Impact Of An Under-Explored Seed Protein: Seinat (Cucumis Melo Var. Tibish) Addition On The Physicochemical, Sensory And Nutritional Characteristics Of Wheat Flour Bread

Azhari Siddeeg, Al-Farga Ammar, Elmuez Alsir, Yanshun-Xu, Qixing-Jiang, Wenshui-Xia

ABSTRACT: The aim of this study was to evaluate the effect of supplementing wheat flour with 5, 10, and 15% seinat seed proteins on physicochemical, nutritional and sensory properties of bread. Seinat seeds protein concentrate (SSPC) and seinat seeds protein isolates (SSPI) were added to wheat flour (WF) to make bread. Dough characteristics were studied using a Brabender Farinograph and Extensograph. Loaves were prepared by using the straight dough procedure. Water absorption was increased ($p<0.05$) as the protein level in the blends increased. The dough stability time decreased ($p<0.05$) with addition of SSPC and SSPI. Addition of 15% SSPC and SSPI to WF did not have any effect on loaf sensory characteristics. WF protein content significantly increased on addition of SSPC and SSPI. The study demonstrated that addition of SSPC and SSPI increases essential amino acids, mineral, biological value and chemical score, thus improving the nutritional value of bread.

KEYWORDS: Seinat seed, protein isolate, protein concentrate, physiochemical properties, nutritional value, sensory evaluation.

INTRODUCTION

Similar to a soybean protein isolates, seinat (*Cucumis melo* var. *tibish*) seed protein isolates contain a high protein content and may therefore be applied as a potential protein fortifier in a variety of food products or even be used as a food ingredient [1]. In our previous study, we reported that seinat seeds contain 31.1% crude oil, 4.2% moisture, 24.7% fiber, 28.5% protein, 4.3% ash and 6.9% total sugars. Furthermore seinat seed is an excellent source of edible oils which comprises the main fatty acids such as linoleic acid 61.10%, oleic acid 18.75%, palmitic acid 10.37%, and stearic acid 9.18% [2]. Seinat seeds are characterized as oilseeds and like other oilseeds such as cotton, peanut, sunflower and sesame seeds, the seeds have varying effects on the dough mixing, loaf volume characteristics and the overall baking qualities compared to oil seed- wheat flour combination. For instance, heating enhances the bread making characteristics of cottonseed and sunflower proteins but has a detrimental effect on the same properties in peanut and sesame proteins [3]. Protein concentrates and isolates play an important role in improving the nutritional and structural properties such as, water holding capacity, solubility, emulsification, gelation and foamability in different processed foods [4]. Nowadays, high-protein wheat flour fortification is being used to increase protein content and to improve essential amino acid balance of bread and to combat worldwide protein malnutrition [5]. In recent years, the increase in urbanization as well as the advancement in baking technology and the change in food habits has seen bakery food products such as bread becoming popular in urban and semi urban areas in most developing countries [6]. Doughnuts exist as fermented fried sweet snacks that are served quite extensively in hotels, restaurants and snack bars in many countries of the world. The addition of high protein content flour when forming yeast raised doughnuts results in a strong visco-elastic dough [7]. Although wheat is considered a good source of energy and other nutrients, wheat protein is deficient in some essential amino acids such as lysine [8]; it therefore requires to be supplemented by other foods rich in such essential amino acids to provide a perfect diet. We therefore set out to determine the effects of supplementation of wheat flour with seinat seed protein concentrate and isolate on the physical properties of dough, baking quality characteristics and chemical composition, mineral contents, amino acid profile as well as to assess the nutritional value of bread made from wheat and seinat seed protein blend preparations.

- *State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, 214122, China.*
- *Department of Food Science and Technology, Faculty of Engineering and Technology, University of Gezira, Wad Medani, P.O. Box 20, Sudan.*
- *Corresponding author: Tel: +8618762651031. Fax: +86-510-85329057.*
- *E-mail Address: xiaws@jiangnan.edu.cn. azhari_siddeeg@yahoo.com.*

MATERIALS AND METHODS

Raw material

Dried seinat fruits were brought from the farm (Wad Medani City, Gezira State, Sudan), and transported to the Food Processing and Ingredients laboratory in Jiangnan University, China. The seeds were removed manually from the fruits and stored in desiccators at room temperature. Wheat flour was obtained from China Oil and Food Corporation (COFCO) Pangthai Co. Ltd, Qinhuangdao, Hebei province, People's Republic of China.

Preparation of defatted and proteins of seinat seed

Defatted seinat seed flour, seinat seed protein concentrate and isolate were prepared as shown in Fig.1. After preparation seinat seed protein concentrate and isolate powder were packed in polyethylene bags and stored at 4°C until used.

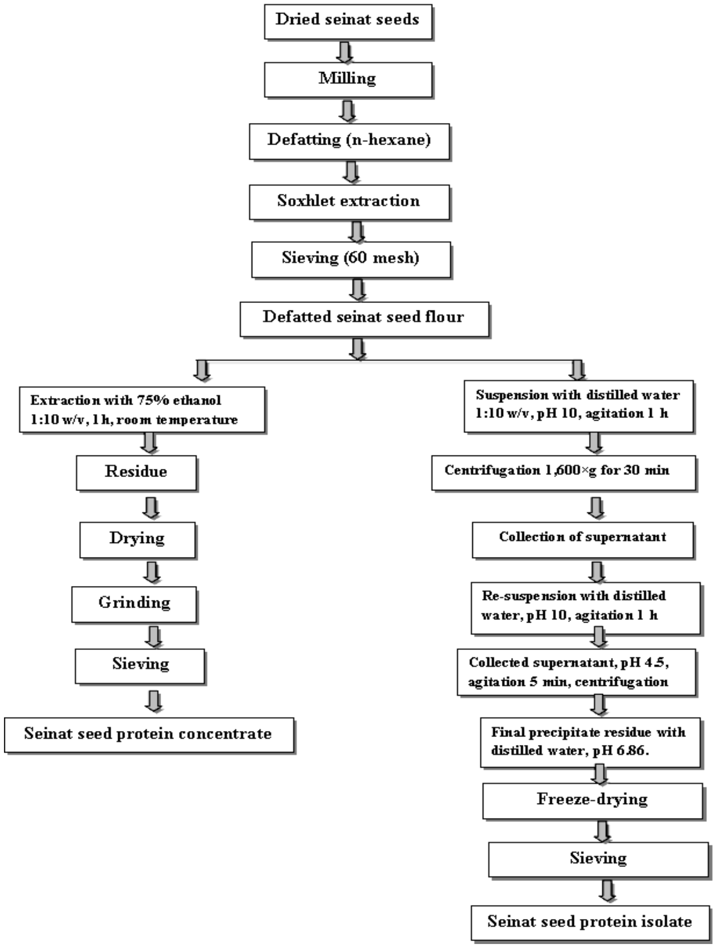

Fig.1 Preparation of protein concentrate and isolate obtained from seinat (*Cucumis melo* var. *tibish*) seed.

Determination of the dough physical properties
Seinat seed protein concentrate and isolate (5, 10 and 15%) were incorporated into wheat flour at different and 300 grams of wheat flour was used as a control. Water absorption, development time and stability time of the dough formed from the blends was determined according to the AACC[9] constant flour method 54-21 using a Barbender Farinograph. The flour blends and the control were mixed to an optimum water absorption level, generating a Farinograph curve centered at the 500 BU line. The extensibility and resistance to extension were determined according to the AACC [9] constant flour method 54-10 using a Barbender Extensograph. All the physical properties of dough were assessed in triplicate.

Preparation of pan bread
Pan bread was prepared using the flour weight based baking formula which constitutes, 100 g of 0-15% SSPI and SSPC supplemented flour, 1.0 g sugar, 1.50 g salt, 1.0 g active dry yeast ,1.0 g shortening and addition of an appropriate amount of water as determined using the farinograph absorption test [3]. This was followed by a straight dough preparation procedure that involved 3-hr fermentation, 55 min proofing at 30°C and 25 min baking at 220°C.

Loaf weight and volume
After baking, the loaf volume was immediately measured using the rapeseed displacement method along with the loaf weight. All measurements were conducted in triplicate and the average values were used for calculating the loaf specific volume as follows; loaf volume divided by loaf weight.

Chemical composition
The total protein content was evaluated using a FOSS nitrogen analyzer (DK-3400 Hilleroed, Denmark) with a 6.25 conversion factor. Fat, moisture, fiber, and ash contents were determined using standard AOAC methods 932.06, 925.09, 985.29, and 923.03, respectively [10], and the carbohydrate content was determined by the difference, based on dry weight.

Mineral analysis
To analyze the mineral composition of the samples, ash was prepared from the flour blends at 550°C, the ash was then dissolved in 6 M HCl and the final volume of the solution was brought to 10 mL. The calcium content was estimated using the titrimetric method by Clark and Collip [11]. The iron content was quantified using UV-Visible spectrophotometry (model UV-160A; Shimadzu, Shanghai, China) at 480 nm [12]. The magnesium content was analyzed according to Ranganna [13], where the blue color solution that was developed was quantified at 650 nm using UV-Visible spectrophotometry (model UV-160A; Shimadzu) and the final amount of magnesium was expressed as magnesium/100 g meal. Other minerals were analyzed using atomic absorption spectroscopy (AA 6701F, Atomic Absorption Flame Emission Spectrophotometer equipped with hollow cathode lamps; Shimadzu).

Amino acid analysis
The samples were digested using 6M HCl at 110°C for 24 h under a nitrogen atmosphere. The presence of amino acids in the treated samples was then assessed using a o-phthaldialdehyde (OPA) derivatized pre-column reversed phase high performance liquid chromatography (RP-HPLC) Agilent 1100 series (Agilent Technologies, Palo Alto, CA, USA) assembly system . Each sample (1 µL) was injected on a Zorbax 80 A C18 column (5 µm 4.0 × 250 mm, Agilent Technologies, Palo Alto, CA, USA) at 40°C with detection set at 338 and 262 nm. Mobile phase A was 7.35mmol/l sodium acetate/ triethylamine/ tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mmol/l sodium acetate/methanol/acetonitrile (1:2:2, v/v/v).

Determination of nutritional characteristics
The biological values of the bread samples were determined on the basis of amino acid profiles. The chemical score (CS) of amino acids was calculated using the FAO/WHO [14] reference pattern. Essential Amino Acid Index (EAAI) was calculated according to Oser [15] using the amino acids composition of the whole egg protein published by Hidvigi and Bikes [16] as a reference. Protein efficiency ratio (PER) was estimated according to the following regression equation proposed by Alsmeyer, Cunningham, and Happich [17]:

$$BV = 1.09 \times EAAI - 11.7$$
$$PER = -0.684 + 0.456\ (Leu) - 0.047\ (Pro).$$

Sensory evaluation

Sensory evaluation was performed using a 10 member panel (trained) to measure crust color, crumb color, crumb texture, flavor, taste and overall acceptability. A hedonic scale of 1 to 9 was used; 1: extremely bad, 2: very bad, 3: bad, 4: fairly bad, 5: satisfactory, 6: fairly good, 7: good, 8: very good, 9: excellent.

Statistical analysis

All experiments were conducted in triplicate and statistical analysis was performed using SPSS version 16.0 software for Windows (SPSS Inc; Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to determine significant differences between means and Duncan's test was used to perform multiple comparisons between means at $p<0.05$significance level.

RESULTS AND DISCUSSION

Chemical composition of seinat seed proteins and wheat flour

The chemical composition evaluation of seinat seed protein concentrate and isolate and wheat flour demonstrated that SSPI had a significant higher ($p<0.05$) protein content than SSPC and WF (Fig. 2). This phenomenon has also been reported in sesame proteins [3] as well as in SSPI [1]. The high protein content was due to the prior extensive defatting of seinat seed flour using hexane and extraction at a high pH value. Therefore, addition of seinat proteins to wheat flour increased both protein and ash contents of the bread. Moisture and crude fat content of WF was significantly higher ($p<0.05$) than SSPC and SSPI. The total carbohydrate content of WF (82.26%) was also significantly higher ($p<0.05$) than SSPC (19.72%) and SSPI (8.55%).

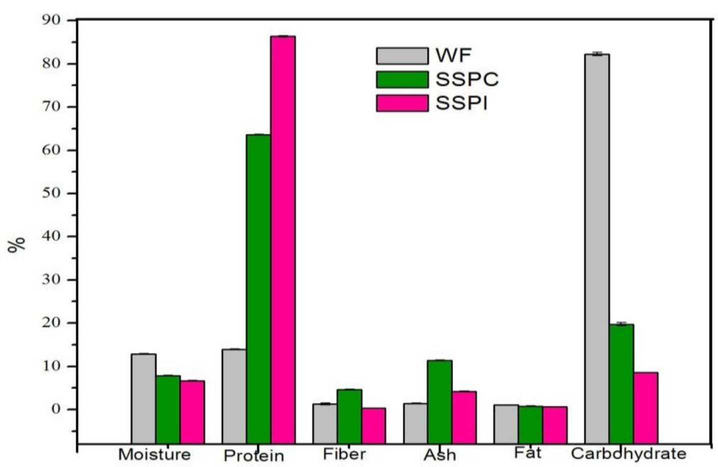

Figure 2 Chemical composition of seinat seed proteins and wheat flour on dry weight basis. Values represent the means±standard deviation of triplicates. WF, SSPC and SSPI denote wheat flour (control), seinat seed protein concentrate and seinat seed protein isolate, respectively.

Physical properties of dough

Farinograph and Extensograph evaluation of the SSPC and SSPI (5, 10 and 15%) supplemented flour samples showed an overall increase in water absorption compared to WF (control) (Table1).

Table 1. Farinograph and Extensograph characteristics of seinat seed proteins with wheat flour blends.

Blends	Water Absorption %	Development time (min)	stability time (min)	Extensibility^{a} (mm)	Resistance to Extension R.BU^{a}	Proportional number^{a}
WF	61.80±0.20^{f}	1.90±0.04^{e}	4.50±0.10^{a}	97.00±0.05^{e}	290.00±0.12^{a}	2.98±0.02^{a}
SSPC (5%)	63.20±0.15^{e}	3.20±0.09^{a}	3.90±0.10^{b}	105.00±0.20^{d}	189.00±0.50^{b}	1.80±0.01^{b}
SSPC (10%)	64.50±0.18^{d}	3.10±0.05^{ab}	3.70±0.08^{b}	109.00±0.62^{ab}	170.00±0.46^{d}	1.56±0.01^{d}
SSPC (15%)	64.90±0.08^{c}	2.90±0.11^{cd}	3.69±0.07^{cd}	110.00±0.89^{a}	160.00±0.59^{e}	1.46±0.02^{e}
SSPI (5%)	65.10±0.05^{c}	3.10±0.08^{ab}	3.90±0.09^{b}	106.00±0.21^{c}	190.00±0.80^{b}	1.79±0.01^{b}
SSPI (10%)	66.80±0.10^{b}	3.00±0.10^{bc}	3.80±0.12^{bc}	108.00±0.09^{b}	175.00±0.90^{c}	1.62±0.03^{c}
SSPI (15%)	67.90±0.09^{a}	2.80±0.07^{d}	3.60±0.11^{e}	109.00±0.20^{ab}	175.00±0.15^{c}	1.60±0.01^{c}

Values represent the means±standard deviation of triplicates. WF, SSPC and SSPI denote wheat flour (control), seinat seed protein concentrate and seinat seed protein isolate, respectively. Means in the same column with different letters as superscripts are significantly different ($p<0.05$). a Proving time: 90 min. The water absorption percentage increased significantly ($p<0.05$) due to the addition of SSPC and SSPI in all the blends. The wheat flour blended with SSPC and SSPI generally had higher water absorption than the WF. The WF-SSPI blends appeared to have the highest ($p<0.05$) water absorption compared to the WF- SSPC blends at all the ratios (Table 1). The increase in water absorption observed in all wheat flour blends was due to an increase in hydration capacity of seinat seed proteins, especially SSPI. These results are supported by El-Soukkary [18] who found that water absorption increased substantially with a 3% addition of native pumpkin protein concentrate and isolate to wheat flour. Furthermore, the farinograph assessment clearly showed that decrease in dough development time of SSPC and SSPI that was directly proportional with increase in seinat protein levels (Table 1). All blends had significantly higher ($p<0.05$) dough development time than the control, however, dough development time decreased with an increase in the amount of seinat seed protein. According to Morad, El-Magoli and, Afifi [19], the increase in dough development time can be attributed to the differences in water absorption capacity of various protein sources and wheat flour. Dough stability time indicated that addition SSPC and SSPI lowered ($p<0.05$) the stability time relative to the control (Table 1). These results are supported by Rehman *et al.* [8], who indicated that the stability time of wheat flour dough was higher than that of wheat flour partially substituted with vetch (*Lathyrus sativus L*) flour. On the other hand, dough stability time decreased with an increase in the amount of seinat protein. As shown in Table 1 also, extensograph parameters (extensibility, resistance to extension and the proportional number) of WF, SSPC and SSPI supplemented wheat flour were analyzed. The results indicate that extensibility increased with an increase in the amount of seinat protein in the flour blends, but SSPI had higher ($p<0.05$) extensibility than SSPC and WF. Resistance to extension and the proportional number decreased with an increase in the amount of seinat protein in the flour blends. Ammar, Hegazi and Bedier, [20], observed the same trends in their study on bread making using wheat flour partially substituted with taro flour.

Weight and volume of loaves

The results for loaf weight, loaf volume and specific volume are shown in Table 2. Loaf weight increased with an increase in the amount of seinat seed protein, while loaf volume and specific volume decreased with an increase in the amount seinat seed protein. The loaf weight of the composite bread samples ranged from 131 to 137 g. Loaf weight reduction during baking has an impact on bread quality attributes as consumers are often attracted to bread with high weight and volume believing that it has more substance for the same price [21]. Loaf volume of the bread samples ranged from 418 to 439 mL. Loaf volume of the control bread was significantly higher ($p<0.05$) than that of SSPC and SSPI supplemented wheat flours. These results are also in agreement with the results obtained by El-Adway [3], in their study on sesame seed protein supplemented wheat flour. As reported by Shittu, Raji and Sanni [22], the specific volume, which is the ratio of the loaf weight and loaf volume, has been generally adopted as a more reliable measure of loaf size.

Sensory evaluation of bread

Sensory evaluation of bread samples was undertaken with consideration of the most acceptable parameters; crust color, crumb color, texture, flavor, taste and the overall acceptability was taken and used as a control (Table 2 and Fig.3). Sensory analysis of seinat seed protein fortified bread samples was evaluated. There were no significant differences ($p>0.05$) in crumb color, taste and overall acceptability among all bread samples. This suggests that bread prepared from 15% SSPC and 15% SSPI substituted wheat flour does not significantly affect desirable sensory attributes of the bread. Crust color and flavor evaluation showed some significance differences ($p<0.05$) among the samples.

Table 2. Sensory evaluation and loaf volume and weight of seinat seed proteins with wheat flour blends

Blends	Crust color	Crumb color	Crumb texture	Flavor	Taste	Overall acceptability	loaf weight (g)[a]	loaf volume (Cm^3)[a]	Specific volume (Cm^3/g)[a]
WF	7.90[ab]	7.80[a]	6.70[b]	7.60[ab]	7.40[a]	7.80[a]	131.40±0.53[d]	439.00±0.25[a]	3.34±0.02[a]
SSPC (5%)	7.80[ab]	7.60[a]	7.50[ab]	7.70[ab]	6.90[a]	7.40[a]	133.00±0.45[c]	425.00±0.86[c]	3.19±0.01[b]
SSPC (10%)	7.70[ab]	7.50[a]	7.40[ab]	6.80[b]	6.90[a]	7.50[a]	134.50±0.60[b]	420.00±0.90[d]	3.12±0.02[c]
SSPC (15%)	7.40[b]	7.30[a]	7.50[ab]	7.70[ab]	7.00[a]	7.30[a]	135.00±0.90[b]	418.00±0.89[e]	3.09±0.03[c]
SSPI (5%)	7.80[ab]	7.60[a]	7.50[ab]	7.90[ab]	6.90[a]	7.40[a]	135.50±0.50[b]	429.00±0.90[b]	3.16±0.01[b]
SSPI (10%)	8.00[ab]	7.30[a]	8.10[a]	7.70[ab]	7.20[a]	8.20[a]	137.00±0.90[a]	426.00±0.91[c]	3.10±0.03[c]
SSPI (15%)	8.50[a]	7.60[a]	7.10[ab]	8.30[a]	7.20[a]	7.80[a]	137.50±0.62[a]	419.00±0.60[d]	3.04±0.02[d]

[a] Values represent the means±standard deviation of triplicates.
WF, SSPC and SSPI denote wheat flour (control), seinat seed protein concentrate and seinat seed protein isolate, respectively.
Means in the same column with different letters as superscripts are significantly different ($p<0.05$).

The results from sensory evaluation revealed; a preference for crust color and flavor of the 15% SSPI-wheat flour blend, taste and crumb color preference for the control and crumb texture and overall acceptability preference for the 10% SSPI-wheat flour blend bread samples.

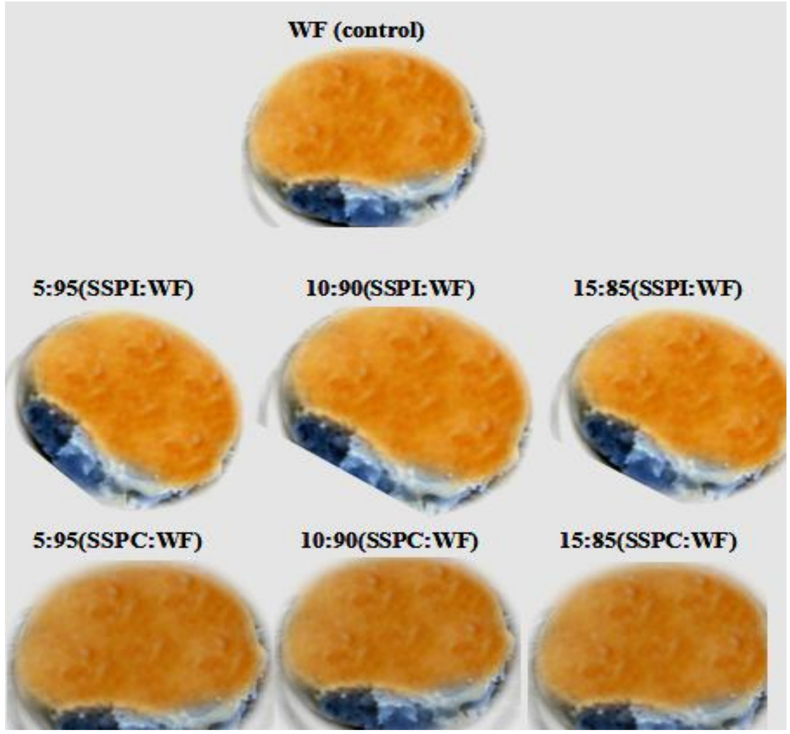

Figure 3. Fortified breads of seinat seed proteins-wheat flour blends. WF, SSPC and SSPI denote wheat flour (control), seinat seed protein concentrate and seinat seed protein isolate, respectively.

Proximate chemical composition of breads

Proximate chemical composition in SSPC, SSPI and WF was evaluated. The findings showed that wheat flour contained 14.08 % protein. The protein content increased after adding SSPC and SSPI, the values ranged from 14.61-16.03% and 18.87-20.02% respectively (Table 3). There was an overall decrease in the total carbohydrate content in the SSPI and SPPC supplemented wheat flour with values ranging from 73.22-75.17 % and 76.16-77.87%, respectively compared to in the WF (80.78%) control sample (Table 3). The SPPC and SSPI supplemented wheat flour samples however, showed an increase in; ash (2.84% and 2.98%), crude fiber (1.95% and 2.45%) as well as crude fat (2.69% and 2.71%) compared to the WF control samples with 1.30% Ash, 1.26% crude fiber and 2.58% crude fat. The changes in proximate composition of bread samples are a result of seinat seed protein addition. Addition of SSPC and SSPI to wheat flour increased protein content significantly. There were significant differences ($p<0.05$) in total carbohydrate content were observed between bread fortified by SSPC and SSPI.

Table 3. Chemical composition of bread fortified with seinat seed proteins

Blend	Crude protein	Ash	Crude fiber	Crude fat	Carbohydrate
WF	14.08±0.13^{d}	1.30±0.02^{d}	1.26±0.05^{e}	2.58±0.04^{e}	80.78±0.11^{a}
SSPC (5%)	14.61±0.76^{d}	2.84±0.19^{b}	1.98±0.11^{b}	2.69±0.01^{b}	77.87±0.76^{b}
SSPC (10%)	15.64±0.31^{c}	2.87±0.14^{a}	2.05±0.10^{ab}	2.70±0.01^{ab}	76.77±0.37^{c}
SSPC (15%)	16.03±0.13^{c}	2.98±0.08^{a}	2.11±0.09^{a}	2.71±0.02^{a}	76.16±0.08^{c}
SSPI (5%)	18.87±0.11^{b}	1.95±0.17^{c}	1.40±0.02^{d}	2.61±0.01^{d}	75.17±0.27^{d}
SSPI (10%)	19.48±0.06^{a}	2.10±0.15^{c}	1.46±0.01^{d}	2.66±0.02^{c}	74.30±0.11^{e}
SSPI (15%)	20.05±0.09^{a}	2.45±0.14^{b}	1.61±0.01^{c}	2.68±0.02^{bc}	73.22±0.17^{f}

Values represent the means±standard deviation of triplicates. WF, SSPC and SSPI denote wheat flour (control), seinat seed protein concentrate and seinat seed protein isolate. Means in the same column with different letters as superscripts are significantly different ($p < 0.05$).

The low carbohydrate content of the seinat protein isolate supplemented bread samples compared to the control and seinat protein concentrate bread samples, could have been due to multiple extraction using alkali and acid. The low carbohydrate content after addition of seinat proteins to wheat flour are in full agreement with those reported by Salama *et al.* [23]. The ash content of seinat seed protein supplemented bread samples were significantly higher ($p<0.05$) than that of the control, therefore, the addition of seinat seed proteins (SSPS and SSPI) to wheat flour increased the ash content of the bread samples. In a study carried out by El-Soukkary [18] on pumpkin seed protein concentrate and isolate and wheat flour blends, it was as observed that the ash content of wheat flour bread (1.53%) was increased after addition of pumpkin seed protein isolate and concentrate (1.61 and 1.93%), respectively. Crude fiber and crude fat of SSPC and SSPI breads were higher ($p<0.05$) than the control; increasing with increase in protein content.

Mineral content of breads

The overall mineral was higher in the bread made from the SPPC and SSPI supplemented wheat flour compared to the WF derived bread (Table 4). Higher mineral contents have also been reported in bread made from sesame supplemented wheat flour [3]. Bread samples baked with the SSPC supplemented flour, had a higher ($p<0.05$) content of minerals, except sodium (Na), than SSPI supplemented and the control flour. There were generally significant differences ($p<0.05$) in mineral content among the SPPC and SSPI supplemented and control bread samples. Potassium and sodium were the most abundant minerals in the bread samples.

Table 4. Mineral content (mg/100 g) of bread fortified with seinat seed protein

Mineral	WF	SSPC[a]	SSPI[b]
Zn	2.13±0.16^{b}	10.59±0.44^{a}	2.34±0.08^{b}
Fe	3.46±0.10^{c}	4.78±0.21^{a}	3.75±0.07^{b}
Cu	0.93±0.09^{b}	2.22±0.12^{a}	0.88±0.10^{b}
Mn	1.60±0.18^{b}	1.90±0.13^{a}	1.80±0.05^{ab}
K	104.53±0.41^{b}	153.20±0.20^{a}	85.40±0.53^{c}
Na	465.07±0.12^{c}	485.21±0.22^{b}	558.40±0.02^{a}
Mg	64.83±0.29^{c}	219.33±0.39^{a}	114.50±0.50^{b}
Ca	93.16±0.15^{b}	139.35±0.33^{a}	86.29±0.34^{c}

Values represent the means±standard deviation of triplicates. WF, SSPC and SSPI denote wheat flour (control), seinat seed protein concentrate and seinat seed protein isolate. [a] Blend of 15 g seinat seed protein concentrate with 85 g wheat flour . [b] Blend of 15 g seinat seed protein isolate with 85 g wheat flour. Means in the same row with different letters as superscripts are significantly different ($p<0.05$).

The highest potassium content (153.20 mg/100g) was observed in the bread samples made from the 15% SSPC supplemented flour while a much lower potassium content (85.40 mg/100g) was registered in the in the bread made from the 15% SSPI supplemented flour. These results further supports earlier findings that indicated that seinat seeds can be used as a natural source of potassium supplementation for pregnant and lactating women, as well as for children and the elderly [1]. Iron, magnesium, zinc and copper contents of bread made from SSPC and SSPI supplemented flour were found higher than that obtained by El-Soukkary [18], for pumpkin seed protein concentrate and isolate supplemented wheat flour.

Amino acids profile of breads

Protein isolates extracted from seinat seed flour have a high protein content and most of their amino acids are present in similar proportions to soybean protein isolate [1]. According to Table 3, the addition of seinat seed proteins to wheat flour increased the concentration of the essential amino acids in the bread samples. The lysine content of seinat seed protein supplemented bread samples was two times more than the control sample (WF).

Table 5. Amino acid composition (g amino acid/16 g nitrogen) of bread fortified with seinat seed proteins.

Amino acid	WF	SSPC	SSPI	FAO/WHO/UNU*
Histidine (His)	3.68±0.02^{a}	4.03±0.03^{a}	4.15±0.01^{a}	-
Threonine (Thr)	3.84±0.03^{a}	3.75±0.02^{a}	3.97±0.02^{a}	4.00
Valine (Val)	3.78±0.01^{b}	4.38±0.04^{a}	4.34±0.01^{ab}	5.00
Methionine (Met)	1.31±0.03^{a}	1.66±0.01^{a}	1.72±0.04^{a}	3.50^{c}
Phenylalanine (Phe)	4.34±0.02^{a}	4.43±0.02^{a}	4.63±0.04^{a}	6.00^{d}

Isoleucine (Ile)	4.10±0.02^{a}	4.16±0.02^{a}	4.09±0.01^{a}	4.00
Leucine (Leu)	7.56±0.01^{a}	7.94±0.01^{a}	7.72±0.03^{a}	7.00
Lysine (Lys)	1.84±0.04^{c}	3.88±0.01^{a}	3.25±0.03^{b}	5.50
Tryptophan (Try)	0.88±0.02^{a}	0.81±0.01^{a}	0.78±0.02^{a}	1.00
Tyrosine (Tyr)	4.69±0.02^{a}	3.56±0.02^{b}	3.41±0.02^{b}	
Cysteine (Cys)	1.85±0.02^{a}	1.44±0.02^{a}	1.50±0.01^{a}	
Aspartic acid (Asp)	5.19±0.02^{b}	6.84±0.01^{a}	6.28±0.02^{a}	
Glutamic acid (Glu)	29.41±0.03^{a}	25.63±0.02^{c}	26.44±0.02^{b}	
Serine (Ser)	3.68±0.04^{a}	3.31±0.02^{a}	3.79±0.01^{b}	
Glycine (Gly)	3.51±0.04^{b}	4.66±0.02^{a}	3.08±0.03^{a}	
Arginine (Arg)	3.94±0.03^{c}	4.75±0.01^{b}	5.38±0.03^{a}	
Alanine (Ala)	3.34±0.01^{b}	4.59±0.03^{a}	4.19±0.03^{a}	
Proline (Pro)	13.06±0.04^{a}	10.18±0.02^{c}	11.28±0.02^{b}	

Values represent the means±standard deviation of triplicates. WF, SSPC and SSPI denote wheat flour (control), seinat seed protein concentrate and seinat seed protein isolate. Means in the same row with different letters as superscripts are significantly different (p< 0.05).
[a] Blend of 15 g seinat seed protein concentrate with 85 g wheat flour .
[b] Blend of 15 g seinat seed protein isolate with 85 g wheat flour.
*Daily requirements for human adult [13].
[c] Methionine + cysteine.
[d] Phenylalanine + tyrosine.

Hansmeyer *et al.* [24] similarly reported that, the amount of lysine in bread constituted with wheat flour- wheat bran protein supplement increased by double, which is in the range of the findings of this study. Lysine is the limiting amino acid in most cereal products and during baking it is suggested that Maillard reactions may affect content and composition of related products lowering its availability, but also their role in taking part in antioxidant capacity properties [25]. The seinat seed protein supplemented bread samples contained similar amino acid levels which were lower than the essential amino acids levels of the standard (FAO/WHO) pattern, but the deficiency of these amino acids was not a result of SSPC and SSPI addition. Among the non-essential amino acids of bread, glutamic acid was found to be the highest, with the control bread sample registering the highest (p<0.05) relative to the SSPC and SSPI supplemented bread samples. The same this trend was reported by El-Soukkary [18], for pumpkin (which also belongs to the same Cucurbitaceae family) seed protein concentrate and isolate supplemented wheat flour.

Nutritional evaluation of breads

Nutritional evaluation such as chemical score, biological value and protein efficiency ratio of the bread samples is given in Table 6. According to their functional groups; hydrophobic, acidic and uncharged polar groups were found the highest percentage in all the bread samples, followed by total aromatic and basic groups (Table 6). Lysine was the first limiting amino acid for the bread samples; however, it was higher in the SSPC and SSPI supplemented bread samples. Valine was the second limiting amino acid, while methionine and cysteine were both the third limiting amino acids in SSPC and the control samples. Valine and theronine were found to be the second and third limiting amino acids in SSPI.

Table 6. Nutritional evaluation of bread fortified with seinat seed proteins

	WF	SSPC[a]	SSPI[b]
First limiting amino acid	Lys (33.55±0.24)^{c}	Lys (70.45±0.16)^{a}	Lys (59.09±0.15)^{b}
Second limiting amino acid	Val (75.70±0.43)^{b}	Val (87.50±0.35)^{b}	Val (86.90±0.26)^{ab}
Third limiting amino acid	Met+Cys(63.20±0.62)^{b}	Met+Cys (61.80±0.02)^{b}	Thr (97.00±0.31)^{a}
Chemical score%	33.55±0.24^{c}	70.45±0.16^{a}	59.09±0.15^{b}
Essential amino acid index%	75.96±0.31^{a}	80.15±0.18^{a}	79.26±0.53^{a}
Biological value%	71.09±0.33^{a}	75.66±0.19^{a}	74.69±0.57^{a}
Protein efficiency ratio	2.34±0.14^{a}	2.46±0.03^{a}	2.31±0.02^{a}
Total essential amino acid	32.36±0.20^{b}	36.04±0.17^{a}	35.67±0.17^{a}
Total non-essential amino acid	70.13±0.25^{a}	66.63±0.16^{b}	66.58±0.19^{b}
Percentage of amino acid with different characteristics[c]			
Basic	9.47±0.21^{b}	12.66±0.48^{a}	12.78±0.31^{a}
Acidic	34.59±0.04^{a}	32.63±0.23^{b}	32.37±0.13^{b}
Hydrophobic	37.52±0.62^{a}	37.34±0.39^{a}	37.97±0.12^{a}
Uncharged polar	17.56±1.02^{a}	16.73±0.01^{ab}	15.75±0.17^{b}
Total Sulfur	3.16±0.31^{a}	3.09±0.04^{a}	3.22±0.04^{a}
Total aromatic	10.04±0.57^{a}	9.00±0.08^{a}	9.04±0.05^{a}

Values represent the means±standard deviation of triplicates. WF, SSPC and SSPI denote wheat flour (control), seinat seed protein concentrate and seinat seed protein isolate. Means in the same row with different letters as superscripts are significantly different (p< 0.05).

[a] Blend of 15 g seinat seed protein concentrate with 85 g wheat flour .
[b] Blend of 15 g seinat seed protein isolate with 85 g wheat flour.
[c] Basic, acidic, hydrophobic, uncharged polar: Lysine, arginine, histidine; Aspartic acid, glutamic acid; Alanine, isoleucine, leucine, methionine, phenylalanine, proline, valine; Glycine, serine, threonine, tyrosine, cysteine, respectively.

Similar results have been reported by El-Adawy, [3] in a study on sesame protein products: sesame meal, roasted sesame meal, autoclaved sesame meal, in addition to sesame protein concentrate and isolate supplementation of wheat flour. The addition of seinat seed proteins to wheat flour improved the essential amino acid index, chemical score and biological value. The chemical score for the SSPC and SSPI supplemented, and control bread samples were calculated with reference to the FAO pattern [14]. The results indicate that the differences in the chemical score were due to the differences in essential amino acid composition among the bread samples. Maciejewicz and Hanczakowski [26] reported that supplementation of wheat flour with 33% green matter barley leaf protein concentrate increased the chemical score from 42 to 50%. The biological value and protein efficiency ratio of the SSPC and SSPI supplemented bread samples increased with an increase in percentage of essential amino acids of seinat seed proteins. The biological values and protein efficiency ratios of SSPC and SSPI supplemented bread were a little similar to the control bread sample. In a study carried out by Mansour *et al.* [27], it was also reported that, wheat flour supplemented with pumpkin seed and canola protein isolate and concentrate enhanced amino acid indices, while biological value and protein efficiency ratio were similar to the control. These findings are also supported by El-Adawy, [28] who reported that, supplementation of wheat flour with detoxified apricot kernel meal improved the chemical score and essential amino acid index of the mixture.

CONCLUSION

In this study, 15% SSPC and SSPI supplemented wheat flour was used to make bread without any observed detrimental effects on sensory properties. The addition of SSPC and SSPI to wheat flour increased dough water absorption and development time, which improved with an increase in the amount of protein, however, a decrease in dough stability, was observed. An overall increase in proximate and nutritional composition was noted namely; protein composition, total essential amino acids, essential amino acid index as well as crude fat, crude fiber, total ash and minerals; with sodium and potassium being the highest quantity. This study also revealed that this seed is an excellent source of lysine which is a major limiting amino acids in most cereals used to supplement baked products. There was also a slight change in the total carbohydrate content with the addition of SSPC and SSPI, a lower carbohydrate content value was observed with the SSPI supplemented flour. These properties make the seinat seed a perfect ingredient that may be used for supplementing bread as well as other baked products like cakes.

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