

## Influence of chickpea, soy flour and protein isolate on pan bread quality and biological parameters in experimental rats

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### ABSTRACT

The aim of this study is to evaluate quality and nutritive value of fortified pan bread with legume flour [chickpea flour (CF) - soybean flour (SF)] and protein isolate [chickpea protein isolate (CPI) - soy protein isolate (SPI)] in addition to their biological effect on experimental rats. Protein isolate preparation was done by isoelectric precipitation method. Trypsin inhibitor activity (TIA) was determined in raw and pretreated legumes seeds, as well as in fortified pan bread. Quality of pan bread samples was evaluated physically and sensory, in addition nutritive value was determined. Biological study (4 wks.) was carried out using male rats of Sprague Dawley strain (n =15), weighing (110±10 g), rats were divided into three experimental groups (each of 5 rats) to determine protein biological effect of legume flour mixture and protein isolate from each types of legumes. Results of the study revealed that SPI had higher protein recovery value (23.5 g/100g) as compared to CPI which found to be (15.2 g/100g). Raw soybean seeds had higher TIA content (28.55 mg/g) than that of chickpea (24.5 mg/g). Soaking process under the present experimental condition reduced TIA content by (11.31 % and 14.57 %) for chickpea and soybean seeds, respectively. Baking temperature at (220°C) for (14 min.) effectively destroyed trypsin inhibitor activity (TIA) in the fortified bread samples. Higher water uptake (ml) was observed during preparation of pan bread dough fortified with SF-CPI (118.5 ml) and with CF-SF (113 ml) as compared to that of the unfortified sample (111 ml). Fortification with legumes' protein isolates CPI-SPI affected negatively physical characteristics of wheat pan bread by reducing loaf weight, height, volume, area and specific volume measurements as compared to fortification with legume flour mixture (CF-SF). There was a noticeable decrease in degree of freshness (mm/sec) of all fortified bread samples as compared to control upon storage (up to 5 days at 25°C). Hunter colorimeter results showed that the lightness color decreased in all fortified bread samples as compared to control. Among all the fortified bread samples, the sensory scores given (mean ± SD) to each of the evaluated characteristics (appearance, internal color, external color, cell uniformity, taste, odor and overall acceptability) for bread fortified with legumes flour mixtures (5% CF + 5% SF) were found to be close to that of the control, followed by sample fortified with (SF-CPI). Pan bread fortified with (5% CF and 5% SPI) demonstrated the highest Iron (Fe) content (11.04 mg/100 g) compared to the other fortified and unfortified bread samples (8.391 to 8.697 mg/100 g). Calcium (Ca) content increased in bread with (5% SF + 5% CPI) and bread with (5% CF + 5% SF) which were found to be (47.63 and 47.19 mg/100 g, respectively). The biological experiment showed that diets supplemented with (SF-CPI) enhanced growth, body weight gain (BWG) and feed efficiency ratio (FER) followed by supplementation with (CF-SFI). Serum albumin and globulin (g/dl) in both groups fed on legumes' flour and protein isolate were found to be significantly (P<0.05) higher than that of the control. There is appears to be a potential market for chickpea-soy flour in bread making. The present investigation recommend that development of such functional foods would be beneficial to improve the nutritional status of consumer. The results presented in this study will be helpful to the food technologists for developing protein enriched formulation for community needs.

**Key words:** soybean, chickpea, protein isolate, legume flour, pan bread, quality evaluation, protein bioavailability.

### Introduction

A change in eating habit and increased population have increased bread consumption in developing countries (Noorfarahzilah *et al.*, 2014). Bread is one of the most important staples in Egypt widely consumed by all age groups across all socio economic groups. Wheat flour (WF), the

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vital ingredient for bread making has low protein content as well as lower protein quality due to deficiency in essential amino acids such as lysine and threonine (Young, 2001; Jideani and Onwubali, 2009). The use of composite flour for improving protein quality of bread and making functional bread has been recommended (Ndife *et al.* 2011; Oluwalana *et al.* 2012; Dooshima *et al.* 2014).

Food fortification is very important process, defined as the addition of one or more nutrients rich ingredients together to improve nutritional composition of food. Usual goal of food fortification is to improve its quality for controlling nutrient deficiency problems (Genenu *et al.*, 2017). Fortification of wheat flour with non-wheat proteins (i.e., high protein legume flours) could provide a good opportunity to improve the nutritional quality of protein (i.e., increase its amino acid profiles) consumed by many people (Stark *et al.*, 1975; Hoover, 1979). In general, legumes have higher amounts of the essential amino acid lysine and improved protein efficiency ratio (PER) compared to wheat. Soybean has a lysine content of (6.34–6.40 g/16 g N) and a PER of (1.60). On the other hand, chickpea contains lower amount of lysine (1.42 g/16 g N), but chickpea protein has been reported to be more digestible and higher in quality (2.8 PER) than that of wheat (2.3 g lysine/16 g N and 0.95 PER) as reported by (Friedman, 1996).

Chickpea (*Cicer arietinum* L.) is an ancient crop, first grown in Turkey about 7,450 B.C. and in India about 4,000 B.C. (Singh, 1997). The production of chickpea ranks third in the world, and first in the Mediterranean. By comparison, soybean is the most commonly used plant protein in the world and ranks first in volume of production (Smartt, 1990). However, soybean proteins are food allergens and a large portion of the world's soybeans have been genetically modified (Chickfood, 2004). Consequently, there is a need to find alternative sources of plant proteins. Friedman (1996) indicated that chickpea protein quality is equivalent to that of soybean meal. Chickpea has powerful nutritive value due to its high content in lysine and sulfur amino acids. 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).

Protease inhibitors,  $\alpha$ -amylase inhibitors, oligosaccharides, phytic acid, saponins, phenolic compounds, and tannins are considered as anti-nutritional factors that are present in chickpea (Wesche-Ebeling *et al.*, 2001). The effects of these anti-nutritional factors are reduced by many processing techniques such as cooking, germination, and fermentation (Chavan *et al.*, 1989). Chymotrypsin and trypsin inhibitors are the main protease inhibitors in chickpea and according to Sumathi and Pattabiraman (1976) reported that the chymotrypsin inhibitor was more heat resistant than trypsin inhibitor and both required heating in acidic conditions for inactivation.

Heat treatment significantly improves protein quality in pulses by destruction or inactivation of heat labile anti-nutritional factors. Studies (FAO/WHO, 1990 and Clemente, *et al.*, 1998) found that in vitro protein digestibility of Kabuli chickpea seed was only (71.8 %) and could be improved significantly to be (83.5 %) after cooking. The storage proteins of chickpea seed have been fractionated into globulin (salt soluble), albumin (water soluble), prolamin (alcohol soluble), glutelin (acid/alkali soluble), and residual proteins (Chavan *et al.*, 1989).

Most of bakery products are used as a source for incorporation of different nutritionally rich ingredients for their diversification (Hooda and Jood 2005; Sudha *et al.*, 2007). The significance of the legume protein addition on wheat was dependent on the flour properties and end-product qualities of the individual wheat cultivars and the type and level of legume protein incorporated (Fenn *et al.*, 2010). In general, soybean protein could be added at the (2%) level to wheat flour without substantial changes to bread quality that would increase lysine content by (28%), a substantial increase in nutritional value of composite flour based foods. Color and qualities of end-products were significantly affected when flours were substituted with greater than (2%) soybean protein or other types of legume proteins. The previous investigators recommended that future studies should include sensory evaluation to determine the upper levels of legume protein addition with consequent change in quality that consumers will accept in order to reap the nutritional benefits of wheat-legume composite flour based foods.

The aim of this study is to evaluate quality and nutritive value of fortified pan bread with legume flour and protein isolate blends from two different legumes source (chickpea and soybean). In addition, the biological effect of that mixture was investigated on experimental rats.

## Materials and Methods

### Materials:

Pan bread quality: Egyptian cultivar of chickpea (*Cicer arietinum* L.), namely 'Giza 195', was procured from Legumes Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Defatted Soy flour (6% fat, 48% protein) (*Glycine max*) was obtained from Food Science and Technology Institute, Agricultural Research Center, Giza, Egypt. Arabic gum, bacterial  $\alpha$ -amylase enzyme (E.C.3.2.1.1) and chemicals for nutrients composition were purchased from El-Gomhouria For Drugs Co., Egypt. Other ingredients include: wheat flour (*Triticum vulgare*) (72% extraction) from El Nile Mill Co., sucrose sugar, corn oil, salt and dry instant yeast were purchased from the local market.

Biological experiment: Fifteen Male albino rats of Sprague Dawley strain weighing (110±10 g) were obtained from Helwan Station farm, Egypt. Casein, minerals and vitamins mixtures were obtained from Misr Scientific Company, Cairo, Egypt. Cellulose was purchased from Morgan Chemical Ind., Cairo, Egypt. Corn starch was obtained from Starch and Glucose Co., Egypt. Chemicals were purchased from Sigma Chemical Company, Egypt.

### Methods:

#### *Pretreatment method of Chickpea Seeds:*

Chickpea (*Cicer arietinum* L.) seeds (300 g) were cleaned and washed to remove foreign materials and then soaked for (12 hrs.) in ordinary water (1:3, w/v) at room temperature (25°C). The soaked seeds were rinsed three times with water and subjected to heat treatment in thermostatically controlled oven with air fan (J.P. Selecta, S.A, Spain) at (45-50° C) according to the reported methods by Ravi and Harte (2008) and Hemedda and Mohamed (2010).

#### *Chickpea flour preparation:*

The pretreated Chickpea seeds (300 g) were crushed to smaller fragments and ground into flour with a Mortar Grinder RM 200 (Retsch GmbH). The obtained chickpea flour was sieved with (80 mesh) screen using vibratory sieve shaker (J-VSS, Nanova Ltd, Korea) to obtain fine flour and then packed in polyethylene bags and stored at 4°C for further usage.

#### *Determination of Trypsin inhibitor activity (TIA):*

The effect of pretreatment process on reducing Trypsin inhibitor activity (TIA) in raw and soaked legume seeds under investigations was determined. Also, TIA in the final fortified product (pan bread) after baking was determined to investigate the effect of oven baking temperature (220°C) on TIA according to method of Mbata *et al.* (2009). Trypsin Inhibitor activity (TIA) in term of milligrams of pure trypsin inhibited per sample (g) was calculated as:

$$TIA = \frac{(2.632 \times D \times A1)}{S(\text{mg})\text{pure trypsin inhibited/g sample}}$$

Where A1 = change in absorbance due to trypsin inhibition/mL diluted sample extract, D = dilution factor and S = weight of sample (g)

#### *Protein isolates preparation:*

The isoelectric precipitation method was used for the preparation of respective protein isolates from chickpea and soybean flour according to method reported by El-Adawy (1996) and modified by Makri *et al.* (2005). Recovery of legume protein isolates was estimated as weight of protein isolates obtained after isoelectric precipitation per (100 g) weight of respective legume as well as protein yield (%) was calculated according to Wang *et al.* (1999).

*Bread making formulas:*

Pan bread was prepared according to the procedures stated by Gisslen (2007) using unfortified and four fortified wheat flour formulas. The ingredients used for each standard formula are presented in Table (1). The products were prepared in the laboratory of Nutrition and Food Science, Faculty of Home Economics, Helwan University. Some modifications were applied to the flour formulas by the addition of 2.5% Arabic gum and 0.005 % bacterial  $\alpha$ -amylase enzyme (on wheat flour weight basis). Water uptake (ml) and fermentation time (min.) during preparation of bread dough were recorded (twice). The fortified and unfortified bread dough weighed (70g) were placed in a mini rectangular loaf pan (10 x 6 x 4 cm) and kept in a warm place (at 37° C) to rise before being baked in a preheated electric oven at (220° C). Baking time (min.) was determined. The bread samples were removed from the pan and left to cool for (2 hrs.) at room temperature. Loaf weight (g) after baking was recorded, percentage (%) change in loaf weigh after baking was calculated, physical and organoleptic properties of the final products were evaluated.

**Table 1:** Pan bread formulas (unfortified and fortified) with different types of legumes (chickpea and soybean) as flour and as protein isolate.

Ingredients (g)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	Control	5 % CF + 5 % SF	5 % CF + 5 % SPI	5 % SF + 5 % CPI	5 % CPI + 5 % SPI
Wheat flour (WF)	243.75	218.75	218.75	218.75	218.75
Chickpea flour (CF)	—	12.50	12.50	—	—
Soybean flour (SF)	—	12.50	—	12.50	—
Chickpea protein isolate (CPI)	—	—	—	12.50	12.50
Soy protein isolate (SPI)	—	—	12.50	—	12.50
Corn oil	25.00	25.00	25.00	25.00	25.00
Sucrose sugar	25.00	25.00	25.00	25.00	25.00
Arabic gum	6.25	6.25	6.25	6.25	6.25
Active dry yeast	5.00	5.00	5.00	5.00	5.00
Salt	2.50	2.50	2.50	2.50	2.50
$\alpha$ -amylase enzyme	0.0125	0.0125	0.0125	0.0125	0.0125

**Pan bread quality evaluation:**

*a) Physical evaluation:*

Height (cm), Area (cm<sup>2</sup>) by using Planimeter apparatus and Volume (cm<sup>3</sup>) by rapeseeds displacement method were determined according to Penfield and Campbell (1990). All measurements were done triplicate and the results were presented as mean value. Specific volume (volume to mass ratio) was calculated using the following equation:

$$\text{Specific volume (cm}^3\text{/g)} = \frac{\text{loaf volume}}{\text{loaf weight}}$$

Percent change (%) for weight, height, area, volume of the fortified products were calculated relative to control values according to the following equation:

$$\% \text{ relative to control} = \frac{\text{fortified value}}{\text{control value}} \times 100$$

*Product degree of Tenderness (mm/sec)* before and after storage (for 5 days at 25° C) was measured using Penetrometer apparatus, Model H-1240 with serial number of 99101240 specs: Ast M, Humboldt MFG, Co., U.S.A. according to Penfield and Campbell (1990) at Nutrition and Food Science laboratory, Faculty of Home Economics, Helwan University.

*Loaf color:*

Color of exterior crust and interior crumb of the (fortified and unfortified) pan bread was determined according to Hunter (1975). Hunter  $a^*$ ,  $b^*$  and  $L^*$  parameters were measured by using a spectro-colorimeter (Tristimulus Color Machine) with CIE lab color scale (Hunter, Lab Scan XE, Germany) calibrated with a white standard tile of Hunter Lab color standard (LXNO. 16379):  $X=77.26$ ,  $Y=81.94$  and  $Z=88.14$  ( $L^*=92.71$ ;  $a^*=-0.89$ ;  $b^*=-0.18$ ) where  $L^*$ =Lightness,  $a^*$ =redness,  $b^*$ =yellowness), using Hunter-Scotfield's equation. Color difference ( $\Delta E$ ) was calculated from  $a$ ,  $b$  and  $L$  parameters, using Hunter-Scotfield's equation:  $\Delta E = (a^2 + b^2 + L^2)^{1/2}$ , where:  $a = a - a^o$ ,  $b = b - b^o$  and  $L = L - L^o$ . Subscript  $^o$  indicates color of control. Hue angle [ $\tan^{-1} b/a$ ] and Saturation index [ $\sqrt{a^2 + b^2}$ ] were also calculated. Color analysis was carried out at Food Technology Department, National Research Centre in Dokki, Cairo, Egypt.

*b) Sensory evaluation:*

Sensory evaluation was carried out by (10) panelists from staff members in both of (Nutrition and Food Science Department at Faculty of Home Economics, Helwan university and Food Technology Department, National Research Centre in Dokki, Cairo, Egypt), using score sheet of [5-points (1=lowest quality to 5= highest quality)] according to methods of Klein (1984) and Penfield and Campbell (1990). Pan bread samples were evaluated for: appearance, color (internal/external), cell uniformity, taste, odor and overall acceptability. The results for each characteristic were calculated and presented as (mean  $\pm$  SE).

*c) Determination of pan bread's nutritive value:*

Fortified and unfortified pan bread samples with flour and protein isolate from chickpea and soybean were chemically analyzed to determine their nutrients composition. Moisture and crude ash were determined according to the method reported by Fennema *et al.* (1996). Crude protein, crude fat, and crude fiber were determined according to the methods of A.O.A.C. (2005). Total carbohydrates were calculated by difference. Minerals: Calcium, Iron and Zinc (Ca, Fe, and Zn) were determined by atomic absorption spectrophotometer (Type Unicomp-929) according to the methods of A.O.A.C. (2005).

**Biological investigation:**

In this phase of study, the fortified flour formulas Fortified with chickpea-soybean flour and protein isolate were subjected to biological evaluation on experimental animals. The experiment was carried out in the animal house of Nutrition and Food Science Department, Faculty of Home Economics, Helwan University.

*a) Experimental design and diet composition*

Male albino rats of Sprague Dawely strain ( $n=15$ ), weighing ( $110\pm 10$  g), were housed in a wire cages at room temperature and kept under hygienic condition. All rats were fed on a standard basal diet for one week before starting the experiment (4 wks.). Food and water were provided *ad-libitum*. After the adaptation period, rats were divided into three experimental groups (each of 5 rats) and preliminary body weights were initially recorded. The basal diet was formulated according to Reeves *et al.* (1993), while 10 % of the diet (based on starch weight) was replaced by the mixture of flour and protein isolate of the two types of legume (chickpea and soybean) as presented in table (2). The experimental animal groups were designed as following:

- Group (1): Control negative; rats fed on basal diet.
- Group (2): Rats fed on diet containing (5% Chickpea flour CF + 5% Soy protein isolate SPI).
- Group (3): Rats fed on diet containing (5% Soybean flour SF + 5% Chickpea protein isolate CPI).

**Table 2:** Chemical composition (%) of experimental diet

Diet components (%)	Group (1)	Group (2)	Group (3)
	Control basal diet	5 % CF + 5 % SPI	5 % SF + 5 % CPI
Corn Starch	55.25	45.25	45.25
Casein	20.00	20.00	20.00
Sucrose Sugar	10.00	10.00	10.00
Corn oil	5.00	5.00	5.00
Cellulose Fibre	5.00	5.00	5.00
Mineral mixture <sup>a</sup>	3.50	3.50	3.50
Vitamin mixture <sup>a</sup>	1.00	1.00	1.00
Choline chloride	0.25	0.25	0.25
Chickpea flour (CF)	--	5.00	--
Soybean flour (SF)	--	--	5.00
Chickpea protein isolate (CPI)	--	--	5.00
Soy protein isolate (SPI)	--	5.00	--

<sup>a</sup> Formulated according to Reeves *et al.* (1993)

*b) Biological assay:*

Feed intake (g/day/rat) was calculated every two days and body weight (g) was recorded at the beginning and weekly till the end of the experimental period (4 weeks). Biological assay was carried out to determine feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) according to Chapman *et al.*, (1959) using the following equations:

$$\text{Body Weight Gain (BWG)} = \frac{\text{Final Body weight} - \text{Initial Body weight}}{\text{Final Body weight}} \times 100$$

$$\text{Feed Efficiency Ratio (FER)} = \frac{\text{Body Weight Gain (g)}}{\text{Feed intake}}$$

At the end of the experimental period, the rats were fasted overnight, then weighed before scarification under ether anaesthetised. Blood samples were collected from the portal vein into dry clean centrifuge tubes and left to clot at room temperature, then centrifuged at 3000 rpm for 15 min. to separate serum (stored at -10 °C) for serum albumin and globulin determination. Organs' weigh (g): heart, spleen, liver and kidneys were recorded and relative organ weight (%) was calculated as following:

$$\text{Relative organ weight (\%)} = \frac{\text{Organ Weight}}{\text{Body Weight}} \times 100$$

*Statistical analysis:*

The statistical analysis was carried out using SAS, PC statistical software for pan bread samples evaluation results: tenderness values (mean ± SD), organoleptic evaluation (mean ± SD) and the biological assays (mean ± SE). The results were expressed as (mean ±SD). Data were analyzed by one way analysis of variance (ANOVA).The differences between means were tested for significance using least significant difference test (LSD) at (P < 0.05) according to SPSS, (1986).

**Results and Discussion**

**Determination of Trypsin inhibitor activity (TIA):**

Results for the effect of pretreatments (soaking and heat treatment) on the levels of trypsin inhibitor activity (TIA) in chickpea and soybean seeds are presented in Table (3). Raw soybean seeds had higher TIA content (28.55 mg/g) than that of chickpea (24.50). Soaking process under the study condition reduced TIA content by (11.31 % and 14.57 %) for soybean and chickpea seeds, respectively. Pramod *et al.* (2014) exhibited the same pattern of reduction in protein based anti-nutritional factors in the eight cultivars of chickpeas that are selected for investigation. They reported

that the soaking treatments have significantly reduced certain anti-nutritional factors as phytates, tannins, trypsin inhibitor and increases antioxidants after 12 hours soaking thus, enhancing its absorption in the body and maintaining the nutritive value of seed intact in terms of trypsin inhibitor.

**Table 3:** Effect of pretreatments (soaking and heat treatment) on the levels of trypsin inhibitor activity (TIA) in chickpea and soybean seeds.

	Trypsin inhibitor activity (mg/g)	Reduction (%)
<b>Chickpea seeds</b>		
Raw	24.50	0.00
Soaking for 12 hrs.	20.93	14.57
<b>Soybean seeds</b>		
Raw	28.55	0.00
Soaking for 12 hrs.	25.32	11.31
<b>Bread samples<sup>a</sup></b>		
5 % CF + 5 % SPI	0.45	—
CF (relative value) <sup>b</sup>	9.00	63.27
5 % SF + 5 % CPI	0.33	—
SF (relative value) <sup>b</sup>	6.60	76.88

<sup>a</sup> CF= Chickpea flour, SF= Soybean flour, CPI= Chickpea protein isolate, SPI= Soy protein isolate

<sup>b</sup> calculated on basis of null (zero content) Trypsin inhibitor activity (TIA) values in both CPI and SPI

Baking temperature at (220 °C) for (14 min.) effectively destroyed trypsin inhibitor activity in the fortified bread samples. The reduction in TIA contents in baked bread fortified with (CF-SPI and SF-CPI) had been detected to be (63.27 % and 76.88 %, respectively). According to Rehman and Shah (2005) and Khattab and Arntfield (2009) the reduction in the level of anti-nutrients, along with an improvement in protein and starch digestibility was observed after cooking some food legumes was detected with a maximum level observed after autoclaving.

#### Protein isolates recovery and yield:

Results of protein isolates recovery (g/100g) and yield (%) from chickpea and soybean are presented in Table (4). Protein isolates were characterized by their high protein content (87.96 and 89.84 % for CPI and SPI, respectively). Moreover, soy protein isolate (SPI) had demonstrated higher recovered value (23.5 g/100g) as compared to chickpea protein isolate (CPI) which was found to be (15.2 g/100g). However, Protein yield (%) of chickpea (67.76%) was found to be greater than that of soybean (43.35%).

**Table 4.** Protein isolates recovery and yield from chickpea and soybean.

Legumes Protein Isolates	Chickpea protein Isolate (CPI)	Soy protein Isolate (SPI)
Protein content (%)	87.96	89.84
Isolate Recovery (g/100 g)	15.20±0.289	23.50±0.404
Protein Yield (%)	67.76±0.035	43.35±0.335

Values are expressed as mean ± standard error of three replicates

Study done by Qayyum *et al.* (2012) reported that protein content, recovery and yield for chickpea protein isolate (CPI) using isoelectric precipitation method were (80.67 %, 20.7 g/100 g and 73.14 %, respectively).

#### Quality evaluation of fortified pan bread

##### a) Physical evaluation:

Physical evaluation results of unfortified and fortified pan bread are represented in Table (5). Water uptake (ml) increased during preparation of dough fortified with SF-CPI (118.5 ml) and with

CF-SF (113 ml) as compared to that of the unfortified sample (111 ml). Whereas; water uptakes for both samples fortified with CF-SPI and CPI-SPI were equal to control. These findings indicated that fortified wheat dough with a mixture of both legumes flour or a mixture of soy flour and chickpea protein isolate had an effect on dough capacity for water absorption. Legumes protein has the ability to compete for water with other constituents in the dough system. The quantity of added water is considered to be very important for the distribution of the dough materials, their hydration and the gluten network development. This observation was in agreement with (Kinsella, 1979; Narayana and Narasinga Rao, 1982) who reported high water absorption capacity (WAC) obtained with the soy flour supplementation. Also, it was found that water uptake increased by 33.3 % as the ratio of soybean increased (10 % of wheat flour) compared to control during the preparation of shortening cake (Hemeda and Mohamed, 2010).

**Table 5.** Objective evaluation of pan bread fortified with different types of legumes (chickpea and soy bean) as flour and protein isolate.

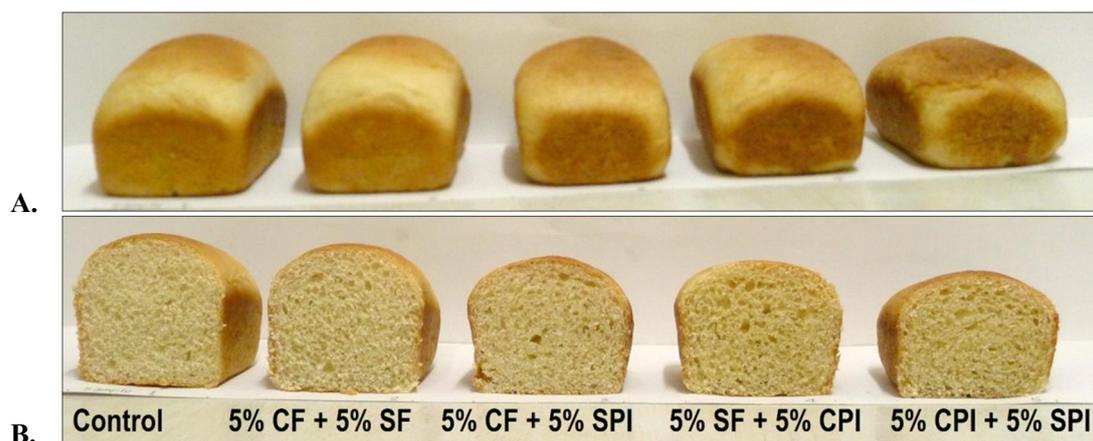
Objective Parameters	Unit.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
		Control	5 % CF + 5 % SF	5 % CF + 5 % SPI	5 % SF + 5 % CPI	5 % CPI + 5 % SPI
Water uptake	ml	111	113	111	118.5	111
1 <sup>st</sup> Fermentation Time	min.	37	37	36.5	38	38
2 <sup>nd</sup> Fermentation Time	min.	29	29	20	22	22
Baking Time	min.	14	12	14	13	13
Weight before baking	g	70	70	70	70	70
Weight after baking	g	63.80±0.16	63.87±0.22	64.51±0.18	64.63±0.22	64.70±0.18
% Change in weight	%	8.68	8.78	7.84	7.67	7.57
% Relative to control	%	100	100.11	101.11	101.30	101.41
Height	cm	5.08±0.04	4.83±0.03	4.50±0.06	4.48±0.04	4.00±0.06
% Relative to control	%	100	95.18	88.58	88.19	78.74
Area	cm <sup>2</sup>	24.40±0.14	23.32±0.13	21.90±0.03	21.56±0.06	19.12±0.02
% Relative to control	%	100	95.57	89.75	88.36	78.36
Volume	cm <sup>3</sup>	210.33±1.33	201.93±0.84	185.33±0.33	185.00±1.50	164.67±1.45
% Relative to control	%	100	96.01	88.11	87.96	78.29
Specific volume	cm <sup>3</sup> /g	3.30	3.16	2.87	2.86	2.55
% Relative to control	%	100	95.76	86.97	86.67	77.27

Values are expressed as mean± standard error of three replicates

Results in Table (5) show that dough fermentation time (1<sup>st</sup> and 2<sup>nd</sup>) did not affected by fortification with flour mixture of (CF-SF) than that of the control samples. However, baking time (min) decreased upon fortification with flour mixture of (CF-SF) as compared to other fortified bread samples and the unfortified sample. These results disagreed with the finding reported by Hemeda and Mohamed (2010) that baking time (min) showed an increase in shortening cake sample fortified with chickpea flour, while decreased upon fortification with soybean flour. Percentages change (%) in bread weight after baking for all samples fortified with mixture included protein isolate (CF-SPI, SF-CPI, SPI-CPI) were found to decrease as compared to bread sample fortified with legumes flour mixture and the control. This finding could be related to water binding capacity of protein isolate which delay dough water evaporation during baking process. This fact was confirmed by the obtained results of height, volume, area and specific volume (as percentage relative to control) for bread fortified with legume flour and protein isolate mixture or the both protein isolates in the present study (CF-SPI, SF-CPI and SPI-CPI). There were decreases in loaves' height (88.58, 88.19 and 78.74 %, respectively), volume (88.11, 87.96 and 78.29 %, respectively), area (89.75, 88.36 and 78.36, respectively) and specific volume (86.97, 86.67 and 77.27%). It can be concluded that fortification with protein isolate affects physical characteristics of wheat pan bread by causing decrease in the values of weight after baking, loaf height, volume, area, and specific volume measurements as compared to fortification with a legume flour mixture (CF-SF). According to El-Adawy (1997), two of the most important causes that have been suggested for loaf volume reduction are the disruptions of the wheat protein-starch interface by non-wheat proteins in the supplemented breads and the dilution

of wheat gluten, the protein matrix that is the primary determinant of baking quality, such that the integrity of the matrix is disturbed and non-functional. Therefore, the present study recommends that it is important to adjust the amounts of the yeast-leavened wheat-legume composite breads to maintain an acceptable loaf volume and height.

Figure 1A and B shows digital image of pan bread (volume, crumb structure and cell uniformity) fortified with different types of legume flour (chickpea and soybean) and protein isolate. Among all the fortified samples, the appearance and cell uniformity of fortified bread with legumes flour mixture was found to be close to that of the control sample. From the previous study results, it could be concluded that the texture of bread is affected by composition of bread, baking condition, and amount of water absorbed during mixing.



**Figure 1:** Digital image of pan bread (A: volume, B: crumb structure and cell uniformity) fortified with different types of legumes flour (chickpea and soybean) and protein isolate (*CF= Chickpea flour, SF= Soybean flour, CPI= Chickpea protein isolate, SPI= Soy protein isolate*).

The same findings were reported by Mohammed *et al.* (2014) who studied the effect of chickpea flour incorporation on the fresh bread characteristics. The volume of the control bread sample was significantly higher than that of samples incorporating chickpea ( $p < 0.05$ ). As the level of chickpea supplementation increased (10 to 30 %), the loaf volume of the corresponding fortified breads gradually decreased. It can be hypothesized that the chickpea flour suppress the amount of steam generated, as a result of their high water absorption capacity, leading thus to reduced loaf volume and greater crumb firmness. Dervas *et al.* (1999) and Doxastakis *et al.* (2002) also, reported a decrease in bread volume with increasing levels of lupine or soy flour and attributed this decrease to the dilution of the wheat gluten by the legume protein. The decrease in bread volume is also consistent with the findings of El-Adawy (1997) who worked with sesame seed protein preparations and reported that sesame protein isolate incorporation provided loaves with a lower specific volume, the extent of reduction depending on the substitution level. The present results are in agreement with the findings of Oluwole *et al.* (2017). They reported that bread samples fortified with soy protein isolate (SPI) had higher loaf weights than the cassava bread. While a significant reduction existed in the loaf volume and specific loaf volume of the soy fortified bread with increased level of soy protein isolate and cassava flour inclusion.

#### b) Pan bread degree of Tenderness:

Degree of Tenderness (mm/sec) results of pan bread prepared with flour and protein isolate from chickpea and soybean at initial time after baking and upon storage period for up to 5 days at room temperature (25°C) are presented in Table (6). The results of the present study indicated that degree of tenderness (freshness) at initial time (after baking) decreased (73.2 mm/sec) significantly ( $p \leq 0.05$ ) in bread fortified with CF-SPI, while increased (89.6 mm/sec) in bread fortified with legumes flour mixture (CF-SF). This increase may be attributed to water holding capacity of fiber content in soy flour (2.15%). The influence of partial substitution of wheat flour with chickpea flour at the levels of

(10, 20 and 30 %) in study done by Mohammed *et al.* (2014) reported that the control bread exhibited good crumb structure than the chickpea-enriched breads, indicating that chickpea addition exhibited a more resistant to deformation crumb. Addition of Chickpea brought a marked increase in crumb hardness probably as a result of the thickening of the crumb walls surrounding the air cells and the strengthening of the crumb structure by the protein particles. After 3 days of storage, there were significant decreases ( $P < 0.05$ ) in degree of tenderness of bread fortified with CF-SPI and SF-CPI (57.2 and 67.2 mm/sec, respectively) as compared to other fortified samples and control. Upon storage period for 5 days at room temperature, there was a noticeable decrease in degree of freshness of all fortified bread samples as compared to control. To counteract the deleterious effects of legumes in composite breads, additions of gluten and/or dough conditioners such as glycolipids, sucrose monolaurate, polyoxyethylene-8-stearate, potassium bromate are recommended (Fenn *et al.*, 2010).

**Table 6:** Degree of tenderness (mm/sec) of pan bread (unfortified and fortified) with different types of legumes (chickpea and soybean) under different storage conditions (at 25 °C for up to 5 days).

Storage time	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	LSD
	Control	5 % CF + 5 % SF	5 % CF + 5 % SPI	5 % SF + 5 % CPI	5 % CPI + 5 % SPI	
Zero Time (after baking)	84.4 ± 6.91 <sup>ab</sup>	89.6 ± 4.51 <sup>a</sup>	73.2 ± 4.71 <sup>b</sup>	85.0 ± 8.69 <sup>ab</sup>	83.33 ± 11.17 <sup>ab</sup>	10.06
3 Days	79.6 ± 5.55 <sup>a</sup>	78.8 ± 6.65 <sup>a</sup>	57.2 ± 4.21 <sup>c</sup>	67.2 ± 12.38 <sup>b</sup>	75.8 ± 5.97 <sup>ab</sup>	9.90
5 Days	64.6 ± 12.21 <sup>a</sup>	43.2 ± 7.82 <sup>bc</sup>	29.4 ± 6.77 <sup>d</sup>	31.2 ± 15.4 <sup>cd</sup>	56.6 ± 6.23 <sup>ab</sup>	13.61

▪ Data are expressed as means ± SD

▪ Means which have different superscript letters within the same row denotes significant differences at ( $P \leq 0.05$ ).

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

Different solutions have been developed to prevent staling bread such as the addition of  $\alpha$ -amylases which reduced the firming rate in bread and the recrystallization of the amylopectin in bread crumb. These enzymes hydrolyze linkages in starch, resulting in short chains which interfere with starch retrogradation and disrupt the continuity of the starch network (Gray and Bemiller, 2003). Others found that Egyptian Balady bread fortified with  $\alpha$ -amylase powder (AAP) or  $\alpha$ -amylase from barley (AAB) individually or combined with other additives such as; lipase enzyme (LE) and locust bean flour powder (LBF) maintained high degree of freshness under storage period up to 7 days at room temperature (Nasef *et al.*, 2016).

### c) Pan bread color evaluation

Color and appearance of baked products are among the important characteristics that indicate their quality acceptability. Figure (2) shows the outer crust color of the unfortified and fortified pan bread. It appeared that the outer crust color of the fortified bread with legumes flour mixture approximately the same as the unfortified sample (control). Hunter colorimeter results of unfortified and fortified bread (Table 7) show that the lightness decreased in the fortified bread samples as compared to the control. Among all the fortified samples, lightness values of outer color of bread with legume flour mixture (57.86) and with SF-CPI (59.38) were close to that of the control (61.63). On the other hand, the redness values increased in all the bread samples upon fortification as compared to control (15.76). The highest value was detected in bread sample fortified with legumes flour mixture (CF-SF) and with protein isolates (CPI-SPI) which were found to be (19.59 and 19.17, respectively).

It could be concluded from these results that different mixture of legumes flour and its protein isolates under the present investigation had an effect on bread color. Therefore, percentage used of legumes in such fortification process should be adjusted to maintain acceptable bread color and appearance. The results of the present study regarding the effect of fortification with legumes under investigation on bread yellow color disagreed with the findings reported by Fenn *et al.* (2010) who reported that supplementation of wheat flours with (5% or 8%) soybean, yellow pea or chickpea protein increased bread crumb yellowness ( $b^*$ ) significantly compared to the controls. Color browning of bakery product is usually related to such chemical reaction called (Maillard reaction), that chemical

reaction involving amino groups and carbonyl groups, which are common in foodstuffs leading to browning and flavor production. As reported by Fukui *et al.* (1993), Maillard reaction occurs between free amino groups of protein and carbonyl groups of reducing sugars, and lead to a decrease in the availability of amino acids involved and in protein digestibility. Basic amino acids are more reactive than neutral or acid amino acids. Lysine appears to be the most reactive amino acid, owing to the fact that it has two available amino groups (O'Brien and Morrissey, 1989). Furthermore, lysine is limiting in cereals, and loss in availability would immediately result in a decrease in protein nutritional value. Lysine may thus serve as an indicator of protein damage during processing. However, arginine, tryptophan, cysteine and histidine might also be affected (Iwe *et al.*, 2001).



**Figure 2:** Digital image of outer crust color of pan bread fortified with different types of legumes flour (chickpea and soybean) and protein isolate (CF= Chickpea flour, SF= Soybean flour, CPI= Chickpea protein isolate, SPI= Soy protein isolate).

**Table 7:** Hunter color values of pan bread (crust and crumb) fortified with different types of legumes flour (chickpea and soybean) and protein isolate.

Bread Samples *	L**	a**	b**	a/b	Saturation Index	Hue Angle	ΔE
<b>Exterior crust (outer surface)</b>							
1 Control	61.63	15.76	40.34	0.39	43.31	68.66	–
2 5 % CF + 5 % SF	57.86	19.59	39.36	0.5	43.97	63.54	5.46
3 5 % CF + 5 % SPI	55.28	18.15	35.34	0.51	39.73	62.82	8.43
4 5 % SF + 5 % CPI	59.38	17.62	38.70	0.46	42.52	65.52	3.35
5 5 % CPI + 5 % SPI	54.83	19.17	36.41	0.53	41.15	62.23	8.56
<b>Interior crumb</b>							
1 Control	79.50	1.74	25.35	0.07	25.41	86.07	–
2 5 % CF + 5 % SF	75.95	2.06	25.16	0.08	25.24	85.32	3.57
3 5 % CF + 5 % SPI	76.67	1.61	25.10	0.06	25.15	86.33	2.84
4 5 % SF + 5 % CPI	74.15	2.92	29.44	0.1	29.91	84.34	6.84
5 5 % CPI + 5 % SPI	69.89	4.66	30.86	0.15	31.21	81.41	11.46

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

\*\* L= Lightness, a= redness, b= yellowness

#### d) Sensory evaluation:

Sensory evaluation results of pan bread samples (unfortified and fortified) with different types of legumes flour (chickpea and soybean) and protein isolate are stated in Table (8). Among all the prepared fortified bread samples, the scores given (mean±SD) to each of the evaluated characteristics (appearance, internal color, external color, cell uniformity, taste, odor and overall acceptability) of bread fortified with legumes flour mixtures (5 % CF + 5 % SF) were found to be close to the control and better than that of the other fortified samples, followed by sample fortified with (SF-CPI). The present results of sensory evaluation disagreed with the finding of Ouazib *et al.* (2016) who stated that regarding bread quality, wheat replacement by (10%) processed chickpea flour led breads with similar technological and sensory characteristics than wheat bread. The increasing replacement (20%) resulted in harder breads, especially in the case of raw and toasted flour.

**Table 8:** Sensory Evaluation of pan bread samples (unfortified and fortified) with different types of legumes flour (chickpea and soybean) and protein isolate.

Sensory attributes	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	LSD
	Control	5 % CF + 5 % SF	5 % CF + 5 % SPI	5 % SF + 5 % CPI	5 % CPI + 5 % SPI	
Appearance	4.8 ± 0.42 <sup>a</sup>	4.5 ± 0.53 <sup>ab</sup>	4.0 ± 0.00 <sup>cd</sup>	4.3 ± 0.48 <sup>bc</sup>	3.8 ± 0.42 <sup>d</sup>	0.38
Internal Color	4.9 ± 0.32 <sup>a</sup>	4.3 ± 0.67 <sup>b</sup>	4.2 ± 0.63 <sup>b</sup>	4.1 ± 0.32 <sup>b</sup>	4.0 ± 0.47 <sup>b</sup>	0.46
External Color	4.7 ± 0.48	4.4 ± 0.70	4.4 ± 0.52	4.2 ± 0.63	4.0 ± 0.47	NS
Cell Uniformity	4.8 ± 0.42 <sup>a</sup>	4.4 ± 0.52 <sup>ab</sup>	4.4 ± 0.52 <sup>ab</sup>	4.2 ± 0.79 <sup>bc</sup>	3.8 ± 0.63 <sup>c</sup>	0.54
Taste	4.9 ± 0.32 <sup>a</sup>	4.4 ± 0.70 <sup>abc</sup>	4.1 ± 0.88 <sup>bc</sup>	4.7 ± 0.67 <sup>ab</sup>	3.9 ± 0.99 <sup>c</sup>	0.68
Odor	4.9 ± 0.32	4.5 ± 0.53	4.3 ± 0.83	4.5 ± 0.85	4.0 ± 0.82	NS
Overall Acceptability	4.7 ± 0.42	4.5 ± 0.71	4.3 ± 0.62	4.5 ± 0.83	4.0 ± 0.75	NS

- NS = Non-Significant.
- Data are expressed as means ± SD
- Means which have different superscript letters within the same row denotes significant differences at ( $P \leq 0.05$ ).
- CF= Chickpea flour, SF= Soybean flour, CPI= Chickpea protein isolate, SPI= Soy protein isolate

e) Nutritive value of pan bread (unfortified and fortified):

Chemical composition (%) of pan bread samples fortified with different types of legumes flour (chickpea and soybean) and protein isolate is presented in Table (9). Results indicated that protein increased gradually in all fortified bread samples as compared to control. Protein levels ranged from (12.8 % to 20.4 %) with the highest ratio recorded for sample containing the two legume protein isolates (5 % CPI + 5 % SPI) and the lowest for the control bread sample. These findings were in agreement with those reported by Shahzadi *et al.* (2005); Alabi and Anuonye (2007); Mishra and Chandra (2012) and Saleh *et al.* (2012). They reported that legumes with high protein content are widely used as composite flour in the production of bakery products, and therefore the protein content and quality of bakery such as biscuits can be improved by substituting cereal with legume as composite flour. Oluwole *et al.* (2017) fortified bread with blends of Soy protein isolate (SPI) and high quality Cassava Flour (HQCF) at (10, 15 and 20%) of wheat flour, the results showed that SPI improved protein content from (4.69-16.54%).

**Table 9:** Nutritive composition of pan bread samples (control and fortified) with different types of legumes (chickpea and soybean) as flour and protein isolate (% on wet weight basis).

Bread Samples *	Chemical composition (%)					
	Protein	Fat	Moisture	Ash	Carbohydrate**	Fiber
1 Control	12.8	2.59	24.23	2.08	57.87	0.43
Fortified samples						
2 5 % CF + 5 % SF	15.2	3.11	22.41	2.02	56.87	0.39
3 5 % CF + 5 % SPI	16.3	2.71	23.91	4.00	52.72	0.36
4 5 % SF + 5 % CPI	18.4	3.03	25.05	3.03	50.18	0.31
5 5 % CPI + 5 % SPI	20.4	2.99	24.42	2.06	49.85	0.28

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

\*\* Carbohydrates were calculated by difference

On the other hand, there was a reduction in carbohydrate and fiber contents in all bread samples in comparison with control as following; (from 57.87 to 49.85 %) and (0.43 % to 0.28 %), respectively. These results are in agreement, regarding carbohydrate content, with those reported by Tariqul-Islam *et al.* (2007); Sanful and Darko (2010) and Oluwole *et al.* (2017). They found that fortification of bread with either the defatted soy flour (DSF) or the chickpea flour (CPF) at different levels led to a decrease in carbohydrates. It could be noticed that, no differences were found in fat content among fortified and unfortified bread samples with flour and isolated protein from chickpea and soybean. It ranged from (2.71 – 3.11 %).

From Table (9), it could be also observed that ash content increased in sample 3 (4.0 %) and sample 4 (3.03 %). Moisture content ranged from 22.41 to 25.05 %, with the highest value in sample (4) and the lowest in sample (2). This increase could be due to the positive influence of increasing levels of protein on water-holding capacity, taking into consideration the high moisture content associated with using the DSF at different replacement levels, due to higher hydration rate that is associated with the high protein content (Rababah *et al.*, 2006). Earlier, Rehman and Shah (2005) worked on beans and legumes as a source of protein, fiber, starch and other nutritional components. They elucidated that legumes provide good quality protein that acts as major contributor amongst phyto-proteins.

Mineral composition (mg/100 g) of pan bread fortified with different types of legumes flour (chickpea and soybean) and protein isolate is shown in Table (10). It could be noticed that pan bread fortified with (5 % CF and 5 % SPI) demonstrated the highest Iron (Fe) content (11.04 mg/100 g) compared to the other fortified and unfortified bread samples (8.391 to 8.697 mg/100 g). Regarding Calcium (Ca) content, there was an increment in bread with (5 % SF + 5 % CPI) and bread with (5 % CF + 5 % SF) which were found to be (47.63 and 47.19 mg/100 g, respectively). This was due to the incorporation of soy flour (SF) that contains very high content of Calcium (Ca) (458.20 mg/100 g). However, Zinc (Zn) levels were found to be very low in fortified and unfortified pan bread with chickpea-soybean flour and/or protein isolate. The same finding was reported by Abou-Arab *et al.*, (2010) who found that mineral contents (K, Ca, Na, Mg, Cu, Fe and Zn) increased in the processed spaghetti samples as the level of replacement (10, 15, 20, 25 and 30 %) with chickpea flour increased.

**Table 10:** Mineral composition (mg/100 g) of pan bread (control and fortified) with different types of legumes (chickpea and soybean) as flour and protein isolate.

Bread Samples**	Mineral composition (mg/100 g)		
	Zinc (Zn)	Iron (Fe)	Calcium (Ca)
1 Control	ND*	8.625	30.20
Fortified samples			
2 5 % CF + 5 % SF	ND*	8.466	47.19
3 5 % CF + 5 % SPI	ND*	11.04	35.80
4 5 % SF + 5 % CPI	ND*	8.697	47.63
5 5 % CPI + 5 % SPI	ND*	8.391	31.37

\* ND = Not Detected

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

### Biological investigation:

The biological investigation results: body weights (g), weight gain (g), body weight gain (BWG), feed intake (FI) (g/day/rat) and feed efficiency ratio (FER), in experimental rats fed with diet containing flour and protein isolate of two types of legumes (chickpea and soybean) are stated in Table (11). Results showed that by the end of the experiment period, body weight gain (%) was greater for rats fed on SF-CPI diet (56.00±4.27), followed by those fed on diets supplemented with CF-SPI (54.00±3.75) as compared to that of group fed on basal diet (41.17±3.70) which were found to be significantly different at (P<0.05). Rats in group fed on a mixture of soybean flour (SF) and chickpea protein isolate (CPI) exhibited higher feed efficiency ratio (FER) which was found to be significantly different at (P<0.05) as compared to animal fed on basal diet (4.87±0.28 and 3.92±0.28, respectively). On the other hand, the study showed no significant difference was found at (P<0.05) with regards to FER between both groups fed on a mixture of two types of legume flour and protein isolate (groups 2 and 3). These findings revealed that (SF-CPI) supplemented diets enhanced growth, BWG and FER followed by supplementation with (CF-SFI). Isolation protein process followed in the present study had a reducing effect on anti-nutrients of the pretreated legumes (soaking and thermal treatments) which caused positive impacts on rats' growth. This finding came in agreement with Siddhuraju and Becker (2005) who stated that hydrothermal processing not only concentrated or isolated proteins, but also destroyed and reduced the level of anti-nutrients.

Moreover, the biological results of the present study (Table 11) revealed that there were increases in organs weigh (%) relative to body weight of both groups (groups 2 and 3) fed on a mixture of two types of legume flour and protein isolate as compared to the control. These differences were found to

be significant ( $P<0.05$ ) with regards to liver and kidneys weight of the control group as compared to groups (2 and 3). In addition, serum albumin and globulin (g/dl) in both groups (2 and 3) were found to be significantly ( $P<0.05$ ) higher than that of the control. These findings disagreed with the study reported by Macarulla *et al.* (2001) who stated that feeding hypercholesterolaemic rats on diets containing faba bean seeds, or the protein isolate prepared by isoelectric precipitation and spray dried significantly lower body weights and energy intakes than that of rats fed on casein diets. Also, the findings of the present study disagreed with the findings of Rubio *et al.* (1998) who reported that inclusion of either chickpea meal or its globulin proteins in the diet significantly increased the amount of Nitrogen excreted, primarily as urea, through the urine. Urea levels in plasma in rats fed diets containing chickpea meal, globulins or soybean meal were significantly higher than in those fed lactalbumin. The low nutritional value of chickpea meal is likely to be due mainly to adverse effects of its globulin proteins on growth and Nitrogen metabolism rather than to the action of any known anti-nutritional factor present in the diet.

**Table 11.** The biological investigation results of experimental rats fed on a mixture of legumes flour and protein isolate as compared to control group (on basal diet).

Biological parameters	Group (1) Control basal diet	Group (2) 5% CF + 5% SPI	Group (3) 5% SF + 5% CPI
Initial weight (g)	108.0±1.95 <sup>a</sup>	109.7±2.56 <sup>a</sup>	110.8±2.28 <sup>a</sup>
Final Weight (g)	152.3±1.37 <sup>b</sup>	169.8±1.10 <sup>a</sup>	172.5±1.75 <sup>a</sup>
Weight Gain (g)	44.2±3.19 <sup>b</sup>	59.0±2.67 <sup>a</sup>	61.7±3.59 <sup>a</sup>
Body Weight Gain (%)	41.17±3.70 <sup>b</sup>	54.00±3.75 <sup>a</sup>	56.00±4.27 <sup>a</sup>
Feed intake (FI) (g/day/rat)	11.27	13.14	12.66
Feed Efficiency Ratio (FER)	3.92±0.28 <sup>b</sup>	4.49±0.20 <sup>ab</sup>	4.87±0.28 <sup>a</sup>
<b>Relative Organs Weight (%)</b>			
Liver	2.76±0.17 <sup>b</sup>	3.48±0.03 <sup>a</sup>	3.65±0.13 <sup>a</sup>
Kidney	1.43±0.02 <sup>b</sup>	1.78±0.05 <sup>a</sup>	1.74±0.04 <sup>a</sup>
Spleen	0.29±0.02 <sup>b</sup>	0.35±0.02 <sup>ab</sup>	0.40±0.03 <sup>a</sup>
Heart	0.45±0.04 <sup>a</sup>	0.46±0.01 <sup>a</sup>	0.46±0.02 <sup>a</sup>
<b>Biochemical Serum analysis</b>			
Albumin (g/dl)	2.58±0.15 <sup>b</sup>	3.52±0.24 <sup>a</sup>	3.27±0.10 <sup>a</sup>
Globulin (g/dl)	2.32±0.18 <sup>b</sup>	2.97±0.04 <sup>a</sup>	3.27±0.22 <sup>a</sup>

Values are expressed as Mean ± Standard Error.

Values at the same row with different letter are significantly different at  $P<0.05$ .

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

In general, it could be concluded that soybean flour-chickpea protein isolate (SF-CPI) as well as chickpea flour-soy protein isolate (CF-SPI) supplemented diets could be useful in growth enhancement and food formulations. Also, in spite of the fact that the pretreatments methods followed with preparation of legumes under the present study did not completely reduced anti-trypsin inhibitors of legumes, but also according to the present results did not affect digestibility and bioavailability of these legumes protein.

## Conclusion

The benefit of producing cereal-legume composite foods may be considered as twofold: (1) There is an overall increase in the protein content of the composite food compared to when only the cereal forms the base, and (2) there are better amino acids balance due to the contribution of lysine by legumes and the contribution of methionine by cereals. 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. The reduction of viscoelastic properties of wheat flour dough, after substitution by legume flour, reduces bread making potential. The ratio of flours in the composite may influence dough rheology and, consequently, physical and sensory characteristics of their baked goods. This directly influences consumer acceptability of final products. In the present study, supplementation of pan bread with a mixture of either both legumes flour or soy protein isolate and chickpea flour caused

better impacts on final product quality. There seems to be a potential market for chickpea flour in the bread industry. The present investigation recommend that development of such functional foods would be beneficial to improve the nutritional status of consumer. Moreover, the results presented in this study will be helpful to the food technologists for developing protein enriched formulation for community needs.

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