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Do production and storage affect the quality of green banana biomass?

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ABSTRACT

The consumption of green banana (GB) products is booming because of their nutritional and physiological benefits to human health. However, there have been no studies on the quality of these products after they are processed and stored. Therefore, this study aimed to investigate whether production and storage affect the sensory and physicochemical characteristics of green banana biomass (GBB). We performed microbiological tests (total coliforms, *E. coli, Salmonella* spp., aerobic psychrotrophic bacteria, molds, yeasts, and *Staphylococcus aureus*); centesimal composition analysis (moisture, protein, ash, lipid, and fiber); and vitamin C, total phenolic content, and resistant starch analysis using official methods. The tests were performed on GBB samples (pressure-cooked for 5 or 10 min) that were stored in freezing (−12 °C) or refrigerated conditions (4 °C). We evaluated the color using a ColorQuestXE Spectrophotometer, and we conducted sensory analysis by the Check-all-that-apply test. We then used statistical tests to analyze the data. Five-minute pressure-cooking preserved the functional components, such as vitamin C, phenolic content, and fiber, better than the longer cooking process (10 min). There was no difference in acceptance attributes between the two cooking times. Refrigeration was better than frozen storage for vitamin C and fiber content, but it did not affect the resistant starch and phenolic content. Refrigerated GBB had the best sensory test results for flavor, texture, and overall acceptance. The refrigerated storage of GBB seems to be the best option for consumer acceptance and maintenance of nutrients and bioactive compounds (vitamin C, resistant starch, and phenolic content).

1. Introduction

Bananas (*Musa* spp.) are the most widely cultivated tropical fruit worldwide. About one-third of all bananas harvested are lost because it is a climacteric fruit that is mainly consumed when ripened. Ripe bananas are prone to mechanical damage and are perishable during the ripening process, so they are difficult to store and transport (Jiang et al., 2015). Therefore, studies have evaluated the optimization of banana processing to reduce the waste and to improve the bioavailability and utilization of nutrients from unripe fruit (Anyasi, Jideani, & Mchau, 2013; Jiang et al., 2015; Zandonadi et al., 2012).

The consumption of green banana (GB) products is booming because of their nutritional and physiological benefits to human health (Zandonadi et al., 2012). GB is a good source of fibers, vitamins, minerals, and bioactive compounds (such as phenolic compounds), and resistant starch (RS) (Chávez-Salazar et al., 2017; LII, CHANG, & YOUNG, 1982). Studies have shown that the consumption of GB could contribute to the control of the glycemic index, cholesterol, gastric fullness, intestinal regularity, and fermentation by intestinal bacteria, which produces short-chain fatty acids that can prevent intestinal cancer (Anyasi et al., 2013; Basso, Silva, Bender, & Silveira, 2011; Bodinham, Frost, & Robertson, 2010; Choo & Aziz, 2010; Costa, Alencar, Rullo, & Taralo, 2017; Dutra-de-Oliveira & Marchini, 2008; Zandonadi et al., 2012). The benefits derived from the consumption of green bananas classify them as a functional food (Anyasi et al., 2013; Padam, Tin, Chye, & Abdullah, 2014; Silva, dos, Barbosa Junior, &

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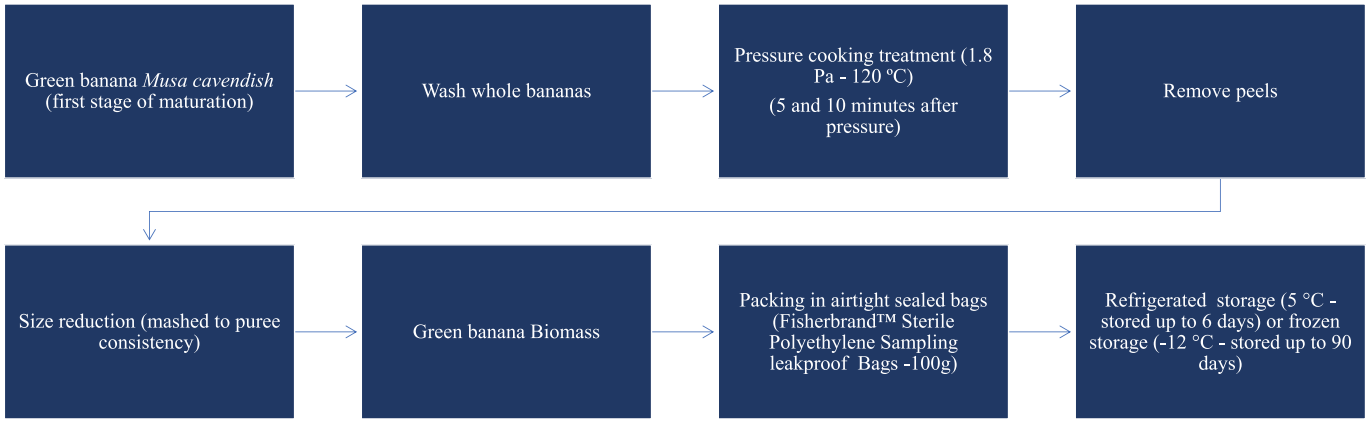

Fig. 1. Green banana biomass production process. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Barbosa, 2015). The term "functional" is defined as "Natural or processed foods that contain known or unknown biologically-active compounds; which, in defined, effective non-toxic amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease" (Martirosyan & Singharaj, 2016), even though the term is not defined by law in the USA (FDA, 2019) and Europe (Comission, 2007).

GB is not usually consumed *in natura*, mainly because of its typical hardness and high astringency due to the presence of soluble phenolic compounds, such as tannins (Sarawong, Schoenlechner, Sekiguchi, Berghofer, & Ng, 2014). Therefore, some studies have used GB as a functional ingredient in different formulations, such as cookies (Fasolin, Almeida, Castanho, & Netto-Oliveira, 2007), pasta (Vernaza, Gularte, & Chang, 2011; Zandonadi et al., 2012), mayonnaise (Izidoro, Scheer, Sierakowski, & Haminiuk, 2008; Ritthiruangdej, Parnbankled, Donchedee, & Wongsagonsup, 2011), cakes (Borges, Pereira, Silva Júnior, Lucena, & Sales, 2010; Oliveira de Souza et al., 2018), and dairy products (Costa et al., 2017; Vogado et al., 2018).

Some studies have examined different GB-derived products used as flour (dried and milled) (Anderson & Guraya, 2006; Bezerra, Amante, de Oliveira, Rodrigues, & da Silva, 2013), and others have used green banana biomass (GBB) (obtained by the trituration of green banana cooked under pressure) (Izidoro et al., 2008; Singh, Singh, Kaur, & Singh, 2016). The use of GBB is widespread in some countries because it is a potential 'functional product' that is easily produced at home at a low cost and stored by freezing or refrigeration (Santeramo et al., 2018).

Most of the studies have used GB flour because it is stable, and this product is well characterized in the scientific literature. However, this product tends to be expensive and inaccessible in some countries (Agama-Acevedo, Nuñez-Santiago, Alvarez-Ramirez, & Bello-Pérez, 2015; Alkarkhi, Ramli, Yong, & Easa, 2011; Bezerra et al., 2013; Borges et al., 2010; Charnchai et al., 2016; da Mota, Lajolo, Cordenunsi, & Ciacco, 2000; Fasolin et al., 2007; Haslinda, Cheng, Chong, & Aziah, 2009; Hoffmann Sardá et al., 2016; Juarez-Garcia, Agama-Acevedo, Sayago-Ayerdi, Rodriguez-Ambriz, & Bello-Pérez, 2006; Juarez-Garcia, Agama-Acevedo, et al., 2006; Liao & Hung, 2015; Pelissari, Andrade-Mahecha, Sobral, & Menegalli, 2012; Vatanasuchart, Butsuwan, & Narasri, 2015; Zandonadi et al., 2012). Despite the use of green banana puree in some studies (Borges et al., 2010; Oliveira de Souza et al., 2018; Vogado et al., 2018) and the growing demand for GBB in several countries (Alkarkhi et al., 2011; Apostolopoulos, Antonipillai, Tangalakis, Ashton, & Stojanovska, 2017; Meneses, Molina, & Vargas, 2016), such as Brazil, there are few studies that have characterized the nutritional, microbiological, and sensorial qualities of GBB after its storage. The characterization of green banana biomass is essential for its application to products to improve its functional characteristics.

Given the high utilization of GBB as an ingredient in preparations and the relevance of food composition data in the provision of accurate information for different situations (such as public policy, dietetic prescription, product developments, menu planning) (Sivakumaran, Huffman, & Sivakumaran, 2018), our study aimed to investigate GBB's microbiological, physicochemical, and sensorial characteristics after production and storage. To our knowledge, this is the first study of its kind that evaluated the quality parameters in an aim to provide information on the safety, sensory, and nutritional aspects of GBB after production and storage.

2. Materials and methods

2.1. Green banana biomass

We collected the green banana (GB) samples (Musa Cavendish) in the first stage of maturation (totally green) (Anyasi et al., 2013) at "Central de Abastecimento de Brasília" (CEASA, Brasília/Brazil). We chose the banana variety (Musa Cavendish) for its high consumption in Brazil and worldwide distribution (IBGE, 2011). We followed the procedures illustrated in Fig. 1 to produce green banana biomass (GBB).

Banana suppliers were selected according to the Brazilian Table of Food Composition methodology (TACO, 2011). GB was acquired on 3 different days (3 different lots of production) from the 3 biggest CEASA suppliers of banana. We acquired 3 kg of GB per day from each supplier. In total, about 30 kg of GB was purchased to produce GBB (TACO, 2011). GBB was produced by washing whole green bananas (GB) and then cooking them under pressure (1.8 Pa, 120 °C) in a pressure cooker for either 5 or 10 min. We stripped the GB, and the pulp was mashed for 5 min in a multiprocessor (Philco®, Brazil).

We prepared samples by the quartet technique (Greenfield & Southgate, 2003) as follows. The puree samples produced were divided into quadrants and, after successive homogenizations and subdivisions into smaller parts, samples of approximately 100 g were packed into individual sterile bags (Fisherbrand™ Sterile Polyethylene Sampling leakproof Bags) and labeled for further analysis. Sample preparation occurred in the laboratory with ambient light (similar to domestic production). We stored part of the GBB samples (50%) under refrigerated conditions at 4 °C and the other part (50%) under frozen conditions at −12 °C under vacuum (with no light inside the storage equipment). We monitored the storage temperature using a thermometer, and we opened the storage equipment only to retrieve a sample (100 g) for analysis.

2.2. Microbiological stability

We performed microbiological analysis with the GBB samples

subjected to the cooking treatment for either 5 or 10 min after preparation and during the storage period under refrigerated conditions at 4 °C (0, 3, 6, and 9 days) or freezing conditions at – 12 °C (0, 30, 60, and 90 days). We used the limits established for thermotolerant coliforms (2 log CFU/g), *Salmonella* spp. (presence or absence in 25 g of food), aerobic psychrotrophic bacteria (3.7 log CFU/g), and molds and yeasts (3.7 log CFU/g) (Codex Alimentarius Comission, 1997). For *S. aureus*, values of $\leq 10^4$ CFU/g or mL were considered acceptable (Fetsch, 2018) since higher numbers indicate that the food was prepared, packaged, or maintained in unhealthy conditions, which can cause foodborne diseases. We diluted the GBB samples (25 g) in 225 mL of 0.85% saline solution. After serial dilutions, we used the counting technique on Petrifilm 3M ™ to quantify the following microorganisms: Total *coliforms* and *E. coli* (6404), *Salmonella* spp. *(6536)*, molds and yeasts (6407), aerobic psychrotrophic bacteria (6400), and *Staphylococcus aureus* (6490) (ANVISA, 2001; AOAC, 2005; Brasil, 2000; Fetsch, 2018). We used microbiological stability to evaluate the safety period of GBB storage for consumption and carried out physicochemical and sensory analyses. The tests were stopped when the samples presented critical microbiological alterations, surpassed reference values, or underwent visual changes (ANVISA, 2001; Brasil, 2000; Codex Alimentarius Comission, 1997).

2.3. Chemical analyses

2.3.1. Centesimal composition

Samples (N = 6) cooked under pressure (5 or 10 min) were withdrawn immediately after cooking (T0), after refrigerated storage (T6: 4 °C; 6 days), and after frozen storage (T90: −12 °C; 90 days) to conduct the centesimal composition analysis. The duration of the storage for composition analysis was based on the microbiological analysis results. We determined the moisture content using the gravimetric method (oven at 105 °C for 24 h) according to the Adolfo Lutz Institute (IAL, 2008). We used the AOAC method (AOAC, 2005) to quantify protein (method 920.152), ash (method 942.05), and crude fiber (method 962.09). We used the MD444/CI Fiber Digester (Marconi, Piracicaba, Brazil) to analyze total fiber. We performed lipid content analysis using petroleum ether extraction using the Ankom Extraction System (Model ANKOM XT10 Extractor, ANKOM Technology, NY, USA) by the Am 5-04 method (AOCS, 2005). We conducted all analyses in triplicate.

We calculated the carbohydrate content by using the differences between the values found for protein, lipids, ash, moisture, and crude fiber (AOAC, 2005), and the total energy value (TEV) was determined from the mean content of fat, carbohydrate, and protein and applied Atwater factors.

2.3.2. Total phenolic and vitamin C content

2.3.2.1. Sample preparation. The samples were frozen immediately after preparation at −12 °C under vacuum. For analyses of the bioactive compounds, frozen samples were lyophilized in a freeze-dryer for 48 h (semi-automatic, brand Cristh®, model Gamma 2–16 LCS Plus). Afterward, samples were milled using a pestle and mortar and then sieved in a 45 mesh (355 μm) granulometry. Then, the samples were stored in plastic bags under vacuum and kept in a freezer at −12 °C until analysis.

2.3.2.2. Sample extraction for total phenolic content determination. Freeze-dried samples (0.3 g) were homogenized in 20 mL of a 70% aqueous methanol solution and shaken for 30 min. After stirring, the solutions were centrifuged for 5 min at 17,600 × *g*, labeled, and stored frozen (−12 °C) in amber glass vials until the analyses were performed.

2.3.2.3. Total phenolic content. We determined total phenolic compounds using the Folin–Ciocalteu reagent (Singleton & Rossi, 1965). In an assay tube, 0.5 mL of extract sample was added to 2.0 mL of water and 1.0 mL of the Folin–Ciocalteu reagent. After 5 min, we added 3.0 mL of 6.0% Na_2CO_3 solution to the tube sample, which remained at room temperature for 1.5 h without illumination. We measured the absorbance at 765 nm (Abs_{765}) with UV–Vis Spectrophotometer (HP8453, Agilent Technologies, USA). Standard solutions of gallic acid (10–100 mg/L) were similarly treated to prepare the calibration curve (Equation (1)). We expressed the results as mg gallic acid equivalent (GAE) per 100 g FW (fresh weight).

$$Gallic\ acid\ (mg\ /L) = 983.107 * Abs_{765} + 3.6363\ R^2 = 0.99 \quad (1)$$

2.3.2.4. Vitamin C. We performed the vitamin C analysis according to the method reported by Terada et al. (Terada, Watanabe, Kunitomo, & Hayashi, 1978) and modified by Nunes et al. (Nunes, Brecht, Morais, & Sargent, 1995) (results expressed in mg/100 g of GBB on a dry basis).

Samples weighing 3 g were extracted with a solution of metaphosphoric acid (6% v/v) in a homogenizer for 1 min with refrigeration and then centrifuged at 10,000 × *g*. After 10 min, we adjusted the volume to 50 mL in a volumetric flask. An aliquot (1 mL) of each sample was added to test tubes containing one drop of 2,6-dichlorophenolindophenol (0.2%) and 1 mL of thiourea (2% w/v). After homogenization, we added 0.5 mL of 2,4-dinitrophenidridrazine (2% w/v) to the tubes, which were placed in a water bath at 96 °C. After 10 min the tubes were refrigerated to room temperature, whose absorbances were measured at 540 nm (Abs_{540}) using a spectrophotometer (HP8453, Agilent Technologies, USA). Dilutions of ascorbic acid standard solution were used (20–140 mg/L) to obtain the equation of the standard curve (Equation (2)).

$$Ascorbic\ acid\ (mg/L) = 152.91 * Abs_{540}\ R^2 = 0.99 \quad (2)$$

2.3.2.5. Resistant starch, non-resistant starch, and total starch content. Resistant starch (RS), non-resistant starch (NRS), and total starch (TS) content of the GBB samples were analyzed using the Starch Assay Resistant Kit (Megazyme®) (AOAC, 2015).

2.4. Sensory analysis

Six samples were used (as mentioned in section 2.3) to perform the sensory analysis. We selected these samples since the microbiological analysis showed that they were safe for consumption. We served the samples in conditions of clear ambient lighting at room temperature since some samples (T0 5' and T0 10") were not stored at the cold temperature. The frozen samples were taken out of the refrigerator/freezer until the temperature reached 24 °C for sensory evaluation. GBB (15 g) was served to the assessors by monadic presentation (with intervals ranging from 5 to 10 min) in a random and balanced order (Bassinello, Rocga, & Cobucci, 2004). We served 6 different samples of GBB to each assessor with filtered water at room temperature (24 °C) to cleanse the palate between each pair of samples. We evaluated the samples for acceptance and sensory characterization with the Check-All-That-Apply (CATA) test as described below.

2.4.1. Descriptor elicitation

Descriptive terms have been previously gathered according to the Repertory Grid method (Foley et al., 2009; Meilgaard, Civille, & Carr, 2007), which was used in this study. Eleven assessors were included in the sensory test. Randomized samples were presented in pairs, with 5 min intervals between each served pair, to elicit attribute descriptions for the appearance, aroma, flavor, and texture of GBB. The evaluators were asked to identify and record the similarities and differences found between sample pairs. After the individual survey of the attributes, the most relevant attributes that characterize the different GBB samples were selected by consensus among the evaluators with the help of a moderator. The total session time was 40 min.

2.4.2. Acceptance and descriptive sensory analysis (check-all-that-apply (CATA))

The acceptance and CATA tests were carried out with 121 consumers (82.6% (n = 100) female and 17.4% (n = 21) male; mean age of 23 ± 7.3 years). Fifteen grams of each GBB sample was served to consumers by monadic presentation in plastic plates that were labeled with random three-digit numbers. For the acceptance test, we used the 9-point hedonic scale in which 1 = disliked extremely and 9 = liked extremely (Peryam & Pilgrim, 1957) for the attributes, namely, appearance, flavor, aroma, texture, and overall acceptance. In the same session, we administered the CATA test. The terms were randomized for each sample and each assessor (Ares et al., 2014) using a complete block design, balanced for presentation order, and generated with the software XLSTAT 2016 (Addinsoft, France) (Ares et al., 2014). The consumer was asked to mark the terms that applied to each sample evaluated.

2.5. Colorimetric analysis

The GBB color evaluation was performed using the ColorQuest XE Spectrophotometer (HunterLab, Reston, United States) to obtain the values of the coordinates L, a, and b of the Hunter system. We evaluated the GBB color immediately after cooking; after 3 and 6 days stored under refrigerated conditions; and after 30, 60, and 90 days stored under freezing conditions. From the values of the coordinates L, a, and b, the parameters were obtained for hue (h, Equation (3)), color saturation or chroma (C, Equation (4)), color difference (ΔE, Equation (5)), and browning index (BI, Equation (6) and Equation (7)) (Buera, Lozano, & Petriella, 1985; Castañón, Argaiz, & López-Malo, 1999).

$$h = \arctan(b/a) \tag{3}$$

$$c = \sqrt{(a^2 + b^2)} \tag{4}$$

$$\Delta E = \sqrt{((L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2)} \tag{5}$$

$$BI = \frac{[100(x - 0.31)]}{0{,}17} \tag{6}$$

where

$$x = \frac{(a + 1.75L)}{(5.645L + a - 3.012b)} \tag{7}$$

2.6. Statistical analysis

For the physicochemical analysis, we used analysis of variance (ANOVA) at a significance level of 5%, followed by Tukey's averages comparison test. We performed all experiments in triplicate. For sensory analysis, the acceptance data were analyzed by Unidirectional Analysis of Variance (ANOVA), in which the source of variation was the sensory scores obtained, followed by multiple comparisons with Tukey ($p < 0.05$). For the descriptive test (CATA), we determined the frequency of each CATA term that was used by counting the number of consumers that used that term to describe each sample.

Non-parametric Cochran's Q test (Jaeger et al., 2015) was carried out for each CATA descriptor to evaluate differences in consumer perception of the GBB samples (Ares et al., 2014, 2013; Nsæ, Varela, & Berget, 2018). CATA data are the most common consumer-based product-profiling method and generate binary data (Ares et al., 2014, 2013; Nsæ et al., 2018). In the CATA test, consumers select the presence/absence of attributes from the predetermined list. The analysis for these types of data involves multiple correspondence analysis and non-parametric data tools, such as Cochran's Q test. Multiple pairwise comparisons were then performed using the Bonferroni procedure. Correspondence analysis was performed on the frequency table from each experimental treatment using chi-square distances. Instrumental, acceptance, and CATA data were associated using Multiple Factor Analysis. We applied the Principal Coordinates Analysis to the correlation coefficients, and the results were visualized in a two-dimensional map. We performed statistical analyses using the XLSTAT 2015 Program (Addinsoft, Paris, France).

3. Results and discussion

GBB is booming as one of the main commercial or home-processed banana products; it is widely used all over the world for the production of foods with a functional appeal (Yap, Fernando, Brennan, Jayasena, & Coorey, 2017). We used green banana at stage 1 of maturation since it presents high antioxidant compound levels, high starch content, and low sugar content (Apostolopoulos et al., 2017).

People usually prepare high amounts of GBB and store it for use afterward since it is a product that requires several steps (Fig. 1) and is time-consuming to prepare. We used 4 °C for storage in accordance with FAO's recommendation (FAO, 2014). For frozen storage, we used a temperature of −12 °C since the temperature of a domestic fridge varies from −5 to −20 °C, depending on the amount of food inside, the frequency of opening the appliance doors, the oscillation of energy at the place of installation, and others reasons (Marklinder, Lindblad, Eriksson, Finnson, & Lindqvist, 2004; Ndraha, Hsiao, Vlajic, Yang, & Lin, 2018).

Since storage conditions affect shelf life, it is necessary to determine the period of storage to ensure the safety of the product to consumers. We evaluated GBB for the presence of pathogenic and deteriorating microorganisms after cooking and during storage treatments of refrigeration and freezing (Table 1). Microbiological tests showed that GBB in refrigerated storage is safe until the 6th day (T6) and up to the 90th day (T90) for frozen storage.

The cooking time (5 and 10 min) did not influence the level of contamination after heating and during the storage period in refrigerated conditions (we did not observe the presence of *Salmonella* spp., total coliforms, *E. coli*, or *S. aureus* in GBB samples stored for 9 days in refrigerated conditions). However, we observed the presence of aerobic psychrotrophic bacteria and molds and yeasts at 6 days of refrigeration, so 6 days was considered the maximum storage period for refrigerated samples.

Brazilian legislation (ANVISA, 2001) does not establish a microbiological standard for deteriorating microorganisms (aerobic psychrotrophic bacteria; molds and yeasts) in fruits subjected to thermal treatment since they are not pathogenic. However, they affect the sensorial characteristics of the product and the product's shelf life. Moreover, according to the *International Commission on Microbiological Specifications for Foods* (ICMSF), conducting microbiological tests on the final products could be a good strategy for verifying its quality, which includes mold and yeast counts (International Commission on Microbiological Specifications & for Foods, 2011). Therefore, after the 12th day of refrigerated storage of GBB, there was no evaluation conducted because there was a visible growth of molds.

A cooking time of 5 or 10 min and appropriate hygienic conditions were enough to prevent the presence of pathogenic microorganisms in GBB. However, storage under refrigerated conditions was not enough to inhibit mold and yeast and aerobic psychrotrophic bacterial growth after 6 days. This result was expected since ready-to-eat foods stored in household refrigerators typically have a shelf life of less than 5 days.

In the case of samples stored under freezing conditions (−12 °C), the shelf life of GBB was longer than that of refrigerated samples as evidenced by the absence of microbial growth for up to 90 days of storage. According to the microbiological analyses, it is possible to prepare GBB in a domestic environment by adopting good practices during all stages of production.

When subjected to heat treatment, the microbiological count tends to decrease significantly, while the occurrence of contamination can result from food contacting contaminated surfaces. Thus, the

Table 1
Microbiological analysis of green banana biomass (GBB) during the storage period under refrigeration (4 °C) and freezing (−12 °C).

Microorganisms (log CFU/g)	Refrigeration							
	Storage period (days)							
	0	3	6	9	0	3	6	9
	Cooking time - 5 min				Cooking time - 10 min			
Salmonella sp.	abs	abs	abs	abs	abs	abs	abs	abs
Staphylococcus aureus	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*
Total coliforms	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*
Mold and yeast	< 1*	< 3*	2.6	> 3.2*	< 1*	< 3*	3.1*	> 4.0*
Psychrotrophic aerobes	< 1*	< 1*	2.7	3.1	< 1*	2.9	> 2.8*	> 5.5*
Microorganisms (log CFU/g)	Freezing							
	Storage period (days)							
	0	30	60	90	0	30	60	90
	Cooking time - 5 min				Cooking time - 10 min			
Salmonella sp.	abs	abs	abs	abs	abs	abs	abs	abs
Staphylococcus aureus	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*
Total coliforms	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*	< 1*
Mold and yeast	< 1*	< 1.4*	< 1*	< 1.5*	< 1*	< 1*	< 1*	< 1*
Psychrotrophic aerobes	< 1	< 1*	< 1*	< 1.5*	< 1*	< 1*	< 1*	< 1*

abs – Absence.
* Estimated data.

microbiological evaluation of GBB helped to verify the appropriate hygiene practices to adopt during its processing and to monitor the microbial growth related to product quality after different storage conditions (Adiani, Gupta, Ambolikar, & Variyar, 2018; Snyder & Worobo, 2018).

The presence of *Staphylococcus aureus* did not occur, confirming the adequacy of the practices adopted during food processing. According to Fetsch (Fetsch, 2018), high numbers of *S. aureus* ($\geq 10^4$ CFU/g or mL) are evidence that food has been prepared, packaged, or maintained in inadequate conditions and may have become unsafe for consumption.

Mold and yeast count in food are useful for evaluating the quality of the product as well as for determining its safety. Our results (Table 1) show a gradual increase in psychrotrophic aerobes, especially in refrigerated biomass (Table 1). These microorganisms can grow at low temperatures and contribute to the deterioration of refrigerated foods (Graça, Esteves, Nunes, Abadias, & Quintas, 2017).

Compared with fresh and whole fruit, we observed a significant reduction in storage life. According to information provided by the FAO, bananas can remain adequate for consumption when packed at 13–15 °C for a period between 7 and 28 days. The biological structure of the banana, the temperature, and its degree of maturation are determinants of the shelf life. However, the ability to prepare GBB and the potential for freezing it are decisive factors for the consumption of the product at this point in its maturation, and utilization of its nutritional components should be considered.

The results of microbiological analyses show that the refrigerated GBB was safe for consumption until the 6th day and the frozen GBB was safe up to the 90th day.

3.1. GBB composition

Table 2 presents the results of the centesimal composition analysis of GBB cooked under pressure (5 or 10 min) and stored by refrigeration (T6) or freezing (T90) methods. The cooking time did not alter the moisture content, ash, protein, carbohydrate, and lipid contents. However, it decreased the crude fiber content ($p < 0.05$), but it only decreased for the frozen sample cooked under pressure for 10 min. Regardless of cooking time, GBB had high moisture, fiber, and carbohydrate content and low protein, ash, and lipid content. Its total energy value (TEV) was about 75 kcal/100 g.

The crude fiber content of GBB ranged from 1.55 g/100 g–2.68 g/100 g (Table 2), which is lower than the concentration found in GB flour (6.3–15.5 g/100 g) because the latter is dehydrated (da Mota et al., 2000; Juarez-Garcia, Agama-Azevedo, Sayago-Ayerdi, Rodriguez-Ambriz, & Bello-Perez, 2006; Tribess et al., 2009).

Food can be considered a source of dietary fiber if it contains 1.5 g of fiber per 100 kcal of food, and it can be regarded as high in fiber if it contains 3 g of fiber per 100 kcal (FAO, 2009). Since the total amount of crude fiber is lower than that of dietary fiber (Pak, Yañez, & Araya, 1987), the GBB samples cooked for 5 min can be considered high in fiber (3.46–3.57 g of crude fiber/100 kcal of food), and the samples cooked for 10 min can be considered a source of fiber (2.06–2.89 g of crude fiber/100 kcal of food). Therefore, in the present study, the longer cooking time reduced the fiber content significantly and converted GBB from high in fiber (5 min pressure-cooked) to a source of fiber (10 min pressure-cooked).

The crude fiber content was affected by the cooking time, and the freezing storage decreased the fiber content (Table 2). The reduction in vegetable fiber could be attributed to the partial degradation of cellulose and hemicellulose (crude fibers) into simple carbohydrates during the cooking process (Rehinan, Rashid, & Shah, 2004). Herranz et al. (Herranz, Vidal-Valverde, & Rojas-Hidalgo, 1983) demonstrated that a high-temperature cooking process decreases crude fiber (cellulose and lignin) content, with a slight change in hemicellulose content.

Wennberg et al. (Wennberg, Engqvist, & Nyman, 2003) studied the effects of boiling on fiber in white cabbage (*Brassica oleracea* var. *capitata*). They demonstrated that the boiling reduced about 10% of the cabbage fiber content. Our study showed a reduction in about 16% of the crude fiber content in GBB cooked for 10 min. It is important to highlight that the samples were cooked under pressure (which results in higher temperatures than traditional boiling) in the present research.

Rehinan et al. (Rehinan et al., 2004) studied the fiber content in legumes cooked by different processes. They showed that there was a greater reduction in fiber content from pressure-cooking than other cooking methods.

The protein content was not affected by the different cooking times or by the freezing or refrigerated storage process. Since bananas do not contain high amounts of either protein or lipids, GBB is not considered to be a source of protein or lipids, and changes in their content do not significantly affect GBB composition. The crude fiber content was

Table 2
Mean and standard deviation of the green banana biomass (GBB) composition cooked under pressure (5 and 10 min) after cooking (T0) and after storage under refrigeration (4 °C – 6 days - T6) and freezing (− 12 °C – 90 days – T90) (N = 6).

Storage time (days)/ Cooking time (min)	Moisture (g/100 gg)	Ash (g/100 gg)	Protein (g/100 gg)	Lipid (g/100 gg)	Crude Fiber (g/ 100 gg)	Carbohydrates (g/ 100 gg)	Resistant Starch (g/100 gg)	Non-resistant starch (g/100 gg)	Total starch (g/ 100 gg)	Vitamin C (mg/ 100 gg)	Phenolic compounds (mg GAE/100 g)
Mean ± Standard deviation											
T0 5′	$78.78^{ab} \pm 0.13$	$0.43^{b} \pm 0.04$	$1.44^{a} \pm 0.13$	$0.20^{c} \pm 0.01$	$2.60^{a} \pm 0.02$	$16.65^{ab} \pm 0.09$	$7.90^{ab} \pm 0.40$	$62.00^{a} \pm 0.30$	$69.90^{a} \pm 0.60$	$54.40^{a} \pm 0.80$	$322.20^{a} \pm 1.80$
T6 5′	$78.79^{ab} \pm 0.17$	$0.71^{a} \pm 0.04$	$1.57^{a} \pm 0.03$	$0.34^{b} \pm 0.01$	$2.68^{a} \pm 0.09$	$15.94^{c} \pm 0.02$	$7.60^{b} \pm 0.30$	$51.90^{b} \pm 1.80$	$59.50^{b} \pm 2.10$	$57.40^{a} \pm 0.80$	$308.30^{a} \pm 9.50$
T0 10′	$78.37^{b} \pm 0.02$	$0.41^{b} \pm 0.02$	$1.46^{a} \pm 0.03$	$0.20^{c} \pm 0.01$	$2.17^{b} \pm 0.02$	$17.39^{a} \pm 0.04$	$9.10^{a} \pm 0.50$	$59.70^{a} \pm 1.50$	$68.80^{a} \pm 2.00$	$23.10^{b} \pm 2.60$	$201.50^{b} \pm 7.80$
T6 10′	$78.96^{a} \pm 0.26$	$0.69^{a} \pm 0.01$	$1.44^{a} \pm 0.09$	$0.49^{a} \pm 0.01$	$1.55^{c} \pm 0.02$	$16.82^{b} \pm 0.34$	$9.10^{a} \pm 0.10$	$58.70^{a} \pm 1.70$	$67.70^{a} \pm 1.80$	$21.20^{b} \pm 2.00$	$189.90^{b} \pm 15.70$
T0 5′	$78.72^{A} \pm 0.11$	$0.43^{B} \pm 0.03$	$1.44^{A} \pm 0.07$	$0.20^{C} \pm 0.01$	$2.60^{B} \pm 0.02$	$16.62^{B} \pm 0.04$	$7.90^{AB} \pm 0.40$	$62.00^{A} \pm 0.30$	$69.90^{A} \pm 0.60$	$54.40^{a} \pm 0.80$	$322.20^{a} \pm 1.80$
T90 5′	$77.89^{AB} \pm 1.02$	$0.67^{A} \pm 0.05$	$1.35^{A} \pm 0.12$	$0.43^{B} \pm 0.02$	$2.66^{B} \pm 0.03$	$16.99^{B} \pm 0.86$	$7.20^{B} \pm 0.10$	$59.60^{A} \pm 1.40$	$66.90^{A} \pm 1.50$	$12.30^{c} \pm 0.50$	$334.40^{a} \pm 1.80$
T0 10′	$78.40^{AB} \pm 0.06$	$0.41^{B} \pm 0.02$	$1.44^{A} \pm 0.02$	$0.21^{C} \pm 0.01$	$2.17^{C} \pm 0.02$	$17.38^{AB} \pm 0.07$	$9.10^{A} \pm 0.50$	$59.70^{A} \pm 1.50$	$68.80^{A} \pm 2.00$	$23.10^{b} \pm 2.60$	$201.50^{b} \pm 7.80$
T90 10′	$76.36^{B} \pm 1.00$	$0.65^{A} \pm 0.07$	$1.35^{A} \pm 0.14$	$0.56^{A} \pm 0.02$	$1.92^{D} \pm 0.01$	$19.16^{A} \pm 1.02$	$8.10^{AB} \pm 0.30$	$46.80^{B} \pm 1.70$	$54.90^{A} \pm 2.10$	$11.60^{c} \pm 0.50$	$312.50^{b} \pm 3.10$

In the columns, averages followed by same lowercase letters and uppercase letters do not differ by Tukey's test ($p > 0.05$).
T0 5′: five minutes of pressured cooking without storage; T0 10′: ten minutes of pressured cooking without storage; T6 5′: five minutes of pressured cooking with refrigeration storage (4 °C) for 6 days; T6 10′: ten minutes of pressured cooking with refrigeration storage (4 °C) for 6 days; T90 5′: five minutes of pressured cooking with frozen storage (− 12 °C) for 90 days; T90 10′: ten minutes of pressured cooking with frozen storage (− 12 °C) for 90 days.

affected by the cooking time and frozen storage. However, in general, the GBB can be considered a source of fiber (cooked for 10 min) or high in fiber (cooked for 5 min).

3.2. Antioxidant compound analysis

Wall (Wall, 2006) reported that the amount of vitamin C in ripened banana ranges from 2.1 to 18.7 mg/100 g. Green bananas tend to contain higher amounts of vitamin C since its content decreases in bananas with increasing maturation and ripeness. Our study showed that the amount of vitamin C in GBB (before storage) ranged from 23 to 54 mg/100 g, depending on the cooking time.

GBB cooked for 5 min presented higher levels of vitamin C and phenolic compounds ($p < 0.05$) when compared with the GBB cooked for 10 min (Table 2). Stable values of vitamin C and phenolic compounds ($p < 0.05$) were maintained during refrigerated storage, regardless of cooking time. Galani et al. (Galani, Patel, Patel, & Talati, 2017) reported the reduction in vitamin C content in ripened bananas stored under refrigerated conditions (4 °C) for 15 days, contrary to our study results (Table 2), which indicate that vitamin C content did not differ with refrigeration. This difference likely occurred because the bananas were processed, which promotes oxidation reactions, in addition to the different conditions between the two studies. The banana varieties, harvest time, and postharvest handling can affect vitamin C content (Mohapatra, Mishra, Singh, & Jayas, 2011). In our study, we used green banana cooked under pressure and stored for 6 days under refrigerated conditions. Also, we evaluated the vitamin C content after the cooking process, which revealed that the loss of vitamin C (to the cooking water) occurred before the analysis of refrigerated samples.

Likewise, according to the study conducted by Galani et al. (Galani et al., 2017), the total phenolic content in ripened banana decreased when stored in refrigerated conditions (4 °C) for 15 days. However, the author stated that the total phenolic content can be influenced by many factors, such as genotype, harvest time, and growing location. In this context, the synthesis of phenolic compounds at low temperatures could be related to the degradation of the polyphenolic compounds as a result of changes in the pattern of enzyme activity in bananas (Galani et al., 2017). Since, in our study, we used high temperatures to cook the green banana samples, the enzymes were inactivated, so there was a lower chance that total phenolic contents would decrease during cold storage.

The storage under freezing conditions showed a significant decrease ($p < 0.05$) in vitamin C content, while the levels of phenolic compounds remained stable ($p < 0.05$) during the GBB storage time (90 days).

Processing and storage can significantly reduce vitamin C content in fruits because it is water-soluble and sensitive to high temperatures and oxidation. GBB is produced by cooking in hot water (100–120 °C). In order to reduce the cooking time, we cooked the banana under pressure (1.8 Pa; 120 °C), as recommended by some studies (Marques, de Oliveira, Aguiar-Oliveira, & Maldonado, 2017; Oliveira de Souza et al., 2018; Zandonadi et al., 2012). No studies have shown the effect of heating or cool storage on vitamin C and phenolic compound content in green banana. However, it is known that cooking in hot water results in enzymatic inactivation, changes in the texture of the fruits, and leaching of soluble compounds in the water, all of which alter the phytochemical composition of foods. Heat causes the breakdown of cell membranes, and the phytochemical compounds are released (Leong & Oey, 2012), which decreases their contents.

Previous studies corroborate our results showing a decrease in vitamin C content and phenolic compounds with increasing time of thermal processing (Alves, Paula, Cunha, Amaral, & Freitas, 2011; dos Reis et al., 2015; Lafarga, Viñas, Bobo, Simó, & Aguiló-Aguayo, 2018; Palma, D'Aquino, Vanadia, Angioni, & Schirra, 2013; Pellegrini et al., 2010). The heat breaks down cell membranes, which causes the release of phytochemical compounds and consequentially decreases their contents. Freezing decreases the vitamin C content in fruits and

vegetables (Oulai, Zoue, Adom, & Niamke, 2015), as we found in the present study.

The FDA states that the claim of "high" Vitamin C applies when the product contains at least 20% (≥18 mg) of the Daily Value (DV) of the nutrient, and it is considered a "good source" when it contains 10–19% of the DV (9–17.9 mg) (FDA, 2005). In this sense, for the daily consumption of 100 g of GBB, the refrigerated products are "high" in Vitamin C (21.2–57.4 mg), and frozen GBB is a "good source" of Vitamin C (11.6–12.3 mg).

3.3. Resistant starch, non-resistant starch, and total starch

The amount of resistant starch (RS) in GBB did not change ($p > 0.05$) throughout the storage period (refrigeration and freezing conditions) or as a result of the cooking time ($p > 0.05$) (Table 2). Most of the benefits attributed to GB consumption are associated with its RS content, which behaves physiologically like fiber to reduce glycemia, prevent intestinal diseases, reduce blood cholesterol levels, and decrease the risk of developing chronic diseases (Basso et al., 2011). RS has little impact on the sensory properties of food or consumer acceptance. The present study showed that the GBB contained high amounts of RS (about 8 g/100 g), which can confer potential health benefits to consumers (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010).

Only one study in Australia evaluated the total starch from green banana puree, and it showed 2.8 g/100 g of total starch in GBB (stage 1 of maturation) (Yap et al., 2017). Our study, which was carried out in Brazil, showed that total GBB starch ranged from 12.62 g/100 g–14.88 g/100 g (without a significant difference). There is a difference in the total starch amount between the two studies since, in the previous study, the tested product was produced with bananas from different countries, and the nutritional composition of a banana at harvest can vary widely depending on cultivar, maturity, climate, soil type, and fertility (Haslinda et al., 2009; Liao & Hung, 2015; Menezes et al., 2011; TACO, 2011). Moreover, Yap et al. (Yap et al., 2017) produced green banana puree instead of GBB (as in our study). They blanched whole unpeeled bananas by placing them in boiling water for 1 min; in contrast, we used pressure-cooking to produce GBB (the way it is produced in Brazil).

There have been no studies on the RS content in GBB, making it difficult to compare our results. In the present study, GBB presented stable RS content ($p > 0.05$) throughout the storage at low temperatures (4 °C and −12 °C), probably because amylopectin is the major fraction of the starch in GBB (da Mota et al., 2000). The polymers of retrograded amylopectin are less tightly bound than those of retrograded amylose, so they have less resistance to enzymatic hydrolysis, thus making the starch more digestible (Colonna, Leloup, & Buléon, 1992). Our results showed that the amount of resistant starch (RS) in GBB was stable throughout the cold storage and cooking process.

3.4. Sensory analysis

3.4.1. Acceptance and CATA

The average acceptance for all attributes was lower than 6.0 (Table 3), which we expected since people are not familiar with consuming isolated GBB. GBB is usually consumed in association with other ingredients to add nutrients, bioactive compounds, and texture-related characteristics (Oliveira de Souza et al., 2018; Ranieri & Delani, 2018; Souza, 2017; Zandonadi et al., 2012). There was no significant ($p > 0.05$) difference between the samples in terms of appearance and odor, indicating that the cooking time and storage conditions of GBB did not affect these sensorial attributes. It is important to mention that no study has evaluated the sensorial aspects of GBB. Only 2 studies have evaluated instrumental textural changes in bananas during ripening (Chauhan, Raju, Dasgupta, & Bawa, 2006; Kajuna, Bilanski, & Mittal, 1997), so it is difficult to compare our results.

The flavor of the frozen samples was significantly worse than that of the other samples, with no difference between the cooking times. Thus, storage conditions could compromise the sensory and technological characteristics of the products to which GBB is added. In light of this result, refrigeration is the best option for GBB storage for later use to obtain better sensory characteristics. The sensorial analysis showed that GBB stored under freezing conditions was less acceptable in terms of flavor, texture, and overall acceptance.

Similarly, the acceptance of the frozen samples' texture was lower ($p < 0.05$) when compared with the other samples, except for the GBB cooked for 10 min and stored under refrigerated conditions. These results suggest that the frozen storage probably modified the structure of the GBB and had an impact on the texture, thus compromising its sensorial quality. The overall acceptance confirmed these results by revealing that the frozen samples had the lowest acceptance, with no difference due to the cooking time.

CATA tests were performed to assess GBB quality resulting from the application of different cooking and storage methods (Table 4, Figs. 2 and 3). For the GBB CATA analysis, a team of evaluators collected the attributes using the Repertory Grid method (Meilgaard et al., 2007). The evaluators found a total of 77 attributes (appearance: n = 15; odor: n = 16; color: n = 10; flavor: n = 19; texture: n = 17) (Table 4). From the Cochran-Q test, it was possible to evaluate whether the consumers detected significant differences between the samples for each one of the terms that were elicited. Of the 77 terms mentioned in the CATA test, no statistical differences were observed in 38 terms (Table 4). Among the terms with a significant statistical difference, 13 were for appearance, 2 were for aroma, 5 were for color, 4 were for flavor, and 15 were for texture.

The most selected appearance attributes ($p < 0.05$) for the samples that were not stored and were cooked under pressure for 5 min (T0 5 ′) were "bright", "creamy", "soft", and "wet". For the "pasty" attribute, samples that were not stored and were cooked for 5 min (T0 5 ′) or 10 min (T0 10′) did not differ significantly and were the most frequently mentioned. The term "compact" was more frequently mentioned for the refrigerated samples (T6 5′ and T6 10′). For the "consistent" attribute, the most frequently mentioned samples were those cooked for 10 min without storage (T0 10 ′) and those stored in refrigerated conditions (T6 10′). The sample cooked for 10 min and refrigerated (T6 10 ′) was characterized as "solid". The term "dry" was more frequently chosen for the sample cooked for 5 min and then frozen (T90 5 ′). For the term "granular", the most frequently mentioned samples were those that were frozen (T90 5′ and T90 10′). The frozen samples were also the least frequently chosen for the terms "cooked", "homogeneous" and "smooth" attributes.

Seventy-seven terms were cited by the tasters (appearance: n = 15; odor: n = 16; color: n = 10; flavor: n = 19; texture: n = 17). We did not find significant differences between samples in 47% (n = 36) of the CATA terms (Table 4). Among the CATA terms with significant differences (53%, n = 41), 13 were appearance terms, 2 were odor terms, 6 were color terms, 5 were flavor terms, and 15 were texture terms. The statistically different appearance terms were "bright", "compact", "consistent", "cooked", "creamy", "granular", "homogeneous", "smooth", "soft", "pasty", "dry", "solid", and "wet".

The cooking of bananas was the most important variable for the item "odor" for the samples cooked without storage; for refrigerated GBB samples, cooking was important for the "odorless" variable. The terms that differed concerning odor were "cooked banana" (T0 10′) and "odorless" (T6 10′) (Table 4). Therefore, cold storage probably reduced the perception of a cooked banana odor among tasters. The other terms evaluated did not present significant differences. The item "soft" was cited with the highest frequency by the tasters, who characterized the GBB odor as soft. It is important to highlight that the acceptance test using the hedonic scale did not show a statistical difference in the odor attribute among the GBB samples.

Six terms were significantly different among GBB samples regarding

Table 3
Mean and standard-deviation of green banana biomass acceptance (9-point hedonic scale; n = 121 tasters).

Storage time (days)/Cooking time	Appearance	Odor	Flavor	Texture	Global acceptance
T0 5′ min.	5.1 ± 2.1[a]	4.6 ± 2.2[a]	4.1 ± 2.3[a]	4.7 ± 2.4[a]	4.7 ± 2.1[a]
T0 10′ min.	5.1 ± 2.2[a]	4.7 ± 2.2[a]	4.0 ± 2.3[a]	4.8 ± 2.3[a]	4.7 ± 2.2[a]
T6 5′ min.	4.9 ± 2.1[a]	4.8 ± 2.2[a]	4.1 ± 2.1[a]	4.9 ± 2.3[a]	4.6 ± 2.0[a]
T6 10′ min.	4.7 ± 2.3[a]	4.9 ± 2.1[a]	4.2 ± 2.2[a]	4.6 ± 2.1[ab]	4.6 ± 2.1[a]
T90 5′ min.	4.5 ± 2.1[a]	4.3 ± 2.1[a]	2.6 ± 2.0[b]	3.8 ± 2.1[b]	3.4 ± 2.1[b]
T90 10′ min.	4.8 ± 2.2[a]	4.5 ± 2.0[a]	3.0 ± 2.1[b]	3.9 ± 2.2[b]	3.8 ± 2.1[b]

In the columns, averages followed by equal letters do not differ by Tukey's test ($p > 0.05$).
T0 5': five minutes of pressured cooking without storage; T0 10': ten minutes of pressured cooking without storage; T6 5': five minutes of pressured cooking with refrigeration storage (4 °C) for 6 days; T6 10': ten minutes of pressured cooking with refrigeration storage (4 °C) for 6 days; T90 5': five minutes of pressured cooking with frozen storage (−12 °C) for 90 days; T90 10': ten minutes of pressured cooking with frozen storage (−12 °C) for 90 days.

color: "yellowish", "light", "whitish", "greenish color", "greenish tone", and "brown". The sample T6 5′ was the most frequently mentioned for the terms "light" and "whitish", and it was less noted as having a "greenish color". The "yellowish" term was most frequently mentioned for sample T0 5′, and the term "greenish tone" was most frequently mentioned for sample T0 10'. The "beige color" term was not significantly different among GBB samples; however, it was the most frequently mentioned term, characterizing the color of GBB as beige.

The flavor was significantly different among samples. The frozen samples (T90 5′ and T90 10′), regardless of the cooking time, were associated with the highest frequencies of the terms "bad", "watery", and rancid. Compared with another sample, the terms cited did not represent good acceptance, and this had an impact on the acceptance average (Table 3). The "neutral" term presented a significant difference among samples and was cited with the highest frequency for samples cooked for 10 min (except frozen samples), showing that the cooking time could interfere with GBB flavor. Sample T6 5 ′ was described most frequently by the term "cooked bean", and sample T90 5′ was less frequently mentioned ($p < 0.05$) for this term. The "neutral flavor" was mentioned with a high frequency for all GBB samples, indicating the neutral characteristic of GBB. GBB's neutral flavor enables its use in several products without having a high impact on the flavor of the product (Zandonadi et al., 2012).

Fifteen texture terms cited presented statistical differences ($p < 0.05$) among GBB samples: "with chunks"; "compact"; "consistent"; "dense"; "hard"; "spongy"; "fibrous"; "firm"; "gummy"; "smooth"; "cooked cassava"; "pasty"; "dry"; "wet", and "viscous". The terms most frequently cited for GBB samples without storage were "with chunks", "consistent", "dense", "firm", "gummy", and "pasty". Among the refrigerated samples, the most cited terms were "compact", "consistent", "dense", "firm", and "smooth"; the least cited terms were "with chunks", "spongy", "fibrous", and "gummy". For frozen samples, the terms most cited were "with chunks", "spongy", "fibrous", and "dry". Regarding the most recognized characteristics among samples, the samples without storage or refrigerated were similar, and the frozen storage led to the most different characteristics regarding texture. These results show that the storage type interferes with GBB characteristics, regardless of the cooking time.

The texture data presented a statistical difference ($p < 0.05$), and the term "with chunks" was more often cited for sample T0 5′ and less often cited for sample T6 10'. The term "compact" was most frequently mentioned for sample T6 10′ and less frequently mentioned for sample T90 10'. The T90 5′ sample was noted less often for the term "consistent". Sample T90 10′ was less frequently cited for the term "dense", and the sample most frequently associated with the term "hard" was T6 10'. The terms "spongy" and "fibrous" further characterized the T 90 5′ and T90 10′ samples. Samples T0 5′ and T0 10′ presented more frequent citations of the term "gummy" and "pasty". The term "smooth" was more frequently noted for sample T6 with a cooking time of 5 or 10 min.

For the terms "firm" and "cooked cassava", the sample cited with the lowest frequency was T90 10'. The term "dry" was more frequently noted for the T90 5 ′ sample, whereas the term "wet" was more frequently noted for sample T90 10′ and less frequently cited for sample T6 10'. Concerning the term "viscous", the most frequently noted sample was T0 5'. Comparing the texture sensory data with the analytical moisture data, we verified that the samples considered to be wetter were T0 5′ and T6 10′, but all samples presented values above 75% humidity. It is noteworthy that the frozen samples were most frequently described as "dry", despite the similarity in moisture content among samples. Despite the samples presenting similar moisture content, the tasters regarded the frozen GBB as the driest samples as a result of water loss while thawing.

There is a correlation between the attributes evaluated and tasters' preferences (Fig. 2). We verified that GBB acceptance was more correlated with the attributes "neutral", "consistent", and "smooth". The correspondence analysis map (Fig. 2) explains 85.73% of the variation in 2 dimensions ($p < 0.0001$). We verified that the refrigerated samples (T6 5′ and T6 10′) were close, and the attributes that were closer ("odorless", "solid", "firm") were depended on the characteristics presented in Table 4. Samples T0 10 ′ and T0 5′ are also close, being characterized by the attributes "cooked banana" and "creamy". The frozen samples exhibited most of the characteristics that are opposite of those used to describe samples T0 10′ and T0 5'. Sample T90 10′ presented some characteristics that are opposite of those used to describe samples T6 5 ′ and T6 10′, for example, "compact". Therefore, from the map, we were able to verify that the tasters probably found similarities among samples under different types of storage conditions, regardless of the cooking time.

Fig. 3 shows the correlation of the attributes with the tasters' preferences. We verified that sample acceptance was more related to the "neutral", "consistent", and "firm" attributes, and rejection was more related to the "bad", "dry", and "granular" attributes. In general, refrigerated storage of GBB seems to be the best option for achieving better sensorial acceptance.

3.5. Colorimetric analysis

Color saturation (chroma, C), color tonality (hue angle, h°), color difference (ΔE), and browning index (BI) showed that there were no significant changes ($p > 0.05$) in GBB color from the interaction between the different cooking times and storage types (Table 5). However, we verified that there was significant variation when the cooking time and storage time were analyzed separately for the chroma, hue angle, and BI variables. The chroma varied significantly with cooking and storage time (in refrigerated and frozen storage). The cooking time for the refrigerated GBB and the cooking and storage time for the frozen GBB significantly affected the hue. The BI varied significantly between refrigeration and freezing with the cooking time and storage time, separately.

The average chroma value of the refrigerated GBB cooked for 5 min (C = 13.43) was lower ($p = 0.002$) than that of refrigerated GBB

Table 4
Cochran's Q test for signed CATA terms and difference between samples for each term.

Terms	p-value	T0 5′	T0 10′	T6 5′	T6 10′	T90 5′	T90 10′
Appearance							
Pleasant	0.422	14^{a}	10^{a}	11^{a}	10^{a}	6^{a}	12^{a}
Bright	< 0.0001	16^{b}	9^{ab}	2^{a}	2^{a}	2^{a}	1^{a}
Compact	< 0.0001	10^{a}	14^{a}	30^{b}	38^{b}	13^{a}	8^{a}
Consistent	< 0.0001	38^{bc}	41^{c}	38^{bc}	45^{c}	19^{ab}	14^{a}
Cooked	< 0.0001	28^{b}	26^{b}	27^{b}	23^{b}	7^{a}	13^{ab}
Creamy	< 0.0001	37^{d}	36^{cd}	13^{b}	15^{bc}	1^{a}	1^{a}
Granular	< 0.0001	21^{b}	20^{b}	8^{ab}	5^{a}	50^{c}	67^{c}
Homogeneous	< 0.0001	18^{b}	23^{b}	23^{b}	21^{b}	1^{a}	3^{a}
Smooth	< 0.0001	7^{ab}	9^{ab}	20^{b}	18^{b}	1^{a}	0^{a}
Soft	< 0.0001	21^{b}	18^{ab}	10^{ab}	5^{a}	4^{a}	8^{ab}
Pasty	< 0.0001	55^{c}	63^{c}	30^{b}	26^{b}	9^{a}	7^{a}
Black dots	0.017	27^{a}	15^{a}	13^{a}	22^{a}	17^{a}	26^{a}
Dry	< 0.0001	6^{a}	8^{a}	27^{b}	30^{b}	63^{c}	46^{bc}
Solid	< 0.0001	17^{ab}	11^{a}	30^{bc}	44^{c}	29^{bc}	13^{a}
Wet	< 0.0001	44^{b}	40^{ab}	22^{a}	23^{a}	32^{ab}	42^{ab}
Odor							
Sweetish	0.131	6^{a}	8^{a}	10^{a}	3^{a}	5^{a}	3^{a}
Buttery	0.106	6^{a}	3^{a}	3^{a}	0^{a}	2^{a}	1^{a}
Sour	0.030	15^{a}	9^{a}	5^{a}	7^{a}	9^{a}	15^{a}
Cooked banana	0.004	22^{b}	21^{b}	18^{ab}	6^{a}	16^{ab}	14^{ab}
Ripe banana	0.722	4^{a}	6^{a}	4^{a}	3^{a}	3^{a}	2^{a}
Very ripe banana	0.014	7^{a}	3^{a}	1^{a}	1^{a}	4^{a}	9^{a}
Unripe banana	0.042	29^{a}	23^{a}	17^{a}	22^{a}	18^{a}	15^{a}
Citric	0.529	3^{a}	5^{a}	3^{a}	3^{a}	6^{a}	6^{a}
Clove	0.008	1^{a}	1^{a}	0^{a}	2^{a}	6^{a}	5^{a}
Unpleasant	0.020	29^{a}	27^{a}	14^{a}	18^{a}	22^{a}	26^{a}
Cooked bean	0.199	8^{a}	8^{a}	13^{a}	6^{a}	11^{a}	7^{a}
Tamarind	0.030	14^{a}	6^{a}	13^{a}	7^{a}	17^{a}	11^{a}
Jaca	0.115	3^{a}	5^{a}	0^{a}	3^{a}	1^{a}	1^{a}
Apple	0.197	1^{a}	3^{a}	0^{a}	1^{a}	3^{a}	2^{a}
Odorless	< 0.0001	16^{a}	24^{a}	34^{ab}	45^{b}	23^{a}	25^{a}
Soft	0.286	27^{a}	27^{a}	38^{a}	29^{a}	26^{a}	30^{a}
Color							
Yellowish	< 0.0001	22^{b}	6^{a}	3^{a}	3^{a}	14^{ab}	17^{ab}
Beige	0.004	37^{a}	36^{a}	53^{a}	55^{a}	46^{a}	53^{a}
Light	0.018	25^{ab}	28^{ab}	39^{b}	19^{a}	29^{ab}	28^{ab}
Whithish	< 0.0001	9^{a}	16^{ab}	32^{b}	14^{ab}	12^{a}	9^{a}
Dark	0.008	2^{a}	4^{a}	1^{a}	10^{a}	3^{a}	2^{a}
Greenish color	< 0.0001	26^{c}	27^{c}	2^{a}	7^{ab}	18^{bc}	14^{bc}
Greenish tone	< 0.0001	11^{ab}	16^{b}	2^{a}	3^{ab}	7^{ab}	3^{a}
Oxidated apple	0.128	9^{a}	7^{a}	2^{a}	9^{a}	7^{a}	4^{a}
Brown	< 0.0001	0^{a}	1^{a}	1^{a}	15^{b}	0^{a}	2^{ab}
Light brown	0.113	18^{a}	17^{a}	18^{a}	27^{a}	18^{a}	27^{a}
Flavor							
Accentuated	0.166	9^{a}	8^{a}	10^{a}	2^{a}	11^{a}	7^{a}
Sweetish	0.278	1^{a}	0^{a}	2^{a}	2^{a}	0^{a}	0^{a}
Adstringent	0.517	5^{a}	4^{a}	4^{a}	2^{a}	3^{a}	2^{a}
Pleasant	0.011	15^{a}	13^{a}	14^{a}	9^{a}	3^{a}	6^{a}
Watery	< 0.0001	8^{a}	9^{a}	11^{ab}	8^{a}	26^{bc}	27^{c}
Bitter	0.015	13^{a}	6^{a}	16^{a}	9^{a}	18^{a}	18^{a}
Sour	0.004	13^{a}	9^{a}	3^{a}	3^{a}	8^{a}	13^{a}
Banana	0.019	12^{a}	12^{a}	9^{a}	3^{a}	6^{a}	4^{a}
Very ripe banana	0.723	3^{a}	1^{a}	3^{a}	4^{a}	5^{a}	3^{a}
Biomass	0.001	20^{a}	16^{a}	15^{a}	20^{a}	7^{a}	7^{a}
Citric	0.284	5^{a}	5^{a}	1^{a}	2^{a}	1^{a}	3^{a}
Clove	0.228	0^{a}	0^{a}	1^{a}	0^{a}	2^{a}	2^{a}
Cooked bean	0.001	8^{ab}	12^{ab}	14^{b}	8^{ab}	2^{a}	3^{ab}
Jaca	0.286	2^{a}	0^{a}	1^{a}	1^{a}	0^{a}	3^{a}
Neutral	< 0.0001	25^{a}	40^{ab}	36^{a}	57^{b}	24^{a}	28^{a}
Rancid	0.003	12^{a}	12^{a}	19^{ab}	17^{ab}	30^{b}	20^{ab}
Bad	0.000	34^{a}	35^{a}	28^{a}	27^{a}	55^{b}	57^{b}
Salty	0.003	8^{a}	7^{a}	1^{a}	0^{a}	5^{a}	4^{a}
Tamarind	0.437	8^{a}	4^{a}	3^{a}	4^{a}	5^{a}	7^{a}
Texture							

(*continued on next page*)

Table 4 (*continued*)

Terms	p-value	T0 5′	T0 10′	T6 5′	T6 10′	T90 5′	T90 10′
		T0 5′	T0 10′	T6 5′	T6 10′	T90 5′	T90 10′
Rubbery	0.441	12^{a}	9^{a}	7^{a}	7^{a}	14^{a}	11^{a}
With chunks	< 0.0001	37^{c}	25^{bc}	14^{ab}	8^{a}	25^{bc}	32^{bc}
Compact	< 0.0001	10^{ab}	11^{ab}	19^{bc}	28^{c}	7^{ab}	4^{a}
Consistant	< 0.0001	34^{bc}	30^{abc}	23^{abc}	37^{c}	14^{a}	16^{ab}
Dense	< 0.0001	17^{b}	18^{b}	21^{b}	23^{b}	8^{ab}	2^{a}
Hard	< 0.0001	4^{a}	5^{a}	12^{ab}	22^{b}	11^{ab}	6^{a}
Spongy	< 0.0001	4^{a}	4^{a}	7^{a}	3^{a}	44^{b}	40^{b}
Fibrous	< 0.0001	3^{a}	1^{a}	2^{a}	3^{a}	17^{b}	17^{b}
Firmm	< 0.0001	24^{bc}	34^{cd}	32^{cd}	45^{d}	13^{ab}	7^{a}
Gummy	< 0.0001	26^{b}	33^{b}	6^{a}	5^{a}	3^{a}	5^{a}
Smooth	< 0.0001	8^{ab}	10^{ab}	23^{b}	17^{b}	0^{a}	0^{a}
Cooked cassava	< 0.0001	7^{ab}	6^{ab}	14^{b}	3^{ab}	2^{ab}	1^{a}
Butter	0.287	3^{a}	3^{a}	3^{a}	3^{a}	0^{a}	0^{a}
Pasty	< 0.0001	55^{c}	54^{c}	38^{bc}	25^{b}	6^{a}	6^{a}
Dry	< 0.0001	4^{a}	4^{a}	21^{b}	26^{b}	56^{c}	38^{bc}
Wet	0.001	42^{ab}	37^{ab}	35^{ab}	25^{a}	42^{ab}	54^{b}
Viscous	0.002	20^{b}	14^{ab}	8^{ab}	5^{a}	7^{ab}	8^{ab}

In rows, frequency counts followed by the same letter do not differ by Cochran's test ($p > 0.05$).
T0 5': five minutes of pressured cooking without storage; T0 10': ten minutes of pressured cooking without storage; T6 5': five minutes of pressured cooking with refrigeration storage (4 °C) for 6 days; T6 10': ten minutes of pressured cooking with refrigeration storage (4 °C) for 6 days; T90 5': five minutes of pressured cooking with frozen storage (−12 °C) for 90 days; T90 10': ten minutes of pressured cooking with frozen storage (−12 °C) for 90 days.

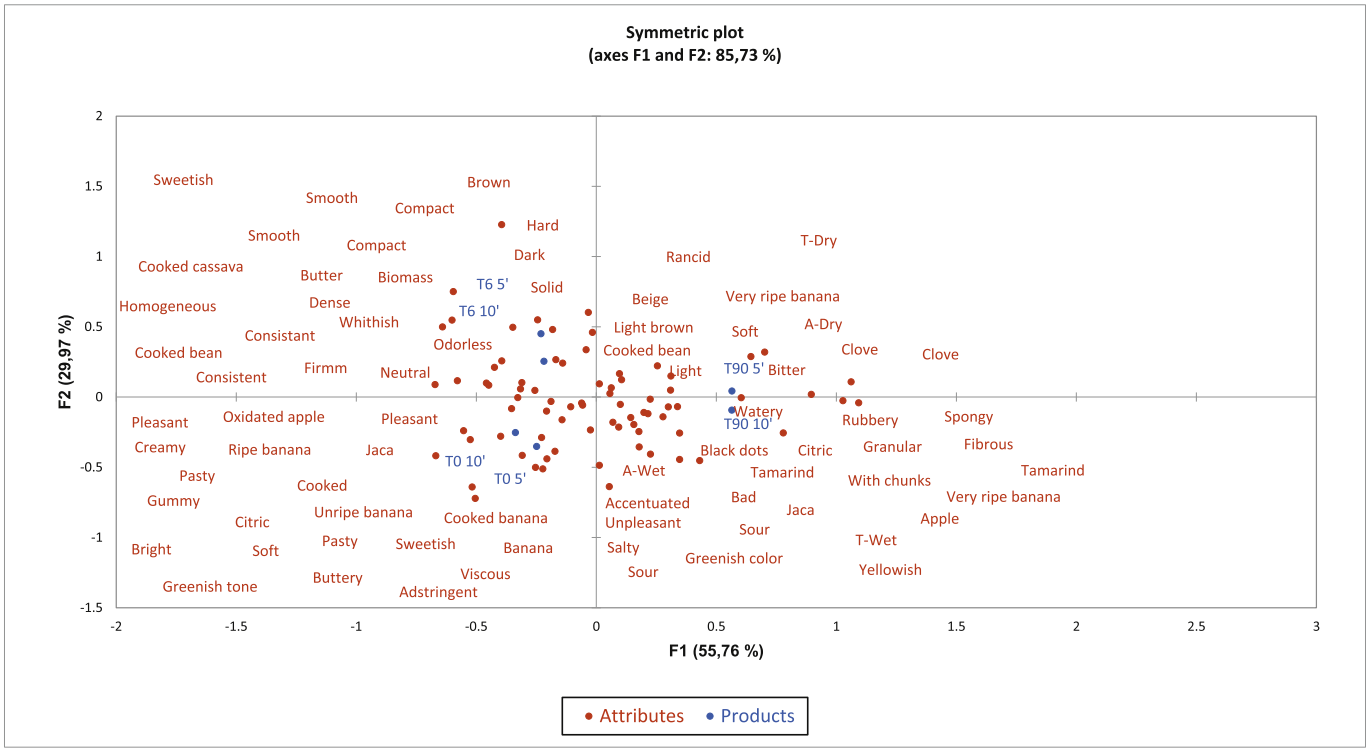

Fig. 2. Descriptive map obtained by means of correspondence analysis of CATA data (n = 121).
T0 5': five minutes of pressured cooking without storage; T0 10': ten minutes of pressured cooking without storage; T6 5': five minutes of pressured cooking with refrigeration storage (4 °C) for 6 days; T6 10': ten minutes of pressured cooking with refrigeration storage (4 °C) for 6 days; T90 5': five minutes of pressured cooking with frozen storage (−12 °C) for 90 days; T90 10': ten minutes of pressured cooking with frozen storage (−12 °C) for 90 days.

cooked for 10 min (C = 14.66). The high values of chroma indicate more intense colors (Jacomino, Mendonça, & Kluge, 2003; Maskan, 2001). The effect of storage time on chroma was analyzed, and there was a significant reduction ($p < 0.01$) as a result of the storage time under refrigerated conditions (mean values: C = 14.69 and C = 13.39 before refrigeration and after 3 days of storage, respectively). The frozen product was analyzed, and the longer cooking time also increased the chroma. Regarding the effect of storage time, the mean value obtained before freezing (C = 14.69) differed statistically ($p < 0.01$) from those obtained when the product stock was analyzed after 30, 60, and 90 days of frozen storage, with mean values equal to C = 19.44, C = 18.99, and C = 19.18, respectively.

The increase in the cooking time reduced the hue angle average value ($p < 0.01$), and the change was independent of the time of refrigerated storage (GBB cooked for 5 min: 89.59; GBB cooked for 10 min: 79.58). A similar result ($p < 0.01$) was found for the GBB under frozen storage (5 min average hue angle: 85.62; 10 min average value: 77.30). In frozen GBB, the hue angle was reduced ($p < 0.01$) during the storage time, regardless of the cooking time. The reduction in the hue angle observed in the GBB cooked for 10 min and stored by

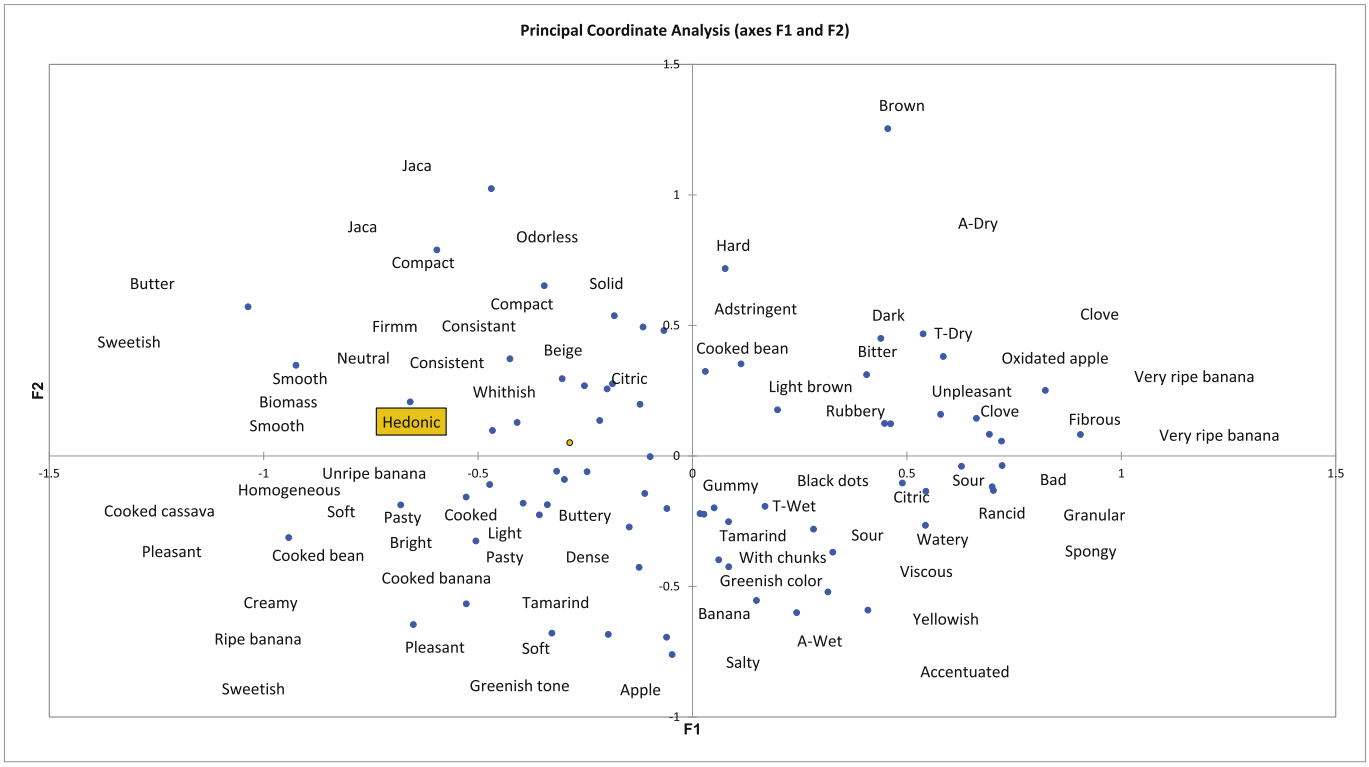

Fig. 3. Analysis of the principal coordinates of the data obtained in the CATA tests and acceptance (n = 121).

freezing (with average values remaining in the first quadrant: $0 \leq h^{\circ} \leq 90^{\circ}$) could be associated with a reddish product (Hernández, Sáenz, Alberdi, & Diñeiro, 2016).

The increase in cooking time increased the BI of GBB ($p < 0.01$), regardless of the freezing time. When we used a 5 min cooking time, the mean value of BI was 38.25, while for a 10 min cooking time, the mean value was 44.83. Regarding the effect of the storage time alone, we verified that freezing caused a significant increase ($p < 0.01$) in the BI. The mean value of the BI before freezing was 32.48. After 30 days (d) of storage of the frozen product, the mean value of the BI was 45.90. It should be noted that the mean BI values obtained after 60 and 90 days were statistically similar ($p > 0.05$) to that obtained after 30 days of storage.

The changes in the chroma and hue angle are associated with the non-enzymatic browning process, which is directly influenced by the freezing storage and the cooking time, as confirmed by the BI results. The cooking time and the freezing increased the BI, which represents the fruit's brown color, and it is an important indicator of non-enzymatic browning (Buera et al., 1985; Palou, Lopez-Malo, Barbosa-Canovas, Welti-Chanes, & Swanson, 1999).

Although it is possible to verify the changes in GBB color from the instrumental analysis, the results of the sensorial analysis generally did not show consumers' perception of this attribute. It is possible that the terms “beige” and “light brown” used by tasters are associated with the

Table 5
Mean values of chroma (C), hue angle (h°), color difference (ΔE) and browning index (BI) of the green banana biomass cooked for 5 and 10 min and stored under refrigeration (4 °C) and freezing (- 12 °C) at different storage periods.

Storage time (days)	Variables							
	C		h°		ΔE		BI	
	Refrigeration (4 °C)							
	Cooking time		Cooking time		Cooking time		Cooking time	
	5 min	10 min	5 min	10 min	5 min	10 min	5 min	10 min
0	14.04 ± 0.27	15.33 ± 0.34	89.88 ± 0.42	80.46 ± 0.49	–	–	29.00 ± 0.83	35.96 ± 1.11
3	12.86 ± 0.09	13.93 ± 0.39	89.90 ± 1.31	78.74 ± 1.18	1.40 ± 0.17	3.70 ± 1.07	26.43 ± 0.68	34.99 ± 2.36
6	13.37 ± 0.04	14.70 ± 0.81	88.97 ± 1.03	79.57 ± 2.10	2.26 ± 1.03	2.42 ± 1.19	26.57 ± 0.95	33.05 ± 2.32
Storage time (days)	Freezing (- 12 °C)							
	Cooking time		Cooking time		Cooking time		Cooking time	
	5 min	10 min	5 min	10 min	5 min	10 min	5 min	10 min
0	14.04 ± 0.27	15.33 ± 0,34	89.88 ± 0.42	80.46 ± 0.49	–	–	29.00 ± 0.83	35.96 ± 3.07
30	19.28 ± 1.08	19.60 ± 0.37	83.76 ± 0.16	75.96 ± 1.25	5.72 ± 1.00	4.68 ± 0.40	43.62 ± 3.06	48.20 ± 1.23
60	18.55 ± 0.52	19.44 ± 0.67	84.43 ± 0.33	76.75 ± 0.56	5.09 ± 0.52	5.02 ± 1.04	41.62 ± 1.19	46.38 ± 2.90
90	18.40 ± 1.15	19.96 ± 1.25	84.52 ± 0.46	76.03 ± 1.72	5.74 ± 1.14	5.11 ± 1.54	39.36 ± 3.07	48.79 ± 3.18

C – Chroma; h° - hue angle; ΔE − Color difference; BI - browning index.

effect of heat and storage time on the GBB color variables, such as hue angle reduction and browning index (BI) increase.

4. Conclusions

This is the first study to investigate the alteration of green banana biomass production and shelf life and the effects of different processing and storage conditions on microbiological, sensory, nutritional, and colorimetric aspects, which are important for consumer acceptance and are related to health benefits from GBB consumption. The results of microbiological analyses show that the refrigerated GBB was safe for consumption until the 6th day and the frozen GBB was safe up to the 90th day. Our results suggest that cooking GBB for 5 min during production is better for preserving several functional components, such as vitamin C, phenolic compounds, and fiber, compared with the longer cooking process. Refrigerated storage was better than frozen storage for maintaining vitamin C and the fiber content, but it did not affect the resistant starch and phenolic compounds. Refrigerated storage of GBB seems to be the best option for achieving better sensorial and nutritional aspects. Further studies are necessary to test the stored GBB when added to different kinds of dishes (usually used) to evaluate whether acceptance would change.

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