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Potential of Turkish Kabuli type chickpea and green and red lentil cultivars as source of soy and animal origin functional protein alternatives

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ABSTRACT

In this study, functional properties of proteins from Turkish Kabuli type chickpea (CPEs) and green and red lentil cultivars (LPEs) were characterized and compared with those of soy and animal proteins. The LPEs and whey protein isolate (WPI) showed higher soluble and total protein content than the other proteins. CPEs showed the highest oil absorption capacity (10.9–14.59 g/g), followed by LPEs (6.90–10.44 g/g), soy protein extract (8.23 g/g), and egg white proteins (6.37 g/g). The highest water absorption capacities were obtained for bovine gelatin (BGEL) (8.84 g/g), CPEs (4.90–7.94 g/g) and soy protein isolate (7.94 g/g). The foaming capacities of BGEL and fish gelatin (FGEL), and emulsifying capacity of WPI were slightly higher than those of CPEs and LPEs, but most stable emulsions and foams were formed by chickpea and lentil proteins. The least gelling concentration of CPEs (5–7 g/100 g) came second after BGEL (3 g/100 g). The 2-D electrophoresis revealed the detailed isoelectric point (between 4.5 and 5.9) and molecular weight patterns of chickpea and lentil proteins. This study clearly showed that the functional properties of Kabuli chickpea proteins are superior than those of lentil proteins and most of the studied soy and animal proteins.

1. Introduction

In different food systems, animal and plant origin proteins are used as ingredients due to their nutritive value, antioxidant activity and many different functional properties such as emulsifying activity, film, foam and gel formation, oil, water and flavor binding, increase of viscosity etc. (Arcan & Yemenicioglu, 2010; Damodaran, 1996). Recently, the consumer demands originated from health concerns, religious limitations and rising trend of vegetarianism have increased the interest of food industry in use of functional plant proteins as alternative to animal proteins (Alvaro, Jose, Maira, Raquel, & Angel, 2006; Dormont, 2002; Jenkins et al., 2002; Karim & Bhat, 2009). Thus, the soybean protein isolates, concentrates and hydrolyzates are currently used extensively in foods such as meat and diary products, infant formulas, functional foods and nutraceuticals (Amigo-Benavent, Silván, Moreno, Villamiel, & del Castillo, 2008; Chove, Grandison, & Lewis, 2007).

Due to the rapidly growing functional plant protein market there is also a great competition to evaluate soy alternative legumes as functional proteins (Arcan & Yemenicioglu, 2007; Boye et al., 2010). Chickpeas and lentils are among main legumes grown in different parts of world including America, Mediterranean Basin, China, Middle East, India and Australia. The major chickpea producers in the world are India, Turkey and Pakistan, while Lentils are produced mainly by India, Turkey and Canada (Roy, Boye, & Simpson, 2010). In India and Pakistan, the chickpeas grown are Desi type, while Kabuli type chickpeas are grown mainly by Turkey. The major types of lentils produced in different parts of world are red and green lentils, but red lentils comprise 2/3 of the world production (Roy et al., 2010). Due to their high protein quality, nutritive value and antioxidant phenolic content chickpeas and lentils have a very important role for the diet in Mediterranean, Middle East, Pakistan and India (Han & Baik, 2008; Mitchell, Lawrence, Hartman, & Curran, 2009). Recently, these pulses and some other legumes have also been strongly suggested by American Dietetic Association to improve diet quality of US population (Mitchell et al., 2009). However, chickpeas and lentils are not extensively grown and used systematically for production of value added products suitable for food industrial applications. Thus, different studies had been conducted on characterization of functional properties for major chickpea and lentil resources in the world at the cultivar level. For example, Kaur and Singh (2007) characterized functional properties of protein from 6 Indian Desi chickpea cultivars. Boye et al. (2010) characterized functional properties of protein in some Canadian lentil (1 green and 1 red) and chickpea (1 Desi and 1 Kabuli) cultivars, while Lee, Htoon,

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Uthayakumaran, and Paterson (2007) studied functional properties of protein in 2 Australian lentil cultivars (1 green and 1 red). However, no studies have been conducted to characterize the functional properties of proteins form chickpea and lentil resources in Turkey, a major center in the world for production of these pulses. In this work, functional properties of protein extracted from different Kabuli type chickpea cultivars (4 cultivars), and different red (4 cultivars) and green (2 cultivars) lentil cultivars have been characterized and compared with those of different commercially important proteins for the first time. This is also the first study conducting 2-D electrophoresis of chickpea and lentil proteins obtained by the classical isoelectric precipitation method.

2. Materials and methods

2.1. Materials

The dry seeds of chickpea cultivars, Cevdetbey-98 and Sarı-98, were obtained from Aegean Agricultural Research Institute in Menemen (Turkey). All other dry chickpeas and lentils were obtained from General Directorate of Agricultural Research in Ankara (Turkey). The seeds were grown in the experimental fields of research Institutes for research purposes. The protein extracts obtained from Kabuli type chickpea cultivars, Canıtez (C-1), Cevdetbey-98 (C-2), Gökçe (C-3), Sarı-98 (C-4), red lentil cultivars, Ali dayı (L-1), Çiftçi (L-2), Fırat (L-3), Kafkas (L-4), and green lentil cultivars, Meyveci (L5), Pul II (L6) were named as CPE-1, CPE-2, CPE-3, CPE-4 and LPE-1, LPE-2, LPE-3, LPE-4, LPE-5, LPE-6, respectively. The dry soybeans (non-GMO) used in soy protein extract (SPE) production (see method given in Section 2.2) were purchased from a supermarket in Izmir (Turkey). Commercial whey protein isolate (WPI) obtained from sweet whey (Product name: BiPRO, Not denatured, Spray dried, Total protein content: 0.98 g/g) was kindly donated by Davisco Foods International, Inc (MN, USA). Commercial soy protein isolate (SPI) obtained from non-GMO defatted and dehulled soybeans (Product name: Dunasoy 90, Total protein content: min 0.90 g/g) and soy protein concentrate (SPC) obtained from non-GMO defatted and dehulled soybeans (Product name: Dunasoy 70, Total protein content: 0.68–0.70 g/g) were from Euroduna Rohstoffe GmbH (Germany). Fish gelatin (FGEL) from cold water fish skin and bovine skin gelatin (BGEL) (Type B, Bloom: 225) were obtained from Sigma Chem. Co. (St. Louis, MO, USA). The egg white protein (EWP) was produced from standard fresh broiler eggs obtained from a supermarket in Izmir (Turkey) by lyophilization of egg whites separated in the laboratory.

2.2. Protein extraction

To remove lipids and phenolic compounds, chickpeas, lentils and soybeans were processed to acetone powder (AP) according to the method given by Arcan and Yemenicioglu (2007). To obtain crude protein extracts, 20 g of AP was suspended in 250 mL deionized water by stirring with a glass rod 100 times. The pH of the mixture was then adjusted to 9.5 with 1 mol/L NaOH. In chickpeas, for inactivation of highly active oxidative enzyme lipoxygenase the extracts were heated to 85 °C and maintained at this temperature for 30 min under continuous magnetic stirring (Arcan & Yemenicioglu, 2007). After that, the extracts were cooled to room temperature in ice water bath by stirring for 15 min. The lentil extracts could not be heated since they showed extreme browning by heating. Thus, they were extracted at room temperature for 45 min under continuous stirring. The soybean extracts were also treated similar to lentil extracts. All extracts were clarified by centrifugation for 30 min at 15,000× *g* (at 4 °C). Part of each extract was separated, lyophilized and kept as crude extract for SDS-PAGE analysis, while the remaining extracts were further purified with the classical isoelectric precipitation (IEP) method. The IEP was applied by adjusting the pH of extracts to 4.5 with 1 mol/L acetic acid. The precipitated proteins were collected with centrifugation and resuspended in distilled water. The pH of the suspensions was once more adjusted to 4.5 and proteins were once more precipitated and collected with centrifugation for 15 min at 15,000× *g* (at 4 °C). Finally, the obtained proteins were suspended in distilled water and lyophilized (Labconco, FreeZone, 6 L, Kansas City, MO, USA), after adjusting their pH to 7.0. The legume protein extracts obtained by the classical isoelectric precipitation method (IEP method) contain mainly globulins. The lyophilized chickpea, lentil and soybean protein extracts were designated as CPE, LPE and SPE, respectively, and stored at −18 °C for several months until they were used for characterization of their functional properties. The CPE and LPE were also characterized for their molecular properties by 2-D electrophoresis.

2.3. Water soluble protein content

The water soluble protein content (WSPC) of extracts was determined by the Bradford method using bovine serum albumin (BSA) as standard. The lyophilized protein extracts were prepared for analysis by dissolving them in deionized water at pH 9.5. The solutions were magnetically stirred for 30 min at room temperature and they were centrifuged at 3500× *g* (at 4 °C) for 20 min to remove insoluble residues. The protein analysis of each sample was conducted with three repetitions and five replicates and results were expressed as g soluble protein per g of protein extract (g/g).

2.4. Total protein content

The total nitrogenous compounds in protein extracts were determined by the Kjeldahl method using an automated testing machine (Gerhard vapodest 50s and Kjeldahl Therm, Germany). The total protein contents (TPC) were calculated by using different conversion factors (5.4 for FGEL, 6 for EWP, and 6.25 for CPEs, LPEs and SPE). The average of three replicates was used to calculate protein content and results were expressed as g protein per g of protein extract (g/g).

2.5. Gel formation capacity

The gel formation capacity of protein extracts was determined by finding the least gelling concentration (LGC). For this purpose, a series of protein solutions were prepared in distilled water (concentrations between 1 and 14 g/100 g). All protein solutions were prepared at room temperature, except BGEL which dissolves at 50 °C. The solutions were then placed into test tubes (1.46 cm in diameter) and they were heated in a water bath at 90 °C for 1 h. The tubes were then cooled immediately to room temperature and incubated for 2 h at 4 °C for gel formation. The gel formation was detected by observing the flow characteristics of tube contents when tubes were turned upside down. The LGC corresponds to the lowest protein concentration (g/100 g) that gives hard gel with no falling or slipping by gravity when tubes are turned upside down.

2.6. Water and oil absorption capacity

To determine the water (WAC) and oil absorption capacities (OAC), 50 mg protein extract and 1.5 mL of distilled water or commercial sunflower oil were mixed at room temperature for 20 s by a vortex in a 2 mL centrifuge tube. After mixing, the lids of tubes were closed and the tubes were incubated at 30 °C for 30 min. The tubes were then centrifuged at 15,000 g (25 °C) for 20 min and the

separated free water or oil in their supernatants was removed carefully. The absorbed water or oil content was determined by weighing of the tubes. WAC and OAC were expressed as g of water or oil absorbed per g of protein extract, respectively. The tests were repeated for three times for each of the protein samples.

2.7. Emulsifying capacity and emulsion stability

Emulsifying capacity (EC) of lyophilized proteins was determined by modification of the method described in Pearce and Kinsella (1978). Briefly, 20 mL of protein solution (10 mg/mL) was prepared in distilled water and its pH was adjusted to 7.0. The solution was stirred for 30 min at 30 °C (at 50 °C to dissolve BGEL) and 6.5 mL of commercial corn oil was added onto it at room temperature. After that, the mixture was emulsified by homogenization at 2300 rad/s for 2 min in a homogenizer–disperser (Yellow Line, DI 18 Basic, Brazil). A 200 μL sample of the emulsion was then taken and mixed with 25 mL of SDS solution (10 mg/mL). The emulsifying capacity was determined by measuring the turbidity of sample as nephelometric turbidity units (NTU) by a turbidity meter (HACH, 2100 AN, U.S.A.). The absorbance of the samples was also determined at 500 nm as in the standard method. However, results of this work were based on turbidity measurements. The emulsion stability (ES) of samples was determined by measuring their turbidities (also absorbance) at 30th and 180th min of emulsification. All measurements were repeated for three times for each of the protein sample and results were expressed as NTU.

2.8. Foaming capacity and foam stability

To determine the foaming capacity (FC), a 25 mL of protein solution (10 mg/mL) was prepared from each protein extract and its pH was set to 7.0. The protein solutions were prepared at room temperature, except BGEL which dissolves at 50 °C. The solutions were then homogenized in the disperser–homogenizer at 2300 rad/s for 1 min to induce foaming. The foaming capacity was determined by measuring volume of the formed foam as mL. The foam stability (FS) was determined by measuring foam volume at 30th and 180th min of foam formation. All measurements were repeated for three times for each of the protein sample.

2.9. SDS-PAGE and 2-D electrophoresis of proteins

The classical SDS-PAGE was conducted for different crude protein extracts collected before isoelectric point precipitation of proteins during extraction (see Section 2.2). The extracts were lyophilized and stored at −20 °C until their SDS-PAGE was conducted on a discontinuous buffered system according to Laemmli method using separating gel (150 mg/mL) and stacking gel (50 mg/mL) (Dunn, 1989). Lyophilized samples were directly solubilized in sample buffer and centrifuged at 15,000× *g* for 30 min. The samples were then heated for 5 min in boiling water before electrophoresis. The electrophoresis was performed at a constant current of 12 mA. Protein fixation was performed with TCA (200 mg/mL). The gel was stained with methanol (12.3 mol/L), acetic acid (1.75 mol/L), Coomassie brilliant blue (R-250) (0.5 mg/mL) solution. The gel destaining was accomplished by using methanol (1.23 mol/L) and acetic acid (2.18 mol/L) solution. Wide molecular weight range molecular marker was used to characterize the obtained protein bands (Sigma Marker™, Sigma–Aldrich).

The two dimensional (2-D) electrophoresis (isoelectric focusing and SDS-PAGE) was applied to CPE with the ReadyPrep™ 2-D Starter Kit (BioRad Laboratories Inc.) according to the method given by Arcan and Yemenicioglu (2010). The sample was prepared in deionized water and then diluted with sample buffer. IPG Strips (ReadyStrip™, BioRad), 17 cm and pH 3–10, were used for isoelectric focusing (IEF). IEF was applied using the Protean IEF cell (BioRad) with 3 step voltage protocol: 250 V for 20 min (linear ramp), 10,000 V for 2.5 h (linear ramp), and total 40,000 V × h rapid ramp step. SDS-PAGE was performed at a two step constant current protocol: 16 mA/gel for 30 min, then 24 mA/gel for 5 h in SDS (120 mg/mL) gel with PROTEAN II XL (BioRad). The protein fixation, staining and destaining were performed as given in the classical SDS-PAGE method above. Protein Ladder, 10–200 kDa (Fermentas International, Inc.) and 2-D SDS-PAGE standard, pI range 4.5–8.5 (BioRad), were used as marker.

2.10. Statistical analysis

Statistical analysis of data for functional properties was carried out by using one-way analysis of variance (ANOVA) with a significance threshold of $P < 0.05$ as determined by Fisher's least significant difference (LSD) method. The correlation coefficients among functional properties of CPE or LPE were calculated by Minitab software (Minitab Inc. PA, USA) with a significance threshold of $P < 0.01$.

3. Results and discussions

3.1. Protein solubilities and contents

The WSPCs of CPEs and LPEs varied between 47 and 52 g/100 g and 56 and 74 g/100 g, respectively (Table 1). The average WSPC of LPEs was almost 1.3 fold higher than that of CPEs. Thus, it seemed that the heat treatment applied for inactivation of oxidative enzymes during extraction caused some limited solubility problems for CPE. The WSPCs of CPEs did not show statistically significant differences by cultivar ($P > 0.05$), but WSPCs of LPEs from different cultivars had some statistically significant differences ($P < 0.05$). Except extract from one cultivar, LPEs had comparable WSPCs with WPI, the most soluble animal protein used in this study, but they showed significantly higher WSPCs than the soy proteins. The CPEs had higher WSPCs than most soy and animal proteins, but SPE and WPI showed higher WSPC than the CPEs.

The TPCs of LPEs and CPEs did not vary considerably for different chickpea and lentil cultivars, but the TPCs determined for LPEs were statistically significantly higher than those of CPEs ($P < 0.05$). The average TPC of LPEs was lower than that of WPI, but similar with those of SPI and SPE. The SPC and other animal proteins contained lower TPCs than LPEs. The CPEs' average TPC was slightly higher than those of SPC and EWP, but lower than those of the remaining proteins. Therefore, considering their total protein contents, the LPEs and CPEs obtained by the IEP procedure could be named as protein isolate and concentrate, respectively. The previous findings of Boye et al. (2010) also showed the higher protein contents of IEP purified (at pH 4.5) lentil proteins than the chickpea proteins. The protein contents determined by these authors for IEP purified protein extracts of green lentils (0.79 g/g), red lentils (0.78 g/g) and Kabuli chickpeas (0.64 g/g) were lower than those determined for Turkish lentil and chickpea cultivars in this study. However, due to the different processes applied to these legumes like dehulling and grinding before protein extraction, and long periods of decantation for extracts (3–12 h) it is hard to attribute these differences to variations in protein contends of seed cultivars.

3.2. Gel forming capacities

The LGC of CPEs ranged between 5 and 7 g/100 g, while only four of LPEs formed hard fixed gels (LGC ranged between 12 and 14 g/

Table 1
Protein content, gelling properties and water and oil absorption capacities of different protein extracts.

Protein extract	WSPC[a] (g/g)[b]	TPC (g/g)	LGC (g/100 g)	WAC (g/g)	OAC (g/g)
CPE-1	0.51 ± 0.02d	0.73 ± 0.06de	6	6.37 ± 0.44c	13.65 ± 0.77b
CPE-2	0.52 ± 0.01cd	0.71 ± 0.02de	5	6.64 ± 0.71c	14.59 ± 0.08a
CPE-3	0.50 ± 0.03d	0.77 ± 0.01d	7	4.90 ± 0.26d	10.93 ± 0.52c
CPE-4	0.47 ± 0.02d	0.73 ± 0.02de	5	7.94 ± 0.52b	14.31 ± 0.45ab
Average	0.50 ± 0.02	0.73 ± 0.03	5.75 ± 2.6	6.46 ± 1.24	13.37 ± 1.68
LPE-1	0.61 ± 0.05b	0.90 ± 0.01abc	12	1.22 ± 0.49ef	6.90 ± 0.10gh
LPE-2	0.56 ± 0.06c	0.95 ± 0.02a	–[d]	1.34 ± 0.09ef	7.57 ± 0.33fg
LPE-3	0.74 ± 0.03a	0.88 ± 0.02bc	12	–[e]	9.67 ± 1.34d
LPE-4	0.62 ± 0.04b	0.93 ± 0.01ab	–[d]	1.47 ± 0.22ef	8.55 ± 0.50e
LPE-5	0.67 ± 0.06ab	0.90 ± 0.02abc	14	1.10 ± 0.14f	8.57 ± 1.20e
LPE-6	0.69 ± 0.02a	0.87 ± 0.05bc	14	1.08 ± 0.51f	10.44 ± 0.24cd
Average	0.65 ± 0.07	0.91 ± 0.03	13 ± 1.2	1.04 ± 0.52	8.62 ± 1.30
BGEL	0.013 ± 0.01f	0.82[c]	3	8.84 ± 0.36a	1.12 ± 0.07i
EWP	0.22 ± 0.01e	0.69 ± 0.02e	10	0.14 ± 0.09g	6.37 ± 0.37h
FGEL	0.09 ± 0.03f	0.85 ± 0.01c	–[d]	0 ± 0h	1.04 ± 0.07i
SPC	0.11 ± 0.01f	0.70[c]	–[d]	4.52 ± 0.19d	1.73 ± 0.36i
SPE	0.57 ± 0.02c	0.92 ± 0.02ab	10	1.69 ± 0.62e	8.23 ± 1.02ef
SPI	0.21 ± 0.01e	0.90[c]	10	7.94 ± 0.44b	1.16 ± 0.05i
WPI	0.70 ± 0.06a	0.98[c]	14	0 ± 0h	1.59 ± 0.15i

[a] WSPC: Water soluble protein content, TPC: Total protein content, LGC: Least gelling concentration, WAC: Water absorption capacity, OAC: Oil absorption capacity.
[b] Different letters in columns indicate statistically significant differences ($P < 0.05$).
[c] Product's manual data.
[d] No hard gel formation between 1 and 14 (g/100 g).
[e] No water absorption at the test conditions.

100 g) at the studied concentration range. These results showed high variations in gelling capacities depending on pulse type and cultivar. The CPEs showed better gelling performance than lentil proteins, soy proteins and animal proteins including FGEL, WPI and EWP. However, the BGEL having LGC of 3 g/100 g showed the best gelling performance. The SPI, SPE and EWP which showed LGC of 10 g/100 g had better gelling capacity than LPEs, but WPI had comparable LGC with those of four LPEs. In the literature, very high LGCs between 11.5 and 18 g/100 mL (10.3–15.3 g/100 g) were reported for chickpea proteins obtained by the IEP method (Boye et al., 2010; Kaur & Singh, 2007; Papalamprou, Doxastakis, Biliaderis, & Kiosseoglou, 2009), but LGC of 12 g/100 mL (10.7 g/100 g) reported by Boye et al. (2010) for two lentil cultivars was only slightly lower than those obtained in this study for LPE-1 and LPE-3. These results clearly indicated the outstanding gelling capacity of proteins in CPE. However, it is hard to attribute this simply to superior properties of protein in the studied material than the others since studies in the literature employed different extraction and testing conditions. In this work the CPE was heat treated during extraction to inactivate off-flavor enzymes, but this procedure was not applied by the other workers. The CPE and LPE used in this work were heated at 90 °C in unsealed tubes to induce gel formation, while workers sited above heated proteins in sealed or unsealed tubes and applied heating at 100 °C or by boiling to induce gel formation.

3.3. Water and oil absorption capacities

The WACs of CPEs showed some statistically significant variations by cultivar ($P < 0.05$), while LPEs had similar WAC values with the exception of LPE-3. The average WAC of CPEs was almost 6-fold higher than those of LPEs. The highest WAC was obtained for BGEL, and this was followed by those of SPI, CPEs and SPC. Three of the LPEs showed comparable WACs with SPE, but one of the LPEs and other animal origin proteins showed inconsiderable WACs.

The OAC values of LPEs showed some statistically significant differences by cultivar ($P < 0.05$), while CPEs showed similar OAC values except that of CPE-3. The average OAC of CPEs was 1.6 fold higher than that of the LPEs. The average OAC of CPEs was also significantly higher than those of soy and animal proteins. The LPEs showed comparable OACs with SPE and EWP, but significantly higher OACs than the remaining soy and animal proteins.

In the literature, chickpea and lentil proteins obtained by the IEP procedure were reported to have WACs between 2.3 and 5.0 g/g, and 0.2 and 4.0 g/g, respectively (Bora, 2002; Boye et al., 2010; Kaur & Singh, 2007; Lee et al., 2007; Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1999). Different reported OAC values of chickpea proteins in the literature ranged between 1.1 and 4.1 g/g (Boye et al., 2010; Kaur & Singh, 2007; Sánchez-Vioque et al., 1999), while the only comparable OACs were reported as 1.15 and 1.25 g/g for red and green lentil types, respectively (Boye et al., 2010). The WAC values of lentil proteins determined in this study were in the range of those reported in the literature. However, WACs of chickpea proteins and OACs of both chickpea and lentil proteins determined in this study were considerably higher than those reported in the literature. Such great differences in functional properties might come from differences in extraction and assay conditions (protein content, rehydration period and oil type) used in WAC and OAC tests, or variations in protein composition of materials due to differences in cultivars, growth conditions and climate. However, it is worth to report that the high OAC of Kabuli type chickpea proteins obtained by the IEP method was also noted by Kaur and Singh (2007). These workers investigated functionality of IEP proteins from 1 Kabuli type and 5 desi type Indian chickpea cultivars attributed the high OAC of Kabuli type chickpea proteins to high non polar amino acid content of these proteins.

3.4. Emulsifying capacities and emulsion stabilities

The ECs of different proteins based on turbidities of emulsions were given in Fig. 1A. The ECs of CPEs and LPEs showed some limited variations by cultivar. The highest ECs were obtained for WPI, CPE-1 and LPE-6, while most other proteins showed comparable ECs (except those of LPE-4, BGEL and SPE). The ECs determined by measuring NTU values of protein emulsions gave highly parallel results with the absorbance measurements (results were not given, but average initial absorbance values of emulsions were 0.760 ± 0.048 for CPEs and 0.691 ± 0.078 for LPEs).

The results of ES tests were given for 30 and 180 min incubation periods (Fig. 1B). The ESs of both CPEs and LPEs showed statistically

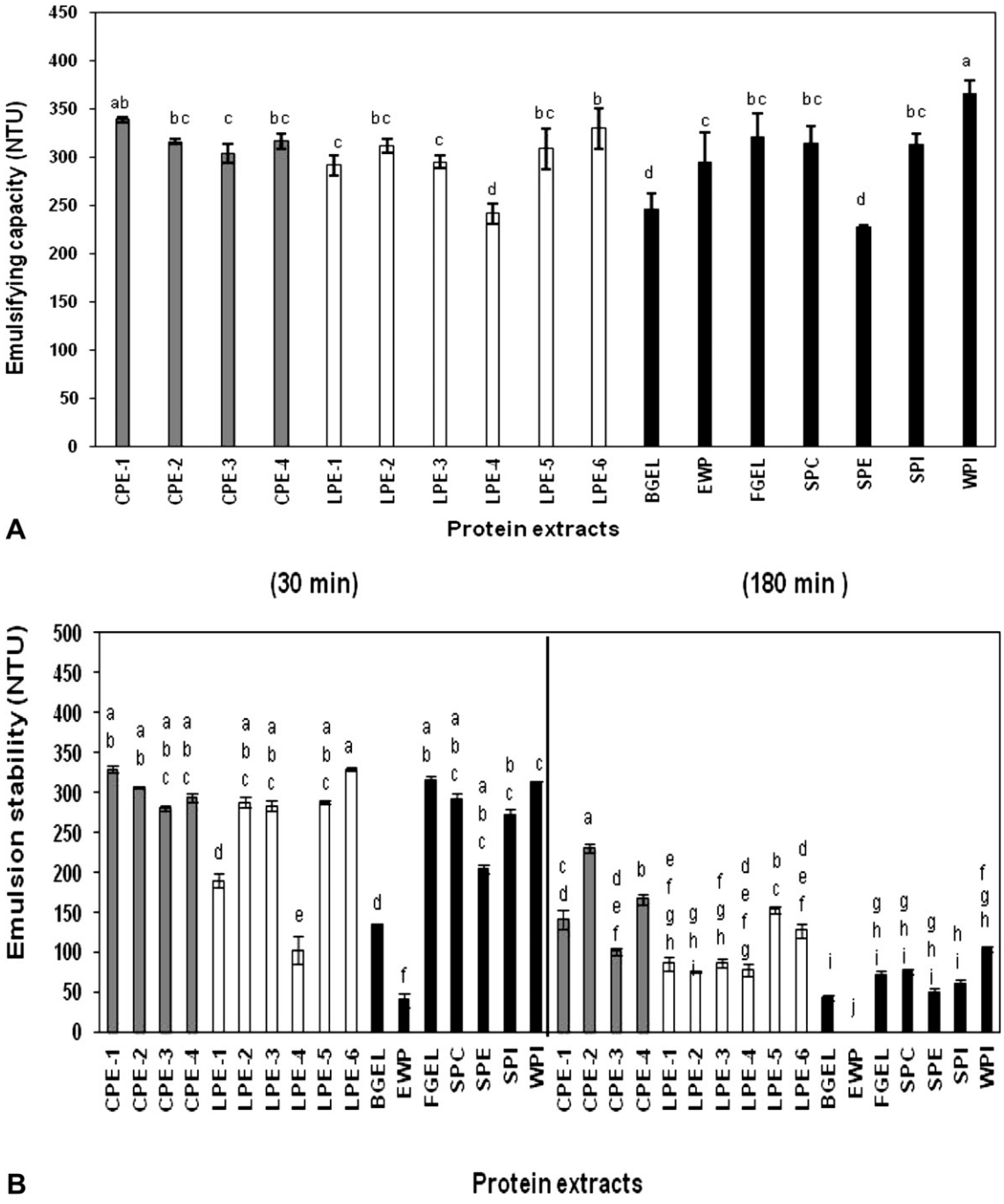

Fig. 1. Emulsifying capacity (A) and emulsion stability (B) of different proteins.

significant variations by cultivar ($P < 0.05$). The rapid destabilization of emulsions formed by LPE-1 and LPE-4 at the end of 30 min clearly showed the lower ES of these proteins than the other lentil proteins. The BGEL and EWP also lost a significant portion of their ESs within 30 min, but the remaining proteins showed quite similar ESs at the end of this short incubation period. The differences among the ESs of proteins were observed more clearly by incubation of emulsions for 180 min. The highest ES values were observed for CPEs and LPEs. However, the average NTUs observed in 180 min for CPEs (160 ± 54) was almost 1.6 fold higher than that of LPEs (102 ± 32). The CPE-2 and CPE-4 showed the highest ES of CPEs, while ES of LPE-5 was the highest of LPEs ($P < 0.05$). Three of the CPEs and one LPE showed statistically significantly higher ESs than all soy and animal proteins ($P < 0.05$). Due to different units and indices used in determination of protein emulsification properties, it is hard to compare our results with those in the literature. However, results of Boye et al. (2010) who determined greater emulsion stability of Canadian Kabuli and desi type chickpea proteins than green and red lentil proteins showed parallelism with results obtained in this study.

3.5. Foaming capacities and foam stabilities

For both CPEs and LPEs the FCs showed some statistically significant variations by cultivar ($P < 0.05$). The average foam volume showing FC of CPEs (10.98 ± 2.61 mL) was slightly higher

than that of LPEs (8.71 ± 2.67 mL). The highest FC was observed for BGEL (Fig. 2A). This was followed by FCs of FGEL, LPE-3 and CPE-4 which were quite similar with each other, but statistically significantly higher than those of other proteins, except WPI, CPE-1 and CPE-2. Three of the CPEs showed similar FC with WPI, but five of the LPEs had statistically significantly lower FCs than the WPI ($P < 0.05$). Most of the LPEs showed similar FC with SPI, SPE and SPC. The lowest FCs were obtained for three of the LPEs, CPE-3, SPE and EWP.

The FS ranking contradicted with FC ranking since foams of most LPEs and CPEs were more stable than those of gelatins (Fig. 2B). The foams of FGEL and SPC showed the least stability and rapidly destabilized by 30 min incubation. With the exception of CPE-1 which showed very low FS, the CPEs and LPEs showed similar FSs and maintained a considerable portion of their stability after 180 min. Although the most stable foams were obtained for CPEs and LPEs, BGEL, SPI, and EWP showed comparable FSs with some of the LPEs and CPEs. In contrast, the remaining soy and animal proteins had inconsiderable foaming stabilities. The FS results obtained in this study for chickpea and lentil proteins contradicted with those of Boye et al. (2010) who reported significantly higher foam stability for Canadian Kabuli and desi chickpea proteins than the green and red lentil proteins. Thus, it is clear that further studies are needed to determine importance of different factors such as cultivar, growth conditions and climate on foaming properties and other functional properties of pulse proteins.

3.6. Identification of cultivars with multiple protein functionalities

From chickpea proteins, the CPE-2 from C-2 and CPE-4 from C-4 had the lowest LGC, highest WAC and OAC, and highest ES values. The CPE-4 also had the highest FC of chickpea proteins. In contrast, CPE-3 showed the lowest WAC, OAC and FC, and highest LGC of chickpea proteins.

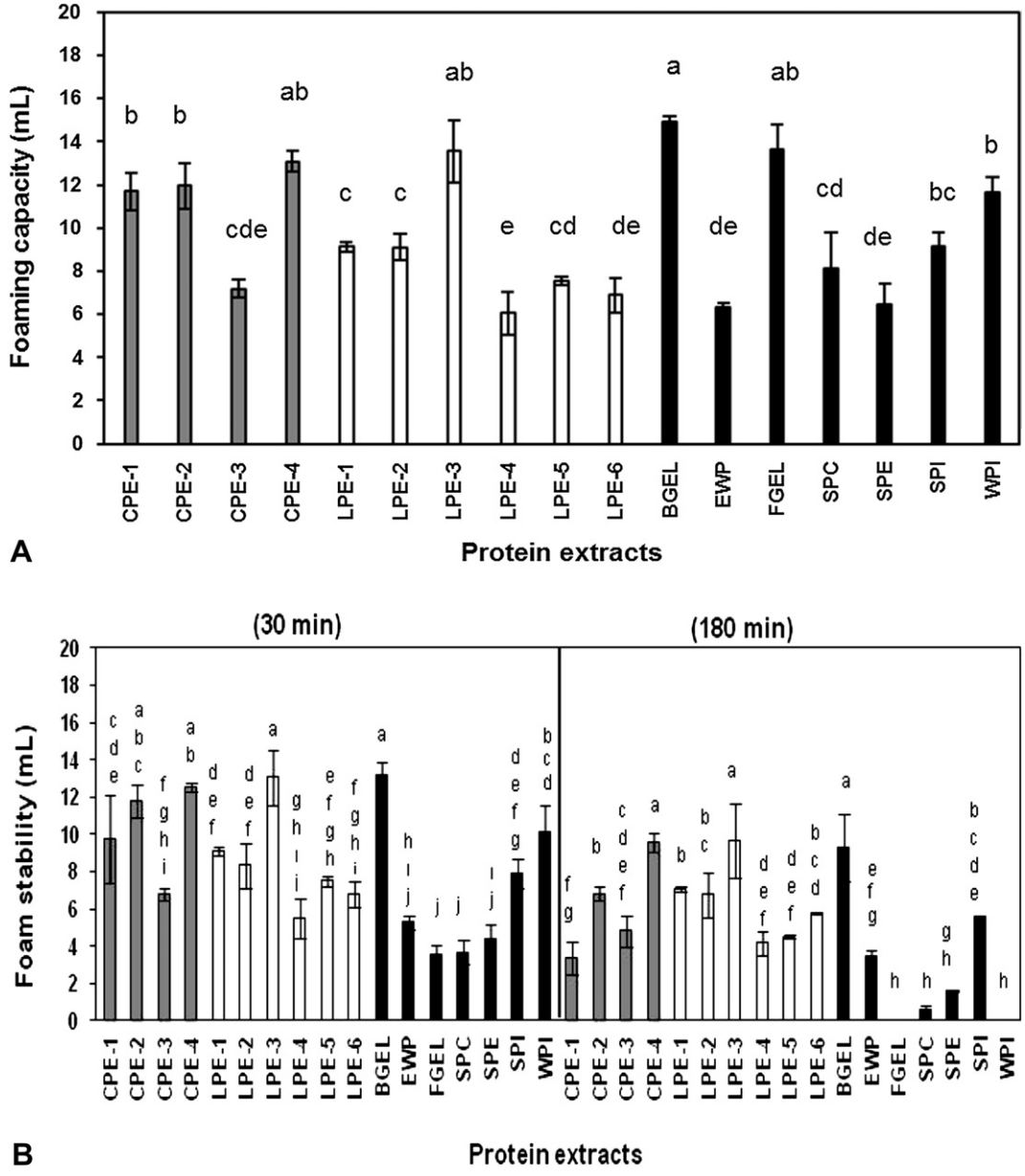

Fig. 2. Foaming capacity (A) and foam stability (B) of different proteins.

In lentils, the proteins did not show multiple very high functionalities as chickpea proteins. All LPEs showed considerably low WACs due to their high solubility in water. The LPE-3 showed the highest solubility in water, thus, lacked any measurable WAC. However, LPE-3 had one of the lowest LGCs and one of the highest OACs of lentil proteins. LPE-3 also showed the best performance in FC and FS tests of LPEs. The LPE-6 had the highest OAC and showed a good performance in EC and ES tests of LPEs. In contrast, the LPE-2 and LPE-4 did not show gelling at the test conditions. LPE-1 and LPE-4 had the worst performances in EC and ES tests of LPEs. The LPE-4 also showed one of the worst performances in FC and FS tests of lentil proteins.

3.7. Correlation of functional properties

The statistical analysis did not indicate any significant positive correlations between WSPC or TPC and any of the measured parameters of LPEs and CPEs. For both LPEs and CPEs, there were also no positive significant correlations among EC, ES, FC, FS, but a significant negative correlation existed between FS and EC ($r^2 = -0.776$) of CPEs. Some significant negative correlations also existed between TPC and WSPC ($r^2 = -0.611$), and WSPC and WAC ($r^2 = -0.612$) of LPEs, and between TPC and OAC ($r^2 = -0.739$) of CPEs. For CPEs, there were also positive correlations between WAC and OAC ($r^2 = 0.816$), and WAC and FC ($r^2 = 0.932$). In contrast, a significant negative correlation was determined between WAC and FC ($r^2 = -0.687$) of LPEs. These results showed the complexity of correlations among different functional properties of LPEs and CPEs. However, it seemed that the determination of TPC might be used to obtain some information about OAC, WAC and FC of chickpea proteins and WSPC, WAC and FC of lentil proteins. The remaining functional properties of chickpea and lentil proteins are not related mainly to protein content or solubility. In the literature, data related to correlations among different functional properties of chickpea and lentil proteins scarce. It was only Kaur and Singh (2007) who reported strong negative correlation of peak denaturation temperature (T_d) determined by differential scanning calorimetry with TPC and OAC of protein extracts obtained from 5 desi and 1 Kabuli chickpea cultivars. Further studies are needed to investigate effect of protein composition, conformation and interactions on protein functionality and understand variations in functional properties of proteins from different cultivars of pulses.

3.8. Molecular properties of proteins

To determine the molecular weight distribution of proteins in chickpeas and lentils and in their different cultivars, classical SDS-PAGE was conducted in crude protein extracts collected before isoelectric precipitation (Fig. 3). The crude chickpea and lentil proteins showed different band patterns. Thus, it is possible to differentiate chickpea and lentil proteins from each other with their SDS-PAGE profiles. In contrast, the band patterns of different cultivars of the same pulse type were quite similar. For crude chickpea proteins, the main protein bands were obtained around 20 kDa and between 30 and 40 kDa, while crude lentil proteins gave dense bands between 15 and 20 kDa, 30 and 40 kDa, and 40 and 70 kDa.

The detailed molecular and isoelectric properties of selected CPE (CPE-3) and LPE (LPE-3, a red lentil) obtained by isoelectric precipitation of crude protein extracts were also determined by 2-D electrophoresis (Figs. 4 and 5). This was done to provide basis for optimal isoelectric precipitation and ultrafiltration used in purification of these proteins. The CPE proteins were distributed mainly between pI 4.5 and 5.9 at MW between 15.0 and 76.0 kDa, while LPE proteins distributed mainly between pI 4.8 and 5.9 at MW below 66.2 kDa. These profiles clearly showed lower pI and MW range of lentil proteins than the chickpea proteins. The most intensive protein spots of CPE concentrated at MW between 24 and

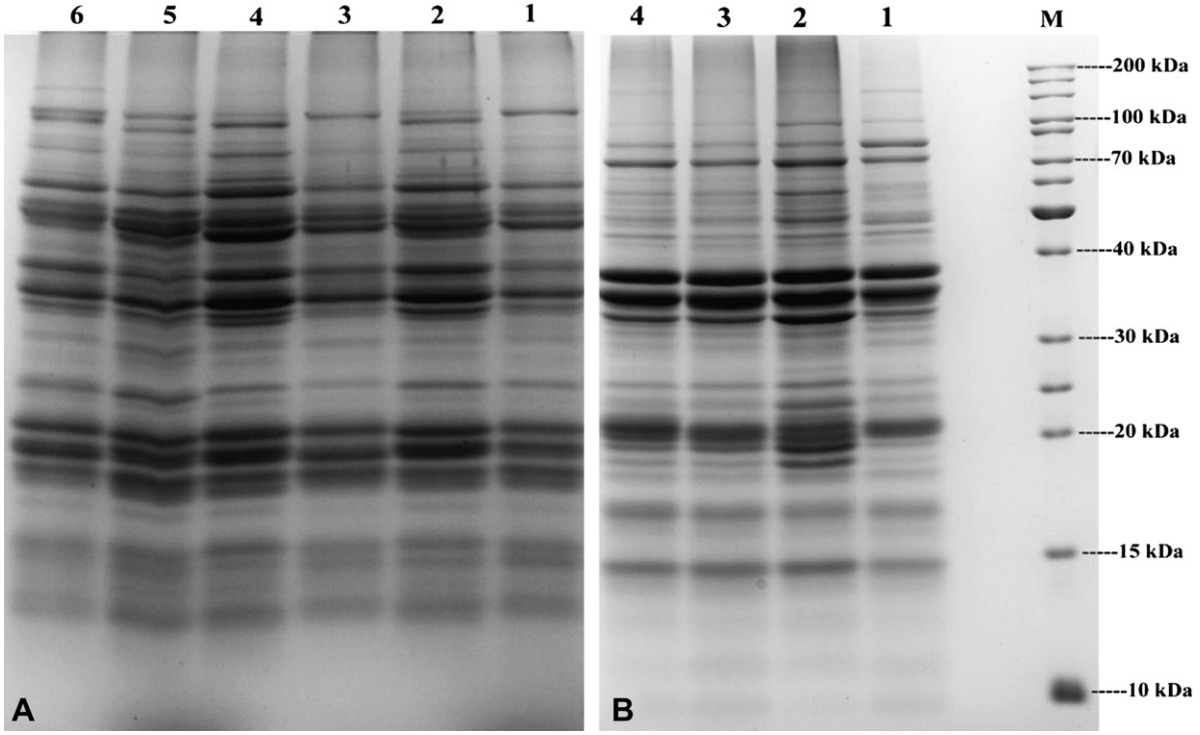

Fig. 3. SDS-PAGE profiles of crude protein extracts before isoelectric precipitation (crude protein extracts in A: 1: L-1, 2: L-2, 3: L-3, 4: L-4, 5: L-5, 6: L-6; in B; 1: C-1, 2: C-2, 3: C-3, 4: C-4).

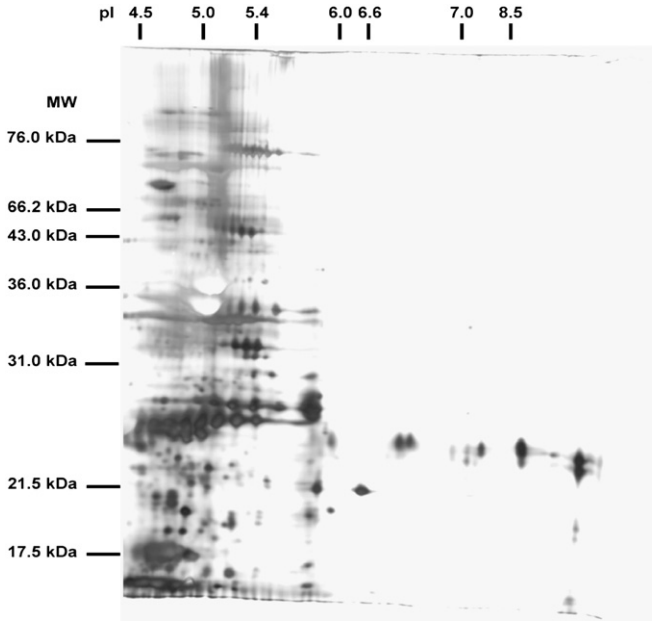

Fig. 4. 2-D electrophoresis of chickpea proteins after isoelectric precipitation (CPE-3).

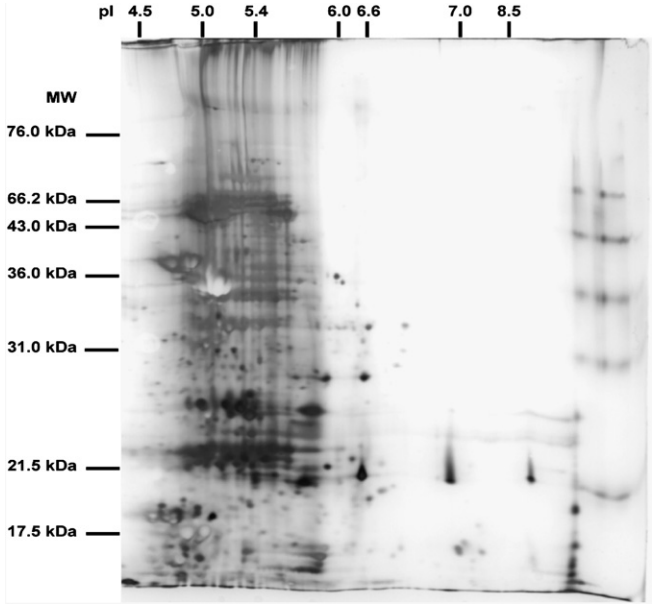

Fig. 5. 2-D electrophoresis of lentil proteins after isoelectric precipitation (LPE-3).

27 kDa, while the most intensive spots of LPE concentrated between 21 and 23 kDa, and around 26 kDa. Both CPE and LPE showed less intense spots above 30 kDa and this suggested low IEP recovery of these proteins appeared as dense bands in classical SDS-PAGE of crude protein extracts.

4. Conclusions

The results of this study clearly showed the potential of chickpea and lentil proteins as commercial functional protein alternatives. Most of the functional properties of chickpea proteins are comparable with or superior than those of soy and animal origin proteins tested in this study. In fact, there was only gelling capacity of bovine gelatin which considerably surpassed a functional property of chickpea proteins. The most outstanding functional properties of studied pulse proteins were extremely high oil and water absorption capacity for chickpea proteins and high oil absorption capacity and solubility of lentil proteins. Both types of pulse proteins were also capable to form highly stable emulsions and foams. In contrast, water absorption capacity and gelling capacity are critical missing functional properties of lentil proteins. Thus, blending of lentil and chickpea proteins could be suggested when high protein solubility is the primary desired functionality, but some water absorption and gelling capacity is also needed. The outstanding chickpea and lentil cultivars with superior protein functionality and cultivars with inferior protein functionality were identified. However, further studies are needed to monitor and characterize the functional properties of protein form different chickpea and lentil cultivars obtained at different growing conditions, harvesting periods and climates and determine magnitude of possible variations in protein functionality at standard extraction and assay conditions.

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