

Preparation and evaluation of a novel therapeutic dairy-based drink for phenylketonuria

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Citation: Abdel-Salam AM, Effat LK. Preparation and evaluation of a novel therapeutic dairy-based drink for phenylketonuria. *North Am J Med Sci* 2010; 2: 66-70.

Doi: 10.4297/najms.2010.266

Availability: www.najms.org

ISSN: 1947 – 2714

Abstract

Background: People with phenylketonuria need to eat a special diet which contains a low level of phenylalanine. Most of these special diets have high protein levels which contain phenylalanine. Control of phenylalanine levels in the early years of life is crucial and remains important throughout childhood, especially for cognitive function and behavior. **Aims:** The current study evaluated the biological and sensory properties of a novel dairy-based drink for patients with phenylketonuria (PKU). **Methods and Materials:** The novel dairy-based drink was prepared by emulsifying corn germ oil with casein glycomacropeptide (GMP) solution in milk permeates. The chemical composition and sensory properties of the dairy-based drink were determined. In addition, the dairy-based drink was nutritionally evaluated using patient volunteers. These patients followed a strict diet limiting phenylalanine in their food. Phenylalanine levels were measured before and after three days of consuming the dairy-based drink. **Results:** The results of the sensory evaluation showed that the product was ranked that there were decreases in “good” and was acceptable by all test panels and volunteers. Serum phenylalanine levels in all volunteers decreased between 30% - 80%. **Conclusions:** The data obtained from the sensory evaluation and the decreases in serum phenylalanine levels encourage us to utilize this formulated dairy-based drink for therapeutic feeding of PKU patients.

Keywords: Phenylketonuria, formulated dairy-based drink, casein glycomacropeptide, therapeutic effect.

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Introduction

Phenylketonuria (PKU) is one of the most common inborn metabolic errors; if not diagnosed and treated early by special diet intervention it will result in mental retardation. The disorder is associated with deficient activity of the enzyme phenylalanine hydroxylase (PAH), which results in elevated concentrations of phenylalanine (Phe) and its metabolites (phenylketones) in the body fluids of affected individuals. Untreated, affected individuals develop severe to profound mental disabilities, behavioral difficulties, seizures, rashes, pigment dilution, and an unusual body

odor [1]. The hallmark of PKU is the very high level of phenylalanine due to the variable deficiency of phenylalanine hydroxylase activity. These high levels of phenylalanine can be modified by the restriction of dietary phenylalanine [2].

Phenylketonuria is an autosomal recessive inborn error of phenylalanine metabolism resulting from deficiency of phenylalanine hydroxylase. Most forms of PKU and hyperphenylalaninaemia (HPA) are caused by mutations in the PAH gene on chromosome 12q23.2 [3]. Initiation and

control of phenylalanine levels in the early years of life are crucial and important throughout childhood, especially for cognitive function and behavior [4].

Milk constituents have become recognized as functional foods, suggesting their use has a direct and measurable effect on health outcomes [5]. Whey, a by-product of cheese manufacture, was early considered as a waste product. Recent discoveries have shown whey to be a functional food with several nutritional applications. Milk contains two primary groups of proteins; the caseins and whey proteins. In cheese making, the caseins are responsible for making curds while in an aqueous environment. The components of cheese whey include β -lactoglobulin, α -lactalbumin, bovine serum albumin, lactoferrin, immunoglobulins, lactoperoxidase enzymes, glycomacropeptides, lactose and minerals [6].

Glycomacropeptide (GMP) is also referred to as casein macropeptide. GMP is present in whey at about 10-15%, due to the action of chymosin on casein during the cheese-making process. However, it is not produced in acid-coagulated cheeses [7]. The κ -casein GMP is an important proteolytic action marker, being useful to ensure the quality control of raw milk as well as to control industrial processes, mainly in the production of cheese and sweet whey. The determination of sialic acid in sweet whey is useful since the concentration of sialic acid reflects the amount of GMP present. Glycomacropeptide is known to be a biologically active compound and is considered to be a potential ingredient for functional foods and pharmaceuticals [8-9]. One of the main characteristics of GMP composition is the lack of aromatic amino acids including phenylalanine. Therefore, GMP has been evaluated as a potential source of protein in the diet of subjects suffering from PKU.

The present work aimed to prepare and evaluate a novel dairy-based drink based on an emulsion of corn oil with GMP in milk permeate for feeding PKU patients. This dairy-based drink would offer a locally produced and affordable product with a low content of phenylalanine for patients suffering from PKU.

Patients and Methods

Buffalo (*Bubalus bubalis*) and cow (*Bos taurus*) milk of healthy lactating animals was obtained from a local farm in the vicinity of Giza, Egypt. Glycomacropeptides (BioPur-GMPTM) were purchased from Davisco, USA. Corn germ oil was obtained from the local market in Egypt.

Preparation and properties of the novel dairy-based drink

Fresh mixed whole buffalo and cow milks (1:1) were ultrafiltered through stainless-steel tubing in a pilot plant equipped with an ultrafiltration inorganic membrane (Carbosep®, France). The temperature was maintained at 50-55°C during processing to get milk retentate and permeate. Ultrafiltered (UF) milk permeate (composed of water, sugar, some minerals and non-protein nitrogen

compounds) was collected and used as an aqueous solution for the dairy-based drink. The dairy-based drink product was prepared by emulsification of 3% corn germ oil (a fat source) in milk permeate (aqueous solution) containing 2.50% glycomacropeptides (a protein source). The final product was then heated to 85°C for 15 minutes, then cooled rapidly and stored at 4°C.

Human subject nutritional studies

The human subject nutritional studies were conducted according to the Helsinki Declaration and approved by the Ethics Committee of the National Research Centre (NRC), Egypt. The subject studies were carried out under the supervision of approved medical staff in the PKU Unit, Medical Practice Units, Division of Medical Sciences, NRC, Egypt. Patients with PKU following the strict diet of limited phenylalanine content volunteered to test the novel dairy-based drink. The volunteer subjects were male and female and included infants, children, and adults.

Other foods consumed by the volunteer subjects, such as low protein breads and another special milk formula containing a low concentration of phenylalanine (resulting from protein hydrolysis and adsorption of phenylalanine) were monitored for phenylalanine level before the trial.

Serum phenylalanine determination

Measurements of serum phenylalanine levels before and after the trial were carried out using the methods described by Aldis et al [10].

Analysis of the novel dairy-based drink for patients with PKU

The total solids, protein, ash, total sugars, and fatty acid content of the novel dairy-based drink were determined according to official methods of the Association of Official Analytical Chemists (AOAC) (11). The pH was measured using a digital pH meter with glass electrodes. Amino acid content of the dairy-based drink was calculated from the amino acid content of the glycomacropeptide obtained from Davisco, USA.

The sensory evaluation of the appearance, color, flavor and overall properties of the novel dairy-based drink were adopted from the National Aeronautics and Space Administration (NASA) (12) using the following scale: (+++) very good, (++) good, (+) acceptable, (-) not acceptable.

Results

A schematic representation of the production of the novel dairy-based drink for PKU patients is shown in Figure 1. The novel dairy-based drink was prepared by emulsifying corn germ oil (a fat source) with casein glycomacropeptide (a protein source) solution in milk permeate.

The chemical composition, nutrient information, and amino acids profile of the novel dairy-based drink are shown in Tables 1 and 2. Each 100 mL of the novel dairy-based drink had 53.80 kilocalories. The sensory

evaluation properties of the dairy-based drink are shown in Table 3. The results showed that the product was ranked “good” and was acceptable to all volunteers.

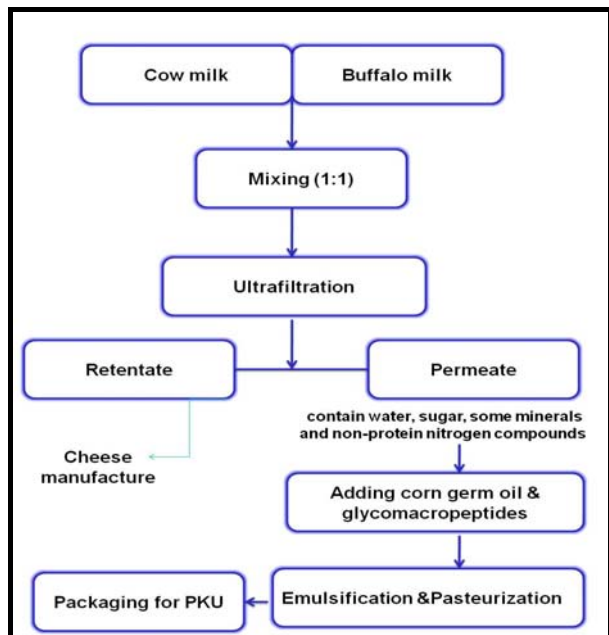


Fig. 1 A schematic representation of the preparation of the novel dairy-based drink for PKU patients

Table 1 Chemical composition and calories per 100 mL of the novel dairy-based drink for patients with PKU

Composition	Value
pH	6.70
Total solids	7.40 %
Lactose	4.20 %
Protein	2.50 %
Fat (%)	3.0 %
Ash (%)	1.09 %
Calories per 100 mL	
Calories from protein	10
Calories from corn oil	27
Calories from carbohydrates	16.80
Total Calories	53.80

Table 2 Amino acid profile of the novel dairy-based drink for patients with PKU

Amino acid profile (mg/100 mL)			
Alanine	152.4	Methionine*	48
Arginine	72	Phenylalanine*	12
Aspartic acid	216	Proline	282
Glutamic acid	504	Threonine*	339
Glycine	27	Tyrosine	15
Histidine*	6	Tryptophan*	3
Isoleucine*‡	249	Serine	153
Leucine*‡	69	Valine*‡	201
Lysine*	150		

*: Essential amino acid; **‡: Branched chain amino acid

Table 3 Sensory evaluation properties of the novel dairy-based drink for patients with PKU

	Sensory evaluation
Appearance	++
Color	+
Odor	++
Flavor	++
Overall	++

(+++) very good; (++) good; (+) acceptable; (-) not acceptable

The phenylalanine levels in patients before and after consumption of the novel dairy-based drink are shown in Table 4. The patients with PKU followed strict diets limiting phenylalanine consumption. The data show that the serum phenylalanine levels were lower after the consumption of the novel dairy-based drink in all patients. The percent reduction in phenylalanine levels ranged from 30 to 80% in all of the groups tested (Table 4).

Table 4 Serum phenylalanine (Phe) levels in patients before and after consumption of the novel dairy-based drink for 3 days

Grps	Phe level before trial mg/dL			Phe level after trial mg/dL			% Reduction
	Mean	SD	C.V. (%)	Mean	SD	C.V. (%)	
1	10.86	0.81	7.43	2.16	0.76	35.25	80
2	16.16	0.76	4.72	9.10	0.91	10.18	44
3	15.83	2.46	15.57	11.0	2.64	24.05	30

SD = standard deviation; C.V. = coefficient of variation; All three groups contained male and female (n= 10). Ages ranged from infants to adults.

The data were used to calculate suggested daily quantities of the novel dairy-based drink for infancy, childhood and adult ages, as shown in Table 5.

Table 5 The suggested daily quantities of the novel dairy-based drink for patients with PKU at different ages

Age	Suggested daily quantities	Phenylalanine mg/day	Protein g/day
Infancy	400 mL	48	10
Childhood	800 mL	96	20
Adult	1000 mL	120	25

Discussion

People with phenylalanine levels of 20.0 mg/dL or higher are considered likely to have "classical" PKU. Infants with these high levels are further tested to confirm the diagnosis before treatment is started. Some infants will have more modest elevations of blood phenylalanine and are said to have "mild hyperphenylalanemia." Many clinicians believe that any child with a phenylalanine level greater than 6 or 8 mg/dL should be treated with a modified phenylalanine restricted diet [13].

Providing products high in protein but low in

phenylalanine for patients with PKU is the focus of many research groups.

Glycomacropeptide (GMP) is a casein-derived whey peptide. When milk is treated with chymosin during cheese making, the milk protein (k-casein) is hydrolyzed into two peptides. The larger peptide containing amino acid residues 1-105 is called para-k-casein, which becomes part of the cheese curd, while the smaller peptide containing amino acid residues 106-169 becomes soluble and part of the whey. The peptide is relatively small, with a molecular weight of 8000 Daltons. There are two major variants of GMP, variant A and variant B, which differ in two amino acids. Different abbreviations are used to identify GMP, but all refer to the same molecule found in whey. GMP is also produced during curd formation by the act of rennin. Many researchers and health experts believe that GMP appears to have many health benefits beyond nutrition and consider it a functional food. Researchers have indicated that GMP can be used as a medical foodstuff for individuals suffering from PKU. Patients with PKU cannot digest phenylalanine, an essential phenolic amino acid, due to a defect in the enzyme hydroxylase. [14-20]. GMP is interesting in that it is naturally low in phenylalanine and has the potential to be used as a protein source in PKU diets.

The novel dairy-based drink that was evaluated in this study contained most of the basic amino acids but had a low level of tryptophan (3mg/100 mL). Fortification of the dairy-based drink with basic amino acids such as tryptophan will be taken into consideration in further studies.

Traditionally, strict adherence to the low phenylalanine diet was recommended for the first four to eight years of life, after which it was felt that a liberalization of protein intake could occur without damage to the developed central nervous system [21]. Early detection of maternal PKU in pregnant women may also be beneficial. If the mother does not follow a restricted phenylalanine diet during pregnancy, there is an overwhelming risk of the birth of an abnormal child. This risk appears to increase as the average maternal levels of phenylalanine during pregnancy increase [22].

In Egypt, people prefer buffalo milk because it is lower in cholesterol and higher in calcium than cow milk. The high milk solids of buffalo milk not only make it ideal for processing into superb dairy products, but also contribute to significant energy savings in conducting that process. Many cheese factories use mixtures of cow and buffalo milk, making this a readily available component for preparing the dairy-based drink for phenylketonuria.

Conclusion

The novel dairy-based drink for phenylketonuria seems a promising therapeutic product for patients. PKU patients are deprived of all animal protein sources rich in phenylalanine content, so an adequate replacement is

mandatory. In addition, this novel dairy-based drink has the advantage of the natural taste and smell of milk, and it is affordable. Longer term evaluation of the effects of this novel dairy-based drink on patients with PKU is recommended in order for it to be commercially developed.

Acknowledgement

The authors thank Prof. Dr. M. H. Abd El-Salam, Professor of Dairy Science, National Research Centre and Editors of Journal of Egyptian Dairy Science for his scientific suggestions, advice, and review of this manuscript. The authors thank Dr. Belinda Peace for assistance of English language editing.

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