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Evaluation of Producing Low Phenylalanine Milk by Formulation Procedure for Phenylketonuria Patients

Samira Hassanpour [1], Kianoush Khosravi-Darani [2,*], Yousef Ramezan [3], Fataneh Hashempour-Baltork [4]

1 Department of Food Science and Technology, Faculty of Pharmacy, Medicinal Sciences Tehran University, Islamic Azad University, Tehran, Iran; samira.hassanpour@rd.nestle.com (S.H.);
2 Research Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran; kiankh@yahoo.com (K.K.D.);
3 Nutrition & Food Sciences Research Center, Medicinal Sciences Tehran University, Islamic Azad University, Tehran, Iran; y.ramezan@iaups.ac.ir (Y.R.);
4 Halal Research Center of IRI, Iran Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran; fhashempour92@halal.ac.ir (F.H.B.);
* Correspondence: kiankh@yahoo.com, k.khosravi@sbmu.ac.ir (K.K.D.);

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Abstract: At present, infant formula is a food that mimics human milk and is intended for infants under 1-year-old. Phenylketonuria (PKU) is a congenital defect in the synthesis of phenylalanine (Phe) and is caused by the failure of phenylalanine hydroxylase to convert phe to tyrosine. Infant formula products produced in Iran contain skimmed milk powder, liquid milk, or whey powder; therefore, the final product contains a large amount of Phe, which prevents infants with PKU from using them. In this study, producing milk with low phe content by formulation procedure was evaluated for PKU patients. It was a combination of the wet & dry mix processes, including evaporation, spray drying, and fluidized bed equipment contributing to the product's suitable physical and sensory properties. Raw materials with low phe content, glycomacropeptide, and essential amino acids were used as part of the recipe. The maximum daily intake of phe for PKU infants < 1 year is 35 mg/kg of the infant's body weight. The amount of the phe in the final product produced according to this study was analyzed by an amino acid analyzer, and it was 0.0098 g/100g. According to the determined serving size and preparation method of the infant formula, infants with PKU can take the product at different times every day, which contains appropriate amounts of essential amino acids and vitamins, which can meet the needs of patients according to their body weight.

Keywords: phenylketonuria; glycomacropeptide; essential amino acids; infant formula; amino acid analyzer; spray drying.

1. Introduction

Infant formula is currently a food that imitates human milk and is intended for use by infants under the age of one either as powdered formula or liquid formula [1]. In addition to the ingredients, it is continuously improved based on new nutritional information and technology. Focusing on nutritional interventions in the early life stages may improve health status [2].

A new formula is required for a particular treatment formula due to the deficiency of phenylalanine hydroxylase in infants. This enzymatic deficiency results in an inborn error of

phe metabolism called PKU, which converts phe to tyrosine (Figure 1). Tyrosine is an essential amino acid in the diet of patients with PKU, which makes it difficult to hydrolyze Phe to tyrosine under normal circumstances. Phe is an essential amino acid to ensure normal anabolism [3].

During the newborn screening, most patients with PKU who need lifelong care are identified [4]. The control of phe levels is crucial throughout childhood, especially for behavioral and mental functions [5]. Growth is one of the main results that need to be considered, and its optimization plays a key role in PKU [3]. Patients' views should be considered when developing and approving new therapies and treatments for PKU [6].

PKU diet management is to limit the combination of natural protein in the diet to reduce the intake of phe, and to supplement special medical formulas to provide enough essential amino acids, energy, vitamins, and minerals [7]. The main purpose of PKU treatment is to strictly control the blood Phe level, mainly during the early stages of life, and new treatment methods are needed to improve the quality of life of PKU patients [8].

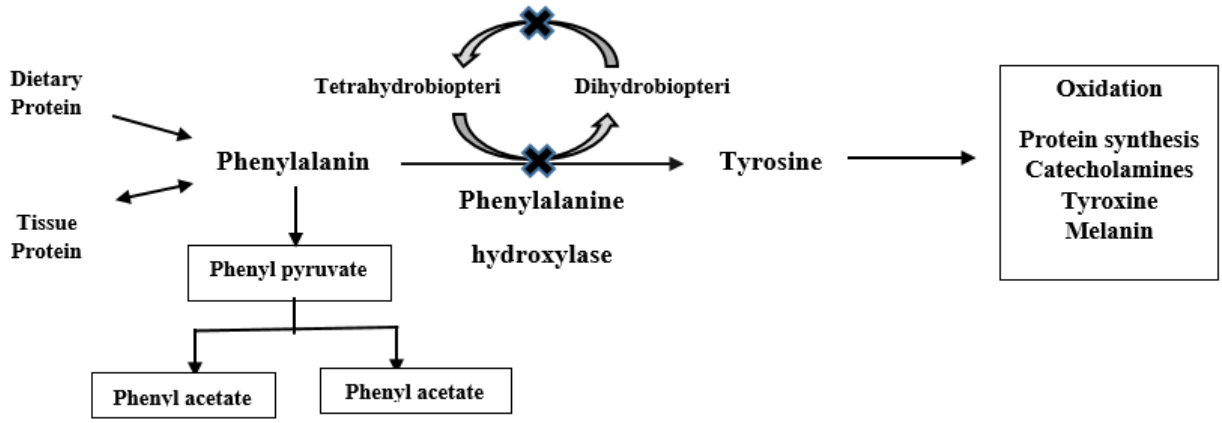

Figure 1. The phenylalanine metabolism in Phenylketonuria.

Glycomacropeptide (GMP) is a plentiful 64-amino-acid glycophosphopeptide produced in the cheese production process. Bovine kappa (κ)-casein is cleaved by chymosin into para-κ-casein remaining with the curd, while GMP remains with the whey. Compared to typical dietary proteins, GMP has a large profile of indispensable or essential amino acids [13]. Pure GMP has a unique amino acid profile that includes a higher concentration of threonine and isoleucine and no aromatic amino acids, including Phe, tyrosine, and tryptophan [3]. GMP is currently being marketed as an ingredient that demonstrates a strong food safety record according to the global supplementation of foods with whey protein and the use of whey-predominant infant formulas [9]. While synthetic amino acids are not suitable for food production, GMP has good functional properties for food manufacturing, including good thermal stability and solubility in acids. GMP has different theoretical advantages, such as enhancing protein retention and nitrogen utilization, strengthening bones, enhancing immunological activities, and improving taste and acceptance [10].

PKU diet management should be appropriate and lifelong to achieve the desired results. Compared with the free synthetic amino acids used as test meals in PKU patients, GMP does not significantly affect any biomarkers. GMP can be used to make palatable, low-Phe foods and beverages to provide an alternative low-Phe protein source in the PKU diet [11].

It is not easy to set a good nutritional status because there are different contributors to the definition. PKU patients have lower intakes of certain micronutrients, such as vitamins A, C, and E, selenium, coenzyme Q_{10}, vitamins B_2, B_6, and B_{12}, folates, iron, zinc, calcium,

carnitine, long-chain polyunsaturated fatty acid and vitamin D [12]. That is why supplementation with micronutrients is essential, and vitamins and minerals should either be added to supplement phenylalanine-free L-amino acids or given alone.

In the past few years, adding free L-amino acids, vitamins, and minerals as supplements to the PKU diet has become a common practice [12]. Comparing medical foods containing GMP with traditional amino acid medical foods, the results show that GMP medical foods are an acceptable and safe source for the nutritional management of PKU and can improve people's lifetime compliance with the essential low-Phe diet [3]. In PKU, biochemical micronutrient deficiencies are common even though micronutrient intake is higher than the reference nutrient intake. Once used in combination with l-amino acid supplements, irregular dose intervals of micronutrients may lead to poor bioavailability [12].

At the clinical level, an obvious "safe" protein: energy ratio should be used to promote optimal body composition and reduce long-term health risks. The concept of protein: energy ratio considers the interdependence between protein and energy intake and describes the ratio of dietary energy obtained from protein [13].

The European PKU guidelines recommend that the total protein intake be 40% more than the FAO/WHO/UNU safe protein intake. However, the amount is arbitrary and has not been confirmed by research. Since most of the available protein substitutes are entirely derived from amino acids that do not contain phe, it is recommended to give higher doses than FAO/WHO/UNU. This extra amount compensates for the ineffective absorption of natural/whole protein (mainly based on plants), poor L-amino acid utilization, and insufficient energy absorption. When determining the dosage of protein substitutes, weight, age, growth, and the prescribed amount of phe/natural protein should be considered. If a person with PKU is obese, protein replacement needs should be based on ideal body weight [14]. The prescribed amount of medical food is designed to meet the protein requirement of each age group in the life cycle shown in Table 1 [7].

PKU is often found in the worldwide population, which is more observed in the Middle East compared to the Western world. Previous studies have been carried out mainly on skimmed milk product powder and also milk to remove phe from these products based on activated carbon usage together with enzymatic hydrolysis by hydrolysates originating from a different microorganism. Lopes *et al*. used activated carbon to remove phe from skim milk powder with enzymatic hydrolysates [15].

Silvestre *et al*. prepared low phe milk hydrolysates by changing enzyme type, enzyme-substrate ratio, and temperature [16]. Silva *et al*. ran research with the aim of Phe removal from skim milk powder by activated carbon and enzymatic hydrolysates using a protease from *Aspergillus oryzae*, isolated or in association with papain [17]. Abdel-Salam *et al*. produced a dairy-based drink by emulsifying corn germ oil with casein GMP solution in milk permeates, leading to a 30% - 80% reduction of serum Phe levels in all PKU patients who consumed this product [18].

Table 1. Recommended daily nutrient intakes (ranges) for infants, children, and adults with PKU [adapted from 7].

Age	**Nutrients**				
	Phe	**Tyrosine**	**Protein**	**Energy**	**Fluid**
Infants	**(mg/kg)**	**(mg/kg)**	**(g/kg)**	**(kcal/kg)**	**(ml/kg)**
0 to < 3 months	25 - 70	300 - 350	3.50 - 3.00	120 (145 - 95)	160 - 135
3 to < 6 months	20 - 45	300 - 350	3.50 - 3.00	120 (145 - 95)	160 - 130
9 to < 12 months	15 - 35	250 - 300	3.00 - 2.50	110 (135 - 80)	145 - 125
7 to < 9 months	10 - 35	250 - 300	3.00 - 2.50	105 (135 - 80)	135 - 120

Age	Nutrients				
	Phe	**Tyrosine**	**Protein**	**Energy**	**Fluid**
Infants	**(mg/kg)**	**(mg/kg)**	**(g/kg)**	**(kcal/kg)**	**(ml/kg)**
Girls and boys	(mg/kg)	(g/day)	(g/day)	(kcal/day)	(ml/day)
1 to < 4 years	200 - 400	1.72 - 3.00	≥ 30	1300 (900 - 1800)	900 - 1800
4 to < 7 years	210 - 450	2.25 - 3.50	≥ 35	1700 (1300 - 2300)	1300 - 2300
7 to < 11 years	220 - 500	2.55 - 4.00	≥ 40	2400 (1650 - 3300)	1650 - 3300
Women					
11 to < 15 years	140 - 750	3.45 - 5.00	≥ 50	2200 (1500 - 3000)	1500 - 3000
15 to < 19 years	230 - 700	3.45 - 5.00	≥ 55	2100 (1200 - 3000)	1200 - 3000
≥ 19 years	220 - 700	3.75 - 5.00	≥ 60	2100 (1400 - 2500)	2100 - 2500
Men					
11 to < 15 years	225 - 900	3.38 - 5.50	≥ 55	2700 (2000 - 3700)	2000 - 3700
15 to < 19 years	295 - 1100	4.42 - 6.50	≥ 65	2800 (2100 - 3900)	2100 - 3900
≥ 19 years	290 - 1200	4.35 - 6.50	≥ 70	2900 (2000 - 3300)	2000 - 3300

This research aims to develop formulated infant formula products according to the selection of the raw materials and adjustment of the formula as per the Phe content for infants from 1st day of birth until 1 year old. Compared to previous research, this study considers different target population ages, product matrices, and manufacturing methods so that PKU babies can be fed healthier from the first day of birth.

2. Materials and Methods

2.1. Material.

L-Carnitine, L-Histidine, L-Isoleucine, L-Leucine, L-Methionine, L-Threonine, L-Tryptophan, L-Valine, and L-Lysine Acetate were purchased from Kyowa Hakko Company in Japan. GMP (Lacprodan® CGMP-20) and Lactose were supplied from Arla Foods Ingredients in Denmark. Fish oil, vegetable oils, soya lecithin, nucleotides, minerals, vitamins, trace elements, and Bifidus culture were supplied from Fuji Oil in Japan and Nestle factory in Switzerland.

2.2. Method.

2.2.1. Recipe adjustment and calculation.

To produce infant formula with low Phe content for infants under the age of 1, the recommended daily intake (RDI) of Phe is considered 15-35 mg/kg (Table 1) in the finished product. In the formula, different raw materials are selected based on their Phe content not to exceed the RDI in the finished product. Except for GMP <0.28 g/100g Phe, no raw materials containing Phe are used in the formula, so the reverse calculation is carried out according to the RDI to define the dosage of GMP in the formula: 15-35 mg/kg and maximum possible content of Phe in the GMP (0.28g/100g). In addition to GMP, essential amino acids were added in pure format.

Table 2. Special equipment and the instrument used for analyses.

Equipment/Instrument	Brand	Country
Amino acid analyser	Hitachi	Germany
Karl-Fischer	Mettler Toledo -DL31	Germany
Balance	Mettler Toledo	Germany
Hygrolab	Rotronic	America
Atomic absorption spectrometer	Varian-GTA96	Germany
Buchi water bath	Buchi461	Switzerland
Spectrophotometer	Hach DR2800	Germany
Buchi distillation unit	Buchi K350	Switzerland

Equipment/Instrument	Brand	Country
pH meter	Metrohm 780	Switzerland
Dosimat	Metrohm 876	Switzerland
Water bath	Memmert	Germany
Buchi digestion unit	Buchi-K424	Switzerland
Centrifuge	Gerber	Switzerland
Hot plate	Rommelsbacher	Germany
Vacuum filter instruction	Gerber	Switzerland
Solumixer	Gerber	Switzerland
Vacuum oven & chamber	LGH	America

2.2.2. Production of powdered infant formula.

2.2.2.1. Wet mix process.

First, some powdered ingredients (such as amino acids, GMP, minerals, vitamins, etc.) are mixed with water in a mixer, Scanima supplied from Denmark, to prepare a liquid phase to form a uniform emulsion, then heated in a plate heat exchanger, and transferred To the evaporator where the oil and lecithin will be added.

2.2.2.2. Evaporation and spray drying.

The total solid in the liquid phase is increased through the evaporation process, and the final heat treatment step is carried out in the falling film evaporator, which is thermal Vapour Recompression developed by TetraPak in Switzerland and Germany. Once the evaporation process is finished, the condensed liquid formulation is dehydrated by spray drying to produce base powder, followed by fluidized bed drying and cooling. The spray drying system is Egron, and the equipment is designed by Nestle Switzerland.

2.2.2.3. Dry mixing step.

After the drying step, the dry blend ingredients are mixed with the produced base powder to protect certain materials from the heat treatment step and obtain a better nutritional status in the final product.

2.2.3. Quality control of the product and required analyses.

To ensure product quality and to take into account the standards of infant formula milk powder and formula milk powder used for special medical purposes, comprehensive microbiological, physical, and chemical amino acid profiles, in particular, phe levels in the final product, vitamin profiles and elemental contaminant analyses of the final product have been carried out [19] using equipment mentioned in Table 2 and all the required chemicals supplied from Sigma, Merck and Fluka. Phe amount in the final product is analyzed by an amino acid analyzer.

3. Results and Discussion

Compared with the previous study, this study is unique in that it seeks to produce formula products for infants on the first day of birth, while the previous products did not directly target infants. Due to this research's target consumer age group, a lot of analysis is required to demonstrate the applicability of the product to infants from a food safety perspective, such as microbiological and chemical contaminant parameters.

Another difference is that in this study, the final product was produced according to the formula, and several ingredients were added to make infant formula, whereas the goal of previous studies was to remove phe from the existing milk matrix. A comprehensive profile of nutrients, chemical, physical, and amino acid parameters have been analyzed to control the formulation, ingredient selection, and production process.

Five samples were taken from the manufactured batch of infant formula, and a full series of analyses were carried out in compliance with the Codex Standard for Infant Foods [19] and the Iran Regulations. As shown in Table 3, both pathogens and non-pathogenic microorganisms are within the acceptable level of the infant products. This is because the direct steam injection in the process contributes to eliminating hazardous microorganisms like Salmonella, Bacillus Cereous, Cronobacter and reducing other microorganisms to an acceptable level. Blanchard *et al*. [20] described wet mix and steam injection in the evaporation process well in their study. Compared to previous research, in this study, a comprehensive microbiological analysis was carried out following all baby food criteria and the current regulations.

Table 3. Microbiological characterization of produced low phenylalanine milk.

Parameter	Content (CFU/g)	Acceptable range
Salmonella	Negative	Negative
E. Sakazakii Cronobacter	Negative	Negative
Yeast and Molds	10	<10
Contaminating microorganisms	100	< 500
Bacillus cereous	<10	<10
Probiotic	20760000 ± 54772.256	Min 10000000 CFU
Enterobacteriaceae	Negative	Negative

As shown in Table 4, combining the wet and dry mixing processes with appropriate process parameters in the evaporation method, spray drying tower, and fluidized bed will provide consumers with appropriate physical properties of infant formula.

Table 4. Physical analysis results produced low phenylalanine milk.

Parameter	Value	Acceptable range
Scoop Delivery	4.04 ± 0.035 g	4 – 4.1 g
Solubility	0.1 ml	Max. 0.5 ml
Dirt Test	1	2
Miscibility	0	2
White specs	1	3
State of dissolution	1	2
Fat Separation	No Separation	1
Phase Separation	No Separation	1

An amino acid analyzer checked the amino acid profile of the product as part of the key performance check of this research. The detailed results are recorded in Table 5 and Figure 2. As shown in the peak in Figure 3, the content of phe in the final product is 0.0098 g / 100 g, which is lower than the detection limit (LOD) of the device (0.01 g / 100 g).

To minimize the phe content in the product, infant-grade ingredients with acceptable levels of phe have been selected in the formula. GMP is used as one of the key ingredients in the formula. In addition, as shown in Table 5, due to the addition of GMP and other pure essential amino acids in the formula, the essential amino acid profile of the final product also meets the needs of patients. According to the research of Lammardo *et al*. [21], it has been a common practice in the last years to supplement the product with L-amino acids. Shehata *et al*. [22] also used pure essential amino acids for product supplementation.

Table 5. Amino acid profile in produced low phenylalanine milk.

Amino acid	Content (g/100g)	Acceptable ranges
L Threonine	1.71 ± 0.017	Min. 0.4
L Valine	2.22 ± 0.024	Min. 0.458
L Isoleucine	1.92 ± 0.027	Min. 0.468
L Leucine	3.42 ± 0.022	Min. 0.864
L Lysine	1.65 ± 0.022	Min. 0.588
L Histidine	0.03 ± 0.003	Min. 0.020
L Tryptophan	0.53 ± 0.001	Min. 0.166
L Cysteine	0.01 ± 0.000	Methionine + Cysteine = Min. 0.317
L Methionine	0.75 ± 0.003	
L Phenylalanine	0.01 ± 0.000	Refer to table 1

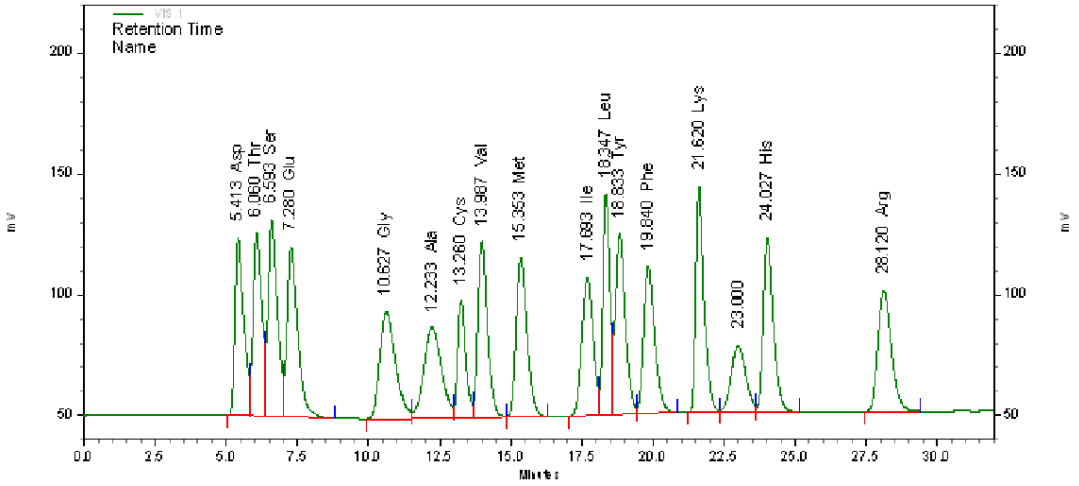

Figure 2. Amino acid analyzer standard curve.

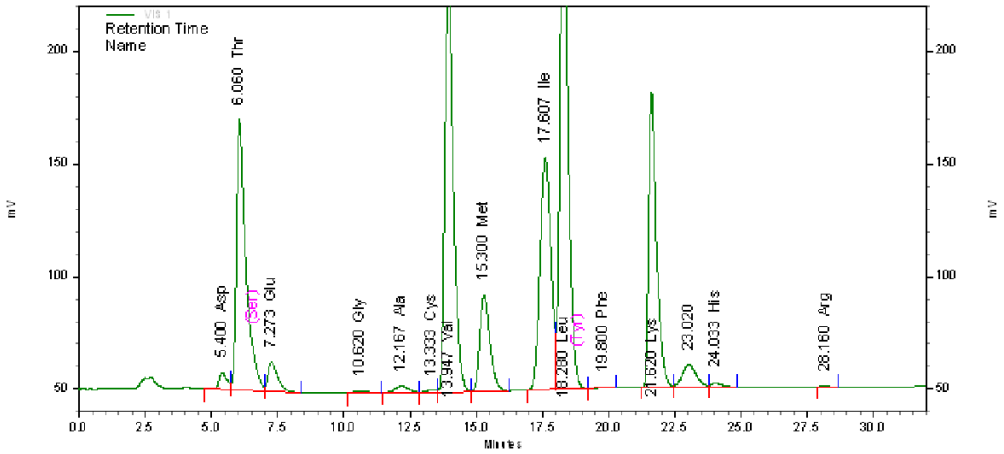

Figure 3. Amino acid analyzer sample curve for produced low phenylalanine milk.

Vitamin premix, probiotics, and trace elements are added in the dry blending process to enrich the final product for PKU infants and meet their needs for vitamins and other micronutrients, as illustrated in Tables 6 and 7, respectively. This product enables patients to rectify vitamin and micronutrient deficiencies considered in the research by Demirkol *et al.* [23].

Table 6. Vitamins analyses profile for produced low phenylalanine milk.

Parameter	Vitamin content (mg/100g)	Acceptable Range
VITAMIN B_6	0.53 ± 0.015	0.238 – 0.68
VITAMIN PP	6.43 ± 0.022	2.73 – 7.8
VITAMIN H	0.02 ± 0	0.009 – 0.027
VITAMIN B_5	4.75 ± 0.030	2.24 – 6.4
Vitamin B_9	0.09 ± 0	0.053 – 0.152
CARNITINE, L	10.70 ± 0	5.6 – 16

Parameter	Vitamin content (mg/100g)	Acceptable Range
ADENOSINE 5'-MONOPHOSPHATE	< 0.02	< 7.8
CYTIDINE 5'-MONOPHOSPHATE	< 0.007	< 13
GUANOSINE 5'-MONOPHOSPHATE	< 0.005	< 2.6
URIDINE 5-MONOPHOSPHATE	< 0.02	<9.1
VITAMIN A	0.64 ± 0.001	0.369 – 0.937
VITAMIN E	4.64 ± 0.024	4.2 – 12
VITAMIN D	0.01 ± 0	0.005 – 0.013
VITAMIN K	0.07 ± 0	0.030 – 0.086
VITAMIN B_{12}	0.00 ± 0	0.001 – 0.004
VITAMIN B_1	0.64 ± 0.023	0.39 – 1.12
Vitamin B_2	1.24 ± 0.018	0.44 – 1.26
Vitamin C	98.08 ± 0.228	60 – 174

Table 7. Chemical characterization of produced low phenylalanine milk.

Parameter	Value (mg/100g)	Acceptable range	Unit
pH	6.69 ± 0.01	6.3 - 7.1	
Moisture	2.46 ± 0.00	< 3	g/100g
Fat	27.28 ± 0.07	23 – 31	g/100g
Total Nitrogen	1.56 ± 0.00	1.5 – 2	g/100g
Water Activity	0.11 ± 0.00	< 0.15	
Sodium	173.80 ± 3.35	112 – 241	mg/100g
Potassium	516.20 ± 2.95	404 – 832	mg/100g
Iron	8.75 ± 0.42	5.2 – 10.4	mg/100g
Magnesium	51.58 ± 0.35	35 – 75	mg/100g
Calcium	279.40 ± 1.34	260 – 528	mg/100g
Phosphorus	133.00 ± 1.41	130 – 277	mg/100g
Chloride	351.80 ± 1.30	260 – 549	mg/100g
Copper	0.49 ± 0.00	0.28 – 0.52	mg/100g
Zinc	6.23 ± 0.04	3.78 – 7.8	mg/100g
Manganese	115.20 ± 0.84	66.5 – 190	µg/100g
Iodine	138.32 ± 0.37	66.5 – 190	µg/100g
Cyanuric Acid	< 0.05	< 0.1	mg/100g
Melamine	<0.05	< 0.1	mg/100g
Aflatoxin M1	<0.025	< 0.1	µg/100g
Lactose	57.52 ±0.08	40.7 – 75.5	g/100g

Infant formula milk powder is a product made to fulfill the specific dietary intake of infants. However, infant formula usually contains harmful substances, such as chemical contaminants and residues, which are usually caused by possible contamination of the raw material or supply chain [24]. As shown in Table 8, a comprehensive elemental contaminants analysis was performed to evaluate the food safety compliance of produced products from the perspective of chemical contaminants. All results meet the requirements of Iranian regulations and infant food CODEX.

Table 8. Elemental contaminants in produced low phenylalanine milk.

Parameter	Content (mg/kg)	Acceptable range
Arsenic	<0.020	< 0.050
Lead	<0.007	< 0.050
Cadmium	<0.005	< 0.011
Mercury	<0.003	< 0.012
Aluminum	0.158 ± 0.004	< 2.10
Selenium	0.078 ± 0.008	0.077 – 0.22
Nitrate	6.000 ± 0.000	< 100
Nitrite	0.840 ± 0.055	< 2

In this study, the “In/Out” method is used to perform the sensory evaluation. Product characterization is used to decide whether a sample meets the target sensory quality (“In”) or not (“Out”) or is at the border to “Out” but still acceptable (“Just In”) for a specific sensory attribute. The concept of consumer-centric sensory specification corresponds to the “Acceptability limits”. Sensory evaluation of a sample is performed against its In/Out sensory

specification and/or physical standard sample that results in an overall In/Out score for the sample by using a 3-point scale ('In', 'Just In', or 'Out'). In order to perform sensory evaluation, 5 different samples are evaluated by 10 panelists based on "In/Out" method as per Eq (1):

$$\text{Defect Score} = ((\text{Number of Out} \times 3) + (\text{Number of Just In} \times 1)) / (\text{Number of Panelists} \times 3)$$
$$\text{In/Out Score} = (1 - \text{Defect Score}) \times 100 \qquad \text{Eq. 1}$$

Where ('In', 'Just In', or 'Out') are as above mentioned description. The sensory evaluation results of different samples are shown in Figures 4 and 5. From the perspective of appearance, smell, taste, texture, and overall acceptability, the acceptance of this product in all test panels is higher than 90%.

This study's sensory results proved that adding GMP to the formula has a positive impact on sensory characteristics, which is consistent with the research conducted by Ney *et al*. [25]. GMP has a range of benefits, such as enhancing the product's sensory characteristics. Appropriate process parameters and the right raw materials selection are crucial to achieving acceptable levels of product sensory and physical properties. In order to determine the acceptability of GMP products, a blind sensory study was carried out (Figure 4).

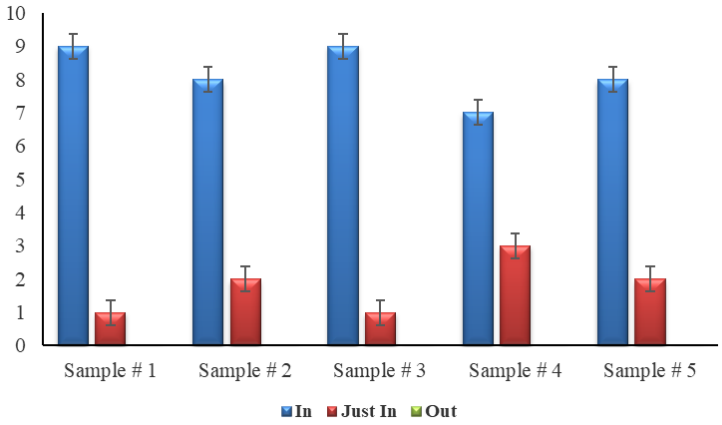

Figure 4. Sensory characterization of 5 samples of produced low phenylalanine milk (based on 10 panelists' evaluation).

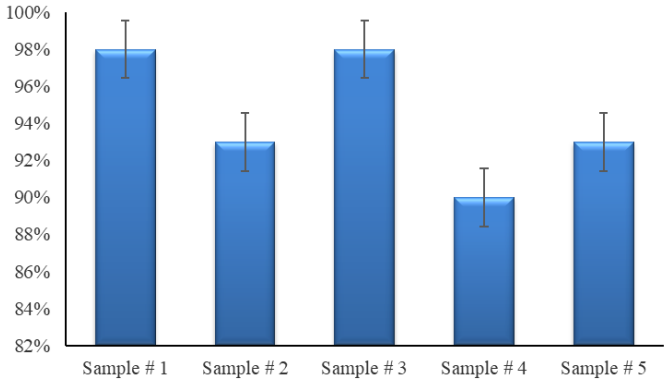

Figure 5. Overall sensory acceptance for 5 samples of produced low phenylalanine milk.

4. Conclusions

According to the 0.0098 g / 100 g phe value reported in the final product, every 100 ml of reconstituted milk contains 0.00117 g phe / 12 g product (1.17 mg Phe / 12 g product). For PKU infants under 1 year of age, the maximum daily intake of phe is 35 mg phe/1 kg of infant

weight. Based on the findings of the final product phe, it can be concluded that PKU infants can take the product at different times of the day depending on their weight. According to this research, the formula method based on the correct selection of the phe content in the raw materials and the dry-wet blending process using infant formula milk powder can effectively produce products with qualified phe content.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Mayer, O.; Cagil, Y.; Kerner, J. *Infant Nutrition in Pediatric Nutrition for Dietitians.* first ed.; Goday, P.; Walia, C. Eds.; CRC Press; **2022**; pp. 65-78, https://doi.org/10.1201/9781003147855.
2. Landry, M.J.; Ruiz, L.D.; Gibbs, K.; Radtke, M.D.; Lerman, J.; Vargas, A.J. Perspective: Early-Life Nutrition Research Supported by the US National Institutes of Health from 2018 to 2020. *Advances in Nutrition* **2022**, *13*, 1395-1401,https://doi.org/10.1093/advances/nmac044.
3. Daly, A.; Pinto, A.; Evans, S.; MacDonald, A. Glycomacropeptide in PKU—Does It Live Up to Its Potential? *Nutrients* **2022**, *14*, 807-824, https://doi.org/10.3390/nu14040807.
4. Al-Bari, A.A. Current Scenario and Future Direction of Newborn Screening and Management Program for Phenylketonuria in Bangladesh. *J. inborn errors metab. screen.* **2022**, *10*, https://doi.org/10.1590/2326-4594-JIEMS-2021-0024.
5. Liemburg, G.B.; Huijbregts, S.C.J.; Rutsch, F.; Feldmann, R.; Jahja, R.; Weglage, J.; Och, U.; Burgerhof, J.G.M.; van Spronsen, F.J. Metabolic control during the neonatal period in phenylketonuria: associations with childhood IQ. *Pediatric Research* **2022**, *91*, 874-878, https://doi.org/10.1038/s41390-021-01728-8.
6. Turner, A.; Glinton, K.E.; Sutton, V.R. Advancements in therapeutics for inborn errors of metabolism. *Current Opinion in Pediatrics* **2022**, *34*, https://doi.org/10.1097/MOP.0000000000001168.
7. van Calcar, S. *Nutrition Management of Phenylketonuria in Nutrition Management of Inherited Metabolic Diseases.* Bernstein, E.L.; Rohr, F.R.; Helm, J. Eds: Springer; **2022**; pp. 127-51, https://doi.org/10.1007/978-3-319-14621-8.
8. de Oliveira Filho, J.G.; Carvalho, A.S.E.S.; Alves, J.d.S.; Egea, M.B. Next-generation probiotics as a therapeutic strategy for the treatment of phenylketonuria: a review. *Nutrition Reviews* **2022**, *80*, 2100-2112, https://doi.org/10.1093/nutrit/nuac024.
9. Guo, M. *Ingredients selection for infant formula in Human Milk Biochemistry and Infant Formula Manufacturing Technology*. second ed. Eds: *Elsevier*; **2021**; pp. 185-221, https://doi.org/10.1533/9780857099150.2.172.
10. Foisy Sauvé, M.; Spahis, S.; Delvin, E.; Levy, E. Glycomacropeptide: A Bioactive Milk Derivative to Alleviate Metabolic Syndrome Outcomes. *Antioxidants & Redox Signaling* **2020**, *34*, 201-222, https://doi.org/10.1089/ars.2019.7994.
11. Pena, M.J.; Pinto, A.; de Almeida, M.F.; de Sousa Barbosa, C.; Ramos, P.C.; Rocha, S.; Guimas, A.; Ribeiro, R.; Martins, E.; Bandeira, A.; Dias, C.C.; MacDonald, A.; Borges, N.; Rocha, J.C. Continuous use of glycomacropeptide in the nutritional management of patients with phenylketonuria: a clinical perspective. *Orphanet Journal of Rare Diseases* **2021**, *16*, 84-94, https://doi.org/10.1186/s13023-021-01721-8.
12. Coşkun, T.; Çoker, M.; Mungan, N.Ö.; Özel, H.G.; Sivri, H.S. Recommendations on phenylketonuria in Turkey. *Turk J Pediatr*. **2022**, *64*, 413-434, https://doi.org/10.24953/turkjped.2021.4098.

13. Veronika, A.P.; Puspitawati, T.; Fitriani, A. Associations between nutrition knowledge, protein-energy intake and nutritional status of adolescents. *J Public Health Res*. **2021** *10*, https://doi.org/10.4081/jphr.2021.2239.
14. Robertson, L.; Adam, S.; Ellerton, C.; Ford, S.; Hill, M.; Randles, G.; Woodall, A.; Young, C.; MacDonald, A. Dietetic Management of Adults with Phenylketonuria (PKU) in the UK: A Care Consensus Document. *Nutrients* **2022**, *14*, https://doi.org/10.3390/nu14030576.
15. Lopes, D.C.F.; Delvivo, F.M.; Silvestre, M.P.C. Use of activated carbon for removing phenylalanine from reconstituted skim milk powder hydrolysates. *LWT - Food Science and Technology* **2005**, *38*, 447-453, https://doi.org/10.1016/j.lwt.2004.07.021.
16. Silvestre, M.P.C.; Lopes Junior, C.O.; Silva, M.V.D.; Aguiar, B.J.M.; Afonso, W.O.; Silva, M.R. Effect of enzyme type, enzyme substrate ratio and temperature on phenylalanine removal from milk. *Am J food technol.* **2012**, *7*, 123-32, https://doi.org/10.3923/ajft.2012.123.132.
17. Silva, D.M.L.; Afonso, W.O.; Lopse, F.D.C.; Januario, J.N.; Aguiar, B.M.J.; Starling, P.A.N.; Silvestre, P.C.M. Preparation of low phenylalanine whey hydrolysate, using papain and pancreatin immobilized on active carbon and alumina. *Am J Food Technol.* **2007,** *2*, 327-41, https://doi.org/10.3923/ajft.2007.327.341.
18. Abdel-Salam, A.M.; Effat, L.K. Preparation and evaluation of a novel therapeutic dairy-based drink for phenylketonuria. *North American journal of medical sciences* **2010**, *2*, 66-70, https://doi.org/10.4297/najms.2010.266.
19. Codex Alimentarius Standards for Infant Formula and Formulas for Special Medical Purposes Intended for Infants, Codex Stan **2007**; pp. 72–1981, https://www.isdi.org/wp-content/uploads/2020/04/CODEX-STAN-72-1981.pdf.
20. Lammardo, A.M.; Robert, M.; Rocha, J.C.; van Rijn, M.; Ahring, K.; Bélanger-Quintana, A.; MacDonald, A.; Dokoupil, K.; Ozel, H.G.; Goyens, P.; Feillet, F. Main issues in micronutrient supplementation in phenylketonuria. *Molecular Genetics and Metabolism* **2013**, *110*, S1-S5, https://doi.org/10.1016/j.ymgme.2013.08.008.
21. Demirkol, M.; Giżewska, M.; Giovannini, M.; Walter, J. Follow up of phenylketonuria patients. *Molecular Genetics and Metabolism* **2011**, *104*, S31-S39, https://doi.org/10.1016/j.ymgme.2011.08.005.
22. de Mendonça Pereira, B.F.; de Almeida, C.C.; Leandro, K.C.; da Costa, M.P.; Conte-Junior, C.A.; Spisso, B.F. Occurrence, sources, and pathways of chemical contaminants in infant formulas. *Comprehensive Reviews in Food Science and Food Safety* **2020**, *19*, 1378-1396, https://doi.org/10.1111/1541-4337.12559.
23. Ney, D.M.; Hull, A.K.; van Calcar, S.C.; Liu, X.; Etzel, M.R. Dietary Glycomacropeptide Supports Growth and Reduces the Concentrations of Phenylalanine in Plasma and Brain in a Murine Model of Phenylketonuria. *The Journal of Nutrition* **2008**, *138*, 316-322, https://doi.org/10.1093/jn/138.2.316.