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SPECIAL ISSUE

MOLECULAR, FUNCTIONAL AND PROCESSING CHARACTERISTICS OF WHOLE PULSES AND PULSE FRACTIONS AND THEIR EMERGING FOOD AND NUTRACEUTICAL APPLICATIONS

Guest Editors
Chantal Bassett, Joyce Boye, Robert Tyler and B. Dave Oomah

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Review

Pulse proteins: Processing, characterization, functional properties and applications in food and feed

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ABSTRACT

Pulses (pea, chickpea, lentil, bean) are an important source of food proteins. They contain high amounts of lysine, leucine, aspartic acid, glutamic acid and arginine and provide well balanced essential amino acid profiles when consumed with cereals and other foods rich in sulphur-containing amino acids and tryptophan. The protein content of most pulse legumes fall within the range of 17–30% (d.w.b.). Apart from their nutritional properties, pulse proteins also possess functional properties that play an important role in food formulation and processing. Examples of such functional properties include solubility, water and fat binding capacity and foaming. Various research studies indicate that some functional properties of pulse proteins may be comparable to those of other frequently used proteins such as soy and whey. The functional properties of pulse proteins have been exploited in the preparation and development of products such as bakery products, soups, extruded products and ready to eat snacks. The growing body of research on the health benefits associated with the consumption of pulses has increased interest in developing innovative technologies to expand the use of pulses in food products. At the same time, there are growing global food security challenges and protein malnutrition continues to be a problem in many countries around the world. Pulses, especially when blended with cereal proteins, may offer a promising alternative source for nutritional and functional proteins. This review provides an overview of the characteristics of pulse proteins, current and emerging techniques for their fractionation, their major functional properties and opportunities for their use in various applications.

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1. Introduction

Pulses constitute an important source of dietary protein for large segments of the world's population particularly in those countries in which the consumption of animal protein is limited by nonavailability or is self-imposed because of religious or cultural habits (Liener, 1962). Pulses provide energy, dietary fibre, protein, minerals and vitamins required for human health. Recent research studies suggest that consumption of pulses may have potential health benefits including reduced risk of cardiovascular disease, cancer, diabetes, osteoporosis, hypertension, gastrointestinal disorders, adrenal disease and reduction of LDL cholesterol (Hu, 2003; Jacobs & Gallaher, 2004; Philanto & Korhonen, 2003; Tharanathan & Mahadevamma, 2003). Such studies have contributed significantly to a growing awareness of the usefulness of including pulses in the diet and a steady rise in interest in using pulses and ingredients derived from them in the development of novel food products, especially in North America. From the nutritional perspective, pulses are of particular interest because they contain high amounts of protein (18–32%). In addition to providing a source of essential amino acids and bioactive peptides, pulse proteins possess functional properties such as water holding, fat binding, foaming and gelation which could expand their potential use in the development of a wide variety of food products.

World protein requirements continue to be a global issue with heightened concerns about food security and protein malnutrition. In 1997 the Food and Agriculture Organization of the United Nations (FAO) estimated that over 800 million people in the developing world were undernourished (Blandford & Viatte, 1997). Today the figure is higher. The World Bank estimates there are currently 967 million malnourished people in the world (http://www.reliefweb.int/rw/rwb.nsf/db900SID/MCOI-7KGM87?OpenDocument). Moreover, an estimated 149.6 million children younger than 5 years are malnourished in terms of weight for age. In south central Asia and eastern Africa, about half the children have growth retardation due to protein–energy–malnutrition (PEM), a syndrome resulting from inadequate supplies of protein and other macro and micronutrients (http://emedicine.medscape.com/article/1104623-overview). Programs such as the World Food Program, the Millennium Hunger Task Force and the New Partnership for Africa's Development (NEPAD)-Hunger Taskforce are undertaking School Feeding Programs aimed at providing adequate nutrition to children and malnourished communities globally. Most hungry and undernourished people live on a mono carbohydrate diet (e.g., maize or rice) and lack the required protein, fat, vitamin A, iodine, zinc and iron. The potential for blending pulses such as pea, chickpea, lentil and beans with other locally grown grains to meet some of the protein malnutrition problem worldwide is, therefore, of tremendous interest.

This paper will attempt to provide a review of the nutritional and functional properties and the current and potential applications of proteins from pulses. The paper will focus specifically on pea, chickpea, lentil and bean. A brief summary of the protein quality of pulse legumes is provided along with the processing technologies available for their fractionation and processing into protein flours, concentrates and isolates. The paper will also briefly address the possible use of pulses as an alternative to priority allergens such as soybean, gluten, dairy, eggs and nuts. As reports in the literature have shown that pulse proteins may themselves be allergenic, a short summary of the body of knowledge on the allergenic properties of pulse proteins is also presented.

2. Composition, molecular characteristics and nutritional quality of pulse proteins

The proximate composition of different pulses is presented in Table 1. Pea, chickpea, bean and lentil contain 17–30% protein with varying concentrations of essential amino acids (Sathe, Deshpande, & Salunkhe, 1984). The major proteins found in pulses are globulins and albumins. Albumins are water soluble and comprise enzymatic proteins, protease inhibitors, amylase inhibitors and lectins and have molecular masses (MM) ranging between 5000 and 80,000 Da. Globulins on the other hand are salt soluble. The major globulins found in pulse legumes are legumin (11S) and vicilin (7S). The 11S and 7S storage proteins of pulses are built of polymorphic subunits encoded by multigene families (Schwenke, 2001 and references within). 11S legumins have hexameric quaternary structures with acidic (MM of ~40,000 Da) and basic (MM of ~20,000 Da) subunits. The 7S vicilins have a trimeric structure with MMs of 175,000–180,000.

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Table 1
Proximate composition of various pulses.

Pulse	Variety	Composition (g/100 g of sample)					Reference
		Protein[a]	Fat	Fibre	Ash	Carbohydrate	
Pea	Pigeon	19.39	3.24[e]	5.56[c]	4.05	–	Nwokolo (1987)
	Cowpea	22.53	1.60[e]	5.33[c]	3.81	–	Nwokolo (1987)
	Lencolen	34.7	2.4	4.25	3.93	54.72	El-Adawy, Rahma, El-Bedawey, and El-Beltagy (2003)
Chickpea	Garbanzo beans	19.30	6.04	17.4[b]	2.48	60.65	USDA
	Kabuli (Iraq)	23.6	4.9	4.4	3.1	–	Sosulski and Gadan (1988)
	Kabuli (India)	20.6	6.6	3.0	3.5	–	Sosulski and Gadan (1988)
	Kabuli (Canada)	22.1	6.5	7.8	2.6	–	Sosulski and Gadan (1988)
	Desi (India)	18.4	5.8	6.2	3.4	–	Sosulski and Gadan (1988)
	Desi (Canada)	25.1	6.1	8.4	2.8	–	Sosulski and Gadan (1988)
	Surutato	21.7	5.6	4.3[d]	3.0	65.4	Fernandez and Berry (1988)
	Surutato	26.2	6.0	5.5[c]	2.8	59.5	Ulloa, Valencia, and Garcia (1988)
	Kabuli	29.0	6.0	6.0	3.0	–	Viveros, Brenes, Elices, Arija, and Canales (2001)
	Desi	25.0	4.5	9.0	3.2	–	Viveros et al. (2001)
Lentil	Giza	27.5	1.16	–	4.03	63.4	El-Nahry, Mourad, Abdel Khalik, and Bassily (1980)
	Family 91	26.7	1.24	–	3.41	64.6	El-Nahry et al. (1980)
	Pakistani	26.4	1.25	–	1.46	64.5	El-Nahry et al. (1980)
	Giza 9	31.4	1.15	6.75	4.16	56.53	El-Adawy et al. (2003)
Bean	Kidney	23.58	0.83	24.9	3.83	60.01	USDA
	Red kidney	16.89	1.64[e]	30.34	1.14	–	Sell, Liuecke, Taylor, and Hamner (1949)
	V.C 2010	26.40	1.75	6.15	4.50	61.20	El-Adawy et al. (2003)

[a] Nx6.25.
[b] Total dietary.
[c] Crude.
[d] Acid detergent.
[e] Ether extract.

A third storage protein, distinct from legumin and vicilin, was purified from the seeds of pea and was named 'convicilin' (Croy, Gatehouse, Tyler, & Boulter, 1980). The protein has a distinctively different amino acid profile and unlike the 7S vicilin, contains very little carbohydrate and has a subunit MM of 71,000 Da and a MM in its native form of 290,000 Da including an N-terminal extension. Qualitative and quantitative variations in convicilin subunits within and between pea lines have been observed. Sulphur-amino acids are present in convicilin but absent in vicilin. The presence of convicilins has been reported in other pulse species of the legume tribe *Vicieae*. Recently, Sáenz de Miera, Ramos, and Pérez de la Vega (2008) analyzed 34 new convicilin gene sequences from 29 different species (*Lathyrus*, *Lens*, *Pisum*, and *Vicia* spp.). The size of the N-terminal extension was found to differ among convicilins from different species. There has been some disagreement, however, about the nomenclature of convicilin. O'Kane, Happe, Vereijken, Gruppen and van Boekel (2004), as an example, have argued that the definition of vicilin heterogeneity be extended to acknowledge the possible oligomeric inclusion of the 70,000 Da polypeptide.

Other minor proteins found in pulses include prolamins and glutelins (Gupta & Dhillon, 1993; Saharan & Khetarpaul, 1994). Prolamins are alcohol soluble and are characterized by a high proportion of proline and glutamine. Glutelins, on the other hand, are soluble in dilute acid or alkali detergents, and in the presence of chaotropic or reducing agents (Osborne, 1924). Glutelins contain higher concentration of methionine and cystine than the globulins and thus are also of nutritional interest. Consequently, some researchers have suggested that the breeding of pulses with higher glutelin content may help to improve their protein quality (Singh & Jambunathan, 1982).

The nutritional quality of food proteins is determined largely by their composition of essential amino acids and digestibility and is often reported as biological value, protein digestibility, protein efficiency ratio or net protein utilization (Young & Pellet, 1994). The amino acid contents and nutritional quality (digestibility) of different pulses are presented in Tables 2–4. Pulse proteins are generally high in lysine, leucine, aspartic acid, glutamic acid and arginine, but lack methionine, cysteine and tryptophan (Swanson, 1990). Proteins with high level of branched-chain amino acids, low level of aromatic amino acids and high Fischer ratio are reportedly beneficial to health (Oomah, 2001). Variation in nutrient composition of different pulses may be due to varietal effects, environmental factors such as geographic location and growing season, as well as differences in the techniques used for analysis (Maheri-Sis, Chamani, Sadeghi, Aghazadeh, & Aghajanzadeh-Golshani, 2008; Sathe et al., 1984).

The albumin:globulin and legumin:vicilin ratios vary markedly for different pulses. Albumins from pulses are generally high in cysteine and methionine whereas legumin proteins generally have higher amounts of the limiting sulphur-containing amino acids (methionine and cysteine) compared to the vicilin fraction (Bulter, 1982). Gupta and Dhillon (1993) reported albumin:globulin ratios of 1:3, 1:6.3, 1:>3 and 1:4 for lentil, black gram, French bean and chickpea, respectively. Similar variations in legumin: vicilin ratio were also reported; 10.5:1, 1:6–9, 1:9, 1–3:1 and 4–6:1 for lentil, French bean, cowpea, pea and chickpea, respectively (Gupta & Dhillon, 1993). Furthermore, the content of sulphur-containing amino acids varies for albumins and globulins from different pulses. Sastry and Murray (1986) reported that the albumin fraction from pea seeds, as an example, had a lower content of essential sulphur-containing amino acids than did the corresponding fraction from chickpea. They found, however, that the pea albumin was a superior source of another essential amino acid, tryptophan and concluded that the albumins enriched in tryptophan are probably different proteins from those responsible for the higher sulphur-amino acid content of albumin fractions compared to globulin fractions. A brief summary of studies conducted on the protein profiles of different pulses is presented below.

2.1. Bean

Among dry beans, *Phaseolus* bean is cultivated and consumed in the greatest quantity on a worldwide basis (Sathe, 2002). Beans in the genus *Vicia* and *Vigna* are also consumed quite widely. In gen-

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Table 2
Amino acid content of various legumes.

Amino acid	Pea *Pisum sativum*		Chickpea *Cicer arietinum*		Lentil *Lens culinaris*		Bean *Phaseolus lunatus*		Soy *Glycine max*	
	Ref.[A]	Ref.[B]	Ref.[C]	Ref.[D]	Ref.[E]	Ref.[F]	Ref.[G]	Ref.[H]	Ref.[I]	Ref.[J]
Essential AA										
Isoleucine	3.33	3.89	0.36	4.1	5.06	9.58	0.54	5.3	1.94	2.28
Leucine	6.58	7.84	0.48	7.0	8.09	15.86	0.72	9.0	3.26	4.10
Lysine	6.84	6.25	0.91	7.7	5.69	12.64	0.83	7.7	2.69	3.23
Methionine	1.03	1.60[d]	0.12	1.6	1.18	1.63	0.23	1.3	0.61	0.80
Phenylalanie	4.19	5.17	0.42	5.9	5.55	10.64	0.69	6.0	2.16	2.66
Threonine	3.59	4.46	0.06	3.6	5.62	7.57	0.26	4.9	1.62	1.67
Tryptophan	0.94	0.61		1.1	ND	ND			0.50	
Valine	3.89	5.11	0.38	3.6	7.24	11.64	0.65	5.9	2.06	2.38
Arginine[a]	6.84	7.93	0.48	10.3	9.10	14.04	0.42	6.9	3.17	3.77
Histidine[a]	2.52	2.33	0.24	3.4	6.84	3.95	0.30	3.2	1.15	1.36
Non-essential AA										
Alanine	4.27	4.83	0.26	4.4	21.32	39.81	0.30	4.7	1.79	2.27
Aspartic acid	10.68	11.16[b]	0.58	11.4	11.17[b]	26.10[b]	1.36	12.0	4.79	6.09[b]
Cystine	1.55	0.35		1.3	0.44	0.39		1.1	0.70	0.86
Glutamic acid	16.92	18.46[c]	1.67	17.3	24.22[c]	42.27[c]	1.88	15.1	7.66	9.39[c]
Glycine	4.32	4.82	0.26	4.1	10.22	12.66	0.43	4.2	1.77	2.20
Proline	3.76	4.64	0.24	4.6	8.88	11.36	0.38	4.7	2.04	2.93
Serine	4.79	5.71	0.12	4.9	11.20	15.60	0.61	7.2	1.92	3.08
Tyrosine	3.16	3.34	0.19	3.7	5.05	7.53	0.45	3.4	1.53	1.75

[a] Conditionally essential.
[b] Aspartic acid + asparagine.
[c] Glutamic acid + glutamine.
[d] Methionine + cysteine.
[A] Unit: g AA per 16 g N (Leterme, Monmart, & Baudart, 1990).
[B] Unit: g/100 g protein (Khattab, Arntfield, & Nyachoti, 2009).
[C] Unit: g/100 g of wet sample (Candela, Astiasaran, & Bello, 1997).
[D] Unit: g/16 g N (Alajaji & El-Adawy, 2006).
[E] Unit: mg/g dry weight basis (Rozan, Yu-Haey, & Lambein, 2001).
[F] Unit: g/16 g N on dry weight basis (Evans & Boulter, 1974).
[G] Unit: percentage of total weight on dry basis (Kovalenko, Rippke, & Hurbugh, 2006).
[H] Unit: percentage on dry weight basis (Karr-Lilienthal, Grieshop, Spears, & Fahey, 2005).

eral, on a dry weight basis, water-soluble albumins and salt-soluble globulins, respectively, account for up to 10–30% and 45–70% of the total proteins in dry beans (Sathe, 2002). The salt-soluble protein fraction of a faba bean isolate was reported by Chakraborty, Sosulski, and Bose (1979) to contain 70.6% of the total flour protein and consisted of 2S (17%), 7S (33%), 11S (45%) and 15S (5%) proteins. El Fiel, El Tinay, and Elsheikh (2002) reported globulin as the major protein fraction of faba bean representing 69.5–78.1% of the total protein. These authors further reported that protein concentration could be increased by phosphorous treatment under wet conditions.

The salt-soluble protein fraction of mung bean isolate contains 75.3% of the total flour protein and consists of 2S (15%), 7S (79%) and 11S (6%) proteins (Chakraborty, Sosulski, & Bose, 1979). For lima bean, the salt-soluble protein fraction contained 59.2% of the total flour protein and consisted of 2S (52%), 7S (21%) and 15S (27%) proteins whereas for Great Northern bean the salt-soluble protein fraction of the isolate contained 62.2% of the total flour protein and consisted of 2S (35%), 7S (57%) and 11S (8%) proteins. Methionine content in whole common beans and isolated protein fractions from seven varieties of *Phaseolus vulgaris* L. ranged between 0.7% and 0.9% (Marquez & Lajolo, 1990).

For further information on the protein profiles of different bean varieties the reader is referred to the following papers: McLeester, Hall, Sun, and Bliss (1973) (characterization of globulin proteins from common bean); Alli, Gibbs, Okoniewska, Konishi, and Dumas (1993) (study of white kidney bean phaseolin polypeptide); Li, Alli, and Kermasha (1989) (characterization of lima bean, white kidney and navy bean proteins); Paaren, Slightom, Hall, Inglis, and Blagrove (1987) (characterization of phaseolin glycoprotein); Sun, McLeester, Bliss, & Hall, 1974 (study of the association–dissociation reactions of common bean globulins); Zirwer, Gast, Welfle, Schlesier, and Schwenke (1985) (characterization of the secondary structure of the 11S and 7S proteins found in different common beans); Plietz, Drescher, and Damaschun (1987) (amino acid sequence and domain structure of faba bean/broad bean 11S); McLeester et al. (1973) (characterization of faba bean globulins) and Bhatty (1982) (characterization of bean albumin proteins).

2.2. Pea

Protein content of pea varieties range from 23.1% to 30.9% (Nx5.6) with albumin and globulin representing 15–25% and 50–60% of the total protein, respectively (Gueguen & Barbot, 1988). Others have reported pea seed albumins to account for roughly 15–40% of the total proteins in the cotyledon (Rao, Costa, Croy, Boulter, & Gatehouse, 1989; Swanson, 1990) with the remaining proteins being largely globulins (legumins and vicilins). The salt-soluble protein fraction of pea isolate was reported by Chakraborty, Sosulski, and Bose. (1979) to contain 70.2% of the total flour protein and consisted of 2S (25%), 7S (43%), 11S (28%) and 15S (4%) proteins. Heterogeneity in the protein composition of different pea varieties as well as in the polypeptide composition of individual proteins from a single variety has been reported. Gueguen, Vu, and Schaeffer (1984), Gueguen and Barbot (1988) conducted large scale purification and characterization of pea globulin and found vicilin: legumin ratio between 0.6 and 3.7 for smooth cultivars and between 2.0 and 6.2 for wrinkled pea (Table 5). O'Kane et al. (2004) found legumin: vicilin ratios varying from 0.2 to 1.5. Compared to the globulins, pea albumins contain more of the essential amino acids tryptophan, lysine, threonine, cysteine and methio-

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Table 3
Protein digestibility of various pulses compared to casein and soybean.

Sample	Protein content (%)	Protein digestibility (%, w/w)	Reference
Casein		98.34	Monsoor and Yusuf (2002)
Casein	70.0	78.3	Prakash and Prakash (1999)
Soy bean	NR	71.8	Han et al. (2007)
Pea	NR	60.4–66.5	Bishnoi and Khetarpaul (1994)
Wrinkle pea	29.32	78.86–80.14	Periago et al. (1996)
Lathyrus pea	29.93	93.39	Monsoor and Yusuf (2002)
Yellow pea	NR	82.0	Han et al. (2007)
Green pea	NR	82.6	Han et al. (2007)
Cowpea	24.4–28	77.2–78.5	Ene-obong (1995)
Pigeon pea	21.2–22.5	76.0–76.7	Ene-obong (1995)
Chickpea	20.40	89.01	Monsoor and Yusuf (2002)
Chickpea	NR	48–53	Jood et al. (1989)
Chickpea	20.42	75.4	Chitra, Singh, and Rao (1996)
Chickpea	27.5	69.1	Chitra et al. (1996)
Chickpea	18.9	69.7–71.8	Clemente, Shanchez-Vioque, Vioque, Bautistab, and Millin (1998)
Chickpea	NR	74.3	Han et al. (2007)
Chickpea	21.0	63.4	Prakash and Prakash (1999)
Lentil	23.23	95.19	Monsoor and Yusuf (2002)
Lentil	NR	81.76–99.88	Sulieman et al. (2008)
Lentil	NR	79.1–79.4	Han et al. (2007)
Lentil	NR	73.5	Melito and Tovar (1995)
Mung bean	25.04	68.54	Abd El-Moniem (1999)
Moth bean	NR	58.69–62.06	Khokhar and Chauhan (1986)
African yambean	21.1–22.5	72.5–74.7	Ene-obong (1995)
Common bean	NR	38.4–44.0	Melito and Tovar (1995)

NR – not reported.

nine while the globulin proteins are rich in arginine, phenylalanine, leucine and isoleucine (Swanson, 1990).

Two major albumin proteins have been identified in peas. The major albumin protein contains two polypeptides with MM of ~25,000 Da whereas the minor albumin protein is a low MM protein containing polypeptides with MM of approximately 6000 Da (Rao et al., 1989). El Fiel et al., 2002 reported that for both pea and faba bean frequent watering could increase albumin, prolamin, and glutelin contents, but could decrease the globulin content.

2.3. Chickpeas

There are two major types of chickpea (*Cicer arietinum*): kabuli and desi. Kabuli varieties are large, cream-coloured and have a thin seed coat whereas desi varieties are smaller, darker, have a thicker seed coat, and generally contain higher amounts of protein than the kabuli varieties (Dhawan, Malhotra, Dahiya, & Singh, 1991). Evaluating six chickpea varieties reported protein contents ranging from 20.9% to 25.27% with albumin, globulin, prolamin and glutelin contents ranging from 8.39% to 12.31%, 53.44% to 60.29%, 3.12% to 6.89%, and 19.38% to 24.40%, respectively. In other studies (da Silva, Neves, & Lourenço, 2001) chickpea globulins were found to represent the largest fraction (41.79%) of the total protein, followed by albumin (16.18%), glutelin (9.99%), and prolamin (0.48%). The salt-soluble protein fraction of chickpea isolate was reported by Chakraborty, Sosulski, and Bose (1979) to contain 50% of the total flour protein and consisted of 2S (36%), 7S (26%), 11S (32%) and 15S (6%) proteins. Dhawan et al. (1991) used electrophoresis to study the protein profiles of different chickpea varieties and reported that the globulins resolved into 19–23 bands whereas albumin proteins resolved into 30–34 bands. The molecular mass of the polypeptides ranged from 10,000 to 91,000 Da. Glutamic acid was present in maximum concentration in the total protein

Table 5
Protein composition of various pea cultivars.[a]

Cultivar	Protein (Nx5.6) g/kg DM[b]	Soluble protein fraction (%)		Vicilin/legumin ratio
		Albumin	Globulin	
Wrinkled				
Winter				
Frogel	263	21.9	59.4	2.5
Fride	295	62.0	15.2	2.0–2.7
Winkossa	263	22.8	60.3	2.8–2.9
Spring				
Alaska Sweet	231	32.9	47.1	3.3–6.2
Erygel	266	60.0	14.6	2.3–2.6
Lincoln	264	60.2	11.6	2.4–2.8
Mini	241	24.6	55.6	3.2
Peter Pan	292	20.0	54.6	2.0–3.0
Sitelle	287	21.5	54.7	3.3–4.3
Smooth				
Winter				
Frijaune	219	24.3	55.7	2.0–2.2
Frimas	273	12.3	61.0	1.3–1.5
Floriver	222	22.5	59.7	1.6–3.7
Spring				
Aldot	189	24.4	54.1	2.8–3.0
Colmo	261	59.3	11.6	0.6–1.2
Finale	272	17.9	62.1	0.8–1.3
Stegholt	225	25.0	58.8	1.8–1.9
Amino 1	195	15.1	58.6	2.1
Amino 3	181	16.1	65.4	2.7–3.2

[a] Extracted from Gueguen and Barbot (1988).
[b] Dry matter.

Table 4
Amino acid characteristics of various legumes.

Legume	Species	BCAA (Val + Leu + Ile)	AAA (Phe + Try)	Fischer ratio	Lys/Arg	Arg + Glu + His	Met + Cys
Pea	*P. sativum*[a]	13.86	5.13**	2.70	1	26.28	2.58
Chickpea	*C. arietinum*[b]	14.7	7.0**	2.1	7.47	31.0	2.9
Lentil	*L. culinaris*[c]	2.04	0.55*	3.71	0.06	4.02	0.16
Bean	*P. lunatus*[d]	20.2	6.0**	3.37	1.12	25.2	2.4
Soy	*G. max*[e]	8.76	2.66*	3.30	0.85	14.52	1.66

[a] Unit: g AA per 16 g N (Leterme et al., 1990).
[b] Unit: g/16 g N (Alajaji & El-Adawy, 2006).
[c] Unit: g/100 g dry weight basis (Rozan et al., 2001).
[d] Unit: g/16 g N dry weight basis (Evans & Boulter, 1974).
[e] Unit: percentage on dry weight basis (Karr-Lilienthal et al., 2005).
* Tryptophan – not detected.
** Tryptophan value not provided; assumed as zero.

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followed by aspartic acid and arginine, whereas sulphur-containing amino acids were deficient.

2.4. Lentil

The crude protein contents of whole seed, defatted seed, cotyledon flour, and defatted cotyledon flour of lentil were reported as 25.9%, 27.8%, 29.1% and 31.1%, respectively (Gupta & Dhillon, 1993). Albumins, legumins, vicilins, glutelins and prolamins were 16.8%, 44.8%, 4.2%, 11.2% and 3.5% of the total proteins, respectively. All lentil protein fractions were found to be glycosylated, particularly the vicilins with about 2.8% carbohydrate. The albumin, globulin, legumin, vicilin, glutelin and prolamin fractions contained 13, 22, 19, 5, 4 and 10 polypeptides, with molecular masses of 20,200 Da, 82,200 Da, 14,600–92,600 Da, 13,800–92,260 Da, 20,200–82,200 Da, 17,000–46,200 Da and 16,980–63,800 Da, respectively (Gupta & Dhillon, 1993).

2.5. Protein digestibility

In addition to beneficial proteins, pulse seeds may contain enzyme inhibitors (e.g., trypsin and chymotrypsin inhibitors) which could have adverse effects on protein digestibility if not properly inactivated during processing. Proteins with higher digestibility are more desirable because they have higher nutritional value. The *in vitro* protein digestibilities of chickpea, mung bean, and pea range between 65.3% and 79.4%, 67.2% and 72.2% and 60.4% and 74.4%, respectively (Chitra, Vimala, Singh, & Geervani, 1995). Jood, Chauhan, and Kapoor (1989) reported protein digestibilities ranging from 52% to 58% for black gram and 48% to 53% for chickpea. Differences in digestibility were also reported as a function of chickpea type and processing. Singh and Jambunathan (1981) found the *in vitro* digestibility of kabuli chickpea (72.7–79.1% for dhal and 52.4–69% for whole seed) to be higher than desi (63.7–76% for dhal and 52.4–69.0% for whole seed). Interestingly, levels of trypsin and chymotrypsin inhibitors were also higher for desi chickpea than for kabuli, and for both types trypsin inhibitor content was found to be higher than chymotrypsin inhibitor content. Marquez and Lajolo (1990) found that isolated globulin from *Phaseolus vulgaris* was highly digestible (89.5%) but had a low NPR value (1.51). Albumin and glutelin fractions had digestibilities of 79.1% and 73.2%, respectively, and NPRs of 3.18 and 2.91, respectively, which was 30% lower than that of casein. Enrichment of whole common beans with the isolated albumin fraction (rich in sulphur-amino acids), did not improve the NPR. This has led to the suggestion that there may be some regions in these proteins with conformational constraints to hydrolysis (Romero & Ryan, 1978). A comparison of the *in vitro* digestibilities of different pulse proteins is presented in Table 3.

The protein digestibility of pulses improves by soaking, cooking, autoclaving, and sprouting (Jood et al., 1989). Autoclaving was found to be most effective followed by cooking and sprouting. Protein digestibility was also higher when soaked instead of unsoaked grains were cooked. In other studies, Carbanaro, Cappelloni, Nicoli, Lucarini, & Carnovale, 1997 reported that while cooking improved *in vitro* digestibility of chickpea and common bean flour/concentrates, it did not increase the digestibility of faba bean and lentil flour. Differences clearly exist in the digestibility and nutritional quality of different pulses and the factors influencing digestibility can vary (Han, Swanson, & Baik, 2007; Yasmin, Zeb, Khalil, Paracha, & Khattak, 2008). Further studies on the digestibility of pulse flours, protein concentrates and protein isolates as a function of different processes and processing conditions would, therefore, be very useful.

3. Techniques for processing pulse protein flours, concentrates and isolates

3.1. Air classification

Air classification is a milling technique that allows the fractionation of grains/seeds into high starch and high protein flours. Milling of pulses results in flours having particles of two discrete sizes and densities. Air classification exploits this phenomenon to separate the light fine fraction (protein) from the heavy coarse fraction (starch). During air classification, whole or de-hulled seed is ground into very fine flour, and the flour is subsequently classified in a spiral air stream to separate the starch from the protein. The process can be repeated several times to improve the separation efficiency, as protein bodies can still adhere to the surface of starch granules after the initial run. Agglomerates present in this initial starch fraction consist of starch granules embedded in a protein matrix, but by repeated pin-milling and air classification, further purification can be obtained (Gueguen et al., 1984). For optimal separation efficiency, the cotyledons of the pulse seeds must be finely ground to achieve complete cellular disruption and maximum protein and starch separation during air classification (Tyler & Panchuk, 1982). The type of milling used for air classification must, therefore, be capable of producing a very fine grind, yet selective enough to break up cells and cell fragments without severely damaging the starch granules (Jones, Taylor, & Senti, 1959). In spite of these efforts, various studies have shown that a portion of the protein cannot be milled free of the starch granules despite repeated milling. The adherent protein is derived from the membranes and stroma of the choloroplasts in which the starch granules developed (Reichert & Young, 1978; Tyler, 1984). The purities of the coarse (starch) and the fine (protein) fractions obtained by air classification are, therefore, often lower than is obtainable with aqueous extraction processes.

Tyler, Youngs, and Sosulski (1981) fractionated cowpea, Great Northern bean, lentil, mung bean, navy bean, faba bean and pea using air classification and measured the protein and starch contents of the fractions. The results obtained varied for the different pulses with the highest and lowest protein purities observed for faba bean and lentil, respectively. For faba bean, the protein content after the first air classification step ranged from 71% to 75%. After re-milling of the high starch fraction (HSF), a second high protein fraction (HPF) containing 64% and 68% protein was obtained. The results for lentil were a protein concentration of 49–65% in the fraction obtained after the first step and 38–54% after re-milling. The protein fractions contained 0–4.6% starch after the first milling and 4–10.4% after the second milling. Elkowicz and Sosulski (1982) also studied the purity of protein fractions obtained from air classification of different pulses (i.e., pea, chickpea, cowpea, faba bean, Great Northern bean, lentil, lima bean, mung bean and navy bean). They obtained a HPF containing 49.5% protein from lentil flour and 75.1% protein from faba bean flour. Protein contents of air classified pulse HPFs reported by other workers range from 40% to 62% (Aguilera, Crisafulli, Lusas, Uebersax, & Zabik, 1984; Gujska & Khan, 1991a, 1991b; Patel, Bedford, & Youngs, 1980).

The purity of the HPF may be associated with the moisture content of the seeds subjected to air classification. The protein content of air classified pea (cv Trapper) and faba bean (cv Maris Bead) HPFs was reported to vary from 46% to 52% and 69% to 74%, respectively, depending on moisture content (Tyler & Panchuk, 1982). Protein separation efficiency (PSE), calculated as the percentage of the total flour protein recovered in the fine fraction, provides a measure of the effectiveness of air classification for fractionating pulse proteins. Tyler (1984) reported PSE values varying between

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78.2% for cowpea and 88.9% for mung bean. On the basis of PSE and protein contents of the HPFs the authors suggested that mung bean and lentil were the most suitable legumes for air classification, whereas lima bean and cowpea were the least suitable (Tyler et al., 1981). It was further suggested that differences among legumes in impact milling efficiency might result from differences in the thickness and structural rigidity of the cell wall and the degree of adhesion between the cell contents and the cell wall and between proteinaceous material and starch granules (Tyler, 1984).

For further information on the processing of legumes by air classification, the reader is referred to Tyler and Panchuk (1982), Tyler (1984), Elkowicz and Sosulski (1982), Aguilera et al. (1984), Patel et al. (1980) and Gujska and Khan (1991a).

3.2. Alkaline extraction/Isoelectric precipitation

Aqueous alkaline extraction followed by isoelectric precipitation (IEP) is another frequently used technique for the extraction of legume proteins (Bahnassey, Khan, & Harrold, 1986; Chakraborty, Sosulski, & Bose, 1979; Duszkiewicz-Reinhard, Khan, Dick, & Holm, 1988; Fernandez-Quintela, Macarulla, Del Barrio, & Martinez, 1997; Flink & Christiansen, 1973; Freitas, Ferreira, & Teixeira, 2000; McCurdy & Knipfel, 1990; Ologhobo, Apata, Oyejide, & Akinpelu, 1993; Paredes-Lopez, Ordorica-Falomir, & Olivares-Vazquez, 1991; Rubio, Grant, Daguid, Brown, & Pusztai, 1999; Szymkiewicz & Jedrychowski, 1998). The technique takes advantage of the solubility of legume proteins which is high at alkaline pH and low at pH values close to their isoelectric point (~pH 4–5) (Fig. 1). The unit operations for this process are quite simple and are presented in Fig. 2. Generally, ground pulse flour (with or without hulls) is dispersed in water using flour:water ratios ranging from 1:5 to 1:20. The pH of the mixture is adjusted to alkaline (pH 8–11) and the mixture is allowed to stand for periods varying from 30 to 180 min to maximize solubilization of the proteins. During this time the pH is maintained at the desired value and the temperature may be elevated (up to 55–65 °C) to further enhance protein solubilization and extraction. The mixture is subsequently filtered to remove any insoluble material and the pH of the extract is adjusted to the isoelectric point to induce protein precipitation, followed by centrifugation to recover the protein, washing to remove salts, neutralization and drying.

The protein contents of pulse protein fractions extracted using this technique has been variable, probably due to differences in the processing conditions. Chakraborty, Sosulski, and Bose (1979) extracted pulse proteins at pH 8.5 (30 min, 40 °C) followed by precipitation at pH 4.5 and obtained isolates containing 90.5% protein for chickpea protein isolate, 90.1% for pea protein isolate, 91.2% for Great Northern bean isolate, 89.3% for lentil protein isolate, 91.2% for lima bean isolate and 88.3% for mung bean isolate. Paredes-Lopez et al. (1991) used similar conditions and obtained a chickpea isolate containing 84.8% protein. In the latter study, chickpea protein isolates were obtained from defatted chickpea flour using an initial aqueous extraction at 10% (w/v) solution and pH 8.5, followed by precipitation at pH 4.5, centrifugation and freeze-drying. In other studies, de-hulled faba bean flour (*var. Muchamiel*) and pea flour (*var. Verde Holanda*) were dispersed in water (1:5 w/v ratio, pH 9, 20 min, room temperature) followed by centrifugation and protein precipitation from the supernatant (pH 4, room temperature, 20 min). The extracts obtained contained 81.2% and 84.9% protein, respectively (Fernandez-Quintela et al., 1997). Flink and Christiansen (1973) produced protein isolates from seeds of *Vicia faba* (*var Kleine Thuringer*) using a 1:5 (w–v) bean to solvent ratio, pH 8–10, 23 °C, 10 min, followed by centrifugation and precipitation of protein

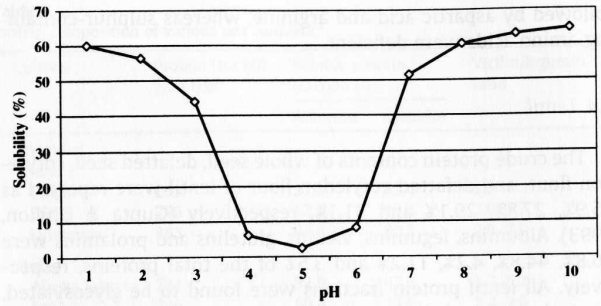


Fig. 1. Solubility of kabuli chickpea protein isolate (prepared by isoelectric precipitation) as a function of pH.

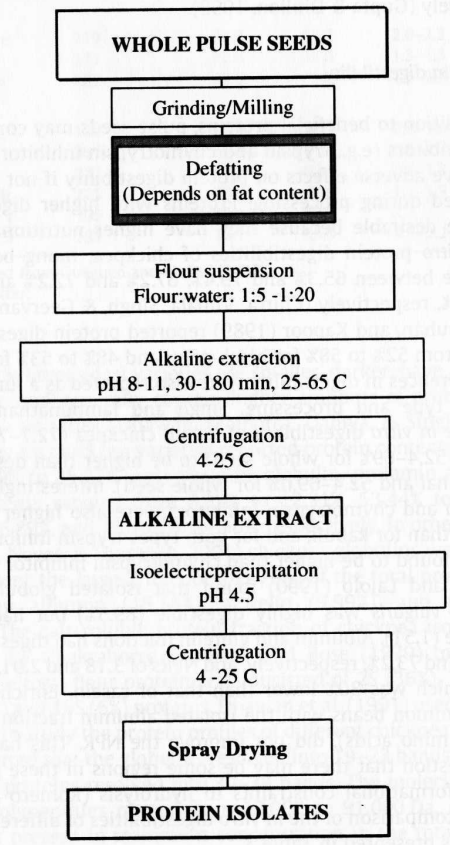


Fig. 2. Schematic diagram of alkaline extraction and isoelectric precipitation process for production of pulse protein.

from the supernatant at pH 3.5. The protein contents of the isolates ranged between 80% and 90%. Using slightly modified methods (extraction at pH 7–10, 30 min, 10 and 20 °C, 1:5 bean to solvent ratio, with and without 0.3 M NaCl, followed by precipitation at pH 4–5.3) isolates with protein contents ranging from 76.4% to 94.0% protein were obtained (McCurdy & Knipfel, 1990). Bahnassey et al. (1986) extracted lentil, pinto bean and navy bean flours with 0.02% (w/v) NaOH (flour to solvent ratio 1:10, 1 h, room temperature) followed by precipitation at pH 4.5 and they reported very low protein contents of 55.3%, 51.63% and 46.41% for the lentil, pinto and navy bean protein ex-

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tracts, respectively (Bahnassey et al., 1986). Ologhobo et al. (1993), also extracted lima bean flour with water (flour to water ratio 1:10, pH 8, 8 h, room temperature) followed by centrifugation, re-extraction, and dialysis and obtained a product with a protein content of 59.3%.

Protein purity and yield are easily affected by processing conditions (e.g., temperature, time, flour:solvent ratio, condition and protein solubility of the starting material, type of equipment and process used, *g* forces used for centrifugation, laboratory vs. pilot-scale extraction, batch vs. continuous extraction, etc.) (Russin, Arcand, & Boye, 2007) which could explain the variation in the reported results. In addition to IEP, protein extracted by alkaline extraction can be precipitated by cryo-precipitation (which refers to the precipitation of proteins under refrigerated conditions) or membrane filtration (discussed later).

3.3. Acid extraction

The principle of acid extraction is similar to that of alkaline extraction except that the initial protein extraction is conducted under acidic conditions. Solubility of pulse proteins (Fig. 1) is also high under very acidic conditions (i.e., pH < 4). This low pH range can, therefore, be used to solubilize proteins prior to their precipitation by IEP, cryo-precipitation (refrigeration) or membrane separation. In one such study, a bipyramidal crystalline protein preparation was obtained from dried seeds of white kidney bean (*Phaseolus vulgaris*) by extraction of the ground seeds with citric acid solution (0.4 N, pH 4.0) followed by refrigeration (4 °C, 18 h) to precipitate the protein material. The isolate obtained contained 95.7% protein and 2.6% total carbohydrate (Alli et al., 1993). Vose (1980) studying the direct acidification (pH 4.4–4.6) of the supernatant from starch extraction of pin-milled faba bean (*Vicia faba equina* L. cv. *Diana*) and pea (*Pisum sativum* L. cv. *Trapper*) obtained protein contents of 91.2% and 91.9% for faba bean and pea, respectively. In a slight deviation of this method, Ologhobo et al. (1993) directly suspended lima bean flour in water (flour to water ratio of 1:10) at pH 4.5 (8 h, room temperature) followed by centrifugation, re-extraction, and dialysis, and obtained a product with 50% protein content. As previously indicated for alkaline extraction, the processing conditions used for acid extraction can influence the yield and purity of the finished product which may explain differences in the reported protein purities. The acid extraction technique is generally used less frequently compared to the alkaline extraction technique.

3.4. Water extraction

Pulse proteins may also be directly extracted with water without the subsequent acid precipitation step. Martin et al. (1995), described protein extraction from seed of five mature dry bean cultivars (*Phaseolus vulgaris*) (*Mwitemania, Canadian Wonder, Mwezi Moja, Rose Coco, Red Haricot*) using a two-time extraction with water. Seeds were blended with water using a 1:10 seed:water ratio at 1–4 °C. After a 16 h resting time, the protein concentration in the supernatants ranged from 5.5–10 g N/kg seed meal. In a similar study, Cai, Klamczynska, and Baik (2001), used water to extract protein from chickpea, common bean, faba bean, lentil, mung bean and smooth pea. They blended 200 g of each seed with 500 mL water and the supernatant was extracted using centrifugation. To increase the recovery, the extraction was repeated a second time. The protein contents of the first extracts ranged from 54% for chickpea to 67% for smooth pea. After the second extraction, the highest protein content of 65.6% was obtained for faba bean and the lowest for chickpea flour with 50.5% protein. Yields for some of these extractions were not reported, and would have been useful to assess the economic relevance of the processes.

3.5. Salt extraction (micellization)

The salt extraction process, sometimes also referred to as micellization (MI), is slightly different from the previously described processes and is based on the salting-in and salting-out phenomenon of food proteins. In this process, after extraction of protein using an appropriate salt solution at desired ionic strength, the solution is diluted, inducing protein precipitation that can then be recovered by centrifugation or filtration, followed by drying. Paredes-Lopez et al. (1991) extracted protein from a 10% (w/v) solution of defatted chickpea with sodium chloride (0.5 M, pH 7.0) and obtained a chickpea protein isolate containing 87.8% protein using this method. After concentration of the extract by ultrafiltration, protein was flocculated by the addition of water (4 °C, pH 7, 1:4 v/v ratio of protein extract:water). Marquez, Barros, and Lajolo (1996) reported protein contents ranging from 74.7% to 84.2% for common bean protein extract using similar methods. For this study bean protein was extracted from meal with a 1:10 (w/v), meal: buffer (0.1 M phosphate and 0.5 M NaCl) ratio at pH 7.6. After centrifugation, the extract was decanted, dialysed against distilled water and freeze-dried to obtain the protein fraction. Albumin was similarly extracted in distilled water at 4 °C for 1 h which yielded an enriched fraction with 53–75.7% protein content.

3.6. Ultrafiltration

Membrane separation is a frequently used alternative to isoelectric precipitation. In this process, the supernatant obtained either after alkaline or acid extraction is subjected to ultrafiltration or ultrafitration/diafiltration to concentrate the proteins. Membranes with specific molecular weight cut-offs must be carefully selected in order to retain the proteins of interest. Vose (1980) using ultrafiltration prepared a protein isolate from faba bean (*Vicia faba equina* L. cv. *Diana*) and pea flour (*Pisum sativum* L. cv. *Trapper*) with 94.1% and 89.5% protein content, respectively. Factors affecting efficient separation of proteins by ultrafiltration include the type of membrane, the molecular weight cut-off, and the volume concentration ratio and diafiltration conditions. An extensive review of membrane separation is outside the scope of this review. For detailed information on membrane separation and its use in the processing of pulse proteins, the reader is referred to Fredrikson, Biot, Alminger, Carlsson, and Sandberg (2001) and Romero-Baranzini, Yanez-Farias, and Barron-Hoyos (1995).

4. Functional properties of pulse protein flours, concentrates and isolates

Functional properties are defined as the physical and chemical properties which affect the behaviour of proteins in food systems during processing, storage, preparation and consumption (Kinsella, 1982). Protein flours (<65% protein, d.w.b.), concentrates (>65% protein, d.w.b.), and isolates (>90% protein, d.w.b.) may be added to foods to increase the nutritional value and provide specific desired functional attributes. Properties of most interest in food processing include solubility, water binding, fat binding, emulsification, foaming, gelation, thickening and flavor binding. Functional properties exhibited by food proteins are determined by their amino acid composition, protein structure and conformation (e.g., surface hydrophobicity, hydrophobicity/hydrophilicity ratio), as well as processing conditions such as pH, temperature and the interactions that occur between proteins and other food components (e.g., salts, fats, carbohydrates and phenolics). A comparison of the functional properties of different pulse protein is provided below.

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4.1. Solubility

The solubility of most pulse proteins (Fig. 1) is highest at low acidic and high alkaline pH values. Solubility markedly decreases near the isoelectric point, generally between pH 4 and pH 6 for most pulses. Torki, Shabana, Attia, and El-Alim (1987) studying the solubility of albumins and globulins extracted from chickpea and lentil flours over a wide pH range reported isoelectric points of 3.9 and 4.2 for the albumin and globulin fractions for chickpea, respectively, and 3.7 and 4.3, respectively, for lentil. The solubility of the chickpea albumin was 79% at pH 2, and 94% at pH 9.5. Solubility for lentil albumin was 95% at pH 7.8 and 77% at pH 1.8, and 89% at pH 8.9 for lentil globulin. Nitrogen solubility of two types of chickpea protein isolates prepared using IEP (84.8% protein) and MI (87.8% protein) was studied by Paredes-Lopez et al. (1991). Solubilities of the extracts at pH 7 were 72.5% and 60.4% for the isolates prepared by MI and IEP, respectively, and were higher than the nitrogen solubility of a commercial soy protein isolate which had a solubility of 21.2%. Fernandez-Quintela et al. (1997) evaluating the solubility of protein isolates from faba bean (*var. Muchamiel*) (81.2% protein) and pea (*var. Verde Holanda*) (84.1% protein) confirmed that the solubility of the isolates was lowest at pHs between 4 and 6 and highest at pHs of 8–9. Pea protein isolates were found to have lower solubilities than faba bean and soy protein isolates at alkaline pH values.

Faba bean (*Vicia faba equina* L. cv. *Diana*) protein isolates prepared by IEP had solubilities of approximately 66% at both pH 3 and 7 (Vose, 1980). The solubilities were comparable to those of IEP protein isolates from smooth-seeded yellow pea (*Pisum sativum* L. cv. *Trapper*) but were lower at pH 3.5 and 5.5 compared to those of the pea isolate. Isolates prepared by ultrafiltration were 22% and 15% more soluble compared to the IEP isolate for the faba bean and pea protein isolates, respectively. Studies conducted by Braudo, Plashchina, and Schwenke (2001) have suggested that interactions of faba bean legumin protein and hydrolysed legumin with polysaccharides (chitosan) could increase their solubility at the isoelectric point and at higher pH values.

4.2. Water binding capacity

Water binding capacity (WBC) (sometimes also referred as water absorption capacity (WAC)) may be defined as the amount of water that can be absorbed per gram of protein material. The methods used for these measurements are sometimes different. WBC/WAC measurements are important for food processing applications. Materials that have low WBC/WAC may not be able to hold water effectively while materials having high WBC/WAC may render food products brittle and dry, especially during storage.

Paredes-Lopez et al. (1991) measured the WAC of chickpea protein isolates prepared using IEP (84.8% protein) and MI (87.8% protein) and soy protein isolate (SPI), and reported values of 4.9, 2.4 and 5.7 g/g for MI, IEP and SPI, respectively. Kaur and Singh (2007) reported values between 2.3 and 3.5 g/g for chickpea protein isolates. Protein isolate from kabuli chickpea, however, had significantly ($P \leqslant 0.05$) lower WACs than those obtained from desi chickpea cultivars. Furthermore, protein isolates from different chickpea cultivars reportedly had higher WAC compared to the whole flours. The WAC of a faba bean (*var. Muchamiel*) protein isolate (81.2% protein) prepared using IEP was reported by Fernandez-Quintela et al. (1997) to be 1.8 g/g. This value was comparable to that of pea protein isolate (*var. Verde Holanda*) prepared using IEP (84.1%, protein) at 1.7 g/g, but was higher than that obtained for SPI (1.3 g/g).

Celik, Basman, Yalsin, and Koskel (2004) investigating the effect of gamma irradiation on the WAC of two dry bean (*Phaseolus vulgaris*) and two kabuli chickpea (*C. arietinum*) varieties applied gamma irradiation at doses of 1, 5, 10 kGy. The lowest dose (1 kGy) did not affect WAC, significantly, but at dosages of 5 and 10 kGy, WAC significantly increased ($P < 0.05$). Obatolu, Fasoyiro, and Ogunsunmi (2007) evaluating the properties of raw, processed, boiled, fermented, roasted and malted yam bean (five mixed varieties), reported WACs ranging from 1.32 g/g for raw flour to 2.19 g/g for boiled flour. These results suggest that heat treatment may increase the WAC of pulse flours. The WAC of Great Northern bean (*Phaseolus vulgaris* L.) flour, albumin fraction and protein isolates was also reported to increase on succinylation and oxidation (Sathe & Salunkhe, 1981a). For this study the crude protein contents of the flour, albumins, globulins, concentrates and isolates were 26%, 82%, 92%, 85%, and 92% (d.w.b.), respectively. WAC of the globulin fraction and protein concentrates, however, decreased on succinylation and oxidation.

4.3. Fat binding capacity

Fat or oil absorption capacity (FAC, OAC), also sometimes referred to as fat or oil binding capacity (FBC, OBC), is calculated as the weight of oil absorbed per weight of protein powder or legume flour. Paredes-Lopez et al. (1991) reported OACs of 1.7, 2.0 and 1.9 g/g for chickpea protein isolates prepared using IEP, MI, and SPI samples, respectively. Kaur and Singh (2007) reported OACs of 2.08–3.96 g/g for protein isolates prepared from chickpea. OACs for the protein isolates were significantly ($P \leqslant 0.05$) higher than those of the corresponding flours (1.05–1.24 g/g). Additionally, OAC for the kabuli chickpea protein isolate was significantly ($P \leqslant 0.05$) higher than that of the desi protein isolate, probably due to the presence of more non polar amino acids in kabuli chickpea protein. OAC of faba bean (*var. Muchamiel*) protein isolate (81.2%, protein) prepared using IEP was 1.6 g/g which was higher than for pea (1.2 g/g) and soy (1.1 g/g) protein isolates (Fernandez-Quintela et al., 1997).

In the study conducted by Obatolu et al. (2007) on raw, processed, boiled, fermented and roasted yam bean varieties, no significant differences in the FACs of flours were observed, except for the one obtained from fermented beans which was significantly lower than that obtained from the boiled bean flour ($P > 0.05$). This may suggest that OAC is not affected greatly by processing. The OACs of Great Northern bean (*Phaseolus vulgaris* L.) flour and protein fractions were, however, reported to increase on succinylation and oxidation (Sathe & Salunkhe, 1981a).

4.4. Emulsifying properties

Emulsifying activity (EA) and emulsifying stability (ES) are two indices often used to evaluate the emulsifying properties of protein flours. Proteins act as emulsifiers by forming a film or skin around oil droplets dispersed in an aqueous medium, thereby, preventing structural changes such as coalescence, creaming, flocculation or sedimentation. Emulsifying properties of proteins are, therefore, affected by their hydrophobicity/hydrophilicity ratio and structural constraints which determine the ease with which they can unfold to form a film or skin around dispersed oil droplets. In a simplified system, EA measures the amount of oil that can be emulsified per unit of protein, whereas ES measures the ability of the emulsion to resist changes to its structure over a defined time period.

The EAs of chickpea protein isolates prepared using IEP (84.8% protein) and MI (87.8% protein) and SPI were 63.7%, 72.9% and 50.8% for the MI and IEP isolates and SPI, respectively (Paredes-Lopez et al., 1991). Corresponding EAs were 94.3%, 85.0% and 99.7%. Vose (1980), however, reported that faba bean (*Vicia faba equina* L. cv. *Diana*) and smooth-seeded yellow peas (*Pisum sativum* L. cv. *Trapper*) protein isolates (prepared by IEP and UF) generally

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had lower emulsifying properties compared to a SPI (Supro 620). Emulsifying properties were, however, found to be comparable in saline solution (4%, w/v, NaCl).

Emulsifying properties of purified pea globulins and vicilin-legumin mixtures investigated by Dagorn-Scaviner, Gueguen, and Lefebvre (1987) indicated that the vicilin proteins generally had better emulsifying properties. Sathe and Salunkhe (1981b) found that albumin proteins from Great Northern bean were better emulsifiers than were globulins. Cserhalmi, Czukor, and Gajzago-Schuster (1998) studied the EA, ES and protein surface hydrophobicity (S_0) of albumin, total globulin, vicilin (7S) and legumin (11S) fractions of five pea varieties. Differences were observed in the emulsifying properties and S_0 of the protein fractions from the different varieties. In general, the total globulin and 7S fractions had better emulsifying properties and higher S_0 than did the albumin and 11S fractions.

Techniques such as hydrolysis as well as processing conditions can impact the emulsifying properties of foods. Hydrolysis improved the EA of a pea protein isolate by approximately twofold with a maximum occurring at a degree of hydrolysis (DH) 3.7 (Sijtsma, Tezera, Hustinx, & Vereijken, 1998). In other studies, hydrolysis plus interaction with chitosan improved the ES of faba bean (Braudo, Plashchina, & Schwenke, 2001). Obatolu et al. (2007) reported emulsifying capacity (EC) values ranging from 50.7% for raw to 20% for roasted yam beans and reported that processing resulted in significant reductions in EC for all the treatments and varieties studied.

4.5. Foaming properties

The most frequently used indices for measuring foaming properties are foam expansion (FE), foam capacity (FC) and foam stability (FS). Foams are formed when proteins unfold to form an interfacial skin that keeps air bubbles in suspension and prevents their collapse. Foam formation is important in food applications such as beverages, mousses, meringue cakes and whipped toppings. Different protocols are reported in the literature for measuring the foaming properties of proteins. In general, for measuring FE and FC, protein dispersions at specified concentrations are homogenized at high speed to induce foam formation. FC or FE is expressed as the volume (%) increase due to whipping whereas foam stability is measured as the change in the volume of foam over a specified period (0–30 min).

Foam expansion (FE) and foam stability (FS) of chickpea protein isolates were 43.3% and 59.2%, respectively, when prepared using MI, and 47.5% and 66.6% for IEP (Paredes-Lopez et al., 1991). These values were higher than those for soy, 41.8% (FE) and 53.2% (FS). Fernandez-Quintela et al. (1997) reported a much lower value for FE (15%) of IEP faba bean protein isolate (*var. Muchamiel*), which was comparable to that of pea protein (15%) but lower than that of SPI (22%). FS was reported as 77% which was lower than that for pea protein (94%) and SPI (93%). Variations in the results from different studies could be due to differences in the protein purity of the samples studied as well as the specific conditions used for the foaming tests.

Il'in, Semenova, Belyakova, Antipova, and Polikarpov (2004) investigated the effect of small-molecule surfactants on the rate of liquid drainage from foams stabilized by faba bean legumin. It was reported that small-molecule surfactants in the presence of denatured legumin increased protein FS. Vose (1980) also found that protein isolates from faba bean (*Vicia faba equina* L. cv. *Diana*) and smooth-seeded yellow pea (*Pisum sativum* L. cv. *Trapper*) prepared using ultrafiltration had superior foaming properties in comparison with skim milk powder, SPI and wheat flour. Furthermore the foaming properties of the faba bean protein isolate were superior to those of the pea isolate. Sathe and Salunkhe (1981b) reported FCs of 32%, 80%, 40%, 64% and 6% for Great Northern bean flour, albumins and protein concentrates and isolates respectively. These values were, except for the bean flour and protein isolate, comparable to or higher than those of egg albumin (40%).

Obatolu et al. (2007) reported foaming capacities ranging from 1.98% for boiled yam bean to 40.2% for raw yam bean. The large difference in FC between the processed and raw bean varieties demonstrates that processing treatments used in the study (i.e., boiling, fermenting, roasting and malting) decreased FC significantly ($P < 0.05$). FS of the raw flour was, however, less than that of the processed bean flours. Hydrolysis was also reported to improve the FE of pea protein isolate nearly threefold at a DH value of 2.5 (Sijtsma et al., 1998).

Akintayo, Oshodi, and Esuoso (1999) reported a FC of 152% at 10% (w/v) concentration compared to 80% at 2% w/v for a pigeon pea concentrate (72% protein). Foaming capacity and stability improved in the acid and alkaline regions, and was better in both regions than in the isoelectric region. The improved FCs in the acid and alkaline regions was explained by the higher solubility of the pigeon pea proteins at these pH values. Additionally, foam stability of pigeon pea concentrate was found to improve as protein concentration and ionic strength increased.

4.6. Gelation (least gelling concentration)

Protein gelation is important in the preparation of many foods (e.g., puddings, jellies and in many dessert and meat applications). An important index of gelling capacity is the least gelling concentration (LGC) which may be defined as the lowest concentration required to form a self-supporting gel. Proteins with lower LGC, therefore, have greater gelling capacity. The effects of pH, sodium chloride and calcium chloride on the gelation properties of chickpea protein isolates (CPI) (91.5% protein) investigated by Zhang, Jiang and Wang (2007) indicated that an LGC of 140 g/L was required to form a gel at pH 7.0 with deionized water, whereas 180 g/L was required at pH 3.0. The LGC decreased to 100 g/L at pH 3.0 when 0.1 M NaCl was added which suggests that environmental conditions such as pH and ionic strength affected the gelation properties of CPI. Furthermore, the authors found that at pH 3.0 the gel strength for the samples containing $CaCl_2$ was stronger than for those with NaCl.

Fernandez-Quintela et al. (1997) reported an LGC of 140 g/kg for faba bean protein isolates (*var. Muchamiel*) prepared using IEP which was lower than for pea (180 g/kg) and soy (160 g/kg) protein isolates. Papalamprou, Doxastakis, Biliaderis, and Kiosseoglou (2009) reported much lower LGC values for chickpea protein isolates prepared from defatted chickpea seed flour by applying alkaline extraction followed by IEP (TpI) or ultrafiltration (TUF), or a combination of protein extraction at a mildly acidic environment followed by ultrafiltration (TF). TpI (92.52%, protein) and TF (90.07%, protein) isolates contained mainly chickpea globulins and albumins, respectively, whereas the TUF (93.43%, protein) isolate consisted of both types of protein with the globulin fraction dominating over the albumin. LGC values for the three samples by visual assessment were 11.5%, 5.5% and 4.5% (w/v) for TpI, TUF and TF, respectively. The authors suggested that it is the method of isolate preparation rather than the composition that determines gelation behaviour.

Kaur and Singh (2007) reported LGCs for various chickpea protein isolates ranging from 14% to 18%. LGCs for the seed flours were much lower (10–14%), which suggested that gelation was not only affected by protein concentration but also the type of protein as well as the presence of non-protein components. An LGC of 6% (w/v) as reported for pigeon pea protein concentrates (72% protein) (Akintayo et al., 1999). Gelation capacity of pigeon pea flour improved in the presence of a moderate NaCl concentration and at

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low ionic strength. Obatolu et al. (2007) analyzed the gelation properties of yam bean subjected to different processing conditions. Gelation significantly ($P < 0.05$) increased with fermentation and decreased with malting of the bean.

LGCs of 10%, 18%, 8% and 12% were reported for Great Northern bean flour, albumin and protein concentrate and isolate, respectively (Sathe & Salunkhe, 1981b). The globulin fraction formed a gel at 20% concentration. LGCs of 12% for cow pea protein isolate and 10% for SPI (Horax, Hettiarachchy, Chen, & Jalaluddin, 2004) and 10% for mung bean protein isolate (Coffman & Garcia, 1977) have been reported. As indicated for the other functional properties, differences in reported LGCs of pulse protein could be due to pulse type, variety, conditions used for protein extraction and drying, protein purity and composition of the extracts, as well as storage conditions.

5. Food applications

Interest in the use of pulses and their constituents in food formulation is growing in many developed countries. Factors contributing to this include their reported nutritional and health benefits, changes in consumer preferences, increasing demand for variety/balance, change in demographics (age, racial diversity), rise in the incidence of food allergies and ongoing research on production and processing technologies. In many developing countries, however, pulses have had a long history of use as human food. Dhal (split chickpea) and food items prepared from besan (dhal flour) and other pulses are regularly consumed in India, Pakistan and Bangladesh (e.g., "Phutna" (roasted grains), "pakora" (oil fried), "kadi" (boiled in buttermilk), "roti" (chickpea flour in combination with wheat flour), "dhokla" (fermented product) and "satu" (roasted, chickpea flour with cereal flours)) (Jambunathan & Singh, 1990). In Thailand, various foods ranging from main dishes and snack foods to desserts are prepared from cowpeas (Ngarmsak, 1989). In many African countries, cowpea is cooked together with vegetables, spices and other ingredients to make a thick soup or gruel which is eaten in association with the basic staple such as cassava, yam, plantain, or cereal or deep-fried as cakes ("akara" balls) prepared from decorticated flour, or as steamed products prepared from decorticated flour (Rachie & Roberts, 1974). In Turkey chickpea is consumed in the form of soup, meals, desserts, baby foods and snacks, such as quadami stamboulich, quadami safra, or quadami bisukar in the Mediterranean region (Williams & Singh, 1988).

A draw back to the use of pulses in both the developing and developed worlds is the length of time it takes to soak, cook and prepare them. With the exception of some lentils and split pea, most pulses require overnight soaking followed by at least 20 min to an hour or more of preparation. Consumers today are striving to have greater variety and healthier foods in the diet with the convenience of ready-to-eat (RTE) and fast cooking meals. Pulse protein flours, concentrates and isolates may, therefore, offer opportunities for novel product development. A brief summary is provided below of current and emerging food application opportunities for pulses and their protein fractions.

5.1. Baby foods

There are a few references in the literature on the use of whole pulses for the preparation of baby foods but very little on the use of pulse extracts (e.g., pulse protein isolates or concentrates). Singh and Singh (1992) used boiled mung bean with rice soup as a protein supplement for baby foods, and demonstrated that a porridge-type weaning food could be prepared from boiled, mashed and sieved mung bean with the addition of selected cereals and sugar. Marero, Payumo, Aguinaldo, Matsumoto, and Homma (1991) showed that germination decreased the level of anti-nutritional factors and increased protein digestibility in an infant feeding trial of gruel (weaning food) made from germinated mung beans and cowpeas.

Abebe, Stoecker, Hinds, and Gates (2006) conducted studies in southern Ethiopia on the nutritive value and sensory acceptability of infant food supplemented with kidney bean. Two complementary foods were formulated based on corn or an indigenous root crop (*Enset ventricosum*) product called kocho supplemented with kidney bean and pumpkin pulp as source of protein and vitamin A, respectively. The kidney bean and corn were soaked, germinated and lightly roasted before grinding. Four formulations were prepared, including two traditional corn- or kocho-based, served as controls. The other two formulae were supplemented to contain either corn: kidney bean: pumpkin (CBP) or kocho: kidney bean: pumpkin (KBP). Sensory acceptability tests were conducted using mother and child pairs on a five-point hedonic scale. The study showed that supplementation with kidney bean and pumpkin was a good way to increase the nutritive value of traditional Ethiopian foods prepared from corn or kocho. The protein content of the CBP and KBP formulations were 14.07 and 13.81 g/100 g, respectively, whereas the traditional corn and kocho products only had 8.82 and 1.46 g/100 g protein d.w.b. Supplementation with kidney bean in both mixtures improved their essential amino acid profiles. The products, however, had to be served as a thin porridge for the infants, which lowered their energy density. The pumpkin in CBP and KBP provided 54 μg RAE per 100 kcal, increasing the Vitamin A value of the mixes by 25- and 180-fold, respectively. Sensory evaluation of CBP by 30 mother and child pairs, and KBP by 28 pairs indicated high acceptability (4.7–4.9 out of 5). Acceptability scores of CBP and KBP were not significantly different ($P > 0.05$) from those of the traditional corn and kocho formulations. This study provides an example of the innovative ways that pulse protein fractions could be introduced into baby foods while using ingredients that can contribute additional beneficial nutrients.

5.2. Imitation milks

Soy proteins have found tremendous success in imitation milk applications. Very few reports can be found in the literature, however, on the use of pulse protein flours in the development of imitation milk products. This may probably be due to the high starch and fibre contents of pulses which make them less suitable for beverage applications where low viscosities are desired. Swanson (1990, and references therein) reported on the use of pea and lentil protein isolates for the preparation of imitation milks. The use of lentil protein isolate resulted in a milk of intermediate quality equivalent to milk prepared from soy protein isolates whereas the use of pea protein isolate gave milk of poorer quality. Successful application of pulse ingredients in imitation milk products will require detailed studies on their rheological and flavor properties under different conditions of processing and the development of effective technologies to remove residual starch.

5.3. Bean curd

One of the most consumed soy foods is tofu, a curd made from extracted soymilk with the appearance of cheese or firm yogurt. Tofu-making begins with the extraction of soymilk from soybeans followed by heating to denature the soy proteins and inactivate trypsin inhibitors. A coagulant (e.g., magnesium chloride, calcium sulphate, glucono-delta-lactone) is subsequently added to induce curd formation. The coagulated soymilk is then separated into

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whey (the uncoagulated soymilk) and curds (the coagulated soymilk), and the curds are subsequently pressed to form tofu.

Cai et al. (2001) conducted studies on bean curd formation using protein extracts of several pulses (chickpea, faba bean, lentil, mung bean, smooth pea, pea, and winged bean) using $CaSO_4$ as coagulant with or without heat denaturation. For all pulse fractions, a protein concentration of 2.3–3% and the use of 1.5% $CaSO_4$ as coagulant yielded the best result for curd formulation. Chickpea and faba beans had comparable textural properties ranking second to soybean. For lentil, mung bean and smooth pea flour, the moisture content of curds was significantly higher than for soy curds. Curds from lentil, mung bean and smooth pea had the lowest springiness and cohesiveness. A laboratory-scale technique was developed by Sri Kantha, Hettiarachchy, and Erdman (1983) for the production of winged bean curd. Preparation of high protein curd from other pulses such as field pea has been reported (Gebre-Egziabher & Summer, 1983). Further studies on the aggregation and gelling properties of pulse proteins will be useful to establish their potential application in the formation of curd-like products such as imitation tofu and cheese.

5.4. Meat products

Pulse flours and fractions have been used successfully as ingredients in the formulation of meat products (e.g., burgers, sausages, nuggets, etc.) (Abu Bakr, Shakib, El Iragi, & Mohammed, 1986; Anjou, Honkanen, Langler, & Ohlson, 1978; McWatters & Heaton, 1979; Modi, Mahendrakar, Narasimha, & Sachindra, 2003; Olsen, 1981; Patel, Caul, & Johnson, 1977; Prinyawiwatkul, McWatters, Beuchat, & Phillips, 1997; Sanjeewa, 2008; Serdaroglu, Yildiz Turp, & Abrodimov, 2005). Incorporation of flours and proteins from pulses such as soy, common bean, cowpea, black gram and green gram, increased the protein content of the meatballs or comminuted meat products (Tömek, Serdaroğlu, & Gönençayoğlu, 1988; Dzudie, Scher, & Hardy, 2002; Kaya & Gokalp, 1990; Prinyawiwatkul, Kay, Larry, & Phillips, 1997).

Modi et al. (2003) conducted studies on the use of Bengal gram flour, black gram flour, green gram flour, and soy flour in buffalo hamburger formulations. Roasted (5 min, 150 °C) and unroasted flours were incorporated at 8 g/100 g raw meat and the chemical composition and physical properties of the products were studied after production and storage. The burgers prepared with black gram gave the highest yield, lowest% shrinkage and lowest fat absorption. Fat absorption was lower for all roasted samples compared to unroasted samples. All products were acceptable when stored for up to four months. Overall, the black gram formulation had the highest scores for all quality attributes.

In another study (Serdaroglu et al., 2005), meatballs were extended using pulse ingredients such as black-eyed bean flour (BBF), chickpea flour (CF), and lentil flour (LF) at a 10% supplementation level. Incorporation of pulse flours slightly increased the toughness and the protein content of the formulation. Moreover, the use of BBF or LF resulted in greater cooking yield and fat and moisture retention whereas the addition of CF increased water holding capacity. All treatments were found to be lighter in colour compared to control samples and had high acceptability in terms of sensory properties.

Abdel-Aal, Youssef, Shehata, and El-Mahdy (1987) used flours, concentrates, powders and protein micellar mass (PMM) concentrates (prepared using a salting-in procedure) from chickpea and faba bean as sausage meat extenders at substitution levels ranging from 20% to 40%. Insignificant differences in sausage taste were observed between the faba bean flour and powder for all substitution levels examined. Products containing faba bean and chickpea PMM were found to be more acceptable at all levels of substitution than the powder-substituted products. Sironi, Sessa, and Duranti (2005) found that the use of lupin flours at substitution levels higher than 5% had detrimental effects on quality of emulsified meat products because of the presence of constituents, such as fibre and oligosaccharides. The type of pulse ingredient used, therefore, needs to be carefully selected for meat applications.

Dzudie et al. (2002) found that meatballs extended with pulse flours had higher water holding capacity than did those extended with rusk probably due to the high protein contents of the pulses. Similarly, the addition of common bean flour increased the water holding capacity of beef sausages (Dzudie et al., 2002). In the selection of the type of pulse ingredient for meat applications, it may be desirable to aim for a stable meat protein matrix which reduces water and fat loss and improves the binding properties of the restructured meat (Carballo, Mota, Barreto, & Colmenero, 1995; Pietrasik & Shand, 2003).

5.5. Baked goods, glazes, frosting and pastes

Spink, Zabik, and Uebersax (1984) formulated a cake doughnut using high protein flours from black bean and navy bean. The flours, containing 44–45% protein were prepared by dry roasting followed by air classification and were used as substitute for all purpose flour by up to 30%. At the 20% and 30% substitution levels, the formulated products were significantly less tender and darker than controls but scored as more desirable than controls for flavor and exterior/interior resistance to biting and chewing. Navy bean flour substitution at 13% yielded tastier and well-liked products. The substituted products were also found to contain less fat when compared to the control samples.

In another study (Faheid & Hegazi, 1991), up to 15% chickpea flour (24.83% protein), lupin flour (26.30% protein) and soybean flour (56.36% protein) were used to replace wheat flour in a cookie formulation. The chemical composition of each formulated products was compared with the control sample. Replacing wheat flour with chickpea or soy bean flour increased protein, ash, fibre and moisture contents and the amino acid scores. Substitution of wheat flour with lupin flour, however, increased the contents of protein and fibre and amino acid scores, and levels of only certain minerals. No data was provided on sensory properties. It would be of interest to repeat these studies with high protein pulse flours as well as concentrates or isolates.

Several researchers have studied the fortification of bread with pulse ingredients (Dalgetty & Baik, 2006; Patel et al., 1977; Sathe, Ponte, Rangekar, & Salunkhe, 1981; Wang, Rosell, & Benedito de Barbera, 2002). While most studies used whole pulse flours, a few have evaluated the suitability of high protein pulse fractions for these applications. In one such study (Abdel-Aal et al., 1987), chickpea and faba bean flours and powders (seeds were cooked, neutralized, blended and dried) were used at 15% and 20% levels to fortify bread. Protein micellar mass (PMM) was prepared from the flours using a salting-in procedure (micellization) and was used at a 15% level of substitution. Protein concentrations of the PMM ranged from 57.5% to 83.8%. Chickpea powder produced more acceptable fortified bread than did wheat flour, and the PMM at a 15% level was more acceptable than the powder. Samples fortified with 20% faba bean powder were rejected. Faba bean-PMM products were preferred over the flour-fortified products. The PMM-substituted breads generally had less beany flavor and yielded acceptable products. Lorenz, Dilsaver, & Wolt, 1979 used faba bean protein concentrate (68.2% protein) processed through air classification as partial replacement for wheat flour in formulations for breads and cookies. Bread and cookie quality were acceptable at up to 10% substitution level. Flavor and taste, although not disliked, were rated inferior to the control wheat flour.

In the orient, whole bean is commonly used as filling in baked goods (e.g., pastries, buns, etc.). The beans are normally cooked

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and used to prepare a sweetened paste. Klamczynska, Czuchajowska, and Baik (2001) prepared sweetened pastes from chickpeas flour, smooth pea flour (*var. Columbian*) and wrinkled pea flour separately, and compared them with a commercial staple food made from azuki bean. The sweetened pastes prepared from chickpea and smooth pea was found to be as acceptable as the commercial staple made from azuki bean. Although no reports were found on the use of pulse protein fractions to prepare fillings and pastes as described above, the commercial availability of pulse fractions could open up new opportunities for such products. One report was found in the literature in which lupin fractions were evaluated for use as egg glazes (Sironi et al., 2005). It may be of interest to extend these studies to other pulses fractions.

5.6. Extruded products

Extrusion technology has become one of the primary techniques for processing food products. Extrusion is generally described as a process by which food materials are forced to flow through a die, under varying conditions of mixing, heating and shear (Riaz, 2000). During extrusion, these food materials, processed by pre-treatment into a dough-like material, are shaped by forcing them through a restriction or die placed at the end of the extruder. Depending on the equipment, the pressure released as the products exit the die causes them to puff into different shapes and dry out. The popularity of extrusion technology is reflected in the variety of extruded foods presently available on the market. These include breakfast cereals, pet foods, snack foods, crisp breads, imitation nuts, croutons, crackers and wafers, amongst many others.

5.6.1. Pastas and noodles

Several studies have reported the use of pulse flours and fractions in the fabrication of pasta products. Bahnassey et al. (1986) supplemented spaghetti with lentil, pinto bean and navy bean flours and protein concentrates at the 10% and 15% levels. They compared the nutritional properties of the treatment samples with the control and found that the supplemented samples had higher nutrient levels. Trypsin inhibitor activity (TIA) in the treatment samples was, however, higher than in the control. The highest TIA was reported for the navy bean products and the lowest was observed with the lentil products. The TIA values decreased when the legumes were roasted. Similar trends were observed for hemagglutinin activity. Taste panels found up to 10% fortification yielded acceptable products.

Durum wheat semolina with added vital wheat gluten (3%, w/v) was fortified with 10% navy bean and pinto flours and protein concentrates and extruded into spaghetti (Duszkiewicz-Reinhard et al., 1988). Cooked weight of the supplemented samples was found to be lower than the control. Firmness scores were highest for samples fortified with protein concentrates followed by flour-fortified samples and then controls. The acidity of the spaghetti increased upon storage, especially for spaghetti made with the pulse protein concentrates. Spaghetti fortified with navy bean flour and concentrates had acceptable sensory analysis scores and shelf stability. Sensory evaluation studies further indicated that spaghetti fortified with legume flours and navy products was preferred by panellists over those fortified with protein concentrates.

Lorenz et al. (1979) used faba bean protein concentrate (68.2% protein) produced by air classification as a partial replacement for wheat flour in noodle formulation and found 40% as the maximum replacement level for noodles. Beyond this value extrusion was difficult. Beans imparted a desirable yellow colour to the noodles and products were also less brittle. Results from sensory studies showed the 20% substituted noodles to be acceptable and had better flavor scores than the control. Mung bean has also been used in noodle preparation and its starch has been reported as the best raw material for preparing transparent starch noodles (Singh, Voraputhaporn, Rao, & Jambunathan, 1989).

5.6.2. Puffed extruded products

In a study conducted by Gujska and Khan (1991a), navy bean high protein fraction (43.75% protein) (HPF) and high starch fraction (HSF), pinto bean HPF (44.66% protein) and HSF blends and corn processed using air classification were extrusion-cooked and the functional properties of the extrudates were studied. Addition of HPF to the HSF decreased the expansion of the extruded blends and the extruded products were also softer and more fragile. Lightness and yellowness increased with increasing protein content. Water absorption index and oil emulsification capacities were better for the corn high protein fractions blended up to the 20% level. In general, extruded blends had lower expansion index and oil absorption capacity and higher protein density and oil emulsification capacities than extruded corn or HSFs. Expansion index, however, increased with increasing protein content.

Filli and Nkama (2007) evaluated the hydration properties of extruded Fura – a legume food consumed in West Africa made from cowpea, soybean, and groundnut flours at 20–30% levels of each flour. The extrusion process compared to the production of traditional Fura was easier to prepare (without the usual rigorous labor). Extruded products had a higher puff ratio than traditional product, although, samples with higher fat contents had lower puff ratios. Significant differences ($P < 0.05$) in the swelling capacities of the Fura products were observed as the amount of water added was varied. Overall extrusion cooking was found to be promising for the commercial manufacture of instant Fura. The use of extrusion technology in food processing is one of the fastest growing trends in the food industry. This trend is likely to continue as consumers continue to demand "designer" foods with high nutritional value. Further studies are, needed to improve our knowledge of how pulse proteins and starches can be used in the development of such products.

6. Allergenicity of pulse proteins

Food allergy is a growing problem around the world. The big eight priority allergens requiring labelling in the European Union (EU) and in countries such as Canada and the USA include soybean, peanut, tree nut, milk, eggs, gluten-containing cereals, fish, and shellfish. Additionally, labelling is required for sesame in Canada, Australia and the EU and for mustard and celery in the EU.

Pea, chickpea, bean and lentil are not classified as major allergens. Although proteins in these pulse crops have been found to be allergenic, allergic reactions have been limited and have been confined mostly to Europe, Asia and the Mediterranean (San Ireneo et al., 2000), probably due to higher consumption of pulses in these populations. Severe anaphylaxis to pulse crops is infrequent (San Ireneo et al., 2000) which may partially explain why pea, chickpea, lentil and bean are not included in the priority allergen list. Multiple proteins in pulses can provoke allergic reactions and these proteins are often thermo-stable. Furthermore, pea, lentil, bean and chickpea allergens have been reported to be cross-reactive (San Ireneo et al., 2000; Szymkiewicz & Jedrychowski, 1998).

A serum pool or individual sera from 18 patients with pea allergy were used to detect IgE binding proteins from pea seeds by immunodetection and immunoblot inhibition assays. Convicilin (63,000 Da), as well as vicilin (44,000 Da) and one of its proteolytic fragments (32,000 Da), reacted with more than 50% of the tested sera. Additional proteolytic subunits of vicilin (36,000, 16,000, and 13,000 Da) bound IgE from approximately 20% of the sera. All these proteins were found to cross-react with the major lentil

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vicilin allergen *Len c 1*. (Sanchez-Monge et al., 2004). In other studies, pea protein fractions with molecular masses of approximately 50,000 Da (vicilin subunits), 70,000 Da (convicilin) and 60,000 Da (legumin proteins) were reported to be allergenic (Szymkiewicz & Jedrychowski, 2006). Cooking the seeds did not decrease their immunogenicity. Hydrolysis with trypsin at 50 °C, however, reduced the immunoreactivity of the legumin and vicilin proteins to 2.6% and 2.0%, respectively, compared to the native proteins (Szymkiewich & Jedrychowski, 2005).

San Ireneo et al. (2000) studied the allergenic composition of raw and boiled chickpea extracts using sera of 29 children with positive skin prick tests to chickpea and a current or past history of allergic reactions after ingestion of chickpea. Multiple IgE binding proteins/peptides were detected in both extracts in the molecular mass range of 10,000–106,000 Da. The authors found no significant differences between specific IgE levels to the raw and boiled chickpea extracts. Patil, Niphadkar, and Bapat (2001) using immunoblot analysis found the 70,000, 64,000, 35,000, and 26,000 Da proteins present in chickpea to be major allergens.

Ibanez Sandin et al. (1999) found approximately 30 IgE reactive bands in a crude lentil extract after immunoblot analysis. Immunoblotting patterns obtained with patients' sera showed IgE-binding bands ranging from 14 to 84,000 Da (Pascual et al., 1999). A 48,600 Da protein, designated *Len c 1.01*, was reported as the major IgE-binding component in lentil (Lopez-Torrejon et al., 2003), and recognized 77% (17/22) of the individual sera from patients with lentil allergy. Three isoforms were identified varying in their degree of N-glycosylation. A comparison of the amino acid sequences showed greater than 50% identity with major peanut (Ara h 1) and soybean (conglutinin subunits) allergens belonging to the vicilin family.

In general, allergic cross-reactions between pulses are highly likely for sensitive individuals. There has been growing concern about the allergenicity of lupin which is a pulse in the *Fabaceae* family and a member of the genus *Lupinus*. Shaw, Roberts, Grimshaw, White, and Hourihane (2008) reported that a small but significant number of children with peanut allergy are allergic to lupin. This, as well as other studies, has raised concerns about whether lupin should be added to the list of priority allergens. For the majority of patients suffering from food allergic reactions to gluten, eggs, milk, fish, shellfish and/or sesame and who do not have cross-reactivities to peanut and soybean, pulses such as pea, chickpea, lentil and bean could serve as alternative sources of nutritional and functional proteins. For sensitized individuals, however, the necessary precautions of ingredient verification and avoidance should be exercised in any food selection. Significant progress has been made in the development of "allergen-free" and "hypoallergenic" foods and already products (e.g., shakes) made from pulse protein isolates and rice ingredients are commercially available (http://www.bargainshopper.ca/nu-life/55.htm; http://www.betterlife.com/prod_home_page.asp?prod_id=26366) as alternatives to the major allergenic foods.

7. Feed applications

A significant proportion of pulse production goes into feed. Feeding trials have been conducted with rats to asses the nutritional quality of pulses for feed and food applications. Earlier studies using uncooked pulses demonstrated deleterious nutritional effects. Higher relative weights of gastrointestinal sections were determined in rats fed pulse seed meal diets compared to those fed lactalbumin (from milk) (Rubio et al., 1999). Cuadrado et al. (2002) reported that the inclusion of whole lentil meal, de-hulled lentil meal or ethanol-extracted lentil meal as the sole source of protein in the diets of rats significantly reduced growth, gain/feed ratio, apparent N digestibility and N retention (even when fully supplemented with essential amino acids). Pure lentil lectin and lectin-depleted albumin proteins had no negative nutritional effects whereas lentil globulins and lentil hulls had significant negative effects. The authors suggested that the low nutritional quality of lentil meal may be due to poor utilization of lentil globulin proteins.

Many of the anti-nutritional factors in pulses can, be eliminated or inactivated to a large degree by appropriate heating and processing during food preparation (Elkowicz & Sosulski, 1982). Treatments may include dehulling, presoaking and diffusion, sterilization, steaming and cooking. Wet milling and processing technique employed during protein concentration and isolation also have been reported to be effective in the detoxification of seed materials. Expander treatment is another technique used in the manufacture of feed which involves treatment with heat, pressure, and shear and which may improve the nutritive value for ruminants (Nielsen, 1994; Prestlokken, 1999), although effects on degradability of protein and starch are not always consistent (Arieli, Bruckental, Kedar, & Sklan, 1995; Goelema, Hof, Van der poel, & Taminga, 1996). Goelema, Smits, Vaessen, and Wemmers (1999) studying the effect of processing on the nutritional properties of a pulse mixture comprised of pea, lupin and faba bean for dairy cow feed applications showed that the type of processing treatment affected rumen protein degradability. Pressure toasting (132 °C, 3 min) decreased total digestibility of protein (TDP) and intestinal digestibility of rumen undegraded protein (DUP), whereas pelleting (80 °C, 10 s) increased TDP and DUP. Expander treatment at 115 °C for 8 s had no significant effect on either TDP or DUP.

Utilization of faba bean as a source of supplementary protein in a formulation reduced egg production for laying hens even when the feed was fortified with methionine (Davidson, 1980). Heat processing, including autoclaving, pelleting and micronization, did not eliminate the anti-nutritive factor. Feeding chickens beans with white skins (low tannin content) or the cotyledon fraction (tannin-containing skin removed) did not increase egg production. Olaboro, Marquardt, Campbell, and Frolich (1981) found that the principal egg weight depressing factor in faba bean was associated with a vicine-reactive fraction which was identified as either vicine or vicine/convicine. They also studied the effect of autoclaving on velvet bean and reported that autoclaving improved the performance of broiler chicks by improving the levels of sulphur-amino acids, particularly methionine, whereas the levels of most other amino acids decreased. Later studies showed that growth was severely hindered in chicks fed diets containing raw lima bean and lima bean fractions (i.e., alkali soluble-protein, acid-soluble protein, defatted lima bean, ether extracted meal) (Ologhobo et al., 1993) Serious histopathological changes within the liver, kidneys, pancreas, spleen and lungs were observed whereas no negative effects were observed with chicks fed soaked and boiled lima bean. The authors concluded that feeding chicks with lima bean has negative health effects on the animals, especially when fractions containing high amounts of trypsin inhibitors and hemagglutinins are used. Evidently, as is the case for human consumption, heat treatment of pulses to inactivate anti-nutritional factors such as enzyme inhibitors is an important processing step prior to their use in feed applications.

8. Industrial applications

8.1. Pest control

Proteinaceous cysteine proteinase inhibitor (CPI), an insecticidal protein found in pulses can be used to control the proteolytic

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activity of endogenous digestive cysteine proteinase in the mid-gut of some insects. CPI has been suggested to be an albumin-like protein and is readily soluble in water. Hines, Osuala, and Nielsen (1992) evaluated black bean, chickpea, common bean, cowpea, lentil, lima bean, mung bean, pea, soybean, lupin, and tepary bean seeds as potential sources of CPI. Soybean, lima bean, lupin and tepary bean seeds had the highest relative CPI activity of 13 different legumes (44 varieties in total) and soybean had the highest CPI activity. A 12,000 Da CPI was isolated from soybeans (using a hypocotyl-rich fraction and water extraction) which inhibited the proteolytic activity of papain and crude gut extract of several bruchid insects. Fakhoury and Woloshuk (2001) reported the presence of a 36,000 Da lectin-like alpha amylase inhibitor in hyacinth bean which inhibits conidial germination and hyphal growth of Aspergillus flavus, a fungal pathogen. Other insecticidal lectins and proteins toxic to cowpea pests have been reported in hyacinth bean. Additionally a patent for the use of a pea protein-rich extract to control insect pests was issued in the United States in 1999 (Bodnaryk, Fields, Xie, & Fulcher, 1999). Pulse proteins may, therefore offer an interesting source of biomass for the development of agricultural bioproducts such as bioinsectides.

9. Conclusion

In the last few years, concern has grown regarding adequate supplies of food for the current (and growing) world population of nearly seven billion. The media has been replete with stories of food crises in many developing countries. At the same time we have witnessed a very unstable world economy, even in the developed world, which has put pressure on many industries including production agriculture and food processing. Protein malnutrition continues to be a major problem in many places in India, Africa, and in pockets of the developed world. At the same time there is growing social consciousness about the impact of climate change and agricultural practices. Food manufacturers, as well as consumers, are increasingly searching for food alternatives that offer variety and which provide functional as well as nutritional benefits and which have reduced carbon footprints. This growing trend offers an opportunity to the pulse industry to identify novel food uses for whole pulses as well as to develop ingredients and products that can be used in complement with other grains and cereals in the development of convenient alternative food and industrial products.

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