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Impact of honey on quality characteristics of goat yogurt containing probiotic *Lactobacillus acidophilus*

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ABSTRACT

This study aimed to analyze the influence of adding stingless bee honey (produced by *Melipona scutellaris* Latrelle - uruçu) on the technological, physicochemical and sensory characteristics of goat yogurt containing probiotic *Lactobacillus acidophilus* La-05 during 28 days of refrigerated storage. Four formulations of goat yogurt were prepared, each varying in the added stingless bee honey amount [(0%, 5%, 10% and 15% (v/v)], but all inoculated with the probiotic *L. acidophilus* La-05 (0.1 g/L of goat milk). The incorporation of stingless bee honey positively affected several characteristics in goat yogurt containing *L. acidophilus* La-05, namely the color, syneresis, viscosity, sensory acceptance and purchase intention. All yogurt formulations presented counts of *L. acidophilus* La-05 above 6.0 log cfu/g by 28 days of storage, but the presence of honey increased the counts (ca. 1 log cfu/g) of *L. acidophilus* La-05 and yogurt starter bacteria until 21 days of storage. The results of this study presented a successful incorporation of both the probiotic *L. acidophilus* La-05 and the honey produced by a native Brazilian stingless bee as ingredients of a new goat dairy product with satisfactory nutritional and sensory quality and added market value because of its potential functional properties.

1. Introduction

The consumer's demand for foods with balanced nutritional composition that may offer additional health benefits has been increasing in recent years. In this context, foods containing probiotic ingredients have gained attention in dairy food industry (Annunziata & Vecchio, 2013; Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013). Studies with probiotics have especially included microorganisms belonging to *Bifidobacterium* and *Lactobacillus* genera, which can be used alone or in co-cultures to achieve a product with satisfactory quality characteristics and good acceptance by consumers (Chapman, Gibson, & Rowland, 2011; Meira et al., 2015). Thus, dairy products are good carriers for probiotics and others bioactive compounds (Balthazar et al., 2016; Batista et al., 2015; Costa, Balthazar, Franco, Cruz, & Conte-Junior, 2014; Esmerino et al., 2015; Morais, Morais, Cruz, & Bolini, 2014; Moriano & Alamprese, 2017).

The addition of flavors and aromas using essences, fruit and/or fruit extracts and honey may be a better option than artificial flavorings for use in the development of new dairy products. This strategy is mostly interesting for use in goat dairy products because should increase nutritional and bioactive values, in addition to become less evident the presence of the goat aroma and aftertaste often associated with a decreased acceptance by consumers (Borba, Silva, Madruga, Queiroga, Souza, & Magnani, 2013). Honey is produced worldwide by over 500 bee species described in 32 genera

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(Michener, 2013, p. 17), and naturally presents some amounts of antioxidants (including flavonoids, phenolics and carotenoids), organic acids, *Maillard* reaction products and amino acids in its composition (Lachman, Orsák, Hejtmánková, & Kovárova, 2010), and specific sugar profile and acidity that bestow unique sensory characteristics (Sousa et al., 2016).

Researchers have shown that honey samples produced by stingless bee (*Melipona* species) have a higher content of antioxidants and different sugar profile when compared to honey samples produced by *Apis* bees (Chuttong, Chanbang, Sringarm, & Burgett, 2016). Among the *Melipona* bee species already identified in the northeastern region of Brazil, special attention has been given to *Melipona scutellaris* Latrelle (uruçu) because this bee species produces different types of honey during the year, according to the availability of botanical species for pollen collection (Sousa et al., 2016).

In recent years, some studies with yogurt made from goat milk (as much as bovine yogurt) have focused on adding artificial sweeteners, fruit juices and pulps (Costa et al., 2015; Ranadheera, Evans, Adams, & Baines, 2012). However, studies about the use of honey as an ingredient in the formulation of these products are still scarce, and to the best of our knowledge, no study is available about the incorporation of honey produced by the stingless bee *M. scutellaris* in a potentially probiotic yogurt. The incorporation of stingless bee honey as an ingredient in goat yogurts may improve their nutritional and sensory characteristics, but the impact on starter cultures must be assessed. Considering these aspects, this study aimed to evaluate the effects of adding honey produced by the stingless bee *M. scutellaris*, at different concentrations, on some technological, physicochemical, microbiological and sensory properties of goat yogurt containing the well-known probiotic strain *L. acidophilus* La-05 during refrigerated storage.

2. Material and methods

2.1. Raw materials

The goat milk was obtained from the Cooperative Farm Capribom® (Monteiro, Paraíba State, Brazil) pasteurized at 65 °C per 30 min and maintained under refrigeration (4 ± 1 °C) until the experiment (maximum period 12 h). Honey from the native stingless bee *M. scutellaris* was collected from a beekeeping facility in the city of Bananeiras - 06° 45′ 00″ S; 35° 38′ 00″ W (Paraíba, Brazil). This honey sample was characterized as a wild multifloral honey produced in a region with characteristic Atlantic forest vegetation (Evangelista-Rodrigues, Silva, Beserra, & Rodrigues, 2005). The honey sample was kept at room temperature overnight before preparing the yogurts. Crystal sugar (União®, Limeira, São Paulo, Brazil) was purchased in a local supermarket (João Pessoa, Paraíba, Brazil), the starter culture (YF-L903 Batch 3212489) comprising *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and the probiotic culture of *Lactobacillus acidophilus* La-05 (Batch 3196076) were freeze dried culture obtained from Christian Hansen® (Valinhos, Minas Gerais, Brazil).

2.2. Characterization of raw materials

The raw goat milk used in preparing the yogurts was analyzed for total solids, fat, protein, lactose, fixed mineral residue, acidity and pH, while the stingless bee honey was analyzed for pH, total acidity, glucose, fructose and fructans content. In the physicochemical evaluation of goat milk, the total solids (method 925.23), proteins (method 939.02), fat (method 2000.18), total sugars (method 923.09), fixed mineral residue (method 930.22), ashes (method 930.30), acidity (lactic acid content) (method 920.124) and pH were determined using standard procedures (A.O.A.C, 2005). For the stingless bee honey, the glucose and fructose content were determined using a high-performance liquid chromatograph with refractive index detector, isocratic pump and oven, equipped with NH2 column (250 × 4.6 mm, 5 μm) Inertsil GL Science, Merck, Luna Phenomenex according to a previously described procedure (Horwitz, Latimer, & George, 2010). The fructans were determined from enzymatic hydrolysis according to a previously described method (Horwitz et al., 2010). The mean values of the assessed physicochemical parameters of goat milk and stingless bee honey used as raw materials for preparing the yogurt are shown in Table 1.

The raw goat milk was also assessed for hygienic sanitary microbiological quality parameters considering the criteria established in the current Brazilian legislation (Brasil, 2000) in terms of numbers of total and thermotolerant coliforms, mold and yeasts and detection of *Salmonella* sp. and *Listeria monocytogenes*. All of these analyses were performed according to standard procedures described elsewhere (A.P.H.A, 2001). The analysis ascertained total and thermotolerant coliform counts <3MPN/mL and mold and yeast counts <1 cfu/mL, in addition to the absence of *Salmonella* sp. and *L. monocytogenes*, confirming the goat milk as being suitable for human consumption and for use as raw material for preparing yogurts.

2.3. Preparation of yogurts

Goat milk was pasteurized (65 °C/30 min), supplemented with 5% (w/v) sucrose and then subjected to an additional heat treatment (91 ± 1 °C/10 min). Next, the milk was cooled to 45 °C, and the cultures were inoculated at a concentration of 0.4 g/L for the starter culture consisting of *S. thermophilus* and *L. bulgaricus,* and a concentration of 0.1 g/L for the probiotic culture containing *L. acidophilus* La-05 defined according to previous testing to guarantee a minimal final count of approximately 7 log cfu/mL. In these previous assays, 12 different goat yogurt formulations were prepared using different concentrations of honey (5, 10, 15, 20 and 25%) *starter* (0.3 and 0.4%) and probiotic cultures (0.01 and 0.02%), and the formulation that presented the best physicochemical and sensory quality characteristics were selected for further studies. Fermentation was performed in an incubator (45 ± 1 °C/4 h), and the end point of yogurt fermentation was based on verification of clot firmness and pH value, which should reach a maximum of 4.5. Subsequently, the product was cooled to 4 ± 1 °C, and the clot was broken by manual stirring with a glass rod. Then, stingless bee honey was added to the different formulations (w/v) at 5% (termed

Table 1
Mean values (±standard deviation) of physicochemical parameters of goat milk and stingless bee honey used as raw material for yogurt preparation.

Parameters	Goat milk
Total solids (g/100 g)	11.48 (±0.02)
Protein (g/100 g)	3.57 (±0.07)
Fat (g/100 g)	3.01 (±0.01)
Lactose (g/100 g)	4.30 (±0.04)
Fixed mineral residue (g/100 g)	0.70 (±0.01)
Acidity (g/100 g)	0.13 (±0.01)
pH	6.78 (±0.03)
Parameters	**Stingless bee honey**
Total solids (g/100 g)	72.50 (±0.02)
pH	4.43 (±0.02)
Total acidity (mEq/kq)	116.19 (±0.50)
Fructose (g/100 g)	32.80 (±0.06)
Glucose (g/100 g)	28.61 (±0.06)
Fructooligosaccharides (g/100 g)	0.45 (±0.03)

YH5), 10% (termed YH10) and 15% (termed YH15) (w/v) under aseptic conditions, and the mixture was softly homogenized. One yogurt formulation without incorporation of honey as an ingredient was termed Y0 (control). Finally, filling was performed in high-density polyethylene bottles, and the product was stored under refrigeration (4 ± 2 °C) until further analysis. All yogurt formulations were prepared in triplicate on three different occasions.

After processing, the yogurts were subjected to technological, physicochemical and microbiological analyses at different time points during 28 days of refrigerated storage, while sensory analyses were performed only on the 14th day of refrigerated storage.

2.4. Technological analyses of yogurts

In technological characteristic assessment, the color, syneresis, water retention capacity (WRC) and apparent viscosity of the different yogurt formulations were determined. The instrumental color was measured using a CR-400 colorimeter (Minolta Co. Osaka, Japan) The CIE Lab colour scale (L*a*b*) was used with a D65 illuminant (standard daylight) and 108 angle. The L*, a* and b* parameters were determined according to International Commission on Illumination (C.I.E, 1986; Balthazar et al., 2015). The values for light and dark are represented by L (luminosity), for red by + a, for green by −a, for yellow by + b and for blue by −b. Using reference plates, the apparatus was calibrated in the reflectance mode with specular reflection excluded. Measurements were performed in triplicate using the inner section of the yogurts immediately after opening the bottles. Susceptibility to syneresis was determined by the drainage method (Hassan, Frank, Schmidt, & Shalabi, 1996). Water retention capacity was assessed using a refrigerated centrifuge (Model CT-5000R) (Harte, Luedecke, Swanson, & Barbosa-Cánovas, 2003). Apparent viscosity was determined using a Thermo Haake (VT550 model, Thermo Haake, Karlsruhe, Germany) with concentric cylinder geometry (MV/MV1) operated manually. Readings were taken at established equilibrium temperature (10 min/10 °C in a thermostatic bath) under a constant shear rate of 106 s^{-1} after 10 s deformation, and the values are expressed in mPa s (Skriver, Holstborg, & Qvist, 1999).

2.5. Physicochemical analysis of yogurts

Physicochemical analysis performed according to standard procedures (A.O.A.C, 2005), consisted of determining the moisture (method 925.09), total solids (method 930.30), fixed mineral residue (FMR), fat (method 2000.18), protein (method 939.02), total sugars (method 923.09), titratable acidity (method 920.124) and pH was determined using a digital pH. Moisture and total solids were determined by drying, the FMR was quantified by carbonization followed by incineration in a muffle furnace, the fat content was determined according to the Gerber method, protein was quantified using the Micro-Kjeldahl method, and total sugars were quantified using the Fehling method. The pH was measured in a digital potentiometer (model Q400AS; Quimis, Diadema, São Paulo, Brazil), and acidity (in lactic acid) was determined by titration and expressed as g/100 g.

2.6. Microbiological analysis of yogurts

The hygienic and sanitary quality was assessed as previously described for milk in section 2.2. and complemented with specific criteria of current regulation (Brasil, 2007). For the lactic acid bacteria counts at each pre-established time, the LC agar (peptone 10 g/L, meat extract 4 g/L, yeast extract 1 g/L, Tween 80 1 mL, KH2PO4 2 g/L, sodium acetate.3H20 3 g/L, tri-ammonium citrate 1 g/L, MgSO4·7H2O0.2 g/L, MnSO4·4H2O 0.05 g/L, acid hydrolysate of casein 1 g/L and bacteriological agar 12 g/L) and *C*-MRS agar (MRS agar plus 0.5 g/L cystein) were used for counting *L. acidophilus* La-05 and the starter bacteria group (*S. thermophilus* and *L. bulgaricus*), respectively. The counts of starter bacteria group were found considering the differences (subtraction) in counts obtained in LC agar from those obtained in *C*-MRS agar (Lima et al., 2009). The counts were expressed as the log of the colony forming units per mL of yogurt (log cfu/mL).

2.7. Sensory analysis of yogurts

Sensory analysis of the different yogurt formulations were performed on the 14th day of refrigerated storage. The yogurts were subjected to tests of acceptance and relative preference among samples as described elsewhere (Faria & Yotsuyanagi, 2002). In the acceptance test, well-known pre-established criteria were used (Stone & Sidel, 1993, p. 338). These tests were performed using 63 non-trained panelists (13 men and 50 women) aged 20–45 years. The tasting panel consisted of students and staff recruited from the Federal University of Paraíba (João Pessoa, Brazil), pre-selected according to interest and with a habit of consuming yogurt. All sensory evaluation assays were performed with the same panelists who worked in individual booths with controlled temperature and lighting conditions. Each panelist was served 20 mL of each yogurt formulation on a small white glass at 6 °C coded with a random three-digit number. All yogurt formulations were served simultaneously using a blind method in a randomized sequence (assuring that each panelist was served with a specific order of samples) immediately after being taken out of the refrigerated storage. The acceptability of appearance, colour, flavour, taste, texture and overall acceptance were evaluated on a nine-point unstructured hedonic scale ranging from 9 (like very much) to 1 (dislike very much) (Gaze et al., 2015). Purchase intention was evaluated using a five point unstructured hedonic scale ranging from 5 (certainly would purchase) to 1 (certainly would not purchase). For the preference ranking test, the panelists were asked to choose the most and least preferred sample based on their overall impressions.

2.8. Statistical analysis

The data from all analysis performed with raw materials (goat milk and honey) were evaluated in triplicate and are expressed as the means and standard deviations. Data of technological, physicochemical, microbiological and sensory acceptance test of the yogurts were subjected to analysis of variance (ANOVA), and the means were compared using Tukey's post hoc test with a $p \leq 0.05$. For this, Statistical Analysis System software, version 8.12 (SAS Institute, Inc. Cary, NC) (SAS Institute 1999) was used. The data of the ordering-preference sensory test of yogurts were analyzed using the Friedman's test and the table of Newell and MacFarlane to determine if the samples differed significantly among them (Faria & Yotsuyanagi, 2002).

3. Results and discussion

3.1. Technological characteristics of yogurts

The mean values for color parameters for different goat yogurt formulations are presented in Table 2. The color parameters results were expressed for brightness L* (0 black, 100 white), a* (− green, + red) and b* (− blue, + yellow) (Anupama, Bhat, & Sapna, 2003). The L* value for all yogurt formulations decreased proportionally with the increased amount of added stingless bee honey,

Table 2
Mean values (±standard deviation) of color parameters of goat yogurt containing *L. acidophilus* La-05 and stingless bee honey at different concentrations, during 28 days of refrigerated storage.

Parameters	Days	Yogurt formulations			
		Y0	YH5	YH10	YH15
L*	1	91.06 (±0.07)^{Aba}	86.77 (±0.04)^{Bb}	84.98 (±0.02)^{Dc}	82.21 (±0.02)^{Ed}
	7	91.23 (±0.13)^{Aa}	89.69 (±0.06)^{Ab}	88.44 (±0.14)^{Ac}	87.36 (±0.05)^{Dd}
	14	91.11 (±0.11)^{Aa}	89.84 (±0.05)^{Ab}	88.29 (±0.18)^{ABc}	87.56 (±0.02)^{Cd}
	21	91.11 (±0.11)^{Aa}	89.62 (±0.25)^{Ab}	88.00 (±0.01)^{Cc}	87.81 (±0.07)^{Bd}
	28	90.85 (±0.03)^{Ba}	89.44 (±0.36)^{Ab}	88.03 (±0.02)^{BCc}	88.35 (±0.03)^{Ac}
a*	1	−3.20 (±0.01)^{Db}	−3.37 (±0.01)^{Ed}	−3.27 (±0.02)^{Cc}	−3.04 (±0.04)^{Da}
	7	−3.12 (±0.02)^{Cc}	−3.04 (±0.00)^{Db}	−2.94 (±0.04)^{Ba}	−2.93 (±0.03)^{Ca}
	14	−3.09 (±0.01)^{Cc}	−2.63 (±0.03)^{Bb}	−2.59 (±0.03)^{Ab}	−2.50 (±0.02)^{Aa}
	21	−2.88 (±0.03)^{Bd}	−2.75 (±0.02)^{Cc}	−2.58 (±0.03)^{Aa}	−2.65 (±0.03)^{Bb}
	28	−2.57 (±0.04)^{Ab}	−2.39 (±0.02)^{Aa}	−2.64 (±0.03)^{Ac}	−2.43 (±0.01)^{Aa}
b*	1	5.79 (±0.06)^{Cd}	6.47 (±0.08)^{Bc}	7.35 (±0.02)^{Cb}	8.22 (±0.03)^{Ca}
	7	5.79 (±0.10)^{Cd}	6.73 (±0.03)^{Bc}	7.62 (±0.03)^{Bb}	8.34 (±0.04)^{Ca}
	14	5.86 (±0.02)^{BCd}	6.72 (±0.03)^{Bc}	7.47 (±0.01)^{Cb}	8.36 (±0.10)^{Ca}
	21	5.95 (±0.03)^{Bd}	6.62 (±0.12)^{Bc}	7.75 (±0.12)^{Bb}	8.63 (±0.03)^{Ba}
	28	6.36 (±0.02)^{Ab}	8.63 (±0.37)^{Aa}	8.77 (±0.01)^{Aa}	8.88 (±0.02)^{Aa}

Y0 - yogurt containing added *L. acidophilus* La-05, without stingless bee honey; YH5 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 5% (v/v); YH10 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 10% (v/v); and YH15 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 15% (v/v).
$^{a\text{-}d}$ Means ± standard deviations with different lowercase letters in the same row denote difference among the different formulations, based on the Tukey's test ($p \le 0.05$).
$^{A\text{–}D}$ Means ± standard deviations with different uppercase letters in the same column denote difference among the different storage periods, based on the Tukey's test ($p \le 0.05$).

probably due to the presence of naturally occurring honey colour compounds. At the end of the assessed storage period, there was an increase in brightness (L* value) in the added honey yogurt formulations; otherwise, the value for brightness decreased in yogurt without added honey. The higher brightness values result in lighter objects, which may be associated with the white color characteristic of goat milk matching the color of honey.

The a* values significantly differed among the assessed yogurt formulations, as these values were higher with the increase of the added honey amounts in yogurts and the course of the assessed storage period interval. This color characteristic in yogurts may be associated with the color characteristics of the added honey, and with the oxidation of fatty acids and protolithic activity naturally occurring in yogurts (Farkye, Smith, & Schonrock, 2001). The values of the component b* also increased in yogurt formulations when the concentration of added honey increased and over the course of the storage time period. However, yogurt formulations containing added honey that presented a difference in b* values among them ($p \le 0.05$) in the early monitored storage time points, showed no difference ($p > 0.05$) at the last assessed storage period (28th day). Such changes in goat yogurt color at the end of the storage period may have occurred because of the color of added honey and the possible presence of Maillard reaction derived compounds (Farkye et al., 2001).

The syneresis behavior observed in goat yogurt formulations is shown in Table 3. At the end of the evaluated storage period, all yogurt formulations, with exception of YH10, presented higher ($p \le 0.05$) syneresis compared to the earliest day of storage. At this storage time point, the yogurt with 15% added honey (Y15) showed lower ($p \le 0.05$) susceptibility to syneresis compared to the other yogurt formulations. This fact may be associated with high osmolarity of honey as an ingredient in the prepared yogurt, which would attract water to the yogurt-forming casein micelles, reducing the water release to the surroundings. Conversely, when the three-dimensional protein networks become denser, it gradually loses the capacity to attract the whey, at which point it is expelled and clearly observed on the surface of fermented dairy beverages (Bezerra, Souza, & Correia, 2012). This behavior may have occurred in the yogurt without added honey at the latest assessed storage period (28th day) because this formulation

Table 3
Mean values (±standard deviation) of syneresis and water retention capacity (WRC) of goat yogurt containing *L. acidophilus* La-05 and stingless bee honey at different concentrations, during 28 days of refrigerated storage.

	Days	Yogurt formulations			
		Y0	YH5	YH10	YH15
Syneresis (%)	1	52.42 (±0.50)^{BCa}	49.66 (±0.08)^{Cb}	53.27 (±0.34)^{Aa}	49.75 (±0.01)^{Cb}
	7	53.36 (±0.33)^{Ba}	52.88 (±0.53)^{Aba}	51.04 (±0.09)^{Cb}	51.01 (±0.58)^{Bb}
	14	51.62 (±0.33)^{Ca}	52.07 (±0.35)^{Ba}	53.28 (±0.58)^{Aa}	51.43 (±0.52)^{ABa}
	21	51.62 (±0.33)^{BCa}	50.40 (±0.27)^{Cb}	52.13 (±0.12)^{Ba}	52.29 (±0.12)^{Aa}
	28	55.45 (±0.48)^{Aa}	53.51 (±0.12)^{Ab}	53.55 (±0.39)^{Ab}	52.45 (±0.19)^{Ac}
WRC (%)	1	49.19 (±0.18)^{Aa}	48.09 (±0.05)^{Bb}	49.40 (±0.18)^{Aa}	49.53 (±0.38)^{Aa}
	7	47.85 (±0.15)^{Bc}	49.51 (±0.30)^{Aa}	48.64 (±0.11)^{Bb}	49.48 (±0.41)^{Aa}
	14	45.62 (±0.36)^{Cd}	46.18 (±0.10)^{Cc}	46.78 (±0.17)^{Cb}	48.17 (±0.12)^{BCa}
	21	44.55 (±0.23)^{Dc}	46.40 (±0.33)^{Cb}	46.91 (±0.10)^{Cab}	47.47 (±0.24)^{Ca}
	28	43.60 (±0.29)^{Ec}	45.71 (±0.44)^{Cb}	45.66 (±0.25)^{Db}	47.51 (±0.22)^{Ca}

Y0 - yogurt containing added *L. acidophilus* La-05, without stingless bee honey; YH5 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 5% (v/v); YH10 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 10% (v/v); and YH15 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 15% (v/v).
$^{a\text{–}c}$ Means ± standard deviations with different lowercase letters in the same row denote difference among the different formulations, based on the Tukey's test ($p \le 0.05$).
$^{A\text{–}D}$ Means ± standard deviations with different uppercase letters in the same column denote difference among the different storage periods, based on the Tukey's test ($p \le 0.05$).

presented the highest syneresis ($p \leq 0.05$); the presence of stingless bee honey seemed to influence the syneresis process in the assessed goat yogurt formulations.

The results for water retention capacity (WRC) in goat yogurt formulations are presented in Table 3. At the 28th day of storage, the WRC was lower ($p \leq 0.05$) when compared to the first day of storage for all yogurt formulations, and the WRC was notably lower ($p \leq 0.05$) in the yogurt without added honey. This WRC behavior was probably associated with the syneresis, as the increasing release of the liquid phase in yogurt was concurrent with smaller WRC in the different yogurt samples over time. This result may also be associated with the metabolism of lactic acid bacteria present in yogurt and other aspects related with the manufacture of this product. In the present study, an increase in acidity content during storage was observed in all assessed yogurt formulations (Fig. 2). This may be associated with the increase in water release (syneresis) in these samples because of possible protein denaturation as a consequence of pH decrease up to the isoelectric point of proteins, when this causes destabilization of casein micelles and consequent loss of liquid (Bezerra et al., 2012).

The results for the estimation of apparent viscosity of goat yogurt formulations are shown in Fig. 1. In this study, the goat yogurt without added honey (Y0) was the only formulation that presented a decrease ($p \leq 0.05$) in apparent viscosity during storage. All the formulations containing different added stingless bee honey amounts experienced an increase ($p \leq 0.05$) in apparent viscosity over time.

Honey is considered a high-viscosity fluid, and at 4 °C (refrigeration temperature), the honey produced by the *M. scutelaris* stingless bee behaves as a pseudo-plastic offering greater resistance (Pereira, 2003) and higher viscosity for yogurts. Furthermore, the addition of honey increase total solids content and increase the consistency of the product. Adding the honey led to an initial increase in the yogurt apparent viscosity that was directly proportional to the added honey amount. However, over the assessed storage period the yogurt formulations containing added honey revealed increased oscillation in viscosity values, which may also be associated with the characteristic of stingless bee honey as a pseudo-plastic fluid, since these fluids are resistant to force applied on them but this resistance is not constant (Pereira, 2003).

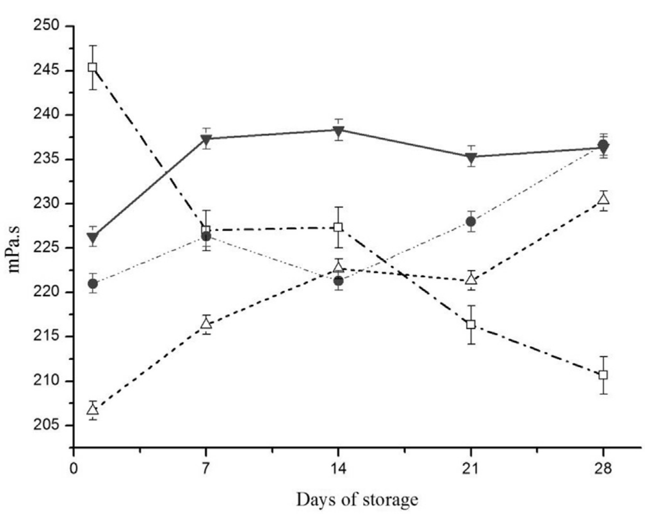

Fig. 1. Apparent viscosity of goat yogurt containing *L. acidophilus* La-05 and stingless bee honey at different concentrations, during 28 days of refrigerated storage. Y0 (□): goat yogurt containing added *L. acidophilus* La-05, without stingless bee honey; YH5 (▼): goat yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 5%; YH10 (△): goat yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 10%; YH15 (●): goat yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 15%.

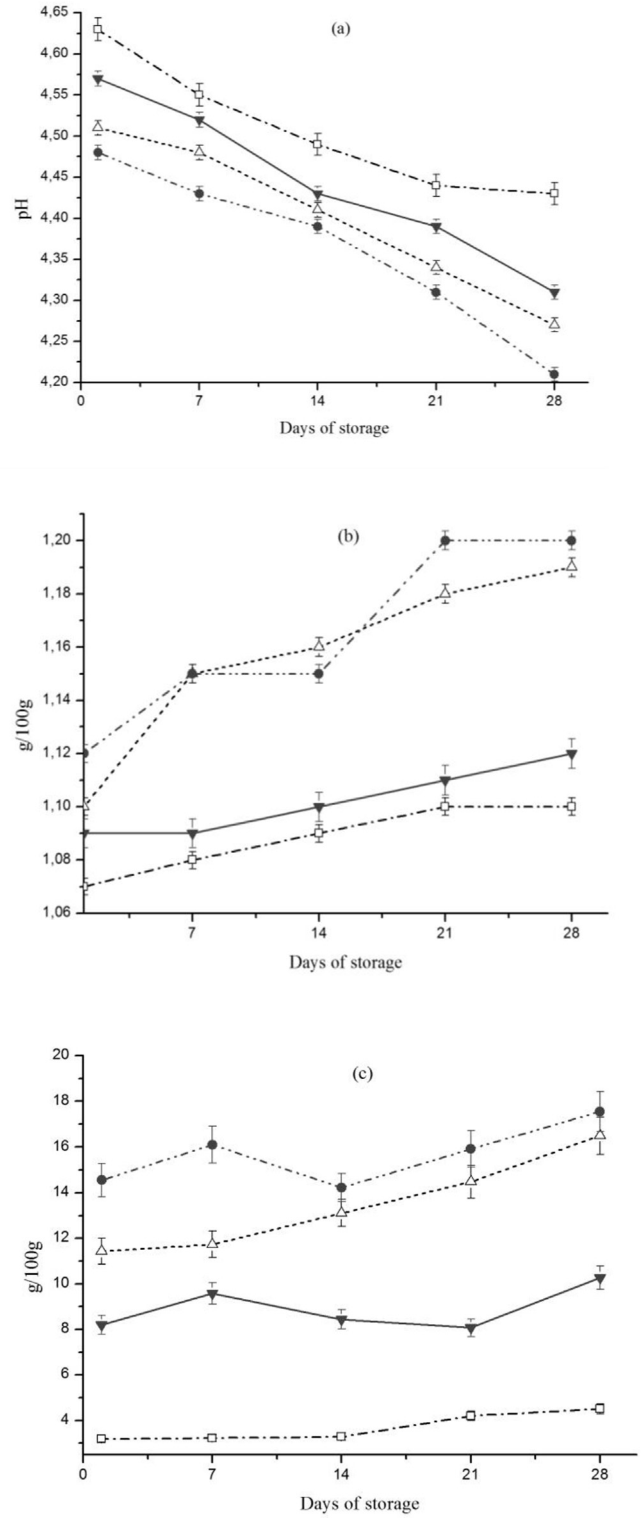

Fig. 2. Mean values of pH (a), acidity (b) and total sugars (c) of goat yogurt containing *L. acidophilus* La-05 and stingless bee honey at different concentrations, during 28 days of refrigerated storage. Y0 (□): goat yogurt containing added *L. acidophilus* La-05, without stingless bee honey; YH5 (▼): goat yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 5%; YH10 (△): goat yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 10%; YH15 (●): goat yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 15%.

Table 4
Mean values (±standard deviation) of physicochemical parameters of goat yogurt containing *L. acidophilus* La-05 and stingless bee honey at different concentrations, during 28 days of refrigerated storage.

Parameters	Days	Yogurt formulations			
		Y0	YH5	YH10	YH15
Total solids (g/100 g)	1	14.18 (±0.04)^{Cd}	16.92 (±0.02)^{Bc}	18.79 (±0.30)^{Bb}	19.90 (±0.05)^{Ba}
	14	15.99 (±0.11)^{Ad}	17.47 (±0.16)^{Ac}	19.62 (±0.02)^{Ab}	20.39 (±0.23)^{Ba}
	28	14.88 (±0.04)^{Bd}	17.27 (±0.11)^{ABc}	19.65 (±0.06)^{Ab}	21.37 (±0.07)^{Aa}
Protein (g/100 g)	1	3.98 (±0.06)^{Aa}	3.93 (±0.01)^{Aa}	3.75 (±0.12)^{Aab}	3.47 (±0.01)^{Cb}
	14	3.80 (±0.00)^{Aa}	3.75 (±0.01)^{Ba}	3.81 (±0.06)^{Aa}	3.76 (±0.01)^{Aa}
	28	3.80 (±0.00)^{Aa}	3.71 (±0.06)^{Ba}	3.75 (±0.01)^{Aa}	3.66 (±0.01)^{Ba}
Fat (g/100 g)	1	3.00 (±0.00)^{Aa}	2.75 (±0.01)^{Ab}	2.80 (±0.00)^{Ab}	2.70 (±0.00)^{Ab}
	14	3.00 (±0.00)^{Aa}	2.75 (±0.01)^{Ab}	2.80 (±0.00)^{Ab}	2.70 (±0.00)^{Ab}
	28	3.00 (±0.00)^{Aa}	2.80 (±0.00)^{Ab}	2.70 (±0.00)^{Abc}	2.65 (±0.01)^{Ac}
Fixed mineral residue (g/100 g)	1	0.82 (±0.01)^{ABb}	0.87 (±0.01)^{Aa}	0.77 (±0.01)^{ABc}	0.64 (±0.03)^{Ad}
	14	0.88 (±0.04)^{Aa}	0.79 (±0.01)^{Ba}	0.74 (±0.03)^{Aa}	0.65 (±0.01)^{Ab}
	28	0.71 (±0.05)^{Ba}	0.68 (±0.02)^{Ca}	0.67 (±0.01)^{Ba}	0.65 (±0.03)^{Aa}

Y0 - yogurt containing added *L. acidophilus* La-05, without stingless bee honey; YH5 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 5% (v/v); YH10 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 10% (v/v); and YH15 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 15% (v/v).
$^{a-d}$ Means ± standard deviations with different lowercase letters in the same row denote difference among the different formulations, based on the Tukey's test ($p \le 0.05$).
$^{A-C}$ Means ± standard deviations with different uppercase letters in the same column denote difference among the different storage periods, based on the Tukey's test ($p \le 0.05$).

3.2. Physicochemical analyses of yogurts

The mean values of the physicochemical parameters of goat yogurts formulations are presented in Table 4. At all assessed storage time points, the yogurt formulations containing 15% added stingless bee honey (YH15) presented higher ($p \le 0.05$) total solids content, likely due to the higher amount of honey contained therein. Furthermore, there were increases ($p \le 0.05$) in total solids content during storage for the goat yogurt formulations Y0, YH10 and YH15. Regarding the FMR content, there was no difference ($p > 0.05$) between the assessed yogurt formulations over the assessed storage period, with exception to YH5. Protein content did not differ ($p > 0.05$) in yogurt formulations Y0 (3.98± 0.06%), YH5 (3.93± 0.01%) and YH10 (3.75± 0.12%), with smaller content ($p \le 0.05$) only in YH15 (3.47± 0.01%) on the 1st day of storage. This finding may be due to the higher amount of honey in YH15 causing a higher dilution in yogurt preparation, therefore reducing the total protein amount. Regarding the fat content, there were no differences ($p > 0.05$) during storage for all yogurt formulations.

The mean values of pH, acidity and total sugars of goat yogurt formulations are shown in Fig. 2. The addition of stingless bee honey, due to its natural acidity (total acidity of 116.19 mEq/kg and low pH of 4.43; Table 1), may have led to the slight reduction of the initial pH (ca. 0.2 units in YH15) in yogurt formulations containing added stingless bee honey. The acidity of honey comes from the naturally occurring organic acids in its composition (Chuttong et al., 2016). The initial pH of the assessed yogurt formulations continuously decreased until the 28th day of storage, and the yogurt samples containing added honey exhibited lesser pH that was proportional to the added honey amount in preparation (Y0 pH from 4.63 ± 0.04 to 4.43 ± 0.01, YH5 from pH 4.57 ± 0.02 to 4.31 ± 0.01, YH10 pH from 4.51 ± 0.01 to 4.27 ± 0.02, YH15 pH from 4.48 ± 0.01 to 4.21 ± 0.01). At the same time, the acidity also increased over the course of the storage period in all yogurt formulations, with a very clear separation of sample YH10 and YH15 presenting the lowest pH values (approx. pH 4.21 ± 0.01–4.51 ± 0.01), followed by sample YH5 presenting pH values closer to those observed for Y0 (approx. pH 4.43 ± 0.01–4.63 ± 0.04).

These changes in pH values observed in yogurt formulations containing added stingless bee honey could be related to the presence of extra fermentable compounds in *M. scutellaris* honey, namely sugars (glucose 28.61 g/100 g, fructose 32.80 g/100 g, fructooligosaccharides 0.45 g/100 g; Table 1); but not exclusively, because the added honey concentration seemed to stimulate the lactic acid metabolism and consequently the acidification of yogurt formulations. This increased acidity in yogurts containing honey may also be associated with the presence of prebiotic oligosaccharides in stingless bee honey because these compounds at such a small amount may promote the growth and/or the metabolic activity of lactic acid bacteria, as previously observed for the effect of sesame honey toward lactobacilli and bifidobacteria (Das, Datta, Mukherjee, Ghosh, & Dhar, 2015). The acidity increase and pH decrease that progressively occurred over the assessed storage period in all goat yogurt formulations may also have contributed to the increased syneresis. A previous study with yogurt containing added fruit observed a reduction in pH values during storage, indicating the increase of the acidity over time (Ranadheera et al., 2012).

Moreover, the fact that the high acidity has not resulted in higher syneresis and lower WRC in goat yogurt formulations should be related with the characteristic honey's physical properties. The honey is a high viscosity fluid, but when maintained under refrigeration temperature it behaves like a pseud-plastic fluid, offering better resistance to yogurt (Pereira, 2003). This property could contribute for the water molecule to be interconnected with carbohydrates and/or proteins, decreasing the possible impact of higher acidity in increasing syneresis.

Additionally, an increase ($p \le 0.05$) in the initial total sugar content was observed at the end of the assessed storage period for all yogurt formulations, but this increase was more evident in formulations YH10 and YH15, and on the 14th or 21st day of storage. The increase in sugar content in honey samples has been associated with the action of enzymes that are capable to catalyse the bee enzyme transferD-glucopyranosyl sucrose to a carbohydrate receptor (Shin & Ustunol, 2005). Still, one could also assume that certain microbial enzymes released to goat yogurt may have similar action toward honey sugars and release greater amounts of reducing sugars in yogurt containing added honey, as detected in this study. Bacteria are capable to use the free glucose originally available in growth substrate, and eventually through the action of enzymes produced by lactic acid bacteria may release additional free sugar molecules from honey in this matrix. These new available sugar molecules may promote the bacterial metabolism and coincide with the overall slight increase of total sugars and increase (or recovery) in counts of *L. acidophilus* La-05 in yogurt formulations

(Fig. 3). The smallest increases in total sugars content in Y0 over the monitored storage period may be related with the use of sucrose (5%, w/v) as an ingredient in this formulation with no honey addition. Sucrose can be hydrolyzed by enzymes of *starter* bacteria with release of glucose and fructose, transforming a non-reducing sugar into reducing sugars that can be quantified by the analytical method used in this study.

3.3. Microbiological analyses

The results of hygienic sanitary microbiological analysis revealed that all prepared goat yogurt formulations were suitable for human consumption throughout the assessed refrigerated storage period (data not shown).

The results of the viable counts of the starter bacteria group (composed of *S. thermophilus* and *L. bulgaricus*) and *L. acidophilus* La-05 in goat yogurt containing or not containing added stingless bee honey during refrigerated storage are presented in Fig. 3. The counts of starter bacteria group at the first day of storage were approximately 8.5 log cfu/mL, and decreased to approximately 7.5 log cfu/mL and 7.2 log cfu/mL on the 14th and 21st days of storage, respectively, in yogurt formulations containing added stingless bee honey. The bacterial counts found after the fermentation (1st day of storage) in goat yogurt containing or not containing added stingless bee honey were higher than the minimum counts recommended to characterize a fermented milk as yogurt (Codex Alimentarius, 2011). High counts in goat yogurt after fermentation were already found to *S. thermophilus* (8.99 log cfu/mL), *L. delbrueckii* ssp. *bulgaricus* (7.83 log cfu/mL) and *L. acidophilus* (9.49 log cfu/mL) (Costa et al., 2014), as well as to *S. thermophilus* (9–11 log cfu/mL) and *L. bulgaricus* (8–9 log cfu/mL) in sheep milk yogurt (Balthazar et al., 2016; Costa et al., 2014).

The counts of starter bacteria group in yogurt formulation without added stingless bee honey dropped to approximately 6.8 log cfu/mL and 6.3 log cfu/mL on the 14th and 21st days of storage, respectively. The starter bacteria group presented a linear decrease in counts over the assessed storage period in all yogurt formulations; however, these counts were higher in yogurts containing stingless bee honey from 7th to 21st day of storage. At the end of the assessed storage period (28th day), the counts of starter bacteria group were close (approx. 6.5 log cfu/mL) in all yogurt formulations regardless of the addition of stingless bee honey. Decreases (1–2 log cycle) in starter bacteria counts has been an expected behavior during yogurt storage (Balthazar et al., 2016; Varga, Süle, & Nagy, 2014).

For *L. acidophilus* La-05, a similar survival/death curve shape was found in all assessed goat yogurt formulations, although these counts were always higher ($p \leq 0.05$) in yogurts containing added stingless bee honey from the 14th day of storage onward. A similar behavior of sharp reduction in counts of *L. acidophilus* La-05 occurred from 7th to 14th day of storage in all yogurt formulations (approximately 8.1 to 7.3 log cfu/mL), followed by an increase and decrease in counts at the 21st and 28th day of storage, respectively (Fig. 3a and b).

In most cases, the counts of *L. acidophilus* La-05 presented a similar pattern to those found for the starter bacteria group, with a clear separation of greater ($p \leq 0.05$) counts in yogurts containing added honey from the 14th day of storage onward, but with no difference ($p > 0.05$) among counts found in yogurts containing the different added stingless bee honey concentrations. For both starter bacteria groups and *L. acidophilus* La-05, the addition of stingless bee honey in yogurts appeared to assure the maintenance of higher counts in comparison to the formulation without added honey over time. These differences in counts of *L. acidophilus* La-05 were more pronounced from the 14th day of storage onward, when an inversion of sugar content coincidentally occurred in the yogurts (mostly in YH15) (Fig. 2c).

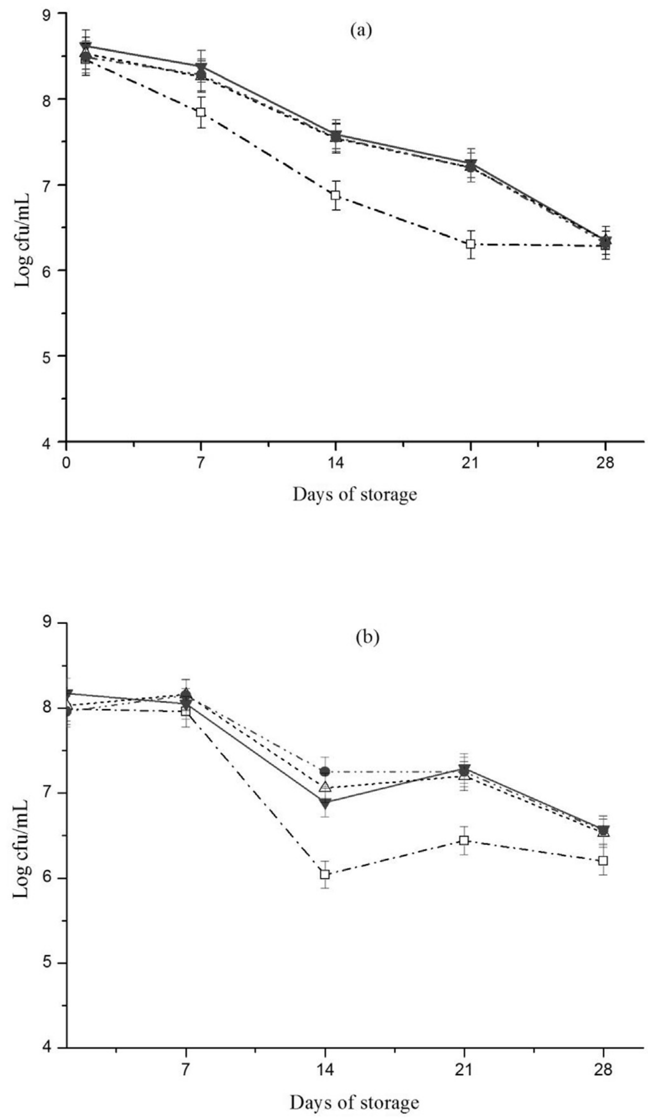

Fig. 3. Viable cell counts of bacteria starter group (a) and *L. acidophilus* La-05 (b) in goat yogurt containing stingless bee honey or not added at different concentrations, during refrigerated storage. Y0 (□): goat yogurt containing added *L. acidophilus* La-05, without stingless bee honey; YH5 (▼): goat yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 5%; YH10 (△): goat yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 10%; YH15 (●): goat yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 15%.

All together, these findings suggest that for *L. acidophilus* La-05, i) a minimal amount of available sugar in assessed yogurt formulations was necessary to promote its growth; ii) from the 14th day of storage onward, some compounds were released in the added-honey yogurts, probably fermentable sugars, that may promote its growth, but this was not enough to maintain or increase the counts between the 21st and 28th day of storage, where they slightly decreased; and iii) the incorporation of added stingless bee honey in the assessed goat yogurts may represent a possible functional advantage, i.e. a probiotic growth promoting effect. Despite all of these aspects, the counts of *L. acidophilus* La-05 were above 6.0 log cfu/mL during the assessed storage period in all yogurt formulations, remaining consistent with the recommendations for counts of probiotics in functional food products. When

Table 5
Mean scores (±standard deviation) of sensory acceptance and purchase intention tests of goat yogurt containing *L. acidophilus* La-05 and stingless bee honey at different concentrations, after 14 days of refrigerated storage.

Attributes	Yogurt formulations			
	Y0	YH5	YH10	YH15
Appearance	6.89 (±1.66)[a]	6.91 (±1.56)[a]	7.14 (±1.29)[a]	7.29 (±1.40)[a]
Color	6.89 (±1.52)[a]	7.10 (±1.27)[a]	7.08 (±1.42)[a]	6.97 (±1.38)[a]
Aroma	6.35 (±1.83)[a]	6.49 (±1.87)[a]	6.57 (±1.86)[a]	6.71 (±1.67)[a]
Flavor	4.02 (±2.20)[c]	5.33 (±2.19)[b]	5.97 (±2.30)[ab]	6.46 (±2.06)[a]
Consistency	6.03 (±2.06)[a]	6.52 (±1.67)[a]	6.70 (±1.83)[a]	6.83 (±1.68)[a]
Overall acceptability	5.06 (±2.01)[b]	6.18 (±1.78)[a]	6.46 (±1.88)[a]	6.84 (±1.80)[a]
Purchase intention	2.05 (±0.94)[c]	2.97 (±1.26)[b]	3.33 (±1.40)[ab]	3.78 (±1.40)[a]

Y0 - yogurt containing added *L. acidophilus* La-05, without stingless bee honey; YH5 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 5% (v/v); YH10 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 10% (v/v); and YH15 - yogurt containing added *L. acidophilus* La-05 and stingless bee honey at 15% (v/v).
[a-c] Means ± standard deviations with different letters in the same row denote difference among the different formulations, based on the Tukey's test ($p \leq 0.05$).

considering a dairy product serve portion of 100 g, it should contain at least 6–7 log cfu/g or mL of viable probiotic bacteria at the time of ingestion to promote health benefits to the consumers (Vinderola Prosello, Ghiberto & Renheimer, 2000).

3.4. Sensory analysis of yogurt

Natural sweeteners are preferable alternative to artificial or refined sources for food industry and consumers because some of them provide other nutrients and biologically-active phytochemicals (Edwards, Rossi, Corpe, Butterworth, & Ellis, 2016). Although substituting the traditional sweeteners (sucrose and glucose) is very relevant for food industry, alternative sweeteners beyond affecting food products' sweetness may also impact on other specific characteristics, such as physical behavior, color and flavor (Balthazar et al., 2016). Thus, the impact of the incorporation of stingless bee honey (as alternative sweeteners) on sensory aspects of goat yogurt containing *L. acidophilus* LA-05 was assessed in order to verify if this use could comply with the growing demand for more natural food products with pleasant sensory characteristics.

The mean scores obtained for goat yogurt in sensory acceptance and the purchase intention test and the score distribution arranged according to the general preference of tasters are shown in Table 5. Significant differences ($p \leq 0.05$) were observed only for the flavor and overall evaluation attributes among the assessed yogurt formulations, wherein goat yogurt containing the greatest added honey amounts, namely YH10 and YH15, showed the highest scores ($p \leq 0.05$) for flavor, whilst YH15 showed highest scores ($p \leq 0.05$) for overall evaluation; when the hedonic terms ranged between "slightly liked" to "moderately liked". In general, the scores for overall acceptability of goat yogurt containing added stingless bee honey were greater ($p \leq 0.05$) than those for goat yogurt without added honey.

Therefore, it could be suggested that the amounts of stingless bee honey incorporated in goat yogurts probably influenced the intention to purchase the yogurt formulations because YH10 and YH15 received the highest ($p \leq 0.05$) scores for purchase intention, with hedonic terms between "maybe would buy/maybe would not buy" and "would buy." These findings reflected directly in preference analysis of the yogurt formulations because YH15 and YH10 were again the most ($p \leq 0.05$) preferred formulations, and the yogurt without added honey was the least ($p \leq 0.05$) preferred formulation. Similarly, a previous study found that the addition of fruit juice positively influenced the acceptance of goat yogurt, improving its taste and suggesting a positive influence of sugars naturally found in fruit on these sensory attributes (Ranadheera et al., 2012). Likewise, the added stingless bee honey may have rendered the goat yogurt containing *L. acidophilus* La-05 more acceptable and attractive due to the presence of these saccharides in the formulation, in addition to the presence of other possible pleasant flavor-forming compounds. Interestingly, the highest acidity observed in goat yogurts containing added stingless bee honey did not impact negatively on the sensory acceptance of these formulations. This could be associated with the dominating honey sweetness that in combination with the higher acidity may provide an enjoyable and desirable flavor to yogurts.

4. Conclusion

The incorporation of honey produced by the stingless bee *M. scutellaris* in goat yogurt containing the probiotic *L. acidophilus* La-05 positively affected some of the assessed physical and mechanical stability characteristics of the product during the 28 days of refrigerated storage; namely color, syneresis, viscosity and water retention capacity. The counts of *L. acidophilus* La-05 in all goat yogurt formulations remained adequate (>6 log cfu/mL) to promote health benefits to the consumer during the assessed storage period. However, the formulation containing stingless bee honey presented the highest counts of *L. acidophilus* La-05, indicating a growth promoting effect. Moreover, the addition of stingless bee honey appeared to directly influence the acidity of the prepared goat yogurt over time, without negatively affecting its acceptance and sensory preference. Otherwise, the yogurts containing stingless bee honey presented the best sensory acceptance and preference. Finally, the results of this study presented a successful incorporation of both the probiotic *L. acidophilus* La-05 and the honey produced by a native Brazilian stingless bee as ingredients of a new goat dairy product with satisfactory nutritional and sensory quality, as well as added market value because of the potential functional properties.

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