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An Evaluation of the Equilibrium Properties in Hexane and Ethanol Extractive Systems for *Moringa oleifera* Seeds and Fatty Acid Profiles of the Extracts

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Abstract: Until the present, oilseed extractions have been mainly performed using hexane: a toxic, non-sustainable solvent. Extraction methods using ethanol have recently been proposed and, to evaluate the suitability of ethanol as an alternative solvent, Moringa seeds with an oil content as high as 40% have been chosen to determine the equilibrium properties in solid–liquid ethanol extractions. The equilibrium constant (K_{eq}) and the specific retained solution (M) of the extractive systems seeds–oil–hexane and seeds–oil–ethanol were determined and validated, following a counter-current multi-stage extraction model. The extractions were carried out at 40 and 50 °C, the mass to solvent ratios used were 1:5, 1:10 and 1:20, and shelled and unshelled seeds (kernels) were tested. The K_{eq} and M of the different kinetics revealed that K_{eq} was not infinite in the hexane systems, whereas the ethanol systems registered slightly lower values. Regarding M, although the seed powder allowed more rapid extractions, particle size was increased to reduce M for an easier phase preparation. Finally, a counter-current multi-stage extraction system was simulated and applied under suitable conditions. The fatty acid profiles for both types of extract were similar regarding their main components. definition:

Keywords: equilibrium properties; fatty acids; multi-stage extraction; Moringa seed

1. Introduction

Moringa oleifera Lam. (Moringa) is considered to be one of the most useful trees we have access to. Its seeds have a high oil content (38.67%) and, similar to olive oil, it is rich in oleic acid (73.56% of the oil). Triglycerides constitute 97–99% of its edible oil content [1], although diacylglycerides and monoacylglycerides with a major polarity can also be found. Phenols, tocopherols, phytosterols and carotenoids are some of the other compounds detected in Moringa seed extracts [2,3]. Solvent extraction is a convenient method for seeds with less than 30% oleaginous material by weight, or seeds that have been mechanically pressed. Various solvents have been employed over the years, but hexane—a mix of hexane and isomers—is generally the preferred solvent because of its convenience, low cost, good diffusivity through cell walls, high oil solubility and low latent heat vaporization [1]. However, other solvents such as ethanol have also been successfully tested for the extraction of soybean, safflower, rice, and wheat germ edible oil [4–13]. Since ethanol is a sustainable and environmentally friendly substance, it could be considered a

preferable extraction solvent. Some authors have reported Moringa oil yields obtained by different methods (supercritical, mechanic, enzymatic or solvent extraction) and different solvents, such as CO_2, hexane or petroleum ether [4–13]. Two extractions methods with a potential industrial interest have been tested: traditional solid–liquid extraction (SLE) by maceration [4,5,11–13] and supercritical fluid extraction (SFE) by fixed column [6–9]. Each one of these processes operates on different principles. Thus, the extraction yields by SLE depend on the equilibrium of the solute concentration in the extract over the maceration stage, which is highly dependent on the solid to solvent ratio. In contrast, the extraction yields through SFE depend on the length of the extraction time in the column, while using a continuous new or recovered solvent flow. SLE yields can be increased by using a counter-current multi-stage configuration, whereas SFE yields can be increased using longer extraction times, with both factors representing a greater energy consumption. Optimization studies for both types of process have been conducted, but many of these studies have been optimized based on surface response methodologies [9,11,14]. However, an adequate optimization procedure that allows for their proper comparison should focus on yield and energy consumption values and be based on rigorous mathematical models. Although SLE is one of the main methods used in chemical engineering, a more thorough general model that rigorously describes the counter-current multi-stage method has recently been proposed [15]. According to this model, the assumption—supported by even the most recent chemical engineering textbooks [16]—of an extracted solute that is entirely transferred to the liquid phase is disputed. In fact, this is not the general behaviour that has been observed in solid–liquid extractive systems; rather, the solute content in its liquid phase (x_{E_2}) remains in thermodynamic equilibrium (equal to Gibbs free energy in both phases) with the solute content in its solid phase (x_{O_2}), which may be represented in terms of Nernst's law Equation (1):

$$x_{E_2} = k_{eq} x_{O_2} \tag{1}$$

Or in a Langmuir relation:

$$x_{E_2} = \frac{k_1 x_{O_2}}{k_2 + x_{O_2}} \tag{2}$$

It is very important to note that x_{O_2} represents the mass fraction of the solute in its solid phase, instead of the mass fraction of the solute in the raffinatte or underflow (x_{R_2}), which is the mixture of the exhaust solid phase with the retained extract. Therefore, another equilibrium property is involved, namely, the amount of retained extract per kg of inert solids (M). Thus, K_{eq} represents the relative affinity of the solute (oil in this case) for both extraction phases, and M represents the affinity of the liquid phase with the solid phase. If both properties are known in a given solute–solid matrix–solvent extractive system, the solid–liquid extraction process, regardless of the operation mode (single stages, multiple stages, cross flow, parallel flow or counter-current flow), can be simulated and optimized. To the best of our knowledge, these two properties have not been reported for any *Moringa oleifera* oil seeds–solvent systems, mainly because the above mentioned complete rigorous model has only been published recently [15]. There are only two extractive systems characterized in terms of K_{eq} and M; namely, oleoresin–vanilla-bean–ethanol 60% [15] and solute–Moringa-leaf–ethanol 80% [17]. Another important thermodynamic aspect of Equation (1) is that the traditional assumption of a total solute transfer to the liquid phase is also considered by Equation (1), i.e., when $K_{eq} \to \infty$.

Hexane, which is toxic and non-friendly to the environment, is the most frequent solvent used for the solid–liquid extraction of oils [18]. However, ethanol has recently been tested on the solid–liquid extraction of oils because of its respectively lower toxicity, and because it is considered a green solvent [19–22]. Subsequently, it has been ascertained that ethanol can extract greater amounts of material from oilseeds than hexane. This is explained by its affinity, as a polar organic solvent, with polar solutes, such as phospholipids, waxes and with some proteins and sugars [20]. In this sense, it should be mentioned that Moringa seeds contain glycosides, and that 35.97% of these seeds are formed by proteins (of which 53% are globulin and 44% albumin). However, these undesirable compounds can be easily

removed by refining the oil [2,23]. Since it has been proven that oils extracted by either hexane or ethanol may present different compositions, it is essential to verify any of the possible differences or similarities between their respective fatty acid profiles.

For this purpose, the present work focuses on the experimental evaluation of the equilibrium properties of the extractive systems: oil–*Moringa-oleifera*-seeds–ethanol, and oil–*Moringa-oleifera*-seeds–hexane. The distribution constant (K_{eq} in Equation (1)), and the specific solution retained by the underflow (M), were the equilibrium properties to be particularly evaluated, in both the ethanol and hexane extraction procedures. Additionally, the fatty oil profiles of the oils extracted from the Moringa oilseeds using either solvent were determined. The equilibrium properties that were obtained were then experimentally validated, with respect to the simulated multi-stage counter-current solid–liquid extractions.

2. Materials and Methods

2.1. Raw Material and Chemicals

Moringa seeds were purchased from a supplier in Navojoa, Sonora, Mexico, in June 2018. The seeds were deshelled to obtain a 500 g batch of kernels. The kernels were milled using a coffee grinder (160 W, KRUPS, Mod. GX410011; Millville, NJ, USA) and subsequently sieved to collect the particles between 0.3 mm (Metallic mesh No. 50) and 0.85 mm (Metallic mesh No. 20) size. The analytical-grade ethanol and hexane for the extractions were purchased from Golden Bell Reagents, CDMX, Mexico.

2.2. Raw Material Characterization

2.2.1. Determining Water Content

The water content was determined by weight difference according to the following procedure: 2 g of samples were placed in a vacuum oven (LabLine Instrument, Mod. 3818−1, Kochi, India) at 60 °C and 6×10^4 Pa until a steady weight was registered. The water content was expressed as the fraction of water mass in the feed (x_{F_3}) according to Equation (3), as follows:

$$x_{F_3} = \frac{w_0 - w_I}{w_0} \tag{3}$$

where w_0 is the initial weight and w_I is the final weight. The analysis was performed in triplicate.

2.2.2. Non-Extractable and Extractable Material

An exhaustive extraction was performed to determine the fraction of non-extractable solids, and thereby the fraction of extractable material available from the Moringa seeds. Eight different set-ups were used to vary the types of sample (whole seeds, kernels), the solvent (ethanol, hexane), and the temperature (40 °C, 50 °C); moreover, two additional set-ups involved extractions at 50 °C, using either kernel–ethanol or kernel–hexane. An amount of 1.5 g was exhaustively extracted using 150 g of solvent in each set-up, so that they would be suitable for K_{eq} and M comparison. The extractions were carried out in triplicate and the extracts were shaken by means of an orbital shaker (Thermo Scientific, Mod. MaxQ 4450; Oakwood, OH, USA), fitted with a temperature controller at 130 rpm (orbital radius: 0.95 cm). The extractions were performed for as long as required to reach the equilibrium of the concentration in the extracts. The equilibrium time was determined based on extraction kinetics data. At the end of the equilibrium time, the underflow (Moringa seeds with solvent) was separated from the extract (extractable material and solvent) by decantation and filtration. Subsequently, the underflow was subjected to the same extraction process five more times using ethanol, and three times using hexane. After each new extraction, the residue was placed in a vacuum oven at 60 °C and 6×10^4 Pa, until a constant weight was reached. The remaining solids constituted the non-extractable material from the Moringa seeds, and were expressed as a mass fraction (x_{F_1}), as in Equation (4):

$$x_{F_1} = \frac{w_0(1 - x_{F_3}) - w_I}{w_0} \quad (4)$$

Then, the extractable mass fraction (x_{F_2}) was calculated based on the difference, as seen in Equation (5):

$$x_{F_2} = 1 - x_{F_1} - x_{F_3} \quad (5)$$

2.3. Kinetics Experiments

The counter-current multi-stage SLE modelling must be performed at the equilibrium [15], and therefore the time required to reach the equilibrium is an important design parameter to establish the extraction tank dimensions, alongside the other properties considered for Equations (1) and (2). Therefore, some experimental extraction kinetics were planned and performed in order to determine the equilibrium time. The experimental extraction kinetics of the kernel samples were determined for different solid to solvent ratios as follows: 1:5, 1:10, and 1:20 (w/w). Then, 4 g samples (F) were placed inside several Erlenmeyer flasks, to which different amounts of solvent were added, stirred at 150 rpm (orbital radius: 0.95 cm), and kept at 40 and 50 °C until the equilibrium was reached. The underflow (R) was carefully separated from the extract (E) at different contact times, by decantation and filtration, and then both phases were weighed. Each R was placed in a vacuum oven at 60 °C and 6×10^4 Pa until a constant weight was reached. The dry residue obtained from R was referred to as D. Each experiment was performed in triplicate.

2.4. Determining the Equilibrium Parameters

Since the thermodynamic equilibrium properties K_{eq} and M could not be directly evaluated, an inverse method was implemented so that K_{eq} and M could be determined by fitting the general SLE model [15] applied to one single stage of the experimental extraction, performed at the equilibrium, using 1:5, 1:10 and 1:20 (w:w) solid to solvent ratios. The extraction methodology was the same as that explained in detail in Section 2.3, where the concentration was determined at the equilibrium. The SLE general model [15] applied to one single stage indicated that the extractable material transferred to the extract (Ex_{E_2}) would be the difference between the total solids in the Moringa seeds (or kernels) and the dry residue (D), as seen in Equation (6):

$$Ex_{E_2} = Fx_{F_{12}} - D \quad (6)$$

where $x_{F_{12}} = x_{F_1} + x_{F_2}$. Since F, E, D, x_{F_1} and x_{F_2} are determined experimentally, the mass fraction of the extractable solids present in E (x_{E_2}) can be calculated. D, which contains a solid phase (O) and certain extractable solids that remain in R because there is a retained solution (L) that has the same concentration as the extract (Lx_{E_2}), can be determined by Equation (7):

$$D = O + Lx_{E_2} \quad (7)$$

whereas R, constituted by O and L, can be determined by Equation (8):

$$R = O + L. \quad (8)$$

As R, D and x_{E_2} were evaluated experimentally, L can be calculated based on the combination of Equations (7) and (8), obtaining Equation (9):

$$L = \frac{R - D}{1 - x_{E_2}} \quad (9)$$

As L was calculated and R was evaluated experimentally, O can be calculated through Equation (7). In addition, it was assumed that O was formed by the feed of non-extractable solids (Fx_{F_1}), as well as by the extractable solids that were not transferred into the extract (Ox_{O_2}) (Equation (10)):

$$O = Fx_{F_1} + Ox_{O_2} \tag{10}$$

Then, the extractable solid mass fraction present in O (x_{O_2}) can be easily calculated from Equation (10), since F and x_{F_1} were experimentally determined. As the equilibrium was reached by the extracts from the three different sample concentrations, K_{eq} was calculated by linear regression without the interception of the x_{E_2} vs. x_{O_2} values, obtained from the three solid to solvent ratios used.

Finally, the specific retained solution of non-extractable solids per kg (M) can be calculated according to Equation (11):

$$M = \frac{L}{Fx_{F_1}} \tag{11}$$

for the three different g Moringa seeds/g solvent extract concentrations (the extractions were conducted using three different ratios).

2.5. Modelling of the Counter-Current Multistage Extractions (CME)

The evaluated thermodynamic properties (K_{eq} and M) described in Section 2.4. can be applied to the counter-current multi-stage SLE general model [15]. For the referred model, it was considered that the extractive system had four components: (1) non-extractable solids, (2) extractable solids, (3) water, and (4) solvent. This model can be applied to both the hexane and ethanol extraction processes; in this sense, the water in the seeds is part of the inert material for hexane and of the extractable material for ethanol. According to the general model, the ideal (at equilibrium) multi-stage counter-current solid–liquid batch extractors, are determined by the macroscopic mass balances at the end of each extraction stage; that is, when the solid–liquid equilibrium has been reached (Equations (12)–(24)).

General balance:

$$E_{j+1} + R_{j-1} - E_j - R_j = 0 \tag{12}$$

Non-extractable solids balance:

$$R_{j-1}x_{Rj-1_1} - R_j x_{Rj_1} = 0 \tag{13}$$

Extractable solids balance:

$$E_{j+1}x_{Ej+1_2} + R_{j-1}x_{Rj-1_2} - E_j x_{Ej_2} - R_j x_{Rj_2} = 0 \tag{14}$$

Water balance:

$$E_{j+1}x_{Ej+1_3} + R_{j-1}x_{Rj-1_3} - E_j x_{Ej_3} - R_j x_{Rj_3} = 0 \tag{15}$$

Extract–solvent balance:

$$E_{j+1}x_{Ej+1_4} + R_{j-1}x_{Rj-1_4} - E_j x_{Ej_4} - R_j x_{Rj_4} = 0 \tag{16}$$

In the underflow:

General balance:

$$R_j - L_j - O_j = 0 \tag{17}$$

Non-extractable solids balance:

$$R_j x_{Rj_1} - O_j x_{Oj_1} = 0 \tag{18}$$

Extractable solids balance:

$$R_j x_{Rj_2} - L_j x_{Ej_2} - O_j x_{Oj_2} = 0 \tag{19}$$

Water balance:

$$R_j x_{Rj_3} - L_j x_{Ej_3} = 0 \tag{20}$$

Extract–solvent balance:

$$R_j x_{Rj_4} - L_j x_{Ej_4} = 0 \tag{21}$$

O definition:

$$O_j - F x_{F1} - O_j x_{Oj_2} = 0 \tag{22}$$

Thermodynamic equilibrium relation:

$$K_{eq} = \frac{x_{Ej_2}}{x_{Oj_2}} \tag{23}$$

Specific retained solution:

$$M_j = \frac{L_j}{R_j x_{Rj_1}} \tag{24}$$

where $F = R_0$ and $S = E_{N+1}$.

The Equations (12)–(24) represent a system of 13 N non-linear equations (N is the number of stages) with 13 N unknown variables. The input variables F, x_{F_1}, x_{F_2}, x_{F_3}, x_{s_3}, x_{S_4} and x_{E1_2}, x_{E1_2} remain at their desired fixed values, which are normally the maximum concentration that can be obtained from one single stage with a low solvent to solid ratio. S and the rest of variables are calculated by applying the 13 N non-linear equations with 13 N unknown variables. The details of the resulting algorithms are reported in [15]. In order to perform an experimental validation, it is necessary to reach stability in the counter-current multi-stage solid–liquid extraction process. That is, each entire stage must have an equilibrated extract from the precedent stage. The stability period applied was in accordance with the description in Figure 1, as suggested by [17].

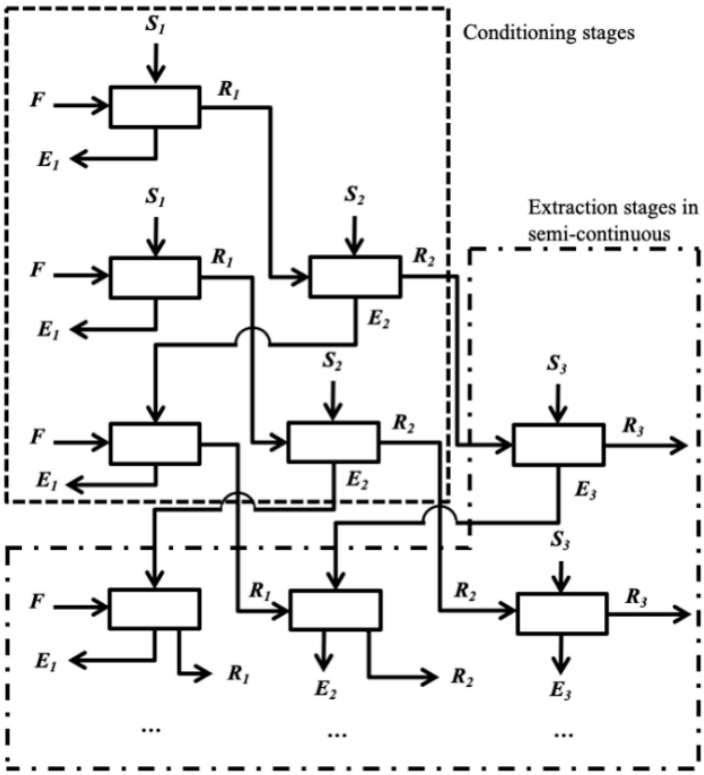

Figure 1. Counter-current multi-stage extraction start-up [15].

An amount of 10 g of Moringa seed kernels were placed in Erlenmeyer flasks, together with the according amount of solvent (hexane or ethanol) to the results obtained from

Equations (12)–(24), and following the sequence indicated in Figure 1. The experimental final extract concentration, x_{E1_2}, obtained from the first stage (the most concerted extract, since the process is counter-current) after 5 sequences, was evaluated by evaporating the extract and placing the resulting oil inside a vacuum oven at 60 °C and 6×10^4 Pa, until a constant weight was reached.

2.6. Derivatization of the Fatty Acids

In addition to the thermodynamic properties, the profiles of the fatty acids in the oil extracted by the two solvents (hexane and ethanol) were evaluated. The fatty acids were converted into their respective fatty acid methyl esters (FAMEs), according to the following procedure. First, in a 10 mL volumetric flask, 1.12 g of KOH was added and diluted with MeOH by stirring, until the complete homogenization of the KOH/MeOH solution (2 M) was achieved. Then, in a vial fitted with a hermetic cap, 100 µL of Moringa seed oil, 200 µL of the KOH/MeOH solution (2 M), and 1 mL of hexane was added. This mixture was stirred for 5 min and then centrifuged at 6810 g, to finally collect approximately 1 mL supernatant. The non-polar phase (upper layer) contained the FAMEs from the Moringa oil together with the hexane.

2.7. Fatty Acid Analysis

The fatty acid composition was determined by gas chromatography [5]. The analyses were performed on a gas chromatograph–mass spectrometer, GC/MS-TQ8040 (Shimadzu, Kyoto, Japan) using a Suprawax 280 capillary column of 60 m × 0.25 mm id. × 0.25 nm film thickness (Teknokroma, Barcelona, Spain) with helium as the carrier gas, at a flow rate of 1.37 mL/min at constant pressure. The analyses were performed in split mode (1:100), using 1 µL volume injections. The GC oven temperature was programmed as follows: 50 °C for 2 min; then, it was heated from 50 °C to 220 °C at a rate of 5.0 °C/min and withheld for 15 min. It was then heated again from 220 to 250 °C at 40 °C/min rate and withheld for 2 min; finally, it was heated from 250 °C to 270 °C at 40 °C/min, and then kept at a constant temperature of 270 °C for 2 min. The samples were prepared and measured in duplicate.

The qualitative analysis was conducted by comparing the mass spectrum data against each fatty acid in the NIST library (NIST v.1.4; Gaithersburg, MD, USA) and by comparison against the fatty acid methyl esters standard Supelco 37 FAME MIX (Merck, Darmstadt, Germany).

2.8. Statistical Analysis

The experimental results were expressed as the mean ± standard deviation and the statistical analyses were performed by Tukey's pairwise test. The differences were considered statistically significant when the probability value was less than 5% ($p < 0.05$).

3. Results and Discussion

3.1. Initial Composition of the Feed

The characterization of the feed is presented in Table 1, where the fractions of inert and total extractable material are considered according to each solvent and each type of feed (kernels or whole seeds) at 40 and 50 °C. Some differences were observed depending on the solvent used, both for grain at 50 °C, and seeds at 40 and 50 °C, revealing greater amounts of extractable material when ethanol was used, compared with hexane. In previous studies, it was reported that the amount of extracted material was greater with hexane than with ethanol for Moringa oil extractions using soxhlet [23], whereas for the extraction from sunflower or wheat germ oil, the amount of extracted material was greater with ethanol. This is because ethanol extracts glycosides, phospholipids and, probably, waxes and proteins [20,24]. Only when kernel feed was extracted at 40 °C were the yields the same from the ethanol and hexane extractions. On the other hand, as the temperature increased, the amount of extracted material was also greater. This effect had already been

observed in other oilseeds and could be attributed to increased solubility and decreased viscosity [19,21,25,26]. However, no relevant differences could be observed between the hexane extractions at 40 and 50 °C. Regarding particle size, only the ethanol extractions presented some reduction in the amount of extracted material as particle size increased. This is explained by a limited rupture of cell membranes [9].

Table 1. Characterization of Moringa seeds.

Sample	Particle Size (mm)	Temperature (°C)	Total Extractable Material (g/g)		Water (g/g)
			Ethanol	Hexane	
Kernel	<0.85	40	0.424 ± 0.010	0.412 ± 0.014	0.039 ± 0.002
	<0.85	50	0.509 ± 0.019	0.424 ± 0.041	0.025 ± 0.001
Kernel	1–4.5	50	0.270 ± 0.014	0.440 ± 0.013	0.035 ± 0.002
Seed	<0.85	40	0.331 ± 0.011	0.281 ± 0.011	0.036 ± 0.001
	<0.85	50	0.410 ± 0.007	0.306 ± 0.046	0.027 ± 0.002

Even if the amount of extractable material (x_{F_2}) for the ethanol and hexane extractions were known, it is the thermodynamic properties of the processes that determine the number of stages in the model developed by Castillo-Santos et al. [15]. This is a mechanistic model based on the balance of the masses after the equilibrium has been reached. Our results indicated that $x_{F_1} > x_{F_2}$ in Moringa seeds (Table 1), and that both fractions, x_{F_1} and x_{F_2}, were key components in defining the equations in the model developed by Castillo-Santos et al.

3.2. Extraction Kinetics

Solid–liquid extractions take place in two stages. Initially, the oil surface is rapidly extracted: this is known as the washing stage. After that, the extraction rate decreases, and the remaining oil is extracted by diffusion until the equilibrium is reached [19,21]. This behaviour can be observed in Figures 2 and 3, where the kernel feed with <0.85 mm particle size reached the equilibrium rapidly, because the oil was exposed on the surface of the particles. On the contrary, when the particle size was increased (1–4.5 mm), the equilibrium time grew longer, as a slower diffusion of the oil took place. Thus, the equilibrium time for 1–4.5 mm size particles was approximately 80 min.

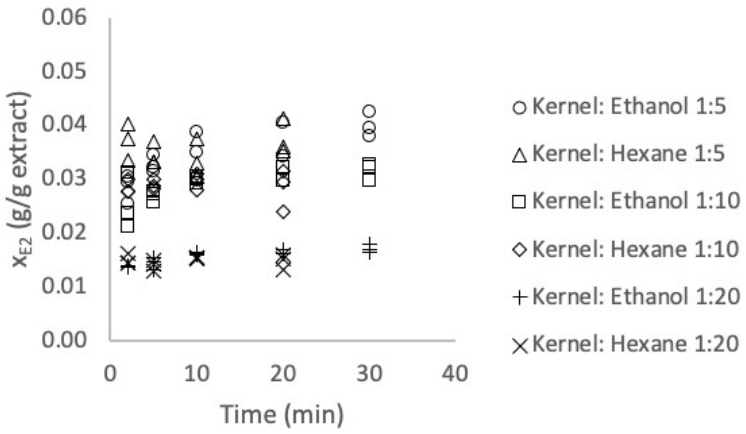

Figure 2. Extract concentrations vs. time in kernel feed extraction at 50 °C (<0.85 mm particle size).

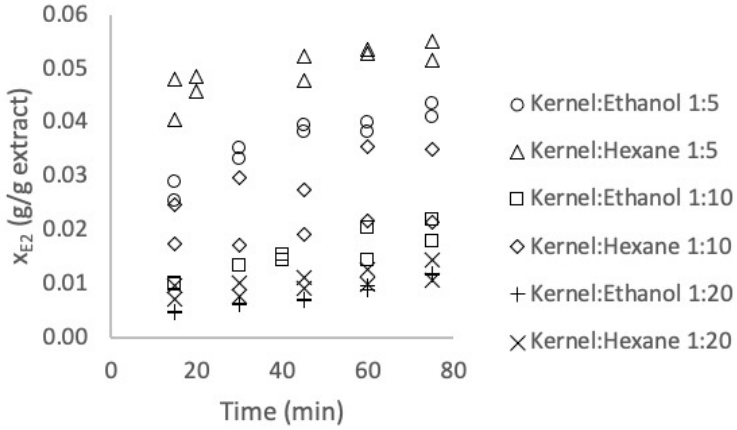

Figure 3. Extract concentrations vs. time in kernel feed extraction at 50 °C (1–4.5 mm particle size).

3.3. Experimental Evaluation of the Equilibrium Parameters

The values of the equilibrium constant K_{eq} and the specific solution retained M obtained at different conditions of the extraction kinetics, are reported in Table 2. Higher K_{eq} values can be observed for hexane, which indicates that Moringa oil is better distributed in this solvent. However, these values did not tend to be infinite, despite the complete solubility of the oil in hexane; this was due to the affinity of the oil with the inert mass. This fact corroborates with the common assumption regarding the complete solution of oil in the solvent, and is not valid to calculate the extractions that would be obtained from the present system [16]. On the other hand, higher K_{eq} values have been reported for ethanol extractions than for hexane in sunflower oilseeds [20]. The specific solution retained M stands out because it represents the amount of extract that is retained after the separation of the phases, and this value will not change from one stage to another in a counter-current solid–liquid extraction process [15,21]. M has also been reported to decrease with increasing temperature. In addition, more viscous extracts would lead to higher retention rates, lower extraction rates, and more stages to exhaust oilseeds [21]. In this research, the M values were lower in hexane extractions. On the other hand, the M values in the ethanol extractions were even higher than the values reported for rice bran oil extractions (0.48 kg of retained solution/kg of inert solids at 80 °C). This behaviour could be attributed to the extraction temperature, in addition to the fact that the presence of teguments or shells increases M in all systems, which is not beneficial for the extraction process. Lower M values were achieved by increasing the particle size and removing the teguments.

Table 2. Equilibrium properties of the extraction process.

Temperature	Sample	Particle Size (mm)	Hexane		Ethanol	
			K_{eq}	M	K_{eq}	M
40 °C	Kernel	<0.85	0.170	1.64 ± 0.25	0.110	2.58 ± 0.27
	Seed	<0.85	0.200	2.28 ± 0.22	0.200	3.72 ± 0.30
50 °C	Kernel	<0.85	0.130	1.73 ± 0.19	0.100	3.15 ± 0.44
	Kernel	1–4.5	0.206	0.96 ± 0.14	0.381	1.017 ± 0.06
	Seed	<0.85	0.200	2.25 ± 0.18	0.120	3.90 ± 0.21

In view of the results that have been obtained, it could be inferred that the efficiency of the separation of the liquid and solid phases by gravity sedimentation and decantation would be affected by the size of the particles. However, a certain number of fine particles were obtained in the alcoholic extracts. Despite this, the separation by decantation was easily carried out when particle sizes were 1–4.5 mm. It is worth mentioning that K_{eq} could be determined from the slope in a graph representing x_{E2} vs. x_{R2} in the equilibrium of the

extracts obtained through three solid–solvent ratios; however, K_{eq} values were defined by minimizing the sum of the squares of the error.

3.4. CME Simulation and Experimental Results

The thermodynamic properties listed in Table 2 were applied to Equations (11)–(23) in order to calculate the solvent requirements to reach x_{E1_2} = 0.0441 for ethanol, and x_{E1_2} = 0.0497 for hexane extractions, at the end of the counter-current multi-stage SLE process using one, two and three stages. The experimental results of the simulated process were applied as detailed in Section 2.5, after the stabilization period indicated in Figure 1. The model predicted the experimentally measured amount of solvent (S), as well as the amount of extract (E_1). The concentrations x_{E2}, predicted and experimental, are listed in Table 3.

Table 3. Experimental and predicted E_1 and x_{E2}.

Solvent	Stages	Solvent (g)	Extract (E_1)		x_{E2}	
			Predicted	Experimental	Simulated	Experimental
Ethanol	1	50	45.39	44.64 ± 0.12	0.0441	0.0364 ± 0.0011
	2	69.5	65.53	63.86 ± 0.30	0.0441	0.0389 ± 0.0004
	3	74.4	70.63	68.49 ± 0.93	0.0441	0.0413 ± 0.0003
Hexane	1	50	47.26	44.90 ± 0.57	0.0497	0.0513 ± 0.0022
	2	76.7	75.17	69.86 ± 1.33	0.0497	0.0431 ± 0.0031
	3	84.8	83.66	76.55 ± 0.26	0.0497	0.0475 ± 0.0028

The average errors between the experimentally measured and predicted values were 4.9% for the extract amounts, and 10.7% for the extract concentrations. The results indicated that a good agreement was obtained between the experimental and predicted values. Furthermore, since it was a pure prediction without any adjustments, the description of the kernel–oil–ethanol and kernel–oil–hexane systems described by the model developed by Castillo-Santos et al. [15] was considered satisfactory.

3.5. Determining Fatty Acid Content

When dealing with vegetable oils, fatty acids are extremely important, since, after their consumption, they enter the organism and form triglycerides that act as energy reservoirs. In this sense, it is of great importance to consume healthy fatty acids, including unsaturated ones. For this reason, it is important to identify the fatty acids that are present in Moringa and to find out how they can be affected by solvent extraction [1]. For this purpose, we decided to evaluate the profile of the fatty acids in the eight systems. The results obtained (Table 4) revealed that there were no statistical differences between the fatty acids identified in each of the eight systems studied. Therefore, ethanol, with a lesser environmental impact than hexane when used for the extraction of Moringa oil, would be the preferred solvent option. The major acid in Moringa oil is oleic acid (*cis*−9-octadecenoic acid), which has been reported to represent up to 73.56% of its content [2]. The benefits of this fatty acid are notorious and mainly associated with a reduction in cholesterol. This fatty acid is formed by the desaturation of the stearic acid that is found in certain oilseeds [3,5]. A lower percentage of this fatty acid (approx. 59%) was obtained in the present work. This low value might be due to the poor concentration of stearic acid found in Moringa (approx. 14%), in addition to some pre-harvest factors such as climate, plant nutrition and soil properties, which have been proven to affect the concentration of oleic acid in other plant matrices [27]. It should also be mentioned that one of the fatty acids could not be identified, although, tentatively, and based on its retention time, could be labelled as vaccenic acid (*cis*−11-octadecenoic acid), an isomer of oleic acid that has previously been reported in Moringa oil [28]. On the other hand, the percentage of palmitic acid was also high (approx. 18%), but this acid has been reported to form large chains including oleic acid [29], which

is consistent with the lower percentages of arachidic and behenic acids. In general, the solubility of vegetable oils in polar organic solvents depends on the size and unsaturation level of the carbon chains [30], where unsaturated fatty acids (commonly oleic acid) are generally in the middle of a triacylglyceride, and saturated ones are at the chain ends [1]. In addition to the triacylglycerides, both solvents extract other compounds such as waxes, phospholipids, tocopherols, sugars (only ethanol), diacylglicerides and monoacylglicerides, according to their polarity and compound–solvent interaction. Their extraction can be favoured using either ethanol or hexane; however, most of them will be removed during the oil refining processes [1,31].

Table 4. Percentage (%) of fatty acids extracted from Moringa according to the treatment employed.

Fatty Acid (%)	KE40	KE50	KH40	KH50	SE40	SE50	SH40	SH50
Caprilic	<0.1 ^{b}	<0.1 ^{a}	<0.1 ^{b}	<0.1 ^{b}	ND	<0.1 ^{b}	<0.1 ^{b}	ND
Myristic	0.4 ^{a}	0.3 ^{a}	0.4 ^{a}	0.4 ^{a}	0.3 ^{a}	0.4 ^{a}	0.4 ^{a}	0.4 ^{a}
Palmitic	18.3 ^{a}	19.2 ^{a}	17.5 ^{a}	18.0 ^{a}	20.1 ^{a}	17.9 ^{a}	17.8 ^{a}	17.9 ^{a}
Palmitoleic	1.5 ^{a}	1.0 ^{a}	1.3 ^{a}	1.4 ^{a}	1.3 ^{a}	1.3 ^{a}	1.3 ^{a}	1.3 ^{a}
Margaric	0.2 ^{a}	0.1 ^{a}	0.3 ^{a}	0.2 ^{a}	0.2 ^{a}	0.2 ^{a}	0.3 ^{a}	0.2 ^{a}
Estearic	13.8 ^{a}	14.1 ^{a}	14.6 ^{a}	14.6 ^{a}	14.5 ^{a}	13.9 ^{a}	14.7 ^{a}	14.6 ^{a}
Oleic	58.9 ^{a}	58.9 ^{a}	59.4 ^{a}	58.5 ^{a}	58.3 ^{a}	59.1 ^{a}	58.7 ^{a}	59.0 ^{a}
Not identified	6.3 ^{a}	5.9 ^{a}	6.0 ^{a}	6.4 ^{a}	4.4 ^{a}	6.5 ^{a}	6.4 ^{a}	6.2 ^{a}
Linoleic	0.6 ^{a}	0.5 ^{a}	0.5 ^{a}	0.6 ^{a}	0.7 ^{a}	0.6 ^{a}	0.6 ^{a}	0.6 ^{a}
ɣ-linolenic	<0.1 ^{b}	<0.1 ^{a}	ND	<0.1 ^{c}	<0.1 ^{a}	<0.1 ^{bc}	<0.1 ^{bc}	<0.1 ^{a}
Linolenic	<0.1 ^{b}	<0.1 ^{a}	ND	<0.1 ^{c}	<0.1 ^{a}	<0.1 ^{bc}	<0.1 ^{bc}	<0.1 ^{a}
Arachidic	ND	<0.1 ^{a}	<0.1 ^{a}	ND	<0.1 ^{a}	<0.1 ^{a}	<0.1 ^{a}	<0.1 ^{a}
Behenic	<0.1 ^{a}	ND	<0.1 ^{a}	<0.1 ^{a}	ND	<0.1 ^{a}	<0.1 ^{a}	<0.1 ^{a}

Different letters (a, b, c) in the same row correspond to statistically different values ($p < 0.05$). Treatment: Sample; Solvent; Temperature Sample: K (kernel) or S (seed); Solvent: E (ethanol) or H (hexane); Temperature: 40 (40 °C) or 50 (50 °C).

4. Conclusions

The hexane and ethanol extractive systems for *Moringa oleifera* seeds were characterized in terms of their thermodynamic equilibrium properties, as required by the general model proposed by Castillo-Santos et al. [15]. The thermodynamic properties were applied to the general model for counter-current multi-stage solid–liquid extractions for both systems, using one, two and three stages. The model successfully reproduced the experimental behaviour with a 9.7% average error, regarding the amount of extract obtained, and a 10.7% average error with respect to extract concentration. The fatty acid profiles obtained for the hexane or ethanol extracts were also similar in terms of their main components (oleic, palmitic and stearic acids). The thermodynamic properties together with the general SLE model can be successfully applied to optimize the process in terms of thermal efficiency (during solvent recuperation). The ethanol extractive system has confirmed its potential as a sustainable alternative to the hexane extractive system.

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