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Optimization of the extraction process of polyphenols from cashew apple agro-industrial residues

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Abstract

The goal of this study was to determine the chemical composition of cashew apples agro-industrial residue and optimize the process of polyphenols extraction in this residue. The extraction process conditions were defined using a 2^{4-1} fractional factorial experimental design using acetone and methanol as solvents. The independent variables were: time (30 to 90 min), temperature (30 to 50 °C), solvent concentrations (50% to 90%), agitation speed (100 to 300 rpm); the dependent variables were: total phenolic content and DPPH scavenging capacity. The optimized process was carried out by applying the Central Composite Rotational Design (CCRD) considering the results obtained with the 2^{4-1} fractional factorial experimental design. The residue presented bioactive compounds in its composition, with emphasis on the content of total phenolic compounds (1975.64 mg/ 100 g). The extraction process was not affected by methanol; however, acetone affected the amounts of extracted phytochemicals. Extracts with high levels of polyphenols and strong DPPH scavenging capacity (> 80%) were obtained using 55% acetone, 30 minutes, 30 °C, and 150 rpm. The results showed that cashew apple residue is a potential natural source of bioactive compounds with strong antioxidant capacity. These compounds could be used partially or totally to replace synthetic antioxidants.

Keywords: polyphenols; cashew apple residue; antioxidant capacity; factorial experimental design.

Practical Application: The factorial design methodology combined with surface response analysis allows the optimization of the process for efficient extraction of phenolic compounds from agro industrial residue cashew.

1 Introduction

The cashew tree (*Anacardium occidental* L.) is a tropical widespread plant that belongs to the Anacardiaceae family. Its fruit is composed of two parts: the nut (fruit itself) and the stalk (pseudo fruit). In Brazil, cashew apples have socioeconomic relevance because they occupy a total planted area of 758,085 hectares and produce 220,505 tons of cashew nuts and 1,592,530 tons of stalks (Instituto Brasileiro de Geografia e Estatistica, 2015; Food And Agriculture Organization Of The United Nations, 2013). The cashew nut is the main exported product, while cashew apples, which are juicy, astringent, and very nutritious, are marketed domestically for consumption *in nature* and production of juice, frozen pulp, and processed deserts (Jayalekshmy & John, 2004).

The diversity of industrialized products derived from cashew apples generates a large amount of waste that can trigger serious environmental problems. In juice and pulp production, about 40% of the weight of most fruits such as mango, acerola, passion fruit, and cashew apple result in agro-industrial residues. Such residues, consisting of peel and seeds, usually concentrate relevant quantities of bioactive phytochemicals, which often contain antioxidant capacity that is higher than that found in the fruit pulp (Ajila et al., 2007).

The food industry uses synthetic antioxidants to inhibit lipid oxidation reactions. However, the safety of these additives has been questioned by studies that suggested their possible mutagenic and carcinogenic effects, stimulating the search for sources of natural antioxidants. Agro-industrial residues have been used to extract compounds with antioxidant properties (Birch et al., 2001; Moure et al., 2001). Potentially strong antioxidant activity has been observed in several tropical fruit residues such as from acerola, passion fruit, pineapple, and guava (Caetano et al., 2009; De Oliveira et al., 2009; Nascimento et al., 2010).

The bioactive compounds contained in such residues present different chemical properties and feature polarities that are dependent on their structures. Polarity affects the extraction process of bioactive compounds; therefore, this process cannot be unique for all raw materials. In addition, the food matrix type, time, and temperature used in the extraction process can influence efficiency. The combination of solvents with different polarities and use of at least two extraction cycles have been recommended for an efficient extraction of phytochemicals (Pérez-Jiménez et al., 2008). The methodological procedure involving aqueous-organic solvents, three extraction cycles, and temperatures of 25 ± 2 °C have been shown to be effective to extract significant amounts of polyphenols from acerola residues (Caetano et al., 2009). In guava residue, most polyphenols were dissolved in 80% acetone and 80% methanol, while statistically

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significant lower amounts were extracted in water (100%) and ethanol (80%) (Nascimento et al., 2010).

Factorial design is a useful tool for optimizing efficiency in the extraction process, providing insight into the influence of variables on the desired response. The methodology allows for efficient assessment of variables such as raw material, types of phytochemical compounds, time, and temperature by simultaneously evaluating variables and reducing the number of experiments. Because of the importance of cashew apples to the agro-industry, volume of its residue resulting from the production of byproducts, and significant amounts of polyphenols in this residue (Rufino et al., 2010), this study aimed to determine the chemical composition of cashew apple apples agro-industrial residue and optimize the process of polyphenols extraction in this residue.

2 Material and methods

Cashew apple residue from fruit processing was donated by a frozen fruit pulp producer industry located in the city of Recife/PE, Brazil. The residue was collected directly from the production line and immediately transported to the laboratory where it was dried in an air-circulating oven (50 °C) until reaching 10% or less in moisture content. The dehydrated residue was ground in a knife mill (Tecnal 631/2) and sieved through an 80 μm mesh to obtain flour with uniform particle sizes; this flour was packaged in high-density polyethylene bags and frozen (–20 °C).

2.1 Analytical determinations

Moisture, ether extract, proteins, fixed mineral residue, total sugars, reducing sugars, non-reducing sugars, and pH were determined in the cashew residue flour according to the AOAC method (Association of Official Analytical Chemists, 2005). Water activity was determined at 25 °C using a water activity analyzer (Aqualab 4TE Decagon Devices). Total carbohydrates were calculated (100 g - ∑ (total moisture grams + protein + ether extract + fixed mineral residue)) and the result was expressed in g/100 g.

2.2 Quantification of the main phytochemicals

Total Flavonoids: The cashew residue flour was solubilized in 80% methanol, shaken for three 20 minute cycles, and filtered. The methanol extract was added to 5% sodium nitrite solution, 10% aluminum chloride, and 1 M sodium hydroxide solution; the final volume was adjusted to 50 mL. Absorbance was read at 510 nm immediately after volume adjustment and according to the method described by Dewanto et al. (2002). The results were expressed in μg of cathechin equivalents per 100 g of sample.

Total flavonols and anthocyanins: The cashew apple residue flour was homogenized in 95% ethanol solution and 1.5 N HCl (85:15 v/v) and incubated for 12 h at 5 °C in the dark. This sample was subsequently filtered, and, after a two hour room temperature incubation, read at 374 nm and 535 nm for flavonols and anthocyanins, respectively. The flavonols and anthocyanins quantifications were determined based on the molar absorptivity coefficient of 76.6 and 98.2, respectively. Flavonol results were expressed in mg of quercetin equivalents per 100 g of sample; anthocyanin results were expressed in mg of cyanide glycoside equivalents per 100 g of sample and according to the method of Lees & Francis (1972).

Condensed Tannins: The extraction and quantification of condensed tannins were performed according to the method described by Julkunen- Tiitto (1985). The cashew apple residue flour was solubilized in 70% acetone, shaken for three 20 minute cycles, and filtered. One aliquot of this extract was added to 4% Vanillin in methanol containing 1.5 ml of concentrated HCl. After 20 minutes incubation, absorbance was read at 500 nm and the results expressed in mg of catechin equivalents per 100 g of sample.

Ascorbic Acid: This was determined by the titrimetric method using 2, 6 dichlorophenolindophenol (Association of Official Analytical Chemists, 2005).

Total carotenoids: The extraction of total carotenoids was performed according to the methodology described by Rodriguez-Amaya (1999). The quantification was performed at 450 nm absorbance with an absorptive coefficient ($E^{1\%}_{cm}$) of 2500 and the following mathematical expression (Equation 1); results were expressed in μg of beta-carotene equivalents per g of sample.

$$\mu g/g = \text{Volume} \times \text{absorbance} \times 10^6 / E^{1\%}_{cm} \times \text{sample weight} \quad (1)$$

Total Phenolics: The extraction of total phenolics was performed through a sequential process using 55% acetone and 50% methanol added to the residue flour; the mixture was shaken for 30 min in the first extracting solvent and centrifuged at 4000 rpm. The supernatant was collected, the precipitate resuspended in the second solvent, shaken for 30 min, and centrifuged at 4000 rpm. The supernatants were combined and concentrated under reduced pressure at 40 °C, and the final volume was adjusted to 50 ml. Total phenolics were quantified according to the methodology described by Wettasinghe & Shahidi (1999) with the result expressed in mg of gallic acid equivalents per 100 g of residue (mg EAG/100 g).

2.3 Application of the experimental designs in the extraction process

The factorial factionary experimental designs (2^{4-1}) were used to screening variable that affect significant the extraction process. This type of experimental design is more indicate for over 03 variables, because with less assays could be identify the significant variable for the next step, as optimization. In this study acetone and methanol as extraction solvents were used to define the best conditions for the polyphenol extraction process. Additional variables included time (30, 60, and 90 min), temperature (30, 40, and 50 °C), concentration of hydroacetonic and hydromethanolic solvents (50%, 70%, and 90%), and agitation speed (100, 200, and 300 rpm). All experiments was conducted the random with one central point after a 04 assays. Considering the results obtained with the factorial factionary, the Central Composition Rotational Design (CCRD) with Response Surface analyze was applied in order to optimize the yield of total phenolic compounds.

The extracts obtained using the experimental designs were evaluated with respect to total phenolics content and DPPH (1.1-diphenyl-2-picrylhydrazyl) scavenging capacity (Wettasinghe & Shahidi, 1999; Brand-Williams et al., 1995). The results were expressed in μg of gallic acid equivalents per mL of extract (μg EAG/ mL) and in percentages of DPPH radical sequestration, respectively.

2.4 Statistical analysis

Experimental design and and the statistical analysis was conducted using the statistic software v7 (Statsoft) with 95% of confidence level. All determinations were carried out in triplicate and the data recorded as mean and standard deviation.

3 Results and discussion

Cashew apple residue flour has a water content of 6.82 g moisture/ 100 g and water activity of 0.36, characteristic of a dehydrated product with a slightly acidic pH that is common in fruits (Table 1). In dehydrated foods, the water activity (Aw) that corresponds to free or active water is in the range of 0 to 0.6, with a moisture content that represents the total water present, below 25%. Low water activity and moisture content promote conservation of the material because, in these conditions, chemical, microbiological, and biochemical reactions are halted or slowed. Pinho et al. (2011) report moisture content in dehydrated cashew apple residues of 6.80%, which is similar to the values observed in this study. Fruits generally contain high sugar contents, particularly fructose and glucose. The obtained values of total reducing and non-reducing sugars in cashew apple residue flour were similar to those reported by Matias et al. (2005). The total sugar content in fresh and dehydrated cashew (peduncle) has been reported as 2.48% and 9.86%, respectively (Pinho et al., 2011).

Table 1. Physicochemical characteristics of cashew apple residue flour.

Parameters	Contents
Water activity	0.36±0.01
pH	4.30±0.01
Moisture (%)	6.82 ±0.55
Ashes (%)	1.42±0.08
Proteins (%)	9.65±0.08
Lipids (%)	5.43±0.76
Carbohydrates (%)	76.68±1.39
Total sugar (%)	16.40±0.17
Reducing sugar (%)	12.20±0.19
Non- reducing sugar (%)	3.99±0.36
Ascorbic acid (mg/ 100g)	78.50±16.74
Condensed tannins (mg CE/100)	313.00±7.69
Flavonols (mg QE/100)	109.03±1.37
Totais Carotenoids (μg β-caroteno equivalent/ g)	67.20±1.15
Anthocyanin (mg cyanidine-3-glycosides equivalent/ 100g)	36.05±1.02
Total Phenolic (mg GAE/100g)	1975.64±24.11

Mean value ± standard deviation (n=3). GAE= gallic acid equivalent. CE= cathequin equivalent. QE=quercetin equivalente.

The approximate composition data determined in cashew apple residue flour is presented in Table 1. This product presents relatively high levels of protein, lipids, and total carbohydrates, similar to those observed by Pinho et al. (2011). The dehydration process concentrates food components, explaining the high contents observed in dried material. That author reports that the protein content of fresh cashew apple is 2.07 mg/100 g; the values obtained in this study were between 9.18 and 10.56 mg/100 g after dehydration.

The major bioactive phytochemicals present in cashew apple residue flour are presented in Table 1. Ascorbic acid and carotenoids contents were, respectively, lower and higher than those reported by Melo et al. (2006) in fresh cashew apple (227.77 mg/100g and 5.34 μg β-carotene/ g, respectively). In addition to ascorbic acid and carotenoids, cashew apple residue flour contains large quantities of polyphenols (1975.64 mg EAG/100g). The values identified for total phenolics were significantly higher than those determined by Rufino et al. (2010) who used the sequential extraction process (50% methanol followed by 70% acetone) and observed 830.00 mg/100 g. However, the values of total phenolics observed in this study were lower than those reported by Sulaiman et al. (2011) who extracted these phytochemicals in 70% acetone (2920.00 mg/100 g).

According to Naczk & Shahidi (2006), solvent type and polarity, extraction time and temperature, and the sample's physical characteristics influence the extraction of polyphenols. Different solvent polarities can influence the solubility of chemical components in a sample (Zhao et al., 2006). The variability among polyphenols identified in the studied residue compared with the levels of those reported in the literature for phytochemicals in dehydrated or lyophilized cashew apples can be explained by methodological differences in the procedures used during extraction and quantification. Sulaiman et al. (2011) reported total flavonoids values of 930 mg/100 g and 2170 mg/100 g when using 70% ethanol and 70% acetone as extracting solvents, respectively. Moreover, intrinsic and extrinsic factors, such as genetic variety, stage of maturation, type of cultivar, weather and culture conditions, and harvesting and post-harvesting conditions along with other variables can contribute to the variability in quantities of extracted phytochemicals (Capecka et al., 2005).

These bioactive phytochemicals exhibit recognized antioxidant activity on mechanisms such as complexation of metal ions, capture of free radicals, decomposition of peroxides, electron and hydrogen donation, inactivation of reactive oxygen species, and absorption of UV radiation among others.

3.1 Factorial experimental designs: hydromethanolic and hydroacetonic extracts

Methanol and acetone were selected as the best solvents for the extraction of phenolics. Using methanol as the extraction solvent in conjunction with the studied variables did not influence the extraction of phenolic compounds. The results demonstrate that, considering the range studied, the variation in phenolic contents observed (from 465.70 to 603.00 μg/ ml) was small regardless of the process conditions (Table 2), however it is observed that for some tests on standard deviation values were

Table 2. Total phenolics and DPPH• scavenging capacity of cashew apple residue flour hydromethanolic and hydroacetonic extracts obtained through fractional planning 2^{4-1}.

Independent variables					Response			
					Hydromethanolic extract		Hydroacetonic extract	
Assays	Time (min.)	Temperature (°C)	Agitation speed (rpm)	Solvent concentration (%)	Total phenolic (μg/ml)	DPPH (%) (5 min.)	Total phenolic (μg/ml)	DPPH (%) (5 min.)
1	−1(30)	−1(30)	−1(100)	−1(50)	498.93±29.43	66.91	1344.00±44,49	92.86
2	+1(90)	−1(30)	−1(100)	+1(90)	511.20±52.58	57.66	745.70± 46,84	70.64
3	−1(30)	+1(50)	−1(100)	+1(90)	466.00±48.08	67.60	710.00± 33,94	57.43
4	+1(90)	+1(50)	−1(100)	−1(50)	574.40±70.76	76.49	1589.20±79,40	91.98
5	−1(30)	−1(30)	+1(300)	+1(90)	516.70±28.93	65.70	747.50± 21,55	78.71
6	+1(90)	−1(30)	+1(300)	−1(50)	513.70±30.48	74.55	1481.30±56,92	91.91
7	−1(30)	+1(50)	+1(300)	−1(50)	570.50±31.04	79.80	1445.50±22,01	93.51
8	+1(90)	+1(50)	+1(300)	+1(90)	465.70±72.06	75.32	777.70± 20,43	75.74
9	0(60)	0(40)	0(200)	0(70)	537.40±15.81	78.68	1372.60±38,92	95.39
10	0(60)	0(40)	0(200)	0(70)	567.10±22.77	81.16	1444.30±30,23	94.87
11	0(60)	0(40)	0(200)	0(70)	603.00±51.04	83.23	1153.10±64,18	92.86
12	0(60)	0(40)	0(200)	0(70)	596.60±11.21	81.62	1294.90±34,65	70.64

high for both types of solvents. Two other assays to determine total phenolics were performed to confirm the previous results. These assays were performed under the following conditions: *a)* time of 90 min., temperature of 50 °C, shaking speed of 100 rpm, and methanol concentration of 70%; and *b)* 60 min. time, temperature of 40 °C, shaking speed of 200 rpm, and methanol concentration of 50%. The influence of time in the process was also evaluated in these assays. The results confirmed that the obtained phenolic content in the extracts using *a* (624.33 μg/ml) and *b* (628.24 μg/ml) conditions does not increase when using methanol, therefore, confirming that methanol does not affect the extraction process. Nevertheless, methanol can be an important extraction component for other residues.

Acetone extraction resulted in higher amounts of phenolic compounds than the methanol extraction. However, the extraction efficiency was dependent on the concentration of acetone. Smaller amounts of phenolics were extracted with increasing acetone concentration. Thus, the maximum extraction efficiency (1,589.20 μg/ml) was achieved using 90 minutes, 50 °C, 100 rpm, and acetone concentration of 50%; the minimum extraction efficiency was achieved using 30 minutes, 50 °C, 100 rpm, and 90% solvent concentration (710.00 μg/ml) (Table 2). The fractional factorial design after analyzes the p-value that acetone concentration ($p=0.0034$) was the only variable with effect significant ($p<0.05$) when compare with others variables as time ($p=0.380$), temperature ($p=0.588$) and speed ($p=0.863$).

To continue the optimization of the extraction process was performed CCDR design based on the results of the fractional planning using a new temperature ranges, acetone concentration, and agitation speed.

The DPPH scavenging capacity of extracts was measured (Table 2). Hydromethanolic extracts, except those from the 01, 02, 03, and 05 assays, reached values greater than 70% and could be considered as displaying strong antioxidant activity according to the classification established by Melo et al. (2008). These authors classify antioxidant action as strong, intermediate, or weak when the DPPH radical scavenging capacity reaches values above 70%, between 60 and 70%, and below 50%, respectively.

The hydroacetonic extracts, except the one from assay 3, demonstrated strong antioxidant activity (> 70%). The lowest DPPH scavenging capacity (57.43%) was observed in the extract that presented the lowest total phenolic content (710 μg/ml) while the highest DPPH scavenging capacity (95.39%) was observed in the extract with total phenolics levels of 1,372.60 μg/ml. Hydroacetonic extracts were superior to hydromethanolic extracts for DPPH radical scavenging capacity except for those extracts obtained under the conditions of assays 3, and 12.

3.2 Experimental design and response surface

In this study, CCDR and Response surfaces was used to determine an optimal region for the extraction of phenolic from agro industrial residue. New conditions were based on results from the fractional planning and investigation of the influence of temperature and agitation speed at an extraction at 30 minutes. The results presented in Table 3 showed that the best conditions for the extraction of phenolic compounds corresponded to assays 1 (1732.47 μg/ml) and 2 (1722.35 μg/ml), which differed only in the temperature used. The statistical analysis confirm that the only variable that has significant effect was solvent concentration ($p=0.0022$, $R^2=0.9614$). Analysis of Variance (ANOVA, Table 4) with 95% of confidence level indicate that results are significant and predictive, could be represented by the model (Equation 2) and response surfaces (Figure 1).

$$[\text{Total phenolic}] = 1609.99 - 196.839[\text{acetone}] - 70.652[\text{acetone}]^2 \quad (2)$$

The antioxidant capacity of extracts obtained in the CCRD assays (Table 3) was measured after the quantification of phenolic compounds. All extracts exhibited strong DPPH scavenging capacity (> 70%) one minute after the reaction start demonstrating that the cashew apple residue still contains considerable amounts

Table 3. Total phenolic and DPPH• scavenging capacity of cashew apple residue flour hydroacetonic extracts obtained through CCRD.

	Independent variables			Response	
Assays	Temperature (°C)	Agitation speed (rpm)	Solvent concentration (%)	Total phenolic (μg/ml)	DPPH (%) (1 min.)
1	−1(30)	−1(150)	−1(55)	1722.35±102.99	84.94
2	1(45)	−1(150)	−1(55)	1732.47±30.35	86.56
3	−1(30)	1(250)	−1(55)	1704.80±38.16	86.84
4	1(45)	1(250)	−1(55)	1668.85±72.56	87.36
5	−1(30)	−1(150)	1(75)	1304.60±16.62	74.11
6	1(45)	−1(150)	1(75)	1286.72±57.10	73.31
7	−1(30)	1(250)	1(75)	1249.10±54.11	71.78
8	1(45)	1(250)	1(75)	1281.13±112.97	77.66
9	−1.68(25)	0(200)	0(65)	1469.68±77.59	81.90
10	1.68(50)	0(200)	0(65)	1626.42±29.09	82.30
11	0(37.5)	−1.68(117.5)	0(65)	1553.80±46.03	83.72
12	0(37.5)	1.68(282)	0(65)	1590.00±30.93	87.63
13	0(37.5)	0(200)	−1.68(48.2)	1709.12±37.17	87.88
14	0(37.5)	0(200)	1.68(81.2)	1125.65±39.94	77.04
15	0(37.5)	0(200)	0(65)	1569.55±87.56	87.79
16	0(37.5)	0(200)	0(65)	1624.97±41.16	86.43
17	0(37.5)	0(200)	0(65)	1632.98±44.16	85.20

Table 4. ANOVA for phenolic concentration with acetone solvent extractor.

	Sum of Squares	Degree of Fredom	Mean Square	F-value	R^2	p
Regress	587499.1	9	65277.68	19.39		0.00001
Residual	23567.4	7	3366.77		0.96143	
Total	611066.5	16				

$F_{0.05;9;7} = 6.72$.

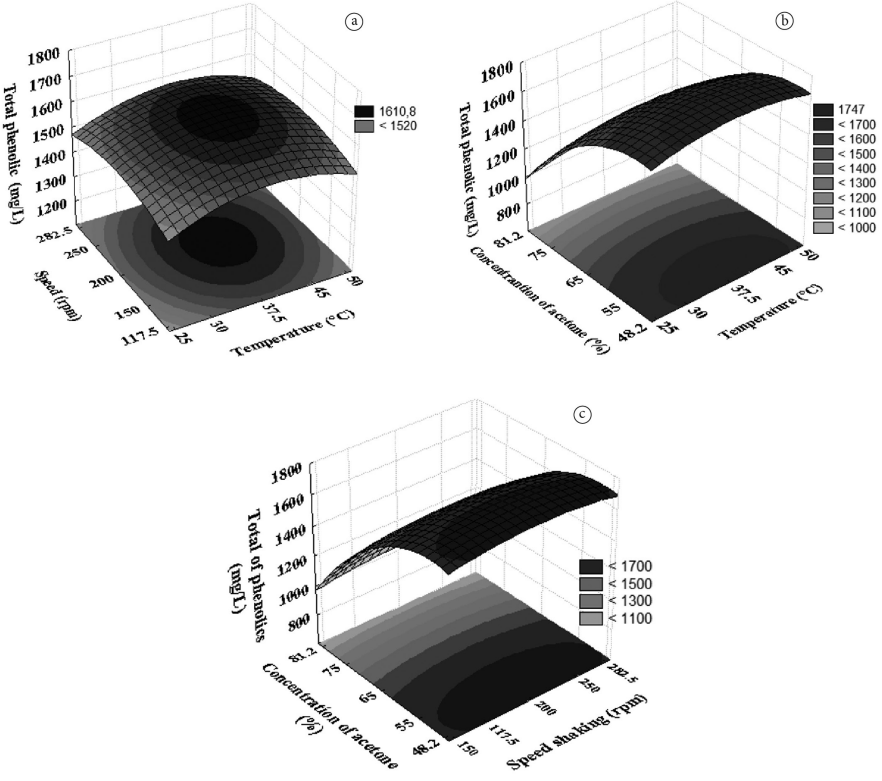

Figure 1. Response surfaces for phenolics contents as a function of (a) agitation speed X temperature, (b) acetone concentration X temperature, and (c) acetone concentration X agitation speed.

of phenolic compounds with significant antioxidant ability and could therefore, be used as a source of natural antioxidants.

Response surfaces shown (Figure 1) phenolic contents as a function of agitation speed and temperature (Figure 1a), acetone concentration and temperature (Figure 1b), and acetone concentration and agitation speed (Figure 1c). In Figure 1a, the extraction reached its highest concentration when the temperature is close to 37.5 °C and agitation speed is approximately 200 rpm. Figure 1b shows results that indicate high extraction concentration values reached at the optimum temperature of 37.5 °C and acetone concentration around 48%. This condition is also demonstrated by the results shown in Figure 1c. Thus, the acetone concentration is a variable that strongly influences the extraction process with an optimal concentration of about 50% at temperatures ranging between 30 and 45 °C and agitation speeds between 150 and 200 rpm. These conditions are economically viable for industrial applications. Therefore, the extract obtained using 55% acetone, 30 °C, and 150 rpm was used for the remaining tests.

4 Conclusions

The cashew apple agro-industrial residue contains macronutrients in relevant quantities and phytochemicals with recognized antioxidant properties. Phenolic compounds are among the phytochemicals present in this residue in great proportion. These phytochemicals can be efficiently extracted in acetone (55%) using a 30-minute extraction time, agitation speed of 150 rpm, and temperature of 30 °C. The hydroacetonic extract obtained under these conditions shows strong DPPH radical scavenging capacity (> 80%). The results from this study demonstrated that cashew apple residue could be considered a potential source of bioactive compounds with strong antioxidant capacity. Total or partial replacement of synthetic antioxidants with these bioactive compounds in processed food may represent cost savings while improving the health value of foods.

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