

## DEVELOPMENT, ANALYSIS AND ACCEPTANCE OF A PETIT SUISSE CHEESE- TYPE DAIRY DESSERT DERIVED FROM EQUINE MILK

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**Abstract:** Cow's milk allergy reaches up to 8% prevalence among children. The consumption of other types of mammalian milk is not recommended due to the high homology between cow's milk proteins, resulting in clinical cross-reactivity. However, mare's milk can be tolerated due to the low homology between proteins. This work aimed to develop and evaluate the acceptance and physical-chemical characteristics of a petit suisse cheese made from fresh equine milk cheese, as an option for people allergic to cow's milk. 14 tests were carried out to verify the best pH condition, type of yeast, fermentation and coagulation time for the production of equine quark cheese. Then, six more experiments were carried out to define the formulation of strawberry petit suisse cheese. The proximate composition and the sensory acceptance test were evaluated with a 9-point hedonic scale and a 5-point purchase scale. The best condition for the production of quark equine cheese led to an average mass yield of  $2.83\pm 0.14$ . The petit suisse cheese was made with equine quark cheese, coconut oil, sugar and freeze-dried strawberries as the main ingredients. The product obtained (%m/m)  $29.8\pm 0.37$  of lipids,  $7.36\pm 0.42$  of proteins and  $45.8\pm 0.10$  of moisture. The sensory analysis included 41 evaluators, with an average age of  $22.56\pm 5.83$  years. The product was well accepted, obtaining frequent value values above 8 (I really liked it) for appearance, odor, flavor, texture and overall score. 97.56% of reviewers stated that they would probably and/or definitely buy the product. The developed petit suisse equine cheese was very well received and can be considered a new dairy dessert option for people with allergies to cow's milk protein, especially children over 2 years of age.

**Keywords:** petit suisse cheese; mare's milk; allergy.

## INTRODUCTION

Cow's milk protein allergy is one of the most common food allergies in children, affecting 8% of the total pediatric population. (LAJNAF et al, 2023) It is an atypical response of the immune system to cow's milk and dairy products. Its main symptoms appear in the gastrointestinal tract, respiratory tract and skin, while clinical manifestations may include urticaria, itching, vomiting, diarrhea, nausea, abdominal pain, angioedema, bronchospasm and intestinal constipation (MORAIS; FAGUNDES-NETO, 2003).

The main allergenic components of cow's milk are glycoproteins with a molecular weight between 10 and 70kDa, among the most common are beta-lactoglobulin and alpha-casein, followed by beta-casein, alpha-lactoglobulin, bovine serum albumin and bovine gamma globulin (BISFELD, 2009). The main treatment currently adopted for cow's milk allergy is the exclusion of this food, its derivatives and any other product that contains it as an ingredient in the formulation. (MORAIS; FAGUNDES-NETO, 2003).

The World Allergy Organization, through DRACMA (Diagnosis and Rationale for Action against Cow's Milk Allergy) considers that equine milk can be considered a valid substitute for cow's milk for individuals with allergies, particularly (but not exclusively) for children with late-onset food allergy (FIOCCHI et al., 2010).

The most recent guidelines point out the need for babies over 2 years of age to have a substitute formula whenever their mother is unable to breastfeed, with cow's milk with an extensively hydrolyzed formula often being the best choice. Milk from other mammals (e.g. donkey, camel, mare, ewe and ewe) must not be used due to incompatibility with the nutritional needs of babies. But it opens up the possibility that in this case, the choice must be based on the context and the values and

preferences of doctors/patients. (FIOCCHI et al., 2022) Equine milk (mare and donkey) is considered as an alternative, however the fat content must be balanced to meet the nutritional needs of children. (MARTORELL-ARAGONÉS et al., 2015).

The high homology between cows, sheep and goats (ruminants) results in high cross-reactivity in cases of cow's milk allergy. However, mare's and donkey's milk have been tolerated by some individuals. Consumption of mare's milk significantly reduced SSAD (Severity Scoring of Atopic Dermatitis) in patients with atopic dermatitis (KATZ et al, 2008; VITA et al., 2007).

Mare's milk proteins are composed of approximately 60% casein and 40% whey proteins (MIRANDA et al., 2004). Thus, equine milk is more similar to human milk with respect to the ratio between casein and whey proteins, which is important for digestibility, in addition to the lower protein content. The high casein content of cow's milk (80%) causes the formation of a firm clot in the stomach, which requires 3 to 5 hours for digestion. Mare's milk forms a thin precipitate layer, and the digestion of proteins that occurs in the stomach lasts around 2 hours (PARK, 2009), similar to what occurs with human milk (LÖNNERDAL, 2010).

In addition to the content and proportion between proteins, a relevant factor is the homology between proteins. The World Allergy Organization (FIOCCHI et al., 2010) shows the low homology between proteins from mare's, donkey's and camel's milk with those from cow's milk and their similarity with human milk, explaining the low cross-reactivity in cases of allergy to cow's milk and horse's milk. This document is undoubtedly a milestone for the recognition of equine milk as an alternative to cow's milk for allergic individuals.

The sugars in equine milk are essentially

represented by lactose, which increases palatability and stimulates intestinal calcium absorption, representing an important factor for bone calcification in the first months of children's lives (ULLREY et al., 1996; BUSINCO et al, 2000). Equine milk also has reduced lipid levels compared to cow's milk (BAYLE-LABOURÉ, 2007), having a higher proportion of polyunsaturated fatty acids and a low concentration of cholesterol (KÜCÜKCETIN et al., 2003).

For Brazilians, the consumption of mare's milk may not be common, however, in Western Europe, in countries such as Russia and Mongolia, mare's milk is consumed daily. In Germany, its consumption has been popular since the beginning of the 20th century (PARK, 2009). In France, consumption is still expanding, being most commonly used for the production of cosmetic products (BAYLE-LABOURÉ, 2007). Around 30 million people in the world consume equine milk regularly (POTOČNIK et al., 2011).

In view of the above, there is a need to develop new products that meet the demands, mainly of children affected by food allergies to cow's milk, given that foods intended for this purpose are still rare. In this context, this research aims to develop, evaluate the acceptance and also the physical-chemical characteristics of a petit suisse cheese-type dairy dessert made from fresh equine milk cheese, as an option for people allergic to cow's milk.

## **METHODOLOGY**

### **ORIGIN OF EQUINE MILK**

Milk from Breton mares was obtained through the "Breton Horse Breeders Association" on a farm in the interior of the state of São Paulo, SP, Brazil, which provided free equine milk samples for this project. Milking was performed manually,

adopting the necessary hygienic care. The milk was stored at 4°C for up to 48 hours and transported under refrigeration to the place where the work was carried out (Experimental Kitchen of ``Universidade Presbiteriana Mackenzie``), where it was processed.

## DEVELOPMENT OF QUARK CHEESE

The equine milk was filtered through a nylon sieve to remove fragments and possible dirt.

Initially, the method for obtaining quark cheese was adapted from Souza et al. (2008): 1) pasteurization (65 °C for 30 min); 2) cooling to 35 °C; 3) addition of lactic yeast (1.5% w/v), calcium chloride (0.001% w/v) and rennet (0,001% p/v) by ``Estrela``; 4) curd cutting; 5) homogenization; 6) desilting. Different concentrations (0%; 1%; 2% and 3%) of two types of yeast were tested (Bela Vista® FERMENTO FX-BV - *Lactococcus lactis subsp. Lactis* and *Lactococcus lactis subsp. Cremoris* e FERMENTO MX-BV - *Lactococcus lactis subsp. Lactis*, *Lactococcus lactis subsp. Cremoris* and *Streptococcus salivarius subsp. Thermophilus*) in triplicate. However, the method was not suitable for equine milk and coagulation was not obtained.

Therefore, new tests were carried out in order to better verify the behavior of equine milk based on the variables of pH, type of yeast and fermentation and coagulation time. 14 tests were carried out (tables 2 and 3), all in triplicate.

After testing and adapting the methodology used by Khan et al. (2004) for the production of cheese from camel milk, whose composition is similar to equine milk, the procedure shown in figure 1 was defined.

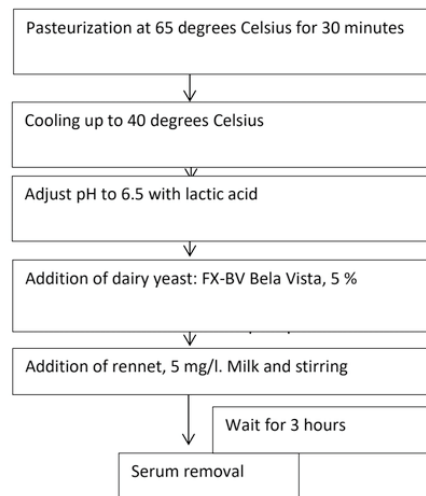


Figure 1. Flowchart for making quark cheese.

## DEVELOPMENT OF PETIT SUISSE CHEESE

The petit suisse cheese was developed based on the methodology developed by Maruyama et al. (2006) with modifications. Thus, to produce the petit suisse type, quark equine cheese, refined coconut fat (MCT®), invert sugar, refined sugar (União®), freeze-dried strawberry, carboxymethylcellulose (Fragon®), xanthan gum (Rhodigel 80®) were used), agar agar gum (Arcolor®), natural cochineal carmine dye (Plury Química®) and natural aroma identical to strawberry (IFF®). The proportion of ingredients is shown below in Table 1.

Ingredients	%(m/m)
Equine cheese	30
Refined coconut oil	30
Inverted sugar	10
Refined sugar	8
Freeze-dried strawberry	1,8
CMC	0,25
Xanthan gum	0,25
Agar agar gum	0,25
Dye	0,07
Smell	0,04
Water	19,34

Table 1. Final formulation of petit suisse equine cheese.

## SENSORY ANALYSIS

The sensory analysis was carried out in the Experimental Kitchen of Universidade Presbiteriana Mackenzie, in individual booths with 41 adults of both sexes selected through contact on the university campus, made up of undergraduate students, teachers and staff, forming a panel of untrained tasters.

The petit suisse cheese sample was served cooled to 10°C in a disposable plastic cup, with an edge of approximately 2 cm. A hedonic scale was used to evaluate product acceptance to analyze food preference and acceptability. The volunteers assigned points (from one to nine) for the degrees of acceptance (from extremely disliked - 1 to extremely liked - 9) for each of the following characteristics: appearance, consistency, odor, flavor and overall score.

The Sensory Analysis form also included the collection of sociodemographic data such as: sex (male and female); age (in years) and level of education (incomplete primary education/complete primary education, incomplete secondary education/complete secondary education, incomplete higher education/complete higher education, postgraduate and did not study).

This work meets the recommendations of Resolution CNS 196/1996 and was approved by CEP/UPM number: 1455/05/2012 and CAAE number: 02764412 8,0000. 0084. Only individuals who accepted and signed the Informed Consent Form participated in the analysis. Volunteers with the flu and/or who smoked in the last hour before the Sensory Analysis test were excluded from the study, as well as individuals who were diabetic, allergic or intolerant to the ingredients in the formulation. The predictable risks in sensory analysis are minimal, however they include allergies or intolerance to any of the food components. To minimize these risks, the components of the product formulation were presented to participants before the sensory

analysis.

## PHYSICOCHEMICAL ANALYSIS

Chemical analyzes were carried out according to LUTZ (2008): acidity in lactic acid, moisture, ash, fat using the Bligh-Dyer method; proteins by micro Kjeldhal. Carbohydrates were calculated by difference.

## ANALYSIS OF RESULTS

The analysis of quantitative data is presented in the form of mean and standard deviation and qualitative data in frequencies and percentages. The collected data were computed and subjected to interpretation and statistical analysis, in the Microsoft Office Excel software, using the Student's T Test, considering  $p > 0.05$  statistically significant.

## RESULTS

Tables 2 and 3 show the main results of the quark cheese development tests. First, it was assessed whether the milk would coagulate with just the addition of rennet, without adjusting the initial pH of the milk (C1), obtaining a negative result for coagulation.

In C2, the pH was adjusted to 5.5 with the addition of lactic acid and without the use of yeast. The milk curdled, however, the cheese had an extremely sensitive texture. In tests C3 to C10, different initial pH values were used and yeasts were added. After one hour of fermentation and one hour of rennet action, it was found that the best yields occurred in the samples that started with a pH between 6.0 and 6.5 (C7, C9, C8 and C10), in addition the texture It was firmer than these.

Test	Initial pH	Yeast (5%, m/v)	pH after 1 hour	Performance (%)
C1	6,79*	-	6,79	-
C2	5,50 (±0,03)	-	5,50 (±0,03)	1,81 (±0,11)
C3	6,79*	MX	6,37 (±0,01)	-
C4	6,79*	FX	6,39 (±0,01)	0,17 (±0,12)
C5	5,50 (±0,05)	MX	5,38 (±0,03)	0,60 (±0,16)
C6	5,50 (±0,05)	FX	5,44 (±0,06)	0,45 (±0,32)
C7	6,00 (±0,01)	MX	5,89 (±0,01)	2,81 (±0,17)
C8	6,00 (±0,01)	FX	5,91 (±0,01)	2,92 (±0,12)
C9	6,50 (±0,01)	MX	6,23 (±0,01)	2,61 (±0,22)
C10	6,50 (±0,01)	FX	6,21 (±0,01)	2,09 (±0,44)

**Table 2:** Evaluation of equine milk for cheese making regarding initial pH and type of yeast. Values in mean and standard deviation. São Paulo, 2013.

Therefore, these tests were repeated applying a longer period of fermentation (three hours) and coagulation (three hours) which consisted of tests C11 to C14, the results of which are presented in Table 3.

Test	Initial pH	Yeast (5%)	pH after 3 hours	Performance (%)
C11	6,00 (±0,02)	MX	5,73 (±0,02)	2,23 <sup>a</sup> (±0,07)
C13	6,50 (±0,01)	MX	5,97 (±0,02)	2,51 <sup>a</sup> (±0,15)
C12	6,00 (±0,02)	FX	5,79 (±0,02)	2,25 <sup>b</sup> (±0,05)
C14	6,50 (±0,01)	FX	6,01 (±0,04)	2,83 <sup>c</sup> (±0,14)

**Table 3.:** Evaluation of equine milk for cheese making regarding initial pH and type of yeast, after three hours of fermentation and three hours of coagulation. Values in mean and standard deviation. Different letters indicate significant differences ( $p < 0,05$ ).

After the tests, the condition that presented the best yield and best texture was C14 (2.83%) with the initial pH adjusted to 6.5, use of FX-BV yeast and rest for three hours, addition

of rennet when the pH was 6.0 and rested for three hours. Increasing the time from one hour to three, both for fermentation and coagulation, directly influenced the taste of the cheese, softening the characteristic flavor of equine milk and improving the texture of the cheese, making it more consistent.

Khan et al. (2004) in Pakistan, developed two fresh cheeses from camel milk using starter culture and acidification with citric acid and obtained yields of 13.22 + 4.49 and 11.70 + 2.35, respectively. Higher yield compared to that found with equine milk.

One of the possible causes for the low yield of cheese produced with equine milk may be due to the difference in size of the K-casein micelles, as those in equine milk are larger compared to cow's milk (FIOCCHI et al., 2010).

Like cow's milk, equine milk has about 50%  $\beta$ -casein, 10%  $\gamma$ -casein and 40%  $\alpha$ s1- and  $\alpha$ s2-casein. Mare's milk, as well as human milk, has a very small amount of k-casein, which directly influences clot formation. For example, during digestion, equine and human milk form a fine precipitate, which facilitates evacuation within two hours. In cow's milk, the high concentration of caseins causes a strong and firm clot at the time of digestion, requiring an average of three to five hours for digestion (PARK, 2009).

After this stage, tests for the production of petit suisse equine cheese began. In Brazil, the legislation for petit suisse cheese is found in Normative Instruction Number 53 of December 29, 2000 – Technical Regulation on Identity and Quality of “Petit Suisse” Cheese. It is defined as fresh, unmaturred cheese, obtained from the coagulation of milk with rennet and/or specific enzymes and/or specific bacteria, whether or not added to other food substances (BRAZIL, 2000).

However, as there is still no specific technical regulation for equine milk products

and no other articles with the same raw material were found, the results of this work were compared with this legislation and works by other authors who developed petit suisse cheeses.

Table 4 presents the results of the physicochemical analyzes.

Composition (g/100g)	Average	Standard deviation
Moisture	45,8	0,10
Protein	7,36	0,42
Lipids	29,8	0,37
Ashes	0,55	0,01
Acidity in lactic acid	0,05	0,01
Carbohydrates	16,51	-

**Table 4.** Results of physicochemical evaluations of equine petit suisse cheese.

Regis et al. (2012) developed a petit suisse using goat's milk and obtained 85% moisture, 9% protein and 8% lipids in the cheese composition. In São Paulo, Veiga and Viotto (2000) produced petit suisse cheese with ultrafiltered cow's milk, which resulted in a cheese with 9.30% proteins and 5.8% lipids. Boatto et al. (2010) manufactured petit suisse cheeses using soybean varieties and found values of 67.53% for moisture, 0.35% for ash, 5.43% for proteins and 4.27% for lipids. The results obtained in the present work are very different, not only because the composition of equine milk is unique, but because in order to have good palatability, the proposed formulation has a high fat content (tables 1 and 4). Brazilian legislation only determines that the amount of proteins cannot be less than 6% (BRAZIL, 2000), which despite being specific for cow's milk derivatives, the result proved to be adequate.

In the sensory analysis, 41 adults participated, 31 females and 10 males. The majority (87.80%) were attending higher education and the average age found was 22,56 + 5.83 years. The petit suisse equine

cheese produced was very well received, as can be seen in Table 5.

Attributes	frequent value	average	Standard deviation
Look	9	8,34	0,69
Smell	9	8,39	0,83
Texture	8	7,71	1,25
Savor	8	8,02	0,88
Global note	8	8,29	0,60

**Table 5.** Results obtained in the sensory analysis of petit suisse equine cheese.

All attributes of petit suisse equine cheese evaluated in the sensory analysis obtained good scores (table 5), equivalent to I liked it regularly (7), I liked it a lot (8) and I liked it extremely (9). In the sensory evaluation with 81 tasters, the petit suisse cheese made with goat's milk prepared by Regis et al. (2012) obtained an overall score of 7. While Veiga and Viotto (2000), in an analysis of 41 tasters, found values of 7.24 for appearance, 7.76 for texture, 6.73 for flavor and 7.32 for the overall score in the evaluation. of petit suisse cheese made from ultrafiltered cow's milk.

Finger et al. (2010), in Paraná, developed a passion fruit and tangerine flavored petit suisse cheese and tested it with 43 untrained people. They received a score of 6.5 for appearance, 6.3 for color, 7.7 for odor, 6.8 for flavor and 7.2 for texture. Furthermore, 77.5% of tasters stated that they would probably buy the product. When purchasing intention for petit suisse equine cheese was assessed, 97.56% of tasters stated that they would definitely and/or probably buy this product, indicating excellent acceptance.

A survey carried out by the consumer observatory, linked to the milk intelligence center/EMBRAPA on the social network Twitter (currently X) evaluated which cheese was most mentioned on Twitter. Petit suisse cheese came in third place in the survey of posts from May 2022. (CILEITE, 2022)

According to the authors, this evidence on the social network could reflect an increase in consumption of the product, opening an important field for insertion of the proposed innovation.

Below are some of the comments made by tasters on the sensory analysis form: “The product is very good, I really liked it.” “The flavor is very good and very close to that of a dairy dessert with cow’s milk. The point that I found different was the texture.” “I found the consistency not very firm, but the flavor is delicious!” “Great flavor! I believe it could be more consistent.” “It’s very good, as it closely resembles the taste of commercial petit suisse

cheese, offering the same pleasure to people with allergies. I loved”. “Quite similar to the original.” “As it does not contain cow’s milk in its composition, it is very tasty, with a very similar taste.”

## CONCLUSION

The petit suisse equine cheese developed was very well received and can be considered a new dairy dessert option for people with food allergies to cow’s milk protein. However, more tests are still needed to improve the yield of equine quark cheese, as the main raw material, and also to adjust the current formulation in terms of possible reduction in fat content.

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