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Review

Proteins from land plants – Potential resources for human nutrition and food security

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Increasing utilisation of plant protein is required to support the production of protein-rich foods that can replace animal proteins in the human diet so as to reduce the strain that intensive animal husbandry poses to the environment. From a nutritional standpoint, with the right combination, plant proteins can supply sufficient amounts of essential amino acids for human health requirements. In addition to their role as a macronutrient, proteins play an integral role in structural formation of foods through processes such as emulsification, foaming, gelation and dough formation. This review aims to provide an overview of the major sources of plant proteins, their physiochemical functionalities and nutritional properties, with emphasis on the research needed to support technology innovation for more plant protein to meet world nutritional requirements and as food sources to feed the growing world population.

Introduction

Land plants have always been part of the human diet to provide energy and nutrients for sustainable living. Although plant proteins are relatively cheap and more abundant than animal proteins, direct consumption of proteins from land plants in conventional human food is still fairly limited. Currently, most plant proteins are used as animal feed to produce functional animal proteins from milk, eggs and meat. However, the conversion of plant proteins (e.g. from grains as feed stock) into animal proteins is inherently inefficient. In some cases, less than 15% of the plant proteins from feed crops are turned into animal proteins for human consumption and 85% are wasted (Aiking, 2011; Pimentel & Pimentel, 2003). As a consequence, meat production is responsible for a disproportionate share of food-related environmental pressure (de Boer & Aiking, 2011; Gilland, 2002). With respect to land use, if the same amount of plant proteins is used directly for human consumption, less than 10% of land will be required to grow food crops as to otherwise feed crops to produce the same amount of meat proteins (de Boer & Aiking, 2011). Furthermore, production of animal proteins requires about 100 times more water than producing an equal amount of plant proteins (Pimentel & Pimentel, 2003). With the rapid growth in the world's population, food security has been seen as the next mega challenge for the agrifood industry. Better and more efficient utilisation of plant-based proteins will become critical when the supply of animal proteins reaches maximum production capacity to feed the growing world population. The shift towards a more sustainable diet necessitates less reliance on foods of animal origin, and thus presents an huge potential for the agrifood industry to explore alternative sources of proteins (Aiking, 2011). For example, the development of new meat analogue products has accelerated in recent years, with some of the most promising alternatives based on proteins from plant sources, such as soybean and peas, and the dairy substitutes market has also expanded. Plant protein-based meat and dairy substitutes can deliver equivalent quality at lower costs, while fulfilling the world's priority of reducing greenhouse gas emissions and limiting destruction of forest land (Dijkstra, Linnemann, & van Boekel, 2003; Linnemann & Dijkstra, 2002). In addition, the right combination of plant proteins can ensure the supply of sufficient amounts of essential amino acids for human health requirements.

Plant protein refers to the protein from land plant origin as opposed to animal origin. In most cases, plant protein resides in the seeds and grains that store most of the nitrogen sources. In the context of human protein nutrition, the most important plant groups are cereal grains and food legumes, including oil-seed legumes, either consumed as part of grain components (e.g. flours milled from grains), or as

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enriched protein ingredients as co-products of oil extraction or starch production (e.g. soy protein and gluten). There are multiple reasons why plant proteins are still underutilised for human food: their lower nutritional values (on a single source basis) as compared with animal proteins; difficulties in maximising their physical functionality due to their large molecular weight and size and poor solubility in water; and the economic cost associated with isolation and recovery of protein fractions. However, there has been considerable development through research and development to improve both the nutritional and functional properties of plant proteins. Soy protein serves an excellent example of how scientific research can increase the consumer's awareness of the nutritional value of proteins from plant sources and how potential technological innovations can add value and diversify the use of plant proteins into a wide variety of food products. While soy protein continues to dominate as an alternative plant protein to replace animal-based protein, a range of new food products is starting to appear, which utilise other grains, legumes and vegetables as sources of proteins (Asgar, Fazilah, Huda, Bhat, & Karim, 2010). From environmental sustainability and food security points of view, there is an urgent need to increase the use of proteins from a wide range of plant sources directly for human food.

Major sources of plant proteins

Soy

Soybean is one of the most important agricultural commodities because of its high protein content which is about 35–40% (Table 1). Soybean is the dominant oil seed crop worldwide and the second largest source of vegetable oil, after palm oil. Currently, global production is estimated to be about 271 million metric tons per annum (USDA-FAS, 2012), and a large amount of defatted soybean meal is produced after extraction of oil. Most defatted soybean meal is used for animal feed. A small portion is further processed into various types of soy protein products for human consumption (Alibhai, Mondor, Moresoli, Ippersiel, & Lamarche, 2006). Although traditional foods made from soybean have been consumed throughout East Asia for more than two thousand years, in Western countries, soybean derived products have only become an economical and high quality vegetable protein source for human diets over the last few decades. Soy's high protein level and well-balanced amino-acid composition makes it an important source of plant protein, with a great potential to replace meat and dairy proteins in our daily diet.

Table 1. Typical protein contents of major cereals, legumes, oilseeds and vegetable sources.

	Protein content	Other constituents
Wheat (flour)	8–15%	~75% starch; 1–2% lipids ~5% non-starch polysaccharides
Rice	7–9%	90% starch
Maize (corn)	9–12%	70–75% starch; 3–18% oil (from the germ)
Barley (dehulled)	8–15%	60–64% starch; 2–3% lipids 3–10% soluble dietary fibre (in which 4–6% β-glucan) and 11–14% insoluble dietary fibre
Sorghum	9–17%	~2% lipids; 70–75% starch
Soybean	35–40%	~20% oil; ~30% non-starch polysaccharides
Pea	20–30%	60–65% starch; ~5% non-starch polysaccharides
Chickpea	20–25%	~60% starch; ~10% non-starch polysaccharides
Lupin	35–40%	~10% oil; 35–40% non-starch polysaccharides
Canola	17–26%	40% oil; 12–30% non-starch polysaccharides

The main soy protein products which are mainly produced in the United States, are soy flour, soy protein concentrate, soy protein isolate, texturised and hydrolysed soy proteins. Fig. 1a illustrates a typical flow chart of the production streams of soy protein ingredients. Soy protein concentrate which is produced from defatted soy flakes, after removing most of the soluble cell wall materials, has greater than 65% protein (Table 2). Soy protein isolate which is produced by alkali extraction and isoelectric precipitation is the most refined form of soy protein with a protein content higher than 90%. Textured soy protein is produced by extrusion to resemble the texture of meat chunks. The principal function of texturised soy protein is to partially or totally replace animal proteins in various food products and is used as a protein source in vegetarian meat alternatives. Soy flour, soy protein concentrate, soy protein isolate and their texturised products, are mainly used as ingredients in formulated foods for their functional properties, such as water and fat binding, emulsification, foaming and gelation.

Soy protein products are used to extend or replace animal proteins. Soy protein is also used as a protein source in infant formulae. Soy milk is used for replacement of cow milk by vegans and persons with intolerance to milk protein. Tofu, which is a traditional soy-protein food product originated from Asia, is a protein curd made by precipitating the protein from soy milk. It may also be produced from soy protein ingredients.

Although soy protein is by far the most utilised plant protein, the growth in soy protein utilisation is not as high as expected; currently only about 2–3% of the soybean production is used for human food with the rest used for animal feed (Johnson, Myers, & Burden, 1992; Johnson, White, & Galloway, 2008). Soy protein for food use continues to be a dynamic growth market worldwide with production reaching 2.77 million metric tons in 2009 (up by more than 50% over the course of the decade). Perhaps the increased uptake of soy proteins and soy flour is largely due to the intensive research and public recognition of the potential health benefits, such as alleviation of chronic diseases (Baier & McClements, 2005; Friedman & Brandon, 2001).

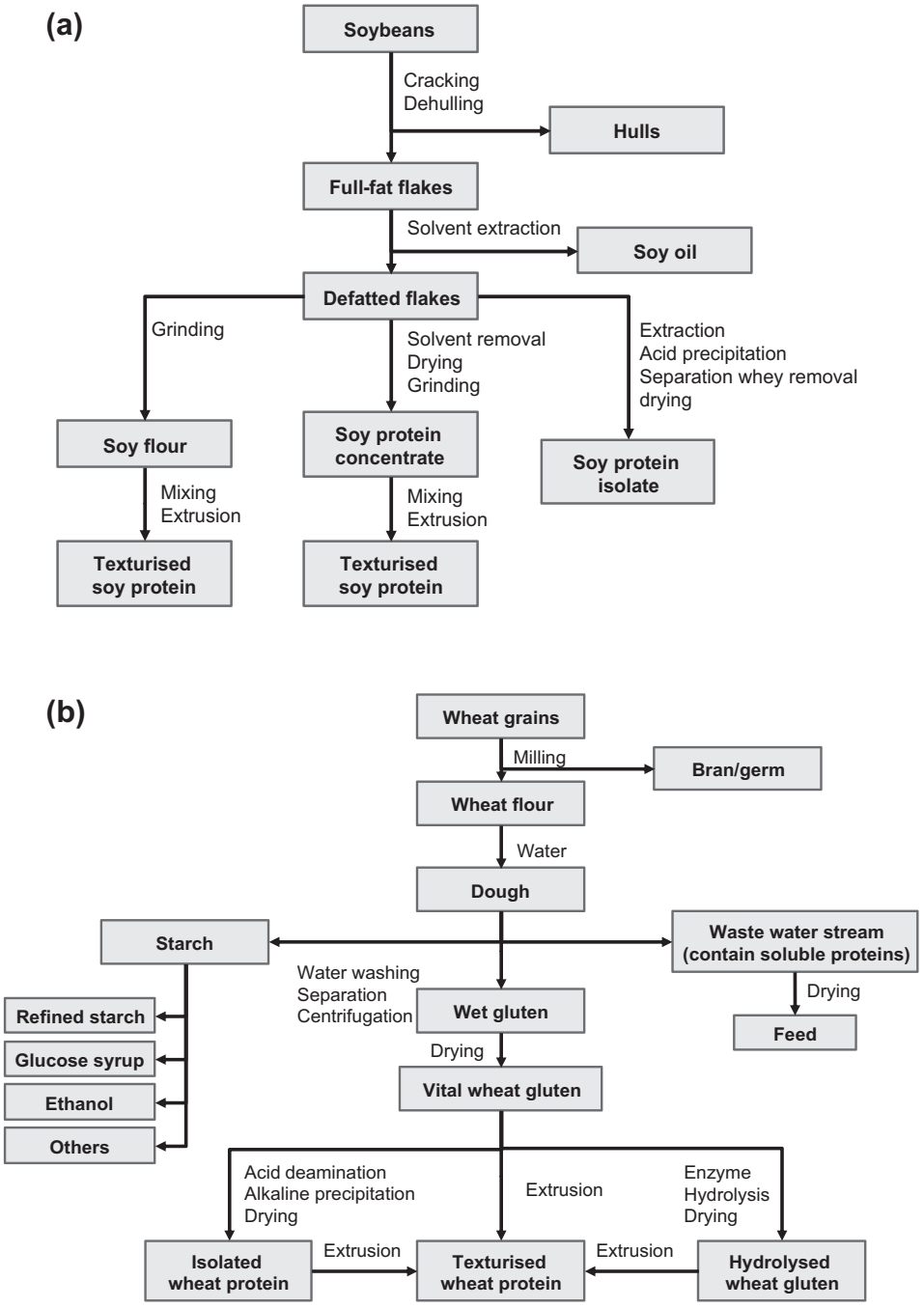

Fig. 1. Protein extraction and processing flow chart from: (a) soybeans; and (b) wheat flour.

Wheat

Wheat is the single most important food crop in the world, eaten in various processed forms by more than one billion people, and making a larger contribution to the calories and protein available to human beings than any other foodstuff (USDA-FAS, 2012). Wheat contains 8–15% protein depending on grain variety (Table 1). The main storage proteins in wheat grains are the gluten proteins. Gluten has a unique physical functional property that none of other plant proteins have Gluten forms a cohesive, visco-elastic proteinaceous network that provides the unique ability of wheat to produce leavened products (Wrigley, 1996). It is this precise ability of forming an insoluble protein mass when wheat flour is mixed with water that allows gluten to be separated from starch through a simple water wash process (Fig. 1b) (Day, Augustin, Batey, & Wrigley, 2006). Commercial gluten (known as vital wheat gluten) with a protein content as high as 75–80% has been produced by this simple physical separation of wheat flour since the 1850s. Nowadays, a variety of modified gluten

Table 2. Major industrially produced protein ingredients from plant sources.

Plant source	Protein products	Protein content	Major manufacturer and/or supplier
Soy	Soy protein concentrates (SPC)	65–70%	www.solae.com
	Soy protein isolates (SPI)	>90%	www.adm.com
	Texturised soy proteins	60%	www.cargill.com
Wheat	Vital wheat gluten (VWG)	75–80%	www.manildra.com.au
	Isolated wheat protein (IWP)	90%	www.mgpingredients.com
	Texturised wheat proteins		www.cargill.com
	Enzyme hydrolysed protein	>90%	
Rice	Rice protein concentrate	~80%	www.foodchem.cn
	Rice protein isolate	90%	
Maize/corn	Zein	88–96%	www.freemanllc.com www.showa-sangyo.co.jp
Peas	Pea protein concentrate	85–90%	www.nutripea.com/company.htm
	Pea protein isolate		www.roquette.com/ www.burcon.ca
Canola	Canola protein isolate	90%	www.bioexx.com
	Hydrolysed protein	83%	www.burcon.ca
Potato	Potato proteins		http://www.solanic.eu

(e.g. isolated wheat protein) is also produced commercially by further chemical or enzymatic treatments of vital wheat gluten in order to obtain modified gluten with enhanced functionality, as well as to increase its protein content (Day, 2011).

The major usage of gluten in western countries has traditionally been in baked products, pasta, noodles and breakfast cereals (Day, 2011). In these product categories, the gluten is used to fortify flours of lower-than-desirable protein content by flour millers and bakers, in order to supplement lower protein local wheats and to improve the quality of the flour to equal those with higher protein content. Gluten is attractive as a fat- and water-binding ingredient in restructured meat, fish and poultry products. A major use of gluten in non-bakery foods is as a meat replacement in vegetarian foods, and in the production of analogues of expensive foods such as seafood and crab meat, particularly in Japan. Texturised wheat protein which is produced *via* extrusion is used increasingly as plant protein to replicate the look and texture of meat products.

Other cereal grains: rice, maize, barley and sorghum

Rice is the second largest cereal crop in the world; however most is grown and consumed in Asian countries. Rice has the lowest protein content of all the major cereals (Table 1), at 7–9% by weight, but it is one of the major sources of protein for those rice-consuming people in South and Southeast Asia (Shih, 2003). High-protein rice ingredients only became commercially available recently, particularly in traditionally low rice-consuming countries such as the United States. They are produced as co-products of rice starch processing from rice bran and broken rice kernels (Fabian & Ju, 2011). However, they are more widely used directly as aquaculture or animal feeds. One of the applications that have seen an increased use of rice powder for human foods is gluten-free food products, due to the hypoallergenicity of rice protein (Shih, 2003).

Maize or corn (*Zea mays*) is one of the most important food and industrial crops, particularly in the USA. The protein content of maize is between 9–12% (Table 1). About half of the production is used directly as animal feed and a quarter is used for ethanol production in the USA. Only a small percentage of total corn production is used for human foods; most typically for products such as corn chips and tortillas, and production of corn syrups. Corn flour is one of the major products generated in the dry milling process of corn and used in diverse food products such as mixes for pancakes, muffins, doughnuts, breadings and batters, as well as baby foods, meat products, cereals and some fermented products. The primary products from the wet milling of corn are starch and oil, with the remaining being the protein by-products, corn gluten meal (with a protein content of ~60%). The corn gluten meal is then used for the production of zein protein which is the major commercial protein from maize (Shukla & Cheryan, 2001). Although zein is one of the few cereal proteins that is industrially produced in a relatively pure form, mostly in China, it is rarely used directly for human consumption due to its poor solubility in water. The major applications of zein are as a polymer material for film, coatings and plastics (Lawton, 2002).

Barley is the fourth most important cereal crop in the world after wheat, rice and corn. Barley was historically one of the most important food grains, but its primary utilisation evolved into animal feed and for malting processes due, in part, to the better quality and mouth-feel of food products prepared from wheat and rice (Baik & Ullrich, 2008). About two-thirds of current barley crops are used for animal feed, about one-third for malting and only 2% is used directly in human food. However, barley remains a major food source for some cultures in the West Asian and North African regions (Newman & Newman, 2006). Barley constitutes 10–17% protein, slightly higher than other cereal grains such as wheat and rice (Table 1). In

Western countries, pearled barley (whole, flaked, or ground) is used in products such as breakfast cereals, soups, porridge and bakery blends (Baik & Ullrich, 2008). The major advantage of incorporating barley into human food is for the potential health benefits of β-glucan in lowering blood cholesterol and glycaemic index. The "soluble barley β-glucan health claim" in food products has recently been approved by US FDA (www.fda.gov).

Sorghum is the fifth most widely grown cereal crop in total world production (FAO, 2009). Sorghum plant can tolerate heat and drought conditions more efficiently compared with other cereal crops such as wheat, barley and maize (Rooney, 2000). It is thus better able to grow and produce in the warmer and drier regions of the world. With increasing world population and decreasing water supplies, sorghum has considerable future potential for further human use. Sorghum is widely used in human diets of populations from Africa, India and China. The main foods prepared from sorghum are tortillas, couscous, porridges and baked goods. In many cases, sorghum is used in combination with wheat, maize or rice. Fermentation is one of the methods used to prepare porridge and baby food in parts of Africa and Asia. However in developed countries, sorghum is primarily used as animal feed and is an underutilised resource for human food. One of the growing potential uses of sorghum is in gluten-free products for people identified with coeliac disease and/or other dietary intolerances to wheat (Taylor, Schober, & Bean, 2006). Sorghum contains about 9–17% protein (Table 1) and is solely used in the form of coarse grains or milled flour. It is known that sorghum proteins become less digestible after cooking due to the extensive cross-linking of disulphide bonds (Duodu, Taylor, Belton, & Hamaker, 2003). One way to improve the digestibility of sorghum protein is perhaps through extrusion processing and other emerging processing technologies such as high pressure in which proteins can be denatured prior to its being heavily cross-linked (Correia, Nunes, Saraiva, Barros, & Delgadillo, 2011).

Pulses: peas, chickpeas and lupins

Pulses including pea, lupin, chickpea, lentil, bean, and other dry edible seeds from the pods of legume plants are important sources of protein in many diets around the world. Pea has also been exploited extensively as an important source of commercial proteins. One of the reasons why pea is more commonly used instead of other pulses for commercial fraction of proteins is that it can be grown extensively all over the world and the hull is easily removed. Peas contain high levels of protein and carbohydrates, relatively high concentrations of insoluble dietary fibre and low concentrations of fat (Table 1). On average, peas contain 25% protein but with a wide variation between plants, species and varieties. Selection of field pea varieties with high protein content and high yield is a major goal of commercial plant breeders. Three forms of pea protein ingredients are produced commercially: pea flour, pea protein concentrate and pea isolate (Table 2). Pea flour is produced by dry milling of dehulled peas. Pea protein concentrate is produced by dry separation methods; whereas pea protein isolate is produced by wet processing using either alkali or acid solubilisation, followed by isoelectric precipitation or an ultrafiltration process which produces a protein fraction with a much higher protein content of 85–95% (Boye *et al*., 2010). Pea proteins have found applications in a range of food products such as cereal and bakery products, nutritional snack bars, pasta, meal replacement beverages, baby food formulations, vegetarian applications, meat and seafood products. The increasing popularity of using pea proteins is largely due to its positive fat- and water-binding capabilities, emulsification properties and gelation, texture and nutritional values (Sandberg, 2011). The use of pea proteins in meat products has mainly been in meat patties, hamburgers and sausages. Pea protein concentrate may be used as an ingredient for producing non-fat dry milk replacement for the bakery industry (Sandberg, 2011). Other uses of pea protein, for example, substitution of milk powder in desserts and as a protein ingredient vegetable in pâtés, have also been explored.

Chickpea is one of the most important pulse crops consumed in the Indian sub-continent. It contains a similar amount of protein compared with peas (Table 1). Both the protein and the starch component of chickpea flours have been regarded as valuable sources due to their versatile functionalities (Ma *et al*., 2011). Concentrating the protein fraction from chickpea flour using techniques similar to those used for pea proteins has been reported and the isolated chickpea protein appears to have good emulsification properties (Boye *et al*., 2010; Karaca, Low, & Nickerson, 2011). Like other flours derived from pulses, chickpea flour is a potential high protein source for use as an extender in emulsified meat products due to its superior technological functionality and minimal effects on flavour (Sanjeewa, Wanasundara, Pietrasik, & Shand, 2010).

Lupins are non-starch leguminous seeds with a similar protein content to soybean, at about 40% (Table 1), and a high fibre content (Evans, Cheung, & Cheetham, 1993). Yellow lupins grown in Europe and South America are mostly used for animal feed. The presence of quinolizidinic alkaloids, which may be removed by soaking, prevents the direct consumption of yellow lupins as food. However, the white lupins, commonly consumed in the Mediterranean countries, are primarily grown for direct food uses. Western Australia has now become the largest grower, producing more than 85% of world's lupin crop and exports most of it as animal feed. Australian sweet lupin (*Lupinus angustifolius*) has a low alkaloid content and hence is more suitable for direct human consumption. When lupin protein is extracted, the resulting protein isolate is free of alkaloids and therefore can be used as a functional ingredient in human food. Lupin flour (i.e. free of alkaloids) and protein

isolates have been successfully tested as ingredients in various food products such as in muffins in which egg and milk proteins are totally substituted; in biscuits to achieve high protein contents; and in dairy, bakery and meat products (Drakos, Doxastakis, & Kiosseoglou, 2007; Pollard, Stoddard, Popineau, Wrigley, & MacRitchie, 2002). Two major protein fractions may be produced by wet milling. The fraction produced by alkaline extraction and isoelectric precipitation has good emulsification properties, whereas the fraction recovered by ultrafiltration of the remaining supernatant has good foaming properties (Duranti, Consonni, Magni, Sessa, & Scarafoni, 2008).

Other oil seeds: canola and sunflower

Canola/rapeseed is commercially the second most important oil seed, after soybean, in the world (USDA-FAS, 2012). Canola's protein content is lower than soybean, in the range of 17–26%. With increasing production of canola oil worldwide, the quantity of canola meal (a by-product after oil extraction) is also increasing. However, protein-rich canola meal is currently almost solely used as a protein source in livestock and aquaculture feedstuffs. The high contents of glucosinolates, phenolics and phytates (which are beneficial to the growing plant) left in canola meal limit its use for human consumption (Tan, Mailer, Blanchard, & Agboola, 2011). However, use of more sophisticated protein extraction and fractionation processes such as ultrafiltration and membrane separation can produce protein isolates with protein contents >80% and with most of the undesirable chemical compounds removed (Logie & Milanova, 2010; Xu & Diosady, 2002). According to the commercial producers (Table 2), canola protein isolates may be used in beverages, dressings and sauces, meat substitutes, baked goods and protein snack bars. The gelling properties of canola protein isolates provide potential for it to be used in comminuted meat products. One of the attractive characteristics of the canola protein is its well-balanced amino acid composition that can be used to complement cereals that tend to be low in lysine and to improve the nutritional quality of baked products (Arntfield, 2011).

Sunflower seed is the third major source of edible oil (after soybean and rapeseed) in the world. Lipids are the major component of sunflower seed. Sunflower seed is also high in protein with the dehulled seed consisting of about 20–40% crude protein. However the protein content is strongly influenced by the sunflower variety. The main use of sunflower seed is in oil extraction and one of the by-products of the oil extraction process is sunflower meal, which has a high protein content. Although the high protein content of the sunflower meal makes it an attractive source of proteins, the suitability of the proteins for food applications depends largely on the oil extraction method. Owing to the solvent extraction and high temperature (during expelling and desolventising/toasting) processing, the proteins are denatured to a large extent, resulting in the meal with a high content of insoluble proteins. Thus, the main use of sunflower proteins is in animal feed (Gonzalez-Perez & Vereijken, 2007).

Proteins from tubers and nuts

Potato is a versatile, carbohydrate-rich food widely consumed worldwide in a variety of ways. Potato is not typically considered to be a good dietary protein source due to its low overall protein content which is around 1–1.5% of tuber fresh weight (Camire, Kubow, & Donnelly, 2009). However potato is also widely used for industrial starch production. The process generates an aqueous by-product, potato fruit juice which contains most of the tuber soluble protein. Potato protein has a relatively high nutritional quality and, therefore, has good potential for utilisation in foods. The process to recover potato protein from such a dilute aqueous system is a challenge in terms of expenses. In addition, mild less harsh isolation process is preferred to recover the protein in a non-denatured form in order to preserve its solubility, foaming and emulsifying properties (Bartova & Barta, 2009; van Koningsveld *et al.*, 2001; Vikelouda & Kiosseoglou, 2004). The potato protein ingredients are now commercially available (Table 2) and can be used in a wide range of food applications including meat-free analogues, gluten-free bakery products, dairy-free ice-cream and toppings and desserts etc.

Nuts are another rich source of plant proteins. They are also important crops for edible oil production. Although the by-products (e.g. meals, skins and hulls) of oil processing contain high levels of compounds, such as protein, fibre and polyphenolics, the use of these by-products as food ingredients has been very limited, largely due to highly allergic nature of proteins from nuts. However nut protein containing products are available, e.g. almond milk, and many nuts are consumed as whole or included in food products.

Plant protein classification

Plant storage proteins were first classified by Osborne (1924) on the basis of their solubility and extractability in various solvents. The four major classes of proteins that have since become known as "Osborne fractions" are: albumins, globulins, prolamins and glutelins. Albumins are soluble in water and coagulatable by heat, whereas globulins are insoluble in water but soluble in saline solutions. Prolamins are insoluble in either water or saline solutions but extractable in concentrated aqueous alcohol solutions (i.e. 60–70% v/v). Glutelins are not soluble in neutral aqueous solutions, saline or alcohol but may be extractable in dilute aqueous acid or alkali solutions. Glutelins are mainly found in cereal grains such as wheat, rice and maize. The Osborne classification remains in use today, but over the years, as methods of protein fractionation have become more refined, it has been recognised that each of these solubility classes contains a complex mixture of proteins and that a large amount of overlap exists between the classes (Shewry &

Tatham, 1990). Most of plant proteins belong to these four protein classes, but the protein contents of each class and their molecular size can vary considerably depending on the plant source (Table 3). These protein fractions exhibit different functional properties for food applications due to the molecular structures of the proteins.

Albumins

Albumins are generally present in relatively small quantities as part of the storage proteins of cereal seeds, but more in oilseeds and legumes (Table 3). Hence they have been more widely studied in the oilseeds (e.g. canola and sunflower) and pulses (e.g. lupins and peas) rather than in cereals. The albumins are compact globular proteins consisting of two polypeptide chains with molecular weight (M*w*) values of 4000 and 9000 Da, to form a disulfide interchain linked protein (Shewry, Napier, & Tatham, 1995). Importantly, the albumin proteins contribute to >50% of the total sulphur in the seeds from leguminous plants, such as peas and lupins, even though they represent only 10–30% of total proteins.

Globulins

Plant storage globulins are the major protein fraction in seeds of leguminous plants including soybeans, peas and lupins, but are low in cereal grains. However the concentration of globulins can vary widely depending on the different cultivars and range between 40 and 80% of total soy proteins, 65–80% of total pea proteins and about 75% of lupin proteins (Table 3). Unlike albumins, globulin proteins from plants contain relatively low levels of the sulphur-containing amino acids, cysteine and methionine. Two types of globulins are found in soybean: glycinin and conglycinin, with sedimentation coefficients of about 11S and 7S, respectively. The 11S glycinin is the major protein in soybean. Pea globulin proteins comprise three distinct major groups; known as legumin (11S), vicilin (7S) and convicilin (7S) (Casey & Domoney, 1999). Similar to pea globulins, three major groups are also present in lupins. They are α-conglutin (11S) which makes up one-third of the total globulins; β-conglutin (7S) present in amounts to about 45% and γ-conglutin which is a minor 7S protein accounting for about 5% of total proteins (Duranti *et al.*, 2008). The globulin 12S cruciferin is the major protein present in canola (Tan *et al.*, 2011).

Table 3. Approximate distribution of the different classes of proteins from different plant sources, according to the Osborne classification (Osborne, 1924; Shewry & Casey, 1999).

Plant source	Albumins	Globulins	Prolamins	Glutelins
Wheat	6–10%	5–8%	35–40%	40%
Rice	2–6%	12%	4%	80%
Barley	3–5%	10–20%	35–45%	35–45%
Maize	4%	4%	60%	26%
Sorghum	2–7%	2–10%	35–60%	20–35%
Soybean		90%		
Pea	15–25%	50–60%		
Chickpea	8–12%	53–60%	3–7%	19–25%
Lupin	25%	75%		
Canola	20%	60%	2–5%	15–20%

Prolamins

Prolamins are the major storage proteins in cereals, accounting for about 50% of the total grain proteins, except in rice where prolamins are minor components and only present at about 4%. The name of prolamins was used by Osborne (1924) to reflect the high contents of proline and glutamine in cereal proteins. Four principal prolamins have been found and they are the gliadins in wheat, hordeins in barley, zeins in maize and kafirins in sorghum. In the original Osborne classification, three fractions were classified as the glutelins: i.e. the glutenins of wheat, the glutelins of maize and oryzenins of rice. The Osborne classifications of prolamins and glutelins have since been modified and revised in the light of scientific advances in the understanding of the structures and relationships of the individual proteins (Shewry, Tatham, Forde, Kreis, & Miflin, 1986). Thus, in wheat the prolamins comprise the gliadins and glutenins, the two major components of wheat gluten. The prolamins proteins in barley are the hordeins, and in maize are zein proteins. The prolamins vary widely in their molecular weights, both within and between plant species.

The most studied prolamins are the gluten proteins from wheat. They represent ~85% of wheat protein. About half of the gluten proteins are monomeric gliadins, with the remainder being disulfide crosslinked polypeptides that form the polymeric glutenin fraction, whose size can range up into the tens of millions of Daltons (Wrigley, 1996). The gliadin fraction contains mainly single polypeptide chains of M*w* in the range of 30–75,000 Da. The gliadins associate with each other and with glutenin proteins through non-covalent hydrogen bonds and hydrophobic interactions. The ω-gliadin contains a large proportion of the amino acids: glutamine, proline and phenylalanine, but no cysteine (Shewry *et al.*, 1995). In contrast, α-, β- and γ-gliadins have less proline, glutamine and phenylalanine, but 2–3 mol.% cysteine plus methionine. The glutenin proteins are divided into two groups, high molecular weight (HMW) and low molecular weight (LMW) subunits. The HMW subunits account for about ~12% of the total gluten protein. Their size (M*w* > 100,000 Da) and their ability to form an intermolecular network give the gluten the framework of its structure. Thus, it is the HMW subunits that are largely responsible for determining gluten's viscoelastic properties (Shewry, Halford, Belton, & Tatham, 2002). The LMW glutenins are structurally similar to the α/β- and γ-gliadins. However their ability to form intermolecular disulphide bonds with each other and/or with HMW glutenins, is important for the formation of the glutenin macropolymer.

Glutelins

Unlike other cereals, which accumulate prolamins as their primary nitrogen reserve, the major storage proteins

in rice are the glutelins which constitute up to 80% of the total rice protein. They have high molecular weights, ranging from 45,000 to 150,000 Da, comprising α polypeptides with M*w* of 34–39,000 Da and β polypeptides of 21–23,000 Da and are homologous at the primary sequence level to the 11S globulins of legumes (Takaiwa, Ogawa, & Okita, 1999). The rice glutelins have been difficult to study because of their extensive aggregation, disulphide bond crosslinking and glycosylation that make them generally insoluble except in dilute alkali, and therefore difficult to extract.

Functional properties of plant proteins

Protein has a number of important functions as part of the human diet. Apart from its essential function to provide amino acids for human nutrition, protein also serves prominent physical functional roles in food preparation, processing, storage and consumption which contribute to the quality and sensory attributes of food products. The most important functional properties of protein in food include its solubility, water- and fat-binding capacities, gel forming and rheological behaviours, emulsifying capabilities, foaming and whipping abilities. These properties relate to the way in which proteins interact with large (carbohydrates, lipids and proteins) and small (gases, salts, volatiles and water) molecules, as well as the molecular size, the structure (primary amino acid sequences, secondary and tertiary conformations), and the charge distribution of the protein molecules. Changes in the protein structure as a result of the environment that the protein is exposed to during food processing or influenced by the food matrix will also affect the functional properties of plant proteins.

Apart from the dough properties of gluten, the physical functional properties of plant proteins have not been studied as extensively as those from animal origin (e.g. dairy protein). One reason for this is that research on plant proteins is complicated by the fact that they are present almost always as mixtures. Purification of these proteins to a single protein has been challenging. The key functionalities that are required from plant proteins for them to be widely adopted into food systems are therefore discussed below.

Solubility

Protein solubility in aqueous solutions is often a prerequisite for its other functional properties such as emulsification and foaming. Factors that affect protein solubility are pH, ionic strength, type of solvent and temperature. Proteins are least soluble at their isoelectric point. A common method used to isolate most soluble plant proteins (largely, albumins and/or globulins) is based on this isoelectric point principle i.e. proteins are solubilised using acid, alkali or solvent (with or with salt) away from their isoelectric point and then precipitated out by adjusting the pH of the protein extract to the target isoelectric point. The isolated proteins (e.g. protein isolates prepared from soy, pea, lupin and canola) have good protein solubility at neutral pH. However, for most plant proteins, particularly those cereal proteins that contain high levels of prolamins and glutelins, their solubility at neutral pH is extremely low due to their low contents of charged amino acid residues. This low solubility has limited the use of these proteins in much wider food applications, except in dough-based products.

Emulsification

Food proteins, particularly those from milk and eggs, are commonly used to stabilise food emulsions and foams. Their amphiphilic nature allows them to be adsorbed at oil/water and air/water interfaces and form an interfacial layer that lowers the surface tension and inhibits the coalescence of oil droplets or air cells during processing and food storage (Damodaran, 2005; Dickinson, 2010; van Vliet, Martin, & Bos, 2002). The ability of a protein to function as a food emulsifier is governed by its structure and properties at colloidal interfaces.

Soy proteins have been studied most amongst the plant proteins for their emulsification properties (Adachi, Ho, & Utsumi, 2004; Keerati-u-rai & Corredig, 2009; van Vliet *et al*., 2002). Recent studies have rendered some new insights into the structure of soy proteins (either individual or as a mixture) adsorbed at emulsion interfaces. *β*-Conglycinin has better emulsifying properties compared with glycinin, due to its smaller molecular mass, higher structural flexibility (more easy to unfold), presence of glycosylated groups and higher hydrophobicity compared with glycinin (Chove, Grandison, & Lewis, 2001; Keerati-u-rai & Corredig, 2010). However, when the two proteins are present in a mixture, e.g. soy protein isolate, glycinin plays a prominent role in determining the behaviour of soy protein isolate-stabilised emulsions (Keerati-u-rai & Corredig, 2009). Similar effects have also been reported for pea proteins. Vicilin was found to be more surface active and led to better emulsifying properties compared with legumin (Dagornscaviner, Gueguen, & Lefebvre, 1987). The vicilin/legumin ratio was found to influence the efficiency of pea isolates as emulsifying agents. Notwithstanding, the ability of plant proteins to stabilise emulsions is highly influenced by the pH, ionic environments, variation in processing pre-treatment of the proteins and thermal processing of emulsion-based foods.

The interfacial structures and properties of plant proteins have not been studied in detail. In general, plant proteins form a relatively thicker interfacial layer at oil/water interfaces, compared with dairy proteins, due to their much larger molecular size and structural constraint by disulphide crosslinks (Wong et al., 2012). This has been demonstrated by soy proteins which form an interfacial layer of 30–40 nm (Keerati-u-rai & Corredig, 2010) and deamidated wheat proteins which form an interfacial layer of ~18 nm (Day, Xu, Lundin, & Wooster, 2009). The weak protein interactions once they are adsorbed at interfaces and the thickness of protein films, at least in part contribute to the superior stability of emulsions stabilised

by some of the plant proteins compared with dairy proteins, against droplet coalescence upon heating and/or in the presence of salt (Day *et al*., 2009; Keerati-u-rai & Corredig, 2009; Palazolo, Mitidieri, & Wagner, 2003). The structure of plant proteins and lack of structural unfolding of these proteins upon adsorption to interfaces may also present a potential opportunity for them to form protein particulates at interfaces, and thus provide better emulsion stability similar to those emulsions stabilised by nano- or microparticles (Dickinson, 2012).

Foaming

The ability of food proteins to stabilise foams is related to the propensity of proteins to be adsorbed onto air/water interfaces and their ability to reduce surface tension and form strong interfacial membranes *via* protein–protein interactions at air/water interfaces. Native plant proteins, because of their compact structure, have limited foaming properties. Plant protein fractions that are rich in albumins (e.g. those fractions from peas and lupins) have shown good foaming properties that are equivalent to that of egg white (Alamanou & Doxastakis, 1997). Similarly, foaming properties of canola meal have been shown to be comparable with, or even better than, those of soybean flour (Aluko & McIntosh, 2001). The foaming properties of soy proteins are by far the most studied compared with proteins from other plant sources. β-conglycinin has better forming properties compared with glycinin, because β-conglycinin is the smaller molecule of the two proteins and has a more flexible molecular structure. The interfacial behaviour of β-conglycinin was found to be similar to those of whey proteins, suggesting there might be a role for β-conglycinin as a replacement for milk proteins in food formulations (Medrano *et al*., 2012). However, structural modification, either by changing the pH or limited enzyme hydrolysis, is often required for plant proteins to achieve their potential as active foaming agents. It should be noted that altering the pH influences the conformation of glycinin at air/water interfaces and this could have a great impact on the interfacial rheological and foaming properties of these proteins.

Gelation

Food proteins, particularly globular proteins, are able to form gels when they are denatured by heat in aqueous solutions when protein–protein and protein–solvent interactions are balanced. Gel formation temperature and the resulting gel properties are determined by the protein molecular structure, protein–protein and protein–solvent interactions (Doi, 1993). Common traditional foods such as tofu and bread doughs are examples of plant proteins forming specific gels or protein networks under specific conditions.

The gelation behaviours of the two major legume proteins, i.e. 11S glycinin (legumin) and 7S conglutin (vicilin), are quite different and result in different gel properties such as gel fracture behaviour, firmness and/or elasticity. Gelation temperatures of β-conglycinin are lower than those of glycinin and are more dependent on protein concentration (Chove, Grandison, & Lewis, 2002). Gelation of 7S protein (β-conglycinin) mainly involves hydrogen bonding and hydrophobic interactions with no covalent disulphide bonds, whereas gelation of 11S protein (glycinin) involves disulphide crosslinks (Shomer, Lookhart, Salomon, Vasiliver, & Bean, 1995). The 7S protein fraction has a softening effect on gels made from whole soy proteins. pH and salt can influence the protein's gelation behaviour greatly as they affect the net charge of the proteins, interactions between protein molecules, stability of protein structure and dissociation of subunit polypeptides. It has been shown that at pH 7.6, protein solutions of β-conglycinin, glycinin or their mixture gelled at a higher temperature than at pH 3.8 (Renkema, Knabben, & van Vliet, 2001). Glycinin forms an aggregated gel structure at 85 °C, but forms an ordered strand structure at 95 °C due to heat induced dissociation of its quaternary structure and reassociation of its subunits into regular strands (Banerjee & Bhattacharya, 2012). In general, gel formation by protein isolates prepared from soy, lupin and peas, is rather similar suggesting a common pattern of protein denaturation and gel formation mechanism.

Dough functionality

One area that plant proteins are mostly utilised for their functional properties is the dough forming properties of gluten. The unique physical properties of gluten set it apart from all other plant proteins. When gluten protein is hydrated, the protein molecules form a cohesive matrix. This matrix is elastic, allowing it to stretch and expand, thus forming the basis of dough viscoelastic properties that are so crucial for the processing of many wheat-based foods. In aerated doughs, the elasticity of gluten network controls the expansion of the gas bubbles that produce the porous texture of the various types of breads and other baked products.

The viscoelastic behaviour of hydrated wheat gluten is determined by its prolamin protein components: gliadin for viscosity and extensibility and glutenin for elasticity; and their interaction in the presence of a given solvent environment, e.g. water (Belton, 1999). Glutenin polypeptides are linked through inter-disulphide bonds to form an extended protein network with Mr sizes ranging into the tens of millions, whereas gliadins form intra-disulphide crosslinked protein monomers that are associated with glutenin polymers *via* non-covalent hydrogen bonds and hydrophobic interactions (Belton, 2005). The low content of charged amino acids in gluten polypeptides makes them associate closely together and resists dispersion in water.

Nutrition

Amino acid composition and protein quality

The nutritive values of various food proteins are to a large extent determined by the concentration and availability of the individual indispensable amino acids and total

nitrogen (Boye, Wijesinha-Bettoni, & Burlingame, 2012; Young & Pellett, 1994). Most animal-based proteins provide these indispensable amino acids in balanced proportions, but many plant-based proteins provide sub-optimal proportions. The typical amino acid compositions of plant proteins from various cereal grains, legumes and oilseeds are shown in Table 4. One of the indispensable amino acids, lysine, is at a lower concentration in most plant proteins compared with animal proteins. In addition, the sulphur-containing amino acids (methionine and cystine) are also relatively lower in legumes compared with amounts found in proteins of animal origin such as dairy, egg and meat. The exceptions are soy and canola proteins which have well balanced amino acid compositions (Table 4).

The amino acid content is only one of the factors determining the overall nutritional quality of dietary proteins. Other factors, such as the digestibility and availability of the protein, can also affect the utilisation of proteins by humans. In general, the digestibility of plant proteins in their natural form is lower than the proteins from animal sources (WHO/FAO/UNU, 2007). However, plant proteins are often consumed after undergoing some degree of processing to enhance their palatability and acceptance. Factors such as temperature, duration of heating and the amount of moisture during processing treatments may reduce the digestibility of proteins. For example, kafirin proteins in sorghum form extensive disulphide crosslinks and non-disulphide interactions when they are heated, resulting in poorer digestibility compared with the proteins of other similarly processed cereals like wheat and maize (Duodu *et al*., 2003).

The protein quality can be evaluated as amino acid score (AAS), nitrogen balance, *in vitro* or *in vivo* protein digestibility, protein efficiency ratio, net protein ratio, protein rating, net protein utilisation, biological value and the so-called protein digestibility-corrected amino acid score (PDCAAS). The detail of these methods and their limitation were recently reviewed by Boye *et al*. (2012). The PDCAAS is a score which is derived by expressing the content of the first limiting indispensable amino acid in the test protein as a fraction of the content of the corresponding amino acid in a reference pattern and multiplied by true faecal N digestibility as measured in a rat assay (Schaafsma, 2012). Scores exceeding 100% are truncated to 100%. The PDCAAS rates the nutritional quality of some plant proteins, e.g. those derived from soy and canola, to be equivalent to animal proteins (FAO/WHO, 1991). Other plant proteins have comparatively low PDCAAS scores (Table 5). One strategy to overcome the unbalanced amino acid profile and low nutritional quality of individual plant proteins is to pair plant-based proteins. Proteins from oilseeds or legumes that are low in sulphur-containing amino acids can be used effectively in combination with most of the proteins from cereal grains which are deficient in lysine. For example, the PDCAAS of sorghum-based foods could be improved by 2–3 fold by supplementing with cowpea flour (Anyango, de Kock, & Taylor, 2011). The improved nutritional quality of sorghum–cowpea

Table 4. Amino acid composition (mg/g protein) of plant proteins from various cereal grains, legumes and oilseeds (Food and Agriculture Organisation, www.fao.org).

	Amino acid content (mg/g protein)									
	Wheat (grain)	Barley (whole seed, dehulled)	Maize (whole meal)	Rice (milled polished)	Sorghum	Soybean	Lupin	Pea	Chickpea	Canola[a]
Indispensable amino acids										
Arginine	48	50	43	79	34	73	98	102	98	58
Histidine	24	22	28	24	22	26	27	25	28	31
Isoleucine	34	38	38	44	41	46	45	46	46	23
Leucine	69	71	128	86	138	79	74	73	78	71
Lysine	30	37	27	38	21	65	55	81	71	56
Methionine	16	18	20	22	14	13	8	10	11	21
Cystine	26	24	16	16	16	13	14	12	12	24
Phenylalanine	47	54	50	50	51	50	38	49	60	38
Tyrosine	31	33	39	33	28	32	37	29	31	32
Threonine	30	35	37	34	31	39	38	44	39	44
Tryptophan	11	16	7	27	13	13	10	10	9	13
Valine	46	53	50	60	52	49	42	51	47	55
Non-indispensable amino acids										
Alanine	37	42	77	59	87	43	37	44	45	44
Aspartic acid[b]	51	60	64	99	65	119	113	118	121	73
Glutamic acid[b]	309	249	194	199	219	190	227	174	165	181
Glycine	41	41	38	45	31	42	43	44	42	49
Proline	103	115	92	49	84	56	42	42	44	60
Serine	48	43	51	48	43	52	52	47	53	40

[a] Data adopted from Wanasundara (2011).
[b] Including asparagine and glutamine respectively.

Table 5. Protein Digestibility Corrected Amino Acid Score (PDCAAS) values of individual plant protein and examples when combined, compared with selected animal proteins (FAO/WHO, 1991).

Protein source	PDCAAS value[a]
Casein	1.00
Egg white	1.00
Beef	0.92
Whole wheat	0.42
Wheat gluten	0.25
Rice	0.47
Barley	
Maize	0.46
Sorghum	0.20–0.30
Soy protein concentrate	1.00
Pea protein concentrate	0.73
Chickpeas	0.71
Lupin	
Canola protein concentrate	0.93
Example of combined plant proteins	
Wheat flour + canola meal (50:50 protein)	0.67
Wheat flour + pea flour	0.82
Wheat flour + soy protein	0.72
Rice + peas	1.00
Sorghum + cowpea	0.35–0.60

[a] Other data sources: Anyango *et al.*, 2011; Hoffman & Falvo, 2004; Rozan *et al.*, 1997; Sarwar & McDonough, 1990.

foods is due to the combined effects of the improvement in the lysine content and protein digestibility. Similarly, the PDCAAS of wheat flour could be improved by addition of protein concentrates from soy, pea or canola (FAO/WHO, 1991). In some cases, the overall nutritive value of the protein mixture exceeds that for each protein source alone when one of the protein sources has a considerably higher concentration of the most limiting amino acid in the other protein, e.g. rice and pea (Table 5). Consequently, mixtures of plant proteins can serve as complete and well-balanced sources of amino acids that effectively meet recommended human nutritional requirements (Young & Pellett, 1994).

However, providing that the dietary protein supply is equal to or above the recommended protein intakes, all of the indispensable amino acids from plant sources, including those sulphur-containing amino acids (in legumes) and lysine (in cereals) are regarded as more than adequate to meet and even exceed adult requirements (WHO/FAO/UNU, 2007). However, this may not be the case for infants and growing children who have relatively higher requirements compared with adults for indispensable amino acids. The requirement for balanced amino acids and easily digestible protein quality is particularly high in the first year of life and is also more important for growing children compared with mature adults.

Studies have shown that high plant protein intake is inversely related to blood pressure, which can potentially reduce the risk of cardiovascular and related diseases (Elliott *et al.*, 2006). Depending on the form of plant proteins being consumed as part of the diet, other constituents in the seeds of plants, such as non-starch polysaccharides, soluble fibre (e.g. β-glucan) and polyphenolic compounds, may also provide supplementary health benefits in addition to the consumption of plant proteins.

Anti-nutritional factors

The presence of high levels of protease inhibitors in soybeans and other legumes and condensed tannins in cereals such as sorghum, can also cause substantial reductions in protein and amino acid digestibility due to their ability to inhibit digestive enzymes or bind to proteins, thus reducing protein solubility and hydrolysis by digestive enzymes.

Plant proteins have also suffered from an unfavourable image with regard to the allergic reactions they cause in 1–2% of the population and the link with coeliac disease from consuming specific proteins from wheat, barley and rye (Mills, Jenkins, Alcocer, & Shewry, 2004; Sollid, 2000). Proteins are able to sensitise an individual and subsequently induce an allergic reaction in which an immunoglobulin E (IgE)-mediated response is generated. Commonly used plant-derived proteins possess an inherently stable native protein structure due to their high cysteine content and conserved disulphide crosslinks. Consequently, these proteins are relatively thermostable and appear to only unfold partially with minor conformational changes after undergoing common food processing. The ability of these proteins to almost retain their native structure makes them more recognisable for IgE-mediated responses (Mills, Sancho, Rigby, Jenkins, & Mackie, 2009).

Coeliac disease is characterised by inflammation of the small intestine resulting from an inappropriate immune response to wheat gluten (and similar proteins from barley and rye) with classic symptoms including chronic diarrhoea, abdominal distension and failure to absorb nutrients from the intestine. The disorder can cause affected persons to lose weight and suffer from deficiencies of vitamins and minerals which can lead to anaemia and skin problems. At present, the only treatment is a strict diet avoiding all products containing gluten and to cater for this market, other non-gluten cereals or legumes are used commercially to produce gluten-free breads, pasta, flour and other foods.

Future research needs to increase the utilisation of plant proteins in human food

Plant breeding

With the advancement of genetic research, many of the plant genes which control the transcription and synthesis of enzymes and storage proteins have been identified in the world germplasm populations for many plant species. For decades, conventional breeding and genetic selection of plant crops to increase both the total protein and the content of essential amino acids, especially lysine, have been explored vigourously. “High protein genes” have been

identified in wheat and breeding to achieve high protein content by incorporating genetic materials from exotic wheat lines or related wild species has had some success (Shewry, 2009).

High lysine mutants of barley, maize and sorghum have been identified and these varieties, which contain 2–3% higher lysine contents than normal, have been bred to increase the nutritional quality of such plant proteins (Baik & Ullrich, 2008; Gibbon & Larkins, 2005). High lysine mutations in all cereals are associated with negative effects on grain size and yield, and thus, they have been commercialised only for animal feed applications. Despite dedicated research efforts, progress in breeding high protein content and high lysine cereals has been slow and has not resulted in commercially viable lines. This is partly due to the complexities of multigenic control and strong environmental impacts which make it very difficult to select viable lines effectively, and more importantly the inverse correlation between yield and protein content is usually commercially unviable. Consequently, there is almost always a yield penalty when growing high protein or high lysine cereals. However, there is considerable potential to breed and develop varieties with improved protein functionality and protein quality for food uses from the cereal grains such as barley and sorghum.

Genetic engineering (GM) approaches have also been explored to increase both total protein and the lysine content in cereal crops by increasing lysine-rich proteins or free amino acids (Shewry, 2007). Recombinant technology and genetic engineering have also been used to alter soybean protein structure to obtain soybean proteins with better physical properties such as solubility, emulsifying ability and gelation (Li, Kong, Zhang, & Hua, 2011). For example, by removing the hydrophilic variable regions or by attaching a hydrophilic domain, modified 11S globulin proteins had increased hydrophobicity and exhibited better emulsifying properties than native glycinin (Tandang, Atsuta, Maruyama, Adachi, & Utsumi, 2005). Molecular design to increase sulphydryl and disulphide bonds to the same molecule resulted in enhanced gel hardness (Adachi *et al*., 2004). GM potato produces 35–45% more protein with 2.5–4-fold increase in lysine, methionine, cysteine, and tyrosine content than non-GM potato has been achieved (Chakraborty, Chakraborty, & Datta, 2000; Gilani & Nasim, 2007). Although the technology to genetically modify seed storage proteins in cereals, legumes and other edible plants with selective quality, physical and nutritional properties has been advanced considerably during the last two decades, the acceptance of GM by consumers is still collectively negative. It is necessary to establish that any transgenic lines are substantially equivalent in terms of grain composition and that the proteins expressed pose no risk to human and animal health in terms of toxicity or allergenicity. The environmental impact of transgenic crop lines also needs to be thoroughly examined before commercialisation.

Cost-effective recovery and separation processing

By their nature, plant proteins are either extracted or concentrated by their (in)solubility in water or various solvents. Although together they constitute up to 30% of the total grain nitrogen, albumin and globulin proteins are essentially washed away when wheat flour is fractionated to produce gluten and starch, due to their high solubility in water. The albumin and globulin proteins in cereals are considered biologically more active proteins and possess far superior physical functionalities such as emulsification, compared with the major cereal prolamin proteins. The recovery of albumin and globulin proteins from the waste streams of current grain processing may add significant value to overall plant protein production. In addition, a considerable proportion of the protein is lost through the loss of aleurone layer in the traditional milling process to produce white flour in the Western world. Thus the cost-effective recovery of those proteins, mostly albumins and globulins from other parts of the grains such as bran and germ of wheat and barley could also add values to grain millers and processors.

Other fractionation technologies that are more commonly employed in the dairy industry for producing protein concentrates such as isoelectric precipitation or ultrafiltration have been explored for concentrating plant proteins. For example, these fractionation technologies have shown potential to obtain protein fractions from lupins and peas with improved gelation properties (Boye *et al*., 2010; Chew, Casey, & Johnson, 2003; Taherian *et al*., 2011).

An alternative method to obtain protein enriched fractions from plant materials is through dry milling or dry fractionation (Schutyser & van der Goot, 2011). Dry milling and air classification are the most commonly used techniques. The separation of protein-enriched particles from starch-enriched particles is achieved according to their densities and sizes. The air classification is controlled by setting a cut size to divide the feed material into a coarse fraction (above the cut size) and a fine fraction. For protein–starch separation, the optimum cut size is around 10 μm, which is just below the size of most starch granules (Schutyser & van der Goot, 2011). Air classification of fine powders for protein separation can be carried out with centrifugal air classifiers with a classifier wheel or rotor (Dijkink, Speranza, Paltsidis, & Vereijken, 2007). However, the yield and protein concentration that have been achieved using dry fractionation techniques have been relatively low compared with the protein fractions produced by wet milling and other fractionation techniques.

Modification of protein structure for new functional properties

Most plant proteins have high levels of the amide-containing amino acids, glutamine and asparagine (Table 4), which render them with low water solubility and low surface activity at neutral pH. One of the approaches that

have been used to modify the plant protein's surface charge distribution to enhance their solubility, and thus their foaming and emulsifying properties, is deamidation. The side chains of the amide-containing amino acids are susceptible to hydrolysis of the amide bonds and are transformed into acidic groups. This conversion of the amide groups to acid groups partially unfolds and increases the protein's surface charge resulting in an amphiphilic molecule with a much better water solubility and surface activity at neutral pH compared with the native protein (Day *et al*., 2009). Due to the unique conserved structure of plant prolamin proteins, upon adsorption to the oil/water interface, deamidated proteins can provide emulsions with a superior interfacial stability against droplet coalescence and heating. The deamidation may be achieved with either an acid treatment or by an enzyme such as transglutaminase. These approaches have been successfully applied to improve the solubility, emulsification and foaming properties of plant proteins such as wheat gluten (Day *et al*., 2009), rice glutelin (Liu *et al*., 2011), zein (Flores, Cabra, Quirasco, Farres, & Galvez, 2010), barley hordein (Zhao, Tian, & Chen, 2010) and soy protein isolate (Suppavorasatit, De Mejia, & Cadwallader, 2011). Deamidated plant proteins can be used in a broad range of food applications, e.g. to provide water-binding and emulsifying properties for meat products, to endow nutritional advantages for sports drinks and medical supplements, to mimic dairy protein functions in products such as infant formula, coffee whitener and calf milk, as an emulsifier for powdered shortenings; and as a milk replacement in bakery applications (Day *et al*., 2006). Other chemical modifications, such as acetylation or succinylation, can also be used to increase the surface hydrophobicity of plant proteins. For example, the alteration of the protein surface charge promotes the formation of a strong network which results in much improved gelation properties of lupin proteins (Krause, Bagger, & Schwenke, 2001). However, chemical modification of proteins may result in some regulatory issues. Although research has shown some success of this approach to modify protein functionalities, particularly plant proteins, it is not an approach favoured by the industry.

Enzymatic modification is another way to enhance the functionality of plant proteins. Limited hydrolysis of proteins by enzymes can be achieved by controlling the hydrolysis conditions. Protease enzymes catalyse the hydrolytic break at peptide linkages and produce smaller peptide units which are more water soluble and with increased amphiphilicity. This approach has been demonstrated for wheat gluten (Popineau, Huchet, Larre, & Berot, 2002), soy protein isolate (Kim, Park, & Rhee, 1990), pea protein isolate (Ribotta, Colombo, & Rosell, 2012), canola proteins (Chabanon, Chevalot, Framboisier, Chenu, & Marc, 2007) and rice protein (Paraman, Hettiarachchy, Schaefer, & Beck, 2007). The success of hydrolysis in improving the emulsifying capabilities and foaming properties of these plant proteins is mainly attributed to decreased molecular size and increased hydrophobicity of hydrolysed polypeptides (Panyam & Kilara, 1996).

There are many proteases available commercially. However, care needs to be taken to choose a specific protease and specific conditions for the hydrolysis reaction to ensure that the protein is not hydrolysed to such an extent that too many small peptides are generated which can lead to a reduction in the protein's emulsification, foaming and gel formation properties. In addition, the release of small peptides by protease action can generate bitter off-flavour taste, even when the fraction of these small peptides is relatively low.

A third approach to modify protein structure is by the use of physical food processing technologies, such as extrusion, high pressure processing and high power ultrasound treatment. Extrusion technology is commercially used to produce fibrous structures from soy proteins, gluten and plant protein mixtures. Alignment of protein molecules during the extrusion process allows them to form thin filaments or microfibrils that assemble into a macroscopic fibrous structure. Hydration of the fibrous strands gives a laminated, fleshy appearance of meat-like texture (Liu & Hsieh, 2007; Yao, Liu, & Hsieh, 2004). Many such plant-based protein structures created by extrusion can simulate meat fibres and are widely used as vegetable meat analogues in restaurants and pre-prepared meals (Anonymous, 1996). Recently, there have also been reports on the use of extrusion technologies in combination with enzyme treatments to produce sorghum protein concentrates (de Mesa-Stonestreet, Alavi, & Gwirtz, 2012). Extrusion changes the molecular structure of proteins resulting in them being more susceptible for further enzyme hydrolysis.

Static high pressure processing is an emerging non-thermal technology that is being increasingly adopted in the preservation and processing of food. This innovative technology has shown great potential as an effective and safe method of modifying protein structures (Galazka, Dickinson, & Ledward, 2000). High pressure treatment (e.g. >400 MPa) can induce a change in the protein conformation that results in an increase in the surface hydrophobicity of the protein. Evidence of the weakening of non-covalent bonds at mild treatment conditions has been demonstrated but, further chemical crosslinks were observed with increasing pressure severity of treatment. Several researchers have studied the effects of high pressure processing conditions on the modification of the structure and functional properties of wheat gluten, soy and pea proteins (Anonymous, de Lamballerie, & Speroni, 2011; Apichartsrangkoon, Ledward, Bell, & Brennan, 1998; Kajiyama, Isobe, Uemura, & Noguchi, 1995; Kieffer, Schurer, Kohler, & Wieser, 2007). For any of these processing technologies to be used effectively to modify plant protein structures, a better understanding of the molecular changes that occur during processing is needed so as to control changes in protein structure to achieve optimal functionalities.

Improving the nutritional values of plant proteins through formulation

Complementation of cereals and legumes as a part of the human diet has been practiced by our ancestors long ago in various parts of the world. For example, soybean was paired with rice in the Far East diet, whereas sorghum and cowpea was probably the most important combination in Africa (Deshpande, 1992). Unknowingly, our ancestors must already have experienced the remarkable complementary nature of cereal and legume proteins in the human diet. With industrial production of proteins from plant sources being available as ingredients, blending of proteins from cereals and legumes has attracted considerable research interest. In recent years, with increasing consumer awareness of food for health, there has been renewed interest in improving the nutritional values of plant proteins, particularly by substituting legume flour (and protein) in wheat-based foods. Flour and protein isolates from soy, chickpea, lupin and pea have been successfully incorporated into wheat flour formulations for cakes (Gomez, Oliete, Rosell, Pando, & Fernandez, 2008), pasta (Martinez-Villaluenga, Torres, Frias, & Vidal-Valverde, 2010; Nielsen, Sumner, & Whalley, 1980), biscuits (Serrem, de Kock, & Taylor, 2011; Singh & Mohamed, 2007) and bread (Angioloni & Collar, 2012; Dhingra & Jood, 2002). Significant amounts (e.g. 15–50%) of legumes to replace wheat flour in foods such as cakes, biscuits and pasta, where gluten functionality is not absolutely essential, are achievable. Such levels of mixed plant protein sources can improve the total plant protein quality in the food by 2–3 fold (Table 5) (Anyango *et al*., 2011; Sarwar & McDonough, 1990). However, supplementing wheat flour with legume proteins in breadmaking applications in which gluten plays a critical role for dough viscoelastic properties has been challenging. The quality of the end-product is often compromised with negative impacts on loaf volume, texture and eating quality of bread (Dhingra & Jood, 2002; Fenn, Lukow, Humphreys, Fields, & Boye, 2010). These negative impacts have restricted the levels of substitution in most cases to no more than 15% flour base or 5% protein base. In another example, peas (and beans) and rice both have incomplete proteins, but together they provide all of the essential amino acids (Table 5). Recently, a website (www.foodwiki.com/vprotein) was set up to provide information on optimal amino acid complements from plant-based foods (Woolf, Fu, & Basu, 2011). The quantity of each food or pair of foods required to satisfy human requirements for indispensable amino acids can be determined. For example pairings of rice to soy protein products suggest an optimal ratio of 25–60% rice, depending on the specific soy ingredient.

Although blending or supplementing each other is a good way to improve the nutritional quality of plant proteins in terms of their amino acid profiles (e.g. consuming a mixture of grains and legumes), it is not necessary that certain combinations of plant foods have to be eaten at the same meal to ensure a sufficient intake of essential amino acids. Provided energy intake is adequate and a variety of plant foods are eaten each day, the body maintains a pool of indispensable amino acids which can be used to complement dietary proteins (Young & Pellett, 1994). There has been considerable work done experimentally to determine the production method and substitution levels for various food applications such as cakes, bread, pasta, extruded and gluten free products. However, most research has taken the formulation and substitution approach, which makes product development a rather lengthy exercise. Since some of the plant proteins have similar molecular structures and belong to the same family of proteins (e.g. albumins and globulins from soy, canola, lupin and pea), further research to understand the structural and functional relationships of major plant protein fractions could provide knowledge for a systematic substitution and formulation approach so as to improve the nutritional quality of plant protein-based foods, without comprising the physical and sensory properties of the food product. One of the areas where a variety of nutritional food products can be created using blends of proteins from a variety of cereals and legumes is extruded snacks and breakfast cereals. Up to 54% soy flour was added to a corn- and oat-based breakfast cereal formulation to produce a high-protein soy-based cereal which achieved a comparable sensory acceptance rating to commercial products (Yeu, Lee, & Lee, 2008). Again, addition of protein to an extrusion formulation requires considerable research to understand the ingredient interactions and the effects of processing on protein structure and aggregation behaviour under the high temperature and low moisture extrusion conditions. Such knowledge can help in the development of processing parameters to produce food products with optimal quality and nutritional value.

Improving the digestibility of some plant proteins such as those from sorghum and rice, will also be an important step in enhancing their quality and nutritional value. More underpinning research is needed to fully understand the chemical and physical changes that occur in plant proteins under various processing conditions in order to develop, in the long-term, optimal procedures for improving the nutrition quality and enhancing the overall utilisation of plant food proteins.

Concluding remarks

With a rapidly rising world population, the demand for protein will also increase. The food industry is actively searching for alternative protein sources and this search is also being partly driven by consumers' concerns about their health and the environmental impact of excessive meat consumption. Traditional plant protein sources like soy, wheat, sorghum, lupin and chickpeas are being examined again as healthy and sustainable solutions to such increasing protein demand. While soy continues to dominate in terms of plant proteins, a range of new products is starting to appear,

based on cereals, other legumes and vegetables. The agri-food industry has becoming more sophisticated since the early days of wheat gluten–starch separation and soy protein concentrate/isolate production. Protein fractions containing largely one group of proteins have been produced to deliver specific functionalities, for example, albumin or globulin-rich fractions can be produced from canola meal or lupin flour. New techniques are also being developed to enhance the texture, juiciness and flavour of meat analogues and proteins.

In spite of continuing research, the allergenicity of plant proteins and those with low digestibility such as the sorghum protein kafirin, remain to be overcome for these proteins to be exploited in wider food applications. Studies in humans concerning the bioavailability and metabolic utilisation of dietary proteins are still lacking. Such studies can potentially provide the strongest evidence and give clear-cut answers with regard to the nutritional values of plant proteins.

Another outstanding challenge which has limited the expanded use of plant proteins, particularly in the Western countries, is the strong 'beany' (proteins of legume sources) or 'grainy' (proteins of cereal sources) flavours associated with them. Although a variety of attempts to remove or mask these flavours have been carried out through targeted processing, it has not been possible to eliminate these undesirable flavours to a satisfactory extent. Effective utilisation of plant proteins in foods for human consumption depends greatly on consumer acceptance. Consequently, further research to find innovative solutions is needed to overcome these flavour issues.

Whereas much is known about the nutritional and health benefits of consumption of plant-based foods, much less is known about the functionalities of plant proteins in terms of processing and food ingredient/product development, compared with the proteins from animal origin and in particular, dairy proteins. The future increased use of plant proteins in food relies on further research innovation and technology developments. There is so much more to be learnt about these proteins at the molecular level as well as their physical intrinsic functionalities that are associated with their structural assembly under given conditions. The diversity and ability of plant proteins to form different food structures, i.e. the fibril structure that mimics meat texture and soft gel particles similar to those of whey proteins with the potential to be used as a substitute for dietary fats, should be further exploited.

In the future, plant proteins can play a more significant role in satisfying food protein demand while contributing to enhanced food security and a sustainable environment. From an environmental sustainability and food security point of view, there is an urgent need to increase the direct use of plant protein for human food. Even though much has been achieved by various extraction and processing techniques for plant-based proteins, fully effective processing methods remain to be developed so as to obtain proteins that have both high nutritional values and functional physical properties from many plant resources which are currently not being fully utilised. More work needs to be done to improve on what has been accomplished so far and to meet the challenge of fully utilising proteins from diverse plant sources which will have the potential and promise to feed the growing world population in the future.

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