

Detection of Lectin Protein Allergen of Kidney Beans (*Phaseolus vulgaris* L.) and Desensitization Food Processing Technology

Yongfei Wang, Shudong He,* Fanlin Zhou, Hanju Sun, Xiaodong Cao, Yongkang Ye, and Jing Li

Cite This: <https://doi.org/10.1021/acs.jafc.1c02801>

Read Online

ACCESS |



Metrics & More



Article Recommendations

ABSTRACT: With the increase of food allergy events related to not properly cooked kidney beans (*Phaseolus vulgaris* L.), more and more researchers are paying attention to the sensitization potential of lectin, one of the major storage and defensive proteins with the specific carbohydrate-binding activity. The immunoglobulin E (IgE), non-IgE, and mixed allergic reactions induced by the lectins were induced in the current paper, and the detection methods of kidney bean lectin, including the purification strategies, hemagglutination activity, specific polysaccharide or glycoprotein interactions, antibody combinations, mass spectrometry methods, and allergomics strategies, were summarized, while various food processing aspects, such as the physical thermal processing, physical non-thermal processing, chemical modifications, and biological treatments, were reviewed in the potential of sensitization reduction. It might be the first comprehensive review on lectin allergen detection from kidney bean and the desensitization strategy in food processing and will provide a basis for food safety control.

KEYWORDS: kidney bean, lectin, allergen, detection, desensitization

INTRODUCTION

Kidney bean (*Phaseolus vulgaris* L.), also called common bean, is one of the oldest vegetables and has been widely cultivated and consumed around the world.¹ In 2020, the global production of *P. vulgaris* L. beans was 34 million ha and 30 million ton, which was a large proportion of the global output of beans of 95 million ha and 92 million ton (<http://www.fao.org/faostat/en/#data/QC>).² However, in recent years, the reports and studies related to undercooked kidney beans (*P. vulgaris* L.) eliciting allergic reactions have been increased sharply in India, Canada, Australia, and the U.K.³ The allergic symptoms usually appear within 1–3 h after eating improperly prepared beans, manifesting as naupathia, vomiting, diarrhea, and abdominal pain.⁴ During the years 2003–2014, there were 124 sensitization events related to kidney beans occurred in China, which affected 7526 individuals.⁵ In 2006, more than 100 people in Japan were hospitalized for eating roasted white kidney bean powder and 1000 people suffered from acute intestinal symptoms.⁶ In Morocco, 51% of children and 39% of adults from a total of 114 patients [76 children and 38 adults, who were consulted for hypersensitivity and had not yet been sensitized or challenged orally to white kidney beans (*P. vulgaris* L.)] exhibited positive values of the specific immunoglobulin E (IgE) level to white kidney bean proteins, indicated that the Moroccan population was also very sensitive to *P. vulgaris* L. bean allergens.⁷

According to the recent literature, the most identified proteins in *P. vulgaris* L. beans, including phaseolin, lectin-related α -amylase inhibitor, and the true lectin proteins, phytohemagglutinins [PHAs, mainly phytohemagglutinin P (PHA-P)], have aroused wide interests among researchers for allergic exploration based on the proteomic studies using two-

dimensional electrophoresis and mass spectrometry [matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF MS) and MALDI–TOF/TOF].^{8,9} In the previous investigation for Indian patients ($n = 355$) with a history of legume allergy, 22.0% of patients were sensitized to kidney bean protein extract by the skin prick test (SPT) for the specific IgE (sIgE) and total IgE determinations, and four identified proteins, named lectin (phytohemagglutinin), phaseolin, α -amylase inhibitor precursor, and group 3 late embryogenesis abundant protein, were confirmed as the potential major allergen proteins in the kidney beans by the liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis and enzyme-linked immunosorbent assay (ELISA) inhibition test.¹⁰ Besides, the non-specific lipid transfer protein (Pha v 3) has been reported as a major allergen in green beans (*P. vulgaris*) and has been proven as a potential risk for lipid-transfer-protein-syndrome patients.¹¹ Subsequently, kidney bean allergen proteins were purified by the anion exchanger, gel filtration, and high-performance liquid chromatography (HPLC). The purified lectin protein, also named PHA, was identified with 88% of the sera IgE binding capacity of patients and 78% SPT-positive reaction to kidney-bean-sensitive patients, and only 102 ng of purified *P. vulgaris* lectin could cause 50% IgE inhibition.¹²

Special Issue: Research Progress of Desensitization Methods in Food Industry

Received: May 11, 2021

Revised: June 22, 2021

Accepted: June 25, 2021



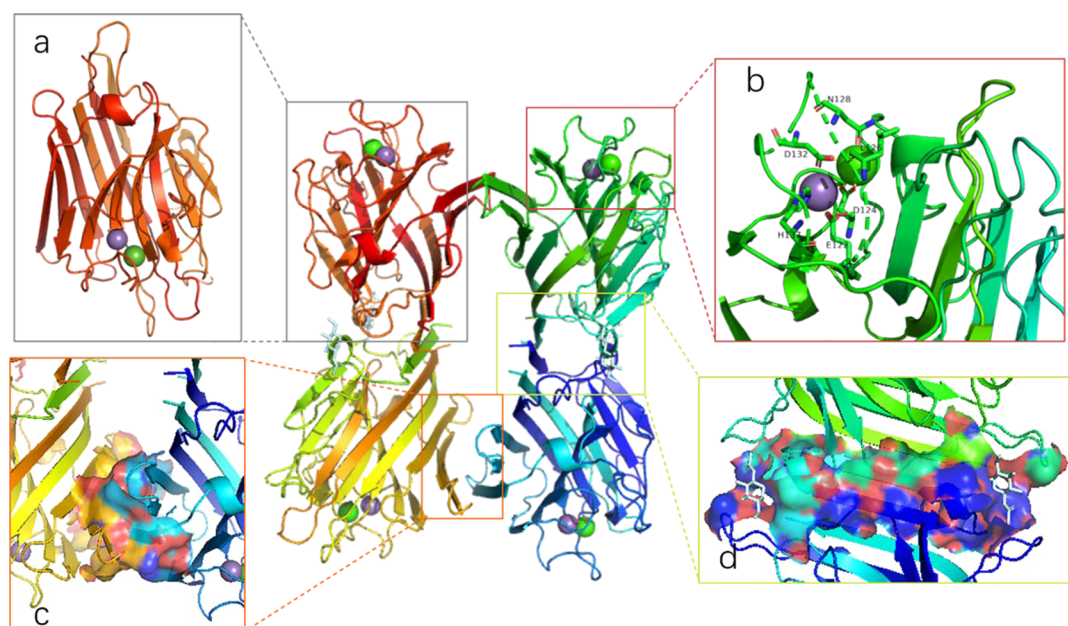


Figure 1. Structure of *P. vulgaris* L. lectin (PDB ID 1FAT). The structures covered by red, yellow, blue, and green represent the four subunits of PHA-L, respectively. The small picture a is the lectin monomer structure, which is comprised of two antiparallel sandwiched β -sheets. Each of the monomers contains two metal-ion-binding sites and carbohydrate-binding sites; the purple ball represents Mn^{2+} ; the green ball represents Ca^{2+} ; and the white-gray molecules are the specific carbohydrates bound by lectin. The small picture b is the distribution of amino acids at the metal-ion-binding sites, and the pictures c and d are the interaction surfaces between lectin subunits, respectively.

According to the previous case of severe anaphylaxis to kidney bean by a 23-year-old female without any atopic history of allergies in France, lectins of edible legume seeds with good resistance to heat denaturation and digestive proteolysis were speculated as potentially allergenic proteins.¹³ Besides, the allergenicity of kidney bean lectin proteins has also been reported in mice, pigs, cattle, etc.^{14,15} In the previous study by feeding kidney beans to rats, the level of immune response of rat to kidney bean lectin was detected to be proportional to the length of feeding time, and there was a typical booster effect when rats had repeated exposure to dietary antigens, while dietary lectin was absorbed continuously throughout each period of bean feeding.¹⁶ In a group of experiments using kidney beans to feed calves, high titers of lectin antibodies were detected in blood samples on the 13th day of feeding by the ELISA method, indicated that kidney bean lectin could selectively pass through the mucosa and enter the systemic circulation to trigger allergic reactions.¹⁷ In addition, similar phenomena have also been observed in experiments on feeding kidney bean lectin to porcine and rabbit.^{18,19}

Lectin is a type of non-enzymatic sugar-binding protein that can non-covalently and reversibly bind to specific monosaccharides and oligosaccharides and plays a key role in pest prevention in the beans as one of the storage proteins (about 12 mg/g of dry red kidney bean seeds).^{20–23} The lectin protein was first found in castor bean (*Ricinus communis* L.) extracts in the year 1888, and the first purified hemagglutinin concanavalin A (Con A) was obtained from jack beans (*Canavalia ensiformis*) in the year 1919.^{24–26} Until in the middle of the 20th century, the purified lectin protein (PHA) of the kidney bean (*P. vulgaris* L.) was obtained using column chromatography, and the hemagglutination characteristics, potential biomedical properties, and sensitizing effects were rapidly explored.²⁷ As shown in the Web of Science by April 30, 2021, about 1153 articles were published concerning the extraction,

characterization, and function analysis of *P. vulgaris* lectins. Since the wide consumption of kidney beans and their related products, more and more researchers are paying attention to the allergenic potential of kidney bean lectins recently; the current review highlights the detection methods of the allergen protein at various aspects and presents the recent food processing technologies devoted to hypoallergenic foods.

■ STRUCTURE AND SPECIFICITY OF KIDNEY BEAN LECTINS

Legume lectins are highly homologous in sequence and have similar physicochemical and structural properties. Previously, our laboratory isolated the lectin from black turtle bean (*P. vulgaris* L.), and the sequence (NCBI accession number AHB17899.1) and the three-dimensional structure presented high homology (97.18% similarity) with phytohemagglutinin E (PHA-E).^{28,29} As shown in Figure 1, most native kidney bean lectins are tetramers composed of four subunits through hydrophobic interactions, hydrogen bonds, and salt bonds. Red kidney bean phytohemagglutinin might be composed of five tetramer isoforms, viz., L_4 , L_3E_1 , L_2E_2 , L_1E_3 , and E_4 , which would be formed by the combination of lymphocyte-specific and red-blood-cell-specific subunits.^{30,31} The relative molecular weight of tetramer phytohemagglutinin L (PHA-L) might be about 120–130 kDa and consisted of four identical subunits [molecular weight (MW) = 29.5–32 kDa],^{14,32–34} while PHA-E might be composed of four 34 kDa subunits.³⁵

As presented in Figure 1, the monomer structure of kidney bean lectin (PHA-L, PDB number 1FAT), comprising two antiparallel sandwiched β -sheets, which are connected by α -turns, β -turns, and bends along with short loops, displays a jelly roll motif best described as a sandwich and contains two divalent cation metal-binding sites (Mn^{2+} and Ca^{2+}) and a carbohydrate recognition domain (CRD).^{36,37} The two antiparallel β -sheets constitute a flat six-strand “back” sheet, a

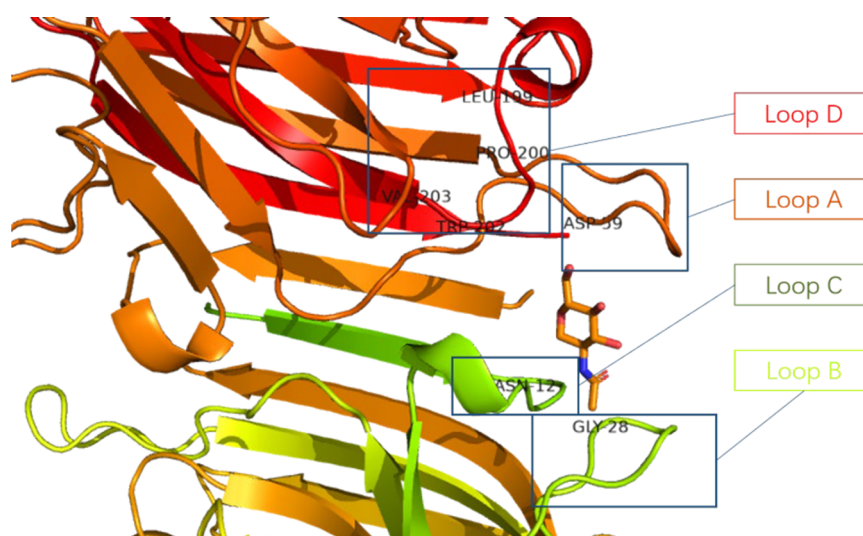


Figure 2. Structure of four binding site loops in the carbohydrate recognition domain. The structure of the carbohydrate recognition domain in *P. vulgaris* lectin consists of four binding site loops A, B, C, and D. The key amino acid residues in loops A, B, C, and D are Asp, Gly, Asn, and an additional interaction provided by amino acids in loop D, respectively.

concave seven-strand “front” sheet, and five short “top” β -sheets, which hold the two pieces together.³⁸ Mn^{2+} and Ca^{2+} are not only necessary for the carbohydrate-binding activity of lectin, but also for functional conformation stability of the monosaccharide binding site, and they are also important for the hemagglutination activity.^{39–41} Previously, when the kidney bean lectin was completely reacted with ethylenediaminetetraacetic acid (EDTA), the coagulation activity of the lectin was almost lost and the activity would be completely restored by re-addition of Ca^{2+} and Mn^{2+} .^{41–43} Mg^{2+} might have a substitution effect on the metal ions.^{44,45} The CRD is a shallow depression located on the top surface of the dome-like structure, which can bind with both monosaccharides and oligosaccharides.⁴⁶ The basic structure of CRD in *P. vulgaris* L. lectins consists of four binding site loops A, B, C, and D, which are adjacent in the three-dimensional structure of the capsule, but they are not close together in sequence, as shown in Figure 2. The bottom of the binding site is composed of a few conserved key amino acid residues in the loops, including Asp in loop A, Gly or Arg in loop B, aromatic residues and Asn in loop C.^{47–49} In general, these residues contribute to hydrogen bonding and van der Waals interaction with sugar, and the changes in loops C and D may be the main determinant of monosaccharide specificity.^{50,51} In addition, the lectin monomer also contains a hydrophobic cavity mainly formed by conserved hydrophobic residues.

Each monomer of PHA-E lectin is involved in two different monomer–monomer interfaces: one is a conventional β -sheet-like contact between two polypeptides, and the other is mainly formed by van der Waals interactions between two dimers. Two curved 12-stranded β -sheets are face to face in the tetramer, resulting in a large channel between them.⁵² The channel is mainly formed by conservative hydrophobic residues and is a hydrophobic cavity, which might be related to the thermal stability of lectin.⁴¹ Besides, the aggregation state of purified lectin might be different under various pH environments. For example, the lectin protein might be in a dimer state at pH 2.5 and would be in a natural tetramer state at pH 7.2, while it might polymerize into the polymer in alkaline conditions.^{53–55}

Although different legume lectins have high similarity in sequence and the primary, secondary, and tertiary structures, the quaternary structure might show considerable changes, which would lead to the changes of the monomer interaction and the presence/absence of protein modification and then might affect the specificity of polyvalent glycan binding. For example, although PHA-E and PHA-L have 70% sequence homology and both are isolated and purified from red kidney beans, PHA-L would bind with β -1,6-GlcNAc moiety from a N-linked glycan core, while binding specificity of PHA-E has been confirmed as bisected N-linked glycan structures, Man- α -1–6-linked part of tri- or tetra-antennary N-glycans.^{56–61} The minimal structural unit required for PHA-L binding was the disaccharide GlcNAc(1 $\rightarrow$ 2)Man.⁵⁸ Besides, there is a cross-reactivity between PHA-E and PHA-L.¹ In addition, kidney bean lectin has cross-reactivity with peanut Ara h 1 and Ara h agglutinin (PNA), black gram, and pigeon pea,^{62,63} while red kidney bean proteins were found to show cross-reactivity with proteins derived from peanut, soybean, chickpea, and black gram.^{9,64}

■ SENSITIZATION OF KIDNEY BEAN LECTINS

The gastrointestinal digestive system is the main route for food allergens to enter the human body. Up to now, several international food security organizations, such as the European Food Safety Authority (EFSA), the international Codex Alimentarius Commission (CAC), the Food and Agriculture Organization of the United Nations (FAO), and the World Health Organization (WHO), have established logical and clear decision systems to evaluate the allergenicity potential of food proteins by simulated digestion.^{65–67} The natural Zihua kidney bean (*P. vulgaris*) lectin gradually decreased in the simulated gastric fluid (SGF) with the extension of the digestion time until to 60 min, while the protein was trypsin-resistant for at least 48 h.⁴¹ In our previous study, black turtle bean (*P. vulgaris*) lectin was relatively stable in both SGF (half-life = 22.71 min) and trypsin digestion (half-life = 90 min) *in vitro* digestibility investigation using the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) dynamic density analysis.⁴³ Furthermore, both heat treatment and

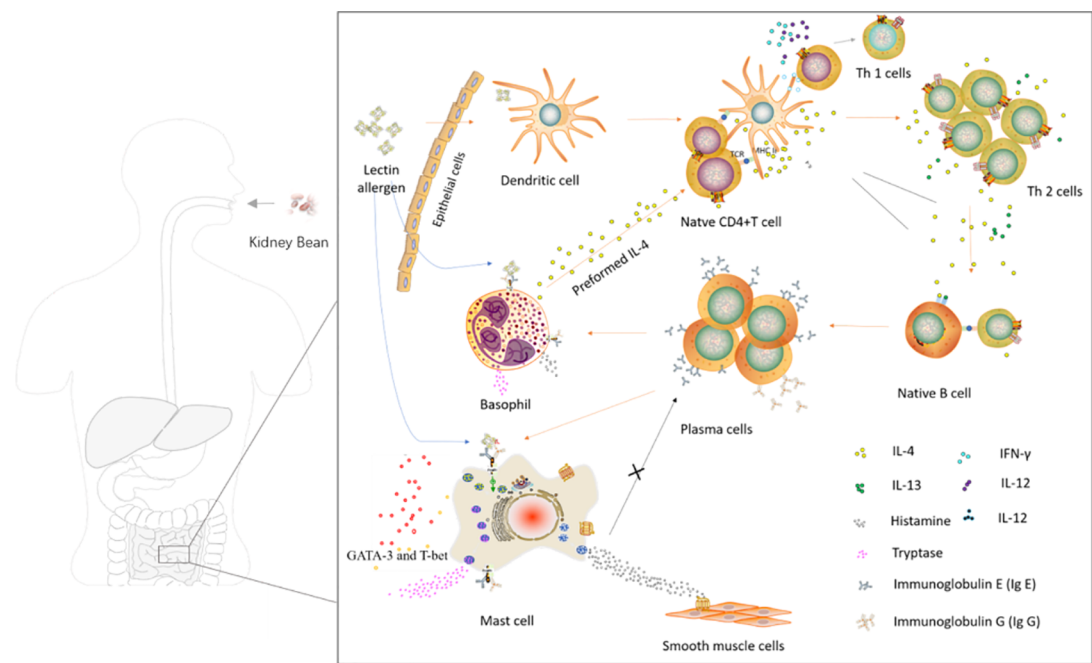


Figure 3. Sensitization mechanism of *P. vulgaris* lectin. After the ingestion of kidney beans, part of the undegraded lectins would pass through the intestinal epithelial cells and be captured by dendritic cells, which, in turn, to trigger the Th₁ and/or Th₂ cell pathway and induce IgE/non-IgE and mixed-mediated allergies. Lectins can also induce basophils and eosinophils to secrete pre-synthesized IL-4, thereby promoting the differentiation and formation of Th₂ cells.

Table 1. Epitope of “*Phaseolus vulgaris*” in IEDB

epitope ID	object peptide	position	non-peptidic epitope accession	antigen name	antigen accession	organism name
20097	linear peptide	418–432		glycine-rich cell wall structural protein 1.8 precursor	P10496.1	<i>Phaseolus vulgaris</i>
122244	non-peptidic		CHEBI_59034	phytohemagglutinin	CAD28837.1	<i>Phaseolus vulgaris</i>
131171	non-peptidic		CHEBI_59031	phytohemagglutinin	CAD28837.1	<i>Phaseolus vulgaris</i>
174332	non-peptidic		CHEBI_63758	phytohemagglutinin	CAD28837.1	<i>Phaseolus vulgaris</i>
174333	non-peptidic		CHEBI_63760	phytohemagglutinin	CAD28837.1	<i>Phaseolus vulgaris</i>
850799	linear peptide	95–103		erythroagglutinating phytohemagglutinin (<i>Phaseolus vulgaris</i>)	AHB17899.1	<i>Phaseolus vulgaris</i>
850837	linear peptide	39–47		erythroagglutinating phytohemagglutinin (<i>Phaseolus vulgaris</i>)	AHB17899.1 AHB17899.1	<i>Phaseolus vulgaris</i>
850841	linear peptide	149–160		erythroagglutinating phytohemagglutinin (<i>Phaseolus vulgaris</i>)	AHB17899.1	<i>Phaseolus vulgaris</i>
850858	linear peptide	131–141		erythroagglutinating phytohemagglutinin (<i>Phaseolus vulgaris</i>)	AHB17899.1	<i>Phaseolus vulgaris</i>
850860	linear peptide	55–66		erythroagglutinating phytohemagglutinin (<i>Phaseolus vulgaris</i>)	AHB17899.1	<i>Phaseolus vulgaris</i>
850891	linear peptide	116–125		erythroagglutinating phytohemagglutinin (<i>Phaseolus vulgaris</i>)	AHB17899.1	<i>Phaseolus vulgaris</i>

removal of metal ions could enhance the sensitivity of lectins to hydrolysis in SGF.⁶⁸ Moreover, more and more researchers are concerning the digestive stability of allergens in the gastrointestinal tract whether using the purified state or the protein embedded in a complex food matrix.⁶⁹ A previous study found that the presence of the food matrix enhanced the immune response to individual allergens. Because nuts and peanuts have a medium protein concentration and high-fat content, the fat components were speculated to enhance the allergenicity of protein allergens by protecting them from being digested or affecting the activation of immune cells.⁷⁰ The

allergenicity reduction was also observed in the complexation of peanut protein with polyphenols from blueberry and cranberry.⁷¹ Furthermore, *in vivo* digestion would partially destroy the allergen epitopes and also expose the allergen epitopes in the natural protein structure. The analysis of antidigestive peptides containing allergen epitopes is a piece of important information for the evaluation of potential allergenicity; however, the related information about the kidney bean lectin is scarce.

Because PHA could resist the decomposition of digestive enzymes and intestinal bacteria *in vivo*, the lectin protein was

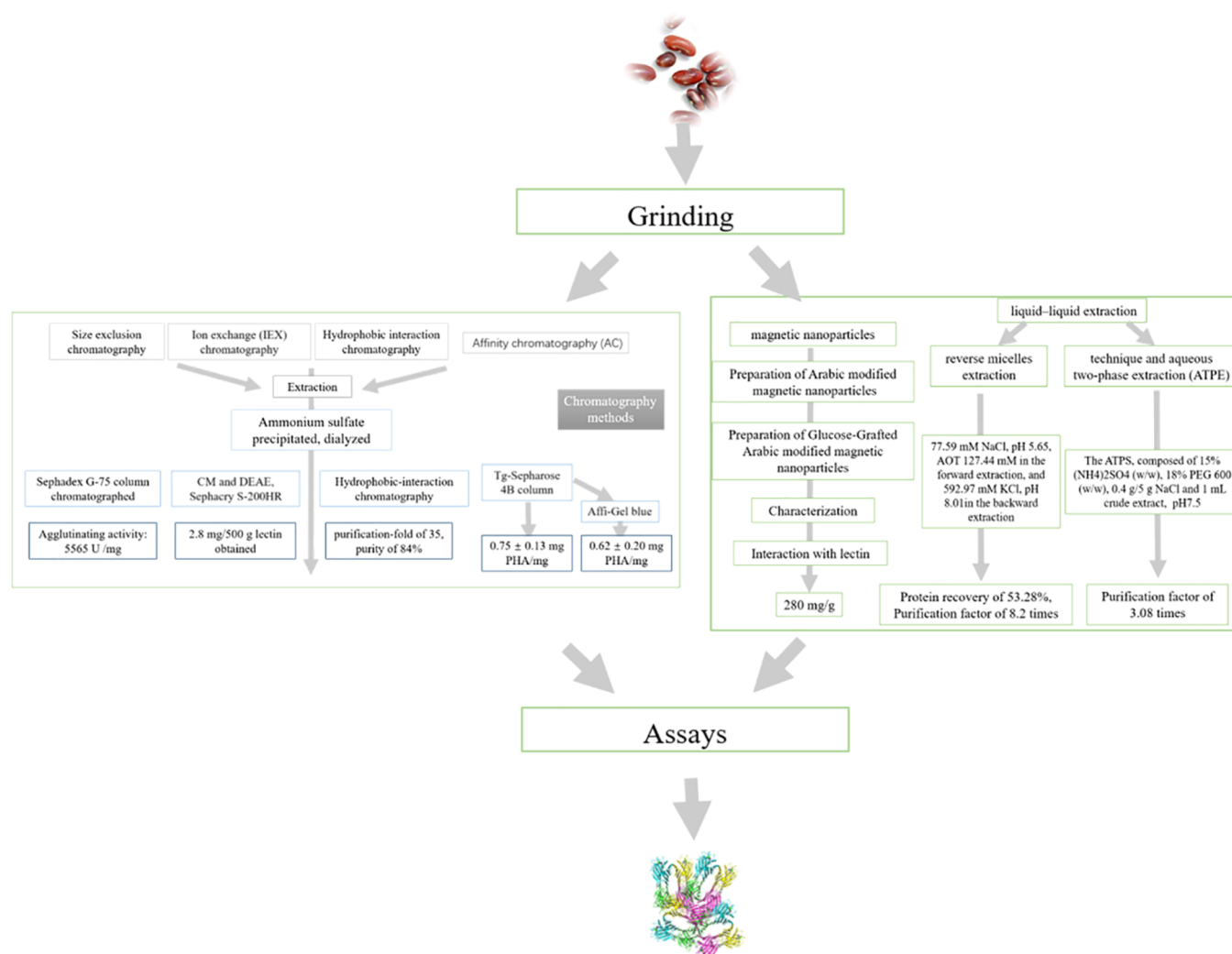


Figure 4. Purification strategies of *P. vulgaris* lectin.

confirmed to specifically bind to the glycoconjugate located on the surface of the intestinal lumen and would be endocytosed into the circulatory system to induce immune responses at the cellular level.⁷² Circulating antibodies to the lectin [immunoglobulin G (IgG) class] were detected in the serum of bean-fed rats by an enzyme-linked immunosorbent assay and suggested that this systemic uptake of lectin could result in hypersensitivity reactions.⁶⁴ However, as shown in Figure 3, the food allergenic mechanism caused by kidney bean lectins was complicated, including IgE-mediated reactions, non-IgE-mediated reactions, and mixed mediated allergic reactions (including both IgE- and cell-mediated immunological reactions).^{1,9,12,15,16,73–75}

The reaction mechanism of IgE-mediated anaphylaxis could be divided into two stages, including the sensitization stage and the induction stage.⁷⁶ The undigested lectin would enter the blood circulation through endocytosis and induce the dendritic cells of the body to present antigen epitopes, leading to the differentiation of T helper 2 (Th₂) cells and the secretion of IgE antibody, which would promote mast cell sensitization during the primary exposure. Then, during the second exposure of lectins, the lectin epitope would cross-link with the IgE antibody, which was bound to the FcεRI receptor, leading to the release of various pro-inflammatory factors and the occurrence of allergic symptoms.⁷⁷ Table 1 showed the *P.*

vulgaris epitopes recorded in the allergen database, Immune Epitope Database (IEDB, <https://www.IEDB.org>). In our previous research, four B cell epitopes and two T cell epitopes (B1, NVNDNGEPTLSS; B2, VGSEPKDKGG; B3, NNY-KYDSNAHT; B4, LYNVHWDPKPRH; T1, LQRDATVSS; and T2, FNIDVPNNS) of black turtle bean (*P. vulgaris* L.) lectin were identified using the immunoinformatic screening and epitope peptide synthesis, ELISA, lymphocyte proliferation experiments, and cytokine spectrum.²⁹

Kidney bean lectin also could trigger non-IgE-mediated food anaphylaxis as the production of IgG antibodies induced by PHA, and it was confirmed by feeding raw kidney beans to rats.¹⁹ Repeated exposure to kidney bean lectin might have a natural promotion effect, and specific serum IgG and immunoglobulin A (IgA) antibodies were produced in mice by oral and intravenous administration of bean lectin.⁷⁸ In addition, total IgA was increased in a dose-dependent manner by feeding different concentrations of pure PHA-E lectin to mice according to the previous studies.⁷⁹ IgA is the most important antibody on the surface of the intestinal mucosa, which might prevent food antigens from entering the human body.

Furthermore, red kidney bean lectin was found to trigger mixed food allergic reactions mediated by IgE and non-IgE in sensitization by culturing BALB/c mice, RBL-2H3 cells, and

bone marrow mast cells (BMMCs).^{15,80} The serum-specific IgE and IgG levels of the PHA-P-treated BALB/c mice group significantly increased with the raising of the levels of interleukin (IL)-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-17, tumor necrosis factor α (TNF- α), and interferon γ (IFN- γ), suggesting the occurrence of the mixed allergic reactions, including both changes of Th1 and Th2 cytokines. Our laboratory previously observed the increase of IL-4 and IFN- γ in Sprague Dawley (SD) rats by intraperitoneal injection of kidney bean lectin.⁸¹ According to the *in vitro* research, kidney bean lectin (PHA) could induce rat spleen cells to secrete IL-2, IL-12, and IFN- γ ,⁶⁰ and PHA-E and PHA-L could cause a significant increase in the levels of GATA-3 and T-bet in BMMCs.¹ On one side, GATA-3 is a GATA gene encoding a human T-cell-specific transcription factor and would promote the secretion of cytokines, such as IL-4, IL-5, and IL-13, by activated Th₂ cells, and on the other side, T-bet is a novel transcription factor belonging to the T-box family, would be selectively expressed in Th₁ cells, would induce the production of IFN- γ , and would play a decisive role in the differentiation of Th₁ cells. Therefore, both the increasing levels of GATA-3 and T-bet indicated that kidney bean lectin not only induced the production of Th₂ cytokines but also induced the differentiation of Th₁ cells. Thus, *P. vulgaris* lectin could affect the balance of Th₁/Th₂ cells through IgE-mediated type I allergic reaction accompanied by the release of IL-4, IFN- γ , histamine, and other cytokines.^{43,82} Furthermore, the secretion of preformed IL-4 was found to be in proportion to the amount of lectin in the *in vitro* experiments using 1 μ M *P. vulgaris* lectins to bind with the purified eosinophils and mast cells.⁸³ IL-4 is an important allergic mediator in the allergic response, which would promote the generation of Th₂ cells, and IL-4 could also induce isotype from immunoglobulin M (IgM) to IgE in B cells and facilitate the extravasation of granulocytes into tissues.^{84,85}

■ PURIFICATION OF KIDNEY BEAN LECTINS

At present, with the rapid demand for the in-depth exploration of lectin in both the immunity and biomedical properties, the need for purified lectins is increasing, and it is also a detection strategy for the lectin proteins. The main processes of various kinds of separation and purification strategies were shown in Figure 4.

In the early stage, the ammonium sulfate precipitation method and solvent precipitations were often used to extract lectin protein; however, the purity was low, and the activity would be greatly lost. The *P. vulgaris* L. lectin protein was usually obtained by precipitation of ammonium sulfate with 60–80% saturation.⁸⁶

Size-exclusion chromatography enables the further separation of proteins based on their relative sizes under physiological conditions. To purify the lectin from tepary bean (*P. acutifolius*) seeds, the crude extract was precipitated with ammonium sulfate, dialyzed, and then chromatographed using a Sephadex G-75 column to obtain the lectin-rich component with an agglutinating activity of 5565 AU/mg (expressed in lectin units per milligram of protein).⁸⁷

Ion exchange (IEX) would be another useful strategy based on the net charge of protein molecules after ammonium sulfate fractional precipitation. Two lectins from Great North bean (*P. vulgaris* L.) were separated using the ammonium sulfate fractional precipitation, carboxymethyl (CM) and diethylaminoethyl (DEAE) cellulose ion-exchange chromatography, and

Sephacry S-200HR gel filtration chromatography, and 2.8 mg of lectin was obtained from 500 g of beans.^{88,89}

The hydrophobicity of the protein could also be used in the hydrophobic interaction chromatography for the protein separation through the hydrophobic interaction between the immobilized hydrophobic ligand and the nonpolar regions on the surface of the protein.^{90,91}

However, the main problem might be difficult to purify the lectin on a large scale. At present, it is still the focus of scientific workers to develop a new high-efficiency, effective, and economical large-scale biological separation technology that can provide a high purity and high recovery rate at the same time. One of the major breakthroughs in lectin purification was the development of the affinity chromatography (AC) method using cross-linked dextran gel to capture lectins based on the specific carbohydrate-binding property.^{26,92} The purification process containing affinity steps could obtain higher recovery and purification factors. The researchers found that the porcine thyroglobulin (Tg)–Sephacry column had a high affinity with the kidney bean lectin, and approximately 700 mg of phytohemagglutinin protein could be obtained by purification of a saline extract of 100 g of beans with 100 mL of porcine Tg–Sephacry.⁹³ Previous studies confirmed that PHA purified by a one-step Tg–Sephacry 4B column processed higher purity (0.75 ± 0.13 mg of PHA/mg of lyophilized powder) than the sample obtained by two-step purification, including Affi-Gel blue and then Tg–Sephacry 4B purification in sequence (0.62 ± 0.20 mg of PHA/mg of lyophilized powder).³⁰ Previously, a high-yield lectin from a dried *P. vulgaris* cultivar (1.1 g of hemagglutinin/100 g of seed) was also obtained using a chromatographic protocol that involved Blue-Sepharose, Q-Sepharose, and Superdex 75.⁹⁴

In general, several chromatographic steps, such as size-exclusion chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, affinity chromatography, and gel filtration chromatography, have been used to purify the lectin in crude extracts according to the arrangement usually.^{95,96} The precipitation and affinity chromatography might be the first step in purification nowadays, while gel filtration and ion exchange were the second steps.

According to the previous studies, glucose or mannose has been successfully grafted onto aminated iron oxide nanoparticles to adsorb the Con A protein with a high affinity of up to 280 mg/g.⁹⁷ The size of magnetic nanoparticles would be controllable and could be easily controlled by the external magnetic field gradient. Later, the gum-arabic-modified magnetic nanoparticles grafted by glucose exhibited a significant specific interaction with Con A using the reducing amine method.⁹⁸ The membrane, such as macroporous cellulose membranes, could also be chemically cross-linking with epoxy groups to immobilize maltose and would be used for affinity purification for lectin protein.⁹⁹ At present, liquid–liquid extraction techniques, including the reverse micelle extraction technique and aqueous two-phase extraction (ATPE), were also attempted for the separation and purification of lectins. The aqueous two-phase system (ATPS) has high biocompatibility and selectivity, easy and reliable amplification, and continuous operation abilities. A lectin extraction method from Zihua snap bean (*P. vulgaris*) was established on the basis of a polyethylene glycol (PEG) 600/ammonium sulfate aqueous two-phase system, indicated that lectin could preferentially be partitioned into the PEG-rich phase, and the ATPS, composed of 15% (NH₄)₂SO₄ (w/w),

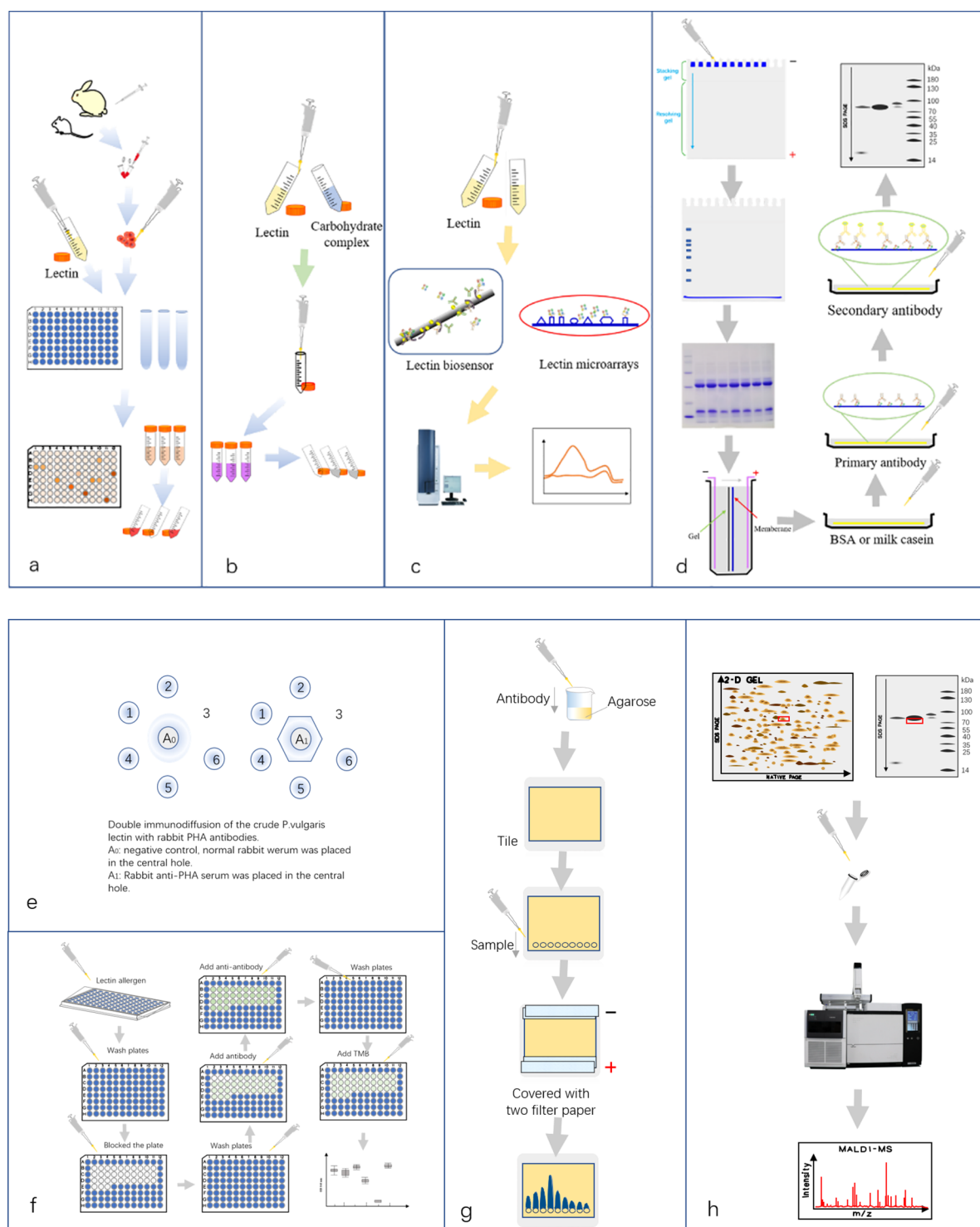


Figure 5. Detection methods for *P. vulgaris* lectin: (a) visual hemagglutination activity detection methods, (b) visual precipitation detection methods, (c) biosensor detection methods, (d) SDS–PAGE and WB, (e) double immunodiffusion methods, (f) ELISA methods, (g) rocket immunoelectrophoresis methods, and (h) mass spectrometry methods.

18% PEG 600 (w/w), 0.4/5 g of NaCl, and 1 mL of crude extract, showed good selectivity for lectin at pH 7.5.¹⁰⁰ Reversed micelle (RM) is an amphiphilic molecule at a nanometer size level in an organic solvent and can be stabilized by the surfactant at the phase interface. The lectin from black turtle bean was successfully extracted using the reversed micellar extraction (RME) with a protein recovery of 53.28% and a purification factor of 8.2 times.^{101,102} In the red kidney

bean lectin extraction system using the RM, the optimal conditions showing better specific hemagglutination activity were extraction at pH 4–6 for 4–6 min, back extraction at pH 9–11 for 8 min, and ion strength at 0.15 M NaCl.¹⁰³

■ DETECTION METHODS OF KIDNEY BEAN LECTINS

Because of the micro-level and various existing forms, it is a challenge to detect allergens in different food matrices. At

present, except for the purification strategy, rapid, cheap, sensitive, and accurate analysis methods, such as immunology, genetics, mass spectrometry, peptidomics, and proteomics, have been quickly developed and widely used for allergen detection. Especially for lectin detection, there are three major strategies: one is based on the protein specificity, such as *N*-acetyl glucose binding specificity and antigen–antibody reaction;^{104,105} one is a combination with the high-throughput instruments, such as nuclear magnetic resonance, mass spectrometry, microarray chip, etc.; and another is the use of bioinformatics software for prediction and analysis. The main detection methods applied in the lectin detection have been shown in Figure 5. However, these methods based on protein specificity need higher requirements for antibodies, and the accuracy might be affected by the existence of cross-reactivity. Besides, the equipment employed in the high-throughput measure might be expensive, and results obtained by the bioinformatics software need to be further verified. Therefore, several detection methods would be rationally arranged in the research.

Visual Hemagglutination Activity Detection. Significant hemagglutination activity of *P. vulgaris* lectin on human blood type and animal erythrocyte has been confirmed in the previous studies.^{35,106–108} Apparently, kidney bean lectin with the hemagglutination activity would lead to the agglutinate of erythrocytes. Actually, the protein could interact with carbohydrate moieties on the surface of erythrocytes based on the specific sugar-binding properties and result in the forming of multiple cross-bridge structures among cells, which would be a visual detection method for lectins.^{35,109} The hemagglutination activity test can be determined by a 96-well hemagglutination plate method, tube method, etc. In the procedure of using a 96-well plate, the red blood cell suspension was prepared first, then the kidney bean lectin with standard dilution times was added in equal volume, and the results of erythrocyte agglutination could be observed directly after standing for a certain time.^{32,86} The unit of hemagglutination activity (U) could be expressed as the reciprocal of the maximal dilution of the sample showing complete agglutination, and the specific activity of the lectin (U/mg) can be defined as the number of hemagglutination units per milligram of protein.^{30,101,110} In the procedure using a tube, the lectin samples were mixed with the red blood cells, then centrifugation would be performed at low speed (about 2000 rpm) after a period of incubation to remove the supernatant, and the adsorbed cell was dried to a constant weight to evaluate the degree of hemagglutination.¹¹¹

On the one side, to improve the sensitivity of the coagulation reaction, trypsin or papain has been applied to treat the red blood cells before determination.¹¹² Furthermore, the sensitivity of the method could also be improved using radiolabeled lectin or using spectrophotometry or the turbidimetric method to detect the results of hemagglutination.¹¹³ On the other side, the hemagglutination test is often affected by various factors, such as the number and molecular size of sugar-binding sites on the lectin, concentration of lectin, source of erythrocytes, blood type, erythrocyte concentration, and accessibility of the surface sites or mobility of the erythrocyte surface film. Meanwhile, the system factors, such as solution pH, ionic strength, and system temperature, also have effects on the coagulation activity of lectin.¹¹⁴ Therefore, because the accuracy, precision, and sensitivity of the results should be further considered, the hemagglutination method

would often be used for a preliminary determination or visual hemagglutination activity measure.

Visual Precipitation Detection of Lectins Based on Sugar Binding. Because the lectin can interact with the carbohydrate complex using the polyvalent glycosyl binding sites, then the precipitation would easily occur under a certain salt concentration at a certain temperature and the amount of labeled conjugate and binding constant could be calculated by detecting the intensity of the radioactive label in the carbohydrate complex by separation of the conjugate using centrifugation or filtration. The intensity of the inhibitor or competitive substrate on the hemagglutination or precipitin reactions could also be detected to reveal the structural differences in binding specificities of lectins.¹¹⁵

Lectin Interaction Signal with Polysaccharide and Glycoprotein for Accurate Detection. The biosensor is a device that can detect different compounds, which consists of three parts, including biological recognition, transduction, and signal processing. Because the biological interaction between lectins and polysaccharides or glycoproteins can be converted into signals, it is easy to quantify and analyze the lectin and lectin complex using various biosensor-based methods.

In the early research, the number of lectin–sugar conjugates and the binding constant could be calculated by detecting the intensity of the radioisotope labeled on the glycoconjugates and the lectin could also be quantified by detecting the radioactive intensity changing using the inhibitors or competitive substrates.¹¹⁶ Electrodes have been used as the sensing surface in the electrochemical biosensor and might be modified with polymers and nanomaterials (carbon nanotubes, gold, magnetic nanoparticles, etc.) to convert the specific recognition signal into a measurable electrical signal, leading to the successful detection of the nanotracing lectin–sugar interaction. In the year 2006, the sensitive amperometric stripping voltammetry was employed to determine the competitive binding degree of the nanocrystal-labeled sugar with lectin, and the voltammetric peak current decreased with the increase of glycan-binding affinity.^{117,118}

At present, more effective detection methods based on the lectin–glycan interaction were developed using fluorescence, isothermal titration calorimetry (ITC),¹¹⁹ surface plasmon resonance (SPR),^{120–122} quartz crystal microbalance (QCM),¹²³ and electrochemical impedance spectroscopy (EIS)¹²⁴ platforms. The fluorophores could be generally conjugated to nanomaterials or lectins through well-established coupling chemistries, like carboxy-amine amidation or click reactions.^{125,126} ITC is a label-free, non-destructive technique that does not require the ligands or substrates, and it can measure the heat absorbed or released during biomolecular interactions at the nanomolar level, which only needs a microgram sample.¹²⁷ SPR is an optical detection technique based on the interactions of light with the surface plasmon.¹²⁸ When the lectin or carbohydrate is binding to the surface of the sensor chip, the refractive index of the medium and the reflection angle of the incident light will change.¹²⁹ QCM relies on a highly sensitive piezoelectric crystal with resonant frequency changes by the mass alteration. Monolayer surface coverage as low as 1 ng cm⁻² Hz⁻¹ can be detected for lectin analysis using glycan-based biosensors.¹³⁰ EIS is based on the change in the amplitude of the alternating current (AC) and the corresponding interfacial characteristic (such as resistance) on the interaction of carbohydrate–lectin.¹³¹

Besides, several fluorescent probes coated with specific polysaccharides were reported, but the emission was located in the short wavelength region.^{132–134} Two novel glycosyl probes (PT-Man and PT-Gal) based on aggregation-induced emission (AIE) have been proposed to detect lectin–sugar interactions in the near-infrared (NIR) region with a good linear relationship between the fluorescence intensity and lectin concentration at a low micromolar range (0.5–2.5 M).¹³⁵ Recently, graphene oxide (GO) was used to assemble the platform for sugar-based probes, which reduced the limits of detection (LODs) to be 8.9×10^{-9} , 8.1×10^{-9} , and 7.9×10^{-9} M for Con A, LcA, and PNA, respectively.¹³⁵ Carboxymethyl chitosan (CM-CHIT) quantum dots have also been recently employed to develop the novel label-free fluorescence “turn off–on” biosensor assembled with CuInS₂ quantum dots, CM-CHIT, and Au nanoparticles (NPs). Then, the fluorescence quenching of CM-CHIT induced by the Au NPs would be avoided by the lectin interaction with specific polyvalent glycoproteins, and the lectin detection limit could be down to 0.08 nmol L⁻¹.¹³⁶

Lectin microarrays have recently been used as a promising tool for glycan analysis by immobilizing lectins with known specificities on a solid surface.¹³⁷ Lectins can quickly and specifically bind to polysaccharides, and then confocal fluorescence and field-induced fluorescence could be recorded with very high accuracy, even for very weak interactions.¹³⁸ Therefore, the development and research of polysaccharide microarrays may be a potential research direction for the quantitative or qualitative detection of lectins.

Immunoassay Methods for Lectin Detection. Nowadays, specific antibodies have been employed to quantitatively detect kidney bean lectin based on the antigen activity.¹³⁹ Then, more and more immunoassay methods have been developed with higher sensitivity and strong selectivity, such as western blot (WB), enzyme immunoassay (EIA), ELISA, rocket immunoelectrophoresis (RIEP), radioimmunoassay (RIA), etc.^{140,141}

SDS–PAGE is first applied to separate and identify proteins with different molecular weights in separation gel according to their mobility in the electric field; the protein band will be transferred from SDS–PAGE to polyvinylidene fluoride (PVDF) or nitrocellulose membrane in the WB; lectin-specific antibody will be applied for incubation; and then the secondary antibody with fluorescence or the color group will be added for the immunological recognition of the antibody-bound protein.

In the procedure of detecting lectin by enzyme immunoassay, most of the lectin-specific antibodies are obtained by establishing an animal sensitization model, and the obtained antiserum or antibody is diluted to a certain concentration to be added to the microtitration plate for coating. Then, the samples are added for incubation, and the rabbit anti-PHA or mouse anti-PHA antiserum is added for the absorbance value determination.⁴² In the previous study, the kidney bean lectin was emulsified in Freund's adjuvant, the sensitization model in rabbits was established by injection on days 14, 28, and 42, the antiserum specific for lectin was collected followed by centrifugation, 40% ammonium sulfate precipitation, and dialysis, and then the lectin content in different tissues of kidney bean plant could be detected as low as 0.3 ng/mL using the antibody.¹⁴² Presently, the lectin antibody was usually purified using protein G Sepharose 4B fast flow column after

the immune serum collection, and the antibody titer would be further evaluated by the ELISA method.¹⁸

The main steps of the ELISA method for the lectin determination are as follows: the samples stored in buffer solution are coated on the ELISA plate for an overnight incubation; then the skim milk is used for sealing; mouse or rabbit anti-lectin serum is added; subsequently, goat anti-mouse or anti-rabbit IgE antibody labeled with horseradish peroxidase (HPR) is added for incubation; and then the absorbance is detected for lectin analysis.^{18,114} According to the published papers, the detection range of the ELISA method to lectins was 30–1000 ng/mL for WGA and 80–1000 ng/mL for both PNA and Con A.¹⁴³ The detection limit of quantification of PHA was 15 ng.¹⁴⁴ Previously, the result of the ELISA inhibitor suggested that the *P. vulgaris* bean lectins could cross-react with the allergens from peanut, black gram, and pigeon pea and required 185, 228, and 1300 ng of lectin proteins for 50% inhibition, respectively.^{12,145} In addition, the serum of peanut allergy patients could interact with peanut lectin and Ara h 1 strongly and could also interact with PHA-E.^{13,146} Competitive inhibition enzyme-linked immunosorbent assay (CI-ELISA) is also commonly used for lectin detection now. According to the published methods, the microtiter plates were sensitized with *P. vulgaris* lectin overnight at 4 °C and filled the well with rabbit anti-PHA serum and inhibitor (rat feces extract or serum), and then peroxidase-conjugated goat anti-rabbit IgG would be loaded. After removal of the soluble secondary antibody, sulfuric acid was added to stop the reaction after incubation in the dark.¹⁴⁷

The double immunodiffusion (Ouchterlony method) can also characterize the reaction between lectin and rabbit anti-lectin antibody, because a precipitation line would be formed on the gel plate when the antigen and antibody meet and reach the optimal ratio.^{19,148} The RIEP method is an antigen detection method that combines electrophoresis and (one-way) immunodiffusion. During electrophoresis, the action of the electric field moves the lectin antigen to the positive electrode, and the antigen and antibody molecules form a rocket-shaped antigen–antibody immunoprecipitation peak. Generally, the greater the lectin concentration, the higher the peak height; therefore, the lectin concentration in the sample can be quantitatively determined according to the standard curve. Immunoelectrophoresis (IEP) can be used to prove the reaction between the antiserum and the commercially available purified PHA and the sample. IEP will be performed in veronal buffer with 1.5% agarose on the microscope glass slide; a prerun of 30 min will be given; the wells will be loaded with purified PHA (10 μ L, 1 mg/mL) and rat biological fluids [such as fecal extract (10 μ L, 100 mg/mL) and sera (10 μ L)] to run the gel at the voltage of 400 V; and then the troughs are separated by cutting and filling with rabbit anti-PHA immunoserum (10 μ L, neat).¹⁴⁹ Recently, a study used the radial double immunodiffusion (ODI) method to screen whether there were lectins in rat serum and feces.¹⁵⁰ First, 1.5% agarose solution in veronal buffer was poured onto the microscope. After the gel was solidified, it was cut into a cylindrical hole with a diameter of 5 mm, the sample [such as rabbit anti-PHA serum (10 μ L), purified PHA (10 μ L, 1 mg/mL), sera (10 μ L), and rat feces extract (10 μ L, 100 mg/mL)] was filled in the wells, the slides were cultured at 24 °C for 24 h and then washed and stained, and the experimental results were observed. In addition, counter immunoelectrophoresis (CIE) has also been used to detect the presence of lectins in

feces and serum specimens of rats fed with kidney beans by the addition of the neat rabbit immunoserum and antigen solutions (1 mg/mL purified PHA, 100 mg/mL rat fecal extract, rat serum, etc.) in the 1% agarose GP 60 gel well.¹⁵¹

However, the disadvantages of these above immunoassay methods are also obvious. First of all, these methods can only detect the immunological properties, and the binding ability with intestinal epithelial cells, red blood cell agglutination ability, and other biological activities cannot be reflected. In addition, the composition and titer of the antibodies might be different depending upon the immunized animal, the preparation process, and the batches, which will affect the detection range and degree of standardization, and the test results are also susceptible to cross-reactions in complex systems.¹³⁹

In recent years, monoclonal antibodies with better specificity and reproducibility have been used instead of polyclonal antibodies, and the detection methods that combine immunoassays with some emerging detection platforms have begun to emerge. Recently, an indirect ELISA detection method based on mouse monoclonal antibodies was established to detect soybean lectin with a detection limit of 30 ng/mL (0.03 ppm) and protein concentration at 50% maximum signal of 786.3 ng/mL.¹³⁹ Our laboratory immobilized the kidney bean lectin antibody on the surface of the electrode and designed a fast and high sensitivity electrochemical immunosensor for the trace amount detection of lectins with immunogenic activity at a detection range of 0.05–100 µg/mL.¹⁸

Allergomics Strategies. In recent years, with the rapidly increased number of recognized allergens and the development of bioinformatics, allergomics is exploring by analyzing the repertoire of allergens, including genetics, proteomics, metabolomics, etc., and would provide more sensitive information for the allergen and epitope prediction with the relatively higher throughput and faster omics characteristics.¹⁵² Except for the sequence analysis, the identification of B cell and T cell epitopes, and the evaluation of allergies and cross-reactivity, bioinformatics tools could also calculate or simulate lectin–protein, lectin–polysaccharide, and lectin–biomolecule interactions, which would provide a theoretical supplement for the explanation of the sensitization alterations.^{153,154}

Mass spectrometry would be the most used method in allergomics, and the concept of targeted quantitative proteomics and absolute quantitative strategy has been actively developed and applied in the trace lectin detections in raw or processed foods.^{155–157} Prior to sample injection into mass spectrometry, one-dimensional gel electrophoresis and two-dimensional gel electrophoresis are more commonly used to reduce the complexity of proteins in biological samples, and now, the capillary electrophoresis and chromatographic methods have become more popular for sample preparation.¹⁵⁸ Reversed-phase high-performance liquid chromatography (RP-HPLC) has been widely used for the separation of proteins and peptides before proteomics applications. All mass spectrometers consist of three main components (ion source, mass analyzer, and ion detector), and the main types of mass spectrometry currently in use include quadrupole time of flight (TOF), linear ion trap, orbitrap, Fourier transform ion cyclotron resonance/mass spectrometry, and ion mobility instruments. For example, mass accuracy below 5 ppm and achievement of mass resolutions of up to 1 000 000 can be provided using the Fourier transform ion cyclotron resonance/mass spectrometry instruments, which is more accurate and

precise, and the whole flow is very fast.¹⁵⁹ Our research team first identified kidney bean lectin from black turtle beans using electrophoretic separation and LC–MS/MS and confirmed the relative monomer molecular weight of 31 kDa.²⁸ In recent years, the development of computer-aided methods has been very popular, and then the mass spectrometry methods for lectin detection will be more intelligent and integrated in the future.¹⁶⁰

According to the published papers, a protein could be regarded as a hypothetical allergen if the protein had a sequence homology of more than 35% with a known allergen in a sliding window of at least 80 amino acids,^{161,162} while some researchers believe that cross-reactivity usually requires more than 50–70% identity in the binding region of the antibody.¹⁶³ Then, the most commonly used allergen databases, such as COMPARE (<https://comparedatabase.org/>), AllergenOnline (<http://www.allergenonline.org/>), Allergome (<http://www.allergome.org/>), National Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov/>), UniProt (<https://www.uniprot.org/>), SDAP (http://Fermi.utmb.edu/SDAP/sdap_src.html), etc., were applied to the comprehensive and detailed electronic search of potential allergen IgE cross-reactions.

With the development of biochemistry and molecular biology, it became possible to use recombinant DNA technology to produce lectin proteins. Most of the recombinant *P. vulgaris* lectins were produced by *Pichia pastoris* because of the high similarity of *N*-oligosaccharides.¹⁶⁴ In the 1970s, both PHA-E and PHA-L have been expressed in *P. pastoris* using *N*-terminal phytohemagglutinin of kidney bean as a signal peptide, and the purified lectin has the same hemagglutination activity as the native protein.¹⁶⁵ *P. vulgaris* lectin PHA-E was expressed in the methylotrophic yeast *P. pastoris* in another study, and purified recombinant PHA-E was confirmed as consisting of four forms with molecular masses between 28.5 and 31.5 kDa.¹⁶⁶

Nowadays, the phage display can be used to determine all of the IgE-binding proteins in the allergenic source, when the phage DNA library has been set up using the cDNA library of a particular food allergen source and allergic serum has been used as the bait molecule. In addition, when the cDNA fragment collection of a particular allergen has been used to set up the phage DNA library, the method can be used to identify the allergen epitopes.¹⁵²

■ DESENSITIZATION PROCESSING TECHNOLOGY OF KIDNEY BEAN LECTINS

In clinical practice, the best way to prevent allergic reactions is to avoid contact with foods containing allergens. However, as a result of the widespread use of kidney beans in various processed foods or because of the mixed use of food processing machines, it is necessary to eliminate allergic ingredients in food or control them within a safe range. According to the previous studies, the allergenicity of kidney bean lectins could be reduced or disappear through physical thermal processing, physical non-thermal processing, chemical modifications, biological treatments, etc.

Physical Thermal Processing. Heat Treatment. Heating is a common food processing method to improve the biological safety of food and extend the shelf life. Heating can induce changes in food allergy protein structures, including the protein unfolding, the loss of secondary and tertiary structures, the formation of intramolecular and/or intermolecular

covalent and non-covalent interactions, etc., which, in turn, would affect the sensitization potential of allergen epitopes.¹⁶⁷ In the investigation of canned red kidney beans, the lectin content in the canned samples was found to be lower than that in the raw kidney beans.¹⁶⁸ Under dry heat conditions, kidney bean agglutination revealed higher heat resistance, but the protein would be more easily inactivated under humid and hot environments.¹⁶⁹ The specific IgE adsorption capacity of kidney bean lectin would be reduced by $74 \pm 6.5\%$ in kidney bean extract after the heat treatment ($121\text{ }^{\circ}\text{C}$ for 15 min).¹⁷⁰ In the family cooking, boiling the black-eyed peas, soybeans, green beans, and red kidney beans at $100\text{ }^{\circ}\text{C}$ for 30 min could completely inactivate the hemagglutinin activity of lectins.¹⁰⁸ Furthermore, to maintain the nutritional value of kidney beans and inactivate the lectin to the greatest extent, steam treatment at $119\text{ }^{\circ}\text{C}$ for 5 or 10 min was recommended, and the hemagglutination activity was reduced by 85 and 98.5%, respectively, while the binding capacity of rabbit serum antibodies was reduced by 98.6 and 99.2%, respectively.¹⁷¹ Direct heating of black kidney beans at $97\text{ }^{\circ}\text{C}$ for 20 min could basically destroy the lectin activity, and the same effect could be achieved by heating at $97\text{ }^{\circ}\text{C}$ for 10 min after soaking for 16 h.¹⁷² According to the published papers, three kinds of pre-soaked kidney beans (red, white, and black kidney beans) combined with heating above $75\text{ }^{\circ}\text{C}$ could significantly reduce the allergenicity.¹⁷³ Studies have pointed out that the allergenicity of lectins from soaked kidney beans could be completely eliminated after boiling for 15 min.¹⁷⁴ In addition, heating would also improve the digestive sensitivity of kidney bean lectin in the gastrointestinal tract, thereby further reducing its allergenicity.¹⁷⁵ However, the lectins might be protected by the whole beans or polysaccharides during the thermal processing; thus, future research should notice the effect of the food matrix on allergen sensitization.

Extrusion Cooking. Because of the huge demand for ready-to-eat foods in the modern lifestyle, extrusion cooking is widely applied using the single-screw extruder and/or twin-screw extruders with high temperature and pressure (up to $200\text{ }^{\circ}\text{C}$ and 20 MPa).¹⁷⁶ Previous studies have demonstrated that low-temperature ($85\text{ }^{\circ}\text{C}$) extrusion could significantly reduce lectin in *P. vulgaris* L. beans by 85–95% over raw beans.¹⁷⁷ In detail, heat, high friction, and shear forces in the extrusion cooking would result in the denaturation of the lectin protein and would improve the accessibility by the exposure of proteolysis-sensitive sites.¹⁷⁸

Physical Non-thermal Processing. The traditional heat treatment process is usually used to reduce the allergenicity of food, but at the same time, the food quality attributes will be changed drastically.¹⁷⁹ Thus, the non-thermal processing technology, such as high-pressure treatment, irradiation, ultrasonication, cold plasma, pulsed light, and pulsed electric field, has been developed to retain the food characteristics and reduce the sensitization potential.

Ultra High-Pressure Treatment. As an emerging non-thermal processing technology in the food industry, high-pressure technology mainly affects non-covalent interactions (hydrogen, ion, and hydrophobic bonds) in protein molecules.^{167,180} Previous studies have shown that the primary and secondary structures of the allergen protein would be reversibly twisted after low-pressure treatment ($<200\text{ MPa}$). When the pressure reached 200 MPa, the tertiary structure and molecular interactions would be changed, and when the pressure further increased to 500 MPa, the molecular structure

of the protein would be unfolded, resulting in the exposure of hydrophobic amino acid residues and intramolecular sulfhydryl groups ($-\text{SH}$).¹⁷⁹ The significant reduction of the IgE binding capacity of the seeds from soybean, dried peanut, chickpea, and lentils was confirmed after the instantaneous high-pressure treatment.^{181–183} The purified red kidney bean lectin began to unfold and then gradually formed a molten spherical state under 50 MPa pressure of high hydrostatic pressure treatment, while the insignificant changes in the coagulation activity under $\leq 150\text{ MPa}$ revealed the integrity of the natural structure, but the kidney bean lectin protein might undergo molecular rearrangement and molecular polymerization when the pressure was $\geq 450\text{ MPa}$, with a complete loss of sensitization potential.^{184,185}

Irradiation Treatment. With a little reduction in the nutrition and sensory of food, irradiation can result in the conformational changes of the proteins, such as fragmentation, aggregation, cross-linking, and amino acid modification, thereby influencing the allergenicity.^{170,186} After the treatment of 25 kGy γ -irradiation, the specific IgE binding rate of kidney bean lectin was reduced by about 34%.¹⁷⁰ Although the kidney bean lectin structure was still intact even at the irradiation of $\leq 30\text{ kGy}$ under dry or 50% moisture conditions, the inactivation of lectin was observed in an aqueous solution at a dose of 10 kGy with the complete destruction in the secondary and tertiary structures, suggesting the significance of moisture conditions.^{187,188} Similarly, structural intactness of PHA was observed below 30 kGy in the dry state (99.5% dry) or in a 50% moisture condition, while a 50% reduction in hemagglutinin and mitogenic activity was observed in the dose range of 30–50 kGy, and soaked kidney beans exhibited 50% loss in biological activity at the radiation doses of 30 kGy; however, purified PHA would be completely inactivated in aqueous solution at a dose of 10 kGy.¹⁸⁹

Ultrasonication. High-power ultrasound can accelerate certain chemical reactions by physically disintegrating or establishing pressure and temperature gradients in the propagating material, thereby causing material modification. Food products could absorb the ultrasonic waves and cause the acoustic cavitation phenomenon in the materials, which could induce structural changes of the native proteins and influence the allergenic features finally.¹⁷⁹ Besides, the high-power ultrasound might induce the physical disintegration or establish pressure and temperature gradients in the propagating media. According to the published papers, ultrasonication was often used in the bean processing combined with other treatments. The IgE-binding capacity of the soybean seeds was reduced by 51.39% after the ultrasonication (300 W) treatment, followed by germination.¹⁹⁰ Continuous ultrasound and enzyme digestion with trypsin and chymotrypsin have been employed in the peanut treatment, and the immunoreactivity of peanut allergens Ara h 1 and Ara h 2 would be reduced by 50%.¹⁹¹ In addition, after the germination for 3 days, the peanut protein allergens would be completely degraded when subjected to high-intensity ultrasound wave (100 kHz) treatment.¹⁹²

Cold Plasma Treatment. Ionized gas, also named the fourth state of matter, is composed of a variety of reactive substances, including ultraviolet (UV) light photons, ions, electrons, free radicals, molecules, excited atoms, etc. These substances would be generated after the gas excitation and ionization and could induce certain interactions with proteins and change the primary conformational structure to incapacitate the binding

sites of antibodies.^{193,194} According to the previous study, soybeans were subjected to the cold plasma treatment, the aggregate state of allergens was observed, and the immune reactivity was significantly reduced, as detected by ELISA.¹⁹⁵

Pulsed Light (PL) Treatment. PL is the white light pulse consisting of a series of very short and high-power wide spectra and contains about 54% UV, 20% infrared (IR), and 26% visible radiation, and then the PL is also the high-energy pulse processing high penetration power with rapid dissipation.¹⁹⁶ Generally, aromatic amino acids, such as phenylalanine and tyrosine, in food proteins easily absorb UV light, which will be accompanied by photooxidation and dissociation and then might be beneficial to the formation of covalent cross-linking among proteins to form larger aggregates.¹⁹⁷ Besides, with the changing of protein integrity, disulfide bond (S–S) oxidation might be induced,¹⁹⁸ and the destruction of the S–S bond might directly lead to the rearrangement of the protein structure and influence the binding ability with IgE.¹⁹¹ It has been proven that PL treatment could significantly reduce the sensitization potential of Ara h 1, Ara h 2, and Ara h 3.¹⁹⁹ Previous studies also exhibited the decrease of glycinin and β -conglycinin in the soy extract after the pulsed ultraviolet (PUV) light for 2, 4, and 6 min, separately.²⁰⁰

Pulsed Electric Field (PEF) Treatment. The PEF will use short-pulse currents to infiltrate and decompose microbial cells while only making small changes to the quality attributes of the food product. PEF treatment will also cause the changes in the protein allergen structures and affect the IgE binding ability, which is similar to other non-thermal treatments.²⁰¹ The effect of PEF treatment on peanut proteins have been explored recently, and the protein structure was altered significantly;²⁰² however, some previous studies also demonstrated that no significant changes in the secondary structures of purified peanut allergy Ara h 2 and Ara h 6 were observed after the PEF treatment.²⁰³ More studies should be carried out in detail.

Chemical Modifications. In the early days, the specific amino acids of lectins were modified by the chemical method to seek the active sites, and the importance of tryptophan in Jalo bean (*P. vulgaris*) lectins was confirmed by tryptophan oxidation with *N*-bromosuccinimide with the elimination of the agglutinating, mitogenic, and toxic activities.²⁰⁴ However, most chemical modifications, such as acylation, phosphorylation, deamination, and alkylation, might induce the formation of toxic reagents and would be difficult to be controlled in food processing. According to the recent literature, the glycosylation, PEG modification, and pH shifting would be the most promising methods to modify the sensitization of lectins.

Glycosylation Modification. The peptide chain of the protein would be broken during the thermal treatment, and the lysine residues on the surface of the lectin would participate in the Maillard reaction with reducing sugars, which might result in the modification of linear epitopes containing these amino acid sites and lead to reduction of sensitization.²⁰⁵ Glycosylation modification was found to be useful to reduce the sensitivity of soybean lectin allergens,¹⁷⁹ but it might be interesting that the IgE binding properties of the peanut agglutinin could not be changed by the glycosylation with maltose and glucose, while it would be eliminated to a certain extent by the glycosylation with fructose and ribose,²⁰⁶ suggesting the significance of sugars.

PEG Modification. The PEG derivatives with a molecular weight above 1000 Da have been approved for pharmaceutical

formulation designs in human use by the U.S. Food and Drug Administration (FDA), and then PEG succinimidyl carbonate (PEG–SC), PEG succinimidyl succinate (PEG–SS), and PEG succinimidyl propionate (PEG–SPA) conjugation have been used to modify the black kidney bean lectin to reduce the hemagglutination activity and allergenicity by the steric hindrance effects as well as improve the gelling properties by the PEG molecules.^{207,208}

pH Shifting. According to the previous studies, significant decreases in the α -helix of *P. vulgaris* lectin were observed at pH < 1.5 incubation accompanied by the dissociation of the polymer, disulfide bond fracture, and increase of hydrophobicity because of the protonation induction; meanwhile, more protease cleavage sites of black kidney bean lectin would be exposed with a significant improvement of digestibility.⁴³ It might be interesting that the unfolding lectin at acidic conditions would be refolded when the pH was adjusted to neutral; structural misarrangement would occur during the pH-shifting process; and the renaturation rate of completely denatured PHA-L was calculated as 85% at pH 7.2 from the gel filtration data after 2 days of renaturation.⁵² According to our studies, the hemagglutination activity of *P. vulgaris* lectin was reduced by 50% after the pH-shifting treatment from pH 1.5 or 1.0 to 7.2, suggesting that the sugar-binding sites might be destroyed as a result of conformational changes, and then the IgE binding ability would also be reduced.²⁰⁹

Molecular Binding. Some molecules, such as polyphenols, could bind with the protein allergen and also inhibit IgE recognition by obstructing IgE epitopes. Previously, procyanidin C1 and chlorogenic acid from *Vaccinium* fruits were used to interact with peanut allergen Ara h 2 and changed the secondary structure of the protein with a significant decrease of the IgE binding capacity.²¹⁰ In addition, another previous study has also demonstrated that the complexation of blueberry and cranberry polyphenols to peanuts could potentially reduce the expression of plasma IgE and spleen CD 63 protein in a peanut-sensitized C3H/HeJ rat.⁷¹

Biological Treatments. Germination. As reported, the lectin content in the black turtle bean samples was also found to decrease by 46% after the germination treatment for 24 h, and it would be reduced by 76% after 48 h.²¹¹ Similarly, after 7 days of germination, the content of white kidney bean (*P. vulgaris* L.) lectin decreased by 85%.²¹² During the germination treatment, the proteolysis and some modifications of the carbohydrate moiety might significantly reduce the agglutinating potential of PHA-P lectin.²¹³

Fermentation. The reduction of *P. vulgaris* L. lectin levels has been found in the natural fermented (NF) and lactic acid fermented (LF) fermentation processes.²¹⁴ During the fermentation process, the linear epitopes and conformational epitopes of the food protein would be destroyed by the conformational change induced by hydrolysis and acidification.²¹⁵ In the *in vivo* study, a significant decrease in allergy symptoms was observed in the mice feeding on the fermented soybeans.²¹⁶

Enzymatic Hydrolysis. In addition, the method of enzymatic hydrolysis mainly destroys linear allergen epitopes by partially or extensively using proteolytic enzymes or cellular enzymes. Previously, the IgE binding ability of the soluble protein extract in kidney bean was reduced by $62.2 \pm 7.7\%$ after treatment with alkaline protease and flavor enzymes, and the release of histamine was decreased significantly in

sensitized basophils, while only 10% of patients showed allergic symptoms in the SPT.²¹⁷

Plant Molecular Breeding. Biological breeding and RNA interference (RNAi) were also used to eliminate or modify allergenic proteins in foods. Differences in the concentration of lectins among cultivars grown in Mexico and the U.S.A. have been reported.²¹⁸ In an investigation for three genotypes of kidney beans, one genotype does not contain phytohemagglutinin, one has a lower content of lectin, and then the kidney bean powder with a low content and without PHA was found to have no erythrocyte agglutination activity, regardless of whether it was fermented or not.²¹⁹ RNAi has been successfully used to inhibit the formation of allergic protein Ara h 2 in peanut.²²⁰

In short, the consumption safety of kidney bean allergies is of great significance. At present, animal models are mostly used to explore related sensitization mechanisms of kidney bean lectin, but research reports related to human sensitization are relatively lacking. Therefore, the sensitization threshold of kidney bean lectin is still unclear. To accurately reflect the immune activity of the lectin, various purification and detection methods have been widely used, and the comprehensive application of several technologies, such as the combination of immunoassay and electrochemical or bioinformation methods, makes the detection method more sensitive, more repeatable, and more efficient. Furthermore, the combination of multiple food processing methods to reduce the allergenicity of lectin protein may be one of the important research directions in this field in the future. Moreover, because the individual differences of the intestinal flora might also play an important role in allergies, then, in the future, the detection of food allergens and the formulation of desensitization strategies may be developed in the direction of personal customization.

AUTHOR INFORMATION

Corresponding Author

Shudong He – Engineering Research Center of Bio-process of Ministry of Education, School of Food and Biological Engineering, Hefei University of Technology, Hefei, Anhui 230009, People's Republic of China; orcid.org/0000-0002-1155-4928; Phone: 86-551-62901505; Email: shudong.he@hfut.edu.cn; Fax: 86-551-62901516

Authors

Yongfei Wang – Engineering Research Center of Bio-process of Ministry of Education, School of Food and Biological Engineering, Hefei University of Technology, Hefei, Anhui 230009, People's Republic of China

Fanlin Zhou – Engineering Research Center of Bio-process of Ministry of Education, School of Food and Biological Engineering, Hefei University of Technology, Hefei, Anhui 230009, People's Republic of China

Hanju Sun – Engineering Research Center of Bio-process of Ministry of Education, School of Food and Biological Engineering, Hefei University of Technology, Hefei, Anhui 230009, People's Republic of China

Xiaodong Cao – Engineering Research Center of Bio-process of Ministry of Education, School of Food and Biological Engineering, Hefei University of Technology, Hefei, Anhui 230009, People's Republic of China

Yongkang Ye – Engineering Research Center of Bio-process of Ministry of Education, School of Food and Biological

Engineering, Hefei University of Technology, Hefei, Anhui 230009, People's Republic of China

Jing Li – College of Biological and Environmental Engineering, Hefei University, Hefei, Anhui 230601, People's Republic of China

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acs.jafc.1c02801>

Funding

The authors are grateful for the financial support from the National Natural Science Foundation of China (31701524), the Major Special Science and Technology Planning Project of Anhui Province, China (202003b06020030), and the Key Research and Development Program of Anhui Province, China (202104a06020016).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are thankful for the hard work and diligence of the predecessors who worked in the food protein allergen area and appreciate the helpful comments from the anonymous reviewers.

ABBREVIATIONS USED

AC, affinity chromatography; AIE, aggregation-induced emission; ATPE, aqueous two-phase extraction; ATPS, aqueous two-phase system; BMMC, bone marrow mast cell; CAC, Codex Alimentarius Commission; CIE, counter immunoelectrophoresis; CM-CHIT, carboxymethyl chitosan; CRD, carbohydrate recognition domain; Con A, concanavalin A; EFSA, European Food Safety Authority; EIA, enzyme immunoassay; EIS, electrochemical impedance spectroscopy; ELISA, enzyme-linked immunosorbent assay; FAO, Food and Agriculture Organization of the United Nations; FDA, Food and Drug Administration; GO, graphene oxide; IEP, immunoelectrophoresis; IEX, ion exchange; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; ITC, isothermal titration calorimetry; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LF, lactic acid fermented; LOD, limit of detection; MS, mass spectrometry; MW, molecular weight; NF, natural fermented; NIR, near-infrared; NP, nanoparticle; ODI, radial double immunodiffusion; PEF, pulsed electric field; PHA, phytohemagglutinin; Pha v 3, lipid transfer protein; PEG, polyethylene glycol; PEG-SC, polyethylene glycol succinimidyl carbonate; PEG-SS, polyethylene glycol succinimidyl succinate; PEG-SA, polyethylene glycol succinimidyl propionate; PHA-P, phytohemagglutinin P; PHA-E, phytohemagglutinin E; PHA-L, phytohemagglutinin L; PL, pulsed light; PNA, Ara h agglutinin; QCM, quartz crystal microbalance; RIA, radioimmunoassay; RIEP, rocket immunoelectrophoresis; RM, reversed micelle; RME, reversed micellar extraction; RNAi, RNA interference; RP-HPLC, reversed-phase high-performance liquid chromatography; SGF, simulated gastric fluid; sIgE, specific immunoglobulin E; SPT, skin prick tested; SPR, surface plasmon resonance; Tg, porcine thyroglobulin; Th₂, T helper 2 cell; TOF, time of flight; UV, ultraviolet; WB, western blot; WHO, World Health Organization

REFERENCES

- (1) Kumar, S.; Verma, A. K.; Sharma, A.; Kumar, D.; Tripathi, A.; Chaudhari, B. P.; Das, M.; Jain, S. K.; Dwivedi, P. D. Phytohemagglutinins augment red kidney bean (*Phaseolus vulgaris* L.) induced allergic manifestations. *J. Proteomics* **2013**, *93*, 50–64.
- (2) Araujo, J.; Urbano, B.; González-Andrés, F. Comparative environmental life cycle and agronomic performance assessments of nitrogen fixing rhizobia and mineral nitrogen fertiliser applications for pulses in the Caribbean region. *J. Cleaner Prod.* **2020**, *267*, 122065.
- (3) Rodhouse, J. C.; Haugh, C. A.; Roberts, D.; Gilbert, R. J. Red kidney bean poisoning in the UK: An analysis of 50 suspected incidents between 1976 and 1989. *Epidemiol. Infect.* **1990**, *105* (3), 485–491.
- (4) Bender, A. E.; Reaidi, G. B. Toxicity of kidney beans (*Phaseolus vulgaris*) with particular reference to lectins. *J. Plant Foods* **1982**, *4* (1), 15–22.
- (5) Sun, Y.; Liu, J.; Huang, Y.; Li, M.; Lu, J.; Jin, N.; He, Y.; Fan, B. Phytohemagglutinin content in fresh kidney bean in China. *Int. J. Food Prop.* **2019**, *22* (1), 405–413.
- (6) Ogawa, H.; Date, K. The “white kidney bean incident” in Japan. *Methods Mol. Biol. (N. Y., NY, U. S.)* **2014**, *1200*, 39–45.
- (7) Amal, B.; Aarab, L. Effect of heat and enzymatic treatments on human IgE and rabbit IgG sensitivity to white bean allergens. *Iran. J. Allergy, Asthma Immunol.* **1970**, *12* (4), 304–311.
- (8) De La Fuente, M.; Borrajo, A.; Bermúdez, J.; Lores, M.; Alonso, J.; López, M.; Santalla, M.; De Ron, A. M.; Zapata, C.; Alvarez, G. 2-DE-based proteomic analysis of common bean (*Phaseolus vulgaris* L.) seeds. *J. Proteomics* **2011**, *74* (2), 262–267.
- (9) Kumar, S.; Verma, A. K.; Das, M.; Jain, S. K.; Dwivedi, P. D. Clinical complications of kidney bean (*Phaseolus vulgaris* L.) consumption. *Nutrition* **2013**, *29* (6), 821–827.
- (10) Kasera, R.; Singh, B. P.; Lavasa, S.; Prasad, K. N.; Sahoo, R. C.; Singh, A. B. Kidney Bean: A major sensitizer among legumes in asthma and rhinitis patients from India. *PLoS One* **2011**, *6* (11), e27193.
- (11) Zoccatelli, G.; Pokoj, S.; Foetisch, K.; Bartra, J.; Valero, A.; del Mar San Miguel-Moncin, M.; Vieths, S.; Scheurer, S. Identification and characterization of the major allergen of green bean (*Phaseolus vulgaris*) as a non-specific lipid transfer protein (Pha v 3). *Mol. Immunol.* **2010**, *47* (7), 1561–1568.
- (12) Kasera, R.; Singh, A. B.; Lavasa, S.; Nagendra, K.; Arora, N. Purification and Immunobiochemical Characterization of a 31 kDa Cross-Reactive Allergen from *Phaseolus vulgaris* (Kidney Bean). *PLoS One* **2013**, *8* (5), e63063.
- (13) Rougé, P.; Culerrier, R.; Thibau, F.; Didier, A.; Barre, A. A case of severe anaphylaxis to kidney bean: Phaseolin (vicilin) and PHA (lectin) identified as putative allergens. *Allergy* **2011**, *66* (2), 301–302.
- (14) Kumar, S.; Sharma, A.; Das, M.; Jain, S. K.; Dwivedi, P. D. Leucoagglutinating phytohemagglutinin: Purification, characterization, proteolytic digestion and assessment for allergenicity potential in BALB/c mice. *Immunopharmacol. Immunotoxicol.* **2014**, *36* (2), 138–144.
- (15) Kumar, S.; Sharma, A.; Verma, A. K.; Chaudhari, B. P.; Das, M.; Jain, S. K.; Dwivedi, P. D. Allergenicity potential of red kidney bean (*Phaseolus vulgaris* L.) proteins in orally treated BALB/c mice and passively sensitized RBL-2H3 cells. *Cell. Immunol.* **2013**, *284* (1), 37–44.
- (16) Grant, G.; Greer, F.; McKenzie, N. H.; Pusztai, A. Nutritional response of mature rats to kidney bean (*Phaseolus vulgaris*) lectins. *J. Sci. Food Agric.* **1985**, *36* (5), 409–414.
- (17) Williams, P. E. V.; Pusztai, A. J.; Macdearmid, A.; Innes, G. M. The use of kidney beans (*Phaseolus vulgaris*) as protein supplements in diets for young rapidly growing beef steers. *Anim. Feed Sci. Technol.* **1984**, *12* (1), 1–10.
- (18) Sun, X.; Ye, Y.; He, S.; Wu, Z.; Yue, J.; Sun, H.; Cao, X. A novel oriented antibody immobilization based voltammetric immunosensor for allergenic activity detection of lectin in kidney bean by using AuNPs-PEI-MWCNTs modified electrode. *Biosens. Bioelectron.* **2019**, *143*, 111607.
- (19) Nciri, N.; Cho, N. New research highlights: Impact of chronic ingestion of white kidney beans (*Phaseolus vulgaris* L. var. Beldia) on small-intestinal disaccharidase activity in Wistar rats. *Toxicology Reports* **2018**, *5*, 46–55.
- (20) Peumans, W. J.; Van Damme, E. J. Lectins as plant defense proteins. *Plant Physiol.* **1995**, *109* (2), 347–352.
- (21) Liu, C.; Zhao, M.; Sun, W.; Ren, J. Effects of high hydrostatic pressure treatments on haemagglutination activity and structural conformations of phytohemagglutinin from red kidney bean (*Phaseolus vulgaris*). *Food Chem.* **2013**, *136* (3), 1358–1363.
- (22) Sharon, N.; Lis, H. History of lectins: From hemagglutinins to biological recognition molecules. *Glycobiology* **2004**, *14* (11), 53R–62R.
- (23) Zhang, J.; Shi, J.; Ilic, S.; Jun Xue, S.; Kakuda, Y. Biological properties and characterization of lectin from red kidney bean (*Phaseolus vulgaris*). *Food Rev. Int.* **2008**, *25* (1), 12–27.
- (24) Bies, C.; Lehr, C.-M.; Woodley, J. F. Lectin-mediated drug targeting: History and applications. *Adv. Drug Delivery Rev.* **2004**, *56* (4), 425–435.
- (25) Spies, J. R.; Coulson, E. J.; Stevens, H. The chemistry of allergens. X. comparison of chemical and immunological properties of CB-1A preparations from domestic castor beans and brazilian castor bean pomace1,2. *J. Am. Chem. Soc.* **1944**, *66* (10), 1798–1799.
- (26) Tsaneva, M.; Van Damme, E. J. M. 130 years of Plant Lectin Research. *Glycoconjugate J.* **2020**, *37* (5), 533–551.
- (27) Nowell, P. C. Phytohemagglutinin: An initiator of mitosis in cultures of normal human leukocytes. *Cancer Res.* **1960**, *20* (4), 462–466.
- (28) He, S.; Shi, J.; Li, X.; Ma, Y.; Xue, S. J. Identification of a lectin protein from black turtle bean (*Phaseolus vulgaris*) using LC-MS/MS and PCR method. *LWT - Food Sci. Technol.* **2015**, *60* (2), 1074–1079.
- (29) He, S.; Zhao, J.; Elfalleh, W.; Jemaà, M.; Sun, H.; Sun, X.; Tang, M.; He, Q.; Wu, Z.; Lang, F. In silico identification and in vitro analysis of B and T-cell epitopes of the black turtle bean (*Phaseolus vulgaris* L.) lectin. *Cell. Physiol. Biochem.* **2018**, *49* (4), 1600–1614.
- (30) Ren, J.; Shi, J.; Kakuda, Y.; Kim, D.; Xue, S. J.; Zhao, M.; Jiang, Y.; Sun, J. Comparison of the phytohaemagglutinin from red kidney bean (*Phaseolus vulgaris*) purified by different affinity chromatography. *Food Chem.* **2008**, *108* (1), 394–401.
- (31) Leavitt, R. D.; Felsted, R. L.; Bachur, N. R. Biological and biochemical properties of *Phaseolus vulgaris* isolectins. *J. Biol. Chem.* **1977**, *252* (9), 2961–2966.
- (32) Wang, P.; Leng, X.; Duan, J.; Zhu, Y.; Wang, J.; Yan, Z.; Min, S.; Wei, D.; Wang, X. Functional component isolated from *Phaseolus vulgaris* lectin exerts in vitro and in vivo anti-tumor activity through potentiation of apoptosis and immunomodulation. *Molecules* **2021**, *26* (2), 498.
- (33) Chrispeels, M. J.; Raikhel, N. V. Lectins, lectin genes, and their role in plant defense. *Plant Cell* **1991**, *3* (1), 1–9.
- (34) Yachnin, S.; Svenson, R. H. The immunological and physicochemical properties of mitogenic proteins derived from *Phaseolus vulgaris*. *Immunology* **1972**, *22* (5), 871–883.
- (35) Einas, H. E. S.; Mahmoud, I. E. A.; Magda; Mervat, M. S. Antimicrobial activities of lectins extracted from some cultivars of *Phaseolus vulgaris* seeds. *J. Microb. Biochem. Technol.* **2017**, *9* (3), 109–116.
- (36) Lagarda-Diaz, I.; Guzman-Partida, A. M.; Vazquez-Moreno, L. Legume lectins: Proteins with diverse applications. *Int. J. Mol. Sci.* **2017**, *18* (6), 1242.
- (37) Hamelryck, T. W.; Dao-Thi, M.-H.; Poortmans, F.; Chrispeels, M. J.; Wyns, L.; Loris, R. The crystallographic structure of phytohemagglutinin-L*. *J. Biol. Chem.* **1996**, *271* (34), 20479–20485.
- (38) Grandhi, N. J.; Mamidi, A. S.; Surolia, A. Pattern recognition in legume lectins to extrapolate amino acid variability to sugar specificity. *Adv. Exp. Med. Biol.* **2015**, *842*, 199–215.

- (39) Agrawal, B. B. L.; Goldstein, I. J. Protein-carbohydrate interaction: VI. Isolation of concanavalin A by specific adsorption on cross-linked dextran gels. *Biochim. Biophys. Acta, Protein Struct.* **1967**, *147* (2), 262–271.
- (40) Mishra, A.; Behura, A.; Mawatwal, S.; Kumar, A.; Naik, L.; Mohanty, S. S.; Manna, D.; Dokania, P.; Mishra, A.; Patra, S. K.; Dhiman, R. Structure-function and application of plant lectins in disease biology and immunity. *Food Chem. Toxicol.* **2019**, *134*, 110827.
- (41) Jiang, B.; Wang, X.; Wang, L.; Lv, X.; Li, D.; Liu, C.; Feng, Z. Two-step isolation, purification, and characterization of lectin from zihua snap bean (*Phaseolus vulgaris*) seeds. *Polymers* **2019**, *11* (5), 785.
- (42) Borrebaeck, C. A. K. Detection and characterization of a lectin from non-seed tissue of *Phaseolus vulgaris*. *Planta* **1984**, *161* (3), 223–228.
- (43) He, S.; Simpson, B. K.; Ngadi, M. O.; Ma, Y. In vitro studies of the digestibility of lectin from black turtle bean (*Phaseolus vulgaris*). *Food Chem.* **2015**, *173*, 397–404.
- (44) Zhao, J. K.; Wang, H. X.; Ng, T. B. Purification and characterization of a novel lectin from the toxic wild mushroom *Inocybe umbrinella*. *Toxicon* **2009**, *53* (3), 360–366.
- (45) Ohtani, K.; Shibata, S.; Misaki, A. Purification and characterization of tora-bean (*Phaseolus vulgaris*) Lectin I. *J. Biochem.* **1980**, *87* (2), 407–416.
- (46) Sharon, N.; Lis, H. How proteins bind carbohydrates: Lessons from legume lectins. *J. Agric. Food Chem.* **2002**, *50* (22), 6586–6591.
- (47) Ambrosi, M.; Cameron, N. R.; Davis, B. G. Lectins: Tools for the molecular understanding of the glycode. *Org. Biomol. Chem.* **2005**, *3* (9), 1593–1608.
- (48) Sharon, N. Lectin-carbohydrate complexes of plants and animals: An atomic view. *Trends Biochem. Sci.* **1993**, *18* (6), 221–226.
- (49) Young, N. M.; Oomen, R. P. Analysis of sequence variation among legume lectins: A ring of hypervariable residues forms the perimeter of the carbohydrate-binding site. *J. Mol. Biol.* **1992**, *228* (3), 924–934.
- (50) Sharma, V.; Surolia, A. Analyses of carbohydrate recognition by legume lectins: Size of the combining site loops and their primary specificity. *J. Mol. Biol.* **1997**, *267* (2), 433–445.
- (51) Rao, V. S. R.; Lam, K.; Qasba, P. K. Architecture of the sugar binding sites in carbohydrate binding proteins—a computer modeling study. *Int. J. Biol. Macromol.* **1998**, *23* (4), 295–307.
- (52) Biswas, S.; Kayastha, A. M. Unfolding and refolding of leucoagglutinin (PHA-L), an oligomeric lectin from kidney beans (*Phaseolus vulgaris*). *Biochim. Biophys. Acta, Gen. Subj.* **2004**, *1674* (1), 40–49.
- (53) He, S.; Shi, J.; Ma, Y.; Xue, S. J.; Zhang, H.; Zhao, S. Kinetics for the thermal stability of lectin from black turtle bean. *J. Food Eng.* **2014**, *142*, 132–137.
- (54) Biswas, S.; Kayastha, A. M. Thermal stability of *Phaseolus vulgaris* leucoagglutinin: A differential scanning calorimetry study. *J. Biochem. Mol. Toxicol.* **2002**, *35* (5), 472–475.
- (55) Shnyrov, V. L.; Marcos, M. J.; Villar, E. Kinetic study on the irreversible thermal denaturation of lentil lectin. *IUBMB Life* **1996**, *39* (4), 647–656.
- (56) Nagae, M.; Soga, K.; Morita-Matsumoto, K.; Hanashima, S.; Ikeda, A.; Yamamoto, K.; Yamaguchi, Y. Phytohemagglutinin from *Phaseolus vulgaris* (PHA-E) displays a novel glycan recognition mode using a common legume lectin fold. *Glycobiology* **2014**, *24* (4), 368–378.
- (57) Hamid, R.; Masood, A. Dietary lectins as disease causing toxicants. *Pak. J. Nutr.* **2009**, *8*, 293–303.
- (58) Hammarström, S.; Hammarström, M. L.; Sundblad, G.; Arnarp, J.; Lönngrén, J. Mitogenic leucoagglutinin from *Phaseolus vulgaris* binds to a pentasaccharide unit in N-acetylglucosamine-type glycoprotein glycans. *Proc. Natl. Acad. Sci. U. S. A.* **1982**, *79* (5), 1611–1615.
- (59) Haas, H.; Falcone, F. H.; Schramm, G.; Haisch, K.; Gibbs, B. F.; Klauke, J.; Pöppelmann, M.; Becker, W.-M.; Gabius, H.-J.; Schlaak, M. Dietary lectins can induce in vitro release of IL-4 and IL-13 from human basophils. *Eur. J. Immunol.* **1999**, *29* (3), 918–927.
- (60) Muraille, E.; Pajak, B.; Urbain, J.; Leo, O. Carbohydrate-bearing cell surface receptors involved in innate immunity: Interleukin-12 induction by mitogenic and nonmitogenic lectins. *Cell. Immunol.* **1999**, *191* (1), 1–9.
- (61) Cummings, R. D.; Kornfeld, S. Characterization of the structural determinants required for the high affinity interaction of asparagine-linked oligosaccharides with immobilized *Phaseolus vulgaris* leucoagglutinating and erythroagglutinating lectins. *J. Biol. Chem.* **1982**, *257* (19), 11230–11234.
- (62) Nagendra, K.; Kasera, R.; Lavasa, S.; Singh, A. B.; Nagendra, K.; Arora, N. Food allergy and anaphylaxis – 2058. Elucidation of a 31 Kda cross-reactive kidney bean. *World Allergy Organ. J.* **2013**, *6*, P141.
- (63) Kasera, R.; Singh, B. P.; Lavasa, S.; Prasad, K. N.; Sahoo, R. C.; Singh, A. B. Kidney bean: A major sensitizer among legumes in asthma and rhinitis patients from India. *PLoS One* **2011**, *6* (11), e27193.
- (64) Kumar, S.; Verma, A. K.; Misra, A.; Tripathi, A.; Chaudhari, B. P.; Prasad, R.; Jain, S. K.; Das, M.; Dwivedi, P. D. Allergic responses of red kidney bean (*Phaseolus vulgaris* cv chitra) polypeptides in BALB/c mice recognized by bronchial asthma and allergic rhinitis patients. *Food Res. Int.* **2011**, *44* (9), 2868–2879.
- (65) Pali-Schöll, I.; Verhoeckx, K.; Mafra, I.; Bavaro, S. L.; Clare Mills, E. N.; Monaci, L. Allergenic and novel food proteins: State of the art and challenges in the allergenicity assessment. *Trends Food Sci. Technol.* **2019**, *84*, 45–48.
- (66) Ladics, G. S. Current codex guidelines for assessment of potential protein allergenicity. *Food Chem. Toxicol.* **2008**, *46* (10), S20–S23.
- (67) Bannon, G.; Fu, T.-J.; Kimber, I.; Hinton, D. M. Protein digestibility and relevance to allergenicity. *Environ. Health Perspect.* **2003**, *111* (8), 1122–1124.
- (68) Morari, D.; Stepurina, T.; Rotari, V. I. Calcium ions make phytohemagglutinin resistant to trypsin proteolysis. *J. Agric. Food Chem.* **2008**, *56* (10), 3764–3771.
- (69) Dupont, D.; Mackie, A. R. Static and dynamic in vitro digestion models to study protein stability in the gastrointestinal tract. *Drug Discovery Today: Dis. Models* **2015**, *17*–18, 23–27.
- (70) Van Wijk, F.; Nierkens, S.; Hassing, I.; Feijen, M.; Koppelman, S. J.; de Jong, G. A. H.; Pieters, R.; Knippels, L. M. J. The effect of the food matrix on in vivo immune responses to purified peanut allergens. *Toxicol. Sci.* **2005**, *86* (2), 333–341.
- (71) Bansode, R. R.; Randolph, P. D.; Plundrich, N. J.; Lila, M. A.; Williams, L. L. Peanut protein-polyphenol aggregate complexation suppresses allergic sensitization to peanut by reducing peanut-specific IgE in C3H/HeJ mice. *Food Chem.* **2019**, *299*, 125025.
- (72) Vojdani, A.; Afar, D.; Vojdani, E. Reaction of lectin-specific antibody with human tissue: Possible contributions to autoimmunity. *J. Immunol. Res.* **2020**, *2020*, 1438957.
- (73) Vasconcelos, I. M.; Oliveira, J. T. A. Antinutritional properties of plant lectins. *Toxicon* **2004**, *44* (4), 385–403.
- (74) Homann, A.; Schramm, G.; Jappe, U. Glycans and glycan-specific IgE in clinical and molecular allergology: Sensitization, diagnostics, and clinical symptoms. *J. Allergy Clin. Immunol.* **2017**, *140* (2), 356–368.
- (75) Calvani, M.; Anania, C.; Cuomo, B.; D'Auria, E.; Decimo, F.; Indirli, G. C.; Marseglia, G.; Mastroianni, V.; Sartorio, M. U. A.; Santoro, A.; Veronelli, E. Non-IgE- or Mixed IgE/Non-IgE-Mediated Gastrointestinal Food Allergies in the First Years of Life: Old and New Tools for Diagnosis. *Nutrients* **2021**, *13* (1), 226.
- (76) Verma, A. K.; Kumar, S.; Das, M.; Dwivedi, P. D. A comprehensive review of legume allergy. *Clin. Rev. Allergy Immunol.* **2013**, *45* (1), 30–46.
- (77) Jimenez-Rodriguez, T. W.; Garcia-Neuer, M.; Alenazy, L. A.; Castells, M. Anaphylaxis in the 21st century: Phenotypes, endotypes, and biomarkers. *J. Asthma Allergy* **2018**, *11*, 121–142.

- (78) Lavelle, E. C.; Grant, G.; Pusztai, A.; Pfüller, U.; O'Hagan, D. T. Mucosal immunogenicity of plant lectins in mice. *Immunology* **2000**, *99* (1), 30–37.
- (79) Kroghsbo, S.; Madsen, C.; Poulsen, M.; Schröder, M.; Kvist, P. H.; Taylor, M.; Gatehouse, A.; Shu, Q.; Knudsen, I. Immunotoxicological studies of genetically modified rice expressing PHA-E lectin or Bt toxin in Wistar rats. *Toxicology* **2008**, *245* (1), 24–34.
- (80) He, S.; Zhao, J.; Zhang, Y.; Zhu, Y.; Li, X.; Cao, X.; Ye, Y.; Li, J.; Sun, H. Effects of low-pH treatment on the allergenicity reduction of black turtle bean (*Phaseolus vulgaris* L.) lectin and its mechanism. *J. Agric. Food Chem.* **2021**, *69* (4), 1379–1390.
- (81) Shudong, H.; Walid, E.; Jing, L.; Jinlong, Z.; Qian, H.; Hanju, S. In vivo anaphylactic assessments of low-dose lectin from the black turtle soup bean (*Phaseolus vulgaris*). *Curr. Top. Nutraceutical Res.* **2018**, *16* (1), 105–112.
- (82) 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. High doses of gamma radiation suppress allergic effect induced by food lectin. *Radiat. Phys. Chem.* **2013**, *85*, 218–226.
- (83) Hoffmann, H. J.; Dahl, C.; Schiötz, P. O.; Berglund, L.; Dahl, R. Lectins interact differentially with purified human eosinophils, cultured cord blood-derived mast cells and the myeloid leukaemic cell line AML14.3D10: Induction of interleukin-4 secretion is conserved among granulocytes, but is not proportional to agglutination or lectin–glycoprotein interaction. *Clin. Exp. Allergy* **2003**, *33* (7), 930–935.
- (84) Vercelli, D.; Geha, R. S. Regulation of IgE synthesis in humans: A tale of two signals. *J. Allergy Clin. Immunol.* **1991**, *88* (3), 285–295.
- (85) Drazen, J. M.; Arm, J. P.; Austen, K. F. Sorting out the cytokines of asthma. *J. Exp. Med.* **1996**, *183* (1), 1–5.
- (86) Nwosu, O. E.; Inuwa, H. M.; Onyike, E.; Dingwoke, J. Detection and isolation of novel lectin gene from *Piliostigma thonningii* seeds. *Am. J. Biochem.* **2016**, *6*, 72–81.
- (87) García-Gasca, T.; García-Cruz, M.; Hernandez-Rivera, E.; López-Martínez, J.; Castañeda-Cuevas, A. L.; Yllescas-Gasca, L.; Rodríguez-Méndez, A. J.; Mendiola-Olaya, E.; Castro-Guillén, J. L.; Blanco-Labra, A. Effects of Tepary Bean (*Phaseolus acutifolius*) protease inhibitor and semipure lectin fractions on cancer cells. *Nutr. Cancer* **2012**, *64* (8), 1269–1278.
- (88) Kamemura, K.; Furuichi, Y.; Umekawa, H.; Takahashi, T. Purification and characterization of novel lectins from Great Northern bean. *Biochim. Biophys. Acta, Gen. Subj.* **1993**, *1158* (2), 181–188.
- (89) Kamemura, K.; Furuichi, Y.; Umekawa, H.; Takahashi, T. Purification and characterization of a pod lectin from Great Northern bean. *Biochim. Biophys. Acta, Gen. Subj.* **1996**, *1289* (1), 87–94.
- (90) Melander, W.; Horváth, C. Salt effects on hydrophobic interactions in precipitation and chromatography of proteins: An interpretation of the lyotropic series. *Arch. Biochem. Biophys.* **1977**, *183* (1), 200–215.
- (91) Queiroz, J. A.; Tomaz, C. T.; Cabral, J. M. S. Hydrophobic interaction chromatography of proteins. *J. Biotechnol.* **2001**, *87* (2), 143–159.
- (92) Agrawal, B. B. L.; Goldstein, I. J. Specific binding of concanavalin A to cross-linked dextran gels. *Biochem. J.* **1965**, *96* (3), 23C–35C.
- (93) Felsted, R. L.; Leavitt, R. D.; Bachur, N. R. Purification of the phytohemagglutinin family of proteins from red kidney beans (*Phaseolus vulgaris*) by affinity chromatography. *Biochim. Biophys. Acta, Protein Struct.* **1975**, *405* (1), 72–81.
- (94) Lam, S. K.; Ng, T. B. Isolation and characterization of a French bean hemagglutinin with antitumor, antifungal, and anti-HIV-1 reverse transcriptase activities and an exceptionally high yield. *Phytomedicine* **2010**, *17* (6), 457–462.
- (95) Nakamura-Tsuruta, S.; Kishimoto, Y.; Nishimura, T.; Suda, Y. One-step purification of lectins from Banana Pulp using sugar-immobilized gold nano-particles. *J. Biochem.* **2007**, *143* (6), 833–839.
- (96) Hamid, R.; Masood, A.; Wani, I. H.; Rafiq, S. Lectins: Proteins with diverse applications. *J. Appl. Pharm. Sci.* **2013**, *3* (4), S93–S103.
- (97) Nascimento, K. S.; Cunha, A. I.; Nascimento, K. S.; Cavada, B. S.; Azevedo, A. M.; Aires-Barros, M. R. An overview of lectins purification strategies. *J. Mol. Recognit.* **2012**, *25* (11), 527–541.
- (98) Banerjee, S. S.; Chen, D.-H. Glucose-grafted gum arabic modified magnetic nanoparticles: Preparation and specific interaction with Concanavalin A. *Chem. Mater.* **2007**, *19* (15), 3667–3672.
- (99) Guo, W.; Ruckenstein, E. A new matrix for membrane affinity chromatography and its application to the purification of concanavalin A. *J. Membr. Sci.* **2001**, *182* (1), 227–234.
- (100) Jiang, B.; Yuan, Y. Q.; Zhang, X. Q.; Feng, Z. B.; Liu, C. H. Separation and enrichment of lectin from Zihua snap-bean (*Phaseolus vulgaris*) seeds by PEG 600-ammonium sulfate aqueous two-phase system. *Molecules* **2017**, *22*, 1596.
- (101) He, S.; Shi, J.; Walid, E.; Ma, Y.; Xue, S. J. Extraction and purification of a lectin from small black kidney bean (*Phaseolus vulgaris*) using a reversed micellar system. *Process Biochem.* **2013**, *48* (4), 746–752.
- (102) He, S.; Shi, J.; Walid, E.; Zhang, H.; Ma, Y.; Xue, S. J. Reverse micellar extraction of lectin from black turtle bean (*Phaseolus vulgaris*): Optimisation of extraction conditions by response surface methodology. *Food Chem.* **2015**, *166*, 93–100.
- (103) Hou, Y.; Hou, Y.; Yanyan, L.; Qin, G.; Li, J. Extraction and purification of a lectin from red kidney bean and preliminary immune function studies of the lectin and four chinese herbal polysaccharides. *J. Biomed. Biotechnol.* **2010**, *2010*, 217342.
- (104) Kaneda, Y.; Whittier, R. F.; Yamanaka, H.; Carredano, E.; Gotoh, M.; Sota, H.; Hasegawa, Y.; Shinohara, Y. The high specificities of *Phaseolus vulgaris* erythro- and leukoagglutinating lectins for bisecting GlcNAc or β 1–6-Linked branch structures, respectively, are attributable to loop B*. *J. Biol. Chem.* **2002**, *277* (19), 16928–16935.
- (105) Lu, G.; Holland, L. A. Profiling the N-Glycan composition of IgG with lectins and capillary nanogel electrophoresis. *Anal. Chem.* **2019**, *91* (2), 1375–1383.
- (106) Zubcevic, N.; Damir, S.; Focak, M.; Rukavina, D. Effects of plant lectins on human erythrocyte agglutination. *Serb. J. Exp. Clin. Res.* **2016**, *17* (3), 207–214.
- (107) Purkait, S.; Koley, S. Identification and characterization of lectins from leguminosae plants. *Int. J. Health Sci. Res.* **2019**, *9* (2), 115–121.
- (108) Eber, U.; Godswill, A. C. Effect of some processing methods on hemagglutinin activity of lectin extracts from selected grains (cereals and legumes). *Int. J. Adv. Acad. Res.* **2016**, *2*, 24–59.
- (109) Lam, S. K.; Ng, T. B. Lectins: Production and practical applications. *Appl. Microbiol. Biotechnol.* **2011**, *89* (1), 45–55.
- (110) Khan, F.; Ahmad, A.; Khan, M. I. Purification and characterization of a lectin from endophytic fungus *Fusarium solani* having complex sugar specificity. *Arch. Biochem. Biophys.* **2007**, *457* (2), 243–251.
- (111) Liener, I. E. The photometric determination of the hemagglutinating activity of soyin and crude soybean extracts. *Arch. Biochem. Biophys.* **1955**, *54* (1), 223–231.
- (112) Vlodavsky, I.; Inbar, M.; Sachs, L. Temperature-sensitive agglutinability of human erythrocytes by lectins. *Biochim. Biophys. Acta, Biomembr.* **1972**, *274* (2), 364–369.
- (113) Duk, M.; Lisowska, E.; Kordowicz, M.; Wasniowska, K. Studies on the specificity of the binding site of vicia graminea anti-N lectin. *Eur. J. Biochem.* **1982**, *123* (1), 105–112.
- (114) Zhao, J.; He, S.; Tang, M.; Sun, X.; Zhang, Z.; Ye, Y.; Cao, X.; Sun, H. Low-pH induced structural changes, allergenicity and in vitro digestibility of lectin from black turtle bean (*Phaseolus vulgaris* L.). *Food Chem.* **2019**, *283*, 183–190.
- (115) Wu, A. M.; Song, S. C.; Sugii, S.; Herp, A. Differential binding properties of Gal/GalNAc specific lectins available for characterization of glycoreceptors. *Indian J. Biochem. Biophys.* **1997**, *34* (1–2), 61–71.
- (116) Pusztai, A.; Watt, W. B.; Stewart, J. C. A comprehensive scheme for the isolation of trypsin inhibitors and the agglutinin from soybean seeds. *J. Agric. Food Chem.* **1991**, *39* (5), 862–866.

- (117) Dai, Z.; Kawde, A.-N.; Xiang, Y.; La Belle, J. T.; Gerlach, J.; Bhavanandan, V. P.; Joshi, L.; Wang, J. Nanoparticle-based sensing of glycan–lectin interactions. *J. Am. Chem. Soc.* **2006**, *128* (31), 10018–10019.
- (118) Coelho, L. C. B. B.; Silva, P. M. d. S.; Lima, V. L. d. M.; Pontual, E. V.; Paiva, P. M. G.; Napoleão, T. H.; Correia, M. T. d. S. Lectins, interconnecting proteins with biotechnological/pharmacological and therapeutic applications. *Evidence-Based Complementary Altern. Med.* **2017**, *2017*, 1594074.
- (119) Liyanage, S. H.; Yan, M. Quantification of binding affinity of glycananomal materials with lectins. *Chem. Commun.* **2020**, *56* (88), 13491–13505.
- (120) Huang, K.-J.; Niu, D.-J.; Xie, W.-Z.; Wang, W. A disposable electrochemical immunosensor for carcinoembryonic antigen based on nano-Au/multi-walled carbon nanotubes–chitosans nanocomposite film modified glassy carbon electrode. *Anal. Chim. Acta* **2010**, *659* (1), 102–108.
- (121) Zhou, J.; Qi, Q.; Wang, C.; Qian, Y.; Liu, G.; Wang, Y.; Fu, L. Surface plasmon resonance (SPR) biosensors for food allergen detection in food matrices. *Biosens. Bioelectron.* **2019**, *142*, 111449.
- (122) Gauthier, L.; Chevallet, M.; Bulteau, F.; Thépaut, M.; Delange, P.; Fieschi, F.; Vivès, C.; Texier, I.; Deniaud, A.; Gateau, C. Lectin recognition and hepatocyte endocytosis of GalNAc-decorated nanostructured lipid carriers. *J. Drug Targeting* **2021**, *29* (1), 99–107.
- (123) Wang, Z.; Chen, G.; Lu, J.; Hong, L.; Ngai, T. Investigation of the factors affecting the carbohydrate–lectin interaction by ITC and QCM-D. *Colloid Polym. Sci.* **2014**, *292* (2), 391–398.
- (124) La Belle, J. T.; Gerlach, J. Q.; Svarovsky, S.; Joshi, L. Label-free impedimetric detection of Glycan–lectin interactions. *Anal. Chem.* **2007**, *79* (18), 6959–6964.
- (125) Eftink, M. R. Fluorescence methods for studying equilibrium macromolecule–ligand interactions. *Methods Enzymol.* **1997**, *278*, 221–257.
- (126) Wang, X.; Ramström, O.; Yan, M. Quantitative analysis of multivalent ligand presentation on gold glycananoparticles and the impact on lectin binding. *Anal. Chem.* **2010**, *82* (21), 9082–9089.
- (127) Wang, X.; Matei, E.; Gronenborn, A. M.; Ramström, O.; Yan, M. Direct measurement of glycananoparticles and lectin interactions by isothermal titration calorimetry. *Anal. Chem.* **2012**, *84* (10), 4248–4252.
- (128) Homola, J. Surface plasmon resonance sensors for detection of chemical and biological species. *Chem. Rev.* **2008**, *108* (2), 462–493.
- (129) Aquino, A.; Conte-Junior, C. A. A systematic review of food allergy: Nanobiosensor and food allergen detection. *Biosensors* **2020**, *10* (12), 194.
- (130) Mi, F.; Guan, M.; Hu, C.; Peng, F.; Sun, S.; Wang, X. Application of lectin-based biosensor technology in the detection of foodborne pathogenic bacteria: A review. *Analyst* **2021**, *146* (2), 429–443.
- (131) Hendrickson, O. D.; Zherdev, A. V. Analytical application of lectins. *Crit. Rev. Anal. Chem.* **2018**, *48* (4), 279–292.
- (132) Ji, D.-K.; Zhang, Y.; He, X.-P.; Chen, G.-R. An insight into graphene oxide associated fluorogenic sensing of glycode–lectin interactions. *J. Mater. Chem. B* **2015**, *3* (32), 6656–6661.
- (133) Sanji, T.; Shiraishi, K.; Nakamura, M.; Tanaka, M. Fluorescence turn-on sensing of lectins with mannose-substituted tetraphenylethenes based on aggregation-induced emission. *Chem. - Asian J.* **2010**, *5* (4), 817–824.
- (134) Sanji, T.; Shiraishi, K.; Tanaka, M. Sugar–phosphole oxide conjugates as “Turn-on” luminescent sensors for lectins. *ACS Appl. Mater. Interfaces* **2009**, *1* (2), 270–273.
- (135) Jiang, T.; Tan, H.; Sun, Y.; Wang, J.; Hang, Y.; Lu, N.; Yang, J.; Qu, X.; Hua, J. Graphene oxide-based NIR fluorescence probe with aggregation-induced emission property for lectins detection and liver cells targeting. *Sens. Actuators, B* **2018**, *261*, 115–126.
- (136) Liu, Z.; Liu, H.; Wang, L.; Su, X. A label-free fluorescence biosensor for highly sensitive detection of lectin based on carboxymethyl chitosan-quantum dots and gold nanoparticles. *Anal. Chim. Acta* **2016**, *932*, 88–97.
- (137) Hirabayashi, J.; Kuno, A.; Tateno, H. Development and applications of the lectin microarray. *Top. Curr. Chem.* **2014**, *367*, 105–124.
- (138) Hirabayashi, J.; Yamada, M.; Kuno, A.; Tateno, H. Lectin microarrays: Concept, principle and applications. *Chem. Soc. Rev.* **2013**, *42* (10), 4443–4458.
- (139) Goodarzi, M. T.; Turner, G. A. A lectin method for investigating the glycosylation of nanogram amounts of purified glycoprotein. *Glycoconjugate J.* **1997**, *14* (4), 493–496.
- (140) Kamada, Y.; Kinoshita, N.; Tsuchiya, Y.; Kobayashi, K.; Fujii, H.; Terao, N.; Kamihagi, K.; Koyama, N.; Yamada, S.; Daigo, Y.; Nakamura, Y.; Taniguchi, N.; Miyoshi, E. Reevaluation of a lectin antibody ELISA kit for measuring fucosylated haptoglobin in various conditions. *Clin. Chim. Acta* **2013**, *417*, 48–53.
- (141) Duk, M.; Mitra, D.; Lisowska, E.; Kabat, E. A.; Sharon, N.; Lis, H. Immunochemical studies on the combining site of the A + N blood type specific *Moluccella laevis* lectin. *Carbohydr. Res.* **1992**, *236*, 245–258.
- (142) Borrebaeck, C. A. Detection and characterization of a lectin from non-seed tissue of *Phaseolus vulgaris*. *Planta* **1984**, *161* (3), 223–8.
- (143) Vincenzi, S.; Zoccatelli, G.; Perbellini, F.; Rizzi, C.; Chignola, R.; Curioni, A.; Peruffo, A. D. B. Quantitative determination of dietary lectin activities by enzyme-linked immunosorbent assay using specific glycoproteins immobilized on microtiter plates. *J. Agric. Food Chem.* **2002**, *50* (22), 6266–6270.
- (144) Boniglia, C.; Fedele, E.; Sanzini, E. Measurement by ELISA of Active Lectin in Dietary Supplements Containing Kidney Bean Protein. *J. Food Sci.* **2003**, *68* (4), 1283–1286.
- (145) Nagendra, K.; Kasera, R.; Lavasa, S.; Singh, A. B.; Nagendra, K.; Arora, N. Food allergy and anaphylaxis – 2058. Elucidation of a 31 Kda cross-reactive kidney bean. *World Allergy Organ. J.* **2013**, *6* (1), P141–P141.
- (146) Rougé, P.; Culerrier, R.; Granier, C.; Rancé, F.; Barre, A. Characterization of IgE-binding epitopes of peanut (*Arachis hypogaea*) PNA lectin allergen cross-reacting with other structurally related legume lectins. *Mol. Immunol.* **2010**, *47* (14), 2359–2366.
- (147) Hajos, G.; Gelencser, E.; Pusztai, A.; Grant, G.; Sakhri, M.; Bardocz, S. Biological effects and survival of trypsin inhibitors and the agglutinin from soybean in the small intestine of the rat. *J. Agric. Food Chem.* **1995**, *43* (1), 165–170.
- (148) Nciri, N.; Shin, T.; Cho, N. Public health hazards associated with naturally occurring toxins in legume seeds-Rapid detection and characterization of a lectin from a Korean cultivated kidney bean (*Phaseolus vulgaris* L.). *Asian J. Pharm. Clin. Res.* **2018**, *11* (Special Issue 3), 76–79.
- (149) Scheidegger, J. J. A micro-method of immuno-electrophoresis. *Int. Arch. Allergy Immunol.* **2004**, *7* (2), 103–110.
- (150) Becker, E. L. Antigen-antibody reactions in gels. *Fed. Proc.* **1953**, *12* (3), 717–22.
- (151) Mackenzie, D. W. R.; Philpot, C. M. Counterimmunoelectrophoresis as a routine mycoserological procedure. *Mycopathologia* **1975**, *57* (1), 1–7.
- (152) Wang, C.; Wang, Y.; Liu, G.; Fu, L. Food allergomics based on high-throughput and bioinformatics technologies. *Food Res. Int.* **2020**, *130*, 108942.
- (153) Fu, Z.; Lin, J. An overview of bioinformatics tools and resources in allergy. *Methods Mol. Biol.* **2017**, *1592*, 223–245.
- (154) Pinto-Junior, V. R.; Santiago, M. Q.; Nobre, C. B.; Osterne, V. J. S.; Leal, R. B.; Cajazeiras, J. B.; Lossio, C. F.; Rocha, B. A. M.; Martins, M. G. Q.; Nobre, C. A. S.; Silva, M. T. L.; Nascimento, K. S.; Cavada, B. S. Crystal structure of *Pisum arvense* seed lectin (PAL) and characterization of its interaction with carbohydrates by molecular docking and dynamics. *Arch. Biochem. Biophys.* **2017**, *630*, 27–37.
- (155) Pan, S.; Aebersold, R.; Chen, R.; Rush, J.; Goodlett, D. R.; McIntosh, M. W.; Zhang, J.; Brentnall, T. A. Mass spectrometry based

targeted protein quantification: Methods and applications. *J. Proteome Res.* **2009**, *8* (2), 787–797.

(156) Haab, B. B. Using lectins in biomarker research: Addressing the limitations of sensitivity and availability. *Proteomics: Clin. Appl.* **2012**, *6* (7–8), 346–350.

(157) Rossi, G. B.; Valentim-Neto, P. A.; Blank, M.; Faria, J. C. d.; Arisi, A. C. M. Comparison of Ggain proteome profiles of four Brazilian common bean (*Phaseolus vulgaris* L.) cultivars. *J. Agric. Food Chem.* **2017**, *65* (34), 7588–7597.

(158) Nasi, A.; Picariello, G.; Ferranti, P. Proteomic approaches to study structure, functions and toxicity of legume seeds lectins. Perspectives for the assessment of food quality and safety. *J. Proteomics* **2009**, *72* (3), 527–538.

(159) Haag, A. M. Mass analyzers and mass spectrometers. *Adv. Exp. Med. Biol.* **2016**, *919*, 157–169.

(160) Roca, L. S.; Schoemaker, S. E.; Pirok, B. W. J.; Gargano, A. F. G.; Schoenmakers, P. J. Accurate modelling of the retention behaviour of peptides in gradient-elution hydrophilic interaction liquid chromatography. *J. Chromatogr. A* **2020**, *1614*, 460650.

(161) World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO). *Foods Derived from Modern Biotechnology*, 2nd ed.; WHO and FAO: Rome, Italy, 2009.

(162) Herman, R. A.; Song, P.; ThirumalaiswamySekhar, A. Value of eight-amino-acid matches in predicting the allergenicity status of proteins: An empirical bioinformatic investigation. *Clin. Mol. Allergy* **2009**, *7* (1), 9.

(163) Aalberse, R. C. Structural biology of allergens. *J. Allergy Clin. Immunol.* **2000**, *106* (2), 228–238.

(164) Cereghino, J. L.; Cregg, J. M. Heterologous protein expression in the methylotrophic yeast *Pichia pastoris*. *FEMS Microbiology Reviews* **2000**, *24* (1), 45–66.

(165) Raemaekers, R. J. M.; de Muro, L.; Gatehouse, J. A.; Fordham-Skelton, A. P. Functional phytohemagglutinin (PHA) and Galanthus nivalis agglutinin (GNA) expressed in *Pichia pastoris*. *Eur. J. Biochem.* **1999**, *265* (1), 394–403.

(166) Baumgartner, P.; Raemaekers, R. J. M.; Durieux, A.; Gatehouse, A.; Davies, H.; Taylor, M. Large-scale production, purification, and characterisation of recombinant *Phaseolus vulgaris* phytohemagglutinin E-form expressed in the methylotrophic yeast *Pichia pastoris*. *Protein Expression Purif.* **2002**, *26* (3), 394–405.

(167) Rahaman, T.; Vasiljevic, T.; Ramchandran, L. Effect of processing on conformational changes of food proteins related to allergenicity. *Trends Food Sci. Technol.* **2016**, *49*, 24–34.

(168) Shi, J.; Xue, S. J.; Kakuda, Y.; Ilic, S.; Kim, D. Isolation and characterization of lectins from kidney beans (*Phaseolus vulgaris*). *Process Biochem.* **2007**, *42* (10), 1436–1442.

(169) Soetan, K. O.; Oyewole, O. The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *Afr. J. Food Sci.* **2009**, *3* (9), 223–232.

(170) Kasera, R.; Singh, A. B.; Kumar, R.; Lavasa, S.; Prasad, K. N.; Arora, N. Effect of thermal processing and γ -irradiation on allergenicity of legume proteins. *Food Chem. Toxicol.* **2012**, *50* (10), 3456–3461.

(171) Van Der Poel, T. F. B.; Blonk, J.; Van Zuilichem, D. J.; Van Oort, M. G. Thermal inactivation of lectins and trypsin inhibitor activity during steam processing of dry beans (*Phaseolus vulgaris*) and effects on protein quality. *J. Sci. Food Agric.* **1990**, *53* (2), 215–228.

(172) Bonorden, W. R.; Swanson, B. G. Thermal stability of black turtle soup bean (*Phaseolus vulgaris*) lectins. *J. Sci. Food Agric.* **1992**, *59* (2), 245–250.

(173) Grant, G.; More, L. J.; McKenzie, N. H.; Pusztai, A. The effect of heating on the haemagglutinating activity and nutritional properties of bean (*Phaseolus vulgaris*) seeds. *J. Sci. Food Agric.* **1982**, *33* (12), 1324–1326.

(174) Venter, F. S.; Thiel, P. G. Red kidney beans—To eat or not to eat? *S. Afr. Med. J.* **1995**, *85* (4), 250–2.

(175) He, S.; Simpson, B. K.; Sun, H.; Ngadi, M. O.; Ma, Y.; Huang, T. *Phaseolus vulgaris* lectins: A systematic review of characteristics and health implications. *Crit. Rev. Food Sci. Nutr.* **2018**, *58* (1), 70–83.

(176) Pasqualone, A.; Costantini, M.; Coldea, T. E.; Summo, C. Use of legumes in extrusion cooking: A review. *Foods* **2020**, *9* (7), 958.

(177) Kelkar, S.; Siddiq, M.; Harte, J. B.; Dolan, K. D.; Nyombaire, G.; Suniaga, H. Use of low-temperature extrusion for reducing phytohemagglutinin activity (PHA) and oligosaccharides in beans (*Phaseolus vulgaris* L.) cv. Navy and Pinto. *Food Chem.* **2012**, *133* (4), 1636–1639.

(178) Alam, M. S.; Kaur, J.; Khaira, H.; Gupta, K. Extrusion and extruded products: Changes in quality attributes as affected by extrusion process parameters: A review. *Crit. Rev. Food Sci. Nutr.* **2016**, *56* (3), 445–473.

(179) Chizoba Ekezie, F.-G.; Cheng, J.-H.; Sun, D.-W. Effects of nonthermal food processing technologies on food allergens: A review of recent research advances. *Trends Food Sci. Technol.* **2018**, *74*, 12–25.

(180) Bañuelos, M. A.; Loira, I.; Escott, C.; Del Fresno, J. M.; Morata, A.; Sanz, P. D.; Otero, L.; Suárez-Lepe, J. A. Grape processing by high hydrostatic pressure: Effect on use of non-saccharomyces in must fermentation. *Food Bioprocess Technol.* **2016**, *9* (10), 1769–1778.

(181) Takács, K.; Guillamon, E.; Pedrosa, M. M.; Cuadrado, C.; Burbano, C.; Muzquiz, M.; Haddad, J.; Allaf, K.; Maczók, A.; Polgár, M.; Gelencsér, É. Study of the effect of instant controlled pressure drop (DIC) treatment on IgE-reactive legume-protein patterns by electrophoresis and immunoblot. *Food Agric. Immunol.* **2014**, *25* (2), 173–185.

(182) Huang, H.-W.; Yang, B. B.; Wang, C.-Y. Effects of high pressure processing on immunoreactivity and microbiological safety of crushed peanuts. *Food Control* **2014**, *42*, 290–295.

(183) Li, H.; Zhu, K.; Zhou, H.; Peng, W. Effects of high hydrostatic pressure treatment on allergenicity and structural properties of soybean protein isolate for infant formula. *Food Chem.* **2012**, *132* (2), 808–814.

(184) Liu, C.; Zhao, M.; Sun, W.; Ren, J. Effects of high hydrostatic pressure treatments on haemagglutination activity and structural conformations of phytohemagglutinin from red kidney bean (*Phaseolus vulgaris*). *Food Chem.* **2013**, *136* (3–4), 1358–1363.

(185) Lu, Y.; Liu, C.; Zhao, M.; Cui, C.; Ren, J. Structure and activity changes of phytohemagglutinin from red kidney bean (*Phaseolus vulgaris*) affected by ultrahigh-pressure treatments. *J. Agric. Food Chem.* **2015**, *63* (43), 9513–9519.

(186) 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. Molecular fragmentation of wheat-germ agglutinin induced by food irradiation reduces its allergenicity in sensitised mice. *Food Chem.* **2012**, *132* (2), 1033–1039.

(187) Mallikarjunan, N.; Marathe, S.; Rajalakshmi, D.; Mahesh, S.; Jamdar, S.; Sharma, A. Effect of ionizing radiation on structural and functional attributes of red kidney bean (*Phaseolus vulgaris* L.) lectin. *LWT-food science and technology* **2014**, *59* (1), 300–307.

(188) Oh, S.; Jang, D.-I.; Lee, J.-W.; Kim, J.-H.; Byun, M.-W.; Lee, S.-Y. Evaluation of reduced allergenicity of irradiated peanut extract using splenocytes from peanut-sensitized mice. *Radiat. Phys. Chem.* **2009**, *78* (7), 615–617.

(189) Mallikarjunan, N.; Marathe, S.; Rajalakshmi, D.; Mahesh, S.; Jamdar, S. N.; Sharma, A. Effect of ionizing radiation on structural and functional attributes of red kidney bean (*Phaseolus vulgaris* L.) lectin. *LWT - Food Science and Technology* **2014**, *59* (1), 300–307.

(190) Yang, H.; Gao, J.; Yang, A.; Chen, H. The ultrasound-treated soybean seeds improve edibility and nutritional quality of soybean sprouts. *Food Res. Int.* **2015**, *77*, 704–710.

(191) Li, H.; Yu, J.; Ahmedna, M.; Goktepe, I. Reduction of major peanut allergens Ara h 1 and Ara h 2, in roasted peanuts by ultrasound assisted enzymatic treatment. *Food Chem.* **2013**, *141* (2), 762–768.

- (192) Yu, M.; Liu, H.; Shi, A.; Liu, L.; Wang, Q. Preparation of resveratrol-enriched and poor allergic protein peanut sprout from ultrasound treated peanut seeds. *Ultrason. Sonochem.* **2016**, *28*, 334–340.
- (193) Tolouie, H.; Mohammadifar, M. A.; Ghomi, H.; Hashemi, M. Cold atmospheric plasma manipulation of proteins in food systems. *Crit. Rev. Food Sci. Nutr.* **2018**, *58* (15), 2583–2597.
- (194) Surowsky, B.; Fischer, A.; Schlueter, O.; Knorr, D. Cold plasma effects on enzyme activity in a model food system. *Innovative Food Sci. Emerging Technol.* **2013**, *19*, 146–152.
- (195) Meinschmidt, P.; Ueberham, E.; Lehmann, J.; Reineke, K.; Schlüter, O.; Schweiggert-Weisz, U.; Eisner, P. The effects of pulsed ultraviolet light, cold atmospheric pressure plasma, and gamma-irradiation on the immunoreactivity of soy protein isolate. *Innovative Food Sci. Emerging Technol.* **2016**, *38*, 374–383.
- (196) Shriver, S.; Yang, W.; Chung, S.-Y.; Percival, S. Pulsed ultraviolet light reduces immunoglobulin E binding to Atlantic White Shrimp (*Litopenaeus Setiferus*) extract. *Int. J. Environ. Res. Public Health* **2011**, *8* (7), 2569–2583.
- (197) Mills, E. N. C.; Huang, L.; Noel, T. R.; Gunning, A. P.; Morris, V. J. Formation of thermally induced aggregates of the soya globulin β -conglycinin. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **2001**, *1547* (2), 339–350.
- (198) Pattison, D. I.; Rahmanto, A. S.; Davies, M. J. Photo-oxidation of proteins. *Photochemical & Photobiological Sciences* **2012**, *11* (1), 38–53.
- (199) Yang, W. W.; Mwakatage, N. R.; Goodrich-Schneider, R.; Krishnamurthy, K.; Rababah, T. M. Mitigation of major peanut allergens by pulsed ultraviolet light. *Food Bioprocess Technol.* **2012**, *5* (7), 2728–2738.
- (200) Yang, W. W.; Chung, S.-Y.; Ajayi, O.; Krishnamurthy, K.; Konan, K.; Goodrich-Schneider, R. Use of Pulsed Ultraviolet Light to Reduce the Allergenic Potency of Soybean Extracts. *Int. J. Food Eng.* **2010**, *6* (3), 11.
- (201) Sun, W.-W.; Yu, S.-J.; Zeng, X.-A.; Yang, X.-Q.; Jia, X. Properties of whey protein isolate–dextran conjugate prepared using pulsed electric field. *Food Res. Int.* **2011**, *44* (4), 1052–1058.
- (202) Vanga, S. K.; Singh, A.; Kalkan, F.; Garipey, Y.; Orsat, V.; Raghavan, V. Effect of Thermal and High Electric Fields on Secondary Structure of Peanut Protein. *Int. J. Food Prop.* **2016**, *19* (6), 1259–1271.
- (203) Johnson, P. E.; Van der Plancken, I.; Balasa, A.; Husband, F. A.; Grauwet, T.; Hendrickx, M.; Knorr, D.; Mills, E. N.; Mackie, A. R. High pressure, thermal and pulsed electric-field-induced structural changes in selected food allergens. *Mol. Nutr. Food Res.* **2010**, *54* (12), 1701–10.
- (204) Figueroa, M.-O. R.; Lajolo, F. M. Effect of chemical modifications of *Phaseolus vulgaris* lectins on their biological properties. *J. Agric. Food Chem.* **1997**, *45* (3), 639–643.
- (205) Taheri-Kafrani, A.; Gaudin, J.-C.; Rabesona, H.; Nioi, C.; Agarwal, D.; Drouet, M.; Chobert, J.-M.; Bordbar, A.-K.; Haertle, T. Effects of heating and glycation of β -lactoglobulin on its recognition by IgE of sera from cow milk allergy patients. *J. Agric. Food Chem.* **2009**, *57* (11), 4974–4982.
- (206) Gruber, P.; Becker, W.-M.; Hofmann, T. 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. *J. Agric. Food Chem.* **2005**, *53* (6), 2289–2296.
- (207) Yang, Y.; He, Q.; Sun, H.; Cao, X.; Elfalleh, W.; Wu, Z.; Zhao, J.; Sun, X.; Zhang, Y.; He, S. PEGylation may reduce allergenicity and improve gelling properties of protein isolate from black kidney bean (*Phaseolus vulgaris* L.). *Food Bioscience* **2018**, *25*, 83–90.
- (208) He, Q.; Sun, X.; He, S.; Wang, T.; Zhao, J.; Yang, L.; Wu, Z.; Sun, H. PEGylation of black kidney bean (*Phaseolus vulgaris* L.) protein isolate with potential functional properties. *Colloids Surf., B* **2018**, *164*, 89–97.
- (209) He, S.; Zhao, J.; Cao, X.; Ye, Y.; Wu, Z.; Yue, J.; Yang, L.; Jin, R.; Sun, H. Low pH-shifting treatment would improve functional properties of black turtle bean (*Phaseolus vulgaris* L.) protein isolate with immunoreactivity reduction. *Food Chem.* **2020**, *330*, 127217.
- (210) Plundrich, N. J.; Cook, B. T.; Maleki, S. J.; Fourches, D.; Lila, M. A. Binding of peanut allergen Ara h 2 with Vaccinium fruit polyphenols. *Food Chem.* **2019**, *284*, 287–295.
- (211) Trugo, L. C.; Muzquiz, M.; Pedrosa, M. M.; Ayet, G.; Burbano, C.; Cuadrado, C.; Cavieres, E. Influence of malting on selected components of soya bean, black bean, chickpea and barley. *Food Chem.* **1999**, *65* (1), 85–90.
- (212) Savelkoul, F. H.; Tamminga, S.; Leenaars, P. P.; Schering, J.; Ter Maat, D. W. The degradation of lectins, phaseolin and trypsin inhibitors during germination of white kidney beans, *Phaseolus vulgaris* L. *Plant Foods Hum. Nutr.* **1994**, *45* (3), 213–22.
- (213) Coffey, D. G.; Uebersax, M. A.; Hosfield, G. L.; Bennink, M. R. Stability of red kidney bean lectin. *J. Food Biochem.* **1992**, *16* (1), 43–57.
- (214) Martín-Cabrejas, M. A.; Sanfiz, B.; Vidal, A.; Mollá, E.; Esteban, R.; López-Andréu, F. J. Effect of fermentation and autoclaving on dietary fiber fractions and antinutritional factors of beans (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* **2004**, *52* (2), 261–266.
- (215) Zhou, H.; Wang, C.; Ye, J.; Tao, R.; Chen, H.; Cao, F. Effects of enzymatic hydrolysis assisted by high hydrostatic pressure processing on the hydrolysis and allergenicity of proteins from Ginkgo seeds. *Food Bioprocess Technol.* **2016**, *9* (5), 839–848.
- (216) Matsuda, A.; Tanaka, A.; Pan, W.; Okamoto, N.; Oida, K.; Kingyo, N.; Amagai, Y.; Xia, Y.; Jang, H.; Nishikawa, S.; Kajiwar, N.; Ahn, G.; Ohmori, K.; Matsuda, H. Supplementation of the fermented soy product ImmuBalance effectively reduces itching behavior of atopic NC/Tnd mice. *J. Dermatol. Sci.* **2012**, *67* (2), 130–139.
- (217) Kase, R.; Singh, A. B.; Lavasa, S.; Prasad, K. N.; Arora, N. Enzymatic hydrolysis: A method in alleviating legume allergenicity. *Food Chem. Toxicol.* **2015**, *76*, 54–60.
- (218) Pusztai, A.; Palmer, R. Nutritional evaluation of kidney beans (*Phaseolus vulgaris*): The toxic principle. *J. Sci. Food Agric.* **1977**, *28* (7), 620–623.
- (219) Gabriele, M.; Sparvoli, F.; Bollini, R.; Lubrano, V.; Longo, V.; Pucci, L. The impact of sourdough fermentation on non-nutritive compounds and antioxidant activities of flours from different *Phaseolus vulgaris* L. Genotypes. *J. Food Sci.* **2019**, *84* (7), 1929–1936.
- (220) Dodo, H. W.; Konan, K. N.; Chen, F. C.; Egnin, M.; Viquez, O. M. Alleviating peanut allergy using genetic engineering: The silencing of the immunodominant allergen Ara h 2 leads to its significant reduction and a decrease in peanut allergenicity. *Plant Biotechnol. J.* **2008**, *6* (2), 135–145.