Journal of Nutrition*, *88*(6), 671–680. https://doi.org/10.1079/BJN2002736

Kopferschmitt, J., Flesch, F., Lugnier, A., Sauder, P., Jaeger, A., & Mantz, J. M. (1983). Acute voluntary intoxication by ricin. *Human Toxicology*, *2*(2), 239–242. https://doi.org/10.1177/096032718300200211

Kornfeld, R., & Ferris, C. (1975). Interaction of immunoglobulin glycopeptides with concanavalin A. *Journal of Biological Chemistry*, *250*(7), 2614–2619. https://doi.org/10.1016/S0021-9258(19)41645-1

Kornfeld, R., & Kornfeld, S. (1970). The structure of a phytohemagglutinin receptor site from human erythrocytes. *Journal of Biological Chemistry*, *245*(10), 2536–2545. https://doi.org/10.1016/S0021-9258(18)63104-7

Koshte, V. L., Aalberse, M., Calkhoven, P. G., & Aalberse, R. C. (1992). The potent IgG4-inducing antigen in banana is a mannose-binding lectin, BanLec-I. *International Archives of Allergy and Immunology*, *97*(1), 17–24. https://doi.org/10.1159/000236090

Krithika, N., Pramod, S. N., Mahesh, P. A., & Venkatesh, Y. P. (2018). Banana lectin (BanLec) induces non-specific activation of basophils and mast cells in atopic subjects. *European Annals of Allergy and Clinical Immunology*, *50*(6), 243–253. https://doi.org/10.23822/EurAnnACI.1764-1489.64

Kroghsbo, S., Madsen, C., Poulsen, M., Schrøder, M., Kvist, P. H., Taylor, M., Gatehouse, G., Shu, Q., & Knudsen, I. (2008). Immunotoxicological studies of genetically modified rice expressing PHA-E lectin or Bt toxin in Wistar rats. *Toxicology*, *245*(1–2), 24–34. https://doi.org/10.1016/j.tox.2007.12.005

Krüpe, M. (1956). *Blutgruppenspezifische pflanzliche Eiweißkörper (Phytagfflutinine)*. Ferdinand Enkverlag.

Kruszewska, D., Kiela, P., Ljungh, A., Erlwanger, K. H., Weström, B. R., Linderoth, A., & Pierzynowski, S. G. (2003). Enteral crude red kidney bean (*Phaseolus vulgaris*) lectin-Phytohemagglutinin-induces maturational changes in the enterocyte membrane proteins of suckling rats. *Biology of the Neonate*, *84*(2), 152–158. https://doi.org/10.1159/000071950

Kuku, A., Togun, R. A., Obuotor, E. M., & Adeyemi, D. O. (2012). Acute toxicity and histopathology study of a galactose-specific lectin from the seeds of *Tetracarpidium conophorum* (African walnut) (Hutch and Dalz). *Toxicological & Environmental Chemistry*, *94*(3), 583–592. https://doi.org/10.1080/02772248.2011.649037

Kumar, S., Kumar Verma, A., Misra, A., Tripathi, A., Chaudhari, B. P., Prasad, R., Jain, S. K., Das, M., & Dwivedi, P. D. (2011). Allergenic responses of red kidney bean (*Phaseolus vulgaris* cv chitra) polypeptides in BALB/c mice recognized by bronchial asthma and allergic rhinitis patients. *Food Research International*, *44*(9), 2868–2879. https://doi.org/10.1016/j.foodres.2011.06.043

Kumar, S., Sharma, A., Das, M., Jain, S. K., & Dwivedi, P. D. (2014). Leucoagglutinating phytohemagglutinin: Purification, characterization, proteolytic digestion and assessment for allergenicity potential in BALB/c mice. *Immunopharmacology and Immunotoxicology*, *36*(2), 138–144. https://doi.org/10.3109/08923973.2014.884136

Kumar, S., Singh, A., Yamini, S., Dey, S., & Singh, T. P. (2015). Crystal structure of Mg(2+) containing hemopexin-fold protein from Kabouli Chana (chickpea-white, CW-25) at 2.45 Å resolution reveals its metal ion transport property. *The Protein Journal*, *34*, 284–290. https://doi.org/10.1007/s10930-015-9624-z

Kumar, S., Verma, A. K., Sharma, A., Kumar, D., Tripathi, A., Chaudhari, B. P., Das, M., Jain, S. K., & Dwivedi, P. D. (2013). Phytohemagglutinins augment red kidney bean (*Phaseolus vulgaris* L.) induced allergic manifestations. *Journal of Proteomics*, *93*, 50–64. https://doi.org/10.1016/j.jprot.2013.02.003

Lafont, J., Rouanet, J. M., Gabrion, J., Assouad, J. L., Zambonino Infante, J. L., & Besançon, P. (1988). Duodenal toxicity of dietary *Phaseolus vulgaris* lectins in the rat: An integrative assay. *Digestion*, *41*(2), 83–93. https://doi.org/10.1159/000199736

Laiseca García, J., Díaz, A., de Durana, M. D., Gonzalez Bravo, L., Pineda de la Losa, F., Macías Iglesias, J., & Rosado, A. (2023). Allergy to white kidney beans with cross-reactivity to red kidney beans: Report of 2 cases in children. *Journal of Investigational Allergology and Clinical Immunology*, *33*(2), 141–142. https://doi.org/10.18176/jiaci.0818

Larson, G., Falk, P., Howard, R., & Banwell, J. G. (1989). Intestinal sphingolipid excretion associated with feeding of phytohemagglutinin lectin (*Phaseolus vulgaris*) to germ-free and conventional rats. *Glycoconjugate Journal*, *6*(4), 539–550. https://doi.org/10.1007/BF01053776

Larue-Achagiotis, C., Picard, M., & Louis-Sylvestre, J. (1992). Feeding behavior in rats on a complete diet containing Concanavalin A. *Reproduction Nutrition Development*, *32*(4), 343–350. https://doi.org/10.1051/rnd:19920404

Lavelle, E. C., Grant, G., Pusztai, A., Pfüller, U., & O'Hagan, D. T. (2000). Mucosal immunogenicity of plant lectins in mice. *Immunology*, *99*(1), 30–37. https://doi.org/10.1046/j.1365-2567.2000.00932.x

Le Berre-Anton, V., Nahoum, V., Santimone, M., Barre, A., Marchis-Mouren, G., Payan, F., & Rougé, P. (1998). Interaction of the bean (*Phaseolus vulgaris*) α-amylase inhibitors with human α-amylases: Structural and functional aspects. In A. J. M. Jansman, G. D. Hill, J. Huisman, & A. F. B. van der Poel (Eds.), *Recent advances of research in Antinutritional factors in legume seeds and rapeseed* (pp. 131–135). Wageningen Academic. https://doi.org/10.1163/9789004683921_018

Lee, X., Thompson, A., Zhang, Z., Ton-that, H., Biesterfeldt, J., Ogata, C., Xu, L., Johnston, R. A., & Young, N. M. (1998). Structure of the complex of *Maclura pomifera* agglutinin and the T-antigen disaccharide, Galβ1, 3GalNAc. *Journal of Biological Chemistry*, *273*(11), 6312–6318. https://doi.org/10.1074/jbc.273.11.6312

Lefever, S., Geerts, I., Vermeulen, E., Vermeersch, P., De Rydt, F., Dobbels, L., Gillis, M., & Croes, K. (2021). Non-lethal intoxication by ingestion of 50 Castor beans: Serial measurement of Ricinine in blood, plasma and urine. *Journal of Analytical Toxicology*, *45*(5), e8–e12. https://doi.org/10.1093/jat/bkaa139

Leoni, C., Volpicella, M., Dileo, M. C. G., Gattulli, B. A. R., & Ceci, L. R. (2019). Chitinases as food allergens. *Molecules*, *24*(11), 2087. https://doi.org/10.3390/molecules24112087

Leontowicz, H., Kostyra, H., Leontowicz, M., & Kulasek, W. (1998). The inactivation of legume seed haemagglutinin and trypsin inhibitors by boiling. In A. J. M. Jansman, G. D. Hill, J. Huisman, & A. F. B. van der Poel (Eds.), *Recent advances of research in antinutritional factors in legume seeds and rapeseed* (pp. 429–432). Wageningen Press.

Leontowicz, H., Leontowicz, M., Kostyra, H., Kulasek, G., Gralak, M. A., Krzemiński, R., & Podgurniak, M. (2001). Effects of raw or extruded legume seeds on some functional and morphological gut parameters in rats. *Journal of Animal and Feed Sciences*, *10*(1), 169–183. https://doi.org/10.22358/jafs/67957/2001

Leontowicz, M., Leontowicz, H., Biernat, M., Gralak, M. A., Kostyra, H., & Czerwiński, J. (2001). The influence of extrusion of faba bean seeds and supplementation of sulphur amino acids on performance, pancreatic trypsin activity, and morphological parameters of the jejunum in rats. *Journal of Animal and Feed Sciences*, *10*(Suppl. 2), 323–329. https://doi.org/10.22358/jafs/70116/2001

Levin, Y., Sherer, Y., Bibi, H., Schlesinger, M., & Hay, E. (2000). Rare *Jatropha multifida* intoxication in two children. *The Journal of Emergency Medicine*, *19*(2), 173–175. https://doi.org/10.1016/s0736-4679(00)00207-9

Li, L., Chu, Z., Ning, K., Zhu, M., Lu, C., Zhai, R., & Xu, P. (2024). An accurate IDMS-based method for absolute quantification of phytohemagglutinin, a major antinutritional component in common bean. *Journal of Food Science*, *90*(1), e17590. https://doi.org/10.1111/1750-3841.17590

Li, Y., Lu, Y., Che, Y., Zhang, P., & Jia, N. Q. (2023). Fullerene-promoted organic-inorganic hybrid electrochemiluminescence biosensor for sensitive detection of concanavalin A. *Sensors and Actuators B: Chemical*, *393*(15), 134311. https://doi.org/10.1016/j.snb.2023.134311

Li, Z., Li, D., & Qiao, S. (2003). Effects of soybean agglutinin on nitrogen metabolism and on characteristics of intestinal tissues and pancreas in rats. *Archiv für Tierernährung*, *57*(5), 369–380. https://doi.org/10.1080/0003942031000160772 5

Li, Z., Li, D., Qiao, S., Zhu, X., & Huang, C. (2003). Anti-nutritional effects of a moderate dose of soybean agglutinin in the rat. *Archiv für Tierernährung*, *57*(4), 267–277. https://doi.org/10.1080/0003942031000159441 4

Liener, I. E. (1986). Nutritional significance of lectins in the diet. In I. E. Liener, N. Sharon, & I. J. Goldstein (Eds.), *The lectins, properties, functions, and applications in biology and medicine* (pp. 527–552). Academic Press.

Lim, H., Kim, H. J., & Cho, Y. S. (2009). A case of ricin poisoning following ingestion of Korean castor bean. *Emergency Medicine Journal*, *26*(4), 301–302. https://doi.org/10.1136/emj.2007.055368

Lin, H., Li, Z., Lin, H., Song, Y., Lv, L., & Hao, Z. (2015). Effect of pH shifts on IgE-binding capacity and conformational structure of tropomyosin from short-neck clam (*Ruditapes philippinarum*). *Food Chemistry*, *188*, 248–255. https://doi.org/10.1016/j.foodchem.2015.05.007

Linderoth, A., Biernat, M., Prykhodko, O., Kornilovska, I., Pusztai, A., Pierzynowski, S. G., & Björn, W. R. (2005). Induced growth and maturation of the gastrointestinal tract after *Phaseolus vulgaris* lectin exposure in suckling rats. *Journal of Pediatric Gastroenterology and Nutrition*, *41*(2), 195–203. https://doi.org/10.1097/01.mpg.0000172262.05297.98

Linderoth, A., Prykhod'ko, O., Ahrén, B., Fåk, F., Pierzynowski, S. G., & Weström, B. R. (2006). Binding and the effect of the red kidney bean lectin, phytohaemagglutinin, in the gastrointestinal tract of suckling rats. *British Journal of Nutrition*, *95*(1), 105–115. https://doi.org/10.1079/bjn20051612

Linderoth, A., Prykhod'ko, O., Pierzynowski, S. G., & Westrom, B. R. (2006). Enterally but not parenterally administered *Phaseolus vulgaris* lectin induces growth and precocious maturation of the gut in suckling rats. *Biology of the Neonate*, *89*(1), 60–68. https://doi.org/10.1159/000088563

Lioi, L., Sparvoli, F., Galasso, I., Lanave, C., & Bollini, R. (2003). Lectin-related resistance factors against bruchids evolved through a number of duplication events. *Theoretische und Angewandte Genetik*, *107*(5), 814–822. https://doi.org/10.1007/s00122-003-1343-8

Liu, C., Zhao, M., Sun, W., & Ren, J. (2013). Effects of high hydrostatic pressure treatments on haemagglutination activity and structural conformations of phytohemagglutinin from red kidney bean (*Phaseolus vulgaris*). *Food Chemistry*, *136*(3–4), 1358–1363. https://doi.org/10.1016/j.foodchem.2012.09.082

Liu, D., Hu, F. X., Zhang, H., Zhang, C., & Chen, S. H. (2019). ECL biosensor for sensitive detection of soybean agglutinin based on AuPt@C_{60} Nanoflowers enhanced N-(aminobutyl)-N-(ethylisoluminol). *Journal of the Electrochemical Society*, *166*(2), B49–B55. https://doi.org/10.1149/2.0541902jes

Liu, Q., Geng, R., Zhao, J., Chen, Q., & Kong, B. (2015). Structural and gel textural properties of soy protein isolate when subjected to extreme acid pH-shifting and mild heating processes. *Journal of Agricultural and Food Chemistry*, *63*(19), 4853–4861. https://doi.org/10.1021/acs.jafc.5b01331

Lopez Nunez OF, Pizon, A. F., & Tamama, K. (2017). Ricin poisoning after Oral ingestion of Castor beans: A case report and review of the literature and laboratory testing. *The Journal of Emergency Medicine*, *53*(5), e67–e71. https://doi.org/10.1016/j.jemermed.2017.08.023

Loris, R., Van Overberge, D., Dao-Thi, M. H., Poortmans, F., Maene, N., & Wyns, L. (1994). Structural analysis of two crystal forms of lentil lectin at 1.8 Å resolution. *Proteins*, *20*(4), 330–346. https://doi.org/10.1002/prot.340200406

Lotan, R., Skutelsky, E., Danon, D., & Sharon, N. (1975). The purification, composition, and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). *Journal of Biological Chemistry*, *250*(21), 8518–8523.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin-phenol reagent. *The Journal of Biological Chemistry*, *193*, 265–275.

Lu, Y., Liu, C., Zhao, M., Cui, C., & Ren, J. (2015). Structure and activity changes of Phytohemagglutinin from red kidney bean (*Phaseolus vulgaris*) affected by ultrahigh-pressure treatments. *Journal of Agricultural and Food Chemistry*, *63*(43), 9513–9519. https://doi.org/10.1021/acs.jafc.5b03337

Luo, L., Yang, J., Li, Z., Xu, H., Guo, L., Wang, L., Wang, Y., Luo, L., Wang, J., Zhang, P., Yang, R., Kang, W., & Xie, J. (2022). Label-free differentiation and quantification of ricin, abrin from their agglutinin biotoxins by surface plasmon resonance. *Talanta*, *238*(Pt 1), 122860. https://doi.org/10.1016/j.talanta.2021.122860

Luo, Y. W., & Xie, W. H. (2013). Effect of different processing methods on certain antinutritional factors and protein digestibility in green and white faba bean (*Vicia faba* L.). *CyTA Journal of Food*, *11*(1), 43–49. https://doi.org/10.1080/19476337.2012.681705

Luther, P., Sehrt, I., Bergmann, K. C., & Reutgen, H. (1978). Allergy and lectins: Action between IgE-mediated histamine release and glycoproteins from *Viscum album* L. (mistletoe). Acta Biologica et Medica. *Germanica*, *37*(10), 1623–1626.

Ma, S., & Yin, J. (2012). Anaphylaxis induced by ingestion of raw garlic. *Foodborne Pathogens and Disease*, *9*(8), 773–775. https://doi.org/10.1089/fpd.2012.1133

Mach, L., Scherf, W., Ammann, M., Poetsch, J., Bertsch, W., März, L., & Glössl, J. (1991). Purification and partial characterization of a novel lectin from elder (*Sambucus nigra* L.) fruit. *Biochemical Journal*, *278*(Pt 3), 667–671. https://doi.org/10.1042/bj2780667

Machado, F. P. P., Queiróz, J. H., Oliveira, M. G. A., Piovesan, N. D., Peluzio, M. C. G., Costa, N. M. B., & Moreira, M. A. (2008). Effects of heating on protein quality of soybean flour devoid of Kunitz inhibitor and lectin. *Food Chemistry*, *107*(2), 649–655. https://doi.org/10.1016/j.foodchem.2007.08.061

Mainz, D. L., Black, O., & Webster, P. D. (1973). Hormonal control of pancreatic growth. *The Journal of Clinical Investigation*, *52*(9), 2300–2304. https://doi.org/10.1172/JCI107418

Makkar, H. P., & Becker, K. (1999). Nutritional studies on rats and fish (carp *Cyprinus carpio*) fed diets containing unheated and heated *Jatropha curcas* meal of a non-toxic provenance. *Plant Foods for Human Nutrition*, *53*(3), 183–192. https://doi.org/10.1023/a:1008087627894

Manen, J. F., & Pusztai, A. (1982). Immunocytochemical localisation of lectins in cells of *Phaseolus vulgaris* L. seeds. *Planta*, *155*, 328–334. https://doi.org/10.1007/BF00429460

Manfredo-Vieira, S., Hiltensperger, M., Kumar, V., Zegarra-Ruiz, D., Dehner, C., Khan, N., Costa, F. R. C., Tiniakou, E., Greiling, T., Ruff, W., Barbieri, A., Kriegel, C., Mehta, S. S., Knight, J. R., Jain, D., Goodman, A. L., & Kriegel, M. A. (2018). Translocation of a gut pathobiont drives autoimmunity in mice and humans. *Science*, *359*(6380), 1156–1161. https://doi.org/10.1126/science.aar7201

Marsh, J. T., Johnson, P., Baumert, J., & Koppelman, S. J. (2024). Peanut lectin: Why it is not a peanut allergen. *Annals of Allergy, Asthma & Immunology*, *133*(2), 224–225. https://doi.org/10.1016/j.anai.2024.05.011

Martín-Cabrejas, M. A., & Esteban, R. M. (1995). Hard-to-cook phenomenon in beans: Changes in antinutrient factors and nitrogenous compounds during storage. *Journal of the Science of Food and Agriculture*, *69*(4), 429–435. https://doi.org/10.1002/jsfa.2740690405

Martín-Cabrejas, M. A., Jaime, L., Karanja, C., Downie, A. J., Parker, M. L., Lopez-Andreu, F. J., Maina, G., Esteban, R. M., Smith, A. C., & Waldron, K. W. (1999). Modifications to physicochemical and nutritional properties of hard-to-cook beans (*Phaseolus vulgaris* L.) by extrusion cooking. *Journal of Agricultural and Food Chemistry*, *47*(3), 1174–1182. https://doi.org/10.1021/jf980850m

Marungruang, E., Arévalo Sureda, E., Lefrançoise, A., Weström, B., Nyman, M., Prykhodko, O., & Fåk Hållenius, F. (2018). Impact of dietary induced precocious gut maturation on cecal microbiota and its relation to the blood-brain barrier during the postnatal period in rats. *Neurogastroenterology and Motility*, *30*(6), e13285. https://doi.org/10.1111/nmo.13285

Marzo, F., Alonso, R., Urdaneta, E., Arricibita, F. J., & Ibáñez, F. (2002). Nutritional quality of extruded kidney bean (*Phaseolus vulgaris* L. var. Pinto) and its effects on growth and skeletal muscle nitrogen fractions in rats. *Journal of Animal Science*, *80*(4), 875–879. https://doi.org/10.2527/2002.804875x

Marzo, F., Milagro, F. I., Urdaneta, E., Barrenetxe, J., & Ibañez, F. C. (2011). Extrusion decreases the negative effects of kidney bean on enzyme and transport activities of the rat small intestine. *Journal of Animal Physiology and Animal Nutrition*, *95*(5), 591–598. https://doi.org/10.1111/j.1439-0396.2010.01088.x

Mateo-Morejón, M., Labrada-Rosado, A., Torralba-Averoff, D., Cruz-Jimenez, R., Oliva-Díaz, Y., Álvarez-Castelló, M., Ciria-Martín, A., Jiménez-Frandín, M., Reyes-Zamora, M. C., Castro-Almarales, R. L., & Tamargo-García, B. (2017). Is peanut causing food allergy in Cuba? Preliminary assessment of allergic sensitization and IgE specificity profile to peanut allergens in Cuban allergic patients. *The World Allergy Organization Journal*, *10*(1), 26. https://doi.org/10.1186/s40413-017-0156-1

Matsui, T., Sato, N., Nakamura, M., Iwawaki, Y., Kitamura, K., Takasato, Y., Sugiura, S., Matsunaga, K., & Ito, K. (2023). A case of white bean allergy. *Asia Pacific Allergy*, *13*(4), 201–204. https://doi.org/10.5415/apallergy.0000000000000111

Matucci, A., Veneri, G., Dalla Pellegrina, C., Zoccatelli, G., Vincenzi, S., Chignola, R., Peruffo, A. D. B., & Rizzi, C. (2004). Temperature-dependent decay of wheat germ agglutinin activity and its implications for food processing and analysis. *Food Control*, *15*(5), 391–395. https://doi.org/10.1016/S0956-7135(03)00104-X

McHugh, J. (2018). Escape of gut microbe to the liver drives autoimmunity. *Nature Reviews Rheumatology*, *14*(5), 247. https://doi.org/10.1038/nrrheum.2018.53

Meagher, J. L., Winter, H. C., Ezell, P., Goldstein, I. J., & Stuckey, J. A. (2005). Crystal structure of banana lectin reveals a novel second sugar binding site. *Glycobiology*, *15*(10), 1033–1042. https://doi.org/10.1093/glycob/cwi088

Menéndez-Rey, A., González-Martos, R., Ye, P., Quiroz-Troncoso, J., Alegría-Aravena, N., Sánchez-Díez, M., Maestu-Unturbe, C., Bensadon-Naeder, L., & Ramírez-Castillejo, C. (2021). Quantification of lectins in *Synsepalum dulcificum* and comparison with reference foods. *Food Chemistry*, *352*, 129341. https://doi.org/10.1016/j.foodchem.2021.129341

Merten, C., Ferrari, P., Bakker, M., Boss, A., Hearty, A., Leclercq, C., Lindtner, O., Tlustos, C., Verger, P., Volatier, J. L., & Arcella, D. (2011). Methodological characteristics of the national dietary surveys carried out in the European Union as included in the European food safety authority (EFSA) comprehensive European food consumption database. *Food Additives & Contaminants Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *28*(8), 975–995. https://doi.org/10.1080/19440049.2011.576440

Mialonier, G., Privat, J. P., Monsigny, M., Kahlen, G., & Durand, R. (1973). Isolation, physico-chemical properties and in vivo localization of a phytohemagglutinin (lectin) from *Phaseolus vulgaris* (red kidney bean). *Physiologie Vegetale*, *11*(3), 519–537.

Mikkola, J. H., Alenius, H., Kalkkinen, N., Turjanmaa, K., Palosuo, T., & Reunala, T. (1998). Hevein-like protein domains as a possible cause for allergen cross-reactivity between latex and banana. *The Journal of Allergy and Clinical Immunology*, *102*(6 Pt 1), 1005–1012. https://doi.org/10.1016/s0091-6749(98)70339-2

Mirkov, T. E., Wahlstron, J. M., Hagiwara, K., Finardi-Filho, F., Kjamtrup, S., & Chrispeels, M. J. (1994). Evolutionary relationships among proteins in the phytohemagglutinin-arcelin-α-amylase inhibitor family of the common bean and its relatives. *Plant Molecular Biology*, *26*, 1103–1113. https://doi.org/10.1007/BF00040692

Mitchell, G. F., & Clarke, A. E. (1979). Allergenicity of concanavalin A in mice. *International Archives of Allergy and Applied Immunology*, *58*(4), 391–401. https://doi.org/10.1159/000232218

Miyake, K., Tanaka, T., & McNeil, P. L. (2007). Lectin-based food poisoning: A new mechanism of protein toxicity. *PLoS One*, *2*(8), e687. https://doi.org/10.1371/journal.pone.0000687

Molla, A. R., & Mandal, D. K. (2013). Trifluoroethanol-induced conformational change of tetrameric and monomeric soybean agglutinin: Role of structural organization and implication for protein folding and stability. *Biochimie*, *95*(2), 204–214. https://doi.org/10.1016/j.biochi.2012.09.011

Mondal, H. A., Chakraborti, D., Majumder, P., Roy, P., Roy, A., Bhattacharya, S. G., & Das, S. (2011). Allergenicity assessment of *Allium sativum* leaf agglutinin, a potential candidate protein for developing sap sucking insect resistant food crops. *PLoS One*, *6*(11), e27716. https://doi.org/10.1371/journal.pone.0027716

Moneret-Vautrin, D. A., Morisset, M., Lemerdy, P., Croizier, A., & Kanny, G. (2002). Food allergy and IgE sensitization caused by spices: CICBAA data (based on 589 cases of food allergy). *Allergy and Immunology*, *34*(4), 135–140.

Monsigny, M., Jeune-Chung, K. H., & Perrodon, Y. (1978). Separation and biological properties of *Phaseolus vulgaris* isolectins. *Biochimie*, *60*(11–12), 1315–1322. https://doi.org/10.1016/s0300-9084(79)80449-6

Montero-Morales, L., Maresch, D., Castilho, A., Turupcu, A., Ilieva, K./. M., Crescioli, S., Karagiannis, S. N., Lupinek, C., Oostenbrink, C., Altmann, F., & Steinkellner, H. (2017). Recombinant plant-derived human IgE glycoproteomics. *Journal of Proteomics*, *161*, 81–87. https://doi.org/10.1016/j.jprot.2017.04.002

Morales, P., Berrios, J. D. J., Varela, A., Burbano, C., Cuadrado, C., Muzquiz, M., & Pedrosa, M. M. (2015). Novel fiber-rich lentil flours as snack-type functional foods: An extrusion cooking effect on bioactive compounds. *Food & Function*, *6*(9), 3135–3143. https://doi.org/10.1039/C5FO00729A

Morales-de León, J. C., Vázquez-Mata, N., Torres, N., Gil-Zenteno, L., & Bressani, R. (2007). Preparation and characterization of protein isolate from fresh and hardened beans (*Phaseolus vulgaris* L.). *Journal of Food Science*, *72*(2), C96–C102. https://doi.org/10.1111/j.1750-3841.2006.00244.x

Moreno, J., & Chrispeels, M. J. (1989). A lectin gene encodes the α-amylase inhibitor of the common bean. *Proceedings of the National Academy of Sciences of the USA*, *86*(20), 7885–7889. https://doi.org/10.1073/pnas.86.20.7885

Mosha, T. C. E., Bennink, M. R., & Ng, P. K. W. (2005). Nutritional quality of drum-processed and extruded composite supplementary foods. *Journal of Food Science*, *70*(2), C138–C144. https://doi.org/10.1111/j.1365-2621.2005.tb07074.x

Mourey, L., Pédelacq, J. D., Birck, C., Fabre, C., Rougé, P., & Samama, J. P. (1998). Crystal structure of the arcelin-1 dimer from *Phaseolus vulgaris* at 1.9-Å resolution. *The Journal of Biological Chemistry*, *273*(21), 12914–12922. https://doi.org/10.1074/jbc.273.21.12914

Mubarak, A. E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. *Food Chemistry*, *89*(4), 489–495. https://doi.org/10.1016/j.foodchem.2004.01.007

Muzquiz, M., Pedrosa, M. M., Trugo, L., Ayet, G., Burnano, C., & Cuadrado, C. (1998). Influence of malting process on levels of non-nutritive compounds. In E. Carnovale (Ed.), *Effects of antinutrients on the nutritional value of legume diets* (pp. 153–159). Commission European Communities.

Nachbar, M. S., & Oppenheim, J. D. (1980). Lectins in the United States diet: A survey of lectins in commonly consumed foods and a review of the literature. *The American Journal of Clinical Nutrition*, *33*(11), 2338–2345. https://doi.org/10.1093/ajcn/33.11.2338

Naeem, A., Khan, R. H., & Saleemuddin, M. (2006). Single step immobilized metal ion affinity precipitation/chromatography based procedures for purification of concanavalin A and *Cajanus cajan* mannose-specific lectin. *Biochemistry*, *71*(1), 56–59. https://doi.org/10.1134/s0006297906010081

Nagae, M., Kanagawa, M., Morita-Matsumoto, K., & Hanashima, S. (2016). Atomic visualization of a flipped-back conformation of bisected glycans bound to specific lectins. *Scientific Reports*, *6*, 22973. https://doi.org/10.1038/srep22973

Nagai, K., & Yamaguchi, H. (1993). Direct demonstration of the essential role of the intramolecular high-mannose oligosaccharide chains in the folding and assembly of soybean (*Glycine max*) lectin polypeptides. *Journal of Biochemistry*, *113*(2), 123–135. https://doi.org/10.1093/oxfordjournals.jbchem.a12401

Naik, S., Rawat, R. S., Khandai, S., Kumar, M., Jena, S. S., Vijayalakshmi, M. A., & Kumar, S. (2017). Biochemical characterisation of lectin from Indian hyacinth plant bulbs with potential inhibitory action against human cancer cells. *International Journal of Biological Macromolecules*, *105*(Pt 1), 1349–1356. https://doi.org/10.1016/j.ijbiomac.2017.07.170

Naismith, J. H., Emmerich, C., Habash, J., Harrop, S. J., Helliwell, J. R., Hunter, W. N., Raftery, J., Kalb, A. J., & Yariv, J. (1994). Refined structure of concanavalin A complexed with methyl a-D-mannopyranoside at 2.0 Å resolution and comparison with saccharide-free structure. *Acta Crystallographica. Section D, Biological Crystallography*, *50*(6), 847–858. https://doi.org/10.1107/S0907444994005287

Nakamura, S., Tanaka, K., & Murakawa, S. (1960). Specific protein of legumes which reacts with animal proteins. *Nature*, *188*, 144–145. https://doi.org/10.1038/188144b0

Nakata, S., & Kimura, T. (1985). Effect of ingested toxic bean lectins on the gastrointestinal tract in the rat. *Journal of Nutrition*, *115*(12), 1621–1629. https://doi.org/10.1093/jn/115.12.1621

Nareddy, P. K., & Swamy, M. J. (2018). Differential scanning calorimetric and spectroscopic studies on the thermal and chemical unfolding of cucumber (*Cucumis sativus*) phloem exudate lectin. *International Journal of Biological Macromolecules*, *106*, 95–100. https://doi.org/10.1016/j.ijbiomac.2017.07.173

Nascimento, K. S., Cunha, A. I., Nascimento, K. S., Cavada, B. S., Azevedo, A. M., & Aires-Barros, M. R. (2012). An overview of lectins purification strategies. *Journal of Molecular Recognition*, *25*(11), 527–541. https://doi.org/10.1002/jmr.2200

Nasi, A., Picariello, G., & Ferranti, P. (2009). Proteomic approaches to study structure, functions and toxicity of legume seeds lectins. Perspectives for the assessment of food quality and safety. *Journal of Proteomics*, *72*(3), 527–538. https://doi.org/10.1016/j.jprot.2009.02.001

Nciri, N., Cho, N., El Mhamdi, F., Ben Ismail, H., Ben Mansour, A., Sassi, F. H., & Ben Aissa-Fennira, F. (2015). Toxicity assessment of common beans (*Phaseolus vulgaris* L.) widely consumed by Tunisian population. *Journal of Medicinal Food*, *18*(9), 1049–1064. https://doi.org/10.1089/jmf.2014.0120

Ninan, E. C., & James, E. (2019). Acute disseminated encephalomyelitis due to *abrus precatorius* poisoning – A case report. *Saudi Pharmaceutical Journal*, *27*(4), 521–524. https://doi.org/10.1016/j.jsps.2018.11.016

Njoroge, D. M., Kinyanjui, P., Makokha, A. O., Christiaens, S., Shpigelman, A., Sila, D. N., & Hendrickx, M. E. (2014). Extraction and characterization of pectic polysaccharides from easy- and hard-to-cook common beans (*Phaseolus vulgaris*). *Food Research International*, *64*, 314–322. https://doi.org/10.1016/j.foodres.2014.06.044

Noah, N. D., Bender, A. E., Reaidi, G., & Gilbert, R. J. (1980). Food poisoning from raw red kidney beans. *British Medical Journal*, *281*(6234), 236–237.

Norozi, M., Rezaei, M., & Kazemifard, M. (2022). Effect of different acid processing methodologies on the nutritional value and reduction of anti-nutrients in soybean meal. *Journal of Food Processing and Preservation*, *46*, e16205. https://doi.org/10.1111/jfpp.16205

NRC (National Research Council). (2012). *Nutrient requirements of swine* (11 Revised ed.). The National Academies Press. https://doi.org/10.17226/13298

Nu, M. A. T., Lupatsch, I., Zannatta, J. S., Schulze, H., & Zijlstra, R. T. (2020). Thermomechanical and enzyme-facilitated processing of soybean meal enhanced in vitro kinetics of protein digestion and protein and amino acid digestibility in weaned pigs. *Journal of Animal Science*, *98*(8), 1–9. https://doi.org/10.1093/jas/skaa224

Oboh, H. A., Muzquiz, M., Burbano, C., Cuadrado, C., Pedrosa, M. M., Ayet, G., & Osagie, A. U. (1998). Anti-nutritional constituents of six underutilized legumes grown in Nigeria. *Journal of Chromatography. A*, *823*(1–2), 307–312. https://doi.org/10.1016/s0021-9673(98)00542-1

Ochoa, J. L., & Kristiansen, T. (1982). Purification and partial characterization of an agglutinin from *Phaseolus coccineus* var. 'alubia'. *Biochimica et Biophysica Acta*, *705*(3), 396–404. https://doi.org/10.1016/0167-4838(82)90262-x

Ogawa, H., & Date, K. (2014). The "white kidney bean incident" in Japan. *Methods in Molecular Biology*, *1200*, 39–45. https://doi.org/10.1007/978-1-4939-1292-6_3

Ojimelukwe, P. C., Onuoha, C. C., & Obanu, Z. A. (1995a). Effects of processing and in vitro proteolytic enzyme action on bambarra groundnut hemagglutinin. *Journal of Food Science and Technology*, *32*(1), 58–61.

Ojimelukwe, P. C., Onuoha, C. C., & Obanu, Z. A. (1995b). Effects of processing and in vitro proteolytic digestion on soybean and yambean hemagglutinin. *Plant Foods for Human Nutrition*, *47*(4), 293–299. https://doi.org/10.1007/BF01088266

Oladunjoye, I. O., Ologhobo, A. D., & Olaniyi, C. O. (2010). Nutrient composition, energy value and residual antinutritional factors in differently processed breadfruit (*Artocarpus altilis*) meal. *African Journal of Biotechnology*, *9*(27), 4259–4263.

Olivera-Castillo, L., Pino-Aguilar, M., Lara-Flores, M., Granados-Puerto, S., Montero-Muñoz, J., Olvera-Novoa, M., & Grant, G. (2011). Substitution of fish meal with raw or treated cowpea (*Vigna unguiculata* L Walp, IT86-D719) meal in diets for Nile tilapia (*Oreochromis niloticus* L.) fry. *Aquaculture Nutrition*, *17*(2), e101–e111. https://doi.org/10.1111/j.1365-2095.2009.00739.x

Olmedilla-Alonso, B., Pedrosa, M. M., Cuadrado, C., Brito, M., Asensio-S-Manzanera, C., & Asensio-Vegas, C. (2013). Composition of two Spanish common dry beans (*Phaseolus vulgaris*), 'Almonga' and 'Curruquilla', and their postprandial effect in type 2 diabetics. *Journal of the Science of Food and Agriculture*, *93*(5), 1076–1082. https://doi.org/10.1002/jsfa.5852

Olsen, L. R., Dessen, A., Gupta, D., Sabesan, S., Sacchettini, J. C., & Brewer, C. F. (1997). X-ray crystallographic studies of unique cross-linked lattices between four isomeric biantennary oligosaccharides and soybean agglutinin. *Biochemistry*, *36*(49), 15073–15080. https://doi.org/10.1021/bi971828+

Olsnes, S., Saltvedt, E., & Pihl, A. (1974). Isolation and comparison of galactose-binding lectins from *Abrus precatorius* and *Ricinus communis*. *Journal of Biological Chemistry*, *249*(3), 803–810. https://doi.org/10.1016/S0021-9258(19)43000-7

Omoikhoje, S. O., Aruna, M. B., & Bamgbose, A. M. (2009). Effect of cooking time on some nutrient and antinutrient components of Bambara groundnut seeds. *Animal Science Journal*, *80*(1), 52–56. https://doi.org/10.1111/j.1740-0929.2008.00599.x

Onwuka, G. I. (2006). Soaking, boiling and antinutritional factors in pigeon peas (*Cajanus cajan*) and cowpeas (*Vigna unguiculata*). *Journal of Food Processing and Preservation*, *30*(5), 616–630. https://doi.org/10.1111/j.1745-4549.2006.00092.x

Orúe, E., Butrón, J., Ibañez, F., Alonso, R., & Marzo, F. (1998). The effect of germination and extrusion on the nutritional quality of peas (*Pisum sativum* L. cv. Ballet). In J. AJM, G. D. Hill, J. Huisman, & V. der Poel AFB (Eds.), *Recent advances of research in antinutritional factors in legume seeds and rapeseed* (pp. 417–420). Wageningen Press.

Osborn, T. C., Burow, M., & Bliss, F. A. (1988). Purification and characterization of arcelin seed protein from common bean. *Plant Physiology*, *86*(2), 399–405. https://doi.org/10.1104/pp.86.2.399

Otte, J. M., Chen, C. X., Brunke, G., Kiehne, K., Schmitz, F., Fölsch, U. R., & Herzig, K. H. (2001). Mechanisms of lectin (phytohemagglutinin)-induced growth in small intestinal epithelial cells. *Digestion*, *64*(3), 169–178. https://doi.org/10.1159/000048858

Ovelgönne, J. H., Koninkx, J. F., Pusztai, A., Bardocz, S., Kok, W., Ewen, S. W., Hendriks, H. G., & van Dijk, J. E. (2000). Decreased levels of heat shock proteins in gut epithelial cells after exposure to plant lectins. *Gut*, *46*(5), 679–687. https://doi.org/10.1136/gut.46.5.680

Pabst, O., & Mowat, A. M. (2012). Oral tolerance to food protein. *Mucosal Immunology*, *5*(3), 232–239. https://doi.org/10.1038/mi.2012.4

Pabst, O., & Slack, E. (2020). IgA and the intestinal microbiota: The importance of being specific. *Mucosal Immunology*, *13*(1), 12–21. https://doi.org/10.1038/s41385-019-0227-4

Padiyappa, S. D., Avalappa, H., Somegowda, M., Sridhara, S., Venkatesh, Y. P., Prabhakar, B. T., Pramod, S. N., Almujaydil, M. S., Shokralla, S., Abdelbacki, A. M. M., Elansary, H. O., El-Sabrout, A. M., & Mahmoud, E. A. (2022). Immunoadjuvant and humoral immune responses of garlic (*Allium sativum* L.) lectin upon systemic and mucosal administration in BALB/c mice. *Molecules*, *27*(4), 1375. https://doi.org/10.3390/molecules27041375

Palatnick, W., & Tenenbein, M. (2000). Hepatotoxicity from castor bean ingestion in a child. *Journal of Toxicology. Clinical Toxicology*, *38*(1), 67–69. https://doi.org/10.1081/clt-100100920

Pan, L., Liu, Y., Lan, H., Bao, N., Zhao, Y., Sun, H., Qin, G., & Farouk, M. H. (2021). Biological mechanisms induced by soybean agglutinin using an intestinal cell model of Monogastric animals. *Frontiers in Veterinary Science*, *8*, 639792. https://doi.org/10.3389/fvets.2021.639792

Park, D., Brune, K. A., Mitra, A., Marusina, A. I., Maverakis, E., & Lebrilla, C. B. (2015). Characteristic changes in cell surface glycosylation accompany intestinal epithelial cell (IEC) differentiation: High mannose structures dominate the cell surface Glycome of undifferentiated enterocytes. *Molecular & Cellular Proteomics*, *14*(11), 2910–2921. https://doi.org/10.1074/mcp.M115.053983

Pascal, J. M., Day, P. J., Monzingo, A. F., Ernst, S. R., Robertus, J. D., Iglesias, R., Pérez, Y., Férreras, J. M., Citores, L., & Girbés, T. (2001). 2.8-Å crystal structure of a nontoxic type-II ribosome-inactivating protein, ebulin I. *Proteins*, *43*(3), 319–326. https://doi.org/10.1002/prot.1043

Pasqualone, A., Costantini, M., Coldea, T. E., & Summo, C. (2020). Use of legumes in extrusion cooking: A review. *Food*, *9*(7), 958. https://doi.org/10.3390/foods9070958

Pastorello, E. A., Farioli, L., Pravettoni, V., Ortolani, C., Fotunato, D., Giuffrida, M. G., Perono Garoffo, L., Calamari, A. M., Brenna, O., & Conti, A. (2003). Identification of grape and wine allergens as an endochitinase 4, a lipid-transfer protein, and a thaumatin. *Journal of Allergy and Clinical Immunology*, *111*(2), 350–359. https://doi.org/10.1067/mai.2003.35

Pastorello, E. A., Farioli, L., Pravettoni, V., Scibilia, J., Conti, A., Fortunato, D., Borgonovo, L., Bonomi, S., Primavesi, L., & Ballmer-Weber, B. (2009). Maize food allergy: Lipid-transfer proteins, endochitinases, and alpha-zein precursor are relevant maize allergens in double-blind placebo-controlled maize-challenge-positive patients. *Analytical and Bioanalytical Chemistry*, *295*(1), 93–102. https://doi.org/10.1007/s00216-009-2945-z

Patil, M. M., Patil, S. V., Akki, A. S., Lakhkar, B., & Badiger, S. (2016). An arrow poison (*Abrus Precatorius*) causing fatal poisoning in a child. *Journal of Clinical and Diagnostic Research*, *10*(3), SD03–SD04. https://doi.org/10.7860/JCDR/2016/18234.7439

Paucar-Menacho, L. M., Berhow, M. A., Gontijo Mandarino, J. M., Gonzalez de Mejia, E., & Chang, Y. K. (2010). Optimisation of germination time and temperature on the concentration of bioactive compounds in Brazilian soybean cultivar BRS 133 using response surface methodology. *Food Chemistry*, *119*(2), 636–642. https://doi.org/10.1016/j.foodchem.2009.07.011

Peddio, S., Lorrai, S., Padiglia, A., Cannea, F. B., Dettori, T., Cristiglio, V., Genovese, L., Zucca, P., & Rescigno, A. (2023). Biochemical and phylogenetic analysis of Italian *Phaseolus vulgaris* cultivars as sources of α-amylase and α-glucosidase inhibitors. *Plants*, *12*(16), 2918. https://doi.org/10.3390/plants12162918

Pedrosa, M. M., Cuadrado, C., Burbano, C., Muzquiz, M., Cabellos, B., Olmedilla-Alonso, B., & Asensio-Vegas, C. (2015). Effects of industrial canning on the proximate composition, bioactive compounds contents and nutritional profile of two Spanish common dry beans (*Phaseolus vulgaris* L.). *Food Chemistry*, *166*, 68–75. https://doi.org/10.1016/j.foodchem.2014.05.158

Pereira, M. E. A., Kabat, E. A., & Sharon, N. (1974). Immunochemical studies on the specificity of soybean agglutinin. *Carbohydrate Research*, *37*(1), 89–102. https://doi.org/10.1016/s0008-6215(00)87066-4

Perera, D., Devkota, L., Garnier, G., Panozzo, J., & Dhital, S. (2023). Hard-to-cook phenomenon in common legumes: Chemistry, mechanisms and utilization. *Food Chemistry*, *415*, 135743. https://doi.org/10.1016/j.foodchem.2023.135743

Pérez-Pimiento, A. J., Moneo, I., Santaolalla, M., de Paz, S., Fernández-Parra, B., & Domínguez-Lázaro, A. R. (1999). Anaphylactic reaction to young garlic. *Allergy*, *54*(6), 626–629. https://doi.org/10.1034/j.1398-9995.1999.00806.x

Petry, N., Rohner, F., Gahutu, J. B., Campion, B., Boy, E., Tugirimana, P. L., Zimmerman, M. B., Zwahlen, C., Wirth, J. P., & Moretti, D. (2016). In Rwandese women with low iron status, iron absorption from low-Phytic acid beans and biofortified beans is comparable, but low-Phytic acid beans cause adverse gastrointestinal symptoms. *The Journal of Nutrition*, *146*(5), 970–975. https://doi.org/10.3945/jn.115.223693

Peumans, W. J., De Ley, M., & Broekaert, W. F. (1984). An unusual lectin from stinging nettle (*Urtica dioica*) rhizomes. *FEBS Letters*, *177*(1), 99–103. https://doi.org/10.1016/0014-5793(84)80989-8

Peumans, W. J., Rougé, P., & Van Damme, E. J. M. (2003). The tomato lectin consists of two homologous chitin-binding modules separated by an extension-like linker. *Biochemical Journal*, *376*(Pt 3), 717–724. https://doi.org/10.1042/BJ20031069

Peumans, W. J., Roy, S., Barre, A., Rougé, P., Van Leuven, F., & Van Damme, E. J. (1998). Elderberry (*Sambucus nigra*) contains truncated Neu5Ac(a-2,6)gal/GalNAc-binding type 2 ribosome-inactivating proteins. *FEBS Letters*, *425*(1), 35–39. https://doi.org/10.1016/s0014-5793(98)00193-8

Peumans, W. J., & Van Damme, E. J. M. (1996). Prevalence, biological activity and genetic manipulation of lectins in foods. *Trends in Food Science and Technology*, *7*(4), 132–138. https://doi.org/10.1016/0924-2244(96)10015-7

Peumans, W. J., & Van Damme, E. J. M. (1998). Plant lectins: Versatile proteins with important perspectives in biotechnology. *Biotechnology and Genetic Engineering Reviews*, *15*(1), 199–228. https://doi.org/10.1080/02648725.1998.10647956

Pita-López, W., Gomez-Garay, M., Blanco-Labra, A., Aguilera-Barreyro, A., Reis-de Souza, T. C., Olvera-Ramírez, A., Ferriz-Martinez, R., & García-Gasca, T. (2020). Tepary bean (*Phaseolus acutifolius*) lectin fraction provokes reversible adverse effects on rats' digestive tract. *Toxicology Research*, *9*(5), 714–725. https://doi.org/10.1093/toxres/tfaa062

Pittschieler, K., Ladinser, B., & Petell, J. K. (1994). Reactivity of gliadin and lectins with celiac intestinal mucosa. *Pediatric Research*, *36*(5), 635–641. https://doi.org/10.1203/00006450-199411000-00018

Poblete, T., Rebolledo, K., Barrera, C., Ulloa, D., Valenzuela, M., Valenzuela, C., Pavez, E., Mendoza, R., Narbona, C., González, J., Estevez, S., Ortega, R., & González, C. (2020). Effect of germination and cooking on iron content, phytic acid and lectins in four varieties of chilean beans (*Phaseolus vulgaris*). *Journal of the Chilean Chemical Society*, *65*(4), 4937–4942. https://doi.org/10.4067/S0717-97072020000404937

Pohleven, J., Štrukelj, B., & Kos, J. (2012). Affinity chromatography of lectins. In S. Magdeldin (Ed.), *Affinity chromatography* (pp. 49–74). IntechOpen. https://doi.org/10.5772/36578

Poola, I., Seshadri, H. S., & Bhavanandan, V. P. (1986). Purification and saccharide-binding characteristics of a rice lectin. *Carbohydrate Research*, *146*(2), 205–217. https://doi.org/10.1016/0008-6215(86)85040-6

Posch, A., Wheeler, C. H., Chen, Z., Flagge, A., Dunn, M. J., Papenfuss, F., Raul-Heimsoth, M., & Baur, X. (1999). Class I endochitinase containing a hevein domain is the causative allergen in latex-associated avocado allergy. *Clinical and Experimental Allergy*, *29*(5), 667–672. https://doi.org/10.1046/j.1365-2222.1999.00502.x

Pramod, S. N., Krishnakantha, T. P., & Venkatesh, Y. P. (2006). Effects of horse gram lectin (*Dolichos biflorus* agglutinin) on degranulation of mast cells and basophils of atopic subjects: Identification as an allergen. *International Immunopharmacology*, *6*(11), 1714–1722. https://doi.org/10.1016/j.intimp.2006.07.006

Pramod, S. N., Venkatesh, Y. P., & Mahesh, P. A. (2007). Potato lectin activates basophils and mast cells of atopic subjects by its interaction with core chitobiose of cell-bound non-specific immunoglobulin E. *Clinical and Experimental Immunology*, *148*(3), 391–401. https://doi.org/10.1111/j.1365-2249.2007.03368.x

Prasthofer, T., Phillips, S. R., Suddath, F. L., & Engler, J. A. (1989). Design, expression, and crystallization of recombinant lectin from the garden pea (*Pisum sativum*). *Journal of Biological Chemistry*, *264*(12), 6793–6796.

Pratap, J. V., Jeyaprakash, A. A., Rani, P. G., Sekar, K., Surolia, A., & Vijayan, M. (2002). Crystal structures of artocarpin, a Moraceae lectin with mannose specificity, and its complex with methyl-α-D-mannose: Implications to the generation of carbohydrate specificity. *Journal of Molecular Biology*, *317*(2), 237–247. https://doi.org/10.1006/jmbi.2001.5432

Prykhod'ko, O., Fed'kiv, O., Linderoth, A., Pierzynowski, S. G., & Weström, B. R. (2009). Precocious gut maturation and immune cell expansion by single dose feeding the lectin phytohaemagglutinin to suckling rats. *The British Journal of Nutrition*, *101*(5), 735–742. https://doi.org/10.1017/S0007114508035940

Prykhod'ko, O., Fed'kiv, O., Pierzynowski, S. G., Svendsen, J., & Weström, B. R. (2010). Hormonal and immune profiles in blood were unaffected by PHA provocation in suckling and weaning pigs. *Livestock Science*, *133*(1–3), 253–256. https://doi.org/10.1016/j.livsci.2010.06.079

Pryme, I. F., & Aarra, T. M. (2021). Exhaustive overview of dietary plant lectins: Prospective importance in the Mediterranean diet. *American Journal of Biomedical Science & Research*, *13*(4), AJBSR.MS.ID.001883. https://doi.org/10.34297/AJBSR.2021.13.001883

Pueppke, S. G. (1979). Distribution of lectins in the jumbo Virginia and Spanish varieties of the peanut, *Arachis Hypogaea* L. *Plant Physiology*, *64*(4), 575–580. https://doi.org/10.1104/pp.64.4.575

Pull, S. P., Pueppke, S. G., Hymowitz, T., & Orf, J. H. (1978). Soybean lines lacking the 120,000-dalton seed lectin. *Science*, *200*(4347), 1277–1279. https://doi.org/10.1126/science.200.4347.1277

Purhonen, A. K., Herzig, K. H., Gabius, H. J., André, S., Ketterer, S., Matzinger, D., Beglinger, C., & Hildebrand, P. (2008). Duodenal phytohaemagglutinin (red kidney bean lectin) stimulates gallbladder contraction in humans. *Acta Physiologica*, *193*(3), 241–247. https://doi.org/10.1111/j.1748-1716.2008.01839.x

Pusztai, A., Clarke, E. M., King, T. P., & Stewart, J. C. (1979). Nutritional evaluation of kidney beans (*Phaseolus vulgaris*): Chemical composition, lectin content and nutritional value of selected cultivars. *Journal of the Science of Food and Agriculture*, *30*(9), 843–848. https://doi.org/10.1002/jsfa.2740300902

Pusztai, A., Ewen, S. W., Grant, G., Brown, D. S., Stewart, J. C., Peumans, W. J., Van Damme, E. J., & Bardocz, S. (1993). Antinutritive effects of wheat-germ agglutinin and other N-acetylglucosamine-specific lectins. *British Journal of Nutrition*, *70*(1), 313–321. https://doi.org/10.1079/bjn19930124

Pusztai, A., Ewen, S. W. B., Grant, G., Peumans, W. J., Vandamme, E. J. M., Rubio, L., & Bardocz, S. (1990). Relationships between survival and binding of plant-lectins during small intestinal passage and their effectiveness as growth-factors. *Digestion*, *46*(2), 308–316. https://doi.org/10.1159/000200402

Pusztai, A., Grant, F., & Grant, G. (1989). Specific uptake of dietary lectins into the systemic circulation of rats. *Biochemical Society Transactions*, *17*(3), 481–482. https://doi.org/10.1042/bst0170481

Pusztai, A., Grant, G., Spencer, R. J., Duguid, T. J., Brown, D. S., Ewen, S. W., Peumans, W. J., Van Damme, E. J., & Bardocz, S. (1993). Kidney bean lectin-induced *Escherichia coli* overgrowth in the small intestine is blocked by GNA, a mannose-specific lectin. *The Journal of Applied Bacteriology*, *75*(4), 360–368. https://doi.org/10.1111/j.1365-2672.1993.tb02788.x

Pusztai, A., Grant, G., & Stewart, J. C. (1981). A new type of *Phaseolus vulgaris* (cv. Pinto III) seed lectin: Isolation and characterization. *Biochimica et Biophysica Acta*, *671*(2), 146–154. https://doi.org/10.1016/0005-2795(81)90128-8

Qayyum, M. M. N., Butt, M. S., Anjum, F. M., & Nawaz, H. (2012). Composition analysis of some selected legumes for protein isolates recovery. *Journal of Animal and Plant Sciences*, *22*(4), 1156–1162.

Qin, G., ter Elst, E. R., Bosch, M. W., & van der Poel, A. F. B. (1996). Thermal processing of whole soya beans: Studies on the inactivation of antinutritional factors and effects on ileal digestibility in piglets. *Animal Feed Science and Technology*, *57*(4), 313–324. https://doi.org/10.1016/0377-8401(95)00863-2

Radauer, C., Adhami, F., Fürtler, I., Wagner, S., Allwardt, D., Scala, E., Ebner, C., Hafner, C., Hemmer, W., Mari, A., & Breiteneder, H. (2011). Latex-allergic patients sensitized to the major allergen hevein and hevein-like domains of class I chitinases show no increased frequency of latex-associated plant food allergy. *Molecular Immunology*, *48*(4), 600–609. https://doi.org/10.1016/j.molimm.2010.10.019

Rahaman, T., Vasiljevic, T., & Ramchandran, L. (2016). Shear, heat and pH induced conformational changes of wheat germ gluten – Impact on antigenicity. *Food Chemistry*, *196*, 180–188. https://doi.org/10.1016/j.foodchem.2015.09.041

Rameshwaram, N. R., & Nadimpalli, S. K. (2008). An efficient method for the purification and quantification of a galactose-specific lectin from vegetative tissues of *Dolichos lablab*. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, *861*(2), 209–217. https://doi.org/10.1016/j.jchromb.2007.09.020

Rani, P. G., Bachhawat, K., Misquith, S., & Surolia, A. (1999). Thermodynamic studies of saccharide binding to artocarpin, a B-cell mitogen, reveals the extended nature of interaction with mannotriose [3,6-Di-O-(a-D-mannopyranosyl)-D-mannose]. *The Journal of Biological Chemistry*, *274*(42), 29694–29698. https://doi.org/10.1074/jbc.274.42.29694

Ravindran, V., Ravindran, G., & Sivakanesan, R. (1996). Evaluation of the nutritive value of jackseed (*Artocarpus heterophyllus*) meal for poultry. *The Journal of Agricultural Science*, *127*(1), 123–130. https://doi.org/10.1017/S0021859600077443

Ravishankar, R., Suguna, K., Surolia, A., & Vijayan, M. (1999). Structures of the complexes of peanut lectin with methyl-b-galactose and N-acetyllactosamine and a comparative study of carbohydrate binding in gal/GalNAc-specific legume lectins. *Acta Crystallographica. Section D, Biological Crystallography*, *55*(Pt 8), 1375–1382. https://doi.org/10.1107/s0907444999006587

Rea, R. L., Thompson, L. U., & Jenkins, D. J. A. (1985). Lectins in food and their relation to starch digestibility. *Nutrition Research*, *5*(9), 919–929. https://doi.org/10.1016/S0271-5317(85)80105-6

Rearick, J. I., Kulczycki, A., Jr., & Kornfeld, S. (1983). Structural studies of oligosaccharides of rat IgE and reexamination of the high-mannose oligosaccharide of human IgE. *Archives of Biochemistry and Biophysics*, *220*(1), 95–105. https://doi.org/10.1016/0003-9861(83)90391-0

Reeke, G. N., Jr., & Becker, J. W. (1986). Three-dimensional structure of favin: Saccharide binding-cyclic permutation in leguminous lectins. *Science*, *234*(4780), 1108–1111. https://doi.org/10.1126/science.3775378

Rehfeld, J. F., Hansen, H. F., Larsson, L. I., Stengaard-Pedersen, K., & Thorn, N. A. (1984). Gastrin and cholecystokinin in pituitary neurons. *Proceedings of the National Academy of Sciences of the USA*, *81*(6), 1902–1905. https://doi.org/10.1073/pnas.81.6.1902

Restum-Miguel, N., & Prouvost-Danon, A. (1985). Effects of multiple oral dosing on IgE synthesis in mice: Oral sensitization by albumin extracts from seeds of Jack fruit (*Artocarpus integrifolia*) containing lectins. *Immunology*, *54*(3), 497–504.

Reyes-Moreno, C., & Paredes-López, O. (1993). Hard-to-cook phenomenon in common beans – A review. *Critical Reviews in Food Science and Nutrition*, *33*(3), 227–286. https://doi.org/10.1080/10408399309527621

Rice, R. H., & Etzler, M. E. (1975). Chemical modification and hybridization of wheat germ agglutinins. *Biochemistry*, *14*(18), 4093–4099. https://doi.org/10.1021/bi00689a027

Rizzi, C., Galeoto, L., Zoccatelli, G., Vincenzi, S., Chignola, R., & Peruffo, A. D. B. (2003). Active soybean lectin in foods: Quantitative determination by ELISA using immobilised asialofetuin. *Food Research International*, *36*(8), 815–821. https://doi.org/10.1016/S0963-9969(03)00076-0

Robinson, S. P., Jacobs, A. K., & Dry, I. B. (1997). A class IV chitinase is highly expressed in grape berries during ripening. *Plant Physiology*, *114*(3), 771–778. https://doi.org/10.1104/pp.114.3.771

Rodhouse, J. C., Haugh, C. A., Roberts, D., & Gilbert, R. J. (1990). Red kidney bean poisoning in the UK: An analysis of 50 suspected incidents between 1976 and 1989. *Epidemiology and Infection*, *105*(3), 485–491. https://doi.org/10.1017/s095026880004810x

Rodríguez-Bürger, A. P., Mason, A., & Nielsen, S. S. (1998). Use of fermented black beans combined with rice to develop a nutritious weaning food. *Journal of Agricultural and Food Chemistry*, *46*(12), 2806–4813. https://doi.org/10.1021/jf980674h

Rolim, L. A., Macêdo, M. F., Sisenando, H. A., Napoleão, T. H., Felzenszwalb, I., Aiub, C. A., Coelho, L. C., Medeiros, S. R., & Paiva, P. M. (2011). Genotoxicity evaluation of *Moringa oleifera* seed extract and lectin. *Journal of Food Science*, *76*(2), T53–T58. https://doi.org/10.1111/j.1750-3841.2010.01990.x

Rooney, A. A., Boyles, A. L., Wolfe, M. S., Bucher, J. R., & Thayer, K. A. (2014). Systematic review and evidence integration for literature-based environmental health science assessments. *Environmental Health Perspectives*, *122*(7), 711–718. https://doi.org/10.1289/ehp.1307972

Rossi, M. A., Mancini Filho, J., & Lajolo, F. M. (1984). Jejunal ultrastructural changes induced by kidney bean (*Phaseolus vulgaris*) lectins in rats. *British Journal of Experimental Pathology*, *65*(1), 117–123.

Rouanet, J. M., Lafont, J., Chalet, M., Creppy, A., & Besancon, P. (1985). Effects of dietary kidney bean (*Phaseolus vulgaris*) lectins in growing rats. *Nutrition Reports International*, *31*(1), 237–244. https://doi.org/10.1093/jn/93.1.77

Rougé, P. (1974a). Study of phytohemagglutinin in lentil seeds during germination and early plant development. Evolution in cotyledons. *Comptes Rendus Hebdomadaires des Séances de L'aAcadémie des Sciences*, *278*, 449–452.

Rougé, P. (1974b). Study of phytohemagglutinin in lentil seeds during germination and early plant development. Evolution in roots, stems and leaves. *Comptes Rendus Hebdomadaires des Séances de L'aAcadémie des Sciences*, *278*, 3083–3086.

Rougé, P. (1975). Fate of phytohemagglutinins from various parts of the seed in young pea sprouts. *Comptes Rendus Hebdomadaires des Séances de L'aAcadémie des Sciences*, *280*, 621–623.

Rougé, P., Barre, A., Causse, H., Chatelain, C., & Porthé, G. (1993). Arcelin and α-amylase inhibitor from the seeds of common bean (*Phaseolus vulgaris*) are truncated lectins. *Biochemical Systematics and Ecology*, *21*(6–7), 695–703. https://doi.org/10.1016/0305-1978(93)90074-2

Rougé, P., Chatelain, C., & Père, D. (1978). Interactions between phytohemagglutinins from various species of Leguminosae and immunoglobulins (IgG, IgA and IgM) of normal human serum. *Annales Pharmaceutiques Françaises*, *36*(3–4), 143–147.

Rougé, P., Culerrier, R., Granier, C., Rancé, F., & Barre, A. (2010). Characterization of IgE-binding epitopes of peanut (*Arachis hypogaea*) PNA lectin allergen cross-reacting with other structurally related legume lectins. *Molecular Immunology*, *47*(14), 2359–2366. https://doi.org/10.1016/j.molimm.2010.05.006

Rougé, P., Culerrier, R., Thibau, F., Didier, A., & Barre, A. (2011). A case of severe anaphylaxis to kidney bean: Phaseolin (vicilin) and PHA (lectin) identified as putative allergens. *Allergy*, *66*(2), 301–302. https://doi.org/10.1111/j.1398-9995.2010.02466.x

Rubi, L. A., Grant, G., Bardocz, S., Dewey, P., & Pusztai, A. (1991). Nutritional response of growing rats to faba beans (*Vicia faba* L., minor) and faba bean fractions. The Bristish. *Journal of Nutrition*, *66*(3), 533–542. https://doi.org/10.1079/bjn19910053

Rubio, L. A., Pedrosa, M. M., Cuadrado, C., Gelencser, E., Clemente, A., Burbano, C., & Muzquiz, M. (2006). Recovery at the terminal ileum of some legume non-nutritional factors in cannulated pigs. *Journal of the Science of Food and Agriculture*, *86*(6), 979–987. https://doi.org/10.1002/jsfa.2446

Rutenber, E., Katzin, B. J., Ernst, S., Collins, E. J., Misna, D., Ready, M. P., & Robertus, J. D. (1991). Crystallographic refinement of ricin to 2.5 Å. *Proteins*, *10*(3), 240–250. https://doi.org/10.1002/prot.340100308

Ryder, S. D., Jacyna, M. R., Levi, A. J., Rizzi, P. M., & Rhodes, J. M. (1998). Peanut ingestion increases rectal proliferation in individuals with mucosal expression of peanut lectin receptor. *Gastroenterology*, *114*(1), 44–49. https://doi.org/10.1016/s0016-5085(98)70631-6

Sahni, P., Sharma, S., & Singh, B. (2021). Impact of different processing treatments on techno and biofunctional characteristics of dhaincha (*Sesbania aculeata*). *Food Science and Technology International*, *27*(3), 251–263. https://doi.org/10.1177/1082013220949812

Sahni, P., Sharma, S., & Surasani, V. K. R. (2020). Influence of processing and pH on amino acid profile, morphology, electrophoretic pattern, bioactive potential and functional characteristics of alfalfa protein isolates. *Food Chemistry*, *333*, 127503. https://doi.org/10.1016/j.foodchem.2020.127503

Sahni, V., Agarwal, S. K., Singh, N. P., & Sikdar, S. (2007). Acute demyelinating encephalitis after jequirity pea ingestion (*Abrus precatorius*). *Clinical Toxicology*, *45*(1), 77–79. https://doi.org/10.1080/15563650601006116

Sahoo, R., Hamide, A., Amalnath, S. D., & Narayana, B. S. (2008). Acute demyelinating encephalitis due to Abrus precatorius poisoning--complete recovery after steroid therapy. *Clinical Toxicology*, *46*(10), 1071–1073. https://doi.org/10.1080/15563650802334671

Sánchez-Monge, R., Blanco, C., Díaz-Perales, A., Collada, C., Carrillo, T., Aragoncillo, C., & Salcedo, G. (1999). Isolation and characterization of major banana allergens: Identification as fruit class I chitinases. *Clinical and Experimental Allergy*, *29*(5), 673–680. https://doi.org/10.1046/j.1365-2222.1999.00526.x

Sánchez-Monge, R., Blanco, C., Díaz-Perales, A., Collada, C., Carrillo, T., Aragoncillo, C., & Salcedo, G. (2000). Class I chitinases, the panallergens responsible for the latex-fruit syndrome, are induced by ethylene treatment and inactivated by heating. *The Journal of Allergy and Clinical Immunology*, *106*(1 Pt 1), 190–195. https://doi.org/10.1067/mai.2000.107599

Sánchez-Monge, R., Blanco, C., López-Torrejón, G., Cumplido, J., Recas, M., Figueroa, J., Carrillo, T., & Salcedo, G. (2006). Differential allergen sensitization patterns in chestnut allergy with or without associated latex-fruit syndrome. *The Journal of Allergy and Clinical Immunology*, *118*(3), 705–710. https://doi.org/10.1016/j.jaci.2006.04.058

Sankaranayanan, R., Sekar, K., Banerjee, R., Sharma, V., Surolia, A., & Vijayan, M. (1996). A novel mode of carbohydrate recognition in jacalin, a *Moraceae* plant lectin with a beta-prism fold. *Nature Structural Biology*, *3*(7), 596–603. https://doi.org/10.1038/nsb0796-596

Santiago, J., Levy-Benshimol, A., & Carmona, A. (1993). Effect of Phaseolus vulgaris lectins on glucose absorption, transport, and metabolism in rat everted intestinal sacs. *The Journal of Nutritional Biochemistry*, *4*(7), 426–430. https://doi.org/10.1016/0955-2863(93)90073-6

Sathya, A., & Siddhuraju, P. (2015). Effect of processing methods on compositional evaluation of underutilized legume, *Parkia roxburghii* G. Don (yongchak) seeds. *Journal of Food Science and Technology*, *52*(10), 6157–6169. https://doi.org/10.1007/s13197-015-1732-4

Schechter, B., Lis, H., Lotan, R., Novogrodsky, A., & Sharon, N. (1976). The requirement for tetravalency of soybean agglutinin for induction of mitogenic stimulation of lymphocytes. *European Journal of Immunology*, *6*(3), 145–149. https://doi.org/10.1002/eji.1830060302

Seena, S., Sridhar, K. R., Arun, A. B., & Young, C. C. (2006). Effect of roasting and pressure-cooking on nutritional and protein quality of seeds of mangrove legume *Canavalia cathartica* from southwest coast of India. *Journal of Food Composition and Analysis*, *19*(4), 284–293. https://doi.org/10.1016/j.jfca.2005.05.004

Seiringer, P., Garzorz-Stark, N., & Eyerich, K. (2022). T-cell–mediated autoimmunity: Mechanisms and future directions. *The Journal of Investigative Dermatology*, *142*(3 Pt B), 804–810. https://doi.org/10.1016/j.jid.2021.04.032

Sela, B. A., Wang, J. L., & Edelman, G. M. (1975). Isolation of lectins of different specificities on a single affinity adsorbent. *Journal of Biological Chemistry*, *250*(18), 7535–7538. https://doi.org/10.1016/S0021-9258(19)40976-9

Sha, H., Zhang, Y., Wang, Y., Ke, H., Xiong, X., & Jia, N. (2019). Electrochemiluminescence resonance energy transfer biosensor between the glucose functionalized MnO(2) and g-C(3)N(4) nanocomposites for ultrasensitive detection of concanavalin A. *Biosensors & Bioelectronics*, *124-125*, 59–65. https://doi.org/10.1016/j.bios.2018.10.023

Shaper, J. H., Barker, R., & Hill, R. L. (1973). Purification of wheat germ agglutinin by affinity chromatography. *Analytical Biochemistry*, *53*(2), 564–570. https://doi.org/10.1016/0003-2697(73)90107-3

Shi, L., Arntfield, S. D., & Nickerson, M. (2018). Changes in levels of phytic acid, lectins and oxalates during soaking and cooking of Canadian pulses. *Food Research International*, *107*, 660–668. https://doi.org/10.1016/j.foodres.2018.02.056

Shibasaki, M., Sumazaki, R., Isoyama, S., & Takita, H. (1992). Interaction of lectins with human IgE: IgE-binding property and histamine-releasing activity of twelve plant lectins. *International Archives of Allergy and Immunology*, *98*(1), 18–25. https://doi.org/10.1159/000236160

Shimelis, E. A., & Rakshit, S. K. (2007). Effect of processing on antinutrients and in vitro protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Food Chemistry*, *103*(1), 161–172. https://doi.org/10.1016/j.foodchem.2006.08.005

Siddhuraju, P., & Becker, K. (2005). Nutritional and antinutritional composition, in vitro amino acid availability, starch digestibility and predicted glycemic index of differentially processed mucuna beans (*Mucuna pruriens* var. *utilis*): An under-utilised legume. *Food Chemistry*, *91*(2), 275–286. https://doi.org/10.1016/j.foodchem.2004.02.044

Sinha, S., Mitra, N., Kumar, G., Bajaj, K., & Surolia, A. (2005). Unfolding studies of soybean agglutinin and concanavalin a tetramers: A comparative account. *Biophysical Journal*, *88*(2), 1300–1310. https://doi.org/10.1529/biophysj.104.051052

Sinha, S., & Surolia, A. (2005). Oligomerization endows enormous stability to soybean agglutinin: A comparison of the stability of monomer and tetramer of soybean agglutinin. *Biophysical Journal*, *88*(6), 4243–4251. https://doi.org/10.1529/biophysj.105.061309

Sinkovič, L., Blažica, V., Blažica, B., Meglič, V., & Pipan, B. (2024). How nutritious are French beans (*Phaseolus vulgaris* L.) From the Citizen Science Experiment? *Plants*, *13*(2), 314. https://doi.org/10.3390/plants13020314

Sletten, K., & Kolberg, J. (1983). The primary structure of the α chain of a mitogenic lectin from the seeds of *Lathyrus sativus*. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie*, *364*(8), 1047–1051. https://doi.org/10.1515/bchm2.1983.364.2.1047

Smith, G. P., Jerome, C., Cushin, B. J., Eterno, R., & Simansky, K. J. (1981). Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science*, *213*(4511), 1036–1037. https://doi.org/10.1126/science.7268408

Smith, J. P., & Solomon, T. E. (2013). Cholecystokinin and pancreatic cancer: The chicken or the egg? *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *306*(2), G91–G101. https://doi.org/10.1152/ajpgi.00301.2013

Sotelo, A., Argote, R. M., Moreno, R. I., Flores, N. I., & Diaz, M. (2003). Nutritive evaluation of the seed, germinated seed, and string bean of *Erythrina americana* and the detoxification of the material by boiling. *Journal of Agricultural and Food Chemistry*, *51*(9), 2821–2825. https://doi.org/10.1021/jf021121g

Sotelo-López, A., Hernández-Infante, M., & Artegaga-Cruz, M. E. (1978). Trypsin inhibitors and hemagglutinins in certain edible leguminosae. *Archivos de Investigación Médica*, *9*(1), 1–14.

Sowka, S., Hsieh, L. S., Kebitz, M., Akasawa, A., Martín, B. M., Starrett, D., Peterbauer, C. K., Scheiner, O., & Breiteneder, H. (1998). Identification and cloning of Pers a 1, a 32-kDa endochitinase and major allergen of avocado, and its expression in yeast *Pichia pastoris*. *The Journal of Biological Chemistry*, *273*(43), 28091–28097. https://doi.org/10.1074/jbc.273.43.28091

Spadoni, I., Zagato, E., Bertocchi, A., Paolinelli, R., Hot, E., Di Sabatino, A., Caprioli, F., Bottiglieri, L., Oldani, A., Viale, G., Penna, G., Dejana, E., & Rescigno, M. (2015). A gut-vascular barrier controls the systemic dissemination of bacteria. *Science*, *350*(6262), 830–834. https://doi.org/10.1126/science.aad0135

Sparvoli, F., Lanave, C., Santucci, A., Bollini, R., & Lioi, L. (2001). Lectin and lectin-related proteins in lima bean (*Phaseolus lunatus* L.) seeds: Biochemical and evolutionary studies. *Plant Molecular Biology*, *45*(5), 587–597. https://doi.org/10.1023/a:1010647310311

Sparvoli, F., Laureati, M., Pilu, R., Pagliarini, E., Toschi, I., Giuberti, G., Fortunati, P., Daminati, M. G., Cominelli, E., & Bollini, R. (2016). Exploitation of common bean flours with low Antinutrient content for making nutritionally enhanced biscuits. *Frontiers in Plant Science*, *7*, 928. https://doi.org/10.3389/fpls.2016.00928

Stanojević, S. P., Barać, M. B., Pešić, M. B., Jankovic, V. S., & Vucelic-Radovic, B. V. (2013). Bioactive proteins and energy value of okara as a byproduct in hydrothermal processing of soy milk. *Journal of Agricultural and Food Chemistry*, *61*(38), 9210–9219. https://doi.org/10.1021/jf4012196

Stanojević, S. P., Barać, M. B., Pešić, M. B., & Vucelic-Radovic, B. V. (2021). Energy value and bioactive proteins of inulin-enriched tofu produced by hydrothermal process with chymosin-pepsin rennet. *International Journal of Food Science and Technology*, *56*(11), 5560–5568. https://doi.org/10.1111/ijfs.15132

Stanojević, S. P., Kostić, A. Ž., Milinčić, D. D., Stanojević, A. B., & Pešić, M. B. (2023). Composition of proteins in fresh whey as waste in tofu processing. *Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes*, *58*(1), 10–20. https://doi.org/10.1080/03601234.2022.2162300

Stern, D., Pauly, D., Zydek, M., Müller, C., Avondet, M. A., Worbs, S., Lisdat, F., Dorner, M. B., & Dorner, B. G. (2016). Simultaneous differentiation and quantification of ricin and agglutinin by an antibody-sandwich surface plasmon resonance sensor. *Biosensors & Bioelectronics*, *78*, 111–117. https://doi.org/10.1016/j.bios.2015.11.020

Stirpe, F. (2004). Ribosome-inactivating proteins. *Toxicon*, *44*(4), 371–383. https://doi.org/10.1016/j.toxicon.2004.05.004

Sun, Y., Liu, J., Huang, Y., Li, M., Lu, J., Jin, N., He, Y., & Fan, B. (2019). Phytohemagglutinin content in fresh kidney bean in China. *International Journal of Food Properties*, *22*(1), 405–413. https://doi.org/10.1080/10942912.2019.1590399

Supriya, P., & Sridhar, K. R. (2019). Impact of electron beam irradiation on the bioactive principles of seeds of coastal sand dune wild legume (*Canavalia* spp.). *Recent Patents on Food, Nutrition & Agriculture*, *10*(1), 57–61. https://doi.org/10.2174/2212798410666180709144200

Suseelan, K. N., Bhatia, C. R., & Mitra, R. (1997). Characteristics of two major lectins from mungbean (*Vigna radiata*) seeds. *Plant Foods for Human Nutrition*, *50*(3), 211–222. https://doi.org/10.1007/BF02436058

Sutton, R., Skerritt, J. H., Baldo, B. A., & Wrigley, C. W. (1984). The diversity of allergens involved in bakers' asthma. *Clinical Allergy*, *14*(1), 93–107. https://doi.org/10.1111/j.1365-2222.1984.tb02196.x

Szalai, K., Schöll, I., Förster-Waldl, E., Polito, L., Bolognesi, A., Untersmayr, E., Riemer, A. B., Boltz-Nitulescu, G., Stirpe, F., & Jensen-Jarolim, E. (2005). Occupational sensitization to ribosome-inactivating proteins in researchers. *Clinical and Experimental Allergy*, *35*(10), 1354–1360. https://doi.org/10.1111/j.1365-2222.2005.02338.x

Tabary, F., Font, J., & Bourrillon, R. (1987). Isolation, molecular and biological properties of a lectin from rice embryo: Relationship with wheat germ agglutinin properties. *Archives of Biochemistry and Biophysics*, *259*(1), 79–88. https://doi.org/10.1016/0003-9861(87)90472-3

Takiguchi, S., Suzuki, S., Sato, Y., Kanai, S., Miyasaka, K., Jimi, A., Shinozaki, H., Takata, Y., Funakoshi, A., Kono, A., Minowa, O., Kobayashi, T., & Noda, T. (2002). Role of CCK-A receptor for pancreatic function in mice: A study in CCK-A receptor knockout mice. *Pancreas*, *24*(3), 276–283. https://doi.org/10.1097/00006676-200204000-00011

Talbot, C. F., & Etzler, M. E. (1978). Development and distribution of *Dolichos biflorus* lectin as measured by radioimmunoassay. *Plant Physiology*, *61*(5), 847–850. https://doi.org/10.1104/pp.61.5.847

Tam, C. C., Henderson, T. D., Stanker, L. H., He, X., & Cheng, L. W. (2017). Abrin toxicity and bioavailability after temperature and pH treatment. *Toxins*, *9*(10), 320. https://doi.org/10.3390/toxins9100320

Tang, S., Qiao, S., Piao, X., & Zang, J. (2006). Effects of purified soybean agglutinin on growth and immune function in rats. *Archives of Animal Nutrition*, *60*(5), 418–426. https://doi.org/10.1080/17450390600884450

Tatham, A. S., & Shewry, P. R. (2008). Allergens to wheat and related cereals. *Clinical and Experimental Allergy*, *38*(11), 1712–1726. https://doi.org/10.1111/j.1365-2222.2008.03101.x

Tchernychev, B., & Wilchek, M. (1996). Natural human antibodies to dietary lectins. *FEBS Letters*, *397*(2–3), 139–142. https://doi.org/10.1016/s0014-5793(96)01154-4

Thompson, H. J. (2019). Improving human dietary choices through understanding of the tolerance and toxicity of pulse crop constituents. *Current Opinion in Food Science*, *30*, 93–97. https://doi.org/10.1016/j.cofs.2019.01.001

Tian, F., Guan, Q., Wang, X., Teng, D., & Wang, J. (2014). Influence of different processing treatments on the detectability of nucleic acid and protein targets in transgenic soybean meal. *Applied Biochemistry and Biotechnology*, *172*(7), 3686–3700. https://doi.org/10.1007/s12010-014-0760-2

Tovar, L. E. R., & Gänzle, M. G. (2021). Degradation of wheat germ agglutinin during sourdough fermentation. *Food*, *10*(2), 340. https://doi.org/10.3390/foods10020340

Tresina, P. S., & Mohan, V. R. (2011). Effect of gamma irradiation on physicochemical properties, proximate composition, vitamins and antinutritional factors of the tribal pulse *Vigna unguiculata* subsp. *unguiculata*. *International Journal of Food Science & Technology*, *46*(8), 1739–1746. https://doi.org/10.1111/j.1365-2621.2011.02678.x

Trugo, L. C., Muzquiz, M., Pedrosa, M. M., Ayet, G., Burbano, C., Cuadrado, C., & Cavieres, E. (1999). Influence of malting on selected components of soya bean, black bean, chickpea and barley. *Food Chemistry*, *65*(1), 85–90. https://doi.org/10.1016/S0308-8146(98)00207-6

Tsaneva, M., & Van Damme, E. J. M. (2020). 130 years of plant lectin research. *Glycoconjugate Journal*, *37*(5), 533–551. https://doi.org/10.1007/s10719-020-09942-y

Tsujino, N., Yamanaka, A., Ichiki, K., Muraki, Y., Kilduff, T. S., Yagami, K., Takahashi, S., Goto, K., & Sakurai, T. (2005). Cholecystokinin activates orexin/hypocretin neurons through the cholecystokinin A receptor. *The Journal of Neuroscience*, *25*(32), 7459–7469. https://doi.org/10.1523/JNEUROSCI.1193-05.2005

Udedibie, A. B. I., & Carlini, C. R. (1998). Crack and cook: A simple and quick process for elimination of concanavalin A (Con A) from *Canavalia* seeds. *Animal Feed Science and Technology*, *74*(2), 179–184. https://doi.org/10.1016/S0377-8401(98)00160-6

Umapathy, E., Makinde, M. O., Erlwanger, K. H., Makkar, P. S., Becker, K., & Skadhauge, E. (1998). Antinutritional factors in raw and heat-treated cowpea (*Vigna unguiculata*) and their effects on growth in weanling pigs. In A. J. M. Jansman, G. D. Hill, J. Huisman, & A. F. B. van der Poel (Eds.), *Recent advances of research in antinutritional factors in legume seeds and rapeseed* (pp. 445–448). Wageningen Press.

Valadez-Vega, C., Guzmán-Partida, A. M., Soto-Cordova, F. J., Alvarez-Manilla, G., Morales-González, J. A., Madrigal-Santillán, E., Villagómez-Ibarra, J. R., Zúñiga-Pérez, C., Gutiérrez-Salinas, J., & Becerril-Flores, M. (2011). Purification, biochemical characterization, and bioactive properties of a lectin purified from the seeds of white tepary bean (*Phaseolus acutifolius* variety *latifolius*). *Molecules*, *16*(3), 2561–2582. https://doi.org/10.3390/molecules16032561

Valadez-Vega, C., Lugo-Magaña, O., Betanzos-Cabrera, G., & Villagómez-Ibarra, J. R. (2022). Partial characterization of lectins purified from the Surco and Vara (furrow and rod) varieties of Black *Phaseolus vulgaris*. *Molecules*, *27*(23), 8436. https://doi.org/10.3390/molecules27238436

Valadez-Vega, C., Lugo-Magaña, O., Figueroa-Hernández, C., Bautista, M., Betanzos-Cabrera, G., Bernardino-Nicanor, A., González-Amaro, R. M., Alonso-Villegas, R., Morales-González, J. A., & González-Cruz, L. (2022). Effects of germination and popping on the anti-nutritional compounds and the digestibility of *Amaranthus hypochondriacus* seeds. *Food*, *11*(14), 2075. https://doi.org/10.3390/foods11142075

Van Damme, E. J., Smeets, K., Torrekens, S., van Leuven, F., Goldstein, I. J., & Peumans, W. J. (1992). The closely related homomeric and heterodimeric mannose-binding lectins from garlic are encoded by one-domain and two-domain lectin genes, respectively. *European Journal of Biochemistry*, *206*(2), 413–420. https://doi.org/10.1111/j.1432-1033.1992.tb16941.x

Van Damme, E. J. M., Barre, A., Rougé, P., & Peumans, W. J. (2004). Potato lectin: An updated model of a unique chimeric plant protein. *The Plant Journal*, *37*(1), 34–45. https://doi.org/10.1046/j.1365-313x.2003.01929.x

Van Damme, E. J. M., Peumans, W. J., Barre, A., & Rougé, P. (1998). Plant lectins: A composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. *Critical Reviews in Plant Sciences*, *17*(6), 575–692. https://doi.org/10.1080/07352689891304276

Van Damme, E. J. M., Roy, S., Barre, A., Rougé, P., Van Leuven, F., & Peumans, W. J. (1997). The major elderberry (*Sambucus nigra*) fruit protein is a lectin derived from a truncated type 2 ribosome-inactivating protein. *The Plant Journal*, *12*(6), 1251–1260. https://doi.org/10.1046/j.1365-313x.1997.12061251.x

Van Damme, E. J. M., Smeets, K., Engelborghs, I., Aelbers, H., Balzarini, J., Pusztai, A., van Leuven, F., Goldstein, I. J., & Peumans, W. J. (1993). Cloning and characterization of the lectin cDNA clones from onion, shallot and leek. *Plant Molecular Biology*, *23*(2), 365–376. https://doi.org/10.1007/BF00029011

Van Driessche, E., Smets, G., Dejaegere, R., & Kanarek, L. (1981). The immune-histochemical localization of lectin in pea seeds (*Pisum sativum* L.). *Planta*, *153*(4), 287–296. https://doi.org/10.1007/BF00384244

Vaz, A. F. M., Souza, M. P., Carneiro-da-Cunha, M. G., Medeiros, P. L., Melo, A. M. M. A., Aguiar, J. S., Silva, T. G., Silva-Lucca, R. A., Oliva, M. L. V., & Correia, M. T. S. (2012). Molecular fragmentation of wheat-germ agglutinin induced by food irradiation reduces its allergenicity in sensitized mice. *Food Chemistry*, *132*(2), 1033–1039. https://doi.org/10.1016/j.foodchem.2011.11.097

Vaz, A. F. M., Souza, M. P., Vieira, L. D., Aguiar, J. S., Silva, T. G., Medeiros, P. L., Melo, A. M. M. A., Silva-Lucca, R. A., Santana, L. A., Oliva, M. L. V., Perez, K. R., Cuccovia, I. M., Coelho, L. C. B. B., & Correia, M. T. S. (2013). High doses of gamma radiation suppress allergic effect induced by food lectin. *Radiation Physics and Chemistry*, *85*, 218–226. https://doi.org/10.1016/j.radphyschem.2012.12.015

Venter, F. S., & Thiel, P. G. (1995). Red kidney beans--to eat or not to eat? *South African Medical Journal*, *85*(4), 250–252.

Vergara-Barberán, M., Catalá-Icardo, M., Simó-Alfonso, E. F., Benavente, F., & Herrero-Martínez, J. M. (2023). Aptamer-functionalized stir bar sorptive extraction for selective isolation, identification, and determination of concanavalin A in food by MALDI-TOF-MS. *Mikrochimica Acta*, *190*(6), 219. https://doi.org/10.1007/s00604-023-05795-y

Vergara-Barberán, M., Simó-Alfonso, E. F., Herrero-Martínez, J. M., & Benavente, F. (2023). Determination of a lectin protein allergen in food by on-line aptamer affinity solid-phase extraction capillary electrophoresis-mass spectrometry. *Microchemical Journal*, *190*, 108746. https://doi.org/10.1016/j.microc.2023.108746

Verlinden, W., Varewyck, M., & Verbeke, T. (2024). Web application for Bayesian benchmark dose modelling. *EFSA Supporting Publications*, *21*(1), EN-8580. https://doi.org/10.2903/sp.efsa.2024.EN-8580

Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. (1995). Effects of various water or hydrothermal treatments on certain antinutritional compounds in the seeds of the tribal pulse, *Dolichos lablab* var. *vulgaris* L. *Plant Foods for Human Nutrition*, *48*(1), 17–29. https://doi.org/10.1007/BF01089196

Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. (1996). Effect of different post-harvest treatments on antinutritional factors in seeds of the tribal pulse, *Mucuna pruriens* (L.) DC. *International Journal of Food Sciences and Nutrition*, *47*(3), 263–272. https://doi.org/10.3109/09637489609012587

Vincenzi, S., Zoccatelli, G., Perbellini, F., Rizzi, C., Chignola, R., Curioni, A., & Peruffo, A. D. (2002). Quantitative determination of dietary lectin activities by enzyme-linked immunosorbent assay using specific glycoproteins immobilized on microtiter plates. *Journal of Agricultural and Food Chemistry*, *50*(22), 6266–6270. https://doi.org/10.1021/jf011585z

Vishweshwaraiah, Y. L., Prakash, B., & Gowda, L. R. (2018). Expression profiling of the *Dolichos lablab* lectin during germination and development of the seed. *Plant Physiology and Biochemistry*, *124*, 10–19. https://doi.org/10.1016/j.plaphy.2017.12.040

Vojdani, A. (2014). A potential link between environmental triggers and autoimmunity. *Autoimmune Diseases*, *2014*, 437231. https://doi.org/10.1155/2014/437231

Vojdani, A. (2015). Immune reactivities to peanut proteins, agglutinins, and oleosins. *Alternative Therapies in Health and Medicine*, *21*(Suppl 1), 73–79.

Vojdani, A., Afar, D., & Vojdani, E. (2020). Reaction of lectin-specific antibody with human tissue: Possible contributions to autoimmunity. *Journal of Immunology Research*, *2020*, 1438957. https://doi.org/10.1155/2020/1438957

Volpicella, M., Leoni, C., Fanizza, I., Distaso, M., Leoni, G., Fareioli, L., Naumann, T., Pastorello, E., & Ceci, L. R. (2017). Characterization of maize chitinase-A, a tough allergenic molecule. *Allergy*, *72*(9), 1423–1429. https://doi.org/10.1111/all.13164

Volpicella, M., Leoni, C., Fanizza, I., Placido, A., Pastorello, E. A., & Ceci, L. R. (2014). Overview of plant chitinases identified as food allergens. *Journal of Agricultural and Food Chemistry*, *62*(25), 5734–5762. https://doi.org/10.1021/jf5007962

Vornholt, W., Hartmann, M., & Keusgen, M. (2007). SPR studies of carbohydrate-lectin interactions as useful tool for screening on lectin sources. *Biosensors & Bioelectronics*, *22*(12), 2983–2988. https://doi.org/10.1016/j.bios.2006.12.021

Vretblad, P. (1976). Purification of lectins by biospecific affinity chromatography. *Biochimica et Biophysica Acta*, *434*(1), 169–176. https://doi.org/10.1016/0005-2795(76)90047-7

Wagner, S., & Breiteneder, H. (2002). The latex-fruit syndrome. *Biochemical Society Transactions*, *30*(Pt 6), 935–940. https://doi.org/10.1042/bst0300935

Wainaina, I., Lugumira, R., Wafula, E., Kyomugasho, C., Sila, D., & Hendrickx, M. (2022). Insight into pectin-cation-phytate theory of hardening in common bean varieties with different sensitivities to hard-to-cook. *Food Research International*, *151*, 110862. https://doi.org/10.1016/j.foodres.2021.110862

Wang, B. X., Zhong, X., Chai, Y. Q., & Yuan, R. (2017). An ECL biosensor for sensitive detection of concanavalin A based on the ECL quenching of Ru complex by MoS_2 nanoflower. *Sensors and Actuators B: Chemical*, *245*, 247–255. https://doi.org/10.1016/j.snb.2017.01.180

Wang, C. F., Nie, X. J., Chen, G. M., Yu, Z. H., Li, Z., Sun, Z. W., Weng, Z. F., Yang, Y. Y., Chen, S. L., Zheng, S. R., Luo, Y. Y., Lu, Y. T., Cao, H. Q., & Zhan, H. X. (2015). Early plasma exchange for treating ricin toxicity in children after castor bean ingestion. *Journal of Clinical Apheresis*, *30*(3), 141–146. https://doi.org/10.1002/jca.21351

Wang, C. Y., & Liu, C. F. (1975). Studies of native plants in Taiwan for lectin content. *Zhonghua Minguo Guo Wei Sheng Wu Xue Za Zhi*, *8*(3), 230–235.

Wang, P., Leng, X., Duan, J., Zhu, Y., Wang, J., Yan, Z., Min, S., Wei, D., & Wang, X. (2021). Functional component isolated from *Phaseolus vulgaris* lectin exerts in vitro and in vivo anti-tumor activity through potentiation of apoptosis and immunomodulation. *Molecules*, *26*(2), 498. https://doi.org/10.3390/molecules26020498

Wang, Q., Yu, L. G., Campbell, B. J., Milton, J. D., & Rhodes, J. M. (1998). Identification of intact peanut lectin in peripheral venous blood. *Lancet*, *352*(9143), 1831–1832. https://doi.org/10.1016/S0140-6736(05)79894-9

Wang, W., Wang, E. Q., & Balthasar, J. P. (2008). Monoclonal antibody pharmacokinetics and pharmacodynamics. *Clinical Pharmacology and Therapeutics*, *84*(5), 548–558. https://doi.org/10.1038/clpt.2008.170

Wang, Z., Chen, M., Zhu, Y., Qian, P., Zhou, Y., Wei, J., Shen, Y., Mijiti, A., Gu, A., Wang, Z., Zhang, H., & Ma, H. (2017). Isolation, identification and characterization of a new type of lectin with α-amylase inhibitory activity in chickpea (*Cicer arietinum* L.). *Protein and Peptide Letters*, *24*(11), 1008–1020. https://doi.org/10.2174/0929866524666170711120501

Watier-Grillot, S., Larréché, S., Mazuet, C., Baudouin, F., Feraudet-Tarisse, C., Holterbach, L., Dia, A., Tong, C., Bourget, L., Hery, S., Pottier, E., Bouilland, O., Tanti, M., Merens, A., Simon, S., Diancourt, L., Chesnay, A., & Pommier de Santi, V. (2023). From foodborne disease outbreak (FBDO) to investigation: The plant toxin trap, Brittany, France, 2018. *Toxins*, *15*(7), 457. https://doi.org/10.3390/toxins15070457

Watzl, B., Neudecker, C., Hänsch, G. M., Rechkemmer, G., & Pool-Zobel, B. L. (2001). Dietary wheat germ agglutinin modulates ovalbumin-induced immune responses in Brown Norway rats. *The British Journal of Nutrition*, *85*(4), 483–490. https://doi.org/10.1017/s0007114501000721

Wedin, G. P., Neal, J. S., Everson, G. W., & Krenzelok, E. P. (1986). Castor bean poisoning. *The American Journal of Emergency Medicine*, *4*(3), 259–261. https://doi.org/10.1016/0735-6757(86)90080-x

Wen, Y., Liu, A., Meng, C., Li, Z., & He, P. (2021). Quantification of lectin in soybeans and soy products by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences*, *1185*, 122987. https://doi.org/10.1016/j.jchromb.2021.122987

WHO/IPCS (World Health Organization/International Programme on Chemical Safety). (2009). Principles and methods for the risk assessment of Chemicals in Food. In *International programme on chemical safety, environmental health criteria 240. Chapter 6: Dietary exposure assessment of Chemicals in Food* https://www.who.int/publications/i/item/9789241572408.

Wiederstein, M., Baumgartner, S., & Lauter, K. (2023). Soybean (*Glycine max*) allergens. A review on an outstanding plant food with allergenic potential. *ACS Food Science & Technology*, *3*(3), 363–378. https://doi.org/10.1021/acsfoodscitech.2c00380

Winter, H. C., Oscarson, S., Slättegård, R., Tian, M., & Goldstein, I. J. (2005). Banana lectin is unique in its recognition of the reducing unit of 3-O-β-glucosyl/mannosyl disaccharides: A calorimetric study. *Glycobiology*, *15*(10), 1043–1050. https://doi.org/10.1093/glycob/cwi074

Wooten, J. V., Pittman, C. T., Blake, T. A., Thomas, J. D., Devlin, J. J., Higgerson, R. A., & Johnson, R. C. (2014). A case of abrin toxin poisoning, confirmed via quantitation of L-abrine (N-methyl-L-tryptophan) biomarker. *Journal of Medical Toxicology*, *10*(4), 392–394. https://doi.org/10.1007/s13181-013-0377-9

Wright, C. S. (1990). 2.2 Å resolution structure analysis of two refined N-acetylneuraminyl-lactose-wheat germ agglutinin isolectin complexes. *Journal of Molecular Biology*, *215*(4), 635–651. https://doi.org/10.1016/S0022-2836(05)80174-3

Wyczolkowska, J., Rydzynski, K., & Prouvost-Danon, A. (1992). Concanavalin A-induced activation of hamster mast cells: Morphological changes and histamine secretion. *International Archives of Allergy and Immunology*, *97*(2), 167–172. https://doi.org/10.1159/000236113

Xiao, J., Zhang, H., Niu, L., Wang, X., & Lu, X. (2011). Evaluation of detoxification methods on toxic and antinutritional composition and nutritional quality of proteins in *Jatropha curcas* meal. *Journal of Agricultural and Food Chemistry*, *59*(8), 4040–4044. https://doi.org/10.1021/jf200104b

Yamashita, K., Hitoi, A., & Kobata, A. (1983). Structural determinants of *Phaseolus vulgaris* erythroagglutinating lectin for oligosaccharides. *Journal of Biological Chemistry*, *258*(24), 14753–14755. https://doi.org/10.1016/S0021-9258(17)43721-5

Yang, S., & Yu, M. (2021). Role of goblet cells in intestinal barrier and mucosal immunity. *Journal of Inflammation Research*, *14*, 3171–3183. https://doi.org/10.2147/JIR.S318327

Yi, J., Njoroge, D. M., Sila, D. N., Kinyanjui, P. K., Christiaens, S., Bi, J., & Hendrickx, M. E. (2016). Detailed analysis of seed coat and cotyledon reveals molecular understanding of the hard-to-cook defect of common beans (*Phaseolus vulgaris* L.). *Food Chemistry*, *210*, 481–490. https://doi.org/10.1016/j.foodchem.2016.05.018

Ylitalo, L., Alenius, H., Turjanmaa, K., Palosuo, T., & Reunala, T. (1998). IgE antibodies to prohevein, hevein, and rubber elongation factor in children with latex allergy. *Journal of Allergy and Clinical Immunology*, *102*(4 Pt 1), 659–664. https://doi.org/10.1016/s0091-6749(98)70284-2

Yurre, A. R., Silva, J. D. F. D., Torres, M. K. D. S., Martins, E. L., Ramos, I. P., Silva, W. S. F. L. D., Sarpa, J. D. S., Guedes, C. C. D. S., Napoleão, T. H., Coelho, L. C. B. B., Paiva, P. M. G., & Medei, E. (2020). Evaluation of the cardiac effects of a water-soluble lectin (Wsmol) from *Moringa Oleifera* seeds. *Arquivos Brasileiros de Cardiologia*, *114*(6), 1029–1037. https://doi.org/10.36660/abc.20190071

Zandalinas, S. I., Felix, B., Fritschi, F. B., & Mittler, R. (2021). Global warming, climate change, and environmental pollution: Recipe for a multifactorial stress combination disaster. *Trends in Plant Science*, *26*(6), 588–599. https://doi.org/10.1016/j.tplants.2021.02.011

Zang, J., Li, D., Piao, X., & Tang, S. (2006). Effects of soybean agglutinin on body composition and organ weights in rats. *Archives of Animal Nutrition*, *60*(3), 245–253. https://doi.org/10.1080/17450390600679082

Zang, J. J., Li, D. F., Wang, J. R., Tang, S. S., Li, X. L., & Guo, P. F. (2006). Soya-bean agglutinin induced both direct and cholecystokinin-mediated pancreatic enzyme synthesis in rats. *Animal Science*, *82*(5), 645–651. https://doi.org/10.1079/ASC200679

Zárate, G., & Chaia, A. P. (2012). Feeding with dairy *Propionibacterium acidipropionici* CRL 1198 reduces the incidence of concanavalin-A induced alterations in mouse small intestinal epithelium. *Food Research International*, *47*(1), 13–22. https://doi.org/10.1016/j.foodres.2012.01.005

Zaremba, S., Banach, M., & Pierscinski, A. (1991). Effect of wheat-germ-agglutinin (WGA) on serum glucose and glycogen contents in liver and muscles of mouse. *Acta Biologica Cracoviensia Serie Zoologia*, *32*, 45–51.

Zhang, Z., Triplett, O. A., Nguyen, K. T., Melchior, W. B., Jr., Taylor, K., Jackson, L. S., & Tolleson, W. H. (2013). Thermal inactivation reaction rates for ricin are influenced by pH and carbohydrates. *Food and Chemical Toxicology*, *58*, 116–123. https://doi.org/10.1016/j.fct.2013.04.012

Zhao, J., He, S., Tang, M., Sun, X., Zhang, Z., Ye, Y., Cao, X., & Sun, H. (2019). Low-pH induced structural changes, allergenicity and in vitro digestibility of lectin from black turtle bean (*Phaseolus vulgaris* L.). *Food Chemistry*, *283*, 183–190. https://doi.org/10.1016/j.foodchem.2018.12.134

Zhao, L., Xie, H., Wang, X., Liu, M., Ma, T., Fu, W., Wang, Y., Wu, D., Feng, Y., Liu, Y., Wu, S., Zhang, X., Wang, H., Luo, F., Guo, B., Wu, H., Fu, L., Valenta, R., Vrtala, S., … Wang, X. (2023). Molecular characterization of allergens and component-resolved diagnosis of IgE-mediated mango fruit allergy. *Allergy*, *78*(6), 1699–1703. https://doi.org/10.1111/all.15664

Zhao, Y., Qin, G., Sun, Z., Che, D., Bao, N., & Zhang, X. (2011). Effects of soybean agglutinin on intestinal barrier permeability and tight junction protein expression in weaned piglets. *International Journal of Molecular Sciences*, *12*(12), 8502–8512. https://doi.org/10.3390/ijms12128502

Zhou, J., Berman, K. H., Breeze, M. L., Nemeth, M. A., Oliveira, W. S., Braga, D. P. V., Berger, G. U., & Harrigan, G. G. (2011). Compositional variability in conventional and glyphosate-tolerant soybean (*Glycine max* L.) varieties grown in different regions in Brazil. *Journal of Agricultural and Food Chemistry*, *59*(21), 11652–11656. https://doi.org/10.1021/jf202781v

Zhu, L., Chen, D., Hendrickx, M., & Kyomugasho, C. (2023). Texture classification and in situ cation measurements by SEM-EDX provide detailed quantitative insights into cell wall cation. Interaction changes during ageing, soaking, and cooking of red kidney beans. *Food Research International*, *174*(Pt 1), 113524. https://doi.org/10.1016/j.foodres.2023.113524

Zhuang, Y., & Dreskin, S. C. (2013). Redefining the major peanut allergens. *Immunologic Research*, *55*(1–3), 125–134. https://doi.org/10.1007/s12026-012-8355-x

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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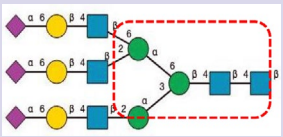
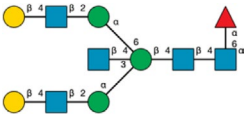
APPENDIX A

Affinity chromatography lectin specificity for ligands

TABLE A.1 Examples of affinity chromatography used for isolation and purification of lectins with various carbohydrate-binding specificities.

Lectin	Plant	Ligand	Immobilised ligand	Reference
LCA	Lentil (*Vicia lens*)	Glc	Sephadex	Howard and Sage (1969)
PSA	Pea (*Pisum sativum*)	Glc	Sephadex	Entlicher et al. (1970)
SBA	Soybean (*Glycine max*)	GalNAc	GalNAc-Sepharose	Vretblad (1976)
PNA	Peanut (*Arachis hypogaea*)	GalNAc	GalNAc-Sepharose	Lotan et al. (1975)
SNA-III	Elderberry (*Sambucus nigra*)	GalNAc	Asialofetuin-Sepharose	Mach et al. (1991)
UEA	Gorse (*Ulex europaeus*)	Fuc	Fucose-Sepharose	Allen and Johnson (1977)
UDA	Stinging nettle (*Urtica dioica)*	GlcNAc	Chitin	Peumans et al. (1984)
Favin	Faba bean (*Vicia faba*)		Fetuin-Sepharose	Sela et al. (1975)
WGA	Wheat (*Triticum aestivum*)	GlcNAc	Chitin-Sepharose	Datta et al. (1984)
		GlcNAc	GlcNAc-Sepharose	Vretblad (1976)
		GlcNAc	GlcNAc-Sepharose	Shaper et al. (1973)
		GlcNAc	Chitin-polyacrylamide gel	Bouchard et al. (1976)
		Fetuin	Fetuin-Sepharose	Sela et al. (1975)
		Chicken ovomucoid	Ovomucoid-agarose	Rice and Etzler (1975)
PHA	Kidney bean (*Phaseolus vulgaris*)		Fetuin-Sepharose	Sela et al. (1975)
			Thyroglobulin-Sepharose	Monsigny et al. (1978)

Ligands: blue circles: glucose (Glc); yellow squares: N-acetyl galactosamine (GalNAc); red triangles: fucose (Fuc); blue squares: N-acetyl glucosamine (GlcNAc); violet rhombus: sialic acid (Sia or NeuAc); yellow circles: galactose (Gal); green circles: mannose (Man).

APPENDIX B

Human studies on lectin effects related to consumption of non-edible plants

TABLE B.1 Accidental intake of abrin. Three studies were identified and L-abrine was quantified in one study.

Author (year)	*N* (age group)	Country	Source (quantity)	Clinical manifestations
Wooten et al. (2014)	1 (Child)	United States	*A. precatorius* (20 seeds)	GI (vomiting)
Patil et al. (2016)	1 (Child)	India	*A. precatorius* (20 seeds)	CNS (agitation, seizures)
Alhamdani et al. (2015)	1 (Child)	United States	*A. precatorius* (NK)	GI (vomiting, diarrhoea, hepatoxicity)

Abbreviation: NK, not known.

TABLE B.2 Intentional consumption (self-harm) of abrin. Five studies were identified and abrin was quantified in one of the identified studies.

Author (year)	*N* (age group)	Country	Source (quantity)	Clinical manifestations
Horowitz et al. (2020)	1 (adult)	United States	*A. precatorius* (1000 seeds)	GI (vomiting, diarrhoea, bleeding), CNS (encephalopathy)
Jang et al. (2010)	1 (adult)	United States	*A. precatorius* (10 seeds)	GI (vomiting, diarrhoea)
Huang et al. (2017)	1 (adolescent)	China	*A. precatorius* (10 seeds)	GI (vomiting, diarrhoea, bleeding)
Sahoo et al. (2008)	1 (adult)	India	*A. precatorius* (10–15 seeds)	GI (vomiting, diarrhoea, abdominal pain, bleeding), CNS (seizures)
Ninan and James (2019)	1 (adult)	India	*A. precatorius* (NK)	GI (vomiting, diarrhoea, bleeding), CNS (encephalopathy)

Abbreviation: NK, not known.

TABLE B.3 Herbal or medicinal preparations with abrin. One study was identified and abrin was not quantified.

Author (year)	*N* (age group)	Country	Source (quantity)	Clinical manifestations
Sahni et al. (2007)	1 (pregnancy)	India	*A. precatorius* (3–4 seeds)	GI (vomiting, diarrhoea, bleeding, hepatotoxicity), CNS (encephalopathy)

TABLE B.4 Accidental intake of curcin/ricin. Eleven studies were identified and curcin/ricin was not quantified in any of the identified studies.

Author (year)	N (age group)	Country	Source (quantity)	Clinical manifestations
Levin et al. (2000)	2 (children)	Israel	*Jatropha multifida* (> 10 fruits)	GI (vomiting, abdominal pain, diarrhoea)
Benamor et al. (2020)	1 (child)	Tunisia	Castor beans (NK)	GI (vomiting, abdominal pain, diarrhoea)
Al-Tamimi and Hegazi (2008)	1 (adult)	Oman	Castor beans (1 fruit)	Sinus bradycardia
Ingle et al. (1966)	57 (children)	India	Castor beans (4-5 seeds)	GI (vomiting, diarrhoea)
Wedin et al. (1986)	2 (adult)	United States	Castor beans (NK)	GI (vomiting, abdominal pain, diarrhoea)
Bispham (1903)	4 (adult)	United States	Castor beans (NK)	GI (vomiting, abdominal pain, diarrhoea)
Al-Eissa et al. (1989)	5 (children)	Saudi Arabia	Castor beans (NK)	GI (vomiting, abdominal pain, diarrhoea)
Challoner and McCarron (1990)	3 (mixed)	United States	Castor beans (1-4 seeds)	GI (vomiting, abdominal pain, diarrhoea)
Wang et al. (2015)	6 (children)	United States	Castor beans (med., 5 seeds)	Sinus bradycardia, GI (vomiting, abdominal pain, diarrhoea)
Palatnick and Tenenbein (2000)	1 (children)	Canada	Castor bean (NK)	Hepatotoxicity
Coattrenec et al. (2017)	1 (adult)	Switzerland	Castor bean (1 seed)	Anaphylactic shock

Abbreviation: NK, not known.

TABLE B.5 Intentional consumption (self-harm) of ricin. Five studies were identified and ricin was quantified in two of the identified studies.

Author (year)	*N* (age group)	Country	Source (quantity)	Clinical manifestations
Lefever et al. (2021)	1 (adult)	Belgium	Castor bean (50 seeds)	GI (vomiting, abdominal pain, diarrhoea), CNS (agitation, anxiety)
Lopez Nunez et al. (2017)	1 (adolescence)	United States	Castor bean (200 seeds)	GI (vomiting, abdominal pain, diarrhoea)
Hamelin et al. (2012)	1 (adult)	United States	Castor bean (6 seeds)	GI (vomiting, abdominal pain, diarrhoea)
de Haan et al. (2016)	1 (adult)	Netherlands	Castor bean (15 seeds)	GI (vomiting, abdominal pain, diarrhoea)
Kopferschmitt et al. (1983)	1 (adult)	France	Castor bean (30 seeds)	GI (vomiting, abdominal pain, diarrhoea)

TABLE B.6 Herbal or medicinal preparations with ricin. Four studies were identified and ricin was not quantified in none of the identified studies.

Author (year)	*N* (age group)	Country	Source (quantity)	Clinical manifestations
El Mauhoub et al. (1983)	1 (pregnancy)	Libya	Castor bean (3 seeds)	Birth defects (moderate growth retardation, convulsions, cranio-facial dysmorphia, absence deformity of limbs and vertebral segmentation defect)
Lim et al. (2009)	1 (adult)	South Korea	Castor beans (5 seeds)	GI (vomiting, abdominal pain, diarrhoea)
Assiri (2012)	1 (adult)	Saudi Arabia	Herbal mixture (NK)	GI (vomiting, abdominal pain, diarrhoea)
Hosseini et al. (2017)	1 (adult)	Iran	Castor beans (8–10 seeds)	CNS (coma)

Abbreviation: NK, not known.

APPENDIX C

Mode of action for the allergenicity of lectins

The N-glycans linked to the different domains of the IgE molecules essentially consist of high-mannose type glycans, sialylated biantennary N-glycans and bisected sialylated biantennary N-glycans of the complex type (Baenziger & Kornfeld, 1974; Montero-Morales et al., 2017; Rearick et al., 1983) (Figure C.1). Both the high-mannose type glycans and the complex N-glycans can interact with different lectins, especially lectins with a Man-binding specificity such as the two-chain Fabeae lectins PSA or LCA, the GNA-related lectins such as the garlic lectin ASA and the jacalin-related lectins such as artocarpin. The galactose-specific lectins such as the soybean lectin SBA can also interact with the complex type N-glycans from IgEs. However, this source of pitfalls due to the lectin activity can easily be avoided by introducing inhibitory concentrations of monosaccharides, e.g. Man for Man-specific lectins, Gal/GalNAc for Gal-specific lectins or GlcNAc/chitobiose for the chitin-binding lectins, in the medium to block the carbohydrate-binding site and prevent the lectins to interact with the IgE and other serum immunoglobulin N-glycans (Pramod et al., 2007, for further results see Section 3.1.5.2.2; Rougé et al., 2010, for further results, see Section 3.1.5.2.1). This ability of Con A to recognise and bind to N-glycan chains associated with the different classes of immunoglobulins has been known for a long time (Nakamura et al., 1960) and has been investigated with many other lectins (Rougé et al., 1978; Dramburg et al., 2023, for further results, see Section 3.1.5.2.2).

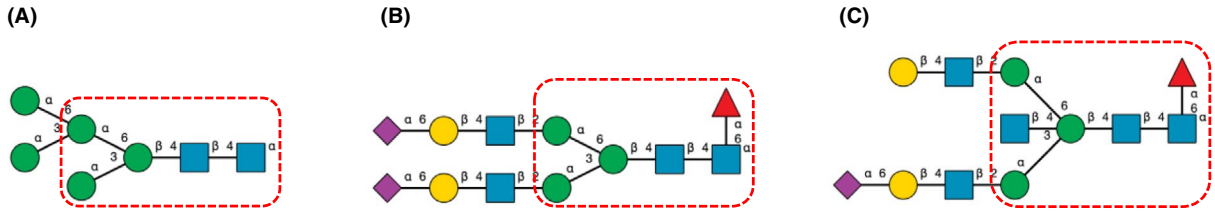


FIGURE C.1 Examples of high-mannose type glycan (A), sialylated biantennary N-glycan (B) and bisected sialylated biantennary N-glycan (C) of the complex type occurring in human IgE (Montero-Morales et al., 2017). Classical coloured symbols were used for representing Fuc (red triangle), Gal (yellow circle), GalNAc (yellow square), Glc (yellow square), GlcNAc (blue square), Man (green circle) and sialic acid/Neu5Ac (purple losange), respectively. The a1,6-fucosylated trimannoside core Man3GlcNAc2 of the N-glycans recognised by Man-specific lectins is red boxed.

A study performed by Haas et al. (1999) confirmed the capacity of lectins with different carbohydrate-binding specificities to interact with the N-glycan moiety of IgE antibodies. Using 16 lectins including 10 plant food lectins (PHA-E and PHA-L, SBA, LCA, PSA, PNA, WGA, AIA from jackfruit and SNA from black elderberry), their capacity to induce the release of IL-4, IL-13 (involved in Th2 response and IgE synthesis) and histamine from basophils was analysed in BAT experiments. Phytohaemagglutinin (PHA-E, PHA-L), LCA, PSA and SNA were the more active in inducing the release of interleukins and histamine from basophils (Table C.1). Other lectins from edible foods were moderately active (WGA) or weakly active (SBA, PNA, AIA). Considering that all active lectins exhibit affinity for the complex N-glycans and high-mannose glycans forming the N-glycan moiety of IgE (Figure C.1), it is likely that their interaction with IgE N-glycans is the trigger for the secretion of interleukins and histamine by basophils (for further results, see Section 3.1.4.2). However, many other N-glycans that exist on the surface including the IgE receptor (FCeRI) of basophils might be recognised by the lectins and thus interfere in this process. In this regard, two receptors for IgE from rat basophilic leukaemia cells bound several lectins including Con A, WGA, PSA (1 receptor only) and RCA from castor beans (Helm & Froese, 1981).

TABLE C.1 Lectins from edible seeds ranked according to their capacity to trigger basophils to release the pro-inflammatory interleukins IL-4 and IL-13 and histamine in BAT experiments performed on human donors ($n = 9–11$). Adapted from Haas et al. (1999). Con A was included in the list as a reference active lectin. The active kidney bean lectins (PHA-E, PHA-L) are shaded in grey and the less active lectins (PNA, SBA and AIA) are shaded in pale grey.

IL-4 release	IL-13 release	Histamine release
Con A	SNA	SNA
PHA-E	PHA-E	Con A
LCA	Con A	LCA
SNA	LCA	PSA
PSA	PHA-L	PHA-L
PHA-L	WGA	PHA-E
WGA	PSA	WGA
PNA	PNA	PNA
AIA	SBA	AIA
SBA	AIA	SBA

The potential allergenicity of different lectins including lectins from jackfruit (*Artocarpus heterophyllus*), seeds (Restum-Miguel and Prouvost-Danon, 1985, for further results, see Section 3.1.4.4), PHA (Haas et al., 2001, for further results, see Section 3.1.4.4; Kumar et al., 2011; Kumar et al., 2013 Kumar et al., 2014; He, Elfalleh, et al., 2018), Con A (Mitchell & Clarke, 1979; Vaz et al., 2013) and garlic lectin (Clement & Venkatesh, 2010, for further results, see Section 3.1.4.4; Padiyappa et al., 2022, for further results, see Section 3.1.6.4) was investigated in animal models, essentially BALB/c mice, using different routes of administration. In addition, the allergenicity of Con A (Wyczolkowska et al., 1992) and PHA (He, Zhao, et al., 2018; Zhao et al., 2019) was investigated in different in vitro tests.

Legume lectins

The peanut lectin (PNA) was identified as a minor allergen from peanut seed (Burks et al., 1994; for further results, see Section 3.1.5.2.1) and was formerly labelled as Ara h agglutinin in the WHO/IUIS database. At present, however, PNA is no longer recognised as a peanut allergen (Marsh et al., 2024) and does not appear in the official list of allergens recognised by the WHO/IUIS Committee (https://allergen.org, accessed on April 2024). However, it is still present in the Allergome (https://www.allergome.org, accessed on April 2024) and COMPARE (https://comparedatabase.org, accessed on April 2024) databases. Nevertheless, PNA interacted with IgE antibodies from patients allergic to peanut in both immunoblotting and surface plasmon resonance experiments, even in the presence of inhibiting sugars (Gal/GalNAc) added to avoid the binding to the N-glycans of IgE antibodies by the lectin (Rougé et al., 2010, for further results, see Section 3.1.5.2.1). Up to six IgE-binding regions were identified on the surface of PNA using a pool of sera from patients allergic to peanut as a probe (Figure C.2). These sequential epitopes are non-glycosylated suggesting a PNA–IgE interaction independent of a lectin recognition of glycans by IgE antibodies. Moreover, the sequential IgE-binding epitopes identified on the molecular surface of PNA exhibited pronounced homologies with the corresponding epitopes from other legume lectins including LCA from lentil, PSA from pea and SBA from soybean suggesting possible cross-reactivity between peanut and other closely related legume lectins.

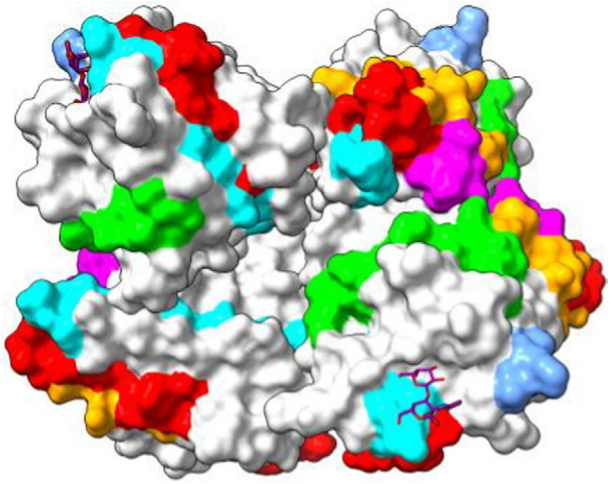


FIGURE C.2 PNA lectin tetramer in complex with Tn antigen (coloured purple), showing the localisation of the IgE-binding epitopic stretches 1 (red), 2 (blue), 3 (green), 4 (magenta), 5 (orange) and 6 (cyan) on the molecular surface of the lectin.

Moreover, ELISA inhibition experiments performed with peanut and black gram (*Vigna mungo*) extracts suggested some cross-reactivity with other legume seed lectins. The possible recognition of the N-glycans decorating the IgE antibodies can be invoked for PHA-E, which preferentially interacts with bisected biantennary N-glycans of the complex type. However, it is excluded for PHA-L, which recognises more complex bisected tri- and tetra-antennary N-glycans of the complex type exhibiting terminal b1-6-linked GlcNAc residues, which apparently are lacking in the IgE molecules (Figure C.3) (Bojar et al., 2022).

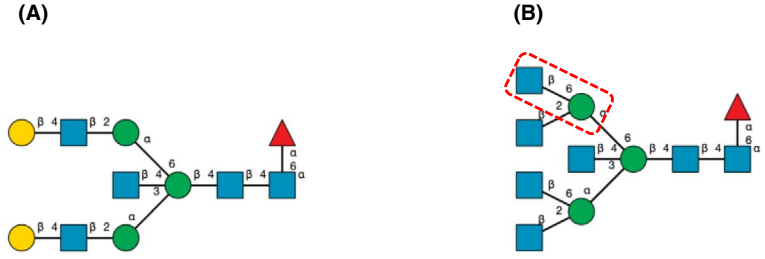


FIGURE C.3 (A) Bisected non-sialylated biantennary N-glycan of the complex type preferentially recognised by PHA-E. (B) Example of bisected non-sialylated tetra-antennary N-glycans of the complex type exhibiting terminal a b1-6-linked GlcNAc residue (red boxed) preferentially recognised by PHA-L.

However, using epitope peptide synthesis together with different proteomics approaches and epitope predictions, He, Zhao, et al. (2018) (for further results, see Section 3.1.4.1) and Wang et al. (2021) identified four B-cell sequential epitopes well exposed on the molecular surface of PHA (Figure C.4), and two T-cell epitopes that could be responsible for the sensitisation of susceptible individuals.

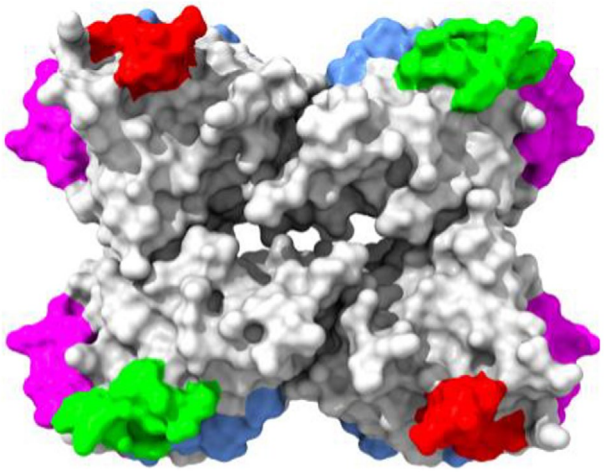


FIGURE C.4 Localisation of four predicted IgE-binding B-cell epitopes B1 (coloured red), B2 (coloured blue), B3 (coloured green) and B4 (coloured magenta) on the molecular surface (coloured white) of the PHA tetramer (PDB code 5AVA).

Hevein-like domain-containing proteins

The wheat lectin, known as wheat germ agglutinin (WGA), was the first lectin containing hevein-like domains identified as a potential allergen for people sensitised to wheat and wheat-derived products (Sutton et al., 1984). In addition, other chitinases of class I and IV containing a hevein-like domain have been recognised as potential food allergens.

These chitinases, widely distributed in fruits (Barre et al., 2023; Leoni et al., 2019), are composed of a N-terminal hevein-like domain, linked by an unstructured peptide segment to a C-terminal domain with chitinase activity. The N-terminal hevein-like domain is closely related to its amino acid sequence and three-dimensional structure to hevein (Hev b 6.02), a small contact IgE-binding allergen of 43 amino acids, from the latex from rubber tree (*Hevea brasiliensis*) (Blanco et al., 1994, for further results, see Section 3.1.5.2.2; Chen et al., 1997; Banerjee et al., 1997; Ylitalo et al., 1998). According to these similarities, the hevein-like domain of chitinases I and IV from many fruits cross-react with Hev b 6.02 and behave as panallergens responsible for the latex-fruit syndrome (Mikkola et al., 1998; Chen et al., 1998; Díaz-Perales et al., 1999, for further information, see Section 3.1.5.2.1; Blanco et al., 1999).

Chitinases which participate in the latex-fruit syndrome as food allergens were identified and characterised in various fleshy fruits including Pers a 1 from avocado fruit (*Persea americana*) (Sowka et al., 1998; for further information, see Section 3.1.5.2.1; Chen et al., 1998; Díaz-Perales et al., 1998; for further information, see Section 3.1.5.2.1; Posch et al., 1999), Mus a 2 from banana (*Musa acuminata*) (Mikkola et al., 1998; Sánchez-Monge et al., 1999), Cas s 5 from chestnut (*Castanea sativa*) (Díaz-Perales et al., 1998; Sánchez-Monge et al., 2006), Man i 1 from mango (*Mangifera indica*) (Zhao et al., 2023; for further information, see Section 3.1.5.2.1), and Zea m 8 from maize (*Zea mays*) (Volpicella et al., 2017). Chitinase allergens were also detected in seeds like Fag e 4 from buckwheat (*Fagopyrum esculentum*) (Geiselhart et al., 2018), and tubers like Bra r 2 from turnip (*Brassica rapa*) (Hänninen et al., 1999); for further information, see Section 3.1.5.2.1.

Although they have not yet been officially recognised as food allergens by the WHO/IUIS Allergen Nomenclature Sub-Committee (https://allergen.org), many other chitinases of class I and IV containing a hevein-like domain have been identified and characterised, especially in fruits, and most of them are listed in the Allergome database (https://www.allergome.org/) (Table C.2).

Monocot GNA-like lectins

Due to their high stability and immunogenicity, the garlic lectins ASA-I and ASA-II have been identified as efficient immunomodulatory proteins (Clement & Venkatesh, 2010, for further results, see Section 3.1.4.4; Clement et al., 2010). In spite of their immunogenic potency, garlic lectins are apparently not effective sensitisers. In fact, food allergies to spices are rather uncommon (2% of food allergies), and allergies to the edible bulbs from garlic (*Allium sativum*) or onion (*Allium cepa*) occupy a negligible place among the responsible spices (Añibarro et al., 1997; Moneret-Vautrin et al., 2002; Pérez-Pimiento et al., 1999). Several case reports of garlic allergy documented by identification of specific IgEs (Ma & Yin, 2012) or positive skin prick tests (SPT) performed with fresh garlic (Asero et al., 1998) were reported in the literature (for further information, see Table C.2). A case of garlic allergy following the first intake was reported in a Spanish 9-month-old breastfeeding infant that could be attributed to garlic lectin, since IgE from the patient serum reacted specifically with fractions of garlic lectin in SDS-PAGE immunoblot experiments (González-de-Olano et al., 2018, for further results, see Section 3.1.5.2.1). In this

case however, the route of sensitisation remains unclear and difficult to identify. In addition, since mannose-specific garlic lectins ASA-I and ASA-II exhibit a monosaccharide-binding activity similar to that of BanLec, some non-specific interaction of the lectin with the N-glycan moiety of IgE susceptible to interfere with the specific interaction of ASA-I and ASA-II with IgE (Krithika et al., 2018) cannot be excluded (for further results, see Section 3.1.5.2.2). Screening for ELISA leaf garlic lectin (ASAL)-IgE reactivity on sera from 216 patients allergic to various plant foods including tomato, spinach, pumpkin and okra and consuming regularly garlic in their diet resulted in an extremely weak interaction with the purified lectin. Similarly, sera from 25 non-atopic volunteers also resulted in a very weak non-specific interaction with ASAL (Mondal et al., 2011). However, this experiment is biased because there is no evidence that the allergic patients recruited in this study are allergic to garlic lectin or to other food lectins capable of cross-reacting with ASAL.

Jacalin-related lectins

Banana consumption was reported to elicit IgG4 antibody production (Koshte et al., 1992), and BanLec was also reported to induce a non-specific activation of basophils and mast cells in BAT performed with atopic subjects (Krithika et al., 2018). The man-specific lectin ArtinM lectin from jackfruit (*Artocarpus heterophyllus)* also activated mast cells and induced the release of newly synthesised IL-4 without affecting the FcεRI degranulation (Barbosa-Lorenzi et al., 2016). Conversely, however, the Gal/GalNAc-specific jacalin from jackfruit does not induce the release of IL-4 and IL-13 interleukins and histamine from activated basophils (Haas et al., 1999).

Type 2 Ribosome-Inactivating Proteins (RIPs)

Occupational sensitisation to RIPs from non-food plants has been reported for researchers working on type 2 RIPs since specific IgE was detected in sera by ELISA with natural RIPs including dianthin-30 from carnation (*Dianthus caryophyllus*), gelonin from *Suregada multiflora*; syn *Gelonium multiflorum*, momordin/a-momorcharin from bitter melon (*Momordica charantia*), saporin from soapwort (*Saponaria officinalis*), volkensin from *Adenia volkensii* and ricin, in sera from four subjects engaged in research on type 2 RIPs (Szalai et al., 2005). The black elderberry (*Sambucus nigra*) type 2 RIP lectin SNA-I was identified as a major allergen in 0.6% of 3668 randomly tested patients experiencing positive SPT or RAST to elderberry (Förster-Waldl et al., 2003). The RIP lectin is present in pollen, flowers and (comestible) berries of the plant. However, since no information is available on both the B and T epitopes present at the surface of type 2 RIPs, it is not known whether it is the a chain (toxic RNAse) or the b chain (lectin) that induces sensitisation. In addition, the ability of type 2 RIPs to non-specifically interact with the N-glycan moiety of IgE was demonstrated with ricin (Bach & Brashler, 1975) and mistletoe (*Viscum album*) lectin (Luther et al., 1978). Mistletoe type 2 RIP lectins inhibited the IgE-mediated histamine release from human leucocytes of patients with atopic asthma by a non-specific interaction with the carbohydrate moiety of the IgE antibodies.

TABLE C.2 List of some lectins identified as potential food allergens. Lectin allergens approved by the WHO/IUIS Allergen Nomenclature Sub-Committee are indicated. Allergens Hev b 6 and Hev b 11 are responsible for contact allergies and for IgE-binding cross-reactivity with hevein-containing food chitinases.

Plant	Plant species	Family	Lectin	WHO/ IUIS	Allergome	COMPARE	Clinical manifestations	Reference
Avocado	*Persea americana*	Lauraceae	Hevein-like	Pers a 1	Pers a 1	Pers a 1	Latex-fruit syndrome OAS Anaphylaxis	Sowka et al. (1998)
Banana	*Musa acuminata*	Musaceae	Hevein-like	Mus a 2	Mus a 2 Mus xp 2	Mus a 2	Latex-fruit syndrome OAS Anaphylaxis	Sánchez-Monge et al. (1999)
Buckwheat	*Fagopyrum esculentum*	Polygonaceae	Hevein-like	Fag e 4	Fag e 4		Latex-fruit syndrome OAS Anaphylaxis	Geiselhart et al. (2018)
Chestnut	*Castanea sativa*	Fagaceae	Hevein-like	Cas s 5	Cas s 5	Cas s 5	Latex-fruit syndrome OAS Anaphylaxis	Díaz-Perales et al. (1998)
Custard apple	*Annona cherimola*	Annonaceae	Hevein-like		Ann c Chitinase		Latex-fruit syndrome OAS	Gamboa et al. (2005)
Garlic	*Allium sativum*	Liliaceae	ASA-I, ASA-II				?	Asero et al. (1998), Ma and Yin (2012)
Grape	*Vitis vinifera*	Vitaceae	Hevein-like		Vit v 5		Latex-fruit syndrome OAS	Pastorello et al. (2003)

(Continues)

TABLE C.2 (Continued)

Plant	Plant species	Family	Lectin	WHO/IUIS	Allergome	COMPARE	Clinical manifestations	Reference
Horse gram	*Macrotyloma uniflorum*	Fabaceae	DBA		Dol b Agglutinin		Anaphylaxis	Pramod et al. (2006)
Kidney bean	*Phaseolus vulgaris*	Fabaceae	PHA		Pha v PHA (PHA-E + PHA-L)		Anaphylaxis FAS	Rougé et al. (2011)
Kiwi	*Actinidia chinensis* var. *deliciosa* *Actinidia chinensis*	Actinidiaceae	Hevein-like		Act d Chitinase Act c Chitinase I Act c Chitinase IV		Latex-fruit syndrome OAS Anaphylaxis	Bublin et al. (2004)
Lentil	*Vicia lens*	Fabaceae	LCA		Lens c Agglutinin		?	Rougé et al. (2010)
Maize	*Zea mays*	Poaceae	Hevein-like	Zea m 8	Zea m 8	Zea m 8	Latex-fruit syndrome	Pastorello et al. (2009)
Mango	*Mangifera indica*	Anacardiaceae	Hevein-like	Man i 1	Man i Chitinase	Man i 1	Latex-fruit syndrome OAS	Zhao et al. (2023)
Papaya	*Carica papaya*	Caricaceae	Hevein-like		Cari p Chitinase		Latex-fruit syndrome OAS	Chen et al. (2007)
Passion fruit	*Passiflora edulis*	Passifloraceae	Hevein-like		Pas e Chitinase		Latex-fruit syndrome OAS	Cabanillas et al. (2009)
Pea	*Pisum sativum*	Fabaceae	PSA		Pis s Agglutinin		?	Rougé et al. (2010)
Peanut	*Arachis hypogaea*	Fabaceae	PNA		Ara h Agglutinin	PNA	Anaphylaxis FAS	Burks et al. (1994)
Soybean	*Glycine max*	Fabaceae	SBA		Gly m Agglutinin			Rougé et al. (2010)
Tomato	*Solanum lycopersicum*	Solanaceae	Hevein-like		Sola l Chitinase		Latex-fruit syndrome	Díaz-Perales et al. (1999)
Turnip	*Brassica rapa*	Brassicaceae	Hevein-like	Bra r 2	Bra r 2		Latex-fruit sundrome	Hänninen et al. (1999)
Wheat	*Triticum aestivum*	Poaceae	WGA	Tri a 18	Tri a 18 Tri a Chitinase		WDEIA Latex-fruit syndrome	Sutton et al. (1984)

Abbreviations: ?, no allergic manifestations clearly related to the lectin have been reported, although some IgE-binding reactivity can be detected; FAS, possible or frequent systemic reactions (anaphylactic shock); OAS, oral allergy syndrome.

Lectins and autoimmunity

Plant-derived dietary lectins have been reported or speculated to be involved in the pathogenesis of several inflammatory diseases, including inflammatory bowel disease, diabetes, rheumatoid arthritis and coeliac disease. Common for these diseases is the involvement of tissue-specific T-lymphocytes and antibodies. Except for coeliac disease, they are all categorised as autoimmune diseases.

Autoimmune disease occurs when the human body loses its tolerance mechanism and its ability to discriminate between self and nonself molecules. This loss of oral, central and peripheral tolerance may ultimately result in the destruction of self-tissue by both autoantibodies and autoreactive T-lymphocytes (Vojdani, 2014). Most autoimmune diseases develop due to genetics and environmental factors such as food antigens, haptenic chemicals that form new antigens not recognised as self by the immune system and infections. For further results, see Section 3.1.6.4.

Some lectins have been suggested to be involved in the pathogenesis of some autoimmune diseases including wheat germ agglutinin (WGA), red kidney bean phytohaemagglutinin (PHA) and soybean agglutinin (SBA).

A causal relation between the lectins and autoimmunity has not been demonstrated in humans.

Interestingly, when biopsies from children with total villous atrophy during active coeliac disease and during remission as well as from healthy children were examined with respect to the binding of gliadin and selected dietary lectins (WGA, SBA, PHA, PSA and SJA from *Sophora japonica*), Pittschieler et al. (1994). Lectins with specificity to xGalNAc/Gal bound to active CD biopsies but not to CD biopsies in remission or from healthy children showing that severe changes in the gut epithelial cells structure are accompanied with changes in the glycosylation pattern in the mucosa and thus in the binding abilities of lectins and microorganisms.

APPENDIX D

Studies on the impact of food processing identified

TABLE D.1 Studies identified reporting the impact of different food processing methods under different conditions on the lectin content or activity.

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Dolichos biflorus seeds	Germination	Germinated for up to 4.5 days in fertiliser and isolation of cotyledons	600–700 ng lectin/µg N	Constant up to 96 h	Radioimmunoassay	Talbot and Etzler (1978)
Peanuts varieties Jumbo Virginia and Spanish	Germination	Germinated at 30°C, constant lighting in vermiculite	NR	**Spanish** 3 days – 2.01 mg in cotyledon 6 days – 0.84 mg in cotyledon 9 days – 0.64 mg in cotyledon 12 days – 0.21 mg in cotyledon 15 days – 0.08 mg in cotyledon ≥ 18 days–≤ 0.01 mg in cotyledon **Jumbo Virginia** 3 days – 4.02 mg in cotyledon 6 days – 4.64 mg in cotyledon 9 days – 3.90 mg in cotyledon 12 days – 2.62 mg in cotyledon 15 days – 2.46 mg in cotyledon 18 days – 0.12 mg in cotyledon 21 days – 0.04 mg in cotyledon 24 days – 0.10 mg in cotyledon 27 days – 0.05 mg in cotyledon 30 days – 0.05 mg in cotyledon	Haemagglutination assay (human type O erythrocytes)	Pueppke (1979)
Soybean (*Glycine max*) cultivar BRS 133	Germination	Rinsed and kept for 8 h at room temperature; germination for 42 h at 25°C	4.2 ± 0.2 mg/g flour	1.5 mg/g flour	ELISA	Paucar-Menacho et al. (2010)
DLL-II lectin from *Dolichos lablab* var *lignosus* seeds	Germination	Submerged in water for 24 h and germinated at 25 ± 2°C for up to 6 days	≈48 mRNA abundance	2 days – 22.4 mRNA abundance ≥ 2 days – negligible	RT-qPCR (DLL-II gene expression)	Vishweshwaraiah et al. (2018)
Chilean bean (*Phaseolus vulgaris*), varieties: Black, Found, White and Miss	Germination Autoclaving	Germinated in incubator at 22°C for 5 days Autoclaved for two 15-min cycles in deionised water (1:10 w/v)	Black – 6.29 ± 0.10 HU/mg bean Found – 6.21 ± 0.04 HU/mg bean White – 6.20 ± 0.17 HU/mg bean Miss – 6.24 ± 0.24 HU/mg bean	Germinated: Black – 5.96 ± 0.13 HU/mg bean Found – 3.17 ± 0.06 HU/mg bean White – 3.06 ± 0.21 HU/mg bean Miss – 3.07 ± 0.01 HU/mg bean Autoclaved (all varieties): n.d.	Haemagglutination assay (rabbit erythrocytes)	Poblete et al. (2020)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Amaranth grains (*Amaranthus hypochondriacus*)	Germination Popping	Soaked for 12 h, softened with 5% sulfuric acid solution for 2 h and germinated for 72 h Heated between 200°C and 240°C for 90 s	Type A erythrocyte: 91.03 ± 0.99 – 11.24 ± 0.14 HA/mg protein	Germinated: Type A: 6.52 ± 0.015 – 3.25 ± 0.005 HA/mg protein Popped: Type A: 3.32 ± 0.013 – 1.66 ± 0.025 HA/mg protein	Haemagglutination assay (type O and A human erythrocytes) *Same trend observed in both human type erythrocytes, only type A reported*	Valadez-Vega, Lugo-Magaña, Figueroa-Hernández, et al. (2022)
Lentil (*Vicia lens* syn. *Lens culinaris*)	Soaking + Germination	Soaking for 6 h; germination at 20°C for 3 or 6 days, watering daily or alternate (dark or 6 h light daily)	250 ± 80 HU	Range: 120 ± 10 HU [3 days (wa,l)] 380 ± 41 HU [3 days (wd,l)]	Haemagglutinating activity (trypsin treated rat erythrocytes)	Cuadrado et al. (2000)
LCA (from *Lens culinaris* var. *vulgaris*)			0.85 ± 0.09 mg/g dw	Range: 0.74 ± 0.09 mg/g dw [3 days (wd,d)] 1.54 ± 0.18 mg/g dw [6 days (wd,l)]	ELISA	
Chickpea (*Cicer arietinum*)	Soaking + Germination Soaking + Boiling Soaking + Autoclaving Soaking + Microwaving	Soaked and germinated in cotton cloth in the dark for 3 days Soaked and boiled at 100°C for 90 min Soaked and autoclaved at 121°C for 35 min Soaked and microwaved on high setting for 15 min *Soaking was done in distilled water for 12 h at ±25°C. Seed/water ratio of 1:10 (w/v) was used for all processes*	6.22 ± 0.22 HU/mg	Germination: 3.73 ± 0.05 HU/mg Boiling, autoclaving and microwave cooking: 0.00 ± 0.00 HU/mg	Haemagglutination assay (rabbit erythrocytes)	El-Adawy (2002)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Soybean (*Glycine max*)	Germination + Boiling (GB) Soaking + Boiling (SB) Soaking + Pressure cooking (SP) Soaking + Microwaving (SM)	Germinated for 3 days and boiled at 100°C for 3, 5, 10 and 15 min Soaked and cooked at 60, 80 and 100°C for 5, 15, 30, 45, 60 and 120 min Soaked and autoclaved at pressure ≥ 1 kgf/m^2 for 1, 2, 3 and 5 min Soaked and microwaved at 2,450 MHz for 0.5, 1, 2, 3 and 5 min *Soaking was done in distilled water for 6 h and seed/water ratio for cooking was 1:5 (w/v)*	Soybean sprouts: 105.14 ± 3.35 HU/mg protein White soybeans: 100% haemagglutinating activity (HU) *(One HU defined as the reciprocal of the highest dilution giving a positive haemagglutination)*	Soybean sprouts (GB): 3 min – 6.80 ± 0.03 HU/mg protein ≥ 5 min – n.d. White soybean (SB): 60°C: 5 min – 104.02% HU 15 min – 105.03% HU 30 min – 30.83% HU 45 min – 9.37% HU 60 min – 2.53% HU 120 min – n.d. 80°C: 5 min – 27.77% HU 15 min – 7.84% HU 30 min – 2.02% HU 45 min – 0.56% HU ≥ 60 min – n.d. 100°C: 5 min – 6.61% HU 15 min – 2.14% HU 30 min – 0.63% HU ≥ 45 min – n.d. White soybean (SP, 120°C): 5 min – n.d. White soybean (SM): 4 min – 50% HA	Haemagglutination assay (rabbit erythrocytes)	Felipe et al. (2006)
Navy and red kidney beans	Soaking Soaking + Cooking Canning	Soaked for 16 h at RT Soaked and cooked in water at 82 and 84°C for 1, 2, 3 and 4 h, at 94°C for 0.5, 1, 1.5 and 2 h, at 100°C for 10, 20, 30, 40, 50 and 60 min Navy beans canned by retort-cooking methods at 121.1°C/14 min and 115.6°C/45 min	Navy: 21.64 ± 1.56 × 10^3 HU/g Red kidney: 45.90 ± 5.66 *10^3 HU/g	**Navy** Soaking: 20.83 ± 0.00 × 10^3 HU/g Soaking+Cooking: 82°C: 1–3 h: 37.7±0.4 to 40.0 ± 0.6 HU/g 4 h: 0 HU/g 88°C: 1-3 h: 20.7 ± 0.3 – 20.9 ± 0.8 HU/g 4 h: 0 HU/g 94°C (0.5–2 h): 22.7±1.4 – 19.7 ± 1.8 HU/g 100°C (10–30 min): 0 HU/g Canning: 0 HU/g **Red kidney** Soaking: 43.59 ± 0.61 × 10^3 HU/g Soaking+Cooking: 82°C (1-4 h): 40.5 ± 0.0 – 41.7 ± 1.7 HU/g 88°C: 1 h – 40.7 ± 0.0 HU/g 2–4 h – 0 HU/g 94°C (0.5–2 h): 0 HU/g 100°C (10–60 min): 0 HU/g	Haemagglutinating activity (trypsin treated and glutaraldehyde-treated rabbit erythrocytes)	Dhurandhar and Chang (1990)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Dolichos lablab var. *vulgaris* seeds	Soaking Cooking Autoclaving	Soaked in distilled water (W) and 0.02% sodium bicarbonate solution (Bi) for 3, 6 and 9 h Cooked at 100°C for 30, 60 and 90 min Autoclaved at 121°C for 15, 30 and 45 min *Soaking, cooking and autoclaving was done in distilled water for in a seed/water ratio of 1:10 (w/v)*	39 HU/mg protein	Soaking W: 3 min – 38 HU/mg 6 min – 37 HU/mg 9 min – 36 HU/mg Soaking Bi: 3 min – 38 HU/mg 6 min – 36 HU/mg 9 min – 30 HU/mg Cooking: 30 min – 33 HU/mg 60 min – 20 HU/mg 90 min – 15 HU/mg Autoclaving: 15 min – 29 HU/mg 30 min – 10 HU/mg 45 min – n.d.	Haemagglutination assay (type A, B and O human erythrocytes) *Same trend observed in all human type erythrocytes, only type A reported*	Vijayakumari et al. (1995)
Velvet bean (*Mucuna pruriens*)	Soaking Cooking Autoclaving	Soaked in distilled water (W) and 0.02% sodium bicarbonate solution (Bi) for 1.5, 3, 4.5, 6, 7.5 and 9 h Cooked in W at 100°C for 15, 30,45, 60, 75 and 90 min Autoclaved at 121°C in W for 15, 30 and 45 min *Seed to water ratios used were 1:10 (w/v)*	148 HU/mg protein	Soaking (1.5–9 h): 148–146 HU/mg protein (W) 148–116 HU/mg protein (Bi) Cooking: 15 min – 194 HU/mg protein 30 min – 166 HU/mg protein 45 min – 146 HU/mg protein 60 min – 105 HU/mg protein 75 min – 34 HU/mg protein 90 min – 5 HU/mg protein Autoclaving: 15 min – 93 HU/mg protein 30 min – 47 HU/mg protein 45 min – 12 HU/mg protein	Haemagglutinin activity (human type A, B and O erythrocytes) *Same trend observed in all human type erythrocytes, only type A reported*	Vijayakumari et al. (1996)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Soybean (*Glycine max*), green and mature peas (*Pisum sativum*), lentil (*Lens esculenta*), faba beans (*Vicia faba*), chickpea (*Cicer arietinum*) and common bean (*Phaseolus vulgaris*) varieties ojo de cabra, black, white and flor de mayo	Soaking Microwaving (M) Soaking + Microwaving (SM) Boiling (B)	Soaked in tap water until reaching 24%–25% moisture level Microwaved at 0.56 KW (2450 MHz) until releasing a cooked smell Soaked and microwaved following previous methods Boiled in water (1:3 w/v) until soft	Beans: Ojo de cabra – 7 (C), 11 (O) Flor de mayo – 5 (C), 12 (O) Black – 7 (C), 12 (O) White – 8 (C), 12 (O) Soybean – 0 (C), 5 (O) Green pea – 0 (C and O) Mature pea – 0 (C), 3 (O) Lentil – 0 (C), 2 (O) Faba bean – 0 (C and O) Chickpea – 0 (C), 3 (O) *(Values expressed as extract maximum dilution causing agglutination of cow (C) and human (O) erythrocytes)*	Microwaving: Ojo de cabra – 3 (C), 7 (O) Flor de mayo – 4 (C), 8 (O) Black – 5 (C), 9 (O) White – 6 (C), 9 (O) Soybean – 0 (C), 3 (O) Green pea – 0 (C and O) Mature pea – 0 (C), 2 (O) Lentil – 0 (C and O) Faba bean – 0 (C and O) Chickpea – 0 (C), 3 (O) Soaking + Microwaving: Ojo de cabra – 2 (C), 9 (O) Flor de mayo – 1 (C), 7 (O) Black – 4 (C), 8 (O) White – 4 (C), 7 (O) Soybean – 0 (C and O) Green pea – 0 (C and O) Mature pea – 0 (C), 2 (O) Lentil – 0 (C and O) Faba bean – 0 (C and O) Chickpea – 0 (C), 3 (O) Boiling: Ojo de cabra – 0 (C), 1 (O) Flor de mayo – 0 (C), 3 (O) Black – 1 (C), 3 (O) White – 0 (C and O) Soybean – 0 (C and O) Green pea – 0 (C and O) Mature pea – 0 (C), 2 (O) Lentil – 0 (C and O) Faba bean – 0 (C and O) Chickpea – 0 (C), 2 (O)	Haemagglutination assay (bovine and human type O erythrocytes)	Hernández-Infante et al. (1998)
Lima bean (*Phaseolus lunatus* L.)	Soaking Autoclaving Toasting	Soaked in distilled water (1:2 w/v) for 3, 6, or 9 h at 2°C Autoclaved at 121°C for 10, 15 or 20 min Heated at 204°C for 10, 15 or 20 min	4.00 HU/mg N	Soaking: n.d. Autoclaving: n.d. Toasting: n.d.	Haemagglutination assay (trypsin-treated rabbit erythrocytes)	Adeparusi (2001)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Mung beans (*Phaesolus aureus*)	Soaking Soaking + Dehulling Soaking + Germination Soaking + Boiling Soaking + Autoclaving Soaking + Microwaving	Soaked for 12 h at 25°C Soaked for 12 h and manually dehulled Soaked and germinated in cotton cloth in the dark for 3 days Soaked and cooked in tap water at 100°C for 90 min Soaked and autoclaved at 121°C for 35 min Soaked and cooked in tap water in microwave for 15 min *Distilled water used in 1:10 (w/v) seed:water ratio for soaking*	2670 HU/g	Soaking: 1360 HU/g Dehulling: 1800 HU/g Germination: 560 HU/g Boiling: 0.00 HU/g Autoclaving: 0.00 HU/g Microwave: 0.00 HU/g	Haemagglutination assay (erythrocytes not reported)	Mubarak (2005)
Pigeon pea *(Cajanus cajan)* and cowpea *(Vigna unguiculata)*	Soaking Cooking Soaking + Cooking	Soaked in water (1:10 w/v) for 6, 12 and 18 h Cooked in water (1:10 w/v) for 40, 60 and 80 min at 100°C Soaked for 12 h, drained and cooked for 40, 60 and 80 min	Pigeon pea: 27.88 HU/mg Vegetable cowpea: 49.50 HU/mg	Pigeon pea Soaking (6–18 h): 17.28–13.94 HU/mg Boiling (40–80 min): 8.22–5.88 HU/mg Soaking + Boiling (40–60 min): 2.48–0.52 HU/mg Cowpea Soaking (6–18 h): 44.25–42.50 HU/mg Boiling (40–80 min): 35.70–30.80 HU/mg Soaking + Boiling (40–80 min): 25.16–9.52 HU/mg	Haemagglutination assay (trypsin-treated rabbit erythrocytes)	Onwuka (2006)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
PHA from kidney bean (*Phaseolus vulgaris* L.) varieties Roba, Awash and Beshbesh	Soaking Germination Cooking Soaking + Cooking Autoclaving Soaking + Autoclaving	Soaked in water (W) and 0.05% sodium bicarbonate solution (pH 8.2) (Bi) for 12 h at RT Soaked in distilled water (1:5 w/v) for 12 h at 25°C and germinated for 24, 48, 72 or 96 h in dark chamber at 25°C Cooked in water at 97°C for 35 min Hydrated for 12 h and cooked at 97°C Autoclaved at 121°C in water for 30 min Soaked in water and 0.05% sodium bicarbonate solution at RT for 12 h and autoclaved at 121°C in water for 20 min *Water (pH 6.9) used in 1:3 (w/v) seed:water ratio*	Roba: 1.92 ± 0.01 g/kg PHA Awash: 4.52 ± 0.01 g/kg PHA Beshbesh: 9.98 ± 0.02 g/kg PHA	Roba variety Soaking (W and Bi): 1.90 mg/g PHA Germination (24–96 h): 1.82 ± 0.01 to 1.65 ± 0.01 mg/g PHA Cooking: 0.24 ± 0.01 mg/g PHA Soaking (W and Bi) + Cooking: n.d. Autoclaving: n.d. Soaking + Autoclaving (W and Bi): n.d. Germination + Autoclaving (W and Bi): n.d. Awash variety Soaking (W and Bi): 4.49 ± 0.03 and 4.45 ± 0.01 mg/g PHA Germination (24–96 h): 4.42 ± 0.01 to 4.22 mg/g PHA Cooking: 0.49 ± 0.02 mg/g PHA Soaking + Cooking (W and Bi): n.d. Autoclaving: n.d. Soaking + Autoclaving (W and Bi): n.d. Germination + Autoclaving (W and Bi): n.d. Beshbesh variety Soaking (W and Bi): 9.90 mg/g PHA and 9.80 ± 0.01 mg/g PHA Germination (24–96 h): 9.29 ± 0.01 to 8.18 ± 0.01 mg/g PHA Cooking: 0.82 ± 0.03 mg/g PHA Soaking + Cooking (W and Bi): n.d. Autoclaving: n.d. Soaking + Autoclaving (W and Bi): n.d. Germination + Autoclaving (W and Bi): n.d.	Competitive indirect ELISA	Shimelis & Rakshit (2007)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Vigna unguiculata subsp. *ungiculata*	Soaking Soaking + Germination Cooking Soaking + Cooking Autoclaving Soaking + Germination + Autoclaving	Soaked in distilled water (W) or sodium bicarbonate solution (Bi) for 12 h at 25°C Soaked and germinated at 30°C for 24, 48, 72 and 96 h Cooked at 100°C for 30 min Soaked and cooked at 100°C for 30 min Autoclaved at 103.4 kPa for 30 min Soaked and germinated seeds were autoclaved at 103.4 kPa for 30 min *A seed/water ratio for cooking was 1:10 (w/v) for soaking and cooking*	38 HU/mg protein	Soaking (W): 34 HU/mg protein Soaking (Bi): 32 HU/mg protein Germination (water): 24 h – 31 HU/mg protein 48 h – 30 HU/mg protein 72 h – 29 HU/mg protein 96 h – 28 HU/mg protein Cooking: 6 HU/mg protein Soaking (W) + Cooking: 4 HU/mg protein Soaking (Bi) + Cooking: 4 HU/mg protein Autoclaving: n.d. Soak (W) + Germ (24–96 h) + Auto: n.d.	Haemagglutination assay (trypsin treated type A, B and O human erythrocytes) *Same trend observed in type A, B and O erythrocytes, only type A reported*	Kalpanadevi & Mohan (2013)
PHA from white bean (*Phaseolus vulgaris* L.) varieties Tunisian Twila, Beldia and Coco	Soaking Cooking Soaking + Cooking Autoclaving Germination	12 h in water Cooking of unsoaked seeds for 35 min at 97°C Cooking of soaked seeds for 35 min at 97°C Autoclaving of unsoaked seeds for 30 min at 121°C Sprouting for 24, 48, 72 and 96 h	9.20 ± 0.16 to 11.50 ± 0.65 g/kg	Soaking: 10.19 ± 0.16 to 12.42 ± 0.17 g/kg Cooking: 0.37 ± 0.02 to 4.87 ± 0.67 g/kg Soaking+Cooking: n.d. Autoclaving: n.d. Sprouting: 9.94 ± 0.19 - 12.47 ± 0.18 g/kg	Indirect competitive ELISA (rabbit anti-PHA IgG antibody and horseradish peroxidase-conjugated goat anti-rabbit IgG)	Nciri et al. (2015)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Whole green pea, Split green pea, Whole yellow pea, Split yellow pea, Whole red lentil, Split red lentil, Football red lentil, Split yellow lentil, Split queen green lentil, Whole fava bean, Split fava bean, Whole chickpea B90, Split chickpea B90, Pinto bean, Dark red kidney bean, Navy bean, French green lentil (CDC Marble), Spanish brown lentil (SB-2 3097-7), Large green lentil (CDC Greenstar), Medium green lentil (CDC Imigreen), Desi chickpea (CDC Covi), Black bean (CDC Expresso), Soybean (TH3303R2Y SB-Sorbia Preston)	Soaking (S) Soaking + Cooking (C)	Soaked in distilled water (1:5 w/v) for 4 h at RT Soaked in distilled water (1:5 w/v) for 4 h at RT, cooked in water (1:5 w/v) for 1 h at 95°C	Whole green pea: 5.68 HU/mg DM Split green pea: 5.53 HU/mg DM Whole yellow pea: 5.64 HU/mg DM Split yellow pea: 5.53 HU/mg DM Whole red lentil: 11.04 HU/mg DM Split red lentil: 10.91 HU/mg DM Football red lentil: 11.04 HU/mg DM Split yellow lentil: 11.07 HU/mg DM Split queen green lentil: 11.07 HU/mg DM Whole fava bean: 5.52 HU/mg DM Split fava bean: 5.55 HU/mg DM Whole chickpea B90: 2.74 HU/mg DM Split chickpea B90: 2.73 HU/mg DM Pinto bean: 88.59 ± 0.04 HU/mg DM Dark red kidney bean: 88.52 HU/mg DM Navy bean: 88.46 ± 0.02 HU/mg DM French green lentil: 11.01 HU/mg DM Spanish brown lentil: 11.02 ± 0.01 HU/mg DM Large green lentil: 11.00 ± 0.01 HU/mg DM Medium green lentil: 11.00 ± 0.01 HU/mg DM Desi chickpea: 2.73 HU/mg DM Black bean: 87.69 ± 0.01 HU/mg DM Soybean: 692.82 ± 0.42 HU/mg DM *(One HU defined as the lowest dilution of haemagglutinin producing a positive agglutination response)*	Whole green pea: 5.49 HU/mg DM (S); 0.17 HU/mg DM (C) Split green pea: 5.30 HU/mg DM (S) 0.17 HU/mg DM (C) Whole yellow pea: 5.46 HU/mg DM (S); 0.17 HU/mg DM (C) Split yellow pea: 5.31 HU/mg DM (S); 0.33 HU/mg DM (C) Whole red lentil: 10.66 HU/mg DM (S); 0.33 HU/mg DM (C) Split red lentil: 10.65 HU/mg DM (S); 0.33 HU/mg DM (C) Football red lentil: 10.71 HU/mg DM (S); 0.33 HU/mg DM (C) Split yellow lentil: 10.59 HU/mg DM (S); 0.33 HU/mg DM (C) Split queen green lentil: 10.64 HU/mg DM (S); 0.33 HU/mg DM (C) Whole fava bean: 5.49 HU/mg DM (S); 0.09 HU/mg DM (C) Split fava bean: 5.26 HU/mg DM (S); 0.08 HU/mg DM (C) Whole chickpea B90: 2.70 HU/mg DM(S); 0.17 HU/mg DM (C) Split chickpea B90: 2.64 HU/mg DM (S); 0.17 HU/mg DM (C) Pinto bean: 87.31 ± 0.01 HU/mg DM (S); 0.17 HU/mg DM (C) Dark red kidney bean: 86.47 HU/mg DM (S); 0.17 HU/mg DM (C) Navy bean: 86.77 ± 0.02 HU/mg DM (S); 0.17 HU/mg DM (C) French green lentil: 10.79 ± 0.01 HU/mg DM (S); 0.33 HU/mg DM (C) Spanish brown lentil: 10.82 ± 0.01 HU/mg DM (S); 0.33 HU/mg DM (C) Large green lentil: 10.79 ± 0.01 HU/mg DM (S); 0.33 HU/mg DM (C) Medium green lentil: 10.85 ± 0.01 HU/mg DM (S); 0.34 HU/mg DM (C) Desi chickpea: 2.72 HU/mg DM (S); 0.17 HU/mg DM (C) Black bean: 87.04 ± 0.05 HU/mg DM (S); 0.17 HU/mg DM (C) Soybean: 687.50 ± 0.66 HU/mg DM (S); 2.70 ± 0.23 HU/mg DM (C)	Haemagglutination assay (rabbit erythrocytes)	Shi et al. (2018)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Soya beans, PHA from black beans	Malting	Steeped for 6 h, germinated for 24 or 48 h at 20°C and kilning at 60°C	Soya bean: 2.56 ± 0.0 g/kg Black bean: 0.166 ± 0.04 mg/100 g	**Soya bean** 1-day malting: 1.60 ± 0.96 g/kg 2-day malting: 0.80 ± 0.48 g/kg **Black bean** 1-day malting: 0.091 ± 0.01 mg/100 g 2-day malting: 0.039 ± 0.01 mg/100 g	Haemagglutination assay (native and trypsin-treated rat erythrocytes) *Only trypsin-treated erythrocytes reported* ELISA (rabbit anti-PHA IgG antibodies and goat anti-rabbit IgG biotin conjugate)	Muzquiz et al. (1998)
Soybean (*Glycine max* cv BR16) PHA from black bean (*Phaseolus vulgaris*)	Malting	Soaked in distilled water for 5 h and germinated during 24 and 48 h in the dark at 30°C	Soybean: 2.56 ± 0.0 mg/g Black bean: 1.7 ± 0.4 mg/g	Soybean: 1 day – 1.60 ±0.96 mg/g 2 day – 0.80 ± 0.481 mg/g Black bean: 1 day – 0.9 ± 0.1 mg/g 2 day – 0.4 ± 0.1 mg/g	Haemagglutination assay (trypsin- and non - trypsin-treated rat erythrocytes) and competitive indirect ELISA (rabbit anti-PHA IgG antibodies) *Same trend observed in haemagglutination assay and ELISA, only ELISA results reported*	Trugo et al. (1999)
Dry bean (*Phaseolus vulgaris* var. Rosinha G2)	Soaking + Boiling Soaking + Autoclaving	Soaked overnight in distilled water and boiled at 97°C up to 60 min Autoclaved at 121°C for 7.5 and 15 min	2000 HU *(One HU was defined as the concentration of the protein that caused haemagglutination of the blood cells)*	Boiling: 2.5 min – 27 HU 5 min – 13 HU 10 min – 1.6 HU 15 min – 0.4 HU 30 min – n.d. Autoclaving: n.d.	Haemagglutination assay (trypsin-treated bovine erythrocytes)	Antunes & Sgarbieri, 1980
White kidney beans (*Phaseolus vulgaris*)	Soaking + Cooking Cooking	Soaking for 16 h and heating in water up to 100°C for up to 6 h Cooking in water at 100°C for up to 6 h	100% HA	Soaking + Cooking: 90°C (≥ 4 h): 0% HA 95°C (≥ 45 min): 0% HA 100°C (4-10 min): 0% HA Cooking: 100°C (90 min): 0% HA	Haemagglutination assay (rabbit erythrocytes)	Grant et al. (1982)
Dark red kidney bean (*Phaseolus vulgaris*)	Soaking +Cooking	Soaked overnight at 4°C in physiological-buffered saline and cooked at 82, 88, 93 and 100°C for times ranging from 10 min to 4 h	NR	Conditions for zero haemagglutinating activity: 88°C – 4 h 93°C – 2 h 100°C – 30 min	Haemagglutination assay (trypsin-treated porcine erythrocytes)	Coffey et al. (1985)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Grass pea (*Lathyrus sativus*) seeds or flour	Soaking + Fermentation + Cooking Roasting Boiling Dry heating Deep frying Soaking + Fermentation + Steaming Soaking + Autoclaving	Dosa Papad roasted Dhal Popped seeds (250°C) Chapathi Papad Muruku Dokla Kichidi	Raw: 1632 HU/g	Dosa: n.d. Papad roasted: 120 HU/g Dhal: 90 HU/g Popped seeds: 30 HU/g Chapathi: 1632 HU/g Papad: 60 HU/g Muruku: n.d. Dokla: n.d. Kichidi: n.d.	Haemagglutination assay (erythrocytes not reported)	Ayyagari et al. (1989)
Soybean (*Glycine max*)	Soaking + Cooking	Soaked in distilled water (1:4 w/v) for 16 h at RT and cooked in distilled water (1:1 w/v) between 60 and 100°C for up to 90 min	9.6 ± 3.2 to 2.4 ± 0.8 (mg lectin equivalents/g defatted soybeans)	Cooking 90 min at 60–70°C: no effect Cooking at 80–90°C: reduced activity Cooking at 100°C for 5 min: abolished activity	Haemagglutination assay (trypsin treated rat erythrocytes)	Armour et al. (1998)
Faba bean (*Vicia faba*), pea (*Pisum sativum* L.) and soybean (*Glycine max* L.)	Soaking+Boiling	Soaked and boiled for 10 or 20 min	Faba bean: 1:100 HA Pea: 1:100 HA Soya bean: 1:800 HA *(One HA defined as the inverse of the maximum lectin dilution having haemagglutinating activity)*	Faba bean: 10 min – 1:50 HA 20 min – 0 HA Pea: 10 min – 1:50 HA 20 min – 0 HA Soya bean: 10 min – 1:600 HA 20 min – 1:200 HA	Haemagglutination assay (human erythrocytes type O, A, B and AB) *Same trend observed in type A, B, AB and O erythrocytes, only type A reported*	Leontowicz et al. (1998)
Black bean (*Phaseolus vulgaris*) Jamapa variety	Soaking + Cooking	Soaked in water for 12 h and boiled until seeds were soft	1:128 HA *(One HA defined as the highest dilution of saline extract causing visible haemagglutination after 1 h)*	1:16 HA	Haemagglutination assay (trypsin-treated human and rabbit erythrocytes) *Same trend observed in human and rabbit erythrocytes, only rabbit reported*	Morales-de León et al. (2007)
Beans (*Phaseolus* vulgaris), 10 varieties; broad bean (*Vicia faba*); lentil (*Lens esculenta*), dry pea (*Pisum sativum*) and ayocote bean (*Phaseolus coccineous*)	Boiling	Boiled in water (1:4 w/v) for 2 h	Beans – 1:50000 Broad bean, lentil and dry pea – 1:15000 Ayocote bean – 1:30000 *(Values defined as the maximal dilution with agglutination present)*	All samples – 1:1000 HA	Haemagglutination assay (trypsin treated rabbit erythrocytes)	Sotelo-López et al. (1978)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Corn–soybean blend	Boiling Hot plate cooking	Corn–soybean blend boiled in limewater (0, 2 and 4% calcium hydroxide) for 15 or 30 min Tortillas cooked on hot plate at 200°C for 15, 30, 45 and 60 sec on each side	Raw corn–soybean blend: 8% soybean – 4 HA 16% soybean – 6 HA Raw corn–soybean tortillas: 8% soybean – 4 HA 16% soybean – 6 HA *(HA defined as the maximum dilution of 1:10 corn-soybean blend dispersion (dry basis) In 1% NaCl solution which still agglutinates red blood cells)*	**Raw corn–soybean blend** Boiling (8% soybean) 0% lime in water: 15 min – 2 HA 30 min – 0 HA 2% lime in water: 15 min – 1 HA 30 min – 0 HA 4% lime in water: 15 min – 1 HA 30 min – 0 HA Boiling (16% soybean) 0% lime in water: 15 min – 2 HA 30 min – 0 HA 2% lime in water: 15 min – 2 HA 30 min – 0 HA 4% lime in water: 15 min – 1 HA 30 min – 0 HA **Hot plate cooking** Hot plate cooking (8% soybean): 15 sec – 3 HA 30 sec – 2 HA 45 sec – 2 HA 60 sec – 2 HA Hot plate cooking (16% soybean): 15 sec – 4 HA 30 sec – 3 HA 45 sec – 3 HA 60 sec – 3 HA	Haemagglutination assay (rabbit blood)	Del Valle et al. (1983)
Bean (*Phaseolus vulgaris*) variety Ojo de cabra	Boiling Toasting	Cooked in boiling water for 140 min Toasted in dry heat at 135°C for 15 min	367.4 sp. act/g *(Specific activity calculated as haemagglutination titre per protein concentration)*	Cooked: 0.8 sp. act/g Toasted: 57.6 sp. act/g	Haemagglutination assay (glutaraldehyde treated erythrocytes)	Almeida et al. (1991)
Whole black turtle soup bean (*Phaseolus vulgaris*)	Cooking Soaking + Cooking	Cooking was done in deionised distilled water (DDW) at 97.8°C Soaked in deionised distilled water for 15 h and boiled at 97.8°C	2.5 ± 0.9 mg/g bean flour	Cooking (> 20 min): n.d. Soaking + Cooking (> 10 min): n.d.	Porcine thyroglobulin (PTG)-Sepharose affinity chromatography coupled with the Fohn-Ciocalteau protein assay	Bonorden & Swanson (1992)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Large white kidney bean (*Phaseolus coccineus*); brown haricot bean (*Phaseolus vulgaris*); small white canning bean (*Phaseolus vulgaris*); red speckled sugar bean (*Phaseolus vulgaris*); local red kidney bean (*Phaseolus vulgaris*); imported red kidney bean (*Phaseolus vulgaris*)	Cooking Soaking + Cooking	Cooked at 85°C or 100°C for 15, 30 and 60 min Soaked overnight in phosphate-buffered saline and cooked at 85°C or 100°C for 15, 30 and 60 min	All samples: 100% HU	Soaking (all samples): 99%–94% HU Cooking (100°C): Large white kidney: 15 min – 99% HU 30 min – 98% HU 60 min – 0% HU Brown haricot: 15 min – 98% HU 30 min – 12% HU 60 min – 0% HU Small white canning: 15 min – 94% HU 30 min – 0% HU 60 min – 0% HU Red speckled sugar: 15 min – 99% HU 30 min – 97% HU 60 min – 0% HU Local red kidney: 15 min – 99% HU 30 min – 93% HU 60 min – 2% HU Imported red kidney: 15 min – 96% HU 30 min – 0% HU 60 min – 0% HU Cooking (85°C): Large white kidney (15 – 60 min): 98%–99% HU Brown haricot: 15 min – 99% HU 30 min – 99% HU 60 min – 5% HU Small white canning: 15 min – 98% H 30 min – 95% HU 60 min – 0% HU Red speckled sugar: 15 min – 99% HU 30 min – 97% HU 60 min – 0% HU Local red kidney (15–60 min): 95%–98% HU Imported red kidney (15–60 min): 98% HUSoaking + Cooking (100°C): Large white kidney: 15 min – 2% HU	Haemagglutination assay (trypsin treated porcine erythrocytes)	Venter & Thiel (1995)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
				30–60 min – 0% HU Brown haricot (15–60 min): 0% HU Small white canning (15–60 min): 0% HU Red speckled sugar (15–60 min): 0% HU Local red kidney: 15 min – 2% HU 30–60 min – 0% HU Imported red kidney: 15 min – 2% HU 30–60 min – 0% HU Soaking + Cooking (85°C): Large white kidney: 15–30 min – 99% HU 60 min – 97% HU Brown haricot: 15 min – 80% HU 30 min – 32% HU 60 min – 3% HU Small white canning: 15 min – 97% HU 30 min – 44% HU 60 min – 0% HU Red speckled sugar bean: 15 min – 99% HU 30 min – 98% HU 60 min – 95% HU Local red kidney: 15 min – 99% HU 30 min – 98% HU 60 min – 97% HU Imported red kidney: 15–30 min – 98% HU 60 min – 65% HU		
Jackfruit seed meal from *Artocarpus heterophyllus*	Cooking Autoclaving	Cooked in boiling water for up to 2 h Autoclaved at 120°C for 5, 10, 15 and 20 min	320 – 40 HU/mg	Cooking: 2 h – ≤ 5% decrease in HU/mg Autoclaving: 5 min – 0 HU/mg	Haemagglutination assay (trypsin-treated type B human erythrocytes)	Ravindran et al. (1996)
Black bean var. Talamanca (*Phaseolus vulgaris*)	Cooking Cooking + Fermentation	Ground beans cooked in deionised water at 100°C for 22 min Ground beans cooked in deionised water at 100°C for 22 min, inoculated with *Rhizopus oligosporus* and fermented for 15, 20 or 25 h	2.2 ± 0.2 HU/mg	Cooking: n.d. Cooking + Fermentation: n.d.	Haemagglutination assay with photometric (trypsin treated rabbit erythrocytes)	Rodríguez-Bürger et al. (1998)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
WGA from wheat-derived foodstuffs (pasta, wholemeal pasta, wholemeal wheat germ-enriched pasta) and ingredients (flours, wholemeal flours and semolina)	Boiling	Boiled in water (1:10 w/v) for 10 min	Pasta 1: ≤ 0.4 ± 0.2 µg/g Pasta 2: 3.2 ± 0.2 µg/g Wholemeal pasta 1: 40 ± 2.7 µg/g Wholemeal pasta 2: 5.7 ± 0.2 µg/g	Pasta 1: ≤ 0.3 ± 0.17 µg/g Pasta 2: ≤ 0.3 ± 0.20 µg/g Wholemeal pasta 1: n.d. Wholemeal pasta 2: n.d.	ELISA (detection with ovoalbumin)	Matucci et al. (2004)
Winged bean seeds (*Psophocarpus tetragonolobus*)	Boiling Roasting Autoclaving	Boiled for 2 h Roasted in sand for 30 min Autoclaved at 121°C for 30 min	4.0 HU/mg N	Boiling: 2.0 HU/mg N Roasting: n.d. HU/mg N Autoclaving:1.0 HU/mg N	Haemagglutination assay (trypsin treated rabbit erythrocytes)	Igene et al. (2006)
Bambara groundnut seeds (*Vigna subterranea*)	Boiling	Cooked in water at 100°C for 30, 60, 90 and 120 min	0.83 HU/mg/N	Boiling: 30 min – 0.05 HU/mg/N ≥ 60 min – 0.00 HU/mg/N	Method not reported	Omoikhoje et al. (2009)
Garlic (*Allium sativum*) Isolated ASA I and ASA II lectins	Boiling pH Heating Chemical denaturation	Boiled in 50 mL PBS for 20 min Incubated 90 µg of ASA I or ASA II in buffers of varying pH (2, 4, 6, 8, 10 and 12) for 1 h at 25°C Heated in oven in PBS for 30 min at 20°C, 40°C, 60°C, 80°C and 100°C Incubated for 30 min in various concentrations of urea (2 M, 4 M, 6 M and 8 M) and guanidine hydrochloride (Gdn.HCl) (2 M, 4 M and 6 M) at RT	Garlic: 23.5 HA ASA I: 100% HA ASA II: 100% HA *(One HA unit is equivalent to the concentration of protein in the last well that completely agglutinates an equal volume of standardised RBC suspension)*	Garlic (boiled): 14.3 HA ASA I: pH (2–12) – 80–100% HA Heating (30 min): 60°C – 100% HA 80°C – 5% HA 100°C – 0% HA Denaturation: Gdn.HCL: 2–4 M – 60%–90% HA 6 M – 50% HA Urea: 2 M – 100% HA 4–6 M – 70% HA 8 M – 40% HA ASA II: pH: (2–4) – 25% HA (6–8) – 100% HA (10–12) – 40% HA Heating (30 min): 60°C – 100% HA 80°C – 60% HA 100°C – 0% HA Denaturation: Gdn.HCl: 2–4 M – 60%–90% HA 6 M – 25% HA Urea (8 M): 30% HA	Haemagglutination assay (trypsin-treated rabbit erythrocytes)	Clement & Venkatesh (2010)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Lupin seeds of two species: bitter (*Lupinus termis*) and sweet (*Lupinus albus*)	Cooking Autoclaving Microwaving Soaking Dehulling	Cooked for 40 min at 100°C Autoclaved for 20 min at 121°C Microwaved for 6 min Bitter raw: soaked for 24, 48, 72 and 96 h Bitter precooked: soaked for 96 h Sweet raw: soaked for 12 and 24 h Sweet precooked: soaked for 24 h Manual dehulling and grinding of raw and processed seeds *Distilled water used in 1:10 (w/v) seed:water ratio for soaking, cooking and microwaving*	Raw bitter: 16 HA/g Raw sweet: 32 HA/g	Cooking bitter: 4 HA/g Cooking sweet: 2 HA/g Autoclaving bitter: 0 HA/g Autoclaving sweet: 2 HA/g Microwave bitter: 4 HA/g Microwave sweet: 4 HA/g Soaking bitter: 16 HA/g (raw, for 24–96 h) 2 HA/g (pre-cooked, for 96 h) 0 HA/g (autoclaved, time NR) 4 HA/g (microwaved, time NR) Soaking sweet: 32 HA/g (raw, for 12–24 h) 0 HA/g (precooked, for 24 h) 0 HA/g (autoclaved, time NR) 4 HA/g (microwaved, NR) Dehulling bitter: 16 HA/g (raw) 2 HA/g (precooked and soaked) 0 HA/g (autoclaved and soaked) 4 HA/g (microwaved and soaked) Dehulling sweet: 32 HA/g (raw) 0 HA/g (precooked and soaked) 0 HA/g (autoclaved and soaked) 4 HA/g (microwaved and soaked)	Haemagglutination assay (trypsin treated rabbit erythrocytes)	Embaby (2010)
Green and white faba beans (*Vicia faba*)	Cooking Autoclaving Microwaving Soaking Dehulling	Cooked in distilled water (1:10 w/v) at 100°C for 30 min Autoclaved in distilled water (1:10 w/v) at 121°C for 20 min Microwaved in water (1:10 w/v) for 6 min Soaked in distilled water (1:10 w/v) for 12, 24, 36 and 48 h Dehulled manually	Green: 28% HA White: 56% HA	**Green** Dehulling: 28% HA Soaking (12–48 h): 28% HA Microwave (+ Soaking + Dehulling): 7% HA Cooking: 7% HA Cooking + Soaking (+Dehulling): 4% HA Autoclaving (+Soaking + Dehulling): 0% HA **White** Dehulling: 56% HA Soaking (12–48 h): 56% HA Microwave (+Soaking + Dehulling): 7% HA Cooking: 4% HA Cooking + Soaking (+Dehulling): 0% HA Autoclaving: 4% HA Autoclaving + Soaking (+Dehulling): 0% HA	Haemagglutination (trypsin-treated rabbit erythrocytes)	Luo & Xie (2013)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Isolated lectin from Zihua snap bean	Heating in water pH	Heated in water at 50, 60, 70, 80 and 90°C for 0, 5, 10, 15, 20, 25 and 30 min Solubilised in a 0.5 mg/mL solution in buffer solutions of glycine-HCl (pH 2.0), citrate (pH 3.0–5.0), phosphate (pH 6.0–8.0) and glycine-sodium hydroxide (pH 9.0–11.0)	pH 7: 100% HA	Heating (90°C): 10 min to n.d. pH: 2–5 – ≈70%–80% HA 6–10 to ≈90%–100% HA 11%–60% HA 12 to n.d.	Haemagglutination assay (rabbit red blood cells)	Jiang et al. (2019)
Isolated lectins from black bean (*Phaseolus vulgaris*) varieties Vara and Surco	Cooking pH	Heated for 30 min at 25, 30, 40, 50, 60, 70, 80, 90 and 100°C Incubated for 60 min at RT with buffers of sodium acetate (pH 3.5, 4.0, 4.5, 5.0 and 5.5), sodium phosphate (pH 6.0, 6.5, 7.0 and 7.5), tris-HCl (pH 8.0 and 8.5) and glycine-NaOH (pH 9.0, 9.5, 10.0 and 10.5)	Vara: 16 ± 0 HU Surco: 2097152 ± 0 HU	Vara: Cooking (80°C): n.d. pH: lowest at 3.5, 4.5 and 8.5 Surco: Cooking (90°C): n.d. pH (range: 3.5–6.5 and 9.5–10.5): n.d.	Haemagglutinating activity (human erythrocytes)	Valadez-Vega, Lugo-Magaña, Betanzos-Cabrera, & Villagómez-Ibarra (2022)
Functional lectins from soybean (*Glycine max*, cullinary grade)	Steaming	Steamed at 102, 120 and 134°C for 1.5, 2, 2.5, 4, 5, 7.5, 10, 20, 40 and 80 min	7638 µg/g	Steamed at 102°C: 10 min – 1005 µg/g 20 min – 105 µg/g 40 min – 14 µg/g Steamed at 120°C: ≥ 2 min – < 0.5 µg/g Steamed at 134°C: n.d.	Immunoassay FLIA-BBM	Qin et al. (1996)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Carbohydrate-binding lectins from soybean (*Glycine max*)	Steaming	Steamed between 90 and 120°C for 5–20 min	Agglutinating lectin: 1.35 mg/g Carbohydrate binding lectin: 2.68 mg/g	Agglutinating lectin: Steaming 90°C: 5 min – 0.12 mg/g 10 min – 0.15 mg/g 15 min – 0.04 mg/g 20 min – 0.06 mg/g Steaming 100°C (5-20 min): 0 mg/g Steaming 110°C (5-20 min): 0 mg/g Carbohydrate binding lectin: Steaming 90°C: 5 min – 1.49 mg/g 10 min – 1.40 mg/g 15 min – 1.38 mg/g 20 min – 1.06 mg/g Steaming 100°C:5 min – 0.13 mg/g 10 min – 0.11 mg/g 15 min – 0.10 mg/g 20 min – 0.10 mg/g Steaming 110°C:5 min – 0.12 mg/g 10 min – 0.14 mg/g 15 min – 0.09 mg/g 20 min – 0.08 mg/g	Agglutinating lectins: brush border lectin agglutination assay (using brush border membrane vesicles from broiler chicks) Carbohydrate binding lectins: protein content measurement of affinity chromatography eluent	Fasina et al. (2003)
Velvet bean (*Mucuna pruriens* var. *utilis)*	Soaking + Microwaving	Soaked in distilled water (1:10 w/v) for 12 h and microwaved at 2450 MHz, 750 watts, 230 volts, for 10 min	Type A erythrocytes: 320–328 HU/mg protein	Microwaving: 20–14 HU/mg protein	Haemagglutination assay (type A, B and O human erythrocytes) *Same trend observed in all human type erythrocytes, only type A reported*	Gurumoorthi et al. (2013)
Soybean (*Glycine max*) variety Rabel	Heating	Heated at 60, 65, 70, 75 and 100°C for 5, 10, 15, 20, 25, 30, 45 and 60 min	100% haemagglutinating activity (HA)	60°C: 15 min – 47.13% HA 30 min – 41.72% HA 45 min – 22.39% HA 60 min – 22.14% HA 65°C: 15 min – 46.59% HA ≥ 30 min – 24.48–22.63% HA 70°C: 15–45 min – 24.07-21.02% HA 60 min – 22.63% HA 75°C: ≥ 30 min – 11.14-10.10% HA 100°C: ≥ 5 min – 0% HA	Haemagglutination assay (fresh erythrocytes of rabbit whole blood)	Hussein et al. (1980)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Defatted meal from spin-blanched Florunner peanut seeds and Bragg soybean seeds	Dry heat Moist heat	Air heated to 177°C for up to 30 min Steamed at 121°C for up to 30 min	**Peanut:** 144.7 ± 2.13 HA **Soybean:** 112.1 ± 2.77 HA	**Peanut** Dry heat: 5 – 20 min: 133.2 ± 0.78 – 108.6 ± 1.09 HA 30 min: 0 HA Moist heat: 5 min – 103.3 ± 0.87 HA 10 min – 37.2 ± 1.29 HA 15 min – 9.2 ± 0.60 HA 20 min – 0.4 ± 0.02 HA 30 min – 0 HA **Soybean** Dry heat: 5 – 10 min: 85.4 ± 2.05 – 70.7 ± 1.65 HA 15 min: 43.5 ± 2.38 20 min – 30 min: 0 HA Moist heat: 5 min – 3.9 ± 0.31 HA 10 min – 3.2 ± 0.57 HA 15 min – 0.6 ± 0.05 HA 20 min – 30 min – 0 HA	Haemagglutination assay (human type A erythrocytes)	Ahmed (1986)
Bambarra groundnut seeds (*Vigna subterranea*)	Dry heat Boiling Autoclaving Soaking Soaking + Fermentation	Heated at 100°C for 30 min, 1, 2, 3 and 4 h in air oven Boiled in water (1:10 w/v) for 30 min, 1 h and 2 h with or without addition of trona Autoclaved at 121°C for 1 h Soaked for 12 h Soaked for 6 h and fermented for 48 h at RT wrapped in banana leaves	NR	Dry heat: 30 min – 1622.82 HU 1 h – 1431.12 HU ≥ 2 h – n.d. Cooking: 30 min – 2051.17 HU 1 h – 1981.39 HU 2 h – 1541.96 HU Cooking + trona (1 h): 3515.95 HU Autoclaving: 2818.42 HU Soaking: 1916.43 HU Fermentation: 2260.14 HU	Haemagglutination assay (trypsin treated cow erythrocytes)	Ojimelukwe et al. (1995a)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Soybean *(Glycine max)* and yam bean (*Sphenostylis stenocarpa*)	Dry heat Boiling Soaking Autoclaving Fermentation	Heated at 100°C for 1, 2, 3 and 4 h Boiled in water (1:10 w/v) for 30 min, 1 h or 2 h and 1 h with the addition of trona Soaked for 12 h in water Autoclaved at 121°C for 1 h NR	Soybean seeds: NR Soybean flour: NR Yam bean seeds: NR Yam bean flour: NR *(One HU is defined as the amount of material required to cause a decrease of 50% in the absorbance of erythrocyte suspension within 2.5 h)*	Soybean seeds Boiling: 30 min – 2340 HU 1 h – 1571 HU 1 h + trona – 2213 HU 2 h – 1946 HU Autoclaving: 2506 HU Soaking: 1641 HU Fermentation (48 h): 1230 HU Soybean flour Dry heat: 30 min – 2260 HU 1 h – 1481 HU > 1 h – n.d. Yam bean seeds Boiling: 30 min – 2834 HU 1 h – 1669 HU 1 h + trona – 3164 HU 2 h – 1477 HU Autoclaving: 2795 HU Soaking: 1042 HU Fermentation (48 h): 1509 HU Yam bean flour Dry heat: 30 min – 1623 HU 1 h – 1078 HU > 1 h – n.d.	Haemagglutination assay (trypsin treated cow erythrocytes)	Ojimelukwe et al. (1995b)
Cowpea (*Vigna unguiculata*)	Dry heating	Dry heated under 1 atm pressure at 120°C for 15 min	25.6 HA (*One HA equivalent to the inverse of the minimum amount in the assay which produced haemagglutination*)	6.4 HA	Haemagglutination assay (erythrocyte not reported)	Umapathy et al. (1998)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Isolated lectins from black turtle bean (*Phaseolus vulgaris*)	Heating	Heated in PBS at 70, 75, 80, 85 and 90°C (±1°C) for 0, 5, 10, 15, 20, 25 and 30 min	1.64 HA	70°C: 5 min – 1.08 ± 0.06 HA 15 min – 0.76 ± 0.08 HA 20 min – 0.54 ± 0.04 HA 25 min – 0.34 ± 0.04 HA 30 min – 0.26 ± 0.03 HA 75°C: 5 min – 1.01 ± 0.08 HA 15 min – 0.50 ± 0.04 HA 20 min – 0.19 ± 0.03 HA 25 min – 0.14 ± 0.04 HA 30 min – n.d. 80°C: 5 min – 0.56 ± 0.05 HA 15 min – 0.29 ± 0.02 HA 85°C: 5 min – 0.44 ± 0.03 HA 15 min – 0.19 ± 0.02 HA 90°C: ≥ 5 min – n.d.	Haemagglutination assay (rabbit red blood cells)	He et al. (2014)
Cowpea (*Vigna unguiculata*) var. IT86-D719 seeds	Soaking+ Dry heating Soaking + Dehulling + Heating	Soaked in 0.2% sodium bisulphite for 16 h at 4°C and heating in oven for 7.5 h at 48°C, for 5 h at 70°C or for 30 min at 119°C Same procedure with dehulling step after soaking	31 µg/g	Soaking (+ Dehulling) + Heating: 48°C – 15 µg/g 70°C – 8 µg/g 119°C – n.d.	Haemagglutinating activity (erythrocytes not reported)	Olivera-Castillo et al. (2011)
Kidney bean (*Phaseolus vulgaris*); cultivar Zihuayoudou (Whole pod)	Stir-frying Braising	Stir-fried for 3, 6, 9, 12, 15 and 18 min Braised for 5, 10, 15, 20, 25 and 30 min	51.2 mg/g	Stir-frying: 3 min – 12.8 mg/g 6 min – 3.2 mg/g 9 min – 1.6 mg/g 12 min – 0.8 mg/g 15 min – 0.2 mg/g 18 min – n.d. Braising: 5 min – 0.4 mg/g ≥ 10 min – n.d.	Haemagglutination assay (rabbit erythrocytes)	Sun et al. (2019)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Peas (*Pisum sativum* L.) cv. Ballet	Extrusion Germination	Extruded in co-rotating twin extruder at constant feed rate 350 g/min and screw speed 100 rpm. Tap water was fed to have a 25% moisture content. Temperature at the outlet die was 140°C Germinated at 25°C in ventilated dark incubator for 2 days	6.0 ± 0.0 *10^6 HU/kg DM	Extrusion: 0.1 ± 0.0 *10^6 HU/kg DM Germination: 6.0 ± 0.0*10^6 HU/kg DM	Haemagglutination assay (trypsin-treated rat erythrocytes)	Orúe et al. (1998)
Bean flours (*P. vulgaris*) cv. Horsehead (HH); Canadian Wonder (CW)	Extrusion	at 140, 160, 170, 180°C and moisture at 25 or 30%	Fresh HH: 4.9 ± 2.4 HTC HH: 9.7 ± 4.8 Fresh CW: 9.7 ± 4.8 HTC CW: 19.4 ± 9.7 (mg of PHA equiv/g)	Fresh HH: 0.2 ± 0 (at 140°C and moisture 30%) HTC HH: n.d. HTC CW: 2.0 ± 0.1 (at 140°C and moisture 25%) (mg of PHA equiv/g)	Haemagglutination assay (rat erythrocytes)	Martín-Cabrejas et al. (1999)
Pea seeds (*Pisum sativum*)	Extrusion	at 145°C; moisture at 25%	6000 HU/kg *(amount of sample in the last dilution which caused 50% agglutination)*	n.d.	Haemagglutination assay (trypsin-treated rabbit erythrocytes)	Alonso, Grant, et al. (2000)
Pea (*Pisum sativum*) and kidney bean (*Phaseolus vulgaris*) meals	Extrusion	at 150°C for *P. sativum* at 155°C for *P. vulgaris*	Peas: 8 HU/100 mg dw Kidney beans: 1.024 HU/100 mg	n.d	Haemagglutination assay (rat erythrocytes, trypsin treated)	Alonso et al. (2001)
Faba bean (*Vicia faba* var. *Nadwislahski*); spring pea (*Pisum sativum* var. Fidelia); soybean (*Glycine max*)	Extrusion	at 150°C; exposure time about 40 s, moisture about 25%	faba bean: 1:100 spring pea: 1:400 soybean: 1:800 *(highest dilution showing full agglutination)*	faba bean: 0 spring pea: 1:200 soybean: 1:200 *(highest dilution showing full agglutination)*	Haemagglutination assay (guinea pig erythrocytes)	Leontowicz, Leontowicz, Kostyra, et al. (2001)
Protein extract from faba bean (*Vicia faba* var. *nadwislahski*)	Extrusion	160°C, exposure time 30-60 s	O: 1:200 A: 1:100 B: 1:50 AB: 1:200 *(highest dilution showing full agglutination)*	O: 0 A: 0 B: 0 AB: 0	Haemagglutination assay (human type A, B, AB and O erythrocytes)	Leontowicz, Leontowicz, Biernat, et al. (2001)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Kidney bean (*Phaseolus vulgaris*)	Extrusion	at 150°C; moisture at 250 g/kg	11,234 HU/kg *(One HU was defined as that contained in the amount of sample in the last dilution that caused 50% agglutination of the blood cells)*	n.d.	Haemagglutination assay (trypsin treated rabbit erythrocytes)	Marzo et al. (2002)
PHA from composite supplementary foods CBSM: corn–bean–sardine meal, BM: bean meal, SBSM: sorghum–bean–sardine meal, RBSM: rice–bean–sardine meal	Extrusion Drum-processing Conventional cooking	at 147°C; moisture 12–14.4% at 135 ± 5°C by steam stirred constantly until boiling for 25 min (CBSM, SBSM, RBSM) and for 35 min (BM)	Results in mg/g of food 2.20 (CBSM) 6.59 (BM) 2.01 (SBSM) 3.51 (RBSM)	Results in mg/g of food Extrusion: 0.17 (CBSM) 0.20 (BM) 0.18 (SBSM) 0.18 (RBSM) Drum-processing: 0.18 (CBSM) 0.31 (BM) 0.20 (SBSM) 0.18 (RBSM) Conventional cooking: 0.19 (CBSM) 0.21 (BM) 0.18 (SBSM) 0.17 (RBSM)	ELISA	Mosha et al. (2005)
Kidney bean (*Phaseolus vulgaris*) diets 1% (KB1) and 6% (KB6)	Extrusion	at 150°C; moisture at 250 g/kg	KB1: 600 ± 89 HU KB6: 3600 ± 201 HU *(One HU was defined as that contained in the amount of sample in the last dilution that caused 50% agglutination of the blood cells)*	n.d	Haemagglutination assay (erythrocytes not reported)	Marzo et al. (2011)
PHA from navy bean (*Phaseolus vulgaris*) and pinto bean (*P. vulgaris*)	Extrusion Steam-cooking	at 85°C at 82°C	2.75 mg/g of dry bean (navy variety) 2.70 mg/g of dry bean (Pinto variety)	Extrusion: 0.30 mg/g of dry bean (navy variety) 0.60 mg/g of dry bean (Pinto variety) Steam-cooking: 0.60 mg/g of dry bean (navy variety) 0.90 mg/g of dry bean (Pinto variety)	ELISA	Kelkar et al. (2012)
LCA from lentil flour (*Lens culinaris*)	Extrusion	at 160°C	1.36 ± 0.14%	n.d.	ELISA	Morales et al. (2015)
PHA (found in rice bean and whole carob fruit flour blends)	Extrusion	at 125°C	Range: 0.035 ± 0.002 to 0.108 ± 0.005%	n.d	ELISA	Arribas et al. (2019a)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Rice, pea and carob fruit, flour blends)	Extrusion	at 125°C	Range: 10.20–20.41 HU *(amount of material (g) causing 50% erythrocyte agglutination was defined as one haemagglutination unit (HU))*	Range: 0.63–10.20 HU	Haemagglutination assay (rat erythrocytes, trypsin treated)	Arribas et al. (2019b)
Lentil flour (*Lens culinaris*)	Extrusion	extrusion at 140°C and at 160°C	333–1250 HU/kg flour	at 140°C: 5.13–10.20 HU/kg flour at 160°C: 3.85–10.20 HU/kg flour	Haemagglutination assay (trypsin-treated rat erythrocytes	Ciudad-Mulero et al. (2020)
LCA from lentils			30.75 ± 2.13 to 37.80 ± 2.16% LCA	at 140°C: 0%–0.11% LCA at 160°C: 0% LCA	Competitive Indirect ELISA	
Soybean flour (*Glycine max*)	Autoclaving	Autoclaved at 121°C for 10, 20, 30 and 60 min	31.5–65 HA *(One HA defined as the reciprocal of the minimum concentration of sample (expressed as mg/mL) that causes agglutination of human red blood cells)*	Autoclaving: 10 min – 8.0 HA 20 min – 3.2–6.5 HA 30 min – 0.85–1.65 HA 60 min – 0.16–0.32 HA	Haemagglutination assay (glutaraldehyde-stabilised human group A erythrocytes)	Friedman et al. (1991)
Cucurbit seed kernels of cucumber var oinsette (*Cucumis sativus*) and bottle gourd var Punjab Round (*Lagenaria vulgaris* (Molina) Standl) and soybean	Autoclaving	Autoclaved for 30 min at 121°C	Number of dilutions performed effecting agglutination: Cucumber: B – 2 R – 2 H – 4 Bottle gourd: B – 2 R – 1 H – 4 Soybean: B – 1 R – 8192 H – 512 *(Bovine erythrocytes (B) and pronase-treated rabbit (R) and hamster (H) erythrocytes)*	Number of dilutions performed effecting agglutination: Cucumber and bottle gourd: 1 (for B, R and H erythrocytes) Soybean: B – 1 R – 256 H – 1	Haemagglutination assay (trypsin- and pronase-treated bovine erythrocytes (B) and pronase-treated rabbit (R) and hamster (H) erythrocytes)	Kanwar et al. (1991)
Jatropha curcas meal	Autoclaving	Autoclaved at 121°C for 15, 30 and 45 min at 66% moisture	51 HU *(One HU was defined as the minimal concentration in mg/mL of the meal that caused haemagglutination of the blood cells)*	15 min – 1.2 HU 30 min – 0.21 HU 45 min – n.d.	Haemagglutination assay (trypsin-treated cattle erythrocytes)	Makkar & Becker (1999)
Soybean flour (*Glycine max*) variety Monarca	Autoclaving	Autoclaved at 121°C (1.7 atm) for 5, 10, 15 and 25 min	≈1.3 mL/mg	Autoclaving (25 min): no activity	Haemagglutinating activity (rabbit erythrocytes)	Machado et al. (2008)
Soybean flour (*Glycine max*) variety Monarca	Autoclaving	Autoclaved at 121°C (1.7 atm) for 5, 10, 15 and 25 min	≈1.3 mL/mg	Autoclaving (25 min): no activity	Haemagglutinating activity (rabbit erythrocytes)	Machado et al. (2008)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Alfalfa seeds (*Medicago sativa*)	Autoclaving	Autoclaved at 110°C for 10 mins	400 HU/g	n.d.	Haemagglutination assay (trypsin-treated rabbit erythrocytes)	Sahni et al. (2020)
Dhaincha *(Sesbania aculeate)* seeds	Autoclaving Microwaving Dry heat Soaking Extrusion	Autoclaved at 110°C 10 min Microwaved at 2450 MHz for 3 min Heating in hot air oven at 120°C for 20 min Soaked in water (1:10 w/v) at 27 ± 2°C for 16 h Extruded at a barrel temperature of 150°C, 25% feed moisture content and 150 r/min screw speed	400 HU/g	Autoclaving: n.d. Microwaving: n.d. Dry heat: 25 HU/g Soaking: 400 HU/g Extrusion: n.d.	Haemagglutination assay (trypsin-treated rabbit erythrocytes)	Sahni et al. (2021)
Common bean (*Phaseolus vulgaris*) and faba bean (*Vicia faba*)	Soaking + Autoclaving	Soaked in water for 2 h (1:4 w/v for common bean and 1:5 w/v for faba bean) and autoclaved for 20 min at 120°C	Common bean: 820 HU/mg DM Faba bean: 51.3 HU/mg DM	Common bean: 3.2 HU/mg DM Faba bean: 6.4 HU/mg DM	Haemagglutination assay (trypsin-treated rat erythrocytes)	Carbonaro et al. (2000)
Velvet bean (*Mucuna pruriens*) seeds	Soaking + Autoclaving	Soaked in waterfor 24 h at RT and autoclaved at 121°C for 20 min	Trace HU/mg sample	n.d.	Haemagglutination assay (trypsin-treated cattle erythrocytes)	Siddhuraju & Becker (2005)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Tree bean (*Parkia roxburghii*)	Soaking + Autoclaving Fermentation Dry heating	Soaked in water (1:10 w/v) with 0.2% ash solution or 0.1% sodium bicarbonate solution or 1% palm sugar solution or distilled water for 12 h and autoclaved in respective soaking solution (1:3 w/v) at 121°C for 15 min Anaerobic fermentation at RT for 36 h of autoclaved kernel slurry with water (1:3 w/v) Hot air oven at 130°C for 20 min	75.3 HU/g protein *(One HU defined as the inverse of the amount of material per mL in the last dilution giving positive agglutination)*	Soaking + Autoclaving: n.d. Fermentation: n.d. Dry heating: n.d.	Haemagglutination assay (trypsin-treated rat erythrocytes)	Sathya & Siddhuraju (2015)
PHA from dry bean (*Phaseolus vulgaris*) varieties Almonga and Curruquilla	Canning	Canned by cooking at 115°C for 40 min at 1800 kg pressure and cooled to 45°C for 25 min	Almonga: 0.7 ± 0.004 mg/g Curruquilla: 12.9 ± 0.79 mg/g	Almonga: n.d. Curruquilla: n.d.	ELISA (rabbit anti-PHA IgG antibodies)	Olmedilla-Alonso et al. (2013)
PHA from dry bean (*Phaseolus vulgaris*) varieties Almonga and Curruquilla	Soaking + Canning	Soaked in decalcified water for 12–14 h (Almonga var) or 16–18 h (Curruquilla var), blanched at 70°C for 9 min, canned and cooked at 116°C for 42 min at 1800 kg pressure and cooled to 38°C for 35 min	Almonga: 0.60 ± 0.004 mg/g Curruquilla: 16.50 ± 0.79 mg/g	Almonga: n.d. Curruquilla: n.d.	ELISA (rabbit anti-PHA IgG antibodies and goat anti-rabbit IgG-biotin conjugate)	Pedrosa et al. (2015)
LCA from lentil *(Lens culinaris)* var. Magda	Fermentation	Lentil flour fermented naturally in sterilised tap water for 4 days without aeration in a stirred fermenter	4.0 ± 1.1 – 6.0 ± 1.2 mg/g	Fermentation: 24 h – 1.4 ± 0.5 mg/g 48 h – 1.1 ± 0.3 mg/g 72 h – 0.008 ± 0.02 mg/g 96 h – 0.09 ± 0.08 mg/g	Competitive indirect ELISA (using rabbit anti-LCA IgG antibodies)	Cuadrado et al. (2002)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
WGA from whole wheat flour (commercial)	Sourdough preparation	Mixing of flour with sterile type water in 1:1 ratio and addition of cultured microbial strains (*Lactobacillus sakei* TMW1.22, *Fructilactobacillus sanfranciscensis* DSM20451 or *Fructilactobacillus sanfranciscensis* DSM20451 ΔgshR) or lactic acid. Doughs were incubated at 30°C for 0.5, 24 or more than 24 h	6.6 ± 0.7 µg WGA/g	*L. sakei* (24 h): 2.7 ± 0.4 µg WGA/g *F. sanfranciscensis* DSM20451 (24 h): 4.3 ± 0.3 µg WGA/g	ELISA (quantification of wheat germ agglutinin (WGA))	Tovar & Gänzle (2021)
Pigeon pea (*Cajanus cajan*)	Soaking + Fermentation Soaking + Germination Soaking + Germination + Fermentation	Soaked in tepid water (1:3 w/v) and fermented naturally for 72 h t 28°C Soaked for 24 h and washed and spread 3 times (at t= 0, 24 and 48 h of germination). Seeds were sprouted for 72 h Soaked for 24 h and washed and spread 3 times (at t = 0, 24 and 48 h of germination). Seeds were sprouted for 72 h and fermented in water for 24 and 48 h	59.89 ± 0.20 mg/g	Fermentation: 18.69 ± 0.10 mg/g Germination: 21.33 ± 0.10 mg/g Germination + Fermentation (24 h): 23.89 ± 0.05 mg/g Germination + Fermentation (48 h): 26.26± 0.07 mg/g	Spectrophotometry (absorbance measurement using 0.9% satrain)	Anaemene & Fadupin (2022)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Vigna racemosa seeds	Open fermentation Controlled fermentation	Bean flour fermented naturally in distilled water at 300 g/L for 48 h Bean flour was autoclaved at 121°C for 15 min and inoculated with *Aspergillus niger* spores (1.064×10^7 spores/25 g flour and incubated at 29 ± 3°C for 48 h	2.60 ± 0.06%	Open fermentation: 2.10 ± 0.06% Controlled fermentation: 24.00 ± 2.03%	Method not reported	Difo et al. (2015)
Pea flour	Fermentation	Fermented for 48 h with lactic acid bacteria (*Pediococcus pentosaceus, Lactococcus raffinolactis, Lactobacillus plantarum*) or fungi (*Rhizopus microspores*, var. *oligosporus, Geotrichum candidum*)	NR	No change in lectin content	ELISA (rabbit anti-native pea protein antibodies)	Barkholt et al. (1998)
Pea seeds (*Pisum sativum*)	Dehulling Soaking Germination Extrusion	Mechanical removal of hulls double-deionised water (1:5 w/v) for 12 h at 30 °C for 24, 48 or 72 h at 25 °C, watering every 12 h, in dark at 148°C; moisture at 25%	6.2 HU/mg dm *(One HU was defined as that contained in the amount of sample in the last dilution that caused 50% agglutination of the blood cells)*	Dehulling or soaking or germination (24, 48 and 72 h): 6.2 HU/mg dm Extrusion: n.d.	Haemagglutination assay (trypsin-treated rabbit erythrocytes)	Alonso et al. (1998)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Faba bean (*Vicia faba*) and kidney bean (*Phaseolus vulgaris*)	Dehulling Soaking Germination Extrusion	Mechanical removal of hulls double-deionised water (1:5 w/v) for 12 h at 30°C for 24, 48 or 72 h at 25 °C, watering every 12 h, in dark at 148°C; moisture at 25%	Faba: 49.3 HU/mg dm Kidney beans: 74.5 HU/mg dm *(One HU was defined as that contained in the amount of sample in the last dilution that caused 50% agglutination of the blood cells)*	Faba bean: Dehulling or soaking or germination (24, 48 and 72 h): 49.3 HU/mg dm Extrusion: n.d. Kidney bean: Dehulling or soaking or germination (24, 48 and 72 h): 74.5 HU/mg dm Extrusion: n.d.	Haemagglutination assay (trypsin-treated rabbit erythrocytes)	Alonso, Aguirre, & Marzo (2000)
Vigna unguiculata subsp. *unguiculata*	Irradiation	Irradiated at different doses of gamma irradiation (2, 5, 10, 15 and 25 kGy) at 25 ± 1°C	Type A: 28 HU/mg Type B: 136 HU/mg Type O: 21 HU/mg	2 kGy: Type A – 32 HU/mg Type B – 120 HU/mg Type O – 21 HU/mg 5 kGy: Type A – 29 HU/mg Type B – 96 HU/mg Type O – 15 HU/mg 10 kGy: Type A – 24 HU/mg Type B – 74 HU/mg Type C – 11 HU/mg 15 kGy: Type A – 18 HU/mg Type B – 52 HU/mg Type C – 9 HU/mg 25 kGY: Type A – 12 HU/mg Type B – 21 HU/mg Type C – 5 HU/mg	Haemagglutination assay (human types A, B and O erythrocyte)	Tresina & Mohan (2011)
Wheat germ agglutinin (commercial source)	Irradiation	Irradiated dry under an O_2 atmosphere by a gamma ray irradiator using doses of 1.0, 10.0 and 25.0 kGy at a rate of 8.25 kGy/h	100% HA	10 kGy: n.d.	Haemagglutination assay (not reported)	Vaz et al. (2012)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Jatropha curcas meal	Hydrolysis Hydrolysis + Washing Washing	Exposure to cellulase (5 mg/g) and pectinase (10 mg/g) at 50°C for 1 h After hydrolysis, sample was washed with 60% methanol (5:1 v/v) and 65% ethanol (5:1 v/v) with constant stirring for 1 h at 50°C Washed by 90% methanol (10:1 v/v) and 90% ethanol (10:1 v/v) with constant stirring for 2 h at 50°C	3.43 ± 0.01 mg/mL	Hydrolysis: 3.41 ± 0.17 mg/mL Hydrolysis + Washing: Methanol 60% – 1.49 ± 0.00 mg/mL Ethanol 65% – 1.35 ± 0.04 mg/mL Washing: Methanol 90% – 1.46 ± 0.12 mg/mL Ethanol 90% – 1.62 ± 0.10 mg/mL	Haemagglutination assay (trypsin-treated rabbit erythrocytes)	Xiao et al. (2011)
Red kidney bean (*Phaseolus vulgaris*)	Ultrahigh pressure (UHP)	Vacuum-sealed packed and subjected to UHP treatment (150, 250, 350 and 450 MPa) at 25°C	2^3 HA *(One HA defined as the reciprocal of the highest dilution exhibiting haemagglutination)*	150 MPa: 2^2 HA 250–350 MPa: 2^1 HA 450 MPa: 2^0 HA	Haemagglutination assay (human type B erythrocytes)	Lu et al. (2015)
SBA from soybean	Ultrahigh-pressure	Soybeans soaked and prepacked in sealed vacuum screw bags. Treated at 350, 400, 450, 500 and 550 MPa for 15 min	100% HA	< 500 MPa: > 50% HA 500 MPa: 40.21% HA 550 MPa: 36.74% HA	ELISA (soybean agglutinin ELISA kit)	Han et al. (2023)
Red kidney bean (*Phaseolus vulgaris*)	High hydrostatic pressure (HHP)	Soaked in 10 mM Tris-HCl buffer (1:10 w/v) at 4°C for 6 h and homogenised in blender. Homogenate vacuum packaged and subjected to HHP (50, 150, 250, 350 and 450 MPa) for 15 min at RT	2^3 HA *(One HA defined as the reciprocal of the highest dilution exhibiting haemagglutination)*	50–150 MPa: 2^3 HA 250–350 MPa: 2^2 HA 450 MPa: 2^1 HA	Haemagglutination assay (trypsin-treated human type B erythrocytes)	Liu et al. (2013)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Breadfruit meal *(Artocarpus altilis)*	Peeled raw breadfruit meal (PRBFM) Unpeeled raw breadfruit meal (URBFM) Peeled soaked breadfruit meal (PSBFM) Unpeeled soaked breadfruit meal (USBFM) Peeled cooked breadfruit meal (PCBFM) Unpeeled cooked breadfruit meal (UCBFM)	Peeling, slicing, sun drying to 13% and milling to 0.5 mm particles Slicing, sun drying to 13% and milling to 0.5 mm particles Peeling, slicing, soaking, sun drying to 13% and milling to 0.5 mm particles Slicing, soaking, sun drying to 13% and milling to 0.5 mm particles Peeling, slicing, cooking, sun drying to 13% and milling to 0.5 mm particles Slicing, cooking, drying and milling to 0.5 mm particles	NR	PRBFM: 11.30 HU/mg URBFM: 11.90 HU/mg PSBFM: 3.00 HU/mg USBFM: 3.50 HU/mg PCBFM: 0.00 HU/mg UCBFM: 0.00 HU/mg	Haemagglutination assay (trypsin treated rabbit erythrocytes)	Oladunjoye et al. (2010)
PHA in pasta made with white rice (*Oryza sativa*), Raw beans (*Phaseolus vulgaris* var. Almonga), Whole carob fruit (*Ceratonia siliqua*)	Pasta preparation	Mixing of different ratios if rice and bean flours and supplementing with 10% whole carob bean. Formulations mixed with hot water (≈46%) for 5 min. Hydrated flours worked for 15 min before cold extrusion (30-40°C) into fettuccine. Cooking of pasta at optimal time	Bean flour: 0.58 ± 0.14% PHA Rice flour: n.d. All uncooked pasta formulations: < 0.59% PHA	Bean flour: n.d. Rice flour: n.d. All cooked pasta formulations: n.d.	ELISA (using rabbit anti-LCA IgG antibody and (HRP)-conjugate goat anti-rabbit IgG)	Arribas et al. (2020)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Defatted soybean meal (*Glycine max*)	Acid treatment	SBM was mixed with acetic (5, 10 and 15%) and citric (0.25, 0.5 and 0.75%) acids in a 1:5 (w/v) ratio and heated at 50°C for 60 min. Then, 80-85% of acids were recovered through washing and rotary vacuum evaporation	≈2 mg lectin/g protein	0.75% citric acid: 0.13 mg/g protein All other treatments: > 0.13 mg/g protein	Haemagglutination assay (human erythrocytes)	Norozi et al. (2022)
Soybean meal (*Glycine max*)	Thermomechanical and enzyme-facilitated processing	Meal conditioned with water and steam and an enzyme mixture. Meal was treated with high temperature and high pressure for a short time during extrusion	339.4 µg/g	3.7 µg/g	ELISA (used antibodies not reported)	Nu et al. (2020)
Common bean (*Phaseolus vulgaris*)	Baking	Bean flour was baked into biscuit form (with sugar, butter, eggs, salt, baking powder and vanilla flavour) at 180°C for 12 or 15 min	NR *(One HAU defined as the reciprocal of the highest dilution still visually showing by agglutination)*	Baking: 12 min – 1:80 to 1:160 HAU 15 min – 1:40 HAU	Haemagglutination assay (human type A erythrocytes)	Sparvoli et al. (2016)
Soybean (*Glycine max*)	Soymilk and Okara (Soaking + Cooking + Filtering)	Soaked in water at 5–7°C for 14 h, ground and cooked by steam injection system at 110°C, 1.8 bar for 8 min and filtered to separate soymilk and okara	5.48–7.74% SBA of total extracted protein *Soybean agglutinin (SBA)*	Soymilk: 5.96%–8.84% SBA of total extracted protein Okara: 0.07–1.73% SBA of total extracted protein	Densitometric analysis of lectin subunit obtained from SDS-PAGE and native-PAGE electrophoresis	Stanojević et al. (2013)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
Soybean (*Glycine max*) of genotypes Lana and Balkan	Tofu making (Soaking + Cooking + Filtering + Enzyme processing)	Soaked in water (1:5 w/v) for 14 h at 5–7°C, ground and cooked by steam injection system at 110°C, 180 kPa for 8 min. Slurry was filtered and squeezed manually to obtain soymilk. Chymosin-pepsin rennet and inulin were added	NR	Tofu (Lana geno.): 0.1 ± 0.02 – 0.2 ± 0.01% SBA of total extracted protein Tofu (Balkan geno.): 6.0 ± 0.02 – 6.8 ± 0.12% SBA of total extracted protein	Densitometric analysis of lectin subunit obtained from SDS-PAGE and native-PAGE electrophoresis	Stanojević et al. (2021)
Soybean (*Glycine max*)	Tofu making (Soaking + Cooking + Filtering + Enzyme processing)	Soaked in water (1:5 w/v) for 14 h at 5–7°C, ground and cooked by steam injection system at 110°C, 180 kPa for 8 min in water (1:6 w/v). Slurry was filtered and squeezed manually to obtain soymilk. Chymosin-pepsin rennet and inulin were added	NR	5.01 – 5.48 ± 0.05% of total extracted protein	Densitometric analysis of lectin subunit obtained from SDS-PAGE and native-PAGE electrophoresis	Stanojević et al. (2023)
Field pea (*Pisum sativum*) V-0 V-I V-II		Exposure to moist heat, organic acids and selected oxides Exposure to 80–90°C for 30 min, the duration of ageing being 40 min Exposure 80–90°C as long as 50 min, the duration of ageing being 60 min	717 ± 376 HA *(One HA defined as the lectin titre at which microscopic evidence for haemagglutination becomes available)*	V-0: 213 ± 60 HA V-I: 461 ± 102 HA V-II: 192 ± 64 HA	Haemagglutination assay (erythrocytes not reported)	Dvořák et al. (2005)

(Continues)

TABLE D.1 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference*
α-Galactosidase-haemagglutinin (high and low molecular weight) from mung bean	pH	Exposure of low molecular weight and high molecular weight α-Galactosidase-haemagglutinin to different pHs	NR	High molecular weight: pH 7.0: visible agglutination pH 5.4: no agglutination Low molecular weight (all pH): no agglutination	Haemagglutination assay (trypsin treated rabbit erythrocytes)	Del Campillo et al. (1981)
Isolated lectins from black turtle bean	pH	Incubated for 0.5, 1, 2, 4, 8, 12 and 24h in buffers with pH 3.5, 3.0, 2.5, 2.0, 1.5 and 1.0	100% HA	pH (1.5–1.0): 50% HA	Haemagglutination assay (rabbit erythrocytes)	Zhao et al. (2019)

Abbreviations: DM, dry matter; EMS, ethyl methane sulfonate; equiv, equivalent; FLIA-BBM, functional lectins analysed by immuno-assay using pig brush border membrane; Gy, gray; HA, haemagglutinating activity; HRP, horseradish peroxidase; HU, haemagglutination units; n.d., not detected; NR, not reported; RT, room temperature; sp. act, specific activity; Tris-HCl, trisaminomethane hydrochloride.

*Studies are ordered by processing method (physical treatments, thermal treatments, mechanical treatments, other) and then by chronological order.

TABLE D.2 Studies identified reporting the impact of different food processing methods under different conditions on **non-edible** lectin content or activity.

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference
Jack bean seeds *Canavalia ensiformis*	Soaking + Germination	Soaking for 6 h; germination at 25°C for 4 days, watering daily (12 h of light daily)	51 ± 0.6 mg of lectin equivalent/g seeds	25 ± 0.6 mg of lectin equivalent/g seeds	Haemagglutinating activity (trypsin-treated rat erythrocytes)	Aguilera et al. (2013)
Abrin toxin	Heating pH	Heated in PBS pH 7.2 + 0.2% phosphate buffer gelatin at 63, 74, 80, 85 and 99°C for 3 min in thermocycler Incubated PBS + 0.2% phosphate buffer gelatin at different pH (pH 2.0–pH 9.0) for 1 h at room temperature	Translation assay: ≈84.5 ± 0.2% TI Cytotoxicity assay: 100% RC Mouse bioassay: ≈1.83 days MSD *Translation inhibition (TI), relative cytotoxicity (RC), median survival (MS)*	Translation assay Heating: 63°C – ~80.43 ± 0.36% TI 74°C – NR. 80°C – NR. 85°C – ≈42.8 ± 0.6% TI 99°C – ≈19.55 ± 0.61% TI pH (2.0-9.0): ≈99.6 – 99.9% TI Cytotoxicity assay Heating: 63°C – 72 ± 4% RC ≥ 74°C – 0% RC pH (2.0-9.0): 99 ± 0 – 86 ± 2% RC IV mouse bioassay Heating: 63°C – 2.167 days MS ≥ 74°C – end of experiment pH (2.0–9.0): 1.94–1.81 days MS	In vitro cell free translation assay (measuring TI) Vero cell cytotoxicity assay (measuring RC) Intravenous route mouse bioassay	Tam et al. (2017)

TABLE D.2 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference
Lectins purified from *Spatholobus parviflorus* seeds	Heating pH Chemical denaturation	Heated for 15 min at 30, 40, 50, 60, 70, 80, 90 and 100°C Dialysed with buffers of pH 2–11 (glycine–HCl for pH 2–3, acetate buffer for pH 4–5, citrate buffer for buffer with pH 6, phosphate buffer for pH 7–8 and glycine–NaOH buffer for pH 9–11) Incubated denaturing solution (urea, thiourea and guanidine hydrochloride at a concentration range of 0.5–8.0 M at 0.5-M interval) at 37°C for 1 h	100% HA	Heating: 70°C – 50% HA 100°C – n.d. pH: ≤ 4 – 40% HA ≥ 10 – 40% HA Chemical denaturation: Urea (4.0 M) – 50% HA Guanidine hypochloride (4.0 M) – 50% HA Thiourea (5.0 M) – 50% HA	Haemagglutination assay (human erythrocytes)	Geethanandan et al. (2013)
Canavalia cathartica seeds	Roasting Soaking + Pressure Cooking	Roasted on sand bath at 180°C for 20 min Soaked in freshwater for 1 h and pressure-cooked for 30 min with 1:3 (v/v) freshwater	Type A: ++++ Type B: ++++ Type O: ++++	Roasting: Type A - ++ Type B - +++ Type O - ++ Soaking + Pressure Cooking: Type A - ++ Type B - +++ Type O - ++	Haemagglutination assay (trypsin-treated type A, B and O human erythrocytes)	Seena et al. (2006)
Cramoll lectin (isolated from *Cratylia mollis* seeds)	Irradiation Irradiation +Heating	Irradiated dry under atmospheric O_2 using doses of 1.0, 10.0 and 25.0 kGy at a rate of 8.25 kGy/h Irradiated and heated at 30–100°C for 30 min	100% SHA *(SHA defined as the relationship between the lowest sample dilution that showed haemagglutination and protein concentration)*	1 kGy – 50% SHA 10 kGy – NR. 25 kGy – NR. Only heating: decrease SHA at 70°C Irradiation + Heating: decrease SHA at 60°C	Haemagglutination assay (glutaraldehyde-treated rabbit erythrocytes)	Vaz et al. (2013)
Purified ricin and abrin extracted from Castor beans (*Ricinus communis*) and Rosary peas (*Abrus precatorius*)	Pasteurisation Fermentation with yogurt starter	Pasteurised in whole and skim milk at 85°C for up to 30 min Added to pasteurised milk with yogurt starter culture and fermented at 42–45°C for 0, 1, 2, 3, 4, 5, 6, and 24 h	100% activity ricin and abrin	Pasteurisation (85°C): 10 min – 13%–15% activity 20 min – n.d. Fermentation (0–24 h): ≈100% activity	ELISA	Jackson et al. (2015)

(Continues)

TABLE D.2 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference
Dry seeds and string beans of *Erythrina americana*	Seeds Boiled Germinated Germinated + Boiled String beans Boiled	Boiled in water (1:5 w/v) for 2 h Soaked in tap water for 6 h and germinated on cotton in the dark at room temperature (25°C) to allow the seeds to sprout Germinated and boiled in water (1:5 w/v) for 30 min String beans with intact pod or seeds, boiled in water (1:5 w/v) for 2 h	Dry seeds: 5 HA String bean: whole pod – 5 HA seeds – 6 HA *(One HA defined as the maximum dilution where agglutination was observed)*	Seed: Boiled – 2 HA Germinated – 3 HA Germinated + Boiled – 1 HA String bean: Boiled (whole pod) – 3 HA Boiled (seeds) – 3 HA	Method not reported	Sotelo et al. (2003)
Ricin standard	Heating in infant formula	Spiked infant formula heated at 60, 70, 75, 80, 85, and 90°C for up to 5 h	NR.	t1/2 values for ricin Heating: 60°C – > 100 min 70°C – 13 ± 0.9 to 17 ± 4 min 75°C – 5.8 ± 0.7 to 8.6 ± 1.1 min 80°C – 5.6 ± 0.9 to 7.1 ± 1.3 min 85°C – 3.0 ± 0.4 to 2.7 ± 0.4 min 90°C – 2.0 ± 0.2 to 2.1 ± 0.2 min	ELISA	Jackson et al. (2006)
Ricin standard, ricin A and B subunits	Heating and pH in food sample	Ricin (0.2–2.0 mol/L) was added to buffer samples (50 mM sodium phosphate and 50 mM sodium citrate) and yogurt fruit juice drink samples adjusted to pH 3.5, 4.5, 5.5, 6.5 or 7.5. Samples were heated to 75, 80, 85 or 90°C	NR.	t1/2 at 75°C Yogurt fruit juice (pH): 3.5 – 30 ± 3 min 4.5 – 146 ± 19 min 5.5 – 63 ± 5 min 6.5 – 10.8 ± 1.4 min 7.5 – 9.6 ± 0.5 min Buffer (pH): 3.5 – 11.7 ± 0.7 min 4.5 – 58 ± 3 min 5.5 – 49 ± 5 min 6.5 – 7.2 ± 0.7 min 7.5 – 3.7 ± 0.2 min t1/2 yogurt fruit juice at pH 4.5 Heating: 75°C – 146 ± 19 min 80°C – 46 ± 4 min 85°C – 22 ± 5 min 90°C – 7.3 ± 1.1 min t1/2 yogurt fruit juice at pH 6.5 Heating: 75°C – 10.8 ± 1.4 min 80°C – 1.66 ± 0.15 min 85°C – 1.23 ± 0.13 min 90°C – 0.24 ± 0.04 min	In vitro cytotoxicity on RAW264.7 mouse macrophage cells	Zhang et al. (2013)

TABLE D.2 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference
Ricin standard	Heating in food sample Clarification + Heating	Apple and orange juices spiked with ricin standard (100 µg/mL) and heated from 60 to 90°C for up to 2 h Clarified by incubating juice with 1 mL of pectinase for 2 h at 50°C and centrifuging. After, heated 80°C and 90°C for up to 12 min	NR.	Apple juice t1/2 cloudy juice Heating: 60°C – > 100 min 70°C – 26 ± 6 min 75°C – 4.2 ± 0.7 min 80°C – 3.3 ± 0.5 min 85°C – 1.45 ± 0.12 min 90°C – 0.96 ± 0.07 min t1/2 clarified juice Heating: 60°C – > 100 min 70°C – 45 ± 10 min 75°C – 9.7 ± 1.6 min 80°C – 3.0 ± 0.3 min 85°C – 1.2 ± 0.1 min 90°C – 1.1 ± 0.1 min Orange juice t1/2 cloudy juice Heating: 60°C – > 100 min 70°C – 60 ± 10 min 75°C – 12 ± 2 min 80°C – 2.1 ± 0.3 min 85°C – 0.51 ± 0.12 min 90°C – 0.50 ± 0.13 min t1/2 clarified juice Heating: 60°C – > 100 min 70°C – 67.0 ± 6.1 min 75°C – 15.8 ± 0.66 min 80°C – 3.06 ± 0.49 min 85°C – 0.80 ± 0.12 min 90°C – 0.45 ± 0.07 min	ELISA In vitro cytotoxicity on RAW264.7 mouse macrophage cells *Same trend observed in ELISA and cytotoxicity assay, only ELISA reported*	Jackson et al. (2010)
Jack bean seeds (*Canavalia ensiformis*)	Cooking Pressure cooking	Cooked at 96°C for 30, 60, 90 and 120 min Pressure cooked for 15, 30 and 45 min *Processing was done to whole and cracked seeds*	Whole seeds: 13,531.8 ± 5.41 HU/g Cracked seeds: 13,531.8 ± 5.41 HU/g	Cooking (whole seeds): 30 min – 102.0 ± 0.18 HU/g 60 min – 12.1 ± 0.22 HU/g 90 min – 9.2 ± 0.04 HU/g 120 min – 5.6 ± 0.06 HU/g Cooking (cracked seeds): 30 min – 14.3 ± 0.13 HU/g ≥ 60 min – n.d. Pressure cooking (whole seeds): 15 min – 16.67 ± 0.17 HU/g 30 min – 4.0 ± 0.01 HU/g 45 min – n.d. Pressure cooking (cracked seeds): 15 min – n.d. 30 min – n.d. 45 min – n.d.	Haemagglutination assay (rabbit erythrocytes)	Udedibie and Carlini (1998)

(Continues)

TABLE D.2 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference
Canavalia ensiformis and *Canavalia braziliensis*	Toasting Soaking (urea) + Cooking Toasting with urea Sequential cooking Boiling Pressure cooking Soaking (water) + Cooking	Toasted at 120°C for 20–25 min Soaked for 1 week in 4% solution of urea and cooked for 1 h in water Mixed with dry urea (2.5% or 5%) and toasted Cooked in stages at 96°C for 1 h (1st stage), 40 min (2nd stage) and 40 min (3rd stage), 40 min (4th stage) Boiled at 96°C for 3 h Pressure cooked for 15, 30, 45, and 60 min Soaked in water for 12, 24, 48, 72, and 96 h and cooked for 30, 60, 90, and 120 min at 96°C	*C. ensiformis*: 13531.8 HU/g *C. brazilensis*: 10204.1 HU/g	*C. ensiformis:* Toasting: 4545.5 HU/g Soaking (urea) + Cooking: 32.3 HU/g Toasting (2.5% urea): 1086.9 HU/g Toasting (5% urea): 526.3 HU/g Sequential cooking: 1st stage – 102.0 HU/g 2nd stage – 12.1 HU/g 3rd stage – 9.2 HU/g 4th stage – 5.6 HU/g Boiling: < 3.6 HU/g Pressure cooking: 15 min – 16.7 HU/g 30 min – 4.0 HU/g 45 min – < 3.6 HU/g 60 min – < 3.9 HU/g Soaking (water, 12–24 h) + Cooking: 30 min: 62.5 HU/g 60 min: 13.3 – 14.3 HU/g 90 min: 7.7 – 8.3 HU/g 120 min: 4.0 – 4.2 HU/g Soaking (water, 48 h) + Cooking: 30 min: 33.3 HU/g 60 min: 14.3 HU/g 90 min: 3.7 HU/g 120 min: < 3.6 HU/g Soaking (water, 72 h) + Cooking: 30 min: 3.2 HU/g 60 min: < 3.7 HU/g 90 min:< 3.3 HU/g 120 min: < 3.6 HU/g Soaking (water, 96 h) + Cooking: 30 min: 4.3 HU/g 60 min: < 4.3 HU/g 90 min: < 4.3 HU/g 120 min: < 3.2 HU/g *C. braziliensis:* Toasting: 1428.6 HU/g Soaking (urea) + Cooking: 216.7 HU/g	Haemagglutination assay (rabbit erythrocytes)	Carlini & Udedibie (1997)

TABLE D.2 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference
				Toasting (2.5% urea): 1076.3 HU/g Toasting (5% urea): 434.2 HU/g Sequential cooking: 1st stage – 285.7 HU/g 2nd stage – 71.4 HU/g 3rd stage – 32.3 HU/g 4th stage – 17.5 HU/g Boiling: < 3.6 HU/g Pressure cooking: 15 min – 12.5 HU/g 30 min – 3.7 HU/g 45 min – < 3.7 HU/g 60 min – < 3.6 HU/g Soaking (water, 12–96 h) + Cooking: 30 min: 7813.5–9005.3 HU/g 60 min: 236.1–301.9 HU/g 90 min: 68.4–81.9 HU/g 120 min: 24.2–34.4 HU/g		
Castor bean (*Ricinus communis*)	Soaking Steaming Boiling Autoclaving Heating Ammonia treatment Formaldehyde treatment Lime treatment Sodium chloride treatment Tannic acid treatment Sodium hydroxide treatment	Soaked in water (1:10 w/v) for 3, 6 and 12 h Moistened with water and steamed for 30 and 60 min Boiled at 100°C for 30 and 60 min Autoclaved at 15 psi for 30 and 60 min Dry heat at 100°C for 30 min and 120°C for 25 min in hot air oven Ammonia solution (25 ml/l) was added to castor cake until a concentration of 7.5 and 12.5 mL ammonia per kg of sample and kept for 7 days Formaldehyde was added until reaching a 5 and 10 g/kg protein concentration and kept for 7 days Calcium hydroxide solution was added until reaching a concentration of 10, 20 and 40 g/kg and kept for 8 h Sodium chloride solution was added to castor cake until a concentration of 5, 10 and 20 g/kg sample and kept overnight	388 µg/g	Soaking: 3 h – 136 µg/g 6 h – 55 µg/g 12 h – 62 µg/g Steaming: 30 min – 103 µg/g 60 min – 57 µg/g Boiling: 30 min – 38 µg/g 60 min – 37 µg/g Autoclaving: 30 min – 58 µg/g 60 min – n.d. Heating (100°C, 30 min): 187 µg/g Heating (120°C, 25 min): 193 µg/g Ammonia: 7.5 ml/kg – 191 µg/g 12.5 ml/kg – 158 µg/g Formaldehyde: 5 g/kg – 236 µg/g 10 g/kg – 75 µg/g Calcium hydroxide: 10 g/kg – 129 µg/g 20 g/kg – 144 µg/g 40 g/kg – n.d. Sodium chloride: 5 g/kg – 78 µg/g 10 g/kg – 85 µg/g 20 g/kg – 70 µg/g Tannic acid: 5 g/kg – 180 µg/g 10 g/kg – 282 µg/g Sodium hydroxide: 2.5 g/kg – 69 µg/g 5 g/kg – 53 µg/g 10 g/kg – 36 µg/g	Haemagglutination assay (human type O erythrocytes)	Anandan et al. (2005)

(Continues)

TABLE D.2 (Continued)

Plant (Lectin)	Method	Conditions	Lectin before treatment	Lectin after treatment	Analytical method	Reference
		Tannic acid solution was added to castor cake until a concentration of 5 and 10 g/kg sample and kept overnight Sodium hydroxide solution was added to castor cake until a concentration of 2.5, 5 and 10 g/kg sample and kept overnight				
Ricin from castor seed (*Ricinus communis*) of cultivars Hale, Lynn, and Brigham	Cold pressing Boiling Autoclaving Chemical treatment + Heating	Seeds driven through a screw press Seeds driven through a screw press at with heating ring at 275°C Whole seeds, milled meal, and cold-pressed meal were boiled at 100°C for 10, 20, 30 40, 50 and 60 min Whole seeds, milled meal, and cold-pressed meal were autoclaved at 121°C for 10, 20, 30 40, 50 and 60 min Solutions of 6 M guanidine–HCl, 8 M urea, and 50 mM calcium hydroxide (pH 12.5) were added to seed meal and heated to 99°C for 1, 2, 3, 4, 5 and 10 min	Whole seeds: + Milled seeds: + (+ indicates the presence of non-denatured lectins)	Cold pressed Boiled: ≥ 10 min – n.d. Autoclaved: ≥ 10 min – n.d. Urea (60 min): + Guanidine (60 min): + Hot pressed: n.d. Whole seeds Boiled: 10 min – + ≥ 20 min – n.d. Autoclaved: 10 min – + 20 min – + ≥ 30 min – n.d. Urea (60 min): + Guanidine (60 min): + Milled seeds Boiled (10-60 min): + Autoclaved (10-60 min): n.d.	Western blotting (Immunodetection using RCA-52B anti-RCA60 mouse IgG primary antibodies)	Barnes et al. (2009)
Ricin from castor plant seeds (*Ricinus communis*)	Heating spiked food samples	Beef, milk and egg aliquots spiked with ricin and heated at 63 or 72°C for 3 min *Beef aliquots were incubated at RT for 5 min before heating*	0% *Loss of lectin activity (%)*	Beef: 63°C – 99.65 ± 1.62% 72°C – 100.00 ± 1.95% Milk: 63°C – 38.76 ± 1.36% 72°C – 60.21 ± 1.12% Egg: 63°C – 89.18 ± 1.37% 72°C – 100.00 ± 5.54%	CFT assay (using nuclease-treated rabbit reticulocyte lysate)	He et al. (2008)

Abbreviations: CFT, cell-free translation; Gy, gray; HA, haemagglutinating activity; HU, haemagglutination units; M, molar; n.d., not detected; NR, not reported; t1/2, apparent half-life.

APPENDIX E

Sources of uncertainty

A systematic approach was used to identify sources of uncertainty affecting the assessment of lectins. A previously prepared list of sources of uncertainty commonly encountered in risk assessments of the CONTAM Panel was reviewed to identify and describe those sources that applied to the present assessment. In addition, the Panel considered each part of the present assessment in turn to identify any additional sources of uncertainty, beyond those in the existing list. Subsequently, the Panel considered which of those sources of uncertainty would have the most impact on the outcome of the hazard identification and characterisation and of the exposure assessment. A complete list of the sources of uncertainty identified is presented in Tables E.1–E.3. The most important uncertainties are listed together with a qualitative evaluation of their potential impact on the assessment in four categories: negligible, low, medium and high. These qualitative ratings were used later in the analysis to prioritise consideration of the main sources of uncertainty and to facilitate the assessment of overall uncertainty.

A critical endpoint is both relevant and adverse for humans and most sensitive among the endpoints for which there are data available to derive a reference point. The critical study for PHA is the study by Bardocz et al. (1995) on and the critical endpoint used for BMD modelling is small intestine dry weight. Uncertainties associated with other endpoints that are relevant and adverse to humans, but not the most sensitive or for which there is not sufficient data are considered in 'Selection of critical studies and endpoints' in Table E.3. Uncertainty related to occurrence data was primarily considered with respect to the exposure assessment by Boniglia et al. (2008) (Table E.2).

TABLE E.1 Sources of uncertainty identified for the hazard assessment.

Group	Description of the sources of uncertainty	Impact on the hazard identification and characterisation[16]
Chemical composition and analytical methods		
Dosing and chemical composition	Uncertainty associated with the dose in the critical studies. Most of the studies were conducted by adding lectins in the diet and some by gavage.	**1 – Low impact**. Tested item in the critical study was administered in the diet.
	Uncertainty associated with the exact composition of the tested items in the critical studies. Tested items are mainly purified using different methods. The WG considered only toxicological studies which are conducted with lectins obtained by reliable methods that lead to lectins of high purity (e.g. affinity chromatography). The WG also considered studies with commercial products. In the majority of the studies lectins are purified and in some cases purity is confirmed by SDS-PAGE, LC-MS. Even in the absence of verification of purity by SDS-PAGE, LC-MS or ELISA, a potential contamination of purified lectin samples would remain very limited and would have only a negligible impact.	**1 – Low impact**. PHA was purified by affinity chromatography on Fetuin-Sepharose-4B, purity not reported.
Analytical methods	Uncertainty associated with lack of certified reference materials, proficiency tests and method validation. Commercial standards are available, but purity may not be reported.	**1 – Low impact**. Commercial reference not used for confirmation of purity of the test item.

(Continues)

[16] **0 – Uncertainty with negligible impact**.
1 – Uncertainty with low impact.
2 – Uncertainty with medium impact.
3 – Uncertainty with high impact.

TABLE E.1 (Continued)

Group	Description of the sources of uncertainty	Impact on the hazard identification and characterisation[16]
Hazard identification and characterisation		
ADME (Absorption, Distribution, Metabolism, Excretion)	Uncertainty associated with insufficient information on absorption of the different lectins. Sufficient information from other articles (no information from the critical studies).	**0 – Negligible impact**. TK data are available for PHA, most studies quite old but body of evidence quite consistent (very low absorption, no metabolites reported, elimination of > 90%)
	Uncertainty associated with accumulation potential. No evidence of accumulation. No studies showing bioaccumulation, effects reversible, high degree of elimination. However, most of the studies are sub-acute studies and long-term exposure effects are unknown.	**1 – Low impact**. No information on ADME in the critical study. Study design sub-acute study thus no conclusion can be drawn. It cannot be excluded that feeding more than one dose/for one day may have stronger effect due to binding of more lectin molecules.
	Uncertainty associated with confounders.	**1 – Low impact**. In the critical study, on final morning of experiment test rats were given 50% of purified PHA by intragastric intubation. Control animals were given only saline, and were not in diet as per the experimental design. The study has an unusual design, but no impact expected for the organ weights.
	Uncertainty associated with insufficient information on the extent of metabolism of the parent compounds.	**1 – Low impact**. Assume no metabolism evidenced from other studies.
	Uncertainty associated with limited relevance in humans, genetic background/susceptibility/sensitive populations.	**0 – Negligible impact**. Study design not realistic for humans. Humans stop eating if they feel bad, at the same time as some people may eat a high amount of food containing lectins. However, there are no non-standard sources of uncertainty affecting the findings.

TABLE E.1 (Continued)

Group	Description of the sources of uncertainty	Impact on the hazard identification and characterisation[16]
Toxicity studies in experimental animals: critical endpoints and critical study design	Limitations in the design of the direct measurements of the effect in experimental animals.	**2 – Moderate impact**. Histological images are used to prove the increase in the weight is linked with damage. Limitations in the method from poor image quality or possibility for quantitative analysis of histological images. Increase in intestine is measured directly on a scale.
	Uncertainty due to studies carried out only in one sex or certain age groups, duration of studies, sample size, biomarkers.	**1/2 – Low to moderate impact**. No sex specified. The impact of the sex on the critical effect is low with an unclear direction. A moderate impact of age, due to changes on the gut maturation, where older animals may need more time to recover from any effect. Study carried out on 30-day-old animals. Relatively short duration of treatment (10 days, sub-acute) and small number of animals per group (5).
	Uncertainty due to dosing regime.	**1 – Low impact**. Unclear dosing regime of the tested items (in diet + 1 gavage of half dose 2h before sacrifice). There is a possibility, albeit low given excretion more than 90%, that lectins binding to the gut may increase the weight.
	Uncertainties in the use of the animal model.	**1 – Low impact**. Limited relevance for humans of the adverse effect.
	Limited information on other effects that could be considered as critical.	**2 – Moderate impact**. The critical study focuses on small intestine, pancreas and nitrogen balance; data on other organs (e.g. liver, kidney) or other metabolic parameters not provided.
	Dietary studies, default factors used for conversion.	**0 – Negligible impact**. No default values used
Genotoxicity	*Insufficient/inconclusive data or inconsistent results*. Non-standard methods used, inconsistency in the findings.	**2 – Moderate impact**. No genotoxcity studies with PHA were retrieved. Long-term exposure is not expected.
Observations in humans	Uncertainty in the study design, sample size, exposure assessment (in the method used), outcome assessment (e.g. histology vs. self-report), statistical analysis including control of confounding factors, missing data, dose response. Variations in human studies regarding exposure (study design).	**1 – Low impact**. Limited data availability; exposure not characterised, thus data not considered further for risk characterisation.
	Inconsistency in human studies (study results).	**1 – Low impact**. Limited data availability; effect consistent.
	Low number of studies per outcome and limited cumulative sample size.	**1 – Low impact**. Limited data availability; no comparative data, thus not considered further for risk characterisation.
	Small sample size of the individual studies.	**1 – Low impact**. Limited data availability; no comparative data, thus not considered further for risk characterisation.

(Continues)

TABLE E.1 (Continued)

Group	Description of the sources of uncertainty	Impact on the hazard identification and characterisation[16]
	Limitations in epidemiological study at the individual study level: e.g. co-exposure, confounding, bias in the study, exposure and outcome assessment.	**1 – Low impact**. Limited data availability; no comparative data, thus not considered further for risk characterisation.
	Limitations in the epidemiological data at the study group level: e.g. research question (correct outcome assessed), detection, reporting and publication bias.	**1 – Low impact**. Outcomes assessed in an appropriate manner.
	Geographical representativeness and consistency.	**1 – Low impact**. Limited data availability; geographical representativeness adequate.
	Consumption patterns, stratification in terms of geographical location and diet.	**1 – Low impact**. Limited data availability; global pattern of consumption.
Mode of action	Uncertainties on the MOA of the substance in animals. Uncertainties in the strength, consistency and specificity of the association of the key events and the critical effect in humans.	**2 – Moderate impact**. The suggested MOA (i.e. that lectins binding to animal gut produce pleiotropic effects).
	Uncertainty on the human relevance of the MOA identified in experimental animals.	**2 – Moderate impact**. Lectins may affect the gut in humans but there are no studies in humans with pure lectins, only with food containing lectins (e.g. beans). The MOA may be similar in animals and humans.
Selection of reference point	Uncertainty on the biological relevance of adversity of the selected BMR.	**2 – Moderate impact**. Unclear whether small intestine weight changes are directly relevant for humans. Sufficient information to justify the selection of the BMR.
Dose response analysis of critical endpoints	Uncertainty regarding the dose response analysis, e.g. trend occurrence, large data variation, possible covariates, lack of raw data points.	**2 – Moderate impact**. Response is not normalised to body weight, which makes the BMDL less conservative.
	No dose-response relationship/not well defined.	**0 – Negligible impact**. Data support a dose-response relationship.

TABLE E.2 Sources of uncertainty identified for occurrence and exposure.

Group	Description of the sources of uncertainty	Impact in the assessment[1]
Occurrence data		
Analytical measurements	Uncertainty due to the performance of the analytical method. Overall, methods used for the quantification of (active) lectins in plants, information on the validation and accreditation of the methods are missing. Note: representativeness considered later.	**1 – Low impact**. Occurrence data selected by Boniglia et al. (2008). ELISA is considered an adequate method to detect active PHA, porcine thyroglobulin used to capture PHA, no validation reported (Boniglia et al., 2003)
	Analytical capability of the method – sensitivity of ELISA method depend on the quality of antibodies used for estimating LOQ and LOD.	**1 – Low impact**. Porcine thyroglobulin used to capture PHA, specific for PHA. LOD = 5 ng and LOQ = 8 ng as reported by the authors.
	Uncertainty due to coeluting proteins. (PHA-related proteins have been identified in the literature)	**1 – Low impact**. Use of glycoprotein binding assay, all PHA-related proteins are expected to be washed away.
	Uncertainty related to consideration of recovery. Efficiency of the lectin extraction process (efficiency of seed grinding, efficiency of lectin solubilisation in appropriate buffer, addition of protease inhibitors during extraction) on lectin recovery. Potential binding to glycoproteins so the bound fraction may not be measured; therefore, higher concentration could be expected. Activity of proteases may lead to underestimation of the concentration of lectins	**1 – Low impact**. Usually, the ripe harvested seeds contain the highest amount of lectin. In addition, the seeds are usually crushed mechanically and the resistance of seed lectins to proteases is generally sufficient to avoid their degradation. No information on the amount of lectins bound to glycoproteins present in the extracts.
	Proportion of left-censored data and magnitude of difference between risk estimates for LB and UB exposures	**Not applicable**. As all samples used for the dietary exposure assessment were quantified (all above LOQ).
	Uncertainty due to lack of certified reference materials and proficiency tests.	**2 – Moderate impact**. PHA consist of a mixture of 5 isomers (E4, E3L, E2L2, EL3 and L4 for PHA) and the proportions of which may vary depending on the bean varieties. Under these conditions, it is difficult to establish certified reference materials of PHA. In fact, best references for pure lectins, e.g. PHA (PHA-L4 or PHA-E4) would be crystallised lectin samples.
Data used	Uncertainty on whether there are errors in the used occurrence data or linked to missing information. Moisture content in fresh commodities was calculated based on literature data. For the occurrence data, information on concentration of PHA in mature beans was considered.	**1 – Low impact**. Moisture content could impact in both directions. No difference in the concentration of lectins in seeds and pods was considered.
	Uncertainty in the information on sampling strategy.	**0 – Negligible impact**. These are not monitoring data, but data from literature. Information missing on the randomisation on the sampling strategy.
	Uncertainty in the form of the food reported (cooked/uncooked, powder/liquid/reconstituted etc).	**1 – Low impact**. There is full certainty on the fact that the initial values for active lectin concentrations refer to raw primary commodities without any processing involved.
	Selection/generation of value for exposure estimation	**3 – High impact**. Extrapolation made from just one type of *Phaseolus* species (and only one sample) to all *Phaseolus* species. 10-fold higher concentration as compared to other results reported in the literature (Table 11) which is considered an adequate value to estimate acute exposure

(Continues)

TABLE E.2 (Continued)

Group	Description of the sources of uncertainty	Impact in the assessment[1]
Representativeness and completeness of the data	Uncertainty in the occurrence data due to limited data availability.	**3 – High impact**. Limited occurrence data produced with reliable methods, collected from few countries and from few years. No data on processed commodities; recipes and factors used from the RPC model to derive the amounts of lectins in processed commodities. The results in the study selected (in which ELISA method was used to quantify PHA in one sample of kidney beans from Italy) are consistent with other studies and reviews.
	Uncertainty in the occurrence data due to lack of data for potentially relevant major food categories.	**Not applicable**. No occurrence values on the presence of active lectins in human milk
Multiple chemicals and metabolites	Uncertainty in the occurrence data due to some **relevant substances** not being reported. Lack of data for some potentially relevant compounds Limited data on the **co-occurrence** for the chemicals belonging to the group of interest Missing information on metabolites or degradation products	**Not applicable**.
Extrapolation or use of models	Uncertainty due to extrapolation or use of models or assumptions to address limitations or gaps in the occurrence data Use of models or assumptions other than those mentioned above (LB/UB estimates, read across between food categories)	**1 – Low impact**. Assumptions on moisture and the extrapolation of the occurrence of PHA from one sample of kidney beans to all Phaseolus species as mentioned above.
Consumption data		
Data reporting	Is there uncertainty in the consumption data due to errors e.g. in classification, body weight, age, memory errors, etc.? Unidentified errors in reporting consumption data (e.g. in the classification of the food, portion size, etc.).	**1 – Low impact**. Generic issue for all CONTAM Opinions.
	Body weight estimation (measured, self-reported or estimated).	**1 – Low impact**. Generic issue for all CONTAM Opinions.
	Memory errors and capacity to report details in dietary surveys, possible under and over reporting.	**1 – Low impact**. Generic issue for all CONTAM Opinions.
	Uncertainty in consumption data. *Dietary survey methodology (dietary record vs 24-h recall), dietary software, interview options (place, face to face vs telephone and background of the interviewers) and use of portion-size measurement aids for the estimation of portion sizes*	**1/2 – Low to moderate impact**. Generic issue for all CONTAM Opinions.
	Uncertainty in consumption data. *Short-term (acute) exposure assessed based on few days of consumption per individual*	**1 – Low impact**. Duration of the dietary surveys (2- to 3-day duration) might have an impact on capturing food commodities that are not typically consumed leading to only few consumers available (e.g. specific types of beans from *Phaseolus* sp.).
	Representativeness over different weekdays and seasons within dietary surveys	**1 – Low impact**. Generic issue for all CONTAM Opinions.
	Sample size and response rate of the dietary surveys	**Not applicable**
	Use of national standard recipes and ingredients factors for composite dishes (e.g. underestimation of minor ingredients, overestimation of standard ingredients, etc.) sampling frame, method and design of the dietary surveys	**Not applicable**
	Use of national standard recipes and ingredients factors for composite dishes (e.g. underestimation of minor ingredients, overestimation of standard ingredients, etc.) Sampling frame, method and design of the dietary surveys	**Not applicable** Studies only on raw primary commodities (see above)
	Uncertainty in the form of the food reported	

TABLE E.2 (Continued)

Group	Description of the sources of uncertainty	Impact in the assessment[1]
		1 – Low impact. The occurrence data used on lectins refer to raw primary commodities and the consumption to processed commodities, there is uncertainty on the effect of processing on lectin activity and therefore in the amounts of active lectins in the processed commodities. Assumptions are made to try to overcome this shortcoming (see dietary exposure estimates methodology).
Group	**Description of the sources of uncertainty**	**Impact in the assessment[1]**
Representativeness of the data	Uncertainty in the representativeness of the consumption data. *Lack of food consumption data for special population groups, including consumers only of specific foods of special interest, or following special diets, countries, etc.*	**1 – Low impact**. Consumption of beans and bean-based products containing PHA is overall well represented in the consumption database.
Dietary exposure estimates methodology	Uncertainty linked to the methodology used for calculating the exposure Assumptions made on the dietary exposure scenarios as regards the inactivation of lectins due to processing/food preparation could impact underestimating/overestimating the true exposure.	**1 – Low impact**. Other than the elimination rate, there were no further assumptions made (elimination is considered as condition)
Non-dietary exposure	Uncertainty in the exposure due to other sources than dietary are not included	Not applicable
Other uncertainties	*Are there any other uncertainties affecting the exposure assessment, which are not addressed in the rows above?*	Not applicable

TABLE E.3 Sources of uncertainty identified for risk characterisation.

Group	Description of the sources of uncertainty	Impact in the assessment
Uncertainty factors (see EFSA Scientific Committee, 2012, 2018b)	Uncertainty about the appropriateness of default UFs used in the risk assessment	**0 – Negligible**. The default factor of 100 was considered to cover inter- and intra-species differences (kinetic and dynamic differences)
	Uncertainty about the appropriateness and magnitude of any case-specific, non-default UFs used in the risk assessment	**Not applicable**. No case-specific UF is used
	Uncertainty about the appropriateness and magnitude of default or non-default UFs which are specific to infants under 16 weeks of age (EFSA Scientific Committee, 2017)	**Not applicable**. Infants under 16 weeks are not expected to be exposed to food containing lectins
Selection of critical studies and endpoints		**3 – High Impact**. The RP might be different if uncertainties affecting non-critical studies and endpoints or uncertainties due to gaps in the toxicological evidence were resolved, e.g. by obtaining more or better data, including acute toxicity studies. One or several studies reporting effects at lower doses than the critical effect which were not selected for setting the critical RP due to insufficient degree of purification, study design unclear, combination of routes of application and limited relevance. There is uncertainty about the reference point due to lack of studies on carcinogenicity, immunotoxicity (including allergy), reproduction and neurotoxicity.
Lack of human data		**3 – High Impact**. Lack of appropriate human data which could be used for the risk assessment, e.g. with purified lectins, quantified lectins consumed, endpoints from the GI system.

APPENDIX F

Overview of the results of the Expert Knowledge Elicitation

Overview of the results of the Expert Knowledge Elicitation Hazard PHA

Parameter	The reference point for the acute hazard assessment to lectins via food														
Stratification	Group of lectins: PHA														
EKE question	If all non-standard sources of uncertainty were resolved, what would be the value of the reference point for the **acute hazard assessment**?														
Unit	mg/kg bw per day														
EKE method	Behavioural aggregation using the quantile method for individual judgements and the probability method for the consensus judgement														
Probabilities	P1%	P2.5%	**P5%**	P10%	P16.7%	P25%	P33.3%	**P50%**	P66.7%	P75%	P83.3%	P90%	**P95%**	P97.5%	P99%
Values	0*		**10**					**20**					**35**		120*
Calculated quantiles	6.7	8.4	10	11.9	13.7	15.4	16.9	20	23.4	25.5	28.2	31.3	34.9	38.2	42.1
Fitted distribution	Skewed normal (location = 12.2, scale = 11.6, slant = 2.79). *limits when fitting the distribution														

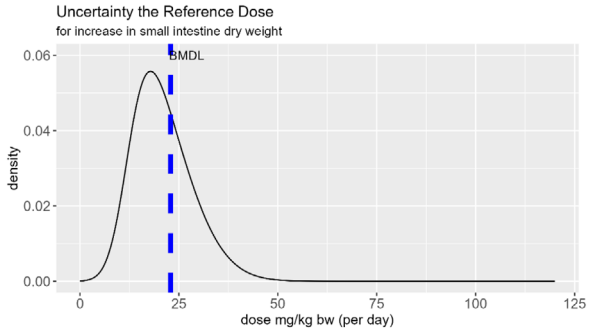


Figure (a): Probability density function (PDF) of the consensus judgement of the parameter

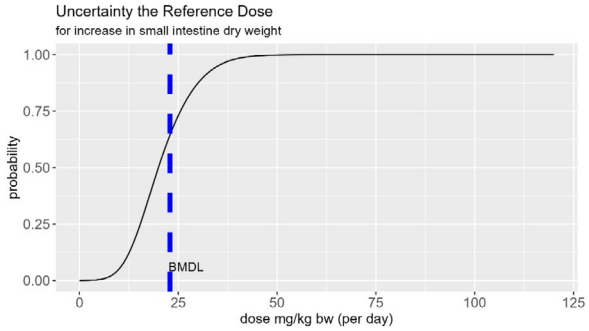


Figure (b): Cumulative distribution function (CDF) expressing the consensus judgement of the parameter

The conclusion from EKE on hazard

After considering the impact of non-standard sources of uncertainty on the reference point for PHA, the experts provided a 50% probability that the reference point is between 15 and 25 mg/kg bw per day, a 90% probability that it is between 10 and 35 mg/kg bw per day and a 50% probability that it is above 20 mg/kg bw per day.

Summary of the evidence used for the elicitation

Reference point estimated from critical study on endpoint increase in small intestine dry weight, BMDL 22.9 mg/kg bw per day (BMR 10%). Further evidence in the drafted Opinion.

Main uncertainties

All identified sources of uncertainty for hazard are listed in Table E.1, Appendix E. The main uncertainties are:

- Limitations in the design of the direct measurements of the effect in experimental animals. **Moderate impact**
- Uncertainty due to studies carried out only in one sex or certain age groups, duration of studies, sample size, biomarkers. **Low to moderate impact**
- Limited information on other effects that could be considered as critical. **Moderate impact**
- Uncertainties on the MOA of the substance in animals and uncertainties in the strength, consistency and specificity of the association of the key events and the critical effect in humans. **Moderate impact**
- Uncertainty on the human relevance of the mode of action identified in experimental animals. **Moderate impact**
- Uncertainty on the biological relevance of adversity of the selected benchmark response (BMR). **Moderate impact**
- Uncertainty regarding the dose response analysis, e.g trend occurrence, large data variation, possible covariates, lack of raw data points. **Moderate impact**

Justification for a lower value	• In the study, body weight over 10 days had decreased over time, which implies that if normalised for body weight would increase the relative effect • BMDL would be lower because it is currently non-representative of differences in sex (sex not reported) and age group (30 days age) • Variability due to biological factors, depending on gut micro biota (facility and diet) could influence the results. Assuming we have representative studies, the lowest will be behind the new reference point.
Justification for both a lower and higher value	• Doses can result in both higher and lower values
Justification for a higher value	• A higher number of animals will reduce uncertainty in BMD, and thereby increase the BMDL • Basing a BMD on acute data (as opposed to subacute data) would result in a higher BMDL
Experts	Pierre ROUGÉ, Hanne FRØKIÆR, Bettina GRASL-KRAUPP, Heather WALLACE
Facilitator/Reporter	Ullrika Sahlin and John Paul Gosling
Observers	
Date and place of the EKE	23 May 2025, Online

Overview of the results of the Expert Knowledge Elicitation Exposure PHA

Parameter	A high exposure for the highest exposed age group after processing by the consumer resulting in an deactivation of 50%. A high exposure is defined as the 99th percentile (P99) of the European population exposure of the selected age group.														
Stratification	Group of lectins: PHA Highest exposed age group: other children														
EKE Question	Assuming the ideal situation: (i) that a representative diet study for daily food consumption of European other children is given; (ii) that all concentrations of lectins are measured with sufficient quality for quantification of the contamination of the (eaten) products before processing at the consumer. What would be the 99th percentile (i.e. the exposure exceeded by 1 person out of 100) of the daily intake of lectins of European other children, when their food has been processed by the consumer (leading to a 50% lectin deactivation)?														
Unit	mg/kg bw per day														
EKE method	Behavioural aggregation using the quantile method for individual judgements and the probability method for the consensus judgement														
Probabilities	P1%	P2.5%	P5%	**P10%**	P16.7%	P25%	P33.3%	**P50%**	P66.7%	P75%	P83.3%	P90%	**P95%**	P97.5%	P99%
Values	0*			**5**				**10**					**20**		40*
Calculated quantiles	2.0	3.0	3.9	5.0	6.0	7.1	8.0	10.0	12.3	13.7	15.5	17.5	20.0	22.2	24.8
Fitted distribution	Skewed normal (location = 4.76, scale = 7.77, slant = 3.56) *limits when fitting the distribution														

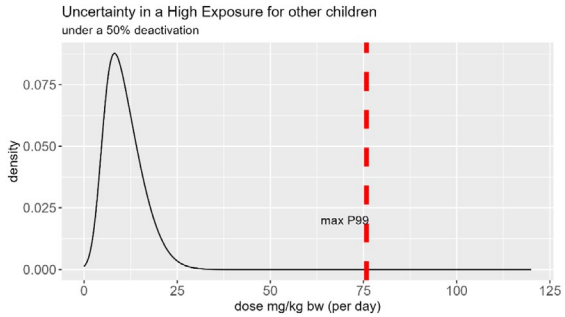


Figure (a): Probability density function (PDF) of the consensus judgement of the parameter

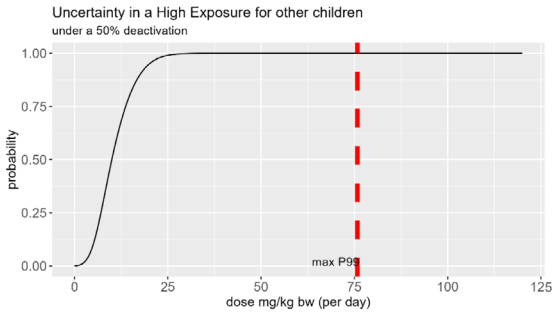


Figure (b): Cumulative distribution function (CDF) expressing the consensus judgement of the parameter

The conclusion from EKE on exposure

After considering the impact of non-standard sources of uncertainty on a high exposure to PHA via food and at a 50% deactivation, the experts provided a 50% probability that a high exposure P99 to PHA for the age group other children is between 7 and 14 mg/kg bw per day, a 90% probability that it is between 4 and 20 mg/kg bw per day and a 50% probability that it is below 10 mg/kg bw per day.

Summary of the evidence used for the elicitation

Overview of exposure estimates for PHA and age group other children. Further evidence in the drafted Opinion.

Foods	Consuming_ days	Mean_ LECTIN_EXP_ LB	Mean_ LECTIN_EXP_ UB	P_EXPO_ LB75	P_EXPO_ UB75	P_EXPO_ LB90	P_EXPO_ UB90	P_EXPO_ LB95	P_EXPO_ UB95	P_EXPO_ LB97_5	P_EXPO_ UB97_5	P_EXPO_ LB99	P_EXPO_ UB99
Beans and vegetables meal	431	27.9	27.9	35.0	35.0	45.4	45.4	58.2	58.2	66.2	66.2	75.8	75.8
Runner beans (with pods)	34	4.7	4.7	5.6	5.6	7.7	7.7						
Navy bean (fresh seeds)	11	4.5	4.5	5.7	5.7								
Lima beans (with pods)	19	4.0	4.0	5.5	5.5								
Navy beans (dry seeds)	107	16.2	16.2	25.6	25.6	42.6	42.6	49.8	49.8				
Lima beans (dry)	15	35.0	35.0	29.6	29.6								
Legumes, nuts, oilseeds and spices	38	14.6	14.6	20.0	20.0	31.1	31.1						
Kidney bean (dry seeds)	43	9.9	9.9	10.6	10.6	18.3	18.3						
Legume (beans) soup	29	22.4	22.4	28.2	28.2	46.2	46.2						
Canned or jarred common beans	283	8.4	8.4	10.0	10.0	20.9	20.9	26.2	26.2	33.9	33.9		

Foods	Consuming_ days	Mean_ LECTIN_EXP_ LB	Mean_ LECTIN_EXP_ UB	P_EXPO_ LB75	P_EXPO_ UB75	P_EXPO_ LB90	P_EXPO_ UB90	P_EXPO_ LB95	P_EXPO_ UB95	P_EXPO_ LB97_5	P_EXPO_ UB97_5	P_EXPO_ LB99	P_EXPO_ UB99
French beans (with pods)	1131	2.7	2.7	4.2	4.2	6.5	6.5	7.9	7.9	9.2	9.2	10.8	10.8
Flageolets (dry seeds)	7	11.5	11.5										
French beans canned	178	5.0	5.0	6.2	6.2	8.7	8.7	11.2	11.2	13.5	13.5		
Borlotti or other common beans (without pods)	52	1.8	1.8	2.0	2.0	4.4	4.4						
Borlotti or other common beans (dry)	296	17.9	17.9	23.4	23.4	37.0	37.0	46.9	46.9	58.9	58.9		

Main uncertainties

All identified sources of uncertainty for exposure are listed in uncertainty (Table 2). The main uncertainties are:

- Uncertainty due to lack of certified reference materials and proficiency tests. **Moderate impact**
- Selection/generation of value for exposure estimation. **High impact**
- Uncertainty in consumption data. **Low to moderate impact**

Justification for a lower value	• Lower reliability in the calculated values since they were derived taking the maximum, and thereby overestimating PHA occurrence. Could result in a reduction by a factor of 10.
Justification for a higher value	• The calculated exposure might be underestimated because in the type of analysis made some of the lectins might bind to sugar and not appear in analysis systems. Could increase by twofold.
Experts	Pierre ROUGÉ, José GÓMEZ, Hanne FRØKIÆR, Jean-Charles LEBLANC
Facilitator/Reporter	Ullrika Sahlin and Kevin Wilson
Observers	Name of any observers at the EKE session, esp. additional adviser etc.
Date and place of the EKE	8 May 2025, Online

Overview of the results of the Expert Knowledge Elicitation Overall Uncertainty PHA	
Outcome	There is a health concern (MOE is below 100)
Stratification and Scenario	Group of lectins: PHA All age groups Deactivation: 50%
EKE question	If all non-standard sources of uncertainty were resolved, what is your probability that the MOE is below the factor100?
Unit	% bounded in 0 and 100
EKE method	Behavioural aggregation using the approximate probability method.
Probability	**> 95%**

The conclusion from EKE on overall uncertainty

The experts agreed that they, after considering all identified sources of uncertainty, are at least 95% certain, i.e. that it is extremely likely, that there is a health concern with PHA under the scenario of 50% elimination/deactivation rate when processing food.

Summary of the evidence used for the elicitation

Uncertainty in the reference point and the P99 exposure of the age group other children were evaluated in EKE hazard and EKE exposure (Figure a). The resulting distributions were combined by Monte Carlo simulation to a preliminary uncertainty in the margin of exposure (Figure b). For the assessment of overall uncertainty, any additional non-standard sources of uncertainty were considered when making judgement of P(MOE < 100).

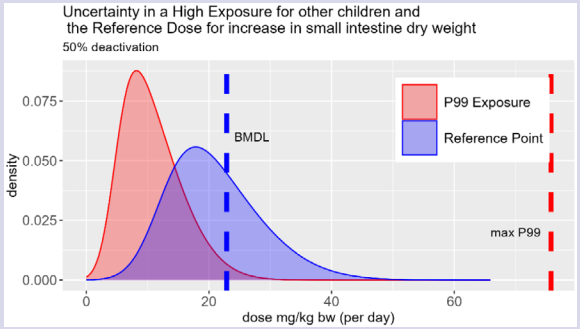


Figure (a): Probability density functions (PDFs) expressing uncertainty in hazard and exposure from considering uncertainties listed in Tables 1 and 2, respectively.

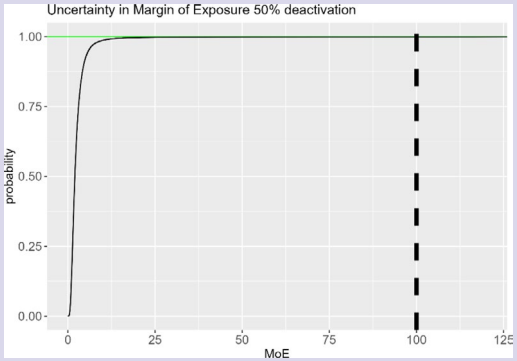


Figure (b): Cumulative probability distribution (CDF) of the margin of exposure resulting from combining uncertainty in hazard and exposure for the critical endpoint and most sensitive age group.

Main uncertainties

All identified additional sources of uncertainty for overall uncertainty are listed in uncertainty (Table 3). The main additional uncertainties are:

- Selection of critical studies and endpoints. **High impact**
- Lack of human data. **High impact**

Justification for a lower probability	• Lack of data on humans • Unclear relevance of animal endpoints to humans • Lack of appropriate occurrence data • Current data is subacute and more conservative than acute data
Justification for a higher probability	The judgement on the lower limit considers that • There can be other more sensitive endpoints, such as allergic reactions, for which no data is available
Experts	Pierre ROUGÉ, José GÓMEZ, Hanne FRØKIÆR, Jean-Charles LEBLANC, Bettina GRASL-KRAUPP, Evangelia NTZANI
Facilitator/Reporter	Ullrika Sahlin and John Paul Gosling
Observers	
Date and place of the EKE	6 June 2025, Online

ANNEXES

Annex A

Protocol for the development of the opinion

The protocol undertaken for the scientific development of this opinion is available under the Supporting Information section on the online version of the scientific output.

Annex B

Search strategies Lectins

The literature search strategies for the scientific development of this opinion is available under the Supporting Information section on the online version of the scientific output.

Annex C

Risk of bias analysis

Results of the risk of bias analysis are available under the Supporting Information section on the online version of the scientific output.

Annex D

Benchmark dose (BMD) modelling

Details of the BMD modelling performed on experimental animal data are available under the Supporting Information section on the online version of the scientific output.

Annex E

Dietary exposure estimations

Acute dietary exposure estimation results are available on EFSA's Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.17899778.

Annex F

Outcome of public consultation

Details of the outcome of the public consultation are available under the Supporting Information section on the online version of the scientific output.