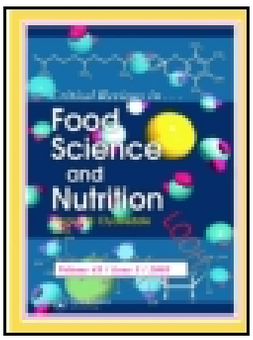

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Z. X. Lu, J. F. He, Y. C. Zhang & D. J. Bing (2019): Composition, physicochemical properties of pea protein and its application in functional foods, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2019.1651248

To link to this article: https://doi.org/10.1080/10408398.2019.1651248

Published online: 20 Aug 2019.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2019.1651248

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REVIEW

Composition, physicochemical properties of pea protein and its application in functional foods

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ABSTRACT

Field pea is one of the most important leguminous crops over the world. Pea protein is a relatively new type of plant proteins and has been used as a functional ingredient in global food industry. Pea protein includes four major classes (globulin, albumin, prolamin, and glutelin), in which globulin and albumin are major storage proteins in pea seeds. Globulin is soluble in salt solutions and can be further classified into legumin and vicilin. Albumin is soluble in water and regarded as metabolic and enzymatic proteins with cytosolic functions. Pea protein has a well-balanced amino acid profile with high level of lysine. The composition and structure of pea protein, as well as the processing conditions, significantly affect its physical and chemical properties, such as hydration, rheological characteristics, and surface characteristics. With its availability, low cost, nutritional values and health benefits, pea protein can be used as a novel and effective alternative to substitute for soybean or animal proteins in functional food applications.

KEYWORDS

Pea; protein; composition; physicochemical property; functional food

Introduction

Legumes in the Fabaceae family are the second most important crops after cereals. The common pea (*Pisum sativum* L.), including field pea and garden pea, is one of the oldest domesticated crops, cultivated for either human foods or livestock feeds. Pea plants can tolerate low temperatures during germination and growth and their cultivation provides an excellent cool season alternative for regions not suitable for soybean or bean production. The garden pea is mainly consumed as a green vegetable with its immature pods and seeds, whereas the field pea is marketed as dry grains and dominates global pea production and commercial pea products. Several market classes of field pea, such as yellow, green, marrowfat, and maple pea, are available in world pea markets. Since field pea and garden pea have significant differences in their genotypes, harvest stages and final products, this review mainly covers the recent research progress on protein composition, properties and utilization of field pea.

As one of the most important leguminous crops, field pea is grown in 84 different countries and constitutes the largest percentage (36%) of total pulse production over the world (Dahl, Foster, and Tyler 2012). Global pea production shows a continuous increase for the last 30 years. In 2008, field pea was cultivated over 10 million hectares worldwide with a total world production of 12.13 million tons (Schatz and Endres 2009). The top 5 countries for pea production are Canada, Russia, China, India and USA. Canada is the largest producer and exporter of green and yellow pea grains over the world. In 2014, Canada used 1.5 million hectares for pea cultivation and produced 3.3 million tons of pea grains, which was more than double that of next largest production country (Russia). Approx. 4.84 and 4.59 million tons of pea grains were produced in Canada in 2016 and 2017, respectively. Total global import of pea grains in 2015 was 4.97 million tons, in which India is the largest importer, followed by China and Bangladesh. Due to significantly high demands on plant proteins and relatively low cost of pea production, global market for pea protein is increasing rapidly and expected to reach 34.8 million US dollars by 2020 (Grand View Research 2015).

Composition and classification of pea protein

Field pea is known as a primary source of nutritional components and can be fractionized into various ingredients and foods products enriched in protein, starch, fiber, etc. (Tharanathan and Mahadevamma 2003; Costa et al. 2006; Tiwari and Singh 2012; Rubio et al. 2014). In general, pea seeds contain 20–25% protein, 40–50% starch and 10–20% fiber (Dahl, Foster, and Tyler 2012; Tulbek et al. 2016). Pea protein is a relatively new type of plant proteins and it becomes more and more popular in global food industry due to its availability, low cost, nutritional values and health benefits (Boye, Zare, and Pletch 2010; Roy, Boye, and Simpson 2010; Lam et al. 2018). Compared to soybean or other plant proteins, pea protein is characterized for its high

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Table 1. Classification of pea protein and its molecular characteristics*.

Class	Content	Solubility	Protein	Svedberg unit	MW	No. of subunit	MW of subunit	Peptide
Globulin	55–65%	salt solution	legumin	11S	320–410 kDa	6	60–65 kDa	40 kDa acidic 20 kDa basic
			vicilin	7S	150 kDa	3	48–50 kDa	n/a
			convicilin	8S	180–210 kDa	3	70 kDa	n/a
Albumin	18–25%	water solution	albumin	2S	68.5 kDa	n/a	n/a	PA1a 53 aa 5.8 kDa PA1b 37 aa 4 kDa
Prolamin	4–5%	alcohol solution	prolamin	n/a	n/a	n/a	n/a	n/a
Glutelin	3–4%	insoluble	glutelin	n/a	n/a	n/a	n/a	n/a

*Cited and summarized from O'Kane et al. (2004a, 2004b); Tzitzikas et al. (2006); Barac et al. (2010); Adebiyi and Aluko (2011); Gressent et al. (2011); Reinkensmeier et al. (2015), etc.

digestibility, relatively less allergenic responses or negative health controversies (Owusu-Ansah and McCurdy 1991; Allred et al. 2004). Variation on protein contents of field pea has been associated with different genotypes and environmental factors (Wang, Hatcher, and Gawalko 2008; Barac et al. 2010; Hood-Niefer et al. 2012). Novel pea germplasm, which contain approx. 30% protein in pea seeds, were identified and used in pea breeding program to improve the content and quality of pea protein. Several advanced lines with 28–30% protein, semi-leafless, earlier maturity, larger seeds, and good disease resistance, have been developed (Bing 2010, Bing 2012, Bing 2015; Bing and Liu 2011; Shen et al. 2016).

Pea protein can be classified into four major groups: globulin, albumin, prolamin, and glutelin (Table 1). Globulin is the main storage protein and accounts for 55–65% of total proteins in field pea (Adebiyi and Aluko 2011). Soluble in salt solutions, globulin can be degraded during the seed germination to provide nutrients for plant growth. Globulin dissociates into subunits at extreme pH values and ionic strength (Henning et al. 1997). Based on its sedimentation coefficients (S as Svedberg unit), globulin can be further classified into two main types (11S legumin and 7S vicilin). The ratio of legumin to vicilin is close to 2:1 and legumin contains more sulphur-containing amino acids than vicilin per unit of protein (O'Kane et al. 2004b; Mertens et al. 2012). Differences in content, composition and structure between legumin and vicilin are exhibited in both nutritional and functional properties, in which their association–dissociation properties and their surface structures are the most important factors for understanding the functionality of pea protein (Barac et al. 2010).

Legumin (11S) is a hexameric protein (320–400 kDa) and consists of six subunits, each (60–65 kDa) has an acidic (~40 kDa) and a basic (~20 kDa) polypeptides linked via a disulfide bond (Barac et al. 2010). The α-chain and β-chain of legumin are linked by disulfide bridges and the hydrophilic α-chains are located at the molecule surface, whereas hydrophobic sections are buried at the interior, minimizing their contact with water (Reinkensmeier et al. 2015). Vicilin (7S) is trimeric proteins (150–180 kDa) and includes a polymorphic type convicilin (8S, 180–210 kDa) (Tzitzikas et al. 2006). Vicilin is a combination of heterogeneous polypeptides with no disulfide bonds and cannot form disulfide bonds due to the absence of cysteine residues (Shewry, Napier, and Tatham 1995). Vicilin subunit (~50 kDa) is glycosylated, has more hydrophilic surface than legumin and can be cleaved into a variety of low molecular weight (MW) fragments (O'Kane et al. 2004a, 2004b). Convicilin subunit (70 kDa) displays about 80% amino acid sequence homology with the uncleaved vicilin subunit, but distinguishable by its highly charged N-terminal extension region and also the absence of in vivo cleavage (Tzitzikas et al. 2006). Moreover, convicilin differs from vicilin in that it does have one cysteine, a sulphur-containing amino acid (Reinkensmeier et al. 2015).

Albumin (2S) is soluble in water and accounts for 18–25% of total protein in pea seeds. Regarded as a metabolic and enzymatic protein with cytosolic function, albumin consists of molecules which have functional roles in seed germination (McCarthy et al. 2016). Two small MW albumins (PA1a and PA1b) have been characterized from pea seeds, in which PA1a has 53 aa with ~6 KDa and PA1b has 37 aa with 4 KDa (Higgins et al. 1986). Gene sequence comparisons revealed some homology between PA1 and a number of low MW proteins from seeds of a wide range of monocotyledonous and dicotyledonous plants. Both PA1a and PA1b have unusually high cysteine contents (7.5 and 16.2%, respectively), in which PA1b can be potentially used as an insecticide in the biocontrol (Gressent et al. 2011; Eyraud et al. 2013). The ratio between globulin and albumin in pea protein isolates (PPI) may show variations due to different genotypes and/or processing methods, which can influence PPI physicochemical properties (Karaca, Low, and Nickerson 2011). Significant difference in composition, structure, and functionality of different pea proteins and their amino acid profiles have been characterized (O'Kane et al. 2004a, 2004b, 2004c; Boye, Aksay et al. 2010).

Prolamin is a group of plant storage proteins and presents a small amount in pea seeds (Tsoukala et al. 2006; Guleria, Dua, and Chongtham 2009; Adebiyi and Aluko 2011). Prolamin is mainly found in seeds of cereals, such as wheat (gliadin), barley (hordein), rye (secalin), corn (zein), sorghum (kafirin), and oats (avenin) (Shewry and Halford 2002). It is characterized by a high glutamine and proline contents, generally soluble only in strong alcohol solutions (70–80%), light acid and alkaline solutions. Prolamin does not coagulate under heat, but can be hydrolyzed into proline and ammonia. Some prolamins, notably gliadin and other similar proteins found in the tribe Triticeae, may induce the celiac disease in genetically predisposed individuals (Shewry and Halford 2002).

As insoluble protein, glutelin is a class of prolamin-like proteins found in the endosperm of certain seeds of the grass family. It is also present a minor amount in pea seeds and constitutes a major component of protein composite as

Table 2. Comparison of essential amino acid profiles in pea, soybean, rice, and wheat*.

Amino acid	Pea	Soybean	Rice	Wheat
Valine (Val)	2.7	2.2	2.8	2.3
Leucine (Leu)	5.7	5.0	5.8	5.0
Isoleucine (Ile)	2.3	1.9	2.0	2.0
Methionine (Met)	0.3	0.3	2.0	0.7
Phenylalanine (Phe)	3.7	3.2	3.7	3.7
Tryptophan (Trp)	0.8	1.6	1.2	1.2
Threonine (Thr)	2.5	2.3	2.3	1.8
Lysine (Lys)	4.7	3.4	1.9	1.1
Histidine (His)	1.6	1.5	1.5	1.4

*Cited from Pownall, Udenigwe, and Aluko (2010) and Gorissen et al. (2018). Values are presented in g per 100 g raw materials, in which total protein content (%) of raw materials are 80% in pea, 72% in soybean, 79% in rice and 81% in wheat.

gluten. Glutenin, the most common type of glutelin, is responsible for some refined baking properties in bread wheat. Glutelin has also been identified in barley and rye (Shang et al. 2005) and it is the primary form of energy storage in the endosperm of rice grains. Only soluble in dilute acids or bases, chaotropic or reducing agents and detergents, glutelin is rich in hydrophobic amino acids, such as phenylalanine, valine, tyrosine and proline. Both high molecular weight (HMW) and low molecular weight glutelins can be typically found in most grass species. A HMW glutenin of the grass Triticeae has been reported as a sensitizing agent for the celiac disease in individuals possessing the HLA-DQ8 class II antigen receptor gene (Dewar et al. 2006).

Pea protein has a well-balanced amino acid profile, containing a high amount of lysine (Schneider and Lacampagne 2000; Nunes, Raymundo, and Sousa 2006). Compared to cereal proteins, pea protein has high levels of lysine, leucine and phenylalanine, but relatively less in sulphur-containing amino acids (methionine and cysteine) (Gruber, Becker, and Hofmann 2005; Pownall, Udenigwe, and Aluko 2010) (Table 2). Amino acid profiles also differ in legumin, vicilin and convicilin of pea protein (Reinkensmeier et al. 2015). Pea globulin tends to be high in arginine, phenylalanine, leucine and isoleucine, whereas the albumin fraction is high in tryptophan, lysine and threonine (Stone et al. 2015). Similar to other grain legumes, pea is relatively less in methionine and therefore its essential amino acid profile is complementary to that of cereal grains (Pownall, Udenigwe, and Aluko 2010; Gorissen et al. 2018).

Physicochemical properties of pea protein

The functionality of pea protein has received much attention in past decade (Shand et al. 2007; Adebiyi and Aluko 2011; Tulbek et al. 2016). Physical and chemical properties of pea protein can significantly influence its behaviors in food processing, storage and consumption (Shevkani et al. 2015). The knowledge on different pea proteins is valuable for the development of new food products or ingredients (Braudo, Plashchina, and Schwenke 2001; Wang et al. 2010). Globulin and albumin are two major types of pea protein and their composition, molecular structure, charge distribution determine PPI physical and chemical properties (Boye, Aksay et al. 2010; Freitas, Ferreira, and Teixeira 2000; Taherian et al. 2011). The processing conditions, such as temperature, pH, ionic strength and/or the presence of other ingredients, can also affect the functional properties of pea protein and their applications in food industry (Rui et al. 2011; Tang and Sun 2011; Day 2013).

Solubility and hydrolysis

Solubility is one of the most commonly measured functional properties of food proteins. PPI is strongly pH-dependent with a minimum solubility between pH 4 and 6, which may diminish its subsequent functional properties (Adebiyi and Aluko 2011). The extraction and dehydration steps during protein processing may affect the protein surface hydrophobicity by exposing hydrophobic residues, leading to increase hydrophobic interactions between proteins and/or peptides (Tsumura et al. 2005; Karaca, Low, and Nickerson 2011). Studies have been conducted to evaluate functional properties of pea protein such as solubility and digestibility (Barac et al. 2010, 2012; Boye, Aksay et al. 2010). For example, the maximum solubility of pea protein has been reported to vary from 20% for commercial PPI to 90% for the laboratory prepared form (Adebiyi and Aluko 2011; Shand et al. 2007), which is comparable to values obtained for soybean protein products. The lower protein solubility of commercial pea protein products was attributed to the heat-induced denaturation and potential aggregation during spray-drying (Shand et al. 2007).

Pea protein tends to form highly viscous solutions under high concentration. Chemical and enzymatic treatments of pea protein have been employed to overcome this viscosity issue as well as improve its functional properties. Compared with chemical modifications, enzymatic digestions on pea protein have many advantages such as the specificity towards substrates, fewer side reactions, mild treatment conditions, and ease of control (Claver and Zhou 2005; Liu, Low, and Nickerson 2009). The use of microbial proteases has been increasing due to their wide specificities and broad actions (Abd El-Salam and El-Shibiny 2015). Enzymatic hydrolysis has been found to reduce the apparent viscosity and improve the processing quality of pea protein-cassava-corn starch gels (Ribotta, Colombo, and Rosell 2012). Bajaj et al. (2017) reported enzymatic hydrolysis of PPI can modify their functional properties, in which hydrolyzed proteins showed higher solubility and lower viscosity.

It is well-known that the effect of enzymatic hydrolysis is dependent on numerous factors such as enzyme types and treatment conditions. The enzymatic hydrolysates of PPI were affected by the degree of hydrolysis and the enzyme used (Tamm et al. 2016). Different enzymes, including trypsin (Guan et al. 2006), papain (Bandyopadhyay and Ghosh 2002), pepsin (Arcan and Yemenicioğlu 2010) and several commercial proteases with different activity (Sijtsma et al. 1998; Betancur-Ancona et al. 2009), have been characterized. Trypsin has been proved to be a suitable enzyme to create PPI hydrolysates possessing superior physicochemical and antioxidant properties (Tamm et al. 2016). As an A1-family

member of protease, chymosin was characterized with the broad specificity similar to that of pepsin A and the PPI functional properties were improved after chymosin treatments (Barac et al. 2011).

Gelation and viscosity

Protein gelation can be viewed as a process during which proteins interact to establish a three dimensional network of molecules structure (Bryant and McClements 1998; Tome et al. 2015). These interactions include protein–water, protein–fat, and protein–protein and they are influenced by several factors such as protein concentration, temperature, pH, ionic strength, additives, endogenous, and exogenous enzymes. The type and composition of pea protein and the different processing procedures have been reported to affect its gelation properties (O'Kane et al. 2004c, 2005; Shand et al. 2007; Sun and Arntfield 2010, 2011, 2012). Unhydrolyzed pea protein forms very viscous emulsions when used at higher concentrations and its content in emulsions is limited to less than 10% due to its tendency to form highly viscous solutions (Bajaj et al. 2017).

Heat-induced gels are well documented on pea protein (O'Kane et al. 2005; Shand et al. 2007; Sun and Arntfield 2010). In addition to the extraction procedure, temperature, pH and salt composition affected the formation of soluble protein aggregates that can rearrange into gelled network and the heat treatment of a pea globulin solution resulted in soluble thermal aggregates (Mession et al. 2013). Pea protein extracted by an ultrafiltration and diafiltration procedure would promote the usefulness of aggregates as "building blocks" for cold-set gels (Mession et al. 2015). The denaturation temperature of pea protein increased from 69 to 77 °C with the increase of legumin content, whereas the disulfide-linked acidic and basic legumin subunits denatured and aggregated in a temperature range of 75–85 °C. Dissociation of legumin oligomers and their rearrangements via hydrophobic interactions and sulfhydryl/disulfide bonds exchange reactions would occur concomitantly during the heat treatment (Mession et al. 2015).

Cold-set gelation of pea protein represents an alternative route to enable a better control of soluble protein aggregation (Bryant and McClements 1998). Acid-induced cold gelation has been reported for whey proteins (Alting et al. 2003) and also for pea protein (Mession et al. 2015). The level of soluble pea protein aggregation was observed to further influence the acid-gel strength. By comparison with soluble and non-covalent vicilin thermal aggregates, legumin thermal aggregates displayed a decreasing solubility that would impair the acid gelation properties (Mession, Roustel, and Saurel 2017a). Studies on PPI gelation properties indicated that the heating rate has no impact on gel formation of pea protein whereas slower cooling rates can influence the gel formation of all pea protein samples (O'Kane et al. 2005; Sun and Arntfield 2011). Gel network formation of a salt-extracted PPI was evaluated by using dynamic rheological measurements, which indicated that the gelling point was dependent on heating rate but not affected by cooling rate (Sun and Arntfield 2011).

Numerous studies have been focused on gelation properties of milk proteins (casein micelles) and plant ones (Guyomarc'h, Law, and Dalgleish 2003a; Guyomarc'h et al. 2003b). The heat-induced protein interaction between pea globulins with the presence of casein micelles (at weight ratio 1:1) was investigated and the formation of protein aggregates was most likely due to the interactions among either denatured pea legumin or vicilin molecules, without the involvement of casein micelles (Mession, Roustel, and Saurel 2017a). Glucono-δ-lactone (GDL), also known as gluconolactone, is a food additive with the E number E575 and can be used as a sequestrant, an acidifier or a curing, pickling or leavening agent (Martin et al. 2009). The GDL acid-induced gelation from the mixture of casein micelles and pea protein indicated that the soluble and sedimentable vicilin aggregates may initiate the acid-gelled network, whereas further strengthening of gel network can result from the involvement of less repulsive casein micelles particles (Mession, Roustel, and Saurel 2017b).

Emulsification and foamability

Pea protein has excellent emulsifying properties for preparing oil in water emulsions (Sijtsma et al. 1998; Franco et al. 2000; Lu, Quillien, and Popineau 2000). As pea protein contains a large amount of reactive amino groups (lysine residue), chemical modification reactions onto the amino group, such as acetylation or succinylation, can be effectively carried out. These reactions were powerful to improve the instance emulsifying properties (Legrand et al. 1997). PPI has less emulsification capacity at the pH values close to its isoelectric point, however, its emulsification capacity was much improved at pH values above pH 7 (Aluko, Mofolasayo, and Watts 2009; Adebiyi and Aluko 2011). Karaca, Low, and Nickerson (2011) reported that the low emulsification capacity of PPI was due to its low surface charge and low solubility. The ratio of the emulsion layer height to the liquid layer height, which was used to calculate the emulsion activity, was reported to range from 38 to 46% (Butt and Batool 2010), whereas the emulsifying stability was reported from 43 to 100% (Aluko, Mofolasayo, and Watts 2009). Graaf et al. (2001) indicated that pea protein hydrolysates were surfactants with good emulsifying and foaming properties. Varied with enzyme types and degree of hydrolysis, the surfactant properties can be tailored toward specific applications. After chymosin treatments, the PPI foaming ability was improved and a positive correlation (0.74) between PPI solubility and emulsifying capacity were characterized (Barac et al. 2011).

Aqueous alkaline extraction followed by isoelectric precipitation and salt extraction processes are used for PPI production (Karaca, Low, and Nickerson 2011; Jiang et al. 2014). Both isoelectric precipitation or salt extraction have significant effects on PPI functionality in emulsion systems, because they may influence the ratio of globulin/albumin or legumin/vicilin and also physicochemical characteristics

(Karaca, Low, and Nickerson 2011). Pea protein carried a net negative charge at neutral pH and the isoelectric precipitation resulted in PPI with higher surface charge and solubility compared to those produced via salt extraction (Karaca, Low, and Nickerson 2011). The emulsifying abilities of PPI, legumin and vicilin at pH 3.0 were found to be generally better than those at other pH values and all pea protein products exhibited the least emulsifying ability at pH 5.0 (Liang and Tang 2013, 2014). Alkaline treatment of pea protein improved the interfacial property and steric hindrance and enhanced the ability of inhibiting oxidation in emulsions (Jiang et al. 2014).

Protein-stabilized emulsions are subjected to thermal processing techniques such as pasteurization and sterilization (McClements 2004). The heat treatment of pea protein resulted in inter-droplet hydrophobic interactions in emulsions, which can increase the droplet flocculation and creaming stability. The oil droplet size, flocculated state and creaming stability of pea protein emulsions were closely associated with heat treatments (Peng et al. 2016). Protein concentration affected the flocculation stability and an increase in protein ingredient significantly improved the creaming stability of emulsions stabilized by soybean protein (Kim, Decker, and McClements 2005; Shao and Tang 2014). The concentration of pea protein as emulsifier greatly influenced the oil droplet size (McClements 2004). Under acidic conditions, the electrostatic attraction between PPI and SSPS (soybean soluble polysaccharide) facilitated the formation of dispersible PPI/SSPS complexes and the emulsions prepared from PPI aggregates exhibited a long term stability against the changes of pH value and NaCl concentration (Yin, Zhang, and Yao 2015).

Allergenicity and inflammation

Pea protein is not commonly considered as a allergenic food or ingredient, however, its allergic reactions have been reported as pea protein or PPI has become more prevalent in the food marketplace (Sanchez-Monge et al. 2004; Barre, Borges, and Rouge 2005; Richard et al. 2015). Several allergens, such as Pis s 1 (50 kDa vicilin), Pis s 2 (64 kDa convicilin), Pis s 5 (profilin), Pis s 6 (17 kDa PR10 protein), Pis s albumin (26 kDa) and an agglutinin (30 kDa), have been identified from pea protein (Sanchez-Monge et al. 2004; Richard et al. 2015). Pis s 1, one of the major allergens in field pea, has a 60% to 65% homologous sequence with peanut Ara h 1 allergen (Burks et al. 1995). The sequence similarity of glycinins among peanut, soybean and pea is in the range of 62–72% (Rabjohn et al. 1999; Beardslee et al. 2000). Pea has shown the broad cross reactivity with chickpea, lentil, lima bean, soybean, peanut, etc. (Duranti 2006; Kumari et al. 2006). Allergen Pis s 1 from pea, Len c 1 from lentil and Ara h 1 from peanut share common epitopes, which can be responsible for the cross reactivity among these legumes (Barre, Borges, and Rouge 2005). Richard et al. (2015) investigated the cross reactivity of dun pea with other legumes and found some cases of clinical reactions to dun pea in patients allergic to glumes or peanut.

Allergenicity induced by different legume proteins demonstrated high degree of immunological cross reactivity because legumes have structurally homologous proteins and share common epitopes (Dadon, Pascual, and Reifen 2014). However, a low cross reactivity has been reported between pea globulins and peanut proteins (Szymkiewicz and Chudzik-Kozłowska 2013), which suggested that pea globulins can be potentially applied in immunotherapy of peanut allergy. Troszynska, Szymkiewicz, and Wolejszo (2007) reported that the germination step (3 days at 20 °C) significantly reduced the immune-reactivity by approx. 40 and 70% in sprouts with cotyledons of pea and soybean, respectively. When the cotyledons were removed, the immune reactivity was significantly reduced by 99.8 and 98% for pea and soybean, respectively. Therefore, the seed germination and cotyledon removal can be a useful approach to produce pea products for people who suffer from food allergic disorders (Richard et al., 2015).

Albumin fraction in pea protein contains bioactive components, including Bowman-Birk inhibitor (BBI), which may exert anti-inflammatory properties within human gastrointestinal tract (Utrilla et al. 2015). It is believed that significant amount of BBIs are not digested by gastric acid or proteolytic enzymes after their oral intake but reach to the large intestine. Utrilla et al. (2015) investigated preventive effects of two pea albumin extracts in the dextran sodium sulfate (DSS)-induced colitis in mice and found that all pea proteins were able to ameliorate the DDS-induced colitis. Mice treated with pea protein extracts and pea legumin isolates demonstrated the decrease of IgE and IgG1 but an increase of IgG2a in their plasma, indicating that pea protein may be used as an immunotherapy to desensitize peanut-induced allergy (Szymkiewicz and Chudzik-Kozłowska 2014). A study on mice anti-inflammatory activities demonstrated that pea protein hydrolysates significantly inhibited nitric oxide, a naturally occurring metabolic by-product which could damage the cells in excessive amounts (Ndiaye et al. 2012). Such inhibition was also shown against the production of TNF-α (tumor necrosis factor alpha) and IL-6 (interleukin-6), the signaling molecules produced in their bodies as part of an immune response to inflammation.

Application of pea protein in functional foods

As a cheap and sustainable source with lower carbon footprint, plant proteins provide a preferred alternative to animal proteins (Dijkink and Langelaan 2002; Adebiyi and Aluko 2011). Moreover, plant-based diets have shown to deliver many health benefits by lowering both cholesterol level and blood pressure, balancing blood sugar and even reducing the risk of certain cancer development (McCarty, 1999). Field pea contains a well-balanced amino acid profile and high level of lysine (Nunes, Raymundo, and Sousa 2006). Because of its availability, low cost, nutritional values and health benefits, pea protein has been widely used as a substitute for soybean or animal proteins in various functional applications (Wang et al. 2003; Iqbal et al. 2006;

Maninder, Sandhu, and Singh 2007; Aluko, Mofolasayo, and Watts 2009; Barac et al. 2010).

Food supplements

Pea protein is popular additive or supplement in global food industry (Shand et al. 2007; Tulbek et al 2016). Commercial pea protein products, such as PPI, are mainly the concentrated forms with <85% protein content (dry weight basis) (Aluko, Mofolasayo, and Watts 2009). In contrast to traditional cereal proteins, PPI does not contain any gluten and can be a useful contribution for the production of gluten-free foods (Han, Janz, and Gerlat 2010; Mariotti et al. 2009). With its excellent physicochemical properties, such as high level of water and oil absorption, excellent gelation capabilities and gel clarity, pea protein provides a novel type of plant proteins for functional foods under new formulations (Agboola et al. 2010; Boye, Zare, and Pletch 2010; Roy, Boye, and Simpson 2010; Stone et al. 2015; Tulbek et al. 2016; Lam et al. 2018).

Pea protein has been incorporated into beef patties (Baugreet et al. 2016), salad dressing (Ma et al. 2016) and encapsulated ingredient powders (Bajaj, Tang, and Sablani 2015) to improve their functional properties. Recent studies showed that addition of PPI to the ground beef produced beef patties that were softer, tenderer and required less force to compress than all-beef patties, the hamburger also presented less fat-retention than regular beef patties (Baugreet et al. 2016; Chao, Jung, and Aluko 2018). The spaghetti fortification with pea protein led to products that had reduced raw noodle strength and cooking time but higher cooking losses (Chao, Jung, and Aluko 2018). Pea protein has been found suitable for preparation of gluten-free muffins with the characteristics comparable to those made from wheat (Shevkani and Singh 2014). Gels made from Cape hake protein showed a softer texture and less rubbery with the addition of pea protein (Tome et al. 2015).

Pea protein can also be used as nutritional supplements for sports and exercises. Leucine, isoleucine and valine are three essential branched-chain amino acids (BCAAs) which have an aliphatic side chain with a branch and can promote muscle growth (Shimomura et al. 2004). Pea protein is an excellent source of BCAAs and has high and balanced contents of leucine, isoleucine and valine. Babault et al. (2015) investigated the impacts of an oral supplementation with pea protein vs. Whey protein and placebo on muscle thickness and strength after a 12-week resistance training program. They found that the supplementation with pea protein promoted a greater increase of muscle thickness as compared to placebo whereas there was no difference between two protein treatments, indicating that pea protein can be used as an effective alternative to whey-based dietary products.

Food emulsifier

The use of plant proteins and polysaccharides as emulsifiers is of great interest to food and beverage industries because of their safety and nutritional values (Zong, Cao, and Wang 2012; Dickinson 2013). To be an effective emulsifier, protein should be adsorbed to the oil-water interface and unfolded at the interface to form a cohesive film around oil droplets through intermolecular interactions (Damodaran 2005). Although proteins possess good emulsifying capacity, the emulsions stabilized by proteins are sensitive to environmental conditions, such as pH, ionic strength and thermal processing (Donsi et al. 2010; Lam and Nickerson 2013). Several polysaccharides, such as pectin, soybean polysaccharide and gum Arabic, are naturally conjugated with hydrophobic proteins and therefore can also be used together as emulsifiers (Nakauma et al. 2008).

The ability of proteins to form and stabilize emulsions is critical to their role as food ingredients in a wide range of applications (Dickinson 2003; McClements 2005, 2007; Gharsallaoui et al. 2009). Pea protein has been used as emulsifier in liquid emulsions (Humiski and Aluko 2007; Aluko, Mofolasayo, and Watts 2009; Barac et al. 2010; Amine et al. 2014) and as emulsifier in spray-dried emulsions for the microencapsulation of oil (Gharsallaoui et al. 2012; Aberkane, Roudaut, and Saurel 2014). Ducel et al. (2004) showed that pea protein was able to decrease the interfacial tension between water and oil and can stabilize emulsions by forming a rigid membrane at the oil–water interface. Pea protein had high surface active properties at the oil–water interface. The capacity of PPI to stabilize food emulsions was optimized and the main processing conditions have been characterized (Nunes, Raymundo, and Sousa 2006).

The capacity of protein to form stable foams is an important property in cakes, souffles, whipped toppings, fudges, etc. (Chao, Jung, and Aluko 2018). Pea protein is a better emulsifier and foaming agent than soybean protein at neutral pH (Aluko, Mofolasayo, and Watts 2009). The interfacial membrane formed by proteins may act as a physical barrier that separates lipid molecules from pro-oxidants in the aqueous phase. Applications for pea protein include vegan style yogurts and nondairy sports products, as well as partial dairy protein replacers for therapeutic beverages and powders (McCarthy et al. 2016). The percentage volume of freeze-dried PPI induced by whipping has been reported to range from 78 to 143%, whereas the foaming stability was between 79 and 98% (Fernandez-Quintela et al. 1997; Butt and Batool 2010).

Fortified beverage

Fortification of beverages involves the process of adding micronutrients to various beverages which are consumed by different consumers. One particular interest and challenge is the application of pea protein in fortified beverages, such as protein shake, sports drink and protein juice blend (Nosworthy, Tulbek, and House 2017). The most important functional properties related with protein fortified beverage include its solubility, thermal stability and rheological behaviors (Lam and Nickerson 2013). In general, protein beverages require thermal processing such as UHT (ultrahigh temperature processing) or retort for safety and shelf life

stability purpose. Currently, protein beverage or protein juice blend beverage are ideally formulated around pH 4–6 to avoid astringency sensorial defects (Wagoner and Foegeding 2017). At neutral pH, pea protein has net negative charge and mutually repels with each other in solution. Pea protein loses its net negative charge during acidification process and has neutral charge rendering the weakest hydration around isoelectric point (pH value around 4.8). Therefore, pea protein will quickly aggregate and be subject to sedimentation in the final products when pea protein products are acidified and heated (Lan, Chen, and Rao 2018).

Soluble complexes of protein and polysaccharide is one promising approach to improve protein solubility and thermal stability at acidic environment (Braudo, Plashchina, and Schwenke 2001). The formation of such soluble biopolymers can potentially lead to superior functional properties (Klassen, Elmer, and Nickerson 2011; Semenova 2017). Recently, the solubility of whey protein in beverage has been enhanced upon the formation of soluble complexes with pectin (Wagoner and Foegeding 2017). This method is based on the pH dependent electrostatic interaction between positive patches on a protein surface and negatively charged polysaccharides (Jones and McClements 2011). Depending on the strength of electrostatic attractions, biopolymer complexes can remain as one phase system (i.e. soluble complexes) or undergo associative phase separation to become insoluble complexes (e.g. complex coacervations or precipitation) (Lan, Chen, and Rao 2018).

The phase behavior and potential factors influencing the formation of soluble complexes in pea protein-polysaccharide systems is of most importance to develop desirable PPI fortified beverage (Lan, Chen, and Rao 2018). The formation of complex coacervations between plant proteins and polysaccharides, such as pea protein-gum Arabic or pea protein-alginate, has been investigated (Liu, Low, and Nickerson 2009; Klemmer et al. 2012; Stone et al. 2015). The high methoxyl pectin (HMP), an anionic polysaccharide with the degree of esterification over 50%, has been selected as polysaccharide because of its widespread application in acidic dairy beverage (Gancz, Alexander, and Corredig 2005). The pKa of carboxyl moieties in HMP ranges from 2.9 to 3.3 and the PPI functionality was enhanced by forming soluble complexes with HMP (Lan, Chen, and Rao 2018).

Protein blends

Proteins from different plant species may have significantly different compositions and physiochemical properties (Boye, Aksay et al. 2010). Pea and rice are excellent protein sources due to their availability and nutritional values (Boye, Zare, and Pletch 2010). Proteins extracted from pea and rice has great potential for use as food ingredients (Cao et al. 2009; Boye, Aksay et al. 2010; Bouasla et al. 2016). Pea protein has high amount of lysine but low in methionine (Boye, Zare, and Pletch 2010), whereas rice protein is rich in methionine and low in lysine, their combination can make a complete protein after they are blended. Furthermore, pea protein has good foaming and emulsifying properties (Aluko, Mofolasayo, and Watts 2009; Taherian et al. 2011) and can form gels (Shand et al. 2007) and bind water and oil (Osen et al. 2014). Rice protein is colorless and tasteless protein source, but its usage in food formulations is limited due to its low solubility (Wang et al. 2016).

Pea and rice mixture (PR blend) or the combination of pea and rice proteins ensure sufficient quantities of all essential amino acids needed in the human diet as recommended by United Nations (2011). However, most commercially available pea and rice protein isolates have poor functional properties, primarily due to the difference in the fractionating process. Exposes of PR blends to high temperatures for short periods of time can improve the functional properties of PR blends without affecting the essential amino acid composition. Several studies (Aluko, Mofolasayo, and Watts 2009; Osen et al. 2014, 2015; Stone et al. 2015) have related the low solubility of protein isolates to more severe processing conditions such as high temperatures during the spray-drying or alcohol decoloration in commercial settings.

The direct steam injection (DSI), a processing method for plant protein blends, provides a potential to improve the PR blend solubility (Ganjyal, Maningat, and Bassi 2011). DSI is assumed to alter disulfide bonds (SS) and sulfhydryl groups (SH) to create cross-linked hybrid proteins from two or more protein sources (Ganjyal, Maningat, and Bassi 2011). The solubility, emulsification, foaming and gelling of proteins treated by DSI can be enhanced (Pietrysiak et al. 2018). The modification of electrostatic properties by adjusting pH combined with heat shock led to protein unfolding and the subsequent cooling period allowed for protein rearrangement (Pietrysiak et al. 2018). The high pressure DSI with pH adjustment can be used to create modified PR blends with enhanced functional properties (Pietrysiak et al. 2018).

Pharmaceutical applications

Compared to animal proteins, plant proteins are widely available and environmentally sustainable and can be used in various pharmaceutic applications (Wan, Guo, and Yang 2015). Moreover, the use of plant proteins as nutraceutical delivery systems also meets the current economic trends in food production and pharmaceutical fields (Wan, Guo, and Yang 2015). Microencapsulation is a process in which bioactive compounds are enclosed within a protective covering to enhance stability during storage, processing and treatment (Ahn et al. 2008; Serfert, Drusch, and Schwarz 2009). There has been a growing interest within pharmaceutics in the emulsion stabilized by food grade particles rather than conventional surfactants. Plant proteins are added to encapsulated materials mainly as emulsifiers or wall materials due to their amphiphilic nature. This type of emulsion is called Pickering emulsion, which can provide outstanding physical and chemical stability to the lipid phase and thus encapsulated bioactives (Dickinson 2012).

In recent years, plant proteins, mainly corn zein and soybean proteins, have been widely used to develop delivery

platforms for encapsulation, protection and controlled release of bioactive compounds, such as micronanoparticles and nanoparticles, fibers, films and hydrogels (Nesterenko et al. 2013; Wan, Guo, and Yang 2015). Soybean protein aggregates (~100 nm) prepared by thermal treatment and the addition of sodium chloride can act as a kind of effective emulsion stabilizer (Liu and Tang 2013). Such emulsions showed extraordinary stability against coalescence and creaming. In another study, the generation of Pickering emulsions using zein colloidal particles as interfacial stabilizer was reported (Folter, Ruijven, and Velikov 2012). Wan, Guo, and Yang (2015) demonstrated the encapsulation of citral and lime flavor in self-assembled core shell structures of zein, which are of interest for encapsulation purposes in pharmaceutical industries.

PPI has been used as film, materix or wall materials for microencapsulation (Tome 2012; Fernandes et al. 2013; Aberkane, Roudaut, and Saurel 2014; Bajaj, Tang, and Sablani 2015). Pea protein films can be prepared by casting from dispersions at pH 7 or pH 10 and by compression molding at 140 °C. Opposite to other proteins, pea protein films combine the strength (5.0–7.5 MPa) with high elongation at break (150%) (Graaf et al. 2001). A protein isolate derived from field pea was applied as matrix material for the microencapsulation of beta-carotene (Graaf et al. 2001). Pea protein is an effective wall material for microencapsulation of ascorbic acid and a-tocoferol (Pierucci et al. 2006; Pereira et al. 2009). The conjugated linoleic acid (CLA) microencapsulation by spray drying with pea protein as wall material and the physical and chemical properties of CLA micro particles were investigated (Costa et al. 2015). The effective stabilization of CLA by microencapsulation in pea protein during two months of storage at room temperature has been characterized (Costa et al. 2015).

Perspectives

In recent years, there has been a growing interest in global food industry towards utilizing pea protein as a new substitute for soybean or animal proteins. Significant research advances on composition, structure, physicochemical properties of pea protein as well as on genetic regulations and metabolic pathways for pea protein synthesis have been achieved. Compared to cereal proteins, pea protein has a well-balanced amino acid profile with high level of lysine, but relatively less in sulphur-containing amino acids. Moreover, pea seeds may contain a number of putative anti-nutritive compounds, such as phytic acid protease inhibitors, lectins and saponins. Pea protein may form weaker and less elastic gels than soybean protein during food processing. More researches will be necessary to address the limitation of pea protein and further improve its nutritional values and processing qualities. More public education will also be essential to promote the acceptability of Western consumers to use pea protein as a healthy food choice. With the increase of consumer awareness on field pea's benefits, it can be expected that global pea market will be continually growing and pea protein will be extensively used in food ingredients, beverages, sport supplements and pharmaceutical applications.

Funding

This work was financially supports by the CAP Stream A Research Project from Agriculture and Agri-Food Canada and the ASC-06 Pulse Cluster Program from Agriculture and Agri-Food Canada and Pulse Canada.

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