Producing bread for phenylketonuria patients

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Abstract

Phenylketonuria (PKU) arises from a deficiency in the activity of the enzyme phenylalanine hydroxylase, which is essential for the conversion of the essential amino acid phenylalanine (phe) to tyrosine. Consequently, a bread product derived from cassava root, termed tapioca bread, was developed. This formulation consisted of tapioca flour and xanthan gum and was compared to locally produced bread intended for individuals with phenylketonuria. Chemical composition and organoleptic properties were analyzed to quantify amino acid content, with a particular focus on phenylalanine, which was found to be 0.99 mg/g in the tapioca bread. Furthermore, the determination of moisture, protein, fat, fiber, and ash content was conducted for both the tapioca bread and the local PKU bread. The protein content in tapioca bread was 2.14 g/100g, while the local PKU bread contained 0.61 g/100g. Fat content was 0.56 g/100g in tapioca bread and 4.82 g/100g in the local PKU bread. Fiber content was 1.86 g/100g in tapioca bread and 0.50 g/100g in the local PKU bread. Carbohydrate content was 64.2 g/100g in tapioca bread and 61.2 g/100g in the local PKU bread. Physical properties of the bread were also assessed, revealing

a weight of 25.26 g, a volume of 177.50 cm^3, and a specific volume of 7.02 cm^3/g for the tapioca bread.

Keywords: phenylketonuria patients, Amino acid, shamy bread

INTRODUCTION

Phenylalanine (Phe) is an aromatic amino acid that provides the carbon skeleton for the phenylpropanoid pathway, making many diverse chemicals used for structure, defense, and yet undiscovered functions **(Perkowski and Warpeha, 2019).** Phenylketonuria Phenylketonuria (PKU) also known as phenylalanine hydroxylase (PAH) deficiency is an autosomal recessive disorder of phenylalanine metabolism **(Van Spronsen, et al.,2021).**

PKU prevalence rate It has a prevalence ratio of 1 in 12,000 babies overall, however the numbers vary from region to region. PKU incidence was reported to be 1.5 per 10,000 and 1.7 per 10,000 for girls and boys respectively suggesting that male and female genders are affected almost equally**(KumarA and Adlakha ,2022a).**

PKU is caused PKU is caused by deficient activity of the enzyme phenylalanine hydroxylase, needed to convert the essential amino acid (AA) phenylalanine (phe) to tyrosine, **(MacLeod et al.,2010**).

PKU requires a lifelong low- phenylalanine diet that provides the majority of protein from a phenylalanine-free amino acid (AA) formula. The goal of PKU therapy is to maintain blood PHE concentrations within a therapeutic range, while providing optimal nutrition to support normal growth and development normal growth and development. (**Cunningham et al.,2023**)

Dietary treatment including the use of Phe-free medical food and the avoidance of high protein foods successfully lowers blood

Phe in most individuals with PKU. **(Brown and Lichter, 2016).** Symptoms of people infected with pku infants with PKU can be identified by newborn screening and placed immediately on a phe-restricted diet. If treated early and aggressively, individuals with PKU avoid severe complications and can achieve normal intellectual functioning (**Bilder et al.,2013).**

Tapioca flour, a widely consumed product from Amazon region (Pará, Brazil), can be classified as a secondary product derived from the production of cassava flour. The cassava roots **(*Manihot sculenta*)** are grinded and then washed with water to partially remove the starch by leaching. The washed cassava grinded mass follows the traditional cassava flour process, and the removed starch will be pressed to remove the excess of moisture, dried and stored in polyethylene plastic bags. Tapioca flour is usually traded in local markets **(Chisté *et al*.,2012)**

Materials and Methods

A. Materials:

Tapioca flour

1-Tapioca flour was obtained from the Agricultural Research Center

2- Xanthan gum will be obtained from the Agricultural Research Center

3-Yeast

4-Baking powder

5-salt , sugar

6-Oil and ghee

7-water

-All other materials that were used in the dough preparation for bread and biscuits were obtained from local markets in Cairo, Egypt.

B.Research Methods

1B.Instruments:

The instruments are separated into two categories. The first are the tools used to make (bread) from Tapioca flour such as agitator, oven, spoon , Household scale. **(Prameswari et al .,2018)**

2B. Ingredients:

Table (1) Products formula made from tapioca flour

Ingredients (g)	Products formula
	Shamy Bread
Tapioca flour	216
Xanthan gum	14
Yeast	10
Baking powder	5
Oil	38
Water(ml)	176
Salt	5
Sugar	5

1.1. Preparation of product:

1-Tapioca bread was made by traditional methods as described by **(Pudjihastuti and Sumardiono., 2018).**with some modification **.**

B-Methods

***B-Chemical composition and organliptic properties* :**

Determination of Amino Acids Content:

Samples measured according to (**Kumar and Adlakha.,2022b)**

4B-Determination of moisture , protein, fat , fiber and ash:

Chemical composition of the resulted product was analyze for protein, fat ,fiber and ash according to the AOAC official method **(AOAC., 2010)**.

5B-Determination of Total Carbohydrates (by differences)

Total carbohydrate = (protein +fat + fiber+Ash +moisture) – 100

6B-Amino acids composition using HPLC:

The amino acids composition of experimental samples were determined using HPLC-Pico-Tag method according to **(Cooperative, 1987)**The Pico-Tag method, was described by **(Heinrikson and Meredith .,1984), (White*.,* 1986)** and **(Cohen *et al* .,198٩).** The Pico-Tag method, was developed commercially by Waters Associates, was an integrated technique for amino-acid analysis. Phenylisothiocyanate (PITC, or Edman's reagent) was used for pre-column derivatization, while reversed-phase gradient elution high-performance liquid chromatography (HPLC) separates the phenylthiocarbamy (PTC) derivatives which were detected by their UV absorbance.. The chromatographic analysis using HPLC was carried out using the following gradient of Pico-Tag solvent A and B (P/N 88108 and 88112). Sample was injected and loaded on Pico-Tag amino acids column (150 x 3.9 mm) stainless steel. Detection of the PTC derivatives is by ultraviolet absorption measurements using a fixed wavelength (254nm) Waters detector. Before injecting of the sample, the illustrated was calibrated by two injections of the standards

C-Evaluation of functional properties

1C-Physical properties of shamy bread

The specific volume **(**cm^3/g) of bread was calculated by dividing volume **(**cm^3) by weight **(**g). Weight after removal from the oven and loaf volume **(**cm^3) was measured by rapeseed displacement. Loaves were placed in a container of known volume into which rapeseeds were run until the container was full. The volume of rapeseeds displaced by the loaf was considered as the loaf volume. Bread diameter **(**cm) was determined by planimeter **(AACC, 2000)**.

D-Statistical Analysis:

The results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data was presented as mean ± standard error (SE). Analysis of Variance test and (independed T test) was used for determining the significances among different groups according to **(Armitage and Berry .,1987).** All differences were considered at a significant level of P-values < 0.05 -P-values < 0.0١.

Results and Discussion

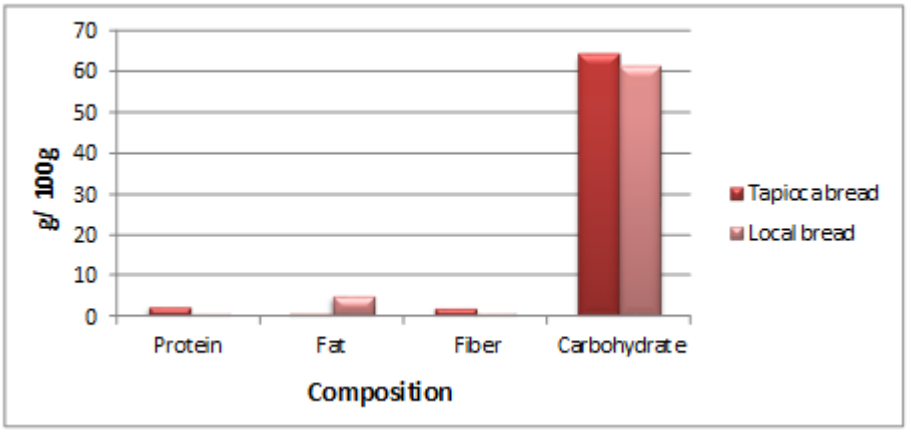
First: Chemical Composition and Organoleptic Properties of the Products A variety of tapioca flour-based products such as bread, biscuits, Syrian bread, noodles, and rusk were developed specifically for patients with phenylketonuria (PKU). These products were compared with locally available PKU products in terms of their chemical composition and organoleptic properties. The chemical analysis included the determination of moisture, fiber, protein, ash, and fat content, as well as the calculation of total carbohydrates. Furthermore, a sensory evaluation was conducted to assess consumer acceptability and product quality based on taste, texture, aroma, and overall appearance.

Table (1) chemical composition of tapioca shamy bread and local bread for PKU patients (g/100g)

Composition Samples	Protein	Fat	Fiber	Total carbohydrate
Tapioca bread	**2.14± 0.30***	**٠.56±.026**	**1.86±.030***	**64.2±.24***
Local shamy bread for PKU patients	**0.61± .010**	**4.82±.010***	**0. 50±.10**	**61.2±.20**

All values are represented as means ± SD

"Values marked with an asterisk (*) indicate a statistically significant difference at (P < 0.01)."



FigureA. chemical composition of tapioca shamy bread and local bread for PKU patients.

Table (1) and Figure (A) present the chemical composition of tapioca shamy bread and local bread for PKU patients (g/100g). The protein content in tapioca shamy bread (2.14 ± 0.30 g/100g) is increases significantly as compared to local bread for PKU patients (0.61 ± 0.10 g/100g).while The fat content in tapioca shamy bread (0.56 ± 0.026 g/100g) is increases significantly as compared to local

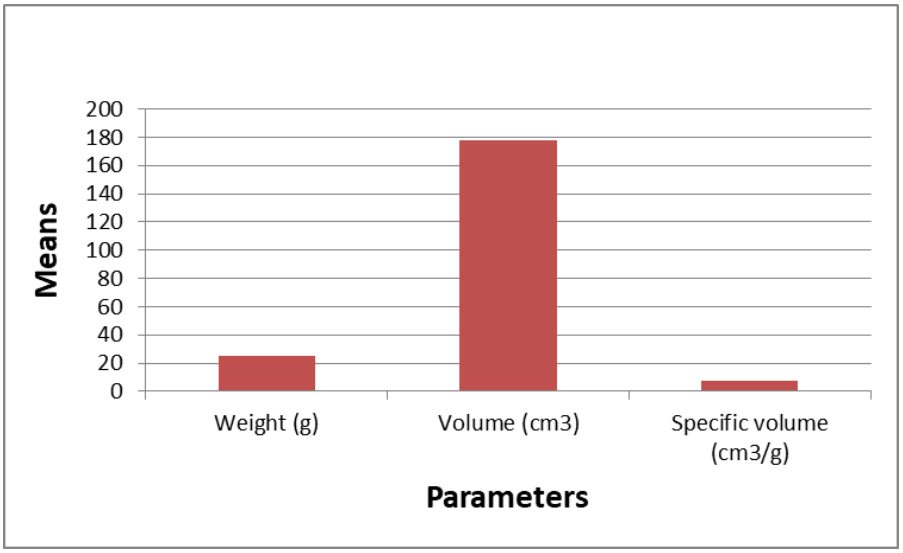
bread for PKU patients (4.82 ± 0.010 g/100g). Additionally, the fiber content in tapioca shamy bread (1.86 ± 0.030 g/100g) is increases significantly compared to local bread for PKU patients (0.50 ± 0.10 g/100g). Finally, the carbohydrate content in tapioca shamy bread (64.2 ± 2.24 g/100g) is increases significantly compared to local bread for PKU patients (61.2 ± 2.20 g/100g). Overall, the results indicated that tapioca bread demonstrated superior nutritional characteristics compared to the locally available bread for PKU patients. These findings suggest that tapioca-based bread could be a more balanced and nutritionally favorable option for PKU patients, aligning better with their specific dietary needs.

These results disagree with **(Kamel *et al* .,2020)**, who found that bread for PKU patients **a** protein content was 1.25%, a fat content was 3.22%, a fiber content was 0.48%, and a carbohydrate content was 94.80%. These results also differ with **(Scortegagna *et al*., 2020),** who found that bread for PKU patients

a protein content a protein was 1.41 g/100g, a fat content was 1.34 g/100g, and a carbohydrate content was 65.28 g/100g.

Table (2):Physical properties of bread

Parameters	**(**Means ± SD**)**
Weight (g)	25.26±0.55
Volume (cm^3)	177.50±3.53
Specific volume (cm^3/g)	7.02±0.01

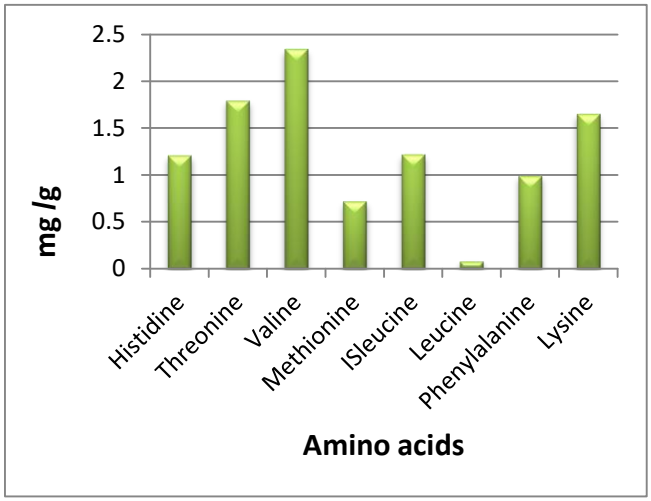


F.g.B Physical properties of bread

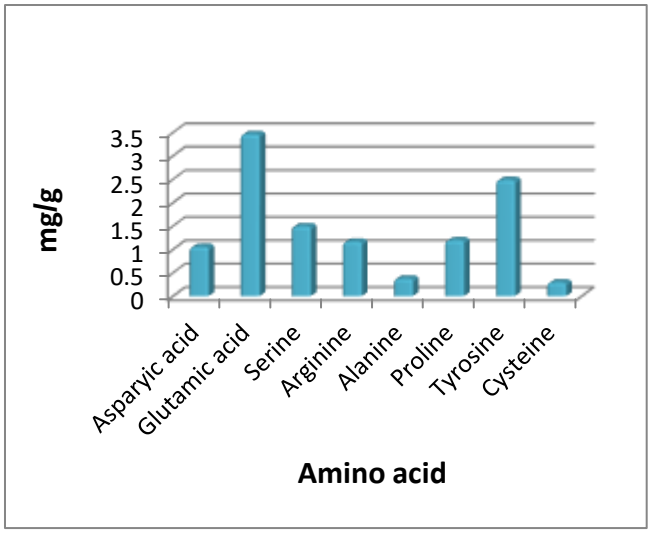
Table(2) and Figure (B) present the physical properties of bread, including weight, volume, and specific volume. The data are expressed as means ± standard deviation (SD), reflecting the variability among the samples. The average weight of the bread is 25.26 ± 0.55 g, indicating a relatively consistent sample size. The volume is 177.50 ± 3.53 cm^3, demonstrating the bread's expansion capability during baking. The specific volume, a key indicator of bread quality, is 7.02 ± 0.01 cm^3/g, which suggests a light and airy crumb structure with minimal variation between replicates.

Table (3):Results (Amounts mg/g sample)		
Amino acids	**unit**	**Shamy Bread**
Asparyic acid	mg/g	١,٠٦
Glutamic acid	mg/g	٣,٤٧
Serine	mg/g	١,٥٠
Glycine	mg/g	0.58
Histidine	mg/g	1.21
Arginine	mg/g	1.17
Threonine	mg/g	1.79
Alanine	mg/g	0.38
Proline	mg/g	١,٢٠
Tyrosine	mg/g	٢,٤٩
Valine	mg/g	٢,٣٤
Methionine	mg/g	٠,٧٢
Cysteine	mg/g	0.30
ISleucine	mg/g	١,٢٢
Leucine	mg/g	٠.83
Phenylalanine	mg/g	٠,٩٩
Lysine	mg/g	١,٦٥
Tryptophan not determined		

The Total Amino Acids were analyzed by Staff member of Amino Acids Units NRC Egypt. The sample was brought by the customer without any liability to the laboratory Concentration of amino acids mg/g sample: Tryptophan has not determined because it is destroyed during the acid hydrolysis reaction.



F.g.C1 refers to the Essential amino acids.



F.g.C 2 refers to the Non-Essential Amino Acids

As illustrated in Table (3) and Figure C1 C 2, it could be observed that the amino acid profile of Shamy Bread reveals significant variations in concentration among the different amino acids analyzed. Glutamic acid exhibits the highest concentration at 3.47 mg/g, notably exceeding the levels of other amino acids. Conversely, Cysteine shows the lowest determined concentration at 0.30 mg/g. The remaining amino acids are present in intermediate concentrations, ranging from 0.38 mg/g for Alanine to 2.34 mg/g for Valine. It is also important to note that Tryptophan was not quantified in this analysis.

The results of your amino acid content analysis in Shamy bread for PKU patients revealed notable discrepancies when compared to the findings reported by **(Yaseen *et al*., 2011).** For instance, your aspartic acid content was 1.06 mg/g, whereas their findings indicated 1.6 mg/g. Similarly, a considerable difference was observed in the glutamic acid content, with your results indicating 3.47 mg/g compared to their 9.6 mg/g. These differences extended to most other analyzed amino acids. Your serine content was found to be 1.50 mg/g compared to their 1.3 mg/g, glycine was 0.58 mg/g versus 1.2 mg/g, histidine was 1.21 mg/g versus 1.0 mg/g, arginine was 1.17 mg/g versus 4.5 mg/g, threonine was 1.79 mg/g versus 5.3 mg/g, alanine was 0.38 mg/g versus 1.1 mg/g, proline was 1.20 mg/g versus 2.7 mg/g, tyrosine was 2.49 mg/g versus 5.3 mg/g, valine was 2.34 mg/g versus 1.3 mg/g, methionine was 0.72 mg/g versus 0.7 mg/g, cysteine was 0.30 mg/g versus 0.8 mg/g, isoleucine was 1.22 mg/g versus 1.1 mg/g, leucine was 0.83 mg/g versus 2.3 mg/g, phenylalanine was 0.99 mg/g versus 1.6 mg/g, and lysine was 1.65 mg/g versus 0.8 mg/g. Notably, the tryptophan level was not determined in your results, consistent with their methodology.

Photo (1) shows tapioca bread for Phenylketonuria patients

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الملخص العربي

ينتج بيلة الفينيل كيتون (PKU) عن نقص في نشاط إنزيم فينيل ألانين هيدروكسيلاز، وهو ضروري لتحويل الحمض الأميني الأساسي فينيل ألانين (phe) إلى التيروزين. ونتيجة لذلك، تم تطوير منتج خبز مصنع من جذور الكسافا، يسمى خبز التابيوكا. تتكون هذه التركيبة من دقيق التابيوكا وصمغ الزانثان، وقد قورنت بخبز منتج محليًا مخصص للأفراد المصابين ببيلة الفينيل كيتون. تم تحليل التركيب الكيميائي وتحليل كيميائي للأحماض الأمينيه لتحديد كمية الأحماض الأمينية، مع التركيز بشكل خاص على الفينيل ألانين، الذي وُجد أنه ٠,٩٩ ملجم/جم في خبز التابيوكا. علاوة على ذلك، تم تحديد محتوى الرطوبة والبروتين والدهون والألياف والرماد لكل من خبز التابيوكا والخبز المحلي الخاص بمرضى بيلة الفينيل كيتون. بلغ محتوى البروتين في خبز التابيوكا ٢,١٤ جم/١٠٠ جم، بينما احتوى الخبز المحلي الخاص بمرضى بيلة الفينيل كيتون على ٠,٦١ جم/١٠٠ جم. وبلغ محتوى الدهون ٠,٥٦ جم/١٠٠ جم في خبز التابيوكا و٤,٨٢ جم/١٠٠ جم في الخبز المحلي الخاص بمرضى بيلة الفينيل كيتون. وبلغ محتوى الألياف ١,٨٦ جم/١٠٠ جم في خبز التابيوكا و٠,٥٠ جم/١٠٠ جم في الخبز المحلي الخاص بمرضى بيلة الفينيل كيتون. وبلغ محتوى الكربوهيدرات ٦٤,٢ جم/١٠٠ جم في خبز التابيوكا و٦١,٢ جم/١٠٠ جم في الخبز المحلي الخاص بمرضى بيلة الفينيل كيتون. كما تم تقييم الخصائص الفيزيائية للخبز، وكشفت عن وزن قدره ٢٥,٢٦ جم، وحجمه ١٧٧,٥٠ سم^3، وحجمه نوعي ٧,٠٢ سم^3/جم لخبز التابيوكا.

الكلمات المفتاحية: الفينيل كيتون يوريا , الأحماض الأمينيه , خبز شامي