Original

"Petit suisse" cheese from *kefir*: an alternative dessert with microorganisms of probiotic activity

Queijo "Petit suisse" de kefir: uma alternativa de sobremesa com microorganismos de ação probiótica

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Abstract

"Petit Suisse" is a creamy cheese. *Kefir* is a symbiotic mixture of lactic acid bacteria and yeasts with probiotic activity including immunomodulation and balance of intestinal microflora. The present study aims to develop "Petit Suisse" cheese from *kefir*. Kefir grains were grown in pasteurized cow milk, and after the separation of *kefir* the serum was discarded and the "Petit Suisse" cheese was prepared using strawberry, mangaba, herbs, and dried tomatoes. The acceptance of the different preparations was evaluated using a nine-point hedonic scale followed by ANOVA. The sweet and salty products were compared by the Student's t-test. Purchase intent was evaluated by the means test and frequency distribution. All products were well accepted by the judges. The product was characterized by low yield, but it can be prepared at home at low cost. The nutritional composition analyses and the variety of flavors as well as the range of age of the judges are alternatives for further studies.

Keywords: *acceptance test; discarded serum; functional foods.*

Resumo

Petit suisse é um queijo de consistência pastosa. O *kefir* é um produto resultante da simbiose entre bactérias ácido-láticas e leveduras, e apresenta ação probiótica, como imunomodulação e balanço da microbiota intestinal. O presente estudo buscou desenvolver *petit suisse* de *kefir*. Os grãos foram cultivados no leite de vaca pasteurizado e, após separação do *kefir,* seguiu-se para dessoragem e preparo do *petit suisse* nos sabores morango, mangaba, ervas e tomate seco. Com escala hedônica estruturada de nove pontos, avaliou-se aceitação pela ANOVA. As amostras doces e salgadas foram comparadas por meio do teste *t* de Student e a intenção de compra foi avaliada por meio do teste de médias e de distribuição das frequências. As amostras apresentaram boa aceitação pelos julgadores. O produto pronto apresentou baixo rendimento, mas pode ser reproduzido em nível domiciliar com baixo custo. A análise da composição nutricional e a variação dos sabores e da faixa etária dos julgadores apresentam-se como alternativas para outros estudos.

Palavras-chave: *teste de aceitação; dessoragem; alimentos funcionais.*

1 Introduction

1.1 "Petit suisse"

"Petit suisse" is a type of fresh cheese with very high water content obtained by coagulating milk with rennet and/or specific enzymes and/or specific bacteria. It is sometimes added with other foods and presents a creamy consistency. It is typically white, but it may change color according to these additional substances; its taste and odor also depend on the substances added. Its preparation includes dairy ingredients such as cream, butter, and milk serum with the addition of fruit, and some other food substances such as cereals, vegetables and spices, sugars and/or carbohydrates, starch, and gelatin and chloride - sodium and calcium. For the preservation of its sensory characteristics, the product should be kept at a temperature not exceeding 10 °C (BRASIL, 2000).

The preparation of "Petit suisse" requires the production of Quark cheese, which is a white, soft, creamy, not aged cheese made with skimmed milk. It has a slightly sour taste and is sold as "Petit suisse" cheese after the addition of sweet or savory ingredients (VAN DENDER, 1985).

In the preparation of Quark cheese, serum separation is a critical step, particularly due to the microbiological risk. Controling this stage of production is of great importance to product quality requiring the application of good manufacturing and hygiene practices, which may be limited at the small-scale production (GONÇALVES, 2009). Accordingly, probiotic foods are presented as alternatives for patients with intestinal disorders, infection by microorganisms resistant to antibiotics, or those who have received large amounts of antibiotics resulting in imbalance of the intestinal microbiota (SANDERS, 1998; ZUBILLAGA et al., 2001; DANIELSEN; WIND, 2003). The "Petit Suisse" cheese prepared in the present study has microorganisms with probiotic activity. There is a difference

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between probiotic microorganisms, which include those isolated from the digestive tract of breast-fed infants and subjected to the selection process, and microorganisms with probiotic activity, which despite showing the same functionality are not native to the digestive tract (DUNNE et al., 2001; BORBA; FERREIRA, 2003; FERREIRA, 2008a).

Probiotics are living microorganisms that confer health benefits when consumed in adequate proportions. They must be of human origin; non-pathogenic; resistant to technological processing, warehousing and storage; resistant to stomach acidity and alkalinity of bile; and viable in the gastrointestinal tract. In addition, they must adhere to the epithelial tissue; produce antibacterial substances; and produce lactase and fatty acid short-chain or vitamins (DUNNE et al., 2001; PINHEIRO et al., 2009).

To prove the viability and probiotic activity of microorganisms, it is necessary to evaluate the product at the end of its shelf life. Additionally, for greater assurance of the effects of probiotic microorganisms, the duration of use and dose of the products should be considered as recommended by the manufacturer (FOOD…; WORLD…, 2001).

Probiotics act in the intestinal mucosa improving, among other factors, the barrier against pathogens due to increased production of IgA, which makes these products effective for treating food allergies and dermatitis (ZUBILLAGA, 2001; LOPITZ-OTSOA, 2006). Other studies have shown additional effects, in which probiotics act in the prevention and control of respiratory infections, in the colonization of pathogenic bacteria by competing for binding sites, and in the change of intestinal microbiota composition (MATTILA-SANDHOLM et al., 1999; WOHLGEMUTH; LOH; BLAUT, 2010).

1.2 Kefir

Kefir grains come in different sizes and texture and are slightly opaque (VAN DENDER et al., 1985). *Kefir,* a product with high probiotic activity, is a product resulting from the symbiosis between groups of lactic acid bacteria and yeasts. The microbial composition can vary in number and species according to the fermentation stage (acid or alcohol) and environmental conditions of inoculation. Thus, different microorganism groups may be found in *kefir* if the analysis is performed with the product under constant activation. This means that the grain or starter culture is often inoculated in milk for 24 hours and then incubated at appropriate temperature, and the new *kefir* is kept under refrigeration (maturation). The grains may also be kept refrigerated, frozen, or even freeze-dried. The quality of *kefir* is therefore dependent on the interaction between yeasts and lactic acid bacteria. This interaction of symbiotic microorganisms results in a slightly sour mass presenting creamy consistency (VECCHI; HAMSANANDA, 1999; WITTHUHN; SCHOEMAN; BRITZ, 2004; LOPITZ-OTSOA et al., 2006; FERREIRA, 2010; PIMENTEL; SANTOS, 2010).

These microorganisms synthesize the soluble matrix of polysaccharides that surrounds them - the kefiran complex, which provides a modulation of product characteristics since it becomes gel-like after cooling. This behavior is also responsible for melting when reaching body temperature, specifically in the mouth, making their use in food preparations quite interesting (DINIZ et al., 2003; PIERMARIA; CANAL; ABRAHAM, 2008).

1.3 Effects of kefir

Kefir has sensory and therapeutic properties such as reducing the effects of lactose intolerance and skin problems, immunity modulation, balance of intestinal flora, as well as antifungal, antibacterial, healing, antitumor, and antioxidant activity (VECCHI; HAMSANANDA, 1999; DINIZ et al., 2003; PIMENTEL; SANTOS, 2010). Its effects on the permeability of the intestinal mucosa also contribute to the suppression of oral sensitization and the development of tolerance in allergic patients (LOPITZ-OTSOA et al., 2006; WRÓBLEWSKA et al., 2009).

The accumulation of organic acids, such as lactic acid, inhibits growth of Gram-positive and Gram-negative bacteria (POWELL, 2006; YESILLIK et al., 2011). Garrote, Abraham and De Antoni (2001) observed more effective inhibition of *Escherichia coli* when *kefir* had a lactic acid concentration between 0.83 and 0.95 $mg.mL^{-1}$. In addition to the minimum concentration of lactic acid that presents antimicrobial activity, it is important to meet the requirements of acidity level as indicated by legislation: below 1.0 g lactic acid/100 *kefir* product (BRASIL, 2007). The CODEX ALIMENTARIUS stablishes minimum 0.6 % lactic acid to *kefir* (FOOD…; WORLD..., 2011).

Kefir also acts as an anti-inflammatory agent and may provide satisfactory results in celiac patients. Celiac disease corresponds to hypersensitivity to gliadin (gluten protein portion) that can be found in wheat, rye, barley, and oats, and it is marked by intense inflammatory processes. In sensitive people, children or adults, there is a reduction of villi, resulting in impaired nutrient absorption, diarrhea, dermatitis, and anemia, as well as other potential manifestations (ANGELIS, 2005; SILVA; MURA, 2007).

For those with celiac disease, *kefir* can also assist in combating nutritional deficiencies resulting from the reduction of villi since it is rich in vitamin B_{12}, thiamin, and potassium, and it increases the absorption of vitamin B complexs (VECCHI; HAMSANANDA, 1999; SILVA; MURA, 2007).

1.4 Kefir applications

Kefir grains can be used with milk, fruit juice, and sugar water. Only the liquid portion resulting from fermentation is ingested; the grains are not eaten (VECCHI; HAMSANANDA, 1999; MOREIRA et al., 2008).

Either fresh, pasteurized, or ultra-high temperature (UHT) milk may be utilized, which is then maintained at room temperature with *kefir* grains for about 24 hours (LOPITZ-OTSOA et al., 2006). *Kefir* should be consumed within seven days since after this period a microbiological succession occurs, and beneficial microorganisms are replaced by proteolytic and lipolytic microorganisms. Acidity plays a role on its shelf-life since it may help inhibit the

development of pathogenic microorganisms that survive milk heat treatment (SALOFF-COSTE, 2000; FERREIRA, 2008b; WESCHENFELDER et al., 2011).

After obtaining the *kefir*, it can be consumed *in natura* or added to soups, sauces, and cakes. Milk preparations containing *kefir* can be used by lactose-intolerant individuals since during fermentation lactose is broken down and consumption does not result in characteristic symptoms such as bloating and diarrhea (VECCHI; HAMSANANDA, 1999; SALOFF-COSTE, 2000).

Individuals with difficulties of digesting certain foods, such as lactose-intolerant individuals and those with celiac disease, may have problems finding healthy food options. The development of a low cost functional product for this kind of pathology may contribute to the adoption of healthy eating habits according to its clinical condition limitations. This study aimed to develop "Petit Suisse" cheese from *kefir* and evaluate its acceptance through judges from the Department of Nutrition and Health – UFV.

2 Materials and methods

2.1 Growing the grain

Kefir grains used in this study were grown in pasteurized cow milk and incubated at 27 to 28 °C for 24 hours without agitation and then rinsed with treated water. At the end of this stage, the grains were recovered using a sieve and replaced in fresh milk for future use, while the *kefir* was separated to prepare the Quark cheese.

2.2 Preparation of the Quark cheese

This step was developed experimentally, as shown in Figure 1.

Acidity was determined by titration with 0.1N NaOH and 1% phenolphthalein and expressed as % lactic acid as described by Tomikawa and Moretti (2009).

2.3 Preparation of the "Petit Suisse" cheese

Four types of samples, two sweet (strawberry and mangaba) and two savory (dried tomatoes and herbs) samples, were prepared.

Quark cheese was added to the cream, sugar, fruit pulp (strawberry and mangaba), and colorless gelatin until a homogeneous mass was formed for the sweet samples. For the savory samples, during the addition of gelatin and colorless cream, dried tomatoes and salt of herbs were also used. All ingredients were homogenized manually, with the exception of the dried tomato "Petit Suisse", for which a blender was used for homogenization prior to addition of cream.

The "Petit Suisse" cheese prepared was kept refrigerated until the day of testing (BRASIL, 2000).

2.4 Characterization of the judges

Each judge completed a survey regarding age, gender, education level, presence of allergy or food restriction, and knowledge of *kefir* (SOUZA, 2006).

2.5 Sensory acceptance test

Each judge received a randomly coded sample(s), water for cleaning the taste buds, the questionnaires, a pencil, and an eraser.

The sensory acceptance test was performed with untrained adult judges at the Laboratory of Experimental Study of the Department of Food Nutrition and Health, Federal University of Viçosa, 1 to 2 hours after a big meal to eliminate the interference of appetite (SOUZA, 2006).

Affective tests were used to verify the subjective reaction of the judges in relation to the different products using the hedonic scale since it is possible to infer whether the product was liked or disliked from the relative acceptance scores (NORONHA, 2003; SAPUCAY et al., 2009). A nine-point hedonic scale was used to verify the overall impression of the product, as well as sensory attributes of appearance and taste (SOUZA, 2003; TOMIKAWA; MORETTI, 2009; MINIM, 2010; WESCHENFELDER et al., 2011).

The judges were also questioned about their intent to purchase with justification. To guide the responses, the following alternatives were listed: (1) price, (2) product characteristics, (3) health claim, (4) others, in this case a specification was requested (SUASSUNA, 2005).

This study was approved by the Ethics Committee for Human Research at the Federal University of Viçosa (case number 0156/2010) in accordance with the provisions of Resolution 196/96 of the National Health Council (BRASIL, 1996).

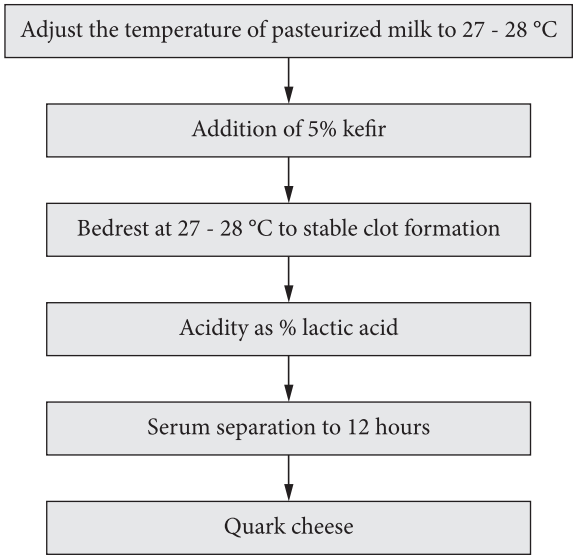

Figure 1. Flowchart for preparation of Quark cheese.

2.6 Statistical analysis

After completing the randomized block design, the data of the sensory analysis were evaluated by analysis of variance (ANOVA) at the 5% significance level. The ratings of sweet and savory samples were compared using the Student's t-test at 5% significance. The characterization of the judges and purchase intent were analyzed by the test of means and frequency distribution. For these tests, the SPSS statistical software version 17 was used.

3 Results

3.1 Preparation of Quark cheese

The formation of a stable clot occurred after twenty-six hours of fermentation, when the *kefir* reached an acidity of 0.96 %. After serum separation, there was a loss of approximately 50 % of the initial serum volume.

3.2 Preparation of the "Petit Suisse" cheese

Considering a portion size of 45 g, the sweet recipe yielded 9 portions, and the savory recipe yielded 8 servings. The cost of the "Petit Suisse" cheese prepared with strawberry, mangaba, dried tomato, and herbs was R US$ 0.44, US$ 0.46, US$ 0.31, and US$ 1.26, respectively.

3.3 Characterization of the judges

Sensory analysis was performed with 64 judges, six males (9.4%) and 58 females (90.6%). Women had a mean age of 25.4 (SD: 7.6) and men 30.2 (SD: 8.7). Regarding schooling, 51.5% of judges (n = 33) reported incomplete higher education, 45.3% (n = 29) were university graduates, 1.6% (n = 1) completed secondary education, and the other 1.6% (n = 1) had completed primary education only. Of the 64 judges, 93.8% (n = 60) said they did not have food allergy or intolerance. The remaining 6.2% (n = 4) reported to have adverse reactions to gluten, lactose, and/or seafood.

Regarding knowledge of *kefir*, 63.5% (n = 40) were aware of the product.

3.4 Acceptance testing

According to the results of ANOVA, it was observed that there was no significant difference between the sweet samples and savory samples ($p > 0.05$).

In the tests for overall acceptance and flavor, the sweet "Petit Suisse" received average scores between the hedonic terms "liked slightly" (6) and "liked moderately" (7). Among the savory samples, the average corresponded to the term "liked moderately" (7). With regard to the attribute appearance, both sweet and savory samples received average scores equivalent to the term "liked moderately" (7).

Figure 2 shows the frequencies of ratings above the term "Indifferent" (5) for overall acceptance, appearance, and taste of each sample. It can be observed that all samples received ratings greater than five in all tests.

With regard to purchase intent, most judges said they would buy the sweet versions because of their health claims. However, they would buy the savory recipe because of its product features (Figure 3).

The savory samples were rated higher than the sweet samples ($p < 0.05$), which is in accordance with the highest scores above the hedonic term "Indifferent" (Figure 2), therefore, indicating greater acceptance.

4 Discussion

Most adult judges who participated in the test had complete or incomplete higher education level and were familiar with *kefir*. In Brazil, marketing of "Petit Suisse" cheese has targeted children; however a sensory analysis with adults may allow for better assessment of consistency and investigation of potential consumer of different age groups (VEIGA et al., 2000), especially when proposing a sensory analysis of savory "Petit suisse".

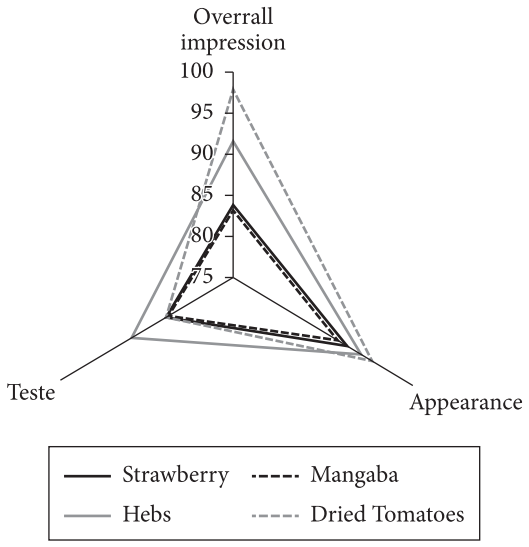

Figure 2. Frequency of ratings above "Indifferent" for the "Petit suisse" cheese samples.

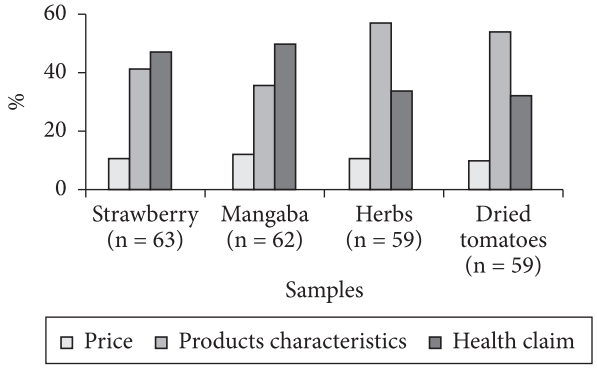

Figure 3. Intent to purchase samples of "Petit suisse" cheese by the judges.

Despite the formation of a stable clot within twenty-six hours, the reproduction of the proposed preparation mode of the Quark cheese can have different fermentation times due to culture variations (CARDARELLI, 2006). Acidity was also a determining variable in the time spent until formation of a stable clot, since the Ministry of Agriculture, Livestock, and Supply - MAPA (2007) establishes values between 0.6 and 2.0 g acid lactic.100 g^{-1} product for fermented milk, and for *kefir* it is recommended that acidity does not exceed 1 g lactic acid.100 g^{-1} product. Therefore, the Quark cheese had developed acidity near the maximum recommended by the MAPA (BRASIL, 2007), and a concentration of lactic acid (9.6 mg.mL^{-1}) of about 10 times greater than the concentration (0.95 mg.mL^{-1}) of lactic acid capable of neutralizing the action of pathogenic microorganisms, as observed in the studies of Garrote, Abraham and De Antoni (2001).

After achieving the recommended acidity, followed by serum separation, the Quark cheese was obtained and refrigerated prior to preparation of the "Petit Suisse" cheese. However, this product cannot be kept under refrigeration for many days after serum separation due to the short shelf life of *kefir* (FERREIRA, 2010). Although the serum separation step is featured for production, Albuquerque, Lima and Andrade (2008) did not consider this step when proposing methods for homemade recipes.

The "Petit Suisse" cheese preparation method used was characterized by a low yield as a result of serum separation, but it is cheap and can be easily replicated at home, besides being an option for lactose intolerant individuals, those with celiac disease, and/or those with health concerns since *kefir* presents several benefits such as anti-inflammatory, immunomodulatory, and antitumor activities (DINIZ et al., 2003; LOPITZ-OTSOA et al., 2006).

It is worth noting that although the product presents high acidity, care should be taken during preparation to prevent microbiological contamination, especially during serum separation which is a critical control point of hygienic sanitation, as indicated by the system of Hazard Analysis and Critical Control Points (HACCP). This system identifies potential hazards (chemical, physical, or biological agents) associated with a particular food, and determines in each production line the points where these hazards could eventually be introduced or reach intolerable levels. Thus, it is necessary to assess the seriousness of these dangers and risks to cause diseases, adopt control measures, and establish the critical control points (BRASIL, 1998; KANE, 1999; FOOD…, WORLD, 2001).

The feasibility analysis of microbiological and nutritional composition of the "Petit Suisse" cheese from *kefir*, although not being the specific goals of the present study, are important to support the recommendation for its use since it is necessary to show the benefits of *kefir* after Quark cheese production and the losses of whey proteins from cow milk with serum separation. However, these proteins can be incorporated into the final product when using ultrafiltration, in which all coagulated milk is converted into cheese (FOX, 2001), a practice widely used on industrial scale in some countries (VEIGA; VIOTTO, 2001).

Calcium may also be lost because with the increasing acidity resulting from fermentation this mineral is removed from casein micelles to the aqueous phase decreasing its concentration in the retentate, which improves the sensory characteristics of the final product (VEIGA; VIOTTO, 2001). Gonçalves (2009) proposed a methodology for reducing these losses; however, it is more complex for home preparation.

The sweet samples (strawberry and mangaba) were less accepted than the savory samples. Since in Brazil "Petit Suisse" cheese is traditionally known to be produced with strawberries, the judges may have rated a sample of this flavor comparing it with the commercial product; therefore, the purchase intent was limited to health claims.

Regarding the mangaba "Petit Suisse", its purchase intent because of health claims may also have arisen from the fact that this is not a fruit characteristic of the region in which the tests were performed (BRASIL, 2002). However, mangaba presents an antioxidant potential due to its vitamin C content (RUFINO, 2008). Moreover, it is a good option for preparation of sophisticated dishes and desserts; and, with globalization, individuals have greater access to consumer habits in other regions increasing the demand for these new flavors. Hence, mangaba fruit can now be found as a frozen pulp during all seasons meeting the demands of consumers outside the regions where the fruit is produced (MOTA; SCHMITZ; SILVA JÚNIOR, 2008).

Although the Technical Regulation of Identity and Quality of "Petit Suisse" Cheese (BRASIL, 2000) allows for the addition of food substances such as vegetables and spices, savory recipes are considered innovative due to its greater acceptance and purchase intent because of their product characteristics.

5 Conclusions

Sweet "Petit suisse" samples with strawberry flavor, such as those found in the domestic market, and a sample with mangaba (a fruit characteristic of the Brazilian Northeast, Midwest, and Amazon regions) flavor were prepared, as well as savory samples, considered to be new flavors for "Petit Suisse" cheese. All samples were well accepted by the judges indicating that *kefir* can be used in preparation of this product. Differences in taste and in age of the judges as well as the determination of shelf life and the characterization and microbiological viability of "Petit Suisse" cheese from *kefir* may be alternatives for future studies.

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