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Research Article

Ultrasound Assisted Extraction of Mexican Mamey Sapote (*Pouteria sapota*) Seed Oil. Fatty Acid Characterization

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Abstract

The objective of this work was to characterize Mexican mamey sapote seed oil using ultrasound assisted extraction and evaluate its fatty acid profile. Optimization was carried out using Response Surface Methodology (RSM) and a Composite Central Design. The extraction parameters were sample/solvent ratio (6 - 20 g/mL), temperature (30 - 40°C) and time (10 - 30 min) and the response variables were yield, extraction efficiency, peroxide and p-anisidine index, Totox value and free fatty acids. The three optimal treatments were determined by a fatty acids profile. In optimized treatment, predominant fatty acid was oleic (52.61%), followed by stearic acid (29.67%). According to results obtained, optimal conditions were: sample/solvent ratio 1:13 (w/v), 40°C and 20 min extraction time.

Keywords: Mamey Sapote Seed Oil; Ultrasound Assisted Extraction; Optimization; Fatty Acids Profile

Introduction

Nowadays the extraction of oils from seeds from unconventional some fruits has been a subject of research. Mamey sapote seed has been considered for this purpose, since oil is extracted from artisanal, which has been used in cosmetics, pharmaceutical and food industry. The mamey sapote (Pouteria sapota (Jacq.) H.E. Moore and Stearn) is a medium-sized fruit that grows in a tropical tree native to Mexico and Central America. The cultivar of this fruit shows potential as an alternative commercial crop for tropical and subtropical regions of the world [1]. Oil extraction through the mechanical expression of the mamey sapote seed was studied [2]. Cravoto., *et al.* (2011) found that it is possible to obtain a high quality oil, free of contaminants and solvent residues, with a yield of up to 18% of the dry mass of the seed. Alvarez-Reyes., *et al.* (2001) [3] and Solis-Fuentes., *et al.* (2015) [4] studied the process of mass transfer during the extraction of seed oil with ganic solvents and found that the process was affected by the particle size, the extraction time and the relationship of seed mass/solvent volume. Under certain conditions, it was possible to extract practically all of the oil contained in the mamey sapote seeds. expensive apparatus and its low oil yield of oil. Alternatively UAE, a relatively new option, adds ultrasonic treatment to traditional Soxhlet extraction (SE) and enhances mass transfer in liquid by using cavitation forces [5]. The high-shear gradients created by the collapsing of explosive bubbles disrupt cell walls and increase the release of intracellular components into the solvent [6]. UAE is seen as an ideal option for the edible oil industry because of improvements in efficiency and speed and because it can be performed at low operation temperatures which avoids termal damage to material [7].

Today mamey oil is being reassessed beyond artisanal applications and has been analyzed in greater detail, for example, it is acquiring an interesting position as a source of triterpene alcohols, whose properties are interesting for applications in cosmetics (it is commonly considered to be effective in skin care and its healing, to improve tolerance to UV radiation, etc.) and pharmacology (anti-inflammatory capacities, erythema and sunburn treatments, etc.) [8]. One aspect, sparsely addressed, is the potential use of this oil in the food sector where, because of its characteristics, it could be an interesting source of vegetable fat. Therefore, the aim of the present study was optimized and to characterize the thermal properties of mamey sapote oil.

Materials and Methods

Mamey sapote fruits were provided by producers at San Juan Bautista Tuxtepec, Oaxaca. All standards of HPLC-grade were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Other reagents used were from J. T. Baker (México City, Mexico).

Extraction of mamey sapote seed oil by ultrasound-assisted solvent extraction

Seed conditioning and oil extraction were performed as described below. Seeds were selected manually, peeled and dried in

an oven at 65°C for 20 h to decrease the moisture content gf w/w) and reduced to particle size of 0.59 mm. An ultrasonic bath (Elmasonic P 30/80 Khz. Singen, Germany) with a frequency of 80 kHz and power of 100% was used, with 20 g of mamey sapote seed flour used for each treatment and hexane as solvent extraction. The extraction conditions were sample/solvent ratio (6 - 20 g/mL), temperature (40 - 60°C) and time (10 - 30 min). After the extraction period, the samples obtained were filtered under vacuum and the oil was recovered in a rotary evaporator (Büchi Rotavapor™ R-100, Thermo Fisher Scientific, USA). Oils recovered were stored in amber bottles under a nitrogen atmosphere at 4°C until analysis.

Yield and efficiency of the extraction of oil

The yield and efficiency of the extraction were calculated with equations 1 and 2

$$Yield(g/kg) = \frac{extracted\ oil\ (g)}{sample-extracted\ oil} x1000 \quad \text{-------(1)}$$

$$Efficiency\ (g/kg) = \frac{yield\ (g)}{total\ lipid\ content} x1000 \quad \text{-------(2)}$$

Oxidative stability

Oxidative stability parameters were calculated according to AOCS (1999) [9]. Peroxide index (PV) (Method Cd 8-53), free fatty acids (FFA) (Method Aa 4-38), p-anisidine (p-A) (Method Cd 18-90) and the TOTOX value (VT) (CG 3-91) was calculated according to the following equation:

TV=p-*A*+ 2PI -------(3)

Characterization of the optimal products

Fatty acid profile

Fatty acids were determined by gas chromatography (GC) according to Rodríguez-Miranda., *et al*. (2019). Then, 100 mg of oil was added to 800 µL of chloroform–methanol (2:1, v/v). To 200 µL of this solution was added 1 mL of 1 mol L^{-1} hydrochloric acid–methanol, and the tube was immediately placed in a dry bath at 80°C for 20 min. The solution was then cooled, 200 µL of distilled water was added, and extracted twice with hexane (1 mL each time). The organic phases were collected, anhydrous sodium sulphate was added to remove the water, and then were centrifuged at 1962 x g for 5 min. The solvent was decanted off, and hexane was evaporated under flowing nitrogen. Finally, the samples were suspended in 1.4 mL of hexane for analysis by GC. The fatty acid profile and CLA content were quantified using an Agilent 6890A GC system fitted with a Supelcowax capillary column (100m × 250 µm i.d., × 0.25 µm film thickness; Agilent Technologies Inc., 5301 Stevens Creek Blvd. Santa Clara, CA 9505, USA) and a flame ionization detector, and the temperature was set at 250 °C, splitless injector (set at 250°C). Nitrogen was employed as carrier gas. Samples were injected in triplicate from each lipid extraction. The methyl esters of the samples were identified by comparison of their retention times with those of commercial standards.

Experimental design and data analysis

A central design composed was performed to determine the optimal conditions of the ultrasound-assisted extraction of mamey sapote seed oil. The experimental design consisted of 20 treatments. The sample/solvent ratio (X_1), extraction temperature (X_2) and extraction time (X_3) were chosen as the independent variables (Table 1). Oil yield extracted and some oxidative stability parameters (IP, p-A value, FFA and VT) were selected as the response variables. The actual and coded levels of the independent variables are given in Table 1. The experimental runs were randomized, and the experimental data were analysed with the Response Surface Methodology (RSM) using the Design Expert statistical package (Design Expert 8.0.2, Stat-Ease Inc., Minneapolis, USA).

Treatments	Proportion (g/mL) (X_1)	Temperature (°C) (X_2)	Time (min) (X_3)	Yield (g/kg)	Eficiency (g/kg)	PI (meq O_2/kg)	*p*-A	FFA (% oleic acid)	Totox value
1	8.84 (-1)	44.05 (-1)	14.05 (-1)	466.63 ± 0.42	946.32 ± 0.95	1.98 ± 0.01	2.90 ± 0.00	0.21 ± 0.00	6.87 ± 0.01
2	17.16 (1)	44.05 (-1)	14.05 (-1)	476.89 ± 0.34	967.13 ± 0.77	1.98 ± 0.00	2.26 ± 0.00	0.21 ± 0.00	6.24 ± 0.00
3	8.84 (-1)	55.95 (1)	14.05 (-1)	467.35 ± 0.06	947.78 ± 0.13	1.97 ± 0.00	2.43 ± 0.04	0.2 ± 0.00	6.38 ± 0.08
4	17.16 (1)	55.95 (1)	14.05 (-1)	478.94 ± 0.03	971.28 ± 0.08	1.98 ± 0.00	3.42 ± 0.01	0.2 ± 0.00	7.41 ± 0.02
5	8.84 (-1)	44.05 (-1)	25.95 (1)	464.89 ± 0.42	942.79 ± 0.96	1.99 ± 0.00	1.68 ± 0.01	0.22 ± 0.03	5.66 ± 0.03
6	17.16 (1)	44.05 (-1)	25.95 (1)	463.84 ± 0.34	940.67 ± 0.78	1.97 ± 0.00	1.52 ± 0.01	0.2 ± 0.00	5.49 ± 0.03
7	8.84 (-1)	55.95 (1)	25.95 (1)	463.96 ± 0.31	940.90 ± 0.71	1.98 ± 0.00	2.09 ± 0.00	0.2 ± 0.00	6.06 ± 0.02
8	17.16 (1)	55.95 (1)	25.95 (1)	460.19 ± 0.01	933.25 ± 0.02	2.0 ± 0.00	3.24 ± 0.02	0.2 ± 0.00	7.23 ± 0.01
9	6 (-1.68)	50 (0)	20 (0)	463.35 ± 0.31	939.67 ± 0.70	1.96 ± 0.00	2.63 ± 0.01	0.18 ± 0.03	6.56 ± 0.02
10	20 (1.68)	50 (0)	20 (0)	471.31 ± 0.00	955.82 ± 0.01	1.99 ± 0.00	2.4 ± 0.03	0.16 ± 0.00	6.39 ± 0.08
11	13 (0)	40 (-1.68)	20 (0)	529.54 ± 0.54	1073.90 ± 1.22	1.99 ± 0.00	4.32 ± 0.01	0.18 ± 0.03	8.21 ± 0.02
12	13 (0)	60 (1.68)	20 (0)	489.26 ± 0.24	992.21 ± 0.54	1.99 ± 0.00	3.45 ± 0.03	0.22 ± 0.03	7.41 ± 0.01
13	13 (0)	50 (0)	10 (-1.68)	462.45 ± 0.12	937.84 ± 0.28	1.99 ± 0.00	1.69 ± 0.02	0.24 ± 0.01	5.67 ± 0.05

14	13 (0)	50 (0)	30 (1.68)	522.82 ± 0.25	1060.28 ± 0.56	1.99 ± 0.00	4.9 ± 0.03	0.24 ± 0.00	8.89 ± 0.04
15	13 (0)	50 (0)	20 (0)	515.89 ± 0.09	1046.21 ± 0.20	1.99 ± 0.00	3.16 ± 0.01	0.2 ± 0.00	7.14 ± 0.02
16	13 (0)	50 (0)	20 (0)	513.36 ± 0.02	1041.10 ± 0.04	1.99 ± 0.00	3.12 ± 0.01	0.2 ± 0.00	7.09 ± 0.00
17	13 (0)	50 (0)	20 (0)	507.10 ± 0.07	1028.38 ± 0.16	1.99 ± 0.00	2.8 ± 0.03	0.26 ± 0.03	6.78 ± 0.03
18	13 (0)	50 (0)	20 (0)	462.55 ± 0.18	938.04 ± 0.40	1.99 ± 0.00	5.44 ± 0.01	0.24 ± 0.00	9.43 ± 0.03
19	13 (0)	50 (0)	20 (0)	485.58 ± 0.09	984.74 ± 0.20	1.98 ± 0.00	3.77 ± 0.03	0.27 ± 0.03	7.74 ± 0.02
20	13 (0)	50 (0)	20 (0)	521.19 ± 0.14	1056.97 ± 0.32	1.99 ± 0.00	3.58 ± 0.04	0.25 ± 0.00	7.57 ± 0.05

Table 1: Experimental design of the independent variables and response of the dependent variables according to the central design composed in the extraction of mamey sapote seed oil.

Values in parentheses are the coded values of the independent variables. PI: Peroxide Index; *p*-AI: *p*-Anisidine Index; FFA: Free Fatty Acid; TV: Totox

Value. The results are the average of 3 determinations ± standard deviation.

The analysis of variance (ANOVA), the mean values were considered significantly different when $p < 0.05$, regression analysis and plotting of response surface plots, allowed establishing the optimal conditions for oil extraction. Statistical analysis was performed by using Design Expert program (10.0. version).

Results and Discussion

Effect of independent variables on oil yield and oxidative stability

Table 1 shows the extraction yield in the different treatments, which was found in a range of 460.19 to 529.54 g/kg, with treatments 11, 14 and 10 having the highest yield of extraction. The conditions where a higher extraction yield was obtained were having a sample/solvent ratio of 13 g of sample per mL of the solvent (hexane), temperature of 40°C and time of 14.05, 20 min.

The correlation coefficient of the experimental data was 0.97 for yield extraction, which indicates that the model had a significant effect ($p < 0.05$) and that it adequately represents the real relationship between the parameters studied. According to the statistical analysis, the oil yield correlates positively with the time and temperature (Figure 1a) and for quadratic terms, the oil yield correlates negatively with sample/solvent ratio and temperature Figure 1b.

Hernández-Santos., *et al.* (2017) [11] reported an extraction of 46.75 g for every 100 g of sample, in this work an extraction yield of 48.35 g of oil was obtained for every 100 g of sample. This is due to the power and frequency of the ultrasonic bath since the use of ultrasound provides a greater contact surface between the phases, due to the vibrations caused by the ultrasound waves and these vibrations allow a better penetration between the solvent and the matrix cellular, in which the oil density decreases making its extraction easier.

Figure 1: Three-dimensional graph of the extraction efficiency of mamey sapote seed oil as a function of (a) temperature (°C), (b) proportion (mL).

Table 1 shows the results of the oil extraction efficiency of mamey sapote seeds, which varied from 937.84 to 1073.90 g/kg with an average of 982.26 g/kg, in the 20 treatments. Treatments 11 and 14 presented a greater efficiency.

Table 2 shows the regression model coefficients, time had a significantly positive effect ($p < 0.05$) and temperature a negative effect on efficiency, a linear behavior was observed which indicates that increasing the temperature decreases the efficiency of the process.

Peroxide index values varied from 1.96 to 2.0 meq O_2/kg of oil, being the treatment T8 the one that showed high values; this variation was dependent on the extraction conditions. However, these results indicated that the oil is stable to the different ultrasound extraction conditions; these samples have relatively low values compared to those allowed by the Mexican Standard Norm NMX [12], which authorizes a maximum value of 20 meq O_2/kg in vegetable oils.

Parameters	Yiel	Eficiency	PI	*p*-AI	FFA	Totox value
Intercept	514.39	1043.17	1.99	3.17	0.23	7.15
X_1	0.38	0.78	0.01	-0.15	-0.01	-0.13
X_2	-13.97	-28.33	0.00	-0.31	0.01	-0.32
X_3	15.97	32.40	0.00	0.87	0.00	0.87
X_1X_2	-2.35	-4.77	0.01	0.69	-0.01	0.71
X_1X_3	-5.20	-10.55	0.00	-0.64	0.00	-0.64
X_2X_3	-17.34	-35.17	0.00	-0.20	0.01	-0.20
X_1^2	-16.62	-33.70	-0.01	-0.23	-0.02	-0.24
X_2^2	-1.77	-3.58	0.00	0.24	-0.01	0.23
X_3^2	-7.70	-15.62	0.00	0.05	0.00	0.05
R^2	0.97	0.97	0.96	0.93	0.93	0.93
F	33.90	33.9	7.75	5.35	3.61	10.37
P>F	0.00	0.00	0.00	0.01	0.03	0.00

Table 2: Regression coefficients of the independent variables and analysis of variance of the central composite model.

*The highlighted values are statistically significant ($p < 0.05$); X_1: Proportion; X_2: Temperature; X_3: Time.

Correlation coefficient of the model adjusted to the experimental data was $R^2 = 0.96$, as shown in Table 2. The temperature and proportion had a significant effect ($p < 0.05$) in the linear term on the PI response. contrary to what happens in the quadratic terms in which when the proportion increases, a positive correlation is observed with negative effects ($p < 0.05$) and in the temperature and time they do not show significant effects ($p > 0.05$) which predicts that when increasing these two variables have higher values.

At high temperatures and prolonged times, mono and polyunsaturated fatty acids are degraded, because they are susceptible to oxidation due to the presence of double ligation, on the other hand peroxides and hydroperoxides are very unstable compounds; They break down easily to secondary oxidation products, resulting in a lower peroxide index (IP). This decomposition is carried out in the middle and final stages of lipid oxidation [13].

The p-anisidine content of the zapote mamey seed oil varied from 1.52 to 5.44 (Table 1). The correlation coefficient was $R^2 = 0.93$. The results revealed that the temperature showed a significant effect ($p < 0.05$) in the linear term, and the interaction of the temperature and ultrasound time variables showed a negative correlation (Table 2) with a significant effect ($p < 0.05$); in the quadratic terms and the combinations it can be observed that they do not have a significant effect ($p > 0.05$), thus indicating that the increase in temperature does affect p-AV, which can cause the deterioration of polyunsaturated fatty acids whose double bonds are more susceptible to oxidation due to thermal effects. This effect has been reported in other studies in which an increase in ultrasound time promotes the oxidation of conjugated double bonds [14]. This effect has been reported in other studies in which an increase in ultrasound time promotes the oxidation of conjugated double bonds [14].

The VT in the oil of the mamey sapote seeds depended on the different extraction conditions used and ranged from 5.66 to 9.43 (Table 1). Table 2 shows the correlation coefficient that was R2=0.93, demonstrating that the model had a significant effect ($p < 0.05$), the significant effects ($p < 0.05$) of the independent variables in the VT were also observed being the time that positively affected in linear term, Because the TOTOX value is associated with both the p-anisidine and peroxide index, this value is also affected by time as mentioned above at higher time, the oxidation of fatty acids and therefore the formation of oxidation compounds.

Regarding the content of free fatty acids Table 2 shows that the coefficient of correlation was $R^2 = 0.73$, indicating that the model had no a significant effect ($p < 0.05$). As shown in Table 2, none of the variables, both linear and quadratic, have a significant effect ($p > 0.05$) on free fatty acids, it is also observed that the interactions do not affect these values.

Optimization and validation of the extraction process

The numerical optimization was carried out based on the experimental results and the statistical analysis, with the objective of maximizing the extraction yield and efficiency as well as minimum values of Index of peroxides, p-anisidine, Totox value and free fatty acids (oxidative stability), in ultrasound extraction. In this study, 7 optimal conditions were obtained, of which 3 (T1-1, T2-1 and T3-1) were chosen based on the most desirable, as shown in Table 3. Experimental values higher in yield and efficiency of extraction than in the values predicted by the model can be observed (Table 3). Of the three treatments selected, the best treatment was T1-1. The extraction conditions were as follows: sample/hexane ratio of 1:13(p/v), temperature 40 °C and extraction time of 20 min with a desirability of 0.8.

Treatments				D	Yield		Efficiency		Peroxide Index (meq O_2/g)		P-anisidine index		Free fatty acids (%)		Totox value	
	X_1	X_2	X_3		P	E	P	E	P	E	P	E	P	E	P	E
T1-1	1:13	40	20	0.80	478.9	580.7	971.2	993.10	1.36	1.99	2.30	3.53	0.99	0.23	4.34	7.45
T2-1	1:13	50	30	0.77	489.3	499.56	992.2	986.36	1.07	3.95	2.57	3.07	1.01	0.22	3.65	6.91
T3-1	1:13	50	20	0.76	485.6	489.98	984.7	985.61	1.65	3.96	1.80	3.10	1.01	0.20	4.85	9.48

Table 3: Optimization and validation of extraction conditions according to the central composite design.

D: Desirability; X_1: Proportion; X_2: Temperature; X_3: Time; P: Predicted Data; E: Experimental Data.

Fatty acid composition

The fatty acid profile of mamey sapote seed oil (Table 4) had a higher content of oleic acid (C18:1), followed by stearic acid (C18:0) and in a lesser amount linolenic acid (C18:3) and gadoleic acid (C20:1). The monounsaturated fatty acids were those that were in greater proportion (531 g kg^{-1}) followed by the saturated (393.6 g kg^{-1}) and to a lesser amount polyunsaturated (75.4 g kg^{-1}). Table 4 shows that these results fall within the values reported by other authors [11,15]. In this work a greater amount of gadoleic acid was found than reporatdo by Hernández-Santos., *et al.* [11] which has also been identified in fish oil, rapeseed oil, melon seed oil and mustard seed oil and mamey sapote seed oil [16,17]. The variation in the content of fatty acids is due to the variety and growth conditions of the mamey sapote, just as the method of extracting the oil by ultrasound could have influenced this proportion. The values found in this work are similar to those found in some vegetable oils for industrial use such as olive and peanut oil, having in common with these oils a high content of oleic acid, also in mamey sapote oil, the acid Stearic acid is the saturated fatty acid found in greater proportion and this fatty acid is found in greater proportion in cocoa butter [11,18].

Fatty acids (g kg^{-1})	This work	Hernández-Santos., *et al.* [11]	Moo-Huchin., *et al.* [15]
Palmitic (C16:0)	96.9 ± 0.15	87.8 ± 0.10	87.1
Stearic (C18:0)	296.7 ± 1.10	223.5 ± 1.30	349.1
Oleic (C18:1)	526.1 ± 1.20	577.3 ± 1.30	470.6
Linoleic (C18:2)	68.9 ± 0.20	101.2 ± 0.30	51.2
Linolenic (C18:3)	6.5 ± 0.10	7.9 ± 0.00	-
Gadoleic (C20:1)	4.9 ± 0.10	2.3 ± 0.10	-
Saturated	393.6	311.3	436.2
Monounsaturated	531	579.6	470.6
Polyunsaturated	75.4	109.1	51.2

Table 4: Fatty acid composition of mamey sapote seed oil

Mean ± standard deviation of three determinations

Conclusion

In this study the optimal conditions for the ultrasound-assisted extraction of mamey sapote seed oil were sample/solvent ratio of 1:13 (p/v), temperature at 40°C and extraction time of 20 min. The profile of fatty acids selected that the oil has a higher content of monounsaturated fatty acids (C18: 1 and C20: 1), with oleic acid being in the highest proportion, which places it in the category of oils rich in oleic acid.

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