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EXTRAÇÃO, CARACTERIZAÇÃO E APLICAÇÃO TECNOLÓGICA DE PECTINA OBTIDA DE SUBPRODUTOS DO PROCESSAMENTO DE FEIJOA (*ACCA SELLOWIANA*)

Extraction, characterization and technological application of pectin obtaining from by-products of feijoa (*Acca sellowiana*) *processing*

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RESUMO: A feijoa (*Acca sellowiana*) é uma fruta nativa brasileira que é cultivada em vários países alem do Brasil, e que apresenta aproximadamente 30% do seu peso em polpa sendo o restante descartado. O objetivo deste estudo foi extrair e caracterizar a pectina da farinha do resíduo do processamento da feijoa e testar a sua aplicação na formulação de geleia empregando a polpa desta fruta. Empregou-se um delineamento composto central rotacional para extração da pectina e os fatores escolhidos para avaliar o rendimento de extração e o grau de metoxilação foram concentração de ácido cítrico (g.L^{-1}) e temperatura de extração (ºC). As propriedades geleificantes foram analisadas por aplicação em geleias do tipo tradicional e Diet. Os resultados mostraram que o rendimento de extração variou entre 11,95 e 49,60 g.100 g^{-1}, sendo o maior rendimento observado com 5 g.100 g^{-1} de ácido cítrico e 95 ºC. O grau de metoxilação variou entre 48,16% e 52,50%. A propriedade geleificante da pectina foi observada na elaboração de geleia do tipo tradicional, sendo que esta pectina foi caracterizada como sendo de alto grau de metoxilação.

Palavras-chave: Tecnología de alimentos. Geléia, Aditivos naturais, Metoxilação

ABSTRACT: Feijoa (*Acca sellowiana*) is a native Brazilian fruit that is grown in several countries besides Brazil, approximately 30% of its weight is the pulp, and the rest is discarded. This study's objective was to extract and characterize the pectin from the feijoa by-products and to test its application in the jelly formulation. A rotational central composite design was used to extract the pectin. The citric acid concentration (g L^{-1}) and extraction temperature (ºC) were used to evaluate the extraction yield and the degree of methoxylation (DM). The gelling properties were analyzed through the application of pectin in traditional and diet jellies. The results showed that the extraction yield ranged from 11.95 to 49.60 g 100 g^{-1}; highest yield was observed to have 5 g 100 g^{-1} citric acid and 95 °C. The DM ranged from 48.16% to 52.50%. The gelling property of pectin was observed in the preparation of traditional jelly, and this pectin was characterized as high DM.

Key words: Food Technology. Jelly. Natural additives. Methoxylation.

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INTRODUCTION

Brazil is the world's third-largest fruit producer, responsible for the production of more than 40 million tons annually. Of this amount, around 30% is waste. To increase profits and avoid losses, the food industry transforms these fruits into products such as juices, pulps, jellies, nectars, soft drinks, and ice creams. However, the industrial process can generate high amounts of waste or other residues that could be considered by-products; these present the potential for technological innovation since they can be alternative sources for various ingredients. One of the focuses of research on the technological potential of industrial fruit by-products is its use as an alternative source for obtaining pectin, a natural polysaccharide widely used in the food industry as a thickening agent (EINHORN-STOLL, 2018).

Pectin is generally composed of homogalacturonan and ramnogalactorunonans. Homogalacturonan is a linear polymer of α-(1,4)-D-galacturonic acid units, which may be methyl-esterified at C-6 and acetylated at O-2 or O-3 (YANG *et al*., 2018) (DM>50%) or low degree of methoxylation (DM<50%) (YAPO, 2011). The primary sources of pectin production are citrus fruits (PUTNIK et al., 2017), apple residue (KUMAR; CHAUHAN, 2010) and beetroot (CHEN et al., 2015). However, pectin can also be extracted from unconventional sources such as coconut shell (CHAN; CHOO, 2013), mulberry branch bark (LIU et al., 2011), fava bean hulls (KORISH, 2015), sisal waste (WANG et al., 2006), watermelon rind (MARAN et al., 2014), pomegranate peels (PEREIRA et al., 2016), mango peels (WANG et al., 2016) and banana peel (OLIVEIRA et al., 2016).

In the food industry, commercial pectin is chemically obtained through an acid extraction. In this context, organic acids, such as citric acid and acetic acid, have shown satisfactory results in the process due to their low cost, low toxicity and environmental safeness (JAFARI et al., 2017; MARIĆ et al., 2018).

Simultaneously, there is a growing interest in the technological potential of Brazilian native fruits. The feijoa [*Acca sellowiana* (O.Berg) Burret] is a specie of the Myrtaceae family, native to southern Brazilian plateau and northeastern Uruguay. It is cultivated internationally as an exotic fruit in countries like New Zealand, Australia, Japan, France, Italy, Russia, Chile, Colombia, the United States, and Spain. In southern Brazil, the specie is adapted to cold climate conditions, more frequently found in areas with altitudes above 800 meters (AMARANTE; SANTOS, 2011; WESTON, 2010). It is also known as goiaba serrana, goiaba crioula, araçá do Rio Grande, guayabo verde, guayabo del país or pineapple-guava (AMARANTE et al., 2017).

In addition to fruit consumption *in natura*, feijoa can be processed, and the pulp used in the production of juices, jellies, ice creams, and beverages. Approximately 70% of its weight is not traditionally used and is often considered a residue. Moreover, research reports that the feijoa peel, the main component of the processing by-product, represents a source of bioactive compounds, such as phenolics and antioxidant vitamins, which have higher vitamin C content than pulp (AMARANTE et al., 2017). However, studies on the valuation of this residue as a source of raw materials for technological use have yet to be performed.

Thus, this work is aimed at evaluating the technological potential of the by-product generated in feijoa processing [*Acca sellowiana* (O.Berg) Burret], through the extraction and characterization of the pectin present in this material, as well as performing its application as a thickening agent in jellies.

MATERIALS AND METHODS

Materials

Feijoa samples were randomly collected at the point of ripeness (identified by the easy detachment of the fruit), in the city of São Joaquim, SC (latitude 28 ° 16 '40.02 "S, longitude 49 ° 56 ' 09.10 "W and altitude of 1400 m), during the 2017 harvest. Following harvest, the fruits were transported in a polyethylene box to the Fruit and Vegetable Processing Laboratory of the Federal Institute of Santa Catarina - Urupema, where they were processed. First, the samples were washed with running water and were sanitized with sodium hypochlorite at 200 ppm for 15 min. The fruits were then cut in half with stainless steel knives, and the pulp was manually separated from the peel and mesocarp, considered as by-products for this study.

Preparation of flour from feijoa by-product

For the flour processing, the feijoa by-product was cut into small slices (approximately 3 mm thick), which were arranged on trays to then be dried in a forced-air circulation oven 310 / 81PID (Adamo, Piracicaba, SP, Brazil) at 45 °C, for approximately 24 h. After drying, the dry by-product was milled with a willey-type knife mill STAR FT 50 (Fortinox, Piracicaba, SP, Brazil) with a 400 mesh particle size, and stored in a hermetically-sealed glass container until analysis.

Pectin extraction

The extraction and titration of pectin from feijoa by-product flour (PF) were performed following a central rotational composite design with four axial points and three replicates at the central point, totaling 11 assays, according to methodology adapted from Munhoz et al. (2010). To evaluate the pectin extraction yield and the degree of methoxylation (DM), two factors were chosen: citric acid concentration (g L^{-1}) and extraction temperature (ºC) —called independent variables— with a constant extraction time of 60 min. The mass yield of pectin extracted in each experiment was considered the response or dependent variable.

First, 2 g of PF was dissolved in 100 mL of distilled water, and the extractions were performed under citric acid concentrations between 3.5 to 7.0% and a temperature of between 45 °C and 95 °C. After acid extraction, the samples were cooled to 4 °C for 2 h and filtered through the polyester fabric to discard the supernatant. To the filtrate containing pectin, ethyl alcohol (96%) was added in the ratio 1:2 (pectin:alcohol, w/w). After one hour the precipitated pectin was filtered off. The pectin obtained was dried in a drying oven at 50 °C until reach a constant weight. The pectin yield

was obtained from the initial amount of the raw material used, according to Eq. 1.

$$\text{Yield (\%)} = (\text{Extracted pectin} / \text{PF}) \times 100 \quad (1)$$

Extracted Pectin - mass of pectin obtained following extraction and drying
PF - mass of the feijoa by-product flour sample on a dry basis.

The pectin samples obtained under the experimental conditions were characterized by titration. Approximately 250 mg of pectin was moistened with 2 mL of ethyl alcohol P.A. and solubilized in 25 mL of deionized water with constant stirring for 30 min in a magnetic stirrer. The solution's pH was then determined. The free carboxylates of the anhydrogalacturonic acids were neutralized with 0.1M NaOH solution. The carboxylates esterified after saponification with 10 mL of 0.25M NaOH solution for 30 min at room temperature were neutralized with 10 mL of 0.25M HCl solution, and neutralized again with 0.1M NaOH solution, obtaining the NaOH mEq values for the two free and esterified carboxyl types respectively represented by mEq' and mEq". The data obtained were the percentages of free anhydrogalacturonic acids (% free mAAG), the percentage of demethoxylated carboxyls (% mAAG), the percentage of methoxylic groups (% MMeO) and the degree of methoxylation (DM), according to Eqs. 2, 3, 4 and 5 respectively.

$$\text{\% free mAAG} = (\text{mEq'} \times \text{M NaOH} \times 178) / 10 \quad (2)$$

$$\text{\% mAAG} = (\text{mEq''} \times \text{M NaOH} \times 178) / 10 \quad (3)$$

$$\text{\% MMeO} = (\text{mEq''} \times \text{M NaOH} \times 31) / 10 \quad (4)$$

$$\text{DM} = [(\text{mAAG})/(\text{free mAAG} + \text{mAAG})] \times 100 \quad (5)$$

free mAAG - free anhydrogalacturonic acids
mAAG demethoxylates - demethoxylates carboxyls
MMeO - methoxylic groups
DM - degree of methoxylation.

Preparation of jellies

The technological application was used to compare the extracted pectin (PF pectin) with the commercial pectin. Diet and traditional jellies ("extra" type) of feijoa were prepared. Fruit pulp was collected during pulping and frozen at -18 ºC until its use in the preparation of jellies. At the time of preparation, the pulp had 12.15 ± 0.23 °Brix, pH of 3.20 ± 0.13, and titratable acidity 1.05 ± 0.01 g 100 g^{-1}. The jelly formulations were defined after the extraction and characterization of pectin (Table 1).

For the preparation of the traditional jellies with high DM commercial pectin (F1) and PF pectin (F2), one-third of the sucrose was added to the pulp during heating. When the blend reached 35 °Brix, the remainder of the sucrose was added. At 54 °Brix, the pre-diluted pectin in distilled water at 70 °C (1:10, w/v) was added. The final point was given at 68 °Brix with the addition of citric acid.

Table 1 - Formulations of traditional and diet jellies of feijoa

	Traditional Jelly		**Diet Jelly**	
Ingredients	**F1**	**F2**	**F3**	**F4**
Pulp (%)	50.0	50.0	78.0	78.0
Sucrose (%)	50.0	50.0	-	-
Lowçucar® (%)	-	-	20.0	20.0
PF Pectin (%)	-	1.0	-	2.0
Commercial Pectin ATM (%)	1.0	-	-	-
Commercial Pectin BTM (%)	-	-	2.0	-
Calcium chloride (mg g^{-1} of pectin)	-	-	50.0	50.0
Citric Acid (%)	0.2	0.2	0.2	0.2

The jelly was bottled at 90 °C in a pre-sterilized glass container with a metal lid. For the elaboration of diet formulations with low DM commercial pectin (F3) and PF pectin (F4), the pulp was mixed with the sweetener and heated. When the blend reached 30 °Brix, the pectin previously diluted in distilled water at 70 °C (1:10, w/v), and calcium chloride were added. Heating continued until the jelly reached 35 °Brix, citric acid was added, and the jelly was jarred under the same conditions as the traditional jelly. After jarring, the containers were inverted for 15 min for sterilization of the cap and continued for cooling under running water.

After preparation, the jellies were stored at room temperature and analyzed for their physicochemical parameters: total soluble solids (TSS) (°Brix), titratable acidity (AT, in g 100 g^{-1} citric acid) and pH, using the methodologies stipulated by the INSTITUTO ADOLFO LUTZ (2008). For evaluation of the instrumental color, a Delta Vista 450G (Delta Color®, São Leopoldo, RS, Brazil) calibrated colorimeter was used, obtaining the parameters L^* for indication of lightness (light/dark), a^* for chromaticity on the green (-) to red (+) axis, and b^* for chromaticity on the blue (-) to yellow (+) axis of the CIELab system.

The syneresis of the jellies was evaluated using the gravimetric method (KHOURYIER et al., 2005). The texture (firmness, cohesiveness and viscosity index) of the jellies was evaluated using a TA-XT Plus (Stable Micro Systems, United Kingdom) universal texturometer, equipped with Exponent Stable Micro Systems software, with the aid of a cylindrical acrylic device with a diameter of 35 mm (Probe A/BE-d35), a pre-test velocity of 2.0 mm s^{-1}, test velocity of 2.0 mm s^{-1} and post-test velocity of 10.0 mm s^{-1}.

Statistical Analysis

The data relative to the analysis of the jellies, obtained in triplicate, were analyzed by calculation of the mean, standard deviation, and analysis of variance (ANOVA). For the comparison of means, the Tukey test was used at the 5% probability level. The pectin extraction yield data (central rotational compound design with four axial points and two replicates at the central point) were also submitted to ANOVA. All analyses were evaluated using Statistica version 8.0 (STATSOFT Inc., United States) software.

RESULTS AND E DISCUSSION

Variations in yield, as well as in the characteristics of pectin extracted, are due to differences inherent to the origins and conditions of the raw plant materials: cultivation, ripening stage, storage, variety, and heat treatment. Also, external factors such as pH, temperature, acid concentration and type, heating time, raw material/solvent ratio, isolation, and precipitation method should also be considered (EINHORN-STOLL, 2018; GRANATO; NUNES, 2016). Therefore, each raw material will present a particular optimal condition for extraction, considering the methodology used and the factors inherent to the sample.

No other studies on the evaluation of feijoa pectin or assessment of the technological potential of this fruit by-product were found in the literature. The average pectin yields extracted from the PF are shown in Table 2; these values ranged between 11.95 g 100 g^{-1} (experiment 7) and 49.6 g 100 g^{-1} (experiment 8).

Table 2. Results of extraction yield and degree of methoxylation of pectin extracted from feijoa by-product flour.

Experiment	Variables		Yield (%)	DM (%)
	Acid (%)	Temperature (ºC)		
1	3.5 (-1)	35 (-1)	18.10	49.11
2	6.5 (1)	35 (-1)	37.75	50.00
3	3.5 (-1)	85 (1)	26.80	48.16
4	6.5 (1)	85 (1)	37.75	49.91
5	3.0 (–1.414)	60 (0)	17.55	50.39
6	7.0 (1.414)	60 (0)	25.15	52.50
7	5.0 (0)	25 (–1.414)	11.95	49.34
8	5.0 (0)	95 (1.414)	49.60	49.96
9	5.0 (0)	60 (0)	18.25	52.33
10	5.0 (0)	60 (0)	22.62	49.12
11	5.0 (0)	60 (0)	22.35	49.20

In the study carried out by Munhoz et al. (2010), the authors reported a maximum yield of 13.24 g 100 g^{-1} of pectin extracted from flour made from guava pulp and peel (*Psidium guajava* L.), a fruit also belonging to the feijoa family. Conventional sources of pectin, such as citrus peel, apple pomace, and beet pulp, have medium extraction rates between 30-35%, 15-20%, and 15-30%, respectively (ADETUNJI et al., 2017). For non-conventional sources, these values range from 5.2% to 12.2% for banana peel (OLIVEIRA et al., 2016), from 2.87% to 28.98% for melon peel (RAJI et al., 2017), and between 3.92% and 11.18% for pomegranate peels (PEREIRA et al., 2016).

Figure 1 shows the 3D response surface graph of the pectin extraction process from PF as a function of the citric acid concentration and temperature, keeping the extraction time constant (60 min). Based on the response surface graph, experiment 8, with 5.0 g 100 g^{-1} of citric acid at 95 ºC, was considered the best combination of factors within the maximum extraction range and obtaining the highest yield of pectin extraction. In the extraction of guava pectin (*Psidium guajava* L.), as well as for feijoa, Munhoz et al. (2010) showed that a citric acid concentration between 5.0% and 6.5% is most efficient in the extraction of pectin, as it guarantees higher levels of this substance.

In the extraction of pectin from carrot cake, Jafari et al. (2017), using a combination of pH (adjusted with different concentrations of citric acid), temperature, time and liquid/solid ratio factors, obtained a maximum yield of 15.6% at a pH of 1.3, a temperature of 90 °C, in 79.8 min of extraction and a proportion of 23.3 v/w. For pectin extracted from grape pomace, Minjares-Fuentes et al. (2014) obtained a maximum yield of 32.3% with extraction at 75 °C for 60 min in a citric acid solution of pH 2.0. For pectin extracted from beet pulp, Li et al. (2015), evaluating the same independent effects for the aforementioned works, found a maximum extraction yield of 23.95% in a citric acid solution of pH 1.0, at 99 ºC, for 166 min and at a liquid/solid (v/w) ratio of 20.

Table 3 presents the results of the ANOVA for the effects of citric acid content (X1) and temperature (X2) on the pectin extraction yield from PF. In Figure 2, the parameters that showed values greater than 4.30 (p=0.05), located to the right of the dashed line, were significant. Thus, from the ANOVA results, the linear effects of acid and temperature (L) and quadratic temperature (Q) were significant (p<0.05); i.e., both the acidity and the temperature positively influenced the pectin extraction yield of the PF, and the increase in one leads to increased extraction.

The acidic pH and the higher extraction temperature hydrolyze insoluble pectin constituents, thus increasing the solubility and diffusion of this substance from the plant to the medium. This effect was also observed in other pectin extraction studies from unconventional sources (Jafari *et al*., 2017; Minjares-Fuentes *et al*., 2014; Li *et al*., 2015; Oliveira *et al*., 2016; Pereira *et al*., 2016). When extracting pectin from the mango peel, Wang *et al*. (2016) reported that at 80 ºC, the extraction percentage was higher (16.70% - 17.15%) than at 20 °C (1.55% to 2.09%). Raji *et al*. (2017) also observed that acid type is an essential factor in the pectin extraction process, with citric acid having the most significant

effect when compared to tartaric, hydrochloric, acetic, lactic, nitric, phosphoric, and sulfuric acids.

Because it is of natural origin, pectin does not constitute a single molecule with known molecular mass and stable characteristics.

Figure 1. Effect of the variables: citric acid concentration (%) and temperature (ºC) on the extraction yield of pectin extracted from the feijoa by-product flour

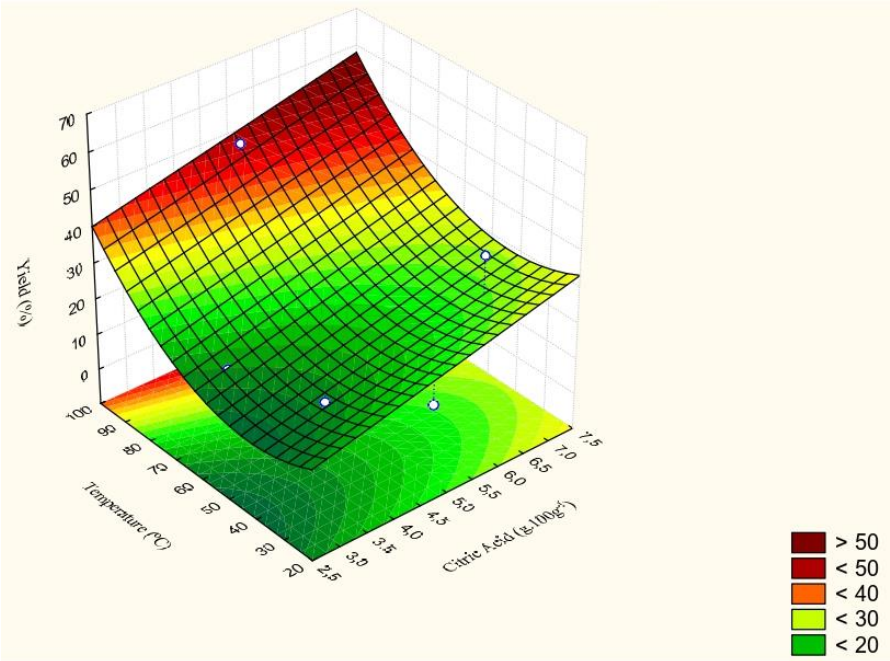
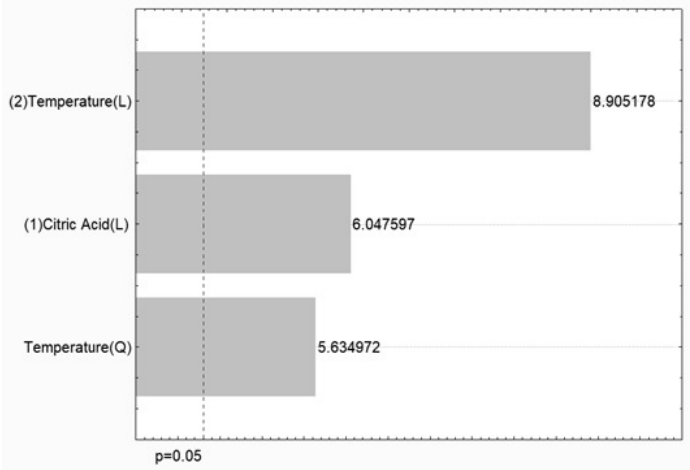


Table 3. Analysis of variance of extraction yield of pectin extracted from feijoa by-product flour as a function of the variables: citric acid concentration (%) and temperature (ºC).

Source of variation	Sum of Squares	Degrees of free	Means Square	F calculated	F tabulated
Regression	881.856179	3	293.9520597	5.58749612	4.3468314
Residue	368.2623437	7	52.60890624	-	-
Lack of fit	356.254	5	71.25071874	11.8664672	19.29641
Pure error	12.009	2	6.004375	-	-
Total	1250.119	10	-	-	-

Figure 2. Graphs levels of yield curves (%) during pectin extraction from the feijoa by-product flour as a function of the variables: citric acid concentration (g 100 g^{-1}) and temperature (ºC)



In addition, the chemical and physical conditions at the time of extraction directly interfere with the extracted pectin molecule (Adetunji *et al*., 2017), since the molar mass and degree of polymerization interfere with gelation capacity, mainly due to the presence and distribution of neutral sugars inside chains (Granato and Nunes, 2016). In our study, the results of DM for each experiment performed with the different combinations of acidity and temperature to extract pectin molecules, ranging from 48.16% to 52.50% (Table 2). Since these values were very close to 50.00%, limiting characterization value between high and low DM pectins, the gelling property of PF pectin was compared to that of commercial pectins by insertion into traditional and diet jelly formulations, as a way to evaluate the gelling property of this pectin molecule through its practical application in jelly preparation.

The physicochemical parameters analyzed in the jellies were used to compare the properties of PF pectin with commercial pectins, which are presented in Table 4. Significant differences ($p<0.05$) were observed between the jellies prepared with commercial pectin and those prepared with PF pectin, for both traditional and diet formulations, as well as in terms of TSS, pH, titratable acidity and syneresis. Experimentally, the jellies showed no difference regarding color, since the color parameters evaluated did not show a significant variation ($p>0.05$).

Table 4. Physicochemical parameters analyzed in traditional and diet jellies elaborated with commercial pectin and pectin extracted from feijoa by-product flour.

	Traditional Jelly		**Diet Jelly**	
Parameter	**F1**	**F2**	**F3**	**F4**
TSS (ºBrix)	69.46 ± 0.30 [b]	73.33 ± 1.75 [a]	33.13 ± 0.11 [b]	39.00 ± 0.00 [a]
pH	3.13 ± 0.00 [a]	2.66 ± 0.02 [b]	3.13 ± 0.00 [a]	2.62 ± 0.02 [b]
AT (g $100g^{-1}$ citric acid)	0.75 ± 0.01 [b]	1.51 ± 0.16 [a]	0.87 ± 0.01 [b]	2.52 ± 0.08 [a]
Syneresis (%)	2.06 ± 0.05 [b]	10.32 ± 0.40 [a]	0.009 ± 0.001 [b]	23.21 ± 0.25 [a]
Color parameters				
L*	15.06 ± 0.11 [a]	15.95 ± 0.40 [a]	28.63 ± 0.85 [a]	27.62 ± 0.02 [a]
a*	2.71 ± 0.11 [a]	3.1 ± 0.80 [a]	1.98 ± 0.23 [a]	2.28 ± 0.03 [a]
b*	13.09 ± 2.18 [a]	13.42 ± 2.18 [a]	16.73 ± 1.31 [a]	20.23 ± 0.07 [a]
Firmness (g)	817.77 ± 3.27 [b]	1726.76 ± 13.26 [a]	912.72 ± 21.62 [a]	31.27 ± 0.13 [b]
Cohesiveness (g)	-408.09 ± 7.91 [b]	-599.585 ± 3.96 [a]	-478.775 ± 12.09 [a]	-23.39 ± 0.24 [b]
IV (g.s)	-1222.43 ± 29.27 [b]	-1757.415 ± 10.45 [a]	-1206.15 ± 52.04 [a]	-67.54 ± 1.26 [b]

TSS - Total soluble solids; AT - total acidity; IV - viscosity index; F1 - formulation of traditional jelly with commercial pectin; F2 - formulation of traditional jelly with PF pectin; F3 - formulation of diet jelly with commercial pectin; F4 - formulation of diet jelly with PF pectin. Averages followed by the same letters, for the same style of jelly, do not differ among themselves by the Tukey test at 5% probability.

The formulations elaborated with PF pectin showed higher TSS, total acidity and syneresis, and lower pH values. These results are related to the acidic characteristic of the raw material, observed in both the fresh fruit and in the physicochemical characterization of the flour (AQUINO et al., 2018), which influenced lowered pH, the consequent increase of overall acidity, and the higher syneresis content of the jellies elaborated with the pectin obtained in this study.

Regarding texture, significant differences were observed between the jellies of the same type for the analyzed parameters of firmness, cohesiveness, and viscosity index ($p<0.05$). The observed values were lower for diet jelly containing PF pectin, due to the non-gel formation, and higher for traditional jelly with this same pectin. Thus, since gel formation only occurs in preparation of traditional jelly, the PF pectin can be classified as high DM pectin. These pectins form gel at pH conditions between 2.0 and 3.5 and sucrose concentrations between 50 - 65%, while pectins with low DM form gel in the absence of sucrose, require the presence of divalent cations, such as calcium, and can jellify in a pH range of between 2.5 and 6.5 (EINHORN-STOLL, 2018; GRANATO; NUNES, 2016). According to Canteri et al. (2012), low DM pectins have, in practice, a DM between 20% and 45%, thus lower than the average DM of pectin obtained in this study (50.0%).

CONCLUSIONS

1. Feijoa is a native Brazilian fruit that has a by-product processing that is rich in pectin and has a high degree of methoxylation.
2. The flour generated with this fruit's by-product can be harnessed for the extraction of this substance, which in turn can be used as a thickening agent in various food products.
3. Further investigation of the chemical structure of the extracted pectin in regards to the presence and distribution of the molecule's side chains is suggested, as is the proposal of the addition of sugars to the side chains for the standardization of the gelation process, a better definition of gel formation rate, and its percentage as an ingredient in the formulations.

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