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Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques

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ABSTRACT

In this study, two methods, isoelectric precipitation (IEP) and ultrafiltration (UF) were optimised for the extraction of proteins from yellow pea, desi and kabuli chickpeas, red and green lentils. For IEP, the following optimal extraction conditions were used: pH 9.5, 1/15 solid/liquid ratio, 35 °C for yellow pea, desi and kabuli chickpeas, and pH 9.0, 1/10 solid/liquid ratio, 25 °C for red and green lentils. UF experiments were performed with a 50 kDa MWCO membrane with diafiltration (4X) at pH 6.0. The initial protein content of the pulses (16.7–24.8%, w/w) was concentrated nearly 4-fold. UF process generated concentrates with slightly higher protein contents (69.1–88.6%, w/w) compared to the IEP process (63.9–81.7%, w/w). Yields for both processes on a protein basis ranged from 50.3% to 69.1% (w/w). All concentrates exhibited good functional properties. However, functional properties varied to some extent as a function of the type of pulse and manufacturing process. For pH ranging from 1 to 3 and from 7 to 10, the red and green lentil concentrates were the most soluble (70–77%) and their UF concentrates were more soluble at all pH values studied compared to the IEP samples which was not the case for the pea and chickpea samples. Water holding capacity was highest for IEP-processed yellow pea and lowest for the UF-processed desi and kabuli chickpeas. Emulsifying properties and foam expansion were generally higher for the chickpea concentrates but they had less foam stability. Protein extracts from green lentils appeared to have the best gelling properties. The results highlight the technological potential of pulse protein extracts for food applications.

1. Introduction

Pulse (peas, chickpeas, lentils, and beans) production is one of the major agricultural sectors of significant importance to Canada. Although pulses are grown in Canada for export, very little is exported in the value-added or processed form. In order to increase the use of pulses in the food industry new opportunities for their application need to be identified. Lentils, peas, chickpeas and beans are mostly consumed whole, split or milled, and have traditionally been used in the preparation of salads, soups, snacks and condiments. Pulses can also be fractionated to obtain fibre, starch and protein concentrates or isolates. These ingredients can be subsequently used in the formulation of different food products.

Techniques for fractionation of pulses include milling, air classification and wet extraction (Tian, Kyle, & Small, 1999; Tyler, Youngs, & Sosulski, 1981; Zheng, Sosulski, & Tyler, 1998). Air classification and pin milling are generally used to fractionate pulses into a light or fine fraction (protein concentrate) and a heavy or coarse fraction (starch concentrate) (Swanson, 1990; Tyler, 1984). The purity of the protein fraction obtained using this process is, however, low (38–65%) and further processing is often required. Alkaline extraction followed by isoelectric precipitation is mostly used for the preparation of extracts with higher protein purity (>70%) (Han & Hamaker, 2002). More recent research findings have shown that techniques such as membrane separation can yield protein isolates with improved functionality (Fredrikson, Biot, Alminger, Carlsson, & Sandberg, 2001; Fuhrmeister & Meuser, 2003). This latter technique can also be effectively used to remove some anti-nutritional components (Mondor et al., 2009). Examples of anti-nutritional factors in legumes such as peas, chickpeas and lentils include protease and amylase inhibitors, lectins and polyphenols (Singh, 1988). An approximate 70% reduction in the concentration of trypsin inhibitors has been observed in the process of extraction and precipitation of isolated soy protein (Waggle, Steinke, & Shen, 1989).

The major proteins found in pulses are globulins and albumins. Globulins represent roughly 70% of legume seed proteins and consist primarily of the 7S, 11S and 15S proteins. Molecular weights of these proteins range from 8000 to 600 000 Da (Freitas, Ferreira, & Teixeira, 2000). These proteins generally have a minimum solubility at pH values between four and five (isoelectric point). By

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manipulating the solubility of the proteins and using filtration techniques that take advantage of their hydrodynamic properties, protein concentrates and isolates with varying purity and functionality can be obtained. Functional properties of food proteins that are of importance in food processing include, solubility, water holding and fat binding capacities, foaming and emulsifying properties, thickening and gel formation. These properties influence food texture and organoleptic characteristics and are essential in the manufacture of products such as confectioneries, beverages, dressings and meat products.

Selection of the appropriate technology and conditions for protein extraction is essential in food processing as these can influence the functional and nutritional properties of the finished product (Paredes-Lopez, Ordorica-Falomir, & Olivares-Vazquez, 1991). Studies conducted on the functional properties of different legume proteins in the last few years have shown that their functional properties can vary (Chakraborty, Sosulsi, & Bose, 1979; Fernandez-Quintela, Macarulla, Del Barrio, & Martinez, 1997; Paredes-Lopez et al., 1991). In spite of this, relatively few studies have been done to compare the functional properties of different pulse protein extracts processed using different techniques in the same study in order to assess the impact of pulse type and processing on functionality. This study was, therefore, undertaken to evaluate the effect of isoelectric precipitation (IEP) and ultrafiltration/diafiltration (UF/DF) extraction methods on the purity and recovery of protein from different pulses (peas, chickpeas and lentils) and pulse varieties (red and green lentils, desi and kabuli chickpeas), and compare the functional properties of the extracted proteins in order to identify potential application opportunities in industry.

2. Materials and methods

2.1. Materials

Yellow pea, desi chickpea, kabuli chickpea, green lentils and red lentils were either purchased or graciously provided by Pulse Growers in Saskatchewan. Specific varieties of certified pulses used and suppliers are as follows: CDC Golden Pea (Wagon Wheel Farms of Churchbridge, Saskatchewan, Canada); CDC Grandora Green Lentil (Simpson Seeds Inc., Saskatchewan, Canada); Common Blaze Red Lentil Seed (Simpson Seeds Inc., Saskatchewan, Canada); Mylese desi chickpea and Xena kabuli chickpea (R Young Seeds Inc., Saskatchewan, Canada). All other materials and chemicals used were purchased from regular suppliers and were of analytical grade.

2.2. Dehulling and milling of seeds

Whole pulse seeds were ground with a Retsch centrifugal grinding mill (Brinkmann Instruments, Ontario, Canada) for proximate composition measurement. Dehulling of whole seeds was performed in two steps by splitting the seeds followed by air separation of the hulls from split seeds. A Quadro mill (Quadro Engineering Inc., Ontario, Canada) was used to split the seeds and an Air Separator (Sullivan Strong Scott, Ontario, Canada) was used for the separation of the hulls. Kabuli chickpeas were soaked in water for 1 h and were dehydrated over night in an oven prior to splitting with the Quadro mill. All pulses were fed into the Quadro mill at a feed rate of 1.2–1.6 cubic feet per hour and into the air separator at a feed rate of 2.8–3.0 cubic feet per hour using a volumetric Feeder (Model 300, AccuRate Whitewater, Wisconsin, USA). After air separation undehulled seeds were separated by using a vibratory screen (KASON Model 18–30, Ingenierie de separateurs Ltee, Pointe Claire, Quebec, Canada) with 4–5 mesh sieves and reprocessed. The dehulled seeds were combined and ground with a Urschel High Speed Chopper (Model 3600, Urschel Laboratories Inc, Indiana, USA) using the 120, 050, 030 and 020 blades in that order.

2.3. Protein extraction

Two methods, ultrafiltration (UF) and isoelectric precipitation (IEP) were developed in this study for the extraction of proteins from the selected pulses. Preliminary studies were conducted to identify optimal conditions for the extraction of proteins from each pulse. For the IEP, the following optimal extraction conditions were used: pH 9.5 with 1/15 solid/liquid ratio at 35 °C for yellow pea, desi and kabuli chickpea and pH 9.0 with 1/10 solid/liquid ratio at 25 °C for the red and green lentils. UF/DF experiments were performed with a 50 kDa MWCO membrane with diafiltration (4X) at pH 6.0. For the ultrafiltration step, a volume concentration ratio (VCR) of five was applied prior to the diafiltration step. The schematic for the processing of the pulse proteins using IEP and UF/DF is presented in Fig. 1. The concentrates obtained after spray-drying were labelled as shown below and stored at 4 °C until analysed: YP-IEP – Yellow pea-isoelectric precipitation; YP-UF – Yellow pea-ultrafiltration; RL-IEP – Red lentil-isoelectric precipitation; RL-UF – Red lentil-ultrafiltration; GL-IEP – Green lentil-isoelectric precipitation; GL-UF – Green lentil-ultrafiltration; DC-IEP – Desi chickpea-isoelectric precipitation; DC-UF – Desi chickpea-ultrafiltration; KC-IEP – Kabuli chickpea-isoelectric precipitation; and KC-UF – Kabuli chickpea-ultrafiltration.

2.4. Physicochemical and functional properties

2.4.1. Proximate and chemical analysis

Pulse flours and extracts were analysed to determine their proximate composition using official methods. Protein content was determined by Leco (Leco FP-428, Leco Corp., St.-Joseph, MI, USA), using the Dumas method (AOAC, 1995) and a nitrogen conversion factor of 6.25. Fat content was determined using a Soxtec apparatus (Foss Tecator Soxtec System HT-6, 1043 extraction unit, Brampton, Ontario, Canada) according to AACC (2003a). Moisture was determined by drying 0.3 g sample in a Fisher Isotemp Vacuum Oven (Fisher Scientific, Montreal, Quecbec, Canada) for 5 h at 100 °C (AACC, 1983). Ash content was determined according to AACC (2003b). Total phenolics was determined using the method of Folin–Ciocalteu reagent (Singleton & Rossi, 1965) as modified by Velioglu, Mazza, Gao, and Oomah (1998). Two hundred milligrams of sample was extracted for 2 h with 4 mL of 80% methanol containing 1% hydrochloric acid at room temperature on a shaker. The mixture was centrifuged at 2000*g* for 15 min and 200 L of the supernatant was mixed with 1.5 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min. 1.5 mL of sodium bicarbonate (60 g/L) solution was added to the mixture. After 90 min at 22 °C, the absorbance was measured at 725 nm and results were calculated as gallic acid equivalent. All measurements were made in at least duplicates and average values were calculated.

2.4.2. Functional properties

2.4.2.1. Protein solubility. Protein solubility indices were determined at various pH values ranging from 1 to 10 according to the modified methods of Betschart (1974) and the AOCS (1974) method Ac4–41. In summary, 100 mg of protein sample was dispersed in 10 mL of water and the pH was adjusted to the desired level using 1 N HCl or 1 N NaOH. The dispersions were continuously stirred for 30 min and centrifuged at 4000*g* for 30 min. The amount of protein in the supernatant was determined by the method of Bradford (1976). Solubility was calculated as the percent ratio of protein in the supernatant to that of the total protein

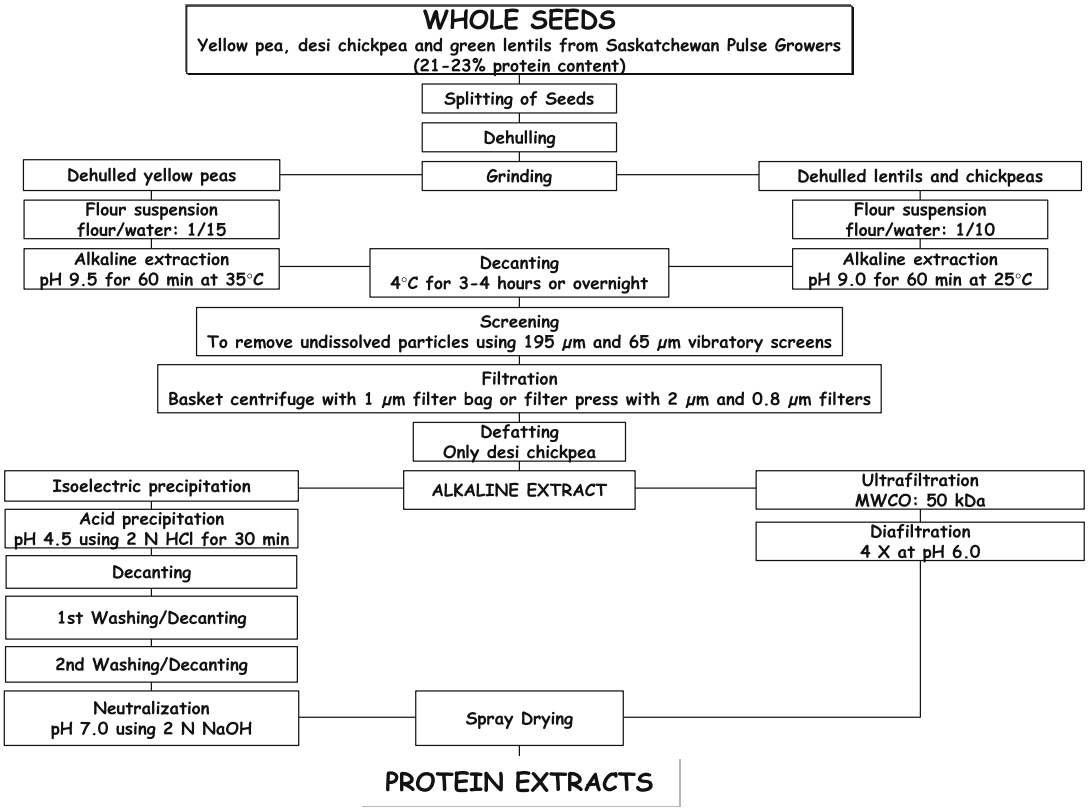

Fig. 1. Schematic of the process used for the pilot scale production of the pulse protein concentrates.

in the initial sample. The solubility profile was obtained by plotting the average protein solubility of duplicate samples vs. pH.

2.4.2.2. Fat absorption capacity (FAC). Fat absorption capacities were determined in triplicate using the procedure of Lin and Humbert (1974) with slight modifications. Samples (0.5 g) were mixed with 3 mL of corn oil in a pre-weighed 15 mL graduated centrifuged tube for 1 min. After centrifugation at 4000*g* for 30 min, the supernatant was discarded and the tubes were re-weighed. The % FAC was calculated as follows:

$$\text{FAC}\ (\%) = 100 \times (\text{weight of fat absorbed by sample/weight of sample}).$$

2.4.2.3. Water holding capacity. Water holding capacity (WHC) was determined in duplicate according to AACC (2000c) method 56–30 with slight modifications. Enough water was added to over saturate the sample but not too much to cause a liquid dispersion to form. The hydrated samples were centrifuged at low speed (2000*g*) and the supernatant was removed. Water holding capacity was expressed as the amount of water absorbed by 1 g of concentrate.

2.4.2.4. Emulsifying properties. Emulsifying properties were studied using the methods of Pearce and Kinsella (1978) with some modifications. 1.5 mL of corn oil was added to 4.5 mL of 0.5% (w/v) protein solution prepared in 0.01 M phosphate buffer (pH 7). The mixtures were homogenized at 20,000 rpm at room temperature for 1 min with a PT 2100 Polytron homogenizer (Kinematica AG, Littau-luzern, Switzerland). 250 L of the emulsion was taken out from the bottom at different times and diluted with 50 mL of 0.1% sodium dodecyl sulfate solution. The absorbance of the diluted emulsion was determined at 500 nm with a Cary 300 Bio, UV–Visible Spectrophotometer (Varian Canada, Inc., St.-Laurent, Quebec city, Canada). Emulsifying activity index (EAI) and emulsifying stability index (ESI) were calculated as described in Pearce and Kinsella (1978). All analysis were conducted in quadruplicate.

2.4.2.5. Foaming properties. Foaming properties were studied in duplicate using the methods of Waniska and Kinsella (1979) with some modifications as described in Achouri, Boye, Yaylayan, and Yeboah (2005). The pulse protein concentrates were dispersed in 0.01M phosphate buffer pH 7 with stirring for 10 min to give final concentrations of 0.5% (w/v). The solutions (15 mL) were then injected into the sparging chamber of a water-jacketed glass condenser via a septum-stoppered inlet. Nitrogen gas was sparged into the protein solution until the foam chamber (55 mL) was filled with foam, while simultaneously maintaining the volume of liquid in the sparging chamber by addition of protein solution. The required time to form 55 mL of foam, and the volume of protein solution added were recorded. After 5 min the volume of liquid drained from the foam was also noted. Foaming properties (foam activity index (Gi) – percent of gas entrapped in 55 mL of foam; foam stability index (R5) – percent of liquid retained in the foam after 5 min, and percent foam expansion (FE)) were calculated as described in the references mentioned above.

2.4.2.6. Gelling properties. The least gelling concentration of the pulse protein concentrates was determined according to the method of Sathe and Salunkhe (1981). Appropriate amounts of pulse protein concentrates were weighed into test tubes containing 5 mL of deionized water to make suspensions ranging in concentration from 2% to 20% (w/v). The samples were vortexed and the tubes were sealed and heated at 100 °C in a water bath for 60 min. The tubes were cooled immediately under tap water and

further cooled at 4 °C overnight. To determine if the suspensions had formed a gel the tubes were inverted. A firm gel was deemed to have occurred when on inverting the tube, the suspensions did not flow. A weak gel was deemed to have been formed when a semi-solid was formed that flowed somewhat on inversion. The least gelling concentration (LGC) was estimated as the critical concentration below which no self-supporting gel was formed. All analysis was conducted in at least duplicate.

2.4.2.7. Statistical analysis. Data were statistically evaluated by one-way analysis of variance (ANOVA) using the PRISM software, version 3.02 (Graph Pad Software, Inc., San Diego, CA, USA). Significant differences between means were determined by the Tukey's Multiple Comparison Test procedure at the 5% significance level.

3. Results

3.1. Pulse flour and protein concentrates composition and yields

The protein content of the whole ground pulses (undehulled) ranged between 16.7% and 25.8% on a wet basis (Table 1). The lowest value was obtained for kabuli chickpea (16.7%) and the highest for red lentils (25.8%). Fat content of the flours was generally low (<2%) except for the desi and kabuli chickpeas which contained 5.23 and 7.34% fat, respectively. Ash content for all pulses ranged between 2.34% and 3.04%.

The highest amount of phenolics was found in the green and red lentil flours (4.69 ± 0.05 and 5.90 ± 0.1 mg/g, respectively) (Fig. 2). After dehulling, the phenolic content decreased to 1.54 ± 0.07 and 1.74 ± 0.03 mg/g, respectively, suggesting that a high amount of the phenolics were present in the hulls. This finding has been reported previously by other workers (Sosulski & Dabrowski, 1984). The desi chickpea had a higher amount of phenolic content compared to the kabuli chickpea as has also been reported by other workers (Maheri-Sis, Chamani, Sadeghi, Mirza-Aghazadeh, & Aghajanzadeh-Golshani, 2008). Phenolic content of the whole yellow pea and chickpea (kabuli and desi) samples ranged between 1.18 ± 0.01 and 2.14 ± 0.01 mg/g. Dehulling and defatting (for chickpea) did not change the phenolic content very much for the kabuli chickpeas and yellow peas. A significant decrease in phenolic content was, however, observed after dehulling of the desi chickpea.

Processing of the flours by both IEP and UF/DF concentrated the proteins nearly 4-fold resulting in concentrates with protein contents varying between 63.9% and 88.6% (w/w) (Table 2). UF/DF process yielded protein concentrates with slightly higher protein contents compared with the IEP process. Studies conducted by Fuhrmeister and Meuser (2003) also found that wrinkled pea concentrates prepared by ultrafiltration (volume concentration ratio 5, 1.05 m s^{-1} cross flow rate, 1 bar transmembrane pressure at 25 °C) had higher protein content (70–80%) and lower fat content (2.3%) than concentrates obtained by isoelectric precipitation (68% and 3.8%, respectively). In our study, for both processes, the kabuli

Table 1
Proximate composition of flours from whole pulses seeds.

Component (%)	Yellow peas	Green lentils	Red lentils	Desi chickpea	Kabuli chickpea
Moisture	14.19 ± 0.03	10.68 ± 0.01	9.27 ± 0.11	9.26 ± 0.04	12.06 ± 0.15
Protein[a]	21.09 ± 0.28 (24.57)	23.03 ± 0.08 (25.78)	25.88 ± 0.12 (28.52)	20.52 ± 0.24 (22.62)	16.71 ± 0.15 (19.00)
Ash	2.42 ± 0.01	2.39 ± 0.03	2.34 ± 0.02	3.04 ± 0.01	2.76 ± 0.01
Fat	2.01 ± 0.28	0.82 ± 0.003	0.53 ± 0.003	5.23 ± 0.15	7.34 ± 0.54
Carbohydrate (calculated by difference)	60.29	63.08	63.10	61.94	61.14

[a] Values in parenthesis are on dry basis.

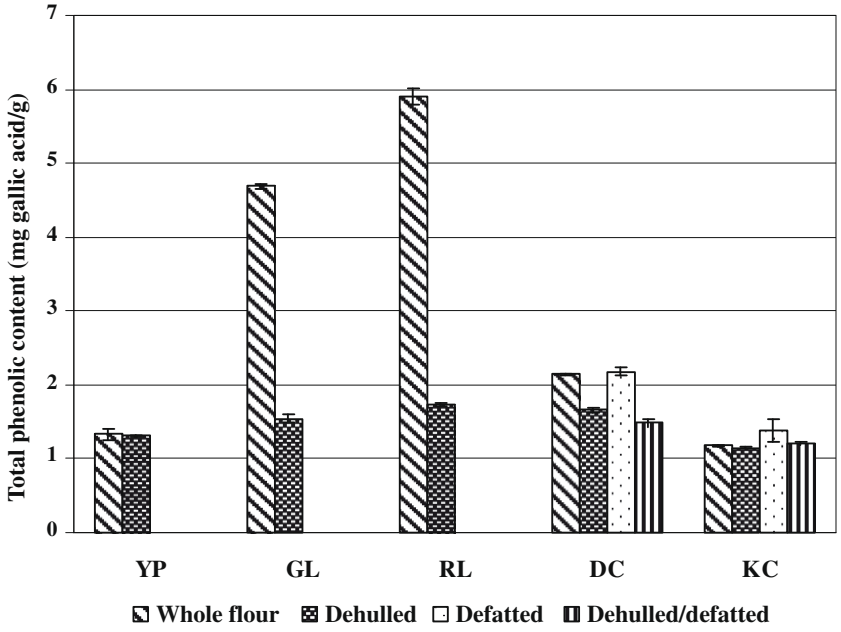

Fig. 2. Total phenolic contents of flour (F), dehulled flour (DH), defatted flour (DF), and dehulled and defatted flour (DDF) of pulses (YP: Yellow pea; GL: Green lentil; RL: Red lentil; DC: Desi chickpea; and KC: Kabuli chickpea).

Table 2
Protein content of isoelectric precipitated (IEP) and ultrafiltered (UF) pulse protein extracts and yield based on the protein content of dehulled flour.

Sample	Protein (%)		Yield[a] (%, based on protein)	
	IEP	UF	IEP	UF
Yellow pea	81.7 ± 0.3	83.9 ± 0.15	55.0	57.1
Green lentil	79.1 ± 0.3	88.6 ± 0.05	50.3	51.9
Red lentil	78.2 ± 0.2	82.7 ± 0.20	62.8	60.5
Desi chickpea	73.6 ± 0.1	76.5 ± 0.05	53.7	54.7
Kabuli chickpea	63.9 ± 1.3	68.5 ± 0.15	69.1	50.3

[a] Yield is calculated based on protein content of dehulled flour.

chickpea concentrate contained the lowest amount of protein (63.9% for IEP and 69.1% for UF). For the concentrates processed by IEP, the yellow pea concentrate contained the highest amount of protein (81.7%, w/w) while for the concentrates processed by UF/DF the green lentil concentrate had the highest protein purity (88.6%, w/w). Fat contents of the isolates were generally low except for the desi and kabuli chickpea protein concentrates which contained high amounts of fat (8.5 ± 0.9% – IEP, 5.2 ± 0.4% – UF for Desi; 12.4 ± 3.17% – IEP, 16.8 ± 0.6% – UF for Kabuli). Our results indicate that processing of the kabuli chickpea protein concentrate by both techniques resulted in a higher concentration of fat in the finished product. Protein recoveries for both processes ranged between 50.3% and 69.1% which is comparable to that reported for other plant proteins processed with similar techniques (Chew, Andrew, & Stuart, 2003; Gueguen, 1983; Papalamprou, Doxastakis, Biliaderis, & Kiosseoglou, 2009).

3.2. Solubility

To provide useful information towards effective utilisation of pulses in various food applications, the solubility of the concentrates was investigated at pH ranging from 1 to 10 (Fig. 3a and b). In general, for all the concentrates, the highest solubility was observed at pH ranging from 1 to 3 and 7 to 10. For most of the concentrates, the solubility was very low at pH 4, 5 and 6 ranging from 2% to 30%. An exception was the red lentil UF concentrate which had a solubility of 58% at pH 4. This high solubility at acidic

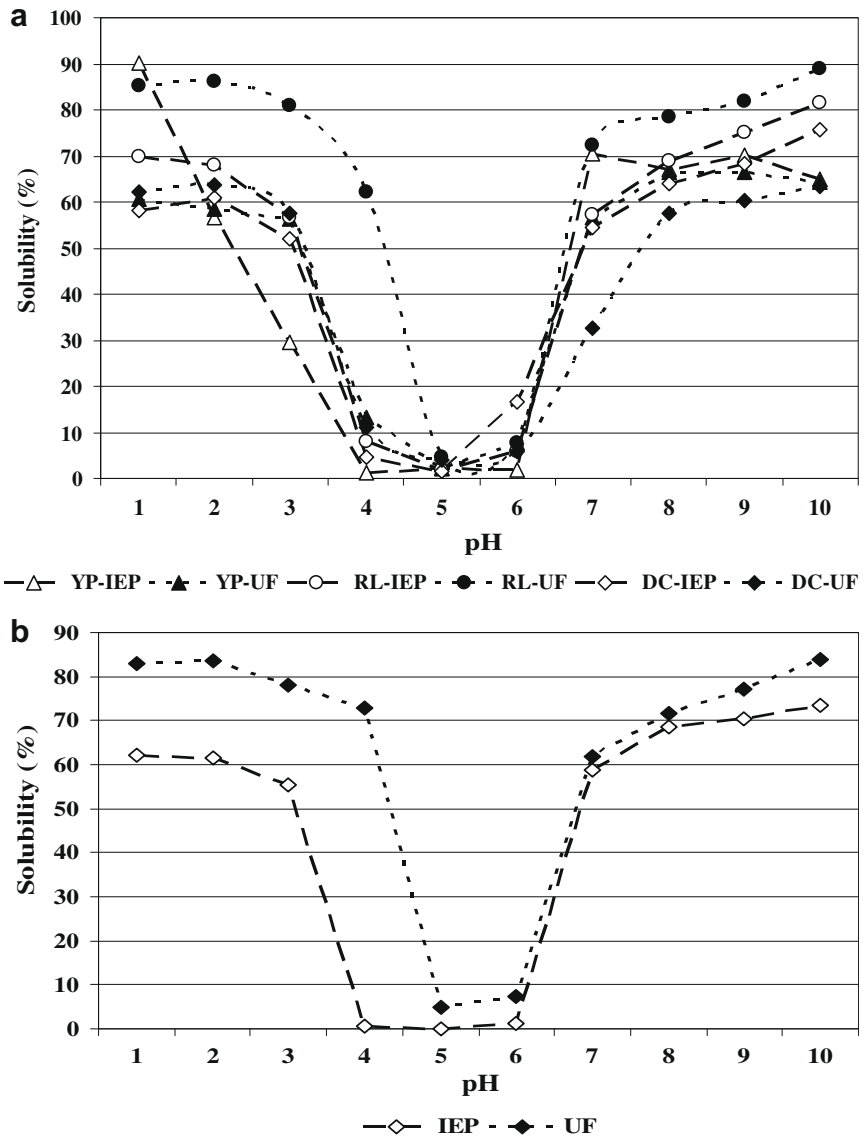

Fig. 3. (a) Solubility of IEP (isoelectric precipitation) and UF (ultrafiltration) pulse protein concentrates (YP: Pea protein concentrate; RL: Red lentil protein concentrate; and DC: Desi chickpea protein concentrate). (b) Solubility of IEP (isoelectric precipitation) and UF (ultrafiltration) green lentil protein concentrate.

pH could make it a very promising candidate for use in acidic beverages. Studies conducted by other workers have also shown the solubility of protein isolates from different legumes (including pea, faba bean and chickpea) to be lowest at pH 4–6 and highest between pH 8–9 (Fernandez-Quintela, Macarulla, Del Barrio, & Martinez, 1997; Fuhrmeister & Meuser, 2003; Paredes-Lopez et al., 1991). At neutral pH (pH 7), the concentrates that had the highest solubility were the yellow pea and red lentil concentrates processed by UF while the desi chickpea processed by UF had the lowest solubility. At pH 3 and within the pH range of 8–10, red lentil concentrates processed by UF had the highest solubility; the desi chickpea processed by UF had the lowest solubility at these pH values. Solubility of the yellow pea concentrate processed by IEP was markedly improved at pH 1–90% which was the highest at that pH. The solubility profiles for kabuli chickpea (not shown) were very similar to that of desi chickpea and that of the green lentil was also similar to the red lentil. For the red and green lentils, the UF treated concentrate generally had higher solubility compared to the IEP concentrate except at pH 5 and 6 where both concentrates had very low solubility. The most remarkable difference was at pH 4 where the UF concentrate had a solubility of 62% while the IEP treated sample had a solubility of 8% a difference of greater than 50%. In the case of the green lentil (Fig. 3b) the difference was over 70% at this pH. Vose (1980) also conducted studies on Horsebean (*Vicia faba equina L. cv. Diana*) and smooth – seeded yellow peas (*Pisum sativum L. cv Trapper*) protein isolates and reported a 22% and 15% increase in solubility for isolates processed by UF compared to IEP, respectively. Similarly, Timmermanns and Breuer (1993) reported that protein isolates from smooth peas extracted using membrane filtration had 10% higher solubility than the IEP counterpart. The effect of the type of process used on the solubility of the yellow pea and desi and kabuli chickpeas was less remarkable. The largest difference was observed at pH 3 for yellow pea where the UF concentrate had a higher solubility (56%) compared to the IEP concentrate (29%) and at pH 1 where the IEP concentrate had a higher solubility (90%) compared to the UF concentrate (60%). Overall, the protein solubility of the pulses was very good when compared with results reported in the literature for some commercial soy proteins (Fernandez-Quintela et al., 1997); this could be of interest as soy is the main competition for pulse proteins.

3.3. Water holding capacity

The water holding capacities for the pulse protein concentrates ranged between 0.6 and 2.7 g/g. These values are similar to those reported for other protein concentrates and isolates produced from legumes including some soy proteins (Fernandez-Quintela et al., 1997; L'Hocine, Boye, & Arcand, 2006; Lee, Htoon, Uthayakumaran, & Paterson, 2007; Obatolu, Fasoyiro, & Ogunsunmi, 2007; Paredes-Lopez et al., 1991; Wong & Kitts 2003). For each pulse, the protein extract produced using IEP had a higher water holding capacity than the one produced using UF (the only exception was the red lentil protein concentrate) (Fig. 4), but the differences were not found to be significant. Significant differences were, however, observed in the WHC of the different pulses. The yellow pea concentrate processed by IEP (YP-IEP) had the highest water holding capacity which was significantly higher than the desi and kabuli chickpeas processed by both IEP and UF. The YP-UF also had higher water holding capacity compared to the desi and kabuli chickpeas processed by both IEP and UF, but the effect was only found to be significant for the DC-UF and KC-UF concentrates. WHC for the green lentil concentrate processed by IEP concentrate was significantly higher compared to the DC-UF and KC-UF concentrates. For the red lentil concentrates, the sample processed by UF was significantly higher than that of the desi and kabuli chickpeas processed by both IEP and UF. Interestingly, the red lentil concentrate processed by IEP was, found to be significantly higher only for the desi and kabuli chickpeas processed by UF. No significant differences were found in the WHCs of different varieties of the same pulse. In general, it could be said that for both UF and IEP processes, yellow pea protein extracts had the highest water holding capacity followed in decreasing order by green and red lentils and desi and kabuli chickpea protein concentrates. Our results suggest a greater effect of variety on WHC than the type of process used.

3.4. Fat absorption capacity (FAC)

Results for fat absorption capacities are presented in Fig. 5. Red lentil and yellow pea protein concentrates processed by ultrafiltration had the highest FAC (226% and 177%, respectively). No significant differences were found between the FAC of all concentrates processed using IEP. For the UF treated samples, the red lentil concentrate had the highest fat absorption capacity, followed by yellow pea, green lentil and kabuli chickpeas and finally desi chickpeas. For green lentils and desi chickpea, the protein extracts produced using IEP or UF exhibited somewhat similar absorption capacities. However, the yellow pea, red lentil and kabuli chickpea protein concentrates produced by UF had significantly higher fat absorption capacities compared to the counterparts produced by IEP. Paredes-Lopez and coworkers (1991) reported fat absorption capacities of chickpea protein isolates prepared by isoelectric precipitation and micellization. The fat binding capacity was 200% for

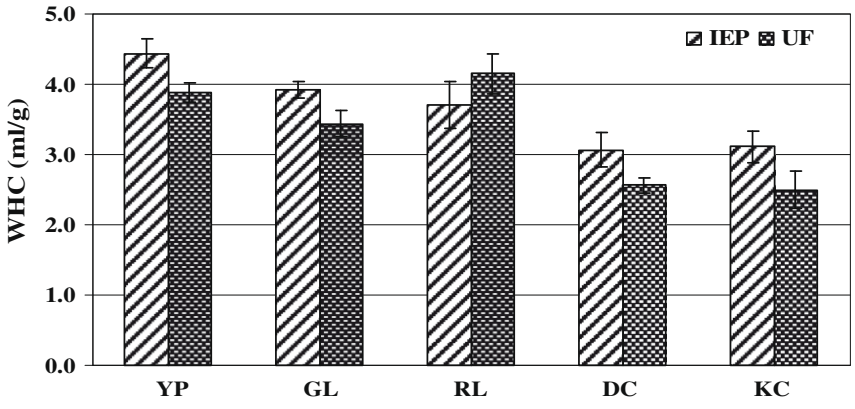

Fig. 4. Water holding capacity of IEP and UF pulse protein concentrates (YP: Yellow pea protein concentrate; GL: Green lentil protein concentrate; RL: Red lentil protein concentrate; DC: Desi chickpea protein concentrate; and KC: Kabuli chickpea protein concentrate).

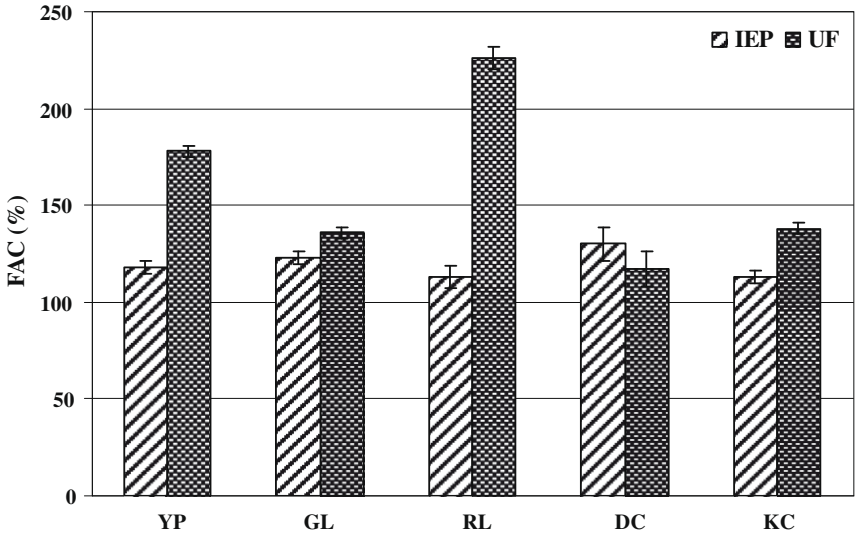

Fig. 5. Fat absorption capacities of IEP and UF pulse protein concentrates (YP: Yellow pea protein concentrate; GL: Green lentil protein concentrate; RL: Red lentil protein concentrate; DC: Desi chickpea protein concentrate; and KC: Kabuli chickpea protein concentrate).

the micellized protein isolate and 170% for the isoelectric precipitated protein isolate. This factor was compared with soy protein isolate which had a FAC of 190%. Other values reported for soy in the literature fall between the range of 254–261% (Tomotake, Shimaoka, Kayashita, Nakajoh, & Kato, 2002; Wong & Kitts, 2003). In earlier studies, Abdel-Aal, Shehata, El-Mahdy, and Youssef (1986) reported FAC of 76.6% for faba bean protein concentrate containing 76% (w/w) protein and 89.5% for chickpea protein concentrate containing 60% protein processed by isoelectric precipitation and 115.2% and 135.3%, respectively, for concentrates processed by micellization. Differences in FAC capacities could be due to pulse type, variety and processing conditions. In this study, the type of processing used and pulse variety appeared to have a greater impact on the fat binding capacity of yellow pea, red lentil and kabuli chickpea compared to the green lentil and desi chickpea.

3.5. Emulsifying properties

Emulsifying properties of food proteins can be described by the emulsifying activity (EAI) and emulsifying stability (ESI) indices. EAI is a measure of the capacity of the protein to aid formation and stabilization of the emulsion created while ESI provides a measure of the ability of the protein to impart strength to the emulsion to resist changes to its structure (e.g., coalescence, creaming, flocculation or sedimentation) over a defined time period (Liu et al., 2008). The emulsifying activity indices for the pulse protein concentrates ranged between (4.6 m^2/g) for YP-UF (lowest) to (5.7 m^2/g) for the DC and KC-IEP (highest) (Fig. 6). The values for green and red lentils ranged between those for the peas and chickpeas. For the ESI, the lowest value (17.8 min) was observed for GL-IEP and the highest (19.7 min) was found for the KC-UF protein concentrate. In general, the process used to produce the pulse protein extracts had little impact on the emulsifying properties. Furthermore, no significant differences were observed in the emulsifying properties of pulse varieties of the same type. Emulsifying properties differed, however, as a function of the type of pulse (pea, chickpea or lentil). The UF and IEP desi and kabuli chickpea protein extracts showed similar emulsifying properties which were significantly higher than for all the other pulse protein concentrates. Very few studies in the literature compare the emulsifying properties of pulse protein concentrates processed using different techniques. In the few studies we found different units and indices were often used to calculate the emulsifying properties which makes a comparison of the results difficult. In one study conducted by Fuhrmeister and Meuser (2003) a higher EAI of 27.4 m^2/g was reported for wrinkled pea protein isolate prepared by ultrafiltration compared to isolates obtained by acid precipitation (pH 3.4 and 4) (EAI of 10.1 and 14.0 m^2/g, respectively). These authors also reported lower EAI (12.1 m^2/g), for a commercially produced pea protein isolate that was substantially denatured. Further studies on the emulsifying properties of pulse proteins are required, especially using different pulses processed at different temperatures. Results from such studies will help to ascertain the role protein denaturation plays on the emulsifying properties of pulse proteins and may explain differences in the results of these workers and those reported in this study. In earlier studies, we reported EAI values of 10.86 m^2/g and ESI of 0.80 min for soy protein isolates prepared in the laboratory by isoelectric precipitation (Achouri et al., 2005). Fuhrmeister and Meuser (2003) reported an EAI of 18.6 m^2/g for soy while Wang and coworkers (2008) and L'Hocine and coworkers (2006) reported values of 11 m^2/g and 45 m^2/g, respectively. Again, differences in protein content, and variations in molecular structure of the soy proteins as a function of the conditions used during processing (e.g., temperature, pH, ionic strength, hydrolysis, etc.) may explain this variability and further studies are clearly needed. Taking the data available into consideration, nonetheless, it could be concluded that the pulse protein concentrates have emulsifying activity indices that are lower than soy protein extracts. The emulsifying stability indices reported in some of the studies (Achouri et al., 2005; Wang et al., 2008), however, suggest that the pulse protein concentrates may have better emulsifying stability compared to soy proteins.

3.6. Foaming properties

The foaming properties of the UF and IEP pulse protein concentrates are presented in Fig. 7. The Gi values (percentage of gas entrapped) of the pulse concentrates, which gives an indication of foaming capacity, ranged between 98% and 106% and were found to be generally not significantly different. The only two samples that had significantly higher Gi values were the desi and kabuli protein concentrates processed by IEP. The volume of liquid retained in the foam after 5 min (R5), an indication of foam stability, was found to be inversely proportional to the percent of foam

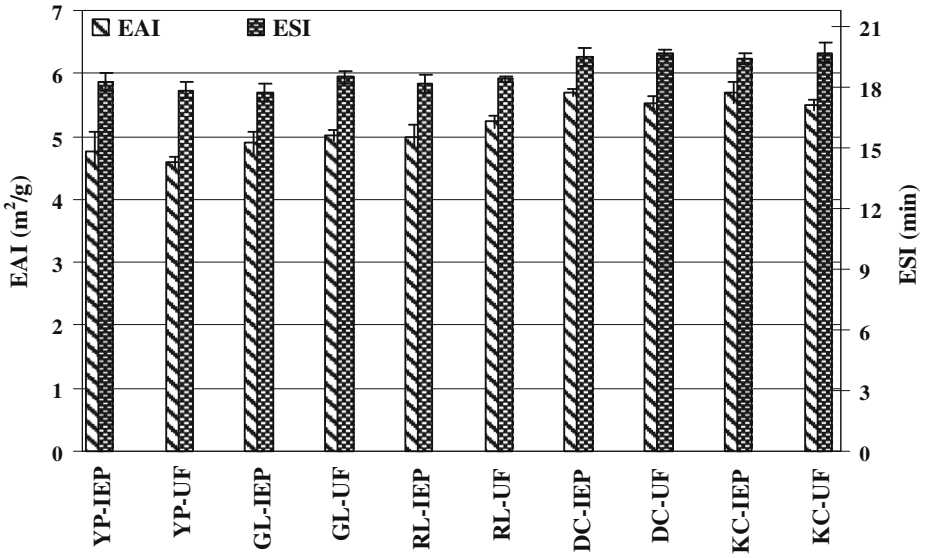

Fig. 6. Emulsifying properties of IEP and UF pulse protein concentrates (0.5% solution at pH 7) (EAI: Emulsion activity index; ESI: Emulsion stability index; YP: Yellow pea protein concentrate; GL: Green lentil protein concentrate; RL: Red lentils protein concentrate; DC: Desi chickpea protein concentrate; KC: Kabuli chickpea protein concentrate; IEP: Isoelectric precipitation; and UF: Ultrafiltration).

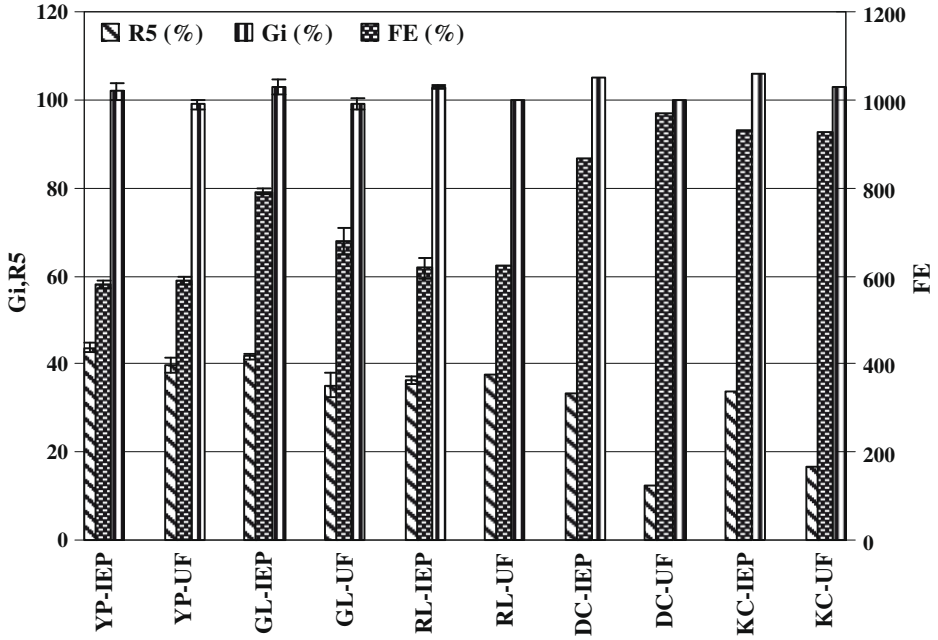

Fig. 7. Foaming properties of IEP and UF pulse protein concentrates (0.5 % solution at pH7) (Gi: Foam activity index; R5: Foam stability index; and FE: Percent foam expansion).

expansion (FE). Thus, the desi and kabuli chickpea concentrates processed by UF had the highest FE values and the lowest R5. In general, the desi and kabuli chickpea protein concentrates had significantly higher foam expansion and lower R5 values compared to yellow pea and the green and red lentil protein concentrates processed by both IEP and UF. No consistent effect of processing was observed. Depending on the pulse, the process used to produce the protein extracts either had no impact or impacted to a different extent their foaming properties. For foam expansion and foaming capacity (Gi), differences between the same variety processed with IEP or UF were not significantly different. For foam stability, however, significant differences were observed between the same variety processed with IEP or UF (i.e., green lentil, desi and kabuli chickpea concentrates processed with IEP had higher R5 values compared to the samples treated by UF). Similar results were reported by Fuhrmeister and Meuser (2003) who observed that pea protein isolates extracted by ultrafiltration had better foam expansion and stability at pH 5 compared to isolates obtained by isoelectric precipitation.

3.7. Gelling properties

The ability of food proteins to form gels on heating is an important functional property in food processing and food formulation. Gelation occurs when proteins form a three-dimensional network that is resistant to flow under pressure. The least gelling concentration (LGC) is often used as an indication of the gelation capacity of food proteins. A lower LGC indicates better capacity for the

Table 3
Gelling behaviour of isoelectric precipitated (IEP) and ultrafiltered (UF) pulse protein extracts at different protein concentrations.

Pulse Concentration (% w/v)	YP IEP	YP UF	GL IEP	GL UF	RL IEP	RL UF	DC IEP	DC UF	KC IEP	KC UF
2	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ
4	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ	ΘΘ
6	ΘΘ	ΘΘ	ΘΘ	±±	ΘΘ	ΘΘ	ΘΘ	Θ±	ΘΘ	ΘΘ
8	ΘΘ	±Θ	±Θ	√√	±±	±±	ΘΘ	±±	ΘΘ	±±
10	±Θ	√ ±	√ ±	√√	√ ±	√√	±±	√√	±Θ	√√
12	√ ±	√√	√√	√√	√√	√√	√√	±±	√ ±	√√
14	√√	√√	√√	√√	√√	√√	√√	√√	√√	√√
16	√√	√√	√√	√√	√√	√√	√√	√√	√√	√√
18	√√	√√	√√	√√	√√	√√	√√	√√	√√	√√
20	√√	√√	√√	√√	√√	√√	√√	√√	√√	√√

Θ – No gel; ± – Weak gel; √√ – Firm gel; and √ √ – Least gelling protein concentration (LGC).

concentrate to form gels. Table 3 summarises the gelling properties of the pulse protein concentrates. For each pulse, the protein extract produced using UF had better gelling properties (i.e., lower LGC) than the one produced using IEP which suggests that the manufacturing processes used to produce the protein extracts had an impact on their gelling properties. Similar findings were reported by Papalamprou et al. (2009). In our study, no gels were formed at a concentration of 2% and 4% (w/v) irrespective of the pulse type, variety or processing technique. At 6% concentration the green lentil concentrate processed by UF formed a weak gel. A strong gel was formed at 8% (LGC); the GL-UF, thus, had the best gelling properties while the yellow peas, desi and kabuli chickpeas processed by IEP had the lowest gelling properties (LGC of 14%, w/v). All other protein extracts demonstrated intermediate gelling properties. Results for LGC in the literature for pulses have been somewhat variable. Zhang, Jiang, and Wang (2007) also reported a LGC of 14% for chickpea protein isolate containing 91.5% (w/w) protein which was prepared by IEP. They found differences in the LGC when the pH and ionic strength was varied. Papalamprou and coworkers (2009) reported LGCs of 11.5%, 5.5% and 4.5% for chickpea protein isolates prepared using IEP, UF and a modified UF procedure (to enrich the albumin fraction), respectively. The protein contents of their extracts ranged between 90.07% and 93.43% (w/w). Some workers have suggested that the method of isolate preparation rather than the composition determines their gelling behaviour (Kiosseoglou, Doxastakis, Alevisopoulos, & Kasapis, 1999; Papalamprou et al., 2009). Our results clearly show an effect of processing and pulse type on gelling capacity. It is evident that further studies on the gelling properties of pulse proteins processed under different conditions and containing different amounts of protein are needed which will help to confirm the findings reported.

4. Conclusions

UF and IEP processing of pulses allowed the concentration of yellow pea, desi and kabuli chickpea and red and green lentil protein content by nearly 4-fold resulting in concentrates with protein contents varying between 63.9% and 88.6%. Manufacturing processes used to produce the protein extracts impacted to different extents their functional properties. All the protein extracts exhibited good functional properties, which in some cases (e.g., solubility, fat binding capacity, emulsifying stability and foaming properties) were comparable to that reported for soy (Fuhrmeister & Meuser, 2003; L'Hocine et al., 2006; Paredes-Lopez et al., 1991; Swanson, 1990). The functional properties, however, varied to some extent as a function of the type of pulse used.

Protein concentrates and isolates used by the food industry today are mostly derived from dairy, soy or wheat. Due to the allergenicity of these foods, food manufacturers as well as consumers are looking for alternative protein sources. There are eight priority allergens (wheat, soy, dairy, peanut, tree nuts, fish, crustaceans and egg) that need to be labelled when they are present in foods. In Canada, sesame also needs to be labelled, and in the European Union, mustard and celery have to be labelled. Elimination of these foods from the diet is the only way to prevent allergic reactions in sensitive individuals. At the same time allergic patients need proteins to satisfy their nutritional requirements. Pulses such as peas, chickpeas and lentils have been consumed for many thousands of years and although they contain allergenic proteins, they are not listed as priority allergens which require labelling and may, therefore, serve as alternatives to the priority allergens that require labelling.

In general pulses are good sources of protein for humans especially when eaten in combination with cereal proteins. Although their nutritional quality compared to animal protein is comparatively lower due to the presence of anti-nutritional factors such as phytate, protease inhibitors and lectins, processing methods like thermal treatment, fermentation, enzyme hydrolysis, etc. can significantly improve their digestibility and nutritional value. Large scale manufacturing of pulse protein concentrates and isolates may, therefore, open up further possibilities for their industrial application, which could allow pulses to be used as a commercial alternative source of functional proteins.

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