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The addition of *Syzygium aromaticum* essential oil preserves the microbiological and physicochemical quality of the fermented milk beverage during storage

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Abstract *— The addition of essential oils (EO) as a natural preservative in dairy products and food matrices has been an alternative to synthetic preservatives. Clove EO (Syzygium aromaticum L.) is a potential possibility because it has therapeutic functions, antimicrobial and antioxidant activity, as well as bioactive compounds. In this study,*

***Keywords* — Clove, Natural Preservative, Dairy Products, Headspace, Eugenol.**

concentrations of S. aromaticum EO were added to a fermented milk drink and the effect on the conservation of the microbiological and physicochemical quality of the product during storage was evaluated. Headspace analysis identified four bioactive chemical compounds from the EO of S. aromaticum in the fermented milk drink, namely eugenol, β-Caryophyllene, α-Humelene and copaene. The microbiological and physicochemical stability parameters of the fermented milk beverage were maintained during storage, indicating that the EO of S. aromaticum contributed to the maintenance of the quality of the manufactured product. In conclusion, the addition of S. aromaticum EO at concentrations of 10, 20 and 30 µL/mL in fermented milk drink helped to preserve the quality of the product in storage. In addition, the milk matrix does not interfere with the presence of bioactive chemical compounds in the oils.

I. INTRODUCTION

In recent years, the general population has been concerned about consuming healthier foods, which favor physical well-being and prevent the risk of diseases [1]. As a consequence, there was a reduction in the intake of processed foods, especially due to the presence of potentially carcinogenic preservatives [2]. In this sense, replacing the use of synthetic preservatives with "natural" components makes essential oils a potential alternative [3,4].

Essential oils (EO), known as volatile or ethereal oils, are volatile, hydrophobic and rarely colored aromatic compounds, originating from the secondary metabolism of plants [2,5]. According to Worwood (2016) [6], EO have several therapeutic functions such as anti-inflammatory, antiseptic, tonic, antispasmodic and diuretic activity. In addition, studies report antimicrobial effects in controlling pathogenic microorganisms [7-9].

Due to their medicinal characteristics, EO have been used as a natural preservative in dairy products and other food matrices [10-12]. Clove (*Syzygium aromaticum* L.) is one of the most valuable spices since antiquity, being widely used in traditional foods and medicines around the world [13]. The *S. aromaticum* EO is extracted from the dried flower bud of the plant and has several activities, demonstrated so far, such as anti-inflammatory, antimicrobial, antibacterial, antiviral [14], antioxidant and antifungal [15], due to the presence of bioactive chemical compounds such as eugenol and other phenolics [2].

Although there are several studies that report the use of EO as a preservative in dairy products and stored foods, so far, there are no scientific reports on the use of EO from *S. aromaticum* in the conservation of fermented milk drinks and, therefore, studies in the area should be carried out. In view of the above, the objective of this study was to evaluate the effect of the addition of EO of *S. aromaticum* on the conservation of the microbiological and physicochemical stability of fermented dairy beverage during storage.

II. MATERIALS AND METHODS

2.1 Materials

Whey and UHT milk were purchased from the local market. For the fermentation of the dairy beverage, a culture Direct Vat Set (DVS) containing lyophilized mixed strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp bulgaricus (YoFlex Harmony 1.0 –C.H, Horsholm, Denmark) was used. The EO of *S. aromaticum* was provided by Ferquima (Vargem Paulista, SP, Brazil), as adopted by Farias et al. (2019) [16]. The other ingredients used in the formulation were purchased from a local establishment. All reagents used in this study were of analytical grade.

2.2 Production of fermented milk drink added with *S. aromaticum* EO

Four formulations were developed according to Figueiredo et al. (2019) [17], with modifications. The developed formulations contained concentrations of 10, 20 and 30 µL/mL of *S. aromaticum* EO and a control formulation added with the preservative potassium sorbate.

The drinks made consisted of 44.5% UHT whole milk, 44.5% reconstituted whey (15%), 10% sucrose and 1% modified starch. This first mixture was heat treated (65 °C for 30 minutes), with subsequent cooling (43 °C) and inoculation of 0.1% of thermophilic DVS lactic culture, containing mixed strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus, in the form of lyophilized granules. Then, the mixture was incubated at 43 °C in a BOD oven (model EL202/4E) until the moment it reached pH 4.60 and clot formation, with subsequent cooling at 5 °C for 5 hours.

Then, the clot was agitated and *S. aromaticum* EO was added, with subsequent homogenization. For the control formulation, 0.003 g/mL of the preservative potassium sorbate was added. Beverages were packaged in one-liter hermetically sealed plastic packages, labeled and stored under refrigeration (5 °C) until analysis.

Stability of the fermented milk drink during storage evaluate the stability of dairy beverages, microbiological and physical-chemical tests were carried out during a 28-day storage period. The analyzes were performed using a randomized block design (RBD), with three replications for the concentration of essential oil formulations and one for the control formulation.

2.3 Microbiological analyzes

The microbiological analyzes were performed according to the methods described by APHA (2015) [18]. The presence of total and thermotolerant coliforms was determined using the series of 3 multiple test tubes, and the results were expressed as the most probable number (MPN) per mL. The viable lactic acid bacteria count was assessed by MRS agar plate counting and incubated in anaerobic conditions at 37 °C for 72 hours. Results were expressed in colony-forming units (CFU) per mL.

2.4 Physicochemical analysis

The physicochemical analyzes were performed according to the methods adopted by AOAC (2000) [19]. The determination of pH was carried out at room temperature (25 ± 2 ºC) using a digital pH meter (model LUCA-210). For acidity, 10 mL of sample was diluted in 10 mL of distilled water and titrated with NaOH solution (0.1M). Results were expressed in g lactic acid/100g sample.

The analysis of protein and fat contents was based on Kjeldahl and Gerber methods, respectively [19]. The syneresis index was determined using the method described by Amaya-Llano et al. (2008) [20].

2.5 Headspace analysis

Product headspace analysis was performed according to Aguiar et al. (2014) [21], with modifications. The headspace flasks (20 mL) containing the product (1 mL) were transferred to the autosampler (HS combi-PAL) where they were homogenized (500 rpm), incubated (75 °C, 5 minutes) and the volatile substances extracted by static headspace. The injection volume was defined (1000 µL) and the syringe was preheated (75 °C).

Agilent Technologies (7890A) system coupled to a mass spectrometer (MS 5975C) equipped with a DB-5 MS fused silica capillary column (30 m x 0.25 mm x 0.25 µm) and helium (flow 1 mL min-1) as carrier gas was used for the identification analysis of volatile chemical compounds. The temperature was programmed from 60 ºC to 240 ºC, with an increment of 3 ºC min-1. The system was operated in scan mode (monitoring) with electronic impact at 70 eV, in a range from 45 to 550 (m/z).

The generated data were analyzed using the MSD Chemstation software together with the NIST library (National Institute of Standards and Technology). The relative abundance (%) of the total ions referring to the compounds was calculated from the peak area of the chromatogram (GC) and organized according to the elution order. The percentage of each component was calculated from the normalized mean of the chromatogram area. The identification of compounds was performed by comparing the mass spectrum with that of the NIST library (2.0, 2009) and compared with information from the literature [22].

2.6 Statistical analysis

Statistical analyzes were performed using the R software. For the microbiological results of total coliforms, thermotolerant coliforms and lactic acid bacteria counts, these were transformed into counts for Log X + 1, and beta regression was used, in the link function (logit). For the comparison of means, the Dunnett test was used at a 5% significance level.

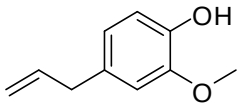
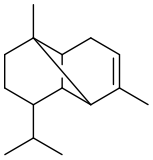
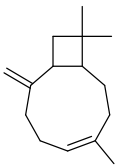
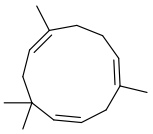
To study the effects of oil concentrations in dairy beverages, the multiple regression methodology was used. In this analysis, the statistical model was considered: $zi = a + bxi + cxi^2 + dyi + fyi^2 + gxiyi + hxiyi^2 + ei$. To estimate the regression coefficients, the least squares method was used, with the help of the lm function of the R software.

For the selection of the best fitted models, the Akaike information criterion (AIC) was considered, in which models with lower estimates are preferable. From the regression models, the values predicted by the predict function were obtained and from these values the response surface graphs were created using the SigmaPlot V11 software.

III. RESULTS

Headspace analysis revealed the presence of four chemical compounds in the fermented milk beverage added with *S. aromaticum* EO during storage for 28 days (Table 1). These results show that the milky matrix of the beverage did not interfere with the presence of bioactive compounds in the oil.

Table 1. HS-GC-MS analysis of the dairy beverage after 1, 14 and 28 days of preparation with the addition of clove essential oil (Syzygium aromaticum).

N°	[a]RT	Compounds	[b]Structure	[e]MF	Characteristic ions (m/z)	Samples detected
1	37,0	Eugenol		$C_{10}H_{12}O_2$	164 (M^+100), 149(36), 137(20), 133(19), 131(19), 104(20), 103(32), 91(27), 77(30), 55(12)	Clove essential oil (control), all analyzed
2	37,6	Copaene		$C_{15}H_{24}$	204(M^+ 24), 161(100), 119(91), 105(89), 93(43), 92(23), 91(39), 81(22), 77(18), 55(12)	Clove essential oil (control), all analyzed
3	39,2	β-Caryophyllene		$C_{15}H_{24}$	204(M^+20), 189(43), 175(15), 121(35), 120(46), 119(50), 109(20), 107(53), 106(35), 105(72)	Clove essential oil (control), all analyzed
4	40,3	α-Humelene		$C_{15}H_{24}$	204(M^+10), 147(20), 122(9), 121(32), 119(12),109(13), 107(20), 105(15), 94(14), 93(100)	Clove essential oil (control), all analyzed

[a]Retention time in minutes; [b]Structure; [e]MF: Molecular formulae. Information obtained from the NITS 2.0 library.

According to Lambert et al. (2020) [23] the main constituent of the EO of *S. aromaticum* is eugenol corresponding to about 61% of the chemical composition. In addition, β-Caryophyllene and α-Humelene compounds are found in smaller amounts of 19.95% and 2.87%, respectively. Chaieb et al. (2007) [24] detected 36 EO compounds from *S. aromaticum*, in which eugenol (88.58%) traces of copaene were found in higher concentration and quantities (<0.01%). Other studies also describe eugenol as the most common bioactive compound in the EO of *S. aromaticum* [2,13].

The effects of *S. aromaticum* EO on the microbiological indicators of the fermented dairy beverage are represented in Figure 1. According to these results, the minimum quality requirements of dairy beverage for total coliforms (Figure 1A) and thermotolerant coliforms (Figure 1B) were maintained, as recommended by Brasil (2005) [25]. The lactic acid bacteria count (Figure 1C) remained above the values of 106 CFU/g, indicating that the oil concentrations did not interfere with the viability of these microorganisms.

Figueiredo et al. (2019) [17], evaluating fermented dairy beverages with pulp from cerrado fruits, found viable lactic cell values greater than 106 CFU/mL at 21 days of storage. According to Kechagia et al. (2013) [26] the minimum concentration of 106 CFU/g of viable cells is generally accepted for probiotic products. In this sense, the results show that the dairy beverage with EO of *S. aromaticam* is a potential probiotic product.

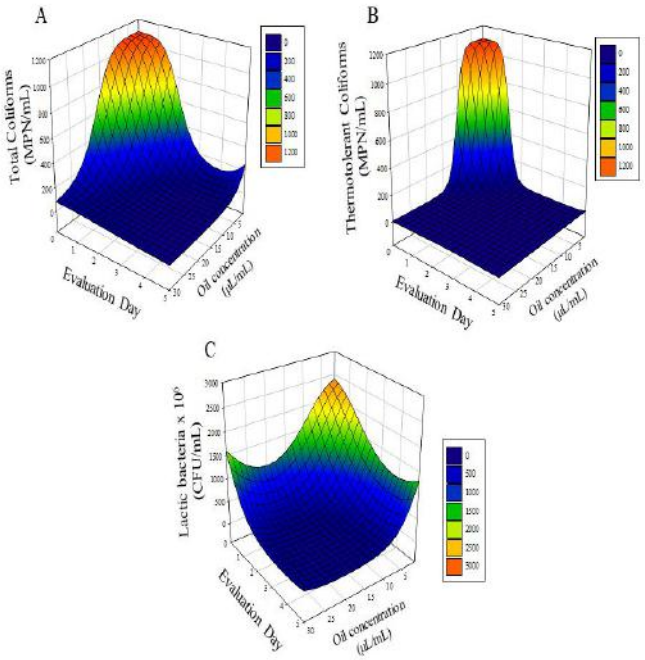


Fig.1. Effect of S. aromaticum EO concentration on microbiological parameters of fermented milk beverage. Total coliforms (MPN/mL) (A), Thermotolerant coliforms (MPN/mL) (B) and Lactic acid bacteria (CFU/mL) (C). Evaluation day: 1= 1st day of storage; 2= 7th day of storage; 3= 14th day of storage; 4= 21st day of storage; 5= 28th day of storage.

Regarding the effect of *S. aromaticum* EO on the physicochemical parameters of the fermented milk beverage, the results are shown in Figure 2. The syneresis values (Figure 2A) ranged from 8 to 26% during storage. Syneresis is an important factor in consumer acceptance of the product. This phenomenon is characterized by the contraction of the gel network resulting in decreased viscosity and gel strength [27].

A value of 25.33% of syneresis in a fermented dairy beverage with cajá-manga pulp was found at 14 days of storage [28]. According to Aportela-Palacios et al. (2005) [29] ideal syneresis values should be below 39%. Taking this into account, the syneresis results (Figure 2A), of the present study, show that the addition of EO from *S. aromaticum* does not cause a high increase for this parameter in the dairy beverage, thus being within the ideal values defined by Aportela-Palacios et al. (2005) [29].

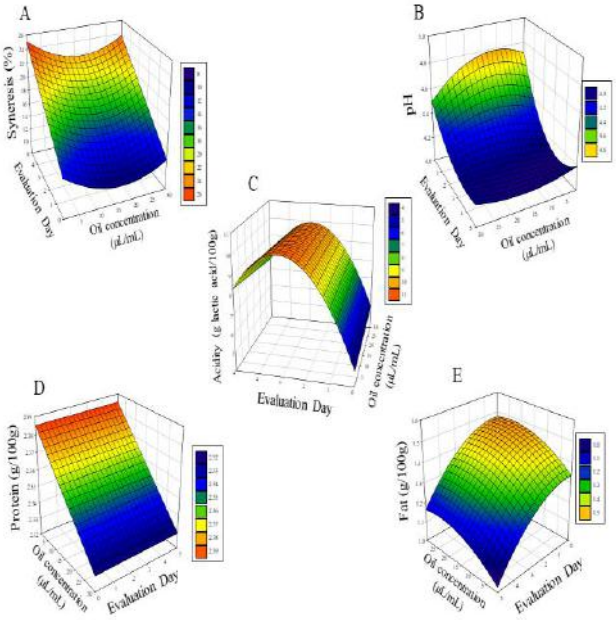


Fig.2. Effect of S. aromaticum EO concentration on the physicochemical parameters of the fermented milk beverage. Syneresis Index (%) (A), pH (B), Acidity (g lactic acid/100g) (C), Protein (g/100g) (D) and Fat (g/100g) (E). Evaluation day: 1= 1st day of storage; 2= 7th day of storage; 3= 14th day of storage; 4= 21st day of storage; 5= 28th day of storage.

The pH values (Figure 2B) ranged from 4.06 to 4.55, being within the appropriate pH range (3.6 to 4.6) for post-processing condition of lactic acid bacteria [30]. Regarding acidity (Figure 2C) the values ranged from 0.66 to 0.95 g of lactic acid/100g. Souza et al. (2020) [28], studying the physicochemical stability of a dairy beverage fermented with cajá-manga pulp, observed pH values ranging from 4.18 to 4.31 and acidity between 0.81 to 0.80%, during storage for 14 days.

The protein values (Figure 2D) observed for dairy drinks were within the acceptable range established by Brasil (2005) [25], which recommends minimum values of 1.7 g/100g of protein for dairy drinks. Dias et al. (2013) [31], evaluating a symbiotic fermented dairy beverage, found a protein value of 1.86 g/100mL of the product. According to Day (2016) [32] proteins are important macronutrients and make up around half of the dry weight of the human body. Accordingly, Cho and Jones (2019) [33] reinforce that proteins are an essential nutrient for the development and maintenance of human beings, in addition, they are considered an alternative in the development excellent food matrices.

Regarding the fats (Figure 2E) the levels varied between 1.0 and 1.5 g/100g of the product. However, fats are nutrients that cannot always be considered as a

negative attribute in food products. According to Luca (2019) [34], fats are important macronutrients, being responsible for providing 35% of total caloric intake, especially in the form of triacylglycerols (TGs). In addition, they provide calories, essential fatty acids (EFA) and contribute to the palatability of foods.

IV. CONCLUSION

The addition of *S. aromaticum* EO at concentrations of 10, 20 and 30 µL/mL in fermented milk drinks helped to preserve the microbiological and physicochemical quality of the product during storage. In addition, the milk matrix does not interfere with the presence of bioactive chemical compounds in the essential oils. However, it is essential to investigate higher EO dosages of *S. aromaticum* in dairy beverages. In addition, sensory tests must be carried out to verify the acceptance and influence of the addition of *S. aromaticum* EO on the flavor of the products.

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