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RECOVERY OF ANTIOXIDANTS FROM PAPAYA (*Carica papaya* L.) PEEL AND PULP BY MICROWAVE-ASSISTED EXTRACTION

RECUPERACIÓN DE ANTIOXIDANTES A PARTIR DE EPICARPIO Y PULPA DE PAPAYA (*Carica papaya* L.) MEDIANTE EXTRACCIÓN ASISTIDA CON MICROONDAS

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Abstract

Papaya (*Carica papaya* L.) has a diversity of bioactive compounds with recognized antioxidant effects. However, the extraction of these compounds presents challenges related to solvent selection, energy consumption, processing time and efficiency. In the present study, the performance of microwave irradiation was evaluated to recover extracts with a high content of polyphenols, flavonoids, and an effective antioxidant activity from a mixture of papaya peel and pulp. The factors solid:solvent ratio, microwave power, and ethanol-water mixture were evaluated. The results showed that the maximum content of bioactive compounds was reached after 3 minutes of extraction with the optimum conditions of 340W for power, 23.34% for mixture ethanol:water and solid:solvent ratio of 1:80.60 (g:mL). The optimal extract presented a concentration of 1186.39 ± 44.49 mg GAE/100gFW for polyphenols and 43.88 ± 6.94 mg CE/100gFW for flavonoids and antioxidant activity of 3920.48 ± 31.97 μmol TE/100gFW, values higher than those reported by other authors. It is confirmed that the application of microwaves in the process of extraction of bioactive compounds reduces time, costs and is efficient to obtain extracts of papaya with an effective antioxidant activity.

Keywords: papaya, microwaves, polyphenols, flavonoids, antioxidant activity.

Resumen

La papaya (*Carica papaya* L.) posee diversidad de compuestos bioactivos con reconocidos efectos antioxidantes. Sin embargo, la extracción de esos compuestos, presenta desafíos relacionados con la selección del solvente, consumo energético, tiempo de procesamiento y eficiencia. En el presente estudio se evaluó el desempeño de las microondas en la obtención de extractos con un alto contenido de polifenoles, flavonoides y con una actividad antioxidante efectiva, a partir de una mezcla de epicarpio-pulpa de papaya. Se evaluaron los factores relación sólido:solvente, potencia de las microondas, y mezcla etanol-agua. Los resultados permitieron establecer que el contenido máximo de compuestos bioactivos se alcanzó a los 3 minutos de extracción con las condiciones óptimas de 340W para potencia, 23,34% para la mezcla etanol: agua y 1:80,60 (g:mL) para la relación sólido:solvente. El extracto óptimo presentó una concentración de 1186,39 ± 44,49 mg GAE/100gFW para polifenoles y 43,88 ± 6,94 mg CE/100gFW para flavonoides y una actividad antioxidante de 3920,48 ± 31,97 μmol TE/100gFW, valores mayores a los reportados por otros autores. Se confirma que la aplicación de microondas en el proceso de extracción de compuestos bioactivos reduce tiempo, costos y es eficiente para obtener extractos de papaya con una actividad antioxidante efectiva.

Palabras clave: papaya, microondas, polifenoles, flavonoides, actividad antioxidante.

1 Introduction

The bioactive compounds from vegetal sources have a high-added value for the cosmetic, food, and pharmaceutical industry due to their functional, antioxidant, or antimicrobial characteristics. From the nutritional viewpoint, these compounds retard or inhibit the oxidation of cellular components caused by the action of free radicals and consequently, protect against the propagation of the oxidative chain (Carocho and Ferreira, 2013; Singh *et al*., 2016).

The principal antioxidants include carotenoids, flavonoids such as rutin, quercetin, and catechin, phenolic acids like caffeic, ferulic and gallic. Specifically, phenolic acids are recognized as the

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main contributors of antioxidant activity in plant and fruit extracts. Its presence in fruits is determined by numerous factors such as variety, ripening conditions, and others (Abbas *et al*., 2017; Hodzic *et al*., 2009; Naczk and Shahidi, 2006).

The papaya world production is around 13 million tons, which India has 40% and Brazil, Dominican Republic, Mexico, and Colombia being the greater producers in America (FAOSTAT, 2017). The production of papaya in Colombia in 2015 was estimated at 105,459 tons (DANE, 2016). The papaya processing industry uses only the pulp, and the seed along with the peel are discarded as processing residues, which correspond to 30% of the weight of the fruit (Gayosso-García Sancho *et al*., 2011; Ikram *et al*., 2015; Nwofia *et al*., 2012). The bioactive compounds present in the papaya fruit (*Carica papaya* L.) are distributed in the pulp, peel, and seeds. These compounds include polyphenols such as gallic, ferulic and caffeic acid derivatives and flavonoids such as rutin and quercetin, which have been proposed to have antiproliferative and anti-inflammatory properties (da Cunha *et al*., 2004; Gayosso-García Sancho *et al*., 2011; Rivera-Pastrana *et al*., 2010).

Phenolic acids and other bioactive compounds are obtained from plant biomass through different extraction and purification processes. Bioactive compound extraction processes based on green chemistry, using non-toxic solvents with low toxicity in humans or the environment, hence the green solvents such as ethanol and water mixtures, are preferred over toxic solvents and non-aqueous conventional technologies (Ameer *et al*., 2017; Capello *et al*., 2007; López-Hernández *et al*., 2018).

Microwave-assisted extraction (MAE) is a green extraction technology, which is suitable when high extraction efficiency is required in a short time. In the MAE, the microwave energy heats the solvent and interacts directly with the free water molecules of the plant material. The heating effect in food materials is a consequence of two mechanisms, dipolar rotation, and ionic conduction (Chan *et al*., 2011; Sparr Eskilsson and Björklund, 2000; Tang *et al*., 2012). Water is the main dipole responsible for heating, which is the main component of most vegetal raw material. Dipole response to an oscillatory field is an increase in vibrational and rotational energies, depending on the molecular symmetry degree, results in the generation of frictional heat. Non-polar molecules which are asymmetrically charged can behave like dipoles in an electric field, however, their response to microwave energy is lower than water. Ions change direction frequently according to field signs, causing a collision between molecules, therefore heat is generated (Alupului *et al*., 2012; Li *et al*., 2013; Tatke and Jaiswal, 2011). The heat produces high pressures, which causes a cell wall rupture, facilitating the penetration of the solvent into the plant matrix and diffusion of the compounds of interest to the bulk solvent. High heating rate is the main advantage of microwaves compared to conventional extraction technologies because extraction time in MAE is of the order of minutes (Dhanani *et al*., 2013; He *et al*., 2015; Milutinović *et al*., 2015). Additionally, microwave application significantly decreases the volume of solvent used, reducing costs and energy in the extraction process, while achieving high efficiency in bioactive compounds extraction (Alupului *et al*., 2012; Ameer *et al*., 2017; Song *et al*., 2011). MAE has been widely used for the extraction of polyphenols, flavonoids, and antioxidants from plants, fruit and vegetable processing residues, due to the low investment cost in equipment and operation compared to other technologies (Dhanani *et al*., 2013; Garcia-Salas *et al*., 2010).

The main objective of this work was to determine optimal conditions of microwave-assisted extraction for maximizing polyphenols and flavonoids extraction efficiency from papaya peel and pulp (*Carica papaya* L.). Furthermore, a correlation between antioxidant activity with polyphenols and flavonoids was established in order to determine the effective antioxidant activity.

2 Materials and methods

2.1 Equipment, standards and chemical reagents

2.1.1 Equipment

Raw material preparation was performed using a laboratory mill (IKA A11 basic, Germany), and sieve shaker (Ro-Tap, WS Tyler, USA). MAE extractions were performed with a microwave oven with a maximum power of 1500 W (LG, MH1143XAR, South Korea). Analytical methods were performed using a laboratory centrifuge (SIGMA, Laborzentrifugen 2-16P, Germany), and spectrophotometer (Thermo Fisher Scientific Inc, Genesys 10 Bio UV-VIS, USA).

2.1.2 Standards and chemical reagents

Sodium nitrite, Folin-Ciocalteu reagent, sodium carbonate, DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), catechin and gallic acid standards were purchased from Sigma-Aldrich (St. Louis, USA). Aluminum chloride, sodium hydroxide, ethanol, and methanol were obtained from Merck (Darmstadt, Germany). All reagents and chemical solvents were analytical grade.

2.2 Raw material and extract preparation

2.2.1 Raw material

Papaya Maradol (*Carica papaya* L.) from a local market was selected by the criteria for the first quality fruit established in the Colombian Technical Standard-NTC 1270 (Instituto Colombiano de Normas Técnicas y Certificación, 1993). The amount of raw material required for all experimental trials was obtained from the same batch of papaya, according to this procedure: the fruit was washed twice in water and rinsed with deionized water to remove dirt (Maisarah *et al*., 2013). The whole fruit was cut into two sections to remove seeds, then cubes of pulp with their peel of approximately 0.5 cm were obtained, these cubes were dried in a laboratory oven at 50 °C to a constant moisture of about 10% in wet basis. Subsequently, dried material was milled by a laboratory mill and the particle size (p.s.) was adjusted to 180 μm with a sieve shaker.

2.2.2 Microwave-assisted extraction (MAE)

MAE process was performed in a microwave oven using a beaker with a final volume of solvent (mixtures of ethanol and deionized water) of 40 mL. The dried and milled material was extracted using different ratios of sample weight to solvent volume (solid:solvent, g:mL). After each extraction, the extract was centrifuged at 10000g and 30 °C for 15 minutes in a laboratory centrifuge, and the obtained supernatant was used for quantification of total polyphenol and flavonoid content.

2.3 Microwave-assisted extraction kinetics of total polyphenol and flavonoid content

The extraction kinetics was carried out at a constant power of 260 W (20% of the maximum power). A mixture of ethanol-water at 50% (v/v) was used in a solid:solvent ratio of 1:60 (g:mL), and the kinetics was carried out until a maximum extraction time to avoid solvent evaporation, these parameters were selected from data reported by Bouras *et al*., 2015; Milutinović *et al*., 2015; Song *et al*., 2011. The samples were subjected to microwave irradiation under the same experimental conditions, i.e., the first sample was subjected to microwave irradiation, then the extraction was stopped at 36 seconds and the extract was centrifuged to perform the measurements; it was continued in the same way for all samples until the extraction kinetics was completed.

A magnetic stirring extraction (MSE) at 350 rpm without microwave irradiation was carried out as a control, with under identical conditions to the microwave extraction.

2.4 Optimization of microwave-assisted extraction of total polyphenol and flavonoid content

According to the preliminary results for the extraction kinetics of total polyphenol and flavonoid content, a central composite design of response surface with axial points was proposed (Table 1), evaluating the factors, power (W), solvent (ethanol-water, % v/v) and solid:solvent ratio (g:mL) in order to maximize the response variables total polyphenol and flavonoid content.

Table 1. Experimental design for polyphenols and flavonoids extraction from papaya peel and pulp using microwave-assisted extraction (MAE).

Factor	**Level**				
	-1.68	**-1**	**0**	**1**	**1.68**
A = Power (W)	41	130	260	390	478
B = Solvent (% v/v)	16.36	30	50	70	83.64
C = solid:solvent ratio (g:mL)	1: 26.36	1:40	1:60	1:80	1:93.64

The general equation for the response surface quadratic model including three different factors is as follows:

$$Y_i = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{1,1} A^2 + \beta_{2,2} B^2 + \beta_{3,3} C^2 + \beta_{1,2} AB + \beta_{1,3} AC + \beta_{2,3} BC \quad (1)$$

In Eq. (1), Y_i indicates the response variable; A, B and C display the independent variables including power, solvent, and solid:solvent ratio, respectively. β_0 is a constant coefficient that fixed the response at the central point of the experiment; β_1, β_2 and β_3 are the coefficients of main effects, $\beta_{1,1}$, $\beta_{2,2}$ and $\beta_{3,3}$ are the quadratic effects coefficients, and $\beta_{1,2}$, $\beta_{1,3}$ and $\beta_{2,3}$, are the interactions coefficients (Bouras *et al*., 2015; Kazemi *et al*., 2016; Xynos *et al*., 2014).

To select the mathematical model that adequately describes the behavior of each response, the highest percentage of coefficient of determination (R^2) was considered. In order to find a combination of factors that maximize the response variables, the results were analyzed according to the multiple response optimization methodology, finding a set of operating conditions that optimize all responses, according to the use of the desirability functions (Felix *et al*., 2018; Montgomery, 2003; Vera Candioti *et al*., 2014), finally an experimental validation was carried out with the optimal conditions for each factor.

2.5 Analytical methods

2.5.1 Extraction efficiency

The extraction efficiency for total polyphenol and flavonoid content was expressed as a percentage based on the total amount of polyphenols and flavonoids in the raw material. The total content of bioactive compounds in the raw material was obtained with a conventional Soxhlet extraction for 4 hours with 2 grams of sample and 180 mL of solvent (ethanol:water 50% v/v) (Galvan D'Alessandro *et al*., 2012).

2.5.2 Total polyphenol content (TPC)

100 μL of extract was mixed with 7.9 mL ultrapure water, then 500 μL of Folin-Ciocalteau reagent and 1.5 mL of a saturated sodium carbonate solution were added and incubated 2h in darkness. The absorbance of this mixture was measured at 760 nm in a spectrophotometer (Galván D'Alessandro *et al*., 2013; Koşar *et al*., 2005; Singleton *et al*., 1999). Gallic acid was used as a standard using a calibration curve between 20 and 1000 mg/L. The concentration of total polyphenol content was expressed in mg of gallic acid equivalents/100g of fresh weight for each measured sample (mg GAE/100g FW).

2.5.3 Total flavonoid content (TFC)

250 μL of extract was mixed with 1.25 mL of ultrapure water, then a solution of 5% sodium nitrite (w/w) was added. Subsequently, a solution of 10% aluminum chloride (w/w) and 1M sodium hydroxide were added. The absorbance of this mixture was measured at 510 nm in a spectrophotometer. Catechin was used as a standard using a calibration curve between 20 and 1200 mg/L. The concentration of total flavonoid content was expressed in mg of catechin equivalents/100g of fresh weight for each measured sample (mg CE/100g FW) (Aliakbarian *et al*., 2012; J. Yang *et al*., 2009).

2.5.4 Antioxidant activity

50 μL of extract was mixed with 1950 μL of a methanolic solution of DPPH radical and incubated 30 minutes in darkness. The absorbance was determined at 517 nm in a spectrophotometer. The antioxidant activity (% AA) was calculated using Eq. (2):

$$\%AA = \frac{(A_{t0} - A_{tf})}{A_{t0}} \times 100 \quad (2)$$

Where, A_{t0}= Initial absorbance of DPPH radical. A_{tf} = Sample absorbance.

Trolox was used as a standard. The results were expressed as a percentage of antioxidant activity (% AA) and mol of Trolox equivalents/100g of fresh weight for each measured sample (μmol TE/100g FW) (Matsusaka and Kawabata, 2010; Servín de la Mora-López *et al*., 2018; Singh *et al*., 2016).

The results of the percentage of antioxidant activity (% AA) are expressed using the EC50 value, which is defined as the extract concentration responsible for a 50% decrease in the initial activity of DPPH. EC50 estimation was calculated by a non-linear regression analysis using a four parameters equation (Eq. (3)) (Chen *et al*., 2013; Garcia-Mendoza *et al*., 2015).

$$Y = A_1 + \frac{A_2 - A_1}{10^{[(\log(EC_{50}) - \log(x))p]}} \quad (3)$$

Where, Y= Antioxidant activity (% AA), A_1 = minimum response (% AA), A_2= maximum response (% AA), p= slope of the curve, x= total polyphenol content (mg GAE/100g FW).

Parameter estimation of Eq. (3) was performed using a curve fitting app from Matlab® R2014a software (The Mathworks Inc., Natick, MA, USA).

2.5.5 *Statistical analysis*

ANOVA and Tukey's mean comparison tests ($p < 0.05$) were used to evaluate the data obtained from the experimental trials using IBM SPSS Statistics® 21.0 statistical software (SPSS, Inc., USA). The analysis of central composite design was carried out with the statistical software Statgraphics 16.1.11 (StatPoint Tecnologies, Inc., USA). All experiments were run in triplicates, and these data were expressed as the mean ± standard deviation.

3 Results and discussion

3.1 *Microwave-assisted extraction kinetics of total polyphenol and flavonoid content*

Extraction kinetics was carried out with a maximum extraction time of 180 seconds because a longer time the solvent was evaporated. Microwave-assisted extraction kinetics of total polyphenol content showed that the mass transfer rate increases rapidly during the first 36 seconds (Fig. 1a). This behavior could be explained due to initially, the driving force of polyphenols concentration gradient between the solid and the bulk of the solvent is high, and as long as the extraction continues the mass transfer is reduced until the driving force tends to zero (Chemat *et al*., 2017; Treybal, 1980). In contrast, in the kinetics extraction of total flavonoid content the mass transfer rate increases to 180 seconds. This could be due to the fact that most of the flavonoids are found in the cell walls linked with hydrogen bonds to proteins or polysaccharides, which causes difficulty in the solubilization of these compounds in the solvent, therefore, long extraction periods are required (Dixon and Paiva, 1995; Galvan D'Alessandro *et al*., 2012; Watson, 2014).

In the case of extraction kinetics without microwave irradiation, a lower mass transfer rate was observed than for microwave irradiation, which indicates a positive effect of microwaves on the extraction rate of these bioactive compounds.

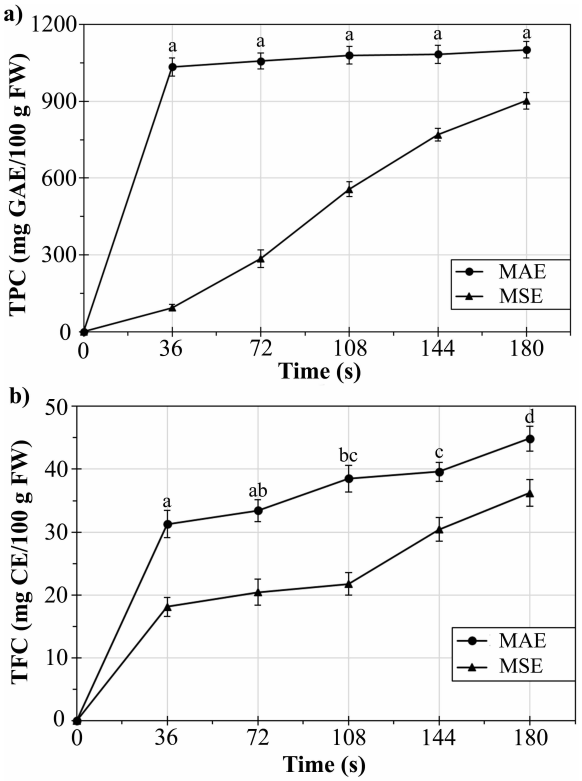

Fig. 1. Effect of microwave irradiation on a) polyphenols, and b) flavonoids extraction kinetics from papaya peel and pulp (p.s. < 180 μm, at 30 °C). Error bars represent standard deviation for triplicate treatments, and the different letters represent the significant differences for the different extraction time, Tukey test ($p < 0.05$).

The microwave-assisted extraction showed an increase of 20% in the recovery of total polyphenol and flavonoid content in comparison with the MSE without microwave irradiation. The positive effect of microwave irradiation has been reported by many investigations related to the extraction of bioactive compounds from different plant materials (Ameer *et al*., 2017; Farhat *et al*., 2011; Tatke and Jaiswal, 2011). As mentioned previously, this effect could be due to the fact that the collision between molecules generates heat, which produces high pressures causing rupture of the cell walls, therefore, the bioactive compounds diffusion to the bulk solvent was increased (He *et al*., 2015; Li *et al*., 2013; Milutinović *et al*., 2015).

The statistical analysis during the Microwave-assisted extraction kinetics of total polyphenol content showed that there were no statistically significant differences (Tukey, $p < 0.05$) from the 36 seconds of extraction. Similarly, other authors found that the

total polyphenol content did not increase significantly with a longer microwave irradiation time, because the maximum content of polyphenols is reached in a short time by this technology (Bouras *et al*., 2015; Veggi *et al*., 2012; Y. Wang *et al*., 2008). In the case of the total flavonoid content, there were statistically significant differences (Tukey, $p < 0.05$) from 144 seconds of extraction, with extraction peak at 180 seconds.

The total polyphenol content after 36 seconds of MAE was 1034.45 ± 35.26 mg GAE / 100g FW and total flavonoid content at 180 seconds of MAE was 44.85 ± 1.98 mg CE / 100g FW (Fig. 1). The extraction efficiency of MAE was greater than 60 and 70% for the extraction of TPC and TFC, respectively. These results show a significant reduction of extraction time with microwave irradiation compared to conventional extraction.

The total polyphenol content extracted by MAE was higher than the values obtained in ripe papaya pulp (*Carica papaya* L.) by Patthamakanokporn *et al*. (2008), Almeida *et al*. (2011) and Maisarah *et al*. (2013), who reported values of 54.00, 53.20, and 249.89 mg GAE/100g FW, respectively. Similarly, the TPC extracted by the MAE are greater than the content obtained by Gayosso-García Sancho *et al*. (2010) in ripe papaya peel (*Carica papaya* L.), who reported values of 358.67 mg GAE/100g FW, however, TPC extracted by the MAE are lower than the content obtained by Matsusaka and Kawabata, (2010), who reported values of 1144.70 mg GAE/100g FW. The total flavonoid content extracted by MAE was higher than the content obtained in ripe papaya pulp (*Carica papaya* L.) by Spínola *et al*. (2015), who reported of 20.47 mg CE/100g FW, however, TFC extracted by the MAE are lower than the content obtained by Maisarah *et al*. (2013), who reported values of 85.19 mg CE/100g FW.

The TPC and TFC obtained in this research were higher than the content reported in literature for papaya pulp, which could be due to the fact that in the mixture of peel with pulp, the peel contributes to a significant amount of polyphenols and flavonoids. Additionally, microwave-assisted extraction is less harsh in comparison with conventional methodologies (organic solvent, acid pH, temperature), allowing the preservation of such compounds in the final extract. Together with the factors mentioned above, these differences with literature may be due to factors associated with harvest time, and ripening stage (Da Silva *et al*., 2014; Gayosso-García Sancho *et al*., 2011, 2010).

According to the results of extraction kinetics of total polyphenol and flavonoid content, the following experimental trials of Microwave-assisted extraction were carried out with a total extraction time of 180 seconds in order to guarantee the maximum extraction of polyphenols and total flavonoids.

3.2 Optimization of microwave-assisted extraction of total polyphenol and flavonoid content

3.2.1 Effect of extraction factors on total polyphenol content

The proposed quadratic mathematical model (Eq. (4)) presented the R^2 coefficient close to 1, a high F-value and p-value < 0.05 (Table 2). Therefore, these results indicated that the model is adequate to describe data from microwave-assisted extraction of total polyphenol content from papaya peel and pulp (*Carica papaya* L.).

$$TPC = 972.79 + 0.30A + 0.93B + 0.92C - 1.75\times10^{-3}A^2 - 1.25\times10^{-3}B^2 - 6.99\times10^{-3}C^2 - 2.06\times10^{-3}AB + 1.37\times10^{-2}AC - 3.07\times10^{-2}BC \quad (4)$$

Table 2. Response surface mathematical models for microwave-assisted extraction of total polyphenol and flavonoid content.

Response variables	F-value	p-value	R^2	Lack of fit (p-value)
TPC (mg GAE/100g FW)	9.21	<0.0040	0.92	0.0543
TFC (mg CE/100g FW)	7.16	<0.0083	0.9	0.058

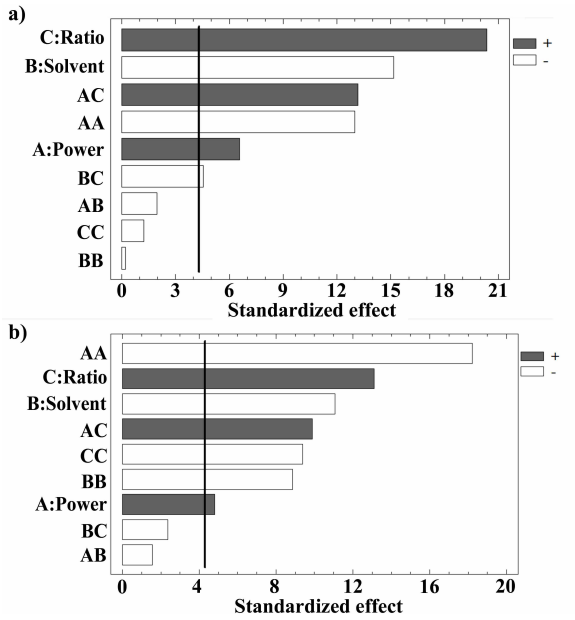

Fig. 2. Pareto diagrams for response variables: a) polyphenol, and b) flavonoid content.

According to Pareto diagram (Fig. 2a), the individual factors power (A), solid:solvent ratio (C), and their interaction (AC) showed a positive and direct effect on the response variable TPC ($p < 0.05$). This could be explained due to a higher solvent volume increases the driving force of polyphenols concentration gradient between the solid and the bulk of the solvent (Luthria, 2008; Pinelo *et al*., 2005; Yang *et al*., 2009).

The application of high microwave power increases the extraction efficiency, because the internal overheating leads to a cell wall rupture, improving the leaching of the bioactive compounds from the solid matrix to the surrounding solvent (Bouras *et al*., 2015; L. Wang and Weller, 2006). However, power values higher than 400 W showed a decrease in TPC, which is evidenced in the negative effect of the AA interaction, additionally, this was observed in the response surface (Fig. 3a and 4a), which could be due to the high microwave power causes an excessive increase in the temperature of the system, causing thermal degradation and oxidation of the polyphenols and flavonoids (Chan *et al*., 2011; Veggi *et al*., 2012; Watson, 2014). Similarly, Bouras *et al*. (2015) and Song *et al*. (2011) found that increasing the irradiation power decreases the total phenolic content, and irradiation power greater than 400 W causes the thermal degradation of the phenolic compounds.

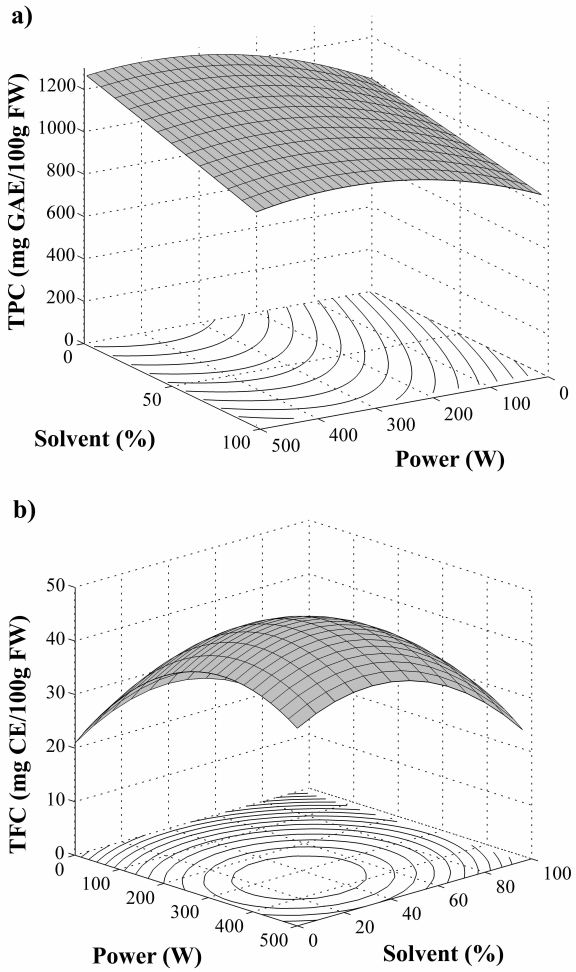

Fig. 3. Response surfaces plot of: a) polyphenol, and b) flavonoid content as a function of microwave power and extraction solvent (The solid:solvent ratio was constant for 1:80.60 g:mL).

The extraction solvent (B), and the interaction with solid:solvent ratio (C) presented a negative and inverse effect on the response variable TPC ($p < 0.05$). The influence of the solvent in the MAE could be due to a mixture of water and low concentrations of ethanol can access the cells, however, high concentration of ethanol can cause cell wall protein denaturation, preventing the dissolution of polyphenols, therefore, the extraction rate is reduced (Dixon and Paiva, 1995; Yang *et al*., 2009). Additionally, the solvent with higher water content improving the extraction because phenolic compounds have a greater solubility in water than in ethanol (Do *et al*., 2014; Turner and Waldebäck, 2010).

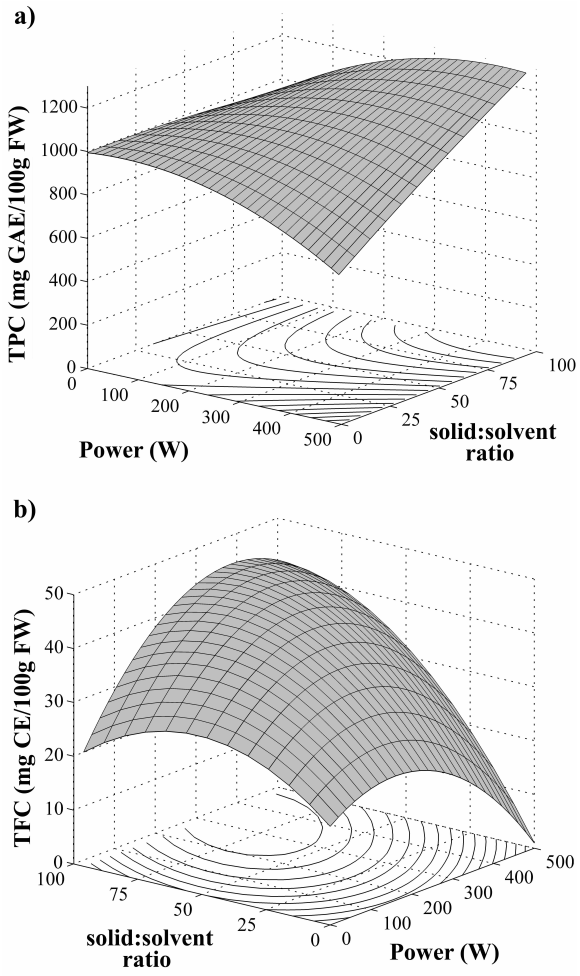

Fig. 4. Response surfaces plot of: a) polyphenol, and b) flavonoid content as a function of microwave power and solid:solvent ratio (The solvent was constant for 23.34%).

3.2.2 *Effect of extraction factors on total flavonoid content*

From a response surface analysis of the experimental data of microwave-assisted extraction of TFC, showed an adequate adjustment with the proposed quadratic mathematical model (Eq. (5)), this model presented an R^2 coefficient close to 1, a high F-value and, a p-value <0.05 (Table 2). Pareto diagram (Fig. 2b), shows similar behavior to TPC extraction, i.e., power, solid:solvent ratio, and their interaction showed a positive and direct effect on the response variable TFC ($p < 0.05$). The interaction effect is evidenced in the response surface plot (Fig. 4b), showing that high values of solid:solvent ratio and power increase the TFC extraction until a maximum is reached.

$$TFC = 10.87 + 6.34\times10^{-2}A + 0.41B + 0.46C - 1.90\times10^{-4}A^2 - 3.91\times10^{-3}B^2 - 4.14\times10^{-3}C^2 - 1.25\times10^{-4}AB + 7.96\times10^{-4}AC - 1.24\times10^{-3}BC \quad (5)$$

The solvent presented a negative and inverse effect on the response variable TFC ($p < 0.05$), however, none of their interactions had a significant effect. This could be due to the fact that high concentrations of ethanol could cause protein denaturation, reducing the solubilization of flavonoids in the solvent, similar to TPC extraction.

Multiple responses optimization was carried out to obtain the overall optimum MAE condition. The significant ($p < 0.05$) interaction effects of MAE factors on total polyphenol, and flavonoid content are indicated by the multiple overlaid contour plot (Fig. 5). The yellow area on the plot represents the suitable range of experimental factors that lead to desirable response variables. The multiple numerical optimization predicted that the most desirable papaya peel and pulp extract can be obtained with the optimum conditions of 340W for microwave power, 23.34% (v/v) for mixture ethanol:water and solid:solvent ratio of 1:80.60 (g:mL).

Under the suggested optimum MAE extraction conditions an experimental validation was carried out, as indicated in Table 3. A difference less than 6% between predicted and experimental values was observed for the extraction of TPC and TFC. Therefore, the multiple responses optimization was suitable and reproducible.

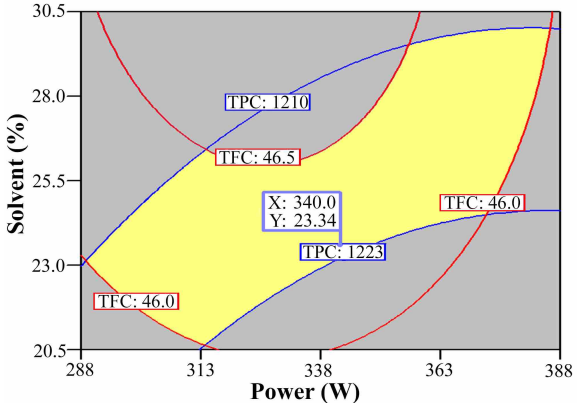

Fig. 5. Overlaid contour plot for MAE extraction of total polyphenol and flavonoid content (The solid:solvent ratio was constant for 1:80.60 g:mL).

Table 3. Predicted and experimental values of responses under optimum MAE conditions and experimental values of responses obtained by conventional extraction.

Response variables	Conventional extraction*	Predicted value	Experimental value*	Extraction efficiency (%)*
TPC (mg GAE/100g FW)	1692.19 ± 36.28	1222.33	1186.39 ± 44.49	70.11 ± 1.42
TFC (mg CE/100g FW)	60.82 ± 5.38	46.28	43.88 ± 6.94	72.15 ± 0.98

* Mean ± standard deviation, n = 3.

The extraction efficiency under the optimum MAE extraction conditions for total polyphenol and flavonoid content was achieved in less than 3 minutes, therefore, this green technology significantly reduce the extraction time compared to conventional extraction. Finally, in these conditions MAE extraction showed an increase greater than 30% in the recovery the total polyphenol content compared to extraction without microwave irradiation.

3.2.3 *Antioxidant activity of papaya peel and pulp extract*

The antioxidant activity under the optimum MAE conditions by the DPPH method was 3920.48 ± 31.97 μmol TE/100g FW. This value of antioxidant activity was higher than the values obtained in ripe papaya pulp (*Carica papaya* L.) by Kelebek *et al*. (2015) and Matsusaka and Kawabata (2010), who reported values of antioxidant activity by the DPPH method of 263.63 and 1934.70 μmol TE/100g FW, respectively. However, in ripe papaya peel (*Carica papaya* L.) Matsusaka and Kawabata (2010) reported values of 5064.40 mol TE/100g FW. The values of antioxidant activity obtained in this research differ from the results presented in literature. As mentioned above, it could be due to various factors associated with the extraction methodology, and physiological aspects of papaya cultivars.

Fig. 6a indicates that the correlation between antioxidant activity (% AA, DPPH) and total polyphenol content showed values for the Pearson's correlation coefficient (R) greater than 0.900 ($p < 0.05$). Similar results have been obtained in tropical papaya pulp (*Carica papaya* L.) by Gayosso-García Sancho *et al*. (2011), (2010), Kelebek *et al*. (2015) and Maisarah *et al*. (2013). On the other hand, the correlation between antioxidant activity (% AA, DPPH) and total flavonoid content showed lower R values ($p < 0.05$) (Fig. 6b). These results are in agreement with other studies from various plants

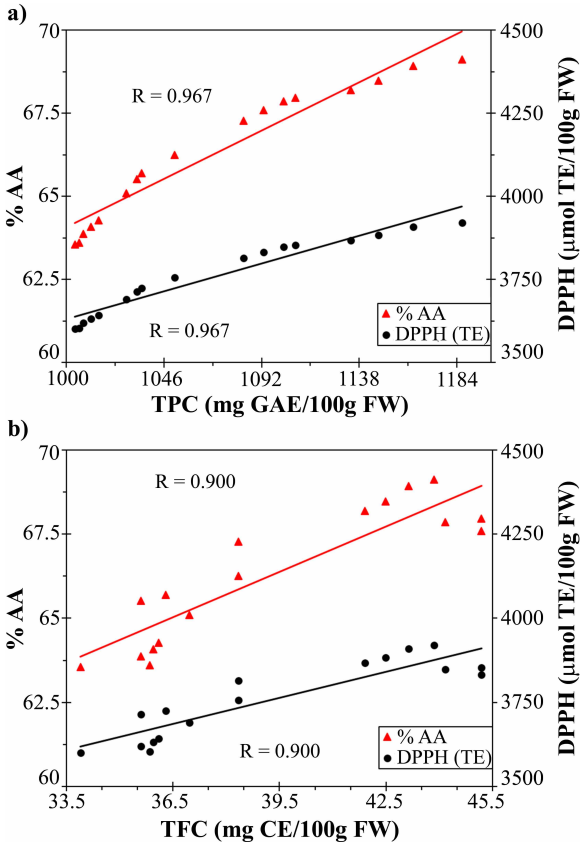

Fig. 6. Correlation between antioxidant activity (% AA, DPPH) with: a) polyphenols, and b) flavonoids.

(Iqbal *et al*., 2012; Singh *et al*., 2016), but differ from the results obtained by Maisarah *et al*., (2013) which reported that the correlation DPPH with flavonoids with was high for tropical papaya pulp. These differences could be due to the cultivar, and the procedures used in the extraction of the samples (Almeida *et al*., 2011; Spínola *et al*., 2015).

Therefore, a high correlation between polyphenol content and antioxidant activity (R = 0.967, $p < 0.05$) indicates that the polyphenols are mainly responsible

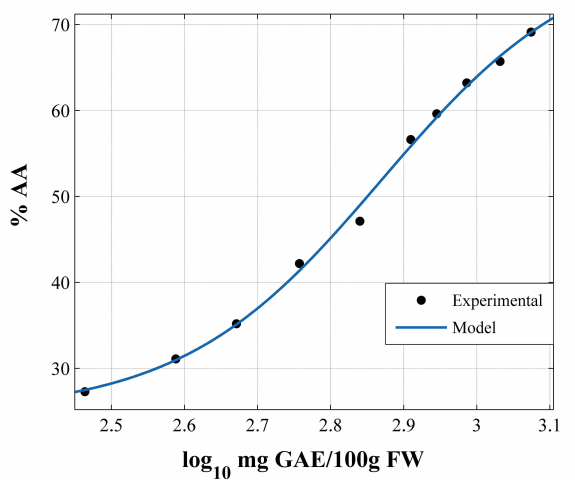

Fig. 7. Effective antioxidant activity (EC50) estimation under the optimum MAE extraction conditions.

for the capacity to eliminate free radicals (Ilaiyaraja *et al*., 2015; Kelebek *et al*., 2015). The contribution of total polyphenols to antioxidant activity is mainly due to the fact that these bioactive compounds neutralize free radicals, and prevent the hydroperoxide decomposition into free radicals (Banerjee *et al*., 2012; Fu and Xu, 2011; Maisarah *et al*., 2013; Watson, 2014).

According to the results, the total polyphenol content (mg GAE/100g FW) corresponding to 50% of antioxidant activity (EC50) was determined. Figure 7 shows the adjustment for the four parameter model (Eq. (3)) with an R^2 coefficient of 0.997, from this model was found a value for $\log(EC_{50}) = 2.87$, which corresponds to 741.31 mg GAE/100g FW. Therefore, to guarantee an effective antioxidant activity a papaya peel and pulp extract with a total polyphenol content higher than the EC50 should be used.

Conclusions

Microwave-assisted extraction of polyphenols and flavonoids from papaya peel and pulp reduces the extraction time compared to conventional extraction, reaching up to 70 % of extraction efficiency. Additionally, the MAE extraction of total polyphenol and flavonoid content were higher compared to the extraction without microwave irradiation. Therefore, this green extraction technology is faster, eco-friendly, and does not require as much energy. The results of MAE optimization indicated that all the factors used in this study had a significant impact on the polyphenols and flavonoids extraction, and the experimental values agreed with the predicted values. Finally, optimum MAE extract showed an effective antioxidant activity, which can be attributed to the total polyphenol content. Therefore, these extracts have potential applications in the food, nutraceutical and pharmaceutical industries. Likewise, papaya peel as an agroindustrial residue represents an alternative for the recovery of high-value bioactive compounds.

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Nomenclature

AA	Antioxidant activity, %
ANOVA	Analysis of variance
CE	Catechin equivalent, mg CE
TFC	Total flavonoid content, mg CE/100g FW
TPC	Total polyphenol content, mg GAE/100g F
W DPPH	2,2-diphenyl-1-picrylhydrazyl
EC50	Total polyphenol content required to reduce 50% of the DPPH radical
GAE	Gallic acid equivalent, mg GAE
MAE	Microwave-assisted extraction
MSE	Magnetic stirring extraction
TE	Trolox equivalent, μmol TE
p.s.	Particle size, μm

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