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EVALUATION OF PUMPKIN SEED PRODUCTS FOR BREAD FORTIFICATION

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ABSTRACT

Pumpkinseed products (raw, roasted, autoclaved, germinated, fermented, pumpkin protein concentrate and pumpkin protein isolate) were incorporated into wheat flour to produce blends with protein levels of 15, 17, 19 and 21%. Dough properties were evaluated by a farinograph; loaves of breads were evaluated by a taste panel for crust color, crumb color, crumb texture, flavor, and overall quality. Results indicated that pumpkin seed products can be added to wheat flour up to a 17% protein level for raw, roasted and autoclaved pumpkin meal, 19% level for germinated, fermented and pumpkin protein concentrate and 21% protein level for pumpkin protein isolate without a detrimental effect on dough or loaf quality. On the other hand, the addition of pumpkinseed proteins resulted in increasing protein, lysine and mineral contents compared to the control. While lysine and tryptophan were the first and second limiting amino acids in the control bread, tryptophan and lysine were the first and second limiting amino acids for raw, roasted, autoclaved, germinated and fermented pumpkin meal; valine and lysine and valine and total sulfur amino acids were the first and second limiting amino acids for pumpkin protein concentrate and isolate, respectively. Also the *in-vitro* protein digestibility improved when the pumpkin seed proteins were added.

Keywords: Amino acid profiles, Fermented pumpkinseed meal, Germinated pumpkinseed meal, Pumpkinseed protein concentrate, and Pumpkinseed protein isolate, Sensory evaluation.

INTRODUCTION

In Egypt wheat flour bread represents the main source of carbohydrates for most of the people. Increasing the protein content of the wheat flour by the addition of legumes and oil seeds (soybean, cotton seed, sesame, sunflower, and peanut flour) can improve the nutritional quality of bread, especially the lysine content. Baking characteristics such as loaf volume and dough mixing (Rooney *et a*l., 1972; Khan *et al.,* 1975; Sosulski and Caden, 1982; Cadden *et al.*, 1983; Gonzalez-Galan *et al.,* 1991; Yue *et al.,* 1991; El-Adawy, 1995 and Mansour *et al.,* 1999) can also be improved. Pumpkin (*Cucurbita moschata,* variety Dickinson) seed could be utilized successfully as a good source of edible protein (320g/Kg) and oil (450g /Kg) for human consumption, as well as food and at the same time, it minimize waste pollution (Lazos, 1986).

The seeds can be cooked and dried and served as snacks (e.g., Egypt) and might also be cooked, ground (west Africa) and fermented for use as a flavor enhancer in gravies and soups (Nwokolo and Sim, 1987).

Mansour and co-workers (Mansour *et al.* 1992, 1993 a, b&c and 1999) investigated the preparation, functional properties and nutritional quality of pumpkin seed meal, pumpkin protein concentrate, and isolate and also canola protein concentrate and isolate. Pumpkin and canola seed proteins exhibit unique functional properties (high water and fat absorption as well as good emulsification properties) and high lysine content, which suggest the ability to

be incorporated in bakery products.

The objective of this study was to determine the effect of partial replacement of wheat flour by raw pumpkin seed meal, roasted and autoclaved pumpkin seed meal, germinated and fermented pumpkin seed meal and pumpkin seed protein concentrate and isolate on the physical properties of the dough, baking quality characteristics and chemical composition, minerals content and amino acid profile of bread made from the various pumpkin seed preparations.

MATERIALS AND METHODS

Wheat flour: Wheat flour (all purpose) (72% extraction rate) was obtained from the Alexandria Milling Company, Alexandria, Egypt. The protein content of wheat flour was 12.5%.

Pumpkin seeds: Pumpkin (*Cucurbita moschata,* variety Dickinson) seeds were obtained from the Agricultural Research Center, Ministry of Agriculture, Alexandria, Egypt. The seeds were cleaned by hand to remove broken seeds and foreign materials. The seeds were peeled by hand.

Autoclaved pumpkin seed. Peeled pumpkin seeds were mixed with tap water (1:10 w/v). The mixture was autoclaved at 121 ^{o}C for 30 min.

Roasted pumpkin seed. Peeled pumpkin seeds were heated at 120 °C for 30 min. in an electric draught oven (VEM MLW Medizinische, Greate, Berlin, Germany).

Germinated pumpkin seed: Whole pumpkin seeds were sterilized by soaking in 75% ethanol for 1 min. The seeds were soaked in tap water for 12 h at room temperature (~ 25 ^{o}C). The soaked seeds were kept between thick layers of cotton cloth and allowed to germinate in the dark at room temperature for 3 days. The germinated seeds were rinsed with tap water and peeled by hand.

Fermented pumpkin seed: Pumpkin seeds were fermented according to the procedure in Fig 1. Saccharomyces Cerevisiae (commercial strain) were obtained from the microbiological laboratory of Bisco Misr Company, Alex, Egypt, and maintained in malt extract broth.

Defatted pumpkin seed meals: Defatted pumpkin seed meals of raw, autoclaved, roasted, germinated and fermented pumpkin seeds were prepared by grinding the seeds (National Matsushita Elec. grinder Ind. Co., LTD., Japan) then the ground seeds were extracted with hexane in a Soxhlet apparatus. Hexane was removed from the extracted ground seeds by heating at 50^{o}C for 2h. The resultant defatted meal was ground again and passed through a 400μm sieve (Brith Standard Screen).

The processed pumpkin seeds were mashed and dried at 50 ^{o} C for 10 h. The resultant defatted meals were kept in refrigerator at 4-5°C until used. The percentage of crude protein in raw, autoclaved, roasted, germinated and fermented pumpkin seed meals were 71.1, 70.2, 72.5, 73.5, and 74.9% respectively.

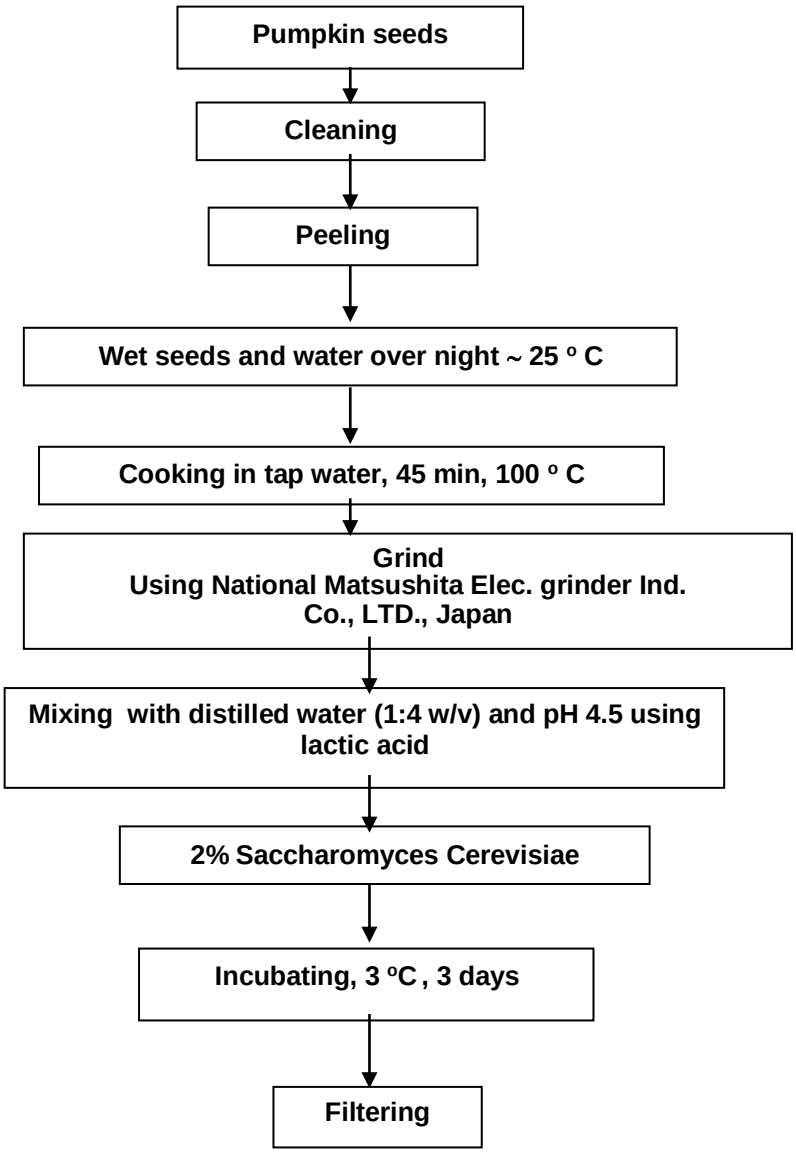

Fig 1: Production of fermented pumpkin seed meal

Pumpkin seed protein concentrate: Pumpkin seed meal was extracted with

petroleum ether for 30 min. (meal to solvent ratio 1:4 w/v) followed by extraction with 75% ethanol for 30 min. (meal to solvent ratio 1:10 w/v). Both extractions were carried out at room temperature. The protein concentrate was dried using a vacuum oven (Type SPT-200, vacuum Dryer, Kraksw, Poland) at 40 ^{o}C for 8 h.

Pumpkin seed protein isolate: The meal was extracted with 0.5% $Na_2 CO_3$ solution (pH 9.5) at room temperature (solid to solvent ratio 1:10 w/v) and the mixture was shaken using a rotary shaker (Julabo D-7633 Seelbach, Germany) for 1h. Insoluble materials were removed by centrifuging at 2515 xg for 15 min. the supernate was acidified to pH 3.0 with 1m HCL. The precipitate was neutralized to pH 7.0 using 2M HCL then precipitate was washed twice with distilled water and dried using a vacuum oven at 40°C for 8 h. Pumpkin seed protein concentrate and isolate were determined according to the method of Mansour and co-workers (Mansour *et al.,* 1993c). Pumpkin protein concentrate and isolate were ground to pass through a 400 μm sieve then kept in refrigerator (4-5°C) until used. The crude protein content of pumpkin protein concentrate and isolate were 78.4 and 94.8%, respectively.

Preparation of pumpkin seed protein-wheat flour blends: Each of the pumpkin seed preparations partially replaced wheat flour to produce blends with final protein concentrations of 15, 17, 19 and 21%. Quantities of pumpkin seed proteins blended with wheat flour are presented in Table 1.

Table 1: Pumpkin wheat flour blends

Product	%Pumpkin seed product in blends (g/100g)			
	15%	[1]17%	[2]19%	[3]21%
Raw pumpkin seed meal[1]	4.30	7.70	11.10	14.50
Roasted pumpkin seed meal[1]	4.20	7.50	10.80	14.20
Autoclaved pumpkin seed meal[1]	4.20	7.80	11.30	14.70
Germinated pumpkin meal[2]	4.10	7.40	10.70	13.90
Fermented pumpkin seed meal[2]	4.00	7.20	10.40	13.60
Pumpkin seed protein concentrate[2]	3.80	6.80	9.90	12.90
Pumpkin seed protein isolate[3]	3.00	5.50	7.90	10.30

1, 2 and 3 were conducted on breads containing these protein levels to evaluate chemical composition, mineral content, amino acid profile and in vitro protein digestibility.

Physical properties of dough. Water absorption, dough stability and dough softening of the various blends were determined using a Barbender Farinograph with a 100 g. mixing bowl, A.A.C.C. (1983) methods were followed.

Pan bread preparation. The procedure of Pollhamer (1981) was used for baking and loaf volume. For each test, 50 g flour, 0.3g dry yeast, 0.5 g salt and water (as flour blend) were incorporated. The dough was kneaded for 3 min in the 100g mixing bowel of the farinograph. The dough was fermented at 30^{o}C for 70 min. in a proofing cabinet. The fermented dough was placed in a lightly oiled glass cylinder and leveled with a cylindrical piece of wood. The dough was left at 30^{o}C for 60 min. in a proofing cabinet. The pan bread was baked in an oven at 260^{o}C for 15 min. Loaf volume was measured by rapeseed displacement after cooling the bread for 1 h. at room temperature

(~ 25°C). Breads were divided into two parts, one for the sensory evaluation and the other for chemical analysis. The latter part was dried at 40-50°C for 12 h in an electric air draught oven, then ground (National Matsushita Elec. grinder Ind. Co., LTD., Japan) and passed through a 400 μm sieve. The ground samples were packed in screw cap glass jars and left at room temperature for chemical analysis.

Sensory evaluation and loaf volume. The freshly sliced bread was cut into 5x5 cm pieces and served to five trained staff members from the university of Minia, Minia, Egypt. Selection of panelists was based on participant interest, taste and flavor acuity and ability to understand test procedures. Instruction score sheets for evaluating samples were given to panelists. They were provided with eight randomly coded samples (control bread, raw pumpkin seed meal bread, roasted pumpkin seed meal bread, autoclaved pumpkin seed meal brad, germinated pumpkin seed meal bread, fermented pumpkin seed meal bread, pumpkin seed protein concentrate bread and pumpkin seed protein isolate) in for sessions (for each concentration). General attribute ranking evaluations were made in individual sensory evaluation tables under fluorescent light at ambient temperature (~25C). Panelists were instructed to rinse their mouth with water before starting and between sample evaluations. Five sensory attributes were evaluated (crust color, crumb color, crumb texture, flavor and overall quality) using a 5-point scale, where 5 = excellent, 4 = good, 3 = satisfactory, 2 = fair, and 1= poor. Accuracy and precision were evaluated statistically.

Analytical methods. The proximate composition of pumpkin seed products, wheat flour and bread was determined using the following AOAC (1980) methods: Moisture content (No. 14.004), crude protein (No. 2.057), crude fat (No. 7.056) and total ash (No. 14.006).

For mineral assays, samples were digested with concentrated sulfuric acid and percholoric acid. Sodium and potassium were measured by flame photometry (Corning 400). Calcium, zinc, manganese, iron, and copper were estimated using an AASI atomic absorption spectrophotometry (Perkin-Elmer Instrument Model 2380).

Amino acids were determined using Beckman amino acid analyzer (Model 118/119CL) according to the method described by Moore and Stein (1963). Flour hydrolysis was performed in the presence of 6 N HCL at 110°C for 22 h in nitrogen atmosphere. Sulfur containing amino acids were determined after performic acid oxidation. Tryptophan was chemically determined by the method of Miller (1967).

In-vitro digestibility and biological value. In vitro protein digestibility was determined as described by Salgo *et al.* (1984) by measuring the change in the sample solution pH after incubation at 37 °C with a trypsin-pancreatin enzyme mixture for 10 min. In vitro protein digestibility was determined in triplicate. Chemical scores of the amino acids were calculated using the FAO/WHO (1973) reference pattern.

Statistical analysis. Physical properties of dough, sensory properties of bread and chemical composition of bread were analyzed using Statistical Analysis System "SAS" (1985). Significant differences between treatments

were determined at the 5% level.

RESULTS AND DISCUSSION

Physical properties of the dough. The physical properties of control (wheat flour) and pumpkin seed blends to give protein level of 15, 17, 19 and 21% are shown in Table 2. Water absorption was increased significantly ($P \leq 0.05$) due to the addition of pumpkin seed products at all protein levels except for raw pumpkin seed meal at 15% protein content. In general, wheat flour fortified with pumpkin protein isolate had the highest water absorption at all levels of blending followed by fermented, germinated, autoclaved, roasted pumpkin seed meal, and pumpkin protein concentrate. The increase in water absorption was mainly due to the high water hydration capacity of pumpkin seed products, especially pumpkin protein isolate. Generally, these results agree well with those reported by Rasco *et al*. (1990), Gonzalez-Galan *et al*. (1991) and Yue *et al*. (1991) who found that water absorption increased substantially by 5-15 percent with the addition of native sunflower protein concentrate and isolate to wheat flour. Also these results are in accordance with those of El-Adawy (1995) and Mansour *et al*. (1999). From Table 2 one can see that all wheat flour-pumpkin seed blends required a significantly higher ($P \leq 0.05$) dough development time than control. However, dough development time was decreased as pumpkin seed product levels increased but was still higher than for wheat flour. Generally, the increment in dough development time may be attributed to the differences in physicochemical properties of pumpkin seed products and that of wheat flour as previously detected by Morad *et al*. (1980) and El-Adawy (1995).

Dough stability time, as a major index of dough strength, was decreased by the addition of pumpkin seed products to wheat flour, except pumpkin protein isolate at the 15% protein level of substitution (Table 2). These results are in accordance with those results of Anjum *et al.* (1991) and Yue *et al.* (1991) who indicated that the high level of replacement of sunflower protein concentrate and isolate may have been responsible for decreasing dough stability time. Roasted and autoclaved pumpkin seed meals had the lowest values for dough stability and the highest values for dough softening which could be attributed to the heat treatment and its effect on protein denaturation. In contrast, Pumpkin seed protein isolate had the lowest amount of softening compared with the others which paralleled dough stability. Table 2 data shows also that the replacement with pumpkin seed products significantly increased the weakening of dough ($P \leq 0.05$). Dough softening was increased with the increase in protein level. The dough weakening could have due to; (a) the presence of sulphhydryl groups in pumpkin seed products which lead to dough softening (El-Farra *et al.*, 1981); (b) the decrease in wheat gluten because of the dilution effect and (c) the competition between proteins of pumpkin products and wheat flour for water absorption (Deshpande *et al.,* 1983). These results also agree well with those of Ranga Rao *et al.* (1980), who reported that supplementation of

wheat flour with 5-20 % wheat germ on weight basis decreased water absorption, stability and softening of bread dough. They also agree with Mansour *et al.* (1999).

Table 2 : Farinograph characteristics of wheat flour pumpkin product blends*

Product	Protein level % (dry weight basis)	Water absorption (%)	Development time (min)	Stability (min)	Softening (BU)
Control	12.5	61.20	1.80	4.20	90
Raw pumpkin seed meal					
	15	61.80	3.10	2.90	100
	17	62.60	3.20	2.80	120
	19	63.10	2.90	2.40	135
	21	63.50	2.90	2.30	155
Roasted pumpkin seed meal					
	15	62.60	3.20	2.70	115
	17	63.50	3.20	2.60	120
	19	64.10	2.80	2.40	130
	21	64.50	2.80	2.20	140
Autoclaved pumpkin seed meal					
	15	62.30	3.40	2.70	110
	17	63.10	3.30	2.50	115
	19	63.10	3.00	2.30	125
	21	64.10	2.80	2.20	135
Germinated pumpkin seed meal					
	15	62.90	3.20	3.40	100
	17	63.80	3.00	3.30	110
	19	64.6	2.90	3.20	120
	21	65.00	2.90	3.20	130
Fermented pumpkin seed meal					
	15	63.30	3.00	3.80	100
	17	64.10	2.80	3.50	115
	19	64.70	2.80	3.40	120
	21	65.40	2.70	3.40	125
Pumpkin seed protein concentrate					
	15	62.20	3.20	3.60	100
	17	62.90	3.10	3.60	110
	19	63.60	2.90	3.40	115
	21	64.00	2.60	3.40	120
Pumpkin seed protein isolate					
	15	64.50	3.00	3.90	95
	17	65.70	2.70	3.80	100
	19	66.40	2.50	3.70	105
	21	67.30	2.50	3.70	110
LSD		1.03	0.44	0.35	2.80

*** Means of three determinations.**

Bread characteristics. Sensory properties of bread fortified with pumpkin seed products are presented in Table 3. There were no significant differences ($p \leq 0.05$) between the control (wheat flour) bread and breads fortified with raw, roasted and autoclaved pumpkin meals (up to 17% protein level), germinated and fermented pumpkin meals and pumpkin protein concentrate (up to 19 % protein level) and pumpkin protein isolate (up to 21% protein

level) as regard to crust and crumb color, flavor and overall acceptability. Mansour *et al.* (1999) indicated that there were no differences in taste and odor among breads containing up to 18 % pumpkin seed protein concentrate or canola protein isolate or up to 20 % pumpkin meal, pumpkin protein isolate or canola protein concentrate. Also, breads containing 22 % pumpkin meal, pumpkin protein isolate or canola protein concentrate were not significantly different in odor from each other or the control bread. The results of loaf volume in Fig 2 and Table 3 show that the volume decreased with increasing level of substitution. The loaf volume of the control was significantly ($P \leq 0.05$) higher than those baked with raw, roasted pumpkin meals (all protein levels), autoclaved pumpkin meal (17, 19 and 21% protein levels), pumpkin protein concentrate (19 and 21% protein levels), germinated pumpkin meal (19 and 21% protein levels), fermented pumpkin meal (19 and 21% protein levels) and pumpkin protein isolate (21% protein level). However, there was no significant depression in loaf volume for breads containing pumpkin protein isolate up to an 21 percent protein level. Also, the same trend was observed for breads containing pumpkin protein concentrate, fermented pumpkin meal, and germinated pumpkin meal at the 19 percent protein level, and autoclaved, roasted and raw pumpkin meals at the 17 percent protein level. These results are much better than those reported by El-Adawy (1995) who found that oil-seeds had varying effect on dough mixing and loaf volume characteristics, and heating enhanced the bread making characteristics of cotton seed and sunflower proteins but was detrimental to these properties in peanut and sesame proteins. Also Hansmeyer *et al.* (1976) and Yue *et al.* (1991) reported a detrimental effect on loaf quality with the addition of succinylaled sunflower protein concentrate and isolate to breads. Flour blends containing 5% native sunflower protein concentrate and isolate produced acceptable breads, but the quality of the breads deteriorated at 10-15% of the same fractions. Our results agree well with those of Mansour *et al*. (1999) who found that addition of canola and pumpkin protein concentrate and isolate greater than 18 to 22% yielded dense, compact and unacceptable breads, and better than those with Talley *et al.* (1972) who found that 17 % and 30 % substitution sunflower meal in wheat flour produced dense, compact loaves; 3 % enrichment resulted in an attractive loaf. From the results in the current study one can conclude that there were no significant differences ($P \leq 0.05$) between control and raw, roasted and autoclaved pumpkin meal breads up to 17% protein level; germinated, fermented pumpkin meals and pumpkin protein concentrate up to a 19% protein level and pumpkin protein isolate up to a 21% protein level. Therefore the rest of this study was conducted using breads containing those protein levels.

Fig2

Table 3 : Sensory properties* of pumpkin seed product-wheat flour breads .

Product	Protein level % (dry weight basis)	Crust color**	Crumb color**	Crumb texture**	Flavor**	Overall quality**	Loaf volume*** (Cm^3)
Control	12.5	4.40	4.50	4.40	4.30	4.40	240
Raw pumpkin seed meal							
	15	4.20	4.40	4.10	4.20	4.20	217
	17	3.80	4.10	3.60	3.80	3.80	188
	19	3.20	3.70	3.10	3.50	3.40	168
	21	2.80	3.20	2.60	3.00	3.00	158
Roasted pumpkin seed meal							
	15	4.30	4.40	4.40	4.30	4.40	222
	17	4.10	4.20	4.10	4.10	4.10	195
	19	3.60	3.80	3.60	3.70	3.70	185
	21	3.20	3.30	3.10	3.40	3.30	169
Autoclaved pumpkin seed meal							
	15	4.30	4.40	4.40	4.30	4.40	225
	17	4.10	4.20	4.10	4.20	4.20	200
	19	3.70	3.90	3.70	3.80	3.70	190
	21	3.20	3.50	3.30	3.40	3.40	175
Germinated pumpkin seed meal							
	15	4.30	4.40	4.40	4.30	4.40	230
	17	4.20	4.30	4.20	4.20	4.20	215
	19	3.80	4.00	3.90	3.90	3.90	205
	21	3.20	3.70	3.70	3.60	3.50	197
Fermented pumpkin seed meal							
	15	4.40	4.50	4.40	4.30	4.40	232
	17	4.20	4.30	4.30	4.20	4.30	220
	19	3.90	4.10	4.00	4.00	4.00	207
	21	3.20	3.90	3.70	3.70	3.60	202
Pumpkin seed protein concentrate							
	15	4.30	4.40	4.40	4.30	4.40	227
	17	4.10	4.20	4.10	4.10	4.10	218
	19	3.40	3.90	3.80	3.70	3.60	203
	21	3.00	3.40	3.50	3.40	3.30	193
Pumpkin seed protein isolate							
	15	4.40	4.50	4.40	4.30	4.40	236
	17	4.40	4.40	4.40	4.30	4.40	228
	19	4.20	4.30	4.20	4.20	4.20	223
	21	3.90	4.20	4.10	4.10	4.10	206
LSD		0.30	0.28	0.29	0.25	0.28	2.17

***Sensory attributes were as follow: 5=excellent, 4=good, 3=satisfactory, 2=fair, 1=poor.**
****Means of five panelist scores. ***Means of three determinations**

Chemical composition of bread: The changes in chemical composition of breads fortified with pumpkin seed products at the 17% protein level of raw, roasted, and autoclaved pumpkin meal; 19% protein of germinated, fermented pumpkin meal and pumpkin protein concentrate, and 21% protein level of pumpkin protein isolate are shown from data presented in Table 4. Addition of pumpkin seed products to wheat flour increased the protein content significantly. No significant differences ($P \leq 0.05$) in crude fat were observed between control and pumpkin seed product breads, with the exception of pumpkin protein isolate which had the lowest value among the products. There were significant ($P \leq 0.05$) differences between the wheat flour and pumpkin seed product substituted breads in ash content, except

fermented pumpkin seed meal and pumpkin seed protein isolate which were the same (1.61%) but lower than the others. Pumpkin protein isolate bread had the lowest carbohydrate content (65%) which could have been due to the multiple extractions with alkali and acid. Longes *et al*. (1983) reported that the composition of defatted pumpkin seed was 69.7% crude protein, 8.2% crude fiber, 6.5% fat, 9.3% ash and 6.3% carbohydrate (dry matter basis). These results are in accordance with the results of Khan *et al*. (1975), Hansmyer *et al*. (1976), Rasco *et al*. (1989), Salama *et al.* (1992), El-Adawy (1995) and Mansour *et al.* (1999).

Table 4 : Proximate composition of bread fortified with pumpkin seed products (dry weight basis)* .

Product	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Carbohydrate[4] (%)
Control	9.60a	13.50a	2.55b	1.53a	72.82d
Raw pumpkin seed meal [1]	10.31a	17.20b	2.72b	1.85c	67.92c
Roasted pumpkin seed meal [1]	9.82a	17.31b	2.64b	1.75bc	68.48c
Autoclaved pumpkin seed meal [1]	9.63a	17.11b	2.69b	1.79bc	68.78c
Germinated pumpkin seed meal [2]	10.11a	19.02c	2.41b	1.81c	66.65b
Fermented pumpkin seed meal [2]	9.93a	19.21c	2.89b	1.61ab	66.36b
Pumpkin seed protein concentrate[2]	9.84a	19.13c	2.75b	1.93c	66.35b
Pumpkin seed protein isolate [3]	10.11a	21.19d	2.11a	1.61ab	64.98a
LSD	0.71	0.95	0.49	0.19	1.12

[a, b, c, d] Means in the same column with different letters are significantly different (p ≤ 0.05).
[1] 17 % protein level, [2] 19 % protein level, [3] 21protein level , [4] calculated by difference .
***Means of three determinations**

Mineral content of breads. Data presented in Table 5 show the mineral content of breads fortified with pumpkin seed products. The results revealed that there was a marked increase in minerals in the final products with the exception of sodium, which was lower in almost all pumpkin supplemented breads. Pumpkin protein isolate breads had approximately one and half times the sodium content of control. Copper was double the control. Fermented meal and pumpkin protein isolate breads had the highest contents of Ca (140 and 145 mg/100g respectively), while roasted and autoclaved pumpkin meal breads had double the content of K^+ relative to the control. Roasted pumpkin meal bread had approximately two and half Mg compared with the control.

Table 5 : Mineral content of bread fortified with pumpkin seed products (dry weight basis)* . (Mg / 100 g)

Product	Na	Ca	K	P	Mg	Cu	Zn	Fe	Mn
Control	485.60	98.30	74.10	85.30	60.60	0.37	3.10	1.90	1.20
Raw pumpkin seed meal [1]	457.50	128.50	100.70	139.80	111.30	0.52	3.20	3.60	2.20
Roasted pumpkin seed meal [1]	413.50	124.20	135.20	154.00	145.20	0.41	3.20	3.80	1.90
Autoclaved pumpkin seed meal [1]	429.20	136.20	142.00	161.30	122.60	0.63	2.80	3.60	1.70
Germinated pumpkin seed meal [2]	498.30	125.90	125.40	180.50	125.80	0.61	3.70	3.90	2.40
Fermented pumpkin seed meal [2]	432.30	140.20	92.80	120.90	115.60	0.42	3.00	4.10	2.20
Pumpkin seed protein concentrate[2]	433.10	120.70	90.00	136.70	81.10	0.51	3.30	3.60	2.20
Pumpkin seed protein isolate [3]	746.90	145.30	78.20	109.20	60.40	0.72	1.50	2.50	0.73

[1] 17 % protein level, [2] 19 % protein level, [3] 21% protein level .
***Average of two determinations.**

Pumpkin protein isolate bread had lower amounts of minerals than those of other pumpkin seed product and control breads, except Na and Cu.

These results are in accordance with Mansour *et al.* (1993a), El-Adawy (1995) and Mansour *et al.* (1999).

Amino acids profiles of breads. Data presented in Table 6 shows the amino acid composition of the breads. The addition of pumpkin seed products to wheat flour appeared to increase the concentration of most of the essential amino acids (isolucine, lysine, cystine, methionine, threonine, tryptophan and valine), compared to control. For lysine the increase was 200-280% compared to the control. Comparison of the amino acid composition of pumpkin seed breads showed that pumpkin seed products contained similar or higher total essential amino acids levels than the FAO/WHO (1973) pattern, except for a few modest deficiencies. The deficiency of these amino acids was not the result of pumpkin seed product addition but is mainly due to the preparation methods. Pumpkin protein isolate and fermented pumpkin meal breads had the highest contents of lysine and total essential amino acids. This apparent increase may have been due to the loss of some protein fractions during their preparation (multiple soaking and mixing with water) which contain plenty of non-essential amino acids. Fermented pumpkin meal bread had a higher content of total sulfur amino acids than the others and the standard; this may have been due to the fermentation process. Hansmeyer *et al.* (1976) reported that the lysine content of breads increased two times with the addition of wheat bran protein concentrate to wheat flour. Carlson *et al.* (1981) reported that supplementation of the wheat flour with 10-20% tomato seed flour increased lysine content by 40-69% in breads. Also, these results agree well with Salama *et al.* (1992), El-Adawy (1995) and Mansour *et al*. (1999)

In vitro digestibility and biological value of breads. In vitro protein digestibilities are given in Table 7. Pumpkin seed product breads had significantly ($P \leq 0.05$) higher protein digestibility than the control bread. Digestibility of bread containing pumpkin protein isolate and fermented pumpkin meal was much better than the others. These results agree well with those reported by Gonzalez Agramon and Serna-Saldivar(1988), who found that soybean isolate fortified tortillas had higher digestibility than 100% wheat flour and soybean meal fortified tortillas. The better of digestibility of fermented meal may have been attributed to the easier attack of the proteolytic enzymes due to the fermentation process. Also the digestibility of both the concentrate and isolate was better than that of the meal which may have been due to the denaturation of protein during preparation. Sathe et al. (1982) reported that heat denaturation of lupin seed protein increased sensitivity into proteolytic attack. In general, the addition of pumpkin seed products to bread improved the protein digestibility. Bookwalter *et al.* (1987) reported that fortification of sorghum with 15% soy meal increased the digestibility from 75 to 85%. Therefore, the low protein digestibility of wheat could be improved by mixing with highly digestible protein such as those of pumpkin seed products. Also, addition of the pumpkin seed products to wheat flour improved the chemical score (CS) percent better than the control. Maciejewicz-Rys and Hanczakowski (1990) reported that supplementation of

wheat flour with 33% leaf protein concentrate from the green matter of barley improved the chemical score from 42 to 50%. The same trend was observed with those reported by El-Adawy (1992 and 1995) who showed that supplementation of wheat with detoxified apricot kernel and sesame seed products improved the chemical score and essential amino acid. Mansour *et al*. (1999) found that wheat flour fortified with pumpkin seed and canola protein isolate and concentrate enhanced amino acid indices; PER and BV were similar to the control. Also, the same trend was observed for our pumpkin seed product breads, except for pumpkin protein isolate and fermented pumpkin meal which had the highest values of PER and BV compared to the others and control (2.408 and 75.251, and 2.386 and 75.030 respectively).

Lysine was the first limiting amino acid for the control bread; it was the second for the pumpkin seed products. Total sulfur amino acids were the second limiting amino acids for pumpkin protein isolate. Tryprophan was the first limiting amino acids for the pumpkin seed products, except pumpkin protein concentrate and isolate which had valine as the first limiting amino acid. These results differ from those in fluted pumpkin reported by Sharma *et al.* (1986) and Longes *et al*. (1983). They found that the sulfur-containing amino acids were the first limiting amino acids, while lysine and threonine were the second limiting amino acids. This difference may have been due to the interspecies variation.

GENERAL CONCLUSION

Pumpkin seed products increased the water absorption, dough development time and softening of but decreased the stability and loaf volume. Pumpkin protein isolate and fermented pumpkin meal had the best results among the pumpkin seed products compared to the control.

Also increasing the protein level beyond a certain point resulted in unacceptable bread quality, therefore there was an upper limit of protein supplementation which was 17% for raw, roasted and autoclaved pumpkin meal, 19% for germinated, fermented and pumpkin protein concentrate and 21% for pumpkin protein isolate.

Also, pumpkin protein isolate, fermented pumpkin meal and pumpkin protein concentrate produced the best overall acceptability.

On the other hand the ash contents and also the macro and micro mineral content of breads fortified with pumpkin seed products especially, Ca, K, P, Mg, Cu, Fe and Mn were increased compared with the control and other oilseeds such as peanut, sunflower and rapeseed meal.

Increasing the lysine, total sulfur amino acids, chemical score, total essential amino acids, crude protein, protein digestibility, crude fat, ash and mineral content make pumpkin seed products are qualified as a good source of protein and nutrients for fortification the baked products, especially bread.

6

On the other hand pumpkin seed protein isolate, fermented pumpkin seed meal and pumpkin seed protein concentrate showed the best results among the pumpkin seed products.

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تقييم منتجات بذور القرع العسلى من أجل تدعيم الخبز

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استخدمت منتجات بذور القرع العسلى (خام ، محمص ، معامل بالأوتوكلاف ، منبت ، متخمر ، مركزات البروتين، تجزؤات البروتين للقرع العسلى) اتدعيم دقيق القمح لإنتاج مخاليط ذات مستويات بروتين تصل إلى 15،17،19، 21%.

وقيمت خصائص العجينة بإستخدام الفارينوجراف وأيضاً خصائص الأرغفة باستخدام اختبار التقييم الحسى للون القصرة ، لون اللبابة ، قوام اللبابة، الطعم وكل خصائص الجودة. وبرهنت النتائج على أن منتجات بذور القرع العسلى يمكن إضافتها لدقيق القمح بنسبة تصل إلى 17% مستوى بروتين للمنتج الخام والمحمص والمعامل بالأوتوكلاف. 19% مستوى بروتين للمنبت ، المتخمر ومركزات بروتين القرع العسلى و 21% مستوى بروتين لتجزؤات بروتين القرع العسلى بدون أى تأثير معنوى على العجينة أو جودة رغيف العيش. وعلى الجانب الآخر تسبب إضافة بروتينات بذور القرع العسلى زيادة البروتين والليسين ومستوى المعادن بالمقارنة بالكونترول بينما كانت أحماض الليسين والتربتوفان هى المحددة الأولى والثانية للقيمة الغائية فى كونترول العيش، كانت أحماض التربتوفان والليسين هى المحددة الأولى والثانية للمنتج الخام والمحمص ، المعامل بالأوتوكلاف ، المنبت والمتخمر لمنتجات بذور القرع العسلى والفالين والليسين والفالين والأحماض الأمينية الكبريتية كانوا هم المحددين الأول والثانى للقيمة الغذائية لمركزات وتجزؤات بروتين القرع العسلى على التوالى. وأيضاً تحسنت القابلية الهضمية للبروتين (خارج جسم الكائن الحى) *in vitro* عند إضافة بروتينات بذور القرع العسلى.

Table 6 : Amino acid composition of bread fortified with pumpkin seed products (g / 16 g nitrogen) .

Amino acid	Control	Raw pumpkin seed meal[1]	Roasted pumpkin seed meal[1]	Autoclaved pumpkin seed meal[1]	Germinated Pumpkin seed meal[2]	Fermented pumpkin seed meal[2]	Pumpkin seed protein concentrate[2]	Pumpkin seed protein isolate[3]	FAO/WHO (1973)
Isoleucine	3.81	3.90	3.99	4.22	3.90	4.35	3.30	3.70	4.00
Leucine	7.93	7.29	7.71	7.54	6.39	7.96	7.30	7.80	7.00
Lysine	2.02	4.82	4.45	4.19	4.15	5.13	3.90	5.20	5.50
Cystine	1.92	1.98	2.08	1.78	1.22	1.95	1.90	1.90	-
Methionine	0.90	1.60	1.77	1.55	1.77	1.82	1.30	1.20	-
Total sulfur amino acid	2.82	3.58	3.45	3.33	2.99	3.77	3.20	3.10	3.50
Tyrosine	4.92	4.01	3.39	3.87	3.61	4.33	5.40	4.90	-
Phenylalanine	5.51	5.07	5.19	5.13	4.98	5.39	5.10	5.50	-
Total aromatic amino acid	10.43	9.08	8.38	9.00	8.59	9.62	10.50	10.40	6.00
Threonine	3.97	3.72	3.95	4.08	3.51	4.17	4.20	4.60	4.00
Tryptophan	0.59	0.83	0.65	0.73	0.62	0.90	0.90	1.00	1.00
Valine	3.71	4.54	4.78	4.85	4.79	4.92	3.20	3.80	5.00
Total essenital amino acid	35.28	37.76	37.56	37.94	34.94	40.82	36.50	39.60	36.00
Histidine	3.33	4.25	3.87	4.42	4.46	4.23	3.40	3.60	-
Arginine	4.15	5.24	5.52	5.39	5.65	4.67	5.10	6.50	-
Aspartic acid	5.62	6.31	6.47	6.83	7.21	6.09	7.30	7.40	-
Glutamic acid	24.57	20.25	20.61	19.86	21.05	17.77	21.90	18.40	-
Serine	6.03	6.11	6.37	5.76	5.98	6.22	5.70	6.10	-
Proline	14.38	12.51	12.50	11.35	12.78	11.90	11.30	9.90	-
Glycine	3.34	3.47	3.21	3.87	3.04	3.91	4.70	4.30	-
Alanine	3.30	4.10	3.89	4.58	4.89	4.40	4.10	4.20	-
Total non-essential amino acids	64.72	62.24	62.44	62.06	65.06	59.18	63.50	60.40	-

[1] 17 % protein level, [2] 19 % protein level, [3] 21 % protein level .

Table 7 : *In Vitro* protein digestibility and biological value of bread fortified with pumpkin seed products (dry weight basis).

Product	*In Vitro* protein digestibility*	Chemical score (CS) %	First limiting amino acid	Second limiting amino acid	Third limiting amino acid	PER**	BV***
Control	72.51a	37.25	Lysine (37.25)	Tryptophan60.29	Valine 75.64	2.240	73.440
Raw pumpkin seed meal [1]	76.22b	97.35	Tryptophan (97.35)	Lysine 83.43	Valine 86.62	2.050	71.510
Roasted pumpkin seed meal [1]	76.73b	62.48	Tryptophan (62.48)	Lysine 77.44	Valine 91.69	2.240	73.530
Autoclaved pumpkin seed meal [1]	78.45bc	69.46	Tryptophan (69.46)	Lysine 72.18	Total sulfur amino acids 90.94	2.220	73.280
Germinated pumpkin seed meal [2]	79.34bc	64.06	Tryptophan (64.06)	Lysine 77.63	Total sulfur amino acids 88.22	1.629	67.060
Fermented pumpkin seed meal [2]	82.56c	79.69	Tryptophan((79.69)	Lysine 82.24	Valine 86.94	2.386	75.030
Pumpkin seed protein concentrate[2]	80.63bc	63.16	Valine (63.16)	Lysine 69.84	Isolucine 81.45	2.114	72.160
Pumpkin seed protein isolate [3]	82.72c	69.66	Valine (69.66)	Total sulfur amino acids 81.36	Isolucine 84.82	2.408	75.251

[a-c] *Means in the same column followed with the same letter are not significantly different ($p \leq 0.05$) using Duncan's Multiple Range Test . Means of three determinations.

[1] 17 % protein level, [2] 19 % protein level, [3] 21% protein level , [4] calculated by difference .

**** PER: -0.684 + 0.456 Leucine - 0.047 Proline (Alsmeyer *et al.,* 1974). *** BV : 49.9 + 10.53 PER (Michel and Block, 1946).**