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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