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APPLICATION OF EXPERIMENTAL DESIGNS TO EVALUATE THE TOTAL PHENOLICS CONTENT AND ANTIOXIDANT ACTIVITY OF CASHEW APPLE BAGASSE

APLICACIÓN DE MODELOS EXPERIMENTALES PARA EVALUAR EL CONTENIDO DE FENÓLICOS TOTALES Y LA ACTIVIDAD ANTIOXIDANTE DEL BAGAZO DE LA MANZANA DEL CAJÚ

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Abstract

Cashew apple (*Anacardium occidentale* L.) bagasse is a waste from the production of cashew apple juice and is generated in large quantities by the pulp juice industry. This is the first time in which such conciliation between cashew apple bagasse and antioxidant total phenolics content was performed. Thus, such a study may provide alternatives for the use of this biomass. The process conditions for the extraction of total phenolics were defined using a centroid-simplex mixture design to find the optimal concentrations of the solvents. Next, a three-level factorial experimental design was applied to evaluate the influence of the variables time and extraction temperature. The best combination of the variables for increasing the yield of total phenolics content and antioxidant activity was obtained with 60% water, 30% acetone and 10% ethanol for 2 h, at 35°C. The yield maximum of total phenolics content found was 343.34 ± 34.76 mg GAE/100 g. The antioxidant capacities were determined by the DPPH and ABTS methods were $5.170.91\pm305$ g/g of DPPH and $13.6\pm1.8 \mu$ M Trolox/g respectively. The study revealed that cashew apple bagasse is a rich source of antioxidants and can be considered an important alternative natural biomass which is cheap for application in bioprocesses.

Keywords: cashew apple, total phenolics, antioxidant activity, biomass, experimental designs.

Resumen

El bagazo de la manzana de cajú (*Anacardium occidentale* L.) es un residuo del proceso de producción del jugo de cajú. Un estudio sobre el contenido de fenólicos totales antioxidantes de ese residuo puede apuntar alternativas para un mejor uso de esa biomasa. Las condiciones experimentales fueron definidas utilizando una planificación experimental de mezcla centroid-simplex para encontrar las concentraciones óptimas de los disolventes. A continuación, se aplicó una planificación experimental factorial de tres niveles para evaluar la influencia de las variables tiempo y temperatura. Las mejores combinaciones de las variables para encontrar un rendimiento óptimo para el contenido de fenólicos totales y la actividad antioxidante se obtuvieron con un 60% de agua, un 30% de acetona y un 10% de etanol, durante 2 h a 35 °C. El rendimiento máximo encontrado para el contenido de fenólicos totales antioxidantes determinadas por los métodos DPPH y ABTS fueron $5.170,91\pm305$ g/g de DPPH y $13,6\pm1,8$ μ M Trolox/g, respectivamente. El estudio reveló que el residuo del bagazo de la manzana del cajú es todavía una fuente rica de antioxidantes y puede ser considerada una importante biomasa natural alternativa y barata para aplicación en bioprocesos.

Palabras clave: manzana del cajú, fenólicos totales, actividad antioxidante, biomasa, planificación experimental.

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1 Introduction

Cashew (*Anacardium occidentale* L.) is an important tropical tree crop native to Brazil, which consists of about 75 genera and 700 species; nowadays, it is widely grown in tropical regions of South, Central and North America as well as in Asia and Africa (de Figueirêdo *et al.*, 2015). The cashew apple is an edible pseudo-fruit developed from the peduncle and is nutritive, juicy and astringent. According to the Food and Agriculture Organisation of the United Nations (FAO, 2017), the world production of cashew apples in 2016 amounted to 2,001.301 metric tons; its production is concentrated in the tropical region of the globe and is widespread in Brazil, India, Mozambique, Tanzania, Kenya, Vietnam, Indonesia and Thailand (Betiku *et al.*, 2016).

The highest production of cashew apples was recorded for Brazil, reaching a total of 1,805.000 tons, which is equivalent to more than 90% of the total world production in 2016. In Brazil, cashew apples have socioeconomic relevance because about 10% of this production, which represents around 180.500 t/year is aimed at agribusinesses of cashew pulp juice, considered one of the most popular juices, and is widely accepted by the population, due to its mild, astringent taste (FAO, 2017).

Moreover, cashew apples and their products contains significant amounts of phenolics compounds generally related as antioxidant (Adou *et al.*, 2012), which play an important role in maintaining human health, since they have a preventive effect against various types of diseases such as cancer, cardiovascular diseases, neuropathies and diabetes (Amara *et al.*, 2015; Parihar *et al.*, 2015; Bagchi *et al.*, 2014; Brito *et al.*, 2007). Salicylic acid, p-cumaric acid and gallic acid are the main phenolics acids found in cashew apples (Trevisan *et al.*, 2006). Indeed, phenolics of food plants have been reported to offer biological benefits, such as a reduced risk of cancer and cardiovascular disease (Honorato *et al.*, 2007).

Cashew apple bagasse is a large source of waste (90-94%) produced by the cashew juice industry (de Albuquerque *et al.*, 2015; Furtado *et al.*, 2014). Such waste, consisting of seeds, peels, husks, and whole pomace, among others, is generated every year and is poorly valued, left to decay on the land or used only as a nutritional supplement in animal feed (Mussatto *et al.*, 2011). This limited use is a result of its rapid degradation, which makes it impossible

to store, thus causing serious environmental problems. The insufficient collection and improper disposal of this waste may generate also a significant loss of biomass which could potentially be used to produce various value-added metabolites. Despite the abundant literature on phenolics content in food, it is the first time that a study involving the biomass potential of the cashew apple bagasse is reported.

Recently, increased attention has been given to these materials as abundantly available and cheap renewable feedstock for the production of valueadded compounds such as polyphenols (Lee *et al.*, 2013; Plaza *et al.*, 2014). The phenolics compounds confer antioxidant, antibacterial, anti-mutagenic, antiinflammatory and anticancer activities (Jiang-ke *et al.*, 2016; Mojzer *et al.*, 2016; Chang *et al.*, 2016; Dinica *et al.*, 2015; Song *et al.*, 2015; Sánchez-Rangel *et al.*, 2014; Tuberoso *et al.*, 2013). As a result, cashew apple bagasse may have great potential for bioprocess to obtain fermented products with high economic value.

The chemical composition of the sample, the polarity of the solvents involved in the process, and time and temperature are the crucial parameters affecting the extraction yield of the total phenolics. Complete extraction is the goal to be achieved, but this is sometimes compromised by the ability of the solvent to fully extract all of the compounds (Palma *et al.*, 2015). A combination of solvents, involving aqueous, alcoholic, ketonic, etc. systems, under different conditions of interactions with the matrices, has been mentioned for the extraction of phenolic compounds in various plant structures (Imeh and Khokbar, 2002). In such cases, mixtures of alcohol-water or acetone-water are suggested (Stalikas, 2007).

Experimental designs combined with response surface methodology (RSM) are a statistical technique to determine the optimum values of independent variables, in order to achieve maximum response and enable the user to investigate the interaction of individual variables, which is considered more efficient than the traditional single parameter optimisation because of time, space, and raw material savings (Altemimi *et al.*, 2016; Flores-Martínez *et al.*, 2016; Wang *et al.*, 2015; Gupta and Sood, 2015; Sousa *et al.*, 2014). Therefore, the presence of total phenolics content and antioxidant activity in cashew apple bagasse was investigated in this study using experimental designs combined with RSM as the optimisation technique.

2 Materials and methods

2.1 Chemicals and reagents

Gallic acid monohydrate (98 %), sodium carbonate P.A, Folin & Ciocalteu's phenol reagent, DPPH (2,2diphenyl-1-picrylhydrazyl), ABTS, 2,2 azino bis (3ethylbenzo thiazoline 6 sulfonic acid), diammonium salt and Trolox (2,5,7,8-tetramethylchroman- 2carboxylic acid) were purchased from Sigma-Aldrich (São Paulo, SP, Brazil). Acetone P.A., distilled water and ethanol 95% P.A. were obtained from Vetec (Rio de Janeiro, RJ, Brazil). Methanol 99.8% P.A. was purchased from Chemis (São Paulo, SP, Brazil).

2.2 Sample

The sample of cashew apple bagasse employed for the development of the extraction method came from a local industry (Frutisol, Jequié, Bahia, Brazil). Bagasse was dried in a drying oven (Solab SL 102, Piracicaba, Brazil) at 50°C for 72 h and ground in a Wiley knife mill (ACB Labor, São Paulo, Brazil) with a 20-mesh size screen until reaching granule size of 2 mm. The powder obtained was stored at room temperature until further analysis.

2.3 Optimization for the extraction total phenolics content

Two experimental designs were carried out for the extraction of total phenolics content present in cashew apple bagasse. The first, involving a mixture planning, evaluated the influence of the proportions of the solvents, ethanol, acetone and water, setting the time and temperature for extraction. Then, a 3-level factorial was applied to evaluate the influence of the time and temperature variables on the extraction process.

A second-order polynomial equation was used to fit the experimental data of the studied variables. The generalised second-order polynomial model used in the response surface analysis is shown in Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^k \sum_{j=i+1}^k \beta_{ij} X_i X_j \quad (1)$$

where *Y* is the predicted response, *k* is the number of variables, β_0 , β_i , β_{ii} and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and X_i , and X_j

are the independent variables (Bruns *et al.*, 2006). The optimised conditions of the independent variables were further applied to validate the model, using the same experimental procedure as reported previously. Triplicate samples of the optimised proportion were prepared and analysed.

2.4 Centroid-simplex mixture design

In order to find the optimum concentrations of the solvents ethanol, acetone and water in the extraction of total phenolics present in cashew apple bagasse, we used the planning of centroid-simplex mixture. The amount of sample used for each experiment was 10 g from the same source, from the same batch. The extraction time was set at 2 h, extraction temperature at 25°C, stirring speed at 200 rpm, centrifugation rate at 5,000 rpm (I 206, equipped with ST-720m rotor, Tecnal, São Paulo, Brazil) and 50 mL extraction solution containing the solvents ethanol, acetone and water. The design consisted of 12 experiments, with 3 replicates at the central point. The results were analysed through the response surface graph to obtain a region representing the optimum concentrations of the solvents for maximum extraction of the total phenolics.

2.5 Three levels factorial experimental design

A level of factorial planning was applied to evaluate the influence of the extraction time and temperature variables, at 3 levels. The design was composed of 11 experiments with three replicates at the central point. The levels of the variables were: extraction time (1, 2 and 3 h) and temperature (25, 35 and 45°C). The results were analysed through the response surface graph to obtain a region representing the optimal time and temperature for maximum extraction of the total phenolics.

2.6 Determination of total phenolics content

The total phenolics contents were determined according to the adapted Folin-Ciocalteu method (Rebayal *et al.*, 2014). Briefly, the extracts (0.5 mL) were mixed with 2.5 mL of Folin-Ciocalteu reagent (1:10) and 2 mL of sodium carbonate solution (4%). The mixture was stirred in a shaker incubator (Solab, SL 222, Piracicaba, Brazil); after stirring, the extract was centrifuged (I 206, equipped with ST-720m rotor,

Tecnal, São Paulo, Brazil) at 5,000 rpm at 25°C for 10 min and kept at 25°C for 2 h in the dark. The sample absorbance was measured by spectrophotometer (UV-340G, Gehaka, Sao Paulo, Brazil) at 750 nm against a blank. Aqueous solutions of Gallic acid were used as a standard and its calibration curve was used to extrapolate the total phenolics content present in the sample. The results were expressed as milligrams of Gallic acid equivalents per 100 gram of waste (mg GAE/100 g). All measurements were performed in triplicate.

2.7 Determination of antioxidant activity

The antioxidant activity was determined using the DPPH and ABTS methods. DPPH was estimated using the method of Berset et al. (1995) with minor modifications. The solution of DPPH (0.06 mM) was diluted with ethanol to obtain an absorbance of 0.70 \pm 0.02 units at 517 nm. The sample extracts (0.1 mL) were allowed to react with 3.9 mL of the DPPH radical solution for 30 minutes in the dark, and the decrease in absorbance from the resulting solution was monitored. The absorbance of the reaction mixture was measured using a spectrophotometer (UV-340G, Gehaka, São Paulo, Brazil) at 517 nm. All assays were carried out in triplicate. The results were expressed as EC₅₀ (g/g of DPPH), corresponding to the sample concentration which reduced the initial absorbance of DPPH at 50%. For the ABTS assay, the procedure followed the method of Evans et al. (1999) with minor modifications. The ABTS radical cation (ABTS⁺⁻) was generated by the reaction of 5 mL of aqueous ABTS solution (7mM) with 88 μ l of 140 mM potassium persulphate (2.45 mM final concentration). The mixture was kept in the dark for 16 h before use and then diluted with ethanol to obtain an absorbance of 0.70±0.05 units at 734 nm using a spectrophotometer (UV-340G, Gehaka, São Paulo, Brazil). Next, 30 μ l of the extract was added in 3.0 mL of diluted ABTS solution. After the addition of 30 μ l of extract, the absorbance at 734 nm was recorded at 6 min after mixing. Ethanolic solutions of known Trolox (Sigma-Aldrich, São Paulo, SP, Brazil) concentrations were used for calibration and all assays were carried out in triplicate. The results were expressed as μM Trolox/g.

2.8 Statistical analysis

The analytical data obtained were subjected to analysis of variance (ANOVA) using a completely randomised design. Experimental design and the statistical analysis were conducted using the statistic software version 10 (Statsoft Inc.) with 95% of confidence level. The terms statistically found to be non-significant were excluded from the initial model and the experimental data were re-fitted only to the significant (p < 0.05) parameters. All determinations were carried out in triplicate and the data recorded as averages and standard deviations.

3 Results and discussion

3.1 Application of the experimental designs in extraction total phenolics content

3.1.1 Centroid-simplex mixture design

Table 1 shows the results obtained by applying a centroid-simplex mixture design matrix for the extraction of total phenolics from cashew apple bagasse varying the concentrations of the solvents.

According to the results in Table 1, the lowest total phenolics content was obtained when only acetone or ethanol was used. Also, the combination of the acetone/ethanol solvents at the concentration of 50% did not result in better extraction efficiencies. However, the extraction efficiency was dependent on the presence of water. In addition, the combination of the three solvents potentially affected the extraction amounts of total phenolics content.

The ANOVA analysis (with p < 0.05) for the obtained results can be observed in Table 2.

The variance due to the regression parameters of the polynomial model was statistically significant at a 95% significance level. The correlation coefficient R^2 was 0.945, which shows that approximately 95% of the results can be explained by the experimental model.

Fig. 1 shows the Pareto graph where the influence of each parameter and its interactions for the mixture design can be observed in descending order of significance of the effects. It is possible to confirm that water was the solvent that presented the greatest contribution to the extraction of total phenolics compounds. Moreover, when evaluating the interactions between the solvents, it is possible to note that only the interaction between the acetone/ethanol solvents was not significant at the 95% confidence level (p < 0.05), thus confirming the results obtained in Table 1.

Run	Acetone concentration (%)	Water concentration (%)	Ethanol concentration (%)	Total phenolic observed (mg GAE/100 g)	Total phenolic predicted (mg GAE/100 g)
1	(1) 100	(0) 0	(0) 0	119.81±3.80	120.4
2	(0) 0	(1) 100	(0) 0	155.77±4.90	153.25
3	(0) 0	(0) 0	(1) 100	124.41±6.90	125.4
4	(1/2) 50	(1/2) 50	(0) 0	170.32±9.30	170.75
5	(1/2) 50	(0) 0	(1/2) 50	124.60±4.20	128.55
6	(0) 0	(1/2) 50	(1/2) 50	168.35±10.4	169.2
7	(2/4) 50	(1/4) 25	(1/4) 25	150.90±10.3	147.6
8	(1/4) 25	(2/4) 50	(1/4) 25	166.10 ± 14.70	172.1
9	(1/4) 25	(1/4) 25	(2/4) 50	153.33±2.00	148.8
10	(1/3) 33.33	(1/3) 33.33	(1/3) 33.33	171.26±13.6	163.9
11	(1/3) 33.33	(1/3) 33.33	(1/3) 33.33	155.96±19.89	163.9
12	(1/3) 33.33	(1/3) 33.33	(1/3) 33.33	167.14±14.50	163.9

Table 1. Centroid-simplex mixture design matrix, real and encoded values (in parenthesis) applied for optimization of the extraction total phenolic content from cashew apple bagasse residue.

Table 2. ANOVA for analysis of variance and regression for the model of the extraction of total phenolic content from cashew apple bagasse residue.

Variation source	SS	df	MS	Fcal	Ftab
Regression	37.62399	5	7.524799	20.54443	4.38
Residual	2.19762	6	0.36627		
Lack-Of-Fit	0.94372	4	0.23593	0.37632	19.24
Pure error	1.2539	2	0.626948		
Total SS	39.82161	11	3.620146		
Predicted R ²	0.945				
Adjusted R ²	0.898				

SS - sum of squares; df - degree of freedom; MS - mean square; Fcal - calculated F value; Ftab - tabulated F value.

A polynomial model of first order considering only the significant parameters was obtained according to Eq. (2), by means of the regression analysis:

Total phenolics =
$$0.090A + 0.207B + 0.108C$$

+ $0.482AB + 0.424BC$ (2)

where A = acetone, B = water, C = ethanol, AB = acetone water interaction and BC = water ethanol interaction.

Based on the mathematical model contained in Eq. (2), Fig. 2 was generated which represents the response surface graph as a function of the

concentrations variables solvents and their interactions on the total phenolics content in cashew apple bagasse.

According to Fig. 2, the optimum region corresponding to the best solvents concentrations for maximum extraction of the total phenolics content were 60% water, 30% acetone and 10% ethanol, by fixing the time and the extraction temperature in 2 hours and 25°C, respectively. Under these conditions, the predicted response was 172.56 mg GAE/100 g. The predicted total phenolics content was confirmed and validated by performing an experiment (in triplicate) under optimised conditions, obtaining a value of 170.32 \pm 9.30 mg GAE/100 g.

Run	Time (h)	Temperature (°C)	Total phenolic observed (mg GAE/100 g)	Total phenolic predicted (mg GAE/100 g)
1	(-1) 1 (-1) 25 256.06 ± 22.32		256.06 ± 22.32	275.4
2	(-1) 1	(0) 35	287.92 ± 6.15	312.3
3	(-1) 1	(+1) 45	339.65 ± 9.99	295.9
4	(0) 2	(-1) 25	280.35 ± 3.73	293.7
5	(0) 2	(0) 35	315.62 ± 14.84	330.5
6	(0) 2	(0) 35	377.81 ± 5.42	330.5
7	(0) 2	(0) 35	359.34 ± 4.90	330.5
8	(+1)3	(-1) 25	330.30 ± 22.28	297.6
9	(+1)3	(0) 35	297.62 ± 14.15	334.35
10	(+1)3	(+1) 45	322.17±25.87	318.05
11	1 (0) 2 (+1) 45		266.31±7.47	314.15

Table 3. Three-level factorial experimental design matrix, real and encoded values (in parenthesis) applied for optimization of the extraction total phenolic content from cashew apple bagasse residue.

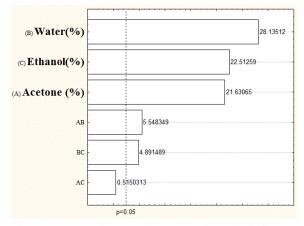


Fig. 1. Pareto diagram demonstrating the influence of solvent concentrations on the extraction of total phenolics content from cashew apple bagasse.

3.1.2 Three levels factorial experimental design

Three-level factorial experimental design was applied in order to optimise the time (1, 2 and 3 h) and temperature $(25, 35 \text{ and } 45^{\circ}\text{C})$ conditions in the extraction of the total phenolics content. Table 3 shows the obtained results.

The new results obtained in Table 3 for the extraction of the total phenolics compounds were

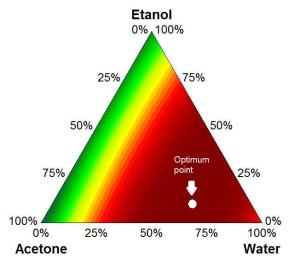


Fig. 2. Response surface plot for optimization of extraction total phenolics content from cashew apple bagasse as a function of the independent variables water, ethanol and acetone.

higher than those found in Table 1. These results were submitted to ANOVA and regression analysis for a confidence level of 95%.

The results of the ANOVA analysis showed that the experimental design presented a lack of adjustment. However, the trends pointed out by the three-level factorial design can be used as optimized values, considering that the adopted planning pointed

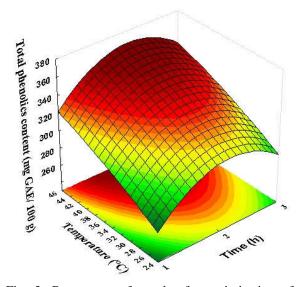


Fig. 3. Response surface plot for optimization of extraction total phenolics content from cashew apple bagasse as a function of the independent variables temperature and time.

out an experimental trend according of Bezerra *et al.* (2008). Thus, the determination of optimal conditions for the modelled values was performed through visual inspection of the response surface according to Novaes *et al.* (2017).

A second-order polynomial model (with p < 0.05) was obtained through the regression analysis, according to eq. (3):

$$Y = 0.49097 + 2.95861t - 1.16330t^{2} + 0.57531T$$
$$- 0.00950T^{2} + 0.04062$$
(3)

where t = time; and T = temperature.

Through the mathematical model contained in Eq. (3), the response surface graph was generated in Figure 3 as a function of time and temperature variables and total phenolics content.

According to Fig. 3, the optimum conditions for the extraction of phenolics compounds are between 1.8 and 2.4 h and between 30 and 40°C, with optimum conditions being 2 h and 34°C for time and temperature, respectively.

Using the optimum conditions found in the experimental designs, the total phenolics content predicted in the present study was 336.79 mg GAE/100 g. The predicted total phenolics content was confirmed and validated by performing an experiment (in triplicate) under optimized conditions, with a value of 343.34 ± 34.76 mg GAE/100 g. This value found for total phenolics content was significantly higher than

the results previously reported in this work using only centroid-simplex mixture design.

The observed and predicted values, along with the computed absolute errors (AE) for total phenolics (mg GAE/100 g) content extraction using the centroidsimplex mixture design and Three-levels factorial experimental design were, respectively: (observed: 170.32 ± 9.30 ; predicted: 172.56; AE = 1.32%) and (observed: 343.34 ± 34.76 ; predicted: 336.79; AE = 1.91%). Due to the low absolute error values obtained by the comparison between observed and predicted values, the proposed models may be used to predict the response value.

According to the international classification concerning the contents of total phenolics proposed by Vasco *et al.* (2009), who tested 17 fruits from Ecuador; the total phenolics content of cashew apple bagasse can be classified as being of medium concentration (100-500 mg GAE/100 g).

In addition, the total phenolics content present in the cashew bagasse waste was higher than the value found for cashew apple pulp fresh reported by Rufino *et al.* (2010), who used the sequential extraction process (50% methanol followed by 70% acetone) and observed 118 \pm 3.7 mg GAE/100 g, and for Mélo *et al.* (2006) who used the extraction process with 80% aqueous methanol and reported 295.25 \pm 25.91 mg GAE/100 g. Therewith, the experimental design performed allowed the optimal conditions for obtaining high extraction yields to be selected and demonstrated that cashew apple bagasse remains a rich source of total phenolics content and can be considered an important alternative biomass.

3.1.3 Antioxidant activity content

The antioxidant activity of cashew apple bagasse was assessed by using two methods: DPPH and ABTS. These methods distinguish themselves from one another by their mechanism of action and are complementary for the study of antioxidant potential. They are largely used as quick, reliable and reproducible assays to search the *in vitro* overall antioxidant activity of pure compounds, as well as that of plant and fruit extracts (Luna-Ramírez *et al.*, 2017; Domínguez-Hernández *et al.*, 2016).

The DPPH method was expressed in EC_{50} , the inhibitory concentration of the cashew bagasse waste required to inhibit 50% of the DPPH radicals, obtained from the standard curve. Indicating that the lower the EC_{50} , the greater the antioxidant activity. The ABTS method was expressed as the total antioxidant

capacity equivalent to Trolox based on the ability of antioxidants to capture ABTS⁺ radical cations.

The values found in this work were an EC₅₀ of 5,170.91±305 g/g DPPH and for ABTS 13.6±1.8 μ M Trolox/g. These results were better than those reported by Rufino *et al.* (2010), who found an EC₅₀ of 7142±205 g/g DPPH and 11.2±0.04 μ M Trolox/g. These results demonstrate that cashew apple bagasse can still be considered a potential source of total phenolics with antioxidant activity and an important biomass for use in bioprocesses.

Conclusion

The application of the experimental designs centroidsimplex mixture and three-level factorial was effective for optimisation of the extraction of total phenolics content and antioxidant activity from cashew apple bagasse. The best combinations of the variables for extraction of total phenolics content and antioxidant activity was obtained with 60% water, 30% acetone and 10% ethanol for 2 h, at 35°C. The results demonstrate that cashew apple bagasse may be considered an important biomass and the exploitation of this abundant and inexpensive renewable resource natural could be performed by industries. However, complementary studies will need to be performed to validate its applicability in this regard.

Nomenclature

- RSM response surface methodology
- DPPH antioxidant capacity (g/g)
- ABTS antioxidant capacity (µM Trolox/g)
- EC₅₀ inhibitory concentration required to inhibit 50% of the DPPH radicals
- t extraction time of total phenolics content (h)
- T extraction temperature of the total phenolics content (°C)

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