LWT - Food Science and Technology 87 (2018) 575–580

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Short communication

Potential of pequi (*Caryocar brasiliense* Camb.) peels as sources of highly esterified pectins obtained by microwave assisted extraction

Daniela P. Leão [a], Bruno G. Botelho [b], Leandro S. Oliveira [c], Adriana S. Franca [c, *]

[a] PPGCA, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, MG, Brazil
[b] DQ, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, MG, Brazil
[c] DEMEC, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:
Received 12 June 2017
Received in revised form
28 August 2017
Accepted 25 September 2017
Available online 25 September 2017

Keywords:
Microwave
Pectin extraction
Pequi peel

ABSTRACT

Extraction of pectin from pequi peel using microwave heating was investigated in this study. Pectin extraction was optimized by a central composite rotational design varying microwave power, extraction time and temperature. Pectin yield ranged from 9.91 to 20.79 g/100 g. Temperature and microwave power significantly affected the yield of pectin extraction employing citric acid, with the highest yield obtained at 108 °C and 600 W. The obtained pectins were of high degree of esterification and the microwave-assisted extraction yields were comparable to other relevant sources of pectin with significantly shorter extraction times, thus confirming the potential of pequi peels as sources of high grade pectins and the extraction technique as quite suitable for such endeavor.

1. Introduction

Pequi (*Caryocar brasiliense* Camb.) is a native fruit of the Brazilian Cerrado that has significant economic relevance for the producing regions (Leão, Franca, Oliveira, Bastos, & Coimbra, 2017). In 2016, over 20,000 metric tons of pequi were produced in Brazil, and Minas Gerais state was responsible for 73% of the total production (Turini, 2016). The peels (exocarp and mesocarp) represent ~ 80% of the mass of the whole fruit and present a high content of dietary fibers (~45%). Furthermore, evaluation of the monosaccharides profile revealed the presence of a large amount of pectic polysaccharides (~56%) (Leão et al., 2017). However, despite the technological potential of this agricultural by-product, they are discarded, and only a few applications have been reported in the literature, such as development of formulations for bread and cookies (Soares Júnior et al., 2009) and mango jelly (Siqueira, Alves, Vasconcelos, Damiani, & Soares Júnior, 2012).

Pectin represents a high-value functional food ingredient widely used as thickener, emulsifier, gelling agent and stabilizer, particularly in jams and jellies (Oliveira, Von Staszewski, Ruiz-Henestrosa, Pintado, & Pilosof, 2016). The pectin that is used in the food industry is commonly obtained from apple and citrus wastes, consisting mainly of D-galacturonic acid polymers bonded by α-1,4 linkages with small fractions of rhamnose (Oliveira et al., 2016; Vasco-Correa & Zapata Zapata, 2017). However, in recent years, pectin has been successfully obtained from various vegetable sources including other types of fruit peels such as passion fruit, mangoes and pomelo (Chen, Hu, Yao, & Liang, 2016; Maran, Swathi, Jeevitha, Jayalakshmi, & Ashvini, 2015; Seixas et al., 2014).

The conventional techniques commonly employed for pectin extraction are time and solvent consuming, as well as thermally unsafe (Bagherian, Ashtiani, Fouladitajar, & Mohtashamy, 2011; Naqash, Masoodi, Rather, Wani, & Gani, 2017). Thus, alternatives that reduce the time of extraction and, consequently, the consumption of solvents, have been investigated, including ultrasound and microwaves (Oliveira et al., 2016; Seixas et al., 2014) as well as enzymatic extraction (Vasco-Correa & Zapata Zapata, 2017). The use of microwaves has shown great potential for pectin extraction, given that reactions are accelerated under microwave irradiation because the energy is dissipated directly to the reaction components, resulting in instantaneously high temperatures. Furthermore, there are other advantages of microwave use in reactions, such as the decrease of undesirable products, higher heating rates (extremely fast heating), energy saving and consequent cost reductions, greater selectivity and yield (Seixas et al., 2014).

Despite the presence of large amounts of pectic polysaccharides in the pequi peel, no studies were found in the literature related to

* Corresponding author.
E-mail addresses: adriana@demec.ufmg.br, drisfranca@gmail.com (A.S. Franca).

https://doi.org/10.1016/j.lwt.2017.09.037
0023-6438/

extraction and characterization of pectin in such waste. Therefore, in the present study, we evaluate the potential of pequi peel as a source of pectin by microwave extraction. The factors influencing the yield of pectin such as intensity of electromagnetic field and exposure time were examined, as well as the degree of esterification.

2. Material and methods

2.1. Material

Pequi (*Caryocar brasilense* Camb.) fruits were obtained in CEASA/Contagem, Minas Gerais State Agricultural Production Distribution Center. Mature fruits were selected, sanitized and the peels were separated into two groups: P1- whole peel, including exocarp and mesocarp; P2- only mesocarp. They were bleached, blended with water, and dried, as detailed in our previous work (Leão et al., 2017).

2.2. Pectin extraction

Pectin was extracted according to the procedure described by Seixas et al. (2014) with modifications. The sample (2 g) was mixed with distilled water (50 mL) and citric acid (50 mL, pH = 2). This mixture (pH = 2.3) was then placed in a microwave reactor under constant stirring (Model *Start Synthesis,* Synth) for selected values of time, power and temperature. The resulting product was filtered while still warm. Ethyl alcohol was added to the filtrate in the ratio of 2:1 (alcohol: filtrate). The mixture was again filtered to obtain the pectin gel, which was partially dehydrated with acetone and dried in a convection oven (Model 420-1DE, Nova Ética, Brazil) at 40 °C for 12 h. The yield of pectin was calculated as:

$$EY = \left(\frac{m_0}{m}\right)x100 \quad (1)$$

where EY represents the extraction yield (%), m_0 (g) is the mass of dry pectin obtained and m (g) is the mass of flour from pequi peel (P1 or P2).

2.3. Experimental design

A Design of Experiment (DOE) strategy was used in order to obtain optimal extraction conditions for the evaluated parameters. Although simpler, a univariate optimization process would take more time and a larger number of experiments, without providing information about the interaction between studied factors. Among all the available DOE strategies, factorial designs such as the 2^k designs are largely used, because of their capacity of evaluate simultaneously the effect of a great number of variable with a reduced number of experiments (Bruns, Iscarminio & De Barros Neto, 2006). Thus, an exploratory full factorial experimental design with 2 levels and 3 variables was performed. After that, a Central Composite Rotational Design (CCRD) was performed in order to determine the optimum conditions for extracting pectin and to build the response surfaces, considering only the significant factors from the full factorial design performed previously. For both designs, three replicates of the central point were performed in order to allow for the estimation of the pure error. *Minitab 17* and *Statistica 10* were employed for data analysis and surface response evaluation, respectively. The significance of the regression and of the studied effects was estimated using ANOVA, using a 95% confidence level and the model adjustment was evaluated trough R^2, adjusted R^2.

The evaluated variables were extraction time (3, 6 and 9 min), microwave power (400, 600 and 800 W) and temperature (60, 80 and 100 °C for temperature). Although pH is a relevant extraction parameter, literature data on microwave-assisted pectin extraction have indicated that pectin yield is maximized at pH values ranging from 1 to 3 (Bagherian et al., 2011; Maran et al., 2015; Seixas et al., 2014). The acidic extraction solvent contacts directly with the insoluble pectin and favors the hydrolysis of the insoluble pectin constituents into soluble pectin, which increases pectin recovery (Maran et al., 2015). The low pH also reduces the molecular weight of pectin, so it will be solubilized from plant tissues with minimum degradation and hence the pectin molecules proneness to precipitation. Beyond the pH value of 3, aggregation of pectin can occur, thus retarding pectin release and decreasing pectin yield. The pH values of our samples were measured (water + flour + citric acid) and we found an average value of 2.3. Since this value was already in the recommended interval, this parameter was not optimized in our study.

2.4. Degree of esterification

The degree of esterification was evaluated by Diffuse Reflectance Fourier Transform Infrared Spectroscopy (IRAffinity-1 equipment, Shimadzu, Japan, DLATGS detector, range of 4000–400 cm^{-1}, 4 cm^{-1} resolution, 20 scans, 20 ± 0.5 °C). The degree of esterification was evaluated as the ratio of the areas of the peaks corresponding to esterified carboxylic groups (~1740 cm^{-1}) and the total peak areas of free (~1612 cm^{-1}) and esterified carboxylic groups (Gnanasambandam & Proctor, 2000).

3. Results and discussion

3.1. Extraction yield

For the exploratory factorial design experiment proposed, pectin yield ranged from 12.75 to 20.79 g/100 g (see Table 1). Experimental design results (Fig. 1) showed that extraction time was not significant ($p > 0.05$). Similarly, second and third order interactions were not significant, showing that variables interfere on the yield extraction separately, without synergistic effects. Temperature presented the greatest influence on the pectin extraction yield, followed by microwave power as shown on the Pareto diagram. The values for R^2 and adjusted R^2 (0.9858 and 0.9290, respectively) indicate a good adjustment of the proposed model to the data. Based on this information, a CCRD design using only the two significant factors (temperature and power) was performed.

Table 1
Central Composite Rotational Design (CCRD) and yield of pectin extracted pequi peels flours under microwave heating using citric acid.

Experiment	Temperature (°C)	Power (W)	Pectin yield (g/100 g)	
			P1	P2
1	52	600	14.56	10.56
2	60	400	12.75	9.91
3	60	800	15.29	12.83
4	80	317	16.45	11.28
5	80	600	13.53	12.11
6	80	600	13.64	13.59
7	80	600	13.64	10.68
8	80	883	16.36	11.25
9	100	400	17.25	16.30
10	100	800	18.25	15.60
11	**108**	**600**	**20.79**	**16.23**

Rotational central composite design with 3 center points and 4 axial points in the cube with extraction time of 3 min (P1- exocarp + mesocarp; P2 - only mesocarp).

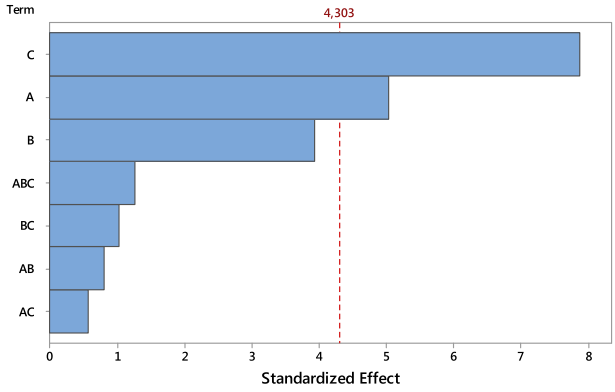


Fig. 1. Pareto diagram for standardized effects (factor name: A: power; B: time; C: temperature).

Fig. 2 (a) and (b) show the response surfaces from pectin extraction yield as a function of power and temperature for P1 and P2 samples, respectively. The CCDR model statistical quality were also assessed using R^2 and adjusted R^2. Both parameters were higher than 0.77 for both models, confirming the statistical quality. Temperature was the most important variable that affected pectin yield. It may be concluded that the best extraction parameters would be temperature of 108 °C and a maximum power of 600 W (Table 1).

It is also possible to notice that further increasing microwave power did not improve pectin extraction. This is attributed to the fact that very high power may cause molecular disorder with consequent degradation of pectic polysaccharides, leading to a reduction in the extracted pectin content. Pectins are arranged in the spaces of microfibrils and between layers of plant cell wall forming a hydrophilic three-dimensional grid. The microwave energy acts as an electromagnetic radiation, which promotes the rapid loosening of the cell wall matrix, improving the penetration of the solvent into the plant matrix and leading to the leaching of the pectin during the heating process (Maran et al., 2015).

P1 samples presented higher yields than P2 for all the evaluated conditions, which is consistent with the results obtained by Leão et al. (2017), who reported higher amounts of polysaccharides in pequi peel flours that included both the exocarp and mesocarp, with the pectic polysaccharide being mainly comprised of rhamnogalacturonans. Our results were in the same range as those reported for microwave-assisted pectin extraction from papaya, passion fruit and mango peels (Maran & Prakash, 2015; Maran et al., 2015; Seixas et al., 2014), and significantly high in comparison to pomelo peels (Chen et al., 2016).

A comparison of the obtained results with respect to pectin extraction by conventional heating indicated that our yields are higher or in the same range of other residues including jackfruit waste (Begum, Aziz, Uddin, & Yusof, 2014) and passion fruit peels (Liew, Chin, & Yusof, 2014). Our results in terms of yield were also similar to those reported for conventional extraction of pectin from pequi peels (68–98 °C/60–80 min) employing citric acid (Siqueira et al., 2012). However, conventional heating required longer extraction time, which lead to degradation of the ester bonds in carboxilic groups and consequently to a low DE product (12–49% DE) in comparison to our results (51–80% DE).

3.2. Degree of esterification

The degree of esterification is used as a pectin classification criteria and measures the proportion of methylated galacturonic acid groups to total galacturonic acid groups in the pectin molecule. The degree of esterification especially affects pectin gel formation mechanism. Pectins obtained from pequi peel flours in this study were of high degree of esterification (>50%) (Fig. 3a). This type of pectins is mostly employed in jams and preserves, but can be also used as stabilizers of acidic dairy products and viscosifiers for beverages (May, 1990; Vasco-Correa & Zapata Zapata, 2017).

The spectra of isolated pectin of P1 and P2 are shown in Fig. 3 (b). A strong O-CH_3 band is observed between 3000 and 2800 cm^{-1} due to the methyl ester group of galacturonic acid in pectin. However, given an intense O-H stretching response occurring in a wide band (3600-2500 cm^{-1}), the intensity of O-CH_3 can be masked, not serving as a good indicator of methoxylation in pectin (Gnanasambandam & Proctor, 2000). A second region (1800-1500 cm^{-1}) has special interest concerning the evaluation of the degree of esterification, since it allows the observation of infrared absorption by the carboxylic acid and the carboxylic ester groups of the pectin molecules (Chatjigakis et al., 1998).

Many studies use the ratio between the area of the band in ~1740 cm^{-1} (absorption of esterified carboxylic groups from pectin molecules) and ~1630 cm^{-1} (absorption of carboxylate anions) for determining the degree of esterification of pectin. However, peaks of other functional groups can occur in the same spectral region, erroneously contributing to increased absorption of peaks of interest for calculating the degree of esterification of pectin (Gnanasambandam & Proctor, 2000). Due to this, the second derivative of the spectra was performed (not shown) for pectin obtained from P1 and P2 samples. Spectral derivation enables a better individualization of the constituents by an increase in number of absorption bands.

In this study, two characteristic absorption bands of carboxylic acids and carboxylic esters of pectin molecules were detected at ~1650 cm^{-1} and ~1740 cm^{-1}, respectively. When the second derivative was applied to the spectral region between 1800 cm^{-1} and 1500 cm^{-1}, 4 relevant peaks were detected: 1612 cm^{-1}, 1632 cm^{-1}, 1649 cm^{-1} and 1747 cm^{-1}.

The peak obtained at 1649 cm^{-1} is characteristic of amides I indicating the presence of protein. The identified peak in 1632 cm^{-1} corresponds to water absorption. Thus, both of the mentioned peaks may contribute to the verified absorption at 1649 cm^{-1}. The third peak which contributes to the band at 1649 cm^{-1} is absorbed in 1612 cm^{-1} and absorption was identified as stretching of the carboxylate ester groups of pectin (Chatjigakis et al., 1998). Thus, only the area of that third peak was used to determine the pectin degree of esterification.

Pectin degree of esterification is a main indicator of its functional properties. Solubility is a parameter strongly influenced by the physicochemical properties of the pectin and tends to increase with the increase of degree of esterification and the molecular weight reduction. In previous studies, Leão et al. (2017) evaluated the hydration properties of pequi peel flours obtaining higher solubility and swelling values for P2 (19.8% and 11.3 mL/g) than for P1 (16.7% and 8.8 mL/g). These results are in accordance with the higher degree of esterification for samples of P2 group.

4. Conclusions

It may be concluded that microwave-assisted extraction of pectin from pequi (*Caryocar brasiliense* Camb.) peel was efficient in a short time (3 min). Temperature was the main factor for increasing pectin yield, and the best results being obtained with 108 °C and a maximum power of 600 W. Pectin content was slightly

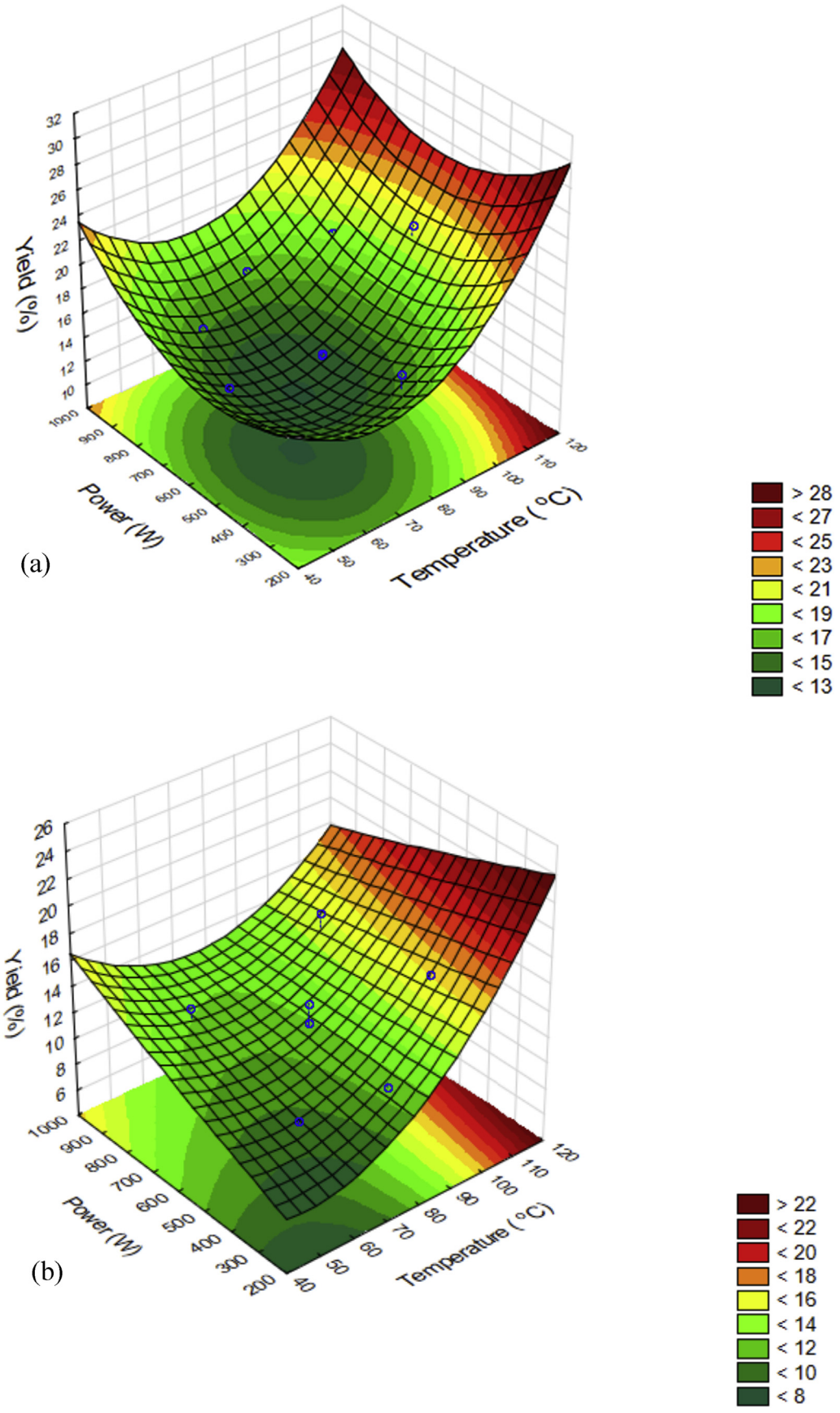
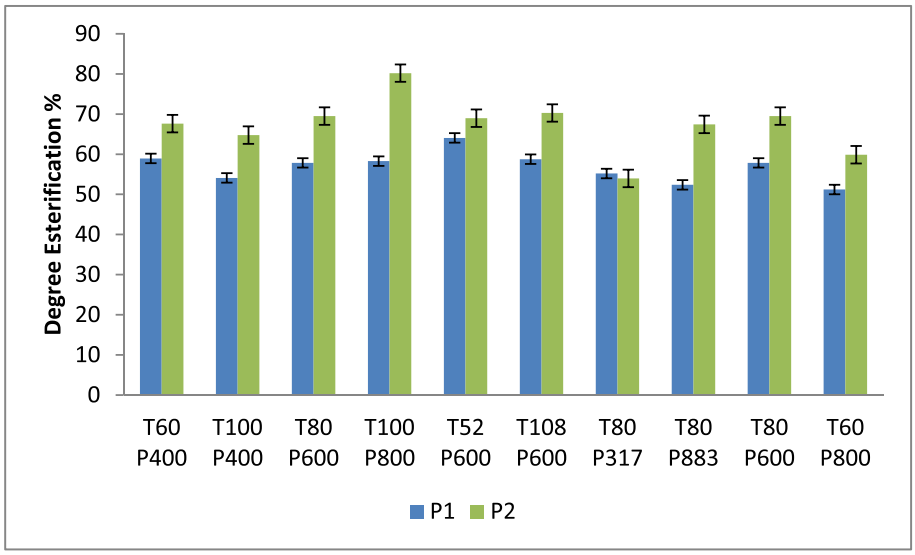
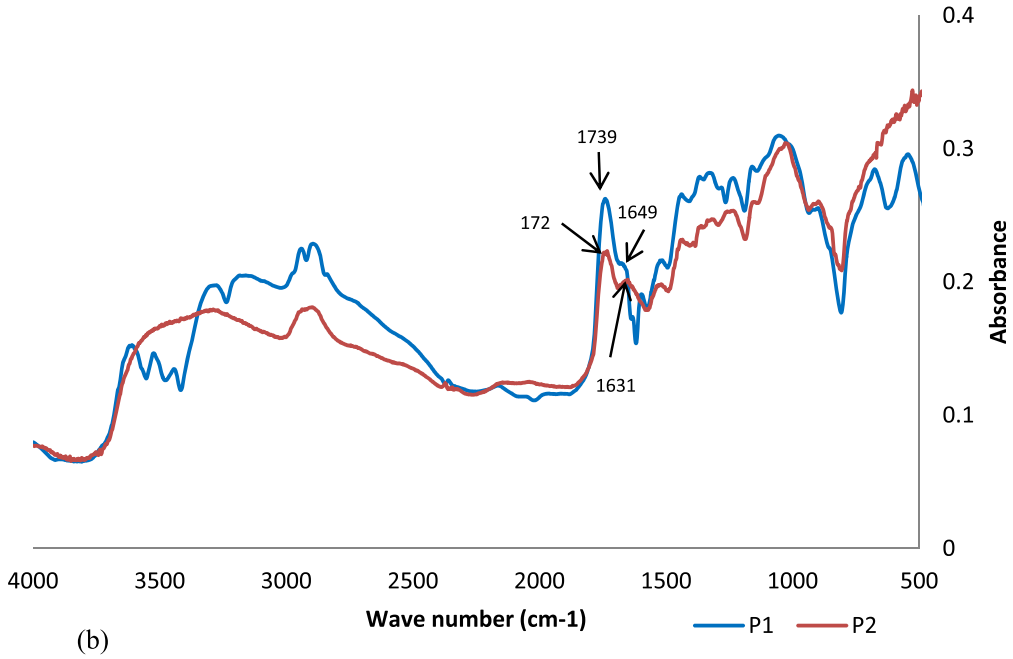


Fig. 2. Response surface showing the effect of temperature and the power on pectin yield; a) P1; b) P2.



(a)



(b)

Fig. 3. (a) Degree of esterification of pectin obtained for P1 and P2 (T: temperature (°C); P: power (W)). (b) Diffuse reflectance Fourier transform infrared spectra of isolated pectin from P1 and P2 samples.

higher if the exocarp was included. However, removal of exocarp improved the degree of pectin esterification. Regardless, all the obtained pectins were of high degree of esterification, confirming the potential of pequi peels as sources of high grade pectin and microwave-assisted extraction as a suitable technique for obtaining higher yields of pectin.

Acknowledgments

The authors gratefully acknowledge financial support from the following Brazilian Agencies: FAPEMIG, CNPq (306139/2013-8) and CAPES.

References

Bagherian, H., Ashtiani, F. Z., Fouladitajar, A., & Mohtashamy, M. (2011). Comparisons between conventional, microwave- and ultrasound-assisted methods for extraction of pectin from grapefruit. *Chemical Engineering and Processing: Process Intensification, 50*, 1237–1243.

Begum, R., Aziz, M. G., Uddin, M. B., & Yusof, Y. A. (2014). Characterization of jackfruit (*Artocarpus Heterophyllus*) waste pectin as influenced by various extraction conditions. *Agriculture and Agricultural Science Procedia, 2*, 244–251.

Bruns, R. E., Iscarminio, I. S., & De Barros Neto, B. (2006). *Statistical design – chemometrics* (p. 412). Amsterdan: Elsevier B.V.

Chatjigakis, A. K., Pappas, C., Proxenia, N., Kalantzi, O., Rodis, P., & Polissiou, M. (1998). FT-IR spectroscopic determination of the degree of esterification of cell wall pectin from stored peaches and correlation to textural changes. *Carbohydrate Polymers, 37*, 395–408.

Chen, Q., Hu, Z., Yao, F. Y.-D., & Liang, H. (2016). Study of two-stage microwave extraction of essential oil and pectin from pomelo peels. *LWT - Food Science and*

Technology, 66, 538–545.
Gnanasambandam, R., & Proctor, A. (2000). Determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy. *Food Chemistry, 68*, 327–332.
Leão, D. P., Franca, A. S., Oliveira, L. S., Bastos, R., & Coimbra, M. A. (2017). Physicochemical characterization, antioxidant capacity, total phenolic and proanthocyanidin content of flours prepared from pequi (*Caryocar brasilense Camb.*) fruit by-products. *Food Chemistry, 225*, 146–153.
Liew, S. Q., Chin, N. L., & Yusof, Y. A. (2014). Extraction and characterization of pectin from passion fruit peels. *Agriculture and Agricultural Science Procedia, 2*, 231–236.
Maran, J. P., & Prakash, K. A. (2015). Process variables influence on microwave assisted extraction of pectin from waste Carcia papaya L. peel. *International Journal of Biological Macromolecules, 73*, 202–206.
Maran, J. P., Swathi, K., Jeevitha, P., Jayalakshmi, J., & Ashvini, G. (2015). Microwave-assisted extraction of pectic polysaccharide from waste mango peel. *Carbohydrate Polymers, 123*, 67–71.
May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate Polymers, 12*, 79–99.
Naqash, F., Masoodi, F. A., Rather, S. A., Wani, S. M., & Gani, A. (2017). Emerging concepts in the nutraceutical and functional properties of pectin—a Review. *Carbohydrate Polymers, 168*, 227–239.
Oliveira, A. L., Von Staszewski, M., Ruiz-Henestrosa, V. M. P., Pintado, M., & Pilosof, A. M. R. (2016). Impact of pectin or chitosan on bulk, interfacial and antioxidant properties of (+)-catechin and β-lactoglobulin ternary mixtures. *Food Hydrocolloids, 55*, 119–127.
Seixas, F. L., Fukuda, D. L., Turbiani, F. R. B., Garcia, P. S., Petkowicz, C. I. de O., Jagadevan, S., et al. (2014). Extraction of pectin from passion fruit peel (*Passiflora edulisf. flavicarpa*) by microwave-induced heating. *Food Hydrocolloids, 38*, 186–192.
Siqueira, B. S., Alves, L. D., Vasconcelos, P. N., Damiani, C., & Soares Júnior, M. S. (2012). Pectina extraída de casca de pequi e aplicação em geleia *light* de manga (Pectin extracted from pequi peels and its application to light mango jelly). *Revista Brasileira de Fruticultura, 34*, 560–567 (in Portuguese).
Soares Júnior, M. S., Reis, R. C., Bassinello, P. Z., Lacerda, D. B. C. L., Koakuzu, S. N., & Caliari, M. (2009). Qualidade de biscoitos formulados com diferentes teores de farinha de casca de pequi (Quality of cookies formulated with different *pequi* peel flour contents). *Pesquisa Agropecuária Tropical, 39*, 98–104 (in portuguese).
Turini, E. (2016). *Conjuntura Mensal de Pequi*. CONAB. Report 16-05-23-17-46-45.
Vasco-Correa, J., & Zapata Zapata, A. D. (2017). Enzymatic extraction of pectin from passion fruit peel (Passiflora edulis f. flavicarpa) at laboratory and bench scale. *LWT - Food Science and Technology, 80*, 280–285.