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Aproveitamento do coproduto de jabuticaba (*Myrciaria* jaboticaba Berg.) para extração de pectina

Utilization of the co-product of jaboticaba (*Myrciaria* jaboticaba Berg.) for pectin extraction

Utilización del coproducto de jaboticaba (*Myrciaria* jaboticaba Berg.) para extracción de pectina

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Resumo

A pectina é um polissacarídeo que pode ser aplicado a vários produtos da indústria de alimentos para modificar a viscosidade dos alimentos. O objetivo deste trabalho foi otimizar a extração de pectina da casca de jabuticaba, modificando o rendimento, o teor de ácido galacturônico (TAG), o teor de esterificação (TE), o teor de pectina (P) e os açúcares neutros (AN). O experimento foi realizado utilizando a técnica de planejamento fatorial de experimentos completos e análise de resposta superficial. A extração pode ser relatada através de planejamento fatorial com coeficientes de determinação (R^2) de 0,92, 0,91, 0,89, 0,88 e 0,90 para a extração de rendimento, TAG, T, P e AN, respectivamente. O conteúdo de TAG variou de 22,82 a 65,35%, o TE variou de 26,72 a 77,50%, a porcentagem de P encontrada na extração de 25,50 a 66,12%. O melhor desempenho foi verificado utilizando os seguintes parâmetros: tempo de extração inferior a 75 min, temperatura de 70 ° C e concentração de ácido cítrico de 0,75 mol. L^{-1}.

Palavras-chave: Ácido cítrico; Conteúdo de esterificação; Fatorial; Jabuticaba; Rendimento.

Abstract

Pectin is a polysaccharide that can be applied to various food industry products to modify the viscosity of food. The objective of this work was to optimize the extraction of pectin of jabuticaba peel by modificating the yield, galacturonic acid content (AUA), esterification content (DE), pectin content (P), and neutral sugars (N). The experiment was performed using the technique of factorial planning of complete experiments and surface response analysis. The extraction can be reported through factorial planning with coefficients of determination (R^2) of 0.92, 0.91, 0.89, 0.88 and 0.90 for the yield extraction, AUA, DE, P, and N, respectively. The content of AUA ranged from 22.82 to 65.35%, the DE varied from 26.72 to 77.50%, the percentage of P found in the extraction 25.50 to 66.12%. The best performance was verified utilizing the following parameters: extraction time less than 75 min, temperature of 70°C, and citric acid concentration of 0.75 mol.L^{-1}.

Keywords: Acid citric; Esterification content; Factorial; Jabuticaba; Yield.

Resumen

La pectina es un polisacárido que se puede aplicar a varios productos en la industria alimentaria para modificar la viscosidad de los alimentos. El objetivo de este trabajo fue optimizar la extracción de pectina de la corteza de jabuticaba, modificando el rendimiento, el contenido de ácido galacturónico (TAG), el contenido de esterificación (TE), el contenido de

pectina (P) y los azúcares neutros (AN). El experimento se llevó a cabo utilizando la técnica de diseño factorial de experimentos completos y análisis de respuesta superficial. La extracción se puede informar a través del diseño factorial con coeficientes de determinación (R^2) de 0.92, 0.91, 0.89, 0.88 y 0.90 para la extracción de rendimiento, TAG, T, P y AN, respectivamente. El contenido de TAG varió de 22.82 a 65.35%, el TE varió de 26.72 a 77.50%, el porcentaje de P encontrado en la extracción de 25.50 a 66.12%. El mejor rendimiento se verificó utilizando los siguientes parámetros: tiempo de extracción inferior a 75 min, temperatura de 70 ° C y concentración de ácido cítrico de 0,75 mol. L^{-1}.
Palavras- clave: Ácido cítrico; Contenido de esterificación; Factorial; Jaboticaba; Rendimento.

1. Introduction

The epicarp of the jabuticaba is rich in nutrients such as fibre, vitamins and minerals. The beneficial nature of this residue and its use as a raw material for new products or to enrich products already commercialized contributes to the increase in the economic potential of this fruit. Due to the desirable presence of nutrients, there is interest in the extraction of its pectin, a fibrous substance, present in considerable quantities in the peel of this fruit (Ferreira et al., 2012).

Pectin can be extracted from fruit peel such as those of cocoa, orange and passion fruit and then analysed through its chemical structure to determine its gelling power, an essential feature indicating the possibility of using this molecular structure and affecting its economic value (Vriesmann et al., 2012, Srivastava & Malviya, 2011; Seixas et al., 2014).

The composition chemical of the pectin chain is dependent on the extraction method, extraction time, chemical characteristic of the acid or base used as the reagent in the analysis, extraction temperature, and raw material studied. These factors will determine the degree of esterification of the molecule, if classified as high degree of esterification the molecule will have higher proportions of methyl groups, otherwise it will have low degree of esterification, with little presence of methyl groups, thus enabling its use in agronomic, food and pharmaceutical technologies (Minjares-Fuentes et al., 2014).

The food industries use pectin with both low and high degrees of esterification. Molecular compounds with a high degree of esterification are suitable for long-term use and are found in jellies, ice cream, cakes, dairy desserts, carbonated drinks, and juices. However, low-grade esterification pectin can be used as an additive to products with a low calorie content so that the products achieve their desired consistency while maintaining a low sugar content (Tian et al., 2016).

Some researchers, including Seixas et al. (2014), Liew et al. (2014) and Oliveira et al. (2015), have studied the extraction of the pectin from fruits and industrial waste frequently. In view of the desirability of pectin.

The objective of this study was to analyse and quantify the extraction of pectin from the peel of jabuticaba to obtain the highest yield and characterize the substances extracted through the content of galacturonic acids, degree of esterification, pectins and neutral sugars.

2. Materials and Methods

In the present study, the quantitative method was used to evaluate the data. This method generates sets or masses of data that can be analyzed using mathematical techniques such as percentages, statistics and probabilities, numerical methods, analytical methods and generation of equations and / or mathematical formulas applicable to any process. (Pereira et al., 2018).

2.1 Materials

The jabuticaba fruits were collected on rural properties in the municipality of Rio Verde, GO. Subsequently, they were transported to the *Fruit and Vegetables Laboratory of the Instituto Federal Goiano - Campus Rio Verde, Goiás, Brazil*, sanitized with chlorinated water at 100 ppm for 15 minutes and allowed to dry naturally. The peel was removed with a knife and dried in a oven with forced air circulation. The jabuticaba peel was dried, in greenhouse a model MA 035 oven (Marconi, Piracicaba, Brazil), at 65°C until it reached a constant weight. After drying, the flour of the jabuticaba peel was obtained by milling three times, for particle size reduction in grinder Diogomaq®. The reagents used in the extraction and characterization of the pectin included Synth citric acid PA, sodium hydroxide PA, acetone PA (all from PA) and 96% ethyl alcohol (Prolab).

2.2 Methods

The pectin was extracted in triplicate with variations in a number of factors. The extraction time (TE) varied between 30, 60 and 90 min. The molar concentrations of citric acid (C) used were 0.25, 0.50 and 0.75 $mol.L^{-1}$. The temperatures (T) examined were 30°C, 50°C and 90°C. The experiment was performed using the technique of factorial planning of complete experiments and surface response analysis. The factorial planning was of the 3^3 order, indicating that it utilized three levels and three variables. Table 1 presents the levels and variables examined utilizing this technique.

Table 1: Levels of the variables studied in the experimental planning of the pectin extraction of the jabuticaba peel.

Levels	**-1**	**0**	**1**
TE (min)	30	60	90
C ($mol.L^{-1}$)	0.25	0.50	0,75
T (°C)	30	50	90

Pectin was extracted as described by Kleimann et al. (2009) with modifications in the acid medium utilizing citric acid in three different concentrations. Samples of 5 g (d.b.) of jabuticaba peel flour were dissolved in 250 mL citric acid and heated in a water bath with pre-set temperatures and agitation with times established by the experimental design. The heated mixture was filtered through filter paper. The contents of the filters were discarded. The filtrate was cooled to 4 ± 0.1°C for one hour. Then, with the solution already cooled, an equal volume of 96% ethyl alcohol was added and allowed to stand at room temperature for 1 hour to allow the pectin to precipitate. The coagulated pectin was filtered and washed twice with 15 mL 96% ethyl alcohol and twice with 15 mL acetone PA to remove the mono- and disaccharides, amino acids, organic acids and salts, as well as some enzymes that can degrade polysaccharides.

Subsequently, the pectin was placed in a forced air circulation oven at 45°C for 24 hours and ground, Diogomaq®, in a mill for further analysis. The pectin yield was calculated by equation 1:

$$\text{Yield} = (\text{extraction pectin (g)} / \text{sample mass (g)}) * 100 \qquad \text{Eq. (1)}$$

The pectin samples were quantified using the titration method (WANG et al., 2002). Approximately 250 mg of the sample was moistened with 2 mL alcohol and solubilized in 25 mL deionized water under constant stirring with a magnetic stirrer for 30 minutes. The pH of this solution was determined at the end of this procedure. The free carboxylates of the anhydrogalacturonic acids were neutralized with 0.1 $mol.L^{-1}$ NaOH until the solution reached pH 7.0. After saponification by the addition of 10 mL 0.25 $mol.L^{-1}$ NaOH, while stirring for

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30 min, 10 mL 0.25 N HCl was added. This solution was titrated again with 0.1 $mol.L^{-1}$ NaOH to neutralize the pH. This enabled the determination of the NaOH mEq values for the two free and esterified types, respectively, represented by mEq' and mEq''. Calculations were performed on these data utilizing the formulas:

$$Z = \frac{mass}{mEqtotal} \quad \text{Eq. (2)}$$

$$AUA = \frac{17600}{Z} \quad \text{Eq. (3)}$$

$$MeO = \frac{(mEq'' \times 31 \times 100)}{mass} \quad \text{Eq. (4)}$$

$$DE = \frac{176}{31} \times \frac{MeO}{AUA} = \frac{mEq''}{(mEq' + mEq'')} \quad \text{Eq. (5)}$$

$$Pectin = AUA(\%) + MeO(\%) \quad \text{Eq. (6)}$$

$$\text{Neutral sugars} = 100 - \text{pectin} \quad \text{Eq. (7)}$$

In which AUA is the content of the galacturonic acids of extracted pectin;

MeO is the methoxyl content of the extracted pectin; and

DE is the degree of esterification of the extracted pectin.

The yield results of pectin extraction were subjected to analysis of variance, multiple regression for the elaboration of mathematical models with the coded variables and surface response analysis comparing the interaction to each two variables studied. The mathematical model used is represented in Equation 8:

$$y = b_0 + b_1 A + b_2 B + b_{11} A^2 + b_{22} B^2 + b_{12} AB \quad \text{Eq. (8)}$$

Where: y = Yield of pectin in dry weight;

b_i = Coefficient of regression for linear effects;

b_{ik} = Coefficient of interaction regression;

b_{ii} = Coefficient for regression of quadratic effects;

A, B = Experimental coding levels of the variable.

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For pectin yield, 5% Tukey test analyzes were performed. The statistical analyzes yield and characterization of pectin were carried out with the aid of Statistical Software 8.0. The optimization occurred with the five observed responses including the yield, galacturonic acid content, degree of esterification, pectin content, and neutral sugars to provide a pectin with desirable chemical characteristics.

The yield results of the pectin extraction were submitted to analysis of variance and desirability considering yield, galacturonic acid content (AUA), esterification content (DE), pectin content (P), and neutral sugars (N). The desirability function is a statistical technique that can be utilized to determine the levels of independent variables that allow the simultaneous optimization of the dependent variables, thus transforming the response variables into a single measure (Derringer & Suich, 1980).

The desirability procedure was first determined by the levels of the independent variables that simultaneously produced desirable responses that factor in the dependent variables. Afterwards, the general desirability analysis was performed considering all the responses of the independent variables as described by Silva et al., (2013).

3. Results and Discussion

The pectin extraction from the jabuticaba peel obtained a mathematical model with linear and quadratic interactions (Equation 9) and coefficient of determination (R^2) of 0.91, indicating that in 91% of the cases the variation of the response can be explained by the determined model.

$$Y=33,63+4,98*a+11,92*b+8,82*c-5,25*c^2-6,60*a*b+5,84*a*b^2+5,25*a*c+5,01*a^2*c-3,5*a*c \quad \text{Eq. (9)}$$

Where: y is the yield of pectin extraction;

A is the extraction time;

B is the extraction temperature;

C is the concentration of citric acid.

The concentration of citric acid in 0.75 M presents considerable results in extracting the shell of the jabuticaba even when using low or high temperatures (Figure 1 (A)), however, the extraction time should be at least 60 min, obtaining a better result with 90 min (Figure 1

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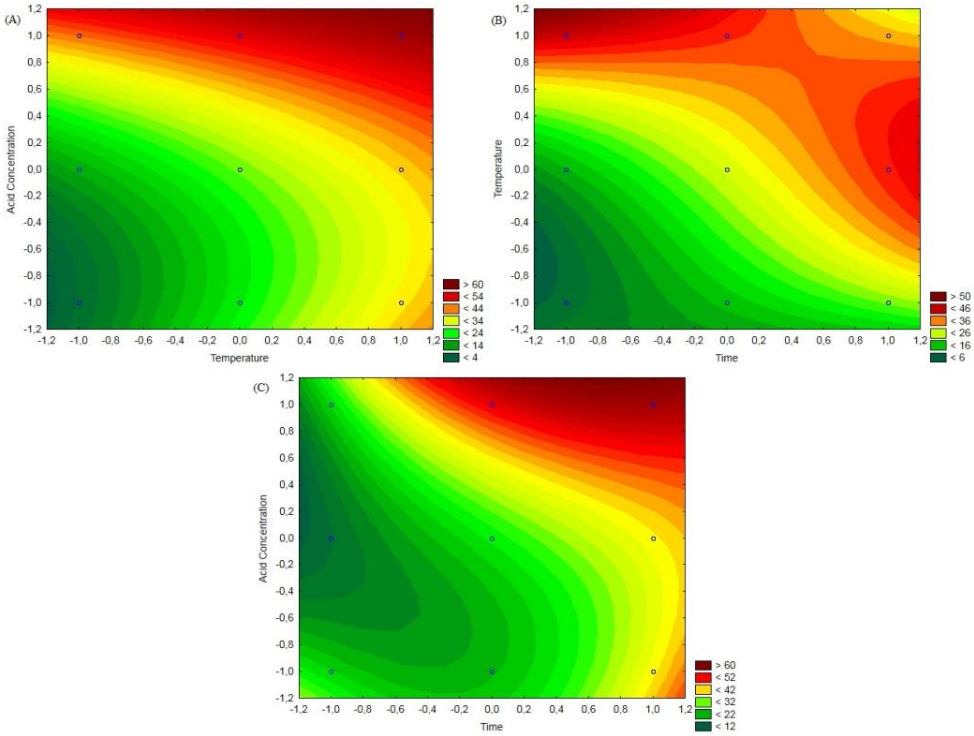
(C)). The time and temperature used in the process have a strong influence on the optimization of this extraction (Figure 1 (B)) due to the linear and quadratic interactions observed by the variable time (A) in Equation 9.

Figure 1: Surface response for the interaction acid concentration and temperature (A), temperature and time (B), and acid concentration and time (C) in the extraction of pectin from jabuticaba peel.



Sayah et al. (2014) reported similar interactions when pectin extraction from orange peel was analysed and that this showed higher yields at high temperatures and time of extraction. As Liew et al. (2014) indicated, in these situations there is a greater dissolution of the flour in the extractive solvent, causing high interaction and better detachment of the macromolecule from the initial system. However, in extreme situations, these parameters could cause degradation of the polymer. Vriesmann et al. (2012) described that the quadratic effect of time and temperature was significant for the extraction of pectin from cocoa fruits and that the increase in temperature during extraction caused an increase in yield.

According to Liew et al. (2014), citric acid has an essential function during this process, contributing to the extraction of the molecule with superior organic acids. According to Canteri et al., (2012), the conditions of molar concentration, temperature, time and pH used during the pectin extraction process are of the almost importance for the yield and nature of the extracted pectin and can affect the gelling capacity and degree of esterification in pectin.

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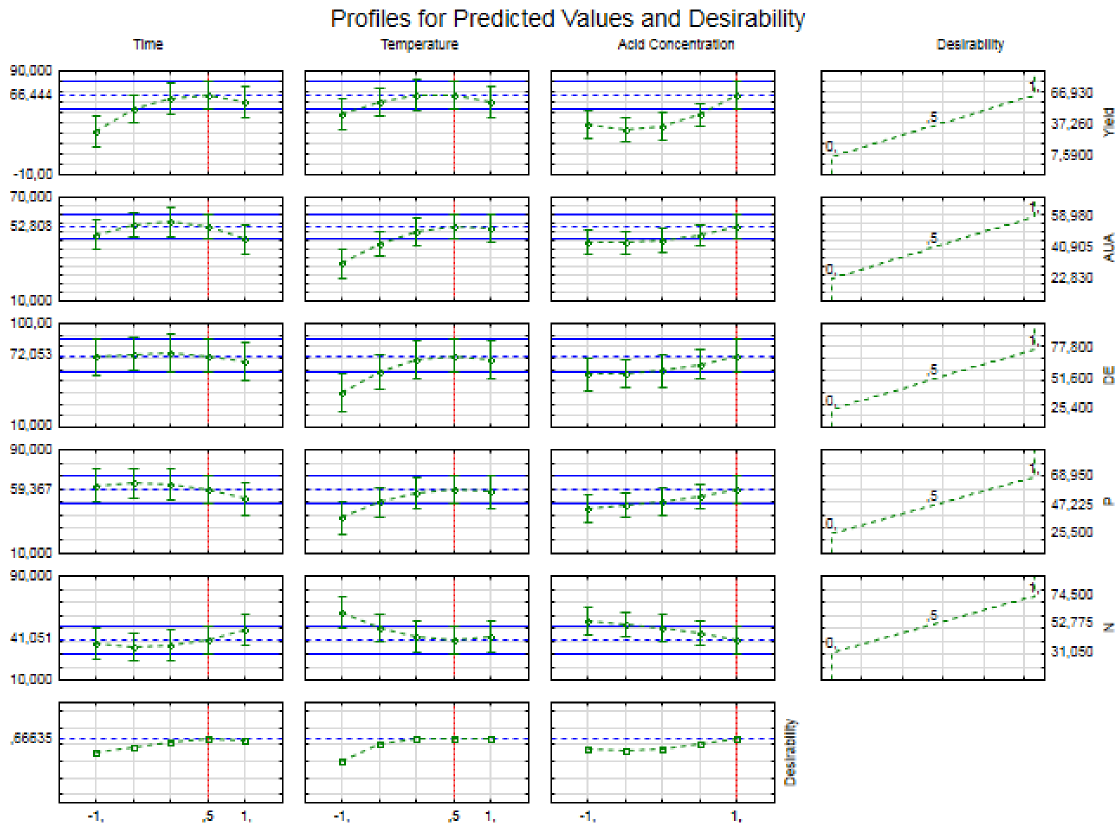
The optimization of the pectin peel extraction can be observed with the desirability calculated with the Statistica 8.0 software considering the optimal values determined in this study (Figure 2).

Figure 2: Desirability of the pectin extraction of the jabuticaba peel by aggregating all of the variables analysed: yield, galacturonic acid content (AUA), esterification content (DE), pectin content (P), and neutral sugars (N).



The results (AUA, DE, P, and N) were adjusted to the models and represented a significant value ($p < 0.05$) with a coefficient of determination (R^2) of 0.92, 0.91, 0.89, 0.88 and 0.90 for the yield, AUA, DE, P, and N, respectively. The smallest mathematical adjustment was for the variable P, but the response was still statistically significant.

The optimal conditions for the extraction of pectin from the peel of the jabuticaba were verified under conditions that included an extraction time of 75 min, a temperature of 70°C, and a citric acid concentration of 0.75 mol.L^{-1}. The parameters of time, temperature, and concentration of the acid are presented as the quadratic profiles for the parameters analysed in the pectin characterization (AUA, DE, P, N). The pectin characterization was performed in the samples that presented the best yield during the process of extraction of this substance from the shell of the jabuticaba. The content of galacturonic acids ranged from 22.82 to 65.35% (Table 2).

Table 2: Characterization of pectin by the content of galacturonic acids (AUA), esterification content (DE), pectin content (P) and neutral sugars (N) considering the best yield of the extract of this substance.

Treatment	TE (min)	C (M)	T (°C)	AUA (%)	DE (%)	P (%)	N (%)
01	30	0,75	50	47,97*	72,09*	55,18*	44,82*
02	30	0,75	90	39,39*	51,67*	44,56*	55,44*
03	60	0,75	50	65,35*	77,50*	66,12*	33,88*
04	60	0,75	90	56,28*	59,50*	53,68*	46,32*
05	90	0,75	50	22,82*	26,72*	25,50*	74,50*
06	90	0,75	90	37,28*	54,39*	42,72*	57,28*

* Significant means through the Tukey test at 5%.

There was a significant difference in the analyzed samples. According to the Food Chemicals Codex (1996) pectins are commercially attractive when they have 65% (w / w) of AUA, since the presence of galacturonic acids in this proportion characterizes pectin purity. Thus, pectins extracted from the shell of jabuticaba are not in the commercial standard, except for sample 03 which was extracted using a concentration of 0.75 M citric acid in the process at 50 ° C for 60 minutes.

Ismail et al. (2012) reported that pectins extracted from Pitaya with oxalic acid, hydrochloric acid and deionized water presented values lower than 65% of AUA, ranging from 42.25 to 54.44%. Seixas et al. (2014) when extracting pectin from passion fruit peel determined 62.5% of AUA for citric acid extraction and 82.3% of AUA for extraction with nitric acid, however, nitric acid is a acid strong influencing the contamination residues.

The degree of esterification (DE) of jabuticaba peel pectins showed significant differences and varied from 26.72 to 77.50% (Table 2). Therefore, the pectin analyzed ranged from low degree of esterification (<50%) to high degree of esterification (> 50%) due to differences in extraction time, temperature and citric acid molar concentration. The selection of the pectin will depend on the desired gelling and texture temperature and the processing of the product. For the formation of edible biofilms or gels in fruits it is necessary that fast gelling occurs, that is, pectin should have a high degree of esterification. In products with low sugar can be used pectins with low DE, as they do not require a large amount of sugar to form gel. In many products there may be aggregation of several pectins with different DEs so that the desired consistency can be achieved (Canteri-Schemin et al., 2005).

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According to Fraye et al. (2010) during the industrial process depolymerization of the pectin molecule which reduces its gelling ability due to the use of strong acids. Seixas et al. (2014) reported the degree of pectin esterification of passion fruit peel of 64.56%. Grassino et al. (2015) determined values of 84.5 to 89.0% of DE for tomato residues extracted by ultrasound, as well as Anese et al. (2013) in which values ranged from 73.3 to 85.4% for this item. It is said by Aina et al. (2012) and Azad et al. (2014) that the characteristics of pectins, especially DE, depend on the nature of the materials and the extraction conditions applied.

The percentage of pectin polymer (P) found in the extraction of this compound from the jabuticaba peel ranged from 25.50 to 66.12% and showed significant differences (Table 2). For treatments that presented lower DE, there was also a lower incidence of pectin and higher neutral sugars (N), which ranged from 33.88 to 74.50%, with significant differences. As for the other pectin characteristics, there is influence of the raw material and the extraction method used, and Yapo et al. (2007) describe that the most present neutral sugars are raminose, arabinose and galactose and that the presence of these indicates a higher degree of branching. During the extraction it can occur the entrainment of other molecules that are not classified as pectin and do not present the same characteristics or also pectins that were degraded during the process, damaging the real yield of this process.

The presence of pectin and neutral sugars is related to the degree of esterification. A higher degree of esterification indicates a higher pectin content and a lower content of neutral sugars. Yapo et al. (2007) reported that the neutral sugars present are raminose, arabinose and galactose and that the presence of these indicates a high degree of branching. During extraction, other molecules can be entrapped and impair the real yield of this process. Such molecules include both those that are not classified as pectin and that do not exhibit the same characteristics and pectins that were degraded during the process.

4. Conclusion

The optimization of pectin extraction from the peel of jabuticaba occurs with the parameters of extraction time less than 75 min, a temperature of 70°C and a citric acid concentration of 0.75 mol.L^{-1}. The extracted pectin with extraction time of 60 min, citric acid concentration of 0.75 M and 50°C showed the best characterization with high degree of esterification. Jabuticaba is a fruit with benefits for application in other foods, either to taste or to add nutritional valu

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