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HEALTH SAFETY ASPECTS OF FOODSTUFFS INTENTED FOR PHENYLKETONURICS

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ABSTRACT

Phenylketonuria is a rare metabolic disorder that occurs due to the lack of liver enzyme phenylalanine hydroxylase. Untreated it leads to mental retardation, delayed development of speech, microcephaly, epilepsy, behavioral problems etc. Its treatment consists of strict diet, very low in phenylalanine content. The aim of this study was to determine the health safety and suitability of 17 selected foods labeled as „low in protein" respectively „foods low in phenylalanine". Analyses were focused on their phenylalanine content and aminoacid representation. The other aim was to compare phenylalanine measured with the content declared by the producers on their label. By product origin, the largest amount of foodstuffs originated from Poland (59%), followed by Germany (23%) and equal representation (6%) had Slovakia, Hungary and Sweden. Automatic analyzer AAA 400, using ion exchange chromatography, analyzed the samples. The lowest determined phenylalanine content was 1 mg.100g^{-1} (in rubber candy) and the highest 299 mg.100g^{-1} (in paté). Powdered egg alternative was monitored as the second highest source of phenylalanine. Phenylalanine content in the monitored foods ranged from 1 to 299.6 mg. 100 g^{-1} respectively, and increased in the following order: Gum candies (1 mg.100g^{-1}) < Cherry jelly (1.4 mg. 100g^{-1}) < Flour (5.8 mg.100g^{-1}) < Flour (5.9 mg.100g^{-1}) < Spaghetti (8.7 mg.100g^{-1}) < Pasta (9.9 mg.100g^{-1}) < Waffle (14.2 mg.100g^{-1}) < Salty sticks (23.8 mg.100g^{-1}) < Chocolate (24.1 mg.100g^{-1}) < Bread (26 mg.100g^{-1}) < Breadcrumbs (27.1 mg.100g^{-1}) < Sticks with salt (30.3 mg.100g^{-1}) < Ice Cornets (30.8 mg.100g^{-1}) < Walnut cookie (37.2 mg.100g^{-1}) < Instant soup with noodles (46.1 mg.100g^{-1}) < Powdered egg alternative (58 mg.100g^{-1}) < Pate (299.6 mg.100g^{-1}). Only seven products of 17 observed showed low phenylalanine content, less than 20 mg.100g^{-1}.

Keywords: phenylketonuria; phenylalanine; low-protein product; acid hydrolysis; chromatography

INTRODUCTION

Phenylketonuria (PKU) is an autosomal recessive inherited disorder of phenylalanine metabolism due to a lack of the enzyme phenylalanine hydroxylase. Most forms of PKU and hyperphenylalaninemia (HPA) are caused by mutations in a gene on chromosome 12 of phenylalanine hydroxylase (PAH) **(Burnett et al., 2008)**. Currently, more than 400 known mutations are identified in the gene for PAH. Lack of or insufficient activity of the phenylalanine hydroxylase causes accumulation of phenylalanine (Phe), and phenyl ketone excretion in the urine. Any increase in the concentration of phenylalanine in the blood results in central nervous system damage **(Strnová and Ürge, 2007)**.

Phenylalanine (Phe) is an essential amino acid that is a precursor of melanin, dopamine, and thyroxine. It is an essential amino acid and its natural occurrence in food is usually sufficient (mean content 3.5%). In protein foods, Phe constitutes about 4-5% **(Velíšek and Hajšlová, 2009)**. The artificial sweetener aspartame is also high source of phenylalanine. The ADI for aspartame for healthy person is 40 mg/kg body weight **(EFSA, 2014)**.

In PKU patients, a restriction of foods rich in protein (meat, fish, eggs, bread, dairy products, nuts and seeds), as well as avoidance of drinks containing aspartame help to control blood phenylalanine levels. The developing fetus of women suffering from PKU is particularly sensitive to their mother's phenylalanine levels **(EFSA, 2014)**. The basic treatments of PKU are mixture preparations of aminoacids. Their use ensures an adequate supply of amino acids without phenylalanine, thereby compensating reduced protein intake in low protein dietary regime. Patients with phenylketonuria usually get these foods by medical prescription as most of these foodstuffs can belong to the group of foods for special medical purposes **(Strnová and Ürge, 2007)**, which safety is defined by the **Commission Directive 1999/21/EC**.

Untreated children with persistent hyperphenylalaninemia (PKU) show brain damage. Symptoms include microcephaly, epilepsy, mental disability and behavioral problems. By the use of neonatal screening and early dietary intervention, children born with PKU can live a relatively normal life **(Burnett et al., 2008; Mitchell, 2000)**.

The application of diet at an early age of life can significantly reduce mental deficiencies associated with phenylketonuria. Dietary measures are based on the foods containing a minimum of Phe, while preserving the normal content of tyrosine and other proteins in food. This is often achieved by substitution of phenylalanine free proteins. Dietary restriction of natural proteins reduces and

maintains the level of Phe in the blood **(Macdonald et al. 2009; Poustie and Wildgoose, 2010; Svačina, 2008)**.
The aim of this work was to analyze selected amino acids (aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine) in seventeen food samples, declared by the producer as "Low-protein foods" respectively "Foods low in phenylalanine content " by automatic analyzer AAA 400 and consequently to evaluate their safety and suitability for a particular nutritional use. To compare the content of phenylalanine declared on the label with content of phenylalanine measured.

MATERIAL AND METHODOLOGY

As material used were the foodstuffs offered for phenylketonurics obtained from internet sale and labeled as food suitable for phenylketonurics as „low in protein" respectively „foods low in phenylalanine". Overview of the material used is given in Table 1.

By origin of products, most of them originated from Poland (59%), followed by Germany (23%) and equal representation (6%) had Slovakia, Hungary and Sweden.

Fifteen amino acids (aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, phenylalanine, tyrosine, histidine, lysine and arginine) were determined in observed samples using ion-exchange chromatography. The total amount of amino

Table 1 Overview of the material tested.

No.	Product	Producer	Main components
1.	Pate	Producer A, Germany	bacon, pork, liver, onion, water, salt, stabilizer E 250, spice, emulsifier
2.	Breadcrumbs	Producer 1, Poland	gluten free wheat starch, corn starch, vegetable oil, glucose, sugar, yeast, salt, guar gum, E 464, fibre, raising agents, E 500, E 575
3.	Spaghetti	Slovakia	corn starch, modified corn starch, emulsifier E 471, dye curcuma (E 100)
4.	Instant soup with noodles	Producer 1, Poland	maltodextrin, gluten free pasta, corn starch, water, salt, mono and diglycerides of fatty acids, E 464, E 575, betacarotene, salt, monosodium glutamate, vegetable oil, carrot, sugar, onion, aroma, celery, leek, black pepper, parsley stalk, curcuma, citric acid
5.	Sticks with salt	Producer 1, Poland	corn starch, salt, wheat starch gluten free, vegetable oil, sugar, yeast, guar gum, E 464, salt, fibre, E 500, E 503, mono and diglycerides of fatty acids, lecithin, E 575
6.	Walnut cookie	Producer 2, Poland	corn starch, gluten free wheat starch, vegetable oil, sugar, walnuts, peanuts, oil, pectin, guar gum, pectin, glucose, butter essence, mono and diglycerides of fatty acids
7.	Powdered egg alternative	Producer B, Germany	corn starch, mono and diglycerides of fatty acids, palm oil, xanthan, rice flour, pea protein
8.	Salty sticks	Producer 2, Poland	margarine, corn starch, gluten free wheat starch, water, sugar, potato starch, yolk, guar gum, yeast, potato syrup, wheat gluten free cellulose, salt, E 503, mono and diglycerides of fatty acids, lecithin, vanillin
9.	Chocolate	Producer C, Germany	cocoa butter, sugar, butter, cocoa matter, powdered whey, vanilla
10.	Pasta	Hungary	corn starch, water, fatty acids, E 471
11.	Waffle	Producer 2, Poland	gluten free wheat starch, corn starch, guar gum, bamboo fibre, glucose, apple pectin, mono and diglycerides of fatty acids
12.	Cherry jelly	Producer 2, Poland	sugar, glucose syrup, water, agar, citric acid, cherry aroma, dye E 124
13.	Ice Cornets	Producer 2, Poland	corn starch, gluten free wheat starch, potato starch, sugar, yolk, guar gum
14.	Bread	Producer 1, Poland	gluten free wheat starch, water, vegetable fat, sugar, glucose, yeast, salt, carob, guar gum, E 464, fibre, E 500, E 575
15.	Gum candies	Producer C, Germany	potato and tapioca starch, citric acid, malic acid, galactic acid, calcium citrate, arome, fruit concentrates, vegetable extracts (blackberries, carrot, pepper, curcuma, spirulina, nettle, spinach), natural dye : chlorophyll, vegetable oil, beeswax, carnauba wax
16.	Flour	Producer 1, Poland	gluten free wheat starch, glucose, corn starch, guar gum, E 464, vegetable fibre, E 500, E 575
17.	Flour	Sweden	wheat starch (max. 0.2 g gluten/100 g), oligofructose, guar gum, vitamins, ferrum

acids and phenylalanine content of food products were monitored after 23-hour acid hydrolysis using HCl (6 mol.dm^{-3}) and 115 ±2 °C. After the hydrolysis, the test tubes were cooled down to 20°C. Hydrochloric acid was evaporated and the ropy residue was diluted in loading buffer in a 25 cm^{-3} volumetric flask. The mixture was filtered through 0.22 μm filter and loaded into an analyser. Amino Acid Analyser AAA400 (Ingos, Prague, Czech Republic) was equipped with a column (370×3.7 mm filled with a ion exchanger Ostion LG ANG – Ingos, Prague, Czech Republic), post-column ninhydrine derivatization and spectrophotometric detection (440 nm for proline and 570 nm for other amino acids). Method was performed according to the **Buňka et al. (2009)**.

Statistical analysis of results

The results were evaluated by calculating the mean, coefficient of variation and standard deviation using the statistical software Statistica 8.0.

RESULTS AND DISCUSSION

Treatment of phenylketonuria should be a combination of several diets that reduce the amount of phenylalanine: gluten-free diet, lactose-free and low proteins diet which is the most important. The principle of the diet is to reduce the amount of phenylalanine in the body and to create a balance between its income and the potential use **(Komárková and Pazdírková, 2010; Dvořák, 2009)**.

Phenylalanine content in the monitored foods in our work was represented in varying amounts, its content ranged from 1 to 299.6 mg. 100 g^{-1} respectively, and increased in the following order:

Gum candies (1 mg.100g^{-1}) < Cherry jelly (1.4 mg.100g^{-1}) < Flour no.16 (5.8 mg.100g^{-1}) < Flour no. 17 (5.9 mg.100g^{-1}) < Spaghetti (8.7 mg.100g^{-1}) < Pasta (9.9 mg.100g^{-1}) < Waffle (14.2 mg.100g^{-1}) < Salty sticks (23.8 mg.100g^{-1}) < Chocolate (24.1 mg.100g^{-1}) < Bread (26 mg.100g^{-1}) < Breadcrumbs (27.1 mg.100g^{-1}) < Sticks with salt (30.3 mg.100g^{-1}) < Ice Cornets (30.8 mg.100g^{-1}) < Walnut cookie (37.2 mg.100g^{-1}) < Instant soup with noodles (46.1 mg.100g^{-1}) < Powdered egg alternative (58 mg.100g^{-1}) < Pate (299.6 mg.100g^{-1}).

Following legislation, it seems that national or European legislation contain no specific requirements for „low protein foods“, or foods low in phenylalanine, so in purpose to evaluate our samples we used as the standard given in the Czech legislation **(Vyhláška Ministerstva zdravotnictví České republiky č. 54/2004 Sb)**, according to which food with no phenylalanine content is defined as food made by special technological process so that the phenylalanine content should not exceed 20 mg per 100 g or 100 cm^{-3} in the food intended for consumption. Under this legislation, suitable foods for phenylketonurics could be classified as follows: Gum candies (1 mg.100g^{-1}) < Cherry jelly (1.4 mg. 100g^{-1}) < Flour no. 16 (5.8 mg.100g^{-1}) < Flour no.17 (5.9 mg.100g^{-1}) < Spaghetti (8.7 mg.100g^{-1}) < Pasta (9.9 mg.100g^{-1}) < Waffle (14.2 mg.100g^{-1}).

Table 2 Aminoacid content (mg.100g^{-1}) determined in sample of pate.

Aminoacids	Content of aminoacids [mg.100g^{-1}]	Standard deviation	CV (%)
Aspartic acid	571.11	0.14	2
Threonine	201.60	0.04	2
Serine	156.48	0.04	3
Glutamic acid	916.62	0.55	6
Proline	546.50	0.27	5
Glycine	762.99	0.12	2
Alanine	471.35	0.02	0
Valine	371.65	0.33	9
Isoleucine	269.77	0.15	5
Leucine	522.17	0.19	4
Tyrosine	121.15	0.03	3
Phenylalanine	**299.59**	0.13	4
Histidine	205.64	0.03	2
Lysine	435.07	0.01	0
Arginine	387.21	0.24	6
Total	**6238.90**		

Table 3 Aminoacid content (mg.100g^{-1}) determined in sample of powdered egg alternative.

Aminoacids	Content of aminoacids [mg.100g^{-1}]	Standard deviation	CV (%)
Aspartic acid	125.69	0.05	4
Threonine	33.60	0.02	7
Serín	55.26	0.03	6
Glutamic acid	163.12	0.08	5
Proline	39.72	0.01	2
Glycine	46.35	0.02	5
Alanine	58.73	0.02	3
Valine	38.43	0.00	0
Isoleucine	29.31	0.02	7
Leucine	87.81	0.04	5
Tyrosine	28.76	0.01	5
Phenylalanine	**58.00**	0.01	1
Histidine	32.71	0.01	4
Lysine	60.59	0.01	1
Arginine	87.63	0.02	3
Total	**945.70**		

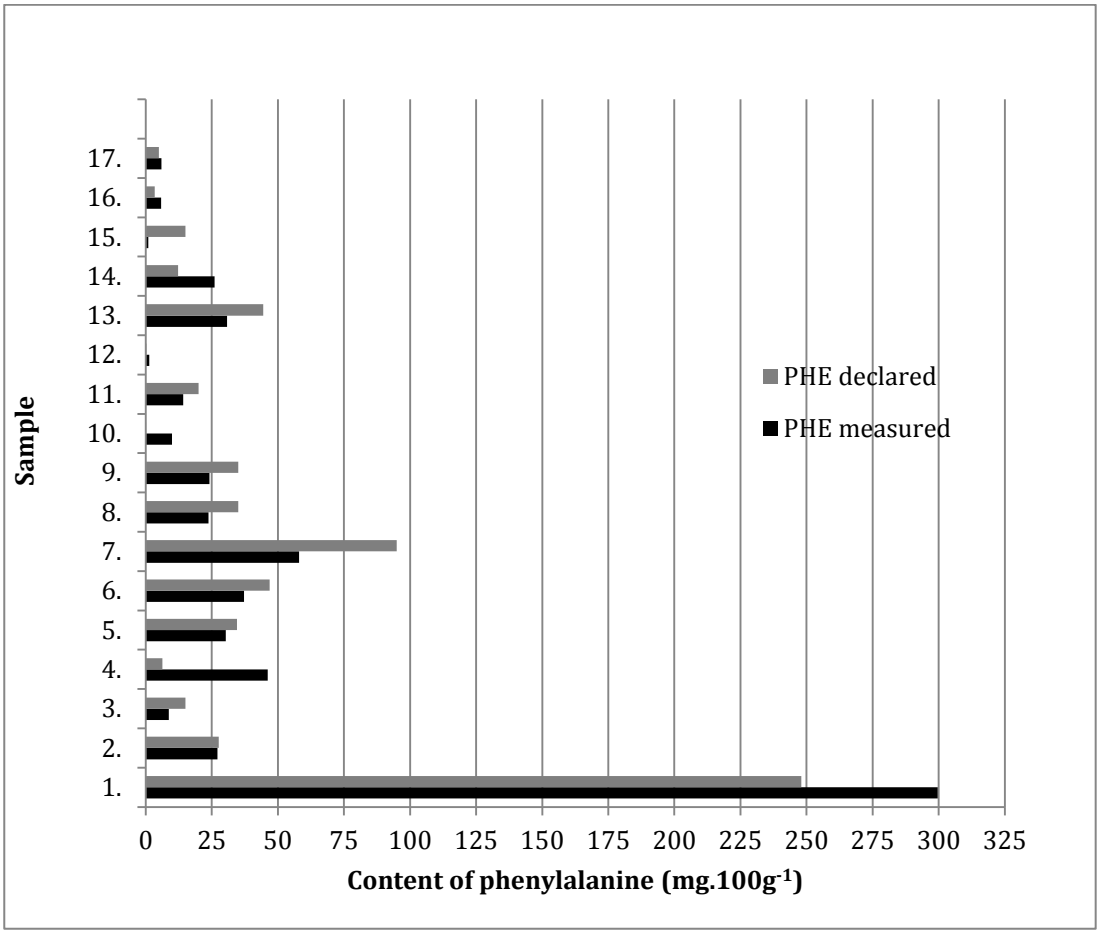

Figure 1 Differences between phenylalanine content measured and labeled by producers (mg.100g^{-1}).

Samples: 1.Pate, 2. Breadcrumbs, 3. Spaghetti, 4. Instant soup with noodles, 5. Sticks with salt, 6. Walnut cookie, 7. Powdered egg alternative, 8. Salty sticks, 9. Chocolate, 10. Pasta, 11. Waffle, 12. Cherry jelly, 13. Ice Cornets, 14. Bread, 15. Gum candies, 16. Flour, 17. Flour.

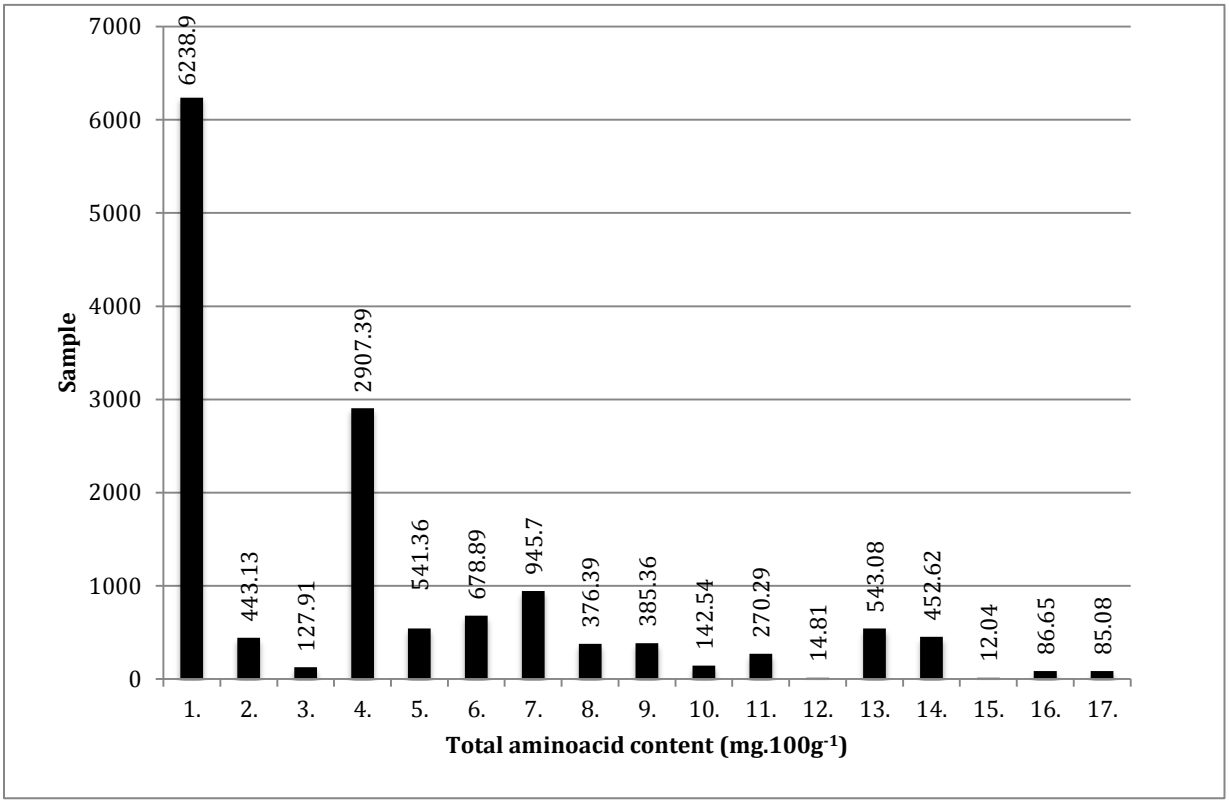

Figure 2 Total aminoacid content (mg.100g^{-1}) determined in selected products.

Samples: 1.Pate, 2. Breadcrumbs, 3. Spaghetti, 4. Instant soup with noodles, 5. Sticks with salt, 6. Walnut cookie, 7. Powdered egg alternative, 8. Salty sticks, 9. Chocolate, 10. Pasta, 11. Waffle, 12. Cherry jelly, 13. Ice Cornets, 14. Bread, 15. Gum candies, 16. Flour, 17. Flour.

Contrary, as sample with the highest content of phenylalanine (299.6 mg.100g^{-1}) was determined pate produced in Germany. The highest amino acid content was also detected (6238.90 mg.100g^{-1}) in this sample probabaly due to meat present, and the highest content of glutamic acid (916.62 mg.100g^{-1}) among amino acids was determined as well.

Powdered egg alternative (sample no.7) was monitored as the second highest source of phenylalanine. It consists of cornstarch, mono and diglycerides of fatty acids, palm oil, xanthan, rice flour and pea protein. Glutamic acid also prevailed in this sample (Table 3).

The only product of Slovak origin was the sample no. 3, spaghetti. Total sum of amino acids 127.91 mg.100g^{-1} was observed, which can be assumed together with the observed phenylalanine content (8.75 mg.100g^{-1}) as low content and therefore this product can be considered as suitable for consumption by phenylketonurics.

As the most suitable for fenylketonurics with the lowest content of phenylalanine were observed gum candies and cherry jelly (less than 2 mg. 100g^{-1}). Total aminoacid content detected in these two products was also the lowest, at gum candies 12.04 mg.100g-1 and cherry jelly 14.81 mg.100g^{-1}.

Sample no. 8 was the sticks of Polish origin. They are comparable with the sample no. 5, which are also sticks with salt with a similar composition but made from other producer. Phenylalanine content determined was 30.0 resp. 23.8 mg.100g^{-1}.

Comparable could be also two samples of flours originating from Poland and Sweden, with detected phenylalanine content at flour no.16 (5.8 mg.100g^{-1}), and flour no.17 (5.9 mg.100g^{-1}). Total aminoacid content were observed as similar (85.08 mg.100g^{-1} vs. 86.65 mg.100g^{-1}) as well.

As it was stated previously, foods for phenylketonurics are often included in the category of foods for special medical purposes that means a category of foods for particular nutritional uses specially processed or formulated and intended for the dietary management of patients and to be used under medical supervision. They are intended for the exclusive or partial feeding of patients with a limited, impaired or disturbed capacity to take, digest, absorb, metabolise or excrete ordinary foodstuffs or certain nutrients contained therein or metabolites, or with other medically-determined nutrient requirements, whose dietary management cannot be achieved only by modification of the normal diet, by other foods for particular nutritional uses, or by a combination of the two **(Commission Directive 1999/21/EC)**. The labelling of foodstuffs intended for particular nutritional uses shall include by the **Directive 2009/39/EC** the particular elements of the qualitative and quantitative composition or the special manufacturing process which gives the product its particular nutritional characteristics. Comparing the results in phenylalanine content measured and the content of phenylalanine labeled by producers, we observed several differences shown in the Fig. 1. Sample no. 2

represented, as breadcrumbs and sample no. 6 were the products with the lowest difference in observed and declared values of phenylalanine in the packaging. Contrary, higher differences in these two amounts were detected in samples 1, 4, 7, 8.

Total aminoacid content determined in selected products is shown in the Figure 2. Its content in selected products ranged from 12.04 (gum candies) to 6238.90 mg.100g^{-1} (in pate) and increased in the order: Gum candies (12.04 mg.100g^{-1}) < Cherry jelly (14.81 mg.100g^{-1}) < Flour (85.08 mg.100g^{-1}) < Flour (86.65 mg.100g^{-1}) < Spaghetti (127. 9 mg.100g^{-1}) < Pasta (142.54 mg.100g^{-1}) < Waffle (270.29 mg.100g^{-1}) < Salty sticks (376.39 mg.100g^{-1}) < Chocolate (385.36 mg.100g^{-1}) < Breadcrumbs (443.13 mg.100g^{-1}) < Bread (452.62 mg.100g^{-1}) < Sticks with salt (541.36 mg.100g^{-1}) < Ice Cornets (543.08 mg.100g^{-1}) < Walnut cookie (678.89 mg.100g^{-1}) < Powdered egg alternative (945.70 mg.100g^{-1}) < Instant soup with noodles (2907.39 mg.100g^{-1}) < Pate (6238.90 mg.100g^{-1}).

CONCLUSION

In this work, amino acids representation, focused on phenylalanine content in selected samples intented for phenylketonurics was observed. We can conclude that may be due to the lack of legislation requirements, most of observed foods was high in content of phenylalanine. Only seven products of 17 observed showed lower phenylalanine content, less than 20 mg.100g^{-1}. The obtained results also suggest that certain foods at the content of phenylalanine differ from those listed on the product label.

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