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Extraction and Characterization of Pectin from Citric Waste

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Brazil is the biggest orange producer in the world and processes about 85% of its production, generating a vast waste quantity. One of the components of these citric by-products is pectin, polymer of great interest to the food industry with possibilities to add value to these wastes. The objective of this study was to determine the optimum extraction conditions and characterize a value-added product, the citric pectin of lime orange waste produced in Santana do Mundaú city, Alagoas, Brazil. These conditions were evaluated through a 2^3 factorial design, with the acid concentration, temperature and extraction time as interest variables and the yield extraction and the esterification degree as answers. Texture analysis and compression profile were also performed. The lime orange residue was dried for about 18 hours, with removal around 80% of moisture, presenting high carbohydrate content (80%) and 8% of pectin. In the experimental conditions studied, it was noticed that at higher heating time (90 min), acid concentration (6%) and temperature (90 °C) it was obtained the highest yield of pectin extraction, around 78 %, with an esterification degree of 8%, that indicates a low degree of pectin methoxylation. While in the highest conditions were generated low pectin content, in low income were obtained high pectin methoxylation. A reason for this esterification loss was the high acid concentrations used, associated with longer heating times. In the texture profile analysis and compression, it was found that the extracted pectins possessed similar characteristics to the commercial one, especially in friability, hardness and elasticity aspects. When grinding, the pectin lost most part of these characteristics, assimilating to a standard without pectin.

1. Introduction

The world population increase has caused a growing concern about the environment, especially in industrial and agricultural activities, with several studies and investments in clean technologies, resource efficiency, recycling and waste reuse (Uenojo and Pastore, 2007). Many fruit waste are studied as potential alternative pectin suppliers, adding value to waste and obtaining a product with similar characteristics to commercially used such as passion fruit (Pinheiro et al., 2006), gabiroba (Santos et al, 2009), guava (Munhoz et al, 2010) and cocoa (Chan and Chao, 2013). Among these technologies, extraction is considered the most reasonable process, due to the low operational cost, resulting molecules used as food additives or nutraceuticals (Shalini and Gupta, 2010).

Brazil is the third largest producer of fresh fruit in the world, with estimated volumes of 43.6 Mt (IBRAF, 2013). In the orange production, since the 80s, the country is the leader, with 25,2% of world production in the 2009/2010 harvest, generating 18 Mt fruit, followed by USA, China, India, Mexico, Egypt and Spain, In this harvest, 86 % of all Brazil's orange was used by industry, being 15% used for NFC (Not From Concentrate) and 85% for FCOJ (Frozen Concentrated Orange Juice) production. Compared to 1995/96 crop, the growth of processed fruit increased by 10%, verifying the great capacity of the Brazilian citrus industry (CitrusBR, 2010).

The citrus fruits residues, formed by pulp debris and membranes, as well as composite fractions of albedo and flavedo, possess 25-30% pectin, making it one of the primary sources of commercial pectin applications (Arslan and Togrul, 1996; Srivastava and Malviya, 2011). In 2002, the worldwide annual pectin consumption was approximately 45 Mt with a global market value of at least 400 million Euros (Savary et al., 2003).

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The pectin physicochemical properties depend mainly on the raw material and the selected conditions for its isolation and purification (Chan and Chao, 2013). Pectic substances are usually extracted by chemical or enzymatic methods, with a process of physical and chemical multiple stages, in which the hydrolysis, extraction and solubilization of macromolecules plant tissue are influenced by several factors such as temperature, pH, acid type and extraction time (Pagan et al., 2001).
In commercial terms, which there are characteristics and various applications, it is customary to classify the pectins in high methoxyl (HM), with values above 50% in esterified carboxylic groups, and low degree of methoxylation or esterification (LM), with results less than 50%. HM pectins are used in gel form for acid applications (2.0 <pH < 3.5) with the high presence of co-solute concentrations (55-75 %), as sucrose or sorbitol. LM pectins can form gels in the co-solutes absence, particularly sucrose, with the addition of divalent calcium ions at a higher pH range (2.0 < pH < 6.0) (Yapo et al, 2007).
This work aimed to characterize the lime orange processing residue obtained in the region of Santana Mundaú, Alagoas, Brazil, to evaluate the best extraction conditions from pectin of their albedos and characterize them from the texture analysis and viscosity.

2. Materials and Methods

The orange lime residues were kindly provided by COOPLAL (Cooperative of Lime Orange Producers Santana Mundaú-AL Ltda.). The collection, reception, cleaning and waste drying have been developed according to the previously work performed (Abud et al., 2007).
For waste characterization, it was determined the carbohydrate content (C), moisture (M), ashes (A), crude protein (P) and lipids (L), followed by analytical standards of Adolfo Lutz Institute (ALI, 2005) and AOAC (2002) The carbohydrate content was taken by the difference between the other constituents by means of Equation 1. The moisture was determined based on the product weight loss subjected to heating at 105 °C until constant weight. The ash content corresponds to the residue obtained by incineration at temperatures of 550 °C to obtain a clear light ash. The total lipids determination was performed directly in Soxhlet, with hexane as the extraction solvent. From the dry and defatted sample of the filter paper lipid analysis it was carried out the fibers analysis according Henneberg method, reported by Winton and Winton (1958). Crude protein was performed by Kjeldahl method, and 6.25 as factor for the protein nitrogen. The acidity was determined by neutralization titration with standard acid solution of alkali (0.1 N NaOH) and using phenolphthalein as indicator (IAL, 2005). The organic acid used was citric acid, with a molecular weight of 192 g/mol and 2 ionizable hydrogens. For the pectin determination was used the method proposed by Carvalho et al. (2002), in which the sample, suitably minced, it was slightly boiled in acid solution, which was solubilized in the amount of present pectin in the sample, followed by precipitation with calcium chloride, filtered, dried and weighed. The calorific value (E) was calculated by Equation 2 (Koziol and Pedersen, 1993).

$$C = 100 - \left[F + M + A + P + L\right] \quad (1)$$

$$E(kcal/100g) = 4\left[C + P\right] + 9L \quad (2)$$

The extraction process was performed according to methodology of Canteri-Schemin et al. (2005), with small modifications from the experiment designs. In a flask, the residue was placed in contact with the citric acid solution at a ratio of 1:50 (w/v), being taken to a heating mantle and temperature during the determined time in the experimental design. After contact time reflux, the system was filtered through cheesecloth and cooled in an ice bath, and it was then centrifuged for 20 min at 3,500 rpm. To the supernatant was added ethanol 92.8 °GL (1:2 v/v) and stirred for 10 min and then allowed to stand for one hour to allow the pectin precipitation. The precipitated pectin was separated by filtration, washed with absolute ethanol and dried in a forced air circulation oven at 50 °C to constant weight. Also the degree of esterification (DE) of pectins samples and it was measured from the potentiometric titration method, using HCl and NaOH. The treatment of the planning data was obtained from the Statistica 8.0 software, building up Pareto charts from variable efficiency and the significant esterification degree and performing ANOVA analysis parameters.
To optimize the extraction process, gels were prepared with pectin extracted and tests performed profile (TPA) and CT3 texturometer in compression. Viscosity measurement was performed according to ASTM D445-09 at 40 °C, where it was used a Ostwald viscosimeter and measure the kinematic viscosity in cSt/s, because each viscosimeter has their own fixed constant times the value of the time in measured seconds. For the gels preparation were used 0.3 g of pectin, 30 g of sucrose and 0.5 g of citric acid. Pectin was mixed with sucrose, taken over low heat and added 20 mL of distilled water. After full dilution and cooling, was added citric acid. The gels were kept in the refrigerator for 24 h before the usage.

3. Results and Discussions

The drying residue lasted about 18 h with removal of approximately 80 % humidity. To reduce and standardize the particles, providing greater surface area, the residues were ground in a slicer type Willye in 30 mesh sieve. By physicochemical characterization present in Table 1 it can be seen that there is in the lime orange residue a high carbohydrate content, which 10 % is composed of pectin.

Table 1: Physical and chemical characteristics of dehydrated waste to 50 °C and milled.

Moisture (%)	Ashes (%)	Lipids (%)	Proteins (%)	Total carbohydrate (%)	Pectin (% calcium pectate)	ART (g/100g)	Caloric value (kcal/100g)
10.05±0.10	3.46±0.07	4.93±1.98	3.38±0.09	78.17±1.30	8.04±0.55	42.52±6.46	3.70

The pectin yield extraction was determined following the 2^3 factorial design, with 3 replications at the central point, with the variable extraction temperature, the citric acid concentration and the time reaction totaling 11 assays, and mass yield of extracted pectin and degree of esterification (DE) as responses (Table 2).

Table 2: Conditions and experimental design responses for pectin extraction.

Assay	Planning			Yield (%)	DE (%)
	T (°C)	C (%)	T (min)		
1	50	2	10	10.38	35.52
2	50	2	70	16.02	20.81
3	50	6	70	5.65	64.66
4	50	6	10	7.47	85.24
5	90	6	70	47.63	7.16
6	90	6	10	34.97	4.72
7	90	2	10	18.20	39.08
8	90	2	70	30.96	11.96
9	70	4	40	23.82	19.42
10	70	4	40	20.40	6.03
11	70	4	40	14.60	25.77

The average yields of extracted pectin from lime orange flour ranged between 5.6 and 47.6%, with higher values observed in experiments 5, 6 and 8, all with the same temperature, 90 ºC. Experiments 1, 3 and 4 had the lowest yields, running at 50 ºC. The data showed that the experiment 5 performed better than the others, indicating better efficiency for changes in higher concentration, time and temperature (6%, 70 min, 90 ºC). Similar results were reported by other authors with different raw materials. Kliemann et al. (2005), extracting pectin from the passion fruit skin at temperatures, times and acid concentrations of 40 to 90 °C, 10 to 90 min and 1 to 5%, respectively, observed that the best condition occurred with 5% of citric acid, 90 minutes and extraction at 90 ºC. Santos et al. (2009) extracted about 31% from the present pectins in gabiroba using 5% citric acid in 60 minutes of heating.

According to the presented data in the planning with lime orange flour, it can be observed an inverse relation between yield and degree of esterification. To the yield lowest experiments (3 and 4), the pectins had higher esterification degree than 50% (64.66 and 85.24, respectively) being ranked as high methoxyl pectin content.

Pinheiro et al. (2006) studying the removal of passion fruit pectin, in citric acid concentrations of 0.086 to 2.9 % and 10-102 min, found that lower concentration of acid after 60 minutes of heating, generated a pectin with high methoxyl content, with an acid concentration the main factor affecting the extraction of pectin, exerting significant influence on the esterification degree. Chao and Chan (2013), using waste from cocoa industry, able to extract between 3.38 and 7.62% of pectin at temperatures from 50 to 95 °C, 1.5 to 3 h, solids concentration 1:10 to 1:25 (w/v) and citric acid pH's in the range of 2.5 to 4.0, checking that at 95 ºC, 3 h and 1:25 (w/v) it was possible to extract more pectin, characterized as low methoxyl grade, indicating that the temperature has a positive factor in solubilizing these pectins as well as the highest concentration of citric acid. Munhoz et al. (2010), extracting pectin from dehydrated guava to time 35-95 min heating and citric acid concentration in the range from 3.5 to 7% observed that the acid concentration and longer extraction has positive influence on the removal of pectin which possessed a low esterification degree (40 %), while the esterification was of high commercial (about 72%).

The fact that at higher acid concentrations, it can be obtained a low methoxylation degree of pectin may be related to the fact that, by combining a high concentration of acid long heating time, accelerates the esterification process (Chan and Chao, 2013). These results can be summarized in Table 3 and Figure 1, by means of variance analysis parameters, Pareto charts and response surface, which shows that the interaction and temperature of concentration with temperature significantly influence the level of 95% , within the ranges studied. For mass yield of extracted pectin, such parameters have a positive effect, while for the degree of

esterification, negatively. The higher extraction yields generated low esterification degree of pectins, whereas smaller values, a high esterification degree, confirming the role of the variables in determining the esterification characteristics of extracted pectin.

Table 3: Analysis of variance from factorial design for the pectin extraction.

Treatment	Variables	Regression Coefficient	R^2	Effect	t (3)	p-value
Mass yield (%)	Average*	42.38917	0.96894	20.80889	16.71419	0.000466
	(1)Temperature (°C) (L)*	-0.81867		23.06000	7.97074	0.004120
	Temperature (min) (Q)	0.00451		-1.80333	-0.65104	0.561439
	(2) Concentration of solids (%) (L)	-8.33000		5.04000	1.74209	0.179856
	(3)Time (min) (L)	-0.13017		7.31000	2.52672	0.085670
	1 L x 2 L*	0.14600		11.68000	4.03722	0.027337
	1 L x 3 L	0.00450		5.40000	1.86652	0.158800
	2 L x 3 L	-0.01575		-1.89000	-0.65328	0.560176
Esterification degree (%)	Average	177.0986	0.94572	28.1203	8.43603	0.003496
	(1)Temperature (°C) (L)*	-5.1246		-35.8275	-4.62528	0.019029
	Temperature (min) (Q)	0.0414		-16.5704	-2.23434	0.111546
	(2) Concentration of solids (%) (L)	30.4611		13.6025	1.75606	0.177338
	(3)Time (min) (L)	-0.6020		-14.9925	-1.93551	0.148363
	1 L x 2 L*	-0.4148		-33.1825	-4.28381	0.023374
	1 L x 3 L	0.0022		2.6525	0.34243	0.754612
	2 L x 3 L	0.0494		5.9225	0.76459	0.500157

* statistically significant factors (95 % of confidence)

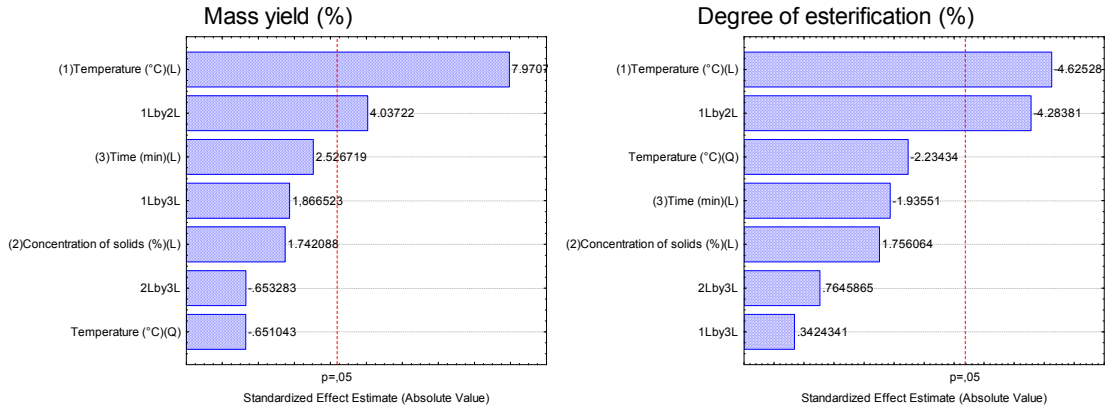

Figure 1: Pareto charts for mass yield and esterification degree.

For viscosity and texture analysis, it was necessary the preparation of gels, differing among themselves with the type of pectin used. For the analysis in texturometer were measured profile test (TPA) and compression. The texture, along with the appearance and taste, are considered quality attributes, which establish the acceptability of food by the consumer (Mohsein, 1986). For the formulations studied gels used were extracted pectin from lime orange albedo and stored for a long period (Old), freshly extracted (New), freshly extracted and crushed to form a powder (milled), commercial pectin (Commercial) and gels prepared with no pectin (absent). The viscosity and texture attributes of each of the five gels formulations are shown in Table 4, according to data provided by the texture profile analysis (TPA) and compression. The texture analysis were performed using a texturometer Brookfield CT3 with stainless steel cylindrical probe.

The texture profile analysis (TPA) shows that pectin gels, old and new, presented with features resembling commercial pectin no significance differences in adhesion and 9% significance in hardness. Sugar is one of those responsible for the ideal texture of fruit candies. When this ingredient is reduced or even excluded from the formulation, the product tends to exhibit brittle texture (Licodiedoff, 2008). As for the adhesive, it can be perceived a greater result with the absence of pectin.

According to Huang et al. (2007), the adhesion depends on the combined effect of cohesion and adhesion forces, showing in this way, the absence of pectin. The adhesiveness and firmness of fruit-banana gels after 165 days of storage decreased, possibly by increasing water activity and increase in acidity (Dias et al., 2011). For compression testing, it was found that the old pectin gel showed the greatest similarity to the commercial pectin gel, especially in friability and elasticity criteria. It was also possible to observe extreme similarity in the results of friability and hardness, showing thus a direct relationship between the two criteria. According to Fennema (2010), concentrations of sugars up to 60% act as dehydrating agents, favoring the formation of

fibrillar networks between pectin and water, increasing the gel hardness. Thus, the higher hardness of the candy, the higher fracture obtained. The tackiness is a secondary parameter derived from a hardness and cohesiveness product, generally having low hardness and a high cohesion degree (Bourne, 2002). The chewability, a secondary attribute of texture, which is evaluated by the chewed number needed to leave the food in a position to be swallowed, showed high positive correlation with the hardness (Silva et al., 2009), demonstrating the gel firmness as resulting of the high sucrose. It is noticed that the milled pectin increased its hardness and decreased its adhesiveness, elasticity, cohesiveness, tackiness and chewiness when compared to other pectins.

Table 4: Results of the texture profile analyzes of gels test and compression.

	TPA					Compression				
	Old	**New**	**Commercial**	**Milled**	**Absent**	**Old**	**New**	**Commercial**	**Milled**	**Absent**
Fracturability (g)	11.9	4.45	5.41	50.70	38.98	3.98	35.23	5.12	49.29	43.26
Adhesiveness (mJ)	0.013	0.103	0.068	0.001	0.232	0.056	0.035	0.119	0.128	0.073
Elasticity (mm)	0.16	-0.07	0.34	0	0	0.05	0.14	-0.37	49.29	43.26
Hardness (g)	11.9	4.45	5.41	50.70	38.98	3.98	35.23	5.12	49.29	43.26
Cohesiveness	0	-5.52	897.48	0	-69.32	-	-	-	-	-
Tackiness (g)	0	-24.58	4855.39	0	0	-	-	-	-	-
Chewability (mJ)	0	0.017	16.189	0	0	-	-	-	-	-

The viscosity results are shown in Table 5, where it is noticed that the milled pectin loses its ability to good viscous properties, reaching a similar value to the gel without pectin, while the former pectin gel prepared with increased viscosity, a fact that also occurred with the fracturability and tackiness. It was not possible to perform this analysis with the commercial pectin gel, because it performed extremely gelatinous and viscous, which may cause clogging device.

Table 5: Kinematic viscosity analysis in gels.

	New	**Old**	**Milled**	**Absent**
Viscosity (cSt/s)	57.38	89.79	32.68	31.98

4. Conclusions

The lime orange residue showed high carbohydrate level (80%) and 8% of pectin. From the experimental conditions studied in the planning, it was noticed that higher heating time (90 min), acid concentration (6%) and temperature (90 ºC) resulted in higher yield of pectin extraction (78%), with 8% of esterification, characteristic pectin value with a low methoxylation degree. Low extraction yields generated pectin with a high methoxylation degree. A reason for the esterification loss was the use of high acid concentrations associated with longer heating times. In the analyzes of texture and compression profiles were realized that extracted pectins, possessed similar characteristics to commercial pectin, mainly in friability, hardness and elasticity, in the grinding aspects, the pectin loses many of these characteristics, assimilating to the standard without pectin.

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