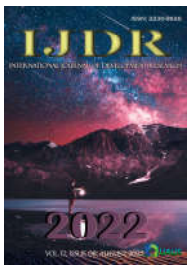
ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 12, Issue, 08, pp. 57902-57906, August, 2022
https://doi.org/10.37118/ijdr.25011.08.2022



RESEARCH ARTICLE OPEN ACCESS

DEVELOPMENT AND SENSORY ANALYSIS OF FERMENTED DAIRY BEVERAGES WITH DIFFERENT CONCENTRATIONS OF *SYZYGIUM AROMATICUM* ESSENTIAL OIL

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ARTICLE INFO

Article History:
Received 05th June, 2022
Received in revised form
28th June, 2022
Accepted 09th July, 2022
Published online 17th August, 2022

Key Words:

Food analysis,
Lactic bacteria,
Milk, Natural essences.

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ABSTRACT

The aim was to develop a dairy drink containing essential oil of *Syzygium aromaticum* as preservative, to evaluate the quality of the elaborated food and its acceptability. The dairy beverage was formulated at concentrations of 10, 20 and 30 µL mL^{-1} of essential oil, and two controls. The physical-chemical and microbiological analyzes were performed, the stability of the essential oil added to the beverage was verified by the headspace methodology. The sensorial analysis was performed by the affective methods of the Hedonic Scale test and Purchase Intention. Microbiological and physico-chemical parameters remained within the legal parameters. No influence of storage time and different concentrations of *Syzygium aromaticum* essential oil was observed. The milk beverage added to the concentration of 30 µL mL^{-1} of the essential oil of *Syzygium aromaticum* presented better results considering all the defined quality parameters. For the sensory analysis, the drinks formulated with 10 and 20 µL mL^{-1} presented better acceptance. The results showed high potential for the application of the essential oil in the dairy drink, providing the use of whey, conservation of lactic acid bacteria and future studies can elucidate functional characteristics for the product.

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Citation: ***Paula Karoline Soares Farias, Francine Souza Alves da Fonseca, Ernane Ronie Martins, Marinilza Soares Mota Sales et al.*** "Development and sensory analysis of fermented dairy beverages with different concentrations of *Syzygium aromaticum* essential oil*", International Journal of Development Research*, 12, (08), 57902-57906.

INTRODUCTION

There is a recurring concern to expand the applicability of whey in different kinds of foods, since about 50% of the whey produced in the world is not used, generating nutritional and financial waste and relevant environmental impacts (Gernigon, Schuck, Jeantet, 2010). Among the alternatives for using whey, one can include the manufacture of products such as dairy drinks (Cancino, Espina, Orellana, 2006), among others. Whey-based beverages have been highlighted as possible substitutes for yogurt, due to the lower market

price and because they have similar sensory and physicochemical characteristics to yogurts, being well accepted by many consumers of different age groups (Andrade *et al*., 2015). In recent years, there has been a growing consumer interest in functional ingredients from natural sources (Miranda *et al*., 2016). Among these natural sources, essential oils stand out due to their long history of use as antimicrobial agents with industrial applications, such as inhibiting the growth of various microorganisms and for presenting a low risk of side effects (Siroli *et al*., 2015). Traditional use of clove essential oil (*Syzygium aromaticum*) is widely accepted due to functional properties such as antioxidant, antibacterial, anti-inflammatory, antifungal capacity (Yang, Wei, Hong, 2014). In this sense, clove essential oil could make food safety viable, since the incorporation of this substance would promote the improvement of microbiological safety, in addition to providing functional properties. The objective was to develop a whey-based beverage containing clove essential oil as a preservative, to evaluate the quality of the product and its acceptability.

MATERIALS AND METHODS

Development of the whey-based beverage: the production of the fermented milk drink was carried out according to the methodology proposed by EPAMIG (2010), with modifications at the Institute of Agricultural Sciences of the Federal University of Minas Gerais. They were formulated at concentrations of 10, 20 and 30 µL/mL of *Syzygium aromaticum* essential oil, a control group was added with the chemical preservative (potassium sorbate) and a dairy drink without preservative and chemical additive. The fermented dairy drinks were stored under refrigeration (5°C) for 28 days. The entire experiment was performed in triplicate (Farias *et al*., 2022). The microbiological analyzes of the fermented milk drinks were carried out according to the recommendations described by the Normative Instruction n° 62 of the Ministry of Agriculture, Livestock and Supply (Brasil, 2003), evaluating the count of lactic acid bacteria (ISO 7889, 2003), presence of *Salmonella* sp. (ISO 6785, 2001), and enumeration of total and thermotolerant coliforms (ISO 4831, 2006; APHA, 2015). Physical-chemical and microbiological tests were carried out, syneresis index and presence of essential oil added to the drinks on the 1st, 7th, 14th, 21st and 28th days after being manufactured, represented with the numbers 1, 2, 3, 4 and 5 in the graphs and tables. The analyzes were performed in a randomized block design (RBD) with three replicates for each oil concentration and control.

Physicochemical analyses: the physicochemical analyzes of the fermented milk drink were carried out in accordance with the Normative Instruction No. 68 of the Ministry of Agriculture, Livestock and Supply (Brasil, 2006). The samples were collected at random and homogenized prior to the determination of pH and total titratable acidity (% lactic acid). The pH was measured in triplicate using a digital pH meter and titratable acidity by titration. The analysis of the protein and fat contents was based on the Kjeldahl and Gerber methods, respectively. The syneresis index was determined using the method described by Gerhardt *et al*. (2013).

Headspace Analysis: the headspace analysis was adapted from the methodology carried out by Aguiar *et al*. (2014). The headspace flasks (20 mL) containing the product (1mL) were transferred to the autosampler (HS combi-PAL) where they were homogenized (500 rpm), incubated (75°C, 5 min) and the volatile substances extracted by the static headspace method. The identification of the compounds was performed by comparing the mass spectrum with that of the NIST library (2.0, 2009) and compared with information from the literature (Adams, 2012).

Sensory evaluation: the sensory evaluation of the elaborate fermented dairy drinks was performed according to the methodology proposed by the Instituto Adolfo Lutz (2008), with modifications, in which the consumers' acceptance and willingness to pay and purchase intention were assessed according to the hedonic scale. The study was approved and supported by the Institute of Agricultural Sciences of the Federal University of Minas Gerais – ICA/UFMG and conducted within the ethical standards established in the Resolution No. 466/2012 of the National Health Council on research with human beings under the Technical Opinion document No. 1,598. 542.

Statistical analysis of results: all statistical analyzes were performed using the R software. For Most Likely Number of total coliforms, thermo tolerant coliforms and lactic acid bacteria counts. To compare the results obtained between the beverages with different oil concentrations and the control group prepared with potassium sorbate, the Dunnett test was used with the significance level (α) set at 0.05. In the sensory analysis, to compare the results obtained between the drinks, the Tukey test and the residual normality test (Shapiro-Wilk) were carried out in the R statistical software.

RESULTS AND DISCUSSION

Microbiological analysis, Quality assessment of fermented milk drink and Physicochemical analysis: the results obtained from the microbiological analyzes (Table 1) carried out for treatments throughout the storage period indicate that the products are fit for consumption, in terms of the recommended quality standards for fermented milk beverages (Brasil, 2005). The concentration of 30 µL/mL of essential oil was the one that presented the most satisfactory results during the entire storage period for the total coliform group, with values similar or lower than the control, except on the 7th day of storage. In relation to the group of thermotolerant Coliforms, it was observed that on the 7th day of evaluation the counts were similar to the ones found at 20 µL/mL essential oil concentration. *Salmonella* sp was absent in all samples and at different analysis times. When analyzing the study of the influence of oil concentrations and the day of evaluation on the count of total coliforms and thermotolerant coliforms, it was observed that at the end of the storage time (28 days), the desirable parameters of conservation of the sanitary quality of the beverages by the oil were maintained. The results obtained allow us to conclude that the essential oil under study showed a preservative effect on groups of indicator microorganisms with similar results and or higher than the activity of the chemical preservative potassium sorbate. These results are important and promising, as they indicate that the organic matter present in the food matrix of dairy beverages did not interfere with the antimicrobial and preservative activity of *Syzygium aromaticum* essential oil, especially eugenol, which is the major compound (Xie *et al*., 2015) with proven antibacterial properties (Hassan, Ibrahim, Raghad, 2014). Some factors can cause a decrease in the viability of lactic acid bacteria, such as the amount of dissolved oxygen, packaging permeability during refrigerated storage, and the toxicity of substances released by bacteria (Ojansivu, Ferreira, Iminen, 2011), which did not occur in this study, even in the presence of essential oil.

Syneresis analyzes ranged from 8 to 26% during the 28 day-evaluation (Table 1). These values are in accordance with Aportela-Palacios, Sosa-Morales and Vélez-Ruiz (2005), who suggest that ideal results should be below 39%. The pH values in this paper ranged from 4.55 to 4.06 (Table 1). Current legislation recommends that fermented dairy products must have a maximum acidity of 6.74, thus the product added with essential oil is within the recommended range (Brasil, 2007). In the study by Erkaya *et al*. (2015) the samples presented a variation from 4.32 to 4.10 and Dias *et al*. (2013) the pH values differed between 4.73 to 4.39 during the 21 days that the research was carried out. The viability of lactic acid bacteria was maintained in this study, indicating that the pH values did not interfere with the quality of the lactic drinks tested. The samples showed titratable acidity values ranging from 0.66 to 0.95 (Table 1) in accordance with the current legislation, which establishes acidity between 0.6 and 2.0g of lactic acid/100g (Brasil, 2007). Içier *et al*. (2015) verified a decrease in the values of titratable acidity on the 21st day of storage. According to the authors, the titratable acidity value of the fermented beverage is reduced with prolonged storage, as the higher solids content and the clotting time can influence the decrease in titratable acidity (Thamer, Penna, 2006).

Table 1. Results obtained from microbiological and physicochemical analyzes of fermented dairy drinks containing *Syzygium aromaticum* essential oil and fermented dairy drinks containing potassium sorbate stored for different periods of times under refrigeration

Concentration of EO*	Total coliforms	Thermotolerant Coliforms	Lactic acid bacteria	Syneresis	pH	Acidity	Protein	Fat
1st day of evaluation								
10	4.0414a	4.0414	10.3979	15.3333	4.3733a	0.7038a	2.2096a	1.2667a
20	3.5492a	1.5299	8.945a	14.6667	4.5267	0.8018	2.4861	1.5
30	3.0182	1.5035	8.69a	14.3333	4.5367	0.8464	2.3415	1.4a
Control	3.789a	2.2998a	9.3942a	18.3333a	4.3633a	0.6919a	2.065a	1.3333a
7th day of evaluation								
10	3.8484	2.2291	10.3979	18.3333	4.21	0.7127	2.4882	1.2333a
20	2.6414	1.6086a	7.737a	18.000	4.2433	0.7988	2.3649	1.4667
30	2.6272	1.5035a	7.5927a	15.3333	4.27	0.8968	2.2777a	1.4
Control	1.889a	1.5035a	7.5474a	21.3333a	4.3767a	0.6682a	2.1437a	1.2667a
14th day of evaluation								
10	3.2579	2.0228	9.3979a	22.6667a	4.1633a	0.7127	2.4223a	1.2
20	2.2271	1.5299a	9.0375a	19.	4.1167	0.8493	2.3627a	1.3667a
30	2.0504a	1.5299a	8.3865	17.6667	4.1867a	0.8761	2.2862a	1.3a
Control	1.6921a	1.5035a	9.3979a	23.3333a	4.2a	0.6682a	2.116a	1.3a
21st day of evaluation								
10	2.3566	1.9067a	9.0487	23.6667a	4.1033	0.7216a	2.5648	1.1333
20	1.4771a	1.4771a	7.9994a	21.3333a	4.1133	0.8018	2.4712	1.3a
30	1.4771a	1.4771a	7.9725a	21.	4.1	0.8968	2.4159	1.3a
Control	1.4771a	1.4771a	8.076a	23.6667a	4.2033a	0.7157a	2.0884a	1.3333a
28th day of evaluation								
10	3.4645	1.8617a	9.6546	25.3333a	4.1767	0.7127a	2.6286	1.0333
20	1.4771a	1.4771a	8.2158a	22.6667	4.1633	0.7899	2.3138a	1.1333a
30	1.4771a	1.4771a	7.6931a	22.6667	4.1567	0.8285	2.3032a	1.0667a
Control	1.4771a	1.4771a	7.8465a	24.3333a	4.27a	0.6771a	2.1352a	1.1333a

Equal letters represent similar responses according to Dunnet test at 5% significance level. Treatments* 10: 10 µL/mL of *Syzygium aromaticum* essential oil; 20: 20 µL/mL of *Syzygium aromaticum* essential oil; 30: 30 µL/mL of *Syzygium aromaticum* essential oil; control: chemical additive potassium sorbate.

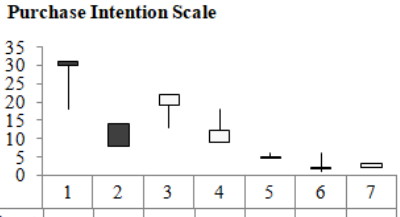
Table 2. Presentation of general data (gender and age), male and female mean values for the Purchase Intention Scale without distinction of sex or age, for the different concentrations tested

Concentration of EO *	Mean Values	Mean Values For Females	Mean Values For Males
S1	5.493976a	5.333333a	6.0
S2	5.421687a	5.301587a	5.8
S3	5.353659a	5.241935a	5.8
S4	4.804878b	4.483871b	5.7

Residual normality test (Shapiro-Wilk). Treatments* S1: 10 µL/mL of *Syzygium aromaticum* essential oil; S2: 20 µL/mL of *Syzygium aromaticum* essential oil; S3: 30 µL/mL of *Syzygium aromaticum* essential oil; S4: chemical preservative potassium sorbate.

Headspace analysis: eugenol is the main compound of the essential oil *Syzygium aromaticum* (Xie *et al*., 2015), and in the fermented milk beverage it was confirmed by the static headspace analysis and it remained in the fermented milk beverage during the 28 days of manufacturing, even after the formulation process without changing the chemical composition of the analyzed products.

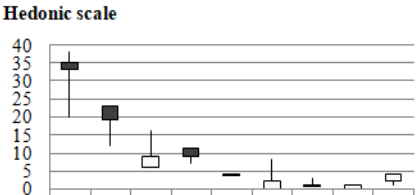
Sensory evaluation: regarding the acceptability test, 83 untrained participants performed the test in the present study, respondents consist of university students and employees of the public educational institution. There was a prevalence of females (76%), and about 60% of respondents were aged between 19 – 27 years old.



	1	2	3	4	5	6	7
Bebida Láctea Sabor A	31	14	19	9	5	2	2
Bebida Láctea Sabor B	30	14	13	16	6	1	2
Bebida Láctea Sabor C	18	12	19	18	6	6	3
Bebida Láctea Sabor D	30	8	22	12	5	2	3

A – Flavored fermented dairy drink A; B – Flavored fermented dairy drink B, C – Flavored fermented dairy drink C; D – Flavored fermented dairy drink D. Caption: 1 – I would always consume; 2 – I would very often consume; 3 – I would frquently consume; 4 – I would occasionally consume; 5 – I would rarely consume; 6 I would very rarely consume; 7 – I would never consume. Flavored fermented dairy drink A - 10 µL/mL of *Syzygium aromaticum* essential oil. Flavored fermented dairy drink B - 20 µL/mL of *Syzygium aromaticum* essential oil. Flavored fermented dairy drink C - 30 µL/mL of *Syzygium aromaticum* essential oil. Flavored fermented dairy drink D – With the chemical additive.

Graph 2. Purchase Intention Scale for fermented dairy beverages added with *Syzygium aromaticum* essential oil and fermented dairy beverages added with potassium sorbate.



	1	2	3	4	5	6	7	8	9
Bebida Láctea Sabor A	35	23	6	11	4	0	1	0	2
Bebida Láctea Sabor B	38	12	12	9	4	3	3	0	1
Bebida Láctea Sabor C	20	22	16	7	4	8	2	1	2
Bebida Láctea Sabor D	33	19	9	9	4	2	1	1	4

A – Flavored fermented dairy drink A; B – Flavored fermented dairy drink B, C – Flavored fermented dairy drink C; D – Flavored fermented dairy drink D. Caption: 1 - Like extremely; 2 - Like very much; 3 - Like moderately; 4 – Like slightly; 5 – Neither like nor dislike; 6 – Dislike slightly; 7 – Dislike moderately; 8 – Dislike very much; 9 – Dislike extremely. Fermented dairy drink A - 10 µL/mL of *Syzygium aromaticum* essential oil. Fermented dairy drink B - 20 µL/mL of *Syzygium aromaticum* essential oil. Fermented dairy drink C - 30 µL/mL of *Syzygium aromaticum* essential oil. Fermented dairy drink – With chemical preservative potassium sorbate.

Graph 3. Hedonic scale for fermented dairy drinks added with *Syzygium aromaticum* essential oil and control preferences.

Regarding Purchase Intention Scale of the fermented dairy drinks produced, it was observed that the concentrations of 10 and 20 µL/mL were the most preferred, with 31 (37.3%) and 30 (36.1%) participants respectively, followed by the dairy beverage containing preservatives, with 30 (36.1%) participants, while the fermented milk beverage with 30 µL/mL was preferred by 18 (21.7%) participants (Graph 1). In the graph 2 a fermented dairy drink B – 20 µL/mL of *Syzygium aromaticum* essential oil was the most preferred. Sensory evaluation revealed statistically significant differences when we take into consideration age, sex and concentrations of 10, 20 and 30 µL/mL of essential oil of *Syzygium aromaticum* and the Tukey range test at 5% probability level was performed, in which treatment S3 (30 µL/mL of essential oil of *Syzygium aromaticum*) differed statistically from all other concentrations tested, for the general average and also among females. For the male purchase intentions scale, this variable was not statistically different according to the analysis of variance 5% significance level (Table 2).

Da Silva *et al*. (2015) found an average equal to 4.5±1.06 in the evaluation of the purchase intention of the fermented dairy beverage added with blueberry juice processing residue, which corresponds to the interval between "would occasionally consume" and "would frequently consume", in the hedonic scale, reinforcing the viability of the product in the consumer market. According to the Hedonic Scale, it is observed that the participants' acceptance of the elaborated product, it can be seen that the flavored fermented milk drinks A, B and D obtained the best acceptance in the item "Liked extremely", followed in the same sequence by "Liked moderately". The flavored fermented milk drink C was the least accepted, as shown in graph 3. Graph 3. Hedonic scale for fermented dairy drinks added with *Syzygium aromaticum* essential oil and control preferences. These good results obtained from the fermented milk drinks tests are related to the changes in eating habits. Consumers are showing concern regarding the purchase of foods without so many additives or chemical preservatives, and the food industry tries to adapt to offer better alternatives for these demanding consumers (Siroli *et al*., 2015).

In the sensory evaluation of the fermented dairy beverage added with blueberry juice processing residue. Da Silva *et al*. (2015) found acceptance averages between 5 and 6 in all beverage attributes with 30% blueberry residue, which correspond, on the hedonic scale, to the range between "like slightly" and "liked moderately". The acceptance rates indicate that the product was accepted, since these values were ≥ 70%. In the present work, the fermented dairy drinks with concentrations of 10 and 20 µL/mL of *Syzygium aromaticum* essential oil obtained the greatest acceptance among the participants, with the options of "like extremely" and "I would consume it often", higher than the concentration of 20 µl/ml.

CONCLUSION

According to the results, it is possible to produce fermented dairy drinks with different concentrations of the essential oil of S. aromaticum, since the production of a food in which there is no addition of preservatives and chemical additives is suitable for a demanding clientele that seeks natural products which offer the benefit of preserving lactic acid bacteria and also be classified as a functional product.

ACKNOWLEDGMENT

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Brasil (CAPES) - Finance Code 001, Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Ministério da Educação/ Secretaria de Educação Superior (MEC/SESu) - EDITAL PROEXT 2015 and Pro- Reitoria de Pesquisa da Universidade Federal de Minas Gerais (PRPq-UFMG), for supporting our research.

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