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Low Phenylalanine Egyptian Shamy Bread

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Four formulas were prepared for production of low phenylalanine Egyptian shamy bread suitable for phenylketonuria (PKU) patients. The formulas were based on partial replacement of first break wheat flour (low protein flour) with different levels of corn starch. Pectin and carboxymethylcellulose (CMC) were used as a texture modifier and to improve staling rate of bread. Shamy bread made of 100% first break wheat flour was also prepared for comparison. Chemical composition, amino acids content, dough characteristics, baking test, sensory evaluation and staling rate of bread were investigated. A remarkable reduction of protein, consequently, phenylalanine of bread was detected. Phenylalanine content of formula "D", which contained 66 g of corn starch, 30 g of first break wheat flour, 2 g of pectin and 2 g of CMC, was reduced by 62% compared with the control. The rheological properties of the dough were affected by starch and hydrocolloids incorporation. Sensory characteristics of bread indicated that all formulas were acceptable, but formula "D" seems to be superior for PKU patients.

INTRODUCTION

The quantity of protein and its amino acids in wheat flour have a great effect on patients who are suffering from phenylketonuria (PKU) [Dwivedi, 1986]. PKU is an autosomal recessive disease caused by phenylalanine-hydroxylase (PAH). The deficiency of this enzyme leads to the accumulation of phenylalanine in tissues and plasma of patients [Hendriksz & Walter, 2004; Sirtori *et al.*, 2005]. Minimal requirements of phenylalanine (9.1 mg/kg/d) are needed to meet the normal growth and development. Excess of phenylalanine (not required for protein anabolism) is mainly metabolised in the liver by PAH. In the United Kingdom, a system of protein exchanges is used, with approximately each 1 g of natural protein representing a phenylalanine load of 50 mg. Most children with PKU can tolerate less than 500 mg of phenylalanine or 10 g of protein exchanges in 24 h [Hendriksz & Walter, 2004; Pencharz *et al.,* 2007].

Since the essential amino acid, phenylalanine, is present in the protein of milk, flour, meat, egg and other common foods, it is difficult to plan a low phenylalanine diet that is adequate in nutritive value and acceptable to the child with PKU. The aim of management in infancy and childhood is to restrict phenylalanine intake to permit an acceptable range of serum phenylalanine, yet provide sufficient phenylalanine to permit normal growth and development. Moderate- to low-cost, low phenylalanine recipes that can be utilized by the whole family are helpful. Dietary management of PKU child is simplified when his diet has minimal differences from the family routine [Dos Santos *et al*., 2006]. According to Anon [2002], low phenylalanine diet consists of three parts:

1. An allowance of a small measured quantity of phenylalanine given in the form of exchange food. Many patients will only be allowed 4–6 exchanges per day.
2. The administration of protein substitute includes all other amino acids except phenylalanine. Some protein substitutes contain added vitamins and minerals, whereas others consist of non-phenylalanine amino acids only. In the last group, vitamins and minerals need to be given in addition to the supplement.
3. Low phenylalanine foods.

Low phenylalanine bakery products may be prepared by selecting ingredients that are low in protein, *i.e.* starch, cellulose derivatives and gums. The functional effects of hydrocolloids stem from their ability to modify dough or batter rheology (or handling) and keeping qualities of finished products [Guarda *et al.,* 2004; Ribotta, *et al.,* 2005; Lazaridou *et al.,* 2007; Shittu *et al.,* 2009]. On the other hand, Shalini & Laxmi [2007] reported that guar gum, CMC and carrageenan can be effectively used to improve the shelf life of bread.

In Egypt, as well as the Middle East, the most popular type of bread is a flat (balady and shamy bread), circular loaf (1 cm in thickness, 10–30 cm in diameter) consisting of two layers. It is commonly made from high extraction flour (82 or 76%) and prepared by straight dough method.

Therefore, the purpose of this study was to formulate different low phenylalanine shamy bread dough. Chosen materials were first break wheat flour, corn starch, pectin and CMC to produce low phenylalanine shamy bread. Chemical com-

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position, amino acids content, rheological properties, baking properties, sensory evaluation and shelf life of the produced bread were studied.

MATERIALS AND METHODS

Materials

Low protein flour (first break wheat flour) was obtained from flour mill, North Cairo Flour Mill Company, Egypt. Pectin and carboxymethylcellulose (CMC) were purchased from Sigma Company, Germany. Other ingredients such as corn starch, salt (sodium chloride) and active dry yeast (*Saccharomyces cerevisiae*) were purchased at the local market.

Formulation and preparation of low phenylalanine Egyptian shamy bread

First break wheat flour was well blended with corn starch. Pectin and CMC were added to first break flour/corn starch blends. Four blends were prepared and a control sample was made with 100% first break wheat flour for comparison. Table 1 summarises blends composition. Flour samples and all ingredients were mixed in a kneader. Dough was kneaded until reaching adequate consistency (3–5 min). The dough was left to ferment for 30 min at 30°C and 85% relative humidity, then divided to 125 g pieces. The pieces were arranged on a wooden board that had been sprinkled with a thin layer of fine bran and were left to re-ferment for about 30 min at the same temperature and relative humidity. The pieces of fermented dough were flattened to about 20 cm in diameter. The flattened loaves were proofed for 10–15 min at 30–35°C and 85% relative humidity and then baked at 400–450°C for 1–2 min. The loaves of bread were allowed to cool on racks for about 1 h before evaluation.

Pasting properties of the dough

Pasting properties of dough were recorded by a Brabender Viscoamylograph (C.W. Brabender Instruments, South Hackensack, NJ) and falling number test according to AACC [2000].

Sensory evaluation of shamy bread

Sensory evaluation of shamy bread was performed by 15 trained panellists as described by El-Farra *et al.* [1982] for general appearance (20), separation of layers (20), roundness (15), distribution of crumb (15), crust colour (10), taste (10) and odour (10).

TABLE 1. Formulas of low phenylalanine shamy bread.

Ingredients* (%)	Control	Shamy bread formulas			
		A	B	C	D
First break wheat flour	100	60	50	40	30
Corn starch	-	36	46	56	66
Pectin	-	2	2	2	2
CMC	-	2	2	2	2
Active dry yeast	0.5	0.5	0.5	0.5	0.5
Salt	1.5	1.5	1.5	1.5	1.5

* Water was added to all formulas as needed.

Freshness of shamy bread

Shamy bread loaves freshness was tested after wrapping using polyethylene bags and storage at room temperature (0, 24 and 72 h) using alkaline water retention capacity test (AWRC) according to the method of Yamazaki [1953], as modified by Kitterman & Rubenthaler [1971].

Amino acids content

Bread samples were hydrolysed in sealed tubes with 10 mL of HCl (6N) for 24 h at 110°C in a sandy bath. The hydrolysed samples were filtered through a 0.45 μm nylon filter, evaporated at 40°C in a rotary evaporator and then dissolved with 1 mL of deionised water and evaporated once again in order to remove the traces of the acid. The residue was reconstituted in 1 mL of deionised water, then 20 μL was injected into the amino acid analyser (Eppendorf LC3000, Germany) for the determination of the amino acid composition of each sample. The amino acids were separated on a cation exchanger resin column (150 mm x 2.6 mm i.d., No. 2619 resin) using citrate buffer at pH 2.2, a column temperature of 53°C, a flow rate of 0.225 mL/min and a postcolumn reaction with ninhydrin (0.3 mL/min ninhydrin flow rate) followed by a photometric detection at 570 nm [Li *et al.,* 2006]. Tryptophan was not determined.

Chemical composition

Moisture, ash, crude protein, fat and crude fibre contents were determined according to the methods outlined in AOAC [2000]. Carbohydrates were calculated by difference.

Statistical analysis

The obtained results were analysed statistically by analysis of variance (ANOVA) and least significant difference (LSD) was calculated according to McClave & Benson [1991].

RESULTS AND DISCUSSION

Chemical composition of raw materials and their blends

Proximate composition of raw materials and their blends are summarised in Table 2. Corn starch was found to be characterised by the lowest protein, ash, fat and fibre content (0.50, 0.28, 0.30 and 0.22%, respectively), compared with first break wheat flour (7.00, 0.52, 1.00 and 0.56%, respectively). So, addition of corn starch to first break wheat flour decreased the previous constituents of the produced blends. For instance, the protein content of the first break wheat flour that was 7.0% being 2.5% for formula "D". However, carbohydrate content showed a reversible trend with respect to the protein, ash and fibre as seems from the same Table. Such findings were observed by El-Bardeny & Moustafa [1993] and Yaseen & Ahmed [1999].

Pasting properties of dough

Bread dough formulas were rheologically evaluated using a viscoamylograph for heat of transition, maximum viscosity, temperature at maximum viscosity, break down viscosity and set back viscosity as presented in Table 3. Results showed that the control sample had 63°C, 370 BU, 85°C, 160 BU and 410 BU for heat of transition, maximum viscosity, tem-

TABLE 2. Chemical composition of raw materials and its blends (% on dry weight basis).

Sample	Protein	Ash	Fat	Fibre	CHO
First break wheat flour	7.0±0.25	0.52±0.03	1.00±0.12	0.56±0.03	90.92±0.35
Corn starch	0.5±0.03	0.28±0.02	0.30±0.03	0.22±0.02	98.70±0.32
Flour mixtures*					
A	4.4±0.25	0.44±0.02	0.70±0.04	0.50±0.04	93.96±1.07
B	3.8±0.30	0.41±0.01	0.65±0.01	0.45±0.01	95.51±0.21
C	3.2±0.11	0.38±0.02	0.60±0.02	0.41±0.03	95.46±0.51
D	2.5±0.21	0.36±0.01	0.56±0.03	0.36±0.03	96.28±0.20

* For abbreviations see Table 1.

TABLE 3. Viscoamylograph parameters of dough formulas.

Sample	Transition temp. (°C)	Maximum viscosity (BU)	Temp. at maximum viscosity (°C)	Breakdown viscosity (BU)	Setback viscosity (BU)
Control	63.0	370	85	160	410
Dough formulas*					
A	67.5	2230	94	1780	3000
B	70.5	2700	94	2000	3300
C	71.0	2900	94	2120	3450
D	71.5	3150	94	2250	3650

BU= Brabender Unit; * For abbreviations see Table 1.

perature at maximum viscosity, break down viscosity and set back viscosity, respectively. Addition of corn starch and hydrocolloids to first break wheat flour at different levels in all formulas gradually increased all measured parameters (67.5––71.5°C), (2230–3150 BU), (1780–2250 BU) and (3000–3650 BU) for heat of transition, maximum viscosity, break down viscosity and set back viscosity. This might be attributed to the higher content of starch in the blends and starch characteristics of corn compared to that of wheat. The gelatinization temperature of wheat starch (54–62°C) is lower than that of corn starch (60–71°C) [Colonna & Mercier, 1985]. Thus, corn starch granules are more rigid than wheat starch granules, they require more heat energy to achieve complete swelling. Shuey [1975] reported that higher amylogram values indicated less amylase activity and conversely lower amylogram values indicated higher activity, whereas extremely low values or higher activity would cause the slackening of the dough, especially during fermentation. The extent of slackening depends on starch damage of the flour.

Falling number

The falling number (FN) determination is commonly used to estimate α-amylase activity in wheat meal and flour [Finney, 2001]. Falling number, falling time and liquefaction number of all dough formulas are shown in Table 4. The results indicated that FN and falling time increased while liquefaction number decreased for all formulas compared with the control. For instance, FN of formula "D" was 590 s with about 58% increase compared to the control dough. Liquefaction number indicated a value of 22.73 for the control sample and this value decreased for all formulas, the reduction was about 45% for formula "D". The increasing of FN is mainly due to the higher starch content of all formulas. This means that the addition

TABLE 4. Falling number parameters of dough formulas.

Sample	Falling number (s)	Falling time (s)	Liquefaction number
Control	374	314	22.73
Dough formulas*			
A	426	366	18.99
B	490	430	15.79
C	550	490	13.66
D	590	530	12.50

* For abbreviations see Table 1.

of corn starch increased the viscosity. Also the effect of hydrocolloids (pectin and CMC) was similar to that of starch. Hydrocolloids modified food texture and consequently increased viscosity. Similar findings were also observed by Boyacioglu & D'Appolonia [1994], they reported that lower levels of α-amylase meant higher falling number values.

As shown in the same table, as falling number increased, falling time increased, while liquefaction number decreased. This trend was observed in all tested samples. The results obtained by the falling number test confirmed those obtained by the viscoamylograph test.

Organoleptic properties of bread

Table 5 represents the mean values of sensory evaluation of bread for general appearance, separation of layers, roundness, distribution of crumb, crust colour, taste and odour of shamy bread. The statistical analysis revealed that all formulas were rated lower than the control sample. Significant differences at >0.05 were noted for all sensory characteristics except taste and odour. The colour of bread crust was changed

TABLE 5. Sensory evaluation of shamy bread.

Characteristics	Control	Bread formulas*				LSD
		A	B	C	D	
General appearance (20)	16.89[a]	14.67[ab]	14.33[bc]	12.11[c]	9.60[d]	2.297
Separation of layers (20)	16.22[a]	14.11[ab]	13.56[b]	11.11[c]	8.33[d]	2.441
Roundness (15)	13.33[a]	11.89[ab]	11.78[b]	10.22[c]	8.65[d]	1.537
Distribution of crumb (15)	13.00[a]	11.44[ab]	11.11[b]	9.33[c]	7.43[d]	1.651
Crust colour (10)	8.3[a]	7.22[ab]	6.89[b]	6.00[c]	4.30[d]	1.405
Taste (10)	8.00	7.11	7.67	6.78	6.20	NS
Odour (10)	8.33	7.78	7.61	7.11	6.00	NS

* For abbreviations see Table 1; NS =Not Significant; There is no significant differences between two mean values (within same property) designed by the same letter.

TABLE 6. Moisture content and staling rate of bread during storage at room temperature*.

Sample	Moisture (%)			Freshness (%)			Loss in freshness(%)	
	Storage period (h)							
	0	24	72	0	24	72	24	72
Control	33.5	32.0	30.0	302	290	270	3.97	10.60
Bread formulas**								
A	34.5	32.0	30.0	305	285	270	6.56	11.48
B	35.0	31.5	29.0	307	280	260	8.80	15.31
C	35.0	31.0	28.0	304	270	250	11.18	17.76
D	34.0	30.0	27.0	300	262	235	12.67	21.67

* Data are average of triplicate analysis; ** For abbreviations see Table 1.

and become whiter by increasing levels of starch in the formulas. Because of browning of regular baked products depends to a large extent on the presence of protein, low protein bread and baked goods have a tendency to brown less than those made from wheat flour. So, we recommended brushing the bread or rolls with a little melted margarine or butter either prior to baking (rolls) or midway through baking the loaves of bread. This enhances the browning process. On the other hand, Bollinger [1992] reported that the addition of apple pectin extract at 2% of dry matter to wheat dough increased intensity of crust browning and gave darker and softer crumb compared with untreated bread. Lazaridou *et al*. [2007] found that the addition of CMC and β-glucans at 1 and 2% gave high porosity values for the produced bread, whereas high crumb elasticity was recorded with the addition of CMC, pectin and xanthan at 2% separately. They also noticed a high sensory characteristics score for bread made from gluten formulation supplemented with 2% CMC.

Freshness and moisture content of shamy bread

Alkaline water retention capacity (AWRC) is a simple and quick test to follow staling of bread. The changes in moisture content and AWRC for different bread samples stored at room temperature for 0, 24 and 72 h are shown in Table 6. It could be observed that the control bread sample had the highest value of AWRC, being 302%, 290% and 270% at 0, 24 and 72 h of storage, respectively. However all bread formulas caused a noticeable decrease in the AWRC values at 24 and 72 h storage compared with the control. The maximum decrease in AWRC values after 72 h of storage was observed in formula "D". This may be due to the presence of high amount of starch and dilution of the protein in all formulas. The obtained results were in agreement with those of Erazo-Castrejon *et al.* [2001], who found that bread firmness increased during the staling experiment for 5 days. They also added that the starch recrystallization has been identified as one of the causes of bread staling. Results showed also that moisture losses and staling rate increased during the storage period, although the addition of hydrocolloids altered these properties.

Yaseen *et al.* [2001] reported that the addition of pectin through dough formulation caused a noticeable increase in AWRC values after 1, 3 and 6 days of bread storage compared to control bread. The maximum increase in AWRC value was recorded at 1.5% pectin. The decrease rate of staling of pectin-treated bread was correlated with decreased starch retrogradation.

Amino acid composition and protein content of bread

Amino acid composition and protein content of all bread samples are shown in Table 7. The results indicated that all formulas of bread had lower total amino acids compared with the control. The total amino acids of the A, B,C and D formulas were 4.25, 3.79, 3.21 and 2.67 g/100 g dry sample constituting about 38%, 44%, 53% and 61% reduction compared to the control bread, respectively. The same findings were noticed when essential and non-essential amino acids were considered. The tested samples showed higher values of glutamic acid, tyrosine, arginine and proline, whereas the lower amino acid values being methionine, cystine and lysine.

TABLE 7. Amino acid composition and protein content of shamy bread (g/100 g sample)*.

Amino acids	Control	Bread formulas			
		A	B	C	D
Essential Amino Acids (EAA)					
Threonine	0.18	0.11	0.10	0.09	0.07
Valine	0.23	0.14	0.13	0.11	0.09
Methionine	0.12	0.08	0.07	0.06	0.05
Isoleucine	0.19	0.12	0.11	0.09	0.08
Leucine	0.41	0.26	0.23	0.19	0.16
Phenylalanine	0.29	0.18	0.16	0.14	0.11
Histidine	0.18	0.11	0.10	0.08	0.07
Lysine	0.15	0.09	0.08	0.07	0.06
Total EAA	1.75	1.09	0.98	0.83	0.69
Non Essential Amino Acids (NEAA)					
Aspartic acid	0.29	0.18	0.16	0.14	0.11
Serine	0.23	0.14	0.13	0.10	0.09
Glutamic acid	1.73	1.08	0.96	0.82	0.68
Proline	0.49	0.31	0.27	0.23	0.19
Glycine	0.21	0.13	0.12	0.10	0.08
Alanine	0.19	0.12	0.11	0.09	0.08
Cystine	0.15	0.09	0.08	0.07	0.06
Tyrosine	0.96	0.60	0.53	0.45	0.37
Arginine	0.81	0.51	0.45	0.38	0.32
Total NEAA	5.05	3.16	2.81	2.38	1.98
Total AA	6.81	4.25	3.79	3.21	2.67
Protein (%)	7.42	4.65	4.10	3.50	2.90

* Tryptophan was not determined.

The results also indicated that all formulated breads were characterised by a lower phenylalanine content by 38%, 45%, 52% and 62% for formulas A, B, C and D compared with the control, respectively. On the other hand, all formulated samples were found to contain the lowest amount of protein compared with the control bread. For instance while the protein content of formulas A, B, C and D was 4.65%, 4.10%, 3.50% and 2.90% that of the control sample was 7.42%. The reduction was 37%, 45%, 53% and 61% for formulas A, B, C and D, respectively.

From the above-mentioned results it could be concluded that one loaf of shamy bread of formula "D" (which contains 66 g of corn starch, 30 g of wheat flour, 2 g of pectin, 2 g of CMC, weight 100 g and 34% moisture) will provide the consumer about 95 mg of phenylalanine. This indicates that about 5 loaves will cover less than 500 mg phenylalanine/day as recorded by Hendriksz & Walter [2004] and consequently be safe.

CONCLUSION

Based on the previous results it could be concluded that all suggested formulas (A, B, C and D) are suitable for the production of low phenylalanine shamy bread, but formula "D" had superior sensory properties and phenylalanine requirements acceptable for PKU patients.

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