Research, Society and Development, v. 9, n. 6, e14962446, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i6.2446

Avaliação de características físico-químicas e microbiológicas de iogurte liofilizado e reidratado

Evaluation of physical-chemical and microbiological characteristics of freeze-dried and reydrated yogurt

Evaluación de las características físico-químicas y microbyológicas del yogur liofilizado y rehidratado

Recebido: 17/12/19 | Revisado: 23/01/2020 | Aceito: 03/04/2020 | Publicado: 09/04/2020

Shana Kimi Farias Yamaguchi
ORCID: https://orcid.org/0000-0002-2074-5865
University of Blumenau, Brazil
E-mail: shanakimi@hotmail.com
Carolina Krebs de Souza
ORCID: https://orcid.org/0000-0003-1340-5085
University of Blumenau, Brazil
E-mail:carolinakrebs@furb.br
Sávio Leandro Bertoli
ORCID: https://orcid.org/0000-0002-6351-4020
University of Blumenau, Brazil
E-mail:savio@furb.br
Lisiane Fernandes de Carvalho
ORCID: https://orcid.org/0000-0002-0816-5200
University of Blumenau, Brazil
E-mail: lfcarvalho@furb.br

Resumo

Este trabalho teve por objetivo avaliar as características físico-químicas e a viabilidade de bactérias lácticas durante o processo fermentativo de iogurte e após a sua liofilização, bem como testar três formulações de espessantes para a reidratação do iogurte em pó. Ao longo do processo fermentativo, foi acompanhada a produção de ácido láctico e o crescimento de bactérias lácticas. Antes e após a liofilização o iogurte foi analisado em relação ao pH, acidez titulável, teor de carboidratos, proteínas, lipídios e bactérias lácticas viáveis. Após liofilização, foram testadas três formulações de espessantes para avaliar a reidratação do iogurte em pó. Ao término do processo fermentativo foi verificado que as bactérias lácticas cresceram até atingir 7,8.10^7 UFC.g^{-1} e a acidez obtida foi de 9,27 g.L^{-1}. A contagem de bactérias lácticas viáveis de iogurte liofilizado e não-liofilizado foi de 5,6.10^7 UFC.g^{-1} e 7,8.10^7 UFC.g^{-1}, respectivamente. Os iogurtes não liofilizados e liofilizados apresentaram um teor de 20,8% e 21,0% de carboidratos, 4,0% e 3,6% de proteínas e 3,7% e 2,7% de lipídios, respectivamente. A combinação de espessantes que proporcionou viscosidade semelhante ao iogurte comercial foi o mix de goma guar, pectina e maltodextrina. Assim, foi possível verificar que o processo de liofilização manteve as características físico-químicas e a viabilidade de bactérias ácido lácticas. Além disso, o iogurte desenvolvido apresentou fácil reconstituição no momento do consumo.

Palavras-chave: Bactérias lácticas; Fermentação; Liofilização; Viscosidade.

Abstract

This study aimed to evaluate the physical-chemical characteristics and the viability of lactic acid bacteria during the fermentation process of the yogurt and after the freeze-dried process, in addition to testing three thickener formulations for the rehydration of the yogurt powder. During the fermentation process, the production of lactic acid and the growth of lactic acid bacteria were accompanied. Before and after freeze-dried process, yogurt was analyzed for pH, titratable acidity, carbohydrates, proteins, lipids and viable lactic acid bacteria. After lyophilization, three thickener formulations were tested to evaluate the rehydration of powdered yogurt. At the end of the fermentation process, it was verified that the lactic acid bacteria grew to reach 7.8.10^7 UFC.g^{-1} and the acidity obtained was 9.27 g.L^{-1}. The viable lactic acid bacteria count of freeze-dried and non-freeze-dried yogurt was 5.6.10^7 CFU.g^{-1} and 7.8.10^7 CFU.g^{-1}, respectively. Non-freeze-dried and freeze-dried yogurts showed a content of 20.8% and 21.0% carbohydrates, 4.0% and 3.6% protein and 3.7% and 2.7% lipids, respectively. The combination of thickeners that provided viscosity similar to commercial yogurts was the guar gum, pectin and maltodextrin mix. Thus, it was possible to verify that the freeze-drying process maintains the physical-chemical characteristics and viability of lactic acid bacteria. In addition, the developed yogurt presented easy reconstitution at the time of consumption.

Keywords: Lactic bacteria; Fermentation; freeze-dry; Viscosity.

Resumen

Este trabajo tuvo como objetivo evaluar las características físico-químicas y la viabilidad de las bacterias del ácido láctico durante el proceso de fermentación del yogur y después de su liofilización, así como probar tres formulaciones de espesantes para la rehidratación del yogurt en polvo. A lo largo del proceso de fermentación, se monitoreó la producción de ácido láctico y el crecimiento de las bacterias. Después de la liofilización, se probaron tres formulaciones de espesante para evaluar la rehidratación del yogur en polvo. Antes y después de la liofilización, se analizó el pH del yogur, la acidez titulable, carbohidratos, proteínas, lípidos y bacterias viables del ácido láctico. También se evaluaron, después de la liofilización, tres formulaciones espesantes para rehidratar yogur en polvo. Al final del proceso de fermentación se verificó que las bacterias lácticas crecieron hasta alcanzar 7,8,10^7 UFC.g^{-1} y la acidez obtenida fue de 9,27 g.L^{-1}. El recuento de las bacterias del ácido láctico viables del yogur liofilizado y no liofilizado fue de 5,6,10^7 UFC.g^{-1} y 7,8,10^7 UFC.g^{-1}, respectivamente. Los yogures no liofilizados y liofilizados tenían un contenido de 20.8% y 21.0% de carbohidratos, 4.0% y 3.6% de proteínas y 3.7% y 2.7% de lípidos, respectivamente. La combinación de espesante que proporcionó una viscosidad similar la yogur comercial fue la mezcla de goma guar, pectina y maltodextrina. Por lo tanto, fue posible verificar que el proceso de liofilización mantiene las características fisico-químicas y las bacterias lácticas viables. Además, el yogur desarrollado presentó una fácil reconstitución en el momento del consumo.

Palabras clave: Bacterias lácticas; Fermentación; Liofilización; Viscosidad.

1. Introduction

The knowledge of the beneficial effects of dairy products allows the production of a range of yogurts with different flavors, textures, and consistencies in response to consumer preferences (Morell et al., 2015). The yogurt offers nutritional benefits from proteins, lactose, minerals and water-soluble vitamins, important in the human diet (Caleja et al. 2016; Ozturkoglu-Budak et al., 2016). According to the Codex Alimentarius Commission (FAO/WHO, 2011), yogurt is defined as a fermented milk with symbiotic yeasts of lactic bacteria such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies *bulgaricus*, which must remain viable in the product until the end of its useful life.

Lactic acid bacteria use lactose as a substrate for fermentation of yogurt, so the final product's low lactose content is advantageous for those who do not digest this carbohydrate

(Silva et al., 2010). In addition, the yogurt is rich in protein, calcium, phosphorus and vitamins and lactic acid bacteria present may exert beneficial effects to the organism (Saad, 2006).

Probiotic microorganisms like *Lactobacillus acidophilus* and bifidobacteria provide health benefits (Brasil, 2002; Brasil 2008). Probiotics contribute to the control of intestinal infections, stimulation of intestinal motility with consequent relief of intestinal constipation, better absorption of certain nutrients, better use of lactose and relief of symptoms of intolerance to this sugar, decrease in cholesterol levels, anticarcinogenic effect and stimulation of the immune system (Espitia et al., 2016).

During the yogurt processing, the lactic bacteria incorporated into milk are responsible for the acidification of the product until it reaches pH close to 4.0, necessary to avoid the development of undesirable bacteria, to provide specific sensorial characteristics, by modifying flavor, texture, aroma and protein content (Dias & Pulzatto, 2009; Piard et al., 2011). In the fermentation process, the bacterium *Lactobacillus bulgaricus* releases amino acids and peptides from the milk protein, which stimulates the growth of *Streptococcus thermophilus*, which grows and releases formic acid and carbon dioxide, thus contributing to the further development of the probiotic *Lactobacillus bulgaricus* (Oliveira & Damin, 2003).

To preserve the nutritional characteristics of the yogurt and to keep the lactic acid bacteria viable, the drying process can be applied by freeze-dried. According to Mata et al. (2005) and Yamaguchi et al. (2017), the freeze-dried process comprises the removal of water from the product by sublimation, allowing the maintenance of biological, nutritional and sensory characteristics similar to its matrix.

Given the above, the objective of this study was to evaluate the physical-chemical characteristics and the viability of lactic acid bacteria during the fermentation process of the yogurt and after the freeze-dried process, in addition to testing three thickener formulations for the rehydration of the yogurt powder.

2. Material and Methods

2.1 Yogurt Preparation

For yogurt formulation whole milk, whole milk powder, sucrose and lactic culture (*Lactobacillus acidophilus* LA-5®, *Bifidobacterium* BB-12® and *Streptococcus thermophilus*) were used. The milk was initially heated to 90 °C for 5 min to promote the growth of the initial culture and partially denature the milk proteins in order to contribute to a better coagulation of the milk and decrease the syneresis after the end of the fermentation (Xu et al., 2008). Subsequently, the milk was cooled to 45 °C and the dried ingredients were added together with the lactic culture previously homogenized. The mixture was incubated at 45 °C for 4 h, cooled to 4 °C and stored in the refrigerator.

2.2 Kinetic study of lactic acid production and growth of lactic acid bacteria during yogurt fermentation

During yogurt fermentation, lactic acid production, pH, and lactic acid bactéria counts were analyzed every 30 min and the specific production rates of lactic acid (μ_P) (1), and cell growth (μ_x), were determined (2), according to Borzani et al. (2001).

$$\mu_P = \frac{1}{X} \cdot \frac{dP}{dt} \quad (1)$$

$$\mu_x = \frac{1}{X} \cdot \frac{dX}{dt} \quad (2)$$

X: cell concentration;

$\frac{dP}{dt}$: instantaneous rate of production of lactic acid;

$\frac{dX}{dt}$: instantaneous rate of cell growth.

2.3 Freeze-dried yogurt

For yogurt freeze-dried, 40 g of the yogurt was used, and the samples were frozen for 10 h at - 18 °C. The containers were then placed in the freeze dryer (Terroni®, Enterprise II, Brazil) for 24 h at - 60 °C.

2.4 Yogurt rehydration

Due to freeze-dried, 85% of yogurt moisture was reduced, 85% filtered water at 10 °C was used for the rehydration of the freeze-dried product, which three formulations of thickeners were evaluated at three different concentrations (Table 1). These thickeners were chosen due to their wide use in dairy products. The formulations were developed according to the recommendation of the use of the manufacturers. The thickeners were weighed and mixed into freeze-dried yogurt for further addition of water, with manual stirring.

After rehydration of the yogurt, the viscosity was evaluated and compared to that of a commercial yogurt. The samples (50 mL) were analyzed by viscometer (model Q860A21, Quimis) using number 4 rotor with a speed of 6 rpm. The results were evaluated through analysis of variance and Tukey's test, with a level of 5% of significance, using *Statistica 7.0.*

Table 1. Formulations of thickeners added to the freeze-dried product.

Formulations	0.7% ($w.w^{-1}$) of thickeners	0.95% ($w.w^{-1}$) of thickeners	1.2% ($w.w^{-1}$) of thickeners
1	0.5% ms+0.2% xg	0.75% ms+0.2% xg	1.0% ms+0.2% xg
2	0.5% ma+0.2% xg	0.75% ma+0.2% xg	1.0% ma+0.2% xg
3	0.7% mix	0.95% mix	1.2% mix

1: Modified starch (ms), xanthan gum (xg);

2: Maltodextrin (ma), xanthan gum (xg);

3: Mix of maltodextrin, pectin and guar gum (mix).

2.5 Characterization of yogurt before and after freeze-dried

Analyses were performed in order to characterize the yogurt before and after the freeze-dried process, being the freeze-dried yogurt previously rehydrated. The viable lactic bacteria count was determined by the depth plating method and overlaid Agar De Man, Rogosa and Sharpe (MRS). This is a culture media that contain polysorbate, acetate, magnesium and manganese, which are known to act as special growth factors for *Lactobacillus*, as well as a rich nutrient base. The incubation temperature was 32 °C for 48 h as a methodology described by Silva et al. (2010).

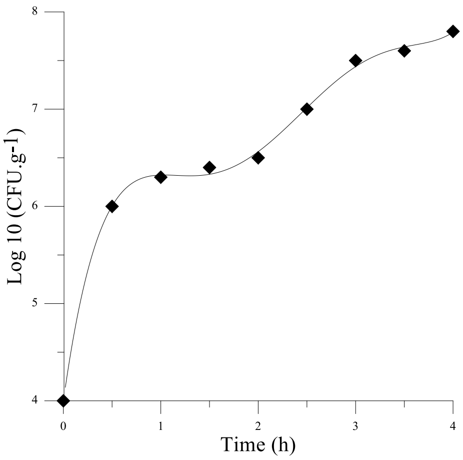
The pH was determined by digital pH meter (Tec-3MP). The acidity, protein, carbohydrate and lipid analyzes were performed according to AOAC (2016).

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i6.2446

3. Results and Discussion

The growth curve of lactic bacteria during yogurt fermentation can be visualized in Figure 1, showing a marked growth in the first 30 minutes of the process, probably due to the higher availability of substrate in the medium; after this period the bacteria grew to reach $7.8.10^7$ $CFU.g^{-1}$.

Figure 1. Growth curve of lactic bacteria.



Source: Own (2019).

The pH decreased from 5.9 to 4.9 after 4 h of yogurt fermentation, possibly due to the formation of lactic acid, and it was favorable for lactic acid bacteria throughout the fermentation process. This result was similar to that obtained by Bett et al. (2017), where the yogurt fermentation time developed with 5% of mangaba pulp was 2.5 h, after incubation at 40 °C, reaching a pH of 4.93. The authors found that the quality of the yogurt was satisfactory during the 28-day shelf life, maintaining the physical-chemical and microbiological quality, with high acceptability during storage. As well as Gonçalves et al. (2018), that developed yogurts with cajá jam, which reached a pH of 4.8 after 6 hours of fermentation and with Acceptability Index above 90%.

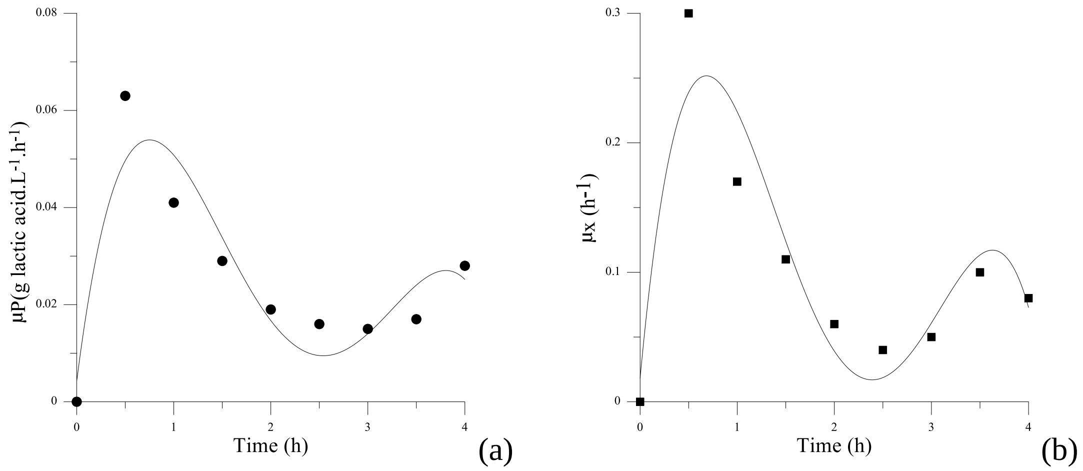
When the acidity reached 9.27 $g.L^{-1}$, the fermentation process was stopped (after 4 h of the process). Dias & Pulzatto (2009) verified acidity of 9.40 $g.L^{-1}$ in 4 h of fermentation for yogurt with soybean addition, a value similar to that obtained in this work.

In around 30 minutes of fermentation, the maximum specific rates of lactic acid production and growth of lactic acid bacteria were 0.063 $g.L^{-1}.h^{-1}$ and 0.301 h^{-1}, respectively.

Afterward, they showed a decrease until 2.5 h and increased again until the end of the process.

According to Silva et al. (2012), at the beginning of the fermentation, the milk acidity (less than 20º D) favors the growth of *S. thermophilus*, stimulated by some free amino acids (especially valine) produced by *L. bulgaricus*, which causes an increase in acidity. Thus, *S. thermophilus* makes the medium more acidic, stimulating the development of *L. bulgaricus*. This fact may explain the behavior of specific lactic acid and lactic acid growth rate curves (Figure 2), since the first peak may have occurred due to fermentation by *S. thermophilus* and the second peak by *L. bulgaricus*.

Figure 2. Kinetics of specific rates of production of lactic acid (a) and growth of lactic acid bacteria (b) throughout the fermentation process.



Source: Own, (2019).

After the freeze-dried process, non freeze-dried and freeze-dried yogurts did not present significant differences ($p > 0.05$) in relation to protein content, with values of 4% and 3.6%, respectively (Table 2).

Increasing the protein content of a food is an effective way to provide greater satiety. Studies indicate that increased protein composition in the diet may lead to a feeling of satiety (Chamberrs et al., 2015). The mechanisms by which amino acids and peptides from protein digestion exert in food intake include the control of intestinal mobility, slowing stomach emptying, stimulating receptors of intestinal hormones and gluconeogenesis (Borreani et al., 2016; Morrel & Fiszman, 2016).

One option is the addition of compounds that provide an increase in protein content in this type of food. As was done by Yamaguchi et al. (2019), which developmented freeze-dried yogurt with added *Spirulina* biomass, which presented 8,1% of protein. According to Becker (2007), *Spirulina's* proteins contain all the essential amino acids and the nutritional value is comparable to the conventional proteins used in food supplementation.

From the results obtained for lipids, a significant difference ($p < 0.05$) was observed, resulting in a reduction for freeze-dried yogurt (2.7%) in relation to non-freeze-dried yogurt (3.7%), according to Table 2. This is due to the lipid content (1%) observed in the aqueous residue removed from the freeze-dryer. The results obtained in this work were similar to those of Costa et al. (2012), who found 2.5% lipids in yogurt with fruit pulp and Oliveira et al. (2011), who found 2.9% of lipids in açai yogurt.

Table 2. Yogurt composition before and after freeze-dried process.

Yogurt	Carbohydrates (%)	Proteins (%)	Lipids (%)
Non freeze-dried	20.8±0.12^{a}	4.0±0.17^{a}	3.7±0.01^{a}
Freeze-dried	21.0±0.21^{a}	3.6±0.02^{a}	2.7±0.09^{b}

*Same letters indicate that the averages do not differ significantly ($p > 0.05$) by Tukey test.

Source: Own, (2019).

As for the carbohydrate concentration, the yogurt did not change after the freeze-dried process, remaining around 21%, as shown in Table 2, a value similar to that found by Oliveira et al. (2011), who found the value of 27.23% in fruit yogurt.

The viable lactic acid bacteria count of freeze-dried yogurt and non freeze-dried yogurt was $5.6.10^{7}$ CFU.g^{-1} and $7.8.10^{7}$ CFU.g^{-1}, respectively, thus demonstrating that the lactic acid bacteria remained viable after the freeze-dried process. In view of this, the product conforms to the standard established by Codex Alimentarius (2011), where the minimum amount of lactic acid bacteria is 10^{6} CFU.g^{-1} in yogurts.

The presence of lactic acid bacteria in fermented foods contributes to increase the shelf life, nutritional value, flavor, aroma, texture and health benefits. The beneficial effects of lactic acid bacteria are attributed to increased digestibility, high levels of B-complex vitamins and some amino acids, better use of lactose, reduced levels of lactose in the product and increased availability of lactase, when comparing fresh milk with yogurt (Gomes & Malcata, 2002; Hong & Marshall, 2001). In addition, other beneficial effects may be cited, such as modulation of the immune system; degradation of carcinogenic potentials; qualitative and quantitative improvement of the intestinal microflora (Mercenier Pavan & Pot, 2003).

Regarding the viscosity, it can be observed that formulations 1, 2 and 3 containing 0.7% (w.w^{-1}) of thickener did not differ statistically between them (p> 0.05) (Table 3), however, they presented viscosity lower than commercial yogurt (40 ± 2.12 Pa.s). At the 1.2% (w.w^{-1}) thickeners concentration, the three formulations showed a significant difference (p <0.05), giving a higher viscosity to the yogurt. Both formulations containing 0.7% and 1.2% (w.w^{-1}) were found to be unsuitable for use in yogurt rehydration due to decharacterization of the product as compared to commercial yogurts.

Table 3. Viscosities of rehydrated yogurts.

Formulations	Viscosity (Pa.s)		
	0.7% (w.w^{-1}) of thickeners	0.95% (w.w^{-1}) of thickeners	1.2% (w.w^{-1}) of thickeners
1	6.25 ± 0.35^{a}	21.00 ± 1.41^{a}	59.00 ± 1.41^{a}
2	7.90 ± 0.14^{a}	34.00 ± 1.41^{b}	73.00 ± 4.24^{b}
3	7.10 ± 0.14^{a}	40.00 ± 0.00^{b}	89.50 ± 3.54^{c}

*Same letters in the same column indicate that the averages do not differ significantly (p> 0.05) in Tukey's test.

1: Modified starch (ms), xanthan gum (xg);

2: Maltodextrin (ma), xanthan gum (xg);

3: Mix of maltodextrin, pectin and guar gum (mix).

Source: Own, (2019).

According to Table 3, formulations 2 and 3 with 0.95% (w.w^{-1}) of thickener did not show any significant difference in relation to commercial yogurt (40 ± 2.12 Pa.s) used as a comparison. Both formulations 2 and 3 contain maltodextrin, confirming what Loret et al. (2004) and Toneli et al. (2005) present in their works, where they demonstrate that the thickener has the capacity to reproduce the sensation caused by the fat due to the three-dimensional network that is formed during the process of gelling, being the same as the fat substitutes.

Cardoso et al. (2017) also highlight that the application of maltodextrin as a fat substitute is more versatile as it allows many of its functional properties to be used simultaneously: ability to thicken, prevent crystallization, promote dispersion and promote the bonding of aromas, pigments and fats, besides being used as an ingredient for the encapsulation of aromas and dyes in baking, dairy products and desserts.

Xanthan gum and guar gum confer a pseudoplastic characteristic to the product of interest regardless of the temperature at the time of mixing (Marcotte et al., 2001). By combining these gums and pectin (Fishman et al., 2000) with maltodextrin the product forms a network (Dias & Pulzatto, 2009), the formulation that showed similar viscosity to commercial yogurt was the one developed with a combination of gum, pectin and maltodextrin.

Yogurt with the highest viscosity (89.50 Pa.s) was obtained when 1.2% of the mix of maltodextrin, pectin and guar gum were used (Table 2). Teles and Flôres (2007) found a similar value (91.62 Pa.s) using 1% powdered milk, 0.8% gelatin, 0.21% xanthan gum and 0.21% guar gum in a total of 2.2% thickeners. For the same authors, the treatment with the lowest viscosity (5.38 Pa.s) was obtained when 1.5% of powdered milk, 0.6% of gelatin, 0.14% of xanthan gum and without the addition of guar gum.

Costa et al. (2013) in fermented dairy beverage using 1% guar gum obtained viscosity of 12.74 Pa.s, within cornstarch treatment (1%) the viscosity was 6.30 Pa.s. Already, Manzano et al. (2008) compared thickeners gelatin (0.5%), modified starch (0.5%) and yam starch (0.5%), and found that modified starch conferred higher viscosity (27.03 Pa.s) in relation to the yam starch which had a viscosity of 20.01 Pa.s.

4. Conclusions

This work presented the applicability of the freeze-dried process in the development of yogurt powder, because it was possible maintain the physical-chemical characteristics and lactic acid bacteria viable in the final product. Also, the lyophilized yogurt presented easy reconstitution at the time of consumption. Bearing in mind that yogurts are consumed by people of all ages, for future study it is suggested the addition of active and/or functional compounds such as antioxidants, vitamins and minerals for food supplementation.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

AOAC. Association of Official Analytical Chemist. (2016). *Official Methods of Analysis International of AOAC International*. 20 ed., EUA.

Becker, E. W. (2007). Micro-algae as a source of proteon. *Biotechnology Advances*, 25, 207-210.

Bett, S. C., Picanço, N. F. M., Faria, R. A. P. G., Nascimento, E. (2017). Elaboração e Qualidade de iogurte com polpa de mangaba. *Higiene Alimentar*, 31, 120-127.

Borzani, W., Aquarone, E., Schmidell, W. & Lima, U. A. (2001). *Biotecnologia industrial*. Engenharia Bioquimica. v.2, ed. Edgard Blücher. São Paulo.

Borreani, J., Lorca, E., Larrea, V., Hernando, I. (2016). Adding neutral or anionic hydrocolloids to dairy proteins under in vitro gastric digestion conditions. *Food Hydrocolloids*, 57, 169-177.

Brasil, Ministério da Saúde. (2002). RDC Nº 02, de 07 de janeiro de 2002. *Technical regulation of isolated bioactive substances and probiotics with functional and health claims.* Brasília.

Brasil, Ministério da Saúde. (2008). *Foods with claims of functional and or health properties, novel foods / ingredients, bioactive substances and probiotics.* Brasília.

Caleja, C., Barros, L., Antonio, A. L., Carocho, M., Oliveira, M. B., Ferreira, I. C. (2016). Fortification of yogurts with different antioxidant preservatives: A comparative study between natural and synthetic additives. *Food Chemistry*, 210, 262-268.

Cardoso, M., Souza, T. T. P., Seabra, E. B. de (2017). Dextrose, Maltodextrin and Waxy Maize: main differences in composition, mechanism of action and recommendations for sports performance. *Cadernos UniFOA*, 33, 101-109.

Costa, A. V. S., Nicolau, E. S., Torres, M. L. T., Fernandes, P. R., Rosa, S. I. R., Nascimento, RC (2013). Development and physicochemical, microbiological and sensory characterization of fermented milk drink made with different stabilizers/thickeners. *Ciências Agrárias*, 34(1), 209-226.

Costa, G. N. S., Mendes, M. F., Araújo, I. O., Pereira, C. S. S. (2012). Development of an Açaí flavored yogurt (*euterpe edulis martius*): Physicochemical and sensory evaluation. *Revista Eletrônica TECCEN*, 5(2), 43-58.

Dias, B. M., Pulzatto, M. E. (2009). Elaboration and evaluation of pectin added yogurt obtained from orange peel (*Citrus sinensis L. Osbeck*). *Instituto de Laticínios Cândido Tostes*, 367/368(64), 26-34.

Espitia, P. J. P., Batista, R. A., Azeredo, H. M. C., Otoni, C. G. (2016). Probiotics and their potential applications in active edible films and coatings. *Food Research International*, 90, 42-52.

FAO/WHO. (2011). Codex alimentarius: codex standards for fermented milks 243- 2003. In: Milk and Milk Products, second ed. World Health Organization, *Food and Agriculture Organization of the United Nations*, Rome, pp. 6-16.

Fishman, M. L., Chau, H. K., Hoagland, P., Ayyad, K. (2000). Characterization of pectin, flashextracted from orange albedo by microwave heating, under pressure. *Carbohydrate Research*, 323(1), 126-138.

Gomes, A. M. P., Malcata, F. X. (2002). Probiotic agents in food: physiological and therapeutic aspects, and technological applications. *Biotecnologia Alimentar*, 101, 12-22.

Gonçalves, N. M., Ferreira, I. M., Oliveira e Silva, A. M., Carvalho, M. G. (2018) Yoghurt with cajá jeleia (Spondias mombin l.) added probiotics: microbiological evaluation and sensory acceptance. *Revista Brasileira de Higiene e Sanidade Animal*, 12(1), 54 – 63.

Hong, S. H., Marshall, R. T. (2001). Natural exopolysaccharides enhance survival of lactic acid bacteria in frozen dairy desserts. *Journal of Dairy Science*, 84(6), 1367-1374.

Loret, C., Meunier, V., Frith, W. J., Fryer, P. J. (2004). Rheological characterization of the gelation behaviour of maltodextrin aqueous solution. *Carbohydrate Polymers*, 57(2), 153-163.

Manzano, G. P. P., Daiuto, E. R., Janzantti, N. S., Rossi, E. A. (2008). Sensory and physicochemical aspects of soy yogurt with yam starch thickeners/stabilizers (*Dioscorea alata*), modified starch and gelatin. *Boletim do Centro de Pesquisa e Processamento de Alimentos*, 26(2), 287-296.

Marcotte, M., Taherian Hoshahili, A. R., Ramaswamy, H. S. (2001). Rheological properties of selected hydrocolloids as function of concentration and temperature. *Food Research International*, 34(8), 695- 703.

Mata, M. E. R. M. C., Duarte, M. E. M., Alsemo, G. C. S., Rodrigues, E., Guedes, M. A., Cavalcanti, A. S. R. de R. M., Oliveira, C. C. A. (2005). Obtaining soursop powder by the freeze drying process. *Revista Brasileira de Produtos Agroindustriais*, 7(2), 165-172.

Mercenier, A., Pavan, S., Pot, B. (2003). Probiotics as biotherapeutic agents: present knowledge and future prospects. *Current Pharmaceutical Design*, 8, 99-110.

Morell, P., Piqueras-Fiszman, B., Hernando, I., Fiszman, S. (2015). How is an ideal satiating yogurt described? A case study with added-protein yogurts. *Food Research International*, 78, 141-147.

Morrel, P., Fiszman, S. (2016). Revisiting the role of protein-induced satiation and satiety. *Food Hydrocolloids*, 68, 199-210.

Oliveira, M. N., Damin, M. R. (2003). Effect of solids content and sucrose concentration on acidification, firmness and viability of yogurt and probiotic bacteria in fermented milk. *Ciência e Tecnologia de Alimentos*, 23, 172-176.

Oliveira, P. D., Lima, S. C. G., Lourenço Júnior, J. B., Araújo, E. A. F. (2011). Sensory evaluation of açaí yogurt (*euterpe oleracea mart*) type "sundae". *Instituto de Laticínios. Cândido Tostes*, 380(66), 5-10.

Ozturkoglu-Budak, S., Akal, C., Yetisemiyen, A. (2016). Effect of dried nut fortification on functional, physicochemical, textural, and microbiological properties of yogurt. *Journal of Dairy Science*, 99(11), 8511-8523.

Piard, J. C., Le Loir, Y., Poquet, I., Langella, P. (2011). Lactic Bacteria: Dairy bacteria at the center of new technological challenges. *Biotecnologia Ciência e Desenvolvimento*. Encarte Especial.

Saad, S. M. I. (2006). Probióticos e prebióticos: o estado da arte. *Revista Brasileira de Ciências Farmacêuticas*, 42, 1-16.

Silva, L. C., Machado, T. B., Silveira, M. L. R., Rosa, C. S., Bertagnolli, S. M. M. (2012). Microbiological aspects, pH and acidity of homemade yogurt compared to industrialized ones from Santa Maria - RS. *Disciplinarum Scientia. Série: Ciências da Saúde*, 13(1), 111-120.

Silva, N., Junqueira, V. C. A., Silveira, N. F. A. (2010). *Manual de Métodos de Análise Microbiológica de Alimentos*. (4ª ed.) Ed Varela. São Paulo.

Teles, C. D., Flôres, S. H. (2007). Influence of the addition of thickeners and milk powder on the rheological characteristics of nonfat yogurt. *Boletim do CEPPA*, 25(2), 247-256.

Toneli, J. T. C. L., Murr, F. E. X., Park, K. J. (2005). Study of rheology of polysaccharides used in the food industry. *Revista Brasileira de Produtos Agroindustriais*, 7(2), 181-204.

Xu, Z. M., Emmanouelidou, D. G., Raphaelides, S. N., Antoniou, K. D. (2008). Effects of heating temperature and fat contente on the structure development of set yogurt. *Journal of Food Engineering*, 85, 590-597.

Yamaguchi, S. K. F., Krebs, C. S., Bertolli, S. L., Carvalho, L.F. (2017). Freeze Dairy Products: A Review. *Revista Espacios*, 38(22), 1-12.

Yamaguchi, S. K. F., Moreira, J. B., Costa, J. A. V., Krebs, C. S., Bertolli, S. L., Carvalho, L.F. (2019). Evaluation of adding Spirulina to freeze-dried yogurts before fermentation and after freeze-dryin. Industrial Biotechnology, 15(2), 89-94.

Porcentagem de contribuição de cada autor no manuscrito

Shana Kimi Farias Yamaguchi – 25%

Carolina Krebs de Souza – 25%

Sávio Leandro Bertoli – 25%

Lisiane Fernandes de Carvalho – 25%