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Preparation of flatbread for phenylketonuria (PKU) patients balanced in protein content and amino acids

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ABSTRACT

An attempt to prepare protein content and essential amino acids balanced flatbread for PKU patients was done in the present study. The novel developed flatbread was done to be below the allowable limit of phenylalanine for this category and its technological properties should be acceptable. The flatbread was prepared by a free phenylalanine mixture of selected amino acids, each separately, and then mixed according to the RDA at a ratio of 10 and 20% were wheat flour (WF), corn starch (CS), cassava starch (CSS) and potatoes powder (PP) chosen in 1and 2 formula. Banana powder (BP), PP, CS and CSS, as 3 and 4 formula, Arabic gum and Pectin, control from WF, CS and CMC, to produce low phenylalanine flatbread. Studding of the chemical composition of flatbread samples showed that protein contents were about two folds in 1 and 3 samples and three times in samples 2 and 4; while total carbohydrates were lowered compared with control samples. Moreover, increases were also found in essential amino acids and total amino acids in flatbread samples fortified with the lower phenylalanine selected amino acids. Besides, the sensory attributes evaluation by PKU patients, also, showed that the flatbread samples were more acceptable compared with control in all properties especially taste and total score. About staling, the best states were in the arabic gum, pectin and fat formula compared with CMC and pectin in the control. In addition, physical and texture properties were also improved by using such materials and free phenylalanine selected amino acids at a ratio of 10 and 20 % of the RDA. These findings may be help to prepare flatbread balanced in protein, amino acids, with a lower phenylalanine and more acceptability for PKU patients.

Keywords: Amino acid, phenylketonuria patients, flat bread, sensory, physical properties.

1. Introduction

The primary macronutrient in our diet, protein serves a variety of structural and functional functions in the body. There are thousands of different protein components, each of which performs a vital purpose; without adequate protein consumption, we would perish. Protein-based ingredients also play many different technological roles in formulated foods, and contribute to texture, colour, flavour, and other properties. Protein is essential for vital bodily processes like growth, cell formation, growth, DNA synthesis, hormones, and enzymes (Loveday, 2019). To properly operate, the human body need 20 different types of amino acids. In combination, these 20 amino acids form There are 20 different varieties of amino acids that are necessary for the human body to function properly. The proteins that make up our bodies are made up of these 20 amino acids in diverse combinations. All nine of the essential amino acids can be produced by the body, but not hundreds of others. The essential amino acids (EAA), also known as indispensable amino acids (IAA), cannot be synthesised by humans or other vertebrates from metabolic intermediates. These essential amino acids (EAA) must be taken from an exogenous diet and consumed through food because the human body is unable to synthesise certain amino acids due to a lack of metabolic pathways. Nine essential amino acids, including phenylalanine, valine, tryptophan, threonine, isoleucine, methionine, histidine, leucine, and lysine, are present in food. Amide bonds, which are also known as peptide connections, join the chains of amino

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acids that make up proteins. The many side-chain groups, or R-groups, are what give each amino acid its own special characteristics (Lopez and Mohiuddin, 2023). According to Yaseen *et al.* (2011), phenylketonuria (PKU) patients are significantly impacted by the amount of protein and its amino acids in their diet. A uncommon metabolic illness called phenylketonuria (PKU) is caused by an insufficient amount of the liver enzyme phenylalanine hydroxylase (PAH), which results in a buildup of phenylalanine in the patient's tissues and plasma (Senemar *et al.,* 2009).

One of the most prevalent dysfunctions brought on by an inherited issue with phenylalanine metabolism is phenylketonuria (PKU). In this metabolic disorder, gene abnormalities in the enzyme phenylalanine hydroxylase (PAH) lead to phenylalanine buildup that causes variable degrees of mental impairment. Phenylalanine needs to be consumed at low levels (9.1mg/kg/d) to support healthy growth and development (Soltanizadeh and Mirmoghtadaie, 2014). Most children whose suffering from PKU can tolerate less than 500mg of phenylalanine or 10g of protein exchanges in 24h (Yaseen *et al*., 2011).

In order to meet the nutritional needs for protein and Phe, which are restricted by the low-Phe diet, Phe-free amino acid supplements and unique or naturally low-Phe foods must be consumed (Ahring *et al.,* 2009).

There are reportedly between 45.000 and 50.000 PKU sufferers globally, according to recent statistics from the National PKU Alliance (Shruti *et al.,* 2020). A PKU diagnosis occurs in roughly 1 in 12,000 births in India. Individuals with PKU must adhere to a reduced Phe diet for the rest of their lives to prevent neurological damage as an emergency treatment. Because natural foods are limited on the low-Phe diet, compliance is frequently poor.

A system of protein exchanges is utilised in the UK, with around 1 g of natural protein reflecting a 50 mg phenylalanine burden. In most PKU patients, fewer than 500 mg of phenylalanine or 10 g of protein swaps in 24 hours can be tolerated (Hendriksz and Walter, 2004; Pencharz *et al.,* 2007).

Because the low-Phe diet limits the consumption of high-protein foods, it is necessary to receive the dietary requirements for protein and Phe from Phe-free amino acid supplements and unique or natural foods that are low in Phe (Ahring *et al.,* 2009). Patients must cut out foods strong in protein, such as meat, poultry, fish, milk, nuts, and eggs, from their diets in order to maintain a diet low in Phe. Small portions of foods with moderate amounts of protein, such as fruits, vegetables, cereals, and potatoes, are therefore advised (Singh 2010). By choosing low-protein ingredients such starch, cellulose derivatives, and gums, low-phenylalanine baked items can be made. The functional impacts of hydrocolloids result from their capacity to alter the rheology (or handling) of batter or dough as well as the keeping properties of final goods (Yaseen *et al.,* 2011).

The world's third-most significant grain crop, behind rice and wheat, is maize. Many food and industrial items are made from corn, including starch, sweeteners, corn oil, drinks, and industries (Yu and Moon, 2021).

Cassava is now being used in items that are gluten-free or have decreased amounts of gluten, such as bread and biscuits. Approximately 80% of the dry weight of cassava roots are made up of the thick storage root and the peel, which are both mostly composed of carbohydrates by Falade and Akingbala (2010). The third-most plentiful source of several nutrients, including vitamins and minerals, is a tuber and root crop called potatoes, which are also quite adaptable (Wang *et al.,* 2016). A number of processed dishes can use potatoes as a raw ingredient.

In tropical and subtropical areas of the world, bananas are an important crop. The fruit is either eaten unripe in some native meals that call for high starch content or ripe due to its high sugar content. Bananas are primarily consumed ripe in Mexico and other Latin American nations. Large amounts of fruits are thus lost during commercialization as a result of inadequate postharvest (Rodriguez-Ambriz *et al.,* 2008).

According to Mahmood *et al.* (2017), hydrocolloids are rich in hydrophilic groups that have strong water binding ability, which changes the rheology of aqueous systems to which they are added. Because they enhance stability, alter textural profile, and slow down starch retrogradation, hydrocolloids (gums) are used in the food industry, the mixture of starch and gums is crucial in improving the functionalities of starch and the quality of products without increasing caloric levels (Von Borries-Medrano *et al.,* 2019).

The manufacturing of baked goods can be facilitated or made simpler by using improvers, which are combinations of ingredients that include additions. They are meant to make up for changes in processing qualities brought on by variations in the raw materials and to affect the quality of baked goods. They are primarily utilised to enhance the quality of bakery goods and industrial processes (Wassermann, 2000).

In contrast, it was observed by Shalini and Laxmi (2007) and Elkhalifa *et al.* (2007) that pectin, guar gum, carrageenan, CMC, and Arabic gum can all be used to extend the shelf life of bread. Arabic gum, which is the most widely used in the Middle East and Egypt, is frequently employed in the food business primarily to add desired features due to its influence on viscosity, body, and texture (Saha and Bhattacharya 2010). According to Shalini and Laxmi (2007), CMC can be used to extend bread's shelf life. The most common bread in Egypt and the Middle East is a round, flat (balady and shamy bread) loaf with two layers that is 1 cm thick and 10–30 cm in diameter.

Fortification of bakery products by amino acids was started before of 75 years by Bender, (1958) who are the first to talk about fortification of bread with amino acids such as lysine, threonine and methionine, which leads to improving amino acids contain, protein efficiency, protein ratio, and quality. Many studies were reported that after that, such as Goyal *et al.* (2017) who reported that the advances in food fortification with essential amino acids led to increasing in total essential amino acids, more improving in chemical score and biological value for food protein.

Therefore, the goal of this study is to limit phenylalanine intake to allow an acceptable range with high contain of other amino acids. To do this, it is planned to prepare protein balance in essential and non-sessional amino acids by using 2 combination forms of free phenylalanine selected amino acids blends, in order to achieve the 10 and 20% from RDA from all needed amino acids. for 7 - 14 old populations with 30 kg weight, and then to add in formulate different low p It had a few carefully chosen ingredients, including wheat flour (WF), maize starch, cassava starch (CSS), potatoes powder (PP), banana powder(BP), Arabic Gum, pectin and CMC to reduce the production of phenylalanine flatbread, to treat PKU.

2. Materials and Methods

2.1. Materials

From the North Cairo Flour Mill Company in Cairo, Egypt, wheat flour 72% extraction was obtained. Banana powder were obtained from Hainan Nicepal Industry Co., Ltd. China, Potatoes powder were obtained from China Mark Foods Trading CO., Ltd. Sigma Company in Germany supplied the CMC, Arabic gum, and Pectin (Food Grade). The Arabic Well Company for Food Additives El-obour in Cairo, Egypt provided the food grade maize and cassava starch. Selected food-grade amino acids were purchased from the Chinese importer XNTAI JIAHE BIOTECH COLTD Company. El-Menstarly House, El-bahr El-Aazam Street is where Menas Incorporation Company is located in Giza, Egypt. All additional ingredients for flatbread creation were purchased from local marketplaces in Cairo, Egypt. El-Gomhoria Company for Chemicals was used to purchase all chemicals.

2.2. Methods

2.2.1. Preparation of flatbread balanced in protein content and amino acids.

According to Yaseen *et al*. (2011) with some modifications, wheat flour and corn starch were thoroughly combined. Pectin and CMC were then added to the wheat/corn starch blends as a control sample, which was advised by the Egyptian Ministry of Health for PKU Patients. According to Table 1, four mixtures (1, 2, 3 and 4) were formed by combining wheat flour with powdered potatoes, maize starch and cassava starch as well as 10 and 20% of the RDA for essential amino acids for people weighing 30 kg and 10 and 20% of the RDA for non-essential amino acids 30 kg. according to (Essari, 2019). While mixtures 3 and 4 were made by combining banana, potato, corn and cassava starches with 10% and 20% of the RDA of essential and non-essential amino acids, respectively. With Arabic gum and pectin, the four mixtures were well blended. In Tables 1 and 2, the suggested formulas are displayed. Hot water (100°C) was added to the flour samples at a rate of 30% 2% to create a partial gelatinization of the starch after mixing the samples with a kneader. With sugar and 10% water at 35°C, active dried yeast was activated. All of the ingredients, baking powder, and activating yeast were added once the dough temperature was around (35°C). The dough was then kneaded for 5-7 minutes, or until it was of an appropriate consistency. The dough was placed within two plastic rolls and flattened to 3 ml after being allowed to ferment for 20 min at 30-35 °C and 75-85% relative humidity. The dough sheet was then divided into 120 g pieces by a die with a 20 cm diameter. The pieces were put together on a wooden board that had been lightly oil-sprinkle-coated, and they were allowed to re-ferment twice, the first time for about 20 minutes at the same temperature and relative humidity, and the second time for about an hour. The flattened loaves were then turned in the opposite direction to create some balance between the layers of the loaves, and they were also lightly oil-sprinkle-coated. At 30-35°C and 85%, the flattened loaves were proofed for 10-15 minutes.

2.2.3. Formulation and preparation of flatbread samples for PKU patients.

Table 1: Recipes of flatbread samples for PKU patients (g/100g).

Ingredient	Control	1	2	3	4
Wheat flour 72% (Wf)	30	15	15	-	-
Corn starch (CS)	70	45	45	45	45
Cassava starch (CSS)	-	25	25	25	25
Potatoes powder (PP)	-	15	15	15	15
Banana power (BP)	-	-	-	15	15
Amino acid/RDA	-	10	20	10	20
Arabic Gum	-	2	2	2	2
Pectin	2	2	2	2	2
CMC	2	-	-	-	-
Active dried yeast	0.5	0.5	0.5	0.5	0.5
Sugar	-	1	1	1	1
Salt	1.5	1.5	1.5	1.5	1.5
Sun flower oil	-	2	2	2	2

Table 2: Recipes of amino acids of flatbread samples for PKU patients (g/100g samples).

Sample (g/100g)	Cont.	1	2	3	4	*FAO/WHO (2007) (g/30 kg BW)
		WF with 10 % RDA of amino acids	WF with 20 % RDA of amino acids	BP with 10 % RDA of amino acids	BP with 20% RDA of amino acids	
		Essential Amino Acids (EAA)Estimated usage, (g/30 kg BW)				
Threonine	-	0.34	0.68	0.34	0.68	3.4
Valine	-	0.35	0.70	0.35	0.70	3.5
Methionine + Cystine	-	0.25	0.50	0.25	0.50	2.5
Isoleucine	-	0.28	0.56	0.28	0.56	2.8
Leucine	-	0.66	1.32	0.66	1.32	6.6
Histidine	-	0.16	0.26	0.16	0.26	1.6
Phenylalanine	--	--	--	--	--	----
Lysine	-	0.58	1.16	0.58	1.16	5.8

Table 2: Continued

Non-essential Amino Acids (NEAA) Estimated usage, (g/30 kg *BW)							
Sample (g/100g)	**Cont.**	**1**	**2**	**3**	**4**	**Estimated NEAA usage g/30 kg *BW**	**RDA of NEAA mg /kg *BW. (Tessari, 2019)**
Serine	-	0.060	0.120	0.060	0.120	0.600	20
Glutamic acid	-	0.111	0.222	0.111	0.222	1.110	37
Proline	-	0.156	0.312	1.56	0.312	1.560	52
Glycine	-	0.147	0.294	1.47	0.294	1.47	49
Alanine	-	0.099	0.198	0.099	0.198	0.990	33
Tyrosine	-	0.027	0.054	0.027	0.054	0.270	9
Arginine	-	0. 090	0.180	0. 090	0.180	0.900	30
Tryptophan	-	0.012	0.24	0.012	0.24	0.120	4
Aspartic acid		0.066	0.132	0.066	0.132	0.660	22

* Body weight

2.3. Chemical composition of flatbread samples for PKU patients.

2.3.1. According to the procedures given in AOAC (2016), the contents of moisture, ash, crude protein, crude fat and crude fibre were determined. Carbohydrates were determined using the difference.

2.3.2. Determination of amino acid profile of flatbread samples for PKU patients.

The produced flatbread samples underwent 24-hour hydrolysis with HCL (6 N) at 110°C. A buffer (pH 2.2) was used to dissolve the residue after the acid had been evaporated. Following the procedure described in AOAC (2016), the soluble amino acids were separated and their concentrations were measured using an amino acid analyzer (Biochrom 30). Tryptophan was not determined.

2.3.3. Physical properties of flatbread samples for PKU patients:

Physical Evaluation of flatbread samples, i.e., Height, weight, volume, specific volume, thickness, Baking loss, Roundness, and Baking time were determined according to the methods described by AACC (2000).

2.3.4. Water activity of flatbread samples for PKU patients:

Water activity (a_w) was measured with arotronic. Hygro Lab EA10-SCS (Switzerland) aw meter. The measurements were performed in triplicates (Czuchajowska *et al.,* 1989).

2.3.5. Freshness of flatbread samples for PKU patients:

According to Yamazaki's (1953) method, which was modified by Kitterman and Rubenthaler (1971) and Licciardello *et al.* (2014), the freshness of flatbread loaves was assessed after wrapping with polyethylene bags and storing them at 30 0C for 0, 24, and 36 and 48 hours: Availability of Alkaline Water (AWRC) as well as staling rate (SR) A bread loaf weighing one gram was placed in 15 mL tubes (W1) after being dried to a constant weight. Five mL of 0.1 N NaHCO3 were then added, stirred for 30 s, and allowed to sit at room temperature for 20 min. The slurry was centrifuged at 3000 rpm for 15 minutes, the supernatant was removed, and tubes were left to drip for 10 minutes while being tilted 150 degrees to the upside down position. The tubes were weighted (W2) after being dried. AWRC (%) was calculated as:

$$[(W2-W1)/W1] \times 100.$$

Where W1 = weight of tube containing the dry sample;
W2= weight of tube containing the dripped sample. Analyses were conducted in duplicate at each day of the experimental period (0, 1, 2 and 3 days).

2.3.5.1. Staling rate (SR) was calculated as follows:

Staling Rate (SR %) =[(AWRC0 – AWRCn)/ AWRC0] x 100

Where:
AWRC0 = AWRC at zero time, AWRC at a specific day of storage (n) by according to Kitterman and Rubenthaler (1971)

2.3.6. Texture profile analysis (TPA) of flatbread samples for PKU patients:

An operating manual for a texture analyzer is available from Stable Micro Systems in the USA under the title Brookfield CT3 Texture Analyzer Operating Instructions Manual, M08-372-C0113. AACC, (2000) was used to measure the flatbread's texture profile in terms of the hardness (N), cohesiveness, chewiness (mj), resilience (N), gumminess (N), and springiness (mm) of the samples. With the use of the parameters Test-TPA, Probe-36 mm (2.5 cm height, 4 cm diameter), the samples were compressed twice to 40% of their initial height. a circular test with a pre-test speed of 2 mm/s, a test speed of 2 mm/s, and a post-test speed of 2 mm. The experiments were conducted in ambient conditions at 0, 24, and 72 hours in the laboratories of the Agricultural Research Centre in Giza, Egypt.

2.3.7. Sensory evaluation of flatbread samples for PKU patients:

As detailed by El-Farra *et al.* (1982), 15 well-trained panellists completed sensory evaluation of flatbread for general appearance (20), separation of layers (20), roundness (15), distribution of crumb (15), crust colour (10), taste (10), and odour (10).

2.3.8. Statistical analysis

Least Significant Difference (LSD) was calculated in accordance with Mc Clave and Benson (1991) for the statistical analysis of the acquired results using analysis of variance (ANOVA).

3. Results And Discussion

3.1. Chemical composition of flatbread samples for PKU patients.

The result in Table (3) shouts the proximate composition crude protein, crude fat, ash, crude fiber, carbohydrate, moisture of the tested materials. It was observed a significant difference in fat and protein, where an increase in protein in all blends from 1 to 4, was detected recorded 6.70, 10.10, 5.61 and 8.60 %, respectively compared to the control sample which recorded 2.70%.

Table 3: Chemical composition of flatbread samples for PKU patients.

Treatments	Moisture (%)	*Protein (%)	*Fat (%)	*Ash (%)	*Crud fiber (%)	*TC
Cont.	22.5c±0.02	2.70e±0.30	0.51b±0.40	1.78a±0.08	0.17 a ±0. 03	94.84 a ±0. 84
1	24.33ab±0.42	6.70c±0.40	1.86a±0.05	1.63a±0.23	0.20 a ±0. 05	89.61 b ±0. 90
2	24.76a±0.23	10.1a±0.68	1.87a ±014	1.65a±0.15	0.20 a ±0. 05	86.18 c ±1. 56
3	23.55bc±0.99	5.61d±0.30	1.83a±0.71	1.58a±0.42	0.16 a ±0. 04	90.82 b±2. 88
4	23.84ab±0.02	8.60b±0.30	1.83a±0.45	1.60a±0.20	0.16 a ±0. 02	87.81c±1.089

Where, TC = total carbohydrates by deference. *= (on dry weight basis).
Control = 30% WF + 66% CS+ 2% CMC and 2% Pectin.
1= 15% WF + 15% PP + 45% CS+25% CSS + 2% Arabic gum+2% Pectin + 10% RDA (EAA& NEAA).
2= 15% WF+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 20% RDA (EAA& NEAA).
3= 15% Bp+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 10% RDA (EAA& NEAA).
4= 15% Bp+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 20% RDA (EAA& NEAA).
* Data are expressed as mean ± standard deviation of three replicates. Numbers followed by different letters within the same column are significantly different *($P < 0.05$)* according to Duncan's multiple range tests.
Means within a column showing the same letters are not significantly different ($P \geq 0.05$).

It could be due to the addition of amino acids (Table 2) on the other hand. there were no significant differences in each of the ash and crud fibers in all blends compared to the control sample. The flat bread prepared by the tested a blends from 1 to 4 were better in protein percentage, and were higher in nutritional values and high nutritionally for patients with phenylketonuria who suffer from malnutrition. Goyal *et al.* (2017) reported that the advances in food fortification with essential amino acids led to increasing in protein percent of food samples. The current results are in the same direction with those reported by Bender, (1958). Who are the first to handle about fortifying bread with amino acids such as lysine, threonine and methionine, which leads to improving the protein efficiency, protein ratio, and quality.

3.2. Amino Acids composition in based of flatbread samples for PKU patients (g/100 sample).

The main characteristic of food is the protein quantity and quality of its amino acid composition. Hence, all amino acids and essential amino acids are the necessary components for human life with values close to those set by WHO (2007). Table 4 illustrates the amino acids' composition of flatbread samples 1,2,3 and 4 made with different percentages of amino acids 10- 20% RDA. The essential amino acids were increased by adding of the mixer of amino acids in formulations, whereas threonine was increased from 0.075 g/100g sample in control to 0.346-0.681 g/100g sample in flatbread blends. Valine was increased from 0.096 g/100g sample in control to 0.350-0.690 g/100 g sample in flatbread blends. Methionine+ Cystine were increased from 0.053g/100g sample in control to 0.259 -0.512g/100g sample in flatbread blends. Isoleucine was increased from 0.082 g/100g sample in control to 0.281-0.563 g/100g sample in flatbread blends, leucine was increased from 0.160 g/100g sample in control to 0.669-1.335 g/100g sample in flatbread blends, phenylalanine was decreased from 0.100 g/100g sample in control to 0.057-0.059 g/100g sample in flatbread blends. Histidine was increased from 0.071 g/100g sample in control to0.161-0.325g/100g sample in flatbread blends and lysine was increased from 0.061 g/100g sample) in control to (0.580-1.152g/100g sample in flatbread blends. However, total essential amino acids (TEAA) were increased from 0.698g/100g sample in control to 2.763g/100g sample in sample (1), 5.317 g/100g sample in sample (2), 2.663g/100g sample in sample (3) and 5.133g/100g sample in sample (4). This increasing in total essential amino acids in flatbread samples may be due to adding of selected amino acids in formulas concurrent with Bender (1958), who found that fortified bread with amino acids such as lysine, threonine and methionine, was improve in quantity and quantity amino acids. Goyal *et al*. (2017), also, reported that the advances in food fortification with essential amino acids led to increasing total essential amino acids, improving chemical score and biological value for food proteins. The same findings were noticed when non-essential amino acids and total non-essential amino acids were considered, as well as Total AA were increased form 2.693 g/100g sample in control to 5.316, 8.799, 4.273 and 7.533 g/100g sample in samples 1,2,3 and 4 respectively. However, such increasing may be come back to same reasons. It was noticed that the adding of selected amino acids in flatbread blends. led to an increasing in total amino acids in flatbread samples compared with control; (WHO 2007). and (Tessari, 2019) for essential and non-essential amino acids.

Table 4. showed that intake of one loaf of 1 and 3 flatbread samples achieved 10% RDA of all required amino acids, while intake of five loaves of (1 and 3) flatbread samples, achieved 50% RDA of all required amino acids. However, intake of (1) loaf of flatbread of (2 and 4) samples were achieved 20% RDA of required amino acids. While intake of five loaves of (3and 4) flatbread samples achieved 100% RDA of all required amino acids. On the other hand, all formulated samples (weight 100 g and 23-24% moisture) found to contain the lowest amount of phenylalanine and will provide the consumer of about 50 mg/100g sample of phenylalanine in 1 and 2 samples and 17 mg/100g sample compared with100 mg/100g sample in control. This suggests that 5 loaves will cover 85–250 mg less than the daily limit of 500 mg phenylalanine, as reported by Hendriksz and Walter (2004), and will therefore be safe in comparison to the control sample of 500 mg/100g. The outcomes also showed that, in comparison to the control, the phenylalanine level of all formed flatbread samples was reduced.

Table 4: Amino acids fraction of flatbread samples for PKU patients (g/100 sample).

Amino acids g/100 sample	Flatbread formulas				
	Cont.	1	2	3	4
			Essential Amino Acids (EAA)		
Threonine	0.075	0.349	0.681	0.346	0.666
Valine	0.096	0.361	0.690	0.350	0.680
Methionine+ Cystine	0.053	0.260	0.512	0.259	0.496
Isoleucine	0.082	0.291	0.563	0.281	0.543
Leucine	0.160	0.682	1.335	0.669	1.300
Phenylalanine	0. 100	0.059	0.059	0.017	0.017
Histidine	0.071	0.170	0.325	0.161	0.311
Lysine	0.061	0.591	1.152	0.580	1.12
Total EAA	0.698	2.763	5.317	2.663	5.133
		Non-Essential Amino Acids (NEAA)			
Serine	0.10	0.136	0.147	0.100	0.160
Glutamic acid	0.63	0.751	0.930	0.456	0.685
Proline	0.21	0.313	0.508	0.132	0.190
Glycine	0.09	0.110	0.252	0.100	0.112
Alanine	0.10	0.200	0.291	0.182	0.221
Tyrosine	0.40	0.482	0.525	0.215	0.373
Arginine	0.320	0.410	0.513	0.259	0.365
Aspartic acid	0.118	0.151	0.216	0.166	0.234
Total NEAA	1.968	2.553	3.382	1.61	2.400
Total AA	2.693	5.316	8.799	4.273	7.533

3.3. Sensory evaluation of flatbread samples for PKU patients.

Sensory evaluation of flatbread blends for PKU patients prepared by different blends of amino acids fortified is given in Table (5). The preliminary evaluation attributes were general appearance, separation of layers, roundness, distribution of crumb, crust color, taste, odor and total score. The results were showed that the best formulas were 1and 2 samples followed by the other and control samples. There are no differences in all the sensory attributes. The best formulas were 1and 2 samples in general appearance where ranged between 13.6 and 13.7, prepared by the control sample 12.5 while the 3 and 4 samples recorded the lower values which ranged between 11 and 11.2. But there is no more significant differences in General appearance of the 3 and 4 flatbread samples values compared with control. As well as, there was a significant difference in the samples 1 and 2 with control. The roundness of flatbread samples was the same direction of general appearance. Consumer acceptance regarding distribution of crumb, crust color, taste and odor was higher in flatbread blends compared to control. This may be due to the use of the amino acids, Arabic gum, pectin and fats that were utilized as in Table (2) in the blends. Utilization of Arabic gum and pectin, which in turn, improved the sensory characteristics, especially crust color, taste, and odor. As they have the ability to retain water for a longer time. These results were consistent with the Yaseen and Shouk (2010) who found that Arabic gum or pectin could be effectively used to improve dough handling, baking quality, sensory acceptability and retarded staling of corn-wheat bread up to 2 and 3%, respectively. It was, also, reported that all the sensory attributes were higher than the control attributes value.

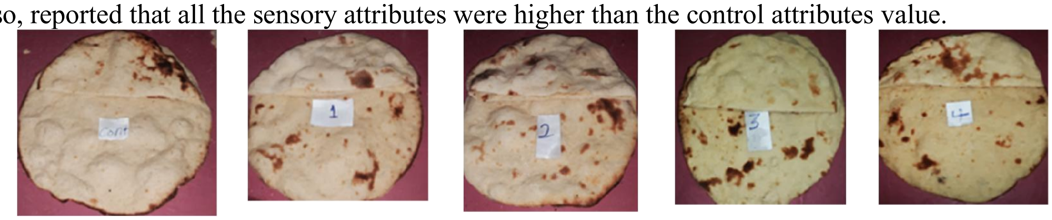

Fig. 1: Illustrate the shape of bread made from four formulae.

Table 5: Sensory evaluation of flatbread samples for PKU patients.

		Flatbread blends			
	Cont.	**1**	**2**	**3**	**4**
General appearance (20)	12.5b ±0.50	13.6 ab±0.60	13.7 a ±0.70	11.0c ±001	11.2c±0.20
Separation of layers (20)	12.8ab±0.80	13.0ab±002	13.5 a ±0.50	11.2b±0.20	11.4b±0.80
Roundness (15)	10.5b ±0.50	11.6 a ±0.10	11.7 a ±0.20	10.8b±0.02	10.8b±0.20
Distribution of crumb (15)	9.2b±0.20	11.6 a ±0.80	11.8 a ±0.80	9.8 b ±0.10	9.9 b ±0.10
Crust color (10)	5.2b±0.40	6.5 a ±0.50	6.6 a ±0.50	6.20a±0.20	6.3a ±0.30
Taste (10)	6.0b±0.50	8.2 a ±0.40	8.5 a ±0.50	7.6a ±0.60	7.8a ±0.80
Odor (10)	6.2b±0.30	7.4 a ±0.40	7.4 a ±0.40	7.0ab ±001	7.2a ±0.20

Control = 30% WF+ 66% CS+ 2% CMC and 2% Pectin.
1= 15% WF + 15% PP + 45% CS+25% CSS + 2% Arabic gum+2% Pectin + 10% RDA (EAA& NEAA).
2= 15% WF+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 20% RDA (EAA& NEAA).
3= 15% Bp+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 10% RDA (EAA& NEAA).
4= 15% Bp+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 20% RDA (EAA& NEAA).
* Data are expressed as mean ± standard deviation of three replicates. Numbers followed by different letters within the same column are significantly different *($P < 0.05$)* according to Duncan's multiple range tests.
Means within a column showing the same letters are not significantly different ($P \geq 0.05$).

3.4. Physical properties of flatbread samples for PKU patients.

The physical properties of the flatbread for PKU patients, were estimated these characteristics the results showed in Table (6). are represented the height, weight, volume, specific volume, thickness, baking loss, roundness, baking time and water activity. The of height (cm) characteristic value the highest in the sample 2, followed by control, then sample 1, and the lowest height (cm) was found in sample 3, then 4. This may be due to the use of wheat flour, pectin, gum Arabic, and essential amino acids that were formulated in Table. (2) while replacement of wheat flour by banana powder led to decreasing the flatbread height. Also, studying of weight, volume and specific volume, showed that there were no significant differences between the samples 3 and 4 compared with control. While, there were a significant difference in the samples, 1 and 2 with control which recorded the highest values in weight, volume and specific volume. This may be due to the use of Arabic gum, pectin and fats in addition to the use of wheat flour, their keep to more water as it was noted in the thickness characteristic, that there is no significant difference between control and 1 and 2 samples values (0.51- 0.56 cm), while the sample 3 and 4 (0.49, 0.50 cm) and significant differences with the control were found. About the baking loss %, it was higher in control which contain CMC and pectin but addition of pectin and Arabic gum in flatbread samples led to decrease of baking loss% to a certain extent, as it recorded 20.83, 20.66, 22.25, and 22 %compared to the control (22.5%). However, it was found that there were no significant differences between all samples compared to the control in roundness (cm). While these differences were seen in the baking time in all samples and control, this may be due to the use of CSS, amino acids, gum Arabic, pectin which works to increase the percentage of water adsorption. While an increase in the baking time was observed in flatbread samples, as it ranged from 127 to 140sec, and 112sec. for control. This may be due to the high protein content (Pecivova *et al.,* 2013). By estimating the water activity, it was observed that the proportion of water activity decreased in the blends, as the blends recorded 0.75, 0.72, 0.78 and 0.75, respectively compared to the control (0.80) and this may be due to the use of pectin and Arabic gum amino acids. As pectin and gum arabic combine to hold water. This results were in agreement with (Pahwa *et al.,* (2016).

Table 6. Physical properties of flatbread samples for PKU patients.

	Height (cm)	Weight (g)	Volume (cm^3)	Specific volume (cm^3/g)	Thickness (cm)
Control	4.20b±0.10	92.9b±0.4	132.91b±0.1	1.43b±0.02	0.51ab±0.04
1	4.00c±0.10	95.0a±0.5	138.50a±0.1	1.45a±0.00	0.53ab±0.03
2	5.45a±0.05	95.3a±0.5	138.81a±0.1	1.45a±0.00	0.56a±0.03
3	3.80d±0.10	93.3b±0.3	131.85b±0.1	1.41b±0.01	0.49b±0.02
4	3.94cd±0.1	93.6b±0.6	131.12b±0.1	1.40b±0.00	0.50b±0.02

Table 6: Cont.

	Baking loss%	Roundness (cm)	Baking time (sec)	Water activity
Control	22.50 a±0.15	10.28a±0.1	112e±0.3	0.80 a ±0.03
1	20.83 b±0.83	10.85a±0.1	132 b ±0.2	0.70b±0.01
2	20.66 b±0.66	11.10a±0.1	140a±0.1	0.65bc±0.05
3	22.25ab±0.75	10.40a±0.4	123d±0.1	0.60c±0.02
4	22.00ab±0.50	10.55a±0.1	127c±0.2	0.62c±0.05

Control = 30% WF + 66% CS + 2% CMC and 2% Pectin.
1= 15% WF + 15% PP + 45% CS+25% CSS + 2% Arabic gum+2% Pectin + 10% RDA (EAA& NEAA).
2= 15% WF+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 20% RDA (EAA& NEAA).
3= 15% Bp+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 10% RDA (EAA& NEAA).
4= 15% Bp+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 20% RDA (EAA& NEAA).
* Data are expressed as mean ± standard deviation of three replicates. Numbers followed by different letters within the same column are significantly different *($P < 0.05$)* according to Duncan's multiple range tests.
Means within a column showing the same letters are not significantly different ($P \geq 0.05$).

3.5. Freshness of flatbread samples for PKU patients.

Alkaline water retention capacity (AWRC) is a simple and quick test to follow staling and freshness of flatbread samples. Higher values of AWRC mean higher freshness of the flatbread (Mahmoud *et al.,* 2013). The changes in moisture and AWRC for different flatbread samples stored at room temperature 25±1°C for 0, 12, 24, and 36h periods storage are shown in Table (7).

Table 7: Effect of storage period at 25°C on staling of flatbread blends for PKU patients.

	AWRC	AWRC	DR %	AWRC	DR %	AWRC	DR %	AWRC	DR %
	Periods storage (hour)								
	0	**12**		**24**		**36**		**48**	
Control	310b±02	298c±02	3.87	280±03c	9.67	260 c±05	16.12	234d±04	24.51
1	321a±03	315ab±02	1.86	305ab±02	4.98	291ab±03	9.34	273ab±03	14.95
2	325a±05	320a±05	1.53	310 a±05	4.61	295a±05	9.23	277a±02	12.92
3	319a±04	312b±02	2.19	301b±02	5.64	284b±04	10.97	265c±05	16.92
4	323a±03	316ab±06	2.16	305ab±05	5.57	287b±02	11.14	269bc±01	16.71

AWRC= alkaline water retention capacity **DR%=** Decreasing rate of AWRC
Control = 30% WF + 66% CS+ 2% CMC and 2% Pectin.
1= 15% WF + 15% PP + 45% CS+25% CSS + 2% Arabic gum+2% Pectin + 10% RDA (EAA& NEAA).
2= 15% WF+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 20% RDA (EAA& NEAA).
3= 15% Bp+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 10% RDA (EAA& NEAA).
4= 15% Bp+15% PP + 45%CS+25% CSS+2% Arabic gum+2% Pectin + 20% RDA (EAA& NEAA).
* Data are expressed as mean ± standard deviation of three replicates. Numbers followed by different letters within the same column are significantly different *($P < 0.05$)* according to Duncan's multiple range tests.
Means within a column showing the same letters are not significantly different ($P \geq 0.05$).

The flatbread samples showed no significant differences in the zero time for all samples in AWRC while recorded significant difference with control, where it was 321, 325, 319 and 323 respectively in samples and 310% in control. But the control sample was higher in decreasing rate %

(DR%) of AWRC after storage to 12h (3.87%) followed by 1.53 and 2.19% in flatbread 2 and 3samples. However, after storing to 24 hours the DR% in the control was increased to (9.67%) while it ranged from (4.61) to (5.57%) in the flatbread samples. As well as after storage in same conditions for 36 hours the DR% was increased to 16.12% in control while it was 9.23-11.14% in the samples. Also, DR% in AWRC was increased to 24.51% in the control sample after storage to the 48-hour in same conditions while it was 12.92-16.71% in flatbread samples. It means that the flatbread samples are better than the control sample in freshness and low staling, while the better samples in freshness were 1and 2 which containing wheat flour, PP, CS, CSS Arabic gum and pectin. This may be due to the use of pectin with gums in addition to the use of fat in the mixture which increasing of water absorption, these results are consistent with (Mohammadi *et al.,* 2014 and Elkhalifa *et al.,* 2007) who used gum and pectin as well as the percentage of fat, as the percentage of fat maintains freshness staling and alkaline water retention capacity (AWRC) such finding were confirmed, also, by Mahmoud *et al.* (2013).

3.6. Textural profile analysis (TPA) of flatbread for phenylketonuria patients.

The textural properties of flatbread for PKU patients are important characteristics as they are related with consumer acceptance. The effect of adding of amino acids to starch material and hydrocolloids on the textural properties (Hardness (N), Cohesiveness, Chewiness (N), Resilience (N), Gumminess (N) and Springiness) of the flatbread samples are shown in Table (8). The results showed that no differences were found between 1 and 2 samples and control in Hardness but the flatbread samples became harder with adding of banana powder in 3 and 4 samples. The hardness and gumminess followed a similar trend and both of them increased with banana powder substitution. The hardness recorded the lowest value in control sample 46.00 N, followed by 1and 2samples (47.8 and 48.5) N, respectively, but it recorded the highest value in samples 4 and 3 (51.6 and 53.0 N respectively). Cohesiveness was higher in control (0.82), but it was decreased (to 0.79-0.73) in samples. Chewiness represent the energy required to chew a solid food into a state for swallowing, values for these characteristics were decreasing in control (5.4N), while was 6.3- 7,4N in other samples. Resilience, were high in control (0.41N) and slowly decreasing in the other samples (0.38-0.31N) but no significant differences were found 1 and 2 samples compared with control. While Springiness showed no significant differences between all samples with control values were (2.36-2.7 N) in springiness. This was similar between 1 and 2 samples and control in most of the textural properties it may be come back to use of wheat flour, starch types and gums. This results are agreed with (Mahmoud, *et al.,* 2013).

Table 8: Texture characteristics of flatbread blends for phenylketonuria patients.

Flatbread blends	Hardness (N)	Cohesivness	Chewiness (N)	Resilience, (N)	Gumminess (N)	Springiness
Control	46.0c±0.5	0.82a±0.02	5.4 c±0.04	0.41 a±0.02	63.62 d ±01	2.36 a ±0.5
1	47.8c±01	0.79ab±0.02	6.3bc±0.03	0.38ab±0.03	65.2cd±01	2.60 a ±0.2
2	48.5c±01	0.76bc±0.01	6.8ab±0.02	0.38ab±0.04	66.8bc±01	2.70 a ±0.5
3	51.6b±01	0.77b±0.02	6.8 ab±0.4	0.33bc±0.03	68.8ab±02	2.55 a ±0.1
4	53.0a±02	0.73c±0.03	7.4a±01	0.31c ±0.03	70.5a ±1.5	2.70 a ±0.2

N =Newton

Control = 30% WF+ 66% CS+ 2% CMC and 2% Pectin.
1=15%WF+15% PP + 45% CS+25% CS + 2% Arabic gum + 2% Pectin + 10% RDA (EAA&NEAA).
2= 15%WF+15% PP + 45% CS+25% CS+2% Arabic gum + 2% Pectin + 20% RDA (EAA&NEAA).
3= 15%Bp+15% PP + 45% CS+25% CS+2% Arabic gum + 2% Pectin + 10% RDA (EAA&NEAA).
4=15%Bp+15% PP + 45% CS+25% CS+2% Arabic gum + 2% Pectin + 20% RDA (EAA&NEAA).
* Data are expressed as mean ± standard deviation of three replicates. Numbers followed by different letters within the same column are significantly different *($P < 0.05$)* according to Duncan's multiple range tests.
Means within a column showing the same letters are not significantly different ($P \geq 0.05$).

4. Conclusion

Based on the previous results, it could be concluded that fortification of flatbread by 2 mixers of selected amino acids free from phenylalanine in formulas from low protein content materials (potatoes powder, banana powder, corn starch, cassava starch and low present of wheat flour 72%with hydrocolloids (Arabic gum and pectin) results in flatbread balance in protein and total amino acids as well as acceptable for PKU patients. All suggested formulas (1, 2, 3 and 4) are suitable for the production of low phenylalanine flatbread and phenylalanine requirements acceptable for PKU patients compared with control. But formulas 1 and 2 which contain 15% wheat flour 72% were superior in technical properties and sensory attributes.

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