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Functional and rheological properties of flour and protein isolate of different types of legumes (chickpea and soybean)

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ABSTRACT

This study was carried out to investigate the influence of two different types of legumes: chickpea (*Cicer arietinium* L.) and defatted soybean as flour and protein isolates on nutritive value and functional attributes of wheat flour dough in the presence of bacterial maltogenic α-amylase and hydrocolloid Arabic gum. The results showed that both types of legumes flour Chickpea flour (CF) and Soybean flour (SF) contain higher values of protein (19.73% and 48.70%, respectively). Ash content in CF and SF was found to be (2.40 and 7.20%, respectively) and crude fiber values were (1.67%) for CF and (2.15%) for SF. Isolation of protein from these two types of legumes: (CPI) and (SPI) by Isoelectric precipitation led to an increase in protein values (87.96% and 89.84%, respectively) in comparison to that of both CF and SF. Defatted soybean flour was found to be a rich source of Zinc (Zn), Iron (Fe) and Calcium (Ca): 5.338, 28.910 and 458.20 mg/100 g, respectively. Total amino acids were higher in the protein isolates either in the essential amino acid contents or in the non-essential ones compared to that of wheat flour. Glutamic acid increased in CPI and SPI (12.42 % to17.46 %, respectively). Protein solubility (as %) at pH ranged from (8-10 pH) showed to be higher for both types of protein isolates, while foam stability (%) of both protein isolate increased at pH ranged from (4-6). The present study revealed that fortification of wheat flour with (CF), (SF), (CPI) and (SPI) individually or as a mixture will be of great value to improve the nutritional status of the general population and helps those suffering from degenerative diseases associated with today's changing life styles and environment. The study recommended that supplementation with legumes under present study investigation is one way to meet the needs for protein, particularly in baked foods.

Key words: soybean, chickpea, protein isolate, legume flour, quality attributes, amino acid composition, minerals

Introduction

Protein energy malnutrition (PEM) is one of the major threats in developing nations where population is escalating at an alarming rate. Stunting (which occurs due to inadequate nutrition over a long period of time or due to chronic illness), is measured by a height for age index and is the most prevalent form of malnutrition among children under five years of age in Egypt. According to FAO (1994) insufficiency of quality foods and hidden hunger has urged the need to utilize indigenous legumes to cope with the problem.

Legumes have a low-energy density and are nutrient dense, making them a valuable food by which the needs of the undernourished or under-served populations could be met. The majority of legumes contain phytochemicals: bioactive compounds, including enzyme inhibitors, phytohaemagglutinins (lectins), phytoestrogens, oligosaccharides, saponins and phenolic compounds. Dietary intake of phytochemicals through frequent legume consumption may provide health benefits, protecting against several diseases (Bouchenak and Lamri-Senhadji, 2013). As a result of their high nutritional properties, legume consumption has been shown to have beneficial effects on the prevention and management of obesity (Papanikolaou and Fulgoni, 2008) and related disorders, such as coronary heart disease (Bazzano *et al.,* 2001), diabetes (Jenkins *et al.,* 2012) and the metabolic syndrome (Mollard *et al.,* 2012).

Legumes are high in fiber, which contributes to lowering the energy density and reducing the glycaemic response, but they are also a good source of protein. A serving of legumes (1⁄2 cup cooked

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dried legumes) contains 2–4 g of fiber and 7–8 g of protein. Most beans are very low in fat, generally containing <5% of energy as fat, with the exception of chickpeas, lupin seeds and soy beans, which contain <15– 47% fat (Messina, 1999). Legumes contain substantial amounts of the B vitamins as well as the nutritionally important minerals, such as iron, calcium and potassium. In addition to its high content in vitamin and mineral, it considered good source of antioxidant like Isoflavones which plays a role in cholesterol reduction, preventing cancer and regulation of menopause (Serrem *et al.,* 2011).

Soybean is one of the most important oil and protein crops of the world. Soybeans contain 30 to 45% protein with a good source of all indispensable amino acids (Serrem *et al.* 2011). The protein content of soybean is about 2 times of other pulses, 4 times of wheat, 6 times of rice grain, 4 times of egg and 12 times of milk. It is also rich in calcium, phosphorous, isoflavones and Vitamins A, B, C and D, and it has been referred to as "the protein hope of the future" (Islam *et al.* 2007).

Chickpea protein is the world's third largest pulse crop in term of area, grown mostly in West Asia and Mediterranean region. According to Dimitrios *et al.,* (2006) and Zhang *et al.,* (2007) the chickpea seed contain 21.1% protein, 3.1% fat, 53.4% carbohydrate, 11.1% fiber, and 5.9% ash. In Egypt, chickpea dry seeds commonly consumed as whole or decorticated after cooking and processing in different ways.

Supplementation of foods is of current interest because of increasing nutritional awareness among consumers. Supplementation with legumes, cereals and pulses is one way to meet the needs for protein particularly baked foods. Wheat flour can be easily fortified with protein rich flours to provide convenient foods, in order to supplement protein in the diet and nutrition (Rebecca *et al.*, 2016).

A complete protein provides all the essential amino acids (EAAs), namely histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. In 2013, the FAO (2013) recommended that proteins be described on the basis of their digestible amino acid contents, with each amino acid being treated as an individual nutrient. Legumes consist of two types of subunits, acidic and basic with a molecular mass of 40 and 20 kDa, respectively.

Legume proteins are known to be low in sulphur-containing amino acids, methionine, cysteine and tryptophan, but they have greater amounts of lysine than cereal grains. Hence, protein complementation can be achieved through combining proteins in which one of the protein sources has a considerably higher concentration, of the most limiting amino acid in the other protein source. Thus, a combination of soy (high in lysine) with a cereal (high in sulphur-containing amino acids) would provide protein quality that is greater than that for either protein source alone (Young and Pellett, 1994). However, the degree of supplementation may also depend upon the second limiting amino acid, namely threonine, in cereals and tryptophan in legumes (Duranti, 2006).

For a long time legumes have been recognized as a valuable and low cost source of high quality protein products such as flour, concentrates and isolates. Nevertheless, the application on an industrial scale has only soybean proteins and to lesser extent pea protein products, in part due to insufficient information relating to functional properties of proteins of other legumes (Paredes-Lopez *et al.*, 2006).

A bacterial maltogenic α-amylase has been found to have anti-staling effect (Olsen, 1990). The effectiveness of α-amylases has been attributed to the inhibition or blocking of starch- protein complexion by dextrin products (Van Dam and Hille, 1992) and to reduce amylopectin crystallization (Hug-Iten *et al.,* 2001). In food industries hydrocolloids exhibit some functions like controlling the pasting properties of foods, improving moisture retention and maintenance of overall product quality during storage (Takahiro *et al.,* 2005 and Ferry *et al.,* 2006).

The present study was undertaken to investigate the functional attributes of two different types of legumes (chickpea and soybean) as flour and as protein isolate on nutritive value and quality attributes of wheat flour dough in the present of bacterial maltogenic α-amylase and hydrocolloid Arabic gum.

Materials and Methods

A. Materials:

Wheat flour (WF) (72% extraction) was obtained from Milled Cairo Company in Giza. Chickpea (*Cicer arietinium* L.) seeds "Giza 195" and Soy flour (SF) (6% fat, 48% protein) were purchased from the Agricultural Research Center, Giza, Egypt**.** Arabic gum, alpha amylase and chemicals used for nutrients composition determination were purchased from Sigma Chemicals Co., in Egypt.

B. Methods:

1. Chickpea flour preparation:

Chickpea (*Cicer arietinium* L.) seeds were soaked in ordinary water (1:3, w/v) for 12 h at room temperature (25°C). The soaked seeds were rinsed three times with water, sun-dried, then oven-dried at 45-50 °C to a constant weight. Dried chickpea seeds were milled twice in a laboratory mill (Braun grinder, ZK 500), then sieved to obtain fine chickpea flour. The flour was packed in polyethylene bags and kept at 4°C for further uses (Ravi and Harte, 2008 and Hemeda and Mohamed, 2010).

2. Protein isolation method:

Protein isolates from chickpea and soy bean flour were prepared following the method reported by El-Adawy (1996) and modified by Makri *et al.* (2005). The isolation process was carried out at Food Technology Dept., National Research Centre in Cairo, Egypt.

3. Determination of protein isolate recovery and yield:

Recovery of legume protein isolates was estimated as weight of protein isolates obtained after isoelectric precipitation per 100 g weight of respective legume. Protein yield (%) was calculated by the following formula using protein content of isolate and parent legume (Wang *et al.,* 1999).

$$\text{Protein yield (\%)} = \frac{\text{wt.(g) of isolated protein}}{\text{100 g (wt. of legume)}} \times \frac{\text{Protein content (isolate)}}{\text{Protein content (legume)}} \times 100$$

4. Chemical composition of legumes Flour and protein isolate:

The wheat flour, the prepared legumes flours and protein isolates were subjected to chemical analysis to determine chemical composition: Moisture content, ash, total protein, total fat and minerals (Ca, Fe, Zn) according to A.O.A.C (2005). Total carbohydrates were determined by difference.

5. Amino acid composition:

Amino acid content was determined as described by Moore *et al*. (1958). This analysis was performed at the Regional Center for Food and Feed, Agricultural Research Center, Egypt using amino-acid analyzer (Biochrom 30).

6. Legume flour and protein isolate color analysis :

Color evaluation of wheat flour, legumes flour under investigation as well as the produced protein isolates were done according to Hunter (1975), spectro-colorimeter (Tristimulus Color Machine) with CIE lab color scale (Hunter, Lab Scan XE, Germany), where L*=Lightness, a*=redness, b*=yellowness).

7. Functional Properties of protein isolates from chickpea and soybean:

Bulk density (g/ml) was determined according to Siddiq *et al.,* (2010), water and oil absorption capacity (g/g) were determined according to Rahma and Narasinga Rao, (1984). Foaming property was determined according to Dipak and Kumar, (1986). Protein solubility was determined according to the procedure of King et al. (1985) and Bera and Mukherjee (1989).

8. Preparation of wheat dough formulas:

Wheat flour (Triticum vulgare) (72%) dough formulas were prepared in the presence of alpha amylase (200 unit) and Arabic gum (2.5 %) as the followings: Control sample (unfortified) and 4 different fortified samples (5% CF + 5% SF), (5% CF + 5% SPI), (5% SF + 5% CPI), (5% CPI + 5 % SPI).

9. Rheological evaluation of Wheat dough :

The rheological properties of different wheat dough, s (fortified and unfortified) were assessed in the National Research Centre, Food Technology Dep., Cairo, Egypt, to determine the effect of each type of legume (soybean and chickpea) as flours and as protein isolates on gluten formation, water absorption and dough stability which all considered being important parameters that reflect the final product quality. Each of the prepared unfortified and fortified wheat dough samples were subjected to total gluten (%) determination (manually) according to AACC, (1962). Farinograph test (AACC, 1995) to determine: water absorption (%), arrival time (min.), dough development time (min.), dough stability (min.) and degree of softening (BU). Also, Extensigraph test (AACC, 1995) was used to assess: Extensibility 'E' (mm), Resistance to Extension (elasticity) 'R' (B.U.), Proportional Number (R/E ratio) and Dough Energy (area under the curve, cm2).

10. Statistical Analysis:

The results were expressed as (mean ± SE) and data collected from the present study was tabulated .

Results and Discussion

Chemical composition :

Chemical composition of Wheat flour (WF) and two different types of legumes: chickpea and soy bean legumes as flour (CF and SF) and as protein isolates (CPI and SPI) are presented in Table (1). Regarding the protein content, the result showed that both types of legume flour (CF and SF) contained higher values of protein (19.73% and 48.70%, respectively) as compared to wheat flour (WF) protein (9.70%). Same findings were detected by other investigators (Saleh et al. 2012). Protein content of CF detected by the previous study was (20.12 %) which was found to be somehow higher than the value detected by the present study (19.73%). The variation in protein values of CF could be related to several reasons; among these are the preparing conditions, method used for protein determination, and environmental factors associated with planting such as choice of species and varieties of the crop, the soil, fertilizers, irrigation system, climate, etc. Variation in the relative proportions of the individual proteins in the seeds of different varieties could definitely alter the nutritive value of the total seed protein.

Table (1) demonstrates that the high level of fat content was found for SF (8.78%) followed by CF the (6.05%) as compared to fat composition of WF (1.46%). On the other hand, moisture and carbohydrate contents of WF as presented in the same table are higher than the values detected for both CF and SF. The ash content in wheat flour was low (0.52%) but increased in CF (2.40%), while the highest amount (7.20%) was found in SF. Concerning crude fiber content, obtained values were (0.77 %) for the WF, (1.67%) for CF and (2.15%) for SF. Moreover, the results of the present study showed that isolation of protein from two types of legumes (CPI and SPI) led to an increase in protein values (87.96% and 89.84%, respectively) in comparison to that of both CF and SF. Protein isolate levels of legumes in the present study came in agreement with the values reported by other investigators (Can Karaca *et al.*, 2011) who used isoelectric precipitation extraction procedures (85.4 % and 87.6 % for CPI and SPI, respectively).

Table 1: Chemical composition of wheat flour, legumes flour and protein isolate from two different types of legumes (chickpea and soy bean), (%, on wet weight basis).

Raw material	Proximate composition (%)					
	Protein	Fat	Moisture	Ash	Carbohydrate**	Fiber
Wheat flour (WF)	9.70	1.46	10.7	0.52	76.85	0.77
Chickpea flour (CF)	19.73	6.05	8.90	2.40	61.25	1.67
Soybean flour (SF)	48.70	8.78	3.30	7.20	29.87	2.15
Chickpea protein isolate (CPI)	87.96	ND*	6.78	5.26	N.D.	N.D.
Soy protein isolate (SPI)	89.84	ND*	4.45	5.71	N.D.	N.D.

** ND = Not Detected* *** Carbohydrates were calculated by difference*

Isoelectric precipitation typically yields mainly globulin proteins (Papalamprou *et al.* 2010), whereas other extraction methods, such as salt extraction, yield isolates comprised of a mixture of globulins and albumins (Liu *et al.* 2008). According to Jay and Michael (2004), Isolate is the most refined form of protein products containing the greatest concentration of protein, but unlike flour and concentrates contains no dietary fiber.

As reported by Bampidis and Christodoulou (2011), variations in proximate composition of different legumes as lentil, chickpea and kidney bean have been observed owing to different environments, genotype and analytical methods. The investigators further reported that protein content was sensitive to rainfall, light intensity, length of growing season, day duration, temperature and agronomic practices. The findings of the present study will be helpful to the food technologists for developing protein enriched formulation for community needs.

Mineral composition:

Minerals composition of wheat flour (WF), chickpea flour (CF), defatted soybean flour (SF), chickpea protein isolate (CPI) and soy protein isolate (SPI) are shown in Table (2). It could be observed that defatted soybean flour was a rich source of Zinc (Zn), Iron (Fe) and Calcium (Ca): 5.338, 28.910 and 458.20 mg/100 g, respectively, on wet basis, which were found to be higher than the values obtained for other samples under the present study investigations. Regarding the wheat flour (WF), it could be seen that it is a poor source of Zinc and contained lower value of Calcium (22.25 mg/100 g) on wet basis. However, it contained a reasonable value of Iron (5.634 mg/100 g) on wet basis. The study done by Al-Kahtani and Abou Arab (1993) concerning Zinc content for soy flour (defatted) and soy protein isolate reported values of (5.8 and 2.45 mg/100 g) for SF and SPI, respectively. However, lower values were found with iron content (8.34 and 3.22 mg/100 g for SF and SPI, respectively) and calcium content (124 mg/100 g). In addition the study done by Abou Arab *et al.* (2010) reported higher levels of Zn, Fe and Ca (3.83, 6.85 and 156.13 mg/100 g, respectively on dry basis) with regard to chickpea flour. The investigators found that content of Zn, Fe and Ca were (2.19, 2.7 and 42.91 mg/100 g, respectively on dry basis) for wheat flour.

Table 2: Mineral composition of wheat flour and two different types of legumes (chickpea and soy bean) as flour and as protein isolate (mg/100 g, on wet weight basis).

Raw material	Mineral composition (mg/100 g)		
	Zinc (Zn)	Iron (Fe)	Calcium (Ca)
Wheat flour (WF)	ND*	5.634	22.25
Chickpea flour (CF)	2.231	5.308	77.26
Soybean flour (SF)	5.338	28.910	458.20
Chickpea protein isolate (CPI)	2.721	18.050	34.702
Soy protein isolate (SPI)	2.514	14.611	94.735

** ND = Not Detected*

Percentage (%) change in minerals composition of the isolated proteins relative to the values for protein flour of legumes (chickpea and soybean) under present investigation are presented in (Table 3). Protein isolation procedures had different effect on the mineral content of legume flour and their corresponding isolated protein. Zinc and Iron content increased by (21.96 % and 240.05 %, respectively), upon isolation process in chickpea however, calcium had decreased by (55.08%). On the other hand, Zinc (Zn), Iron (Fe) and Calcium (Ca) had decreased by (52.9%, 49.46% and 79.32%, respectively), in soybean upon isolation process. Regarding the effects of isolation process on minerals composition, it was found great reductions in soy protein composition of Zn, Fe and Ca, whereas there was found a reduction in Ca composition of Chickpea protein isolate. The same trend in mineral reduction which occurred after soy protein isolation was in agreement to that observed by Al-Kahtani and Abou Arab (1993). Zn, Fe and Ca had decreased by (57.78 %, 61.15 % and 9.68 %, respectively) in soy isolate.

Table 3: Percentage (%) change in mineral composition of the isolated proteins relative to protein values of legumes flour (Chickpea and Soybean).

Source of Protein isolate	% Change in mineral composition		
	Zn (%)	Fe (%)	Ca (%)
Chickpea	21.96	240.05	- 55.08
Soybean	- 52.9	- 49.46	- 79.32

It could be concluded that the reduction in minerals composition detected by the present study after isolation process (isoelectric precipitation of protein) may be due to leaching of minerals to water during centrifugation process from legume flours to the residue or the supernatant produced.

Amino Acid composition:

Amino Acid composition of wheat flour, legume flour and protein isolate from chickpea and soy bean are reported as (% on wet basis) in Table (4). From the presented results, it could be observed that, almost total amino acids were higher in the protein isolates than their respective legume seeds, either in the essential amino acid contents or in the non-essential ones compared to that of wheat flour. This could be due to non-protein nitrogen (NPN), which may influence the estimated seed protein content by at least 2–4% on a seed weight basis, leading to erroneous protein determinations (Deshpande and Nielsen, 1987).

Soybean flour (SF) had scored higher content of all the determined amino acids (42.05 %) compared to chickpea (16.84 %) and wheat flour (9.63 %). Total essential amino acids were higher in chickpea protein isolate (CPI) than that of soy protein isolate (SPI): 34.36% and 30.04%, respectively. However, non-essential amino acids increased in soy protein isolate (SPI) than chickpea protein isolate (CPI): 53.56% and 48.52%, respectively. For example, Glutamic acid increased from (12.42 % to 17.46 %) in CPI and SPI, respectively. The amino acid profiles of the protein isolates were close to those reported by Johnston *et al.* (2015). Chickpea and soybean flour and their protein isolates are rich in essential amino acids (Isoleucine, Leucine and Lysine) compared with cereals as wheat flour. Lysine acid increased from (0.22 %) in WF to (1.20 %, 2.76 %, 5.24 % and 5.87 %) in CF, SF, SPI and CPI respectively.

Amino acid composition is characterized by low methionine content. Methionine contents for the wheat flour (WF), chickpea flour (CF), defatted soybean flour (SF), chickpea protein isolate (CPI) and soy protein isolate (SPI) were found to be (0.39 %, 0.30 %, 0.66 %, 1.22% and 1.00 % respectively). Legume seeds are rich in lysine and poorer in sulfur-containing amino acids (methionine and cysteine) compared to cereals. Lysine is the first limiting amino acid so it is important that legumes complement cereals in lysine balance. Legume proteins are composed of several thousand specific proteins. About (70 to 80 %) of the crude protein in legume seeds is storage protein. The non-storage proteins are enzymes, enzyme inhibitors, hormones, transporting, structural and recognition proteins. The main protein fractions of legume seeds are albumin and globulin, which can be separated into two major fractions, vicilin and legumin. The relative proportion of legumin to vicilin varies with genotype, but vicilin is the major protein fraction in all legumes. Albumins and globulins fractions are different in their amino acid composition, molecular weight and physico-chemical properties (Schuster-Gajzágó, 1998).

Table 4: Amino acid composition of wheat flour and of two different types of legumes (chickpea and soy bean) as flour and as protein isolate (%, on wet weight basis [a]).

Amino Acid (%) [b]	Wheat flour	Chickpea flour	Soybean flour	Chickpea protein isolate	Soy protein isolate
	Essential amino acids (EAAs) [c]				
Histidine (His)	0.17	0.50	1.31	2.84	2.30
Isoleucine (Ile)	0.35	0.74	1.98	4.36	3.96
Leucine (Leu)	0.72	1.33	2.97	7.11	6.24
Lysine (Lys)	0.22	1.20	2.76	5.87	5.24
Methionine (Met)	0.39	0.30	0.66	1.22	1.00
Phenylalanine (Phe)	0.51	1.04	1.89	5.81	4.44
Threonine (Thr)	0.29	0.69	1.80	3.07	2.88
Valine (Val)	0.45	0.78	1.76	4.08	3.98
Total EAAs	3.1	6.58	15.13	34.36	30.04
	Non-Essential amino acids (Non-EAAs)				
Alanine (Ala)	0.23	0.89	1.92	3.71	3.50
Arginine (Arg)	0.39	1.45	2.36	7.32	6.38
Aspartic (Asp)	0.45	1.92	5.02	9.55	9.67
Cysteine (Cys)	0.26	0.30	0.72	1.26	1.28
Glutamic (Glu)	3.03	2.92	8.88	12.42	17.46
Glycine (Gly)	0.38	0.70	1.83	3.30	3.39
Proline (Pro)	1.00	0.78	2.46	3.76	4.35
Serine (Ser)	0.47	0.81	2.03	4.07	4.18
Tyrosine (Tyr)	0.32	0.49	1.70	3.13	3.35
Total Non-EAAs	6.53	10.26	26.92	48.52	53.56
Total determined Amino Acids	9.63	16.84	42.05	82.88	83.6

[a] By amino acid analyzer (Biochrom 30), [b] Amino Acid (%) adjusted on the basis of protein content, [c] Tryptophan was not determined.

Digestibility of protein and bioavailability of its constituent amino acid are very important factors in determining protein quality (FAO/ WHO, 1990 and Clemente *et al.*, 1998). They found in vitro protein digestibility of Kabuli chickpea seed was only (71.8 %) and could be improved significantly to (83.5 %) after cooking. Cooked chickpea seeds had a decrease in methionine, cysteine, tyrosine and leucine (Clemente *et al.*, 1998). The highest reductions being in cysteine (15 %) and lysine (13.20 %). According to Abou Arab *et al.*, (2010) chickpea seed have been considered a suitable source of dietary proteins due to their good balance of amino acid and high protein bioavailability.

Hunter Color values:

Results in Table (5) present color values for wheat flour, legume flour and protein isolate from two different types of legumes (chickpea and soy bean) as measured by Hunter colorimeter instrument. The results indicated that the color of both chickpea and soybean flours tended to be white with a little yellowish color, while the isolated protein from the same type of legumes tended to be a little white color with more degree of yellowish color than that of flour form.

Functional attributes of protein isolates from chickpea and soy bean:

As stated in table (6), the isolation process used in the present study resulted in 67.76% protein yield from chickpea and 43.35% yield from soybean. The study done by Qayyum *et al.*, (2012) indicated that the isoelectric precipitation method was used for the recovery of respective protein isolates from defatted legume samples (chickpea, lentil, broad and kidney beans). Lentil and chickpea showed higher protein isolates recovery (84.66 and 80.67 g/100 g, respectively) and yield (80.47%

and 73.14%, respectively) as compared to other legumes. It is deduced that lentil and chickpea had better protein isolates recovery and yield thus suitable for the preparation of protein enriched food formulations.

Table 5: Hunter Color values for wheat flour, legume flour and protein isolate from two different types of legumes (chickpea and soy bean).

	Hunter Color values (Parameters)					
Raw material	L*	a*	b*	a/b	Saturation index	Hue angle
Wheat flour (WF)	92.71	0.54	11.07	0.05	11.08	87.21
Chickpea flour (CF)	88.28	2.34	22.69	0.1	22.81	84.11
Soybean flour (SF)	81.84	- 1.72	26.3	- 0.07	26.36	93.74
Chickpea protein isolate (CPI)	75.27	4.55	30.81	0.15	31.14	81.6
Soy protein isolate (SPI)	76.34	2.97	33.78	0.09	33.91	84.98

** L= Lightness, a= Redness, b= Yellowness*

Table 6: Functional attributes (mean± standard error) of protein isolates from chickpea and soy bean.

Legumes Protein Isolates	Chickpea protein Isolate (CPI)	Soy protein Isolate (SPI)
Isolate Recovery (g/100 g)	15.20±0.289	23.50±0.404
Protein Yield (%)	67.76±0.035	43.35±0.335
Bulk Density (g/ml)	0.704±0.003	0.694±0.017
Water Absorption Capacity (g/g)	1.30±0.012	1.41±0.173
Oil Absorption Capacity (g/g)	0.94±0.023	0.77±0.046
Protein Solubility (%)		
At different pHs: 2.0	46	43
4.0	7	5
6.0	10	9
8.0	69	61
10.0	88	85

Values were expressed as mean ± standard error.

Water absorption capacity (g/g) was found to be a little higher in soy protein isolate. On the other hand, oil absorption capacity (g/g) was found to be higher in protein isolate from chickpea than that of soy protein isolate. While, protein solubility (%) at different pH (2-10) showed to be higher for both types of protein isolates especially as the pH increased (be alkaline) at (8-10 pH). Foam Capacity (%) and Foam Stability (% over 2 hrs.) of Chickpea (CPI) and soy (SPI) protein isolates at different pH are stated in Table (7). Foam stability (%) of both protein isolate increased at pH ranged from (4-6) and decreased below and higher than that range of pH. The same finding was reported by Abou Arab *et al.*, (2010) with regard to functional properties of chickpea flours. The increase utilization of grain legumes in composite flours for various food formulations, their functional properties (water absorption, foaming capacity and foaming stability, etc.) are assuming greater significance.

Table 7: Foam Capacity (%) and Foam Stability (% over 2 hrs.) of chickpea (CPI) and soy (SPI) protein isolates at different pH.

	Chickpea protein isolate (CPI)		Soy protein isolate (SPI)	
pH	Foaming Capacity (%)	Foam Stability (%)	Foaming Capacity (%)	Foam Stability (%)
2.0	460	25	370	16
4.0	250	95	150	90
6.0	230	100	200	100
8.0	400	60	300	50
10.0	480	50	400	25

Rheological evaluation of wheat dough fortified with legumes' (flour and protein isolate):

The rheological properties of fortified wheat flour with flour blends and protein isolate from two different types of legumes (chickpea and soybean) were investigated for gluten determination of the prepared four formulas: (5% CF + 5% SF), (5% CF + 5% SPI), (5% SF + 5% CPI), (5% CPI + 5% SPI) in addition to control sample (unfortified).

Table (8) shows the obtained results for gluten determination (%) of wheat flour formulas fortified with chickpea and soybean as flour and as protein isolate. The most important component of wheat flour is the gluten protein, that plays a deceive role in dough formation, gas retention and the structure of crumb.

Table 8: Gluten determination (%) of wheat flour formulas fortified with chickpea and soybean.

Wheat Flour Formulas *		Wet gluten (%)	Dry gluten (%)
1	Control	32.93	11.93
2	5 % CF + 5 % SF	28.99	10.86
3	5 % CF + 5 % SPI	29.52	11.78
4	5 % SF + 5 % CPI	30.21	11.89
5	5 % CPI + 5 % SPI	30.94	12.09

** CF= Chickpea flour, SF= Soybean flour, CPI= Chickpea protein isolate, SPI= Soy protein isolate*

The wet gluten (%) was found to be at the highest value (32.93%) in wheat flour (control). Since chickpea or soybean flours are gluten-free, wheat flour substitution with other legume flours or protein isolate resulted in the reduction of wet gluten (%) formation. The incorporation of chickpea and soy protein isolates with 5% (sample 5) resulted in increasing percentage of wet gluten to (30.94%), compared to the fortification with legume flour only, as in the second treatment which was 28.99 %. Dry gluten (%) decreased in all formulas except for the fifth formula which contained a mixture of the two protein isolates (5% CPI + 5% SPI), was higher than that of control sample (unfortified sample); due to the higher water absorption capacity (WAC) of protein isolates than that of wheat flour. Fortification with legume flours (chickpea and soy bean) (sample 2) lead to a reduction in gluten formation among all different prepared samples. The incorporation with 5, 10, and 15% chickpea flour resulted in decreasing the amount of wet and dry gluten as compared to wheat flour (Sulieman, *et al.*, 2013).

Farinograph evaluation:

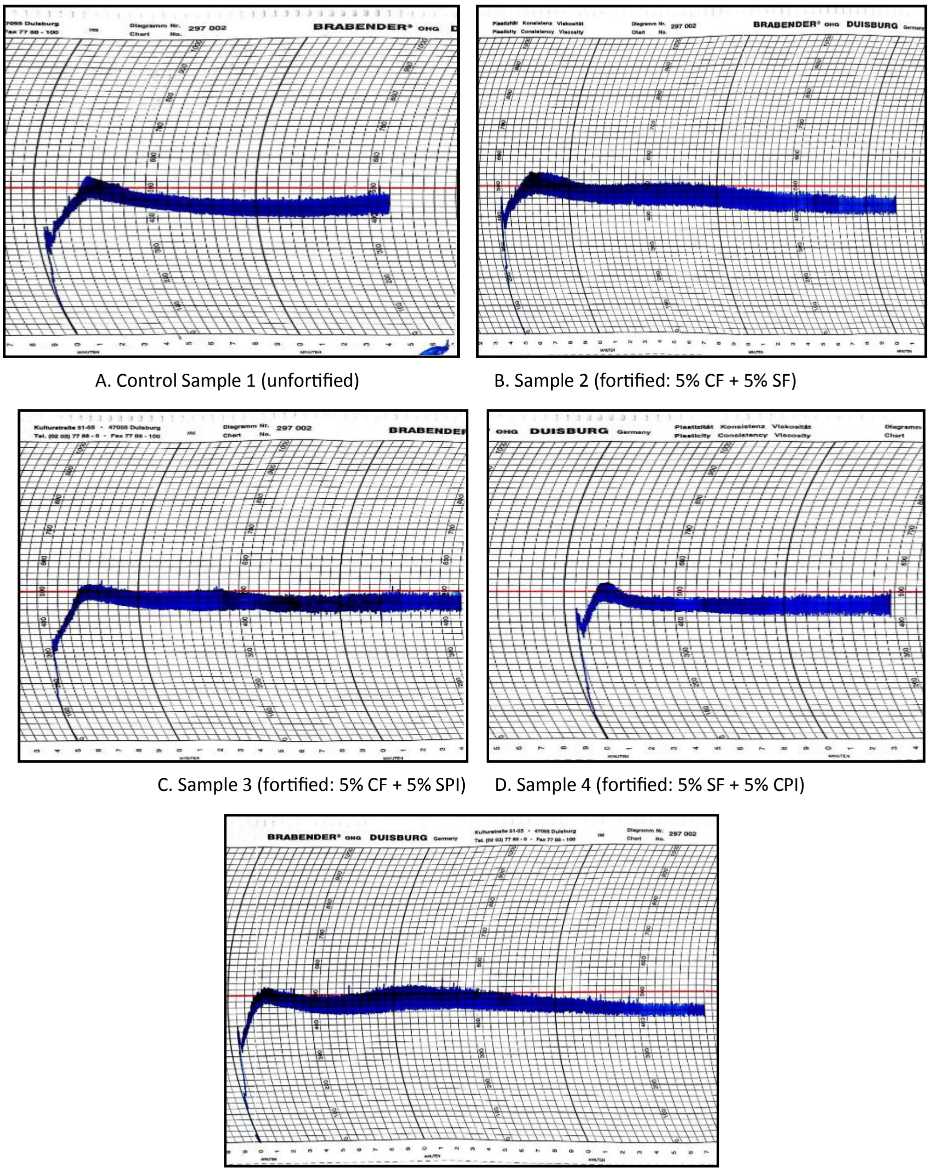
The results of Farinograph test for wheat dough (unfortified and fortified with chickpea and soybean as flour and as protein isolate) are presented in table (9) and Figure (1).

Water absorption (%) of wheat flour upon fortification with flour mixture of both chickpea and soybean (5% each) showed to increase as compared to that of the unfortified sample (control). Also, a mixture of protein isolate of each type of legume with legume flour caused an increase in water absorption of wheat dough than that of the unfortified samples but less than what observed with the addition of a mixture of legumes flour (5% each) together.

Table 9: Farinograph characteristics of Wheat flour dough fortified with different types of legume (chickpea and soy bean) in the presence of Alpha amylase and Arabic gum.

Wheat Flour Formulas *		Water absorption (%)	Arrival time (min)	Development time (min)	Dough stability (min)	Degree of softening (B.U.)
1	Control	60.5	2.0	2.5	2.0	60
2	5 % CF + 5 % SF	62.0	1.5	3.0	13.5	35
3	5 % CF + 5 % SPI	61.3	2.0	2.5	7.5	40
4	5 % SF + 5 % CPI	61.5	1.5	2.0	1.0	40
5	5 % CPI + 5 % SPI	60.0	1.5	2.5	15.0	30

** CF= Chickpea flour, SF= Soybean flour, CPI= Chickpea protein isolate, SPI= Soy protein isolate*



A. Control Sample 1 (unfortified) B. Sample 2 (fortified: 5% CF + 5% SF)

C. Sample 3 (fortified: 5% CF + 5% SPI) D. Sample 4 (fortified: 5% SF + 5% CPI)

E. Sample 5 (fortified: 5% CPI + 5% SPI)

Fig. 1: Farinogram of wheat flour dough fortified (B, C, D and E samples) with different types of legume (chickpea and soy bean). (CF= Chickpea flour, SF= Soybean flour, CPI= Chickpea protein isolate, SPI= Soy protein isolate)

These results are in agreement with those found by Dodok *et al.* (1993); Fenn *et al.* (2010); Saleh *et al.* (2012) and Mohammed *et al.* (2014) who reported that fortification of wheat flour with soybean and chickpea flours at different levels caused an increase in water absorption in all blends. The increase in water absorption was attributed to the increase in total protein content of the composite flours as well as pentosan content, especially ribose and deoxyribose (non-starch polysaccharides) (Ribotta *et al*., 2005 and Anton *et al*., 2008). The ability of these proteins to absorb high quantities of water results in doughs which exhibit increased farinograph water absorption values (Doxastakis *et al*., 2002).

Dough development time (min) and dough stability (min.) increased (3 and 13.5 min., respectively) upon fortification with a mixture of both legumes flour (5% each) than that of the unfortified samples and the other fortified samples with protein isolate. Dough stability (min.) on the other hand, decreased (1 min.) upon fortification with (5 % SF + 5 % CPI) as compared with the unfortified sample (control); due to weakening of the gluten network configuration during the kneading. This is attributed to an intense incompatibility between the protein spectrum of chickpea-soy and wheat gluten protein. This conclusion is relevant with the results of study done by Roccia *et al.* (2009) who found that the substitution of wheat protein by soy protein decreased mixture elasticity, indicating dough network weakening; due to the substitution of gluten proteins by the non-gluten-forming vegetable proteins that causes a dilution effect and consequently weakens the dough.

Extensigraph characteristics:

Results of wheat flour dough fortified with different types of legume (chickpea and soy bean) as flour and as protein isolate in the presence of α-amylase and Arabic gum are presented in table (10). Dough extensibility (mm) was found to be greater (130 mm) upon fortification with a legumes flour mixture (5 % each) than that of the other fortified samples and the control (96 mm). However, dough resistance to extension (B.U) 'elasticity' values was found to be greater in all the fortified samples (flours and protein isolate) as compared to that of the unfortified sample. The greatest values among all the prepared samples were obtained with fortification by a mixture of legume flour and protein isolate of the investigated legumes: (5% SF + 5 % CPI) and (5% CF + 5 % SPI), which represented about 164 and 160, respectively. Dough Energy (cm^2) was found to be higher than the control as the fortification was done with either mixture of (5 % CF + 5 % SF) and (5 % CF + 5 % SPI).

Table 10: Extensigraph characteristics of Wheat flour dough fortified with different types of legume (chickpea and soy bean) in the presence of Alpha amylase and Arabic gum.

Wheat Flour Formulas *		Resistance to Extension (Max.) (R) (B.U.)	Dough Extensibility (E) (mm)	Proportional Number (Max.) R/E**	Dough Energy (cm^2)
1	Control	116	96	1.21	15.42
2	5 % CF + 5 % SF	120	130	0.92	20.24
3	5 % CF + 5 % SPI	160	83	1.93	18.76
4	5 % SF + 5 % CPI	164	57	2.88	12.2
5	5 % CPI + 5 % SPI	148	73	2.03	14.32

** CF: Chickpea flour, SF: Soybean flour, CPI: Chickpea protein isolate, SPI: Soy protein isolate*
*** R/E = Resistance to Extension / Extensibility*

From the previous results it could be concluded that a mixture of (5 % CF + 5 % SF) caused an increase in Dough Extensibility (mm) and Dough Energy (cm^2). These results came in agreement with those reported by Saleh *et al.* (2012) who found that the addition of defatted soybean flour DSF caused an increase in the resistance to extension, proportional number, and energy and diminished extensibility of dough.

Conclusion:

The present study indicates the beneficial use of Chickpea flour (CF), Soybean flour (SF), Chickpea protein isolate (CPI) and Soy protein isolate (SPI) individually or as a mixture to fortify wheat flour. This will not only improves the nutritional status of the general population but also helps those suffering from degenerative diseases associated with today's changing life styles and environment. Dough rheology directly affects the baking performance of wheat flours, and rheological analyses have been made in order to optimize dough formulation. The unique bread making properties of wheat flour can be attributed mainly to the ability of its gluten proteins to form a viscoelastic network when mixed with water. Study chemical composition, functional properties and amino acids content of legumes under the present investigations (chickpea and soybean) as well as understanding their rheological behavior on wheat flour will help in maximizing their application in food system to provide product not only high in nutritional value but also with good quality characteristics. Supplementation with legumes, cereals and pulses is one way to meet the needs for protein particularly baked foods.

References

AACC. American Association of Cereal Chemists, 1962. Approved Methods of the American Association of Cereal Chemists. 7th Ed. Method 38-10. The Association: St. Paul, MN. USA.

AACC. American Association of Cereal Chemists, 1995. Approved Methods of the American Association of Cereal Chemists. 9th Ed. Method 54-21 and 54-10. The Association: St. Paul, MN. USA.

Abou Arab, E. A., I. M. F. Helmy and G. F. Bareh, 2010. Nutritional Evaluation and Functional Properties of Chickpea (*Cicer arietinum* L.) Flour and the Improvement of Spaghetti Produced from its. Journal of American Science, 6:1055-1072.

Al-Kahtani, H. A. and A. A. Abou-Arab, 1993. Comparison of physical, chemical and functional properties of *Moringa peregrina* (Al-Yassar or Al-Ban) and Soybean Proteins. Cereal Chem. 70(6): 619-626.

Anton, A.A., K.A. Ross, O.M. Lukow, R.G. Fulcher and S.D. Arntfield, 2008. Influence of added bean flour (*Phaseolus vulgaris* L.) on some physical and nutritional properties of wheat flour tortillas. Food Chem 109(1):33–41.

AOAC, 2005. Official Methods of Analysis of AOAC International. 18th Ed., AOAC International, Gaithersburg, MD, USA.

Bampidis, V. A. and V. Christodoulou, 2011. Chickpeas (*Cicer arietinum*) in animal nutrition: A review. Anim. Food Sci. Technol. 168:1-20.

Bazzano, L. A., J. He and L. G. Ogden, 2001. Legume consumption and risk of coronary heart disease in US men and women: NHANES I epidemiologic follow-up study. Arch Intern Med. 161: 2573–2578.

Bera, M. B. and R. K. Mukherjee, 1989. Solubility, emulsifying, and foaming properties of rice bran protein concentrates. J. Food Sci. 54: 142–145.

Bouchenak, M. and J. I. M. Lamri-Senhad, 2013. Nutritional quality of legumes, and their role in cardiometabolic risk prevention: a review. J Med Food; 16: 185–198.

Can Karaca, A., N. Low, and M. Nickerson, 2011. Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. Food Res. Int. 44(9): 2742–2750

Clemente, A., R. S. Vioque, J. Vioque, J. Bautista and F. Millan, 1998. Effect of cooking on protein quality of chickpea (*Cicer arietinum* L.) seeds. Food Chemistry. 62, 1-6.

Deshpande, S. S. and S. S. Nielsen, 1987. Nitrogenous constituents of selected grain legumes. J Food Sci 52: 1321–1325.

Dimitrios, S., M. Eleousa and D. Georgios, 2006. Effect of durum flour enrichment with chickpea flour on the characteristics of dough and lasagna. J. the Sci. of Food and Agric., 86(12): 1938-1944.

Dipak, K. and K. D. Kumar, 1986. Functional properties of rapeseed protein products with varying phytic acid content. J. Agric. Food Chem. 34:775.

Dodok, L., A.A. Modhir, B. Hozova, G. Halasova and I. Polacek, 1993. Importance and utilization of chickpea in cereal technology. Acta alimentaria 22(2):119–129.

Doxastakis, G., I. Zafiriadis, M. Irakli and C. Tananaki, 2002. Lupin, soyaand triticale addition to wheat flour doughs and their effect on rheological properties. Food Chem 77(2):219–227.

Duranti, M. 2006. Grain legume proteins and nutraceutical properties. Fitoterapia 77: 67–82.

El-Adawy, T. A. 1996. Chemical, nutritional and functional properties of mung bean protein isolate and concentrate. Menofiya Journal of Agricultural Research, 21(1), pp. 657– 672.

FAO/WHO. 1990. Protein quality evaluation Report of joint FAO/WHO expert consultation, Bethesda, MD, FAO/WHO, Rome, Italy.

FAO. 1994. Pulses and derived products. Definition and classification of commodities. FAO, Rome.

FAO. 2013. Dietary protein quality evaluation in human nutrition: report of an FAO expert consultation.http://www.fao.org/ag/humannutrition/35978-02317b979a686a57 aa4593304 ff c. Last accessed November 2017.

Fenn, D., O. M. Lukow, G. Hummphreys, P.G. Fields and J. I. Boye, 2010. Wheat-legume composite flour quality. International Journal of Food properties, 13: 381-393.

Ferry, A.L., J.J. Hort, R.D. Mitchell, J. Cook, S. Lagarrigueand Valles and B. Pamies, 2006. Viscosity and flavour perception: "Why is starch different from hydrocolloids? Food Hydrocolloids. *20:* 855-862.

Hemeda. H. M. and E. F. Mohamed, 2010. Functional attributes of chickpea and defatted soybean flour blends on quality characteristics of shortening cake. European Journal of Applied Sciences, 2 (2): 44-50.

Hug-Iten, S., F. Escher and B. Conde-Petit, 2001. Structural properties of starch in bread and bread model systems: Influence of an antistaling α-amylase. Cereal Chem. 78:421-428.

Hunter, R.S., 1975. Scales for measurements of color differences In Measurement of Appearance, J. Wiley Ed., P. 133. Interscience, New York.

Islam, T., A. Chowdhury, M. Islam and S. Islam, 2007. Standardization of Bread Preparation from Soy Flour. Int. J. Sustain. Crop Prod 2(6): 15-20.

Jay, R. H. and J. F. Michael, 2004. Macronutrient Utilization during Exercise: Implications for performance and supplementation. *Journal of Sports Science and Medicine* 3: 118-130.

Jenkins, D.J., C.W. Kendall and L.S. Augustin, 2012. Effect of legumes as part of a low glycemic index diet on glycemic control and cardiovascular risk factors in type 2 diabetes mellitus: a randomized controlled trial. Arch Intern Med 2012; 172: 1653–1660.

Johnston, S. P., M. T. Nickerson and N. H. Low, 2015.The physicochemical properties of legume protein isolates and their ability to stabilize oil-in-water emulsions with and without genipin. J. Food. Sci. Technol. 52(7):4135–4145.

King, J., C. Aquirre and O. Depablo, 1985. Functional properties of lupin seed protein isolates (*Lupinus albus* cv. *multolupa*). J. Food Sci. 50:82.

Liu, L.H., T.V. Hung and N. Bennett, 2008. Extraction and characterization of chickpea (*Cicer arietinum*) albumin and globulin. J. Food Sci. 73(5):C299–C305.

Makri, E., E. Papalamprou and G. Doxastakis, 2005. Study of functional properties of seed storage proteins from indigenous European legume crops (Lupin, Pea, Broad bean) in admixture with polysaccharides. Food Hydrocolloid. 19:583- 594.

Messina, M.J. 1999. Legumes and soybeans: overview of their nutritional profiles and health effects. Am. J. Clin. Nutr. 1999; 70 (suppl.):439S-450S.

Mohammed, I., R. Abdelrahman and A. B. Senge, 2014. Effects of chickpea flour on wheat pasting properties and bread making quality. Food Sci. Technol. 51(9):1902–1910.

Mollard, R.C., B.L. Luhovyy, S. Panahi, M. Nunez, A. Hanley and G.H. Anderson, 2012. Regular consumption of pulses for 8 weeks reduces metabolic syndrome risk factors in overweight and obese adults. Br J Nutr 108(Suppl. 1): S111–S122.Nutr 70: 439S–50S.

Moore, S., D. H. Spackman and WH. Stein 1958. Chromatography of amino acids on sulphonated polystyrene resins: An improved system. Anal. Chem. 30:1185-1190.

Olsen, T., 1990. Anti staling process and agent. European patent EP0494233.

Papalamprou, E. M., G. I. Doxastakis, and V. Kiosseoglou, 2010. Chickpea protein isolates obtained by wet extraction as emulsifying agents. J. Sci. Food Agric. 90(2):304–313.

Papanikolaou, Y. and V.L. III. Fulgoni, 2008. Bean consumption is associated with greater nutrient intake, reduced systolic blood pressure, lower body weight, and a smaller waist circumference in adults: results from the National Health and Nutrition Examination Survey 1999–2002. *J Am Coll Nutr;* 27: 569–576.

Paredes-Lopez, O., C. Ordorica-Falomir and M. R. Olivares-vazquez, 2006. Chickpea protein isolates: physicochemical, functional and nutritional characterization. J. Food Sci. 56(30):726-729.

Qayyum, M. M. N., M. S. Butt, F. M. Anjum and H. Nawaz, 2012. Composition analysis of some selected legumes for protein isolates recovery. J. Anim. Plant Sci., 22(4): 1156- 1162.

Rahma, E. H. and M. S. Narasinga Rao, 1984. Gossypol removal and functional properties of protein produced by extraction of glanded cottonseed with different solvents. J. Food Sci., 49: 1057.

Ravi, R. and J.B. Harte, 2008. Milling and physicochemical properties of chickpea (*Cicer arietinum* L.) varieties. Journal of Science Food Agric. 89:258–266.

Rebecca, L. J., C. Seshiah and D. Sharmila, 2016. Fortification of Cupcakes with Cereals and Pulses. International Journal of Novel Research in Life Sciences. 3(3), pp: (1-6).

Ribotta, P. D., S. A. Arnulphi, A. E. León and M. C. Añón, 2005. Effect of soybean addition on the rheological properties and bread making quality of wheat flour. J. Sci. Food Agric., 85: 1889–1896.

Roccia, P., P. D. Ribotta, G.T. Prez and A. E. Leôn, 2009. Influence of soy protein on rheological properties and water retention capacity of wheat gluten. LWT- Food Sci. Technol. 42(1):358–362.

Saleh, A. M., A. E. Salama, S. H. Bedeir, and E. I. Abd-Elazim, 2012. Effect of partial substitution of wheat flour with either defatted soybean or chickpea flours at different ratios on rheological and physical properties of dough, and quality characteristics of biscuits. J. Appl. Sci. Res., 8(12): 5806-5817.

Schuster-Gajzágó, I., 1998. Nutritional aspects of legumes. Cultivated plants, primarily as food sources. Vol. I Encyclopedia of Life Support Systems (EOLSS)

Serrem, C., H. Kock and J. Taylor, 2011. Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. Int. J. Food Sci. Technol., 46: 74-83.

Siddiq, M., R. Ravi, J.B. Harte and K.D. Dolan, 2010. Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris* L.) flours. LWT- Food Sci. Tech. 43:232-237.

Sulieman, A. E., E. A. Sinada and A. O. Ali, 2013. Quality Characteristics of Wheat Bread Supplemented with Chickpea (*Cicer arietinum*) Flour. International Journal of Food Science and Nutrition Engineering, 3(5): 85-90.

Takahiro, F., K. Yohei, O. Toshio, G. Yasunori, A. lwaoand and N. Katsuyoshi, 2005. Effect of non-ionic polysaccharides on the gelatinization and retrogradation behavior of wheat starch. Food Hydrocolloids, 19: 1-13.

Van Dam, H. W. and J. D. Hille, 1992. Yeast and enzymes in bread making. Cereal Foods World 37:245-251.

Wang, M., N. S. Hettiarachchy, M. Qi, W. Burks and T. Siebenmorgen, 1999. Preparation and functional properties of rice bran protein isolate. J. Agric. Food Chem. 47(2):411-416.

Young, V.R. and P.L. Pellett, 1994. Plant proteins in relation to human protein and amino acid nutrition. Am J Clin Nutr 59: 1203S–12S.

Zhang, T., B. Jiang and Z. Wang, 2007. Gelation properties of chickpea protein isolates. Food Hydrocolloids, 21:280–286.