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Aqueous Extraction of Seed Oil from Mamey Sapote (*Pouteria sapota*) after Viscozyme L Treatment

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Abstract: In this study, aqueous enzymatic extraction (AEE) was evaluated during the process of obtaining oil from mamey sapote seed (OMSS). Viscozyme L enzyme complex was used at pH 4 and 50 °C during the optimization of the extraction process by central composite design and response surface methodology. Optimal conditions were: 3.5% (*w*/*w*) of enzyme (regarding the seed weight), 5.5 h of incubation time, 235 rpm of agitation rate, and 1:3.5 of solid-to-liquid ratio. These conditions enabled us to obtain an OMSS yield of 66%. No statistically significant differences were found in the fatty acid profile and physicochemical properties, such as the acid and iodine values and the percentage of free fatty acids, between the oil obtained by AEE or by the conventional solvent extraction (SE). However, the oxidative stability of the oil obtained by AEE (11 h) was higher than that obtained by SE (9.33 h), therefore, AEE, in addition to being an environmentally friendly method, produces a superior quality oil in terms of oxidative stability. Finally, the high oil content in mamey sapote seed, and the high percentage of oleic acid (around 50% of the total fatty acid) found in this oil, make it a useful edible vegetable oil.

Keywords: mamey sapote seed oil; Viscozyme L; fatty acid composition; oxidative stability

1. Introduction

Mamey sapote (*Pouteria sapota* H.E. Moore & Stearn) is a tropical fruit from the *Sapotaceae* family [1–8]. This fruit presents good sensory characteristics and high nutritional value (it contains vitamins A, C, and E, minerals, fiber, carbohydrates, and some carotenoids) [9–12]. The fruit flesh pulp is soft, with a smooth texture, and sweet flavor, and with an attractive orange or red color when ripe; it is often used as an ingredient in products like bakery, desserts, yogurt, ice pop, sorbet, and others [13]. Mamey sapote shape changes from round to ovoid or elliptic, its size is in the range of 10–20 cm in length and its weight ranges from 0.2 to 3 kg; it has a firm, dark-brown, rough, leathery semi-woody rind [1,14–16]. This contains one-to-four bright brown oily seeds (40–60% of lipids) with a characteristic almond odor [1,6,15,17]. Traditional indigenous medicine employs the oil from the mamey sapote seed (OMSS) to treat ear pain (due to its analgesic properties)

and alopecia, and the milled seed to treat digestive disorder, rheumatism, and kidney stones. The cosmetic industry uses this oil to produce hair dyes, shampoos, soaps, and other products [1,15,17,18]. The chemical composition of crude OMSS might be mixed with some natural fats, such as cocoa butter or mango seed fat, to improve their nutritional features [15,18]. Additionally, the possibility of using OMSS for biodiesel production has been explored, obtaining a yield of 92.48%, employing 0.760% of catalyst, 6 methanol equivalents per mol of triglyceride, at 60°C and after 90 min of reaction [19].

OMSS presents a high content of oleic acid (48.62–53.59%) and a similar fatty acid composition to other edible vegetable oils; this composition is influenced not only by the state of the fruit maturity, but also by the variety of the mamey, the cultivation conditions (e.g., soil, climate), and the oil extraction method used [15,20]. In this sense, when evaluating new extraction methods, it is necessary to show that they do not negatively alter the composition of fatty acids. Some studies on its potential applications, physicochemical properties, chemical composition, and extraction methods have been reported [15,21]. Thus, the extraction of OMSS is a process of great interest in diverse areas such as the production of edible vegetable oils, natural drugs, food substitutes, and biodiesel.

The main extraction methods of vegetable oils are mechanical pressing and solvent extraction (SE), mainly using hexane [22,23]. The main disadvantage of mechanical pressing is its low oil yield, generally necessitating the use of a second solvent extraction step. Solvent extraction generates high extraction yields (higher than 90%) being the most efficient industrial oil extraction method [24,25]. In this regard, Solís-Fuentes et al. (2001) studied the process of mass transfer during the extraction of seed oil with organic solvents, finding that the process was affected by the extraction time, seed particle size, and the seed mass/solvent volume relationship [26]. However, in addition to its high cost, the utilization of organic solvent pollutes the environment and is dangerous for human health [27,28]. Moreover, after oil extraction, the defatted seed kernels, which still contain important nutrients (such as carbohydrates and proteins), must receive a subsequent treatment to eliminate all solvent residues, or must be discarded, which limits the integral use of the seeds [29–31]. In this context, it is obviously interesting to develop and apply efficient methods of oil extraction that allow obtaining oil yields similar to conventional methods, but which are also environmentally friendly and safe for people, and promote the development of the circular economy [32–36].

Aqueous extraction (AE) methods are gaining attention in this context [37–39]. However, compared to SE, AE oil yields are lower, as oil is trapped in the rigid cell wall structure of the seed [37,40,41]. Considering that the vegetable cell wall mainly consists of proteins, lignin, hemicellulose, cellulose, and pectic substances [42,43], enzymes like hemicellulase, cellulase, and pectinase can be used to destroy the cell wall structure and permeabilize the liposome membranes, to facilitate the release of oil from the lipid bodies to the medium [44–47]. AE has been improved using enzymes to enhance oil extraction yields; this strategy is named aqueous enzymatic extraction (AEE), and it does not produce residual deleterious solvents, allows the simultaneous extraction of proteins [48,49], and preserves the nutritional and bioactive features of the extracted natural products, including oils [47,50–53]. The produced defatted oilseed kernels (which will not contain toxic residues) can be employed as raw material in feed and/or food industries [42,44,45] or in the production of other valuable products such as bioactive peptides after proteolysis [54–58].

Several studies have been reported regarding the evaluation of AEE in obtaining oils from various seeds such as tiger nut (*Cyperus esculentus* L.) [59], sacha inchi (*Plukenetia volubilis* L.) [60], *Jatropha curcas* [61], Jicaro [62], pomegranate [63], castor oil [64], *Pinus pumila* [25], *Moringa oleifera* [65], Pumpkin (*Cucurbita pepo*) [50], sunflower [66], soybean [67], almond [49], etc. Although the enzymatic oil extraction has been widely explored, in the reported studies, different oil yields are observed for each seed; this is due to the fact that the composition of the cell wall of the seed, which is quite heterogeneous, is different for each seed, so different enzymes and conditions are necessary in each case. In addition,

AEE results are still lower than that achieved using SE, which limits its use [64,68–70]. In this context, AEE efficiency has been improved by the utilization of technologies like microwave irradiation and ultrasounds [71,72].

Some variables that determine the efficiency of AEE are the agitation rate, seed particle size, liquid-to-solid ratio, pH, temperature, incubation time, and the amount of enzyme [63,65,73]. However, the selection of the utilized enzyme or enzyme cocktail is crucial to success [52]. Several studies report that the use of enzymatic cocktails allows one to obtain better oil extraction yields, compared to that obtained by a single enzyme [25,53,72]. If the modification of a single and monofunctional compound may already be improved when using several enzymes [74], now that very different compounds must be modified, the use of an enzyme cocktail seems to be highly recommendable. For instance, Latif et al. (2009), reported that compared to Alcalase 2.4 L, Kemzyme, Natuzyme and Protex 7 L, Viscozyme L (a multienzymatic complex composed of xylanase, cellulase, arabanase, β-glucanase, and hemicellulase) [62] produced the most elevated oil yield from sunflower seeds (*Helianthus annuus* L.) [46]. Liu et al. (2020) reported that the oil and protein extraction must be closely related to the degree of degradation of the main components of the cell wall; in this sense, Viscozyme L degrades the hemicellulose, cellulose and pectin molecules, and destroys the cell wall structure acting on the C–C stretching, C–O stretching and CH_2 symmetrical bending of cellulose, the C–O stretching, and O–C–O asymmetrical bending of hemicellulose, and the C–C stretching and C–O stretching of pectin, which facilitate the release of oil bodies and proteins from cells [48].

We did not find any previous communications in the literature on the application of AEE to produce OMSS; for this reason, the objective of this study was to optimize, by response surface methodology coupled to central composite design (CCD), the aqueous enzymatic extraction of OMSS using the enzymatic complex Viscozyme L, comparing the fatty acid composition and physicochemical features of the oil that has been obtained by AEE with the one obtained by traditional SE. In addition, the changes of the aspect of the seeds before and after AEE and SE were investigated.

2. Results and Discussion

2.1. Solvent Extraction

SE got 2.23 ± 0.06 g of OMSS from 5 g of dried seeds, representing an OMSS content of 44.63 ± 1.2%, which is quite similar to that previously reported by Moo-Huchin et al. (2013) and Solís-Hernández et al. (2015), who found an OMSS content of 40 ± 1.7% [18] and 44.41% [15], respectively, but lower than that obtained by Hernández-Santos et al. (2017), who reported an OMSS content of 55.9% [20]. These differences can be attributed to differences in the fruit maturity and variety, or environmental factors of the geographical location (temperature, soil type, humidity, etc.) [20]. Some traditional sources of vegetable oils have similar or lower oil contents, e.g., 12–50% in olive [15,18], 40–48% in rapeseed [75], 42–49% in peanuts [76], 30% in safflower [77], 20% in cotton [78], and 18–23% in soybeans [79].

The oil content obtained by SE was considered the total oil present in the mamey seed, and was used to calculate the yields of the AEE for comparison.

2.2. Optimization of Aqueous Enzymatic Extraction

AEE variables, such as incubation time, amount of enzyme, solid/liquid ratio, and agitation rate were investigated using a central composite design in order to determine the linear, quadratic, and combination of factors effect on the extraction performance of OMSS. Table 1 presents the conditions and results of the 28 treatments of the experimental design. AEE yielded an OMSS recovery in the range of 48–69% which is higher than the 38% yield obtained in the control extraction where no enzyme was added. The highest oil yield (69%) was obtained in run 11 (3.5% of Viscozyme L, 1:3 solid/liquid ratio, 5 h of incubation time, and 250 rpm) followed by run 20 with 68% (3% of Viscozyme L, 1:4 solid/liquid ratio, 8 h of incubation time and 200 rpm). Although there are no previous reports of AEE of OMSS, the results obtained are comparable with those reported using other seeds, for example

those reported by Nolasco-Arroyo et al. (2019), who obtained an oil yield of 69.71% using Viscozyme L (3% of enzyme, 1:6 solid/liquid ratio, 5 h, and 200 rpm) in the extraction of oil from Jicaro seeds [62]. However, the yield obtained in this study was lower than that reported by Latif and Anwar (2009) (87.5%) and Agarwal and Bosco (2014) (86.14%) in the extraction of sunflower oil [46] and coconut oil [80], respectively, and higher than that reported for peanut oil with a yield of 42.37% [48] when Viscozyme L was used; these differences can be due mainly to the different chemical composition of the wall cells of the used seeds. The enzymatic cocktail, Viscozyme L, seems to successfully break down the polysaccharides (e.g., cellulose, hemicellulose) of the structure to the cell walls that trap the lipid bodies, thereby liberating the oil to the aqueous media [46,48,81,82].

Table 1. Central composite design experiments matrix and the results of the OMSS yield for AEE (with respect to the total oil content present in the mamey seed measured by solvent extraction).

Treatment	Amount of Enzyme (%)	Time (h)	Agitation Rate (rpm)	Solid-to-Liquid Ratio (*w*/*w*)	Oil Yield (%)
1	2.5	5	150	1:3	57 ± 0.36
2	2.5	5	150	1:5	56 ± 0.22
3	2.5	5	250	1:3	66 ± 0.43
4	2.5	5	250	1:5	60 ± 0.37
5	2.5	7	150	1:3	49 ± 0.32
6	2.5	7	150	1:5	48 ± 0.12
7	2.5	7	250	1:3	56 ± 0.26
8	2.5	7	250	1:5	64 ± 0.28
9	3.5	5	150	1:3	51 ± 0.57
10	3.5	5	150	1:5	53 ± 0.58
11	3.5	5	250	1:3	69 ± 0.02
12	3.5	5	250	1:5	65 ± 0.69
13	3.5	7	150	1:3	59 ± 0.14
14	3.5	7	150	1:5	55 ± 0.12
15	3.5	7	250	1:3	63 ± 0.48
16	3.5	7	250	1:5	58 ± 0.23
17	2	6	200	1:4	65 ± 0.02
18	4	6	200	1:4	64 ± 0.43
19	3	4	200	1:4	61 ± 0.17
20	3	8	200	1:4	68 ± 0.30
21	3	6	100	1:4	51 ± 0.47
22	3	6	300	1:4	58 ± 0.43
23	3	6	200	1:2	61 ± 0.03
24	3	6	200	1:6	57 ± 0.29
25	3	6	200	1:4	60 ± 0.03
26	3	6	200	1:4	66 ± 0.25
27	3	6	200	1:4	66 ± 0.35
28	3	6	200	1:4	64 ± 0.47

Table 2 shows the linear, quadratic, and interaction effects of the studied factors (amount of enzyme (%), incubation time (h), agitation rate (rpm), and solid-to-liquid ratio (*w*/*w*)) on OMSS extraction yield. The regression coefficients of the significant effects (linear, quadratic, and interaction) were obtained through multiple regression analysis to empirically determine a relationship between the response variable (oil yield %) and the independent variables (A, B, C, and D) of the model.

The second order polynomial equation, formulated with the regression coefficients significant at 95%, is:

$$Y = 64.12 + 0.72X_1 - 0.52X_2 + 3.64X_3 - 2.77{X_3}^2 - 0.84X_4 - 1.76{X_4}^2 + 1.23X_1X_2 - 0.62X_1X_4 - 0.83X_2X_3 + 0.53X_3X_4 \quad (1)$$

where Y is the oil yield (%), and X_1, X_2, X_3, and X_4 are the coded values of amount of enzyme, incubation time, agitation rate, and solid-to-liquid ratio, respectively.

Table 2. Effects of the parameters of the central composite design.

Variable	Effect	*p*-Value
Mean	64.12	<0.0001 *
X_1	1.44	0.0009 *
X_1^2	−0.76	0.0626
X_2	−1.03	0.0129 *
X_2^2	−0.55	0.1737
X_3	7.28	<0.0001 *
X_3^2	−5.54	<0.0001 *
X_4	−1.68	0.0002 *
X_4^2	−3.53	<0.0001 *
X_1X_2	2.46	<0.0001 *
X_1X_3	−0.07	0.8881
X_1X_4	−1.23	0.0154 *
X_2X_3	−1.65	0.0017 *
X_2X_4	1.07	0.0333 *
X_3X_4	−0.59	0.2308

* Statistically significant at 95% of confidence level.

The determination coefficient (R^2) of the model for the extraction yield was 0.68 and the correlation coefficient (R) was 0.82. The correlation coefficient suggests a satisfactory representation of the process model and a good correlation between the experimental data and the theoretical values predicted by the model equation. The F-test showed that the extraction performance in the model was predictable with an F-value of 6.32 (*p*-value < 0.0001).

The effects of the variables are presented in Table 2. All linear parameters were statistically significant. The amount of enzyme and the agitation rate presented positive effects while the incubation time and solid-to-liquid ratio presented negative effects. The positive effect of the agitation rate on the oil extraction yield in AEE was the highest among the linear effects. It can be attributed to the fact that this variable is important to maintain a homogeneous reaction medium, a multiphasic system (solid, aqueous, and oily phases), avoiding concentration gradients, and thus enabling the release of the oil [64]. In the case of the amount of enzyme, its positive effect can be attributed to the fact that as the amount of enzyme molecules increases, there will be more interactions with the substrate, that is, greater destruction of the cell wall, and consequently, greater oil release [60,83]. Regarding to the solid-to-liquid ratio, this parameter plays an important role in the maintenance of an adequate homogeneity, viscosity, and concentration of the reaction medium that favors the interaction between the enzyme and the seed particles; however, an excessive volume of water would lower the concentration of substrate and enzyme, reducing the effectiveness of the enzyme treatment, and brought difficulties in the subsequent downstream processing, generating a negative influence on oil extraction [70,84]—as can be observed in this study. With respect to incubation time, it presented a negative effect, which means that by increasing incubation time, the extraction yield was decreased. This result could be considered unexpected, since it is expected to find that the longer the incubation time is, the greater the degradation of the components of the seed cell wall, and therefore, the higher the oil extraction yield. However, it has been described that an excessive incubation time can promote the formation of increasingly thick and stable oil in water emulsions, generated by the emulsifying activity of some peptides and polysaccharides that are released during the destruction of the cell wall, reducing the final extraction of the oil [65,85].

The optimal conditions for the AEE of OMSS determined by the statistical analysis were: 3.5% of enzyme; 5.5 h; 235 rpm; and a solid-to-liquid ratio of 1:3.5. Under the optimal conditions, it was possible to obtain 66% of OMSS yield. The contour figure (Figure 1) shows the relationship among the variables (variables that were not represented in the figure were fixed at their optimum value). These optimal conditions were used to obtain the OMSS used in the following experiments.

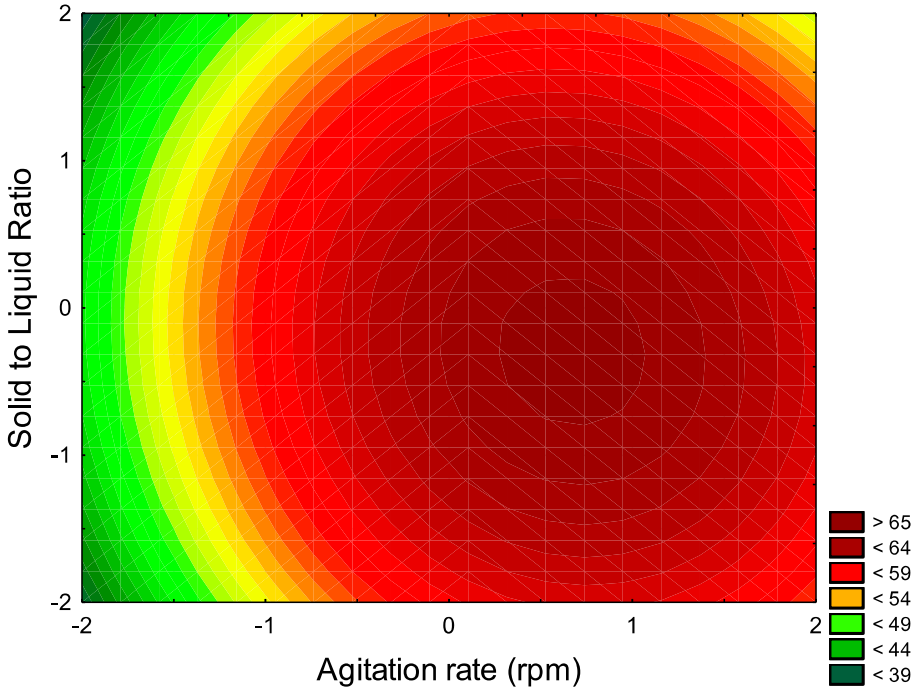


Figure 1. Response surface contour plot of OMSS yields for aqueous enzymatic extraction.

2.3. Analysis of the Oil Obtained by SE or AEE

2.3.1. Some Physicochemical Properties of the Obtained Oils

Some physicochemical properties of the OMSS obtained by SE and AEE under the optimal conditions are shown in Table 3.

Table 3. Physicochemical properties of OMSS obtained by solvent extraction (SE), and aqueous enzymatic extraction (AEE).

Physicochemical Properties	SE	AEE
Acid value (mg KOH/g)	1.12 ± 0.11 [a]	1.09 ± 0.06 [a]
Free fatty acid content (%)	0.57 ± 0.06 [a]	0.55 ± 0.03 [a]
Iodine value (g/100 g)	57.40 ± 0.62 [a]	56.77 ± 1.43 [a]
Oxidative stability (h)	9.33 ± 0.18 [b]	11.00 ± 1.17 [a]

[a,b] Different letters on the same line indicate a significant difference ($p < 0.05$) by Student's *t*-test.

No statistically significant differences were found in the acid value, free fatty acid, and iodine value. Acid value of OMSS (1.09–1.12 mg KOH/g) was lower than that reported by Moo-Huchin et al. (2013), who obtained an acid value of 4.44 ± 2.19 mg KOH/g [18], but in both cases, the acidity of OMSS can be considered low for unrefined oils, and show that they are potentially edible oils [18]. In the case of free fatty acid content (0.55–0.57%), the results are lower than those reported by Hernández-Santos et al. (2017); they found 1.88% of free fatty acid in OMSS [20]. This is an excellent result for an oil which has not been subjected to a refining process, especially considering that the degree of edibility of an oil is generally considered to be inversely proportional to the total amount of free fatty acids [20,86]. In the case of the iodine values (56.77–57.40 g/100 g), the results obtained were lower than those reported by Moo-Huchin et al. (2103) (174 g/100 g) [18], which suggest a lower content of unsaturated fatty acid [71,87], but in general, OMSS contains a high proportion of monounsaturated and saturated fatty acids in comparison with polyunsaturated fatty acids, so low iodine values are to be expected [20]. The differences found between the results of the OMSS properties studied in this work and the few studies reported in the literature can be attributed to differences in the plant variety or environmental conditions [20].

The oxidative stability of the OMSS extracted by AEE (11 h) was significantly higher than the OMSS obtained by SE (9.33 h), which indicates that the AEE extracted oil will be more stable over long periods of time than the SE extracted oil, with no deterioration caused by rancidity [20]. This could be related to the release of antioxidant phenolic compounds, α-tocopherol, and β-carotene, which naturally occurred in plants; thus, the presence of these bioactive compounds can improve the oxidative stability of the AEE extracted oil [46]. On the other hand, the elevated temperature and prolonged duration of the SE method (95 °C and 8 h) could promote the destruction of these antioxidant compounds, decreasing the resistance of the oil to oxidation, thereby affecting its final quality [33,46,70,87–89]. Moreover, these antioxidant compounds are positive in many of the oil applications.

2.3.2. Analysis of the Fatty Acid Composition of the Different Extracted Oils through Gas Chromatography

Fatty acid compositions of the AEE- and SE-extracted OMSS are shown in Table 4. As can be observed, three saturated fatty acids (SFAs), one monounsaturated fatty acid (MUFA) and one polyunsaturated fatty acid (PUFA) were identified as the main components, and there were no statistically significant differences in the percentages of these fatty acids obtained for the two evaluated extraction methods (with the exception of linoleic acid, which presented a very slight difference, which cannot be considered relevant for the quality of the oil) [64]. As can be seen in Figure 2 and Table 4, the SFAs were palmitic acid C16:0 (8.7–9.6%), stearic acid C18:0 (27.8–28.1%), and arachidic acid C20:0 (0.8%). On the other hand, the MUFA oleic acid C18:1 (49.6–50.5%) was the most predominant fatty acid presented in OMSS, while the main PUFA was linoleic acid C18:2 (11–13%). A similar composition with small differences was reported for OMSS by Hernández-Santos et al. (2017), Moo-Huchin et al. (2013), and by Solís-Fuentes et al. (2015). These last ones found palmitic (10.50 ± 2.63%), stearic (28.65 ± 1.82%), oleic (48.62 ± 1.95%), linoleic (10.77 ± 0.01%), linolenic (0.58 ± 0.21%), arachidic (0.36 ± 0.19%), and behenic (0.40 ± 04%) acids. The differences found in the composition of fatty acids between the aforementioned work and this study can be attributed to the mamey variety, harvesting, fruit maturity, and the geographical location (which may affect the soil, climatic conditions) [72,90]. All these parameters may affect the lipid profile in OMSS [15]. As reported by Hernández-Santos et al. (2017), the fatty acid composition found in OMSS is similar in its main fatty acids, to those found in some edible oils such as soybean and olive oil, having in common with these oils a high content of oleic acid, which is desirable for oil stability during the frying process, and mainly in terms of nutrition, because it has been demonstrated that oleic acid consumption reduces coronary heart disease risk, mainly via LDL-cholesterol reduction, and has beneficial effects on risk factors for cardiovascular diseases (such in vitro LDL oxidative susceptibility, thrombogenesis, and insulin sensitivity) [91]. High oleic acid content places this oil in the category of high oleic oils, making it able to compete with highly demanded commercial edible vegetable oils [18,20].

2.4. Scanning Electron Micrographs (SEM) of the Seed before and after Extraction

Figure 3a shows that before the extraction, the cells are intact and the surface of the seed is smooth and oily; but after SE (Figure 3b), the surface of the seed is full of pores, which were generated by the entry of hexane [72]. Finally, Figure 3c shows the seed structure after AEE, which causes significant damage to it due to the enzymatic hydrolysis of cellulose and hemicellulose, which generates a disorganization and destruction of cell walls and liposome membranes, thus facilitating the release of oil from the seed [71].

Table 4. Fatty acid composition of OMSS obtained by aqueous enzymatic extraction (AEE) and solvent extraction (SE), in comparison with soybean and olive oils.

Fatty Acids	Fatty Acids Average Composition (%)			
	OMSS in This Study		Soybean Oil [92]	Olive Oil [93]
	SE	AEE		
Palmitic acid (C16:0)	8.7 ± 0.6 [a]	9.6 ± 0.4 [a]	10.9	5.13
Stearic acid (C18:0)	27.8 ± 0.3 [a]	28.1 ± 0.6 [a]	3.5	2.8
Oleic acid (C18:1)	49.6 ± 0.6 [a]	50.5 ± 0.3 [a]	20.7	89
Linoleic acid (C18:2)	13.0 ± 0.2 [a]	11.0 ± 0.1 [b]	57.5	-
Linolenic acid (C18:3)	-	-	7.4	-
Arachidic acid (C20:0)	0.8 ± 0.0 [a]	0.8 ± 0.1 [a]	-	0.6
Eicosenic acid (C20:1)	-	-	-	1.3
Docosanoic acid (C22:0)	-	-	-	0.4
Saturated fatty acid (SFA)	37.3	37.7	14.4	8.93
Monounsaturated fatty acid (MUFA)	49.6	50.5	20.7	90.3
Polyunsaturated fatty acid (PUFA)	13.0	11.0	64.9	-

[a,b] Different letters on the same line indicate a significant difference ($p < 0.05$) by Student's *t*-test.

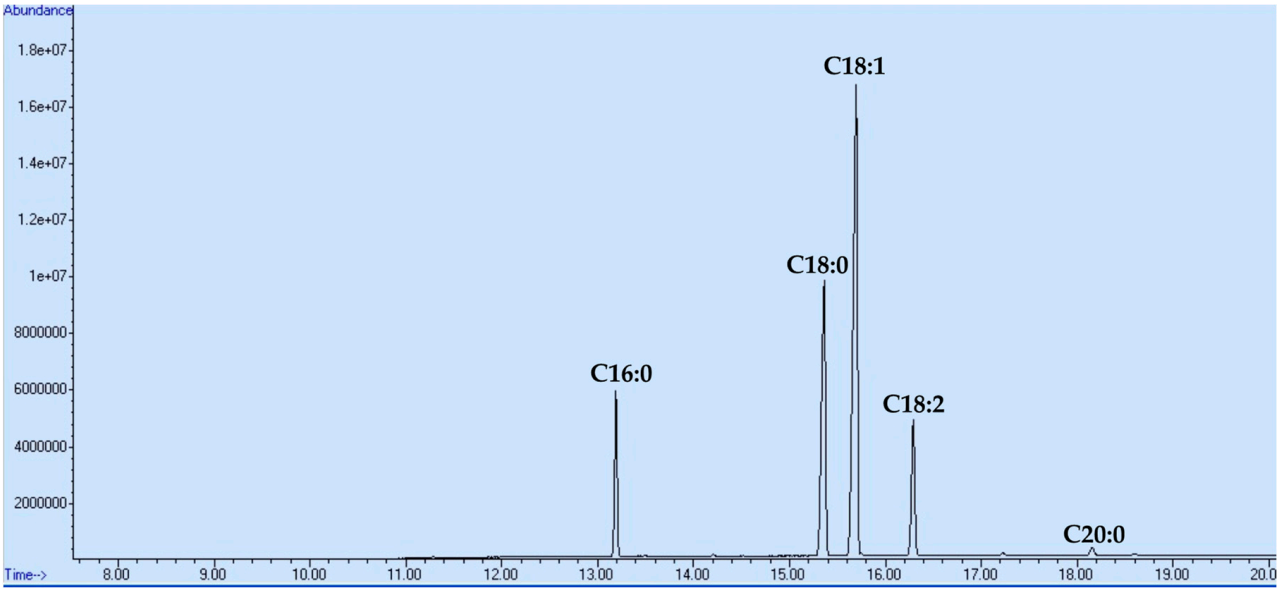
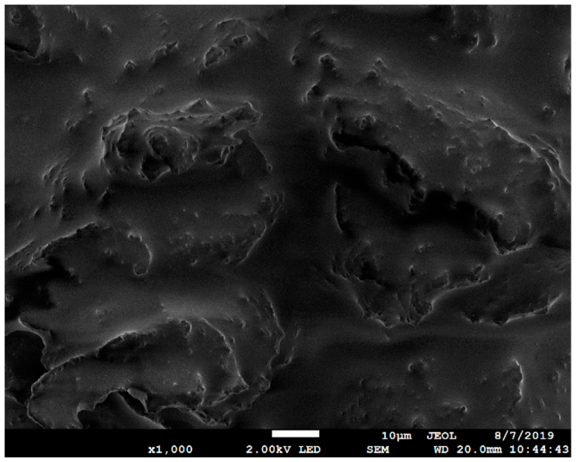
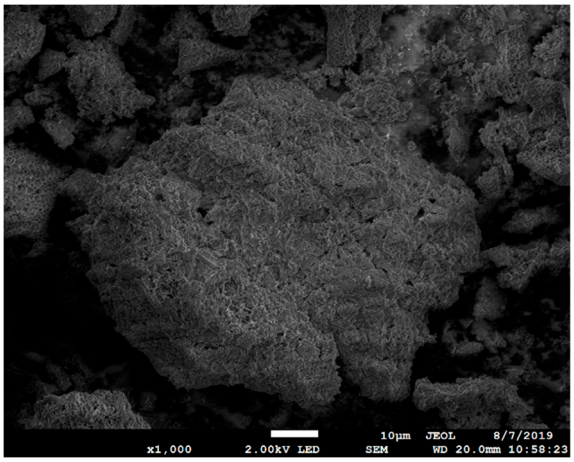


Figure 2. GC analysis of chromatogram of OMSS extracted by AEE.

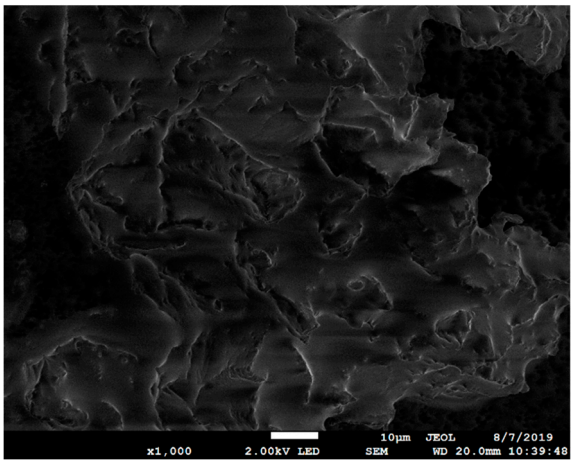


(**a**)



(**b**)

Figure 3. *Cont.*



(**c**)

Figure 3. Scanning electron microscopy (SEM) of cells' surface morphological alterations of the mamey seed samples by (**a**) non-extraction; (**b**) solvent extraction; and (**c**) aqueous enzymatic extraction.

3. Materials and Methods

3.1. Materials

Mature mamey sapote fruits were purchased from a local market from Tuxtla Gutiérrez, Chiapas, Mexico. The fruits were opened to obtain the seeds, and they were manually dehulled to extract the kernels, which were cut into thin slices and dried at 70 °C for 24 h (in an oven). After that, the dried slices were ground in a blender (Moulinex type 643), and then stored in amber flasks at 4 °C.

Fatty acid methyl ester mixture (FAME MIX) standard and Viscozyme L was purchased from Sigma Aldrich, Mexico. Viscozyme L had a claimed the enzymatic activity of 120 fungal beta-glucanase units (FBGs) mL^{-1}, in which 1 FBG is the amount of enzyme required under the standard conditions (30 °C, pH 5.0 and 30 min of reaction time) to hydrolyze barley β-glucan to reducing carbohydrates, with a reducing power corresponding to 1 µmol glucose min^{-1} (according to the supplier). Hexane, ethanol, and other chemicals were of analytical or HPLC grade (supplied from J.K. Baker).

3.2. Solvent Extraction

Solvent extraction (SE) was carried out according to the protocol recommended by the Association of Official Analytical Chemists (AOAC) [94]. Briefly, 200 mL of n-hexane and 5 g of ground mamey sapote kernel were placed in a Soxhlet extraction equipment at 95 °C for 8 h. After the oil extraction, hexane was eliminated using a rotary evaporator under reduced pressure at 50 °C. The extracted OMSS was dried at 70 °C for 24 h, and then stored in amber flasks at 4 °C until its use. The percentage of OMSS obtained by SE was considered as the total oil (100%) contained in the mamey sapote seed, and this value was used for subsequent calculations of the performance of the AEE [95]. Each experiment was performed in triplicate, and the results are presented as the mean values ± SD.

3.3. Aqueous Enzymatic Extraction (AEE)

AEE was performed in 50 mL screw cap Erlenmeyer flasks, which contained 5 g of ground and dried mamey sapote seeds and distilled water (solid-to-liquid ratio according to the experimental design). The mixture was subjected to boiling for 10 min, and then allowed to cool down to 25 °C [64]. The pH of the reaction mixture was set to 4 using 0.5 N aqueous HCl or NaOH solutions, and then an amount of Viscozyme L (% by seed weight) (according to the experimental design) was added. The enzymatic treatments were carried out at 50 °C [62], under an orbital stirring and for the incubation time indicated in the experimental design. At the end of the experiments, the reaction mixture was centrifuged at 4500 rpm during 30 min; after that, the oily phase was withdrawn using

a pipette and then centrifuged at 10,000 rpm for 30 min. The obtained clean OMSS was exposed to 70 °C during 24 h to dry, and then it was weighted. The yield of extraction was expressed as a percentage ratio (% *w/w*) with respect to the oil obtained by SE, according to Equation (2). Control samples were prepared and treated identically, but without the addition of enzymes. Each experiment was performed in triplicate, and the results are presented as the mean values ± SD:

$$Oil\ Yield\ (\%) = \frac{g\ of\ oil\ obtained\ in\ AEE}{g\ of\ oil\ obtained\ in\ SE} \times 100 \quad (2)$$

Optimization of the AEE

A central composite design with four variables was employed to determine the optimal conditions for AEE of OMSS. The variables, amount of enzyme (% with respect to the weight of the seed), incubation time (h), agitation rate (rpm), solid-to-liquid ratio (*w*/*w*), and their coded and uncoded values are presented in Table 5.

Table 5. AEE process variables and their levels used in CCD.

Variables	Name	Coded Levels				
		−2	**−1**	**0**	**1**	**2**
X1	Amount of enzyme (%)	2.0	2.5	3.0	3.5	4.0
X2	Incubation time (h)	4	5	6	7	8
X3	Agitation rate (rpm)	100	150	200	250	300
X4	Solid-to-liquid ratio (*w/w*)	1:2	1:3	1:4	1:5	1:6

Table 1 shows the matrix with the 28 treatments of the four variables, each at five levels. In each case, the yields of oil extraction were determined and fitted to a second-order polynomial equation:

$$Y = B_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X^2 + \sum_{i>j}^{k} B_{ij} X_j + E \quad (3)$$

where Y represents the response variable; B_0 is the constant coefficient; B_i, B_{ii}, and B_{ij} are the linear, quadratic, and interactive coefficients terms of the regression, respectively; and finally, the independent variables are represented by X_i and X_j. The determination of regression coefficients of individual linear, quadratic, and interaction terms was performed employing an analysis of variance (ANOVA) and *p*-value. A 3D surface graph was generated from the fitted polynomial equation using the regression coefficients to present a graphical representation of the response variable (oil yield %).

3.4. Analysis of the Extracted Oil

3.4.1. Determination of Some Physicochemical Properties

The OMSS obtained by AEE and SE methods were analyzed in terms of FFA percentage and iodine and acid value, which were determined by American Oil Chemists' Society (AOCS) methods [96]. Additionally, oxidative stability was estimated by measuring the oxidation induction time on a Rancimat equipment (Metrohm CH series 679). In this process, air was bubbled (20 L/h) through the OMSS (3.0 g) heated at 110 ± 0.2 °C, and the volatile compounds produced were trapped in water, and the changes in the electrical conductivity of this water were recorded, until reaching the conductivity inflection [97]. Each measurement was performed in triplicate, and the results were presented as the mean values ± SD.

3.4.2. Fatty Acid Composition through Gas Chromatography

Fatty acid compositions of the OMSS extracted by AEE and SE were determined by gas chromatography–mass spectrometry (GC–MS) in an Agilent Technologies chromatograph model 5975 inert XL Net Work GC system equipped with a DB-WAX capillary column (60 m × 250 mm × 0.25 mm). Prior to the chromatographic analysis, the OMSS were separately converted into its corresponding fatty acid methyl esters (FAME) [98]. After that, 1 µL of FAME samples were injected (split ratio of 1:100) at 250 °C. The initial temperature in the oven was 60 °C, which was maintained during 5 min and then increased as follows: first 20 °C/min to 210 °C, then 1 °C/min to 213 °C and finally 20 °C/min until 225 °C. The carrier gas was helium at a constant flow rate of 1 mL/min [99]. The identification of the fatty acid components of OMSS was carried out by comparing their mass spectral fragmentation patterns with those of the similar compounds stored in the GC–MS system software database (NIST Mass spectral Finder 2.0 Library, NIST/EPA/NIH). Relative oil composition percentages were expressed as the average percentage (%) of individual fatty acids relative to total determined fatty acids. All measurements were performed in triplicate, and the results are presented as the mean values ± SD.

3.5. Scanning Electron Micrographs (SEM)

Scanning electron micrograph (SEM) was used to analyze the microstructure of the mamey sapote seed before and after the application of the SE or AEE. The analysis was carried out by sticking the previously dried samples on a double-sided adhesive conductive carbon tape which was coated with gold at high vacuum. The morphology of the samples was observed by the analysis of images obtained with a JEOL model JSM7100F field emission scanning electron microscope system (JEOL Company, Tokio, Japan).

3.6. Statistical Analysis

The experimental design and analysis of the results were performed using Statistica 13.0 (TIBCO Software Inc., Tulsa, OK, USA). The statistical analysis of the model was carried out as analysis of variance (ANOVA). The significance of the regression coefficients and the associated probabilities, p(t), were determined by Student's t-test; the second order model equation significance was determined by Fisher's F-test. The quadratic model was represented as surface contour plots (2D).

4. Conclusions

The use of the multienzyme complex Viscozyme L generated an OMSS extraction yield of 66% under optimal extraction conditions (amount of enzyme, 3.5%; incubation time, 5.5 h; agitation rate, 235 rpm; solid-to-liquid ratio, 1:3.5) at pH 4 and 50 °C. No significant statistical differences were found in the acidity and iodine value, free fatty acids content or in the fatty acid composition of the OMSS obtained by AEE compared to SE. However, the oxidative stability was 9.33 h for the oil obtained by SE and 11 h for that obtained by AEE. In addition, SEM images show that Viscozyme L destroys the cell wall. Finally, the fatty acid composition of this OMSS reveals its high oleic acid content (around 50% of the total fatty acids presents in the OMSS), which is comparable to many edible vegetable oils, making it an interesting potential source as a commercially edible vegetable oil.

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