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Development of prebiotic yogurt with addition of green-banana biomass (*Musa spp.*)

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Abstract: This study evaluated the technological viability of yogurt with the addition of green-banana biomass (*Musa spp.*) considering the resistant starch (BBV) as a potential prebiotic ingredient and texture agent. Four yogurt formulations were prepared: control; 3% BBV; 5% BBV; and 10% BBV. They were subjected to analysis of resistant starch, lactose, fat, total dry extract, defatted dry extract, moisture, ash, proteins, pH and titratable acidity; syneresis analysis, instrumental texture and instrumental color. All four formulations met the requirements of the identity and quality regulation for fermented milks regarding the physicochemical and microbiological parameters. In the instrumental color analysis, in all treatments with added BBV, darkening was observed after 21 days, with a reduction of a* coordinate and an increase of b* coordinate. In the instrumental texture analysis, the yogurt in the Control treatment had the highest firmness (0.430 N) at 21 days among these treatments. Among the treatments with added BBV, the yogurt with 5% added BBV showed the best results for increasing the viability of lactic bacteria. It was found that yogurt with added BBV is a promising alternative in the elaboration of functional dairy products, adding value to the banana production chain by reducing the green fruit waste.

Key words: Fermented milk, functional food, resistant starch, texture.

INTRODUCTION

In recent years, the world population has adopted new eating habits due to the scarcity of time to prepare meals at home, in addition to having greater access to ready-to-eat food delivered at home. Thus, functional foods have become a research priority in the field of food technology, considering the interest of consumers in adopting a healthy diet, a fact exacerbated by the worldwide reflection on food safety during the Covid-19 pandemic.

Yogurt is more digestible than milk. The heating step in the processing of yogurt denatures the whey proteins. Subsequent fermentation results in partial digestion of casein and whey protein by the hydrolytic enzymes of the yogurt milk culture. Its content of lactic acid and vitamin B improves digestion in general. In addition, yogurt is a source of biologically active peptides, which provide benefits to the immune, nervous, gastrointestinal and cardiovascular systems, thus, a health promoter (Spadoti et al. 2011, Chandan et al. 2017).

Banana, from the Musaceae family, is a plant widely cultivated in countries with tropical and subtropical climate. However, almost a third of its production is lost, since the main market demand is for ripe bananas. However, these ripe fruits are perishable during maturation and prone to mechanical damage, which makes them difficult to be stored and transported to consumer centers. The optimization of banana

processing was studied to reduce waste generation (rejection is about 1/4 of the banana) improving the bioavailability and utilization of nutrients of that fruit, with emphasis on the use of green banana (Anyasi et al. 2013, Jiang et al. 2015).

The green banana is extremely versatile and can be used in flour and biomass forms, due to its emulsification technological properties, and as a thickener, with increased nutritional value of phosphorus, iron, manganese, magnesium, potassium and zinc. The green banana is rich in resistant starch (RS) — present in the pulp and peel fibers - vitamin B6, vitamin C, beta-carotene (provitamin A) and phenolic compounds with antioxidant function (Bianchi 2010, Falcomer et al. 2019). In addition to antioxidant activity, several authors report that phenolic compounds have several biological activities, including antibacterial, antithrombotic, vasodilating, anti-inflammatory and anticarcinogenic effects, mediated by different mechanisms of action (Gibellini et al. 2011).

The green banana is a source of carbohydrates for food consumption, since its starches are relatively low in amylose content with high resistance to heat and amylase action, low swelling and low retrogradation, which provides its application as gelling, thickener and stabilizer agents in the food industry. Such characteristics are relevant to the dairy industry, since the green banana is considered a tasteless, odorless and soluble ingredient which could be used in the formulation of Functional Foods (Padam et al. 2014).

The interest in green banana's RS is due to its physiological role. As it is not digested in the small intestine, RS is fermented by the microbiota of the intestinal colon. Thus, RS may be considered a prebiotic functional ingredient (Fuentes-Zaragoza et al. 2010). The green-banana biomass (GBB) is obtained by cooking the green banana with peels immersed in water, followed by the separation of the peels and pulp to obtain the puree, known as biomass (Oliveira et al. 2015).

Prebiotic is a substrate that is selectively used by host microorganisms and confer health benefits (Gibson et al. 2017). The consumption of dairy products with prebiotics was associated with anti-diabetic and anti-hypertensive properties, and improvements in the blood lipid profile, immunity, and intestinal health. (Rosa et al. 2021). The prebiotic components could be used as fat replacers in dairy products, resulting in non-fat products with rheological, physicochemical, and sensory properties similar to the full-fat products (Rosa et al. 2021). According to Rosa et al. (2021), previous works demonstrated improvements in the quality properties observed for ice creams, cheeses, yoghurts, whey beverages, processed cheese, and dairy dessert. According to Farias et al. (2019), prebiotics can be found in several plant and animal sources, but to meet global demand the development of strategies that increase the production of these compounds at the industrial level is necessary. In this sense, the use of microorganisms and enzymes can be an alternative to increase the variety and availability of prebiotics such as non-digestible carbohydrates, besides increasing the use of these compounds in the food industry (Farias et al. 2019).

Prebiotics, detected as soluble fibre, could be listed as fibre on the nutrition facts label. Under the new regulations, this listing will not be allowed. Fibre has been redefined to be soluble and insoluble non-digestible carbohydrates (with three or more monomeric units) and lignin that are intrinsic and intact in plants, and certain isolated and synthetic non-digestible carbohydrates (with three or more monomeric units) (Gibson et al. 2017).

According to Pérez-Chabela et al. (2022), fermented dairy foods as yogurt are an opportunity to counter deficiencies in nutrients intake as a good source of calcium, protein, and live bacteria, providing health benefits to the host. In the same way, dairy products as yogurt also contain considerable amounts of other macronutrients, vitamins, and minerals, being in general nutrient-dense foods, where yogurt is as well a vehicle to fortification and a tool to improve nutrient deficiencies of vitamin D (Pérez-Chabela et al. 2022). Thus, the objective of the present study was to evaluate the potential of yogurt added with green banana biomass (*Musa spp.*) as a prebiotic agent.

MATERIALS AND METHODS

Place of study

The experimental procedures for the manufacture of yogurts and microbiological and physicochemical analyses were carried out at the Laboratory of Food Technology (LTA) of Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). Centesimal composition analysis was performed at the Applied Chemistry Laboratory of the Instituto Federal do Espírito Santo (Ifes-Alegre) and instrumental texture analysis was performed at the Laboratory of Biochemistry and post-harvest physiology of UENF.

Material

Green bananas (*Musa spp.*) cultivar 'Silver ' were obtained directly from the rural producer of Campos dos Goytacazes, Rio de Janeiro, Brazil, immediately after its harvest, without any treatment. Bananas were chosen according to their stage of maturation, according to Von Loesecke's maturation scale (1950), i.e., Stage 1 with totally green skin (PBMH & PIF 2006). Lyophilized milk culture "Ricaferm YR03®" was used, along with mixed strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus*, acquired directly from the company Rica Nata®, industry and Commerce Ltda. and intended for the manufacture of yogurts with rapid fermentation.

For the manufacture of yogurt, pasteurized homogenized whole milk of Fiore® brand, registered within the Federal Inspection Service of the Ministry of Agriculture, Livestock and supply (MAPA) (SIF 1300), was purchased in local trade and transported to the UENF Food Technology Laboratory in an isothermal box to guarantee its temperature up to 7°C. The instant skimmed milk powder of Itambé® Alimentos S/A (SIF 769), another product used in the manufacture of yogurt, was also purchased in local trade.

Methods

Green-banana biomass

The steps to obtain GBB were taken according to the methodology of Mendonça et al. (2017). The fruits were received at UENF-LTA and selected according to their integrity and Stage 1 of maturation (PBMH & PIF 2006). The green bananas were washed with Peel in running drinking water with the aid of a soft bristle brush to remove possible dirt. Subsequently, they were sanitized with sodium hypochlorite solution at 150 ppm for 15 minutes, followed by rinsing in running drinking water to have chlorine residues removed. Then, the unpeeled bananas were immersed in drinking water inside a pressure cooker and cooked for eight minutes (after starting of the pressure). After that period, the heating was interrupted and the bananas were left to rest until the pressure was lost. The cooked bananas were peeled while still hot with the aid of a stainless-steel handle and cut into slices with the help of a stainless-steel

knife. The pulp was processed in a Mixer (High Power Oster®} - 550 W) until the formation of a homogeneous and thick mass. The GBB was packaged in transparent polypropylene plastic jars (250 mL) previously sanitized with 1% sodium hypochlorite solution, identified and stored at an average temperature of 0°C. Then, the biomass was preheated to a temperature of 42°C in a B. O. D. (Biochemical Oxygen Demand) incubator, in order to facilitate its homogenization in the yogurts of the respective treatments.

Manufacture of yogurt

The manufacture of yogurt was determined according to preliminary laboratory tests and based on the methodologies adopted by Costa et al. (2017) with modifications. The lyophilized mixed lactic culture "Ricaferm YR03®" was used to ferment milk and obtain yogurts. Due to the small volume of milk used in each processing (3.2 liters) and, consequently, due to the small amount of lactic culture required for coagulation, the freeze-dried culture was activated and a mother culture was prepared for the manufacture of the yogurts used in the present study.

The culture was activated in skimmed milk powder and incubated at 42°C for 5 hours until its pH reached 4.6±0.1, according to the manufacturer's recommendations. Then, it was kept under refrigeration at 0°C and used for the manufacture of yogurt. For each repetition of the experiment, a new mother culture was prepared following the same protocol.

For the manufacturing processing of yogurts, pasteurized homogenized whole Fiore® milk (3.2 liters) added with 3% instant skimmed milk powder was used. The mix underwent heat treatment (90°C for 5 minutes), cooled to 42°C and divided into four portions of 800 mL each, to which 2% of the activated "Ricaferm YR03®" dairy culture was added. From that initial processing, the portions were divided into four groups with their respective percentages of GBB addition (before fermentation):

- 2% of the dairy culture "Ricaferm YR03®" (Control);
- 2% of the dairy culture "Ricaferm YR03®" + 3% of green banana biomass (F3);
- 2% of the dairy culture "Ricaferm YR03®" + 5% of green banana biomass (F5);
- 2% of the dairy culture "Ricaferm YR03® + 10% of green banana biomass (F10).

The batches of each group were stored in their corresponding Becker glass cups (1 liter) with lid and taken for fermentation in the B. O. D. incubator at 42°C ±1°C. To evaluate the fermentation time, samples were collected every 60 Minutes from each treatment for pH evaluation with portable digital pH meter Akso® AK 90 and titratable acidity evaluation. The fermentation time of the yogurts was enough for the product to reach pH 4.6 ±0.1.

At the end of fermentation, yogurts underwent cooling in two stages. In the first stage, Becker cups were immersed in a thermal box with ice water, which was replaced until the yogurt temperature reached around 24°C in order to inhibit the production of lactic acid. However, the cooling was slow, around 40 minutes, to avoid thermal shock. In that stage, the clot was broken with a glass stick (maximum of 20 seconds) to homogenize each treatment and avoid the occurrence of syneresis. After that process, yogurts were packaged in round polyethylene jars with lid (250 mL), previously washed with distilled water and sterilized with an ultraviolet irradiation stick (wavelength of 254 nm) for 60 seconds at a distance of no more than 10 cm (Alexandre et al. 2008). The containers used for the storage of yogurts were identified with self-adhesive labels with the group description.

The second stage was carried out in a refrigerator at temperature of 4°C ±1°C until the yogurt temperature was reduced to 10°C within a maximum period of 1 hour. Such samples were stored in refrigerator at 4°C ±1°C during the 21 days of evaluation. The whole experiment was repeated four times and in each repetition four samples were produced from each experimental group (treatment).

Quantification of resistant starch (RS) in green banana biomass

The determination of RS was quantified based on AOAC method (2002.02), according to the "Association of official Analytical Chemists" (AOAC), (McCleary & Monaghan 2002), with modifications. The determination was made in triplicate, and the results were expressed in % of sample on the integral basis. The resistant starch content was 23%.

Centesimal composition

Yogurt samples with four days of storage at 4°C±1°C were analyzed, in triplicate, regarding the contents of lactose, fat, moisture, ash, protein and total and dry degreased extracts.

The lactose content was determined according to the DNS (dinitrosalicylic acid) method (Miller 1959) based on the reaction between reducing sugar and 3,5-dinitrosalicylic acid (yellow color), reduced to 3-amino-5-nitrosalicylic acid (reddish). Absorbance reading was performed on a spectrophotometer at 540 nm and the DNS test was performed (Cecchi 2003, Maldonade et al. 2013).

Fat content was determined according to Gerber's method with butyrometer and centrifuge (AOAC 2019). The total dry extract (TDE) was determined by stove drying at 105°C (AOAC 2019) and the dry degreased extract (DDE) was determined by the difference between the total dry extract (TDE) and the fat content (AOAC 2019).

The moisture content was determined by the gravimetric method through heating in oven at 105°C until constant weight was obtained and the ash content was determined by the gravimetric method through incineration in a muffle furnace at 550°C until light ash remained (AOAC 2019).

The protein content was determined by the Kjeldahl method, in which the protein fraction was obtained by determining the percentage of total nitrogen in the sample, and the result, multiplied by the conversion factor (6.38) for dairy proteins (AOAC 2019).

Determination of PH and titratable acidity

Yogurts' pH and titratable acidity were determined, in triplicate, after the periods of 1, 7, 14 and 21 days of storage at 4°C±1°C. The pH was determined with a portable digital potentiometer (Akso-AK 90) calibrated at pH 7 and pH 4. The titratable acidity was determined by the amount of lactic acid (g / 100g) of each sample according to the following equation: lactic acid (g/100g) = V × f × 0.9/m (V = volume of sodium hydroxide solution 0.1 N spent on titration, in mL; f = correction factor of sodium hydroxide solution 0.1 N; 0.9 = conversion factor to lactic acid; m = sample mass, in ml) (Brazil 2019).

Syneresis analysis

Spontaneous Syneresis was determined by collection of the spontaneously released serum from 100 g of yogurt to obtain the syneresis index = 100 x (aspirated serum mass/100 g of the product) (Fiszman et al. 1999).

Syneresis by drainage was determined by the amount of serum drained for 30 minutes at 4°C ± 1°C using a 120-mesh stainless steel sieve (De Wit 1988) to obtain the syneresis index (%)

= 100 x (mass of the drained serum/mass of the sample).

Syneresis by centrifugation was determined by the expulsion of the serum under centrifugal force according to the methodology of Farnsworth et al. (2006) with some modifications. Samples of 10 grams of yogurt (4±1°C) underwent 1422 g centrifugation for 10 minutes (Quimis® centrifuge, model Q222T). The supernatant serum was aspirated and weighed to provide the syneresis index (%) = 100 x (supernatant serum mass/sample mass). All syneresis analyses were performed in triplicate, on Days 1, 7, 14 and 21 of yogurt storage.

Instrumental texture analysis

The instrumental texture analysis of the samples (8°C±1°C) was performed on Days 1, 7, 14 and 21 of storage. Texture profile analysis (TPA) was adapted from the methodology of Ramos et al. (2009) and carried in a TA.XT Express texturometer (Stable Micro Systems, Godalming, UK) through rear extrusion method (aluminum back Extrusion Cell – probe a/BE with 35 mm disc and extender with 5kg load cell). Sample aliquots (100 mL) were separated and inserted into 125 mL Becker glass cups (diameter 50 mm) with about 75% of its volume occupied. The parameters adopted were: pre-test speed: 2.0 mm/s; test speed: 2.0 mm/s; post-test speed: 2.0 mm/s; distance which the device penetrates the sample: 20 mm; distance traveled in sample penetration: 20 mm; contact time: 5s and contact Force: 1 g (0.009807 N). Eight replicates were done for each group and the following texture parameters were taken: adhesiveness, cohesiveness, firmness and gumminess (Szczesniak 1963).

Instrumental color analysis

The instrumental color analysis of the samples was carried out in triplicate, on the first and 21st day of yogurt storage at 4°C ± 1°C, with the use of a portable spectrophotometer model miniscan CFL XE Plus – HunterLab, using illuminant D65, observation angle of 10 C°, following CIELAB system. The results were expressed through the angular coordinates L* = Luminosity (0 = black and 100 = white), a* (-80 to zero = Green, from zero to + 100 = red) and b* (-100 to zero = blue, from zero to + 70 = yellow). The samples were homogenized and dispersed over a borosilicate optical glass bucket in enough quantity to cover the base of the bucket and be analyzed.

Microbiological analysis

The microbiological analyses of the samples were carried out according to the protocols described by the Manual of official methods for the analysis of foods of Animal origin adopted by MAPA (Brazil 2019) and proposed by Silva et al. (2010), on the first day and 21st day of storage. The results were compared with the acceptable standards regulated by normative instruction n. FFM 46 (Brazil 2007) for coliforms at 35 °C, coliforms at 45 °C, molds and yeasts and evaluation of the viability of the dairy culture "Ricaferm YR03®" during its shelf life.

According to current legislation (Brazil 2007), the analysis of coliforms at 45 °C using the method of the American Public Health Association (APHA) was required. Thus, 1 ml aliquots of each dilution were inoculated in a series of five tubes containing 10 ml of lactosed broth (LST), with an inverted Durham tube (presumptive test). Tubes were incubated at 35°C for 24-48 hours. After this period, the tubes were read. Tubes that showed gas production due to fermentation of lactose in the medium were considered positive in the presumptive test. Then, with the aid of a loop, inoculations were performed from positive tubes of the presumptive test to tubes containing brilliant green bile broth (BVB) and tubes with EC broth. The BVB were incubated in an oven at 35°C for

24-48 h for confirmatory tests of total coliforms and EC tubes in a water bath at 44.5-45°C for 24 h for confirmatory tests of coliforms at 45°C. After the incubation periods, the production of gas was verified in the Durham tubes placed in the culture media of the confirmatory test and, from these tests, the positive tubes (gas). Queries were made in the table to estimate the MPN of coliforms at 30°C and coliforms at 45 °C (Silva et al. 2010).

For the determination of molds and yeasts, Potato Dextrose Agar (PDA) medium was used, following the instructions given by the manufacturer, subsequent homogenization and heating on a hotplate, with subsequent autoclaving. After being acidified with 10% tartaric acid (1 ml of acid for each 100 ml of medium), the culture medium was distributed in an amount of 20 ml in sterile Petri dishes. The method of direct plating on the surface of serial dilutions (10^{-1} to 10^{-3}) previously prepared was used. For this purpose, 0.1 ml of each dilution was inoculated on the surface of the solidified PDA medium in Petri dishes. With the aid of a Drigalski loop, the inoculum was carefully spread over its entire surface until complete absorption. The plates were incubated in an oven at 25 °C for 5 days, and the result was expressed by the number of colony forming units per ml of sample (Silva et al. 2010).

The opening of the yogurt pots took place inside the laminar flow chamber to prevent any environmental contamination of the sample. A 1 ml aliquot of sample was transferred to a screw tube containing 9 ml of sterile 0.1% peptone water solution. From this dilution, subsequent dilutions necessary for this analysis were made. After the incubation time required for each culture medium, the count was performed in Petri dishes, which showed between 25 and 250 colonies. The selective media were prepared with the intention of favoring the growth of the bacteria of interest, preventing the growth of the others. For the enumeration of Streptococcus salivarius subsp. Thermophilus, the M 17 agar medium was used. Inoculation was performed by depth. After inoculation, the Petri dishes were incubated inverted in aerobic conditions at 37°C for 48 hours. Typical colonies of *Streptococcus thermophilus* are lentil-shaped and have a diameter of between 1 and 2 millimeters. For the enumeration of *Lactobacillus delbrueckii subsp. Bulgaricus*, acidified MRS glucose agar medium was used. Inoculation was performed by depth. After inoculation, Petri dishes were incubated inverted in jars containing Anaerobac anaerobic generator (Probac) at 37 °C for 72 hours (IDF 1997).

Statistical analyses

The results of the microbiological analyses (count of: coliforms at 35 °C, coliforms at 45 °C, molds and yeasts, *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*), physicochemical analysis (%AR, lactose content, fat content, total dry extract, dry degreased extract, moisture content, ash content, proteins, pH determination and titratable acidity), syneresis analysis and instrumental analysis of color and texture were submitted to the analysis of variance (Anova) and the Student-Newman-Keuls Test (SNK), at a level of 5% significance, through the statistical analysis software SAS University Edition (SAS Institute Inc 2018).

RESULTS AND DISCUSSION

Centesimal composition

The centesimal composition of the formulations analyzed meets the standards of the current Brazilian legislation regarding its constituents, especially in relation to the content of milk proteins in fermented milks of at least 2.9 g/100g. In Table I, the yogurt fat content is shown to vary

Table I. Centesimal composition of yogurts with green banana biomass.

Parameters (g/100g)	Group			
	Control	F3	F5	F10
Total dry extract	13.55 ± 0.04[b]	13.71 ± 0.19[b]	13.64 ± 0.21[b]	14.35 ± 0.41[a]
Fat	5.00 ± 0.00[a]	5.00 ± 0.00[a]	5.00 ± 0.00[a]	5.00 ± 0.00[a]
Dry degreased extract	8.55 ± 0.04[b]	8.71 ± 0.19[b]	8.64 ± 0.21[b]	9.35 ± 0.41[a]
Moisture	86.34 ± 0.02[a]	86.09 ± 0.18[b]	86.05 ± 0.04[b]	85.66 ± 0.15[c]
Ashes	0.90 ± 0.01[a]	0.90 ± 0.00[a]	0.89 ± 0.00[a]	0.87 ± 0.01[b]
Proteins	4.44 ± 0.24[a]	4.31 ± 0.21[a]	4.60 ± 0.24[a]	4.22 ± 0.23[a]
Lactose	5.22 ± 0.07[b]	6.33 ± 0.06[a]	6.23 ± 0.10[a]	5.00 ± 0.09[c]

Note: Means ± standard deviation, on the same line, followed by distinct lowercase letters, indicate significant differences between treatments according to the Student Newman-Keuls Test at 5% significance.

between 0.93 and 0.97 g/100g, which makes it a partially skimmed yogurt, since the legislation establishes a range of 0.6 to 2.9 g/100g of fat in this category (Brazil 2007). Homogenized whole milk with 4.00 g/100g fat was used in this study.

Costa et al. (2017), when analyzing the physicochemical parameters of probiotic yogurt with addition of GBB (3, 5 and 10%), reported fat content ranging between 2.6 and 3.2 g/100g, which differ from those observed in the present study. When verifying the physicochemical parameters of yogurt with the addition of GBB (5, 10 and 15%), Silveira et al. (2017) observed fat content around 2.5 g/100g. Such values differ from those found in the present study (between 0.93 and 0.97 g/100g), with no difference ($p > 0.05$) between groups. Such lack is justifiable, since the fat content in the added GBB is not significant (less than 0.05 g/100g) to change the fat concentration between the different formulations. Leonel et al. (2011) carried out physicochemical analysis of GBB from seven different cultivars and reported fat content ranging from 0.08 to 0.2 g/100g, variations similar to those of the present study.

Table I shows the protein contents ranging between 4.60 g/100g (5% GBB) and 4.22 g/100g (10% GBB) with no difference ($p>0.05$) between groups. Such results demonstrate the addition of GBB does not represent an increase in the protein value in the formulations analyzed. Costa et al. (2017) observed protein content of 4.4 g/100g in yogurt with 3% GBB, a value similar to the present study, which was 4.31 g/100g (3% GBB). However, the protein content values for 5% and 10% GBB found by the authors were 2.7 and 2.6 g/100g, respectively, which differ from the protein contents of our study on 5% and 10% GBB.

The protein contents observed in the present study are higher than those found by Silveira et al. (2017), which ranged between 3.81 g/100g (15% GBB) and 3.92 g/100g (5% GBB). Such divergence is expected, since in our study, 3% of skimmed milk powder were added to increase the solid content in the formulations. Such increase leads to the formation of a firmer clot, which mitigate the occurrence of syneresis, a recurrent fact observed in preliminary tests without the addition of skimmed milk powder.

Difference of ash content ($p > 0.05$) occurred between the groups, with F10 10% GBB (0.87 g/100g) showing lower content compared to the other groups. Such fact may be explained by the higher percentage of organic matter from GBB (F10) among the evaluated groups, which was degraded during the incineration process at 550 °C and resulted in lower ash content, while the other groups present higher percentage of minerals, such as calcium, from milk present in the ash. Costa et al. (2017) found the lowest ash content in the treatment with 10% GBB, a fact similar to what was observed in the present study. However, there was no difference ($p>0.05$) between the treatments with probiotic yogurt with GBB. Lower ash values were verified by Silveira et al. (2017), between 0.63 and 0.73 g/100g, and Lucatto (2013), between 0.74 and 0.72 g/100.

Regarding the values of total dry extract (TDE) and dry degreased extract (DDE), there was a difference ($p > 0.05$) between the groups, with F10 10% GBB (14.35 g/100g and 13.41 g/100g, respectively) presenting the highest values compared to the other groups (Table I). In both cases, group F10 presented the highest values of TDE and DDE due to the greater addition of GBB (organic solids), not degraded in drying oven at 105 C°. When analyzing a symbiotic yogurt with green banana pulp (8%), Lucatto (2013) reported values of TDE - 19.88 to 20.35 g/100g and DDE - 18.63 to 19.09 g/100g, which are above those found in our study. Such difference may be justified by the use of reconstituted whole milk powder added with sugar (12%), which increased the solid content of their symbiotic yogurts. However, in our study, yogurts were prepared without addition of sugar, proportionally presenting lower solid content in the formulations.

Moisture levels varied ($p<0.05$) between 86.34 g/100g (Control) and 85.66 g/100g (10% GBB) (Table I). The lower moisture content in the group with 10% GBB is due to a higher index of syneresis that occurs over time with the outflow of water from the gel, consequence of the retrogradation of the starch present in a higher percentage of GBB added. Costa et al. (2017) found moisture contents ranging between 86.0 g/100g (10% GBB) and 88.0 g/100g (Control), a range of values similar to those found in the present study. In addition, there was no difference ($p > 0.05$) between the 3% and 5% GBB groups, same fact reported in our study.

The lactose content observed in the milk used was 5 g/100g, a value similar to that found by Lucatto (2013) (4.94g/100g) in the cow's milk used for symbiotic yogurt with green banana pulp. The lactose content (Table I) varied between 5.00 g/100g (F5 - % GBB) and 6.33 g/100g (F3 - 3% GBB), with difference between groups. Both formulations (3% and 5% GBB) are suggested to present greater solubilization of GBB, which would present greater contact surface for the action of lactic acid bacteria (LAB) and the consequent use of resistant starch (RS) present in GBB as an energy substrate, to the detriment of using lactose as an energy source. Such fact can be corroborated by the lower lactose content in the group with 10% GBB, since, due to the higher concentration of GBB, there was a sedimentation forming insoluble dense lumps of the probable retrograded starch, decreasing the contact surface of the GBB, which hindered a possible amylolytic activity of the LAB on the air, propitiating its action in the degradation of lactose.

Alves (2014) presented several studies reporting the existence of some LAB species capable of converting starch directly into lactic acid, and divided these into two groups: amylolytic and non-amylolytic. Amylolytics produce enzymes which hydrolyze starch into fermentable sugar, and non-amylolytics require

a prior hydrolysis treatment. We suggest future studies to analyze the amylolytic capacity of LAB (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus*) in order to obtain detailed information on new technologies for the food industry. The lactose contents (Table I) are similar to those reported by Borges et al. (2010) when analyzing yogurts with pieces (4.85 g/100g), however, there were low levels of lactose in flavored (4.20 g/100g) and liquid (3.41 g/100g) yogurts.

Determination of PH and titratable acidity

The PH drop curve of GBB yogurts over time, during the yogurts manufacturing stage and during the pre-established periods (1, 7, 14 and 21 days). All groups with the addition of GBB reach the pH of 4.4 (21 days), with the exception of the control group, that reaches pH 4.5 at 21 days.

There were differences ($p < 0.05$) between the periods, with stability of final PH at 4.5 for the control group from 7 days of storage, with no differences ($p > 0.05$) until 21 days of Storage (Table II). Such fact demonstrates that, even during the refrigerated period of yogurt, dairy cultures remain active, fermenting lactose and producing small amounts of lactic acid, resulting in a decrease in pH and increased acidity (Soares et al. 2011).

In Table II, with 3 hours of fermentation, the three groups added with GBB reach pH 4.6. However, the control group only reaches that pH value with 3.5 hours of fermentation.

In conclusion, GBB provides an adequate medium for the activity of LAB by increasing its metabolic activity, considering the lower pH measured in the groups with GBB, which allows the acceleration of the yogurt manufacturing. Such fact is extremely relevant, since it could allow a production in less time, which would represent profits due to greater efficiency in the productive scale in the food industry.

Table II. PH values of GBB yogurts during the manufacturing process and in the periods: 1. 7. 14 and 21 days.

Periods	Group			
	Control	F3	F5	F10
1 h	$^{A}6.4 \pm 0.0^{a}$	$^{A}6.2 \pm 0.0^{b}$	$^{A}6.2 \pm 0.0^{b}$	$^{A}6.2 \pm 0.0^{b}$
2 h	$^{B}5.3 \pm 0.0^{a}$	$^{B}5.0 \pm 0.0^{c}$	$^{B}5.1 \pm 0.0^{b}$	$^{B}5.0 \pm 0.0^{c}$
3 h	$^{C}4.8 \pm 0.0^{a}$	$^{D}4.6 \pm 0.0^{b}$	$^{C}4.6 \pm 0.0^{b}$	$^{D}4.6 \pm 0.0^{b}$
3.5 h	$^{D}4.6 \pm 0.0^{a}$	$^{D}4.6 \pm 0.0^{a}$	$^{C}4.6 \pm 0.0^{a}$	$^{D}4.6 \pm 0.0^{a}$
1 d	$^{C}4.8 \pm 0.0^{a}$	$^{C}4.8 \pm 0.0^{a}$	$^{C}4.6 \pm 0.0^{c}$	$^{C}4.7 \pm 0.0^{b}$
7 d	$^{E}4.5 \pm 0.0^{a}$	$^{E}4.5 \pm 0.0^{a}$	$^{D}4.5 \pm 0.0^{a}$	$^{E}4.5 \pm 0.0^{a}$
14 d	$^{E}4.5 \pm 0.0^{a}$	$^{F}4.4 \pm 0.0^{b}$	$^{E}4.4 \pm 0.0^{b}$	$^{F}4.4 \pm 0.0^{b}$
21 d	$^{E}4.5 \pm 0.0^{a}$	$^{F}4.4 \pm 0.0^{b}$	$^{E}4.4 \pm 0.0^{b}$	$^{F}4.4 \pm 0.0^{b}$

Notes: results expressed as mean ± standard deviation. Means, in the same row, followed by distinct lowercase letters indicate significant differences between the groups; and means, in the same column, preceded by distinct capital letters, indicate significant differences between the periods according to the StudentNewman-Keuls Test at 5% significance. h (hours) - d (days).

The groups with the addition of 3, 5 and 10% of green banana biomass showed stability in the final pH at 4.4 only within 14 days of storage, with no differences ($p > 0.05$) from this period until the 21 days of evaluation in the present study.

The evaluation of pH values during their shelf life is important in quality control, because when a yogurt has low acidity (pH > 4.6), the separation of the serum occurs due to the deficiency of formation of the gel structure with susceptibility to syneresis. As in the case of pH values < 4.0, clot contraction occurs due to a reduction in protein hydration, making syneresis susceptible (Thamer & Penna 2006). In all groups, the pH dropped during the shelf life of all formulations analyzed within the traditional technological standards of yogurt manufacturing. However, all other groups with

the addition of GBB (pH = 4.4) show difference ($p<0.05$) in relation to the control group (pH = 4.5). The lower pH in the groups with addition of GBB may be related to the availability of air present in GBB, which could have prebiotic action for LAB, increasing the activity of lactic acid production. Similar pH values are found by Lucatto (2013), between 4.33 and 4.38, and by Furlani et al. (2020) with marketed yogurts presenting pH = 4.33. When analyzing probiotic yogurts with the addition of GBB (3%, 5% and 10%), Costa et al. (2017) found values similar to those of the present study, ranging between 4.5 and 4.3.

According to Caldeira et al. (2010), pH is a fundamental parameter related to the visual aspect of the dairy product, therefore, dairy products must be submitted to strict quality control programs so there is no phase separation, high acidification influenced by the fermentation time, in addition to changes in sensory characteristics that may make a dairy product undesirable.

There is an increase in lactic acid levels over the shelf life and with variation in titratable acidity levels between 0.20 and 1.22 g/100g (Table III).

The variation range of titratable acidity levels of all groups in the present study are similar to those reported by Furlani et al. (2020) (0.59 to 0.94 g/100g) and by Mendonça et al. (2017) (0.61 to 1.13 g/100g). In Table III, the titratable acidity values during the yogurt manufacturing showed difference ($p < 0.05$) between groups with GBB. However, with 3 hours of fermentation, the three groups added with GBB reached the titratable acidity content of 0.73 g/100g. However, the control group only reaches that value with 3.5 hours of fermentation.

However, the titratable acidity of the group with 10% GBB ranged from 0.20 g/100g (1 hour) to 1.08 g/100g (21 days). Among the analyzed groups, the lowest titratable acidity content at the beginning of yogurt manufacturing and the lowest final pH detected were from the group with 10% GBB. The results of the present study fit the legal standard that determines yogurt with acidity ranging from 0.6 to 1.5 (g lactic acid/100g) (Brazil 2007).

Table III. Titratable acidity values (g/100g) of GBB yogurts during the manufacturing process and in the periods: 1, 7, 14 and 21 days.

Periods	Group			
	Control	F3	F5	F10
1 h	^{E} 0.22 ± 0.00^{b}	^{E} 0.23 ± 0.00^{a}	^{E} 0.23 ± 0.00^{a}	^{E} 0.20 ± 0.00^{c}
2 h	^{D} 0.55 ± 0.00^{c}	^{D} 0.64 ± 0.00^{b}	^{D} 0.68 ± 0.00^{a}	^{D} 0.68 ± 0.00^{a}
3 h	^{D} 0.55 ± 0.00^{b}	^{C} 0.73 ± 0.00^{a}	^{C} 0.73 ± 0.00^{a}	^{C} 0.73 ± 0.00^{a}
3.5 h	^{C} 0.73 ± 0.00^{a}	^{C} 0.73 ± 0.00^{a}	^{C} 0.73 ± 0.00^{a}	^{C} 0.73 ± 0.00^{a}
1 d	^{B} 1.03 ± 0.03^{b}	^{B} 0.97 ± 0.03^{c}	^{B} 1.12 ± 0.03^{a}	^{B} 0.88 ± 0.03^{d}
7 d	^{B} 1.02 ± 0.03^{b}	^{B} 0.93 ± 0.01^{c}	^{B} 1.08 ± 0.02^{a}	^{B} 0.92 ± 0.03^{c}
14 d	^{B} 1.03 ± 0.03^{c}	^{A} 1.12 ± 0.05^{a}	^{B} 1.09 ± 0.00^{ab}	^{A} 1.07 ± 0.02^{ab}
21 d	^{A} 1.16 ± 0.02^{a}	^{A} 1.19 ± 0.05^{a}	^{A} 1.22 ± 0.05^{a}	^{A} 1.08 ± 0.03^{b}

Notes: results expressed as mean (g/100g) ± standard deviation. Means, in the same row, followed by distinct lowercase letters, indicate significant differences between the groups; and means, in the same column, preceded by distinct capital letters, indicate significant differences between the periods according to the Student-Newman-Keuls Test at 5% significance. h (hours) - d (days).

Not all the fruit peels affect yogurt fermentation. At this respect, whereas no difference in yogurt pH with papaya peel flour was detected during storage (Manzoor et al. 2019), titratable acidity in yogurts containing passion fruit peel powder was significantly higher than in their respective control, behavior expected by the homolactic metabolism of the lactic acid bacteria (do Espírito Santo et al. 2012). This is the reason why determinate the prebiotic activity score is important. Although the concomitant effect of green-banana biomass on the growth of lactic acid bacteria in yogurt during storage, reflected in lower pH due to lactic acid produced, the syneresis in samples containing 10% of this agro-industrial co-product was greater (Table V) besides present higher values of adhesiveness and cohesiveness (Table VI). The continuous growth of lactic acid bacteria that produce lactic acid through storage is responsible for the decrease in pH, increasing the tendency to exhibit syneresis, affecting yogurt structure (Pérez-Chabela et al. 2022).

Instrumental color analysis

Regarding the coordinate L* (luminosity), Table IV shows a difference ($p < 0.05$) between the periods. In Control group, the value of L* coordinate ranged from 92.40 (1 day) to 92.96 (21 days). The color of that yogurt became lighter, since over time, due to the pH below the isoelectric point of the milk proteins (4.6), the clot got firm in the course of its shelf life. In addition, the absence of GBB in the control group provided greater luminosity, since its presence darkens the formulation because of the retrograded starch with granular structure, which increases the amount of light absorbed and decreases the amount of reflected light.

The values of L* coordinate indicate a darkening in the course of periods with the addition of GBB (Table IV). Such is corroborated by Riquette et al. (2019), who evaluated the influence of refrigerated and frozen storage time under GBB darkening index. The researchers noted freezing storage significantly increased the rate of darkening of the product after 30 days. The L* values obtained in the present study on the first day and 21st, respectively, for treatments with 3% GBB (91.71 and 91.01), 5% GBB (91.82 and 90.88) and 10% GBB (90.76 and 90.62) are similar to the values found by Silveira et al. (2017) for the treatment with 5% GBB (87.12) and 10% GBB (88).

On the first day, as the concentration of GBB increases, a green color is indicated. Silveira et al. (2017) found values of coordinate a* in the treatment with 5% GBB (0.28) and 10% GBB (0.27) close to the values of the present study. The coordinate a* showed difference ($p < 0.05$)

Table IV. L*, a* and b * coordinate values of GBB yogurts on the 1st and 21st days.

Day	Group			
	Control	F3	F5	F10
L*				
1st day	^{B} 92.40 ± 0.14^{a}	^{A} 91.71 ± 0.04^{c}	^{A} 91.82 ± 0.08^{b}	^{A} 90.76 ± 0.03^{d}
21st day	^{A} 92.96 ± 0.09^{a}	^{B} 91.01 ± 0.08^{b}	^{B} 90.88 ± 0.15^{c}	^{B} 90.62 ± 0.02^{d}
a*				
1st day	^{A} -2.08 ± 0.06^{c}	^{A} -1.57 ± 0.03^{a}	^{A} -1.70 ± 0.06^{b}	^{A} -1.72 ± 0.03^{b}
21st day	^{B} -2.24 ± 0.04^{c}	^{B} -1.37 ± 0.04^{b}	^{B} -1.19 ± 0.04^{a}	^{B} -1.38 ± 0.03^{b}
b*				
1st day	^{A} 10.11 ± 0.04^{b}	^{B} 10.43 ± 0.02^{a}	^{B} 10.02 ± 0.11^{c}	^{B} 10.41 ± 0.04^{a}
21st day	^{B} 9.93 ± 0.14^{c}	^{A} 10.90 ± 0.03^{a}	^{A} 10.50 ± 0.03^{b}	^{A} 10.61 ± 0.17^{b}

between the first day and 21st day of the control group (-2.08 to -2.24) (Table IV). However, the green coloration increased in the control group, which is an opposite trend.

Silveira et al. (2017) found b* coordinate values approximate to the values of the present study in the groups with 5% GBB (12.63) and 10% GBB (13.31) (Table IV). Regarding the b* coordinate, there is a difference ($p < 0.05$) between the first day and the 21st day in control group (10.11 and 9.93).

Syneresis analysis

There were no spontaneous syneresis values in any of the groups, a fact that can be explained by the data collection of that analysis, during which the samples were less disturbed physically, which kept the clots firm and able to retain free water and avoid syneresis. The values were extremely different from those presented in comparison to the other two methods analyzed (by drainage and centrifugation). In a study for the development of a method to characterize the separation of serum spontaneously, the method of spontaneous syneresis was shown to be dependent on the incubation container (Hatanaka 2009).

Analysis of the data from Table V shows a difference ($p < 0.05$) between the periods in the control group and with 3% of GBB related to the progressive decrease in syneresis by drainage, during the shelf life, due to the formation of a firm clot capable of retaining the free water of this solution. In the results of syneresis by drainage (Table V), a difference was observed ($p < 0.05$), with the highest syneresis index of the group with 10% GBB (20.80%) followed by the group 5% GBB (17.12%).

However, in the case of groups with 10% GBB and 5% GBB, the results showed a difference ($p < 0.05$) between the periods (Table V). In those cases, with the progressive increase in syneresis by drainage over the shelf life, GBB sedimentation happened in the treatment with 10% GBB with the decrease in the water holding capacity of those samples and the highest rates of syneresis at 21 days. On one hand, fruit peels flour increased yogurt viscosity and increased water retention, decreasing syneresis during storage time (Vieira et al. 2015, Manzoor et al. 2019).

Table V. Values of syneresis by drainage and centrifugation of yogurts with GBB in the periods: 1, 7, 14 and 21 days.

Day	Group			
	Controle	F3	F5	F10
Drainage (%)				
1 day	$^{B}16.66 \pm 0.27^{c}$	$^{A}17.34 \pm 0.21^{b}$	$^{B}14.26 \pm 0.12^{d}$	$^{B}18.34 \pm 0.44^{a}$
7 days	$^{A}18.50 \pm 0.27^{a}$	$^{B}16.85 \pm 0.11^{c}$	$^{C}13.54 \pm 0.11^{d}$	$^{C}17.48 \pm 0.16^{b}$
14 days	$^{C}16.26 \pm 0.06^{c}$	$^{C}16.32 \pm 0.16^{c}$	$^{A}17.37 \pm 0.31^{b}$	$^{A}21.09 \pm 0.54^{a}$
21 days	$^{D}13.73 \pm 0.70^{c}$	$^{D}13.59 \pm 0.10^{c}$	$^{A}17.12 \pm 0.39^{b}$	$^{A}20.80 \pm 0.56^{a}$
Centrifugation (%)				
1 day	$^{D}32.52 \pm 0.20^{d}$	$^{B}36.21 \pm 0.16^{c}$	$^{A}43.74 \pm 0.27^{b}$	$^{A}46.34 \pm 0.29^{a}$
7 days	$^{C}36.78 \pm 0.09^{c}$	$^{C}35.64 \pm 0.14^{d}$	$^{B}41.48 \pm 0.22^{b}$	$^{C}42.25 \pm 0.16^{a}$
14 days	$^{B}37.66 \pm 0.12^{b}$	$^{B}36.50 \pm 0.28^{c}$	$^{A}43.63 \pm 0.24^{a}$	$^{B}43.46 \pm 0.18^{a}$
21 days	$^{A}42.38 \pm 0.42^{c}$	$^{A}40.65 \pm 0.17^{d}$	$^{A}43.47 \pm 0.27^{b}$	$^{A}46.97 \pm 0.52^{a}$

Notes: results expressed as mean ± standard deviation. Means, in the same row, followed by distinct lowercase letters, indicate significant differences between the groups; and means, in the same column, preceded by distinct capital letters, indicate significant differences between the periods according to the Student-Newman-Keuls Test at 5% significance. h (hours) - d (days).

The presence of native starch in GBB provides some technological drawbacks, since characteristics such as low shear and decomposition resistance, high retrogradation and syneresis, instability of its structure in different conditions of temperature, pH and pressure, hinder the use of native starches (Zieba et al. 2011). However, modifications can be made prior to the application of native starches for industrial purposes. Syneresis was greatly reduced in modified banana starches (Almeida 2013). Other ingredients as pine honey had been employed to reduce syneresis, and producers can use it in yogurt formulations decreasing serum separation (Coskun & Karabulut Dirican 2019).

In the results of Table V, there was a difference ($p < 0.05$) between the periods in the control groups and with 3% of GBB in relation to the progressive increase in syneresis by centrifugation method. In the results of such method (Table V), a difference was noted ($p < 0.05$) with the highest syneresis index coming from group with 10% GBB (46.97%) followed by 5% GBB (43.47%), control (42.78%) and 3% GBB (40.65%) at 21 days. Such facts are related to the phenomenon that occurs after GBB cooling, when some solubilized amylose and amylopectin polymers begin to reassociate and form a precipitate or gel, resulting in increase in GBB opacity and cohesion (retrogradation) (Albuquerque 2011). As a consequence of this phenomenon, syneresis occurred in the group with 10% GBB (46.97%), which presented a higher percentage of GBB added in its formulation among the evaluated groups.

In the groups with 10% GBB and 5% GBB, the results did not show difference ($p > 0.05$) (Table V) between the first and the 21st storage days due to the progressive increase in syneresis by centrifugation over time. Lower values were reported by Cavalcanti (2016) 23.60% and Hatanaka (2009) 23.70%.

Instrumental texture analysis

Table VI shows the results of adhesiveness in which all analyzed groups showed a difference ($p < 0.05$) at 21 days, with the exception of the group with 10% GBB. Nevertheless, in all groups, there was a progressive increase in the following adhesiveness intervals in descending order: control (-1.835 to -1.161 N. s); 5% GBB (-1.514 to -1.389 N. s); 3% GBB (-1.553 to -1.318 N. s); and 10% GBB (-1.514 to -1.389 N. s).

The lower values of adhesiveness in GBB yogurts at 21 days show the interference in adhesiveness, since the control yogurt presented greater adhesiveness. Regarding the values of adhesiveness at 21 days, control group showed a difference ($p < 0.05$) in relation to the other groups. Results similar to those found in the present study were reported by Silveira et al. (2016) (0.59 to 0.74), but lower cohesiveness values were also reported (Table VI).

All analyzed groups showed a difference ($p < 0.05$) between periods, with the exception of the group 10% GBB (Table VI). However, there was an increase in all groups, with the following firmness intervals in descending order: control (0.430 to 0.314 N); 3% GBB (0.389 to 0.317 N); 5% GBB (0.374 to 0.316 N); and 10% GBB (0.367 to 0.329 N). The decrease in the firmness values, as GBB is added in the treatments, demonstrates the interference of GBB, since the control yogurt showed the greatest firmness. Regarding the firmness values at 21 days, the Control group showed a difference ($p < 0.05$) in relation to the other groups. Lillford (2018) found firmness values (0.112 to 0.221 N) below the values of the present study.

The gumminess results of all analyzed groups showed a difference ($p < 0.05$) between periods, with the exception of the group with

Table VI. Values of adhesiveness, cohesiveness, firmness and gumminess of GBB yogurts in the periods: 1, 7, 14 and 21 days.

Day	Group			
	Control	F3	F5	F10
Adhesiveness (N.s)				
1 day	^{A} -1.161 ± 0.221^{a}	^{AB} -1.318 ±0.167^{a}	^{A} -1.329 ± 0.165^{a}	^{A} -1.389 ± 0.254^{a}
7 days	^{A} -1.438 ± 0.194^{a}	^{B} -1.504 ± 0.113^{a}	^{B} -1.720 ± 0.065^{b}	^{A} -1.349 ± 0.119^{a}
14 days	^{A} -1.290 ± 0.184^{b}	^{A} -1.238 ± 0.100^{b}	^{AB} -1.633 ±0.164^{a}	^{A} -1.478 ± 0.183^{a}
21 days	^{B} -1.835 ± 0.054^{b}	^{B} -1.553 ± 0.093^{a}	^{B} -1.605 ± 0.062^{a}	^{A} -1.514 ± 0.157^{a}
Cohesiveness				
1 day	^{A} 0.71 ± 0.05^{a}	^{A} 0.69 ± 0.02^{a}	^{A} 0.70 ± 0.03^{a}	^{A} 0.70 ± 0.03^{a}
7 days	^{A} 0.69 ± 0.01^{ab}	^{A} 0.67 ± 0.02^{c}	^{A} 0.68 ± 0.02^{bc}	^{A} 0.70 ± 0.01^{a}
14 days	^{A} 0.70 ± 0.02^{a}	^{A} 0.69 ± 0.02^{ab}	^{A} 0.67 ± 0.02^{b}	^{A} 0.68 ± 0.01^{ab}
21 days	^{A} 0.67 ± 0.02^{a}	^{A} 0.69 ± 0.02^{a}	^{A} 0.70 ± 0.02^{a}	^{A} 0.69 ± 0.04^{a}
Firmness (N)				
1 day	^{B} 0.314 ± 0.014^{a}	^{B} 0.317 ± 0.025^{a}	^{B} 0.316 ± 0.020^{a}	^{A} 0.329 ± 0.032^{a}
7 days	^{B} 0.342 ± 0.025^{b}	^{B} 0.349 ± 0.015^{b}	^{A} 0.376 ± 0.013^{a}	^{A} 0.337 ± 0.015^{b}
14 days	^{B} 0.331 ± 0.015^{bc}	^{B} 0.316 ± 0.019^{c}	^{A} 0.372 ± 0.023^{a}	^{A} 0.343 ± 0.021^{b}
21 days	^{A} 0.430 ± 0.021^{a}	^{A} 0.389 ± 0.019^{b}	^{A} 0.374 ± 0.020^{b}	^{A} 0.367 ± 0.045^{b}
Gumminess (N)				
1 day	^{B} 22.83 ± 2.00^{a}	^{B} 22.23 ± 1.61^{a}	^{B} 22.44 ± 0.83^{a}	^{A} 23.27 ± 1.66^{a}
7 days	^{B} 24.06 ± 1.51^{b}	^{B} 24.07 ± 1.11^{b}	^{A} 25.93 ± 0.91^{a}	^{A} 23.61 ± 1.05^{b}
14 days	^{B} 23.41 ± 1.00^{b}	^{B} 21.95 ± 1.31^{c}	^{A} 25.34 ± 1.01^{a}	^{A} 23.86 ± 1.08^{b}
21 days	^{A} 29.40 ± 0.52^{a}	^{A} 26.69 ± 0.60^{b}	^{A} 25.99 ± 0.71^{c}	^{A} 24.12 ± 0.84^{d}

Notes: results expressed as mean ± standard deviation. Means, in the same row, followed by distinct lowercase letters, indicate significant differences between the groups; and means, in the same column, preceded by distinct capital letters, indicate significant differences between the periods according to the Student-Newman-Keuls Test at 5% significance. h (hours) - d (days).

10% GBB. In all groups, there was a progressive increase, with the following gumminess intervals in descending order: control (29.40 to 22.83 N); 3% GBB (26.69 to 22.23 n); 5% GBB (25.99 to 22.44 N); and 10% GBB (24.12 to 23.27 N). Regarding the gumminess values (21 days), there is a difference ($p < 0.05$) between the groups. The decrease in gumminess values, as GBB is added in the treatments, demonstrates its interference in gumminess, which is the energy required to chew a semisolid. In the present study, yogurt can be classified as a non-Newtonian and pseudoplastic fluid, so the analyses were carried up to the temperature of 10 °C, since yogurt can undergo texture changes with conservation temperature variations (Table VI).

Microbiological analysis

Table VII shows the values of the microbiological analyses performed in the control yogurt and in the other groups with the addition of GBB.

The results indicate the absence of coliforms at 35°C and 45°C (Table VII). According to the acceptance criteria of the three-class sampling plan, all groups are considered acceptable and are in compliance with current legal requirements (Brazil 2007), which establish a maximum limit of coliforms at 45°C up to 10 NMP/g and 100 NMP/G for coliforms at 35°C/g as microbiological criteria for fermented milks.

The results obtained for the analysis of molds and yeasts classify all groups with acceptable hygienic-sanitary quality, since none

Table VII. Mean results of microbiological analysis performed in GBB yogurts.

Parameters	Group			
	Control	F3	F5	F10
Coliforms at 35 °C (NMP/mL)	< 1.8	< 1.8	< 1.8	< 1.8
Coliformes a 45 °C (MLN/mL)	< 1.8	< 1.8	< 1.8	< 1.8
Molds and yeasts (CFU/mL)	$2.1 \times 10^{3\ b}$	$1.5 \times 10^{2\ c}$	$2.1 \times 10^{1\ d}$	$2.7 \times 10^{4\ a}$
Streptococcus thermophilus (CFU/mL - 1 day)	$7.5 \times 10^{11\ a}$	$4.0 \times 10^{11\ c}$	$6.2 \times 10^{11\ b}$	$2.4 \times 10^{9\ d}$
Streptococcus thermophilus (CFU/mL - 21 days)	$5.3 \times 10^{8\ d}$	$9.3 \times 10^{9\ b}$	$2.5 \times 10^{12\ a}$	$3.2 \times 10^{9\ c}$
Lactobacillus delbrueckii sub sp. Bulgaricus (CFU/mL - 1 day)	$1.3 \times 10^{12\ b}$	$4.3 \times 10^{11\ d}$	$1.4 \times 10^{12\ a}$	$1.2 \times 10^{12\ c}$
Lactobacillus delbrueckii sub sp. Bulgaricus (CFU/mL - 21 days)	$4.1 \times 10^{8\ a}$	$3.6 \times 10^{8\ b}$	$2.5 \times 10^{8\ d}$	$3.4 \times 10^{8\ c}$

Notes: CFU:-colony forming unit; MLN: most likely number. Means, in the same line, followed by distinct lowercase letters indicate significant differences between treatments according to the Student-Newman-Keuls Test at 5% significance.

of the samples reaches the minimum limit of 50 CFU/g as established by the microbiological acceptance criteria according to the normative instruction (IN) N 46 (Brazil 2007). It is worth noting that prebiotic yogurts with 3 and 5% GBB presented the lowest CFU/ml numbers in relation to the other groups. As already reported, the GBB used in the present study, with 23% of RS, may have provided the proliferation of LAB and created an inadequate medium for the growth of molds and yeasts, therefore, the control groups and with 10% of GBB presented the highest fungal counts. Another important factor is the sedimentation and the formation of lumps in the group with 10% GBB that may have decreased the availability of air for lactic acid bacteria.

Regarding the count of *Streptococcus thermophilus* (Table VII) there was difference ($p < 0.05$) between all groups. On the first day, the highest count was from the control group (7.5 Feb 1011 CFU / mL), followed by similar counts in 5% GBB (6.2 Feb 1011 CFU/mL), 3% GBB (4.0 Feb 1011 CFU / mL) and 10% GBB (2.4 Feb 109 CFU / mL). The groups with addition of 3 and 5% GBB favored the viability of the *Streptococcus thermophilus*, however, as mentioned, GBB sedimentation hindered the development of GBB. At 21 days, the highest count of *Streptococcus thermophilus* was from the group 5% GBB (2.5 Feb 1012 CFU / mL), this being the most recommended for the maintenance of viable cells.

Regarding the count of *Lactobacillus delbrueckii subsp. Bulgaricus* (Table VII), there was a difference ($p < 0.05$) between all groups. On the first day, the highest count was 5% GBB (1.4 Feb 1012 CFU / mL), followed by similar counts in the control (1.3 Feb 1012 CFU / mL), 10% of GBB (1.2 Feb 1012 CFU / mL) and 3% of GBB (4.3 Feb 1011 CFU / mL). The addition of GBB was favorable to the initial proliferation of *Lactobacillus delbrueckii subsp. Bulgaricus*. At 21 days, the highest count of *Lactobacillus delbrueckii subsp. Bulgaricus* was from the control group (4.1 Feb 108 CFU / mL). It is worth noting all groups met the legal requirement of viable total lactic acid bacteria counting, of at least 107, (CFU/g), on the final product during its expiration date (Brazil 2007).

CONCLUSIONS

The proximate composition of the formulations with GBB meets the standards of the current Brazilian legislation regarding its constituents. The pH (4.4) of all GBB-containing formulations was reached after 14 days of storage, causing these yogurts to have higher Titratable acidity

values. Yogurts with 10% GBB were darker and had higher syneresis values. In general, yogurts produced with GBB presented texture parameters (adhesiveness, cohesiveness, firmness and gumminess) adequate to the commercialization standard.

The present study suggests the addition of GBB presents prebiotic potential, since it provides an efficient initial proliferation of *Lactobacillus delbrueckii subsp. bulgaricus* and the maintenance of its viability during the 21 days of yogurt storage. However, GBB provides a greater viability of *Streptococcus thermophilus* due to the higher count of colony forming units at 21 days, in relation to the *Lactobacillus delbrueckii subsp. bulgaricus*. All groups have acceptable hygienic and sanitary conditions, since all the results of microbiological analysis are in accordance with the microbiological standards established by the laws in force in Brazil. However, new research is needed to develop new technologies to enable the industrial use of GBB's native starch, minimizing technological drawbacks.

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