Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.88021

Structure and nutrition of dark chocolate with pequi mesocarp (*Caryocar villosum* (Alb.) Pers.)

Natasha Dantas LORENZO[1], Orquídea Vasconcelos dos SANTOS[2], Suzana Caetano da Silva LANNES[1*]

Abstract

In response to consumer's claims, the aim of this study was the addition of lyophilized pequi mesocarp (pulp) in dark chocolate formulation to improve nutritional value. The lyophilized pequi mesocarp was analyzed and granulometry, centesimal composition, carotenoids, and scanning electron microscopy were verified. Lyophilized mesocarp was added (1.5%) (F2) to the dark chocolate (F1) formulation, and particle size, temper index, physicochemical parameters, rheology, thermal analysis, total antioxidant capacity by iron reduction method, and total phenolics were determined. The addition of 1.5% of pequi lyophilized mesocarp caused no significant increase ($p < 0.05$) in the particle size of the formulated chocolate mass, without increasing its viscosity and the initial tension. For the formulated chocolate's tempering curve relative to the control there was an increase in the initial temper temperature and a decrease in the total process time. Infrared showed the presence of fatty acids in the pequi pulp, with 47% fat content and energy value of 519.71 kcal.100 g^{-1}. Pequi pulp oil content was added (0.7%) to the formulated chocolate by adding the oil properties as high contents of oleic, palmitic and linoleic acids. The energy value of F1 and F2 formulations were 557.5 and 559.73 kcal.100 g^{-1}, respectively. The thermal analyzes of the control and added chocolates showed that both withstood until 50 °C, not having interference of pequi pulp in the chocolate. The analysis of total phenolics showed an increase in the concentration of these compounds in the chocolate formulation added with lyophilized pequi mesocarp (813.49 μg GAE.mL^{-1}) compared to the dark chocolate control formulation (235.98 μg GAE.mL^{-1}). The use of lyophilized pequi pulp as an alternative for the enrichment of nutritional properties in dark chocolate was confirmed in this work. The products obtained have desirable nutritional quality and physical properties, being suitable for industrial production.

Keywords: bioactive compounds; fruit products; cocoa; thermal analysis; rheology.

Practical Application: The results show the structuring of dark chocolate with addition of powder fruit. The nutritional fruits addition in chocolate has positive aspects. The first is related to public health since it boosts good nutritional, and the second is related to the greater stability of this material. This study will reveal the industrial feasibility of applying and provide health benefits in dark chocolate with a good, accepted structure. Pequi pulp is an alternative for the enrichment of nutritional properties in dark chocolate, suitable for industrial production. Accordingly, based on the way of market demand for healthier indulgent food products, and nutritional characteristics of pequi, the present study has an opportunity to the industrial production for dark chocolate.

1 Introduction

The pequi of the species *Caryocar villosum* (Alb.) Pers., also known as piquiá in the Para state, Brazil, comes from South and Central America, being quite common in Guianas and Amazonia because it is a non-traditional fruit. Pequi in natura form has expressive concentrations of phenolic compounds and total carotenoids and its composition of fatty acids is considered similar to the mesocarp of dende oil (Rufino et al., 2010; Oliveira & Scariot, 2010; Xavier et al., 2011; Pessoa et al. 2015; Lorenzo et al., 2018).

In vitro studies have shown that anti-inflammatory effects and inhibition of tumor capacity in tissues have been shown to be protective against oxidative stress (Oliveira et al., 2020). This ability is due to the fact that the genotype's pulp of the fruit, which is the most abundant in the pequi fruit, and its oil, has expressive amounts of antioxidants and unsaturated and polyunsaturated fatty acids (Miranda-Vilela et al., 2009).

Antioxidants and unsaturated and polyunsaturated fatty acids have the effect of retarding free radicals, which are commonly linked in the oxidation of biomolecules (DNA, vitamins, lipids, proteins), resulting in cell death and tissue damage and leading to reactions linked to diseases such as cancer, atherosclerosis, diabetes, arthritis, and cardiovascular diseases (Santos et al., 2019).

In search of delayed action of free radicals, research look for plant sources that contain an expressive amount of antioxidant compounds to be incorporated in the elaboration of products for consumption. Chocolate is a potential product with a considerable amount of antioxidant since it has polyphenols in its composition that have, among others, antioxidant effects

Received 13 Sept., 2021
Accepted 24 Sept., 2021
[1] *Biochemical-Pharmaceutical Technology Department, Pharmaceutical Sciences School, Universidade de São Paulo – USP, São Paulo, SP, Brazil*
[2] *Nutrition School, Institute of Health Sciences, Universidade Federal do Pará – UFPA, Belém, PA, Brazil*
*Corresponding author: scslan@usp.br

and the presence of methylxanthines, including caffeine and theobromine, which in turn play a role in the central nervous system (CNS) stimulation (Fernández-Murga et al., 2011; Todorovic et al., 2015; Lannes, 2016).

Although several studies indicate that chocolate can reduce risks related to coronary heart disease and stroke, it must be known that there are uncertainties regarding the type of chocolate ingested (Morze et al., 2020).

Due to the presence of these compounds in their composition and the growing need for products with health benefits appeal, previous studies have been able to increase the relevant benefits of the antioxidant concentration of chocolates with the addition of fruits with high antioxidant capacity. Gültekin-Özgüven et al. (Gültekin-Özgüven et al., 2016), fortified dark chocolate with the blackberry extract (*Morus nigra*) encapsulated with chitosan coating using a spraying method, showing that the chitosan used to coat the encapsulated material provided better protection of anthocyanin content, enhancing bioaccessibility of the anthocyanins present in chocolate. Some works added dry fruits to the chocolate formulations in order to obtain greater antioxidant capacity, being well accepted sensorially. Carvalho et al. (2018) added grape and kale to improve the milk chocolate nutritional role. Agibert & Lannes (2018) improved the nutritional value of chocolate adding high oleic peanut oil microcapsule.

The objective of the present study was to incorporate the lyophilized pulp of pequi mesocarp (*Caryocar villosum* (Alb.) Pers), with a view to nutritional enhancement in dark chocolate.

2 Materials and methods

The pequi fruits (*Caryocar villosum* (Aubl) pers.) used for this study were harvested from adult trees, after their natural fall of the tree - which was a requirement for the harvest of all fruits -, located in the city of Bragança – PA – Brazil, crop of 2018. In the herbarium "João Murça Pires" (MG), denominated Herbarium Amazonicum Musei Paraensis, they received the codification MG n°. 205226, latitude 01 ° 22'55.8 "S and longitude 46 ° 43'50.3" w. The fruits were selected according to the stage of maturation in which they are consumed (yellowish/orange coloring). At harvest the satisfactory integrity of the samples was considered by their preservation status.

The samples were transported in plastic bags of low-density polyethylene (LDPE) and sent to the Laboratory of operations and separation of the Federal University of Pará, Belem – Brazil, where the fruits were washed in running water, immersed in chlorinated water solution at 150 ppm for 5 min. The samples were transferred to the Pharmaceutical Sciences School, Laboratory of Food Technology, University of São Paulo (USP).

The pulp was ground in a food processor (ARNO, Brazil) where it was again stored in plates and frozen at -86 °C in ultrafreezer (TectalMaq, Brazil), and later lyophilized in the freeze drier equipment (Edwars vacuum, Brazil) for 3 days under a pressure of 40 mbar. Then, it was packed in LDPE bags and sealed under vacuum to maintain the material integrity.

Microbiological analyzes were carried out on pequi in natura according to the methodology described by Vanderzant & Splittstoesser (1992) for coliforms 35 °C and 45 °C by the most probable number (NMP), salmonella sp, for the detection of salmonella sp, and molds and yeasts by counting plates.

A Produtest sieves agitator (Granutest, Brazil) was used for the granulometric distribution test of the lyophilized pequi pulp. The sieves used are shown in Table 1.

The sieves were placed in descending order of aperture opening, from top to bottom, with their rheostat in the vibration or stirring position Nº. 7 for 10 minutes for complete separation of the particles.

The percentage of material retained was determined by Equation 1:

$$Mi = \frac{mi}{mo} x100 \quad (1)$$

Mi = Material retained in the sieve i;

mi = mass of the material retained in the sieve i;

m_0 = sample mass.

Soluble solids were determined by refractometry, using the manual refractometer Tecnal, model AR200 digital (Tecnal, São Paulo, Brazil). Titratable total acidity, according to AOAC method Nº 22.058 (Association of Official Analytical Chemists, 2005) which measures the substances' titratable acidity.

Physicochemical analyzes of pequi mesocarp and chocolate were determined by Association of Official Analytical Chemists (2005) methods: pH: AOAC method Nº 981.12 (2000), using a potentiometer (QUIMIS, Brazil), previously calibrated with buffer solutions pH 4 and 7; Protein: micro Kjeldahl method Nº 950.48, which is based on the determination of the amount of total nitrogen in the sample. Crude protein content was calculated by multiplying total nitrogen by factor 6.25 (% N x 6.25); Humidity: Method No. 920,151 with an oven regulated to 105 °C; Fixed mineral residue: Method No. 930.05 with muffle at 550 °C.

Total Carbohydrate amount was calculated by difference (100 g - total grams of moisture, proteins, lipids, fibers, and ashes), according to Resolution RDC 360 (Brasil, 2003).

For carotenoids analysis, the samples were ground with grade and pistil and diluted with 5 mL of acetone, whereafter the cold method described by Moretti et al. (1998) was used.

Table 1. Sieves used in the particle size analysis of the lyophilized pequi pulp.

Tyler standard	Aperture size (mm)
20	0.85
24	0.71
60	0.250
100	0.150
150	0.106

Sugars determination was conducted according to Association of Official Analytical Chemists (2005), the Fehling A and Fehling B reagents were duly standardized with glucose solution.

Energy values of pequi mesocarp and the samples of chocolate were obtained by applying the factors 4 - 9 - 4 kcal/g to the values of proteins, lipids, and total carbohydrates, respectively, according to Resolution RDC 360 (Brasil, 2003).

Transmittance infrared analysis: The sample was compressed with KBr for the formation of a pellet, where it was placed in Alpha model infrared spectrum (Bruker, Germany), and read by the transmittance technique in the range of 400 to 4000 cm^{-1}.

The morphological analyzes of the lyophilized pequi pulp were performed by Scanning Electron Microscopy (SEM), with samples previously dried in air circulation greenhouses at 105 °C for 24 hours, after which a quantity of the powder was deposited on a carrier and sampled with the aid of carbon tape. The samples were metalized with gold to allow the electrical conductivity necessary in the process of image formation in the equipment (S 150, Edwards, USA). The images or electromicrographs were performed in Scanning Electron Microscope (VEGA3, TESCAN, Fuveau, France), with an electron beam current of 85-90 μA.

Thermal analysis was performed on DSC-Differential Scanning Instrument Specialists, I Series (USA) in a nitrogen atmosphere, with alumina crucible, at a temperature ranging from 25-200 °C. Thermogravimetric and differential analyzes (TG-DTA) were performed in a Shimadzu - DTG - 60H (Japan) thermobalance, using air atmosphere, at a flow rate of 50 mL/min. Heating ramp: 10 °C in the temperature range of 600 °C, alumina crucible 5 mg ± 0, 5.

Infrared analysis by transmittance was led by sample pressed with KBr to form a pellet, where it was placed in an infrared spectrum Alpha model (Bruker, Germany) read by the transmittance technique in the range of 400 to 4000 cm^{-1}.

Universal equipment with ball mill coupled WA-FA 20 - SERIE 2872 (Mazzetti, Milan, Italy) was used for chocolate production, with the capacity to process 5 kg. The processing time was 2 h, and the quantity processed in each batch was 3 kg, at a working temperature of 45 °C, added in the following order: 7.5% cocoa butter, cocoa liquor; sugar, 2.5% cocoa butter, soy lecithin, vanillin, run flavor. At the end of the process the lyophilized pequi mesocarp was added manually, a step that took 5 min for each chocolate. The formulations used are shown in Table 2.

Tempering was done manually. The samples were heated to 45 °C and cooled by mixing with a spreader on a marble countertop until they reach 29 °C. They were quickly shaped and refrigerated at 9 °C for 30 min, unmolded, packed in aluminum sheet, arranged in trays, and covered by PVC film. The percentage of each raw material was determined from the fat content to obtain an approximate value of 35% of fat in the final product. Formulation tests were performed in the laboratory with additions of 5%, 2%, 1.5% and 1%. The formulation with 1.5% was chosen since it was the one that best fit the objective of the work in comparison with the formulation of 1% and the one that felt less sandiness and bitter taste coming from the lyophilized mesocarp.

The determination of the cocoa butter cooling curve and the chocolate formulations temper curve were conducted in Multitherm TC Thermometer (Bühler AG, Uzwil, Switzerland). The butter was preheated to a temperature of 50 °C and put into proper capsules of the equipment. The result was evaluated by the Buhler Crystallization Index™ (BCI™). The chocolates were heated to 45 °C and tempered manually to evaluate the validity of the tempering procedure performed, and the result was evaluated by the Buhler Temper Index™ (TI™) (MultiThermTM, 2011).

The particle size of chocolates was carried out in Digimatic Mitutoyo - digital Micrometer Model 293 (Mitutoyo, Kawasaki, USA).

The rheological parameters were obtained on a Haake Mars III oscillatory/rotational MARS III rheometer (Thermo Scientific, Waltham, MA, USA). The equipment was coupled to a thermostated bath. The Casson parameters were calculated by the equipment's computer program at 40 °C after 10 min of rest and the sample preconditioning was made at 55 °C for 75 min based on the official method of the IOCCC (International Office of Cocoa, 2000). The C35 / 2 cone-plate sensor was used with the following parameters: three-step controlled rate rotational test (0.00 1/s - 65.00 1/s, t = 180 s, 65.00 1/s, t = 60 s, 65.00 1/s, - 0.00 1/s, t =180 s), to obtain a thixotropy curve. The amount of sample was sufficient to fill the space between the plates. Data regarding plastic viscosity and yield stress were adjusted to the Casson equation.

Lipid determination after acid hydrolysis of the chocolate and ether extraction was conducted by Soxhlet, the method described and adapted by Lannes (1997).

Table 2. Chocolate formulations.

Ingredients	F1 (control) (%)	F2 (%)
Refined Sugar (União, Brasil) (União, Sertãozinho, São Paulo, Brazil)	43.30	43.30
Cocoa liquor (Cargill, São Paulo, Brazil)	46.10	46.10
Cocoa butter (Cargill, São Paulo, Brazil)	9.80	9.80
Soy lecithin (Tovani Benzaquen Ingredients, São Paulo, Brazil)	0.50	0.50
Vanillin powder (Daxia Ingredients and Additives, São Paulo, Brazil	0.30	0.30
Run flavor powder (Daxia Ingredients and Additives, São Paulo, Brazil)	---	0.05
Lyophilized pequi pulp	---	1.5

Determination of water activity was conducted in Novasina water activity equipment model LabMaster (Novasina, Switzerland).

Thermal analysis was performed in DSC-Differential Scanning Equipment - I Series (Instrument Specialists Incorporated, Twin Lakes, WI, USA) in a nitrogen atmosphere, with alumina crucible, at a temperature ranging from 25 °C to 250 °C, based on data acquired by the Acquire Program (Instrument Specialists Incorporated). The calorimetric properties (melting onset, peak melting, and melting end, as well as the peaks of the caramelization and carbonization temperatures) were identified on the graphic plotted by Origin, version 8 (OriginLabCorp, Northampton, MA, USA).

Determination of antioxidant capacity: The extract was prepared following the methodology described by Genovese & Lannes (2010), in which, using a ultraturrax (Marconi, BRASIL), 0.5 g of the sample was added to 20 mL of methanol/water in the ratio 70:30 for 1 min at speed 4 and ice bath. The extract was filtered using filter paper and the resulting extract was stored in amber glass. It was used for the formulations of pequi lyophilized mesocarp, cocoa liquor and chocolate.

- Total phenolics for lyophilized pequi mesocarp pulp, cocoa liquor and chocolate formulations: it was performed based on the method of Folin Ciocauteau described by Singleton et al. (1999), with modified volume. The standard curve was built with solutions of gallic acid (50, 100, 150 and 250 mg/L). To determine the phenolic compounds, 20 µL of the sample was added to a 5 mL volumetric flask. Subsequently, 200 µL of the Folin-Ciocauteau reagent was added and the mixture was homogenized. After 1 to 8 min, 750 µL of 20% sodium carbonate was added and the volume was adjusted. The reading was performed in a visible UV spectrophotometer (Spectrum Meter, Brazil) at 760 nm.
- Total antioxidant activity by iron reduction method (FRAP) for pequi lyophilized mesocarp, cocoa liquor and chocolate formulations: the methodology described by Rufino et al. (2006) was followed. A standard curve was made with ferrous sulfate from the standard solution of ferrous sulfate (2000 µM), prepared in 10 mL volumetric flasks, with solutions ranging from 500 µM to 1500 µM. From that point, the absorbance of 1000 mM of ferrous sulfate was calculated based on the line equation. From the absorbances obtained from the extracts' different dilutions, the absorbance was plotted on the Y axis and the dilution (mg/L) was plotted on the X axis. Then, the equation of the line was determined. For the calculation of AAT (Total Antioxidant Activity), the absorbance equivalent to 1,000 µM of the ferrous sulfate standard was substituted in the straight line's equation.

The analysis was performed in triplicate, and the means and standard deviation were calculated. The results were analyzed by ANOVA and later by Tukey, in 5% of significance, with Statistica version 11 software (StatSoft, USA) and Microsoft Excel (Microsoft, USA).

3 Results and discussion

3.1 Microbiological analysis

The results of the microbiological analyzes of the pequi fruit are presented according to the standards determined by the Brazilian legislation in force. They demonstrated a product suitable for consumption due to the absence of Salmonella sp. in 25 g. Regarding the presented value of coliforms at 45 °C and 35 °C, it indicates the presence of these microorganisms with results well below the tolerance levels allowed by the legislation: maximum of 102/g (Brasil, 2001). The values are shown in Table 3.

However, since it was a fruit with a high moisture content in the pulp, an analysis for molds and yeasts was also carried out, in which the fruit had 3.7 x 10 CFU/g, demonstrating good condition - according to the legislation, the food becomes unfit for consumption from 106 CFU/g (Brasil, 2001).

3.2 Particle size

Table 4 shows the particle size mean result of the lyophilized pequi mesocarp pulp with the average opening of the sieves and the variance by the algebric method, classifying the approximate size.

As observed in Table 4, the lyophilized pulp was concentrated in the screen opening of 0.567 mm, this being the maximum particle size, and the mass retained in the sieves with their respective openings is shown in Figure 1.

Table 3. Microbiology of pequi pulp.

Coliforms 35 °C e 45 °C	*Salmonella* sp.	Molds and yeasts (UFC)
< 3 nmp/g	Absent	3.7 x 10

Table 4. Results of particle size determination by algebraic method of lyophilized pequi pulp.

	Average opening (AO)	CV (%)
Algebric method	0.567 ± 0.005	42.518 ± 0.005

n : 3.

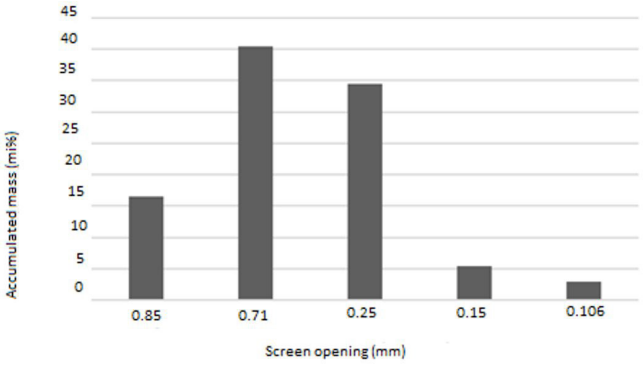

Figure 1. Masses of lyophilized pulp retained in the sieves with their respective openings.

If we consider normative instruction No. 52, of November 7, 2011 (Brasil, 2011) for cassava flour, pequi flour would fit in a thick type, since the product was retained in a value higher than 10% in the 2mm opening-sieve.

3.3 Nutritional composition

Table 5 shows the results obtained in relation to the mesocarp of pequi on a dry basis, which will be used to compare results obtained by other authors.It was verified that the pequi presented low moisture content in comparison to the values obtained by Berto et al. (2015) and Chisté & Mercadante (2012). However, the result of 27.715 ± 0.017 is still high and unfavorable in terms of conservation. Even more in a fruit that is considered oleaginous, which can provide oil rancidity.

The obtained proteins values are similar to those obtained by Chisté & Mercadante (2012). According to Marx et al. (1997), when quantifying the amino acids of the pequi mesocarp, considerable amounts in mg/100 g of valine (11.80), leucine (14.62), asparagine (18.16), glutamine (10.36), and alanine (17.20) were found.

Lipid values were higher than those obtained by Chisté & Mercadante (2012) (25.5%) and Berto et al. (2015) (1.80%), possibly by the latter using lipid extraction such as Bligh and Dyer. There is a difference of 5% when comparing the results of total sugars with those of carbohydrates of Table 6. These values can be explained when choosing the calculation of the difference for carbohydrates by the legislation, which showed the total value coming from the losses of the glycoproteins and the carbohydrates present in the fibers obtained from the acid reactions.

The total energy value (VET) shows that the consumption of 100 g of pequi pulp can supply approximately 26% of the caloric needs of an adult based on a 2.000 kcal diet.

Table 5. Nutritional composition of pequi pulp (*C. villosum*).

(%)	Results
Humidity	27.72 ± 0.02
Ash	0.83 ± 0.01
Protein	3.08 ± 0.01
Lipid	46.78 ± 0.69
Carbohydrate	21.59
Total sugars	13.37 ± 0.21
Reducing sugar	10.77 ± 0.17
No reducing sugar	2.6
Sucrose	2.47
Energy value (kcal.100 g^{-1})	519.71

n : 3 dry basis.

Table 6. Values of carotenoids of pequi pulp (*C. villosum*).

Carotenoids (μg β-carotene/g)	Carotenoids (μg lutein/g)	Total Carotenoids (μg/g)
3.296 ± 0.439	3.3 ± 0.439	6.60 ± 0.44

n : 3 Dry basis.

From an industrial point of view, a low-acid food is conducive to the development of pathogenic microorganisms and deterioration. The pulp's pH was 3.4, measured at a temperature of 25 °C, lower than the result obtained by Souza et al. (2013) – 5.21 in the species *Caryocar coriaceum* Wittm.

When the acidity was related to SST, the ratio index was 21.78. There is still no industrial determination regarding this index that provides higher juice quality for this fruit, as is the case with orange, for example, where its favorable maturation index is located between 11 and 14 (Stuch et al., 2009).

The compounds belonging to the group of carotenoids have antioxidant properties. The β-carotene is the major provitamin A precursor, as well as other carotenoids (lycopene, lutein and zeaxanthin) (Ferrari & Torres, 2002).

Carotenoids (μg / g for β-carotene and lutein) found in the fruit mesocarp (pequi pulp) were 3.296 ± 0.439 and 3.3 ± 0.439 (Table 6), respectively, which were lower than the results of 4.42 ± 0.15 μg β- carotene/ g found by Machado et al. (2013). These divergences may be related to the possible solvents used and/or their extraction methods employed, with the maturation point and the edaphoclimatic conditions of the plant.

3.4 Scanning electron microscopy (SEM) analysis

In Figures 2AB and Figure 3, micrographs are performed by the defatted and lyophilized pequi mesocarp scanning electron microscopy.

The study of the microscopic structures of the pequi mesocarp aims to broaden and ratify the knowledge about the composition and characterization of the morphological structures of this raw material, and also to develop parameters to identify its microscopic structure in foods made from it.

Figure 2AB shows an overview of the irregular pore structure and the cavernous interior, noting the presence of non-uniformly distributed starch granules and oval shapes.

The microscopy (Figure 3) shows a smooth fibrous structure, possibly due to the temperature and the use of solvents in the lipid extraction.

The characteristics observed in the pequi starch granules are similar to those found by Cunha (2016) in arrowroot starch granules used in confectionery and baking, making this material an additional increment in the diversification of compounds and matrices combined for use in various sectors of the food industry.

3.5 Infrared analysis of lyophilized pequi mesocarp pulp.

Figure 4 shows the infrared analysis by the transmittance module of the lyophilized pequi pulp in order to verify the compounds present in its structure.

In the broad band of 3500 cm^{-1} to 3300 cm^{-1}, characteristic of the amines and amides groups (N-H), and it can also be attributed the presence of adsorbed water in the band centered at 3410 cm^{-1}, besides the contribution in this region to the stretching of the amide bond (NH). Peaks at about 2928 cm^{-1} and 2818 cm^{-1} were attributed to the methyl and methylene (CH)

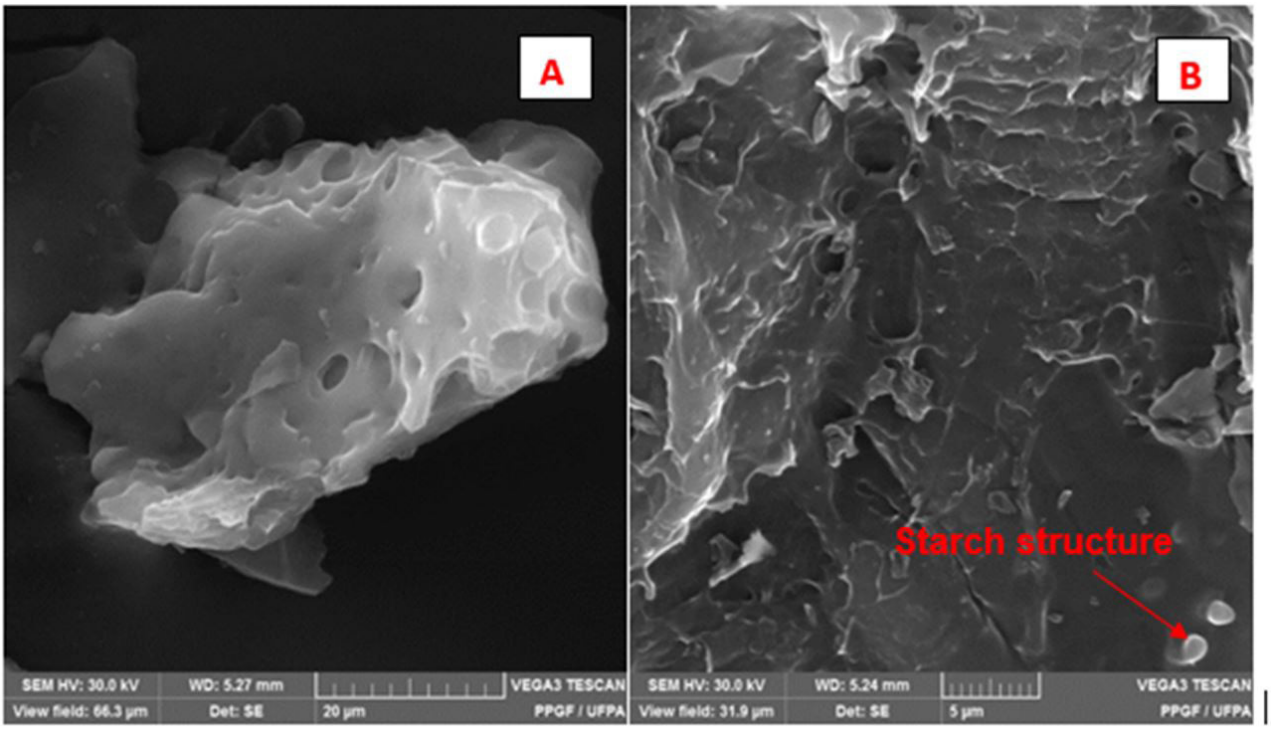

Figure 2. General structure of pequi pulp (A) and structure with starch granule (B).

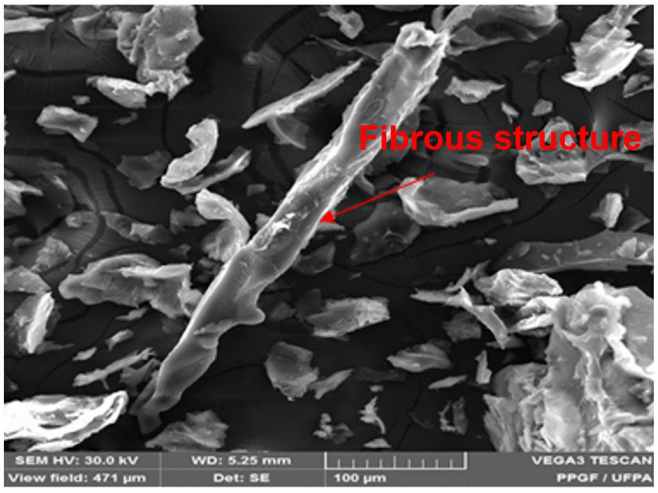

Figure 3. Fibrous structure of pequi pulp found in spectroscopy at 100 µm.

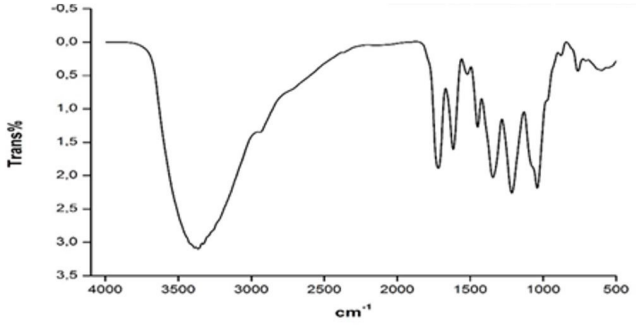

Figure 4. Infrared of the lyophilized pequi pulp by transmittance.

groups and the presence of the carbonyl groups (C = O) found in 1730 cm^{-1} confirmed the presence of fatty acids. The band near 1000 cm^{-1} was attributed to C-OH elongation vibration from cellulose and hemicelulose (Figure 4).

Bands around 1650 and 1500 cm^{-1} can also be observed, being characteristic of the presence of aromatic rings (C = C). In the 1750 cm^{-1} range there is the presence of carbonyl group (C = O), belonging to methyl esters, ketones, aldehydes common in long chain fatty acids found in oils such as patauá and buriti (Figueira, 2012) and macaúba (Del Río et al., 2016).

There are still bands characteristic of saturated esters (C-O-C) in the bands between 1000 cm^{-1} and 1300 cm^{-1}; the range of 1290 to 1050 cm^{-1} is characteristic of alcohols, esters, ethers, carboxylic acids, and fatty acids. The lowest verified bands are around 690 cm^{-1} and may be linked to the alkenes (C-H), which may be the sequence of aliphatic fatty acid chains (Figure 4).

3.6 Cooling curve of cocoa butter and tempering curve of (F1) control chocolate and (F2) chocolate added with lyophilized pequi pulp.

The aim of the cooling curve of cocoa butter was to verify the production of cocoa butter crystals suitable to produce chocolate. The cooling curve is shown in Figure 5.

Cooling curve means the identity of a fat. The results of the cooling curve of the cocoa butter expressed in Buhler Crystallization Index ™ (BCI ™) are, in triplicate: Tn : 20.5 ± 0.15 °C; tn: 8.5 ± 0.45 min; Q : 0.2 ± 0.01 °C/min; BCI : 3.7 ± 0.00. Where: Tn: crystallization; tn: time in minutes; Q: Cooling rate; BCI: Buhler Crystallization Index ™ (Figure 5).

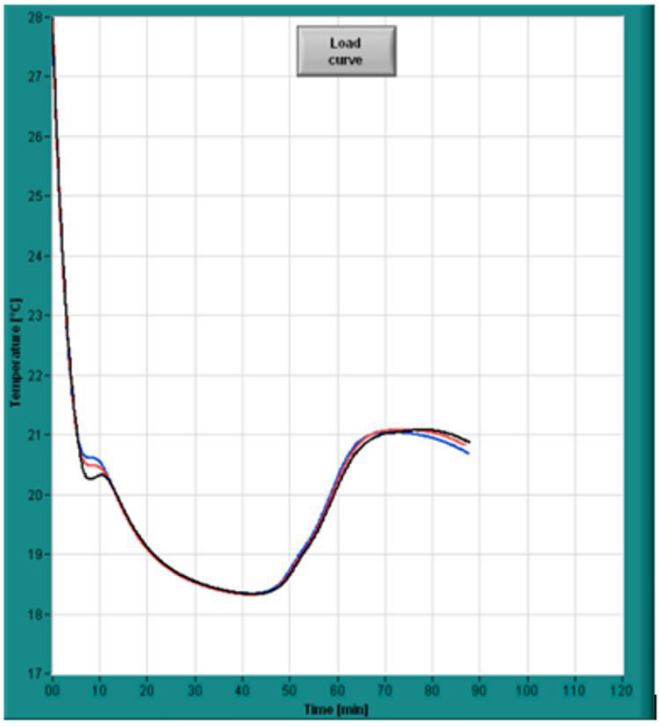

Figure 5. Cooling curves of cocoa butter.

The cooling curve of the cocoa butter demonstrates its characteristics of melting and the rate of crystallization, presenting the adequacy of the butter for use in chocolates. The manufacturer of the equipment (Buhler), using cooling curve, known as Buhler Crystallization Index (BCI), has an index for assessing the quality of cocoa butter. According to the manufacturer, the BCI found for the butter analyzed (3.7 ± 0.00) is in compliance with the equipment's quality standard, which should have an oscillation between 3 and 5 in the BCI value.

The tempering curve was used to verify the tempering performed on the chocolate's formulations and, if concomitantly, the formation of the appropriate crystals of the cocoa butter in their stable form were formed. The tempering curve of the standard and added chocolates of pequi lyophilized pulp are shown in Figure 6.

Table 7 shows the values obtained in the respective chocolate temperature curves, where TI_{BR} represents the temperature curve of the control chocolate (F1) and TI_{AD} the temperature curve of the chocolate added with lyophilized pulp (F2). As can be observed, the temperature is lower and the time is higher for F1, showing the influences of pulp addition in the chocolate's temper process.

The curve generated has an adequate linearity, and it can be stated that an appropriate temperature was obtained in the

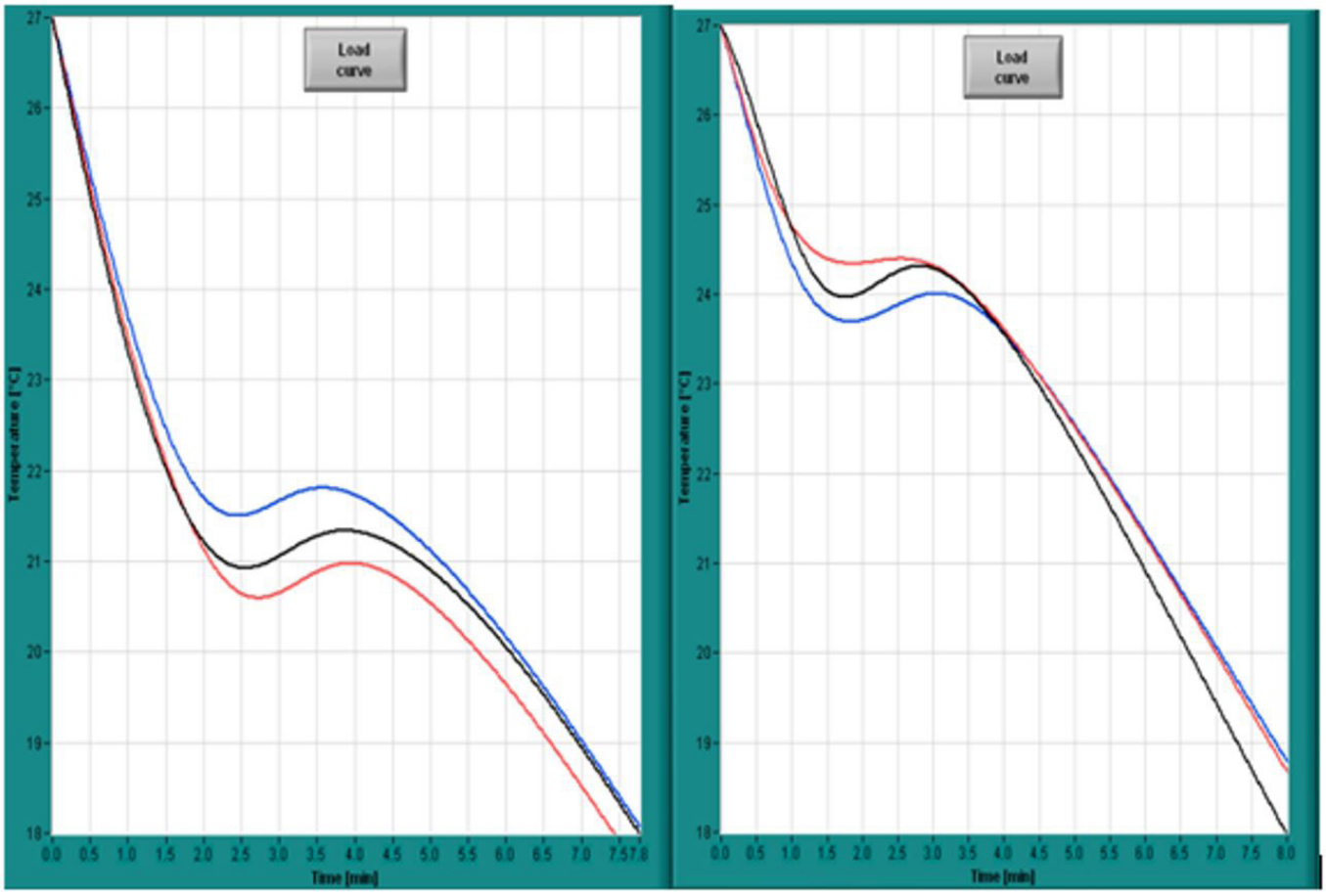

Figure 6. Temper curves of control chocolates (left) and chocolates added lyophilized pequi pulp (right).

Table 7. Results of the tempering curves of the control chocolate (F1) and the chocolate added of lyophilized pequi pulp (F2).

	Temperature (°C)	Time (min)	Slope (°C/min)	TI
TI_{BR}	21.2 ± 0.4	3.1 ± 0.2	0.47 ± 0.04	3.4 ± 0.12
TI_{AD}	24.1 ± 0.3	2.3 ± 0.12	0.34 ± 0.21	3.8 ± 0.7

n : 3. TI_{BR} control chocolate; TI_{AD} chocolate added of lyophilized pequi pulp; TI Temper Index.

chocolate mass: 0.47 °C/min in 3.1 min and 21.2 °C. The Temper index (TI), used by the equipment manufacturer (Buhler) to evaluate the performance of the tempering, resulted in 3.4, a value considered acceptable for dark chocolate. The same was considered for chocolate added with TI of 3.8, in which, according to the equipment's quality standard, tempered chocolates should have an oscillation between 3 and 5 in the TI value.

3.7 Physicochemical analyzes of chocolate

The results of the physicochemical analyze of the control chocolate (F1) and the one with 1.5% of lyophilized pulp (F2) with their respective mean and standard deviations were also presented in Table 8. The ANOVA and Tukey tests were also performed at 5% significance level to evaluate possible significant differences between samples. The water activity in chocolates is relatively low and usually has values from 0.3 to 0.6. The study revealed a water activity value of 0.42. The water activity in the present work is in the indicated range, and the addition of lyophilized product did not influence the increase of the activity. This result is mainly due to the low water activity in the lyophilized raw material. There was a significant difference between the pH of the chocolates due to the addition of the lyophilized raw material (pH of 3.4), which, despite the small percentage of the addition, interfered significantly in the chocolate's pH.

3.8 Nutritional composition of formulated dark chocolates

The results of the nutritional composition of control chocolate (F1) and chocolate added with 1.5% of lyophilized pulp (F2) are represented in Table 9 with their respective means and deviations and with the ANOVA and Tukey tests at 5% significance.

Both the standard chocolate and the chocolate added with lyophilized pequi pulp showed a significant difference between the humidity and the ash concentration, while the other analyzes did not register significant differences possibly because the amount of lyophilized material added in 1,5%, does not lead to the decrease or increase of these compositions. Lipid quantification showed no significant difference between the formulations. Such results can be explained due to the low addition (1.5%) of lyophilized pulp to the chocolate, although the addition of 0.7% of pulp oil of pequi to chocolate include the natural properties of this oil, like high levels of oleic, palmitic, and linoleic acids, besides the health benefits based on the oil functionality index. The mineral content presented significant difference with the pequi added formulation. This result was expected due to the addition of solid/mineral material to the product, which is nutrient and protein-poor in its composition.

Table 8. Physicochemical analysis of chocolates: (F1) Control and (F2) chocolate added with 1.5% lyophilized pequi pulp.

.	Control (F1)	(F2)
Water activity (Aw)	0.43^{a} ± 0.02	0.46^{a} ± 0.00
pH	7.07^{a} ± 0.03	6.32^{b} ± 0.01

n : 3 Same letter, in the same line, indicates that there is no difference in 5% of significance.

Table 9. Nutritional composition of chocolates: Control (F1) and chocolate added with 1.5% lyophilized pequi pulp (F2).

(%)	Control (F1)	(F2)
Moisture	1.06 ± 0.07^{b}	1.20 ± 0.01^{a}
Ash	2.47 ± 0.03^{a}	2.21 ± 0.02^{b}
Proteins	14.40 ± 0.28^{a}	14.30 ± 0.25^{a}
Lipids	34.32 ± 0.11^{a}	34.67 ± 0.39^{a}
Carbohydrates	47.8	47.62
Energy value (kcal.100 g^{-1})	557.5	559.73

n : 3 Same letter, in the same line, indicates that there is no difference in 5% of significance.

3.9 Rheology

Figure 7 shows the viscosity and initial stress of Casson to attest to the quality of standard chocolates and added during their production. These were evaluated in the present work for all the chocolates and the results are found in Table 10.

Based on the results presented in Table 10, there was no significant difference between the chocolates' viscosities. The samples presented thixotropy, as can be showed by the hysteresis curve. The particle size distribution influences the product rheology, affecting its viscosity – the larger the particle size, the smaller its viscosity will be. By reducing the size, there is an increase of friction between the particles, moreover, particles larger than 30 μm are perceived on the palate, leading to a sandy texture, while the ones closer to 20 μm are more sensorially pleasing, as they do not alter their ideal viscosity (Beckett, 2009; Gonçalves & Lannes, 2010; Afoakwa, 2016).

According to the statistical analysis performed, the addition of pequi lyophilizate significantly alters the chocolate particle size. Such a divergence of the chocolate with the pattern can be explained because the pequi pulp has been added after completion of the chocolate mass manufacture, under manual agitation. Therefore, it has not undergone the refining process. This difference in the process was certainly reflected in the particle size of the added chocolate, which suggests that these particles could be sensed sensory.

High amounts of fat (34.32 g/100 g in F2-chocolate, 34.67 g/100 g in F1-chocolate with addition of lyophilized pulp) were found in all formulations. According to Afoakwa (2016), the higher the amount of fat, the emptier spaces are filled in the structure of the chocolate, leading to a greater ease of flow and, consequently, lower viscosity. The yield stress did not present significant difference (5%) among the samples.

Industry's coverture chocolates can present lower values of apparent viscosity (1.3-2.5 Pa.s) and yield value (2.5-9.0 Pa). Although the chocolate with 1.5% addition of pequi had undergone

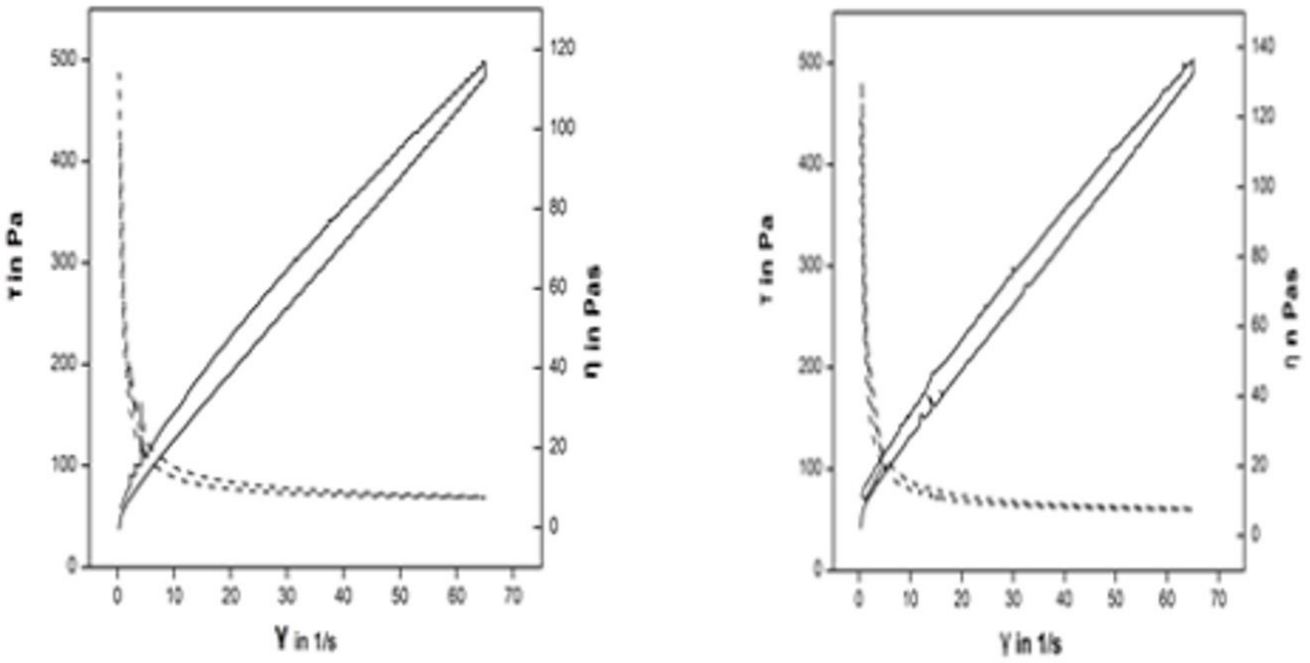

Figure 7. Rheology of the F1 - Control (left) and F2 - added with 1.5% lyophilized pequi pulp (right).

Table 10. Casson parameters for dark chocolate formulations.

	Control F1	F2
Particle size	22.5[b] ± 0.26	62.0[a] ± 2.4
Yield stress (Pa)	28.04[a] ± 0.21	30.65[a] ± 1.69
Viscosity (Pa.s)	4.32[a] ± 0.40	3.98[a] ± 0.83
Determination Coefficient	0.97 ± 0,00	0.96 ± 0.00

n : 3 Same letter, in the same line, indicates that there is no difference in 5% of significance ($p < 5$).

the addition of pulp mechanically, it is noteworthy that the amount of sample and the particle size of the pequi pulp were not enough to significantly affect the rheology of F2 chocolate in comparison with F1 chocolate. Both chocolates presented tixotropy (Figure 7).

3.10 Thermal analysis of chocolate formulations

Figure 8 shows the differential scanning thermal analysis (DSC) curve of standard chocolate.

The F1 chocolate was subjected to a temperature of up to 250 °C, where it had two endothermic curves, reaching all the events up to the established temperature. This graph (Figure 8) shows that the product is stable at temperatures up to 50 °C, as demonstrated in first peak with enthalpy change ΔH = -23.01 J/g; the second is the melting reaction of the sugar crystals and the total carbonization of the sample with ΔH = -97.90 J/g.

Glicerina et al. (2010) found DSC curve peaks for tempered chocolate, in which, fat was represented between 25 °C and 35 °C and sugar crystals between 174 °C and 190 °C . Agibert & Lannes (2018) found similar results.

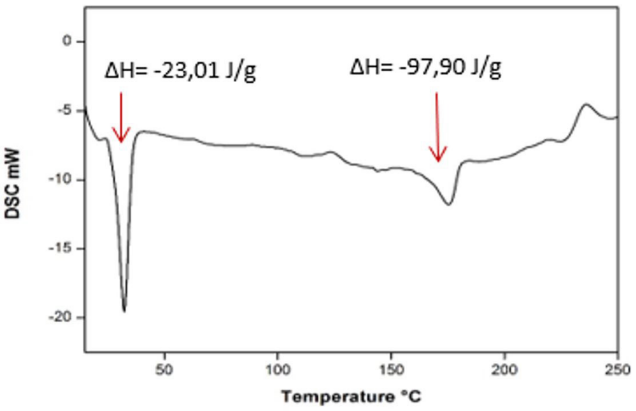

Figure 8. Differential Scanning Calorimetry (DSC) of control chocolate (F1).

Figure 9 shows the differential scanning thermal analysis (DSC) graph of chocolate added with lyophilized pequi pulp (F2).

The chocolate added with pulp was subjected to a temperature of up to 250 °C. It had five endothermic curves, reaching all events up to the set temperature - this graph (Figure 9) shows that the product is stable at temperatures up to 50 °C, as demonstrated in the first peak with enthalpy change ΔH = - 36.73 J/g; the second to third peak corresponding to the areas from 100 °C to 140 °C; the fourth peak correspond to the oxidation and degradation of the lyophilized pequi pulp added to the chocolate, a differential compared to the standard chocolate; and the fifth peak is the total sample carbonization with ΔH = -78.2 J/g.

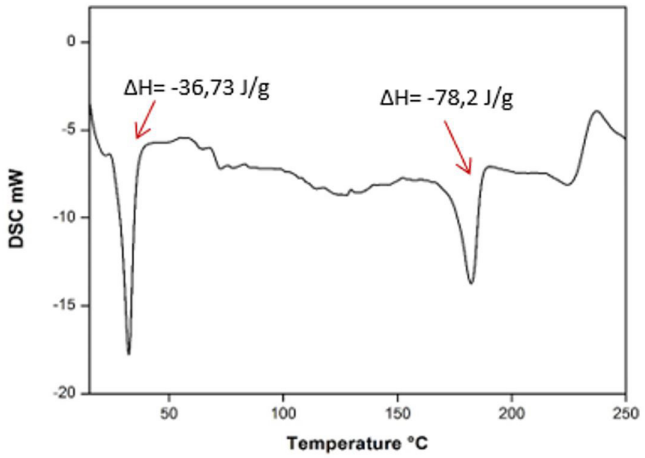

Figure 9. Differential Scanning Calorimetry (DSC) curve of chocolate added with 1.5% of lyophilized pequi pulp (F2).

3.11 Quantification of total phenolics.

From the standard curve of gallic acid, a line equation was obtained and used to calculate the concentrations of each extract. Table 11 shows the results, in which the ANOVA and Tukey tests were performed with 5% significance. There was a significant difference in all samples showing the highest concentration of phenolics in the added chocolate, followed by the CSF and pequi pulp. The result obtained for the lyophilized pequi pulp extract was higher than the one found by Machado et al. (2013), 196.23 ± 3.26 mg GAE.mL^{-1} in methanolic extract and 216.69 ± 0.69 mg GAE.mL^{-1} in an aqueous extract. Machado et al. (2015), with 277.25, still registered inferior values to the one obtained. Socio-climatic factors of the periods in which the fruits of pequi were collected can influence the results of each research. Carvalho et al. (2019) found 259.94 ± 2.46 mg/g GAE to cocoa liquor. If we compare the result of the cocoa liquor with the control chocolate (F1), we can observe a decrease in phenolic concentration due to the addition of other ingredients in the manufacture of chocolate. Campos (2019) analyzed the content of total phenolic compounds of different types of cocoa and found a value of 16.48 ± 0.63 mg GAE g^{-1} B.U. for organic liquor. Komes et al. (2013) found that compared to milk chocolate, bitter chocolate exhibited higher polyphenolic content, while in relation to plain ones, the addition of dried cranberries and raisins to chocolates contributed to the increase of total polyphenols.

3.12 Analysis of antioxidant activity FRAP

The results of the antioxidant activity FRAP radical are found in Table 12.

In the extract of the lyophilized pequi, pulp was superior to that found by Machado et al. (2013), FRAP (mM FeSO4.mL^{-1}) 2.47 ± 0.050 in methanolic extract and 2.62 ± 0.007 in aqueous extract, and in its study, in which Machado et al. (2015) state 2.62 ± 0.01 mMmL^{-1}. In this analysis there was also a decrease in liquor concentrations in relation to the control chocolate (F1). However, when compared to the added chocolate version (F2), it was noted that there was no significant difference in the results of the antioxidants. Such a result is possible due to the interaction capacity of the lyophilized pequi extract with the TPTZ moiety used in the FRAP analysis, possibly showing that the pequi antioxidants are not overly reactive to the TPTZ.

Table 11. Results of total phenolics obtained from the standard curve of gallic acid in the samples of lyophilized pequi pulp, cocoa liquor, control chocolate (F1), and chocolate added with lyophilized pequi pulp (F2).

	Total phenolics content (mg GAE.mL^{-1})
Lyophilized pequi pulp	$358.43^{c} \pm 0.01$
Cocoa liquor	$523.80^{b} \pm 0.01$
F1 (Control)	$235.98^{d} \pm 0.05$
F2 (Chocolate added with lyophilized pequi pulp)	$813.49^{a} \pm 0.03$

n : 3 Same letter, in the same line, indicates that there is no difference in 5% of significance ($p < 5$).

Table 12. Results of antioxidant activity FRAP radical.

	ferrous sulphate mM/g*
Lyophilized pequi pulp extract	$8.67^{b} \pm 0.01$
Cocoa liquor extract	$25.68^{a} \pm 0.03$
F1 (Control chocolate extract)	$23.62^{ab} \pm 0.05$
F2 (Chocolate added with lyophilized pequi pulp extract)	$23.51^{ab} \pm 0.02$

*n : 3 Same letter, in the same line, indicates that there is no difference in 5% of significance ($p < 5$).

5 Conclusion

There was no change in the taste and color of the dark chocolate (F1) formulated, with the addition of 1.5% of pequi lyophilized mesocarp in dark chocolate (F2). The increase in particle size of the formulated chocolate mass was not sufficient to increase its viscosity or the initial tension. Infrared showed the presence of fatty acids in the pequi pulp. Pequi pulp oil (0.7%) was added to the formulated chocolate through the oil properties as high contents of oleic, palmitic, and linoleic acids. The energy value of F1 and F2 formulations presented small difference. For the formulated chocolate tempering curve of relative to the control there was an increase in the initial temper temperature and a decrease in the total process time. The thermal analyzes of the control and pequi pulp added chocolates showed that both withstood temperatures until 50 °C, not having interference of pequi pulp in the chocolate. Total phenolics was bigger in F2 than F1. Antioxidant activity showed no significative difference to F1 nor F2. The use of lyophilized pequi pulp as an alternative for the enrichment of nutritional properties in dark chocolate was confirmed in this work.

Conflict of interest

The authors declare that they do not have any conflict of interest.

Acknowledgements

This work was supported by CAPES--*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*, Brazilian Government.

References

Afoakwa, E. O. (2016). *Chocolate science and technology* (2nd ed.) UK: Jonh Wiley & Sons Ltd. http://dx.doi.org/10.1002/9781118913758.

Agibert, S. A. C., & Lannes, S. C. S. (2018). Dark chocolate with a high oleic peanut oil microcapsule content. *Journal of the Science of Food and Agriculture*, 98(15), 5591-5597. http://dx.doi.org/10.1002/jsfa.9102. PMid:29696663.

Association of Official Analytical Chemists - AOAC. (2005). *Official methods of analysis of AOAC international* (18th ed.). Gaithersburg: AOAC Internacional.

Beckett, S. T. (2009). *Industrial chocolate manufacture and use* (4th ed.). Chichester: Wiley-Blackwell.

Berto, A., Silva, A. F., Visentainer, J. V., Matsushita, M., & Souza, N. E. (2015). Proximate compositions, mineral contents and fatty acid compositions of native Amazonian fruits. *Food Research International*, 77(Part 3), 441-449. http://dx.doi.org/10.1016/j.foodres.2015.08.018.

Brasil, Agência Nacional De Vigilância Sanitária. (2001, January 2). Resolução RDC n.12 de 2 de janeiro de 2001. Regulamento técnico sobre os padrões microbiológicos para alimentos. *Diário Oficial [da] República Federativa do Brasil*.

Brasil, Agência Nacional De Vigilância Sanitária. (2003, December 23). Resolução RDC n.360, de 23 de dezembro de 2003. Regulamento técnico sobre rotulagem nutricinal de alimentos embalados. *Diário Oficial [da] República Federativa do Brasil*.

Brasil, Agência Nacional De Vigilância Sanitária. (2011, November 7).)Instrução normativa n.52, de 7 de novembro de 2011. Regulamento Técnico da Farinha de Mandioca. *Diário Oficial [da] República Federativa do Brasil*.

Campos, I. C. C. (2019). *Desenvolvimento de achocolatados em pó com adição de subprodutos de frutas, processados por spray dryer e com modificador reológico* (Thesis). Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo.

Carvalho, J. C. S., Romoff, P., & Lannes, S. C. S. (2018). Improvement of nutritional and physicochemical proprieties of milk chocolates enriched with kale (Brassica olereacea var. acephala) and grape (Vitis vinífera). *Food Sci Technol*, 38(3), 551-560. http://dx.doi.org/10.1590/fst.15018.

Chisté, R. C., & Mercadante, A. Z. (2012). Identification and quantification, by HPLC –DAD-MS/MS, of carotenoids and phenolic compouds from the Amazonia fruit Caryocar villosum.⁋ *Journal of Agricultural and Food Chemistry*, 60(23), 5884-5892. http://dx.doi.org/10.1021/jf301904f. PMid:22612541.

Cunha, A. L. (2016). *Caracterização do amido da araruta tipos seta e redonda, visando utilização na indústria de alimentos* (Dissertação de Mestrado). Universidade Federal de Lavras, Lavras.

Del Río, J. C., Evaristo, A. B., Marques, G., Martín-Ramos, P., Martín-Gil, J., & Gutiérrez, A. (2016). Chemical composition and thermal behavior of the pulp and kernel oils from macaúba palm (*Acrocomia aculeata*) fruit. *Industrial Crops and Products*, 84, 294-304. http://dx.doi.org/10.1016/j.indcrop.2016.02.018.

Fernández-Murga, L., Tarín, J. J., Gárcia-Perez, M. A., & Cano, A. (2011). The impact of chocolate on cardiovascular health. *Maturitas*, 69(4), 312-321. http://dx.doi.org/10.1016/j.maturitas.2011.05.011. PMid:21665390.

Ferrari, C. K. B., & Torres, E A F. S. (2002). Novos compostos dietéticos com propriedades anticarcinogênicas. *Revista Brasileira de Cancerologia*, 48(3), 375-382. http://dx.doi.org/10.32635/2176-9745.RBC.2002v48n3.2212.

Figueira, L. C. 2012. *Espectroscopia vibracional (no infravermelho e raman) e espectrofotometria de absorção uv-vis dos óleos de buriti (Mauritia flexuosa) e de patauá (Oenocarpus bataua)* (Dissertação de Mestrado). Universidade Federal do Oeste do Pará, Santarém.

Genovese, M. I., & Lannes, S. C. S. (2010). Comparison of total phenolic content and antiradical capacity of powders and "chocolates" from cocoa and cupuassu. *Food Sci Technol*, 29(4), 810-814. http://dx.doi.org/10.1590/S0101-20612009000400017.

Glicerina, V., Balestra, F., Rosa, M. D., & Romani, S. (2010). Rheological, textural and calorimetric modifications of dark chocolate during process. *Journal of Food Engineering*, 30, 845-885.

Gonçalves, E. V., & Lannes, S. C. S. (2010). Chocolate rheology: a review. *Food Sci Technol*, 30(4), 845-885. http://dx.doi.org/10.1590/S0101-20612010000400002.

Gültekin-Özgüven, M., Karadağ, A., Duman, S., Özkal, B., & Özçelik, B. (2016). Fortification of dark chocolate with spray dried black mulberry (*Morus nigra*) waste extract encapsulated in chitosan-coated liposomes and bioccessabilitity studies. *Food Chemistry*, 201, 205-212. http://dx.doi.org/10.1016/j.foodchem.2016.01.091. PMid:26868567.

International Office of Cocoa - IOCCC. (2000). *Chocolate and sugar confectionery, viscosity of cocoa and chocolate product, analytical method*. Hardricourt: IOCCC.

Komes, D., Belščak-Cvitanović, A., Škrabal, S., Vojvodić, A., & Bušić, A. (2013). The influence of dried fruits enrichment on sensory properties of bitter and milk chocolates and bioactive content of their extracts affected by different solvents. *Lebensmittel-Wissenschaft + Technologie*, 53(1), 360-369. http://dx.doi.org/10.1016/j.lwt.2013.02.016.

Lannes, S. C. S. (1997). *Estudo das propriedades físico-químicas e de textura de chocolates* (Tese de Doutorado). Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo. http://dx.doi.org/10.11606/T.9.1997.tde-18102007-143658.

Lannes, S. C. S. (2016). Chocolate and its products: health approach. In G. V. Barbosa-Cánovas, G. M. Pastore, K. Candoğan, I. G. M. Meza, S. C. S. Lannes, K. Buckle, R. Y. Yada & A. Rosenthal (Eds.), *Food security and wellness* (p. 175-194). New York: Springer.

Lorenzo, N. D., Santos, O. V., & Lannes, S. C. S. (2018). Piquiá (Caryocar villosum (Aubl) pers.). In S. D. Todorov & F. A. Pieri (Eds.), *Tropical fruits: from cultivation to consumption and health benefits, fruits from the amazon* (1st ed, pp. 279-304). Hauppauge: Nova Science Publishers.

Machado, M. T. C., Mello, B. C. B. S., & Hubinger, M. D. (2013). Study of alcoholic and aqueous extraction of pequi (Caryocar brasiliense Camb.) natural antioxidants and extracts concentration by nanofiltration. *Journal of Food Engineering*, 117, 450-457. http://dx.doi.org/10.1016/j.jfoodeng.2012.12.007.

Machado, M. T. C., Mello, B. C. B. S., & Hubinger, M. D. (2015). Evaluation of pequi (Caryocar Brasiliense Camb.) aqueous extract quality processed by membranes. *Food and Bioproducts Processing*, 95, 304-312. http://dx.doi.org/10.1016/j.fbp.2014.10.013.

Marx, F., Andrade, E. H. A., & Maia, J. G. (1997). Chemical composition of the fruit pulp of Caryocar villosum. *Food Res Technol*, 204(6), 442-444. http://dx.doi.org/10.1007/s002170050110.

Miranda-Vilela, A. L., Pereira, L. C. S., Gonçalves, C. A., & Grisoli, C. K. (2009). Pequi fruit (*Caryocar brasiliense* Camb.) pulp oil reduces exercise-induced inflammatory markers and blood pressure of male

and female runners. *Nutrition Research (New York, N.Y.)*, 29(12), 850-858. http://dx.doi.org/10.1016/j.nutres.2009.10.022. PMid:19963158.

Moretti, C. L., Sargent, S. A., Huber, D. J., Calbo, A. G., & Puschmann, R. (1998). Chemical composition and physical properties of pericarp, locule and placental tissues of tomatoes with internal bruising. *Journal of the American Society for Horticultural Science*, 123(4), 656-660. http://dx.doi.org/10.21273/JASHS.123.4.656.

Morze, J., Schwedhelm, C., Bencic, A., Hoffmann, G., Boeing, H., Przybylowicz, K., & Schwingshackl, K. (2020). Chocolate and risk of chronic disease: a systematic review and doseresponse meta-analysis. *European Journal of Nutrition*, 59(1), 389-397. http://dx.doi.org/10.1007/s00394-019-01914-9. PMid:30805697.

MultiThermTM. (2011). *Operating Manual*. Uzwil: Bühler AG.

Oliveira, V. S., Augusta, I. M., Braz, M. V. C., Riger, C. J., Prudêncio, E. R., Sawaya, A. C. H. F., Sampaio, G. R., Torres, E A F. S., & Saldanha, T. (2020). Aroeira fruit (*Schinus terebinthifolius* Raddi) as a natural antioxidant: chemical constituents, bioactive compounds and in vitro and in vivo antioxidant capacity. *Food Chemistry*, 315, 126274. http://dx.doi.org/10.1016/j.foodchem.2020.126274. PMid:32007814.

Oliveira, W. L., & Scariot, A. 2010. *Boas práticas de manejo para o extrativismo sustentável do Pequi*. Brasília: Embrapa Recursos Genéticos e Biotecnologia, 84 p.

Pessoa, A. S., Podestá, R., Block, J. M., Franceschi, E., Dariva, C., & Lanza, M. (2015). Extraction of pequi (*Caryocar coriaceum*) pulp oil using subcritical propane: determination of process yield and fatty acid profile. *The Journal of Supercritical Fluids*, 101, 95-103. http://dx.doi.org/10.1016/j.supflu.2015.03.006.

Rufino, M. S. M., Alves, R. E., Brito, E. S., Morais, S. M., Sampaio, C. G., Pérez-Jiménez, J., & Saura-Calixto, F. D. (2006). Metodologia científica: determinação da atividade antioxidante total em frutas pelo Método de Redução do Ferro (FRAP). *Embrapa Comunicado Técnico on line*, 125, 1-4.

Rufino, M. S. M., Alves, R. E., Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, 121(4), 996-1002. http://dx.doi.org/10.1016/j.foodchem.2010.01.037.

Santos, O. V., Carvalho, R. N. Jr, Costa, C. E. F., & Lannes, S. C. S. (2019). Chemical, chromatographic-functional, thermogravimetric-differential and spectroscopic parameters of the sapucaia oil obtained by different extraction methods. *Industrial Crops and Products*, 132, 487-496. http://dx.doi.org/10.1016/j.indcrop.2019.02.043.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocaltue reagent. *Methods in Enzymology*, 299, 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1.

Souza, J. P., Alves, R. E., Brito, E. S., Nogueira, D. H., & Lima, J. R. (2013). Estabilidade de produtos de pequi (*Caryocar coriaceum* Wittm) sob congelamento em diferentes tipos de embalagens. *Revista Brasileira de Fruticultura*, 35(4), 971-976. http://dx.doi.org/10.1590/S0100-29452013000400007.

Stuch, E. S., Sempionato, O. R., & Silva, J A A. (2009). Influência dos porta-enxertos na qualidade dos frutos cítricos. *Laranja*, 17(1), 143-158.

Todorovic, V., Redovnikovic, I. R., Todorovic, Z., Jankovi, G., Dodevska, M., & Sobajic, S. (2015). Polyphenols, methylxanthines, and antioxidant capacity of chocolates produced in Serbia. *J Food Compos*, 41, 137-143. http://dx.doi.org/10.1016/j.jfca.2015.01.018.

Vanderzant, C., & Splittstoesser, D. F. (1992). *Compendium of methods for the microbiological examination of foods* (3rd ed). Washington: APHA.

Xavier, W. K. S., Medeiros, B. J., Lima, C. S., Favacho, H. A., Andrade, E. H. A., Araújo, R. N. M., Santos, L. S., & Carvalho, J. C. T. (2011). Topical anti-inflammatory action of *Caryocar villosum* (Aubl) Pers oil. *Journal of Applied Pharmaceutical Science*, 1(3), 62-67.