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Original Article

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# Analog kefir production with a low phenylalanine for Phenylketonuria



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## Abstract

Phenylketonuria (PKU) is one of the most prevalent types of hereditary metabolic disorders which is caused due to an absence or reduction of the activity of the Phenylalanine hydroxylase enzyme in the liver which in turn, inhibits the transformation of phenylalanine (Phe) to tyrosine. This research was aimed at development of a highly nutrient and acceptable suitable analogue kefir drink for these patients. The mentioned drink is based on milk permeate, cream powder and includes glycomacropeptide (GMP) as a source of protein, starter as a fermentation source, the transglutaminase (TG) enzyme, dough stabilizer and modified corn starch as tissue maker, salt and water. GMP used in this analogue drink is intended for enrichment of the product and therefore it was added by 3% to one formula. Results of chemical analysis indicated that the sample was enriched with GMP had a significantly ($p<0.05$) higher levels of proteins, phenylalanine, salt, dry matter and acidity in comparison to the other 16 samples which lacked GMP. Sample enriched with GMP had a lower calculated amount of pH and alcohol percentage in comparison with the other samples, also this sample had the highest overall acceptance in sensory evaluation. The results of this study showed that the analog kefir has a low level of phenylalanine (30.40 mg/100g) and in this regard; it can be considered to be useful for patients with PKU.

**Keywords:** Permeate, Analogue kefir, Glycomacropeptide, Phenylketonuria, Phenylalanine

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## Introduction

The history of using living microorganisms in food, especially lactic acid bacteria for maintenance and improvement of human health is very long. Dairies were the first type of fermentative products which were used by human being. The history of consumption of dairies dates back to the beginning of human civilization (Rodas, 1986). Kefir is an unnatural fermentative drink produced from milk and it's originated from Caucasus Mountains in Russia (et al., 2009). The benefits of kefir are due to lactic acid, carbon dioxide, alcohol ethyl and aromatic combinations during the fermentation process (Robinson, 1991). In addition, the elements that form the unique sensory properties of kefir, are its sour acidic taste and refreshment effect. Kefir is as dense as yoghurt and its pleasant scent is due to presence of diacetyl and acetaldehyde. Diacetyl is produced by a Leuconostoc ssp. (Arslan, 2015).by using carbohydrates, fats and proteins; kefir promotes the growth and maintenance of cellular activity. Kefir has a high nutritional value and promotes the health and therefore, its consumption is recommended for premature infants, children, pregnant women, the sick and the elderly and patients with lactose intolerance (Ot1es & Cagindi, 2003). Kefir is enriched with vitamins, minerals, necessary amino-acids and proteins with easy digestibility and it also helps healing and maintenance of the human body. Kefir includes high amounts of B12 and B1 vitamins, calcium, and amino-acids, methionine, folic acid, vitamin K, magnesium, phosphorus and copper are helpful for autolysis of carbohydrates, fats and proteins and they also, promote cell growth and energy production (Sarkar, 2007).

PKU is an autosomal recessive disorder which is resulted from a mutation in the Phenylalanine hydroxylase Gene. This disorder results in accumulation of Phe which is an important amino acid and is metabolized in liver by the Phenylalanine hydroxylase system. This enzyme hydroxylases the Phe to tyrosine which requires Tetrahydrobiopterin (BH4) as a cofactor (Blau et al., 2010). A defect in hydroxylase Phe or in the production and recycling process of (BH4) leads to increased levels of Phe which if not remedied, causes intellectual disabilities. The casual results of this phenomena are severe mental retardation and emission of mold smell, reduced hair growth and skin hives, brightening of hair and eye color, reduced growth, small brain and head round and neurological symptoms of epilepsy including repeated legs and hands movements are also other effects of this illness(Enns et al., 2010). The patients who do not get remedied will probably develop behavioral problems such as hyper activity and anxiety. The clinical severance of the phenotype of this disorder is directly correlated with the level of blood Phe which in turn shows the severity of the enzyme defect (Pietro & Concolino, 2014). Phe is an amino-acid which is present in every protein containing food. PKU patients have a low tolerance for Phe and therefore, the dietary therapy of PKU patients requires high precision. This diet lacks any protein containing food. These foods include dairy, red meat, fish, egg, cheese, walnut, cereals and bread dough, pasta, biscuits and cakes (NSPKU, 2010). In fact, dietary therapy of PKU patients is based on limitations of protein digestion which reduce the Phe level to its minimum and improves the growth of patients with other nutritionals (Lopez Bajonero et al., 1991).

The main treatment which is nowadays applied for PKU is Phe absent diets. People with a Phe level of more than 20 mg/dL are known as PKU patients (Abdel Salam & Effat, 2010). In general, the desirable level of blood Phe is between 2 to 6 mg/dL. Foods with lower levels of Phe such as fruits, vegetables and grains should be consumed in appropriate amounts. Modern dietary therapies include formulations which result in better tastes and improved calorie volume which in turn, simplify compliance with these diets (Outinen et al., 1996). The amount of daily intake of Phe in the form of an exchange of 50 mg of Phe is approximately equal to 1 g of protein from foods such as potatoes, breakfast cereals and some vegetables. The level of Phe tolerance is low is usually about 4-6 g of protein per day (NSPKU, 2010). GMP is a glycosylated 64 amino acid peptide which is naturally found in cow milk and also in cheese water. After the milk is consumed by an infant or a mature, GMP is released along the gastric intestinal tract through proteolysis and with mediation of pepsin (Chabance et al., 1998). The commercially available GMP is a product which is produced when the kappa casein is divided to para kappa-casein which remains in the clot and GMP which remains in

whey (Etzel, 2004).
For the PKU diet, GMP is a highly suitable alternative formula for amino acid, because pure GMP has no aromatic amino-acid including Phe (Van calcar and Ney, 2012). Isolating GMP from whey leads to pollution with other clotted proteins such as Alpha-lactalbumin and beta-lactoglobulin which all contain Phe (Lim et al., 2007). Therefore, the publicly available GMP contains about 0.2 to 0.5 mg of Phe (Ney et al., 2009).
The majority of applied methods for elimination of Phe from hydrolyzed proteins are based on releasing a sufficient amount of Phe by enzyme hydrolysis, gel filtration of the released Phe and absorption of the released Phe by activated carbons or resins (Silva et al., 2007). Still, diets should contain low and controlled amounts of Phe in order to achieve a normal growth in PKU children. Synthesis of proteins and Neurotransmitters are both required Phe (Lara et al., 2005).
The method of eliminating Phe through enzyme hydrolysis, in addition to having a high cost of operation, lacks adequate practical and industrial applications and is only restrained to laboratory operations. Therefore, this article investigates a solution for implementation of this operation in industry. The formulation method is the method we are intended to study. In this method, by imposing a change or alteration in the formulation of milk which dairy base, it is tried to produce dairies containing minimum amounts of Phe. With the implementation of this method, the high costs of enzyme hydrolysis are avoided and at the same time, production of these products will become industrially feasible.

**Table 1: Glycomacropeptide (GMP) chemical specification (%)**

| Content | Percentage |
|---|---|
| Protein | 81 |
| Lactose | 1 |
| Fat | 1 |
| Ash | 9 |
| Moisture | 5.5 |
| Phenylalanine (of protein) | 0.28 |

## Material and methods

In order to perform the experiment, the following items were purchased: glycomacropeptide (CGMP- 20) from Arla food ingredients company (Denmark) (Table 1), cream powder (P350) from PulseGuard Company (Denmark), the stabilizer was supplied from the Hann Company and the starter was also supplied from the Sacco Company. Furthermore, the other materials including low fat pasteurized milk, milk permeate from microfiltration (PMF), margarine, modified corn starch, transglutaminase (TG) enzyme and salt were purchased from Iranian Companies. 6 M HCl, phosphate buffer (pH 3.5) and acetonitrile (98:2) from Merck Company (Germany).

### Sample preparation method:

Beforehand to production of the product, the required analog milk for this process is made from PMF (5%), modified corn starch (1.5%), cream powder (2%) and margarine (1.5%).
At the first step, the entire ingredients were weighted (Andek-300i digital scale, Germany). Next, the low-fat pasteurized milk (1.5%) is diluted with a 1:10 ratio with water. At the next step, the entire ingredients were mixed (Kepler mixer, China) and homogenized in order to produce analog milk with 8% MSNF. After these processes, the pasteurizer (APV4102, Denmark) was used for pasteurization of the analog milk.
This research includes four variables with two levels of high and low. These variables include yoghurt stabilizer, TG enzyme, starter and salt which have led to formulation of 16 formulas. In addition, GMP powder (3%) was added for enrichment of the product and increasing its nutritional value. The total formulation of the product is described in the following Table (Table 2).

**Table 2: Kefir ingredients for 17 formulations (%)**

| Sample | D. milk [a] | Sta. against two phases [b] | Sta. yoghurt [c] | M. C. starch [d] | TG enzyme [e] | Starter [f] | Salt [g] | Water |
|---|---|---|---|---|---|---|---|---|
| 1 | 70 | 0.2 | 0.1 | 0.5 | 0.00 | 0.5 | 0.46 | 28 |
| 2 | 70 | 0.2 | 0.1 | 0.5 | 0.00 | 0.7 | 0.46 | 28 |
| 3 | 70 | 0.2 | 0.1 | 0.5 | 0.00 | 0.5 | 0.5 | 28 |
| 4 | 70 | 0.2 | 0.1 | 0.5 | 0.00 | 0.7 | 0.5 | 28 |
| 5 | 70 | 0.2 | 0.1 | 0.5 | 0.04 | 0.5 | 0.46 | 28 |
| 6 | 70 | 0.2 | 0.1 | 0.5 | 0.04 | 0.7 | 0.46 | 28 |
| 7 | 70 | 0.2 | 0.1 | 0.5 | 0.04 | 0.5 | 0.5 | 28 |
| 8 | 70 | 0.2 | 0.1 | 0.5 | 0.04 | 0.7 | 0.5 | 28 |
| 9 | 70 | 0.2 | 0.3 | 0.5 | 0.00 | 0.5 | 0.46 | 28 |
| 10 | 70 | 0.2 | 0.3 | 0.5 | 0.00 | 0.7 | 0.46 | 28 |
| 11 | 70 | 0.2 | 0.3 | 0.5 | 0.00 | 0.5 | 0.5 | 28 |
| 12 | 70 | 0.2 | 0.3 | 0.5 | 0.00 | 0.7 | 0.5 | 28 |
| 13 | 70 | 0.2 | 0.3 | 0.5 | 0.04 | 0.5 | 0.46 | 28 |
| 14 | 70 | 0.2 | 0.3 | 0.5 | 0.04 | 0.7 | 0.46 | 28 |
| 15 | 70 | 0.2 | 0.3 | 0.5 | 0.04 | 0.5 | 0.5 | 28 |
| 16 | 70 | 0.2 | 0.3 | 0.5 | 0.04 | 0.7 | 0.5 | 28 |
| 17 * | 67.7 | 0.2 | 0.1 | 0 | 0.04 | 0.5 | 0.46 | 28 |

*[a] Diluted milk: milk/water (1:10, v/v); [b] Stabilizer against two phases; [c] Yoghurt Stabilizer; [d] Modified corn starch; [e] Transglutaminase; [f] Starter ; [g] salt; * sample enriched with 3% GMP powder.*

### Analog kefir production

First, the entire ingredients were weighted then the milk was heated up to 60 °C. afterwards, the modified corn starch and yoghurt stabilizer were added to the analog milk and the mixture was mixed. The mixture was homogenized with a Mettler Toledo PT3100 (Switzerland). The analog milk is pasteurized with APV-4102 pasteurizer at 85 °C for 15 S (Bylund, 2003). After pasteurization, the temperature of the mixture was reduced to 45-50 °C. At the next step, the TG enzyme was added and the mixture was mixed for 5 min. Next, the starter was added to the mixture at 40 °C and the mixture was again mixed for another 3 min. Samples were put in an incubator at 42 °C for 18 h. After incubation time, salt and water were added to the mixture and finally physical and chemical tests were performed on the samples.

### Chemical tests

Experiments on kefir quality features which include, acidity (ISO 11869, 2012), pH (ISO 26323, 2009), proteins (ISO 8968-1, 2014), dry matter (ISO 1736, 2008), salt percentage (ISO 1738, 2004), fats (ISO 2446, 2008) and alcohol percentage (AOAC 983.12, 1985) were determined.

### Phenylalanine measurement

For the purpose of extracting Phe, the method of hydrolysis of proteins by acids was used. In this regard, 5 g of the sample was hydrolyzed by 8 ml of 6 M HCl in an oven at 110 °C for 24 h. The prepared hydrolyses were then centrifuged at 5000 rpm for 10 min to get prepared for being injected to the HPLC. Supernatants were also filtered with Acrodisc filters (Piecyk et al., 2007).

### HPLC conditions

For the purpose of measuring the amount of Phe, an RP C18 column with a length of 150 × 3.9 mm was used (Waters, USA). The injection volume was 20 $\mu$l and the time of the chromatographic resolution of samples was 26 min for both samples and external standard (Fig. 1 & 2) using the mobile phase flow of 0.8 mL/min and the applied pressure was equal to 107-109 kgf/cm2. For the mobile phase, the combination of phosphates buffer (pH 3.5) and acetonitrile (98:2) was used and in addition, measurement was performed at the wavelength of $\lambda$=214 nm

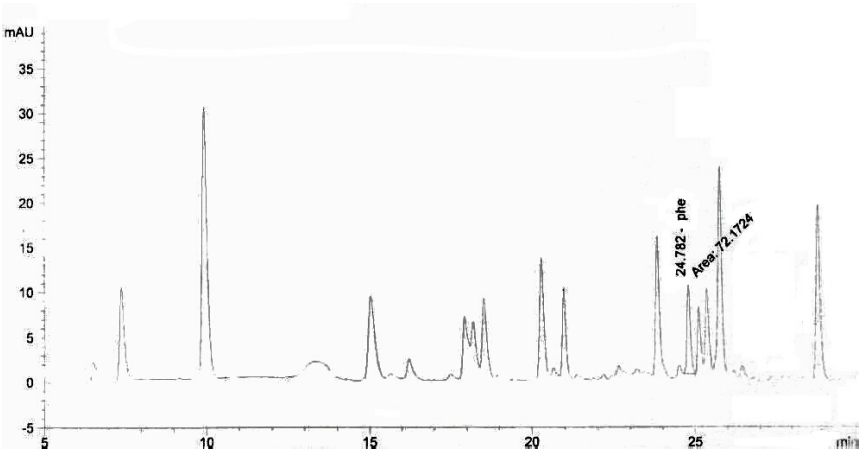


**Figure 1:**

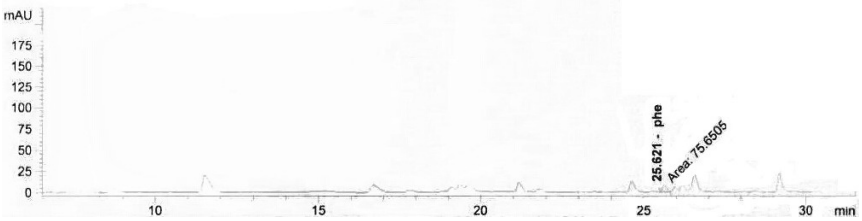


**Figure 2:**

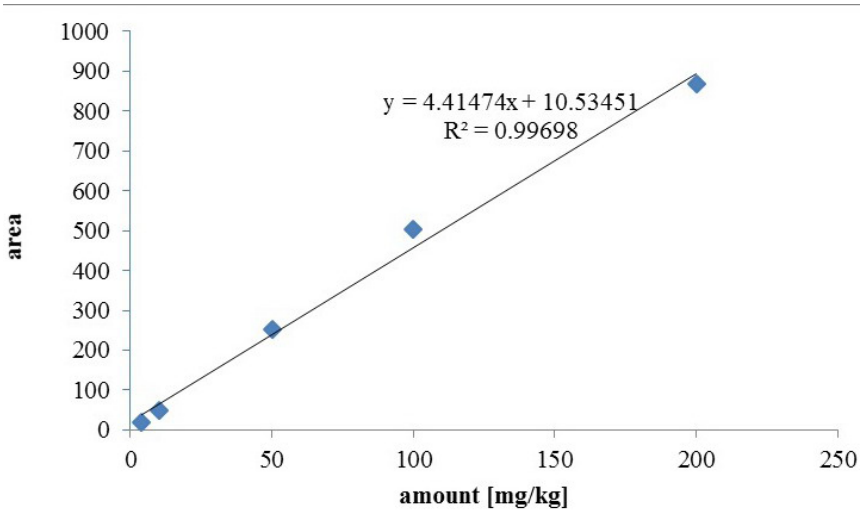


**Figure 3:**

with a UV detector (2487 model) at 25-30°C. For the preparation of calibration curve the standard L-phenylalanines (99%, FLUKA) in different concentrations was used (Fig. 3) (Piecyk et al., 2007).

### Sensory evaluation

Color, aroma, taste, texture, mouthfeel and overall acceptance of the entire kefir samples were determined by the panelists and through the application of consumer tendency test and the five point hedonic method. A number of 20 evaluators were selected through the quality level assessment test. A number of 20 similar samples were collected from each treatment and were handed to evaluators along with a

form which they were asked to fill according to their own taste. In this regard, the score of five was considered for a desirable quality and consequently, the score of one was considered for undesired quality. The evaluators have also used water for washing their mouth after consuming each sample (IDF, 1987).

### Statistical design

In this research, the SPSS V23.0 software was used for analysis of data resulted from chemical and sensory tests. On this basis, for the purpose of significance determination the ANOVA method was used and additionally, the Duncan test at a confidence level of 95% was used for comparing the averages ($p<0.05$). All the experiments were performed in triplicate orders.

### Results and discussion

The test results were collected in the Table 3 after all the chemical tests.

### Fats

Samples Nos. 7, 3 and 4 respectively contained least and most amounts of fat. With respect to the Table 3 and the percentage of fat in the formula, no great alterations have been imposed on the obtained averages. In addition, by enriching the mixture with GMP powder, no significant modifications have been recorded in comparison with the other 16 non-GMP containing samples. On the other hand, there are significant difference between samples No. 7, 3 and 4 as the least and most fatty samples ($p<0.05$).

### Acidity

Samples Nos. 5 and 6 and the GMP containing sample respectively contained least and most level of acidity. Acidity changes have had an ascending trend which is due to presence of starter bacteria and heat resistance lactic bacteria in the pasteurized milk such as Streptococcus and Lactobacillus. These bacteria slowly grow in refrigerated conditions and by fermentation of lactose and production of lactic acid, lead to increase acidity. By enriching the mixture with GMP and altering the formulation, the GMP containing sample showed an increase in its acidity level compared to the 16 non-GMP containing samples and this increase could be related to presence of lactose in GMP powder. On the other hand, there are significant difference ($p<0.05$) between the GMP-containing sample and samples number 5 and 6 as the most and least acidic samples. With respect to the obtained data, it can be observed that the acidity of the produced kefir

**Table 3: Chemical Composition of kefir samples [a]**

| | Fat | Acidity | Dry matter | (%) Alcohol | Salt | pH | Protein | Phe (mg/100 g) |
|---|---|---|---|---|---|---|---|---|
| 1 | 2.07±0.03$^{ef}$ | 28.67±1.15$^{ab}$ | 5.80±0.01$^{a}$ | 0.51±0.01$^{bcd}$ | 0.7003±0.00$^{a}$ | 4.67±0.01$^{h}$ | 0.55±0.03$^{a}$ | 21.36±1.19$^{a}$ |
| 2 | 1.97±0.06$^{cde}$ | 28.33±0.58$^{a}$ | 6.00±0.03$^{d}$ | 0.57±0.01$^{e}$ | 0.7003±0.00$^{a}$ | 4.65±0.02$^{gh}$ | 0.59±0.02$^{ab}$ | 22.23±0.61$^{abc}$ |
| 3 | 2.18±0.28$^{f}$ | 28.33±1.15$^{a}$ | 5.96±0.03$^{bcd}$ | 0.50±0.01$^{abcd}$ | 0.7310±0.00$^{b}$ | 4.64±0.01$^{g}$ | 0.58±0.01$^{ab}$ | 23.66±0.60$^{d}$ |
| 4 | 2.18±0.28$^{f}$ | 28.66±1.53$^{ab}$ | 5.91±0.03$^{b}$ | 0.57±0.00$^{e}$ | 0.7310±0.00$^{b}$ | 4.64±0.01$^{g}$ | 0.58±0.01$^{ab}$ | 23.53±0.90$^{d}$ |
| 5 | 1.72±0.03$^{ab}$ | 27.66±0.58$^{a}$ | 5.97±0.02$^{bcd}$ | 0.52±0.01$^{d}$ | 0.7003±0.00$^{a}$ | 4.65±0.00$^{gh}$ | 0.57±0.01$^{ab}$ | 22.90±0.45$^{cd}$ |
| 6 | 1.92±0.03$^{bcde}$ | 27.66±0.58$^{a}$ | 5.92±0.03$^{bc}$ | 0.57±0.01$^{e}$ | 0.7003±0.00$^{a}$ | 4.63±0.00$^{g}$ | 0.58±0.02$^{ab}$ | 22.03±0.41$^{abc}$ |
| 7 | 1.58±0.08$^{a}$ | 29.00±1.73$^{abc}$ | 6.08±0.05$^{e}$ | 0.48±0.01$^{a}$ | 0.7310±0.00$^{b}$ | 4.55±0.02$^{f}$ | 0.61±0.02$^{b}$ | 21.80±0.30$^{abc}$ |
| 8 | 1.72±0.03$^{ab}$ | 30.00±2.00$^{abc}$ | 6.15±0.35$^{ef}$ | 0.57±0.01$^{e}$ | 0.7310±0.00$^{b}$ | 4.50±0.03$^{cde}$ | 0.57±0.01$^{ab}$ | 21.86±0.80$^{abc}$ |
| 9 | 2.08±0.08$^{ef}$ | 29.00±2.00$^{abc}$ | 5.83±0.25$^{a}$ | 0.51±0.01$^{cd}$ | 0.7003±0.00$^{a}$ | 4.52±0.00$^{e}$ | 0.59±0.00$^{ab}$ | 22.70±0.70$^{abcd}$ |
| 10 | 1.97±0.03$^{cde}$ | 29.33±1.15$^{abc}$ | 6.00±0.006$^{d}$ | 0.57±0.01$^{e}$ | 0.7003±0.00$^{a}$ | 4.50±0.00$^{de}$ | 0.57±0.01$^{ab}$ | 21.73±0.47$^{abc}$ |
| 11 | 2.03±0.03$^{def}$ | 29.66±1.15$^{abc}$ | 5.82±0.03$^{a}$ | 0.48±0.01$^{a}$ | 0.7310±0.00$^{b}$ | 4.50±0.01$^{de}$ | 0.59±0.01$^{ab}$ | 21.86±0.40$^{abc}$ |
| 12 | 1.92±0.08$^{bcde}$ | 29.00±1.00$^{abc}$ | 6.11±0.06$^{e}$ | 0.56±0.01$^{e}$ | 0.7310±0.00$^{b}$ | 4.48±0.00$^{bc}$ | 0.59±0.02$^{ab}$ | 22.13±1.33$^{abc}$ |
| 13 | 1.83±0.08$^{bcd}$ | 28.66±1.15$^{ab}$ | 5.98±0.08$^{cd}$ | 0.49±0.01$^{abc}$ | 0.7003±0.00$^{a}$ | 4.48±0.00$^{bcd}$ | 0.58±0.01$^{ab}$ | 22.63±0.40$^{abcd}$ |
| 14 | 1.90±0.00$^{bcde}$ | 29.00±1.00$^{abc}$ | 6.11±0.02$^{e}$ | 0.58±0.02$^{e}$ | 0.7003±0.00$^{a}$ | 4.48±0.01$^{bc}$ | 0.57±0.01$^{ab}$ | 21.50±0.40$^{ab}$ |
| 15 | 1.82±0.03$^{bc}$ | 31.00±1.00$^{bc}$ | 6.19±0.03$^{fg}$ | 0.48±0.02$^{a}$ | 0.7310±0.00$^{b}$ | 4.47±0.01$^{b}$ | 0.56±0.01$^{a}$ | 22.76±0.11$^{bcd}$ |
| 16 | 2.02±0.08$^{cdef}$ | 31.33±1.15$^{c}$ | 6.22±0.03$^{g}$ | 0.57±0.01$^{e}$ | 0.7310±0.00$^{b}$ | 4.46±0.00$^{b}$ | 0.57±0.01$^{ab}$ | 23.56±0.97$^{d}$ |
| 17* | 2.07±0.08$^{ef}$ | 34.33±0.58$^{d}$ | 7.90±0.01$^{h}$ | 0.49±0.02$^{ab}$ | 1.1000±0.01$^{c}$ | 4.43±0.00$^{a}$ | 2.95±0.08$^{c}$ | 30.40±0.30$^{e}$ |

*a Each value in the table represents the mean value± standard deviation of triplicate analyses. Means within each column with different letters are significantly ($p<0.05$) different; b Phenylalanine; * Sample enriched with 3% GMP powder.*

product is not accordable with the acidity range of commercially available kefir drinks, however by using different starters and edible lactic acids, the acidity level could be matched with standard levels.

**Dry matter**

Samples Nos. 1 and 17 (sample enriched with GMP) respectively contained the most and least percentage of dry matter. In terms of the samples No. 1, as a result of insufficiency of the applied material, and low amount of used dry matter, this issue is justifiable. With enriching the mixture with GMP powder and altering the formulation, the amount of the dry matter in the GMP containing sample was increased compared to the other 16 non-GMP containing samples. In addition, the results obtained from other samples are also acceptable with respect to the formula and obtained averages. Nevertheless, there is a significant difference between samples No. 1 and the GMP containing sample as the highest and lowest dry matter containing samples ($p<0.05$).

**pH**

Samples No. 1 and the GMP containing sample respectively contained the most and least pH levels. With respect to the formula, the change trend of this factor in other samples is descending and it can be considered due to production of lactic acid as a result of fermentation of lactose by the starter bacteria and other microorganisms which survive the pasteurization process. In addition, by enriching the mixture with GMP powder and imposing alterations on the formula, the GMP containing sample possessed the least level of pH in comparison to the remaining 16 non-GMP containing samples. Nevertheless, there is a significant difference ($p<0.05$) between sample No. 1 and the GMP containing sample as the highest and lowest pH containing samples.

**Salt**

With respect to the formula, it was observed an increase salt amount in highly salty samples with 0.5% salt, compared to low salt containing samples with 0.46% salt. Considering the amount of the salt in formula, the other results are acceptable and justifiable. By enriching the mixture with GMP powder, the amount of salt has increased which can be due to presence of a 1.5% sodium in GMP powder. There also is a significance difference between the GMP containing sample and the other 16 samples in terms of salinity ($p<0.05$).

**Proteins**

With respect to the obtained data from the 17 samples, the level of protein of each sample is either high or low. However, by enriching the mixture with GMP powder, the percentage of proteins is increased which can be related to presence of large amount of proteins in GMP powder (Table 3). Nevertheless, there is a significant difference between samples No. 1 and the GMP containing sample as the highest and lowest protein containing samples ($p<0.05$).

**Phenylalanine**

Samples Nos. 1 and 17 (sample enriched with GMP) were respectively the lowest and highest protein containing samples. The level of Phe of each sample is either high or low. However, by enriching the mixture with GMP powder, the percentage of Phe is increased which can be related to presence of a large amount of Phe in GMP powder (Table 3). With respect to recommendations regarding the daily uptake of Phe by PKU patients, although that there is not a clear agreeing point between different articles in this context, it has been mostly stated that blood Phe concentration must be between 2 to 6 mg/dL (Wappner et al., 1999) and that the amount of Phe in the diet should change according to individuals blood Phe levels. In this regard, final concentration of Phe amino acid in the kefir drink produced for PKU patients, should be reported precisely so that the uptake amount of this amino acid is set according to the needs of each person. In this regard, considering the clinical aspects of PKU, it can be concluded that with respect to the allowed dosage of consumed Phe in different ages, the average of obtained data are in an acceptable range and are approved for every age group (MacLeod & Ney, 2010).

**Alcohol**

The alcohol level of each sample is either high (0.7% of the starter) or low (0.5% of the starter). With respect to the original formula, enriching the mixture with GMP powder has not resulted in significant changes. There also exited a significant difference between samples No. 7 and 11, 15 and 14 as respectively least and most alcohol containing samples. Since this research was performed in Iran, it was tried to keep the alcohol level in the allowed range of Iran's alcohol standard. Because according to Iranian national standards organization, the

**Table 4: Sensory evaluation for 17 kefir samples a**

| Sample | Color | Aroma | Taste | Texture | Mouth feel | O. acceptance [b] |
|---|---|---|---|---|---|---|
| 1 | 4.25±0.64$^{bcd}$ | 3.65±0.81$^{bc}$ | 4.00±0.65$^{bcd}$ | 4.40±0.68b | 3.35±0.74$^{bc}$ | 3.80±0.69$^{a}$ |
| 2 | 3.60±1.14$^{a}$ | 2.75±0.97$^{a}$ | 2.60±0.82$^{a}$ | 3.65±0.87$^{a}$ | 2.70±1.08$^{a}$ | 3.85±0.81$^{a}$ |
| 3 | 4.15±0.74$^{bc}$ | 4.25±0.44$^{cd}$ | 3.50±0.51$^{bc}$ | 4.15±0.74$^{b}$ | 3.30±0.57$^{bc}$ | 3.85±0.74$^{a}$ |
| 4 | 4.30±0.47$^{bcd}$ | 4.10±0.79$^{bcd}$ | 3.50±0.94$^{bc}$ | 4.10±0.64$^{b}$ | 3.45±0.60$^{bcd}$ | 3.70±0.65$^{a}$ |
| 5 | 4.10±0.91$^{bc}$ | 4.20±0.61$^{cd}$ | 4.25±0.72$^{d}$ | 4.15±0.67$^{b}$ | 4.05±0.82$^{e}$ | 3.65±0.67$^{a}$ |
| 6 | 4.10±0.79$^{bc}$ | 3.85±0.67$^{bcd}$ | 3.95±0.69$^{bcd}$ | 4.30±0.86$^{b}$ | 3.60±0.68$^{bcde}$ | 3.60±0.82$^{a}$ |
| 7 | 4.10±0.72$^{bc}$ | 3.90±0.64$^{bcd}$ | 3.85±0.74$^{bcd}$ | 4.25±0.85$^{b}$ | 3.80±0.83$^{bcde}$ | 3.70±0.73$^{a}$ |
| 8 | 3.90±0.79$^{ab}$ | 3.70±0.86$^{bcd}$ | 3.70±1.03$^{bcd}$ | 4.30±0.57$^{b}$ | 3.45±0.82$^{bcd}$ | 3.80±0.77$^{a}$ |
| 9 | 4.35±0.49$^{bcd}$ | 3.65±1.18$^{bc}$ | 3.95±0.10$^{bcd}$ | 4.30±0.66$^{b}$ | 3.55±0.82$^{bcde}$ | 3.70±0.65$^{a}$ |
| 10 | 4.30±0.66$^{bcd}$ | 4.35±0.59$^{d}$ | 4.15±0.81$^{d}$ | 4.45±0.51$^{b}$ | 3.95±0.82$^{de}$ | 3.85±0.87$^{a}$ |
| 11 | 4.55±0.51$^{cd}$ | 3.75±1.02$^{bcd}$ | 3.95±0.82$^{bcd}$ | 4.55±0.51$^{b}$ | 4.05±0.82$^{e}$ | 3.55±0.68$^{a}$ |
| 12 | 4.50±0.61$^{cd}$ | 4.25±0.79$^{cd}$ | 4.30±0.66$^{d}$ | 4.55±0.60$^{b}$ | 3.85±0.74$^{cde}$ | 3.85±0.74$^{a}$ |
| 13 | 4.60±0.50$^{cd}$ | 3.45±1.14$^{b}$ | 3.45±0.94$^{b}$ | 4.45±0.51$^{b}$ | 3.25±0.79$^{b}$ | 3.75±0.71$^{a}$ |
| 14 | 4.55±0.60$^{cd}$ | 3.65±1.04$^{bc}$ | 3.90±0.91$^{bcd}$ | 4.40±0.68$^{b}$ | 3.75±0.97$^{bcde}$ | 3.75±0.78$^{a}$ |
| 15 | 4.70±0.47$^{d}$ | 4.10±0.91$^{bcd}$ | 4.10±0.85$^{cd}$ | 4.30±0.57$^{b}$ | 3.75±0.64$^{bcde}$ | 3.85±0.81$^{a}$ |
| 16 | 4.55±0.51$^{cd}$ | 3.60±1.23$^{bc}$ | 3.40±0.88$^{b}$ | 4.50±0.51$^{b}$ | 3.55±0.76$^{bcde}$ | 3.75±0.78$^{a}$ |
| 17* | 4.10±0.79$^{bc}$ | 3.70±0.80$^{bcd}$ | 3.95±0.76$^{bcd}$ | 4.40±0.60$^{b}$ | 3.75±0.79$^{bcde}$ | 4.45±0.51$^{b}$ |

*a Each value in the table represents the mean value± standard deviation of triplicate analyses. Means within each column with different letters are significantly ($p<0.05$) different; b Overall acceptance; * Sample enriched with 3% GMP powder.*

level of alcohol in kefir drink should be lower than the amount of alcohol in kefir produced by other countries (Table 3).

### Sensory evaluation

Results of the sensory evaluation indicate that enriching the mixture with GMP has no significant effects on the sensory characteristics of the product ($p>0.05$). Sample enriched with GMP (No. 17) had the highest overall acceptance (4.45±0.51) in comparison with other samples (Table 4).

## Conclusion

Severe functional and neurological disorders of PKU can be remedied by dietary therapies which are aimed at lowering the blood Phe level to unofficial levels. With respect of high rate of existence of this illness in Asian countries and developing countries and as a result of family marriages and lack of proper screening in these countries and societies, a well-advised and correct planning is required for avoiding and reducing the damages caused by this disorder. The most important way of reducing the damages of this illness is to use suitable diets. The aforementioned drink is a suitable and appropriate nutritional product for PKU patients. Since protein sources have high levels of Phe, PKU patients who have low tolerance against Phe cannot use these sources. Therefore, this product is a suitable and necessary alternative. With respect to the results obtained from this research, it can be concluded that it is possible to produce dairies with limited amount of Phe which at the same time possess the entire other characteristics commercially available products such as protein texture, sensory characteristics and other chemical indexes. Patients who have a defect in their Phe hydroxylase enzyme, can purchase and consume this product with a moderate price. Because not only its Phe amount is in an acceptable range, but also producing this product or similar products is not associated with high costs and is economically affordable.

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## Conflicts of Interest

None of the authors have any conflict of interest associated with this study.

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