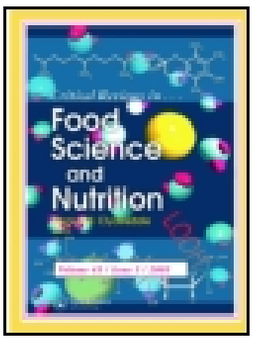

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Phaseolus vulgaris lectins: a systematic review of characteristics and health implications

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Abstract

Legume lectins are carbohydrate-binding proteins of non-immune origin. Significant amounts of lectins have been found in *Phaseolus vulgaris* beans as far back as in the last century;

however, many questions about their potential biological roles still remain obscure. Studies have shown that lectins are anti-nutritional factors that can cause intestinal disorders. Owing to their ability to act as toxic allergens and hemagglutinins, the *Phaseolus vulgaris* lectins are of grave concern for human health and safety. Nonetheless, their potential beneficial health effects, such as anti-cancer, anti-human immunodeficiency virus (anti-HIV), anti-microbial infection, preventing mucosal atrophy, reducing type 2 diabetes and obesity, promoting nutrients absorption and targeting drugs, are of immense interest. The significance of *Phaseolus vulgaris* lectins in biological researches and the potential biomedical applications have placed tremendous emphasis on the development of purification strategies to obtain the protein in pure and stable forms. These purification strategies entail considerations such as effects of proteolysis, heating, gamma radiation and high-hydrostatic-pressure that can have crucial outcomes in either eliminating or improving bioactivities of the lectins. Thus, up-to-date research findings of *Phaseolus vulgaris* lectins on different aspects such as anti-nutritional and health impacts, purification strategies and novel processing trends, are systematically reviewed.

Keywords: Lectin, *Phaseolus vulgaris* bean, beneficial and detrimental properties, purification strategy, processing

INTRODUCTION

Phaseolus vulgaris denotes the common bean, and includes types such as black turtle bean, string bean, flageolet bean, kidney bean, pea bean, pink bean, pinto bean, white bean, yellow bean, cranberry and borlotti bean and so on. This kind of beans constitutes one of the most important varieties of cultivated grain legumes, and is widely used throughout the world for direct human consumption, especially in tropical and subtropical countries of the Americas, Europe, Africa and Asia. *Phaseolus vulgaris* beans are rich in proteins (22-27% of seed weight) and carbohydrates (39-47% of seed weight), thus, making them valuable food ingredient for over half a billion people (Gepts, 2001; Van der Poel et al., 1990). However, the high culinary use of *Phaseolus vulgaris* beans is currently a source of significant concern for human health and wellness due to their potential to elicit toxic and allergenic reactions (Kumar et al., 2013a; Fu et al., 2002). This has therefore stimulated great interest in the toxicological evaluation of the components in the beans.

Lectins are the sugar-binding proteins of non-immune origin with the capacity to recognize specific carbohydrates or glycoproteins and/or agglutinate cells without modifying them (Franz et al., 1982; Lis and Sharon, 1998; He et al., 2015d). They are ubiquitous macromolecules widespread in nature – not only in plants, but also in animals and microorganisms (Esko and Sharon, 2009), where they perform various functions such as regulation of (i) cell adhesion during glycoprotein synthesis and (ii) protein levels in blood receptors in animals; and for (iii)

cell attachment and tissue colonization in microorganisms. Lectins are invariably resistant to degradation by heating and digestive enzymes, and they can bind to the surface of epithelial cells in the digestive system because of their high affinity for carbohydrates, and can result in toxic reactions with changes in intestinal permeability (Menard et al., 2010; Miyake et al., 2007). There have also been extensive studies in recent times on the potential health benefits of lectins. It has been demonstrated that plant lectins may be involved in the defense mechanisms of plants via antifungal activities (Rüdiger and Gabius, 2001; De Hoff et al., 2009; Etzler, 1985). Plant lectins have also been reported to have promising biologically and medical uses for the isolation of glycoconjugates from cells, recognition of microorganisms, monitoring changes of carbohydrate expression on living cells, mitogenic simulation, anti-proliferative effects, anti-tumor, HIV reverse transcriptase inhibition, blood typing and targeting of drugs to the gastrointestinal tract (Lehr, 1993; He et al., 2013; Gabius et al., 2011; Zhang et al., 2008). Because of the general ability to bind with specific carbohydrates or glycoproteins, the exploration of plant lectins for novel biological action is both timely and relevant.

Although different reviews of the occurrence and structural characteristics of plant lectins have been published (Rüdiger and Gabius, 2001; Zhang et al., 2008; Lis and Sharon, 1998; Gabius et al., 2011; Liu et al., 2010), there is a paucity of information providing a more comprehensive and balanced approach to understand the potential health implications of *Phaseolus vulgaris* lectins. Thus, a better understanding of both the beneficial and detrimental

effects of lectins is needed.

The general methods used in the preparation of lectins include precipitation with salts or acids, followed by several chromatographic purification strategies (He et al., 2013; Nascimento et al., 2012). Although affinity chromatography has been widely used in the laboratory practice, the entire purification process achieves low recoveries, and is tedious and time-consuming (Zhang et al., 2008). Due to the growing importance of the *Phaseolus vulgaris* lectins, the development of more cost-effective purification methods has become one of the focal points of lectin research.

It is also necessary to study the effects of processing on the integrity of lectins in order to justify the development of procedures to eliminate or inactivate the legume protein to enhance consumer safety (Sathe et al., 2005). Although it is well known that the conformation of protein molecules in general can be altered by thermal and/or non-thermal treatments, there is no such direct information on *Phaseolus vulgaris* lectins to verify the effectiveness of such treatments on these particular molecules.

A SHORT OVERVIEW ON THE PHASEOLUS VULGARIS LECTINS

Toward the middle of the 20th century, Nowell (1960) made the discovery at the University of Pennsylvania, Philadelphia, that lectin of the red kidney bean (*Phaseolus vulgaris*), known as phytohemagglutinin (PHA), is mitogenic. This finding brought lectins into the limelight (Sharon and Lis, 2004), and since then, there has been great strides in the knowledge of lectin and

lectinology.

Phaseolus vulgaris lectins exhibit remarkable sequence homologies with other legume lectins (He et al., 2015b). The tertiary structures of this kind of lectins are now well known as the 'jelly roll' fold, similar to the jelly roll topology commonly found in viral coat proteins (Loris et al., 1998). The monomer of *Phaseolus vulgaris* lectins is formed largely of 3 β-sheets, i.e., an antiparallel 6-stranded back sheet, a concave 7-stranded front sheet, and a smaller 5-stranded sheet (Banerjee et al., 1996). In contrast with other legume lectins, short α-helices are usually found in the structures of the *Phaseolus vulgaris* lectins (Nagae et al., 2014). In general, PHA consists of two types of polypeptide chains called E and L reflecting their preferential binding to erythrocytes and leukocytes, respectively. Thus, five possible tetrameric isolectins (~ 120 kDa), i.e., E_4, E_3L_1, E_2L_2, E_1L_3 and L_4 can be formed at random, and a larger channel is created in the middle of the tetramer by the stranded back sheet, and may thus protect the protein from proteolytic degradation (Loris et al., 1998).

The carbohydrate-binding sites of lectins are located mostly in the β folds of the 7-chain sheet, which can recognize not only monosaccharides, but also oligo- and polysaccharides. Both PHA subunits contain the characteristic N-glycosylation binding sites, and PHA-E is specific for bisected complex-type N-glycans with an outer Gal and bisecting Glc*N*Ac, while PHA-L specifically binds tetra- and triantennary complex-type *N*-glycans with β6-branching (Hirabayashi et al., 2011; Gabius et al., 2011). The metal ions Ca^{2+} and Mn^{2+}, are in close

proximity to the carbohydrate binding sites, which can help form an active stable conformation called 'locked', having a much greater affinity for saccharides (Brewer et al., 1983).

THE POTENTIAL HEALTH IMPLICATIONS OF PHASEOLUS VULGARIS LECTINS

The *Phaseolus vulgaris* beans are rich sources of lectins in human diet, and it has become clear that this kind of proteins can cause diseases, as their biologically and immunologically intact forms are resistant to digestion in the body (Woodley, 1999; Bardocz et al., 1995). Although complete proof is still lacking in most cases, lectins have been considered by most biomedical scientists for use in therapy in a variety of diseases (Freed, 1991; Mascanfroni et al., 2011; Lam and Ng, 2011b; Melnykova et al., 2013). Thus, increasing concerns are currently established about the safety and efficacy of the *Phaseolus vulgaris* lectins as shown in Table 1.

Toxicity

In general, the oral acute toxicity of *Phaseolus vulgaris* lectins manifests as nausea, vomiting, bloating and diarrhea in humans when they are exposed to high concentrations of the protein; and lectins may have reversible effects on the growth and metabolism of experimental animals (Burbano et al., 1999; Vasconcelos and Oliveira, 2004). The literature is replete with data on the toxic effects of PHA (Buul and Brouns, 2014; Kumar et al., 2013a; Fantini et al., 2009; Vasconcelos and Oliveira, 2004). It has been suggested that PHA has a high oral toxicity for rats, and hemagglutinating effects of the protein were found for normal and enzyme treated erythrocytes (Grant et al., 1985). However, there is a lack of substantial and credible evidence

that can firmly establish the relationship between hemagglutinating ability and oral toxicity (Vasconcelos and Oliveira, 2004).

There is strong binding of PHA to the brush border membrane of the small intestine, and it has been shown that undigested PHA has profound effects on hyperblastosis, and tissue growth was even more extensive at a higher concentration (42 mg PHA/day/rat) (Bardocz et al., 1995). The disruption and abnormal development of intestinal microvilli was also observed when rats were fed high-lectin ground beans (300 mg beans/rat) (King et al., 1980). Furthermore, because of the affinity of undigested lectins to the surface of gut epithelia cells, plasma membrane repair was inhibited, resulting in necrotic cell death of the wounded cell (Miyake et al., 2007).

However, not all *Phaseolus vulgaris* beans are known for their toxicity, e.g., the small white navy beans or white kidney beans (*Phaseolus vulgaris*) have negligible levels of lectins, and the "no-observed-adverse-effect level" (NOAEL) of the bean extracts was established as 175 g/day/70 kg body weight (2500 mg/day/kg rat), which is much higher than the recommended safety daily amount (85 mg/day/70 kg body weight) (Chokshi, 2007). It has been demonstrated that the toxicity of PHA could be destroyed by adequate cooking (FDA/CFSAN, 2012). Even so, in considering the application of natural lectins in drug delivery systems or other therapeutics for humans, the toxicity of lectins should be carefully evaluated by rigorous toxicological testing, although only microgram quantities of the lectins can enter into the GI tract (He et al., 2015d). In addition, it is time consuming and costly to use the *in vivo* feeding methods to predict the oral

toxicity of the protein, therefore, establishing a quick screening procedure would be useful.

Immunogenicity

The administration of *Phaseolus vulgaris* lectins such as PHA to rats, was found to induce immunoglobulin (Ig) E-mediated reactions, and sometimes simultaneously with IgG-mediated reactions (Rougé et al., 2010; Kumar et al., 2013b).

A continuous immune response observation suggested that the normal defense mechanisms were repressed by feeding kidney beans (*Phaseolus vulgaris*) to mature rats, resulting in an insufficient secretion of IgA, and inability to block the absorption of the lectins (Grant et al., 1985). Hence, lectins may effectively stimulate immune responses after oral administration. After intranasal or oral immunization of PHA, although only IgG1 was measured at a significant level in the IgG subclasses, the systemic and mucosal antibody responses were triggered with the detection of specific IgG in immunized BLAB/c mice (Lavelle et al., 2000). However, the selective humoral response of the IgG-type was also unable to prevent the uptake of the lectin into the systemic circulation (Vasconcelos and Oliveira, 2004).

Because of the strong binding of lectins to glycoproteins, the systemically absorbed *Phaseolus vulgaris* lectins may bind to the serum basophils *in vivo* after absorption through mucosa. Consequently, the type 2 helper T cells are activated. After the primary exposure of the lectin to lymphocytes and monocytes, interleukin-4 (IL-4) and IL-13 are secreted by the activated human basophils, following the IgE synthesis (Kumar et al., 2013a). The mechanism of

the release of mediators such as interleukin-(IL-)4 and IL-13 by basophils via the trigger of lectins is still not completely understood. It is speculated that several allergic mediators, such as histamine, leukotrienes, and prostaglandin D2 are released upon IgE cross-linking the Fcε receptors (FcεRI) mediated by lectins or via direct cross-linking between the lectins and this receptor (Haas et al., 1999; Shibasaki et al., 1992; Kumar et al., 2013b). Hence, an immediate hypersensitivity reaction towards a Th2 response and type I allergy is expressed because of the participation of these mediators.

In addition, it has been suggested that there was a difference between male and female mice in the modulations of IgE responses by PHA treatment, as sex hormones might be involved in the IgE synthetic regulatory pathways (Astorquiza et al., 1987). Furthermore, in contrast with a previous study using 42 mg/day/rat, a low dose (6 mg/day/rat) of PHA was reported not to induce the IgE-mediated allergy after intragastric administration to Sprague-Dawley (SD) rats, which might be because of the antagonism effect on the IL-4 and IL-13 caused by the release of both interferon-γ and IL-12 from peripheral blood mononuclear cells (Haas et al., 2001). Other studies have confirmed that heat inactivated *Phaseolus vulgaris* lectins do not play a role in the initiation of an allergic immune response (Jasin and Ziff, 1968). Since much of the studies were carried out in rats or *in vitro*, the applicability of the immune responses in humans should be evaluated extensively. Thus, much more information on human immune profiles to *Phaseolus vulgaris* lectins is specially required.

Anticarcinogenic potential

Since the first discovery by Liener and Seto (1955) about 60 years ago suggested that soybean agglutinin (SBA) might be a therapeutic agent against the Walker tumor, considerable knowledge has accumulated confirming plant lectins as having anticarcinogenic activity; and biomedical application in cancer treatment is gaining impetus in the field of lectinology. Although the therapeutic pathways are still unclear on the molecular basis, numerous recent studies have showed the beneficial effects of *Phaseolus vulgaris* lectins in the possible therapies for liver carcinoma, lung cancer, nasopharyngeal carcinoma, breast cancer, melanoma, myeloma, lymphoma and so on (Fang et al., 2011; Ang et al., 2014; Lam and Ng, 2011a; Kuo et al., 2011; De Mejía and Prisecaru, 2005).

The affinity of *Phaseolus vulgaris* lectins for N-linked sugars in the cell membrane glycocalyx may imply the inhibition possibility for tumor malignancy. Owing to the affinity properties, lectins can bind to cancer cell membranes or their receptors, inducing cytotoxicity and apoptosis or autophagy (De Mejía and Prisecaru, 2005; Liu et al., 2010). In one recent study, two new *Phaseolus vulgaris* lectins were purified from extra-long autumn purple beans and blue tiger king beans. Upon their use both in dose- and time- dependent ways to inhibit the proliferation of human tumor cells, both lectins could elicit production of nitric oxide (NO) probably via the up-regulation of inducible NO synthase (iNOS), which is anticarcinogenic, to produce the apoptotic bodies (Fang et al., 2010; Fang et al., 2011). PHA-L, one of the isoforms

of PHA that can bind mannose, was observed to promote autophagic cell death of hepatoma cells in a BCL2/adenovirus E1B 19 kd-interacting protein 3 (BNIP3)- mediated mitochondria pathway (Lei and Chang, 2007). After the membrane glycoprotein agglutination, the lectins are internalized into the hepatoma cells, and the BNIP3 is gradually induced with the rapid accumulation effect of the lectins onto the mitochondria (Liu et al., 2010). Furthermore, undigested *Phaseolus vulgaris* lectins can simultaneously activate the immune system that secrete various interleukins, including IL-1b, Il-2, TNF-a and TNF-γ, involved in the apoptosis (Fang et al., 2011).

In addition, the administration of polyethylene glycol-modified lectin to mice showed that the anti-tumor cytotoxicity of peripheral lymphocytes against melanoma cells was enhanced by the chemical modification (Ueno et al., 1999). Due to the selective targeting of lectins to malignant cells, the utilization of the lectins as carriers for targeted drug delivery was found to be effective for cancer therapy against tumor cells (Khopade et al., 1998). However, compared with the studies of other lectin families, such as Con A, BSA and so on, studies on the anticarcinogenic potential of *Phaseolus vulgaris* lectins is still limited. Therefore, the potential of *Phaseolus vulgaris* lectins ought to be elucidated on the molecular level as well as in clinical trials.

Anti-human immunodeficiency virus (HIV) activity

It has been demonstrated that plant lectins, particularly *Phaseolus vulgaris* lectins, can

potently and selectively inhibit leukemia viruses (Hirsch et al., 1972), the acquired immune deficiency syndrome (AIDS) virus (Koyanagi et al., 1987), myxoviruses (Vlietinck et al., 1998) and coronaviruses (Keyaerts et al., 2007). Thus, the remarkable anti-HIV activity of *Phaseolus vulgaris* lectins has been a hot area of research in recent years.

Considering the rapid spread of the AIDS epidemic and the attendant high mortality, much effort has been devoted to search for new agent that can be used to treat and prevent HIV virus and AIDS infections. *Phaseolus vulgaris* lectins were also found to block syncytium formation between HIV-infected cells and uninfected cells, which efficiently prevented the virus transmission to the normal T-lymphocytes (Balzarini, 2006). It is estimated that the entry of HIV into its target cells is mediated by the glycoproteins gp120 and gp41 which abundantly contain the N-linked carbohydrates on the envelope, thus the lectins are presumed to directly bind with the specific glycosylation sites (Vlietinck et al., 1998). It has also been demonstrated that *Phaseolus vulgaris* lectins can hinder HIV replication directly inhibiting HIV-1 reverse transcriptase (HIV-1 RT), which is a crucial enzyme for the viral replication (Ng et al., 2002; Lam and Ng, 2011b; Zhang et al., 2008). PHA exerted a strong inhibitory effect on HIV-1 protease and HIV-1 integrase, whereby, about 95.4% HIV-1 RT were inhibited by 5 mg/ml red kidney bean lectin with an EC_{50} of 2.19 mg/ml (Wang and Ng, 2001). Furthermore, lectins found in other *Phaseolus vulgaris* beans, such as the extra-long autumn purple bean lectin, showed stronger anti-HIV-1 RT activities (Fang et al., 2010). Because of the efficacy of these lectins,

further studies on the anti-HIV activity should be conducted in early AIDS clinical trials.

Implications for anti-microbial infective therapy

Investigation of microbial infections is cutting edge research, which benefits the health impacts of bacterial and fungal pathogens. Recent researches have verified that pattern recognition receptors (PRRs) can signal the presence of infection to the host, and the Toll-like receptor (TLR) family is one of the best-studied PRRs in mammalian species (Iwasaki and Medzhitov, 2004). TLRs have a central role in innate immunity, and they can also trigger antimicrobial host defense responses for inflammatory therapeutic effects (O'Neill et al., 2013).

PHAs are known to be potent lymphocyte mitogen (Nowell, 1960), and research have demonstrated pro-inflammatory cytokine production based on the ability of PHA for immunoregulation. Thus, PHA-L has been proposed as a specific human TLR4 agonist during a plant lectins screening, and PHA-P (mixtures of PHA that include PHA-L and PHA-E isolectin subunits) seemed to have much more intrinsic activities in stimulating TLR4 activity than PHA-L. PHA-L also stimulated extracellular TLRs2/6, 4 and 5 against a suit of hrTLR cell reporter assays (2/6, 3, 4, 5, 7, 8 and 9) (Unitt and Hornigold, 2011). Considering that each lectin family has different sugar specificity, the recognition motifs of lectins to the appropriate oligosaccharide surface expression on different TLRs play a decisive role in TLR agonism (Silva and Correia, 2013; Unitt and Hornigold, 2011).

In recent years, the host-derived nucleic acids have been recognized as endogenous ligands

for TLRs (O'Neill et al., 2013). It was speculated that the capacity of *Phaseolus vulgaris* lectins to modulate the host-derived nucleic acids might be explored to activate TLRs, and then lead to autoimmunity (Marshak-Rothstein, 2006). Therefore, lectins act by increasing the immune response of the host against microbial infections and this can be used to develop novel anti-inflammatory and immunomodulatory agents, which are useful in therapy.

Other possible beneficial effects

As knowledge of the carbohydrate-binding properties and mitogenic activities of *Phaseolus vulgaris* lectins has increased, the possible beneficial effects have arisen for the exploitation of the biomedical applications of the proteins.

Total parenteral nutrition (TPN) is a valuable way in medical care by supplying all the nutritional needs of the body via bypassing the digestive system and dripping nutrient solution directly into a vein. However, it often causes important clinical complications, such as gastrointestinal atrophy, which can result in malabsorption, diarrhea, intestinal permeability increasing and bacterial translocation (Deitch et al., 1995). Recently, *Phaseolus vulgaris* lectins were suggested as agents to prevent mucosal atrophy and its associated complications when patients are receiving TPN, as lectins can bind in different regions of the gastrointestinal tract and stimulate epithelial proliferation (Lajolo and Genovese, 2002). It was found that PHA significantly increased proliferation in the gastric fundus and small intestine, but had little effect in the mid-colon (Jordinson et al., 1999; Sasaki et al., 2002).

Since type 2 diabetes and obesity are now becoming a pandemic that can lead to serious health problems, medical attentions to these patients are urgent. Extracts of *Phaseolus vulgaris* beans seem to be promising remedies for the reductions of body weight, lipid accumulation and glycemia (Spadafranca et al., 2013). Except the actions of α-amylase in the *Phaseolus vulgaris* beans to inhibit carbohydrate metabolism and absorption, PHA may play a key role to simulate the release of cholecystokinin and glucagon-like peptides, which are two critical hormones in the regulation of digestive processes and appetite, via binding to the stomach epithelial cells or the brush border membrane of small intestine, cecum and colon (Carai et al., 2009). Furthermore, no loss of body protein or skeletal muscle was observed, even by increasing to a high lectin dose of intake (≥ 0.4 g/day/kg obese Zucker (fafa) rat), suggesting that it may also be possible to use *Phaseolus vulgaris* lectins as a dietary adjunct or therapeutic agent to control the risks of extra weight gain (Pusztai et al., 1998).

Additionally, although high intake of lectins has been demonstrated to damage the gut leading to various nutritional disorders, it was shown that at least up to 200 μg/ml dose of plant lectins had no apparent cytotoxicity against Caco-2 cells, and at the same time, it could modulate the transports of food factors via improving the permeability of Caco-2 cell monolayers (Ohno et al., 2006; Ohno et al., 2004). Although the regulatory mechanism(s) of lectins still remains to be elucidated, their implication(s) for the intestinal absorption of nutrients seems to be of great significance.

Also, in order to improve the safety and effectiveness of the administration of peptides, proteins and other macromolecular drugs, the lectin-mediated delivery systems have been developed in recent years, as lectins can specifically recognize the receptor-like structures of the cell membrane, and bind directly to the epithelial cells, and subsequently trigger the active transport of the medicinal ingredients (Lehr, 2000). *Phaseolus vulgaris* lectins could have potential as 'natural' mucoadhesives, however, they should be considered carefully with respect to the problems of toxicity (Woodley, 1999).

The consumption of *Phaseolus vulgaris* lectins has been both associated with numerous health risks and important pharmacological effects to humans. Therefore, the purification of the actual active proteins from crude extracts has gained tremendous emphasis in recent years (Sharon and Lis, 2004).

THE PURIFICATION STRATEGIES OF PHASEOLUS VULGARIS LECTINS

Up to now, nearly 3,000 articles have reported on the purification of *Phaseolus vulgaris* lectins as the main subject. The strategies commonly used for lectin purification are summarized in Figure 1. In particular, great strides have been made with the introduction of affinity chromatography, to bind lectins on the basis of specific carbohydrate affinity via cross-linked dextran gels (Agrawal and Goldstein, 1965). In this section, the developments of purification strategies using various protocols are discussed.

Chromatographic purification

The cotyledons or endosperm of the bean are usually rich in lectins, while the lectin content is low in the embryo and in the seed coat. Although it is difficult to remove the seed coat before purification, it is highly recommended to do this because the tannins, dyes and detrimental substances contained in the seed coats may disturb the specific precipitation of lectins, leading to brownish colored products, or resulting in the irreversible damage to the column materials via adsorbing to them and spoiling them (Rüdiger, 1988).

In general, the purification of lectins starts with an extraction step with buffer solutions and water, as the lectins are water-soluble proteins. In this regard, the slightly alkaline buffer solutions of not too low ionic strengths (≥ 10 mM) have been confirmed to be more suitable, because the pH and ionic strength are crucial for complete extraction (Zhang et al., 2008). This aqueous extraction step is followed by salt precipitation, and then typically by one to three or four chromatographic steps, such as ion exchange chromatography, size exclusion chromatography, hydrophobic interaction chromatography and affinity chromatography (He et al., 2015c). A general procedure for the purification of *Phaseolus vulgaris* lectins is shown in Figure 2.

The yields and the purification factor (PF, based on the specific activity of lectin) are the focal points of the lectin purification strategy. Experience has shown that the desired level of lectin purity could be easily achieved by simply adding or repeating chromatographic steps, however, it is to be expected that the loss of protein will increase with each additional step

introduced in the purification scheme. In an analysis of the relationship between average recovery yields of lectins and the number of purification steps, the yield was sharply reduced to only about 5% after 4 processing steps, compared to the yield of just one step based on affinity chromatography which was about 38% (Nascimento et al., 2012).

It seems that the combination of ion exchange chromatography and size exclusion chromatography was the most popular purification procedure used in earlier studies to achieve successful insolation and purification of *Phaseolus vulgaris* isolectins (Leavitt et al., 1977; Felsted et al., 1977). Even as at now, ion exchange chromatography is still employed as one of the common lectin purification steps, being used in about 21.7% of the cases based on the statistical data from 46 recently published articles, followed by ammonium sulfate precipitation, mainly because of economic considerations (Nascimento et al., 2012). Nevertheless, since the target lectins cannot bind to gel filtration or size exclusion chromatography and volume capacity is limited, this particular chromatographic technique is always used as a follow up step after any of the selective and concentrating chromatographic techniques. Furthermore, both ion exchange chromatography and size exclusion chromatography can only process small amounts of the proteins and long periods of time would be needed to complete the process if not performed in the HPLC mode.

Nowadays, lectins from *Phaseolus vulgaris* beans have been widely purified by affinity chromatography using adsorbents of immobilized matrix-bound glycoproteins and glycopeptides,

after salt precipitation (Zhang et al., 2008; Felsted et al., 1975). The protocol is efficient to meet the requirement for higher yield and purity with a minimum number of chromatographic steps, based on the special ability of *Phaseolus vulgaris* lectins to bind to carbohydrates. The Affi-Gel blue gel (Bio-Rad) has been reported to be successfully used in the isolation of *Phaseolus vulgaris* lectins (Ye et al., 2001; Shi et al., 2007; Wong et al., 2010; Chan et al., 2012). However, considering the purity and hemagglutinating activity of the target samples, the Affi-Gel blue gel exhibited less affinity for the *Phaseolus vulgaris* lectins than the thyroglobulin (Tg)-Sepharose 4B matrix, as the Affi-Gel blue gel seemed to also bind non-lectin proteins. Furthermore, a larger variation in the yields has been reported in the above comparison, ranging from as low as 0.25 mg lectin/g bean meal by two chromatographic affinity steps to 2.26 mg lectin/g bean meal by Affi-Gel blue gel or 2.55 mg lectin/g bean meal by Tg-Sepharose for a single-step affinity chromatography. The one-step Tg-Sepharose purification seemed to be a promising method for the effective and time-saving preparation of *Phaseolus vulgaris* lectins (Ren et al., 2008). However, the relative high cost of the affinity absorbents and the natural defects of the insoluble polysaccharides materials both prevent the lectin purification from running in long columns or in batch procedures (He et al., 2013). Thus, there has been a growing interest in the development of effective large scale purification methods.

Membranes adsorption

Considering the valuable applications of lectins in research and the requirement of gram

quantities for the studies, membrane technology is perceived to be of great value to researchers, as it allows for the isolation and purification of a large amount of biomolecules and biopharmaceuticals in a relatively short time owing to the its finely organized and well-controlled macroporous polymeric stationary phases (Tennikova et al., 1990; Zou et al., 2001).

A semi-pilot-scale procedure has been designed to purify bean lectins, using the combination of microfiltration (with a molecular weight cutoff above 200 kDa), affinity chromatography and ultrafiltration (with a molecular weight cutoff in the range of 1-200 kDa depending on the molecular weight of target lectin). Although the yield of the lectin extracted (about 1.42 mg lectin/g bean meal) was similar with the small-scale affinity chromatography procedures, the obviously larger scale, low cost and faster speed for the processing of large quantities of samples make the membrane separation approach as a superior technique for purifying gram quantities of the lectins (Fasina et al., 2003). Hence, the semi-pilot-scale procedure should be further modified in a more logical sequence to use as few purification steps as possible to avoid the loss of the target protein.

The recently introduced high-performance membrane chromatography (HPMC) via the integration of membrane and column chromatography (such as ion exchange, hydrophobic interaction, reverse phase and affinity chromatography) provides a number of advantages in protein extraction, with regards to time-saving and higher activity recoveries (Tennikova and

Svec, 1993; Boi et al., 2006). Affinity-membrane chromatography has quickly become the stationary phase of choice for the separation and purification of lectin (Zeng and Ruckenstein, 1999b). The wheat germ agglutinin (WGA) was separated from a wheat germ extract in high purity and good yield (0.5 mg WGA/g wheat germ) via macroporous chitin affinity membranes (Zeng and Ruckenstein, 1999a). *Momordica charantia* seed lectins have also been successfully purified using the affinity membranes prepared by chemical modification of a cellulose matrix, while arabinogalactan displayed good binding capacity with galactose-specific lectins as ligand (Boi et al., 2006). Considering the biodegradability of the natural polymers, nanofibrous mats (polyacrylonitrile-based copolymers) represent another option for affinity separation. Based on this, a new kind of glycosylated nanofibrous membrane (GNM) was developed for the separation and purification of lectins on the basis of carbohydrate ligands that were coated on the nanofiber surface (Che et al., 2011).

Affinity precipitation

Salt precipitation, such as ammonium sulfate precipitation, is still a widely used method for fast and convenient protein purification, however, it lacks specificity. Thus, affinity precipitation was developed in the late 1970s to utilize a smart affinity macroligand to isolate the target protein (Hilbrig and Freitag, 2003). Since there is no resistance to mass transfer in the affinity interaction step and no steric hindrance in the association step, the ligands can bind to the target protein rapidly and have a higher available capacity, which can overcome the well-known

limitations of affinity chromatography, such as expensive, tedious and time-consuming (Senstad and Mattiasson, 1989).

The low binding constant between carbohydrate and target lectin, which often is of the order of 10^4 M^{-1}, well indicates the suitability of the affinity precipitation employed in the lectin purification. An affinity precipitation procedure was also developed using chitosan as a natural polyligand for the extraction and purification of WGA, and the overall yield of 70% and the high SDS-PAGE purity of the product clearly demonstrated that affinity precipitation was an efficient purification process, even for large-scale production (Senstad and Mattiasson, 1989). Furthermore, *p*-aminophenyl-α-D-glucopyranoside coupled to the polymer Eudragit S-100 was used to purify Con A in a model system, and the polymer-ligand could be recycled and used five times (Larsson and Mattiasson, 1994). Later, the polymer-ligand used in affinity precipitation was evaluated through the purification of bean lectin from extracts of soya flour. Although the purification and yield of the protein by the procedure was less than that by affinity chromatography, the co-precipitation process was confirmed to be easily used without too many pre-separation steps (Larsson and Mattiasson, 1996). The thyroglobulin extract was also designed to separate and purify PHA-E from *Phaseolus vulgaris* beans by the co-precipitation process, and protein of high purity was obtained after an ultrafiltration step (Zhang et al., 2011).

Recently, the aqueous two-phase affinity extraction (ATPAE) and macro-affinity ligand facilitated three-phase partitioning (MLFTPP) were explored as powerful extensions of affinity

precipitation. The affinity ligands linked to PEG were incorporated in PEG phase in the ATPAE, and the complex between the macroligand and the target protein was formed by the affinity partitioning. However, in either affinity precipitation procedures, it is crucial point to solve the difficulties to harvest the target lectin and to recover the ligand-polymer from the mixed precipitates (Larsson and Mattiasson, 1996); thus, the dissociation process should be considered carefully towards the development of a rational purification protocol.

Liquid-liquid extraction

It also has been demonstrated that liquid-liquid extraction, such as the aqueous two-phase system (ATPS) mentioned above, could serve as a useful process for the separation of lectins (Nascimento et al., 2012). When the immiscible or partially soluble liquid phases are brought into contact, an interface is formed, and the target lectin can transfer from one phase to another by the liquid-liquid extraction. So the ease of scale up and the high partition coefficients allow its application in downstream processing (Kilikian et al., 2000). There are two different well established systems of liquid-liquid extraction; one is the ATPS utilizing two immiscible aqueous phases of simple electrolytes and water soluble polymers or of two incompatible water soluble polymers, while the other is the reverse micellar extraction (RME) using the water in oil microemulsion (Pires et al., 1996).

Each phase in the ATPS generally contains 80-90% water, and provides gentle environments to preserve the biological activities of lectins. The polyethylene glycol

(PEG)/dextran and PEG/potassium phosphate systems have been well studied, and citrates could be used as the inorganic salts in the ATPS (Tubio et al., 2009). Although mass transfer is fast and equilibrium can be reached in a matter of seconds, there are several parameters, such as the properties of the target protein, the pH of the system, both the type and concentration of salts and polymers used in the system, to be adjusted for optimized protein purification (Raghavarao et al., 1995). The PEG/sodium sulfate systems have been selected to purify ricin B (RTB), the unstable non-toxic lectin subunit of ricin secreted from hairy root cultures, and it suggested that the ATPE could be effective in initial recovery/purification, as no apparent RTB degradation was observed in the top phase (Zhang et al., 2005). As a first step in the lectin extraction for commercial applications, the PEG-phosphate system may hold promise for the selective isolation of *Canavalia brasilienses* lectin (ConBr) from crude extracts of the seeds, and it indicated that only pH and PEG concentration had a significant effect on purity by ANOVA (Nascimento et al., 2010). In the PEG/sodium citrate ATPS, citrate concentration and PEG molar mass were shown to significantly affect lectin yield from *Canavalia grandiflora* Benth (ConGF) partitioning due to salting out and the volume exclusion effects, and the system proved to be efficient as a first step in the lectin purification process with a high recovery (Porto et al., 2011).

Few studies have been published on the purification of lectins from crude plant extracts using RME (He et al., 2013). However, RME has shown particular promise in the isolation of proteins/enzymes in the past two decades (Roy and Gupta, 2000). Reverse micelles (RM) are

thermodynamically stable nanometer sized droplets formed by self-aggregation of surfactant molecules in organic solvents containing an aqueous solution; hence they can prevent protein denaturation (Sii and Sadana, 1991; He et al., 2015c). The selective separation of the target protein from the crude extracts can transfer to the inner core of the RMs based on various factors such as pH, ionic strength of the aqueous phase, type and concentration of the surfactants, charge of the protein, temperature, etc., and the desired protein can be "back-extracted" into the aqueous phase under certain conditions of pH and ionic strength (Pires et al., 1996). The application of RME for the separation and purification of lectin was firstly demonstrated in a model system using the purified lectin from *Cratylia mollis* seeds via sodium bis (2-ethylhexyl) sulfosuccinate (AOT)/isooctane micelles, and the yields of 38% forward extraction and 100% backward extraction were obtained by the adjustment of pH (Nascimento et al., 2002). Recently, lectin from a crude extract of black turtle bean (*Phaseolus vulgaris*) was successfully extracted using the AOT/isooctane micelles as the first step of the purification. Although non-lectin proteins could crowd into the RMs, the total recovery of lectin content was high (about 9.48 mg lectin/g bean meal) with high purity as determined by SDS-PAGE and RP-HPLC analysis. Furthermore, FTIR spectra results indicated the lectin extracted by RME had similar structure to that obtained by the conventional methods. Thus, RME can be a valuable protocol for the purification of lectin (He et al., 2013; He et al., 2015c).

New trends in the lectin purification

It is possible to demonstrate that the affinity techniques are commonly used as the first purification step in the preparation of lectin. However, it is also clear that affinity chromatography requires the application of pre-purified samples to eliminate column clogging and increase of back-pressure, resulting in the small yield extractions, which are time-consuming and costly. Therefore, novel affinity methods need to be designed to be relatively easy and selective to purify the target lectin with high yields, purity and activity to meet the requirements for the industrial production and widespread commercialization of the proteins. Then non-chromatographic alternative techniques, such as the membrane technology and precipitation isolation, are integrated with the affinity ligands and developed. Considering the easy use in large-scale operations and feasibility for the recovery of the affinity ligands, magnetic affinity separation might be another choice. The magnetic affinity particles are currently mainly employed in the separation of nucleic acids, target cells and selected analytes (Safarik and Safarikova, 2004). The magnetic derivatives of chitosan and acetylated chitosan were firstly proved to absorb the N-acetylglucosamine specific lectins well. Also, it could be cheaper and simpler to elute the adsorbed target compounds with a change of pH and ionic strength of the elution buffer, followed by separation of the magnetic absorbents from the mixtures using a magnetic separator (Šafaříková and Šafařík, 2000). Recently, the grafting of glucose or mannose to aminated iron oxide nanoparticles and gum Arabic coated nanoparticles were both employed to adsorb Con A (Nascimento et al., 2012).

Interest in lectin research has dramatically increased the development of downstream purification processes with cost-effective applications. However, the production of lectins in the upstream process should not be ignored. With the introduction of recombinant techniques, the yield of lectins is higher (up to 20 mg/l culture medium) compared with the previous studies (0.1-5 mg/l culture medium) (Lam and Ng, 2011b). In general, *Escherichia coli* are the most popular expression systems used to produce a sizeable quantity of lectin. In a previous study, relatively high concentrations of recombinant PHA-E was achieved by cloning the cDNA in the methylotrophic yeast *Pichia pastoris* expression system, and approximately 100mg/l at the 2- and 200-l scale of proteins were successfully produced (Baumgartner et al., 2002). Furthermore, after further purification steps for the recombinant lectins, the valuable purified proteins could be used for in-depth studies of the effects of processing on the lectins to explore its biological and biomedical applications.

EFFECT OF PROCESSING ON STABILITY OF PHASEOLUS VULGARIS LECTINS

Both the detrimental and beneficial effects of *Phaseolus vulgaris* lectins have made it important to study the stability of the lectin after processing. However, the properties have not been properly assessed in view of the various practical processing applications of livestock consumption. The available information of the consequences of the processing on the lectin is summarized below:

Effect of proteolysis

Considering the allergenicity and bio-adhesion applications of lectins, it is necessary to assess the degradation of the proteins in the GI tract or other application environments. Many plant lectins have been found to be resistant to proteolysis, which has been considered as the main reason for their poor nutritional value *in vivo* (Vasconcelos and Oliveira, 2004). *Phaseolus vulgaris* lectins in particular were shown to have resistance to breakdown by proteolytic enzymes in both *in vivo* and *in vitro* situations (Lajolo and Genovese, 2002). Over 90% of the ingested PHA was still found in the feces of the study rats and were fully reactive towards rabbit antilectin antibodies by rocket immunoelectrophoresis, following intragastric administration of known amounts of pure lectins (Vasconcelos and Oliveira, 2004). In a peptic hydrolysis assay using simulated gastric fluid (SGF, pH 2.0), PHA-L showed resistance up to 60 min with a remnant of 56.5%, indicating the lectin can reach to intestinal mucosa (Kumar et al., 2014). The content of initial PHA decreased at a slower rate in the tryptic digestion (pH 8.0), and a large part of protein could still be observed by SDS-PAGE even after 48 h of hydrolysis at an enzyme to protein ratio of 1:50 (Morari et al., 2008). Similar *in vitro* digestion results were also obtained in the assessment of lectin from black turtle bean (*Phaseolus vulgaris*), which demonstrated resistance of *Phaseolus vulgaris* lectins to the action of proteolytic enzymes (He et al., 2015d).

However, the *in vitro* experiments proposed for lectin resistance to proteolysis are controversial, as the techniques of measuring the *in vitro* protein digestibility are not standardized. For this, the reproducibility of a common digestion protocol was assessed in nine

different laboratories (Thomas et al., 2004), and an appropriate kinetic model was developed to quantify the band densities on SDS-PGAE gels (Herman et al., 2006; Herman et al., 2007; He et al., 2015d). But more than that, considering the comparison of substrate ratios between the enzymatic digestion protocols and *in vivo* digestions, it is clearly found that the ratios used *in vitro* are likely to be several orders of magnitude greater than *in vivo* (Wickham et al., 2009). Furthermore, many of the current studies are based on the weight of the digestive enzymes rather than the activity, which make it difficult to compare the digestion protocols (Moreno, 2007).

It is also noteworthy that there is an obvious protection for the *Phaseolus vulgaris* lectins from proteolytic degradation in the presence of Ca^{2+}. Only 10% of the PHA was hydrolyzed by trypsin in the presence of Ca^{2+} in 48 h, versus 75% protein hydrolyzed in the absence of Ca^{2+} over the same time period (Morari et al., 2008). Meanwhile, only 3.45% of the residual lectin from black turtle bean (*Phaseolus vulgaris*) was visually detected using kinetic densitometric analysis after the final pepsinolysis of 60 min by the removal of metal ions, while the unhydrolyzed lectin fraction from tryptic digestion significantly decreased to 40.09% after 90 min in the demetallized state (He et al., 2015d). Since Ca^{2+} and Mn^{2+} are the metal ions essential for the stability of the lectin structures, it is possible that the folding of the protein is less tightly packed upon demetallization, and thus, more protease cleavage sites may be exposed to improve the susceptibility to the proteolytic enzymes.

Effect of thermal treatments

Similar with other antinutritional factors (ANFs), lectins are heat-stable proteins. Thereby, how to improve the nutritive value of legume seeds by thermal treatments was well studied in previous researches, and it has been well established that the lectins can be inactivated after special heating, such as cooking (around 100^{o}C), with an improvement of the accessibility of the protein to enzymatic attack (Coffey et al., 1992). The total lectin activity (hemagglutination activity (HA)) as a function of time and temperature has been commonly used for the assessment of the effectiveness of heat treatment (He et al., 2014). *Phaseolus vulgaris* lectins in the whole beans or protected by their specific carbohydrates exhibited a first-order reaction kinetics during the thermal inactivation process with a biphasic mechanism (Bonorden and Swanson, 1992; Boufassa et al., 1986). Furthermore, the thermal inactivation of *Phaseolus vulgaris* lectin from black turtle bean also followed a first-order reaction kinetics, and could be well described by the Arrhenius model with a relatively high enthalpy increment, accompanied by a striking loss of definable HA of the lectin at 90^{o}C for 5 min, which attested to the importance of temperature/time setting, in particular of high temperature, short time (HTST) treatment (He et al., 2014). Moreover, the residual activities of *Phaseolus vulgaris* lectins after the industrial and experimental heating processing, such as autoclaving, steam heating, extrusion and dry roasting, have been studied to improve the utilization of the beans. It was demonstrated that the HA of navy bean (*Phaseolus vulgaris*) lectin could be fully eliminated at 121^{o}C for 5 min after the autoclaving treatment, and white bean (*Phaseolus vulgaris*) lectin could be fully inactivated after

steam treatment of 100 ^{o}C for 15 min; while the extrusion treatment of 145^{o}C for 16 min resulted in 98% reduction in the HA of small red bean (*Phaseolus vulgaris*) lectin, as well as 99% reduction in the HA of navy bean (*Phaseolus vulgaris*) lectin observed with the dry roasting treatment at 200^{o}C for 23 min (Van der Poel, 1990; Shimelis and Rakshit, 2007).

The enormous interests in the practical applications of lectins in biotechnology on the basis of their physico-chemical properties require spectroscopic and thermodynamic techniques to gain insight into the thermal denaturation of the proteins. In this regard, differential scanning calorimetry (DSC) in particular, appears to be the most useful approach for these purposes (Marcos et al., 1999). Contrary to the previous reports on legume lectins, the thermal denaturation studies showed that the PHA-L exhibited irreversible thermal unfolding at neutral pH and remarkable thermostability, as the thermal transition obtained at about 80^{o}C at pH 2.3 suggested there existed a compact-protein structure in the acidic pH environment (Biswas and Kayastha, 2002). Irreversible thermal denaturation of lectin from black turtle bean (*Phaseolus vulgaris*) was also observed at various pHs (2.0-10.0), and the first-order two-state kinetic model was applied to explain the scan-rate dependent DSC transitions (He et al., 2015a).

Considering the significance of Mn^{2+} and Ca^{2+} on the stable feature in the legume lectin crystal structures, thermal stability of Mn^{2+} binding sites in the lectin from kidney bean (*Phaseolus vulgaris*) were studied using electron spin resonance (ESR) spectroscopy in the temperature range of -153 to 127^{o}C. It was demonstrated that the irreversible changes in the

nature of the lectin was dependent on the atmosphere under which the annealing was performed, and that could also influence the activation energy computed. Nevertheless, it was confirmed that the Mn^{2+} binding sites were rapidly degraded above approximately $80^{o}C$ (Coşkun and Korkmaz, 2009).

Effect of gamma radiation

Since non-thermal processing methods, such as the gamma radiation, have been employed to control foodborne pathogens and extend the shelf of the food products, it is to be wondered whether the approach could also reduce the allergenicity of the *Phaseolus vulgaris* lectins without altering the nutritional values. It has been demonstrated that the specific IgE binding of kidney bean lectins was reduced by 34% followed by 25 kGy gamma radiation treatment (Kasera et al., 2012). Moreover, considering that lectin is one of the major allergens, the efficacy in attenuating allergenicity of legume bean lectins indicated that the gamma radiation may have a significant effect on the structure and function of the protein.

The relationship of allergenicity and molecular structure of the lectin from *Cratylia mollis* seeds after gamma irradiation was evaluated. It was confirmed that the exposure of the lectin to high doses (10-25 kGy) of gamma irradiation produced alterations to the structures by a breakdown of polypeptide chains, and resulted in the formation of molten globule states, as neither tertiary nor secondary structure was observed with a lack of native β-rich structures as measured by the fluorescence and far-UV CD; and the lectin was proved to be harmless as a

result of conformational changes. This could also be another research approach to study protein unfolding, misfolding and aggregation. Meanwhile, a minimal impact of the low dose radiation (1 kGy) on the allergic response of the lectin was also obtained, however, it was speculated that the low doses of radiation could destroy epitopes and generate new ones which could make it even more detrimental (Vaz et al., 2013).

In a recent study, the effect of gamma irradiation on the *Phaseolus vulgaris* lectins was evaluated. It was observed that the radiation damaging effect on PHA contained in the *Phaseolus vulgaris* bean under different moisture conditions started at gamma irradiation doses of 30-50 kGy, while the obvious decrease in the content of the protein was exhibited at 100 and 200 kGy. It also suggested that the hydrophobic core of the PHA was destroyed by the gamma irradiation, which was favorable to the denaturation and aggregation of the protein. Since a pronounced inactivation of purified PHA in aqueous solution was obtained at a dose of 10 kGy, the presence of other food components in the *Phaseolus vulgaris* bean such as carbohydrates was suggested to protect the protein from radiation damage, besides moisture. In the aqueous system, the collapse of the structure of the purified lectin was shown to start at 1 kGy, and complete destruction was achieved at 10 kGy (Mallikarjunan et al., 2014). Thus, radiation may play a role not only in abolishing allergenicity in the bean food, but also in the regulations of the lectins on the immune responses and biomedical applications.

Effect of high hydrostatic pressure

More recently, high hydrostatic pressure (HHP) has been widely applied within the food industry with the outstanding advantages for extending shelf-life of food products without a reduction in nutritional value and flavor (San Martin et al., 2002). To date, few studies have been carried out to investigate the effect of high pressure treatment on the lectins. Thus, the recent systematic study demonstrating practical application of HHP in abolishing lectin activity in *Phaseolus vulgaris* bean system is noteworthy. After HHP-treatment of *Phaseolus vulgaris* beans at ambient temperature, PHA was purified using the Affi-Gel blue gel column, followed by PAGE gels, SEC, FTIR and DSC analyses. In the work, the hemagglutination activity of PHA was not significantly changed at low pressure treatment of 150 MPa, nevertheless, the unfolding process seemed to be started at 50 MPa to form the molten globule state. However, it clearly indicated that the native structure of PHA had not been completely lost with the HHP treatment. It is compelling that the higher pressure (450 MPa) was found to induce rearrangements and aggregation of the protein, with a noticeable decrease in the hemagglutination activity. Considering the formation of unknown components of a larger molecular weight, the PHA in the bean system might be protected by the other low-molecular-weight proteins in the HHP treatment (Liu et al., 2013). Furthermore, more studies are needed to focus on the effect of HHP on the purified lectin in the future.

CONCLUSION

Due to its low fat, high fiber and rich protein content, *Phaseolus vulgaris* beans are

commonly recommended to be one of the major food sources for good health. Nonetheless, the wide consumption of *Phaseolus vulgaris* lectins continue to pose grave public health concerns because of the perceived adverse effects. The current scientific evidence is strong and consistent to indicate that the lectin can cause much gastrointestinal damage or disease and possible deleterious health effects via reacting with the surface epitheliums on the basis of specific carbohydrate affinity. The evidence we have presented shows the autoimmunity and IgE-mediated allergy may be ideally suited to explain the effect of lectin on the immune system, endocrine system and nervous system for a rational therapy. Since the *Phaseolus vulgaris* lectins have become the focus of intense interest in particular for developing pharmaceutical preparation and health care services, more of this kind of proteins should be purified, to permit further studies with respect to toxicity and immunogenicity. Affinity technologies in the past decades have led to major developments in the isolation and purification of lectins from crude extracts, which is meeting the faster research developments for the protein. More rapid and highly selective purification strategies must be developed to enable separation and purification of lectins under gentle and mild reaction conditions to protect the integrity of the molecules. Considering the beneficial and potential properties of lectins, the utilization and effects of various processing methods on the bioactivity of the molecules should be re-evaluated, as they may lead to conformational changes in the protein, result in the changes in bioactivities. Therefore, there is a critical need to evaluate the current available treatments on lectins, to assist the rational

processing designs by food scientists, biologists and pharmaceutical researchers.

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Table 1 Examples of *Phaseolus vulgaris* lectins with potential health implications [a]

Plant sources	Lectins	Biological effects	Mechanisms	References
Red kidney bean	PHA-P	High oral toxicity, allergenicity, anti-HIV, immunoregulation	Hemagglutinating ability, cell adhesion, up regulated expression of GATA-3 and T-bet, inhibition reverse transcriptase of HIV, stimulation TLR4 activity	Buul and Brouns (2014), Kumar (2013a), Kumar et al. (2013b), Unitt and Hornigold (2011), Zhang et al. (2008)
	PHA-L	High oral toxicity, allergenicity, anti-tumor, immunoregulation	Hemagglutinating ability, cell adhesion, up regulated expression of GATA-3 and T-bet, promotion autophagic cell death, induction cell apoptosis, stimulation TLRs2/6, 4 and 5 activities	Buul and Brouns (2014), Kumar (2013a), Kumar et al. (2013b), Unitt and Hornigold (2011), Fang et al. (2011), Liu et al. (2010), Lei and Chang (2007)
	PHA-E	High oral toxicity, allergenicity	Hemagglutinating ability, cell adhesion, up regulated	Kumar (2013a), Kumar et al. (2013b)

			expression of GATA-3 and T-bet	
Extra-long autumn purple bean	EAPL	Anti-HIV, anti-tumor	Induction the expression of iNOS mRNA	Fang et al. (2010)
Blue tiger king bean	BTKL	Anti-tumor	Induction of DNA fragmentation, production of apoptotic bodies and chromatin condensation, triggering of cell apoptosis and necrosis, depolarization of mitochondrial membrane	Fang et al. (2011)

[a] The data are selected from the published articles that both the specific plant source and lectin name were given.

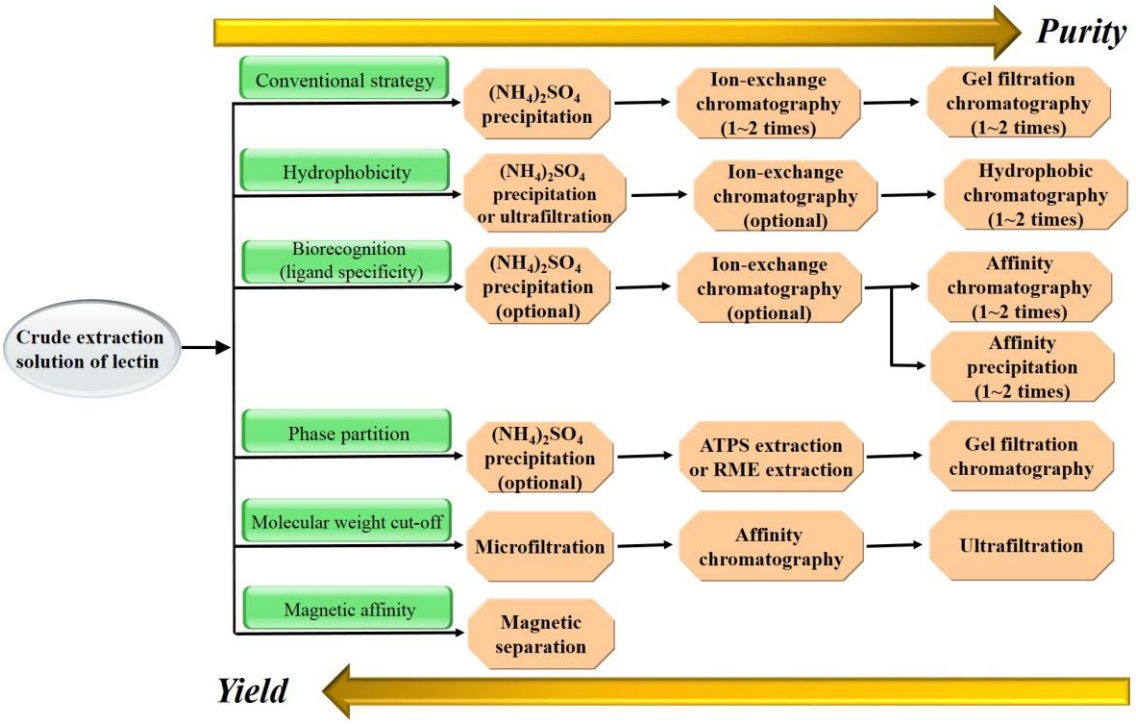

Figure 1. The purification strategies for separation of *Phaseolus vulgaris* lectins.

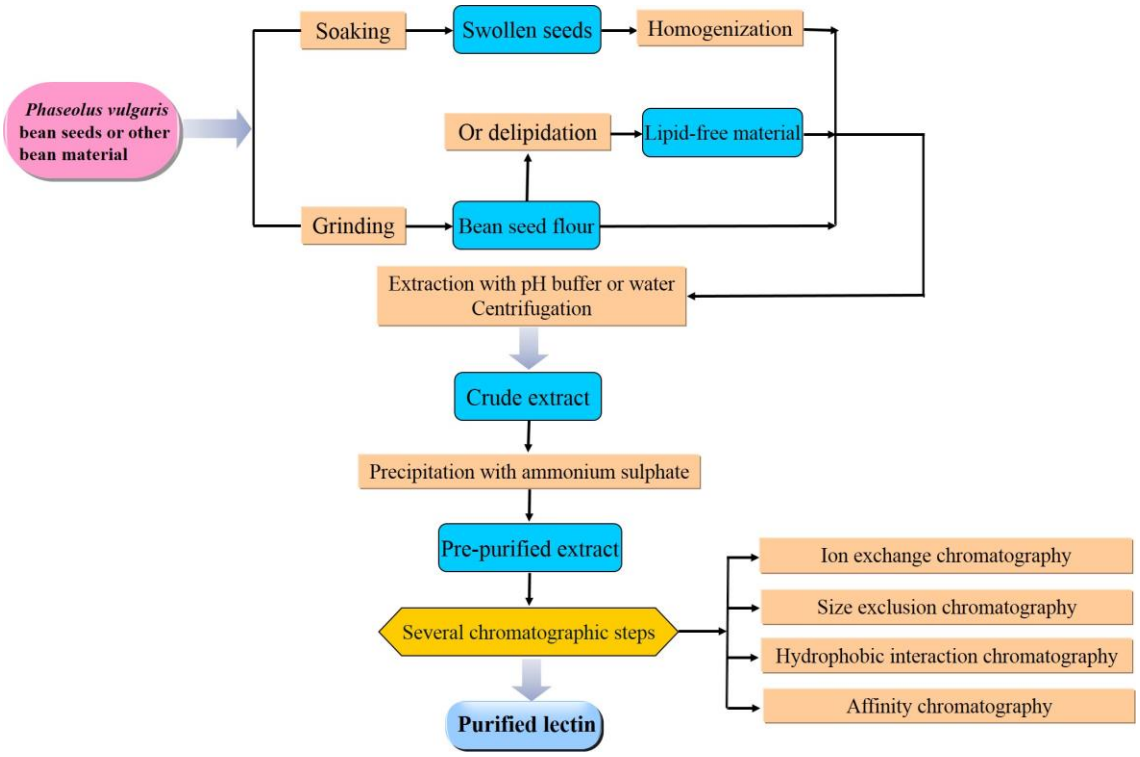

Figure 2. A general procedure for the purification of *Phaseolus vulgaris* lectins (modified from (Zhang et al., 2008)).