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Aqueous extraction of pectin from sisal waste

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ABSTRACT

In this work, sisal waste was used as a source of pectin. Sisal is known worldwide as a source of hard fibres, and Brazil is the largest producer of sisal, producing more than 246,000 tonnes. However, the process of removing the fibres of the sisal leaf generates 95% waste. This study investigated the effect of the liquid/solid ratio (%), time (min), and temperature (°C) on the yield of the pectin obtained from sisal waste by attractive environmentally friendly process. A statistical Box–Behnken design was applied to determine the important effects and interactions of these independent variables on the yield of pectin, the dependent variable. Significant models were obtained. The yield of the extracted pectin ranged from 4.61 to 19.2%. The conditions that produced the highest yield (19.2%) were a temperature of 85 °C, extraction time of 60 min and a liquid/solid ratio of 2%.

1. Introduction

Pectins are a family of complex heteropolysaccharides that constitute a significant proportion (35%) of the primary cell wall of dicotyledonous plants and non-graminaceous plants, with important functions in their development, growth and maturation (Mohnen, 2008; Morris & Ralet, 2012). These polysaccharides have multifunctional properties *in muro*, as well as *out of muro*, such as the control of cell wall integrity and porosity, the protection of plants against phytopathogens; they also have gelling, emulsifying, stabilising, and thickening properties and provide several health benefits (Yapo, 2011).

Pectins consist of at least eight different types of polysaccharides, of which homogalacturonan, rhamnogalacturonan types I and II, and to a lesser extent xilogalacturonan, are the most common. Homogalacturonan (HG) is the most abundant cell wall pectic polysaccharide, corresponding to approximately 50–90% of the total pectin (Mohnen, 2008; Yapo, 2011). HG is a linear polymer of α-1,4-linked D-galacturonic acid units, partially methyl-esterified at the C-6 carboxyl, and sometimes O-acetylated at the O-2 or O-3 positions, depending on the plant source (Mohnen, 2008; Vriesmann, Teófilo, & Petkowicza, 2011).

The second most abundant type of pectic polysaccharide is rhamnogalacturonan I (RG-I), comprising between 20% and 35% of the total pectin. This polysaccharide shows a high degree of cell specialisation and development that are dependent on the expression in the type and number of simple sugars, oligosaccharides, and branched oligosaccharides that are attached to its backbone. The chain length may vary considerably, and the sugar composition of RG-I may be highly heterogeneous (Willats, Knox, & Mikkelsen, 2006).

The degree of methoxylation (DM) of the pectins is an important parameter for the determination of their use. It is defined as the number of moles of methanol per 100 moles of galacturonic acid. Pectins with a high DM, with more than 50% of the carboxyl groups esterified, form gels when heated in solutions with a $\text{pH} < 3.5$ and at sugar concentrations above 55%. Low methoxy pectin (LM less than 50%) is used in gel form for a wide range of pH values. Their gel-forming properties, with or without a small amount of sugar and in the presence of Ca^{2+}, is an important factor for efficient binding both at low and high pH values (Lofgren & Hermansson, 2007).

In general, pectin is extracted via a physicochemical process. This process begins with an extraction step with hot dilute mineral acid followed by recovery by precipitation in alcohol (Seggiani, Puccini, Pierini, Giovando, & Forneris, 2009). However, the use of strong mineral acids (e.g., H_2SO_4, HCl) can be harmful to the environment and require additional steps to remove toxic elements (Yapo, 2009).

The majority of commercially available pectin is extracted from citrus peels, as well as beet and apple pomace (Kurita, Fujiwara, & Yamazaki, 2008; Mesbahi, Jamalian, & Farahnaky, 2005). However,

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non-traditional sources of pectin have been investigated. The pectin from alternative sources such as fresh peach pomace, sunflower seeds, the bark of the mango tree and red fruit pulps have been studied (Torralbo, Batista, Medeiros, & Fernandes, 2012).

Pectin has several industrial applications. Specifically, in the food industry, pectic polysaccharides provide increased viscosity and function as stabilisers in foods and beverages. The prevention of fruit flotation, stabilisation of bread products, stabilisation of proteins, softening of textures, and increasing of volumes are among the numerous other applications of pectin in the food industry. In the pharmaceutical industry, pectic substances reduce total cholesterol and glucose uptake. They also bind heavy metals and toxic microorganisms in the colon, thus preventing the resorption of toxins produced by pathogens, reducing the incidence of cancer (Ele-Ekouna, Pau-Roblot, Courtois, & Courtois, 2011; Vriesmann et al., 2011).

Sisal, *Agave sisalana* Perrine, is known worldwide as a source of hard fibres and Brazil comprising more than 68% of the world's production (FAOSTAT, 2012). However, only 3–5% of the leaves are utilised in the production of fibres; the remainder (95–97%) of the processing waste is used as organic fertiliser and animal feeds and has applications in the pharmaceutical industries (Branco et al., 2010).

Sisal waste is composed of water (85%), parenchymal tissue, short fibres, primary and secondary metabolites and inorganic compounds (Silva & Beltrão, 1999). Some studies have indicated the presence of pectin in sisal leaves (Aspinall & Cañas-Rodriguez, 1958; Silva & Beltrão, 1999). Pectin extraction without the use of mineral acids is an attractive environmentally friendly process. In this work, we investigated the aqueous extraction of pectin from sisal waste using the response surface methodology.

2. Materials and methods

2.1. Obtainment of sisal waste

The sisal waste utilised in this study was collected directly from a decortication machine on a sisal farm in the city of Valente, Bahia State, Brazil (S 11°24′53.4″). The processed sisal leaves were from plants six years of age, collected in May 2011. One kilogram of sisal waste was dried in a forced circulation oven at 50 °C until a constant weight (yield: 21.2%) was achieved then stored in containers at 4 °C.

2.2. Aqueous extraction of pectin

The dried samples were extracted with 200 mL of distilled water. The extractions were conducted at different temperatures (75, 85 and 95 °C) with different liquid/solid ratios (2, 4 and 6%) for various extraction times (20, 40 and 60 min). After extraction, the samples were filtered through cotton. Ethanol (95%) was added to the filtrate in a 2:1 ratio and stored at 4 °C for 24 h. The polysaccharides containing pectin were removed by centrifugation (10,000 rpm, 10 min) and then washed twice with ethanol. After one hour, the pectin was separated as a precipitate by filtration. The pectin was dried in a forced circulation oven at 55 °C until a constant weight was achieved. The yield of pectin was calculated from the initial amount of raw material (Wai, Alkarkhi, & Easa, 2010): Yield (%) = Mass of extracted pectin/Mass of waste dehydrated × 100.

2.3. Characterisation of the precipitate from experiments

The precipitates from the assays were analysed by Fourier transform-infrared (FT-IR) spectroscopy in a Perkin-Elmer FTIR Spectrometer (Model Spectra 100) using KBr pellets. Citric pectin (VETEC, Brazil) was used as a reference.

Table 1
Independent variables of the experimental design for the extraction of pectin from sisal waste.

Assay	Variables		
	Liquid/solid ratio (%) (X_1)	Time (min) (X_2)	T (°C) (X_3)
1	6 (1)	40 (0)	75 (−1)
2	6 (1)	20 (−1)	85 (0)
3	6 (1)	60 (1)	85 (0)
4	6 (1)	40 (0)	95 (1)
5	4 (0)	20 (−1)	75 (−1)
6	4 (0)	60 (1)	75 (−1)
7c	4 (0)	40 (0)	85 (0)
14c	4 (0)	40(0)	85(0)
15c	4 (0)	40(0)	85(0)
8	4 (0)	20 (−1)	95 (1)
9	4 (0)	60 (1)	95 (1)
10	2 (−1)	40 (0)	75 (−1)
11	2 (−1)	20 (−1)	85 (0)
12	2 (−1)	60 (1)	85 (0)
13	2 (−1)	40 (0)	95 (1)

2.4. Determination of the degree of esterification (DE)

The DE of the pectin was determined by the titrimetric method (USP 26 NF 21, 2003) with minor modifications. The dehydrated sample was moistened with 2 mL of ethanol and dissolved in 25 mL of distilled water free of carbon dioxide. After complete dissolution of the sample, two drops of phenolphthalein were added to start the process of titration with 0.25 M sodium hydroxide to neutralise the free carboxyl acids from anhydro-galacturonic acid (V1). Then, 10 mL of 0.25 M sodium hydroxide was added to the sample and stirred for 30 min for hydrolysis, followed by the addition of 10 mL of 0.25 M hydrochloric acid and stirring until the complete disappearance of the pink colour of the solution. Finally, the sample was titrated again with 0.25 M sodium hydroxide with vigorous stirring until the solution turned pink (V2). The volumes were used for determining the titration equivalent of the carboxyl esterified pectins. The DE of the pectin was calculated using the following formula:

$$\%\,\mathrm{DE}\frac{\mathrm{V2}}{\mathrm{V1}+\mathrm{V2}}\times 100$$

2.5. Experimental design

The variables were monitored using a 3^3 fractional factorial Box–Behnken design (Table 1). The variables used in this design were the liquid/solid ratio, extraction temperature (T) and extraction duration (t) (Wai et al., 2010; Yapo, 2009, 2011). Three repetitions of the central point (c) were performed to estimate the possible pure error, and the experimental treatments were varied randomly to detect the presence of possible systematic errors. The response measured was the pectin yield (%) of the aqueous extraction of sisal waste. All the calculations and graphics in this work were performed using the software Statistica®, including the analysis of variance (ANOVA) for the responses of this study.

3. Results and discussion

The response surface methodology (RSM) comprises a combination of techniques of experimental design, regression analysis and optimisation methods. It has wide applications in situations where a large number of variables of a system influence a key feature of this system. The Box–Behnken factorial design is an experimental design used for optimisation procedures, and the model consists of the repetition of the central point to measure

Table 2
Yield of the aqueous extract of pectin from *Agave sisalana* waste.

Assay	Pectin (g)	Yield (%)
1	0.6199	5.14
2	0.5681	4.71
3	0.5534	4.61
4	0.7963	6.63
5	0.4407	5.50
6	0.5111	6.36
7	0.5087	6.36
8	0.5039	6.30
9	0.529	6.58
10	0.4871	6.03
11	0.4661	5.77
12	0.7761	19.21
13	0.2454	6.03
14	0.2367	5.90
15	0.2339	5.78

Table 3
ANOVA of the pectin yield.

Variation	SS[a]	df[b]	MS[c]	*F*[d]	*p*[e]
Regression	0.372028	9	0.041336	8.37	4.77
Residues	0.024670	5	0.004934		
Lack of fit	0.024315	3	0.008105	45.6555	0.021510
Pure error	0.000355	2	0.000178		
Total SS	0.396697	14			

[a] SS: sum of squares.
[b] df: degree of freedom.
[c] MS: mean squares.
[d] *F*: *F* distribution.
[e] *p*: *p* value.

the experimental variability, plus a set of points anchored in the central factor defining the region of interest.

The total extraction yield reflects the extraction efficiency of pectin; however, some impurities such as degraded pectin can be present. The highest pectin yield was obtained when sisal waste was extracted at 85 °C at a concentration of 2% for 60 min (Table 2). The quadratic model that represents the behaviour of the yield of pectin was obtained, describing the response surface contour curves (Eq. (1)). The ANOVA results are shown in Table 3. The calculated *F* value (8.37) obtained from the ratio 0.041336/0.004934 was greater than the tabulated *F* value (4.77) for 9 and 5 degrees of freedom at the 95% confidence level, indicating that treatments are significantly different.

$$\text{Pectin (g)} = 8.91 + 0.0343X_1 - 0.000304X_1^2 - 0.7139X_2 + 0.00216X_2^2 - 0.0000375X_1X_2 \quad (1)$$

The contour lines on the top surface (Fig. 1), corresponding to 1–4 experiments (Table 2) show that the pectin values, predicted by the model, increases substantially with increasing liquid/solid ratio. This is consistent with the positive value of the coefficient X_1. However, the significant negative influence of X_2 (extraction time), observed in Eq. (1), explains the low yields observed at the extremes of the design and suggests the use of 40 min (mean) for the extraction process. The negative interaction X_1X_2 indicates that there is no synergism between the time of extraction and liquid/solid ratio and that the linear model (Eq. (1)) was superior to quadratic model.

Fig. 2 shows the graph of predicted by Eq. (1) from the corresponding experimental results. The points are randomly distributed near the line, showing excellent agreement and that the model does not present a significant lack of fit. The predicted optimum values of time and liquid/solid ratio obtained by differentiating Eq. (1) were equal to 41 min and 6% liquid/solid ratio, respectively.

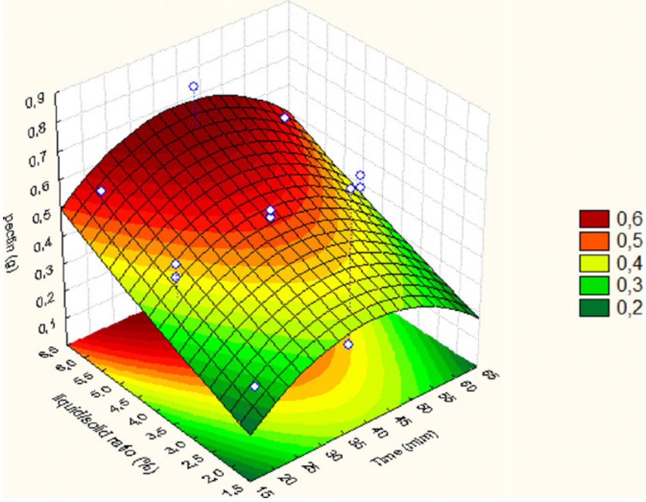

Fig. 1. Response surface of the pectin yield with respect to extraction time (min) and the liquid/solid ratio (%).

Fig. 3 shows the response surface for obtaining pectin related to temperature and liquid/solid ratio. The quadratic model that represents the behaviour of the yield of pectin, which describes the surface response is shown in Eq. (2).

$$\text{Pectin (g)} = 8.91 - 0.7139(X_3) + 0.0021(X_3)^2 - 0.1787(X_1) + 0.000840(X_1)^2 + 0.008982(X_1X_3) \quad (2)$$

The contour lines at the top surface correspond (Fig. 3) to experiment 10 (Table 1), showing that the values of pectin yield decreases with increased temperature. This is consistent with the negative value of the coefficient X_3. The absolute difference between the coefficients X_3 and X_1 indicates that the temperature contributes negatively, about three times more with respect to liquid/solid ratio. The positive interaction X_1X_3 indicates that there is synergism (weak) between temperature and liquid/solid ratio; and that the quadratic model (Eq. (2)) was superior to linear model.

The predicted optimum values of temperature and mass obtained by differentiating Eq. (2) were 76 °C and a liquid/solid ratio of 6%, respectively.

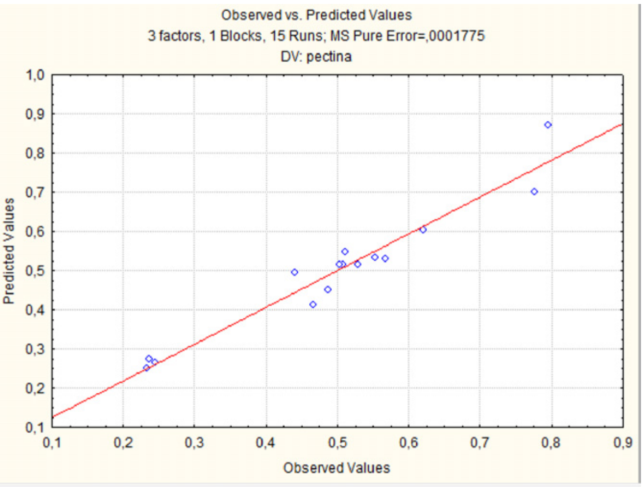

Fig. 2. Experimentally determined values of pectin yield versus the predicted values determined by a quadratic model equation. The diagonal line represents the exact agreement.

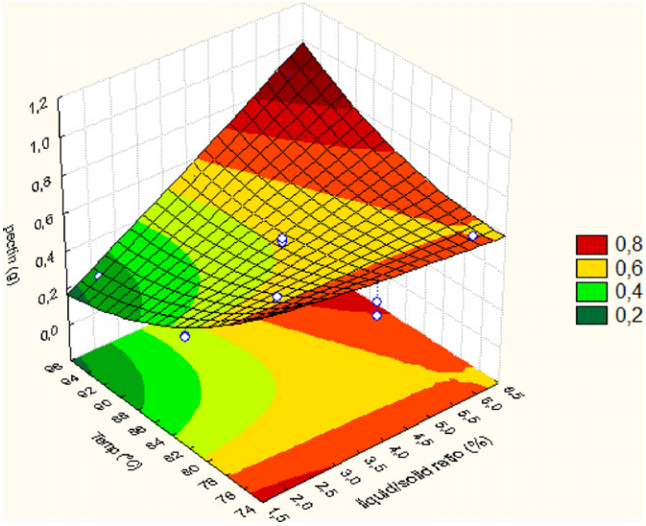

Fig. 3. Response surface of the pectin yield with respect to the liquid/solid ratio (%) and temperature (°C).

Compared with other non-commercial sources of pectins, the yield from sisal waste was higher than those from sunflower head residues (Iglesias & Lozano, 2004), cocoa bean husks (Mollea, Chiampo, & Conti, 2008), durian rings (*Durio zibethinus*) (Wai et al., 2010), cacao pod husks (*Theobroma cacao* L.) (Vriesmann et al., 2011), banana peels (Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008) and mango peels (Koubala et al., 2008); but inferior to that of *Solanum lycocarpum* (Torralbo et al., 2012).

Several studies describe the use of mineral acids in the process of extracting pectin (Yapo, 2011). However, the absence of mineral acid in the extraction of pectin from mango peels resulted in a yield similar to experiments with acid (Koubala et al., 2008), which may explain the observed pectin yields from sisal waste. Mollea et al. (2008) described various processes of extracting pectin from cocoa husks from different continents. Experiments using cocoa husk pieces were observed to produce a higher yield compared to using the whole husk. In this sense, higher yields of pectin from sisal waste may be due to the increased contact area with the solvent extractor. This finding also explains the difference in pectin yields observed between experiments three and twelve.

The degree of esterification of pectin from experiment twelve showed a 50% degree of methoxylation. The characterisation of the other experiments was not performed due to the low yields observed.

The analysis of the infrared spectra of the precipitates obtained from the experiments was performed to identify the main functional groups. Fig. 4 shows the infrared spectra of the precipitate from experiment eight and a citrus pectin pattern. The other precipitates of the present study showed spectra similar to the precipitate of experiment eight. Among the absorption bands common to the two spectra, the band between 3300 and 3500 cm^{-1} is due to OH stretching. Signals between 2919 and 2940 cm^{-1} correspond to the stretching of the CH and CH_2 groups, and the two bands at 1598 and 1417 cm^{-1} correspond to the asymmetric and symmetric vibration of the COO (carboxylate) structure, respectively. Carbohydrates showing a high absorbance in the region between 1200 and 950 cm^{-1} are the 'fingerprint' specific polysaccharides. A strong absorbance at 1075 cm^{-1} is characteristic of the type β glycosidic linkages between the sugar units (Kozarski et al., 2011).

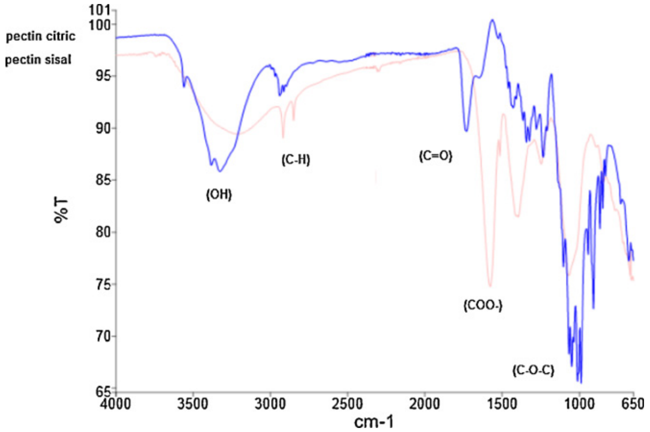

Fig. 4. Fourier transform infrared spectra of a commercial pectin standard and the pectin obtained from sisal waste.

The band at 1740 cm^{-1} is attributable to the stretching vibration of the C=O group of the carboxylic acid methyl ester (or protonated carboxylic acid), representing a characteristic differential spectra. Manrique and Lajolo (2002) reported that the infrared spectra of aliphatic carboxylic acids (anionic form) exhibiting a pair of bands with high intensities between two ranges, 1610–1550 and 1410–1300 cm^{-1}, correspond to the asymmetric and symmetric stretching of the carboxylate group, respectively. These bands can be observed between 1623 and 1428 cm^{-1}, corresponding to the characteristic wavelengths for polygalacturonic acid. The effect of the displacement values of the wavelengths observed is the result of several factors derived from the vicinity where the polymer COO- group of galacturonic acid are inserted and the physical state of the sample. The data analysis indicates that the precipitate is a polysaccharide-rich polygalacturonic acid in the salt form.

4. Conclusions

The use of experimental design and response surface analysis allowed for the investigation and optimisation of the influence of several variables and their interactions on the yield of pectin from sisal by attractive environmentally friendly process. In this work, it was observed that the liquid/solid ratio of the residue sisal significantly influenced of the aqueous extraction of pectin. The temperature and liquid/solid ratio were variables that contributed negatively to the extraction of pectin from sisal waste; the temperature variable reduced the yield of pectin approximately three times more than liquid/solid ratio.

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