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ORIGINAL PAPER

Comparison of Conventional Microwave and Focused Microwave-assisted Extraction to Enhance the Efficiency of the Extraction of Antioxidant Flavonols from Jocote Pomace (*Spondias purpurea* L.)

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Abstract Jocote (*Spondias purpurea* L.) is rich in phenolic compounds which have antioxidant properties. The focused microwave-assisted extraction (FMAE) was compared with the conventional microwave-assisted extraction (MAE) to obtain flavonols from jocote pomace. The effects of parameters such as the extraction time, the temperature and the composition of the solvent mixture (*i.e.*, the ethanol to water ration) were evaluated and optimized using a statistical experimental design approach. Response surface methodology (RSM) was applied to determine the important effects and interactions of these independent variables on the extractive yield and quantification of some flavonoids. In addition, the antioxidant activity was analyzed. The total phenolic and flavonoid content was determined according to the Folin–Ciocalteu and aluminum chloride methods, respectively. The free radical scavenging activity of the extract was evaluated according to the DPPH assay. The results showed that the optimum extracting parameters used FMAE with extraction time of 20 min, temperature of 68 °C and ethanol composition of 80 % in water. Under these conditions, a yield of 3.42 % was obtained. Rutin and quercetin were quantified (0.19 mg/mL and 0.024 mg/mL, respectively) through HPLC-DAD. The total phenol and flavonoid contents were found to be 0.897 g GAE/g and 1.271 g QE/g, respectively. In the DPPH scavenging assay, the IC_{50} value of the extract occurred at 43.10 μg/mL. This study shows that FMAE is suitable as an efficient extraction procedure for the extraction of flavonols from jocote pomace.

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Introduction

Jocote (*Spondias purpurea* L.) is a native fruit of Central America, dispersed in Mexico, Guatemala, the Caribbean and in the northeastern region of Brazil [1]. The processing of the fruit generates approximately 30–40 % pomace, consisting of bark and seed or seed and mulch, traditionally considered as an environmental problem [2, 3], but that is being increasingly recognized as source for obtaining high-phenolic products and related health-promoting benefits [4–7]. Engels et al. [8] characterized the profile of the phenolic compounds of jocote peels using ultra-high-performance liquid chromatography coupled with a diode array and an electrospray ionization mass spectrometer, and in addition to phenolic acids, detected several O-glycosides of quercetin, kaempferol, kaempferide and rhamnetin.

In recent years, more and more investigators focus on flavonoids and efficient extraction technique is key issue for its widely application [9]. Modern extraction methods that are economically and environmentally viable and efficient include microwave-assisted extraction (MAE) [10] and focused microwave-assisted extraction (FMAE). MAE is one of the novel extraction techniques which gives better extraction yield, enhances the quality of extracts while decreasing the extraction time and the solvent consumption in comparison to conventional techniques [11]. MAE allows fast extractions, without the degradation of thermolabile compounds, with considerable savings in time and energy. This technique is already used for the extraction of bioactive substances which

are of interest for the food and pharmaceutical industry. Also, another important advantage of the microwave is its applicability in a laboratory, on a pilot and industry scale [12, 13]. The use of a household microwave oven as an extraction apparatus makes this technique less reliable when compared with a FMAE oven. This disadvantage occurs because a household microwave oven cannot monitor the temperature, pressure and energy absorbed inside the vessel during irradiation; these can only be measured after the reaction is completed [14, 15]. However, with the recent development of the FMAE technique, it became possible to monitor several parameters in the extraction procedure, such as the temperature and the pressure during irradiation. Therefore, to obtain an efficient extraction of target compounds, the optimization of experimental conditions is a critical step in developing an extraction method. Response surface methodology (RSM) is a collection of statistical and mathematical techniques which is effective for responses that are influenced by many factors and their interactions, because it allows more efficient and easier arrangement and interpretation of experiments compared to other methods [16]. In addition, it is less laborious and time-consuming than other approaches that are applied to optimize a process. It is widely used for optimization of the extraction process of the bioactive ingredients [17]. Consequently, the aim of this study was to investigate the effect of microwave-assisted techniques on the extraction of flavonols from jocote pomace (*i.e.*, peels, seeds and fibers) by using RSM to determine the optimum extraction conditions in terms of extractive yield and the determination of rutin and quercetin content. In addition, the antioxidant activity of the extract was analyzed.

Materials and Methods

Reagents and Standard

Analytical grade ethanol was used (Synth-Brazil®). Rutin and quercetin standards were purchased from Sigma-Aldrich®. Analytical grade solvents used in the high performance liquid chromatography were purchased from Merck®. A Milli-Q System® (Bedford, MA, USA) was used to purify water. The reactions were performed in shaker flasks (model TE 424, TECNAL®). 2,2-diphenyl-1-picryl hydrazyl (DPPH), ascorbic acid, gallic acid, $NaNO_2$ solution, $AlCl_3.6H_2O$ solution, NaOH and Folin–Ciocalteu's phenol reagent were obtained from Sigma-Aldrich®.

Equipment

The jocote pomace was produced in an electric depulper (Bonina). FMAE was performed using a Discover microwave system (CEM), which operates at a maximum power of 300 W. MAE was performed using an Electrolux ME21S microwave at a power of 100 W. The HPLC apparatus was equipped with a VWR HITACHI L- 2130 pump, a VWR HITACHI L-2300 Diode-array detector, and an auto sampler with a 100 μL loop. The data were acquired and processed using Ezchrom Elite software. All other analyses were performed using a spectrophotometer (QUIMIS, Brazil).

Plant Material

Jocote fruits were collected in São José, State of Bahia, Brazil, in February 2012. Jocote pomace was extracted using an electric depulper, stored in jars, and frozen immediately at −20 °C prior to their use. These were subsequently dried to constant weight at 40 °C and ground to a fine powder, yielding 38.6 % dry pomace.

Microwave Extraction Techniques

For FMAE, samples (1 g) of powdered jocote pomace were placed in a 100 mL flat-bottomed narrow-neck flask topped by a vapor condenser and suspended in 30 mL of extracting solvent. For MAE, the powdered jocote pomace (1 g) was subjected to extraction with 30 mL of the extracting solvent. At the end of each test, the extract was filtered through a vacuum filtration system and concentrated. The extracts were filtered through a 0.45 μm membrane prior to HPLC analysis. A complete factorial experimental design was applied to enable perception of the influence of different independent variables. The FMAE was optimized using a three-level full factorial 2^3 with 3 center points (17 runs). For MAE, the extraction was optimized using a two-level full factorial 2^2 with 3 center points (11 runs) (Table 1). The efficiency of the extraction was calculated as follows: percent extraction yield (*w*/*w*)=mass of extracts/mass of dried material x 100.

HPLC-DAD Analysis

The chromatographic conditions were based on those used in previous studies; with some modifications [18]. A Purospher 100 RP-18 (250 mm×4.6 mm i.d., 5 μm) column (Merck) was used. The mobile phase was composed by solvent (A) H_2O/H_3PO_4 0.1 % and solvent (B) MeOH. The solvent gradient was programmed as A (75–0 %) and B (25–100 %) for 20 min, then 100 % B for 4 min, then 75 % A and 25 % B for 10 min. A flow rate of 1.0 mL/min was used in a 30 °C oven, and 20 μL of each sample was injected. Mobile phases were filtered through a 0.22 μm Millipore filter prior to HPLC injection. Spectral data from 200 to 400 nm were recorded during the entire run. The eluate was monitored at a detection wavelength of 360 nm. Precisely, weighed samples obtained using the microwave-assisted techniques were dissolved in

Table 1 Coded and real values of extraction parameters by microwave-assisted techniques according to a (2^3) experiment design (FMAE) and (2^2) experiment design (MAE) with three central points

	Time (X_1, min)	Ethanol concentration (X_2, %)	Temperature (X_3,°C)	Extraction yield (%)
FMAE				
1	−1.00 (20.00)	−1.00 (20.50)	−1.00 (68.00)	3.14±0.02
2	+1.00 (50.00)	−1.00 (20.50)	−1.00 (68.00)	3.10±0.02
3	−1.00 (20.00)	+1.00 (80.00)	−1.00 (68.00)	3.42±0.06
4	+1.00 (50.00)	+1.00 (80.00)	−1.00 (68.00)	3.38±0.04
5	−1.00 (20.00)	−1.00 (20.50)	+1.00 (92.00)	3.01±0.05
6	+1.00 (50.00)	−1.00 (20.50)	+1.00 (92.00)	2.98±0.07
7	−1.00 (20.00)	+1.00 (80.00)	+1.00 (92.00)	3.04±0.05
8	+1.00 (50.00)	+1.00 (80.00)	+1.00 (92.00)	3.02±0.08
9	−1.68 (10.00)	0.00 (50.00)	0.00 (80.00)	3.22±0.07
10	+1.68 (60.00)	0.00 (50.00)	0.00 (80.00)	3.20±0.09
11	0.00 (35.00)	−1.68 (0.00)	0.00 (80.00)	2.96±0.07
12	0.00 (35.00)	+1.68 (100.00)	0.00 (80.00)	3.18±0.08
13	0.00 (35.00)	0.00 (50.00)	−1.68 (60.00)	3.36±0.09
14	0.00 (35.00)	0.00 (50.00)	+1.68 (100.00)	2.90±0.09
15	0.00 (35.00)	0.00 (50.00)	0.00 (80.00)	3.30±0.09
16	0.00 (35.00)	0.00 (50.00)	0.00 (80.00)	3.26±0.08
17	0.00 (35.00)	0.00 (50.00)	0.00 (80.00)	2.95±0.04
MAE				
1	−1.00 (4.00)	−1.00 (15.00)	–	1.24±0.05
2	−1.00 (4.00)	1.00 (85.00)	–	1.35±0.04
3	1.00 (26.00)	−1.00 (15.00)	–	1.28±0.02
4	1.00 (26.00)	1.00 (85.00)	–	1.60±0.06
5	−1.41 (2.00)	0.00 (50.00)	–	1.18±0.01
6	1.41 (30.00)	0.00 (50.00)	–	1.48±0.05
7	0.00 (16.00)	−1.41 (0.00)	–	1.08±0.04
8	0.00 (16.00)	1.41 (100.00)	–	1.51±0.01
9	0.00 (16.00)	0.00 (50.00)	–	1.42±0.05
10	0.00 (16.00)	0.00 (50.00)	–	1.56±0.07
11	0.00 (16.00)	0.00 (50.00)	–	1.30±0.05

Extraction yield obtained under experimental conditions by microwave techniques

methanol (10 mg/mL) in an ultrasonic bath for 10 min and filtered through a 0.45 μm filter before injection. The method was validated by an external calibration curve using standard solutions of two flavonols, of rutin and quercetin (Mix), prepared in methanol in eight different concentrations, ranging from 0.01 to 0.2 mg/mL (rutin) and 0.002–0.04 mg/mL (quercetin). The Mix was injected three times, and the curve was constructed in Microsoft Office Excel 2007 using the average of the area. Intra-day measurements were used to determine the precision of the developed assay method; these measurements were conducted using three different working solutions prepared with the rutin and quercetin standards. Each solution was injected into the HPLC apparatus in triplicate, and variations were expressed by the relative standard deviations (RSD). The accuracy of the developed assay method was also determined using three different working solutions prepared with rutin and quercetin standards, in triplicate. The accuracy was expressed as the agreement between the experimentally measured value and the set reference value. The precision and accuracy were calculated according to the formula, respectively: RSD (%)=(SD×100)/C, where RSD (%) is the precision, SD is the standard deviation and C is the mean calculated concentrations; Accuracy (%)=(C_{exp}×100)/TC, where C_{exp} is the total concentration of polyphenols from the extract and TC is the theoretical concentration of the standard reference.

The detection limit (LOD) and quantification limit (LOQ) were estimated by the slope and the mean standard deviation of the concentrations used to construct the analytical curve, according to the formula, respectively: LOD=3σ/S, where LOD is the estimated detection limit (mg/mL), σ is the mean standard deviation and S is the slope of the analytical curve; LOQ=10σ/S, where LOQ is the estimated quantification limit (mg/mL), σ is the mean standard deviation and S is the slope of the analytical curve.

Antioxidant Activity

Total Phenolic Content (TPC) Based on the method reported by Slinkard and Singleton [19], the TPC was assayed with reduced volumes of the Folin-Ciocalteu reagent. A plot of absorbance vs. concentration was made based on the measurements. TPC of the extracts were expressed as g gallic acid equivalents per gram (g GAE/g) based on a gallic acid calibration curve.

Determination of Total Flavonoid Content (TFC) The TFC was determined by using a colorimetric method as previously described with a few modifications [20]. The results were expressed as g of quercetin equivalents per gram of extracts (g QE/g) based on the quercetin calibration curve.

DPPH Free Radical Scavenging Assay The free radical scavenging activity was measured using the 2.2-diphenyl-1-picrylhydrazil (DPPH) assay [21, 22]. The absorbance values were measured at 518 nm and converted into the percent antioxidant activity (AA) using the following formula: AA% = [(absorbance of the control − absorbance of the sample)/absorbance of the control] × 100. The IC_{50} values were calculated from a linear regression of the data using the GraphPad Prism 5.0 program.

Statistical Analysis

A CCD design was used to evaluate the influence of the microwave extraction parameters on the efficiency of the experiments (Table 1). A variance analysis was performed for the comparison of means at a 5 % significance level using the Statistica 7.0 software (Minneapolis, USA). The software was also used to plot a Pareto chart and estimate the response surface. Analyses were conducted in triplicate, and the data are expressed as the means±SD. Values were considered significantly different for $p<0.05$.

Results and Discussion

Microwave-Assisted Techniques of Flavonol Extraction

The yield for each set of extraction conditions is shown in Table 1. For the parameters, ANOVA indicated that the differences between the formulations were not statistically significant ($p>0.05$). These results show that FMAE is a rapid and efficient procedure for the extraction of jocote pomace when compared with MAE. The yield (%) varies from 2.90 to 3.42 and 1.08 to 1.60 respectively. This study shows that FMAE doubles the efficiency of extraction of flavonols from jocote pomace. That can be explained because when microwave radiation is focused directly onto the sample, heating is more efficient and thus homogeneity and reproducibility improve greatly. Thus, FMAE has been a selective extraction method and offers a significant improvement in the analysis time and reduces solvent consumption because the system provides a constant feedback control of the extraction temperature through the continuous monitoring of the solvent temperature in the control vessel. However, significant advantages show that FMAE provides a very good and consistent extraction method of bioactive metabolite from natural plants in comparison with MAE.

A microwave-assisted extraction procedure has been optimized to isolate flavonoids from cultivated *Epimedium sagittatum* [23]. In this study, both pure water (0 % ethanol solution) and absolute ethanol (100 % ethanol solution) led to comparatively low extraction yield. When ethanol concentration increased from 0 to 60 % (*v*/*v*), extraction yield improved dramatically. One of the possible reasons is that the affinity of 60 % ethanol solution with flavonoids is closer than that of pure water and absolute ethanol. Karabegovic et al. [12] studied the operational conditions of the microwave-assisted extraction of phenolic compounds in the extracts of fresh cherry laurel leaves and showed a great increase in extraction yield with an increase in extraction time from 10 to 28 min, and then a slight decrease from 28 to 30 min, which indicates 28 min is required to achieve maximum increase.

Microwave-assisted extraction of flavonoids from *Cyclocarya paliurus* (Batal.) Iljinskaja leaves was investigated by Xie et al. [24]. The results showed that increasing the temperature from 40 to 80 °C significantly increased the extraction efficiency. That may be because higher temperature causes intermolecular interactions within the solvent to decrease, giving rise to higher molecular motion, and increasing the solubility. However, higher extraction temperatures beyond 80 °C did not show any significant improvement in the extraction yield. Moreover, the flavonoid yield increased as the extraction time was increased from 3 to 25 min. Further increases in extraction time resulted in little change in the yield of flavonoids.

Rutin and Quercetin Quantification by HPLC-DAD

The chromatographic peaks in the samples were confirmed by comparing the retention time and UV spectra using standard samples. The retention times for rutin and quercetin were 18.2 and 21.5 min, respectively. The linearity was confirmed by preparing standard solutions of rutin and quercetin solutions in methanol at eight concentrations. Calibration curves were plotted and determined using the standards data: for rutin, $y=200000000x+192739$ and $R^2=0.9999$, and for quercetin, $y=300000000x+29774$ and $R^2=0.9996$, which were linear for specified concentration ranges. The detection limit was 0.002891 mg/mL for rutin and 0.00029774 mg/mL for quercetin. The quantification limit was 0.009637 mg/mL for rutin

and 0.000992467 mg/mL for quercetin. The quantification results show that the amounts of rutin and quercetin present in all samples were high above the detection and quantification limits, further emphasizing the reliability of the method. The precision and accuracy of the measurements were within allowable values; the accuracy did not allow values that exceeded 15 %. The concentrations of rutin and quercetin present in all extracts ranged from, for FMAE, 0.074 to 0.19 mg/mL and 0.004 to 0.024 mg/mL; for MAE 0.0266 to 0.052 mg/mL and 0.0021 to 0.0064 mg/mL, respectively (Table 2).

Pérez-Gregorio et al. [25] determined the flavonol and anthocyanin concentrations in different varieties of red and white onions. They found that flavonols (quercetin 7,4-diglucoside, quercetin 3,4-diglucoside, quercetin 3-glucoside, quercetin 4-glucoside) were the predominant polyphenolic compounds. White onion cultivars had the lowest total flavonol content (89.3 and 101.0 mg quercetin/kg fresh weight for Branca da Póvoa and the hybrid SK409, respectively), whereas red onions presented the highest levels of flavonols, with 280.2 and 304.3 mg quercetin/kg fresh weight for Vermelha da Póvoa and Red Creole, respectively.

Table 2 Concentrations of rutin and quercetin present in extracts from jocote pomace using microwave-assisted techniques

Experiment	Flavonol concentration			
	Rutin (mg/mL)	Rutin (mg/g)	Quercetin (mg/mL)	Quercetin (mg/mg)
FMAE1	0.089±0.002	0.2794±0.0005	0.012±0.004	0.0376±0.004
FMAE2	0.088±0.006	0.2728±0.0004	0.011±0.004	0.0341±0.005
FMAE3	0.190±0.065	0.6498±0.001	0.024±0.006	0.0820±0.001
FMAE4	0.157±0.062	0.5306±0.002	0.022±0.001	0.0743±0.002
FMAE5	0.084±0.001	0.2528±0.0002	0.009±0.001	0.0270±0.004
FMAE6	0.080±0.007	0.2384±0.0002	0.008±0.001	0.0238±0.004
FMAE7	0.086±0.004	0.2614±0.0002	0.010±0.005	0.0304±0.004
FMAE8	0.085±0.004	0.2567±0.0002	0.009±0.003	0.0271±0.004
FMAE9	0.105±0.053	0.3381±0.0001	0.012±0.001	0.0386±0.003
FMAE10	0.101±0.061	0.3232±0.0002	0.012±0.006	0.0384±0.002
FMAE11	0.076±0.008	0.2249±0.0005	0.006±0.001	0.0177±0.005
FMAE12	0.099±0.004	0.3148±0.0002	0.012±0.005	0.0381±0.002
FMAE13	0.138±0.062	0.4636±0.002	0.017±0.002	0.0571±0.001
FMAE14	0.074±0.006	0.2146±0.0004	0.004±0.001	0.0116±0.004
FMAE15	0.122±0.047	0.4026±0.001	0.015±0.007	0.0495±0.001
FMAE16	0.118±0.045	0.3846±0.002	0.013±0.006	0.0423±0.002
FMAE17	0.110±0.031	0.3245±0.002	0.018±0.002	0.0531±0.001
ME*	$Y=0.116-0.003X_1+0.015X_2-0.021X_3-0.003X_1X_2+0.003X_1X_3-0.020X_2X_3-0.002X_1^2-0.008X_2^2-0.001X_3^2$		$Y=0.015-0.0003X_1+0.0025X_2-0.004X_3-0.0001X_1X_2+0.0001X_1X_3-0.0026X_2X_3-0.0005X_1^2-0.0016X_2^2-0.001X_3^2$	
	($R^2=0.91$)		($R^2=0.90$)	
MAE1	0.0302±0.003	0.0374±0.004	0.0040±0.0001	0.0049±0.0003
MAE2	0.0305±0.001	0.0411±0.001	0.0050±0.0001	0.0067±0.0001
MAE3	0.0303±0.001	0.0387±0.003	0.0041±0.0001	0.0052±0.0002
MAE4	0.0520±0.005	0.0832±0.002	0.0064±0.0002	0.0102±0.0002
MAE5	0.0296±0.003	0.0349±0.003	0.0057±0.0002	0.0067±0.0001
MAE6	0.0354±0.005	0.0523±0.004	0.0046±0.0001	0.0068±0.0004
MAE7	0.0266±0.004	0.0287±0.004	0.0021±0.0001	0.0022±0.0001
MAE8	0.0380±0.001	0.0573±0.001	0.0059±0.0001	0.0089±0.0003
MAE9	0.0404±0.002	0.0573±0.002	0.0050±0.0002	0.0071±0.0001
MAE10	0.0375±0.003	0.0585±0.003	0.0042±0.0001	0.0065±0.0002
MAE11	0.0412±0.002	0.0535±0.001	0.0045±0.0001	0.0058±0.0001
ME*	$Y=0.039+0.003X_1-0.002X_1^2+0.004X_2-0.0028X_2^2+0.0053X_1X_2$		$Y=0.0045-0.000006X_1+0.0003X_1^2+0.0010X_2-0.0002X_2^2+0.0003X_1X_2$	
	($R^2=0.90$)		($R^2=0.90$)	

*ME model equations

Antioxidant Activity

The results showed that the tests using FMAE were more efficient than the extraction performed through MAE (Table 3). However, there was no significant difference in antioxidant activities in the different extraction parameters ($p>0.05$). The TPC and TFC is reported in Table 3 and shows that the values of FMAE ranged from 0.863 to 0.897 g GAE/g and 1.200 to 1.271 g QE/g, respectively. That highlights not only the potential of extracting more antioxidants from the plant using a focused microwave-assisted extraction technique but also its potential as an antioxidant compound source. The parameters using a conventional microwave had lower levels of TPC and TFC; the values ranged from 0.309 to 0.336 g GAE/g and 0.602 to 0.629 g QE/g, respectively.

Compared with other works that conducted similar fruit extract analyses, the extraction yield in this study was fairly high for both the TPC and TFC. In the analyses of phenolic and flavonoid compounds, Luximon-Ramma et al. [26] found seventeen fruit phenolic compounds with concentrations ranging from 11.8 to 563.8 mg GAE/100 g pulp and flavonoid compounds with concentrations between 2.1 and 71.2 mg quercetin/100 g of pulp. The yellow mombin (*Spondias mombin* L.) pulp presented a total phenolic content of 260.21 mg GAE/100 g [27], which was superior to that found in most fruit pulps consumed in Brazil. Phenolic composition of plant extracts is affected by different factors—variety, climate, storage [28], processing etc. However, the discordance in phenolic content of different groups of plants could be due to varietal, seasonal, agronomical and genomic differences, moisture content, method of extraction and standards used, and so forth [29]. Interestingly, dry fruits had higher activity than did fresh fruits probably due to their low moisture content [30]. A study by Omena et al. [31] demonstrated that the

Table 3 Comparison of total phenolic content (TPC). Total flavonoid content (TFC) and DPPH free radical scavenging assay of extracts from jocote pomace through microwave-assisted techniques

Experiment	TPC[a]	TFC[b]	DPPH Assay	
			%AA	IC_{50} (μg/mL)
FMAE1	0.884±0.0005	1.237±0.002	90.21±1.04	46.93±0.58
FMAE2	0.874±0.003	1.236±0.003	88.69±1.09	47.21±1.06
FMAE3	0.897±0.001	1.271±0.001	94.73±1.07	43.10±1.04
FMAE4	0.896±0.001	1.270±0.001	93.15±1.08	43.90±0.46
FMAE5	0.865±0.0005	1.219±0.002	87.52±1.69	48.73±0.63
FMAE6	0.864±0.002	1.210±0.002	87.16±0.95	49.10±0.68
FMAE7	0.867±0.001	1.226±0.001	88.05±1.47	48.09±1.36
FMAE8	0.866±0.003	1.220±0.003	87.96±0.58	48.64±1.05
FMAE9	0.893±0.002	1.246±0.001	90.90±1.39	46.52±1.08
FMAE10	0.893±0.0005	1.246±0.002	90.88±1.05	46.53±1.05
FMAE11	0.863±0.001	1.202±0.002	86.24±0.68	49.66±0.77
FMAE12	0.893±0.001	1.246±0.003	90.87±1.05	46.55±1.02
FMAE13	0.895±0.003	1.269±0.001	93.04±1.09	44.52±0.95
FMAE14	0.884±0.002	1.200±0.002	85.14±1.04	50.54±0.78
FMAE15	0.894±0.001	1.267±0.003	92.59±1.06	44.98±0.88
FMAE16	0.894±0.0005	1.261±0.002	92.10±1.02	45.78±0.94
FMAE17	0.870±0.001	1.222±0.001	83.21±0.86	40.04±0.98
MAE1	0.317±0.001	0.610±0.004	32.15±1.04	95.64±0.43
MAE2	0.327±0.001	0.620±0.002	33.07±1.09	97.56±0.60
MAE3	0.320±0.002	0.613±0.001	32.41±1.07	98.64±0.23
MAE4	0.336±0.001	0.629±0.004	35.70±1.05	92.34±0.57
MAE5	0.315±0.001	0.608±0.001	31.56±1.03	94.65±0.71
MAE6	0.329±0.002	0.622±0.002	33.39±1.02	96.57±0.63
MAE7	0.309±0.002	0.602±0.003	30.24±0.80	98.74±0.73
MAE8	0.335±0.004	0.628±0.001	34.96±0.98	95.24±0.56
MAE9	0.331±0.001	0.625±0.002	33.68±1.04	96.15±0.42
MAE10	0.330±0.002	0.623±0.003	35.14±1.05	95.41±0.63
MAE11	0.312±0.004	0.610±0.001	32.38±0.94	92.45±0.53
Standard AA[c]	–	–	**95.13±0.04**	**2.41±0.02**

The data are presented as means±SD for three data points; *%AA* percent antioxidant activity (IC50=the effective concentration of the jocote residue extract at which 50 % of DPPH radicals are reduced)

[a] *g GAE/g – GAE* gallic acid equivalent, [b] *g QE/g – QE* quercetin equivalence, [c] standard *AA* ascorbic acid

ethanol extracts of seeds and peels of both jocote and umbu fruits showed the highest antioxidant activities, among other fruits. The jocote pomace extract obtained through focused microwave showed antioxidant activity in the DPPH assay with high antioxidant capacity (the % AA ranged from 83.21 to 94.73). The lower IC_{50} occurred at 43.10 μg/mL extract, represented by FMAE 3. The scavenging effect of the plant extract was compared with a known antioxidant (*i.e.*, ascorbic acid); the result was comparable as shown in Table 3. Such observations suggest that the jocote pomace extract is rich in phenolics which have the potential to be value-added products [29].

Statistical Treatment of Experimental Data

Experimental results for the different extraction conditions showed a significant difference ($p<0.05$) between the concentration of rutin and quercetin. An analysis of variance (ANOVA) was performed to determine the significance. The correlation coefficient (R^2) of the model (Table 2) confirms that the model can adequately represent the true relationship between the parameters chosen. A non-significant lack of fit ($p>0.05$) showed that the quadratic model is valid to the spatial influence of variables and their mutual interactions on the flavonol concentration of the extract can be seen on the three-dimensional response surface curves. The data were used to develop a model second-order polynomial equation to evaluate flavonol concentrations in the extract through MAE as a function of the extraction time (min; X_1), ethanol concentration (%; X_2), and the interaction between them (X_1 and X_2) (Table 2). The Pareto chart (Fig. 1a) demonstrated that the linear function of the ethanol concentration variable showed a slight positive effect on the increase of flavonol concentration, indicating important influence in extraction. Pareto chart also showed that the interaction between both independent variables (X_1 and X_2) does not influence the quercetin concentration.

The effects of the independent variables and their interaction on the increases in rutin and quercetin concentration in jocote pomace extracts can be seen on three dimensional

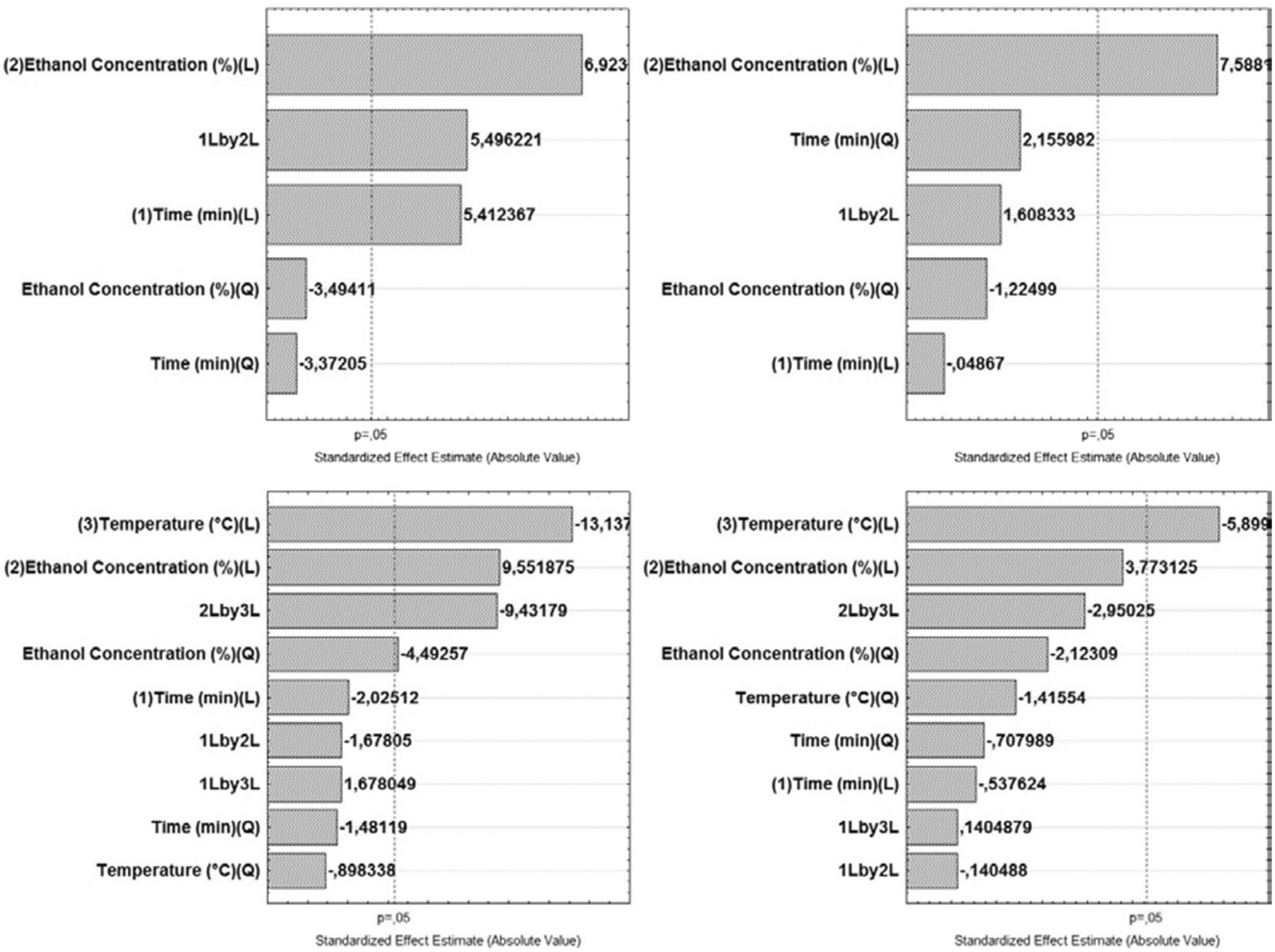

Fig. 1 Standardized main effect of the Pareto chart of rutin and quercetin in jocote pomace extract of MAE (*upper*) and FMAE (*down*), respectively. The *vertical line* in the chart indicates a 95 % confidence level

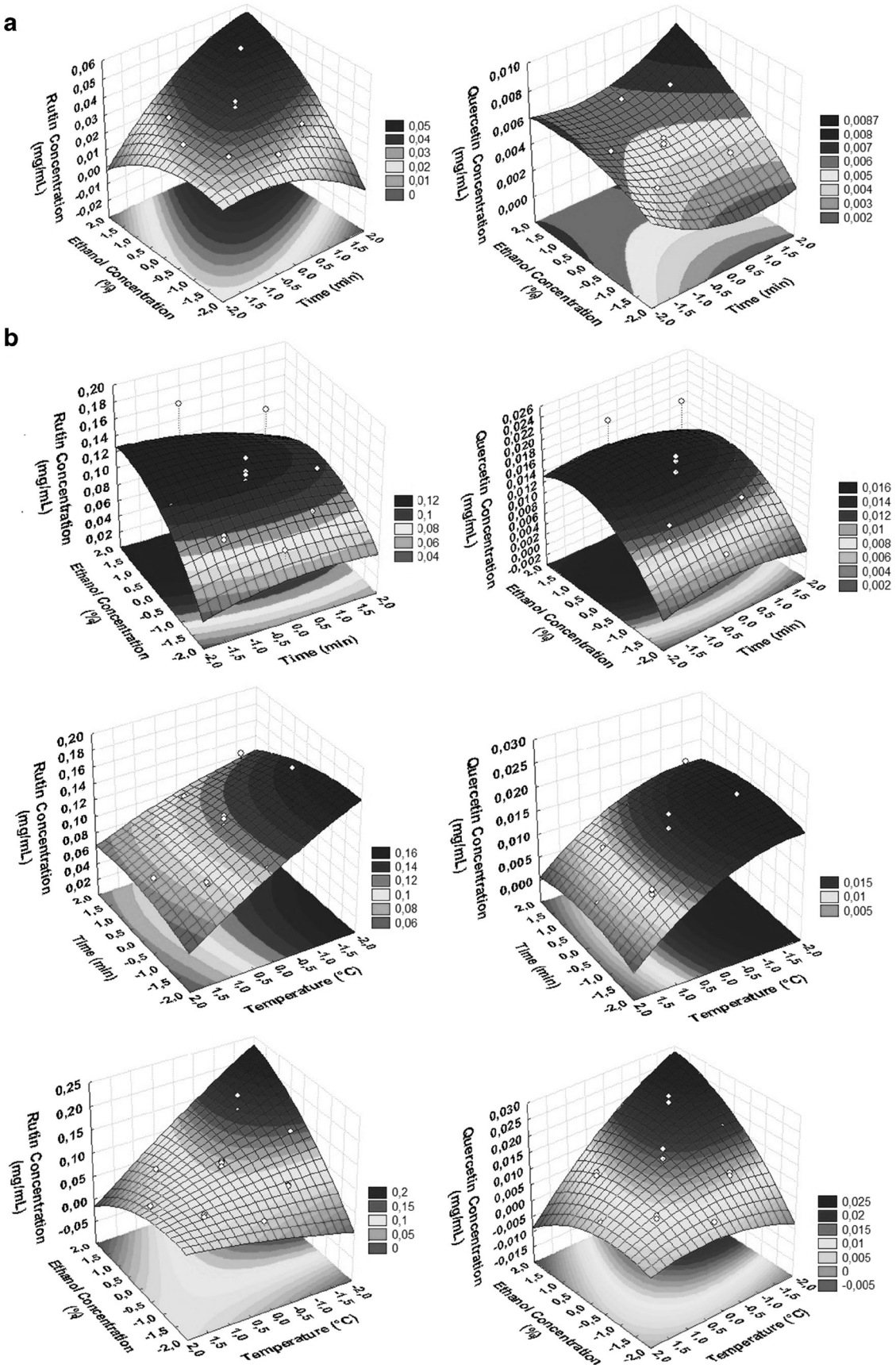

Fig. 2 Response surface representation for rutin and quercetin concentration in jocote pomace extracts of MAE (**a**) and FMAE (**b**)

response surface curves shown on the Fig. 2. The effects of ethanol concentration and extraction time to conventional microwave-assisted extraction (MAE) are shown in Fig. 2a. The response surface graphs presented in Fig. 2b for the independent variables (extraction time, temperature, ethanol concentration) were obtained by keeping one of the variables constant, which indicated the changes in rutin and quercetin concentration under different FMAE conditions.

By analyzing the response surface graphs (Fig. 2a), it was observed that the combined effect of time and concentration of ethanol provides data that can be used to determine the maximum values of these parameters. In this case, there is an apparent linear relationship between a time of 26 min and 85 % ethanol and the increased concentration of active compounds. Normally, if the extraction time is increased, the mass or quantity of metabolites extracted is increased, although sometimes there is the risk that chemical degradation may take place. The extraction of mangiferin from *Mangifera indica* L. leaves with MAE technique was reported by Salomon et al. [32]. The concentration of mangiferin first increased at 4.5 min and then decreased at 5 min. Therefore, very high irradiation time is not appropriate for mangiferin extraction using microwave technique. Analogous results were observed in the withdrawal of flavonoid from *Radix astragali* [33]. In this work, the best microwave radiation time was 25 min. Ethanol is reported to be an effective solvent for the recovery of phenolic compounds, usually used for the production of nutraceuticals and foods, and is related to its GRAS (generally recognized as safe) classification [34]. A few authors reported that the effectiveness of the phenolic compound recovery through solvent extraction with ethanol could be increased by the addition of different amounts of water [35]. One possible reason for the increased efficiency with the presence of water might be the increase in the swelling of the plant material by the water, which increased the contact surface area between the plant matrix and the solvent [33].

Under the different FMAE extraction conditions, a total of 17 runs were used to optimize the three individual parameters in the CCD applied to the extraction of flavonols from jocote pomace. By applying multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial model equation (ME) (Table 2). The significance of each coefficient was determined using the *F*-test and *p*-values. The corresponding variables are more significant if the absolute *F*-value becomes greater and the *p*-value becomes smaller. It can be seen that the variables with the largest effect were the linear terms of extraction temperature (X_3), ethanol concentration (X_2) and the quadratic term of ethanol concentration (X_2^2), followed by the interaction effects of extraction temperature and ethanol concentration (X_2X_3). The results suggest that the change of ethanol concentration and extraction temperature had highly significant effects on the flavonol concentration of the extract. The coefficient of determination (R^2) of the predicted model was 0.91 (rutin) and 0.90 (quercetin), suggesting a good fit; the predicted model seemed to reasonably represent the observed values. Thus, we conclude that the response was sufficiently explained by the model. As shown in Fig. 2b, the increased time (X_1) and extraction temperature (X_3) up to a threshold level led to increased flavonol concentration. Beyond this level, the flavonol concentration slightly decreased, which indicated that a greater extraction could be achieved if the moderate X_1 and X_3 were selected. The Pareto chart (Fig. 1b) shows that the rutin and quercetin concentrations have both linear and quadratic dependencies on the time, temperature, concentration of the ethanol solvent, and the interaction between the independent variables; however, it showed the magnitude of the negative effect of temperature on increasing flavonol concentration, thus reinforcing the idea that this factor does not have a favorable influence on that variable. At temperatures above 90 °C, the FMAE rutin and quercetin concentration were lower than the other parameters, suggesting that this was not an effective extraction temperature. That can be explained because the amount of focused microwave energy applied in the reaction can easily reach and bypass the solvent boiling point. At this moment, most of the solvent would be located in the condenser area, leaving the plant material directly under the microwave energy. It is known that an overheated environment can cause compound loss, which would decrease the flavonoid concentration.

Meanwhile, ethanol concentration (X_2) had a positive impact on the extraction. There is an increase in the flavonol concentration with an increase in ethanol concentration. Therefore, it could be concluded that the optimal conditions for microwave-assisted extraction of rutin and quercetin concentrations from jocote pomace were a microwave extraction temperature of 68 °C, ethanol concentration (80 %) and extraction time of 20 min.

Conclusions

This study proves that FMAE is suitable as a rapid and efficient extraction procedure. The optimum temperature (68 °C), solvent composition (80 % ethanol) and extraction time (20 min) resulted in a maximum flavonol extraction. The plant pomace can be considered as a potential source for flavonoids and phenolic compounds that may be used in several fields, such as nutraceuticals, cosmetics and agro-food industry.

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Conflict of Interest The authors declare that they have no conflict of interest.

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