J Food Sci Technol (May 2017) 54(6):1395–1405
DOI 10.1007/s13197-017-2551-6

ORIGINAL ARTICLE

Preparation of mayonnaise from extracted plant protein isolates of chickpea, broad bean and lupin flour: chemical, physiochemical, nutritional and therapeutic properties

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Revised: 11 February 2017 / Accepted: 14 February 2017 / Published online: 10 March 2017

Abstract This investigation was aimed to study the molecular, physico-chemical, and biofunctional health properties of mayonnaise prepared using proteins isolated from broad bean, lupin and chickpea flour. Proteins were isolated from chickpea (CPPI), broad bean (BBPI) and lupin (LPPI) flour and assessed for molecular, physico-chemical, biofunctional, and protein yield. The highest water holding capacity, foaming stability, emulsion stability as well as protein yield and protein content of 44.0, 70.8, 37.5, 81.2, and 36.4, respectively were observed for BBPI. Mayonnaise prepared from the isolated plant proteins was evaluated for chemical composition, molecular properties of the protein subunits, and potential nutraceutical properties. Preparation of mayonnaise using BBPI or a mixture of either BBPI and CPPI or BBPI and LPPI showed superior values for lightness and lowered values for redness. Mayonnaise prepared from either BBPI or the BBPI and CPPI mixture showed the best antioxidant, antihypertensive and antidiabetic properties. The present study results indicated that the use of the BBPI and CPPI mixture can be a novel technological approach for the development of a mayonnaise with improved health promoting properties.

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Keywords Mayonnaise · Legumes · Protein isolates · Antioxidant · ACE · Amylase

Introduction

There has been longstanding consumer demand for more nutritious natural food products, including foods that can provide additional health benefits beyond basic nutrition (McClements and Demetriades 1998). One of the most widely consumed products worldwide is mayonnaise for use in condiments or sauces (Harrison and Cunningham 1985). Mayonnaise is a semi-solid product since it is an oil-in-water emulsion with oil representing around 70–80% of the total composition by weight (McClements 2005). Mayonnaise is prepared by mixing several food ingredients such as egg yolk, vinegar, oil and spices such as mustard (Liu et al. 2007). Legume grains occupy an important role in nutrition as they serve as a relatively inexpensive dietary source of protein, especially in developing countries due to their low annual incomes (Tharanathan and Mahadevamma 2003). Legume crops are an important source of phytochemicals as well as certain vitamins and essential minerals (Makri et al. 2005). Legume grain products can provide health promoting benefits such as antioxidant and anti-diabetic properties. For example, Sreerama et al. (2012) showed significant antioxidant activity and inhibitory activities of α-amylase and α-glucosidase for phenolics associated with chickpea, cowpea, and horse gram flours. Legumes are also of interest for individuals with specific dietary needs or restraints such as vegans, vegetarians and hyperlipidemic individuals with strict dietary fat restrictions (Arozarena et al. 2001). In addition to their nutritional quality, legume proteins have properties for their use as

functional ingredients that can improve the texture and stability of foods (Makri et al. 2005).

The use of unpasteurized eggs in the manufacture of mayonnaise is considered as a health problem due to the risk of *Salmonella* infection. In addition, the high caloric content of traditional mayonnaise is a nutritional disadvantage. Production of a low fat mayonnaise can take place via the use of various agents such as modified starch, pectin, microcrystalline cellulose, carrageenan gum and thickeners. These compounds function in low fat mayonnaise to decrease the dispersed phase and increase the water content as well as stabilize the emulsion and increase its viscosity. In that regard, proteins derived from plant sources can provide several aspects useful in the manufacture of low fat mayonnaise including emulsification properties in addition to microbiological, physico-chemical, nutritional and biofunctional health benefits. To our knowledge, there has been no previous research that has demonstrated the use of proteins derived from plant sources as stabilizing and emulsifying agents instead of egg yolk. The incorporation of mayonnaise with different mixtures of protein isolates from chickpea, broad bean and lupin flour could enhance its physico-chemical, nutritional and functional health properties. The objectives of the present study were to evaluate the effect of egg yolk replacement in the form of different plant protein isolates (chickpea, broad bean and lupin) on the physico-chemical, nutritional and functional health properties of mayonnaise.

Materials and methods

Materials

Three dried selected legume grain products (chickpea, broad bean and lupin flour) were obtained from Irbid Governorate-Jordan. Dried plants (legume grains) were ground/milled into flour by the Private Milling Company, Irbid. The dried plants and flour were then stored in plastic bags at 4 °C until further use.

Extraction of protein isolates from chickpea, broad bean and lupin flour

Protein isolates were extracted from 100 g full-fat flour legume grains (chickpea, broad bean and lupin) according to the method described by Alu'datt et al. (2012) with some modifications and then were mixed with 1000 ml distilled water and shaken in water bath for 1 h. The aqueous solution was centrifuged at 10,000×*g* for 15 min (Z32HK, Hermle Labortechnik GmbH, Germany). The extract was filtered using filter paper (101 FAST, 125 mm, China). The pH of the extract was adjusted to 4.5–4.6 using diluted acetic acid (20%, v/v). The proteins were isolated by centrifugation at 10,000×*g* for 15 min followed by freeze drying at −50 °C for 48 h (LFD-5508, Korea) for storage until further analysis. The protein isolates were assigned according to the protein source using the following designations: chickpea protein isolate (CPPI), broad bean protein isolate (BBPI) and lupin protein isolate (LPPI).

Protein isolate yield

Protein isolate yield from each legume grain source was calculated according to the method described by Alu'datt et al. (2012) using the following equation:

$$\%\ \text{Yield (protein basis)} = \frac{\text{Weight of protein isolate} \times \text{Protein content in protein isolate} \times 100\%}{\%\ \text{Protein content in flour} \times \text{Weight of flour}}$$

Functional properties of chickpea, broad bean, and lupin flour and their isolates

Aqueous dispersions (16% w/v) of chickpea, broad bean, and lupin flour and their protein isolates were prepared by adding 4 g from each sample to 25 ml of distilled water and mixed well in beakers using a magnetic stirrer for 5 min.

Water holding capacities of prepared aqueous dispersions were evaluated according the method of Alu'datt et al. (2012). The process of gelation took place by heating the dispersions in beakers at 95 °C for 30 min in a water bath. Aluminium foil was used in order to avoid loss of moisture. The gels were cooled at 4 °C in refrigerator for 24 h. The gels were subjected to centrifugation at 10,000×*g* for 15 min followed by measuring of separated supernatants after centrifugation. Water holding capacity was expressed as water retained in the residue after centrifugation (i.e., g of water retained to total water added).

Emulsion stabilities of the above prepared samples were measured (Alu'datt et al. 2012). Two grams of aqueous dispersions were prepared by mixing with 20 ml of distilled water and 20 ml of olive oil followed by vigorous shaking for 2 min. The emulsion was centrifuged at 2000×*g* for 5 min. The emulsion stability was measured by heating the emulsion in a shaker water bath at 80 °C for 30 min followed by cooling to 25 °C by running tap water for 15 min and centrifuging at 2000×*g* for 15 min. The emulsion stability calculated by the ratio of the volume or height of the emulsified phase to the total volume or height of the solution and emulsified phases.

Foaming stabilities of the above prepared aqueous dispersions were determined as previously described by Alu'datt et al. (2012) with some modifications. Two grams of each sample was mixed with 40 ml distilled water at

30 °C in a 100 ml measuring graduated cylinder. The dispersion was mixed and shaken for 5 min to produce layer of foam. Foam stability was measured as the ratio of foam volume obtained at 0 min to foam volume at 60 min.

Preparation of mayonnaise using protein isolates

Mayonnaise was prepared according to slightly modified method described by Depree and Savage (2001) using protein isolates from three legume sources (broad bean, chickpea and lupin) in combination with other ingredients (oil, salt, xanthan gum, mustard, vinegar, garlic, onion, citric acid, water, potassium sorbate and sodium benzoate) as listed in Table 1. Salt, mustard, garlic, xanthan gum, onion, potassium sorbate and sodium benzoate were mixed with vinegar and water via use of an electrical mixer (Depose, LM 207, France) for 1 min. Oil was added to the mixture slowly at the beginning and then more rapidly after the mixture began to thicken. Finally, all ingredients were mixed in the blender for 5 min.

Chemical composition of mayonnaise prepared using protein isolates

Protein, moisture, ash and fat were evaluated as the method described by the AOAC (1984). The carbohydrate content was measured by difference by subtracting the content of protein, moisture, fat and ash from 100.

Extraction of phenolic compounds

One gram of eggless mayonnaise was subjected to methanol extraction as previously described by Mohamed et al. (2007) using 25 ml methanol at 60°C for 1 h and the supernatants were centrifuged at 10,000×*g* for 10 min.

Table 1 Formula for the preparation of mayonnaise from isolated proteins of broad bean, chickpea and lupin flour

Ingredients	Weight (g)
Protein	3.00
Vinegar	0.30
Soy oil	75.00–76.00
Salt	1.10
Xanthan gum	0.10–0.15
Garlic powder	0.05
Potassium sorbate	0.04
sodium benzoate	0.04
Mustard	0.25
Onion	0.03
Citric acid	0.30
Water (ml)	19.74

Methanolic extracts of phenolic compounds were filtered in cheese cloth followed by flushing with a stream of nitrogen and stored at −40°C.

Total phenolic content

The total phenolic content for each extract was determined by the spectrophotometric method of Folin–Ciocalteu method with some modifications (Hoff and Singleton 1977). A stock solution using 5 mg of gallic acid in 50 ml of distilled water was used to prepare a standard curve. Solutions were prepared containing 500, 100 and 8400 µl of Folin reagent, phenolic extract and distilled water, respectively. The solution mixed at room temperate for 4 min in the test tube and then mixed with 1 ml of 5% sodium carbonate. The resulting content was mixed using vortex (Reamix 2789, 50 Hz). The absorbance colour was measured 1 h later at wavelength of 725 nm using spectrophotometer (UV 1800, 50 Hz, UK). The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry matter. The analysis was conducted in duplicate for each sample.

Antioxidant activity

Antioxidant activity of phenolic compounds was measured determined by the method described by Emmons et al. (1999) with some modifications. Five milligrams of β-carotene solution (Sigma Chemical Co.) was prepared by dissolving in 50 ml of chloroform. Three millilitres of β-carotene solution was mixed with 50 µl of linoleic acid (Sigma Chemical Co.) and 400 mg of Tween 20 (Sigma Chemical Co.). Chloroform was evaporated by nitrogen gas and then was shaken with 100 ml of distilled water. Three millilitres of β-carotene/linoleic acid emulsion was mixed with 100 µl of phenolic extract. The colour absorbance of the emulsion was measured at 470 nm followed by incubation at 50 °C for 60 min in a water bath. The degree of oxidation of the emulsion was calculated by measuring after 60 min the colour absorbance at 470 nm using a spectrophotometer (UV 1800, 50 Hz, UK). Control samples contained 100 µl of solvent and the β-carotene/linoleic acid emulsion. For each sample, measurement was conducted in duplicate. The antioxidant activity (AA%) was calculated according to the following equation:

$$AA\% = [(DR_b - DR_e)/DR_b] \times 100$$

AA% antioxidant activity percentage; Dr_b degradation rate of the blank ($Dr_b = (\text{In } (A_0/A_{60})/60)$); DR_e degradation rate of the extracts $DR_e = (\text{In } (A_0/A_{60})/60)$; A_0 absorbance at 0 min; A_{60} absorbance at 60 min.

Angiotensin-converting enzyme (ACE) inhibitory activity

The in vitro inhibitory activity of ACE was evaluated using a modified method described by Cushman and Cheung (1971). HEPES-HCl buffer was prepared by mixing of 1.3014 g from HEPES-sodium salt with 1.75329 g of sodium chloride in 100 ml of distilled water. Hippuryl-histidyl-leucine (HHL) (Sigma-Chemical Co.) solution was prepared by dissolving 6 µl in 2 ml HEPES-HCl buffer (Sigma Chemical Co.). Two hundred microliters of HHL solution was mixed with 100 µl of phenolic extract. A solution of 2 U of ACE enzymes (Sigma Chemical Co. A6778-2UN) was prepared by dissolving this concentration in 6.06 ml of distilled water. A 50 µl ACE solution was incubated at 37 °C for 15 min. A solution containing 250 µl of 1 M of HCl was used to stop the activity of the ACE enzyme. The hippuric acid liberated from the enzymatic reaction was extracted by addition of 2 ml of ethyl acetate. The ethyl acetate layer (1 ml) was separated via centrifugation (50 Hz, USA) followed by evaporation at 100 °C for 15 min in a water bath followed by mixing with 3 ml of distilled water. The sample absorbance was measured by spectrophotometry (UV 1800) at 228 nm. Two hundred microliters of HHL and 50 µl of ACE were mixed in 100 µl distilled water as either the control or the phenolic extract containing sample. The inhibition of ACE was measured in duplicate for each sample extract and calculated using the following equation:

$$\text{ACE Inhibition}(\%) = [(A_{228}\,\text{Blank} - A_{228}\,\text{Sample})/A_{228}\,\text{Blank}) * 100]$$

Inhibitory activity of alpha-amylase

The inhibitory activity of α-amylase was estimated by a colorimetric method according to the method described by McCue et al. (2005). A sample of 0.125 g of potato starch (Sigma Chemical Co.) was mixed with 25 ml of distilled water at 65 °C for 20 min. The α-amylase solution (Sigma Chemical Co., 99.9% activity) was prepared by mixing of 0.03 g of α-amylase enzyme in 100 ml of distilled water. Colorimetric reagent was prepared by mixing 19.8 g of sodium hydroxide with 10.6 g of 3,5-dinitrosalicyclic acid solution (DNS) in 1416 ml of distilled water. The solution was mixed with 3.06 g of sodium potassium tartrate, 7.6 g of phenol and 8.3 g of sodium metabisulfite. One hundred microliter sample of phenolic extract was mixed with 500 µl of phosphate buffer (pH 7) and 500 µl of α-amylase solution followed by mixing and incubation at 25 °C for 10 min. A 500 µl aliquot of the starch solution was added to the mixture followed by incubation for 10 min at 25 °C. one milliliter of the colorimetric reagent (DNS) was added to the mixture and boiled for 5 min at 100 °C. The mixture was cooled and mixed with 7.4 ml of distilled water at room temperature. Maltose concentration was measured using spectrophotometer at wavelength absorbance of 540 nm (UV 1800). The inhibition (%) of α-amylase was determined using the following equations:

$$\text{Inhibition}\,(\%) = [(A_{540}\,\text{Blank} - A_{540}\,\text{Sample})/A_{540}\,\text{Blank}) * 100].$$

Inhibitory activity of alpha-glucosidase

Inhibitory activity of α-glucosidase was determined using colorimetry as described by the method of McCue et al. (2005) with modifications. Alpha glucosidase solution was prepared by mixing of 1 mg of α-glucosidase (Sigma Chemical Co. G5003-100UN) in 5.7 ml of phosphate buffer (pH 6.9). A 0.151 g sample of nitrophenyl-α-D-glycopyranoside (Sigma Chemical Co.) was dissolved in 100 ml phosphate buffer (pH 6.9) in volumetric flask. Fifteen microliters of phenolic extracts was mixed with 100 µl of α-glucosidase solution and digested for 10 min at room temperature. A 50 µl sample of the nitrophenyl-α-D-glycopyranoside solution was added to the mixture and absorbance was measured at 405 nm using spectrophotometer (Dynatech, MR5000) against the control sample that replaced the extract with extraction solvent. Percentage of inhibition activity of α-glucosidase was measured in duplicate. The inhibitory activity of α-glucosidase was calculated using the following equation:

$$\text{Inhibition}\,(\%) = [(A_{405}\,\text{Blank} - A_{405}\,\text{Sample})/A_{405}\text{Blank}) * 100].$$

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) was prepared using the BioRad Mini Protean apparatus (6 cm × 8 cm × 0.75 mm) according to the original method described by Laemmli (1970). Twelve percent resolving gel was prepared from 2 ml of acrylamide/bisacrylamide solution (30 and 0.8% w/v, respectively), 1.25 ml Tris HCl (1.5 M, pH 8.8), 1.68 ml H_2O, 50 µl SDS (10%), 20 µl ammonium persulfate (10%) and 5 µl TEMED. Four percent stacking gel was prepared from 325 µl of acrylamide (30% w/v) and bisacrylamide solution (0.8% w/v), 625 µl Tris HCl (0.5 M, pH 8.8), 1.5 ml H_2O, 25 µl 10% SDS, 10 µl ammonium persulfate (10%) and 5 µl TEMED. Protein samples were prepared by mixing with buffer in 1:1 ratio of 0.6 ml Tris HCl (1 M, pH 6.8), 5 ml glycerol (50%), 2 ml SDS (10%), 0.5 ml

mercaptoethanol, 1 ml bromophenol blue (1%) and 0.9 ml distilled water. A 20 µl of sample mixture were loaded in each well. SDS-PAGE was run at a constant 80 V for 30 min followed by run at 160 V for 45 min. The gel was removed from apparatus and immersed in fixing solution (50% ethanol and 12% acetic acid) followed by silver staining. Gel was rinsed three times with 50% ethanol. Sensitization with sodium thiosulfate (0.2 g/l) was performed by rinsing for 1 min and then washing three times with distilled water. Silver staining (2 g/l Ag_2NO_3 and 0.75 ml/l formaldehyde) was carried out for 20 min and then was washed two times with distilled water. Developing solution was used for 15 min (60 g/l sodium bicarbonate, 0.5 ml/l formaldehyde and 0.004 g/l sodium thiosulfate). The reaction was stopped using 0.05 M EDTA solution. Gel documentation was performed using GS-800 Densitometer and Quantity One Software (Bio Rad, USA).

Colour value measurement

Colour values were estimated by using a colorimeter (Minolta, Ramsey, CR-300, NJ, USA) and the measurements of L^*, a^*, b^* were recorded with the Minolta colour system. The values of L^*, a^*, b^* colour system involves of lightness component (L^*) and 2 chromatic components (a^* component (green (−a) to red (+a)) and the b^* component (blue (−b) to yellow (+b)) values. Colour values of the samples were estimated at two positions.

Statistical analysis

Collected data were subjected to statistical analysis using the procedure of general linear model (GLM) with SAS software package (Version 8.2, SAS 2002 Institute Inc., Cary, NC, USA). Means were calculated and separated using LSD analysis with a *P* value less than 0.05 signifying significance.

Results and discussion

Protein yields from chickpea, broad bean and lupin flour

Table 2 shows the protein content and yields of CPPI, BBPI and LPPI. The highest yield was obtained for BBPI with a value of 36.41% followed by CPPI and LPPI with values of 32.66 and 19.12%, respectively. The yield values did not vary significantly between BBPI and CPPI, while the yields in LPPI were significantly lower as compared to both CPPI and BBPI. The present results indicate that the protein isolates from broad bean could be recommended for further applications in food industry due to their relatively higher protein content and yield. The yield values of protein isolates from broad bean, chickpea and lupin flour have previously been characterized as 92% (Vioque et al. 2012), 65.9–62.1% (Sanchez-Vioque et al. 1999) and 13.13–79.7% (Wang et al. 2012; Osman and Simon-Sarkadi 1991), respectively. The protein yield has also been shown to range from 63 to 74% in protein isolates from chickpea (Boye et al. 2010). The variation in yield and protein solubility among the three different protein isolates was likely due to the variations in ratio of hydrophilic to hydrophilic amino acids, chemical structure, amino acid sequences, and content of acidic and basic amino acids.

Table 2 Yield (based on protein content) and content of protein isolates from broad bean, chickpea and lupin flour

Protein isolates	Protein content (%)**	Yield (%)**
BBPI	81.16[a*]	36.41[a]
CPPI	48.13[c]	32.66[a]
LPPI	58.75[b]	19.12[b]
SE	1.73	1.45

SE standard error, *CPPI* chickpea protein isolate, *BBPI* broad bean protein isolate, *LPPI* lupin protein isolate

* Means with different letters in the same Column are significantly different at $P \leq 0.05$

** Means are average of two replicates and expressed as % based on dry weight

Colour of protein isolates from broad bean, chickpea and lupin

The lightness (L^*), redness (a^*), and yellowness (b^*) values of different protein isolates are shown in Table 3. The result showed that the LPPI had the highest value of lightness (L^*) (79.07), which differed significantly as compared to values of either BBPI or CPPI. The value of lightness L^* for BBPI did not vary significantly compared to CPPI. The value of redness (a^*) for CPPI had the highest value (−1.12) and varied significantly as compared to values of either BBPI or LPPI. The value of redness (a^*) in BBPI did not differ significantly in comparison to the value of LPPI. The values of yellowness (b^*) among LPPI, BBPI and CPPI varied significantly amongst each other. The highest value for yellowness (b^*) was obtained in LPPI followed by CPPI and BBPI with values of 3.89, 3.33 and 2.82, respectively. The highest values of lightness and yellowness were obtained for LPPI. Kaur and Singh (2005) have reported that the protein isolate from chickpea had lower L^* value and higher a^* value (darker and reddish in colour) compared to chickpea flour. Kaur and Singh (2007) found that the values of a^* and b^* for protein isolates from

Table 3 Color values and functional properties of protein isolates from broad bean, chickpea and lupin flour

Treatments	L*,***	a*,***	b*,***	WHC (%)****	Emulsion stability (%)****	Foaming stability (%)****
BBPI	72.90^{b**}	−2.15^{b}	28.20^{c}	44.0^{c}	37.5^{a}	70.8^{ab}
CPPI	74.35^{b}	−1.12^{a}	33.26^{b}	42.0^{c}	36.3^{a}	50.0^{c}
LPPI	79.07^{a}	−1.91^{b}	38.88^{a}	33.0^{d}	33.8^{ab}	50.0^{c}
SE	0.68	0.11	0.64	1.96	1.22	3.09

SE standard error, *CPPI* chickpea protein isolate, *BBPI* broad bean protein isolate, *LPPI* lupin protein isolate

** Means with different letters in the same column are significantly different at $P \leq 0.05$

*** Means are average of triplicates for color values

**** Means are average of two replicates for functional properties

chickpea were in the range of 1.88–2.21 and 22.46–24.95, respectively. Paredes-Lopez et al. (1991) reported that the L*, a* and b* for protein isolates from chickpea were 56.8, 3.5 and 17.2, respectively. The variations in colour values among LPPI, BBPI and CPPI may be related to protein-mineral, protein–polyphenol and protein-pigment interactions.

Functional proprieties of protein isolates from broad bean, chickpea and lupin

Table 3 shows water holding stability, foaming stability and emulsion stability of BBPI, CPPI and LPPI. The water holding capacity of BBPI (44%) did not vary significantly as compared to CPPI (42%). The lowest values of water holding capacity and foaming stability were found for LPPI with values of 33% and 50%, respectively. The foaming stability did not vary significantly among LPPI and CPPI (50%). The emulsion stability among protein isolates from chickpea, broad bean and lupin did not differ significantly and ranged from 33.8 to 37.5%. The foam stability of Kabuli chickpea protein isolate has been shown to have the highest value of 94.7% after 120 min of storage (Kaur and Singh 2007). The foaming stability of lupine proteins was previously demonstrated to be in range of 50–60% (Pozani et al. 2002). Kaur and Singh (2007) reported that the foaming stability of protein isolates from different varieties of chickpea ranged from 85 to 100%. The water holding capacity and foaming stability have been shown to range between 71–84 and 15–29% for protein isolates of different varieties of chickpea (Thushan Sanjeewa et al. 2010). The protein isolates from broad bean have shown better emulsifying and foaming properties as compared to protein isolates from lupin flour (Makri et al. 2005). The variations in functional properties in protein isolates described in the present study could be due to a variety of factors that include difference in protein content, protein-lipid interactions, the overall chemical composition, degree of purification as well as intrinsic factors such as molecular properties of the proteins (i.e., size, shape and conformation). Shevkani and Singh (2014) and Shevkani et al. (2015a) reported that incorporation of protein isolates from cowpea, kidney bean, field pea and amaranth enhanced the functional properties of muffins more as compared to those prepared using wheat gluten. Foaming, solubility and emulsification of white cowpea protein isolates were higher than red cowpea protein isolate (Shevkani et al. 2015a). Broad bean proteins showed higher foaming properties than chick pea and lupin proteins (Table 3). The significant variations in these functional properties are likely due to the variation in protein content (Shevkani et al. 2014).

Protein subunit characterization by SDS-PAGE for chickpea, broad bean and lupin flour and their protein isolates

Figure 1a illustrated SDS-PAGE patterns of protein subunits from lupin, chickpea and broad bean flour and their protein isolates. Seven major protein subunits of legumin (11S) corresponding to 22.3, 24, 25.8, 37.5, 39.8, 43.5 and 43.5 kDa, and two major protein subunits of vicilin (7S) corresponding to molecular weights of 33.8 and 50.7 kDa were observed in protein isolates from lupin flour (LPPI). Previous SDS-PAGE results have shown α-subunits of legumin with molecular weight of 40.6 and 39.5 kDa and β-subunits of legumin with molecular weights of 23.5 and 22.5 kDa, and vicilin subunits with molecular weights of 70.2, 50.7, 35, 33.6, 18.9 and 15.5 kDa (Chang et al. 2012; Wang et al. 2010). Lupin proteins consist from three globulin proteins including 12 protein subunits of β-conglutin (7S), 4 protein subunits of α-conglutin (11S) and a minor γ-conglutin (2S) of 170, 315 and 17 kDa molecular weight (Chapleau and de Lamballerie-Anton 2003). In the present study, two major predominant protein fractions were separated from CPPI with molecular weights of 170 and 110 kDa, respectively. Earlier SDS-PAGE studies of CPPI showed protein subunits with molecular weights of 14.9–84.2 kDa (Paredes-Lopez et al. 1991) and molecular

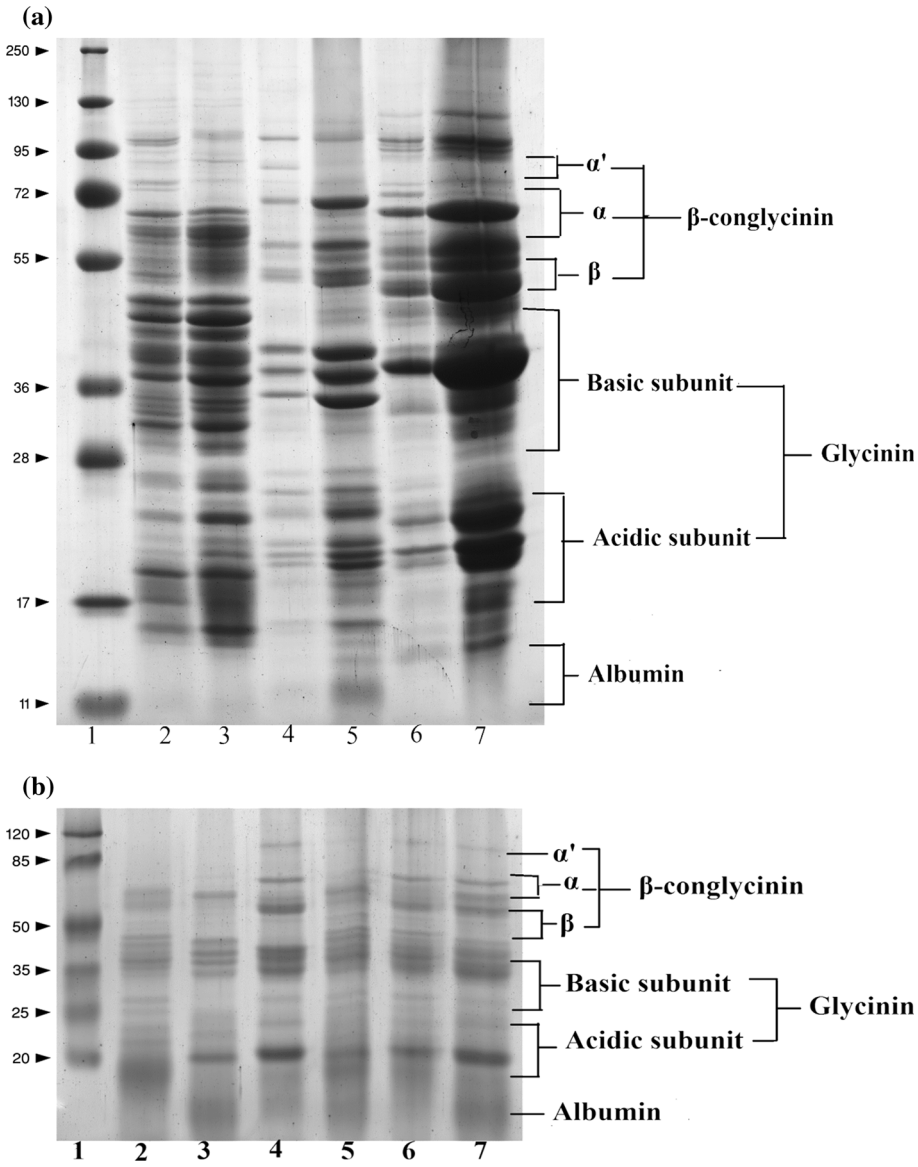

Fig. 1 **a** SDS-PAGE of lupin (LP), chickpea (CP) and broad bean (BB) flour (F), and their protein isolates (PI): *Lane 1* Standard Marker, *Lane 2* LPF, *Lane 3* LPPI, *Lane 4* CPF, *Lane 5* CPPI, *Lane 6* BBF, *Lane 7* BBPI. **b** SDS-PAGE of mayonnaise prepared from individual and mixture of protein isolate (PI) of lupin, chickpea (CP) and broadbean (BB). *Lane 1* Standard marker, *Lane 2* LPPI, *Lane 3* CPPI, *Lane 4* BBPI, *Lane 5* LPPI + CPPI, *Lane 6* LPPI + BBPI, *Lane 7* CPPI + BBPI

weight ranges of 20–70 kDa (Neves et al. 2006). SDS-PAGE of CPPI demonstrated four major protein subunits of legumin (11S), which correspond to molecular weights of 22.5, 22.8, 23 and 24.1 kDa and seven major protein subunits of vicilin (7S) with molecular weight of 33.7, 34.5, 37.3, 53.4, 58.4, 59.1 and 71.1 kDa. Wang et al. (2010) and Chang et al. (2012) have found that the globulin protein 11S (legumins) and 7S (vicilins) were the predominant major protein subunits in CPPI using isoelectric and cryo-precipitation techniques, while the 2S albumin proteins was the minor protein subunits CPPI using isoelectric and cryo-precipitation techniques. BBPI showed four major protein subunits of legumin (11S) with molecular weights of 22.4, 22.8, 23.5 and 24.2 kDa and five major protein subunits of vicilin (7S) corresponding to molecular weights of 50.7, 53.5, 55.1, 55.7 and 69.1 kDa. The major protein subunits in faba beans were 7S and 11S globulins (Cai et al. 2002). Shevkani et al. (2015b) reported that the molecular weights of major protein subunits in kidney beans were 21, 32, 46–40 and 55 kDa.

Chemical composition of mayonnaise prepared from protein isolates of chickpea, broad bean and lupin

The chemical composition of mayonnaise prepared using BBPI, CPPI, and LPPI is illustrated in Table 4. The protein content in mayonnaise prepared from BBPI did not vary significantly as compared to the protein values of mayonnaise prepared from LPPI or mixture of either

Table 4 Gross chemical composition of mayonnaise prepared from protein isolates of broad bean, chickpea and lupin flour

Type of mayonnaise	Protein (%)**	Fat (%)**	Moisture (%)**	Ash (%)**	Carbohydrate (%)**
Egg	1.99^{c*}	14.29^{a}	15.33^{bc}	0.98^{bc}	55.08^{cd}
BBPI	2.67^{a}	10.63^{b}	13.79^{d}	1.04^{b}	63.88^{ab}
CPPI	2.43^{b}	10.15^{b}	14.26^{cd}	1.04^{b}	64.38^{ab}
LPPI	2.56^{ab}	7.14^{c}	14.71^{cd}	1.29^{a}	69.71^{a}
BBPI and CPPI (50:50)	2.68^{a}	15.57^{a}	17.71^{a}	1.01^{bc}	50.12^{d}
BBPI and LPPI (50:50)	2.71^{a}	14.89^{a}	17.04^{a}	0.97^{bc}	52.18^{d}
CPPI and LPPI (50:50)	2.57^{ab}	11.23^{b}	16.37^{ab}	0.83^{c}	60.31^{bc}
SE	0.04	0.72	0.45	0.06	1.79

SE standard error, *CPPI* chickpea protein isolate, *BBPI* broad bean protein isolate, *LPPI* lupin protein isolate

* Means with different letters in the same column are significantly different at $P \leq 0.05$

** Means are average of two replicates and expressed as % based on wet weight

BBPI and CPPI or BBPI and LPPI, which ranged from 2.56 to 2.71%. Protein content did not differ significantly in mayonnaise prepared from either CPPI or LPPI (2.43–2.56%). The content of protein in mayonnaise prepared from egg (1.99%) did not vary significantly as compared to mayonnaise prepared from the mixture of CPPI and LPPI (2.16%), which indicated that mayonnaise prepared from those protein isolates provided similar protein content in the mayonnaise. The fat content in mayonnaise prepared from LPPI was significantly lower than from mayonnaise prepared from BBPI and CPPI. For mayonnaise prepared from mixture of CPPI and LPPI, the fat content did not vary significantly when compared to the fat content of mayonnaise prepared from CPPI. The mayonnaise prepared from mixture of BBPI and CPPI did not show any difference in fat content from mayonnaise prepared from the mixture of BBPI and LPPI. These latter results suggested that the preparation of mayonnaise from the mixture of BBPI with either CPPI or LPPI mixture had similar fat content of mayonnaise along with good solubility and emulsification properties. The moisture content of mayonnaise prepared from CPPI was similar to that of mayonnaise prepared from BBPI or LPPI (13.79–14.71%). Likewise, mayonnaise prepared from mixture of BBPI and CPPI did not show differences in moisture content from mayonnaise prepared from mixture of either BBPI and LPPI or a mixture containing CPPI and LPPI (16.37–17.71%). The moisture value in mayonnaise prepared from egg differed significantly as compared to the mayonnaises produced from BPPI and from the mixtures of protein isolates apart from the mayonnaise prepared from mixture of CPPI and LPPI. For mayonnaise prepared from mixture of BBPI and LPPI, the ash content was similar to the mayonnaise prepared from BBPI or CPPI, the CPPI and LPPI mixture and the mixture with CPPI and LPPI (0.83–1.04%). The ash content of egg-containing mayonnaise did not vary significantly as compared to all the prepared mayonnaises from the protein isolates except for mayonnaise prepared from LPPI, which had significantly higher ash content relative to all of the other prepared mayonnaises.

Protein subunit characterization by SDS-PAGE of mayonnaise prepared from chickpea, broad bean and lupin flour and their protein isolates

Figure 1b demonstrates SDS-PAGE of the prepared mayonnaise from BBPI, CPPI, and LPPI. SDS-PAGE for mayonnaise prepared with LPPI had six major protein subunits corresponding to molecular weights of 17.5, 37.5, 44.8, 47.4, 56, and 58.5 kDa, while mayonnaise prepared from CPPI gave seven major protein subunits corresponding to molecular weights of 12.5, 21.5, 34.2, 36.5, 38, 46.6 and 75.1 kDa. Six major protein subunits identified in mayonnaise prepared from BBPI corresponded to molecular weights of 23.5, 34.5, 36.8, 38.2, 56.9 and 79.4 kDa. Mayonnaise prepared from CPPI and LPPI had 5 major protein subunits, which corresponded to subunits of CPPI (12.5, 21.5 and 36.5 kDa) and LPPI (17.5 and 58.5 kDa). Mayonnaise prepared from BBPI and LPPI had 6 major protein subunits that corresponded to subunits of BBPI (23.5, 34.5, 36.8 and 79.4 kDa) and LPPI (17.5 and 58.5 kDa). For mayonnaise prepared from CPPI and BBPI, SDS-PAGE had 11 major protein subunits corresponding to subunits of CPPI (12.5, 21.5, 34.2, 36.5, 38 and 75.1 kDa) and BBPI (23.5, 34.5, 36.8, 56.9 and 79.4 kDa).

Effect of protein isolates from broad bean, chickpea and lupin on the colour values of mayonnaise

Table 5 shows the effect of protein isolates from broad bean, chickpea and lupin on lightness (L^*), redness (a^*) and yellowness (b^*) values of mayonnaise. The highest value of

Table 5 Color values of mayonnaise prepared from protein isolates of broad bean, chickpea and lupin flour

Treatment	L*	a*	b*
Egg	73.38[c**]	7.115[a]	35.53[a]
BBPI	76.61[ab]	5.15[bc]	26.24[b]
CPPI	73.78[c]	4.82[c]	25.94[b]
LPPI	72.92[c]	5.91[b]	27.40[b]
BBPI and CPPI (50:50)	77.15[ab]	4.07[c]	25.88[b]
BBPI and LPPI (50:50)	77.27[a]	4.54[c]	25.69[b]
CPPI and LPPI (50:50)	75.93[b]	4.59[c]	25.85[b]
SE	0.43	0.25	0.84

SE standard error, *CPPI* chickpea protein isolate, *BBPI* broad bean protein isolate, *LPPI* lupin protein isolate

** Means with different letters in the same column are significantly different at $P \leq 0.05$; Means are average of two replicates

lightness (L*) was obtained for mayonnaise prepared from mixture of BBPI and LPPI with value of 77.27. The value of lightness L* for mayonnaise prepared from BBPI did not differ to mayonnaise prepared from mixture of the BBPI and CPPI or the BBPI and LPPI mixture or the mixture of CPPI and LPPI. The lowest lightness (L*) value was found for mayonnaise prepared from LPPI that did not varied significantly to the values of mayonnaise prepared from either CPPI or egg, which ranged from 72.92 to 73.78. It thus appeared that the presence BBPI in mayonnaise enhanced the lightness of mayonnaise whereas LPPI and CPPI decreased the lightness. The maximum value of redness (a*) was found in mayonnaise prepared from egg (7.12) followed by mayonnaise prepared from either of LPPI (5.91) or BBPI (5.15) that were not varied significantly between each other. The lowest value of redness (a*) was found in the mayonnaise prepared from mixture of BBPI and CPPI (4.07) that did not vary significantly with the value of mayonnaise prepared from either the mixture of LPPI and CPPI (4.59) or the mixture containing BBPI together with either LPPI (4.54) or CPPI (4.07). The present findings thus suggested that the preparation of mayonnaise using either the mixture of BBPI and LPPI or the BBPI and CPPI mixture decreased redness as compared to mayonnaise prepared from any of the individual protein isolates or egg. The highest value of yellowness (b*) was found in mayonnaise prepared from egg with a value of 35.53. The values of yellowness (b*) among mayonnaise prepared from individual protein isolates or mixtures of protein isolates containing LPPI, BBPI and CPPI did not differ significantly. Overall, the above findings indicated that mayonnaise prepared from either the BBPI and LPPI mixture or a mixture of BBPI and CPPI can be used for food industry applications due to their high degree of lightness and lower values of redness and yellowness.

Effect of protein isolates of broad bean, chickpea and lupin on the bioactive properties of mayonnaise

Table 6 illustrates the effect of BBPI, CPPI, and LPPI on the potential bioactive properties of mayonnaise imparted by the presence of phenolic compounds in terms of antioxidant activities as well as ACE, α-amylase and α-glucosidase inhibitory activities. The total phenolic content was significantly higher for LPPI mayonnaise in comparison to all the other prepared mayonnaise products. The total phenolic content did not vary significantly among mayonnaises prepared from CPPI, the mixture of BBPI and CPPI or the mixture of BBPI and LPPI (12.04–13.28 mg/g), while significant differences were observed in phenolic content in mayonnaise prepared from BBPI, CPPI and LPPI. The content of phenolic compounds was similar for mayonnaise prepared from BBPI and the mixture of CPPI and LPPI, which corresponded to a range of 14.83–15.45 mg/g. The total phenolic content in lupin varieties has been shown to range from 0.3 to 1.7 mg/g (Siger et al. 2012) whereas the content of phenolic compounds in chickpea flour ranged from 0.98 to 10.8 mg/g (Xu et al. 2007; Sreerama et al. 2012). The antioxidant activity of extracted phenolic compounds from mayonnaise prepared using LPPI was significantly lower than mayonnaises prepared from either BBPI or CPPI. The antioxidant activity of extracted phenolic compounds from mayonnaise prepared using BBPI did not differ from mayonnaise prepared using CPPI. The antioxidant activity of extracted phenolic compounds from mayonnaise prepared using mixture of CPPI and LPPI was significantly lower as compared to mayonnaise prepared from any of the individual protein isolates as well as the mixture of BBPI and LPPI or the mixture of CPPI and BBPI. The value of antioxidant activity of extracted phenolic compounds from mayonnaise prepared using mixture of BBPI and LPPI was similar to that observed from the mayonnaise prepared from CPPI. The ACE inhibitory activities values were significantly higher in mayonnaise prepared from BBPI or the mixtures containing BBPI with CPPI in comparison to all other tested mayonnaise products. The ACE inhibitory activities for extracted phenolic compounds from mayonnaise prepared from LPPI (13%) did not vary from mayonnaise prepared from the mixture of CPPI and LPPI (14.64%) but were significantly higher than the mayonnaise containing both BPPI and LPPI. The lowest ACE inhibitory activity was associated with the LPPI mayonnaise. In terms of the α-amylase inhibitory activities, mayonnaise prepared with BBPI and CPPI showed significantly higher activity than all other prepared mayonnaise products. The CPPI mayonnaise demonstrated higher α-amylase inhibitory activity in comparison to all other mayonnaises apart from the BBPI and CPPI mayonnaise.

Table 6 Total content, antioxidant activity, ACE inhibitory activity, α-amylase inhibitory activity and α-glucosidase inhibitory activity of phenolic compounds extracted from mayonnaise prepared from protein isolates of broad bean, chickpea and lupin

Treatment	Total phenolics (mg/g)**	Antioxidant activity (%)**	ACE inhibitory activity (%)**	α-amylase inhibitory activity (%)**	α-glucosidase inhibitory activity (%)**
BBPI	14.83[b*]	88.32[a]	18.97[a]	41.60[d]	35.07[b]
CPPI	13.28[c]	86.35[ab]	7.03[d]	56.97[b]	55.97[a]
LPPI	17.52[a]	72.77[e]	13.00[b]	43.14[d]	26.11[c]
BBPI and CPPI (50:50)	12.04[c]	82.30[c]	19.17[a]	75.41[a]	9.70[d]
BBPI and LPPI (50:50)	12.04[c]	83.70[bc]	9.24[c]	49.28[c]	6.71[d]
CPPI and LPPI (50:50)	15.45[b]	75.85[d]	14.64[b]	50.82[c]	9.70[d]
SE	0.35	0.76	0.67	1.58	2.43

SE standard error, *CPPI* chickpea protein isolate, *BBPI* broad bean protein isolate, *LPPI* lupin protein isolate

* Column values with the same letters were not significant different ($P < 0.05$)

** Means are average of two replicates

The α-amylase inhibitory activity of extracted phenolic compounds in mayonnaise prepared from mixture of BBPI and LPPI did not vary in comparison to the prepared mayonnaise from the mixture of CPPI and LPPI. The LPPI mayonnaise did not differ from mayonnaise prepared with BBPI, which were the two mayonnaises showing the lowest α-amylase inhibitory activity. The inhibitory activity of α-glucosidase was highest for the CPPI mayonnaise. The next highest inhibitory activity of α-glucosidase was associated with the BBPI mayonnaise, which was higher than the other mayonnaises except for the CPPI mayonnaise. The mayonnaise with LPPI had significantly higher α-glucosidase inhibitory activity than the mayonnaises prepared from mixtures of BBPI and CPPI, BBPI and LPPI or CPPI and LPPI. Previous work has shown that α-amylase and α-glucosidase inhibitory activities are shown to typically increase with an increase in the content of phenolic compounds of chickpeas with IC50 values of antioxidant, α-amylase and α-glucosidase inhibitory activities of 108.3, 92.2 and 61.3 μg/ml, respectively (Sreerama et al. 2012). The present results thereby indicate that the mayonnaise prepared either from BBPI or CPPI could be recommended as possible health promoting ingredients for mayonnaise in terms of their antioxidant, antihypertensive and antidiabetic properties.

Conclusion

The findings from the present study indicate that protein isolates from broad bean have higher protein content and yield as well as superior functional properties as compared to protein isolates from lupin and chickpea flour. The preparation of mayonnaise from either BBPI or the mixture of BBPI and CPPI could be recommended due to superior values of lightness and redness associated with this mixture in addition to possible antioxidant, antihypertensive and antidiabetic properties. Future studies are needed to study the effect of mayonnaise prepared from the various legume protein isolates in terms of their essential fatty acid and amino acid content as well as their sensory attributes and consumer acceptability. In vivo feeding trials would also be needed to evaluate the potential health attributes of mayonnaise prepared from the protein isolates in terms of their bifunctional properties in order to support the findings of the present in vitro studies.

Acknowledgements The authors would like to thank the Deanship of Scientific Research at the Jordan University of Science and Technology (JUST) for their financial support.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors. All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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