Emirates Journal of Food and Agriculture. 2021. 33(4): 328-341
doi: 10.9755/ejfa.2021.v33.i4.2687
http://www.ejfa.me/

RESEARCH ARTICLE

Influence of extracts of different Feijoa (*Acca sellowiana*) leaf genotypes on the lactic fermentation performed by a mixed culture

Luniele Beilke[1], Eduarda Heck Sumny[1], Liziane Schittler Moroni[1], Cassandro Vidal Talamini do Amarante[2], Aniela Pinto Kempka[1*]

[1]Santa Catarina State University – UDESC, Department of Food Engineering and Chemical Engineering. Pinhalzinho, SC, Brazil. lunielebeilke@gmail.com, sumnyduda@gmail.com, liziane.schittler@udesc.br, aniela.kempka@udesc.br.
[1]Santa Catarina State University – UDESC, Department of Agronomy. Lages , SC, Brazil. cassandro.amarante@udesc.br

ABSTRACT

The objective of this research was to evaluate the influence of the addition of aqueous extracts of Feijoa leaf genotypes (*Acca Selowiana*) (Access 2316, Alcântara, Helena, Mattos and Nonante) on the lactic fermentation performed by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*, in MRS broth and milk. The total content of phenolic compounds (TPC), the antioxidant activity (AA) and the antimicrobial activity were determined. For the samples fermented in MRS broth, with and without the presence of extracts, the parameters analyzed were the kinetics of cell growth, TPC, AA, pH, and bacterial microscopy. For the samples fermented in whole milk, the parameters analyzed were pH, titratable acidity, moisture, ash, protein, total solids, and sensory profile. There was inhibition of the mixed culture's growth in the dilution of 80% of the extracts (TPC 75.82 - 110.90 mg EAG g^{-1}), the other experiments having been performed with TPC below 70 mg EAG g^{-1}. The kinetics of growth was 16 hours. For the fermented samples, there were changes to the TPC and antioxidant activity, except for the one fermented with the Access 2316 extract, for which activity remained the same. The microscopy showed predominance of *Streptococcus thermophilus*. In the fermented milks, pH decreased, and acidity increased, and the sensory profile was modified by the addition of the extracts. The addition of Feijoa leaf extracts in fermentation enriches the fermented product with phenolic compounds and antioxidant activity and can be a viable alternative for the provision of bioactive compounds.

Keywords: *Streptococcus thermophilus*; *Lactobacillus delbrueckii* subsp. *Bulgaricus*; growth; fermented milk; phenolic compounds.

INTRODUCTION

Feijoa [*Acca sellowiana* (O. Berg) Burret or *Feijoa sellowiana* Berg] belongs to the *Myrtaceae* family, which comprises 130 genera and approximately 4,000 species, being the largest family of the *Myrtales* order (Souza and Lorenzi, 2005). Popularly known as Feijoa, *goiabeira-do-mato*, *goiabeira-da-serra*, pineapple guava, *guayabo verde* or *guayabo del país* (Weston, 2010; Mokhtari et al., 2018), it is native to the southern Brazilian plateau and the northeast region of Uruguay (Amarante and Santos, 2011). In Brazil, the commercial production of Feijoa is negligible, and its fruits are not widely known. The largest producers are Colombia, Russia, New Zealand, and the United States (Moretto et al., 2014).

In Brazil, four new cultivars of Feijoa were released in 2007 and 2008, by the EPAGRI of São Joaquim, Santa Catarina, named Alcântara, Helena, Mattos and Nonante (Ducroquet et al., 2008). Alcântara was obtained from the selection of seedlings originated from the collection of seeds, Helena and Nonante derive from genetic crossing, and Mattos is a wild genotype's clone (Ducroquet et al., 2008; Santos et al., 2017). In addition to these four genotypes, other accesses present in the Active Germplasm Bank (EPAGRI) have also been studied, such as Access 2316, which has been included in several Brazilian studies for its potential to be released as cultivar (Amarante et al., 2017). The release of these genotypes promotes greater availability of fruits to be harvested throughout the year, providing a more regular supply of Feijoa (Ducroquet et al., 2008).

The Mattos genotype has large fruits (100 and 150 g) and medium-sized, oblong-shaped leaves, with their inner part colored silver-green. The Nonante genotype has good

***Corresponding author:**
Dr. Aniela Pinto Kempka, Department of Food Engineering and Chemical Engineering, Fernando de Noronha Street, BR 282, Km 573,7, Pinhalzinho, SC, Brazil, Zip Code 89870-000. Phone: +55 49 20499594. **E-mail:** aniela.kempka@udesc.br

Received: 28 August 2020; **Accepted:** 12 March 2021

production stability due to the self-compatibility of its flowers; its medium-sized fruits (90g) have a favorable appearance, in addition to a sweet-acidulous taste, fruitier than that of the others, and its leaves have the same coloring of the Mattos cultivar on the underside, but a more oval shape and smaller size. The Alcântara genotype has leaves of shape and size like those of Nonante, however, with a whitish color on the underside, and fruits (50 and 120 g) with early maturation. The Helena genotype stands out because of its fruits (150 g), which have a soft skin, and its leaves are also larger and have a shape that is like Nonante's (Ducroquet et al., 2008).

In general, the fruits are like common guava (*Psidium guajava*), but their taste and aroma is sweet-acidulous, and their weight can vary between 20 and 250 g. They are low in calories and high in fiber, minerals, and vitamin C (approximately 9 mg) (Ducroquet and Hickel, 1991; Weston, 2010). The skin of mature fruits is green. Feijoa's pulp can be consumed *in natura* or used in the production of wines, juices, ice creams, jams, breads, sweets, chocolates, yogurt, among others (Isobe et al., 2003; Sun-Waterhouse et al., 2013).

It has been shown that this plant is capable of promoting benefits to human health because it also has secondary metabolites such as tannins, terpenes and phenolic compounds, present in its flowers, leaves and fruit, with antidiabetic, antioxidant and antimicrobial potential, among other nutraceutical properties (Romero-Rodriguez et al., 1994; Amarante and Santos, 2011; Amarante et al., 2013; Manabe and Isobe, 2005; Beyhan et al., 2010; Belous et al., 2014; Monforte et al., 2014).

Feijoa leaves are used by the population in the form of infusion, to treat digestive problems, associated with severe diarrhea, gastrointestinal and respiratory disorders, and ulcers. These and other properties, already known by common sense, have been proven in several studies (Ojewole, 2005, OH et al., 2005; Beyhan et al., 2010; El-Shenawy et al., 2008; Mosbah et al., 2018). In relation to its mineral and vitamin content, the leaves' composition may be affected by factors associated with their environment, genetics, management, and with the soil's fertilization. The leaves' macronutrient (Ca, Mg, K, and P) and micronutrient (Fe, Mn, Zn, Cu) levels are higher than those of the fruits' skin and pulp. The vitamin E content of the Feijoa leaves' methanolic extract corresponds to 33 mg g^{-1} of dry matter (α and β-tocopherol) (Ruberto and Trigali, 2004; Souza, 2015). The total content of phenolic compounds also varies depending on genotype, extraction method and time of year, among other factors. Various phenolic compounds have been reported, including α-tocopherol, flavones, stigmasterol and β-carotene, an inseparable mixture of tyrosol esters of lignoceric, cerotic and montanic acids, and a new galactolipid identified as (2S) 1, 2, 6 '-tri-O-[(9Z, 12Z, 15, Z)-octadec-9, 12,15-trienoil]-3-O-βD-galactopyranosyl glycerol; also, gallic acid, catechin, quercetin, rutin, apigenin and ferric acid, ferulic acid, and ellagic acid (Aoyama et al., 2018; Mousavi et al., 2018; Poodi et al., 2018; Ruberto and Tringali, 2004). As for the presence of volatile compounds, Mosbah et al. (2018) found as main volatile compounds of ethanolic extracts that were rota-evaporated and subsequently resuspended in water, limonene (36.2%), β-caryophyllene and (27.8%), aromadendrene (12.5%) and α-copaene (6.6%).

The leaves also have antioxidant, analgesic, anti-inflammatory, antiulcer, hepatoprotective (Ebrahimzadeh et al., 2008, El-Shenawy et al., 2008), antimicrobial (Gram-positive and Gram-negative) and antifungal activity. The extracts can inhibit key enzymes related to diabetes (α-glucosidase and α-amylase) (Mosbah et al., 2018). Mosbah et al. (2018) evaluated the leave extracts' toxicity at 200, 500 and 2000 mg kg-1 concentrations. They did not detect toxic effects of these extracts in albino Wistar and Swiss rats, and skin abnormalities, changes in behavior, salivation, tremors, diarrhea, sleep, coma, or death were not detected, demonstrating the potential for application of these extracts in foods such as fermented dairy products.

In lactic fermentation, lactic acid bacteria (LAB) metabolize sugars and other compounds and produce lactic acid. The LAB comprises 11 genera of bacteria, of which *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus* and *Streptococcus* stand out (Poffo and Silva, 2011). They are bacteria Generally Recognized as Safe (GRAS), and have been used for a long time in fermented products with plant and animal origins (meat and dairy products). Fermentation promotes changes in the foods' nutritional profile, develops their sensory and rheological characteristics (Settanni and Moschetti, 2010; Filannino et al., 2018) and ensures greater conservation due to the ability of these bacteria to produce compounds with bactericidal or bacteriostatic action (Heredia-Castro et al., 2017). *Streptococcus thermophillus* and *L. delbrueckii subsp. bulgaricus* are the main starter cultures, recognized worldwide to produce yoghurt, being typically used as mixed cultures for developing in symbiosis (Sun-Waterhouse et al., 2013).

S. thermophillus is characterized as Gram-positive cocci, thermophilic and homofermenters (lactic acid is the only product formed) of lactose and glucose. It quickly starts the production of lactic acid up to a concentration of approximately 1.1%, and produces formic acid and carbon dioxide (Bergy, 1974; Walstra et al., 2006). Its compounds are used by *L. delbrueckii ssp. bulgaricus*, a rod-shaped Gram-positive bacterium that is thermophilic and

heterofermenter (produces lactic acid, carbon dioxide, acetic acid and alcohol) of lactose.

The fermented milks are known for their ability to provide improvements in the intestinal microbiota and its stabilization after the use of antibiotics, control of pathogenic microorganisms due to the production of acetic acid and lactic acid, improvements in the immune system and, constipation relief (Saad, 2006). Other benefits are related to the antimicrobial, antioxidant, antihypertensive and immunomodulatory activity of the bioactive peptides, released in the process of fermentation by the proteolytic activity of casein and other proteins (Parvez et al., 2006; Torino et al., 2013).

Studies associating lactic fermentation with the development of new products, with addition of bioactive compounds, have been carried out over the years, being of interest for the food, chemical and pharmaceutical industries (Martins et al., 2011; López et al., 2013; Chavan et al., 2018). These include the assessment of the influence of the addition of leaves or fruits and their plant extracts, rich in polyphenols, in liquid or solid-state fermentation, on the ability of microbial growth, on the changes in the content of phenolic compounds, on antioxidant activity and on the formation of new compounds via fermentation and their nutraceutical action (Martins et al., 2011; Torino et al., 2013; López et al., 2013; Limón et al., 2015; Kaprasob et al., 2017; Pacheco-Ordaz et al., 2018; Chavan et al., 2018). Some examples include fermented food products supplemented with extracts that are rich in phenolic compounds and natural antioxidants originated from mulberry leaves (Kemsawasd and Chaikham, 2018), pomegranate juice (Valero-Cases et al., 2017), hazelnut (Bertolino et al., 2014), lentil (Agil et al., 2013), pistachio (Mandalari et al., 2013), stevia leaves (Vital et al., 2015) and green tea (Lamothe et al., 2014).

Despite the wide variety of works that propose the addition of phenolic compounds in products fermented by lactic acid bacteria, there are yet no works in the literature using Feijoa leaves for this purpose. Therefore, the objective of this research was to verify the influence of the addition of aqueous extracts of different Feijoa genotypes in the lactic fermentation of De Man, Rogosa and Sharpe (MRS) broth, and subsequently, in the fermentation of whole milk, to produce a fermented milk derivative containing phenolic compounds that can contribute to human health.

MATERIAL AND METHODS

Collection and selection of the Feijoa leaves

The Access 2316, Alcântara, Helena, Mattos and Nonante Feijoa leaves were collected in March 2018, from the Active Germplasm Bank (BAG) of EPAGRI, Experimental Station of São Joaquim-SC (28° 16' 40, 02" S latitude, 49° 56' 09,10" W longitude, and 1.400 m height). The collection was held in the middle third of the internal and external branches, equally in all four sides of the plants. The leaves were placed in labeled plastic bags and transported in a Styrofoam box containing ice blocks. The leaves that had injuries or defects were discarded. The other leaves were washed with distilled water, frozen (Thermo Fisher Scientific, 910, USA) at -86° C, lyophilized (Ilshin Lab. Co. Ltd., TFD5503, Korea) at 50 mtorr and -60±1°C for 48 h, macerated with the aid of liquid nitrogen in mortar and pistil, and stored in plastic containers at -83° C, protected from light.

Obtaining the extracts

The leaves' extracts were obtained using the methodology adapted from Larrauri et al. (1997). Firstly, 0.5 g of the leaves and 50 mL of distilled water were mixed, and this mixture was subjected to extraction in an ultrasound bath (Quimis, Q335D, Brazil) for 60 min, at a 50/60 Hz frequency and with 135Watts RMS ultrasonic power. The extracts were filtered on qualitative filter paper ('Whatman' No. 40) and stored in Falcon tubes, protected from light and frozen (Indrel, IULT 335 D, Brazil) at -86° C, until the time of analysis.

Total Phenolic Compounds (TPC)

The TPC was determined in the aqueous extracts and over the time of the fermentations in the MRS broth containing the extracts, using the colorimetric method with Folin-Ciocalteau, according to Bonoli et al. (2004), with modifications. Then, 0.5 mL of extract or fermented medium were diluted in 9.5 mL of distilled water. Of this mixture, 0.1 mL of the extract were removed, and 500 mL of Folin-Ciocalteau and 6 mL of distilled water were added. The solution was stirred for 1 min, and then 2 mL of the Na2CO3 solution at 20% were added. The mixture was stirred for 30 seconds and left to rest for 2 h. The readings were held in a spectrophotometer (Hach, DR3900, Germany) at 720 nm. The analyses were performed in triplicate. The TPC was expressed as equivalent gallic acid milligram per gram of dry weight (mg EAG g^{-1}DM). Gallic acid was used as standard and the curve was built in concentrations ranging from 10 to 100 mg mL^{-1}.

Antioxidant activity

The antioxidant activity against the DPPH radical was determined in the aqueous extracts and over the time of the fermentation of the MRS broth containing the extracts. The methodology of Brand-Williams et al., (1995), based on the samples' capacity of scavenging the 1.1-diphenyl-2-picrylhydrazyl radical, was adopted, with modifications. In a dark environment, test tubes containing 0.3 mL of the

extract at different concentrations (0.01 to 0.09 mL) and 2.7 mL of the DPPH radical (40 μg mL^{-1} of methanol) were used. The readings were held in a spectrophotometer (Hach, DR3900, Germany) with 515 nm absorbance after 1 h. The mixture of water (2.7 mL) and concentrations of the samples (extracts and fermented media) (0.3 mL) was used as blank. The antioxidant activity was presented in IC_{50} (μ g mL^{-1}), defined as the concentration needed to eliminate 50% of the DPPH present in the test solution.

Reactivation and propagation of the dairy culture

For the fermentation tests, a mixed culture of *Streptococcus Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Delvo YOG VV 231 1 U, Globalfood) was used. The Molico skimmed milk powder (Nestlé) was reconstituted according to the manufacturer's recommendations, and sterilized in autoclave (Phoenix Luferco, analog AV, Brazil) at 121°C for 15 min. After cooling to a temperature below 30°C, 10 mL of milk were inoculated with 20 mg of the freeze-dried culture and kept in an oven at 37° C for 24 h, to obtain the reactivated pre-inoculum. Then, 1 mL of the reactivated pre-inoculum was added to 100 mL of MRS broth, previously sterilized at 121°C for 15 min and put again in an oven at 37° C for 24 more hours, obtaining the inoculum. The inoculum was kept in a fridge (Electrolux, DFW35, Sweden) at a cooling temperature (below 10°C and above 2°C) for no more than 10 days, until the time of analysis (Kempka et al., 2008).

Antimicrobial activity

The extracts were assessed at 100%, 90%, 80%, 70%, 60%, 50% and 40% concentrations in relation to their antimicrobial activity against the mixed culture's bacteria, based on the methodologies proposed by Ostrosky et al. (2008) and Pacheco-Ordáz et al. (2018). Antimicrobial activity was defined as the lowest extract concentration (TPC) that inhibited the visible growth of bacteria (purple color).

Microbial growth curves in the MRS broths with and without addition of the extracts

To obtain the microbial growth curve as well as the time of fermentation, the kinetics of the culture's growth in MRS broth with and without the addition of the leaf extracts was performed, considering the results of antimicrobial activity. For the MRS broth containing the extracts, a 1:5 (extract/MRS) ratio of each extract was used, totaling 150 mL sterile fermentation medium (extract + MRS). A ratio of 1 mL of inoculum for each 100 mL of the fermentation medium was used in all experiments. After adding and mixing the components (fermentation medium and inoculum), the vials were incubated at 37° C for 24 h. For each sample removed every 2 h for 24 h, optical density (O.D.) was determined with a spectrophotometer at 650 nm (Kempka et al., 2008).

Lactic fermentation in MRS broth with and without addition of the extracts

The lactic fermentation in MRS broth without (control) and with addition of the leaf extracts was performed while taking into consideration the results of antimicrobial activity, the microbial growth curves and the TPC present in the extracts. The addition of the extracts was held to obtain a TPC between 50 -70 mg EAG g^{-1} DM, MRS broth, previously sterilized at 121°C for 15 min, having been used to increase the volume up to 250 mL. The inoculum ratios and incubation conditions were the same as those previously described, with removal of the samples every 4 h, for 16 h. For each removed sample, pH, mesophilic bacteria count and TPC were determined. For the control sample, the soluble protein content was also determined. The antioxidant activity against the DPPH radical was evaluated at the start and at the end of fermentation.

Scanning electron microscopy (SEM)

To verify the morphology of the lactic bacteria, grown in the MRS broths (containing the extracts or not), SEM was performed. The lyophilized samples were fixed on a conductive adhesive surface and covered with gold in a sputter coater (SCD 050, Baltech, Brazil). A Field Emission Scanning Electron Microscopy (FESEM) (JSM6701F, JEOL, Brazil), equipped with a system of microanalysis via x-ray spectrometry (SEM), was then used. The analyses were performed with 15 kV electron acceleration voltage and analyzed in SEM based on images formed by secondary electrons.

Lactic fermentation in whole milk with and without addition of the extracts

The fermentation of whole milk, without (control) and with addition of the leaf extracts, was carried out under the same conditions of fermentation adopted for the MRS broth. The samples were removed at times 0 h and 16, and had their pH, titratable acidity, count of mesophilic microorganisms, total solids, total proteins (using the Kjeldahl method), moisture and total ashes determined.

Flavor profiles with the use of an electronic tongue

The flavor profiles of the extracts and fermented milks with and without addition of the extracts were evaluated by an electronic tongue (Alpha MOS ASTREE II, USA) equipped with a set of 7 sensors for sour, metallic, salty, umami, spicy, sweet, and sour flavors, a reference silver/silver chloride (Ag/AgCl) electrode, and an auto-sampler with 16 positions for 150 mL beakers. The measure consists in the potentiometric difference between each sensor and the reference electrode in a state of equilibrium at room temperature. The sensors were conditioned and calibrated in a standard 0.01 mol L^{-1} hydrochloric acid solution. The

sensory profiles were subsequently assessed using 10 g of sample, to which distilled water was added until obtaining a 100 mL volume. Of this total, 80 mL were added in beakers, and submitted to analysis. The data acquisition and analysis times were 120 s and 180 s, respectively. The sensors were cleaned with deionized water between determinations.

Physical-chemical and microbiological determinations

The pH values were determined using a digital potentiometer. The soluble protein content of the fermented MRS broth was estimated according to Lowry's methodology (Macedo et al., 2005), based on a standard curve obtained with different concentrations of egg albumin (mg albumin mL^{-1}). The titratable acidity was determined at the end of the fermentation and expressed in lactic acid percentage (AOAC, 2016). The fermented milk samples' moisture content was determined by drying them at 105° C, until reaching constant weight (AOAC, 2000). The ashes were determined by incinerating the samples in a muffle furnace at 550 ºC, until reaching constant weight and a greyish color (AOAC, 2000). The total protein content was determined according to the Kjeldahl method (AOAC, 2016). Plate count agar was used to determine the total number of mesophilic microorganisms (Brazil, 2003) and the results having been expressed in Log CFU mL^{-1}.

Statistical analysis

The results were expressed as mean and standard deviation. The analyses of ash, moisture and acidity were carried out in duplicate, the sensory analysis was carried out in quadruplicate, and the others in triplicate. The significance ($p<0.05$) was obtained with the ANOVA and Tukey tests, and using the Statistica® 13.3 software.

RESULTS AND DISCUSSION

Total phenolic content and antioxidant activity of the aqueous Feijoa leaf extracts

The results obtained for the extracts' TPC and antioxidant activity are shown in Table 1.

The TPC showed statistically significant difference ($p<0.05$), with the highest content having been obtained by the Nonante leaves, and the lowest, by the Alcântara leaves. Benincá (2014), using solutions containing methanol and acetone for the extraction of these same genotypes, also found differences in the TPC, the highest concentrations having been obtained by Nonante and Access 2316 (9.10 and 9.09 mg EAG g^{-1} DM). The results found by Ebrahimzadeh et al. (2008) for aqueous and methanolic extracts of Feijoa leaves were 92.09 mg EAG g^{-1} and 44.17 mg EAG g^{-1}, respectively. Mosbah et al. (2018) evaluated the TPC of ethanolic extracts of Feijoa leaves from Tunisia and obtained 179.43 mg EAG g^{-1} of powdered extract. Amarante et al. (2017) evaluated the TPC of hydroalcoholic and aqueous extracts of Feijoa pulp and skin using the same genotypes evaluated in this study and verified that those that showed the highest TPC levels were Mattos and Nonante for the skin, and Nonante and Access 2316 for the pulp. For the present study, considering that factors such as soil, climate, collection, and preparation conditions were the same, the different values observed between genotypes are probably due to genetic differences (Amarante et al., 2017). The differences found in relation to other studies can also be justified by the way of extracting the compounds. Ultrasound, which was the extraction method used in this study, contributes to the material's hydration and to the cell wall's disruption by forming bubbles and cavitation, causing the transfer of mass and greater ease of removal of the intracellular bioactive compounds (Moraes et al., 2009; Barba et al., 2016; Poodi et al., 2018).

Table 1: Total phenolic compounds and antioxidant activity of the leaf extracts from Feijoa.

Feijoa leaf Extract	Total phenolic compounds (mg EAG g^{-1} DM)	DPPH (IC_{50} µg mL^{-1})
Access 2316	122.32 ± 2.23^{b}	0.106 ± 0.001^{c}
Alcântara	94.77 ± 2.19^{d}	0.133 ± 0.002^{a}
Helena	107.58 ± 0.74^{c}	0.108 ± 0.001^{bc}
Mattos	109.51 ± 1.22^{c}	0.112 ± 0.003^{b}
Nonante	138.63 ± 1.82^{a}	0.109 ± 0.002^{bc}

Means followed by different lowercase letters, in the same column, differ statistically by the Tukey test ($p<0.05$).

The phenolic compounds have one or more hydroxyl-containing aromatic rings and are generally categorized as phenolic acids, flavonoids, anthocyanins, and tannins (Masisi et al., 2016). As for microbial growth, the phenolic compounds can behave as activators or inhibitors of bacteria, depending on the bacterial concentration, the cell wall's structure, the dose tested and the chemical structure of these compounds (Rozès et al., 2003; Taylor, 2013). According to Kemperman et al. (2010), phenolic compounds can bind themselves to the bacterial cell membrane and interact with lipids and proteins, changing the membrane's permeability and interfering with the detection of the *quorum sensing*, which is the mechanism of communication between bacteria, based on the production and diffusion of small chemical or signaling molecules through bacterial membranes (Yin et al., 2015).

Pacheco-Ordáz et al. (2018) found that the phenolic compounds can selectively inhibit pathogenic bacteria without affecting the viability of lactic bacteria. In this research, the commercial phenolic compounds of catechin, gallic, protocatechuic, vanillic and ferulic acid, when evaluated together and in isolation, showed antimicrobial action against pathogenic *Salmonella Thyphimurium* and *E. coli* 0157:H7 bacteria at concentrations ranging between 15 and

30 mmol L^{-1}. For catechin only, concentrations greater than 35 mmol L^{-1} were required. However, these compounds did not inhibit the growth of *Lactobacillus rhamnosus* and *Lactobacillus acidophilus*. The combination of catechin and protocatechuic or vanillic acid in the MRS broth without dextrose discreetly allowed the growth of both probiotics.

Like for the TPC, the Ancântara leaf extract showed the lowest antioxidant activity, statistically differing ($p<0.05$) from the others. Extracts Access 2316, Nonante and Helena, which were statistically equal ($p>0.05$), showed the highest antioxidant activity (a lower IC_{50} value corresponds to greater antioxidant activity). Similarly, extracts Access 2316 and Nonante showed the highest TPC levels. This result may be related to the phenolic compounds present in the extracts, which can grant antioxidant properties (Mittler et al., 2011).

The phenolic compounds' antioxidant properties are related to the action of these compounds as reducing agents of reactive oxygen species (ROS), such as superoxide anion (O2 •-), hydroxyl (•OH), the peroxyl radical (ROO•) and hydrogen peroxide (H_2O_2), as well as other compounds such as nitrogen, carbon-centered radicals, and sulfur. The presence of these compounds may cause disturbances at the cellular level when reacting with proteins, lipids, carbohydrates, and nucleic acids, damaging health. Antioxidants are molecules that can slow or preventing oxidation, stopping chain reactions by removing the free radical intermediates and inhibiting the occurrence of other oxidation reactions (Mittler et al., 2011).

Mosbah et al. (2018) also presented important results for antioxidant activity in ethanolic Feijoa leaf extracts from Tunisia, based on other methodologies and on the inhibition properties of the key enzymes of diabetes (α-glucosidase and α-amylase), as well as their antimicrobial and antifungal activity. It should be noted that the authors also found that the Feijoa leaf extracts have no toxicity at 200, 500 and 2000 mg kg^{-1} concentrations when ingested by albino Wistar and Swiss rats, demonstrating the possibility of further studies on their application in food and pharmaceutical products.

Antimicrobial activity of the extracts against the lactic culture of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus

The antimicrobial activity test showed that starting from 80%, the dilutions of the extracts inhibited the lactic bacteria, so there is no need for the extracts to be diluted until obtaining a TPC lower than 80% of the total present in them. The 80% dilution corresponded to 97.67 mg EAG g^{-1} DM, 75.82 mg EAG g^{-1} DM, 86.06 mg EAG g^{-1} DM, 87.61 mg EAG g^{-1} DM and 110.90 mg EAG g^{-1} DM TPC, for Access 2316, Alcântara, Helena, Mattos and Nonante, respectively. In general, the phenolic compounds' antimicrobial action depends on the structure of the bacteria's cell wall. Gram-positive bacteria typically have higher resistance than Gram-negative bacteria to phenolic compounds (Lou et al., 2012), as is the case of *Streptococcus thermophilus* e *Lactobacillus delbrueckii* subsp. *Bulgaricus*. It should be noted that, for the cultures here evaluated, there are no records in the literature of antimicrobial activity tests using Feijoa leaf extracts.

According to Mosbah et al. (2018), Feijoa leaf extracts have phenolic compounds in the form of flavonoids, flavonols, tannins and phenolic acids in their composition, and, volatile compounds with antimicrobial properties, such as limonene and β-caryophyllene. The extracts' antimicrobial action may be related to the type and quantity of bioactive compounds, and it may be triggered by these compounds' synergistic effect, seeing as isoflavones and terpenoids, among others, are able to bind themselves to the bacterial cell membrane and interact with lipids and proteins, changing the membrane's permeability, breaking it down and binding themselves to the DNA, thus inhibiting cellular function and preventing bacterial growth (Kemperman et al., 2010; Lou et al., 2012). In addition, these compounds may interfere with the detection of the *quorum sensing*, which is the mechanism of communication between bacteria, based on the production and diffusion of small chemical or signaling molecules through bacterial membranes, with the purpose of regulating a diverse set of biological activities (Yin et al., 2015).

Therefore, a 50-70 mg EAG g^{-1} DM concentration range was established for the next MRS broth fermentations, inferior to the antimicrobial activity shown against the lactic bacteria, so as to add the maximum possible TPC content without preventing microbial growth.

Growth curves of the lactic culture over time in MRS medium with and without addition of the extracts

The lactic culture's growth curves are shown in Fig. 1.

The concentration of phenolic compounds, added through the extracts to the fermentation broth, did not inhibit the mixed culture's growth over time. The adaptation stages lasted 6 h for the control and for the fermented media containing Access 2316, Alcântara and Mattos. After these periods, the fermented media underwent the stages of acceleration, exponential and deceleration, until hour 16, where they entered the stage of stability. As the leaf extracts have different phenolic compounds due to genetic variations (Amarante et al., 2017), these may interfere with the composition of the media and consequently with the fermentation process. The initial differences in optical density (time zero) occurred due to the extracts' staining, which browned the media when compared to the Control

experiment. The increase in optical density, due to microbial growth, estimated by the difference between the mean values of the absorbance at time zero and at hour 16, was greater for the Control experiment (1.088), followed by the MRS broths fermented in the presence of the Alcântara (0.961), Helena (0.913), Nonante (0.651), Mattos (0.553) and Access 2216 leaf extracts. Although microbial growth was not inhibited, interference of the compounds present in the extracts on the bacteria's growth was detected.

A similar behavior was shown by Pacheco-Ordáz et al. (2018), who evaluated the addition of different phenolic compounds (catechin and gallic, ferulic, vanillica, and protocatechuic acids, added in isolation) in the fermentation of MRS broth. The authors mention that the phenolic compounds' chemical characteristics may interfere with the lactic culture's growth. Strains *L. acidophilus* and *L. ramnosus* showed better adaptation to the gallic, protocatechuic, vanillic acids and catechin, than to ferulic acid; in addition, catechin and gallic acid reduced the lag stage, improving the microorganisms' growth rate. When simultaneously adding two phenolic compounds, they noted an increase in the lag stage's time, but with an optical density that was like that of the end of fermentation.

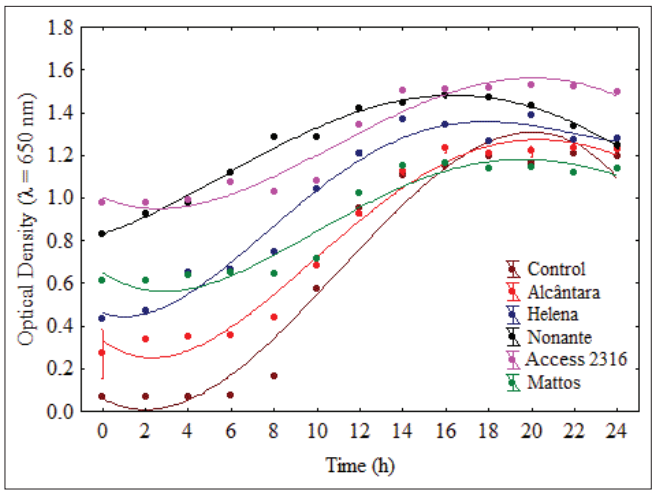

Fig 1. Microbial growth curves of the mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, in MRS broth without and with the addition of the aqueous extracts of Feijoa leaves.

Kempka et al. (2008) verified results like those of the Control sample in the fermentation of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus* e *Bifidobacterium* in MRS broth, at 36°C, for which the highest optical density results reached approximately 1.5. Kemsawasd and Chaikham (2018), having added *L. casei* and *L. acidophilus* cultures in MRS broth (separately) and incubated them at 37°C, found that they reached the stationary stage after 12 and 16 hours, respectively.

TPC, antioxidant activity, cellular growth, and pH of the fermented media

Based on the cellular growth and antimicrobial activity data collected, fermentation experiments were carried out in MRS broth during 16 h, for determination of the TPC, antioxidant activity, cellular growth, and pH of the fermented media. Table 2 shows the TPC results of the media fermented for 16 h, with and without addition of the Feijoa leaf extracts. There was no statistical difference ($p>0.05$) in the mean TPC results between time 0 and hour 16, except for the fermented medium containing the Helena extract, for which the final value was lower than the initial. Over the 16 hours, there was oscillation in the TPC values of some of the fermented media. In the comparison between the genotypes for a same time, it may be noted that at hour 16, the fermented media containing the Access 2316 and Helena extracts showed statistical difference ($p<0.05$), with higher TPC for the one containing the Access 2316 extract. It should be noted that at time zero, the TPC values of the fermented media containing extracts Access 2316 and Helena were statistically equal ($p<0.05$), demonstrating the change in the TPC of the one containing the Helena extract.

The TPC oscillations over time may be related to degradation or the formation of new compounds. Valero-Cases et al. (2017) verified changes in antioxidant activity and formation of new phenolic compounds during the fermentation of pomegranate juice by lactic acid bacteria *B. bifidum, L. plantarum, B. longum* subsp. *infantis* e *L. acidophilus*. The authors identified the presence of a new derivative catechin and α-punicalagin, both absent in the control sample. The compounds are formed due to the LAB's metabolism in the presence of other phenolic compounds

Table 2: Total phenolic compounds, for the times 0, 4, 8, 12 and 16 hours of fermentation, of the fermented media with addition of Feijoa leaf extracts.

Time (h)	Total phenolic compounds (mg EAG g^{-1}DM)				
	MRS + extract Access 2316	MRS + extract Alcântara	MRS + extract Helena	MRS + extract Mattos	MRS + extract Nonante
0	67.40 ± 2.65^{bA}	54.77 ± 2.19^{abC}	61.79 ± 3.65^{aAB}	57.23 ± 2.19^{abBC}	55.82 ± 1.22^{aBC}
4	76.53 ± 2.11^{aA}	58.98 ± 1.61^{aB}	49.86 ± 2.43^{bC}	62.49 ± 5.19^{aB}	48.11 ± 1.05^{cC}
8	81.79 ± 1.82^{aA}	47.40 ± 3.04^{bD}	57.58 ± 2.11^{aBC}	56.53 ± 1.05^{abC}	62.84 ± 2.79^{abB}
12	65.47 ± 0.74^{bA}	58.98 ± 1.22^{aB}	54.77 ± 3.04^{abB}	52.67 ± 3.80^{bB}	55.47 ± 2.79^{aB}
16	61.09 ± 1.61^{bA}	57.93 ± 0.74^{aAB}	48.11 ± 2.23^{bB}	56.53 ± 1.49^{abAB}	60.39 ± 3.72^{abA}

Means followed by different lowercase letters, in the same column, differ statistically ($p<0.05$) (between times). Means followed by different uppercase letters, in the same row, differ statistically ($p<0.05$) (between fermented medias), by Tukey test.

such as epicatechin and catechin, present in the control, but degraded with fermentation. A similar effect may have occurred in the fermented media containing the Feijoa extracts, because catechin is one of the main phenolic compounds found in phenolic compound profile studies performed on the leaves (Mousavi et al. 2018; Poodi et al., 2018).

Pereira et al. (2017), found that the TPC of cupuaçu juice fermented by *L. casei* showed oscillation over 18 hours of fermentation, decreasing in the first few hours, and increasing from hour 14 on, and that even with this behavior, the final TPC were lower than the initial ones. Sun-Waterhouse et al. (2013) found that the TPC was 3.5-3.9 times higher for yogurt containing polyphenols present in *Ribes nigrum* spp. in pre-fermentation in relation to those to which polyphenols were added after fermentation. They found that the addition before fermentation provided the presence of small phenolic polar molecules (such as protocatechuic acid and a derivative of catechin) as well as larger molecules (ferulic, salicylic and coumaric acids).

The interest in maintaining the number of phenolic compounds or increasing it with lactic fermentation is related to the decrease in the concentration of these compounds in vegetables after simulated digestion and has thus the purpose of ensuring that these compounds show greater bioavailability for ingestion (Rodríguez-Roque et al., 2013). Furthermore, fermented beverages are largely consumed by the population, so the addition of bioactive compounds may grant beneficial properties to the consumers' health, such as antioxidant properties, also contributing to the prevention of neurodegenerative diseases, cardiovascular diseases, and cancer (Septembre-Malaterre et al., 2018).

The antioxidant activity of the fermented MRS broths containing the Feijoa leaf extracts is shown in Table 3.

Statistically, only the fermented medium containing the Alcântara leaf extract had no changes in antioxidant activity between time 0 and after 16 hours of fermentation ($p > 0.05$). However, for the others, the values of which statistically differed between themselves, the only significant change occurred for the fermented medium containing the Helena extract. For hour 16, the fermented medium that showed the lowest antioxidant activity, statistically differing from the others ($p<0.05$), was the one containing Helena leaf extracts, which also showed the lowest TPC.

Kaprasob et al. (2017) verified that the antioxidant activity in cashew juice fermented by *L. plantarum*, *L. casei* and *L. acidophilus* (separately for 48 h at 37° C) decreased by approximately 4% (DPPH and ABTS methods). The reduction in antioxidant activity may be associated with oxidation or degradation of antioxidant compounds (Johnson et al., 2011). Muniandy et al. (2016) state that plant extracts rich in phenolic compounds have been applied in fermented milks to obtain a functional food, because phenolic compounds derived from the extracts can offer major health benefits when consumed regularly, being considered potent sources of antioxidants.

In Fig. 2 and 3, the results of the count of mesophilic microorganisms and pH over the fermentation time are respectively presented.

The count of mesophilic microorganisms significantly increased ($p<0.05$) over time for all experiments, with initial counts that varied between 3.07 (Access 2316) and 3.92 (Control) Log CFU mL^{-1}, and final counts that varied between 7.48 (Access 2316) and 7.75 (Nonante) Log CFU mL^{-1}. Lactic acid bacteria have high tolerance to antimicrobial phenolic acids and their resistance is partially dependent on the ability to convert phenolic acids into metabolites with reduced metabolic activity (Sánchez-Maldonado et al., 2011).

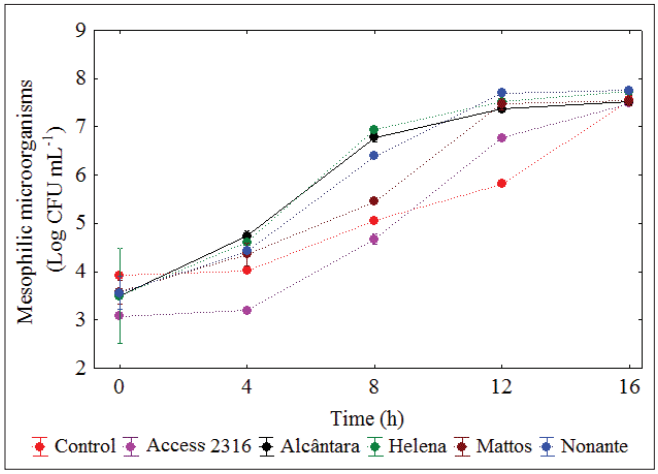

Fig 2. Count of mesophilic microorganisms of the fermented MRS broths without and with the addition of the aqueous extracts of the Feijoa leaves, in times of 0, 4, 8, 12 and 16 h.

Table 3: IC_{50} (µg mL^{-1}) for the times 0h and 16 hours of fermentation, of the fermented media with addition of Feijoa leaf extracts.

Tempo (h)	MRS + extract Access 2316	MRS + extract Alcântara	MRS + extract Helena	MRS + extract Mattos	MRS + extract Nonante
0	0.324 ± 0.004^{aA}	0.525 ± 0.080^{aC}	0.402 ± 0.026^{bAB}	0.507 ± 0.013^{bBC}	0.394 ± 0.012^{aA}
16	0.422 ± 0.013^{bAB}	0.527 ± 0.033^{aAB}	0.958 ± 0.148^{aC}	0.592 ± 0.036^{aB}	0.351 ± 0.010^{bA}

Means followed by different lowercase letters, in the same column, differ statistically ($p <0.05$) (between times). Means followed by different uppercase letters, in the same row, differ statistically ($p <0.05$) (between fermented medias), by Tukey test.

Reduction in pH occurs because of microbial development, being associated with the production of organic acids such as citric, acetic or lactic acid by lactic acid bacteria as part of metabolism and energetic growth (Kaprasob et al., 2017). The pH of the broths fermented in the presence of the Feijoa leaf extracts showed reduction over time for all experiments. From hour 4 onwards there was a more pronounced decline in pH values, at the same time greater microbial growth starts to occur. After hour 16, the pH values showed statistical difference ($p<0.05$) between the Control and Access 2316 samples and the others, varying from 5.21 ± 0.01 to 4.93 ± 0.01.

Pereira et al. (2017), using optimized conditions in the fermentation of Cupuaçu juice by *L. casei* and an initial 5.8 pH, decreased the pH's value down to 4.3. The initial viable cell counts corresponded to 7.5 Log CFU mL^{-1}, and after fermentation, they increased by approximately 2 Log, with 9.34 ± 0.06 log CFU mL^{-1} counts. Given this study's initial and final concentrations, the cell increments were similar.

In lactic acid fermentation, *S. thermophillus* quickly starts the production of lactic acid up to concentrations of about 1.1%, and produces formic acid and carbon dioxide, reducing the oxygen that stimulates the growth of *Lactobacillus*. Subsequently, their compounds are used by *L. delbrueckii* subsp. *bulgaricus* which, being more proteolytic, promotes the release of small peptides and amino acids that are used by *S. thermophillus*, stimulating their growth (Bergy, 1974, Walstra et al., 2006, Bezerra et al., 2011).

This generation of peptides can be verified in the Control experiment's results of soluble proteins, where 3.58 ± 0.02 mg g^{-1} was obtained for time zero, 2.94 ± 0.18 mg g^{-1} for hour 4, 3.53 ± 0.85 mg g^{-1} for hour 8, 12.99 ± 0.77 mg g^{-1} for hour 12, and 13.41 ± 0.34 mg g^{-1} for hour 16. The changes in the control's soluble protein content over time may be related with the formation of new polypeptides, due to the lactic acid bacteria's proteolytic activity in relation to enzymes such as proteinase of the cell wall and several intracellular peptidases (Eça and Implvo, 2006).

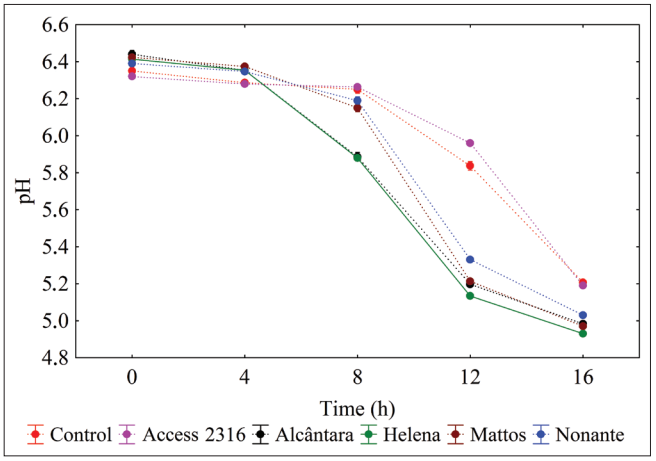

Fig 3. pH of the fermented MRS broths without and with the addition of the aqueous extracts of the Feijoa leaves, in times of 0, 4, 8, 12 and 16 h.

In Fig. 4, the morphological aspect of the mixed culture (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) after 16 hours of fermentation with and without addition of the Feijoa leaf extracts obtained by SEM is shown.

It may be observed that the predominating morphology is that of cocci. The final pH of the fermented media may indicate that *S. thermophilus* showed an even more pronounced growth than *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Count of mesophilic microorganisms, physicochemical characterization and sensory profile of whole milk fermented in the presence of the extracts

In Fig. 5, the results of the initial and final counts of mesophilic microorganisms for milk fermented with and without addition of the aqueous Feijoa leaf extracts are shown.

The results of the count of mesophilic microorganisms in the fermentations with and without addition of the aqueous Feijoa extract did not statistically differ ($p>0.05$) for time zero, and showed no statistical difference ($p>0.05$) for hour 4, indicating that the application of the extracts, regardless of genotype, leads to an equal development of the LAB in fermentation. The counts at time zero ranged from 4.10 ± 0.17 Log CFU mL^{-1} (Control) to 4.58 ± 0.32 Log CFU mL^{-1} (Alcântara), and the counts at hour 16 ranged from 6.15 ± 0.13 Log CFU mL^{-1} (Alcântara) to 6.39 ± 0.11 Log CFU

Table 4: pH, in the initial and final fermentation times, and titratable acidity after the fermentation of the milk added from Feijoa leaves extracts.

	pH		Titratable acidity (g lactic acid 100g^{-1} sample) in 16h
	0 h	16 h	
Milk	6.69 ± 0.01^{bcA}	4.27 ± 0.01^{cB}	0.725 ± 0.005^{b}
Milk + extract Access 2316	6.75 ± 0.02^{aA}	4.39 ± 0.02^{aB}	0.690 ± 0.007^{b}
Milk + extract Alcântara	6.71 ± 0.03^{abA}	4.37 ± 0.03^{bB}	0.711 ± 0.025^{b}
Milk + extract Helena	6.67 ± 0.01^{bcA}	4.24 ± 0.00^{eB}	0.811 ± 0.001^{ab}
Milk + extract Mattos	6.66 ± 0.01^{bA}	4.26 ± 0.01^{dB}	0.808 ± 0.077^{b}
Milk + extract Nonante	6.65 ± 0.01^{bA}	4.36 ± 0.01^{bB}	0.894 ± 0.002^{a}

For pH results: means followed by different lowercase letters, in the same column, differ statistically ($p<0.05$) (between extracts) and means followed by different capital letters, in the same row, differ statistically ($p<0.05$) (between the times). For titratable acidity, means followed by different lowercase letters, in the column, differ statistically ($p<0.05$) (between extracts).

mL^{-1} (Mattos). By comparing these results with those of the fermentation in MRS broth, under the same conditions, it may be noted that, in general, the growth rates in whole milk were lower, showing that the change in fermentation medium may also influence the LAB's growth.

The pH results at the initial and final times and the titratable acidity results are shown in Table 4.

For pH, a reduction deemed as desirable in lactic fermentations may be noted, with final pHs below 4.5, statistically differing from each other ($p<0.05$). It may also be noted that there was greater reduction in the fermentation's pH when using milk, compared to fermentation in MRS broth. For fermented milks, the ideal pH is near 4.5, as it inhibits the growth of pathogen and sporulated microorganisms. Values lower than 4.0 may favor the coagulum's contraction due to the reduction in the proteins' hydration, and cause the loss of serum, leading to rejection by consumers. pH values above 4.6 are also not indicated, because they favor the serum's separation due to the influence of the gel formed (Vodnar et al., 2010; Azevedo et al*.,* 2018).

In relation to titratable acidity, all the fermented media are in accordance with Normative Instruction No. 46 of the Brazilian Technical Regulations on the Identity and Quality of Fermented Milk (BRAZIL, 2007), which establishes a level of acidity between 0.6 and 2 g of lactic acid for every $100g^{-1}$ of sample. Lactic acid is recognized as the main metabolite of lactic acid bacteria, and acidification is one of the most desirable effects of their growth (Lima et al., 2012).

The fermented milks' results of moisture, ash, total protein, and total solids, with and without addition of the aqueous Feijoa leaf extracts, are shown in Table 5.

Both for humidity and for total solids, there was statistical difference ($p<0.05$) between the Control and other samples. However, statistical difference was not verified ($p>0.05$) between the samples with addition of the aqueous Feijoa leaf extracts. Despite this significant difference between the control and other samples, the numerical values are still very similar. No statistical difference was detected between the fermented media for ash content.

The highest total protein values were found for the Control, Access 2316, Helena and Mattos samples, which were statistically equal ($p>0.05$), and the smallest value was found for the Alcântara sample, which statistically differed ($p<0.05$) from the others. For the latter, the mean value obtained is below the one established by the Brazilian legislation for fermented products (Brazil, 2007), which determines a minimum 2.9 g $100g^{-1}$ value. However, the legislation also says that the protein levels may be lower in milks fermented with aggregates and must not be reduced to a smaller percentage than that of non-dairy food substances. It should be noted that the fermentation process may improve the digestibility of proteins compared to milk *in natura*, due to the peptides formed.

In Fig. 6 and 7, the sensory profiles of the aqueous Feijoa leaf extracts and those of the milks fermented with the presence of Feijoa leaf extracts are shown, respectively.

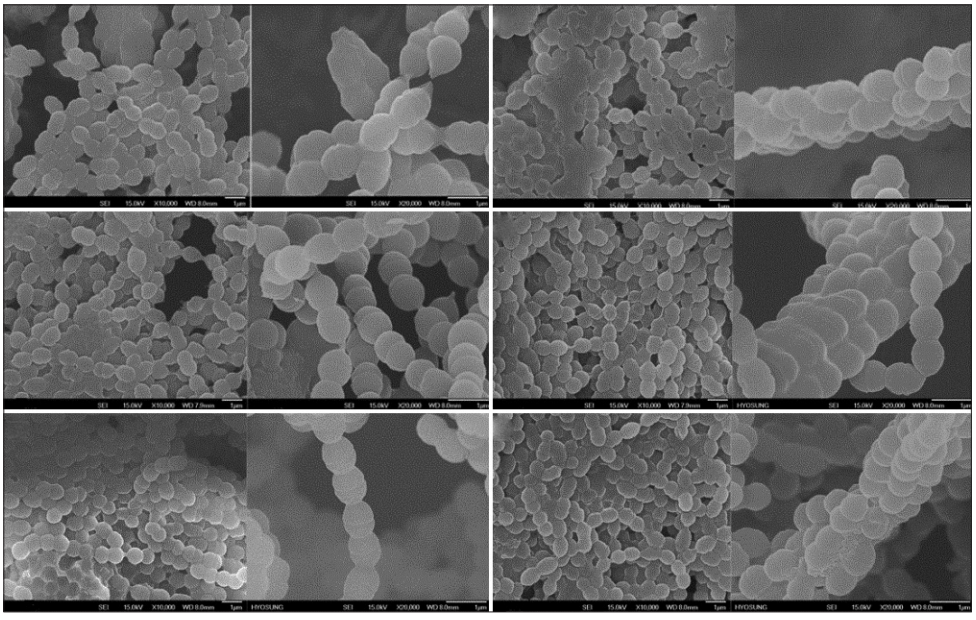

Fig 4. Scanning Electron Microscopy, with 10,000-fold and 20,000-fold increases in mixed culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) for Control (A), Acesso 2416 (B), Alcântara (C), Helena (D), Mattos (E), Nonante (F).

Table 5: Moisture, ashes, total proteins and total solids of fermented milks, without and with the addition of aqueous extracts of Feijoa leaves.

Parameters	Fermented milks					
	Milk (Control)	Milk + Access 2316	Milk + Alcântara	Milk + Helena	Milk + Mattos	Milk + Nonante
Moisture (%)	88.48 ± 0.01[a]	88.88 ± 0.11[b]	89.14 ± 0.08[b]	89.14 ± 0.05[b]	89.19 ± 0.08[b]	89.17 ± 0.01[b]
Ash (%)	0.72 ± 0.11[a]	0.80 ± 0.01[a]	0.70 ± 0.35[a]	0.94 ± 0.08[a]	0.62 ± 0.00[a]	0.68 ± 0.03[a]
Proteins (g 100g-1)	3.17 ± 0.01[a]	2.97 ± 0.08[ab]	2.58 ± 0.19[c]	2.92 ± 0.13[ab]	3.12 ± 0.01[a]	2.90 ± 0.08[ab]
Total solids (%)	11.53 ± 0.01[a]	11.12 ± 0.11[b]	10.86 ± 0.08[b]	10.86 ± 0.05[b]	10.81 ± 0.08[b]	10.83 ± 0.14[b]

Means followed by different letters in the row differ statistically (p <0.05) (between the fermented milks).

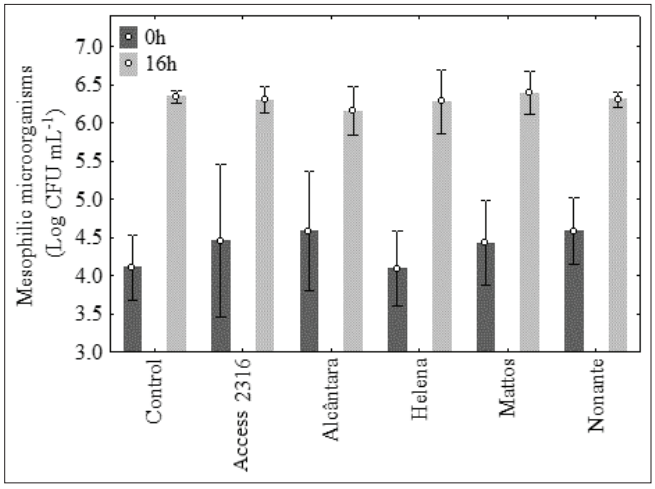

Fig 5. Initial and final counts of mesophilic microorganisms (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) of the milk fermented with the aqueous extracts of Feijoa leaves.

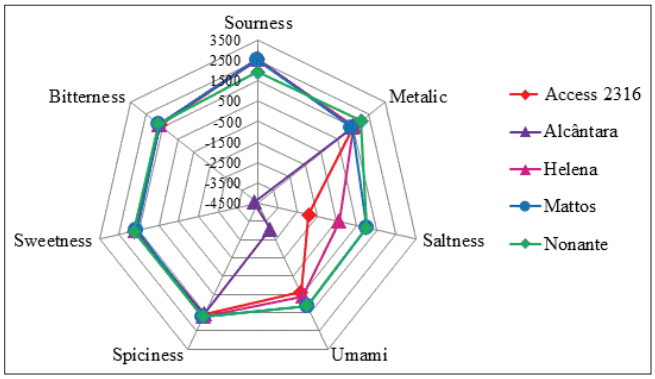

Fig 6. Sensory profiles of the aqueous extracts of Feijoa leaves.

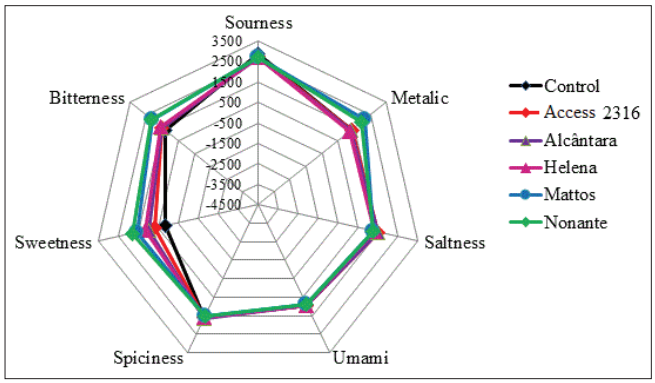

Fig 7. Sensory profiles of the fermented milks without and with of aqueous Feijoa leaves extract.

In relation to the extracts' sensory profile (Fig. 6), it may be noted that differences in the metallic, savory and umami flavors occur for the Access 2315, Alcântara and Helena leaf extracts. For the other flavors, there was no difference between the extracts. The fermented media's sensory profile (Fig. 7) showed differences in the sweet, bitter, and metallic flavors, again with sweet and bitter intensity equal to or greater to that of the control, for all the fermented media containing the Feijoa leaf extracts.

The phenolic compounds present in the extracts contribute to the formation of aroma, mainly due to the presence of volatile phenols (Naczk and Shahidi, 2004). As for the presence of volatile compounds, Mosbah et al. (2018) observed a plethora of these in ethanolic extracts of Feijoa leaves that were rota-evaporated and subsequently resuspended in water via gas chromatography. Limonene (36.2%), β-caryophyllene (27.8%), aromadendrene (12.5%) and α-copaene (6.6%) were present in greater amounts, in addition to various other compounds detected in lower quantities.

Volatile phenols can also be produced by the hydrolysis of alcohols or by the metabolism of microorganisms, yeast, and lactic acid bacteria, granting characteristic aromas (Raven et al., 2007; Heleno et al., 2015). The fermented milks have a typical flavor due to the LAB's metabolism and to the presence of compounds such as lactic acid, carbonyl compounds (acetaldehyde, acetone, diacetyl), non-volatile acids (oxalic, succinic, pyruvic), and volatile acids (formic, propionic, acetic) (Rosenthal, 1991).

CONCLUSION

The present study presents important additional records on the influence of the addition of leaf extracts of five Brazilian genotypes of Feijoa (*Acca sellowiana*) on lactic fermentation. The extracts showed high content of phenolic compounds and antioxidant activity for all genotypes. In the fermentation process using *S.thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in MRS broth, added of extract, there were alterations in the values of phenolic compounds and antioxidant activity. Microscopy showed a higher presence of *S. thermophilus*, indicating a possible interference of

extracts in the development of microorganisms, and it may be associated with final pH conditions. In milk, the fermentation occurs in a satisfactory way, demonstrating the potential of the Feijoa leaf extracts to produce fermented milk beverages.

ACKNOWLEDGMENTS

The work described in this article was supported by the Food and Chemical Engineering Department of the Santa Catarina State University - UDESC, especially by the Biolab (Bioprocess laboratory) and Microlab (Microbiology laboratory). This study was financed in part by the Coordination of Improvement of Higher Education Personnel - Brazil (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - CAPES) - Finance Code 001, and Foundation for Research and Innovation Support of the Santa Catarina State (*Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina*, FAPESC - 2017TR721). Registration Number in SisGen (*Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado*) AC525B6.

Conflict of interest

The authors declare that they have no conflict of interest.

CONTRIBUTION OF EACH AUTHOR

Luniele Beilke: adapted the methodologies, investigated, and wrote the work.

Eduarda Heck Sumny: adapted the methodologies and investigated.

Liziane Schittler Moroni: co-supervised the work.

Cassandro Vidal Talamini do Amarante: co-supervised the work.

Aniela Pinto Kempka: conceptualized and supervised the work, co-administrated the project, wrote, and revised the work.

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