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Screening of stability of *Moringa oleifera* seed oil under extended frying conditions

Determinación de la estabilidad del aceite de semilla de *Moringa oleifera* bajo condiciones de freído prolongadas

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Abstract
Moringa oleifera is a crop that has spread throughout the tropics worldwide and its seeds are a non-conventional source of edible oil. The objective of this study was to evaluate the oxidative stability and fatty acid profile of *M. oleifera* oil under frying conditions for an extended time. Typical parameters used to characterize and assess the quality of edible oils were evaluated following standard methods such as the acid and saponification value. In addition, oxidative stability was monitored by measuring conjugated dienes and thiobarbituric acid reactive substances (TBARS) for primary and secondary oxidation, respectively. The fatty acid profile was determined by gas-liquid chromatography. The results showed 1-2% and 170-340 mg NaOH/g, for the acid and saponification indexes, respectively. In addition, the oxidation values for conjugated dienes were below 10 and less than 10 μmol of malondialdehyde equivalents per g for TBARS. On the other hand, eight primary fatty acids were identified. Generally, the fatty acid profile was not affected during the evaluated frying conditions. Finally, the results show that *M. oleifera* oil has significant stability to oxidation and hydrolysis under prolonged thermal conditions.
Keywords: Fats and oils, oxidative stability, oilseeds, crop production and agronomy.

Resumen
Moringa oleifera es un cultivo que se ha extendido por los trópicos a nivel mundial y sus semillas son una fuente no convencional de aceite comestible. El objetivo del estudio fue evaluar la estabilidad oxidativa y el perfil de ácidos grasos del aceite de *M. oleifera* en condiciones de freído durante un tiempo prolongado. Además, se monitorearon los índices de acidez y saponificación como parámetros típicos de la calidad de los aceites comestibles siguiendo métodos estándares. Se determinaron los dienos conjugados y las sustancias reactivas al ácido tiobarbitúrico (TBARS) para la oxidación primaria y secundaria, respectivamente. El perfil de ácidos grasos se determinó por cromatografía gas-líquido. Los resultados mostraron 1-2% y 170-340 mg NaOH/g, para los índices de acidez y saponificación, respectivamente. Además, los valores de oxidación de los dienos conjugados estaban por debajo de 10 y menos de 10 μmol de equivalentes de malondialdehído por g para TBARS. Por otro lado, se identificaron ocho ácidos grasos primarios. En general, el perfil de ácidos grasos no se vio afectado durante las condiciones de fritura evaluadas. Finalmente, los resultados obtenidos muestran que el aceite de *M. oleifera* tiene una estabilidad significativa a la oxidación e hidrólisis en condiciones térmicas prolongadas.
Palabras clave: Grasas y aceites, estabilidad oxidativa, semillas oleaginosas, producción de cultivos y agronomía.

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1 Introduction

There is an imperative need to find new or unconventional sources of food, nutrients, and micronutrients (Alpizar-Reyes *et al*., 2022; Franco-Vásquez *et al*., 2023). One of the most demanded inputs in the food industry is the use of edible oils, especially those that provide unsaturated fatty acids. It is mainly due to the growing demand and, more importantly, the impact on human health caused by the deterioration of lipids. It is also worth mentioning the preponderant role of oils and fats in the food economy (Zargaraan *et al*., 2019). In this sense, the increase in fast food establishments where the frying process is dominant and prolonged requires sources of edible oils with high oxidative stability (Esfarjani *et al*., 2019).

Moringa oleifera (moringa) is a plant native to India that has acclimated to many countries, mainly in the tropics. *M. oleifera* belongs to the Moringaceae family, which includes 13 species. It is a fast-growing deciduous tree, resistant to drought, and optimally grows at a temperature of 25 to 35 ºC; it adequately performs in various types of soil and up to 2000 meters above sea level. Although it is a North India native species, it develops in tropical climates, so its cultivation has spread to North Africa, Southeast Asia, Central and North America (Paliwal *et al*., 2011), there is underuse of its fruits, specifically of its seed-contained oil, representing more than 30% (Servín de la Mora-López *et al*., 2018). The plant productivity has enormous potential since harvests can be above 2 tons/ha, and the orchards can maintain production for up to 20 years (Ledea-Rodríguez *et al*., 2018).

The oil from the *M. oleifera* seeds has been extensively studied due to its similarity in composition to olive oil, mainly the percentage of oleic acid (~75%) and the presence of omega-3 fatty acids. In addition to its composition, its physicochemical behavior in refining stages has been characterized as well as its oxidative stability during both storage and frying conditions for periods of up to 10 h (Tsaknis *et al*., 1998; Tsaknis *et al*., 1999; Lalas and Tsaknis, 2002; Abuzaytoun and Shahidi (2006); Anwar and Rashid, 2007; Sánchez-Machado *et al*., 2015). This information suggests that *M. oleifera* oil is a viable alternative to edible oil for domestic use. Furthermore, unconventional vegetable oils have unique physicochemical properties. For instance, products derived from alternative sources, such as moringa, may provide health benefits as they contain phytochemicals with important bioactive properties (Uluata and Özdemir, 2012).

The hypothesis of this work was that it is possible to extend the oil heating time during the frying conditions to simulate the processes performed by fast food establishments and/or industrial processes, where the oil used for cooking may maintain its fundamental properties for up to 24 h. Therefore, the objective of the study was to evaluate the oxidative stability, fatty acid profile and basic parameters (acidity and saponification values) of *M. oleifera* seed oil maintained for 24 h under frying conditions.

2 Materials and methods

2.1 Collection samples and sample preparation

Mature pods of *M. oleifera* were collected in the municipality of Etchojoa, Sonora, Mexico (36°54’39"N, 109°37’31"W). They were air-dried for 3 days and the seeds were then manually removed. In order to increase the contact surface area during oil extraction, the seeds were ground using a commercial mill (Chef Solutions© CE22369, IL, USA).

Unless otherwise indicated, all reagents used in this work were obtained from Sigma (St. Louis, MI, USA). The protocols proposed by Sánchez-Machado *et al*. (2015) were followed for the oil extraction and refining. First, batches of 50 g of seed powder were weighed into a 500 mL flask, 250 mL of hexane (1:5) (HYCEL, Mexico City, Mexico) were added, and the mixture was vortexed for 3 min. It was then sonicated (BRANSON 5800, St. Louis, MO, USA) for 15 min, trying to shake the flask’s contents manually every 3 min. Then, the mixture was vacuum filtered through a Whatman # 4 filter (Maidstone, UK) to recover the supernatant. The residue was re-extracted under the same conditions and both supernatants were mixed. To isolate the oil, hexane was recovered by vacuum distillation (BUCHI R-3, Flawil, Switzerland) at 40 °C and 40 mmHg. After the solvent was recovered, the extracted oil was stored in a container and kept in the dark. To refine the oil, only the degumming and neutralization processes proposed by Sánchez-Machado *et al*. (2015) were used.

2.2 *Frying conditions*

The frying process was carried out following exactly the methodology proposed by Tsaknis *et al*. (1999). First, batches of 150 g of oil were weighed, poured into a 250 mL beaker and heated on a CORNING PC-420D hot plate (Corning, NY, USA) at 175 °C. Potato sliced discs (0.5 thickness and 2.5 cm diameter) were used as frying products. Next, batches of 100 g of fried potatoes were made for 6 min every 2 h, obtaining a total of 12 sets. Aliquots of 5 mL of oil were taken at 0, 6, 12, 18 and 24 h. After sampling, the oil was cooled down, and the analyses described below were performed. The assay was conducted three times including three technical replicates.

2.3 *Oxidative stability of M. oleifera seed oil*

To determine the acidity and saponification values, the Ca 5a-40 and Cd 3-25 methods of the AOCS (2004) were used. The acid and saponification values were mentioned in the text as a physicochemical parameters. In addition, the conjugated diene method proposed by Abuzaytoun and Shahidi (2006) was used with minor modifications to identify primary oxidation products. The oil (0.02 g) was weighed and brought to 25 mL using iso-octane (Fisher Chemical, Hampton, NH, USA). Absorbance was measured in a UV-Visible spectrophotometer (Agilent 8453, Agilent Technologies, Santa Clara, CA, USA) at 234 nm. Pure iso-octane was used as blank and the content of conjugated dienes was calculated following the equation: $CD = (A/C) \times (d)$: Where: CD is conjugated dienes, A is absorbance, C is solution concentration in g/100 mL and d is cell length in cm.

In the case of detecting secondary oxidation products, the content of thiobarbituric acid reactive substances (TBARS) was determined according to Abuzaytoun and Shahidi (2006). The oil (0.05 g) was weighed in volumetric flasks, diluted and brought to 25 mL with 1-butanol (Jalmek, Nuevo Leon, Mexico). Then 2.5 mL of this mixture was taken and transferred to a test tube containing 2.5 mL of 2-thiobarbituric acid (Sigma-Aldrich, St. Louis, MO, USA). The mixture was homogenized and placed in an incubator (SHEL LAB 1212, Cornelius, OR, USA) at 95 °C for 2 h and the absorbance at 532 nm was registered. To calculate the concentration of TBARS, the absorbance was multiplied by the factor of 0.415, which was determined using 1,1,3,3-tetramethoxypropane as standard and results were expressed in μmol of malondialdehyde equivalents per g of oil (MA Eq./g).

2.4 *Analysis of fatty acids profile*

The analysis of fatty acids profile was done according to the methodology proposed by Núñez-Gastélum *et al*. (2011). 50 mg of oil were weighed into a screw cap tube, 2 mL of toluene (Sigma Aldrich, St. Louis, MO, USA) and 3 mL of freshly prepared 5% methanolic HCl were added. This mixture was vortexed and placed in a water bath at 70°C for 2 h. The mixture was cooled to room temperature and 3 mL of potassium carbonate (K_2CO_3) (Sigma-Aldrich, St. Louis, MO, USA) at 6% and 2 mL of toluene were added, immediately vortexed. The mixture was centrifuged at 2,400 rpm for 5 min (Eppendorf 5018 R, Berlin, Germany), then the organic phase was separated and dried with anhydrous Na_2SO_4 (Sigma-Aldrich, St. Louis, MO, USA). A VARIAN 3800 gas chromatograph (Melbourne, Victoria, Australia) equipped with a flame ionization detector (FID) and a CP-Sil 88 capillary column (60 m × 0.25 mm i.d., 0.25 μm thickness, VARIAN, Melbourne, Victoria, Australia). The operating conditions were: injection volume, 1 μL; injector temperature, 220 °C; carrier gas, helium; detector temperature, 235 °C. The temperature gradient in the column oven started at 120 °C for 1 min, followed by increases of 3 °C/min up to 170 °C, is maintained at this temperature for 1 min, and finally rised to 235 °C with a rate 6 °C/min. Finally, 235 °C were kept for 5 min. Fatty acid peaks were identified by comparing the fatty acid retention times of the reference standard 37 Component FAME MIX (Sigma-Aldrich, St. Louis, MO, USA). The relative amount of each fatty acid is estimated in proportion to the peak area with respect to the total area of the identified fatty acids.

2.5 *Statistical analysis*

Data were analyzed using program SPSS version 24 (SPSS Inc., Chicago, IL). A one-way ANOVA was conducted to compares the means of groups (Tukey test $p<0.05$).

Table 1. Acidity and saponification values of *M. oleifera* oil during frying process.

Time (h)	Acidity (% as oleic acid)	Saponification value (mg NaOH/g oil)
0	1.6 ± 0.11^{a}	178 ± 10.0^{bc}
6	1.3 ± 0.17^{ab}	322 ± 8.49^{a}
12	1.1 ± 0.0^{b}	252 ± 33.9^{ab}
18	1.3 ± 0.17^{ab}	150 ± 25.5^{c}
24	1.3 ± 0.17^{ab}	332 ± 32.0^{a}

The values correspond to the average of three replicates ± standard deviation. Different superscripts represent significant differences between the values of the same column, according to a Tukey test ($P \leq 0.05$).

3 Results and discussion

3.1 *Physicochemical properties of M. oleifera seeds oil during frying treatment*

A yield of 31% oil was obtained from the collected *M. oleifera* seeds. The oil presented a characteristic golden-yellow color and a mild foliage smell (Leone *et al*., 2016). Table 1 shows the acidity and saponification index values during the 24 h frying. For the acidity value, values between 1 and 2% were obtained (Sánchez-Machado *et al*., 2015); there was no significant difference after 18 h, and the acidity was maintained. Saponification value data is generally difficult to explain, especially during frying. White (1991) and Firestone (2007), mentioned that this oscillation is possibly due to polymerization and depolymerization of the triglycerides by a Diels-Alder reaction involving conjugated dienes. Casal *et al*. (2010), subjected different olive oils to frying conditions for 27 h, divided into 3 daily periods of 9 h. The acidity value remained below 1%. In our study, values higher than 1% were obtained during a constant heater for 24 h, however, these values are within the range recommended by the FAO/WHO (1999) for edible oils (≤ 2%).

3.2 *Oxidative stability of M. oleifera oil*

Due the continuos heating time in this study was long (24 h), it is difficult to compare with the various studies found in the literature. For this reason, an attempt was made to make a general comparison based on the ranges considered normal regarding the oxidation values of edible oils during frying. Figure 1 shows the behavior of CD and TBARS during the frying process of *M. oleifera* seed oil. A relatively stable behavior of the primary oxidation products is observed during

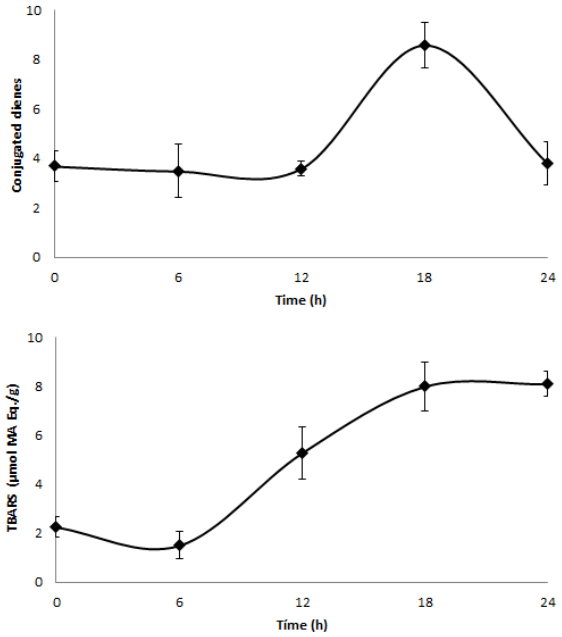

Figure 1. Oxidative stability values (average ± SD of three experiments by triplicate) of the *M. oleifera* oil during frying.

the first 12 h of the heat treatment. The peak in the concentration of these compounds is located between 12 and 24 h. Meanwhile, the behavior of the secondary oxidation products concurs with the production of a greater number of their precursors; this is since their concentration increases after 12 h.

It is widely demonstrated that CD is a representative oxidation parameter during frying to characterize the oxidation state of the oil. Likewise, it has been reported that CD values initially increase to then reach a plateau during frying (Farhoosh and Moosavi, 2009). In general, the initial CD values found in this work are similar to those reported for *M. oleifera* oil where the same method of analysis was used, regardless of the cultivation region and the extraction process (Tsaknis *et al*. 1998; Tsaknis *et*

al. 1999; Lalas and Tsaknis, 2002; Abuzaytoun and Shahidi, 2006; Anwar and Rashid, 2007; Sánchez-Machado *et al*., 2015). The stability shown during the first half of the heat treatment is due to the content of pigments, tocopherols and phenolic compounds, which remain in the oil since it was only degummed and neutralized (Abuzaytoun and Shahidi, 2006). Tsaknis *et al*. (1999) and Abdulkarim *et al*. (2007) obtained lower CD values by keeping the oil in frying conditions for less time than in this study, 4.84 (10 h) and 6.07 (2.91 h), respectively. Generally, vegetable oils will exhibit CD values between 0-6, depending on the source and oil processing. The leading causes of CD accumulation in vegetable oils are the high linoleic acid content (C18:2) and/or polyunsaturated fatty acids, in addition to increments in time and temperature (White, 1995). Likewise, olive oil is generally a comparative reference for evaluating new edible oils. For olive oil and extra-virgin olive oil heated at 180 °C for 1 h, CD values of 0.05 and 0.025, respectively, have been reported (Silva *et al*., 2010). Sunflower oil intermittently subjected to frying processes three times a week for two weeks presented CD values around 8 (Ní Eidhin and O'Beirne, 2010); in contrast, a 6-h intermittent heating over 4 days yielded CD values of 0.52 (Smith *et al*., 2007).

The hydroperoxides produced in the primary oxidation of lipids are mainly transformed into aldehydes; these are the main products of secondary oxidation. The most representative compounds are hexanal, 4-hydroxynonenal and malondialdehyde (MDA). To quantify MDA in food, the spectrophotometric method is mainly used, based on the measurement of the pink product resulting from the reaction of MDA with 2-thiobarbituric acid (TBA) (Abuzaytoun and Shahidi, 2006; Papastergiadis *et al*., 2012). An acceptable range for TBARS for edible vegetable oils has not been established; however, it is accepted that the lower the values, the better the oil's oxidative stability and, therefore, the better its quality. In this work, the TBARS values registered are lower than those reported in oxidative acceleration tests (Tsaknis *et al*., 1998; Lalas and Tsaknis, 2002; Abuzaytoun and Shahidi, 2006; Anwar and Rashid, 2007; Sánchez-Machado *et al*., 2015). It should be noted that, in addition to heat treatment, forced air circulation is used in the mentioned process. It is well known that oxygen in the air is fuel for lipid oxidation (Hoppenreijs *et al*., 2021).

On the other hand, lower TBARS values have been reported for olive oil treated under the same conditions for 9 h, without subjecting samples to the frying process (~0.038 μmol MA Eq./g) (Berasategi *et al*., 2012); while the oil showed a value of 0.0005 μmol MA Eq./g during intermittent frying processes and less prolonged than that of the present study. (Ní Eidhin and O'Beirne, 2010). Furthermore, Zargaraan *et al*. (2019) established that a good oil is resistant to hydrolysis and oxidation reactions. Non-conventional and unheated oils obtained from stinging nettle (*Urtica dioica* L.), laurel (*Laurus nobilis*), terebinth (*Pistacia terebinthus*), hemp (*Cannabis sativa*), and radish (*Raphanus sativus*) showed much higher TBARS values to those of moringa oil of this study (Uluata and Özdemir, 2012). This study sought to emulate the frying conditions of homes and commercial establishments.

3.3 Fatty acids profile of oil during frying conditions

The fatty acid profile of *M. oleifera* oil during frying is presented in Table 2. Eight main fatty acids were identified in the oil. Significant differences were found in the relative abundance of oleic (C18:1n9), linoleic (C18:2n6), linolenic (C18:3n3), and eicosenoic (C20:1n9) acids according to frying time; however, the profile remained stable throughout the frying process. In general, oleic acid had the highest proportion, its counterpart being linolenic. The second fatty acid in a more significant proportion was palmitic (C16:0). It is widely reported that the seed oil of *M. oleifera* contains, on average, 70-75% oleic acid, a similar content to olive oil (Tsaknis *et al*. 1998; Tsaknis *et al*., 1999; Lalas and Tsaknis, 2002; Abuzaytoun and Shahidi 2006; Anwar and Rashid, 2007; Sánchez-Machado *et al*., 2015). Generally speaking, although most of the fatty acids were maintained, some were reduced in their relative amount.

An essential nutritional factor of the oil is the presence of oleic, linoleic, and linolenic acids since a synergistic effect of these oils have been reported to reduce total cholesterol, lipoproteins, and apolipoproteins in plasma (Chan *et al*., 1991). Moreover, the proportion of oleic acid was maintained during the frying time, indicating the oil's substantial stability to hydrolysis (Zargaraan *et al*., 2019). This is a significant indicator of the feasibility of the oil to be used in fast-food restaurants, even in processes at an industrial level, where the replacement of frying oil is limited.

Table 2. Fatty acids profile of *M. oleifera* seeds oil during frying process.

	Time (h)				
Fatty acid	0	6	12	18	24
Palmitic (C16:0)	6.60 ± 0.05^{a}	6.88 ± 0.67^{a}	7.64 ± 0.36^{a}	6.43 ± 0.01^{a}	7.23 ± 0.26^{a}
Palmitoleic (C16:1n7)	1.70 ± 0.03^{a}	1.68 ± 0.24^{a}	1.42 ± 0.08^{a}	1.60 ± 0.09^{a}	1.69 ± 0.15^{a}
Oleic (C18:1n9)	77.8 ± 0.13^{ab}	81.14 ± 5.90^{a}	72.46 ± 0.29^{b}	78.44 ± 0.19^{ab}	74.13 ± 2.64^{ab}
Linoleic (C18:2n6)	0.64 ± 0.05^{a}	0.58 ± 0.06^{abc}	0.25 ± 0.00^{d}	0.60 ± 0.01^{ab}	0.47 ± 0.03^{bc}
Linolenic (C18:3n3)	0.13 ± 0.00^{bc}	0.10 ± 0.01^{cd}	0.16 ± 0.02^{b}	0.13 ± 0.00^{bc}	0.08 ± 0.00^{d}
Araquidic (C20:0)	3.76 ± 0.04^{a}	4.13 ± 0.30^{a}	4.35 ± 0.16^{a}	3.79 ± 0.07^{a}	4.59 ± 0.62^{a}
Eicosenoic (C20:1n9)	1.90 ± 0.02^{a}	1.99 ± 0.15^{a}	2.00 ± 0.03^{a}	1.93 ± 0.00^{a}	2.21 ± 0.24^{a}
Behenic (C22:0)	5.68 ± 0.09^{a}	6.24 ± 0.37^{a}	6.57 ± 0.49^{a}	5.59 ± 0.02^{a}	7.03 ± 0.98^{a}

The values are the average of three replicates ± standard deviation. Different superscripts letters stand for significant differences among the values of the same column, as per Tukey test ($P \leq 0.05$).

Conclusions

According to what was found in the bibliography, this study is the first to evaluate the chemical characteristics of *M. oleifera* seed oil (degummed and neutralized) subjected to frying conditions for 24 h. In general, the oil undergoes minor changes in its composition due to prolonged frying conditions. Likewise, it showed a stable behavior in the oxidative stability parameters, as supported by the behavior of the CD and TBARS values for primary and secondary oxidation, respectively. Furthermore, although an increase in the production of TBARS was registered, the values obtained are considered low for oils subjected to the specified frying conditions. Finally, a tolerance of the oil to thermal hydrolysis was observed since the fatty acid profile was regularly preserved during frying.

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