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Nutritional value and acceptability of chocolate with high cocoa content and green banana biomass

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ABSTRACT

Chocolate and green banana biomass (GBB) are nutritious foods that benefit well-being and health. GBB adds nutritional value, acceptance, and benefits due to its fiber content, resistant starch, and low glycemic index. Adding GBB to chocolate with high cocoa content (approx. 70%) was evaluated by physicochemical, microbiological, and sensory properties. Samples were analyzed for composition, plastic viscosity, sensory analysis, particle size, and water activity. The results showed that chocolates added with GBB have moisture, lipids, and mineral content values by the current legislation. Viscosity analysis showed that the studied formulations presented expected values for chocolate (2.44–4.79 Pa), not compromising the processing of tempering, molding, cooling, and demolding, demonstrating the feasibility of incorporating GBB into chocolates. The values of the average particle sizes were less than 25 μm in the chocolates and recommended in terms of the sensory aspect. The rheological measurements showed that the Casson plastic viscosity values are in the suggested chocolate range. Sensory analysis revealed that the sample with the highest content of GBB showed the highest acceptance and obtained 56% of purchase intent. Adding GBB to chocolate did not change the physicochemical, microbiological, or sensory characteristics and made the product healthier for consumers.

1. Introduction

Cocoa is a powder made from ground cacao seeds from the cacao tree (*Theobroma cacao* L.), a plant native to the Amazon region; the seeds and fats are the raw material for the manufacture of chocolates (Moda, Boteon, & Ribeiro, 2019). Brazilian cocoa production in 2017 was 204,000 tons (seventh largest world production) (ICCO, 2020), with Bahia responsible for most of the production. Cocoa is sold as dried beans and derivatives (cocoa mass or liquor, cake, cocoa powder, and butter), and used as raw material to produce chocolate and sweets (dosSantos & Fontes, 2020).

Chocolate is one of the most appreciated sweets in the world due to its taste, texture, and color characteristics (Owusu, 2010). In Brazil, the production and consumption of traditional and gourmet chocolate are economically significant, especially in the southern region of Bahia (Moda et al., 2019), with a trend of increasing consumption. In recent years, there has been increased pressure on the food industry for healthier products, which demands healthier preparations of chocolate.

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From this perception, chocolate can become more attractive by adding fiber and resistant starch (RS) from integral green banana biomass (GBB).

Banana is one of the most consumed fruits in Brazil and the world; easily accessible, cheap, and used as a viable ingredient in preparations of healthy foods (Souza, 2017). GBB is a commercial paste that does not change the taste of food but adds nutritional value due to its composition, high amount of fiber, and resistant starch (Cabral, de Quadros, & de Sá Silva, 2020). RS is responsible for the physiological benefits of GBB (Cassettari, Machado, Lourenção, Carvalho, & Ortolan, 2019; Walter, Silva, & Emanuelli, 2005).

Studies carried out adding GBB in different chocolate formulations (chocolate truffles (Almeida & Gherardi, 2018), "brigadeiros" (Cabral et al., 2020; Marques, de Oliveira, Aguiar-Oliveira, & Maldonado, 2017), ice cream (Marques, Antunes, & Gama, 2018), cheese bread (Marques et al., 2017), energy juice (Marques et al., 2017), biscuit (da Silva, Bezerra, et al., 2017a), "bombom" (da Cruz & Guimarães, 2020) and others) showed that it makes them healthier.

Green banana biomass (GBB) contains dietary fiber and a high concentration of amylase-resistant starch (approximately 74% of the composition) (Cassettari et al., 2019). The composition of GBB performs several functions in the body, such as regulating intestinal motility and delaying gastric emptying, in addition to helping reduce blood cholesterol levels; it can also be used as a substrate for fermentation by aerobic bacterial colonies (Hongpattarakere & Uraipan, 2015).

GBB can be added in chocolate formulations, partially replacing sugar, as ingesting RS can minimize postprandial glucose and insulin concentrations (Rebello, Greenway, & Dhurandhar, 2014). Adding GBB can also increase the feeling of satiation and control liver and gastric problems (Eleazu & Okafor, 2015). Adding GBB to chocolate would be valuable in weight loss or body weight maintenance diets.

Resistant starch resists hydrolysis by digestive enzymes (amylase and pullulanase); unlike fast-digesting starch, RS is a fraction of the starch that resists digestion in the small intestine, reaching the large intestine intact (Pereira, 2007).

RS has a behavior similar to dietary fibers, as RS and the degradation products are not absorbed in the small intestine of healthy individuals. In this way, this starch fraction becomes a substrate for fermentation by anaerobic bacteria colonies, being considered a prebiotic agent (Almeida-Junior, Curimbaba, Chagas, Quaglio & di Stati, 2017; Scarminio, Fruet, Witaicenis, Rall, & Di Stasi, 2012).

The addition of GBB in chocolate formulations contributes to nutritional and non-nutritional properties since the starch and dietary fibers are responsible for physicochemical properties that characterize partially processed products, contributing to texture properties, thickening, colloid stabilizer, gelling and bulking agents, adhesives, retention/structuring of water molecules, among others (Kaur, Dhull, Kumar, & Singh, 2020).

Structurally, starch is a homopolysaccharide composed of amylose and amylopectin (Emaga, Robert, Ronkart, Wathelet, & Paquot, 2008). Amylose is formed by glucose units joined by α-1,4 glycosidic bonds, creating a linear chain. Amylopectin is a polymer of glucose units joined at α-1,4 and α-1,6 glycosidic bonds, forming a branched structure. This structure presents a degree of polymerization between 500 and 2000 glucose units, and the chain branching is found in plant cells as micrometric granules with molecular porosity. Starches' structural and functional differences are attributed to morphology, granule size, and composition. There is a relationship between starches' molecular structures and their physicochemical properties regarding the amylose content, the length distribution of amylopectin chains (size and shape), and the degree of crystallinity in the starch granules. The size and shape variation of the constituents in the amorphous and crystalline regions strongly influence the functional properties of the starch granules (Lopez-Silva, Bello-Perez, Agama-Acevedo, & Alvarez-Ramirez, 2019). These characteristics are associated with gelatinization, retrogradation, granule swelling, leaching of amylose and/or amylopectin, loss of radial structure (birefringence), and enzymatic degradation, which consequently affects their absorption by the body.

RS can be classified into five types. Type 1: physically inaccessible starch with size and composition that delays the action of digestive enzymes; Type 2: resistant starch that presents slow digestibility due to the size, shape, and crystal structure of the granules; Type 3: retrograde starch composed of amylose, when the food starch is cooked and cooled; Type 4: modified starch after changing the chemical structure (Scarminio et a., 2012; Olawuni, Uruakpa, & Uzoma, 2018); and Type 5: is formed by combining long and unbranched starch chains with free fatty acids, forming a helical structure that is difficult to digest as well as resistant maltodextrin, which is a new, non-viscous type of dietary fiber, produced by the intentional rearrangement of starch molecules (Bojarczuk, 2002).

The green banana is the primary source of RS in the human diet; the starch is converted into simple sugar and sucrose during the ripening of the fruit, so only the green banana has a significant amount of starch (types 1 and 2). The benefits of green banana biomass (GBB) are mainly attributed to RS, as it is fermented in the intestinal colon by microorganisms, reducing the pH, making the environment unsuitable for pathogenic microorganisms, and reducing the risk of cancerous cell formation (Khoozani, Birch, Bekhit, 2019). In addition, GBB improves immunity, controls cholesterol levels, prevents diabetes, and avoids accumulating abdominal fat (Silva et al., 2020). GBB does not have trans fat, sodium, and preservatives and contains fibers and proteins; its addition in the formulation of chocolates is beneficial for health if consumed consciously and in a balanced diet.

The objective of this study was to evaluate the physicochemical, sensorial (CATA), rheological, microbiological, and health benefits of formulations of chocolate with high cocoa content (approx. 70%) added with green banana biomass (GBB).

2. Materials and methods

2.1. Raw material

The integral green banana biomass (GBB) (La Pianezza, Santa Barbara do Oeste, SP) was acquired in a local market; according to the manufacturer, this biomass is "organic, whole and made with banana pulp and peel and presented as a paste".

The cocoa beans used were blends of 3 cocoa varieties ("trinitário", "catongo", and "parazinho") without a defined compositional percentage and obtained at Fazenda Conjunto Estrela Guia, municipality of Itajuípe, southern Bahia.

Deodorized cocoa butter, soy lecithin (0.25%), commercial refined sugar, and polyglycerol polyricinoleate (PGPR) (0.5%) were provided by the Food Technology Institute (ITAL).

2.2. Chocolate production

The chocolates were produced at ITAL's Cereal and Chocolate Technology Center (Cereal Chocotec), as shown in Fig. 1.

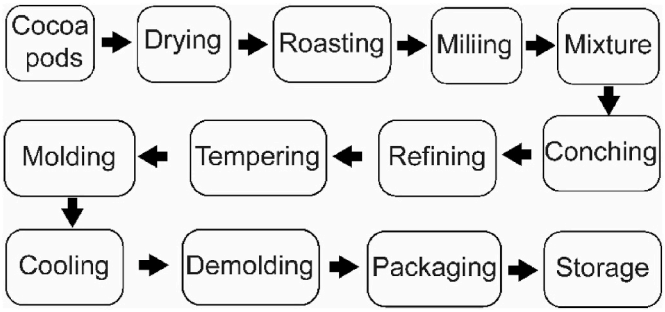

Fig. 1. Flowchart of the chocolate manufacturing process.

The dried cocoa beans were roasted in an oven at 130 °C for 50 min, then were manually broken, and the husks were separated from the cocoa nibs. After that, the nibs were ground in a knife mill (JAF Inox, São Paulo, Brazil) to obtain the cocoa liquor. The refined mass of cocoa liquor went on to the conching process, carried out in a ladle (Inco, Brazil) at 60 °C for 24 h at 48 rpm; in this step, the different concentrations of GBB were added, as shown in Table 1.

After conching, the mass was taken to the final refining process in a 6 mm steel ball mill (Caotech, Netherlands) until obtaining a bar of chocolate with a maximum particle size of 25 μm. In this step, refined sugar, cocoa butter, soy lecithin (0.25%), and PGPR (0.5%) were added. Then, the chocolate was taken to the manual tempering process on a granite bench to reduce the temperature (from 45 °C to 27 ± 1 °C), followed by reheating to 31 °C using a heat gun. Subsequently, it was molded in polycarbonate molds and subjected to a vibrating table to remove air bubbles. Finally, the product passed through the cooling tunnel (Siaht) at 15 to 18 °C (inlet and outlet of the equipment) and 10 to 12 °C (center of the equipment), being later packed in aluminum foils and stored in a BOD oven at 20 °C.

2.3. Physicochemical characterization of chocolate bars

2.3.1. Determination of the maximum size of particles

Determining the maximum particle size of chocolate was performed in triplicate using a Mitutoyo digital micrometer (Mitutoyo, USA). Five portions were taken from different sample regions (approximately 0.15 g) dispersed in pure mineral oil at 2:1 (w/w). Two repetitions were performed for each portion, so ten measurements for each sample were done (Luccas, 2001).

2.3.2. Water activity (Wa)

Water activity analyses were determined in triplicate using the Aqualab Series 3 TE equipment (Decagon, USA) by direct reading at 25 ± 1 °C.

2.3.3. Ash content and mineral composition

The ash content was performed in triplicate. The chocolate and GBB samples were heated to 550 °C to decompose the organic components, leaving only the mineral content in oxide and/or carbonate forms. Approximately 5 g of the samples were weighed in a porcelain crucible (previously oven-dried). The samples were taken to a muffle furnace under 550 °C for 8 h, and white ash or slightly gray ash was obtained, and after cooling, they were weighed. To calculate the ash content, Equation (1) was employed.

$$\%\ Ash = \frac{N}{P}\ x\ 100 \tag{1}$$

where:

N = weight of ash;
P = sample weight.

Table 1
Composition of the chocolate bars.

	Control (GBB0), %	Formulation 1 (GBB10), %	Formulation 2 (GBB15), %
Components		Quantity (%)	
GBB	0	9.93	14.89
Sugar	29.77	19.85	14.89
Cocoa butter	9.93	9.93	9.93
Cocoa liquor	59.55	59.55	59.55
Lecithin	0.25	0.25	0.25
PGPR	0.50	0.50	0.50

Added in the formulation soy lecithin (0.25%) and polyglycerol polyricinoleate (PGPR) (0.5%)/Food Technology Institute (ITAL).

The samples were digested in a Borghof SpeedWare microwave oven with Teflon digestion tubes. The digester solution comprised 3 mL of nitric acid and 2 mL of hydrogen peroxide. The digestion time was 38 min, according to the schedule suggested by the manufacturer. After digestion, the samples were swollen with ultrapure water to a volume of 25 mL and stored in 50 mL tubes until mineral readings were taken.

The minerals P, Ca, Mg, K, Fe, Mn, Ni, Na, and Zn were analyzed in ICP-OES (PerkinElmer model Optima 8300) using a multi-elementary analytical curve and the data obtained was then processed to obtain the results. All samples were digested and analyzed in triplicates and the results presented with mean and standard deviation.

2.3.4. Protein content

Protein content was determined using the Kjeldahl method in triplicate. Approximately 0.25 g of the sample was weighed and transferred to the digestion tube, followed by adding sulfuric acid (10 mL) and 6 g of the catalytic mixture (Na_2SO_4:$CuSO_4$/10:1). It was kept under heating in a digester block at 350 °C until a blue-greenish solution was obtained. After cooling, enough concentrated sodium hydroxide solution (50%) was added, and the steam distillation process began, receiving the distillate in 50 mL of 4% (m/v) boric acid solution. It was distilled to obtain 150 mL of distillate with excess ammonia. The excess ammonia was titrated with 0.1 M hydrochloric acid until the solution (yellow) turned red. To calculate the protein content, Equation (2) was used.

$$\%\ Protein = \frac{V\ x\ 0,14\ x\ f}{P} \tag{2}$$

where:

V = volume of sodium hydroxide (0.1 mol L^{-1}) spent in the titration
f = conversion factor (Used 6.25)
P = sample weight

2.3.5. Humidity

Moisture was determined in triplicate by the desiccation loss method in an oven at 105 °C. Samples (5 g, pulverized) were weighed in a Petri dish, left for 8 h in an oven, cooled in a desiccator, and then weighed on an analytical balance. The humidity was calculated using Equation (3).

$$\%\ Moisture = \frac{P_f}{P_i}\ x\ 100 \tag{3}$$

where:

Pi = initial weight (wet sample) in grams (discounting the filter weight)
Pf = final weight (dry sample) in grams (discounting the filter weight)

2.3.6. pH determination

The methodology of Instituto Adolfo Lutz (1985) was used and carried out in triplicate to determine the pH. The samples (10 g) were weighed in a beaker and diluted with 100 mL of water. Then, the contents were stirred until homogenized. The pH value was evaluated by direct reading on a previously calibrated portable digital pHmeter (Instrutherm, PH-2000).

2.3.7. Color determination

Color analyses were performed directly on the chocolate surface, using a Hunterlab colorimeter (Colorquest II, Fairfax, VA, USA). Each sample's parameters L*, a*, and b* were determined five times to generate an average of each chocolate formulation.

2.3.8. Plastic viscosity and Casson yield limit

The rheological measurements were performed using a programmable digital rheometer (Brookfield, LVDV-III), with an adapter for

small samples to store the previously melted chocolate. The system temperature was controlled using a thermostatic bath (Brookfield, TC500). The spindle used was cylindrical type S15 (maximum torque of 90). Spindle rotations were established according to Vissotto et al. (1999). Three determinations were made for each sample analyzed.

2.3.9. Determination of lipids

The continuous extraction methodology was used in a Soxhlet apparatus in triplicate to determine the lipid content. Approximately 5 g of samples were weighed in a degreased paper cartridge and transferred to the Soxhlet extractor. Then, the solvent (petroleum ether) was added to the extractor. Continuous extraction was maintained (under heating) for 8 h; then, the residue was dried in an oven at 105 °C for 1 h. After being cooled, the weight of the residue was determined through Equation (4).

$$\% \ Lipids = \frac{P_r}{P_a} \ x \ 100 \quad (4)$$

where:

Pr = weight of residue
Pa = sample weight

2.4. Antioxidant activity

Samples were prepared with the addition of 2 g of ground material (GBB and chocolates) and 20 mL of methanol/water solution (70/30 v/v) to an ultrasonic bath for 60 min at 30 °C, followed by centrifugation (2000 rpm for 20 min at 4 °C). The supernatants (methanol and water) were evaporated to obtain the crude methanolic extracts (Hammi, Jdey, Abdelly, Majdoub, & Ksouri, 2015). The analyses were performed in triplicate. Yield of extracts: GBB, GBB10, GBB15, GBB0: 7.4%, 10%, 12%, and 14%, respectively.

The free radical scavenging activity of the samples was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Genovese & Lannes, 2009) in triplicate. A stock solution of DPPH in 0.004% methanol was prepared, and 50 μL of concentrations 10.0; 5.0; 2.5; 1.25; 0.625, and 0.312 μg mL^{-1} of the samples were added to 5 mL of the DPPH solution.

After 30 min of incubation in a dark environment at room temperature, the absorbance was measured at 517 nm. Butyl hydroxy toluol (BHT) and rutin were used as standard. To calculate the DPPH inhibition (I%), equation (5) was used:

$$\% \ I = \frac{A_a - A_b}{A_a} \ x \ 100 \quad (5)$$

where:

Aa = control absorbance
Ab = absorbance of the reaction

The concentration of extracts that caused 50% inhibition of DPPH (IC_{50}, in μg mL^{-1}) was obtained by linear regression analysis.

2.5. Microbiological assays

To carry out the microbiological tests, each chocolate sample packed in its packaging (GBB0, GBB10, and GBB15) was externally sanitized with 70% ethanol, then fractionated into portions of 25 g in a laminar flow, with the aid of Petri dishes, sterilized knives, and tweezers. Subsequently, for the analysis of *Staphylococcus aureus*, total coliforms, thermotolerant coliforms, molds, and yeasts, 25 g of the samples were homogenized in 225 mL of 0.1% peptone water, followed by serial dilutions up to 104 times. For *Salmonella* spp. analysis, 25 g of samples were homogenized in 225 mL of buffered peptone water (BPW) for pre-enrich for 24 h at 37 °C (da Silva, Junqueira, et al., 2017b).

2.5.1. Staphylococcus aureus

To evaluate *S. aureus* in chocolate samples, the methodology adapted from the American Public Health Association (APHA) 39.63:2015 was performed in triplicate. After serial dilutions, 0.1 mL of each dilution (10^1 to 10^4) were plated on plates containing Baird-Parker Agar (BP), followed by incubation at 37 °C for 24 h. Then, typical colonies were counted, expressing the results in CFU/g (da Silva, Junqueira, et al., 2017b).

2.5.2. Total and thermotolerant coliforms

To evaluate the total and thermotolerant coliforms in chocolate samples, the official method of Most Likely Number (MLN) of the American Public Health Association (APHA) 9:2015 (da Silva, Junqueira, et al., 2017b) was used in triplicate. In this methodology, according to the number of positive tubes and considering the dilutions performed, the most likely number of coliforms per gram of chocolate is estimated using tables, and the value obtained is expressed in MLN/g. From each selected dilution (10^1 to 10^4), 1 mL was inoculated into three tubes containing 10 mL of Lauryl Sulfate Tryptose Broth (LST), which were incubated at 35 °C for 24 h. After this period, growth through gas production was observed. If they produce gas, the tubes move to the subsequent step; if not (no gas production), the tubes are re-incubated until completing 48 h (da Silva, Junqueira, et al., 2017c). Brilliant Green Bile 2% broth (BGB) was used and incubated at 35 °C for 24 h to confirm total coliforms. The tubes with gas production confirmed the presence of total coliforms using the most likely number table (MLN). *E. coli* broth (EC) was used to confirm thermotolerant coliforms, incubated in a water bath at 45.5 °C for 24 h. After this period, the growth through gas production was verified, and the tubes with gas production were confirmed for the presence of thermotolerant coliforms using the most likely number table (MLN) (da Silva, Junqueira, et al., 2017a).

2.5.3. Molds and yeasts

To evaluate molds and yeasts in the chocolate samples, the methodology adapted from the International Organization Standard (ISO) 21,527–1:2008 was carried out in triplicate. After the serial dilution, 0.1 mL of each dilution (10^1 to 10^4) was added to plates containing Potato Dextrose Agar (PDA), followed by incubation at 25 °C for 5–7 days. The colonies were then counted, expressing the results in CFU/g (da Silva, Junqueira, et al., 2017a).

2.5.4. Salmonella spp

Salmonella spp. analysis was performed using the methodology adapted from the Food and Drug Administration (FDA), BAM/FDA:2016, in triplicate. After 24 h, 1 mL of the pre-enriched product was placed in 10 mL of tetrathionate (TT) broth and 10 mL of Cystine Selenite (CS), and 0.1 mL in 10 mL of Rappaport-Vassiliadis broth Modified (RV). TT and CS selective enrichment media were incubated at 35 °C/24 h and RV at 42 °C/24 h. After this period, differential selective plating was performed on Xylose Lysine Decarboxylase Agar (XLD) with the help of the platinum loop through the stripping method. The plates were incubated at 35 °C for 24 h and later verified the presence of typical colonies (da Silva, Junqueira, et al., 2017a).

2.6. Sensory evaluation

Sensory analyses were carried out by 100 tasters guiding these tasters to mark the characteristics that describe an ideal chocolate. Participants signed the consent via the statement “I am aware that my responses are confidential, and I agree to participate in this survey,” where an affirmative reply was required to enter the survey. They could withdraw from the survey at any time without giving a reason. The products tested were safe for consumption. The sensory evaluation of acceptance employed a 9-point structured hedonic scale, where 9 = I liked it very much, 8 = I liked it a lot, 7 = I liked it moderately, 6 = I liked it a little, 5 = I neither liked nor disliked, 4 = I disliked a little, 3 =

disliked, 2 = disliked very much, 1 = disliked extremely, adapted from the methodology of Meilgaard, Civille, and Carr (1999).

The sequence of presentation of samples to the tasters was random, and the acceptance test was carried out in a closed cabin in the cold kitchen laboratory of the Centro Universitário da Grande Dourados (UNIGRAN). The taster was asked about the appearance of the sample, taste, texture, aroma, color, and overall mood.

A five-point scale was used regarding purchase intention, where 5 = would certainly buy, 4 = would probably buy, 3 = maybe would buy, maybe not buy, 2 = probably would not buy, 1 = certainly would not buy (Ferreira et al., 2000).

CATA (check all that apply) methodology consists of presenting to the taster a list of terms and/or phrases related to the product to identify the sensory characteristics of the treatments performed. The evaluation form was composed of 27 attributes: milk chocolate color; uniform appearance; dark or semisweet; chocolate color; opaque surface; shiny surface; little bitter taste; medium bitter taste; high bitter taste; little sweet taste; medium sweet taste; very sweet taste; crunchy texture; melts in the mouth; "sandy" structure; good snap; sour taste; no acidic taste; chocolate flavor; citrus flavor; fruity taste; roasted aroma; chocolate flavor; raisin fruit aroma; tasty; rich in nutrients and lower sugar content. Statistical analysis of CATA was performed using the XLStat program. The frequency of use for each term was determined by counting the number of consumers who used each term, and then the Q-Cochran test was performed at a 5% significance level.

2.7. Statistical analysis

The analysis data (when no specific method is described) of the chocolate bars were evaluated by analysis of variance (ANOVA) and the means compared by Tukey's test, considering a 5% significance level. All statistical analyses were performed using a statistical program.

3. Results and discussion

3.1. Physicochemical characterization of chocolates

Determining the particle size is essential to control the quality of chocolates, as it influences the rheology of the product and the sensory analysis. Chocolates with particles larger than 30 μm are persistent in the mouth, as they have a sandy texture, while particles smaller than 20 μm have a softer texture and a creamier mass (Afoawka, 2010). The ideal particle size for chocolates is between 20 and 30 μm or smaller (Carvalho, 2016). Chocolates with particle sizes smaller than 15 μm are creamier but with greater viscosity, which must be corrected by adding fat (ITAL, 1998).

According to Fig. 2, the three formulations presented values very close to the maximum particle size, below 25 μm, indicating that the chocolates will not present sensorial problems of sandiness or particle perception during the tasting. In addition, the close values of maximum particle size associated with low humidity values (Fig. 3) indicate that the refining conditions (load and size of the balls; mill rotation and refining time) and conching (time and temperature) were suitable for the product (see Fig. 4).

Water activity is the parameter that measures the availability of water in a food or solution. Wa values range from 0 to 1, with values close to 1.0 more favorable for microbial development (Franco & Landgraf, 2008). Table 2 presents the values of water activity. The GBB exhibited a Wa of 0.99, while the formulations with and without GBB showed values below 0.44.

Previous studies found Wa values for GBB that corroborate the findings of this study. Oliveira (2007) found Wa values of 0.982 and 0.990. Santos, Silva, Santos & Oliveira Júnior (2010) found a water activity value of 0.99 for green bananas and Souza et al. (2020) found a mean value of 0.99 for GBB.

The formulation without GBB (standard chocolate; GBB0) had an

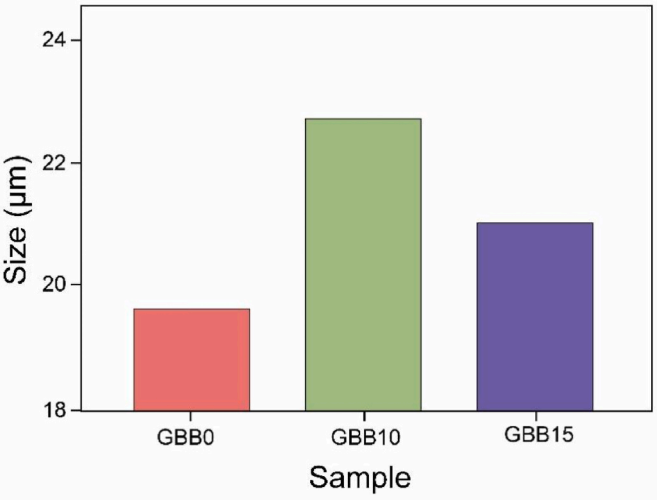

Fig. 2. The maximum particle size of samples with and without GBB.

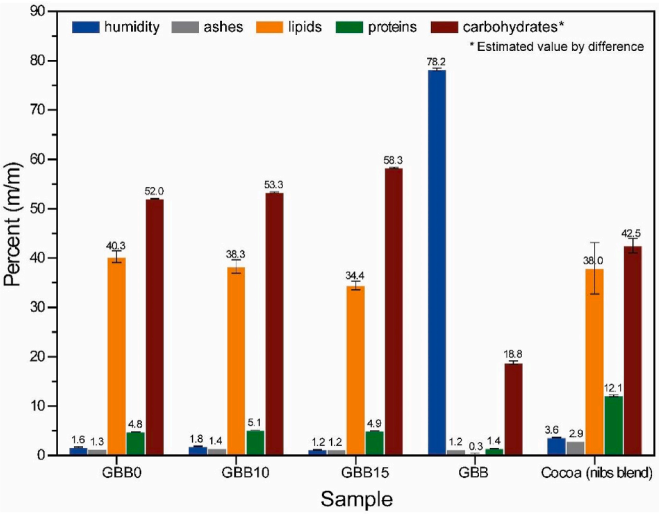

Fig. 3. Centesimal composition (wet basis) of chocolate samples.

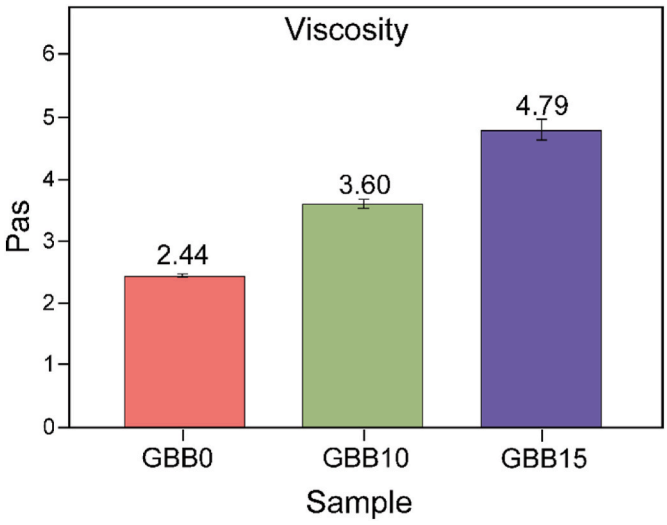

Fig. 4. Casson plastic viscosity of chocolate samples with and without GBB.

Table 2
Water activity (Wa) and pH of the formulations of chocolates without and with GBB.

Parameter	Samples			
	GBB	**GBB0**	**GBB10**	**GBB15**
Wa	0.9944 ± 0.002[a]	0.4386 ± 0.009[b]	0.4190 ± 0.000[b]	0.4056 ± 0.016[b]
pH	-	5.06 ± 0.01	5.04 ± 0,01	5.06 ± 0.00

Means followed by the same letter do not differ from each other at 5% probability ($P > 0.05$) by Tukey's test.

average Wa value of 0.4386. Similar values were found for white chocolate (0.44), semisweet chocolate (0.42), milk chocolate (0.44), and powdered chocolate (0.49). Chocolate is considered a low microbiological risk product due to its low water activity (<0.60) (Hiramatsu, Matsumoto, Sakae, & Miyazaki, 2005).

Adding GBB did not significantly interfere with the results for Wa, which showed an average value lower than the standard chocolate, allowing the elaboration of microbe-free chocolate with storage stability.

The pH did not show a difference between samples with and without GBB, which indicates that adding GBB did not affect the pH of the final product. The pH was above 4.5, characteristic of low acidity for chocolates.

According to Dimick and Hoskin (1981), efficient conching can increase pH from 4.95 to 5.70 due to the loss of volatile organic acids. The low pH value and a high content of organic acids, mainly acetic, from the fermentation of cocoa beans can undesirably affect the taste of chocolate.

The values for the color analysis of samples with and without GBB are shown in Table 3. Color analysis is a very important parameter for foods since it is the first to be perceived by consumers. Product color change may be related to manufacturing or packaging/storage defects, which may show bleaching because of sugar or fat bloom or the migration of these constituents to the surface (Wu & Sun, 2013).

The analysis was performed with untampered chocolates. The value of L* indicates luminosity; the closer to zero, the darker the sample. The average values of L* for the samples showed no difference. However, samples containing GBB showed the most intense color. The intensity of red (a*) showed the lowest value for GBB15 and the highest for GBB10. The highest yellow intensity value (b*) was obtained for sample GBB15, while samples GBB0 and GBB10 did not differ.

The data obtained by Salvi (2018) corroborate the values found herein. The author evaluated the color of traditional and diet-type chocolates and observed that the values of the a* coordinate varied between 9.38 and 10.30, indicating a tendency to red, as expected for brown color chocolates. The b* coordinate presented values between 12.40 and 14.30, denoting a tendency to yellow, also expected for the coloring of chocolates. The luminosity (L*) varied between 35.90 and 39.80, indicating that the chocolates presented a darker color, as expected for the samples.

Fig. 3 shows the physicochemical characterization of the chocolate samples.

The moisture content of GBB was determined at 78.20%; concomitantly, the samples with GBB had higher moisture than the control sample. This result corroborates the study by Franco (2000) in which the GBB presented 71.30% of humidity. In addition, the content of ash, lipids, and proteins in the samples of chocolate were similar to those of the abovementioned study, which showed values of 0.80% for ash, 38% for lipids, and 1.40% for protein.

Formulations GBB10 and GBB15 presented 1.6% and 1.8% moisture, respectively, acceptable values for chocolates. Values greater than 3% can increase the initial tension and viscosity of the product (Afoawka, Paterson, & Fowler, 2007), in addition to being related to product failures during the shelf life (Afoawka, 2010).

Regarding the lipid content, all samples presented results in accordance with the legislation, which stipulates a minimum value of 30%. The GBB0 sample showed 34.4%, GBB10 was 40.3%, and GBB15 indicated 38.3% of lipids. These results showed that the extraction and quantification of lipids were not homogeneous in the samples because the solvent extraction was not exhaustive for all the samples. Therefore, it is necessary to use different solvents to extract lipids. This could be evidence of the interaction of the lipid content with a microstructure of the chocolate constituents with the fibers and starch of GBB. Thus, the non-homogeneity of the lipid content by the method used, with variations of 34–40% between samples, is justified.

Cocoa liquor (or mass) was also evaluated. The moisture, ash, and protein values corroborate the results from Leite (2012), who analyzed three cocoa varieties. Pimentel (2016) studied the proximate composition of 3 clonal varieties of cacao and Catongo, and the values of lipids, ash, and moisture were similar to those found in this study. The author concluded that there was no significant difference between the studied varieties for the analysis of proximate composition.

The mineral content for samples with and without GBB showed no significant difference, and both have acceptable values for chocolate, with a maximum limit of 2.5% for ash. Adding GBB increases the nutrient content of chocolate, both in protein and ash, making the product an alternative for increasing protein and mineral consumption. Analyzing Table 4, the increase in ash content occurs mainly due to the presence of phosphorus, potassium, magnesium, and nickel in GBB. Cr, Co, Cu, Pb, and Cd were not detected in the samples of GBB and GBB0.

Chocolate is rich in essential minerals that can contribute to a healthy diet. According to the data in Table 5, chocolate is a source of mainly phosphorus, potassium, magnesium, and nickel. Minerals assist in activating enzymes and hormonal secretions in cells and are essential for the absorption of vitamins (Gupta & Gupta, 2014).

The rheological analyses showed that the data from the three chocolate formulations, with and without GBB, follow the Casson model, with a superior fit of 0.99 (R^2). The mean yield point was 1.15 ± 0.16. Although there was an increase both in viscosity and in the yield point for GBB15, the values obtained are still within the expected for chocolates and did not compromise the following steps, such as tempering, molding, cooling, and demolding, showing the feasibility of incorporating GBB in chocolates.

The expected values for chocolates regarding Casson plastic viscosity are 1–20 Pas (Beckett, 2009). The viscosities found in the work for GBB0 (control) (2.44 Pa), GBB10 (3.60 Pa), and GBB15 (4.79 Pa) are within the range (Fig. 3).

Gonçalves and Lannes (2010) emphasize that high-viscosity chocolates have a pasty sensation in the mouth that lasts longer. Furthermore,

Table 3
Values of color parameters of samples with and without GBB.

Parameters	GBB0	GBB10	GBB15
L*	38.83 ± 3.58	39.42 ± 2.47	39.33 ± 4.58
a*	10.52 ± 1.24	10.68 ± 0.86	10.00 ± 1.39
b*	13.52 ± 2.21	13.45 ± 1.70	14.24 ± 1.98

Table 4
Micro and macronutrient mineral (ash) composition of GBB0 and GBB (mg kg^{-1}).

Element	GBB0	GBB
P	4.93 ± 0.36	1.24 ± 0.10
Ca	0.25 ± 0.19	0.00 ± 0.00
Mg	2.04 ± 0.13	0.41 ± 0.04
K	7.27 ± 1.29	4.30 ± 0.29
Fe	0.06 ± 0.03	0.02 ± 0.00
Mn	0.00 ± 0.00	0.02 ± 0.00
Ni	2.91 ± 0.11	2.06 ± 0.25
Na	1.08 ± 0.40	0.84 ± 0.51
Zn	0.11 ± 0.15	0.00 ± 0.00

Table 5
Antioxidant potential of chocolate samples, GBB formulations and controls.

Samples	IC_{50} (μg mL^{-1})
GBB0	46.8 ± 6.5
GBB10	74.2 ± 5.7
GBB15	52.2 ± 5.4
GBB	70.8 ± 0.1
Rutin	3.3 ± 0.3
BHA	1.6 ± 0.1

this parameter is related to the distribution of particle size, fat content, moisture content, and temperature (Cohen & Jackix, 2009). The particle size distribution directly influences the product's viscosity; thus, the more significant the particle size, the lower the viscosity (Beckett, 2009). The results corroborate this information since GBB15 had a smaller particle size than GBB10 and, consequently, higher viscosity. The formulation GBB10 had an elevated amount of fat than GBB15 and, consequently, lower viscosity; thus, the greater the amount of fat, the lower the viscosity (Chevalley, 1994). As for the influence of moisture on viscosity, Efraim (2009) explains that an increase in moisture content from 1.0 to 2.9% can lead to an increase in viscosity of 200%. This information corroborates the results because the GBB15 formulation presented higher moisture and viscosity than the other formulations.

3.2. Antioxidant activity

Table 5 shows that the control samples (rutin and BHA) showed a greater capacity to scavenge the DPPH free radical, presenting more significant antioxidant potential. All samples showed antioxidant activity, evidenced by the discoloration of the solution.

The extracts obtained from GBB0, GBB10, GBB15, and GBB were bioactive, and the influence of constituents in the formulations was observed regarding the biological activity. Samples GBB10 and GBB15, prepared with mixtures of refined sugar, cocoa liquor, GBB, and cocoa butter, showed activity with IC_{50} values of 74.2 and 52.2 μg mL^{-1}, respectively. These results demonstrate that the sample GBB15 was below expectations but still contributed to the antioxidant activity of chocolate formulations.

Notably, the GBB extract showed bioactivity when tested alone with IC_{50} of 70.8 μg mL^{-1}. The extract from GBB0 had the lowest IC_{50} (46.8 μg mL^{-1}). The best antioxidant activity points to the formulated samples containing cocoa, which are more effective for the antioxidant activity than only the formulations containing GBB. Values close to IC_{50} were found in extracts from GBB0 and GBB15 formulations.

Fruits are natural sources of bioactive compounds with antioxidant activity, such as phenolic compounds, carotenoids, and vitamin C, among others. The antioxidant potential contributes to food conservation due to the elimination of reactive oxygen species and other compounds related to degradation, and it can inhibit or reduce damage caused by free radicals in cells (Vieira, Sousa, Mancini-Filho, & Lima, 2011). The phenolic compounds present in GBB collaborate in the antioxidation process of foods (Silva, 2014).

In addition to the benefits of food, antioxidant activity has been studied for its impact on cognitive ability. Baroni, Sarni, and Zuliani (2021) conducted a literature review in this regard and observed that most studies showed associations with significant beneficial effects on cognitive functions. Frequent chocolate consumption, for example, was significantly associated with better performance on all tests except working memory.

For this reason, adding GBB in chocolates is a biological alternative with antioxidant action and essential in replacing sugars, without causing significant sensory changes in the products. Moreover, because it is rich in fibers, vitamins, minerals, flavonoids, and resistant starch, it is considered a functional food and can increase the quality of chocolates.

3.3. Microbiological analysis

Chocolate is an appreciated product and considering the possibility of food poisoning caused by the consumption of chocolate, it is extremely important to verify the microbiological conditions of the product (Tejada, Dias, Conceição, & Timm, 2012).

As shown in Table 6, the chocolates produced and evaluated in this work are within the standards of identity and quality according to ANVISA Normative Instruction No. 60, which establishes a maximum value of 102 g for enterobacteria and an absence of 25 g for *Salmonella* spp. (Brasil, 2019). Thus, the microbiological health of the samples proves the correct method in their preparation and the Good Manufacturing Practices linked to the process (Zanchett, Mignoni, Barro, & Rosa, 2016). Storage stability must also consider the total count of aerobic mesophilic bacteria (AMB). This is a regulated parameter in many countries; therefore, the total AMB count must be additionally researched.

3.4. Sensory evaluation

The results obtained in the sensory evaluation of the acceptance test for the attributes of appearance, flavor, color, brightness, consistency, and crunchiness are shown in Fig. 5.

For the attributes of global appearance, color, brightness, and crispness, the samples GBB0, GBB10, and GBB15 did not show significant differences. The averages obtained for the evaluated attributes ranged from "I liked it moderately" (score 8) and "I liked it extremely" (score 9). In a study carried out by Braga, Moraes, Freire, Lima, and Portela (2009), sweets made with banana pulp and peel presented acceptance averages between 7.76 and 8.20, which correspond to the scores "I liked it moderately" and "I liked it a lot". When preparing "brigadeiro" with GBB and rice flour, Silva, Costa, Araujo, and Cavalcanti (2014) obtained averages from 6 (I liked it moderately) to 8 (I liked it a lot) and an acceptance rate above 70%.

Sample GBB10 differed from sample GBB15 in terms of flavor and consistency. The sample with the highest percentage of GBB (GBB15) had a greater overall acceptance in all aspects. Adding 15% of GBB containing resistant starch did not affect the sensory properties of the chocolates. Panelists were unable to discern between the samples. Walter et al. (2005) emphasize that this type of starch can complement or replace the fiber fraction of certain foods without significantly altering the organoleptic characteristics, thus being an option to enrich preparations with this food matrix.

The crispness characteristic had the lowest sensory average for the three samples. The change in chocolate texture can explain this fact due to the addition of GBB. These results corroborate those found by da Silva, Bezerra, et al. (2017c) in the preparation of cookies from GBB.

The data obtained in the purchase intent test are shown in Fig. 6.

It is observed that both formulations showed a high percentage of purchase intent, with emphasis on GBB15, which obtained 56% of purchase intent and better performance in the acceptance test. The sample with 10% of GBB and the control showed an equal percentage of 47%. The percentage of undecided respondents ("maybe I would buy it,

Table 6
Microbiological analysis of *Salmonella* ssp, *Staphylococcus aureus*, total and thermotolerant coliforms, molds, and yeasts in chocolate samples.

Samples	*Salmonella*	*S. aureus*	Total coliforms (NMP/g)	Thermotolerant coliforms (NMP/g)	Molds and Yeasts (UFC/g)
GBB0	Absence	Absence	Absence	Absence	2.33×10^2
GBB10	Absence	Absence	Absence	Absence	5.33×10^2
GBB15	Absence	Absence	Absence	Absence	Absence

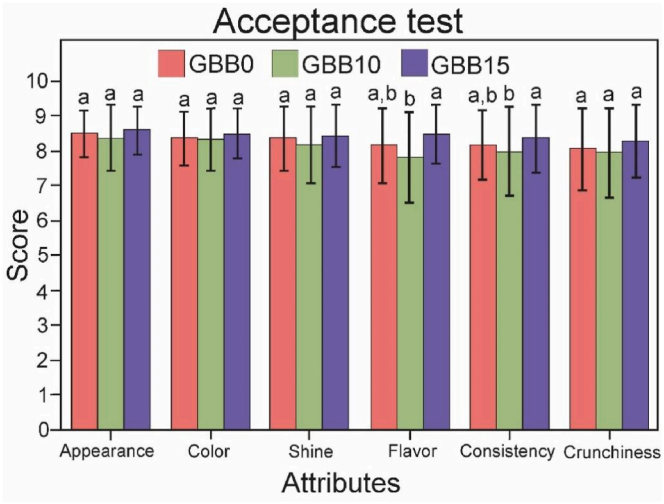

Fig. 5. Sensory evaluation of acceptance test for chocolates with green banana biomass.

maybe I wouldn't buy it") was 21% for the control samples, 18% for the GBB10, and 12% for GBB15. The rejection rate ("probably would not buy" and "certainly would not buy") was higher for control samples.

Almeida and Gherardi (2018) also obtained a high purchase intention index when developing a truffle filling with GBB. da Silva (2018) developed muffin-type cakes based on *Cassava puba* dough with fat replacement by GBB, in which the tasters showed 50% purchase intention.

Overall, samples containing GBB had a higher acceptance rate than samples without GBB, indicating that these products have good market potential. In addition, product rejection was low, less than 3% for samples with GBB and less than 1% for GBB15.

3.5. Sensory assessment "CATA"

Sensory analysis based on the CATA methodology was conducted with 100 participants, aged between 18 and 59 years, with 64% women and 36% men. All participants admitted to liking chocolate. The statistical results obtained, considering the descriptor terms using the CATA methodology for the formulations, are presented in Table 7.

It can be observed that the attribute "milk chocolate color" did not present a significant difference between the GBB0 and GBB10 formulations. While the sample GBB15 showed a lower value for this attribute and higher frequency than the other formulations in the attribute "sweet or semisweet chocolate color".

The GBB samples showed a lower frequency in the attribute "sandy texture" than the control (GBB0). The other attributes showed no significant difference between the formulations at the significance level adopted in the test.

In Table 7, we can verify the terms most and least mentioned by the panelists through the analysis of frequency by the Cochran test. It appears that "tasty" was the most cited descriptor for GBB10 and GBB15, while for GBB0, the most cited descriptor was "uniform appearance". The least cited term for GBB0, GBB10, and GBB15 was "very sweet taste", which can be explained by the amount of cocoa (70%) in the formulations. The term "dark or semisweet chocolate color" was most cited for GBB15.

Principal Component Analyses (PCA) of the control and GBB samples were performed. PCA of chocolates with and without GBB, with a profile considered ideal by tasters for attributes and products. The first principal component (F1) concentrates most of the total variation found in the original data (69.93%), and the second principal component (F2) explains the rest of the variation (23.56%), with 93.50% of the total variance of the data.

By PCA, it can be verified that GBB0 and GBB15 are more similar to each other when compared to GBB10. This similarity is mainly explained by the attributes: "dark or semisweet chocolate color", "no acidic taste", "little sweet taste", "shiny surface", and "toasted aroma". On the other hand, GBB10 was distinguished from the others, especially by the attributes: "uniform appearance", "opaque surface", "high bitter taste", "acidic taste", "raised fruit aroma", and "sandy texture". The condition indicated as ideal by the tasters was associated with attributes such as "crunchy taste", "very sweet taste", "tasty", "chocolate aroma", "chocolate flavor", and "melts in the mouth".

When analyzing the main component of PCA of chocolates with and without GBB overall appearance and tastes, it is observed that the appearance and flavor of the treatments are related to the attributes: "lower sugar content", "milk chocolate color", "medium sweet taste", "no acidic taste", "little bitter taste", and "uniform appearance". These characteristics are more noticeable in GBB0. However, it is essential to point out that, according to Table 7, these attributes did not differ significantly ($p \geq 0.05$) between the chocolate formulations.

4. Conclusion

The experimental results allowed answers to the study's central questions with chocolate and GBB content. Apart from analyzing the nutritional benefits, the study demonstrated the feasibility of incorporating GBB into commercially available chocolate pastes with a 70% cocoa content. Formulations with GBB did not interfere with the physicochemical properties of the chocolates but increased the nutritional value, prebiotics, acceptability, and healthiness. Additionally, it showed evidence of possible interactions between the fiber and starch content and the constituents of the chocolate formulation. It can be suggested

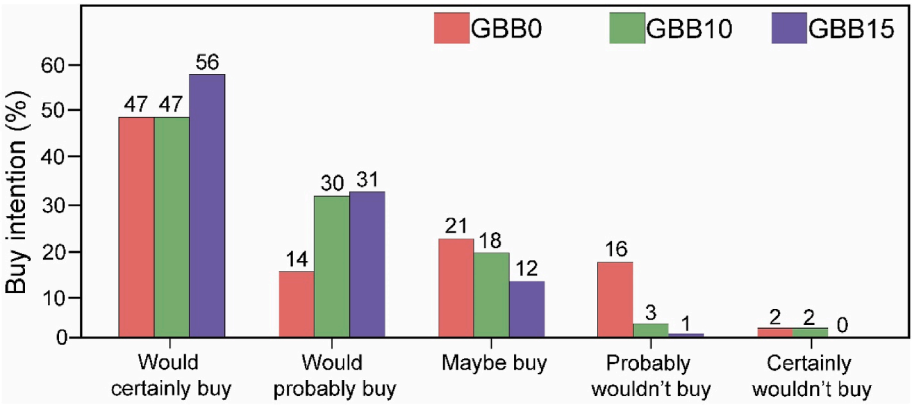

Fig. 6. Purchase intention of chocolate samples with and without GBB.

Table 7
Cochran test for chocolate formulations with and without GBB.

Attributes	p-values	GBB0	GBB10	GBB15
milk chocolate color	0.002	0.430[b]	0.470[b]	0.280[a]
uniform appearance	0.595	0.850[a]	0.840[a]	0.810[a]
dark or semisweet chocolate color	0.059	0.660[a]	0.590[a]	0.720[a]
opaque surface	0.266	0.300[a]	0.270[a]	0.220[a]
shiny surface	0.627	0.710[a]	0.700[a]	0.750[a]
little bitter taste	0.521	0.460[a]	0.390[a]	0.450[a]
medium sweet taste	0.131	0.370[a]	0.350[a]	0.470[a]
high bitter taste	0.728	0.260[a]	0.290[a]	0.250[a]
little sweet taste	0.907	0.550[a]	0.550[a]	0.570[a]
medium sweet taste	0.045	0.110[a]	0.220[a]	0.150[a]
very sweet taste	0.526	0.080[a]	0.080[a]	0.050[a]
crunchy texture	0.428	0.250[a]	0.310[a]	0.290[a]
melts in your mouth	0.057	0.750[a]	0.660[a]	0.770[a]
"sand" structure	0.000	0.160[a]	0.310[b]	0.150[a]
good snap	0.619	0.460[a]	0.440[a]	0.480[a]
sour taste	0.110	0.190[a]	0.290[a]	0.210[a]
no acid taste	0.218	0.660[a]	0.570[a]	0.610[a]
chocolate flavor	0.195	0.650[a]	0.680[a]	0.730[a]
citrus flavor	0.558	0.250[a]	0.260[a]	0.300[a]
fruity flavor	0.402	0.230[a]	0.240[a]	0.290 [a]
toasty aroma	0.975	0.350[a]	0.340[a]	0.350[a]
chocolate aroma	0.405	0.710[a]	0.690[a]	0.750[a]
raisin fruit aroma	0.033	0.290[a]	0.330[a]	0.200[a]
tasty	0.129	0.810[a]	0.870[a]	0.880[a]
rich in nutrients	0.581	0.530[a]	0.480[a]	0.510[a]
lower sugar content	0.024	0.670[a]	0.550[a]	0.690[a]

*Different letters in the same line show a significant difference ($p < 0.05$) between the samples. GBB0: no green banana biomass; GBB10: 10% green banana biomass; GBB15: 15% green banana biomass.

the formation of a network of GBB fibers that could interact and/or microstructure the constituents of the formulation and contribute to increasing the stability of the product on the shelf based on the mechanical and aesthetic properties and reduce fat migration processes (fat bloom) and sugar bloom that could mischaracterize the product and reduce the nutritional value and acceptability.

Additionally, the results of the physicochemical evaluations showed that the pH was not different between the control and GBB samples, indicating that adding GBB did not interfere with the pH of the final product or change the product's characteristics. Color analysis showed the characteristic values for chocolates. Chocolate is an appreciated product and considering the possibility of food poisoning caused by the consumption of chocolate, it is extremely important to verify the microbiological conditions of the product (Tejada et al., 2012). As shown in Table 6, the chocolates produced and evaluated in this work are within the standards of identity and quality according to ANVISA Normative Instruction No. 60/2019, which establishes a maximum value of 102 g for *Enterobacteria* and an absence of 25 g for *Salmonella* spp. Thus, the microbiological health of the samples proves the correct method in their preparation and the Good Manufacturing Practices linked to the process. Storage stability must also consider the total count of aerobic mesophilic bacteria (AMB). This is a regulated parameter in many countries; therefore, the total AMB count must be additionally researched. The size of particles smaller than 25 μm indicated that the chocolates did not present sensorial problems in the tasting. Mineral content, lipids, and moisture are in accordance with current legislation. The viscosity was in accordance with the recommended intervals for chocolates. Thus, chocolates with a high cocoa content and GBB were suitable and safe for consumption with the absence or low levels of microorganisms, within the parameters of current legislation, innovative, and with greater nutritional value.

In the chocolate formulations, GBB15 showed a higher average of acceptance in all attributes and 56% of purchase intention, presenting characteristics closer to what is considered "ideal chocolate". Chocolate bars with GBB are a healthier food alternative, rich in fiber and resistant starch, with GBB15 standing out as the best option in all evaluated properties. It can be considered that GBB in the form of a commercial paste is an alternative for replacing refined sugar, reducing the caloric value, and possibly being suggested as an indication for a different public, including diabetics, the elderly, and athletes, as these chocolates presented characteristics of a prebiotic food, nutritionally differentiated in antioxidant content, high content of cocoa solids, fibers and resistant starch and low amount of sugar in the evaluated formulations.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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