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A Pilot-Scale Supercritical Carbon Dioxide Extraction to Valorize Colombian Mango Seed Kernel

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Abstract: Colombian mango production, which exceeded 261,000 t in 2020, generates about 40% of the whole fruit as solid waste, of which more than 50% are seed kernels (over 52,000 t solid by-product); though none is currently used for commercial purposes. This study reports the results of the supercritical carbon dioxide ($scCO_2$) extraction of an oil rich in essential fatty acids (EFAs) from revalorized mango seed kernels and the optimization of the process by the Response Surface Methodology (RSM). In pilot-scale $scCO_2$ experiments, pressure (23–37 MPa) and temperature (52–73 °C) were varied, using 4.5 kg of CO_2. The highest experimental oil extraction yield was 83 g/kg (37 MPa and 63 °C); while RSM predicted that 84 g/kg would be extracted at 35 MPa and 65 °C. Moreover, by fine-tuning pressure and temperature it was possible to obtain an EFA-rich lipid fraction in linoleic (37 g/kg) and α-linolenic (4 g/kg) acids, along with a high oleic acid content (155 g/kg), by using a relatively low extraction pressure (23 MPa), which makes the process a promising approach for the extraction of oil from mango waste on an industrial scale, based on a circular economy model.

Keywords: *Mangifera indica* L.; circular economy; valorization strategy; fruit by-products; response surface methodology; essential fatty acids

1. Introduction

The concept of sustainability relies on the circular economy model, which focuses on resource efficiency, waste reduction, recycling, and valorization [1,2]. Food waste is a key element of the circular economy concept, and the valorization of these wastes constitutes a promising alternative to turn waste into valuable bio-based products [2,3]. In this regard, the extraction of high-value functional compounds offers one of the many valorization strategies for a sustainable and efficient waste management approach, with possible benefits for the food, pharmaceutical, and cosmetic industries [2].

Mango (*Mangifera indica* L.) is one of the most important and popular tropical fruits worldwide [4,5], ranking second in terms of production and cultivated area, after bananas. Mango had a global production volume of over 55 million t in 2020 and is projected to reach 65 million t by 2028, increasing at an annual rate of 2.1% over this decade [6]. Approximately 99% of mango production takes place in developing countries, with Latin America contributing 26% of production, led by Peru, Brazil, and Colombia [6,7].

The mango processing industry produces a large amount of solid waste as peels, pomace, and seed kernels, which altogether account for 40% to 60% of the whole fresh

fruit, of which up to half may be seed kernels [8]. Colombian mango production exceeded 261,000 t in 2020 [9], which indicates that the country had a production capacity of more than 156,000 t of solid waste last year. Up to 78,000 t of those residues were seed kernels, none currently used for commercial purposes, even though the mango seed kernel contains several families of health-promoting compounds such as fatty acids [10] and other substances (triacylglycerols, gallotannins, xanthones, flavonoids, phenolic acids, among others) with related health benefits and interesting techno-functional properties for applications in the food [8,11] and pharmaceutical industries [12]. Therefore, through the use of revalorization strategies, based on the circular economy model, these by-products could be used as a source of important bioactive compounds with high added value.

Likewise, the use of supercritical fluids as alternative solvents for oil extraction has been attracting widespread interest due to their particular properties (e.g., liquid-like solvent power, negligible surface tension, and gas-like transport properties) and changes in environmental regulations, which foster the utilization of green solvents [13]. In this field, carbon dioxide (CO_2) has been specially employed [14,15] since it is essentially non-toxic, non-flammable, inexpensive at the industrial level, can be recycled, has easily accessible supercritical conditions, and is dissipated from extracts at atmospheric pressure, avoiding the necessity of further expensive and harmful refining treatments [16]. Moreover, extractions with supercritical CO_2 ($scCO_2$) have been successfully recognized in the obtention of valuable extracts from agro-industrial by-products [17].

On the other hand, fatty acids are divided into two main groups according to their structural characteristics: saturated (SFAs) and unsaturated fatty acids (UFAs). The latter, depending on their degree of unsaturation, can be classified as mono (MUFAs) and polyunsaturated fatty acids (PUFAs). Depending on the position of the double bond, counting from the extreme carbon to the carboxylic functional group, MUFAs and PUFAs can be classified into three main series: omega-9 (ω-9, first double bond in carbon 9), omega-6 (ω-6, first double bond in carbon 6), and omega-3 fatty acids (ω-3, first double bond in carbon 3) [18]. Essential fatty acids (EFAs) are those PUFAs that must be provided by foods because these cannot be synthesized in the body yet are necessary for health. There are two families of EFAs: ω-6 and ω-3. Linoleic ($C_{18:2}$ *cis*-9,12) and α-linolenic acids ($C_{18:3}$ *cis*-9,12,15) are examples of ω-6 and ω-3, respectively [19]. Currently, an awareness of the role of these EFAs in human health and disease prevention has been unremittingly increasing among people, and many studies have positively correlated these compounds with infant development, cancer prevention, optimal brain and vision functioning, and the reduction of arthritis, hypertension, cardiovascular morbidity and mortality, diabetes mellitus, and neurological/neuropsychiatric disorders [20]. Therefore, the search for sustainable resources for these compounds is paramount. Moreover, the global market for natural fatty acids was US $13.5 billion in 2018 and is expected to reach US $ 17.5 billion by 2023. A growing demand for naturally derived products in the food, beverage, and dietary supplement industries is expected to be a significant driving force in the future global market [21].

Some studies have employed the special properties of $scCO_2$ to achieve the valorization of mango seed kernels [22–24]. As an example, Awolu et al. [22] reported an oil extraction yield between 25 g extract/kg biomass and 36 g extract/kg biomass by $scCO_2$ extraction from the agri-food industrial by-product. Other studies also reported low oil extraction yields at the same conditions used in this work [23,24]; and to the best of our knowledge, no study has focused on the pilot-scale $scCO_2$ production of lipid fractions rich in EFAs from mango seed kernels.

The search for natural sources of bioactive compounds with nutritional interest has received widespread attention, so research in this field has explored the valorization of residues from conventional processes [8,23,25]. These target compounds have usually been extracted using organic solvents; however, this technique is not selective and has become less attractive as it generates products with traces of the residual solvent [26]. The aforementioned, along with the growing awareness of the environmental and health problems

arising from the use of organic solvents has led to the implementation of tougher regulations; hence, the use of $scCO_2$ extractions has become a commercial and environmentally friendly alternative [14].

In this work, first, the main operating conditions were optimized to achieve a high oil extraction yield from mango seed kernels in a pilot-scale $scCO_2$ plant. Afterward, the focus was placed on a selective extraction of a lipid fraction rich in EFAs.

2. Results and Discussions

The current work proposed a valorization strategy of mango seed kernels. For this, $scCO_2$ experiments at a pilot-scale were performed in a wide range of pressures (from 23 up to 37 MPa) and temperatures (from 52 to 73 °C) by using a solvent ratio of 22.5 kg CO_2/kg biomass. Pressure and temperature were analyzed as the main operating conditions of the procedure and optimized by Response Surface Methodology (RSM) to reach a high oil extraction yield with a fatty acid profile rich in EFAs (mainly linoleic and α-linolenic acids). Table 1 shows the rotatable central composite design (RCCD) conditions and $scCO_2$ extraction yields from the studied biomass.

Table 1. Design conditions and supercritical extraction yields from mango seed kernels.

Pressure (MPa)	Temperature (°C)	Extraction Yield (g Extract/kg Biomass)
25 (−1)	55 (−1)	50 ± 3 [b]
25 (−1)	70 (+1)	35 ± 3 [a]
35 (+1)	55 (−1)	69 ± 1 [cd]
35 (+1)	70 (+1)	74 ± 2 [cd]
23 (−1.41)	63 (0)	24 ± 1 [a]
37 (+1.41)	63 (0)	83 ± 2 [de]
30 (0)	52 (−1.41)	63 ± 2 [bc]
30 (0)	73 (+1.41)	65 ± 1 [bc]
30 (0)	63 (0)	73 ± 3 [cd]

Different letters (a, b, c, d, and e) in the same column represent statistically significant differences at 5% significance.

2.1. Total Extraction Yield Analysis

As can be seen in Table 1, the extraction yield reached at the most adequate experimental conditions with supercritical extraction (37 MPa and 63 °C) was 83 g extract/kg mango seed kernels. Supercritical extracts were semi-solid at ambient temperature and light white in color, physically cleaner than the Soxhlet extracts. Meanwhile, the conventional Soxhlet extraction with hexane had a total extraction yield of 90 g extract/kg mango seed kernels. This slightly higher oil extraction yield with the organic solvent was probably achieved due to the lower selectivity of the Soxhlet procedure since the extract obtained with hexane contained neutral and polar lipids and pigments like chlorophyll [25]. This explains the differences in appearance between an extract obtained by one method ($scCO_2$) and the other (Soxhlet). Besides, it should be noted that the plant material was not completely exhausted during the $scCO_2$ extraction, which also explains the oil extraction yield differences.

2.2. Influence of Solid Conditions on Extraction Yield

The highest extraction yield value found in this work (83 g extract/kg mango seed kernels) was within the range of oil extraction values reported by other authors for $scCO_2$ extractions from mango seed kernels [23,24]. Jahurul et al. [23] reported an extract yield of 110 g extract/kg mango seed kernels using 50 MPa and 60 °C. Meanwhile, in another study [24], the same authors reported that the highest yield was roughly 130 g extract/kg biomass at 42.2 MPa and 72 °C. However, those other studies involved higher pressures (and therefore more energy expenditure) to achieve an extraction yield close to the one found in the current work, which used a lower pressure (37 MPa). The aforementioned could be due to the improved solid conditions of the biomass subjected to supercritical

extraction in this study [25,27], since an optimal particle size and moisture content were pursued in prior experiments, which were then applied during the high-pressure procedure.

During the $scCO_2$ extraction from a solid substrate the mass transfer depends heavily on the transport rate in the solid phase and the length of the transport path through the solid [27,28]. Solid particles with a diameter greater than 0.80 mm hinder the penetration of the supercritical solvent and the solubilization of the solute, while particles smaller than 0.4 mm could represent a risk of formation of preferential channels and blockages during extraction [25,28]. Therefore, seed kernels with a particle size of 0.6 mm were subjected to supercritical extractions during the current work. In contrast, particles smaller than 0.2 mm were used in the studies of Jahurul et al. [23,24].

On the other hand, the excess of water can create a barrier to mass transfer when the raw material is subjected to $scCO_2$ extraction [27,28]. Therefore, the initial water content of the biomass was also optimized. In our case, preliminary studies showed that the fastest extraction was achieved when the sample had a water mass fraction of 6.8%, which was the equilibrium moisture with the environmental humidity in Pasto. However, to improve the extraction rate it was necessary to dry the raw material, which should be taken into account for the economic viability of the whole process.

2.3. Effect of Process Parameters on Oil Extraction Yield

The statistical significance of $scCO_2$ pressure and temperature, and their interactions, on mango seed kernel oil extraction yield, was assessed by analysis of variance (ANOVA). Statistically significant effects ($p \leq 0.05$) on the extraction yield were found for the pressure levels employed, the pressure-temperature interaction, and squares of both pressure and temperature. According to ANOVA, the temperature did not show a significant effect under the levels evaluated.

As expected, extraction yields significantly increased with pressure (see Figure 1). As shown in Figure 1, by increasing pressure from 20 to 35 MPa, the oil extraction yield increased significantly at the temperature levels evaluated, which was related to the increase in solvent density and solvent capacity of $scCO_2$ [27]. Also, by increasing pressure, the solvent penetration into seed pores was enhanced, thus achieving an efficient contact between $scCO_2$ and the substrate [28].
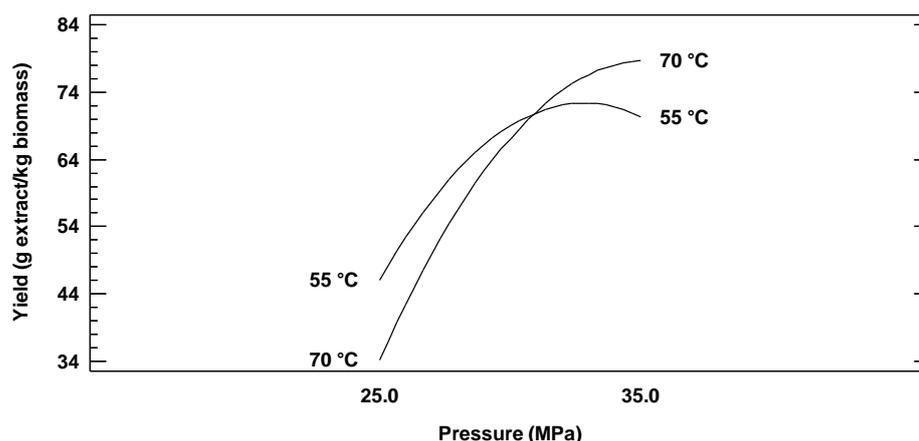

Figure 1. Effect of pressure and temperature on oil extraction yield from mango seed kernels.

As seen in Figure 1, the temperature-pressure interaction played a special role during $scCO_2$ extractions from mango seed kernels in this study. The isotherms shown in Figure 1 intersected at a point called the crossover point (slightly over 30 MPa), which represents the pressure at which the increase of the operating temperature benefited the extraction [28]. As a result, the pressure-temperature interaction during the $scCO_2$ extraction of this study had a statistically significant effect ($p \leq 0.05$). For vegetable oils the crossover pressure is

about 30 MPa [29], so the crossover point found for mango seed kernel oil in this study was within the values reported in the literature.

During a scCO$_2$ extraction, working at constant pressure, the change in temperature affects the density of CO$_2$, the vapor pressure of the solutes involved in the extraction procedure, and their desorption from the biomass [25]. At higher temperatures, the solute becomes more volatile; however, the scCO$_2$ density decreases [28,29]. In Figure 1, at a relatively low pressure (25 MPa), and below the crossover point, a decrease of density and solvent power with increasing temperature prevailed, while an increase in vapor pressure predominated at a fairly high pressure (35 MPa). This phenomenon is an example of how the optimal temperature to increase oil yield should be well established. It is not possible to anticipate the temperature impact, as it affects many different aspects of the thermodynamics and mass transfer during the high-pressure procedure [25,28].

Even though the extraction yield obtained by the conventional solvent extraction did not differ significantly from the scCO$_2$ extraction, it should be noted that quite a high extraction pressure (37 MPa) was required during the extraction to achieve an extract yield of 83 g extract/kg biomass (see Table 1). Considering that the pilot extraction of this study constitutes preliminary data for a future profitable industrial extraction from mango seed kernels, this matter should be addressed.

2.4. Oil Extraction Yield Optimization

RSM predicted a maximum oil extraction yield from mango seed kernels of 84 g extract/kg biomass, operating at 35 MPa and 65 °C (see Figure 2). It is worth noting that, according to the prediction, with a reduction in operating pressure and a minor reduction in temperature, an oil yield slightly higher than that already obtained experimentally could be achieved. The possible explanation for the above phenomenon is that excessive pressures hinder mass transfer and thus extraction of the extract [28].
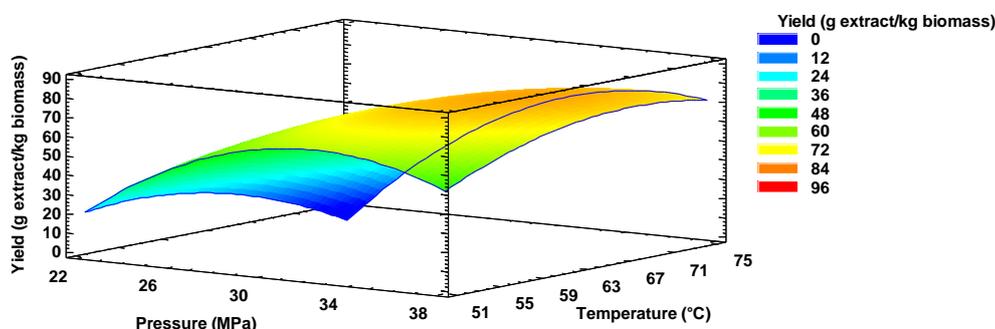

Figure 2. Optimization of the mango seed kernel oil extraction yield with scCO$_2$ by Response Surface Methodology (RSM).

The optimization provided the second-order mathematical model for the response surface of Figure 2, as expressed in Equation (1):

$$Y = -51.9706 + 1.96210X_1 + 0.782824X_2 + 0.0121X_1X_2 - 0.0393747X_1^2 - 0.0091667X_2^2 \quad (1)$$

where Y is the experimental extraction yield (g/kg); and X$_1$ and X$_2$ are the operating pressure and temperature, respectively. This model fitted the factors studied at their actual physical levels and explained 96% of the variability of the mango seed kernel oil yield data. The optimal statistical extraction conditions were within the range of the experimentally evaluated levels. Finally, the lack-of-fit test was not significant (*p* > 0.05); and therefore, the model was adequate to describe the observed data.

2.5. Fatty Acid Profile

Table 2 contains the fatty acid profile of the lipid extracts obtained by supercritical extraction and by conventional extraction with hexane. The fatty acid profile of the lipid

fraction revealed the presence of palmitic, stearic, oleic, linoleic, and α-linolenic acids. The content of each fatty acid in the obtained extract depended on the method and the supercritical extraction conditions.

Table 2. Fatty acid profile of mango seed kernel extracts obtained by supercritical and conventional solvent extraction.

Pressure (MPa)	Temperature (°C)	g Fatty Acid/kg Extract					SFA/UFA
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	
25	55	32 ± 1 [abc]	61 ± 2 [ab]	93 ± 1 [ab]	24 ± 1 [ab]	3 ± 0 [bcd]	1.29 [a]
25	70	38 ± 2 [cd]	63 ± 1 [ab]	105 ± 1 [abc]	31 ± 1 [ab]	4 ± 0 [de]	1.41 [a]
35	55	28 ± 1 [abc]	45 ± 1 [a]	139 ± 1 [bc]	25 ± 0 [ab]	2 ± 0 [abc]	2.45 [a]
35	70	23 ± 1 [ab]	54 ± 1 [ab]	69 ± 0 [a]	16 ± 1 [a]	1 ± 0 [a]	1.12 [a]
23	63	50 ± 1 [d]	71 ± 1 [ab]	155 ± 1 [c]	37 ± 2 [b]	4 ± 0 [e]	1.62 [a]
37	63	23 ± 1 [ab]	55 ± 2 [ab]	69 ± 1 [a]	16 ± 1 [ab]	2 ± 0 [ab]	1.09 [a]
30	52	35 ± 1 [bc]	84 ± 1 [b]	111 ± 1 [abc]	24 ± 1 [ab]	2 ± 0 [ab]	1.14 [a]
30	73	41 ± 1 [cd]	64 ± 1 [ab]	111 ± 2 [abc]	33 ± 1 [ab]	3 ± 0 [cde]	1.41 [a]
30	63	30 ± 1 [abc]	62 ± 1 [ab]	93 ± 1 [abc]	22 ± 1 [ab]	2 ± 0 [abc]	1.25 [a]
Soxhlet extraction		21 ± 1 [a]	47 ± 1 [a]	61 ± 2 [a]	17 ± 1 [ab]	2 ± 0 [ab]	1.15 [a]

Palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and α-linolenic ($C_{18:3}$) acid. Ratio saturated/unsaturated fatty acids. Different letters (a, b, c, d, and e) in the same column represent statistically significant differences at 5% significance.

The mango seed kernel oil obtained with $scCO_2$ was, on average, made up of a higher proportion of UFAs (about 58%) than SFAs (about 42%); with a variation in the ratio of unsaturated/saturated fatty acids from 1.09 to 2.45, depending on the extraction conditions and extraction method. Table 2 shows that, regardless of the extraction method or condition, oleic acid was the major constituent of the extracts. Oleic acid content was much higher in supercritical extracts than in those obtained with hexane, representing up to almost 50% of the lipid fraction.

Oleic acid is an ω-9 fatty acid. Unlike ω-3 and ω-6, ω-9 fatty acids are not essential since humans can introduce an unsaturation to an SFA in that position. Therefore, oleic acid, to which beneficial nutritional properties are attributed, does not need to be present in our diet [18]. However, it is beneficial to obtain oleic acid from food because similar to other ω-9 fatty acids, oleic acid can help to reduce the risk of heart diseases by raising levels of high-density lipoprotein and lowering low-density lipoprotein [30]. It is not the same with ω-3 and ω-6 fatty acids since our organism cannot introduce unsaturations in these positions. In this way, fatty acids such as linoleic and α-linolenic acids are essential; so our diet requires them in well-determined proportions as their lack or imbalance in intake produces serious metabolic alterations [31].

As seen in Table 2, extracts obtained in this study contained a considerable fraction of both linoleic and α-linolenic acids, which provides additional value in terms of nutritional quality to the lipid fractions obtained in this proposal for the revalorization of mango seed kernels. Table 2 shows that the profile of these acids in the supercritical extracts exceeded that of the extracts obtained with hexane, and the supercritical extracts obtained at 23 MPa and 63 °C were particularly noteworthy. At first, the good fatty acid profile of the supercritical extracts obtained in preliminary tests was surprising, which was another reason for the decision to explore different extraction conditions with an RCCD experimental design; and thus, to find the best-operating conditions to obtain an extract rich in these two EFAs. $scCO_2$ at 23 MPa and 63 °C yielded the best results. More importantly, no excessive pressures were needed to achieve this selective extraction.

Although previous studies claimed the presence of linoleic and α-linolenic acids in supercritical extracts from mango seed kernels [23,24,32]; those studies reported lower values than these found in this study in terms of both compounds. Indeed, studies such

as that of Ballesteros-Vivas et al. [8] did not report the presence of α-linolenic acid in their extracts. The above could be due to the optimization of the operating and the solid conditions of this study [25,27]; but it could also be due to the mango variety involved in each study [23].

Finally, palmitic and stearic acids accounted for about 15% and 22% of the fatty acid fraction of the supercritical extracts, respectively (see Table 2). The presence of these compounds as major fatty acids in mango seed kernel extracts has been previously reported [23,24,32]. In terms of future industrial applications of the obtained extracts, their presence offers stability, yielding a cocoa butter analogy fat and a product with characteristics of vegetable butter, suitable for use in the confectionery industry [23,24,33].

2.6. Extraction Optimization of Targeted Fatty Acids

The extraction pressure, along with the pressure-temperature interaction, had a statistically significant effect ($p \leq 0.05$) on the extraction of oleic, linoleic, and α-linolenic acids. Temperature, on the other hand, had a statistically significant effect ($p \leq 0.05$) only on the extraction of oleic acid.

For all the analyzed fatty acids, the pressure exerted a negative effect on the extraction since the increase in the $scCO_2$ extraction pressure led to a decrease in the diffusion coefficient of the extracted fatty acids. According to López-Padilla et al. [34], when solvent density increases due to high pressure, diffusion becomes more difficult due to a higher number of molecular collisions. According to those authors [34], intermolecular interactions also increase, since the average intermolecular distance decreases as the density increases, which interferes with the extraction of fatty acids.

As already mentioned, the pressure-temperature interaction was also significant ($p \leq 0.05$) during the extraction of oleic, linoleic, and α-linolenic fatty acids. It was established that the effect of temperature at low pressures (25 MPa) was positive, while at high pressure (35 MPa), an increase in temperature led to a decrease in the extraction yield of fatty acids. This was since the diffusion coefficient and solubility of fatty acids increased with increasing temperature at a constant pressure. This effect was less significant at higher pressures [34].

Response surfaces in Figure 3 show the influence of extraction pressure and temperature on the oleic, linoleic, and α-linolenic acid contents in supercritical extracts. According to Figure 3, it could be established that the highest concentration of the three fatty acids could be obtained in lipid fractions extracted at a relatively low pressure of 23 MPa and a temperature of 73 °C. At those conditions, a concentration of 172 g oleic acid/kg extract, 46 g linoleic acid/kg extract, and 5 g α-linolenic acid/kg extract would be achieved. For the benefit of extraction and our proposal, the optimal extraction conditions for the three fatty acids analyzed were the same, because the three target compounds belong to the same chemical family (fatty acids with 18 carbons) [30], with similar chemical structure, molecular weight, volatility [18], and diffusion coefficients [34].

As a final remark, and for the future feasibility of this work at an industrial scale, authors would like to mention that when the yield or the selectivity of the supercritical extraction is low, or the operating pressure or CO_2 consumption is to be reduced, a cosolvent (entrainer or modifier) may be added to overcome the poor solvent nature of $scCO_2$ [35–37]. However, considering the economic and practical difficulties involved in choosing a cosolvent at supercritical conditions, theoretical evaluations must be implemented. In this regard, the Hansen solubility theory (HST) has arisen as a suitable tool to reduce the number of experiments for the selection of a proper cosolvent for $scCO_2$ [37]. The Hansen approach can be used to predict the best cosolvent for $scCO_2$ in the solubilization of bioactive compounds from natural matrices within a specific interval of operating conditions [35–37]. In this regard, and in order to increase the lipid fraction rich in fatty acids such as linoleic and α-linolenic acids, some studies have made calculations with several organic solvents used in food and pharmaceutical industries [35,36]. In their previous reports, Professor Lourdes Calvo's Research Group in Madrid [35,36] reached a selective

extraction of oleic, linoleic, and α-linolenic acids by using the Hansen approach with a supercritical homogeneous mixture with a cosolvent volume fraction of 5% (0.05 m^3/m^3). The authors correctly predicted the order of the cosolvent ability in a wide range of operating conditions suitable for supercritical extraction, finding that the cosolvent order depended on the fatty acid, but in general, ethanol was the best cosolvent to solubilize and therefore to selectively extract oleic, linoleic and α-linolenic acids with scCO$_2$. The above, along with the valorization strategy proposed in this study, could represent a chance to improve the commercial opportunity of the process. Finally, on a possible industrial scale, CO$_2$ is recirculated to the process, which, as it is well known [28], undoubtedly lowers the operating costs of the high-pressure process.
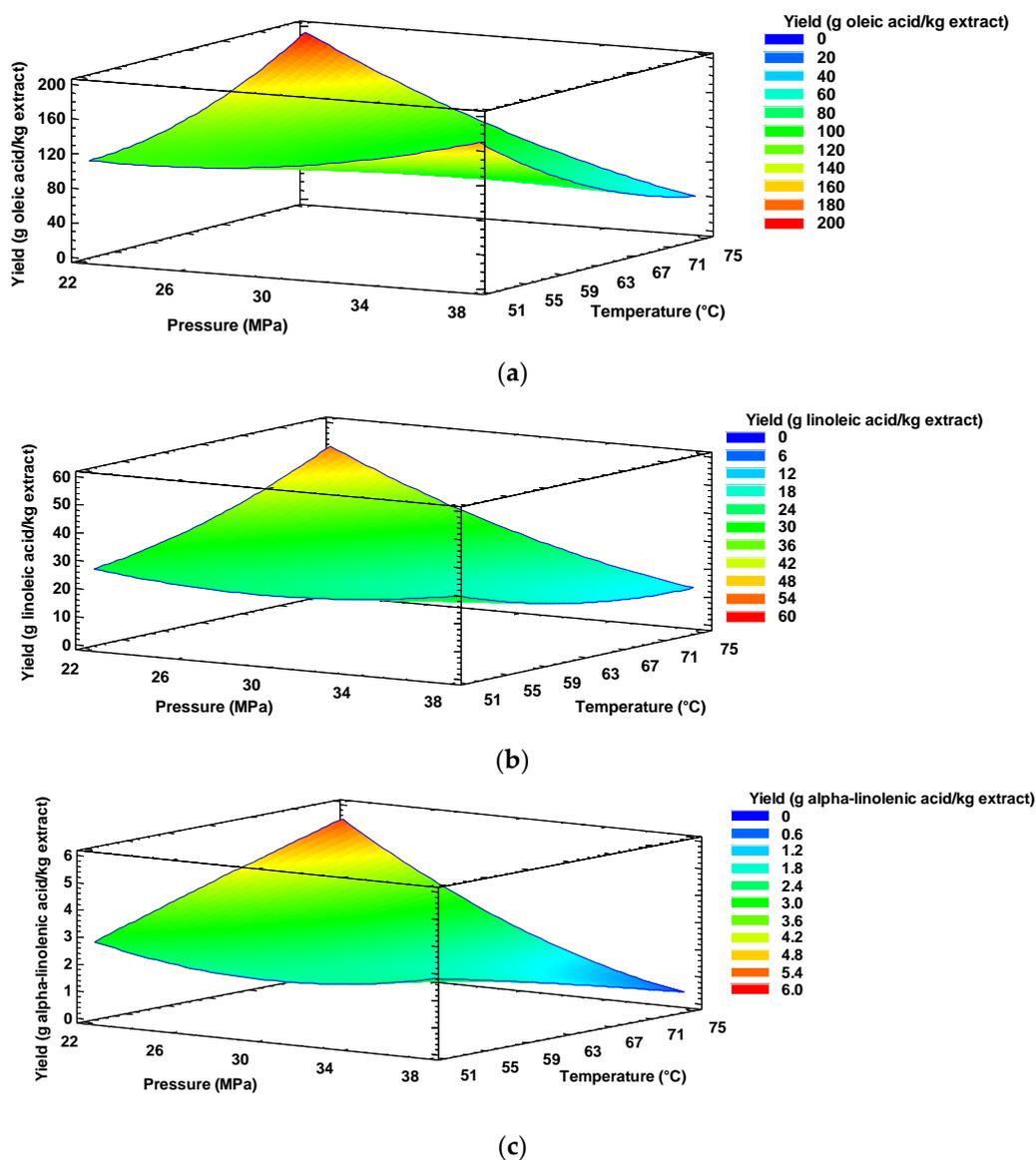

(a)

(b)

(c)

Figure 3. Effect of temperature and pressure on the (**a**) oleic, (**b**) linoleic, and (**c**) α-linolenic acid extraction yield from mango seed kernels.

3. Materials and Methods

3.1. Reagents

Boron trifluoride (BF$_3$, 1.3M in methanol, Sigma-Aldrich, Pasto, Colombia), hexane (99%, Sigma-Aldrich, Pasto, Colombia), methanol (HPLC, $\geq$99.9%, Sigma-Aldrich, Pasto, Colombia), sodium hydroxide (NaOH, $\geq$98%, pellets anhydrous, Sigma-Aldrich, Pasto,

Colombia), sodium sulfate anhydrous (Na_2SO_4, Sigma-Aldrich, Pasto, Colombia), un-decanoic acid (98%, Sigma-Aldrich, Pasto, Colombia), CO_2 (99.9% purity, CRYOGAS, Pasto, Colombia), helium ($\geq$99.99%, CRYOGAS, Pasto, Colombia), and nitrogen ($\geq$99.99%, CRYOGAS, Pasto, Colombia) were used for this study. All materials were used as received.

3.2. Biomass

Mango (*M. indica* L.; *var.* Tommy Atkins) seed kernels were obtained as a by-product from INPADENA, a company devoted to the production of fruit pulp in the city of Pasto (Nariño, Colombia). Fresh seed kernels were provided with an approximate water mass fraction of 44% (0.44kg water/kg seed kernel). The moisture was measured in an oven (Digiheat, J.P. Selecta, S.A., Pasto, Colombia) at 103 °C until the weight did not vary more than 0.1%.

Considering that the initial solid conditions of the raw material to be submitted to supercritical fluid extraction are especially important to maximize the extraction yield and to improve the quality of the extract [25], the raw material was dried and milled before being subjected to the extraction process. For drying, a CST-800 tray dryer (FIQ S.A.S., Pasto, Colombia) was used at 50 °C for 8 h until a humidity of 6.8% in mass fraction was reached, which corresponded to the equilibrium moisture of the raw material with the air in Pasto. Afterward, seed kernels were milled with a hammer mill (TRAPP, Vieira, Jaraguá do Sul, Brazil). The average particle size of the ground kernels was 0.6 mm, which was measured using a sieve (PS-35 series 1182, Pinzuar, Pasto, Colombia) with the ASTM E11 sieve series, mesh 0.10–0.80 mm.

3.3. Supercritical Fluid Extraction

3.3.1. Experimental Installation

$scCO_2$ extractions from mango seed kernels were carried out in an SFE 500 system, which is an automated supercritical fluid extraction apparatus from the Waters® line of pilot-scale systems (Waters Acquires Thar Instruments, Milford, CT, USA). The experimental installation consists of a CO_2 pump with an operating pressure capacity of up to 60 MPa, which is fed with CO_2 at about 5.7 MPa from a cylinder with a dip tube. A cooling heat exchanger is used to cool and liquefy CO_2 before it enters the pump, to avoid CO_2 cavitation during pumping, and to guarantee maximum efficiency. An electrical heating heat exchanger is located upstream from a 0.5 L stainless steel vessel, to ensure that the CO_2 is heated up before entering the extractor, which is equipped with a high-pressure valve to isolate the vessel from high pressures. It also has a cap with a spring-loaded seal that enhances safety and lends to automation for efficient loading and unloading. A mass flow meter, located on the inlet of the CO_2 pump, measures liquified CO_2 mass output. The system controls pressure by using a motor-driven automated back pressure regulator (ABPR), which is temperature-controlled to compensate for cooling during depressurization due to the Joule-Thomson effect. A built-in pressure sensor provides closed-loop feedback for control and pressure alarm monitoring through the software Process Suite V 5.9. Meanwhile, a temperature control monitors and controls up to six temperature zones independently. Moreover, the SFE 500 system is equipped with a high-pressure collection vessel.

CO_2 is a non-toxic gas; however, it will displace the air in the room and can lead to suffocation if not properly ventilated. Therefore, the system also provides for venting to a fume hood through a 1/4″ compression fitting and line. A schematic representation of the equipment is shown in Figure 4 and complete details of the equipment have been described in a previous report [38].
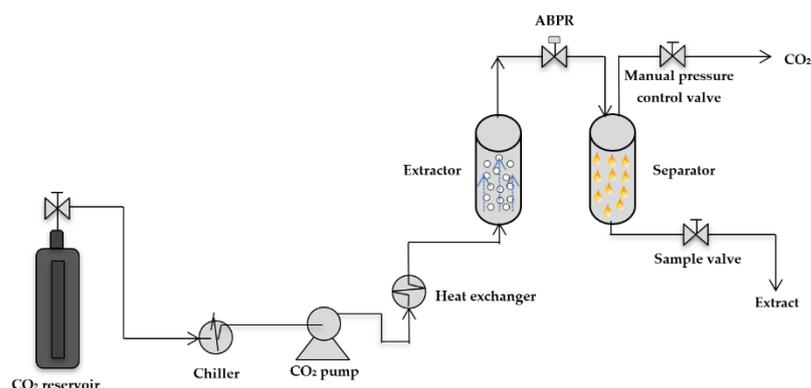
Figure 4. Schematic representation of the equipment used for supercritical fluid extraction.

3.3.2. Operation Procedure

The extractor was filled with 0.2 kg of dried and milled seed kernels, forming a fixed bed. The extractor was then closed, and the fluid was pumped in to achieve working conditions. After this, the ABPR was opened to provide a continuous flow of 30 g CO_2/min through the bed for 150 min (4.5 kg of CO_2). Previous experiments performed at the central point of the experimental design (30 MPa and 63 °C) revealed that a solvent ratio of 22.5 kg CO_2/kg biomass was enough to deplete the plant material by up to 80% of its total oily extract content. After the ABPR, the fluid was depressurized; the solvent power of the CO_2 dropped, the extract was collected in pre-weighed amber vials and kept at 4 °C until further analysis.

All the experiments were carried out at least two times and the experimental error was deduced from selected tests that were repeated six times. The oil extraction yield was expressed in g extract/kg biomass.

3.3.3. Optimization

A Box–Wilson RCCD, containing an embedded factorial design 2^2, with center points augmented with a group of star points allowed the estimation of curvature (RSM) to obtain the highest oil and EFAs yield. The independent factors studied to optimize the extraction yield (Y), along with their operating ranges, were pressure (X_1; from 25–35 MPa) and temperature (X_2; 55–70 °C). Levels of each factor were established from preliminary tests and coded as Table 3 shows.

Table 3. Factors and levels of the rotatable central composite design.

Factor	Level		
	Low (−1)	Central (0)	High (+1)
Pressure (MPa, X_1)	25	30	35
Temperature (°C, X_2)	55	63	70

Four (4) points from the factorial design, four (4) star points (α = 1.414), and four (4) central points (30 MPa and 63 °C in Table 1) were used for a total of twelve (12) experimental runs. All experiments were performed in randomized order to minimize the effect of unexplained variability in the observed responses due to the extraneous factors. A second-order polynomial regression equation was used to predict the response variable (Y) according to Equation (2) as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \tag{2}$$

where Y is the response variable (extraction yield, g/kg); X_1 and X_2 are the independent factors pressure and temperature, respectively; β_0 is the intercept; β_1 and β_2 are linear effect coefficients; β_{11} and β_{22} are quadratic effect coefficients; β_{12} is the coefficient for

the interaction of factors; and ε is the random error. STATGRAPHICS Centurion XV.II (Statpoint Technologies, Inc., The Plains, VA, USA) was used for the optimization, analysis of experimental data, as well as for the creation of the design matrix. Data were submitted to ANOVA and the statistical significance of the factors was set at a significance level of 5% ($p \leq 0.05$).

3.4. Conventional Solvent Extraction

To obtain the total extract yield and for comparison purposes, a Soxhlet extraction was performed using 0.01kg of dried and milled seed kernels. The sample was transferred into a cellulose thimble and inserted into a Soxhlet assembly fitted with a 250 mL flask. Then, 100 mL of hexane was added and the whole assembly was heated for 8h using a heating jacket. Afterwards, the extract was concentrated using a rotary evaporator Re 121 (BUCHI, Flawil, Switzerland). Solvent traces were removed in an oven at 103 °C for 2 h; and finally, the extract was stored in a refrigerator at 4 °C until further analysis.

3.5. Fatty Acids Analysis

To analyze the fatty acid profile of lipidic fractions from both supercritical and conventional extraction, fatty acids were first, transesterified and then related to its respective fatty acid methyl ester (FAME, Restek, Bellefonte, PA, USA). The derivatization was conducted following the method described by the AOAC Official Method 969.33 [39]. For this, approximately 50 mg of mango seed kernel oil was placed into a screw cap glass tube. As much as 10 mg of undecanoic acid (internal standard) and 1 mL of NaOH in methanol (0.5 N) were added into the tube and then nitrogen was blown for 15 s. The tube was covered tightly, vortexed, heated in a water bath for 5 min at 85 °C, and then cooled. Afterward, 1 mL of BF_3 solution was added, vortexed and then nitrogen was blown to the tube. The tube was covered tightly, heated for 15 min at 85 °C, and then cooled. In this stage, fatty acids were converted to FAMEs. The extracting solvent, 3 mL of hexane, was added and then the mix was vortexed. Then 3 mL of saturated NaCl solution was added, and the solution was vortexed. Finally, the upper layer was transferred through a small amount of anhydrous Na_2SO_4 (placed on the top of a filter liner) into a test tube with a Pasteur pipette.

Subsequently, the samples were analyzed by gas chromatography (GC) with a Shimadzu GC-17A Version 3 (Shimadzu Corporation, Kyoto, Japan) equipped with a Flame Ionization Detector (FID) and a DB-WAX capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, Inc., Folsom, CA, USA) 5% phenyl-methylpolysiloxane. The separation was carried out with helium (1 mL/min) as a carrier gas. The column temperature was set at 40 °C for 5 min and then heated to 250 °C at 5 °C/min. A split injector (1:10) at 250 °C was used. The FID was also heated up to 280 °C. The injection volume was 1 μL.

The chromatographic data were obtained and processed with the Shimadzu Class Vp 4.3 Software and the compounds were quantified by relating them to the area of the internal standard.

4. Conclusions and Perspectives

This work explored the possibility of taking advantage of mango seed kernels as a by-product of the Colombian agroindustrial sector, to obtain a lipid fraction rich in both linoleic and α-linolenic acids, along with oleic acid. This could be a potentially valuable product for use in the cosmetic, pharmaceutical, and perhaps food industry since it would be obtained without the intervention of organic solvents. These supercritical extracts, which can be considered premium grade [33], could be used in the food industry as antimicrobial extracts and thus increase the shelf life of foods; but there is also a clear potential use as vegetable oil or additive in the confectionery industry for chocolates and bakery products [40]. Not to mention that a mixture of supercritical mango seed kernel extracts and palm stearin could also be used in countries with warm climates by chocolate manufacturers for its resistance to high temperatures [33]. The aforementioned gives a bioactive potential to

mango seed kernels, developing a green valorization strategy and posing a great challenge and a unique opportunity for the mango processing industry in Colombia to deliver a value-added product to the market with health-promoting properties, based on a circular economy model.

The proposal made in this study required drying and milling of the raw material, expensive operations that associated with the high installation costs of the supercritical plant could jeopardize the economic viability of the process. Nonetheless, a quality oil with a clear potential use in nutraceutical formulations, cosmetics, or even high value-added drugs was obtained. Such an oil would not require downstream processing, and a revalorized biomass would be used, which, along with CO_2 recycling on an industrial scale, would represent a strong contribution to the feasibility of the current proposal.

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