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Optimization of the inulin aqueous extraction process from the açaí (*Euterpe oleracea,* Mart.) seed

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Abstract

Inulin is a resistant fructooligosaccharide, synthesized by a wide variety of plants. Due to the importance of fibers intake and the high amount of residues of açaí, the objective of this study was to optimize the inulin extraction process from açaí seed flour (ASF) and subsequently, from this biomass, to develop inulin purification technology. Açaí seeds were dehydrated in a ventilated oven. Aiming the inulin extraction, from ASF, an aqueous extract was elaborated. It went through several stages of centrifugation and filtration with alcohol, acetone and water, until the constitution of a precipitate that was lyophilized. The analysis of the solvent concentrations effects and the precipitation temperatures on the responses was performed using the Designer Expert Program. A model with a response surface and contour was generated, allowing temperature, time and water: ASF relation selection, what provided the highest inulin concentrations, in other words, the extraction treatment with 80 °C and proportion of water: ASF of 4:1, for 20 minutes, presented the best performance. Although this extraction output is inferior when compared to other sources, it must be considered as relevant, since the açaí seed is evaluated as a waste product.

Keywords: fiber; residues; fructooligosaccharide.

Practical Application: Optimization of the inulin aqueous extraction process.

1 Introduction

The açaí palm (*Euterpe oleracea*, Mart.) is a typical palm tree from Amazônia, with natural occurrence in the states of Pará, Amapá, Maranhão and east of Amazonas (Amazonas, 2003; Tavares et al, 2017). The açaí palm fruit is a round drupe, circumference of about 1-2 cm and an average weight of 1.5 grams (Siqueira et al., 2017). The epicarp can vary between purple and green when ripe. The mesocarp, which together with the epicard corresponds to 7% of the fruit, surrounds the voluminous and hard endocarp, is responsible for the fruit shape, and holds the seed. What is popularly known as seed is the pyrene, since the seed is still surrounded by the endocarp (Nascimento et al., 2008; Cavalcante, 2010).

Currently, the production of açaí runs around 1.1 million of tons per year and the consumption of its pulp has been rising. However, the residue, constituted of seeds and thick layers, generated during the pulping process, corresponds to more than 90% of the fruit volume produced. Despite its lignocellulosic composition, this residue is commonly left, irregularly, in the natural environment, becoming harmful when discharged along the water sources, due to a reduction of dissolved oxygen tax in the water and eutrophication (Lima, 2007; Martins et al., 2009; Maranho & Paiva, 2012; Pereira & Rodrigues, 2013; Instituto Brasileiro de Geografia e Estatística, 2017).

Inulin is an important storage carbohydrate in plants. It belongs to the fructane group and is synthesized by a variety of plants, approximately 36.000 species, that represent 10 different families (Roberfroid, 1993; Gibson & Roberfroid, 1995; Roberfroid et al, 1993). It is a fructooligosaccharide with a molecular configuration that makes it resistant to the intestinal and salivary enzyme activity, reaching the colon completely intact. Therefore, it is considered a prebiotic (Bahia, 2005; Rosa & Cruz, 2017).

Traditionally, inulin extraction has been based in methods that include washing, slicing and milling of tubers; inulin extraction with water; extract treatment with carbon dioxide and lime; filtration and inulin recovery by precipitation and evaporation (Laurenzo et al., 1999). This aqueous inulin extraction method is based on inulin water solubility that is temperature highly dependent. As the temperature decreases the solubility also reduces: at 10 °C it presents a solubility of 6%, while at 90 °C it increases to 35% (Silva et al., 2008). Therefore, several researches have been held aiming the optimization and development of inulin extraction methods from different

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sources (Carvalho et al., 1998; Park et al., 2000; Leite et al., 2002; Park et al., 2003; Oliveira et al., 2004; López-Molina et al., 2005; Silva et al., 2008; Galante, 2008).

Cichorium intybus, belonging to *Composit*ae family, popularly known as chicory, is the only plant species, until now, that has been industrially used for the extraction of inulin (Roberfroid, 2005). This major source is not found in Brazil, consequently, there is a high demand to obtain new sources from Brazilian food products, in particular by-products residue. Therefore, the objective of this research was to optimize the inulin extraction process from açaí seeds using the aqueous hot extraction method, and subsequently, from this biomass, to develop an inulin purification technology.

2 Materials and methods

2.1 Raw material

The Açaí seeds were supplied from a rural property in Pará, place where the fruit was pulped, sun-dried and conditioned in cardboard boxes. Subsequently, the material was transported to the Food Processing Laboratory (LAPAL-Laboratório de Processamento de Alimentos) at the Institute of Nutrition Josué de Castro (Instituto de Nutrição Josué de Castro (INJC)) – UFRJ.

2.2 Açaí Seed Flour (ASF) production

Firstly, the açaí seeds were sanitized with potable water and dehydrated in a ventilated oven at 60 °C for 40 hours. In the sequence, they were grinded in a hammer mill (TECNAL TE-360) and the obtained flour was conditioned in plastic bags of nylon and polyethylene, holding high protection against water vapor and atmospheric oxygen. After being vacuum sealed they were labeled and stored under refrigeration.

2.3 Inulin extraction

Inulin extraction was conducted according to a methodology determined in advance at LAPAL, INJC/UFRJ, adapted from Hauly et al. (1992), Fontana et al. (1994) and Grzybowski (2008).

The previously prepared açaí seed flour (ASF) was homogenized in an L-40 Mondial Blender with distilled water. Next, the suspension was heated and under agitation of 2.500 rpm, the pH was adjusted between 6.5 and 7.0 with a phosphate buffer solution (pH=7). Thereafter, the solution was clarified through consecutive vacuum filtrations in cotton filters to hold the insoluble cellulose fibers and subsequently in layers of diatomaceous earth.

Toluene drops, bacteriostatic and fungistatic, were added to the filtered solution, which was stored in a cold chamber (-18 °C) for 72 hours to precipitate the higher molecular mass polydisperse fraction. Afterwards, the sample was centrifuged and the supernatant was treated with two volumes of anhydrous ethanol to precipitate (temperature of 8 °C) the lower molecular mass fraction.

The two precipitates, after addition of acetone, were centrifuged and assembled composing the crude inulin (a light cream powder) that when dissolved again in hot water was rapidly filtered (vacuum) through a layer of DEAE-cellulose to retain colored pigment, acid heteroxylans and any other component of anionic character or susceptible to adsorption in derivatized cellulose.

The percolate was precipitated again with two volumes of anhydrous ethanol and centrifuged. The inulin mass was frozen and subsequently dried in a lyophilizer, constituting the purified inulin (whitish powder). The obtained inulin was then transferred by an ABNT/ASTM 200 sieve and stored in glass flasks.

2.4 Inulin extraction process optimization

Experimental planning

Aiming the assessment of the process variables influence on the inulin production, the experimental tests were conducted according to the factorial planning, including 6 duplicated central points (level 0), 8 factorial points represented by the vertices of the cube and 6 axial points, totalizing 20 tests (Table 1).

Table 1. Inulin extraction experimental planning from açaí seed (coded levels).

TESTS	TEMPERATURE (°C)	CONCENTRATION (WATER: ASF)	TIME (Min)
1	60	08:01	10
2	100	08:01	10
3	60	02:01	10
4	100	02:01	10
5	60	08:01	30
6	100	08:01	30
7	60	02:01	30
8	100	02:01	30
9	60	04:01	20
10	100	04:01	20
11	80	08:01	20
12	80	02:01	20
13	80	04:01	10
14	80	04:01	30
15	80	04:01	20
16	80	04:01	20
17	80	04:01	20
18	80	04:01	20
19	80	04:01	20
20	80	04:01	20

Time evaluation at 10, 20 and 30 minutes.

The studied variables were based on the solvent/substratum proportion and ASF cooking temperature and time.

Variable 1: solvent/substratum proportion, water volume per gram of ASF sample. Evaluation of 8:1; 4:1 and 2:1 proportions.

Variable 2: inulin extraction temperature. Temperature evaluation at 60, 80 and 100 °C.

Variable 3: Predetermined temperature maintenance time during inulin extraction.

The analyses were based on the solid precipitation mass production. Each test used 100 g of ASF.

Effect analysis of solvent concentration and precipitation temperature on the results were performed through the response surface methodology of the Designer Expert program. The variables that presented $p< 0.05$ were considered significant.

2.5 Mass production from different inulin extraction tests

The obtained inulin precipitate from the different tests were classified according to the calculated mass produced as the ratio between the total solid mass in the precipitate and the total solid mass in the concentrated extract that was centrifuged. Each test used 100 g of ASF.

3 Results and discussion

The Experiment Planning structure is important to validate the processes through documented evidence capable of assuring that a specific process will consistently generate a product according to specifications and characteristics of predetermined quality (Silva & Sant'Anna, 2007).

According to the obtained results of the 20 different inulin extraction tests using ASF, it was possible to observe a variation of 0.003-4.6 g/kg ASF (Table 2). Test 15 (80 °C, 4:1 water proportion: ASF, for 20 min) reported the best performance.

The best obtained model was the quadratic one, as shown in the following Equation 1:

$$ln\left(yield\right) = -1.77537 - \left(0.16258*T\right) + \left(0.25366*C\right) + \left(0.54471*t\right)\left(+9.29681E-004*T\ 2\right) - \left(2.31101E-003*c\ 2\right) - \left(0.020894*t\ 2\right) - \left(1.60107E-003*T*c\right) + \left(2.98168E-003*T*t\right) + \left(1.14251E-003*c*t\right) \quad (1)$$

In this adjustment, the considered production range was of 0.039 until 4.75605, that is, all the tests were considered (Table 3).

The response surface charts (Figure 1 and 2) demonstrate that the higher performance was obtained with a temperature of 80 °C, ASF concentration of 12% and extraction time of 20 minutes. The quadratic model with lambda equals to zero shows the interactions of temperature, time and ASF concentration. During the inulin extraction process from ASF, it was necessary to double the volumes of ethanol and acetone to precipitate the high and low molecular weight inulin, what increased the process costs and time. It is also important to highlight that the inulin extraction was higher when the extraction temperature was between 80 and 100 °C, obtaining a darker product, probably due to the caramelization of carbohydrates implicated in the process. This fact can directly influence the final acceptance of the product.

Table 2. Performance of Inulin extraction from ASF in 20 different tests.

Test	Production (g/1000 g of ASF)
1	0.21
2	0.38
3	1.20
4	0.04
5	0.05
6	0.20
7	0.14
8	0.24
9	3.22
10	3.74
11	0.90
12	1.20
13	0.21
14	0.42
15	4.76
16	1.48
17	3.17
18	2.11
19	1.26
20	2.69

The obtained results were inserted in the *Designer Exp*ert program to optimize the process. The statistical solutions proposed are at Table 4.

The efficiency of the inulin extraction process from ASF was of approximately 4.5%, a higher percentage than the one found by Godoy & Wagner (2017) who extracted inulin from chicory roots in an autoclave. Nogueira (2002) obtained an extraction efficiency of 0.70% from inulin powder, using chicory. Oliveira et al. (2004) researched inulin extraction from chicory and achieved a mass extraction of 0.90%. Dalonso et al. (2009) reached 2.65% of inulin extraction from garlic. Leite et al. (2004) observed a 40.8% efficiency for inulin extraction from chicory roots that were extracted through hot diffusion, concentrated by evaporation and precipitated through temperature reduction. Moreover, Cataldo et al. (2005) obtained, approximately, an efficiency of 10% in the inulin extraction from chicory with supercritical carbon dioxide. Toneli et al (2006) extracted (moist) inulin from chicory roots and observed an efficiency of 61%. Silva et al. (2008) extracted inulin with hot water from chicory roots and observed an inulin concentration of 38%. The results obtained in this research extracting inulin from ASF, despite lower than in other studies, has its significance because the seeds of açaí constitute 93% of fruit. Therefore, a better usage of this residue for the production of higher commercial value food product is of extreme importance.

Table 3. Statistical diagnosis for the quadratic model, lambda equal to zero.

Factors	Estimated coefficient	GL	Standard error	Estimate by interval (95%)	
				Inferior limit	Superior limit
Mean	0.86	1	0.19	0.42	1.29
A-TEMPERATURE	-0.077	1	0.18	-0.48	0.32
B-SEED CONCENTRATION	0.098	1	0.18	-0.30	0.50
C-TIME	-0.17	1	0.18	-0.57	0.23
A2	0.37	1	0.34	-0.39	1.13
B2	-0.83	1	0.34	-1.60	-0.072
C2	-2.09	1	0.34	-2.85	-1.33
AB	-0.61	1	0.20	-1.06	-0.16
AC	0.60	1	0.20	0.15	1.04
BC	0.22	1	0.20	-0.23	0.66
AB	-0.61	1	0.20	-1.06	-0.16
AC	0.60	1	0.20	0.15	1.04
BC	0.22	1	0.20	-0.23	0.66

Temperature (A), Seed concentration (B) and Heating time (C). GL = degree of fredom.

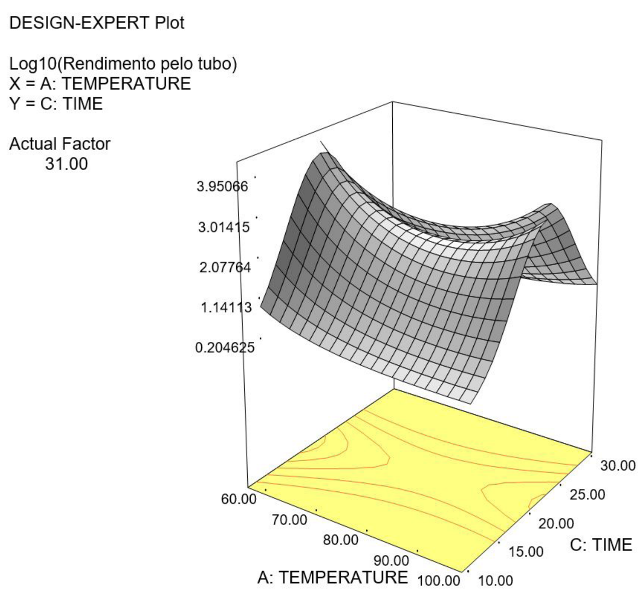

Figure 1. Response surface for inulin production according to Temperature (X=A) and Heating Time (Y=C). Log10.

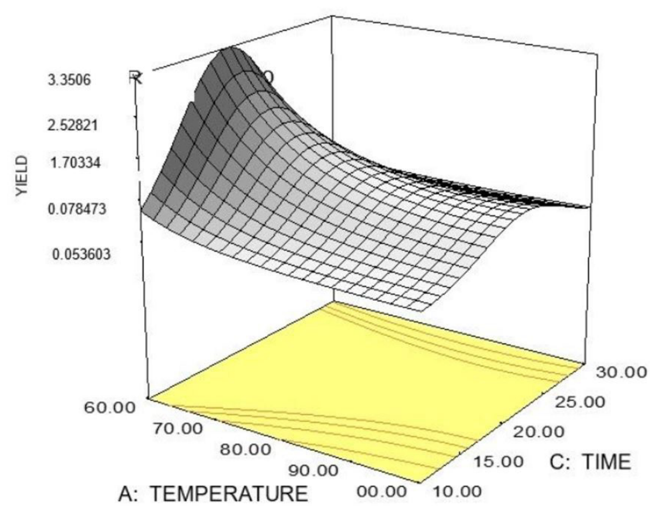

Figure 2. Response surface adjusted for inulin production according to Temperature (X=A) and Heating Time (Y=C). Optimization of the process, temperature of 60 °C and Seed Concentration of 31%. Log10.

Table 4. Optimizaton of inulin extraction process from açaí seed flour.

Optimized treatment	Temperature (°C)	ASF concentration	Time (Min)	Expected production (g/1000 g ASF)
1	60	38.65	18.36	4.51918
2	60	38.80	18.42	4.51877
3	60	39.30	18.91	4.48958
4	60	39.27	19.76	4.34195

ASF = açaí seed flour.

4 Conclusion

The process employed to obtain ASF generated a high extraction of flour from açaí seeds. The experimental planning used to optimize the inulin extraction process was adequate and presented satisfactory results.

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