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Chemistry

Development and characterization of probiotic pectin candy with jussara and passion fruit

Desenvolvimento e caracterização de bala de pectina probiótica com jussara e maracujá

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ABSTRACT

Functional foods are being developed in different industries. This study evaluated the survival of the probiotic *Bacillus coagulans* GBI-30 6086 in pectin candies containing jussara and passion fruit pulps. Physicochemical analyses, anthocyanin content, antioxidant capacity, microbiological quality, probiotic viability, and in vitro gastrointestinal resistance of B. *coagulans* were carried out during 30 days (28 °C). Sensory acceptance was done after elaboration of the candies. The addition of probiotics and fruit pulps did not modify the physicochemical characteristics of the pectin candies during storage ($p > 0.05$). The pH of the candies was 3.77, and the acidity was approximately 1.0% citric acid during storage. Aw was 0.70, and the candies showed a red / purple color due to the addition of the jussara pulp. Values of 45 mg.100g-1 and 360 µM Trolox.g-1 were found for anthocyanins and antioxidant capacity, respectively, at the end of the shelf life. The product was safe to consume, and the average viability of B. *coagulans* was 6.57 log CFU/g from 15 days to 30 days of storage. At the end of the gastrointestinal simulation, 6.72 log CFU/g of B. *coagulans* remained viable, indicating that the pectin candies have the potential to be probiotic. The candies had excellent acceptance by consumers. The inclusion of the tropical fruit mix had a positive impact on the formulation of pectin candies, reducing the need for artificial additives while combining the desirable characteristics of the fruits with the probiotic, enhancing the functional appeal of the product.

Keywords: Pectin candy; Fruit pulp; Gastrointestinal resistance

RESUMO

Alimentos funcionais estão sendo desenvolvidos em diferentes indústrias. Este estudo avaliou a sobrevivência do probiótico *Bacillus coagulans* GBI-30 6086 em balas de pectina contendo polpas de jussara e maracujá. Análises físico-químicas, teor de antocianinas, capacidade antioxidante, qualidade microbiológica, viabilidade do probiótico e resistência gastrointestinal *in vitro* de B. *coagulans* foram realizadas durante 30 dias (28 °C). A aceitação sensorial foi feita após a elaboração das balas. A adição de probiótico e polpas de frutas não modificou as características físico-químicas das balas de pectina durante o armazenamento (p>0,05). O pH das balas foi de 3,77 e a acidez foi de aproximadamente 1,0% de ácido cítrico durante o armazenamento. A Aw foi de 0,70 e as balas apresentaram coloração vermelha/roxa, devido à adição da polpa de jussara. Valores de 45 mg.100g-1 e 360 μM Trolox.g-1 foram encontrados para antocianinas e capacidade antioxidante, respectivamente, no final da vida de prateleira. O produto mostrou-se seguro para consumo e a viabilidade média de B. *coagulans* foi de 6,57 log UFC/g de 15 a 30 dias de armazenamento. Ao final da simulação gastrointestinal, 6,72 log UFC/g de B. *coagulans* permaneceram viáveis, indicando que as balas de pectina são potencialmente probióticas. As balas apresentaram excelente aceitação pelos consumidores. A inclusão do mix de frutas tropicais teve um impacto positivo na formulação das balas de pectina, reduzindo a necessidade de aditivos artificiais e combinando as características desejáveis das frutas com o probiótico, aumentando o apelo funcional do produto.

Palavras-chave: Bala de pectina; Polpa de fruta; Resistência gastrointestinal

1 INTRODUCTION

Different kinds of confectionery products are produced and gummy candies represent approximately 50% of confectionery market (Garcia, 2000), with a heterogeneous group of consumers, characterized as eventual consumption (Sessler et al., 2013). Gummy candies are essentially composed by sugars, gelling agents, as gelatine, pectin, guar and xanthan gums, starch and derivatives (Charoen et al., 2015; Habilla & Cheng, 2015; Riedel et al., 2015; Utomo et al., 2014; Miranda et al., 2020), as well as flavouring and coloring agents.

In recent years, there has been a growing emphasis on natural ingredients that promote health, mood, sensory properties and convenience as factors influencing food preferences and consumer motivation (Souza et al., 2020). When it comes to confectionery products, the main motivational factors are “satisfying hedonic hunger” and “pleasure”. Despite the increasing demand for healthier food options (Schieber et

al., 2001), there has not been a significant decrease in the consumption of confectionery products (Bracale & Vaccaro, 2020; Khan & Smith, 2020; Laguna et al.,2020; Konar et al., 2022). Therefore, these products continue to be attractive and popular, primarily due to their sensory appeal and convenience (Sessler et al., 2013; De Moura et al., 2019). Consequently, they serve as promising matrices for incorporating functional components (De Moura et al., 2019).

There are some strategies to develop healthier products, such as, incorporating vitamins, probiotics and fruits to the formulations (Silva et al., 2016; Altinok et al., 2020; Miranda et al., 2020). Probiotics are living organisms that when administered in adequate amounts, confer benefits to the health of the host (FAO & WHO, 2006). These microorganisms have been added in different matrices and confectionery products are an innovative option. In addition, Brazil has a large fruity diversity (Rigueira et al., 2013). Júnior et al. (2020) appointed that, the industrial production of brazilian candies is mainly related to fruits such as guava, banana and cashew. Some of Brazilian fruity are unexplored potential, such as, Jussara fruit (*Euterpe edulis* M.), a palm widely distributed in the Brazilian Atlantic Forest and produces jussara fruit, with dark purple pulp (Borges et al., 2013). This fruit has a high concentration of phenolic compounds and antioxidant activity, being able to act as an additive natural in the development of new functional products (Bicudo et al., 2014). It is similar açai (*Euterpe Oleracea* Mart.), however, it still has low economic interest. Other fruit of interest and typical of Brazil is yellow passion fruit (*Passiflora edulis*), that presents peculiar flavor (Oliveira, 2015), antioxidant activity, vitamins (A and C), phenolic compounds, carotenoids, and mineral salts (Zeraik, 2010; Rotili, 2013).

Considering the importance of consuming probiotics and the potential of unexplored Brazilian fruits, as jussara, besides the predominance of probiotics in dairy products and limitations on the consumption by lactose intolerant individuals, allergic to milk proteins and vegetarian individuals (Martins et al., 2013; Perricone et al., 2015), it is believed that the incorporation of probiotics and fruit pulps in pectin candies

presents itself as a potential alternative for the food industries to develop products more health, attracting the most demanding people who value well-being.

Thus, the present study aimed to develop jussara and passion fruit pectin candies, supplemented with *Bacillus coagulans* GBI-30 6086 and to evaluate their physical-chemical characteristics, antioxidant capacity, microbiological characteristics and probiotic viability during storage. In addition, the probiotic resistance to the conditions of the gastrointestinal tract (GTI) simulated *in vitro* and sensorial acceptance were assessed.

2 MATERIAL AND METHODS

2.1 Material

The following material were used: sucrose (Union, Brazil); glucose syrup (Glucogill 40 DE, Cargill, Brazil); high methoxyl pectin (Rica Nata, Brazil); sodium citrate (Synth, Brazil); citric acid (Proregi, Brazil) and probiotic *Bacillus coagulans* GBI-30 6086 (Schiff®, USA).

2.2 Methods

2.2.1 Obtaining fruit pulp

The fruit pulp of jussara palm (*Euterpe edulis* Martius) was obtained from the farmers in the region of Rio Pomba city (Minas Gerais, Brazil). Passion fruit (*Passiflora edulis*) purchased in the same city were washed in running water and sanitized by immersion in sodium hypochlorite solution (200 $mg.L^{-1}$/10 minutes). The passion fruit pulp was obtained in an industrial blender (KD Eletro, Brazil), filtered, pasteurized (82 °C/1min) and frozen in a cold chamber (-18 °C) until use.

2.2.2 Pectin gummy candy production enriched with *Bacillus coagulans* GBI-30 6086

The texture agent of gummy candy matrix was the pectin. The pectin (1.5%) was hydrated in hot water (22.4%) at a temperature of 70 to 90 °C, vigorously stirring using a mixer. The sucrose (21.1%), glucose syrup (24.9%) and sodium citrate (0.1%) were manually homogenized and mixed to hydrated pectin. This syrup was concentrated under atmospheric pressure until reached 95 °C and the fruit pulps were added to a concentration of 72-74 °Brix. The mass was cooled to 80 °C by manual stirring, and citric acid was added (0.1%).

After adding citric acid, the mass was cooled to 55 °C and capsules containing 10^9 spores of the probiotic *Bacillus coagulans* GBI-30 6086 (Schiff®, USA) was added in 1000g of syrup in order to obtain about 10^6 cells in the product. The syrup was immediately deposited in silicone molds for drying in an oven (25 ºC/48h), until reached the water activity (Aw) of approximately 0.70. . The gummy candies were unmolded and stored in closed glass bottles protected from light.

2.2.3 Determination of the physicochemical characteristics

The pH value (GEHAKA, São Paulo, Brazil), titratable acidity (citric acid/100g of product) and the water activity (Aw/Aqualab, Decagon Devices Aqualab Lite) of the pectin candies were determined according to AOAC (2016).

Colour analysis was evaluated using a Konica Minolta CR10 (Tecnal, São Paulo, Brazil) colorimeter. The colour was determined by direct reading of the reflectance of the coordinates L* (lightness and varies from 0 (black) to 100 (white) and b* (saturation and varies from +b (yellow) to -b (blue)) using the CIELAB L* scale adopted as standard by the International Commission on Illumination.

All analyzes of pH, acidity, water activity and color were performed immediately after the elaboration of the candies (time zero) and at times 15 and 30 days of storage at 28 °C ± 2.

2.2.4 Determination of anthocyanin content and antioxidant capacity of the candies

The anthocyanin contents of the pectin candies were determined according to Lees and Francis (1972) after the elaboration of the candies (time zero) and at 30 days of storage. The results were expressed in mg anthocyanin per 100 g of product. The pectin gummy candies were evaluated for their antioxidant capacity according to the ABTS radical cations [2,2'-azinobis (3-ethylbenzthiazoline sulfonic acid-6)] capture method described by Re et al. (1999) and the results expressed in μM Trolox/g of product. The analyze was carried out in the time zero, and subsequently after 15 and 30 days of storage at 28 °C ± 2.

2.2.5 Microbiological quality of the pectin candies

Thermotolerant coliforms were determined by the Most Probable Number (MPN) technique according to Kornacki and Johnson (2001). The presence or absence of *Salmonella* sp. was determined in 25 g of the samples, homogenized in 225 mL of lactose broth (MicroMed/Isofar, Duque de Caxias, Rio de Janeiro, Brazil) using the methodology of Andrews et al. (2001). The pectin candies were evaluated immediately after processing (time 0) and after 30 days of storage.

2.2.6 Evaluation of the viability of *B. coagulans* GBI-30 6086

The viability of *B. coagulans* was determined immediately after elaborating the pectin candies (time “0 day”) and after 15 and 30 days of storage. This probiotic bacterium was quantified in the Tryptic Soy Agar (TSA, Merck Millipore, Brazil) by the pour plate technique, and the Petri dishes were incubated at 50 °C for 72 h. Gram staining and morphology evaluation were performed to confirm *B. coagulans*.

2.2.7 *In vitro* simulation of *B. coagulans* GBI-30 6086 to the gastrointestinal conditions

The methodology described by Bedani et al. (2013) was used, simulating the gastric, enteric I and enteric II phases, and applying soon after preparation of the pectin candies (“0 day”) and after 30 days of storage of the candies maintained at 28 °C ± 2.

2.2.8 Sensorial acceptance

A total of 100 consumers (> 18 years old) carried out the sensory test to evaluated pectin candy (time "0 day"). The Ethics Committee at the Federal Institute of Education, Science and Technology of Southeast of Minas Gerais, Brazil approved the study (CAAE: 92732418.2.0000.5588). The hedonic scale of nine points was used, varying from "extremely liked" (score 9) to "disliked extremely" (score 1) for the following attributes: sweetness, aroma, taste, texture, color and overall liking.

2.3. Data analyses

The analyzes of pH, acidity, water activity, color, antioxidant capacity and viability of *B. coagulans* were evaluated using a completely randomized design with three replications and 1x3 factorial scheme, being one treatment and three times of storage. Anthocyanins and survival of *B. coagulans* to the gastrointestinal conditions were evaluated using a completely randomized design with three replications and 1x2 factorial scheme, being one treatment and two times of storage.

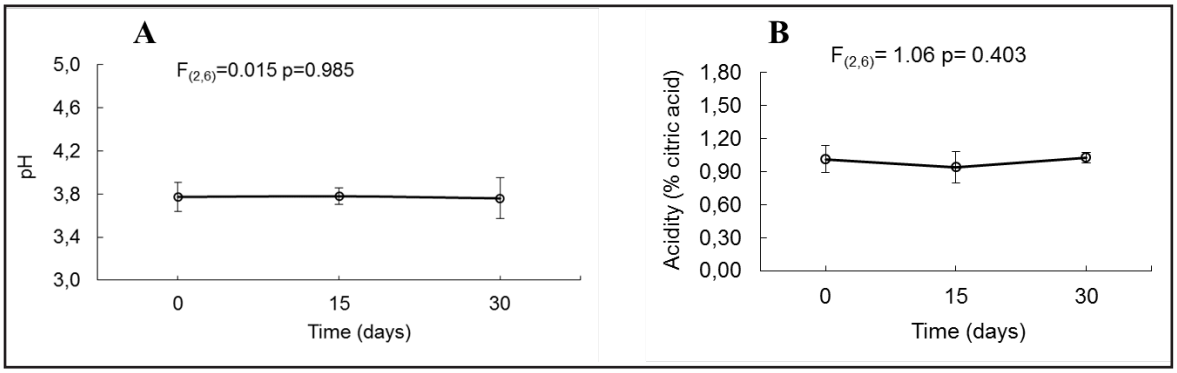
After performing the analysis of variance (ANOVA), the averages of the different treatments were compared by Tukey test. Acceptance data were evaluated by media test. All statistical procedures were carried out considering a 5% level of probability and using STATISTICA 13.0 software (TIBCO Software Inc, 2017).

3 RESULTS AND DISCUSSION

3.3.1 Physicochemical analysis

There was no significant change (p> 0.05) of pH and acidity during storage and average pH value was 3.77 and the acidity value was, approximately, 1.0 %. Considering that pectin gels are formed at pH below 3.8 (Avelar & Efraim, 2020; Toft et al., 1986), the pH in 3.77 was essential for gel formation, an important e expected sensory parameter, similar results were presented by Avelar and Efraim (2020) in pectin candies.

Figure 1– pH (A) and acidity (B) obtained during the storage period at 28 °C for jussara and passion fruit pectin candies. Erro bars represent ± standard deviation (n=3)



Source: research data

The average water activity (Aw) value of the candies was 0.702, during the 30 days of storage, (p> 0.05). For better stability, this kind of candy should present A_w range between 0.5-0.75 (Ergun et al., 2010), which contribute to a longer shelf-life. Jellies and gummies are products whose characteristics are determined by the gelling agent and the final water content (Demars & Ziegler, 2001).

It was found that the pectin candies maintained their color over the 30 days of storage (p> 0.05), with no variation in the coordinates L * (brightness – average value of 19.45), a * (red-green– 1.04) and b * (yellow – blue–1.33). The candies color presented no discoloration, showing the jussara as potential natural color in gummy candy, maintaining the original color during the storage.

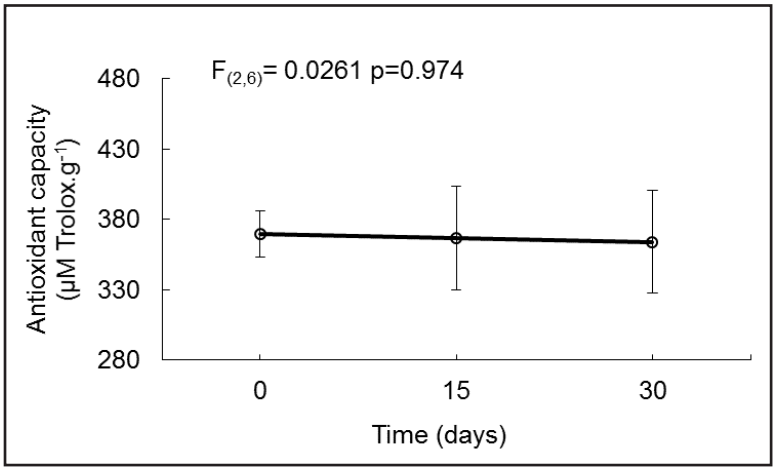
The low average values of the L* coordinate associated with the positive values of a* and b* characterize a dark reddish color of the candies, which was expected due to the use of jussara pulp.

The substitution of artificial colors for fruit pulps adds natural color to the product, in addition to compounds beneficial to health, such as antioxidant pigments that promote well-being, considering that some synthetic and artificial colors are harmful to consumers' health. Bearing in mind that children are among the main consumers of these products and that artificial colors, in addition to not having nutritional value, can still cause allergies in this public, the use of fruit pulps is very important.

Anthocyanins are natural pigments present in vegetables, responsible for the colors blue, purple, violet, orange and for almost all shades of red found in these products (Blando et al., 2004). There was a significant reduction (p<0.05) between anthocyanins from the beginning (time 0 - 54.64 mg. $100g^{-1}$) to the end of storage period of pectin candies (30 days - 46.00 mg $100g^{-1}$). According to Bastos et al. (2017), factors such as temperature, exposure to light and the presence of oxygen influence the stability of these pigments, especially temperature and luminosity that accelerates the degradation of these compounds.

On the other hand, the storage period did not change (p> 0.05) the antioxidant capacity of the pectin candies analyzed (Figure 2), which reveals the stability of these chemical compounds.

Figure 2 – Antioxidant capacity of pectin candies over the storage period at 28 °C. Erro bars represent ± standard deviation (n=3)



Source: research data

Jussara and passion fruit are fruits with powerful antioxidant action. Then, the determination of antioxidant capacity is useful to evaluate the action that bioactive compounds exert on the body's protection against harmful effects (Borges et al., 2013). There are no published studies in the literature involving the use of jussara and passion fruit pulps in confectionery products. Therefore, we believe in the promising

potential of using these pulps in candies and derivatives, since in addition to meeting the need for the use of artificial colors, they promote flavor due to their soluble solids content and provide nutritional value to the products.

3.3.2 Microbiological analyzes

The analyzed candies are safe for human consumption, with a thermotolerant coliform count of the <3.0 NMP.g^{-1} and absence of *Salmonella* sp. in 25 g of sample. These results show that the fruits used were healthy and correctly sanitized, and that the processing was adequate. Other factors that also contribute to microbiological safety are the use of heat in the processing and the high acidity of the product, besides Aw of approximately 0.70.

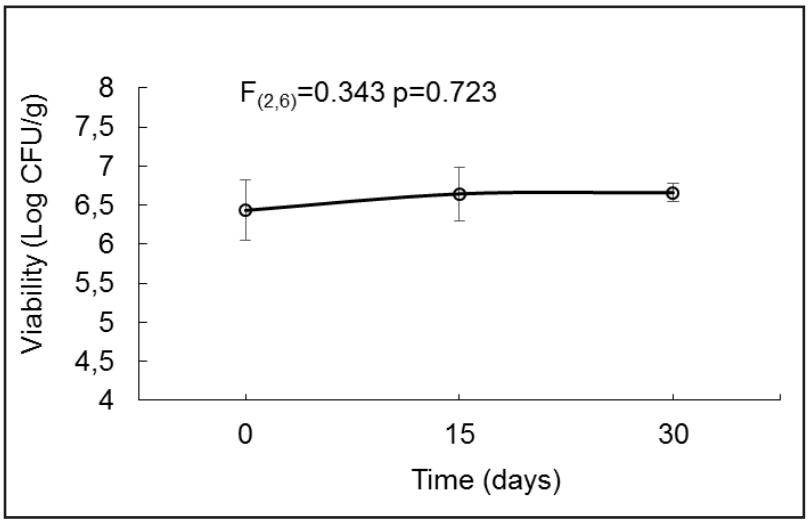
B. coagulans GBI-30 6086 remained viable during the storage period of the candies, with an average of 6.57 log CFU/g during the 30 days of storage, with no influence of time on the counting over the shelf life (Figure 3), which demonstrates the stability of the culture in the food matrix.

The international literature reports that it is necessary > 6.0 CFU of probiotic bacterium per serving of food to be considered a probiotic (Madureira et al., 2011; Argyri et al., 2013). Based on these results, the gummy candies showed promising counts. According to Martins et al. (2016), to guarantee the survival of probiotic microorganisms during storage time is a technological challenge, since most probiotic bacteria are sensitive to exposure to oxygen, heat and acids. This problem can be solved using strains of probiotic bacilli, as used in the present study.

Miranda et al. (2020), when evaluating the viability of *B. coagulans* in gelatin candy, found counts greater than 6.4 log CFU/g over the 90-day storage period. The viability results confirm that this probiotic is a viable option to be used in the production of functional foods that are subjected to high temperatures during processing, as they showed good counts in the final product and throughout the shelf life.

However, the incorporation of probiotic microorganisms into a food matrix also requires studies on their survival during industrial processing and storage conditions, and during the stress conditions imposed during gastrointestinal transit to the site of action (Bernat et al., 2014), in order to guarantee the effective action of the probiotic bacteria (Botta et al., 2013).

Figure 3 – Viability of *B. coagulans* GBI-30 6086 in jussara and passion fruit pectin candies during the storage period at 28 °C. Erro bars represent ± standard deviation (n=3)



Source: research data

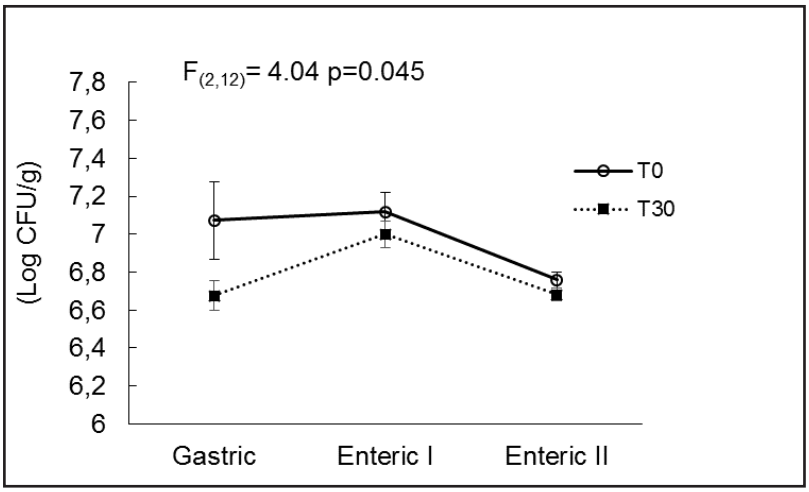
According to Minekus et al. (2014), during digestion, the food is submitted to the action of gastric pepsin at an acidic pH, finally reaching the intestine, where it again encounters a pH value close to neutrality and with the bile salts.

When evaluating the behavior of the microorganism in the different phases, gastric, enteric I e enteric II, it was verified that *B. coagulans* reached the end of the gastrointestinal simulation with an average count of 6.72 log CFU/g (Figure 4). Although viability has differed over time (p<0.05), *B. coagulans* reached the end of the shelf life of the gummy, in the enteric phase II, with counts that ensure the effective action of the probiotic for the consumer. Therefore, the gummy candies can be considered a potentially probiotic product.

Hong et al. (2005) highlighted the resistance of *Bacillus* strains to many food processing operations, as well as survival in adverse gastric and intestinal conditions, due to their sporulated characteristic. Sporulated bacteria can resist high temperatures, including pasteurization and low pH (Evelyn & Silva, 2019). Therefore, the use of *B. coagulans* in the preparation of pectin candies is a viable alternative.

Thus, the selection of a suitable strain is essential to obtain success in the elaboration of a functional probiotic product, since the interaction between the microorganism and the matrix must help in the resistance of the bacteria during the passage through the gastrointestinal tract (Do Espírito-Santo et al., 2011; Burns et al., 2014).

Figure 4 – Viability of *B. coagulans* GBI-30 6086 in pectin candies after the different phases of *in vitro* simulation of gastrointestinal conditions. Erro bars represent ± standard deviation (n=3)



Source: research data

3.3.3 Sensorial acceptance

The results of the sensory acceptance indicate that the pectin candy containing the probiotic had excellent acceptance, meeting the expectations of consumers, since most identified the aroma, flavor, and texture as pleasant. The purple and attractive

color, characteristic of jussara pulp, was evidenced by approximately 90 consumers, as well as the nice overall rating. The overall evaluation obtained an average score of 8.38 on the nine-point hedonic scale, which is equivalent to I liked it very much and I liked it extremely.

4 CONCLUSIONS

The mix of jussara and passion fruit pulp, as well as the addition of the probiotic, contributed positively to the development of functional pectin candies and the product meets the expectation of consumers and food industry, since developing solutions for healthy confectionery is a market trend. Jussara and passion fruit pulps acted as a natural additive in this study, contributing with color and antioxidant activity, which maintained during storage. Furthermore, the candies can be potentially probiotic due to the high counts of *Bacillus coagulans* GBI-30 6086 during shelf life and at the end of the gastrointestinal simulation. The high acceptability of pectin candies suggests the product's market potential in the confectionery industry.

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