

# Sensory, Physicochemical and Microbiological Analysis of Fermented Milk Piima

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## ABSTRACT

Fermented milk is a product that positive influences on human health. Different fermented milks are development for to please the palate of the people and increase your life. This work produced a fermented milk Piima type with honey and made physicochemical and microbiological tests demonstrated that the product is can be consumed and, the sensorial analysis demonstrated high acceptability of the consumer. Therefore, product development has a high probability of the market insert.

**Keywords:** *Lactococcus lactis*; Probiotic; Fermented milk; Piima-type; Consume

## INTRODUCTION

Nutrition quality is an important factor in the equilibrium and maintenance of human health. The growth in people's concern in knowing the characteristics of the food they consume has raised the development of products that, other than their function of nutrition, promote health and well-being, denominated functional foods [1].

The word probiotic was introduced by Lilley and Stillwell to describe microorganisms that perform beneficial activities to the human organism. Probiotics are defined as living microorganisms capable of improving the intestinal microbial balance, producing beneficial effects on the individual's health [2]. The regular consumption of these foods can be associated with different beneficial health effects, such as improving the intestinal transit of food, facilitating digestion, relieving symptoms of lactose intolerance, decreasing infantile colic, increasing the resistance of the gastrointestinal tract to the colonization of pathogens, stimulating the immune system, reducing cholesterol and preventing or suppressing colon cancer [3].

Fermented milk is products added or not with other food substances, obtained by coagulating and lowering the pH of the milk, or reconstituted milk, added or not with other dairy products, by lactic fermentation through the action of specific microorganisms, which must be viable, active and abundant in the final product during the shelf life. According to legislation, the cultures or microorganisms used in fermentation define the name

of the product, which can be yogurt, fermented milk, acidophilic milk, kefir, kumys and curd [4].

The Piima fermented milk, of Scandinavian origin, have a finer consistency, a milder flavour and is less acid than other yogurts. It is mesophilic fermented milk, which means that it can be fermented at room temperature [5]. It is described as a mixture of bacteria of the genera *Streptococcus* and *Lactococcus* however other bacteria and yeasts can be associated with the culture [6]. The probiotic *Lactococcus lactis* is a non-pathogenic Gram-positive bacterium, related to the genus *Streptococcus*, is also the best characterized lactic acid bacterium [7]. The *Lactococcus lactis* are of great importance in the dairy industry, mainly cheese and butter production [8]. Thus, the purpose of this work was to prepare a fermented milk piima with honey and to analyze the physicochemical, (carbohydrate, protein, lipids, moisture, ash, acidity and pH), elaborate the nutrition facts table of the product, besides microbiological analysis, molecular identification and sensory evaluation.

## MATERIALS AND METHODS

### Fermented milk Piima preparation

The Piima type fermented milk was obtained per donation and kept viable through daily cultivation. One tablespoon (about 20 g) of fermented milk Piima was added on a bottle containing 150 mL of whole milk; the mixture was homogenized and kept at room temperature for 12 hours. The result is creamy fermented milk, low

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acidity, with a texture close to plain yogurt. The fermented milk of this new flask was used to produce the yogurt of the next analyzes.

Was used 250 ml of fermented milk Piima in 2 litres of whole milk and fermented for 12 hours at room temperature. After this period, it was kept refrigerated ( $4 \pm 1^\circ\text{C}$ ) for 12 hours and then added with 5% honey, homogenized and refrigerated again until the analysis.

### Molecular identification

The identification of bacteria was performed using high-performance sequencing of the V3/V4 regions of the 16S rRNA gene. For sequencing, it was necessary to amplify the gene with the primers 341F CCTACGGGGRSGCAGCAG and 80R GGACTACHVGGGTWTCTAAT [9]. The DNA extraction using magnetic beads was performed according to the manufacturer's protocol (Neoprosperta Microbiome Technologies, Brazil). The libraries were sequenced using the Mi Seq Sequencing System (Illumin Inc., USA) and the kit V2, with 300 cycles and single-end sequencing. The sequences were analyzed using a proprietary pipeline (Neoprosperta Microbiome Technologies, Brazil).

### Microbiological analysis

Microbiological analysis was performed 1 day after yogurt production in order to guarantee the food safety of samples to be used on sensory analysis. Thermo tolerant coliforms, yeasts and moulds were analyzed; since they are those who have presence limits established by current Brazilian legislation [4]. The MRS agar culture medium was used for the *Lactococcus* analysis, since this is the main microorganism in this fermented milk.

A total of 25 mL of the sample was transferred into a stomacher containing 225 mL of sterile 0.1% w/v peptone water (Himedia Ltd., Curitiba, Brazil). Further dilutions were made from this original dilution. For yeasts and moulds quantification 0.1 mL from dilutions was inoculated on dry 2% potato glucose agar surface (Himedia Ltd., Curitiba, Brazil), acidified to pH 3.5. The plates were incubated without inverting at  $25 \pm 1^\circ\text{C}$ , for 5 days [10]. For *Lactococcus* quantification, 1 mL from dilutions was inoculated on MRS agar plates. The plates were incubated in an oven at  $43^\circ\text{C}$  for 72 hours.

Thermo tolerant coliforms were quantified in EC broth (Himedia Ltd., Curitiba, Brazil) using colonies from total coliform plates, after incubation at  $45^\circ\text{C}$  for 48 h [11].

### Physicochemical analysis

Protein, fat, moisture and, ashes were performed within 2 days of manufacture. Crude protein was determined via the Kjeldahl method as nitrogen (%) and then multiplied by a conversion factor (6.38). Fat was determined by Soxhlet [12]. Moisture was determined by drying (MA033, Marconi, Brazil) the samples at  $105^\circ\text{C}$  to a constant weight (BRASIL, 2006). For the ash determination, samples were previously dried at  $105^\circ\text{C}$  and then incinerated at  $550^\circ\text{C}$  (CE-800/R, Cienlab, São Paulo, Brazil). The ash content was measured gravimetrically. All samples were measured in triplicate.

The pH and titratable acidity were performed after 1 day of refrigerated storage. The pH values were measured using a pH meter (Digimed DM-20, SPLabor, Presidente Prudente, Brazil) by direct insertion of the electrode into the sample. Titratable acidity was determined after mixing 10 mL sample with 40 mL distilled

water and titrating with 0.1 N NaOH using a 1% phenolphthalein indicator to an endpoint of faint pink colour (AOAC, 2005). Amount of titratable acidity (a lactic acid percentage) was calculated. All samples were measured in triplicate.

Total carbohydrate was calculated using Eq. 1:

$$\text{Total carbohydrate} = [100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ fat})]$$

### Sensory analysis

Sensory evaluation was carried out in the Sensory Laboratory, at Tuiuti University of Paraná, PR, Brazil. The selection criterion was the nonexistence of allergic reactions to milk and consumption of fermented milk. Analyses were performed 6 days after production and lasted 2 days.

Sixty untrained sensory assessors evaluated the sample in individual sensory booths. Sample (25 ml) was served at refrigeration temperature in white plastic cups. An effective (hedonic) sensory test and a purchase intent test were performed. For the effective test the appearance, flavour, taste and, the texture was investigated on a 9-point hedonic scale (1=dislike extremely, 5=neither like nor a dislike, 9=like extremely). The consume intent test was performed to better understand the likely consumers of the products and was evaluated using a five-point structured scale (1= certainly would not consume and 5=certainly would consume) according to the methodology proposed by Dutcoski [13].

For the calculation of the (AI) of the product, the expression was measured using Eq. 2:

$$AI (\%) = A \times 100 / B$$

Where, A = average grade obtained for the product, and B = maximum grade given to the product.

The coefficient of variation allows comparisons between variables of different natures and provides an idea of the accuracy of the data. At first it is considered that the smaller the CV, the more homogeneous the data are (Garcia, 1989). The CV was calculated using Eq. 3:

$$CV = (\text{standard deviation} / \text{mean}) \times 100$$

The AI, CV and graphics formulations were performed using GraphPad Prims 7.05 versio program.

## RESULTS AND DISCUSSION

### Molecular identification

The sequences that passed through the initial procedures and that had 100% identity were grouped into phylotypes/clusters and were used for taxonomic identification, by comparison with a database of accurate sequences of 16S rRNA (Neoprosperta, 2017). Results of molecular identification show that 85% of the strains presented on the fermented milk piima analyzed are *Lactococcus lactis*, while other strains represent values lower than 1% (they are *Lactobacillus* and *Streptococcus* species).

Lactic acid bacteria used in the fermentation of foods are able to inhibit or reduce contamination by deteriorating and/or pathogenic microorganisms through the production of antimicrobial compounds, such as a reduction in pH (acidification), production of diacetyl, carbon dioxide, peroxide, ethanol and

bacteriocins that can exert an inhibitory action on different groups of microorganisms [14].

Among the bacteriocins used, the most used is nisin, which is used by several lines of *Lactococcus lactis* subsp. *Lactis*. Considered as a safe and non-toxic substance, it was used in the food industry of countries as an antimicrobial agent in cheeses, liquid eggs, sauces and canned foods, extending the shelf life of products [14].

### Microbiological analysis

It was not detected the presence of coliforms at 45°C, moulds nor yeasts during analysis. That indicates adequate handling of the product and fits it as within the microbiological standards required by the current Brazilian legislation [4].

The results obtained in the *Lactococcus* count showed a value of  $9,1 \times 10^7$  CFU/g, where the consumption of adequate amounts of the desired probiotic microorganisms in the bioproducts is of 0, 77 UFC/g sufficient to maintain the physiologically active concentration [15]. The probiotic cells should survive during the time until reaching the consumer [16] and after ingestion, at adverse conditions present in the gastrointestinal tract, maintain their effectiveness to get metabolically active in the intestine and exercise beneficial functions in the host [17-19]. When the concentration found was compared with the Brazilian legislation, we can be fixing the attention, but it is not worrisome because the high concentrations can be used in the dairy industry [20].

### Physicochemical and nutritional analysis

The nutritional analysis resulted in 88.18% of moisture, 3.12% of protein, 4.74% of carbohydrates and 3.15% of total fat and can see in Table 1. The results obtained have a CV of more than 25% when compared with other products, except moisture with 8.77%, showing that different ingredients in composition alter the nutritional value [21,22]. Many ingredients can be used for yogurt development to improve nutritional quality, the buffalo milk and goat milk are can be to use for the production of the yogurt [19] or the addition of fruit jelly [21] result in yogurts with different physicochemical characteristics. This work produced a fermented milk Piima type with honey than reached characteristics shows in other development and accepted by the tasters.

### Sensorial evaluation

The final product of this work was considered acceptable by the evaluators, since an AI above 70% indicates the acceptance of the food, on different categories, shown in Figure 1. The values are similar of others yogurts development where, the addition of honey is demonstrated good acceptability and the addition of others ingredients how the fruits with the attention for the amount, can be to attract the consumer for the product [23].

Garcia and Travassos [24] developed the umbu-flavoured goat fermented milk, which in their sensory attributes obtained good acceptance, registering average values between 6.07 to 7.17, which corresponds to the " I slightly liked it "and the hedonic scale "I moderately liked it". They obtained results like those found for fermented milk Piima type with honey. Among the comments of the tasters, there was a positive highlight about the taste of honey that pleased them a lot, as Barroso et al. [25] in his work on the acceptability of the Cajá pulp used as a flavour, softened the

Table 1: Physico-chemical analysis of fermented milk Piima type.

Variables	Author Lima, 2020	Nóbrega, 2020	Cunha et al, 2018	CV (%)	
Moisture (%)	88,18	88	72,75	81,91	8,77
Protein (%)	3,12	4,3	5,07	2,23	34,13
Carbohydrates (%)	4,74	1,83	9,34	13,29	89,98
Total Fat (%)	3,15	4,89	3,05	1,91	37,83

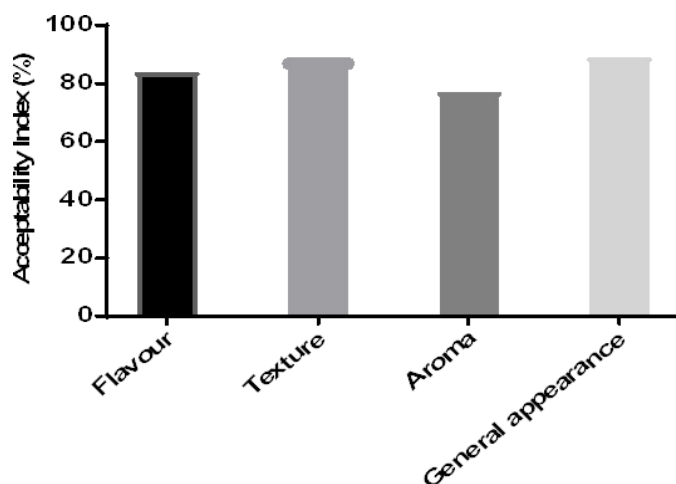


Figure 1: Acceptability Index (IA) of fermented Milk Piima type.

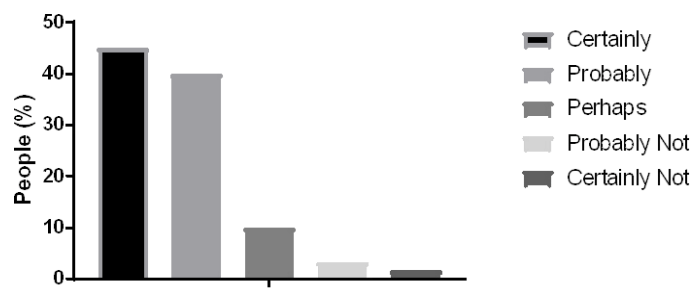


Figure 2: Consume intent of fermented milk Piima type.

taste and texture of goat's milk. The flavouring used also helped the acceptability of fermented milk of the Piima type to which honey was added. Garcia and Travassos [24] developed a goat fermented milk umbu flavour. The samples of fermented milk with additions of umbu candy obtained good acceptance and might be an interesting option for the dairy industry. With scored 85% of 'would consume' answers (Figure 2), the fermented milk Piima type with honey presented a greater potential for consumers if the product is marketed.

### CONCLUSION

The results obtained by the present study were satisfactory when compared to other studies. In addition, its nutritional composition points this product as a positive alternative for the dairy market. The fermented milk Piima type with honey was accepted by the evaluators, with the results obtained in the affective acceptance test, it was concluded that all the attributes presented (aroma, flavour, texture and general appearance) were accepted by the untrained tasters who contributed to the realization. from this analysis, the effective test of consumption intention resulted in a

large percentage of consumers if the product were available, among the comments of the tasters there was a positive highlight about the taste of honey, which pleased them a lot.

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