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Fermented Milk Drinks Produced with Sheep's Milk and the Impact of Physicochemical Differences on Sensory Perception

Bebidas lácteas fermentadas produzidas com leite de ovelha e o impacto das diferenças físico-químicas na percepção sensorial

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Three different fermented milk drink formulations were developed with sheep's milk and whey supplied by a producer in the region of Bragança, Portugal. The variation of the formulations occurred in the proportions of whey, with $FORM_1$ with 25% whey; $FORM_2$, 40% and $FORM_3$, 60%. The fermented dairy drinks were evaluated for fat, protein, pH determination and titratable acidity, followed by the preference and acceptance test between the three samples. The fermented dairy drinks showed average fat results of 6.1% in $FORM_1$, 5.0% in $FORM_2$ and 2.0% in $FORM_3$. Protein results were 5.3%, 4.5%, and 3.6% on $FORM_1$, $FORM_2$, and $FORM_3$, respectively. The acidity and pH values did not differ in the three formulations evaluated. There was no significant difference in the acceptance of $FORM_1$ and $FORM_2$, allowing a serum addition of up to 40%. Thus, dairy drinks produced with sheep's milk in different concentrations of whey were obtained acceptance of 4.3 and 4.23, respectively for $FORM_1$ and $FORM_2$, and these samples also obtained purchase intent represented by the terms “I would definitely buy” or “I would probably buy”.
Keywords: lactic acid bacteria, quality attributes, sensory acceptance.

Com o objetivo de desenvolver uma bebida láctea à base de soro de leite de ovelha e apresentá-la como forma de aproveitamento de recursos, foram desenvolvidas três diferentes formulações de bebida láctea fermentada com leite e soro de leite de ovelha fornecidos por um produtor da região de Bragança, Portugal. A variação das formulações ocorreu nas proporções de soro de leite, com $FORM_1$ com 25% de soro de leite; $FORM_2$, 40% e $FORM_3$, 60%. As bebidas lácteas fermentadas foram avaliadas quanto à gordura, proteína, determinação do pH e acidez titulável, seguido do teste de preferência e aceitação entre as três amostras. As bebidas lácteas fermentadas apresentaram resultados médios de gordura de 6,1% na $FORM_1$, 5,0% na $FORM_2$ e 2,0% na $FORM_3$. Os resultados de proteína foram 5,3%, 4,5% e 3,6% em $FORM_1$, $FORM_2$ e $FORM_3$, respectivamente. Os valores de acidez e pH não diferiram nas três formulações avaliadas. Não houve diferença significativa na aceitação de $FORM_1$ e $FORM_2$, permitindo adição de soro de até 40%. Assim, as bebidas lácteas produzidas com leite de ovelha em diferentes concentrações de soro obtiveram aceitação de 4,3 e 4,23, respectivamente para $FORM_1$ e $FORM_2$, e essas amostras também obtiveram intenção de compra representada pelos termos “com certeza compraria” ou “provavelmente compraria”.
Palavras-chave: bactérias láticas, atributos de qualidade, aceitação sensorial.

1. INTRODUCTION

Milk-based products that are fermented with lactic acid bacteria (LAB) have been defined as "superfoods", claimed to improve health, defy aging and prevent the progression of changes that lead to diabetes, hypertension, Alzheimer's and cancer [1, 2].

Among the milks used for the manufacture of fermented products, sheep's milk is becoming increasingly attractive to consumers [3, 4]. Compared to goat and cow's milk, sheep's milk has a higher content of protein, lipids, minerals and vitamins, conjugated linolenic acid, short and medium chain fatty acids and fatty acids n-3 [4, 5], making it interesting from a nutritional and technological point of view.

Dairy drinks as fermented or non fermented are the result of mixing milk and whey, with the addition or not of other ingredients, and may be added with other types of milk, fruits, starch and sucrose, and must be exempt from heat treatment after fermentation. Some laws stipulate the dairy base must make up at least 51% of the product's total ingredients [6].

But in countries like Portugal, there is no specific legislation for the elaboration of milk drinks, only fermented milk is defined as the coagulated product obtained by fermentation due to the action of specific microorganisms on the milk, and the microbiota must be alive and abundant on the final product [7].

Whey, used as a raw material in the manufacture of dairy drinks, retains about 55% of the nutrients in milk, which is relevant, considering the volume produced and its nutritional composition [8]. According to Magalhães et al. (2011) [9], approximately 50% of the whey of different animal species produced in the cheese dairies is not used for any purpose, generating environmental damage, waste and economic losses. These factors elucidate the important to find alternatives to use whey in food products, thus avoiding inappropriate disposal and overcoming the challenge imposed on small and medium-sized industries regarding the destination of this by-product.

The objective of this research was to develop a dairy drink based on sheep's milk whey and present it as a way of using resources that would otherwise be discarded, starting to be used as raw material.

2. MATERIALS AND METHODS

The research was carried out at the Milk Science Laboratory of the Instituto Politécnico de Bragança (IPB), Portugal. A dairy drink made with milk from Lacaune sheep, supplied by a local producer, was developed, being the property characterized as small, with about 150 total sheep and 100 lactating sheep, confined due to the time of year in which the research was carried out (winter).

2.1. Feedstock

The sheep's milk was pasteurized at 72 ºC for 15 seconds according to the Regulation (CE) nº 1662/2006 [10]. Then, the sheep's milk was cooled and maintained to a temperature of 4 °C until the preparation of the milk cultures, which took place on the same day. For the formulation of the dairy beverage, sheep's milk and pasteurized sheep's milk whey obtained from the production of cheeses, sugar (7%), red fruits (3%) and dairy cultures (3%) were used. The lactic acid bacteria *Streptococcus thermophilus* e *Lactobacillus bulgaricus* were acquired from the company Chr-Hansen® (Portugal). Red fruits and sugar were obtained from the local market.

2.2. Preparation of dairy culture

For the preparation of the milk culture, from the envelope composed of lyophilized lactic acid bacteria, the lactic cultures were diluted and subcultured, according to Thamer and Penna (2006) [11], with changes.

2.3. Preparation of fermented milk drinks

Milk and whey were used to prepare three different formulations, with variations in their concentrations adding up to 100% dairy base in all cases. The amount of each ingredient and the concentrations were defined based on similar works, such as Araújo and Barbosa (2015) [12], Mendes et al. (2017) [13] and Gajo et al. (2010) [14]. Three formulations were used where the variation occurred in the proportions of whey and milk from sheep, being $FORM_1$ the formulation composed of 25% whey; $FORM_2$, 40% of whey and $FORM_3$, 60% of whey. The volumes of dairy-based ingredients add up to 100% and the other ingredients are calculated from that value.

After cooling, the whey together with the sheep's milk were transferred to glass containers and 7% sugar was added to the three formulations, and then 3% of the reactivated milk culture was inoculated. Samples were then incubated at 42 °C. Fermentation took place until the samples reached 70 ºD, a process that took an average of four hours of incubation. After this period, the samples were cooled to 5 ºC to stop the activity of lactic acid bacteria, thus avoiding excessive acidification. After cooling, the clot was broken, with subsequent addition of red fruits (3%) and, finally, storage at 4 °C.

2.4. Physicochemical analysis

After the manufacture of the fermented milk drink, the samples, as well as the milk, were submitted to physicochemical analyzes to determine the pH [15], fat content [16], protein 17] and titratable acidity [15]. Dairy beverages were evaluated in triplicate. The measurements of the pH values were performed in a digital potentiometer and the fat contents were determined by the Gerber method, as described Brazil (2018) [18]. Protein analyzes were performed based on the titration with formalin and the protein content was calculated by multiplying the value used in the titration by 1.7. Determinations of acidity contents were carried out by acid-alkalimetric titration, using phenolphthalein as an indicator [18].

2.5. Sensory evaluation

After the production of the fermented milk drink and refrigeration at 4 °C, the samples were transferred to 20 mL flasks sterilized by irradiation, duly identified with the corresponding serum concentration. The three sample fermented milks drinks was analyzed by volunteers belonging to the academic community of the Instituto Politécnico de Bragança (IPB), Portugal, who have shown interest in the product.

The sensory evaluation, by affective test, aimed to identify how much the volunteers liked the lactea drinks. The volunteers were then asked to taste the three different formulations of fermented milk drinks ($FORM_1$, $FORM_2$ e $FORM_3$) and then, express their opinion, in the acceptance test performed using a five-point structured hedonic scale, where number 5 meant "I liked it a lot"; 4-"Likes moderately"; 3-" Did not like nor disliked"; 2-" Disliked moderately" and 1-" Disliked a lot".

Then the volunteers were asked about their purchase intention using a structured 5-point scale, specified in "I would definitely buy", "I would probably buy", "I doubt I would buy it", "I would probably not buy it" and "I would certainly not buy it".

2.6. Statistical analysis

Statistical analysis was performed using SPSS Statistics software, using Analysis of Variance (ANOVA) to verify if there was a statistically significant effect ($p< 0,05$). Tukey's test was also used as a comparison test, with a 5% probability level. In addition, the Chi-Square test of independence ($p<0.05$) was also performed in the same software to verify the existence of significant differences between the sensory responses.

3. RESULTS AND DISCUSSION

3.1. Physical chemical characterization of dairy beverage

Five samples were analyzed for fat, protein, pH and titratable acidity of sheep's milk and of the three different formulations of fermented milk drink tested, with the averages of the results shown in the Table 1. The fermented dairy drinks presented average results of fat of 6.1% in the $FORM_1$, 5,0% in the $FORM_2$ and 2,0% in the $FORM_3$. The protein results were 5.3%, 4.5% and 3.6% in the $FORM_1$, $FORM_2$ and $FORM_3$, respectively. The gradual reduction in the levels of fat and protein is related to the higher content of whey and the lower content of sheep's milk added to the $FORM_2$ and $FORM_3$.

Table 1: Centesimal composition of samples of sheep's milk and dairy drinks in the three formulations tested.

ANALYSIS	**SHEEP'S MILK**	**MILK BEVERAGE FORMULATIONS**		
		$FORM_1$	**$FORM_2$**	**$FORM_3$**
Fat	6.5%	6.1%	5.0%	2.0%
Protein	6.0%	5.3%	4.5%	3.6%
pH	6.6	4.4	4.3	4.3
Titratable acidity	25°D	70°D	70°D	70°D

$FORM_1$: 25% of *sheep* whey; $FORM_2$: 40% of *sheep* whey; $FORM_3$: 60% of *sheep* whey.

For dairy drinks, it was noted a gradual reduction in the protein and lipid levels according to the increase of the concentration of whey in the product, that can be justified by the fact that the whey used in the manufacture of the dairy drink has a lower protein content compared to milk, which by dilution effect, it reduces the protein concentration in the product. The same is true for the lipid content.

Using Brazilian legislation as reference, the minimum protein content in the fermented milk drink must be 1.0% and the fat content of 2.0% [6]. Although the regulation deals with dairy drinks produced from cow's milk, the data presented in the work show that the products meet the criteria of the legislation, even in the sample with 60% whey. It is worth mentioning that, to date, there is no technical regulation in Brazil and Portugal that defines the parameters that must be followed for the production of dairy beverages fermented with sheep's milk.

The fat content observed in sheep's milk (6.5%) is close to the average values described in the literature. Gomes et al. (2017) [19] describes values similar to the present study, with an average of 6.79% of lipid content. Gajo et al. (2010) [14] identified a percentage of fat in sheep's milk of 5.6%, as well as Balthazar et al. (2016) [4], that identified 5.79% of fat in the milk of Brazilian herds with blood level up to 7/8 Lacaune x Texel. Revers (2016) [20] found fat contents in sheep milk of approximately 9% fat.

The protein content in sheep's milk of 6.0% is close to those found in the literature. Balthazar et al. (2016) [4] and Gajo et al. (2010) [14] described higher values, both 6.45% protein. Although Revers et al. (2016) [20] describes a higher fat content in his work, the same does not happen in relation to the level of proteins, presenting 5.8%, as well as Gomes et al. (2017) [19], who found an average of 5.37% of content protein in sheep's milk.

The differentiated protein content contributes to the increase in the viscosity of fermented milks, beside in the pasteurization process these proteins have their structure modified, increasing their water absorption capacity in the elaborated product [21, 14].

It is also important to note that the availability of lactose in milk causes microbial action with the production of lactic acid, increasing acidity and decreasing the availability of lactose. For this reason, titratable acidity can be used to check the degree of conservation of milk [22, 23]. The average pH value of sheep's milk identified at 6.6 is in agreement with the values described by Park et al. (2007) [24], which varied between 6.5 e 6.8. The same happens in relation to the titratable acidity (25 ºD), which is described as normal by the author, between 16 and 28 °D. Rivers (2016) [20] showed similar values for sheep’s milk, that presenting pH of 6.7 and acidity of 30 ºD. In the dairy beverage, the values of acidity (70 ºD) and pH (4.3-4.4) were similar in the three formulations evaluated, probably due to the control of these parameters during the fermentation of the product.

3.2. Affective evidence of milk drinks

Regarding the sensory evaluations, for the acceptance test it was possible to observe that the formulation $FORM_2$ showed an indication on scale 5 (I liked it a lot) more frequently, followed by $FORM_1$ and $FORM_3$ (Figure 1). The milk drink $FORM_3$ it was the sample that showed the highest frequency in scales 1 (Disliked a lot), 2 (Disliked moderately) and 3 (Did not like nor disliked) and the lowest record in scale 5 (Figure 1) The sample $FORM_1$ did not get any indication on the scales 1, 2 and 3.

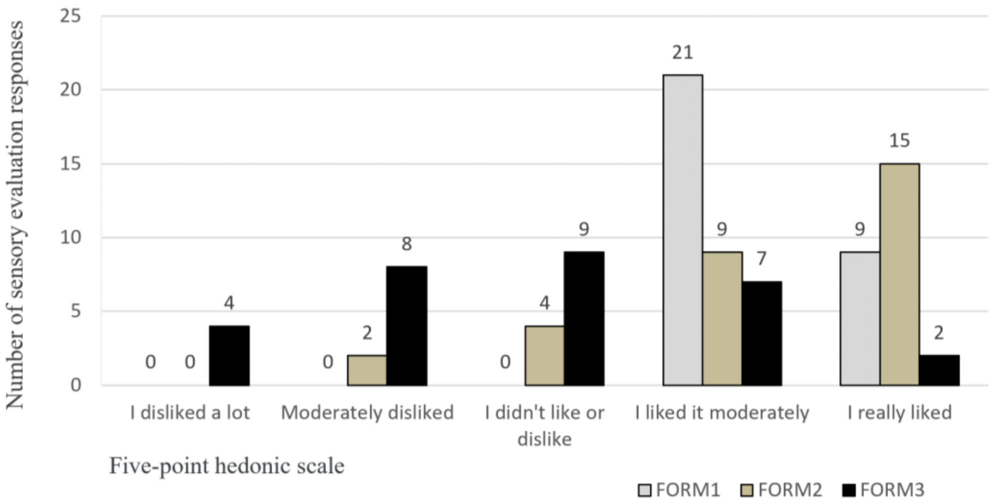

Figure 1: Acceptance of the three different samples of dairy drinks. $FORM_1$: 25% of sheep whey; $FORM_2$: 40% of sheep whey; $FORM_3$: 60% of sheep whey.

In Table 2, it is possible to verify that there is no significant difference in the acceptance of the $FORM_1$ and $FORM_2$, which allows a serum addition of up to 40%, according to the data collected.

Table 2: Median acceptance test responses performed on samples $FORM_1$, $FORM_2$ and $FORM_3$.

FORMULATION	MEDIAN
$FORM_1$	4.30^a
$FORM_2$	4.23^a
$FORM_3$	2.83^b

Different letters in the same column indicate significant differences by Chi-Square test ($p<0,05$). $FORM_1$: 25% of sheep whey; $FORM_2$: 40% of sheep whey; $FORM_3$: 60% of sheep whey.

The sample $FORM_2$ showed a higher percentage of indications on the I liked it a lot scale (50%). Only 7% (2) of the participants did not like it and 13% (4) were "Did not like nor disliked" about the acceptance of this sample. Santos et al. (2008) [17], working with sensorial acceptance of milky beverage fermented with mango pulp in concentrations of 20%, 40%, 60%, and 80% of cow's milk whey, also found satisfactory results with the formulation that contained 40% of whey, this being described as the best, followed by concentrations of 20%, 60% and 80%.

Santos et al. (2008) [17] worked with different formulations of cow's milk whey and, although they presented lower acceptability as the amount of whey increased, the author chose the formulation with 60% whey as the best one because it presents sensory acceptance equal to the best formulations and takes advantage of a large amount of whey in its elaboration. Since the sensory characteristics were statistically similar, with the exception of the formulation with 80% whey, which differed from the others, the author's choice was based on an economic and sustainable issue.

Likewise our results, Araújo and Barbosa (2015) [12] also noticed that the volunteers had greater acceptance of the product with less whey, as well as in the present study. Figure 2 represents the purchase intention for the sample chosen by the volunteer.

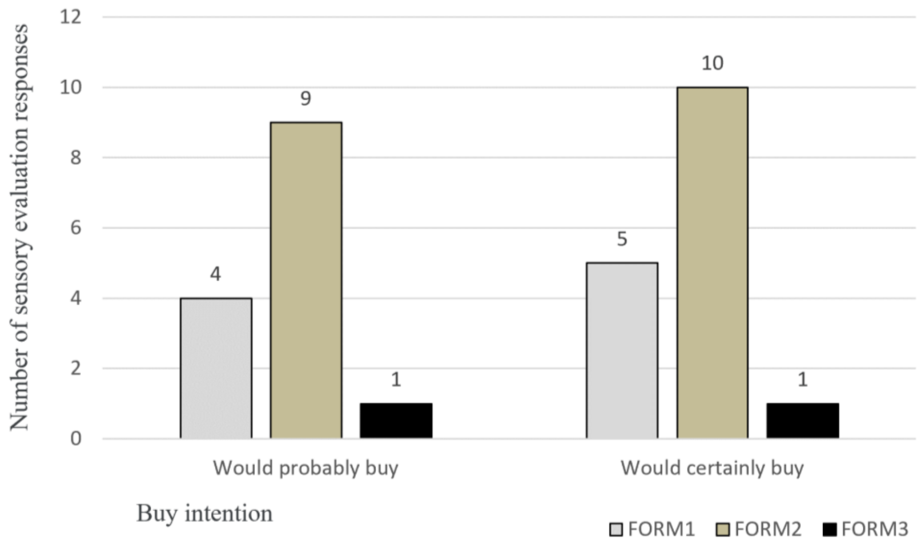

Figure 2. Purchase intention of the three different samples of dairy drinks. FORM$_1$: 25% of sheep whey; FORM$_2$: 40% of sheep whey; FORM$_3$: 60% of sheep whey.

Regardless of the sample chosen, all participants claimed to the possibility of purchasing the product if the selected sample was on sale in supermarkets, with just over half of the evaluators saying that they would certainly buy the product and the rest said that they would probably buy.

The volunteers were instructed to declare their purchase intention to the chosen sample. Therefore, it is not possible to say that the same would happen if the sample that was available for sale was different from the one chosen at the time of analysis.

In research carried out by Gajo et al. (2010) [14], purchase intention was higher for dairy drinks prepared with the addition of 35% and 45% whey compared to the drink added with 25%. Mendes et al. (2017) [13] identified that the most accepted product was the one made with a higher percentage of cow's milk whey (49%) compared to another formulation with 38.46% of whey.

4. CONCLUSION

With the result obtained in the present research, it becomes possible to apply technologies to fill a gap in the dairy segment with the development of a new product based on sheep's milk whey, in addition, of course, to offering the consumer the possibility of a product with properties that confer health and flavor to whoever comes to consume it.

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