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Short communication

Effect of the refining process on *Moringa oleifera* seed oil quality

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ABSTRACT

We evaluated the physicochemical properties and oxidative stability of the oil extracted from the seeds of *Moringa oleifera* during its refining process. Refining is accomplished in three stages: neutralization, degumming, and bleaching. Four samples were analyzed, corresponding to each step of the processed and crude oil. Increases in the density, viscosity, saponification value and oxidation of the oil were detected during the refining, while the peroxide value and carotenoid content diminished. Moreover, the refractive index and iodine content were stable throughout the refining. Nine fatty acids were detected in all four samples, and there were no significant differences in their composition. Oleic acid was found in the largest amount, followed by palmitic acid and behenic acid. The crude, neutralized, and degummed oils showed high primary oxidation stability, while the bleached oil had a low incidence of secondary oxidation.

1. Introduction

Vegetable oils are an important part of human sustenance worldwide. However, fats and oils, crude vegetable oils in particular, contain various kinds of minor constituents such as dirt, moisture, gums, waxes, carbohydrates, proteinaceous materials, pigments, flavoring substances, trace metals, antioxidants, and free fatty acids (Gustone, 2005, chap. 6). The term crude oil is assigned to oil that is not processed after being extracted from the animal or plant material. Crude oil is usually refined to remove the majority of these unwanted components and achieve the desired color and a mild taste. In general, refined oil is clear, odorless, and resistant to rancidity (Ranken, Kill, & Baker, 1997). The refining process generally includes the steps of degumming, neutralization, bleaching, and vacuum deodorizing (Ortega-Nieblas & Vázquez-Moreno, 1993).

However, the lipids in both refined and crude oil may deteriorate over periods of prolonged storage and exposure to elevated temperatures (Shahidi & Zhong, 2005, chap. 8). The problem is further complicated if one takes into account that oxidation reactions can be initiated, modified, or inhibited by factors such as temperature, light, pH, and the presence of enzymes, metals, and antioxidants (Akoh & Min, 2008, chap. 12). However, oils rich in oleic acid, which is a monounsaturated fatty acid, have a high oxidative stability compared to oils containing polyunsaturated fatty acids.

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The increase in demand for food implies the need to increase the overall production of alternative sources of edible oils (Manzoor, Anwar, & Iqbal, 2007). Therefore, we focused our attention on the *Moringa oleifera* tree, the fruit of which has many health benefits. Tsaknis, Lalas, Gergis, and Spiliotis (1998) gave an excellent description of the morphological generalities of the tree. This plant has many potential uses in food, cosmetics, and other industrial applications (Anwar, Latif, Ashraf, & Gilani, 2007). The oil extracted from the seeds of *M. oleifera* is composed of 82% unsaturated fatty acids, 70% of which is oleic acid. This oil contains the same fatty acid profile as olive oil except for linoleic acid (Tsaknis et al., 1998). But, currently, seeds from *M. oleifera* are not used widely for extraction, processing, and marketing of edible oil.

The main purpose of this work was to study the physicochemical properties of *M. oleifera* seed oil during the different stages of refining and its oxidative stability throughout the process, and to determine its fatty acid profile.

2. Materials and methods

2.1. Collection and sample preparation

Ripe pods of *M. oleifera* were collected from a field in South Sonora, Mexico. The seeds were extracted manually and ground in a hand mill, before being stored in plastic bags in the dark until the extraction of the oil.

2.2. Extraction of the oil from the seeds of M. oleifera

A solvent extraction was performed using sonication. Specifically, 50 g of sample was weighed into a 500 mL flask, and 250 mL hexane was added. The mixture was stirred until it was homogeneous, and the flask was then sonicated for 15 min at room temperature (Branson 1510, Danbury, CT, USA). Afterwards, the mixture was filtered under vacuum, and the residue was subjected to the same extraction procedure again. The oil-hexane mixture was concentrated in a rotary evaporator (Büchi 124, Flawil, Switzerland) at 40 °C to obtain the oil. In order to remove traces of hexane, the oil was heated for 1 h at 60 °C under vacuum (Ortega-Nieblas & Vázquez-Moreno, 1993).

2.3. Oil refining process

The refining process was conducted according to the report from Ortega-Nieblas and Vázquez-Moreno (1993), with some modifications. The crude oil (CO) was neutralized by adding the required amount of 3 mol/L NaOH at room temperature with stirring. Subsequently, the mixture was heated to 65 °C under reduced pressure for 15 min, cooled in a separating funnel at room temperature to facilitate decantation, and filtered. The neutralized oil (NO) was washed with deionized water (20 wt%), and the water was separated from the oil with the help of a separating funnel. For the degumming process, the oil was heated in a water bath at 70 °C, boiling water (20 vol%) was added, and the mixture was stirred for 10 min. After cooling the mixture, the degummed oil (DO) was recovered by centrifugation at 3000 rpm for 15 min (Harrier 18/80, Greenwich, CT, USA). Trace moisture was removed with anhydrous sodium sulfate, and the oil filtered under vacuum with Whatman No. 1 filter paper. The bleaching was performed by adding 3 wt% activated carbon and heating to 110 °C for 10 min. The charcoal and the bleached oil (BO) were separated by vacuum filtration on a 0.45 μm membrane.

2.4. Physicochemical properties of the oils

The refractive index was determined according to method Cc-7-25 of AOCS (1989). The density was estimated by weighing a known volume of oil at 20 °C. The viscosity was analyzed with a DV-E viscometer at 20 °C (Brookfield, Middleboro, MA, USA) (Núñez-Gastélum et al., 2011). For determination of the acid value, iodine value, and saponification, methods Ca 5a-40, Cd 1–25, and Cd 3–25, respectively, were employed (AOCS, 1989). Carotenoids were quantified spectrophotometrically at 450 nm using the following equation:

$$\frac{\text{mg of carotenoid}}{\text{g of sample}} = \frac{A \times V \times \text{FD} \times 10}{g * E^{1\%}_{1\text{cm}}}$$

where A is the absorbance at 450 nm, V is the volume of extract, FD is the dilution factor, g is the sample mass, and $E^{1\%}_{1\text{cm}}$ is the specific extinction coefficient of β-carotene in hexane 3450 (Strati & Oreopoulou, 2011).

2.5. Oxidation indexes

To establish the presence of primary oxidation in the samples during the refining process, the peroxide value (Cd 8–53, AOCS, 1989) and the conjugated dienes content (Paquot & Hautfenne, 1987) were determined. Meanwhile, the method for *p*-anisidine (Cd 18–90) was used to establish the presence of secondary oxidation products of lipids (AOCS, 1989).

2.6. Determination of the fatty acid profile

The fatty acid profile was determined by gas chromatography in accordance with the procedure of Núñez-Gastélum et al. (2011). Specifically, 0.5 g dry sample was weighed in a tube with a screw cap and treated with 2 mL of toluene and 3 mL 5 vol% methanolic HCl. The mixtures were vortexed and placed into a water bath for 2 h. After the samples had cooled to room temperature, 3 mL 6% K_2CO_3 solution and 2 mL toluene were added to the sample, followed by agitation in the vortex. The samples were then centrifuged for 5 min at 2400 rpm (Clay Adams Compact II Centrifuge, Parsippany, NJ, USA). After the organic phase was separated and dried with anhydrous Na_2SO_4, 1 mL of the organic phase was filtered through a 0.45 μm membrane. All the samples were analyzed in duplicate. The equipment consisted of a gas chromatograph 3800 with a flame ionization detector, a capillary column CP-Sil 88 (60 m × 0.25 mm i.d., thickness of 0.25 μm), and a CP-8410 auto-injector, all from Varian Inc. (Palo Alto, CA, USA). The injection volume was 1 μL (at 220 °C), the carrier gas was helium (1 mL/min), and the detector temperature was held constant at 235 °C. The column temperature was held at 120 °C for 1 min and was then increased to 170 °C at a rate of 3 °C/min, where it was held for 1 min, and then finally to 235 °C and maintained for 5 min. Peak identifications were based on comparing the retention times with those of known standards obtained from Sigma (St. Louis, MI, USA). The areas of the peaks were quantified using the software Galaxie Workstation (Varian Inc., Palo Alto, CA, USA). The relative amount of each fatty acid (% of a fatty acid of the total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids.

2.7. Oxidative stability of the oils

Tests on the oxidative stability of the oils were performed according to the methodology proposed by Abuzaytoun and Shahidi (2006), with minor modifications. Specifically, 0.5 g of oil