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Chickpea Protein Isolation, Characterization and Application in Muffin Enrichment

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Abstract

The aim of this study was to enhance the nutritional value and the functional characteristics of muffins by enriching with chickpea protein isolate, while keeping their rheological characteristics. Chickpea Protein isolate (CPI) was prepared by alkaline solubilization (pH 11), followed by isoelectric precipitation at pH 4.5. SDS-PAGE revealed three subunits with molecular weights of 47, 30 and 85 kDa; representing the globulin fractions, legumin-like and vicilin-like proteins. Maximum protein solubility (83.32%) was obtained at pH 11. CPI exhibited an emulsifying activity index of 25.17 m^2 g^{-1}, emulsion stability index of 14.09 min. The foaming capacity and stability were 62% and 94.49%, respectively. Water and oil absorption were 3.65 and 2.30 mL g^{-1}, respectively. CPI was added to muffin batter at 0, 2.5, 5, 7.5 and 10%. CPI fortified muffins showed reduction in moisture content, which influenced texture profile analysis through increasing hardness, gumminess and chewiness values. Additionally, both protein content and protein digestibility of muffins increased to 22.2 and 94.08%, respectively. CPI-enriched muffins were darker (lower L) with yellowish crumbs (higher b). Finally, preliminary sensory evaluation showed high consumer acceptance for CPI-enriched muffins.

Keywords: Chickpea proteins isolate; Enriched muffins; *In-Vitro* Protein digestibility; Colour analysis; Texture Profile Analysis; Sensory evaluation

1 Introduction

Consumers throughout the world enjoy baked food products, especially muffins, due to their organoleptic characteristics (Gao, Brennan, Mason & Brennan, 2016, 2017). Their high-level consumption makes them useful as potential carriers of bioactive compounds. Recently, consumers appreciate improvements in product flavor but they do not neglect their health benefits (Valmorida & Castillo-Israel, 2018; Wardy et al., 2018). Chickpeas (*Cicer arietinum* L.) are an oldworld pulse and were first grown in the Levant and ancient Egypt. They have a nutlike flavor and are used to complement grains (such as whole grains); to form a complete protein. The protein

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quality of legumes such as chickpeas are significantly improved by heat treatment since heat destroys and/or inactivates anti-nutritional factors. Therefore, this might be important for vegans, individuals adhering to variations of plant based diets or low socio-economic individuals (O'Neil, Nicklas & Fulgoni III, 2014; Wallace, Murray & Zelman, 2016). Like other legumes, chickpea's albumins and globulins represent the two major protein fractions. The albumin fraction constitutes up to 15-25% whereas the globulins, represented mainly by vicinin and legumin, reach up to 60-80% of the extractable proteins. Albumins display a higher nutritive value due to their high content of lysine and sulfur amino acids. Chickpea proteins are appreciated due to their high biological value, well balanced amino acid content and low content of anti-nutritional factors. However, there have been concerns about chickpea protein isolates due to their low fat content among other reasons (Aloweidat, 2014; Carbonaro, Cappelloni, Nicoli, Lucarini & Carnovale, 1997). Earlier researchers have investigated the physicochemical properties of chickpea protein isolates and their use in food enrichment as a dietetic alternative for individuals with special caloric or metabolic requirements (Aguilar & Vélez-Ruiz, 2016). Herein, this study aims to enrich muffins with chickpea isolate proteins to potentially increase their nutritional and functional qualities while preserving rheological characteristics.

2 Materials and Methods

2.1 Raw materials

Organic chickpea flour (*Cicer arietinum* L.) (aka Besan flour, ACO, Australia); 25% protein, 4.5% fat and 9.7% moisture. The ingredients used for muffin preparation are: wheat flour (Chantal, NZ); 10.5% protein, 1.4% fat and 70.1% carbohydrates, skimmed milk powder (Go Milk, NZ); 8.3% protein, 0.3 % fat, 13.8% carbohydrates, 125mg sodium and 300 mg calcium, margarine from (Anchor, NZ); protein <1.0 g, fat 2g, carbohydrates <1g and sodium 24 mg, sugar (Chelsea White sugar NZ), baking powder (Edmonds, NZ), and salt (Essentials, Australia). All ingredients were obtained from local markets located in Christchurch, New Zealand.

2.2 Preparation of defatted chickpea flour

Defatting of chickpea flour was carried out according to Folch, Lees and Stanley (1957) with some modifications. The chickpea samples were homogenized in chloroform/methanol (v/v; 2/1); the final volume was 20 fold the sample volume (1 g in 20 ml of solvent mixture). After dispersion, the whole mixture was agitated for 60 min in an orbital shaker at room temperature. Then, the homogenate was filtered with a folded filter paper to recover the liquid phase. Finally, the chickpea flour was dried at 43^{o}C/ 36 h in a hot air flow.

2.3 Preparation of chickpea protein isolate

The chickpea protein isolate was prepared according to Chang, Alli, Konishi and Ziomek (2011), El-Sohaimy, Sitohy and El-Masry (2007). Fifty grams of defatted chickpea flour were suspended in 1000 mL deionized water (1:20, w/v), the pH values varied in the range of 3.0 to 12.0 using 0.1 N NaOH and 0.1 N HCl. The suspensions at different pH values were stirred for 1 h to assess optimum solubility. The soluble isolate fractions (at the desired pH) were centrifuged at 6,000 x g for 30 min 20^{o}C. The supernatant was collected and acidified to pH values ranging from 1 to 6 to facilitate protein precipitation and determine the isoelectric point. Precipitates were then centrifuged at 10,000 x g for 45 min at 4^{o}C. The precipitated protein fractions were collected, neutralized and freeze dried. The total protein content in the isolates was determined by Kjeldahl method.

2.4 Characterization of chickpea protein isolate

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The protein isolate was dissolved in aqueous solution at six different concentrations (0.015, 0.03, 0.062, 0.125, 0.5 and 1 mg mL^{-1}) and applied to the gel for better resolution of the bands. SDS-PAGE was carried out by the technique reported by Laemmli (1970); using 4% stacking gel and 12% separating gel. Sample solutions (20 μL) were prepared by dissolving 10 mg of freeze dried protein extract in 1 mL sample buffer [distilled water, 0.5 M Tri HCl (pH 6.8), glycerol, SDS (10%), bromophenol blue (1%) and β-mercaptoethanol]. The samples were heated at 98 ^{o}C for 10 min, then applied to the sample wells. The standard protein marker (260, 160, 110, 80, 60, 50, 40, 30, 20, 15, 10 and 3.5 KDa) (Bio-Rad Hercules, USA) was used for molecular weight estimation. Electrophoretic migration was monitored at constant current (14 mA/gel) for 1.5 to 2 h. Gels were fixed with a fixing solution [water/methanol/acetic acid, 700: 200: 100 mL] for 30 min and then stained with commassie brilliant blue R-250 for 1 h. The stained gels were destained by frequent change of the fixing solution.

Protein solubility

Chickpea protein isolate solubility (5% suspension) was determined at pH values ranging from 1.0 to 12.0 according to Klompong, Benjakul, Kantachote and Shahidi (2007). For better solubilization, the suspensions were stirred at room temperature for 1 h, using a magnetic stirrer. The pH values were adjusted using HCl (0.1 N) and NaOH (0.1 N). The suspensions at different pH values were centrifuged at 6,000 x g for 30 min. The total protein was determined in the supernatants by Kjeldahl method. Protein solubility (PS) was calculated using the following equation. Samples were tested in triplicate.

$$PS(\%) = \left(\frac{Protein\ content\ in\ supernatant}{Total\ protein\ content\ in\ sample}\right) \times 100 \quad (1)$$

A protein solubility curve was constructed by using the average of soluble protein percentage values calculated at each pH value.

Functional properties of protein isolate

Emulsifying activity index (EAI) and emulsion stability index (ESI)

EAI and ESI were measured using the method of Pearce and Kinsella (1978) with some modifications. Fifteen mL of 1% neutralized protein solution was mixed with 5 ml of commercial sunflower oil. The mixture was homogenized at 7,500 rpm for 1 min, using homogenizer (MZIP Model 114, China). Then, 50 μL aliquots were taken from emulsions at 0 and 10 min from the bottom of the tube and mixed with 10 mL of 0.1% sodium dodecyl sulphate (SDS) (1:200 dilution). The absorbance of the diluted solutions was measured at 500 nm immediately after emulsion formation (A_0) and at 10 min (A_{10}). EAI and ESI were calculated using the following equations:

$$EAI\frac{m^2}{g} = \frac{2T \times F \times A_0}{C \times \theta \times 10,000} \quad (2)$$

$$ESI = A_0 \times \frac{\Delta t}{\Delta A} \quad (3)$$

$$\Delta A = A_0 - A_{10} \text{ and } \Delta t = 10min \quad (4)$$

Where: T= 2.303; F: dilution factor (200); A_0: absorbance measured at 500 nm immediately after emulsion form ation; A_t: absorbance measured at 500 nm after 10 min of emulsion formation c: protein concentration (0.01 g/mL) and θ: dispersed phase (oil) volume fraction (15).

Foaming capacity (FC) and foaming stability (FS)

FC and FS were assessed according to the method described by Tsutsui (1988) with some modifications. The protein solution was agitated in a blender at high speed (Breville, platinum, China) for 5 min and then transferred into graduated cylinders. Foam capacity was calculated according to following equation:

$$FC(\%) = \left(\frac{V_{after\ agitation} - V_{prior\ agitation}}{V_{prior\ agitation}}\right) \times 100 \quad (5)$$

Similarly, FS value was determined, however samples were allowed to stand at room temperature for 30 min and the residual foam volume ($V_{Residual\ foam}$) was calculated according to the following equation:

$$FS(\%) = \left(\frac{V_{Residual\ foam}}{V_{Total\ foam}}\right) \times 100 \qquad (6)$$

Water and oil absorption

The water/oil absorption capacity of chickpea protein isolate was determined by the method of Chandra and Samsher (2013). One gram of the isolate was mixed with 10 mL of distilled water/sunflower oil (specific gravity: 0.88) and allowed to stand at ambient temperature (30± 2^{o}C) for 30 min, then was centrifuged at 3,500 x g for 30 min. Water/oil absorption (WOA) in mg/l was calculated according to the equation:

$$WOA = V_{water\ (oil)\ Initial} - V_{supernatant} \qquad (7)$$

2.5 Batter and muffin preparation

Four muffin batter formulations were prepared by replacing a percent of the wheat flour with chickpea protein isolate (CPI) according to Rahman, Hiregoudar, Veeranagouda, Ramachandra et al. (2015) with some modifications. The samples were identified as [control, M1 (CPI 2.5), M2 (CPI 5), M3 (CPI 7.5) and M4 (CPI 10)]. The recipes used for different muffin preparations were exhibited in (w/w %) as shown in table 1. The ingredients were weighed using a kern 572 balance (Scout™ Pro SP602, OHAUS Corporations, USA). Egg and margarine were mixed in a laboratory scale kitchen mixer (Kitchen aid, St. Joseph, USA) at speed 4 then speed 8 for 10 and 50 s, respectively. Flour, sugar, salt, milk powder and water were mixed at speed 2 for 10 s, then speed 8 for 50 s. Forty-five grams of batter was filled into paper cups in a muffin pan and baked in the oven (MIWE condo) at 180^{o}C for 20 min. Baked muffins were left to cool at room temperature for 1 h in order to avoid moisture condensation on their undersurface, and finally packed in polypropylene bags and stored in a dry environment prior to analysis.

2.6 Muffin characteristics

Muffin properties (moisture content, height, specific volume and colour) were assessed following (Rahman, Hiregoudar, Veeranagouda, Ramachandra et al., 2015) procedures. Moisture content of different muffin recipes was determined according to AOAC (1990). Height was measured with a digital caliper, from the bottom to the highest point of the muffin. Specific volume was determined by milletseed displacement method and was expressed as specific volume (cm^3 g^{-1}). Colour parameters were determined for muffin crust and crumb via Ultrascan VIS Hunter Lab (MiniScan XE Plus, Model 45/0-L, Hunter Associates Inc, Reston, VA, USA). Values were expressed by Hunter (L, a, and b) values which correspond, respectively, to: value of lightness (0-100 representing dark to light), value of redness and greenness degree (higher positive indicating more red) and value of yellowness and blueness degree (higher value indicating more yellow).

2.7 Protein content and *In-Vitro* protein digestibility of muffins

Total protein content of enriched muffins was determined by Kjeldahl method as described in AOAC (1990). One g of muffin sample was placed into a digestion flask, along with 15 mL of concentrated sulfuric acid (H_2SO_4). Additionally, seven grams of potassium sulfate and a catalyst, usually copper, were added to the flask. The mixture was transferred to a digestion tube and boiled at 400^{o}C using a heating block until formation of white fumes, then heating was continued for about 60-90 min. The tube was cooled then water (250 mL) was cautiously added. The pH of the mixture was raised using sodium hydroxide (45% NaOH solution); this converts the ammonium (NH_4^+) ions, which are dissolved in the liquid, to the ammonia gas (NH_3). The nitrogen has been separating away from the digestion mixture by distilling the ammonia (converting it to a volatile gas, by raising the temperature to boiling point). The distilled vapor has been trapped in a special trapping solution of about 15 mL HCl in 70 mL of water, and then the trapping flask

Table 1: Chickpea protein enriched muffin batter recipes (CPI, Chickpea Protein Isolate)

Ingredients (w/w)%	Control	M1 (CPI 2.5)	M2 (CPI 5)	M3 (CPI 7)	M4 (CPI 10)
Wheat flour	32.94	32.12	31.29	30.47	29.65
Chickpea protein isolate	0.00	0.82	1.65	2.47	3.29
White sugar	19.70	19.70	19.70	19.70	19.70
Salt	0.40	0.40	0.40	0.40	0.40
Baking powder	1.66	1.66	1.66	1.66	1.66
Fat (margarine)	16.46	16.46	16.46	16.46	16.46
Skimmed milk powder	2.49	2.49	2.49	2.49	2.49
Liquid whole eggs	9.89	9.89	9.89	9.89	9.89
Water	16.46	16.46	16.46	16.46	16.46

was removed. As the ammonia dissolves in the acid trapping solution, it neutralizes some of the present HCl. The excess HCl was then back titrated with a standard NaOH. The indicator dye was added to the acid/ammonia trapping solution. In this way the amount of ammonia distilled off from the digestive solution could be calculated; this amount corresponds to the nitrogen content of the protein. The volume of sodium hydroxide solution was noted, and the nitrogen was calculated by the following equation:

$$\%N = \frac{(ml\ standard\ acid\ -\ ml\ blank) \times N\ of\ acid \times 1.4007}{weight\ of\ sample\ in\ grams} \quad (8)$$

$$\%P = \%NX5.7 \quad (9)$$

In-vitro protein digestibility was carried out for CPI-enriched muffins by the multienzyme method of Bodwell, Satterlee and Hackler (1980), Carbonaro et al. (1997). Porcine pancreatic trypsin (type IX, 15 310 units/mg protein), bovine pancreatic chymotrypsin (type II, 48 units/mg of solid), porcine intestinal peptidase (P-7500, 115 units/g of solid) and bacterial protease (type XIV, 4.4 units /mg of solid) (Sigma-Aldrich, Germany) were employed for the enzymatic digestion. In-vitro protein digestibility was calculated according to the equation:

$$Y = 234.84 - 22.56X \quad (10)$$

Where, Y is the *In-vitro* digestibility of protein (%) and X is the pH of the suspension after 20 min of digestion.

2.8 Texture Profile Analysis (TPA) of muffins

The texture profile analysis was carried out using texture analyzer (TA/TX-plus texture analyzer; Texture analyzer, Stable Micro system, Surrey, UK) equipped with a 5-kg load cell. Exponent software was used for testing procedures, presentation formats and data analysis to provide the most powerful and flexible testing analysis solution available. The muffin samples were placed at the center of a heavy-duty platform (HD P/90), and subjected to compression (50%) using a 75 mm diameter flat aluminum probe (P/75) at test speed of 1 mm/s. Firmness is the maximum peak force during the first compression cycle. Springiness (the height that the muffin sample recovered during the time elapsed between the end of the first compression and the start of the second one) and cohesiveness (the ratio of the peak area during the second compression to the one during the first compression) were calculated from the force time curve (Bourne, 2002).

2.9 Sensory evaluation

Ten well trained panelists from staff members of Food Technology Department, City of Scientific Research, and Technological Applications, Alexandria, Egypt carried out preliminary sensory evaluation of enriched muffins. Samples were randomly assigned to each panelist. The panelists were asked to evaluate each sample: shape, mouth feel, flavour, crumb texture, crumb col-

our, crust colour and crust texture, through a nine point hedonic scale according to (Ihekoronye & Ngoddy, 1985). The ratings are: Dislike extremely (1), Dislike very much (2), Dislike moderately (3), Dislike slightly (4), Neither like nor dislike (5) Like slightly (6) Like moderately (7), Like very much (8) and Like extremely (9).

2.10 Statistical analysis

Statistical factorial analysis was performed using analytical software SPSS® 13.0 (Statistical Package for the Social Sciences, 2005). Differences were considered significant at $P < 0.05$.

3 Results and Discussion

3.1 Chickpea protein isolate average yield

Protein isolates from chickpea defatted flour were prepared in two steps: the first was the solubilization and extraction of protein from chickpea flour at alkaline pH. The optimum pH for the extraction of maximum amount of protein was 11; maximum chickpea protein extraction rate was 80 %. The protein was precipitated at isoelectric point (pH 4.5), which recovered maximum soluble protein (78%), as described by El-Sohaimy et al. (2007). Extraction of chickpea protein reached a recovery percent of 82.94% yield.

3.2 Characterization of chickpea protein isolate

Protein profile

Figure 1 illustrates the SDS-PAGE profiles of six different concentrations of chickpea protein isolate (0.015, 0.03, 0.062, 0.125, 0.5 and 1 mg ml^{-1}). SDS-PAGE gel analysis revealed that the chickpea protein profiles were composed mainly of three bands; the major protein subunit (MW 47 kDa), followed by a 30 KDa protein and finally an 85 kDa protein. Similar observation was reported by Papalamprou, Doxastakis and Kiosseoglou (2010), who stated that these protein constituents belong to the globulin fractions, legumin like and vicilin like proteins. Different concentrations aid the confirmation of band positions with increased intensity at higher concentrations.

Proteins solubility

The solubility of isolated proteins at different pH values is presented in figure 2. A sharp minimum solubility (24.92 and 28.65 %) was observed at acidic pH values (4 and 5). On the other hand, the protein isolate showed the highest protein solubility at pH 11 (83.32 %). This observation illustrates that the major isolated chickpea proteins are acidic and are soluble in alkaline medium. The protein solubility profile is similar to those reported for several legume proteins in earlier studies (Carbonaro et al., 1997; Liu, Hung & Bennett, 2008). These results suggest that alkaline medium, pH 11, is the optimum pH for solubilization of most chickpea proteins.

3.3 Functional properties of protein isolate

Functionality is any property of a food ingredient, except its nutritional values, that has a great impact on its utilization. Chickpea protein isolate functional properties are presented in table 2.

Emulsifying activity index (EAI) and emulsion stability index (ESI)

EAI and ESI were determined for the chickpea protein isolate to support its applications in food industry. Table 2 shows the EAI and ESI of chickpea protein isolate. Chickpea protein isolate exhibited EAI and ESI values of 25.17±0.07 m^2/ g^{-1} and 14.09±0.40 min, respectively. Several studies have reported the emulsifying properties of chickpea protein isolate (Alvarez, Cuesta, Herranz & Canet, 2017; Ladjal-Ettoumi, Boudries, Chibane & Romero, 2016). To form emulsions, proteins migrate to the oil-water interface and re-align to allow positioning of hydrophobic groups towards the oil phase and hydrophilic groups towards the aqueous phase. Reducing interfacial tension between oil and water phases enables emulsion

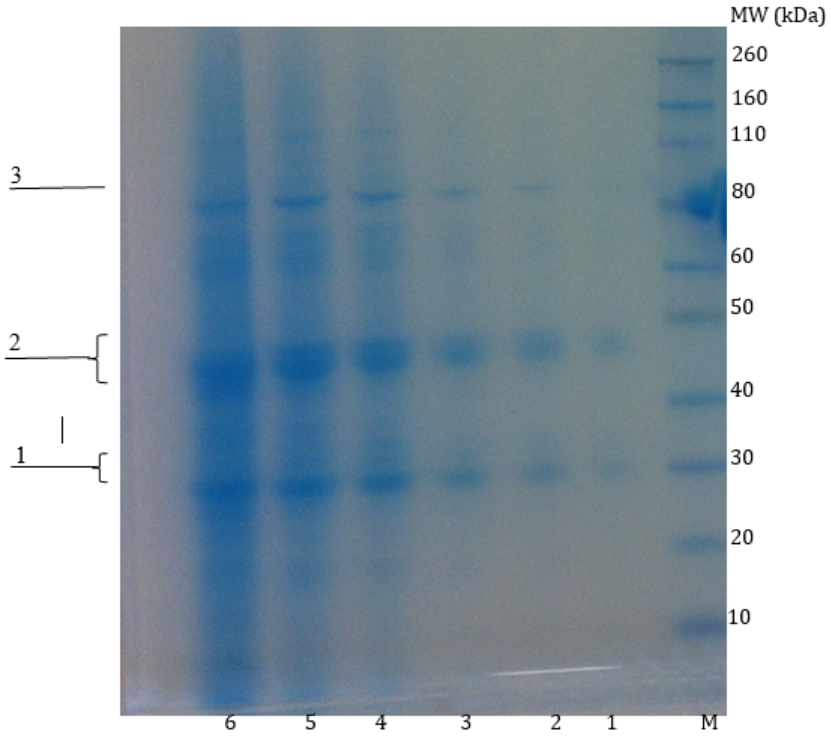

Figure 1: SDS-PAGE for Chickpea Protein Isolate Lane M: protein marker; Lane 1: 0.015 mg ml^{-1}; lane 2: 0.03 mg mL^{-1}; lane 3: 0.062 mg mL^{-1}; lane 4: 0.125 mg mL^{-1}; lane 5: 0.5 mg $mL^{-1}$and lane 6: 1mg mL^{-1}.

Table 2: Chickpea protein isolate functional properties

Parameter	**Unit**	**Value**
Emulsifying activity index (EAI)	m^2g^{-1}	25.17±0.07
Emulsion stability index (ESI)	min	14.09±0.40
Foaming capacity (FC)	%	62.00±2.83
Foaming stability (FS)	%	94.49±1.67
Water absorption	mlg^{-1}	3.65±0.07
Oil absorption	mlg^{-1}	2.30±0.14

Data presented as mean±SD (samples were run in duplicate)

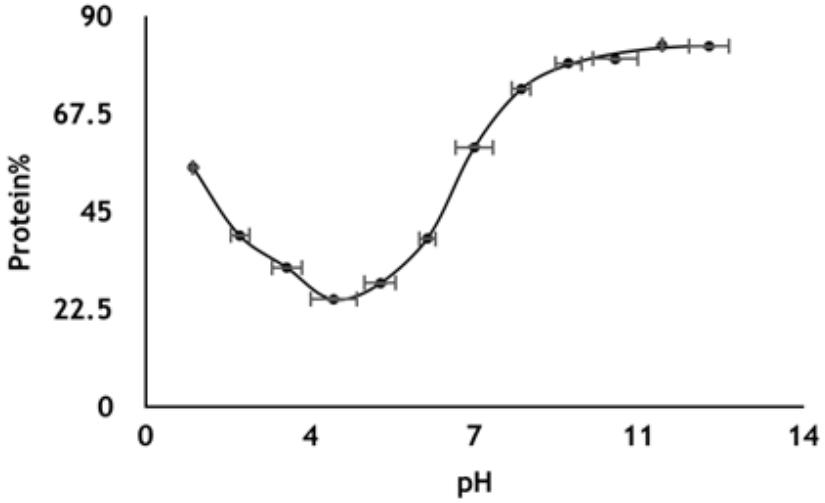

Figure 2: Chickpea protein isolate solubility profile. Data presented as the mean±SD value (samples were run in triplicate)

droplets to form, which subsequently leads to higher emulsion stability (Johnston, Nickerson & Low, 2015).

Foaming properties (Foaming capacity and foaming stability)

Foam capacity and foam stability of chickpea protein isolate are shown in table 2. The protein isolate showed foam capacity of 62.00±2.83 %, which might be related to the presence of globulin fractions, which can encapsulate and retain air. Therefore, rapid migration, unfolding and rearranging of the air-water interface are necessary to exhibit good foam capacity (Alleoni, 2006). On the other hand, chickpea showed relatively high foaming stability (94.49±1.67 %), based on air retaining, which could support its recommendation in food industries, such as bakery products and ice creams. Similar foaming properties of chickpea proteins have been reported earlier by Boye et al. (2010).

Water and oil absorption

The water/oil absorption properties of chickpea protein isolate are shown in table 2. The isolated protein showed water and oil absorption of 3.65±0.07 and 2.30±0.14 mL/g respectively. These values are similar to those previously reported by Aloweidat (2014), who also reported that oil absorption capacity of protein is partially related to the physical confinement of oil by means of the protein matrix. Therefore, the source of the protein might be important.

3.4 Characteristics of muffins enriched with chickpea protein

The moisture content, colour, height and volume properties of baked CPI-enriched muffins are illustrated in tables 3 and 4. Enrichment of muffins with chickpea protein caused a reduction in **moisture content** of fortified muffins compared to control, and this reduction correlates with the increase in CPI concentration. However, this decrease was not significant in the first two blends (CPI 2.5 and CPI 5.0%). Rahman,

Table 3: Properties of chickpea protein enriched muffins

Sample	Moisture (%)	Height (mm)	SP. Volume (cm^3g^{-1})
Control	23.05±2.05^a	42.11±1.80^c	92.00±4.00^a
M1 (PI 2.5)	22.95±1.03^a	43.42±1.69^a	88.00±2.00^c
M2 (PI 5)	22.75±0.62^a	42.75±0.51^b	90.00±3.06^b
M3 (PI 7.5)	21.83±1.28^b	43.08±0.25^{ab}	92.00±2.00^a
M4 (PI 10)	21.41±1.02^c	40.11±0.64^d	33.00±1.16^a

Data presented as mean±SD (samples were run in triplicate), Mean in the same column followed by different superscript letters are significantly different (p>0.05)

Table 4: Colour properties of chickpea protein enriched muffins

Sample	L		A		b	
	Crust	Crumb	Crust	Crumb	Crust	Crumb
Control	64.03±0.85^a	80.24±1.59^b	16.92±0.75^b	3.58±0.17^c	45.24±0.87^a	29.90±0.56^e
M1 (CPI 2.5)	62.23±2.13^c	80.15±1.41^b	17.65±0.81^a	3.94±0.50bc	44.51±1.02^b	31.06±0.94^d
M2 (CPI 5)	63.12±1.76^b	80.74±2.24^a	16.07±2.65^c	4.11±0.08^b	43.88±1.31^c	32.83±0.42^c
M3 (CPI 7.5)	55.03±3.15^e	78.51±1.09^c	16.41±0.49^c	4.85±0.06^{bc}	41.07±2.09^e	33.68±0.70^b
M4 (CPI 10)	56.95±2.42^d	77.84±0.76^d	16.79±0.63^b	4.87±0.20^a	42.54±2.12^d	34.82±0.43^a

Data presented as mean±SD (samples were run in duplicate), Means in the same column followed by different superscript letters are significantly different (p>0.05) CPI= Chickpea protein isolate
Colour parameters by Ultrascan VIS Hunter Lab (MiniScan XE Plus, Model 45/0-L, Hunter Associates Inc, Reston, VA, USA). *L, a,* and *b* represent: value of the lightness (0-100 representing dark to light), value of redness and greenness degree (higher positive indicating more red) and value of yellowness and bluenessdegree (higher value indicating more yellow) respectively.

Hiregoudar, Veeranagouda, C T et al. (2015) reported similar behavior with increasing levels of enrichment in muffin batter. There was no significant variation in either the height or the specific volumes of the four enrichment treatments in comparison with the control, except for M1 (CPI 2.5%) which showed a decrease in **specific volume** with a value close to control. These results could help to improve muffin quality (height and volume) like control muffins that consumers are familiar with. This emphasizes that the enrichment of muffins with chickpea protein didn't negatively affect the physical properties of the product. Colour characteristics of fortified muffins are presented in table 4. The colour properties were more pronounced in the crust compared to the crumb for all parameters and all combinations. Crusts tended to be darker (lower L values; 56.95±2.4), more reddish (higher values; 16.79±0.63) and yellowish (lower b values; 42.54±2.12) than crumbs. L (Lightness) results illustrate a significant impact of protein enrichment on the muffins. This is because the interaction between the ingredients during baking, possibly due to increasing of Millard browning reactions concurrent with higher protein enrichment percent, subsequently resulted in darker muffins in both crust and crumb. Similar observations have been recorded by Bhaduri (2013). Comparing the redness degree to control, the CPI enrichment did not cause high variations in crumb and crust despite their significance. The yellow colour of CPI affected the crumb colour, b values in the muffins increased with higher substi-

tutions of CPI, however, b values decreased for the crust. This could be attributed to Millard reactions that might cause consumption of the protein amino acids and reducing sugars to produce a brown colour that diverts the tendency for lightness (to become darker) rather than yellowish. Generally, similar patterns were reported by Bhaduri (2013), (Rahman, Hiregoudar, Veeranagouda, C T et al., 2015) and (Wardy et al., 2018).

3.5 Protein content and *In-Vitro* Protein Digestibility

Figure 3 and 4 exhibits the protein content and *in vitro* protein digestibility of CPI-enriched muffins. Figure (3) shows a significant increase in protein content of enriched muffins compared to control, as a function of CPI concentration. The increasing of protein content in enriched muffin results, consequently, in increasing its nutritional quality, due to the quality of chickpea protein, compared to the control muffins (89.47%).
The protein digestibility (%) of chickpea protein in muffins is represented in figure (4). It is directly proportional to the enrichment percentage of protein isolate with scores of 91.44, 92.97, 93.40 and 94.08% for M1, M2, M3 and M4 respectively. Unlike other legume proteins, chickpea protein has been shown to have improved digestibility upon heating, which can be mainly ascribed to protein denaturation and inactivation of protease inhibitors (Carbonaro et al., 1997).

3.6 Texture Profile Analysis (TPA) of muffins

Table 5 illustrates the Texture Profile Analysis (TPA) of CPI-enriched muffins showing: hardness, cohesiveness, springiness, gumminess and chewiness parameters. Hardness, springiness and cohesiveness are crucial textural parameters for consumers (Shevkani & Singh, 2014). Chickpea protein isolate enrichment resulted in a significant increase in hardness, gumminess and chewiness of produced muffins as a function of increasing levels of protein isolate substitution. These results could be referred to increased protein that starts crosslinking during batter preparation. The reason for that is mixed networks which form with proteins during baking based on hydrophobic interactions and S-S bonds, which determine volume and texture (Deleu, Wilderjans, Van Haesendonck, Brijs & Delcour, 2016; Deleu, Wilderjans, Vanhaesendonck, Brijs & Delcour, 2017). Moreover, different types of proteins can impact each other's network formation (Lambrecht, Rombouts, Nivelle & Delcour, 2017).

3.7 Sensory evaluation

The mean sensory score of chickpea protein fortified muffins is illustrated in table 6. Muffin shape had a high acceptability score with up to 10% of protein isolate (similar to the shape of control, 100% wheat flour). The mouth feel of the product showed significant differences among control and all blends, with sensory score gradually decreasing with higher CPI inclusion; the score in control was (like very much) while in 10 % CPI was (like moderately). The low score at high level of protein isolate in muffins might be due to the higher value of gumminess and chewiness of protein isolate compared with wheat flour. The muffin samples (2.50, 7.50 and 10 % of protein isolate) showed a higher score in flavour, which resulted in good acceptance (like very much) for panelists. All blends of protein isolate had a score of 8.50 to 7.50, in accordance with sensorial acceptance (like moderately). The supplemented muffins had a yellow reddish crust colour. It is clear from data, that crust colour of fortified muffins in the ratio range (2.5% - 10% CPI) had almost the same score (like very much), meaning that fortification of muffins with chickpea protein isolate didn't negatively affect the crust colour. The same trend in crust colour was obtained for crumb colour of fortified muffins. No significant differences among control and all blends; sensory score was the same (Like very much). The results presented in table 6 show the texture of crust and crumb, there are no significant differences among control and all blends from 2.5% to 10% CPI, which means that wheat flour can be substituted with chickpea protein isolates with up to 10 % without any negative effect on

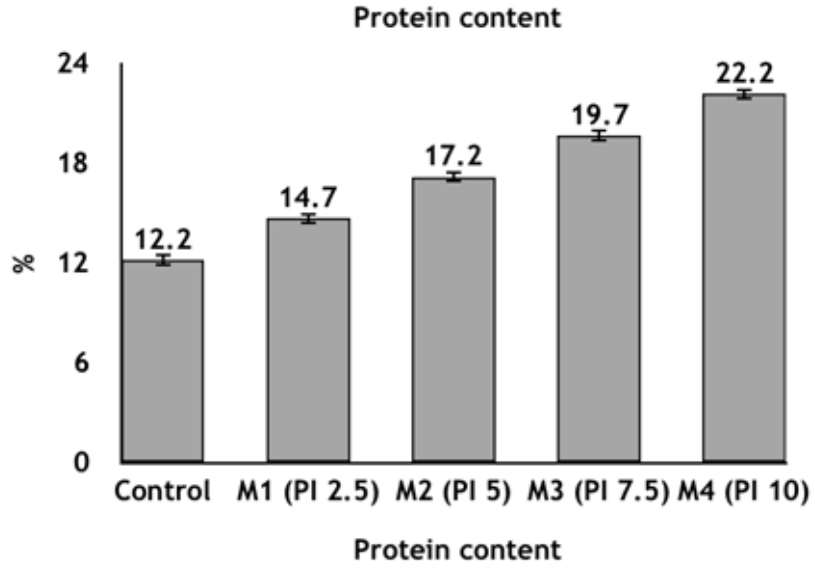

Figure 3: Protein content of enriched muffins. Data presented as mean±SD (samples were run in triplicate), [a,b]Means followed by different superscript letters differ significantly (p>0.05)

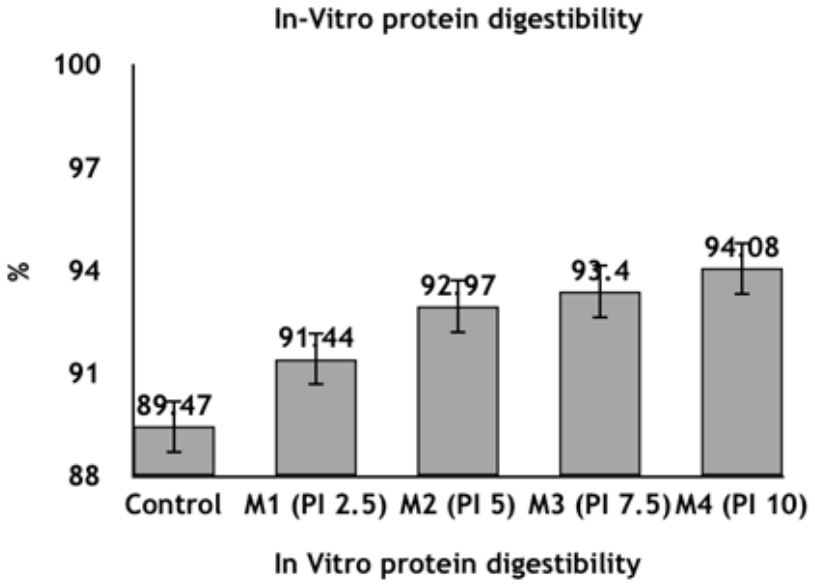

Figure 4: *In Vitro* protein digestibility of enriched muffins. Data presented as mean±SD (samples were run in triplicate), [i,j]Means followed by different superscript letters differ significantly (p>0.05)

Table 5: Texture Profile Analysis (TPA) of chickpea protein enriched muffins

Sample	Hardness (g)	Cohesiveness	Springiness (cm)	Gumminess (N)	Chewiness (g cm)
Control	176.67±19.66^{e}	1.05±0.14^{a}	1.02±0.08^{a}	183.11±15.99^{e}	187.05±20.44^{e}
M1 (CPI 2.5%)	180.00±10.00^{d}	1.07±0.06^{a}	1.05±0.06^{a}	187.73±3.21^{d}	196.16±8.71^{d}
M2 (CPI 5%)	200.00±10.00^{c}	1.05±0.01^{a}	1.00±0.00^{a}	205.00±7.07^{c}	205.00±7.07^{c}
M3 (CPI 7.5%)	213.33±11.55^{b}	0.95±0.06^{a}	1.00±0.00^{a}	210.00±14.14^{b}	210.00±14.14^{b}
M4 (CPI 10%)	296.67±32.15^{a}	1.11±0.11^{a}	1.00±0.00^{a}	315.00±7.07^{a}	315.00±7.07^{a}

Data presented as mean±SD (samples were run in triplicate),
Means in the same column followed by different superscript letters are significantly different (p>0.05)
CPI= Chickpea protein isolate

Table 6: Sensory evaluation of chickpea protein enriched muffins

Organoleptic properties	Control	M1 (2.5% CPI)	M2 (5% CPI)	M3 (7.5% CPI)	M4 (10% CPI)
Shape	8.15^{a}±0.63	8.08^{ab}±0.73	8.00^{b}±0.66	7.80^{ab}±0.84	7.60^{ab}±0.84
Mouth feel	8.40^{a}±0.70	8.20^{ab}±0.63	7.80^{abc}±1.03	7.80^{bc}±0.79	7.50^{bc}±0.52
Flavor	8.50^{a}±0.70	8.60^{a}±0.70	8.30^{ab}±0.73	8.00^{ab}±0.67	7.50^{b}±0.85
Crust colour	7.90^{ab}±0.99	7.70^{ab}±1.16	8.20^{a}±0.73	7.70^{ab}±0.74	7.45^{ab}±0.95
Crust texture	7.80^{a}±0.92	7.70^{a}±0.82	7.90^{a}±0.92	8.30^{a}±0.73	8.07^{a}±0.96
Crumb colour	8.70^{a}±0.53	8.20^{a}±0.79	8.10^{a}±0.87	7.80^{a}±0.63	8.04^{a}±0.66
Crumb texture	8.90^{a}±0.71	8.10^{a}±0.74	7.70^{a}±1.33	8.00^{a}±0.47	8.36^{a}±1.05
Overall acceptance	8.55^{a}±0.49	8.15^{ab}±0.69	7.73^{abc}±0.97	7.79^{abc}±0.76	7.47^{bc}±0.33

Means in the same column followed by different superscript letters are significantly different (p>0.05)
CPI= Chickpea protein isolate

the consumer perception of texture characteristics of muffins. The overall acceptance in control was 8.90±0.49 while in 10% protein isolate was 8.36±0.57. There is no reduction in overall acceptance in all blends with chickpea protein isolate and a high organoleptic score (from like moderately to like very much). The fortification of muffins with up to 10% chickpea protein isolate shows no negative effect on overall acceptance of the final product. These results agree with Herranz, Canet, Jose Jimenez, Fuentes and Dolores Alvarez (2016) who reveled that fortification of muffins with chickpea flour resulted in a chickpea like taste which was not a driver of disliking for the panelists.

4 Conclusion

The present study was designed to increase the nutritional and rheological properties of muffins by adding chickpea protein isolate in different blends (up to 10 %). Chickpea protein profile on SDS-PAGE revealed protein subunits with molecular weights of (47, 30 and 85 kDa) in descending order, which could belong to globulin fractions, legumin-like and vicilin-like protein. Chickpea protein isolate (CPI) showed relatively high emulsifying activity index of 25.17 m^2 g^{-1}, emulsion stability index 14.09 min, foaming capacity 62% and foaming stability 94.49%. Water and oil absorption scored 3.65 and 2.30 mL g^{-1}, respectively. Chickpea protein isolate incorporation resulted in increases in hardness, gumminess and chewiness of baked muffins due to

increasing protein concentration. Sensory evaluation showed consumer acceptance of enriched muffins where they achieved high scores. Since consumers enjoy baked food products; the fortification of muffins with up to 10% chickpea protein isolate has no negative effect on the overall acceptance of the final product. Our results encourage the employment of chickpea protein isolate as a vehicle to produce higher nutritional value products. More research is going on to achieve the maximum replacement of wheat flour with protein isolate and chickpea flour in muffins to develop a high nutritionally valued and glutenfree muffin that does not affect the rheological properties

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