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DEVELOPMENT AND CHARACTERIZATION OF CONCENTRATED AND FERMENTED WHEY BEVERAGES

Desenvolvimento e caracterização de bebidas concentradas e fermentadas a base de soro de leite

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ABSTRACT

The work aimed to develop and characterize the physical-chemical, microbiological, and sensory properties of concentrated and fermented beverages, made with whey. Whey, lyophilized thermophilic culture, sucrose, sodium bicarbonate, and xanthan gum were used. The treatments were: T1 (concentrated and fermented whey), T2 (concentrated and fermented whey added with 8% w/w of sucrose), T3 (concentrated and fermented whey, added with sodium bicarbonate), and T4 (concentrated and fermented whey added with 8% w/w of sucrose and sodium bicarbonate). The preparations were inoculated, incubated (45 ºC/0.75% lactic acid v/v), and added with 0.05% of xanthan gum. The beverages were subjected to physical-chemical, microbiological evaluation, and sensory acceptability. The physical-chemical compositions found for the beverages were: T1 - lactose 9.7%, lipid 2.3%, and protein 2.4%, T2 - lactose 10.6%, lipid 2.0%, and protein 2.8%, T3 - lactose 9.9%, lipid 2.0%, and protein 2.3%, and T4 - lactose 10.8%, lipid 2.1%, and protein 2.3%. Microbiological characterization indicated the most probable number (MPN) for coliforms below 3 NMP/mL in treatments. The treatments T2 and T4 did not present a significant difference between them ($p > 0.05$), but they were better accepted than the other treatments. The addition of sucrose improved the acceptance of beverages and reflected positively on the purchase intention. Beverages proved to be suitable for consumption, within the conditions evaluated.

Keywords: dairy; vacuum evaporation; new product.

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RESUMO

O trabalho teve como objetivo desenvolver e caracterizar as propriedades físico-químicas, microbiológicas e sensoriais de bebidas concentradas e fermentadas, feitas com soro de leite. Foram utilizados: soro de leite, cultura termofílica liofilizada, sacarose, bicarbonato de sódio e goma xantana. Os tratamentos foram: T1 (soro concentrado e fermentado), T2 (soro concentrado e fermentado adicionado com 8% p/p de sacarose), T3 (soro concentrado e fermentado adicionado com bicarbonato de sódio) e T4 (soro concentrado e fermentado adicionado com 8% p/p de sacarose e bicarbonato de sódio). As preparações foram inoculadas, incubadas (45 ºC/ácido lático 0,75% v/v) e adicionadas com 0,05% de goma xantana. As bebidas foram submetidas a avaliações físico-químicas, microbiológicas e de aceitabilidade sensorial. As composições físico-químicas encontradas para as bebidas foram: T1 - lactose 9,7%, gordura 2,3% e proteína 2,4%, T2 - lactose 10,6%, gordura 2,0% e proteína 2,8%, T3 - lactose 9,9%, gordura 2,0% e proteína 2,3% e T4 - lactose 10,8%, gordura 2,1% e proteína 2,3%. A caracterização microbiológica indicou o número mais provável (NMP) para coliformes abaixo de 3 NMP/mL nos tratamentos. Os tratamentos T2 e T4 não apresentaram diferença significativa entre si ($p > 0,05$), mas foram mais bem aceitos que os demais tratamentos. A adição de sacarose melhorou a aceitação das bebidas e refletiu positivamente na intenção de compra. As bebidas mostraram-se adequadas ao consumo, dentro das condições avaliadas

Palavras-chave: lácteos; evaporação a vácuo; novo produto.

INTRODUCTION

Brazil is the fourth largest producer of milk and cheese in the world, which makes it a great generator of whey. Whey is a product that has about 55% of milk nutrients, being: lactose, soluble proteins, vitamins, minerals, and fat (CARDOSO *et al*., 2018; VASCONCELOS *et al*., 2018; EC, 2020).

According to Carvalho; Carneiro (2016), in 2016 approximately 4 billion liters of whey were produced in Brazil and, although the industries already know the nutritional value and economic advantages of this food, there is still a large number of industries that incorrectly dispose of whey. The fact that this product has high nutritional value makes it a strong polluting agent of the environment since for its degradation there is a decrease in dissolved oxygen in the territory where it is released and a consequent increase in the chemical and biochemical oxygen demand (NUNES *et al*., 2018).

Concentration by vacuum evaporation (VE) is an interesting alternative for the use of whey, either as food for direct consumption or as an ingredient for food formulations. VE is a technique applied to the food industry which consists of the removal of water and has the advantages of reducing the volume facilitating transport, enhancing the flavor and mainly increasing the shelf life of the food, reducing microbial development and the activity of some enzymes (KHETNI, 2018; JORISCH, 2014).

Lactic fermentation is another process widely used in the dairy industry that consists of the conversion of lactose into lactic acid and other organic substances carried out by lactic acid bacteria. Fermentation brings several benefits to the product, increasing its conservation, nutritional values, and the

functionality of the food. In addition, this process improves sensory characteristics, simplifying the more complex nutrients, and providing greater added value (MARCO *et al.*, 2017).

In general, consumers have become increasingly demanding regard to food, while looking for healthy, attractive, innovative, safe, sustainable, and practical products (PAULA *et al.*, 2019). One of the alternatives that industries have found to meet this demand is the elaboration of beverages based on dairy products. According to the Clúster Alimentario de Galicia (2020), a considerable growth in the number of innovations in fermented milk products is expected for the coming years.

Aiming to reduce the indiscriminate disposal of whey and obtain a food with different characteristics from this co-product, this work aimed to develop and characterize the physical-chemical, microbiological, and sensory properties of a concentrated and fermented beverage made with whey.

MATHERIAL AND METHODS

The work was developed at the Federal Institute of Education, Science and Technology of the Southeast of Minas Gerais – *Campus* Rio Pomba (IF Sudeste MG – *Campus* Rio Pomba). Whey derived from the production of Minas Padrão cheese was used. Thermophilic culture, lyophilized, containing the microorganisms *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* were used. Sucrose, sodium bicarbonate, and the thickener xanthan gum (Proregi, Rio Pomba, Brazil) were added to the beverages produced.

Product development

The formulations of mixtures for the production of beverages are shown in Table 1.

The concentrated whey (18 ºBrix) was divided into four containers and subjected to acidity analysis, to define the proportion of bicarbonate to be added in the T3 and T4 treatments. Sucrose was added in treatments T2 and T4. All treatments were inoculated with the thermophilic culture and incubated at 45 ºC until the acidity reached between 75 and 80 grams of lactic acid per liter of whey. Upon reaching the desired acidity the products were subjected to refrigeration (4 ºC) and seven days later they were analyzed for their physical-chemical, microbiological, and sensory characteristics. The seven-day period was defined empirically, since the initial objective of the work was to verify the feasibility of producing the beverages and provide support for the development of future work. The use of whey is not mentioned in the current legislation for beverages in general (BRASIL, 2009). Thus, for the

Table 1 – Formulation of mixtures for the production of concentrated fermented beverages

Treatments	Whey (L)	Sucrose (g/100g)	Latic culture (g/100g)	Xanthan gum (g/100g)	Sodium Bicarbonate
T1	60	-	1.5	0.05	-
T2	60	8.0	1.5	0.05	-
T3	60	-	1.5	0.05	+
T4	60	8.0	1.5	0.05	+

* The sign (-) means absence and the sign (+) means presence.

purpose of comparison, the physical-chemical and microbiological requirements stipulated specifically for fermented dairy drinks were used as standards (BRASIL, 2005).

Physico-chemical analyzes

The lactose content of the samples was analyzed using the Lane-Eynon method (BRASIL, 2006), used for the quantification of reducing glycides, adapted to the quantification of lactose. The lipid content was analyzed using the Gerber method (ISO, 2008). The total protein content was analyzed by the Kjeldahl methodology, adopting a conversion factor of nitrogen to protein of 6.38 (HORWITZ; LATIMER, 2005).

Microbiological analyzes

Microbiological analyzes were carried out in accordance with the current legislation for fermented dairy milk beverages, using as acceptance criteria the most probable number count (MPN/mL) of coliforms at 30/35 ºC and 45 ºC (BRASIL, 2005).

Sensory analysis

For the sensory analysis, the acceptance test and the purchase intention test were applied. The acceptance test was performed using the 9 points hedonic scale which ranges from 1 (I really disliked) to 9 (I really liked it) in which the attributes of acidity, aroma, color, global impression, and flavor were analyzed. For the purchase intention analysis, a 5 points scale was used, which ranges from 1 (decidedly would not buy) to 5 (decidedly would buy).

Sixty-three evaluators were recruited following the standard NBR ISO 11136:2016 (ABNT, 2016). However, the responses of 8 of the evaluators were inconsistent and were discarded, using data from 55 in total. Evaluators were potential consumers of fermented beverages and have been selected in an entirely casual manner with ages varying from 18 to 60 years. These evaluators had predominance ages between 18 and 25 years and belonged to the academic community. The quantity of 30 mL of each sample was prepared and served randomly to the evaluators in individual booths under white light.

Statistical analysis

The experiment was carried out in three repetitions and the data were interpreted through analysis of variance (ANOVA) and the means were compared using the Tukey test at 5% probability using the Sisvar Program version 5.3 (FERREIRA, 2014).

In the statistical analysis of sensory acceptance, the forms were collected and the responses converted into scores (1 to 9). The arithmetic means of the scores obtained for each product were calculated and they were submitted to analysis of variance (ANOVA) by Randomized Block Design (DBC) and to the Tukey test to compare the means at the level of 5% probability using the Sisvar Program version 5.3 (FERREIRA, 2014).

Principal Component Analysis (PCA) was also carried out for acceptability tests in the construction of Internal Preference Maps, using the Senso Maker Program, version 1.9 of MatLab® (PINHEIRO *et al.*, 2013).

RESULTS AND DISCUSSION

Physico-chemical characteristics of concentrated and fermented beverages

According to the results expressed in Table 2, there was no difference ($p > 0.05$) between treatments in relation to the content of lactose and lipids. The current legislation for dairy beverages does not specify values for both components (BRASIL, 2005).

Ferreira *et al.* (2013) and Almeida *et al.* (2001) elaborated dairy drinks in different whey proportions and obtained average values for lactose content at least twice lower than those found in this work. The presence of lactose in foods is an interesting factor from the biological and nutritional point of view as it provides energy for consumers in addition to functioning as a substrate for the microorganisms present in lactic cultures, responsible for the production of acidity and flavor in dairy products (ROWLAND, 2017; VARELA *et al.*, 2019).

The lipid contents found in this work are above those described by Souza *et al*. (2020), Costa *et al*. (2013), and Santos *et al*. (2006), which obtained values of 1.2, 1.63, and 0.78% for lipids, respectively. These authors carried out studies with fermented dairy drinks produced based on whey but without using the concentration technique, which explains the greater proportions of this constituent in the present work.

As for protein concentration, T4 showed a similar mean ($p > 0.05$) only to T3. The values found for protein content in all treatments were above the minimum required by the current legislation for fermented dairy drinks which recommends the presence of at least 1.7 g of milk protein per 100 g of product (BRASIL, 2005). The values found in this work are slightly above those presented by Souza et al. (2020) and Thamer; Penna (2006), whose works characterized fermented dairy drinks containing a mixture of whey and milk. However, the results obtained in this work were similar to those presented by Costa *et al*. (2013), who characterized fermented dairy drinks with whey in different concentrations of stabilizers/ thickeners and found an average of 2.41% protein.

It is important to note that the treatments used in this experiment contain only whey proteins and these proteins have bioactive peptides containing a high content of essential amino acids, especially branched-chain ones, such as leucine, isoleucine, and valine which are related to factors of muscle growth, reconstruction, and repair. The soluble proteins present in milk do not participate in the coagulation process in cheese production, therefore, they are contained in the whey. These proteins are divided into different fractions, which are: β-lactoglobulin, α-lactoalbumin, bovine serum albumin (BSA), immunoglobulins, glycomacropeptides (GMP), peptone, proteases, and in a smaller amount or subfractions: lactoferrin and lactoperoxidase (LE MAUX *et al*., 2016; SOUSA *et al*., 2012).

This protein blend that forms whey proteins is responsible for all the physiological and technical or functional characteristics of this class of protein, such as anti-microbial activity against pathogenic bacteria, anti-cancer, anti-hypertensive, antioxidant,

Table 2 – Physical-chemical characteristics of concentrated fermented beverages*

Constituents (g/100g)	T1	T2	T3	T4
Lactose	9.7 ± 1.0^{a}	10.6 ± 0.6^{a}	9.9 ± 0.0^{a}	10.8 ± 0.9^{a}
Lipids	2.3 ± 1.1^{a}	2.0 ± 0.1^{a}	2.0 ± 1.1^{a}	2.1 ± 1.1^{a}
Protein	2.4 ± 0.0^{a}	2.4 ± 0.0^{a}	2.3 ± 0.2^{ab}	2.3 ± 0.0^{b}

* Average values ± standard deviation. Equal letters on the same line indicates that there was no difference by the Tukey test ($p > 0.05$); T1 – concentrated and fermented whey; T2 – concentrated whey, fermented, and added with sucrose; T3 – concentrated whey, fermented, and added with sodium bicarbonate, and T4 – concentrated whey, fermented, and added with sucrose and sodium bicarbonate.

immunostimulant, and hypocholesterolemic (BATISTA *et al*., 2018; DISSANAYAKE *et al*., 2013). In addition to the various physiological properties, whey proteins also have several technical functional properties, such as gel-forming capacity, viscosity enhancer, and emulsifying power (KEPPLER; SCHWARZ, 2017; BELLARDE, 2005).

Microbiological quality of concentrated and fermented beverages

All beverages had the most probable number (MPN) for coliforms below 3 MPN/g, therefore being within the values required by current legislation (BRASIL, 2005). These results are similar to those obtained by López *et al*. (2018) and Tebaldi *et al*. (2007), who developed milk beverages based on whey. For this last author, the absence of coliforms in dairy drinks, among other factors, is attributed to the acidity in the product, which acted in an inhibitory way on unwanted microorganisms.

The results allow us to say that the products of the present work were obtained in satisfactory hygienic-sanitary conditions, respecting the rules for good manufacturing practices (BRASIL, 2004). Along with this, it is important to highlight the good microbiological quality of the raw materials used.

In addition to the pasteurization applied to milk used in the preparation of the Minas Padrão cheese, the whey was also subjected to the binomial time/temperature of 65 ºC for 30 minutes (low temperature, long time pasteurization), during the concentration by EV, which also impacts positively on the product's microbiological characteristics (ANDRADE *et al*., 2008; VASCONCELOS; MELO FILHO, 2016).

Sensory quality of concentrated and fermented products

The results of the sensory evaluation performed with concentrated fermented beverages are shown in Table 3.

A product of yellow/green color with a tone similar to that of fluid whey, however, less translucent, was obtained from the concentration and fermentation processes. For this attribute, there was no difference ($p > 0.05$) between the four treatments developed, indicating that the addition of sugar and sodium bicarbonate to the concentrated whey did not affect the color of the product.

For aroma, there was a difference ($p < 0.05$) only between T3 and T4 showing that the addition of sucrose improved this attribute in products containing sodium bicarbonate. The odor was characteristic of fermented dairy

Table 3 – Scores of sensory evaluation of concentrated fermented beverage attributes*

Attributes	T1	T2	T3	T4
Color	7.2 ± 1.3^{a}	7.4 ± 1.3^{a}	7.1 ± 1.7^{a}	7.2 ± 1.4^{a}
Aroma	6.9 ± 1.6^{ab}	7.3 ± 1.3^{ab}	6.6 ± 1.9^{b}	7.3 ± 1.5^{a}
Flavor	5.0 ± 2.1^{b}	7.4 ± 1.7^{a}	4.4 ± 2.3^{b}	6.3 ± 2.2^{a}
Acidity	5.3 ± 2.1^{b}	7.2 ± 1.7^{a}	5.0 ± 2.3^{b}	6.7 ± 1.9^{a}
Global Impression	5.6 ± 1.9^{b}	7.4 ± 1.6^{a}	5.0 ± 2.3^{b}	6.6 ± 2.0^{a}

* Average scores ± standard deviation. Equal letters on the same line indicates that there was no difference by the Tukey test ($p > 0.05$); T1 – concentrated and fermented whey; T2 – concentrated whey, fermented, and added with sucrose; T3 – concentrated whey, fermented, and added with sodium bicarbonate, and T4 – concentrated whey, fermented, and added with sucrose and sodium bicarbonate.

products, certainly coming from metabolic products, developed during fermentation by *Lactobacillus*, and also by the presence of lactic acid, without the disappearance of the characteristic odor of whey (ABREU, 2014).

The results showed low acceptance of the T1 and T3 treatments as they obtained scores lower than 6. It is important to note that whey contains mineral salts, which can give the product an unwanted flavor and can be intensified by the process of concentration by vacuum evaporation. On the other hand, it was noticed that the addition of sucrose improved ($p < 0.05$) the acceptance of the products (T2 and T4), concerning taste, acidity, and overall impression. Sucrose is the substance most used as a sweetener for drinks, due to its unique flavor characteristics. Sweeteners have several properties when added to products, but the persistence of sweet taste, the presence of residual taste, and the degree of fruit flavor intensification depend on the characteristics of the food to which the sweetener is added (ANDRADE JÚNIOR *et al*., 2016; CAVALLINI; BOLINI, 2005).

In this work, the acceptance of concentrated fermented products added with sugar is in line with the results presented by Oliveira (2006), who obtained good acceptance in fermented dairy drinks containing different concentrations of whey.

The intention to purchase the fermented and concentrated beverages made was evaluated and the results are shown in Table 4.

According to Table 4, the products added with sucrose (T2 and T4) had a higher purchase intention ($p < 0.05$) compared to T1 and T3. However, when comparing only products containing sucrose there was no difference ($p > 0.05$) in the purchase intention. Samples added with sucrose obtained the highest acceptance for the flavor attribute. This preference may explain the greater purchase intention for these treatments. Santos *et al*. (2017) described that taste, among the main characteristics of a food, is the most determinant in choosing decision.

Internal preference maps (IPM)

It is assumed as a desirable criterion for the success of the IPM methodology that the sum of the first two main components (PC) totals a percentage of the explained variance equal to or greater than 70% (MINIM, 2013). According to Figure 1, this value was reached for all the attributes evaluated in this work.

The acidity attribute presents a higher number of evaluators in its graph preferring the no added sugar samples (T1 and T3). Three groups can be identified: the first group formed by samples T1 and T3, which was the most accepted group for this attribute by consumers, the second group for sample T2, and the third group for T4, which was less accepted. As for the aroma and color, the evaluators' preference as shown in the graph was more evenly distributed among the samples. For aroma there was the formation of three groups: a first group formed by samples T1 and T4, a second group for sample T2, and a third group for T3, these being equally

Table 4 – Scores for the purchase intention evaluation of concentrated fermented beverages*

Treatments	T1	T2	T3	T4
Purchase Intention	2.5 ± 1.2^{b}	3.9 ± 1.1^{a}	2.0 ± 1.2^{b}	3.5 ± 1.3^{a}

* Average scores ± standard deviation. Equal letters on the same line indicates that there was no difference by the Tukey test ($p > 0.05$); T1 – concentrated and fermented whey; T2 – concentrated whey, fermented, and added with sucrose; T3 – concentrated whey, fermented, and added with sodium bicarbonate, and T4 – concentrated whey, fermented, and added with sucrose and sodium bicarbonate.

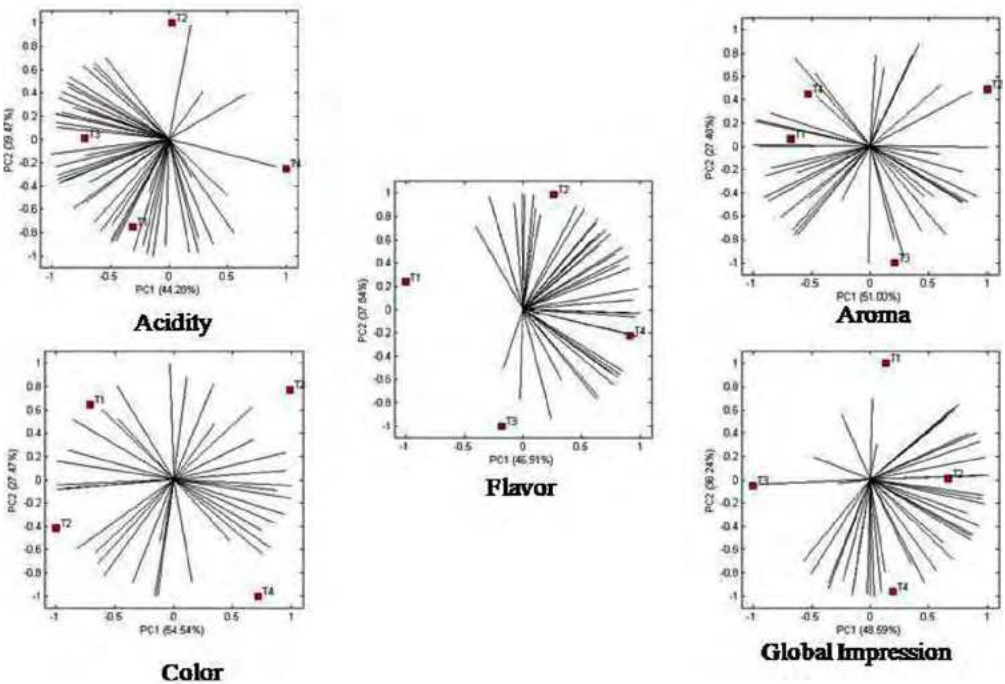

Figure 1 – Internal preference maps for the attributes: acidity, aroma, color, global impression, and flavor

accepted. As for color, there was the formation of four distinct groups, one for each sample, with no preference being observed between them.

Regarding taste and general impression, the greater preference for treatments with added sugar was more evident due to the number of evaluators, since almost all tasters are distributed between T2 and T4 samples. Thus, three groups were identified: the first group formed by samples T2 and T4, which was the group most accepted for this attribute by consumers, the second group for sample T1, and the third group for T3, which were the least accepted.

CONCLUSION

Concentrated and fermented beverages showed good nutritional and microbiological quality, being under the conditions evaluated suitable for human consumption. Sucrose improved the sensory acceptance of the beverages, and its presence positively influenced the purchase intention. This work proved to be an interesting proposal for the elaboration of beverages from the concentration and fermentation of whey and opened the opportunity for the development of other foods based on this methodology and technique.

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