Viability of probiotic bacteria in bioyogurt with the addition of honey from Jataí and Africanized bees

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Abstract – The objective of this work was to evaluate the viability of probiotic bacteria in bioyogurt with the addition of honey from Africanized and Jataí bees, in different concentrations. To prepare the fermented milk, reconstituted powdered milk and lactic acid starter culture were used. The bioyogurt was evaluated at 0, 7, 14, 21, 28, and 35 days of storage. Analyzes of pH, titratable acidity, and selective count of the *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* BB-12 microorganisms were carried out. Counting was done, respectively, on MRS agar, in aerobiosis, and MRS-LP agar, in anaerobiosis, with plates incubated at 37°C for 72 hours. Treatments were arranged in a completely randomized design in split plot, with five treatments: without honey; 5 and 10% honey from Africanized bees, and 5 and 10% honey from Jataí bees. Storage times were evaluated in the split plots. In all treatments, bioyogurt showed counts of 10^7 CFU g^{-1} lactic acid bacteria. Probiotic cultures remained viable for 35 days under refrigeration (2–4°C). The interaction between the variation factors affected the probiotic concentration in the bioyogurt. The honeys have a favorable effect on the cell counts of the evaluated microorganisms.

Index terms: *Bifidobacterium*, *Lactobacillus acidophilus*, functional food, prebiotic.

Viabilidade de bactérias probióticas em bioiogurte adicionado de mel de abelhas Jataí e africanizadas

Resumo – O objetivo deste trabalho foi avaliar a viabilidade de bactérias probióticas em bioiogurte adicionado de mel de abelhas africanizadas e Jataí, em diferentes concentrações. Para a elaboração do leite fermentado, utilizou-se leite em pó reconstituído e fermento lácteo. O bioiogurte foi avaliado com 0, 7, 14, 21, 28 e 35 dias de armazenamento. Foram realizadas análises de pH, acidez titulável e contagem seletiva dos microrganismos *Lactobacillus acidophilus* LA-5 e *Bifidobacterium* BB-12. As contagens foram realizadas, respectivamente, em ágar MRS, em aerobiose, e ágar MRS-LP, em anaerobiose, com placas incubadas a 37ºC por 72 horas. Os tratamentos foram arranjados em delineamento inteiramente casualizado, com parcelas subdivididas e cinco tratamentos: sem mel; 5 e 10% de mel de abelha africanizada; e 5 e 10% de mel de abelha Jataí. Os tempos de estocagem foram avaliados nas subparcelas. O bioiogurte apresentou contagens de bactérias láticas de 10^7 UFC g^{-1}, em todos os tratamentos. As culturas probióticas mantiveram-se viáveis por 35 dias sob refrigeração (2–4°C). Houve efeito da interação entre os fatores de variação sobre a concentração dos probióticos no bioiogurte. Os méis têm efeito favorável sobre a contagem de células dos microrganismos avaliados.

Termos para indexação: *Bifidobacterium*, *Lactobacillus acidophilus*, alimento funcional, prebiótico.

Introduction

The concern with health and life quality has led people to eat healthier foods with some functional properties. In this scenario, the dairy industry stands out with the largest number of functional products, such as bioyogurt and other fermented milk, obtained with the addition of probiotics and prebiotics (Antunes et al., 2007; Granato et al., 2010).

The consumption of fermented milk has been based, for a long time, on yogurt traditionally

produced with cultures of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. As time went by, however, the use of probiotic microorganisms, associated or not with traditional bacteria, became usual. In this sense, bioyogurt is being prepared with microorganisms that promote beneficial effects to the consumers, such as *L. acidophilus* and *Bifidobacterium* spp. (Lourens-Hattingh & Viljoen, 2001).

The development of probiotics is favored by prebiotics, which are selectively fermentable ingredients that promote bacteria maintenance (Wang, 2009; Bindels et al., 2015). The main prebiotics used in the food industry are oligosaccharides – especially inulin and oligofructose (Akalin & Erisir, 2008) –, which are sugars found in most of the foods such as vegetables, fruits, milk, and honey (Dwivedi et al., 2014; García-Cayuela et al., 2014).

According to Ustunol & Gandhi (2001), honey is a food with prebiotic activity due to the variety of oligosaccharides present in it. However, the prebiotic effect may vary with the composition of different types of honey, which is a factor that should be investigated. Crane (1985) pointed out that these variations depend on the plant species visited by the bees, on environmental conditions, and also on the bee species.

The objective of this work was to evaluate the viability of probiotic bacteria in bioyogurt with the addition of honey from Africanized and Jataí bees, in different concentrations.

Materials and Methods

The raw materials used for the preparation of the bioyogurt were: Molico (Nestle Brasil Ltda., São Paulo, SP, Brazil) skimmed powder milk; BioRich (Chr. Hansen Ind. e Com. Ltda., Valinhos, SP, Brazil) probiotic culture containing *L. acidophilus* LA-5, *Bifidobacterium* BB-12, and *S. thermophilus*; and the honeys – purchased in the retail market – from Africanized (*Apis mellifera*) and "stingless" Jataí (*Tetragonisca angustula*) bees, after pasteurization at 78°C, for 6 min in water bath (Gonnet et al., 1964).

The pH from the reconstituted milk (12%) used to make the bioyogurt was determined using the P100 benchtop pH meter (PHOX, Colombo, PR, Brazil) and by acidity titration with Dornic solution (0.1111 N NaOH). The count of aerobic mesophilic microorganisms was performed starting at 10^{-1} dilution up to 10^{-5} decimal dilutions in peptone water. Inoculation was carried out on plate count agar (PCA), with incubation at 35ºC for 48 hours (Silva et al., 2010).

For the characterization of the honeys, the following analyses were performed in duplicate: moisture, determined with a refractometric method using the PAL-22S digital refractometer (Atago CO., Ltd., Tokyo, Japan), specific for honey; pH, with the P100 benchtop pH meter (PHOX, Colombo, PR, Brazil); water activity (aw), by readings in the 3TE Aqualab equipment (Meter Group, Inc., Pullman, WA, USA); and instrumental color evaluation, i.e., luminosity (L*) and coordinates a* and b*, using the Miniscan EZ colorimeter (HunterLab, Reston, VA, USA). The microbiological analysis was performed by yeast and mold counts, using a 10^{-1} dilution, with decimal dilutions up to 10^{-5}, in peptone water. Inoculations were made on plates of acidified potato dextrose agar (PDA), with incubation at 22–25ºC, for five days (Silva et al., 2010).

For the preparation of fermented milk (bioyogurt), the skimmed powder milk was reconstituted to 12% (m/v) of total solids-not-fat, subjected to slow pasteurization (65ºC for 30 min), and then cooled (42°C) for the inoculation of lactic yeast. Culturing was done by seeding lactic yeast to milk at a concentration of 400 mg L^{-1}, followed by manual homogenization and incubation at 40–42ºC for 5 hours. To obtain the treatments, the formulations of the bioyogurt were prepared as described in Table 1.

After fermentation, the bioyogurt was kept under refrigeration at 2–4°C for 12 hours and then analyzed after 0, 7, 14, 21, 28, and 35 days of storage. The following analyses were carried out in duplicate in the bioyogurt: pH, using the P100 benchtop pH meter (PHOX, Colombo, PR, Brazil); and titratable acidity, by calculating the percentage of lactic acid in the

Table 1. Raw materials and concentrations used in the different formulations of the bioyogurt.

Treatment	Reconstituted skimmed milk (%)	Africanized bee honey (%)	Jataí bee honey (%)
1	100	0	0
2	95	0	5
3	90	0	10
4	95	5	0
5	90	10	0

sample with titration performed with NaOH 0.1 N. For the selective count of *L. acidophilus* LA-5, MRS agar was used, with plates incubated at 37ºC for 72 hours, in aerobiosis. For *Bifidobacterium* BB-12, counting was done on MRS-LP agar, with incubation at 37ºC for 72 hours, in anaerobiosis, using the Anaerobac atmospheric generator (Probac do Brasil Produtos Bacteriológicos Ltda., Santa Cecília, SP, Brazil). In the two culture media, inoculation in depth was carried out with overlay. For the preparation of the MRS-LP agar, 0.3% sodium propionate and 0.2% lithium chloride were used (Vinderola & Reinheimer, 1999).

Plates with 25 to 250 colonies of *L. acidophilus* LA-5 and *Bifidobacterium* BB-12 were selected for the counts. The tests of Gram stain and catalase were performed to identify and confirm the colonies.

A completely randomized design arranged in split plots was used. The treatments (without honey, 5 and 10% Africanized bee honey, and 5 and 10% Jataí bee honey) were placed in the plots, and storage times (0, 7, 14, 21, 28, and 35 days) in the subplots. The data were subjected to the analysis of variance, and, when significant, the averages were compared by Tukey's test, at 5% probability. For the variable pH, which, in the analysis of variance, was only affected by storage times, the regression analysis was performed.

Results and Discussion

The reconstituted powdered milk used to prepare the bioyogurt presented high acidity levels (24ºD) in relation to those established by legislation, i.e., between 14 and 18ºD (Brasil, 2011). This probably occurred because of the reconstitution of the product, which was standardized to have a content of solids-not-fat of 12% in skimmed milk, and of the concentration of proteins, which can acidify the medium. According to Reis et al. (2011), the increase in milk solids results in an increase in titratable acidity, due to the higher content of proteins, citrates, and phosphates. Therefore, an adjustment in the contents of milk solids favors the stability of fermented milk and improves the consistency of the product. The pH showed an average value of 6.7, within the normal range for milk, which lies from 6.6 to 6.8.

The assessment of milk microbiological quality regarding mesophilic aerobic microorganisms showed 3,200 colony-forming unity (CFU) per mL^{-1}, within the limit permitted by law, which establishes a maximum value of 80,000 CFU mL^{-1} (Brasil, 2011). These low counts were probably due to the heat treatment to which the milk was subjected to.

The honeys differed significantly regarding pH, water activity, moisture, L*, and the b* color coordinate (Table 2). In addition, the honey from the Jataí bee was more acid than that from the Africanized bee. Abadio Finco et al. (2010) found that the pH of the Jataí bee honey varies from 3.39 to 4.63 and of the Africanized bee honey, from 3.4 to 4.2. However, the Brazilian legislation does not establish limits for pH values in honey. According to Crane (1985), this parameter can be directly associated with the floristic composition in the collection areas.

The moisture content of the Jataí bee honey (26.06%) was also greater than that of Africanized bee honey (15.43%). The law allows moisture contents of up to 20% for Africanized bee honey, and the values for Jataí bee honey were within the normal range observed in Brazil (Anacleto et al., 2009; Lira et al., 2014). This parameter can greatly affect honey quality, since greater contents favor the growth of microorganisms and may lead to undesirable honey fermentation during storage (Saxena et al., 2010). According to Silva et al. (2010), the limit of water activity for the multiplication of the majority of molds and yeasts is around 0.75. In the present study, the values found for water activity were 0.48 for Africanized bee honey and 0.70 for Jataí bee honey.

No statistical difference was observed in the quantification of molds and yeasts. The two types of honey assessed showed average values (Table 3) below

Table 2. Characteristics of the Jataí (*Tetragonisca angustula*) and Africanized (*Apis mellifera*) bee honeys used to make the bioyogurt[(1)].

Parameter	Africanized bee honey	Jataí bee honey
pH	5.04a	3.67b
Water activity	0.48b	0.70a
Moisture (%)	15.43b	26.06a
L*	0.07b	0.17a
a*	0.18a	0.15a
b*	0.05b	0.38a
Mold and yeast (CFU g^{-1})	36.6a	70.0a

[(1)]Means followed by equal letters do not differ by Tukey's test, at 5% probability. L*, luminosity; a* and b*, color coordinates; and CFU, colony-forming unit.

the one of 100 CFU g^{-1} allowed by legislation (Brasil, 2000). Therefore, both types are suitable for use as bioyogurt ingredients.

Regarding L*, the honey from the Jataí bee was lighter (0.17) than that from the Africanized bee (0.07), and, as to parameter b*, Jataí bee honey had a more yellow coloring. The honeys, however, did not differ as to a*. According to the literature, Jataí bee honey has a predominance of bright tones, when compared with Africanized bee honey; the color of honeys varies according to the bee species and to factors such as mineral and flavonoid contents and Maillard reaction products (Sant'Anna et al., 2012).

Several requirements should be met for a microorganism to be classified as a probiotic, and viability is one of the most important. Viable cells should be present at the time of consumption until the last day of the validity date, at least with the minimum concentrations required by legislation (Macedo et al., 2008). The lactic acid bacteria counts in the bioyogurt were within law requirements in all treatments. According to Brazilian legislation, fermented milk must present counts of total lactic bacteria of at least 10^6 CFU g^{-1}; however, the requirement for bifidobacteria alone is 10^6 (Brasil, 2007). All treatments showed 10^7 CFU g^{-1}.

The effects of types of honey and of the different concentrations interacted with those of storage times, significantly affecting cell counts. Moreover, both types of honey favored *L. acidophilus* LA-5 and *Bifidobacterium* BB-12 (Table 3). The control treatment, without the addition of honey, showed the lowest concentration of *L. acidophilus* LA-5 at the end of 35 days of storage, whereas the addition of both types of honey, regardless of the concentration, favored the maintenance of *L. acidophilus* LA-5. This result contradicts those of Macedo et al. (2008), who did not observe honey (*A. mellifera*) prebiotic effect on the growth and viability of *Lactobacillus* spp. in milk.

Treatments 3 and 5 (10% Jataí and Africanized bee honey, respectively), with the highest honey concentrations, had a significant favorable effect on *Bifidobacterium* BB-12 counts, compared with treatments 1, 2, and 4 (no honey, 5% Jataí and 5% Africanized bee honey, respectively). According to Silva et al. (2006), honey oligosaccharides vary according to their floral origin; therefore, the prebiotic effect of different honeys should differ.

Jan Mei et al. (2010), when evaluating *B. longum* BB 536 in skimmed milk with the addition of 5% honey, reported an increase of more than three logarithmic cycles in an interval of 24 hours. Macedo et al. (2008) also found favorable effects of honey on bacterial counts in milk, with significantly higher overall average growth and viability of *Bifidobacterium* lineages compared with the control (without honey).

Despite their favorable effect on bacterial counts (Table 3), the studied honeys also had a detrimental effect on the viability of these microorganisms over the

Table 3. Means (log CFU mL^{-1}) of the counts of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* BB-12 obtained in the evaluated bioyogurt during storage times[(1)].

Treatment	Storage time (days)					
	0	7	14	21	28	35
			Lactobacillus acidophilus LA-5			
Control	7.78Aa	7.66Aa	7.64Aa	7.49Cab	7.21Bb	7.28Bb
Jataí bee honey (5%)	7.78Aa	7.70Aab	7.78Aa	7.62BCab	7.38ABb	7.54Aab
Jataí bee honey (10%)	7.74Aa	7.70Aa	7.75Aa	7.84Aa	7.64Aa	7.57Aa
Africanized bee honey (5%)	7.83Aa	7.76Aab	7.74Aab	7.68ABb	7.45ABc	7.43ABc
Africanized bee honey (10%)	7.85Aa	7.75Aab	7.79Aa	7.73ABabc	7.60ABbc	7.59Ac
			Bifidobacterium BB-12			
Control	7.67Aa	7.59Ba	7.53Ba	7.51Ca	7.53Da	7.50Ba
Jataí bee honey (5%)	7.87Aa	7.86Aa	7.85Aa	7.67Bb	7.64BCbc	7.56Bc
Jataí bee honey (10%)	7.88Aab	7.90Aab	7.92Aa	7.84Ab	7.84Ab	7.86Aab
Africanized bee honey (5%)	7.69Aa	7.67Ba	7.60Ba	7.71ABa	7.56CDa	7.52Ba
Africanized bee honey (10%)	7.90Aab	7.92Aa	7.87Aab	7.79ABcd	7.73Bd	7.84Abc

[(1)]Means followed by equal letters, lowercase in the lines and uppercase in the columns, do not differ by Tukey's test, at 5% probability. CFU, colony-forming unit.

storage periods. The viability of *L. acidophilus* LA-5 decreased in all treatments, except in that with 10% Jataí bee honey (treatment 3). *Bifidobacterium* BB-12 had its viability decreased in treatments 2, 3, and 5 (5 and 10% Jataí bee honey, and 10% Africanized bee honey, respectively). According to Cruz et al. (2011), the probiotic microorganisms in yogurt face adverse conditions during the storage period, such as stresses caused by cold, oxidation due to the exposure to oxygen, and post-acidification.

Neither treatments nor storage times influenced bioyogurt acidity. The average values observed were 0.58, 0.65, 0.66, 0.65, and 0.62% lactic acid, for treatments 1, 2, 3, 4, and 5, respectively. Most values were within the titratable acidity range of 0.6 to 2.0% lactic acid established for fermented milk by the Brazilian legislation; however, the control treatment did not comply with this minimum requirement. The interaction between microorganisms and honey types suggests that a fermentation time greater than 5 hours should be used. Regarding pH, no significant difference was observed between treatments (Figure 1); however, storage times had a significant effect on this variable.

It is essential to control the pH and acidity of the product in order to avoid phase separation and great alterations in other sensory characteristics caused by high acidification (Vinderola et al., 2000). The conditions observed in the present study probably can provide a proper maintenance of the probiotic microorganisms evaluated.

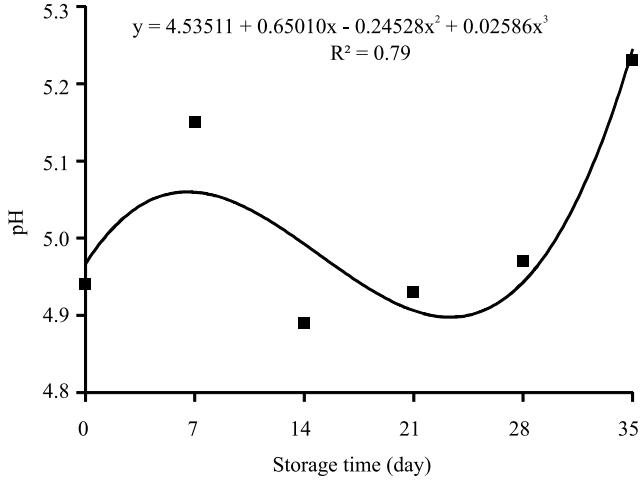

Figure 1. Bioyogurt pH over the period of 35 days of storage.

Conclusions

1. Both Jataí (*Tetragonisca angustula*) and Africanized (*Apis mellifera*) bee honeys favor the viability of probiotic cultures obtained with *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* BB-12 in bioyogurt.

2. Both honey types provide lactic bacteria counts within the range required by Brazilian legislation, regardless of the honey concentration used, and the probiotic cultures remain viable after 35 days of storage under refrigeration (2–4°C).

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References

ABADIO FINCO, F.D.B.; MOURA, L.L.; SILVA, I.G. Propriedades físicas e químicas do mel de *Apis mellifera* L. **Ciência e Tecnologia de Alimentos**, v.30, p.706-712, 2010. DOI: 10.1590/S0101-20612010000300022.

AKALIN, S.; ERISIR, D. Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream. **Journal of Food Science**, v.73, p.184-188, 2008. DOI: 10.1111/j.1750-3841.2008.00728.x.

ANACLETO, D. de A.; SOUZA, B. de A.; MARCHINI, L.C.; MORETI, A.C. de C.C. Composição de amostras de mel de abelha Jataí (*Tetragonisca angustula* latreille, 1811). **Ciência e Tecnologia de Alimentos**, v.29, p.535-541, 2009. DOI: 10.1590/S0101-20612009000300013.

ANTUNES, A.E.C.; MARASCA, E.T.G.; MORENO, I.; DOURADO, F.M.; RODRIGUES, L.G.; LERAYER, A.L.S. Desenvolvimento de buttermilk probiótico. **Ciência e Tecnologia de Alimentos**, v.27, p.83-90, 2007. DOI: 10.1590/S0101-20612007000100015.

BINDELS, L.B.; DELZENNE, N.M.; CANI, P.D.; WALTER, J. Towards a more comprehensive concept for prebiotics. **Nature Reviews, Gastroenterology & Hepatology**, v.2, p.303-310, 2015. DOI: 10.1038/nrgastro.2015.47.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 11, de 20 de outubro de 2000. Aprova o Regulamento Técnico de Identidade e Qualidade do Mel. **Diário Oficial [da] República Federativa do Brasil**, 23 out. 2000. Seção 1, p. 23.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 46, de 23 de outubro de 2007. Adota

o Regulamento Técnico de Identidade e Qualidade de Leites Fermentados. **Diário Oficial da União**, 24 out. 2007. Seção 1, p. 4.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 62, de 29 de dezembro de 2011. Aprova o Regulamento Técnico de Produção, Identidade e Qualidade do Leite Tipo A, o Regulamento Técnico de Identidade e Qualidade de Leite Cru Refrigerado, o Regulamento Técnico de Identidade e Qualidade de Leite Pasteurizado e o Regulamento Técnico da Coleta de Leite Cru Refrigerado e seu Transporte a Granel. **Diário Oficial da União**, 30 dez. 2011. Seção 1.

CRANE, E. O livro do mel. In: CRANE, E. **Constituintes e características do mel**. 2.ed. São Paulo: Nobel, 1985. p.55-80.

CRUZ, A.G.; ANTUNES, A.E.C.; FARIA, J.A.F.; CHAVES, A.C.S.D.; CARVALHO, L.M.J.; SAAD, S.M.I. Leites fermentados e iogurtes probióticos e prebióticos. In: SAAD, S.M.I.; CRUZ, A.G. da; FARIA, J. de A.F. **Probióticos e prebióticos em alimentos**: fundamentos e aplicações tecnológicas. São Paulo: Varela, 2011. p.271-304.

DWIVEDI, S.; SAHRAWAT, K.; PUPPALA, N.; ORTIZ, R. Plant prebiotics and human health: biotechnology to breed prebiotic-rich nutritious food crops. **Electronic Journal of Biotechnology**, v.17, p.238-245, 2014. DOI: 10.1016/j.ejbt.2014.07.004.

GARCÍA-CAYUELA, T.; DÍEZ-MUNICIO, M.; HERRERO, M.; MARTÍNEZ-CUESTA, M.C.; PELÁEZ, C.; REQUENA, T.; MORENO, F.J. Selective fermentation of potential prebiotic lactose-derived oligosaccharides by probiotic bacteria. **International Dairy Journal**, v.38, p.11-15, 2014. DOI: 10.1016/j.idairyj.2014.03.012.

GONNET, M.; LAVIE, P.; LOUVEAUX, J. La pasteurization des miels. **Annals of Abeilles**, v.7, p.81-102, 1964. DOI: 10.1051/apido:19640201.

GRANATO, D.; BRANCO, G.F.; CRUZ, A.G.; FARIA, J. de A.F.; SHAH, N.P. Probiotic dairy products as functional foods. **Comprehensive Reviews in Food Science and Food Safety**, v.9, p.455-470, 2010. DOI: 10.1111/j.1541-4337.2010.00120.x.

JAN MEI, S.; MOHD NORDIN, M.S.; NORRAKIAH, A.S. Fructooligosaccharides in honey and effects of honey on growth of *Bifidobacterium longum* BB 536. **International Food Research Journal**, v.*17,* p.557-561, 2010.

LIRA, A.F.; SOUSA, J.P.L. de M.; LORENZON, M.C.A.; VIANNA, C.A.F.J.; CASTRO, R.N. Estudo comparativo do mel de *Apis mellifera* com méis de meliponíneos de diferentes regiões. **Acta Veterinaria Brasilica**, v.8, p.169-178, 2014. DOI: 10.21708/avb.2014.8.3.3560.

LOURENS-HATTINGH, A.; VILJOEN, B.C. Yogurt as probiotic carrier food. **International Dairy Journal**, v.11, p.1-17, 2001. DOI: 10.1016/S0958-6946(01)00036-X.

MACEDO, L.N.; LUCHESE, R.H.; GUERRA, A.F.; BARBOSA, C.G. Efeito prebiótico do mel sobre o crescimento e viabilidade de *Bifidobacterium* spp. e *Lactobacillus* spp. em leite. **Ciência e Tecnologia de Alimentos**, v.28, p.935-942, 2008. DOI: 10.1590/S0101-20612008000400027.

REIS, S. de M.; PINTO, M.S.; BRANDI, I.V. Efeito do teor de sólidos não gordurosos e da concentração de sacarose na acidificação de iogurte por bactérias láticas. **Revista do Instituto de Laticínios Cândido Tostes**, v.66, p.34-39, 2011.

SANT'ANA, L.D'O.; SOUSA J.P.L.M.; SALGUEIRO, F.B.; LORENZON, M.C.A.; CASTRO, R.N. Characterization of monofloral honeys with multivariate analysis of their chemical profile and antioxidant activity. **Journal of Food Science**, v.71, p.135-140, 2012. DOI: 10.1111/j.1750-3841.2011.02490.x.

SAXENA, S.; GAUTAM, S.; SHARMA, A. Physical, biochemical and antioxidant properties of some Indian honeys. **Food Chemistry**, v.118, p.391-397, 2010. DOI: 10.1016/j.foodchem.2009.05.001.

SILVA, R.A.; MAIA, G.A.; SOUSA, P.H.M.; COSTA, J.M.C. Composição e propriedades terapêuticas do mel de abelha. **Alimentos e Nutrição**, v.17, p.113-120, 2006.

SILVA, N. da; JUNQUEIRA, V.C.A.; SILVEIRA, N.F. de A. **Manual de métodos de análise microbiológica de alimentos e água**. 4.ed. São Paulo: Varela, 2010. 624p.

USTUNOL, Z.; GANDHI, H. Growth and viability of commercial *Bifidobacterium* spp. on honey-sweetened skim milk. **Journal of Food Protection**, v.64, p.1775-1779, 2001. DOI: 10.4315/0362-028X-64.11.1775.

VINDEROLA, C.G.; BAILO, N.; REINHEIMER, J.A. Survival of probiotic in Argentina yogurts during refrigerated storage. **Food Research International**, v.33, p.97-102, 2000. DOI: 10.1016/S0963-9969(00)00011-9.

VINDEROLA, C.G.; REINHEIMER, J.A. Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. **International Dairy Journal**, v.9, p.497-505, 1999. DOI: 10.1016/S0958-6946(99)00120-X.

WANG, Y. Prebiotics: present and future in food science and technology. **Food Research Internationa**l, v.42, p.8-12, 2009. DOI: 10.1016/j.foodres.2008.09.001.

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