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Extraction and characterization of *Oecopetalum mexicanum* seed oil

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ABSTRACT

The aim of this work was to extract and characterize the oil obtained from the seeds of the *Oecopetalum mexicanum* tree. The results show that the seeds contained 11.20% moisture, 19.37% protein, 1.68% ash, 7.70% crude fiber and 60.02% fat. A preliminary phytochemical analysis revealed the presence of saponins, coumarins and sterols. The physical properties of the oily extract showed it to be a light yellow color at ambient temperature. The oil contained high levels of polyunsaturated fatty acids, especially linoleic (Ω-6, 48%), palmitic (25%) stearic (12.1%), oleic (Ω-9, 7.17%) and linolenic (Ω-3, 5.62%) acids. Additionally, the following volatile compounds were identified during storage for six months from the seed: 3-methyl-butanal (16.48%), benzaldehyde (14.3%), 2,3-pentanedione (8.50%), 2-furanmethane (7.12%) and 2,6-dimethylpyrazine (4.08%). The iodine index was 48.56 g I_2/100 g oil, which classifies this as a non-drying oil, therefore unfit for human consumption. We investigated the oxidative stability of the oil and found a peroxide index of 15.80 meq O_2 kg^{-1} oil. The results show that this seed could be considered as a food supplement and could also be used for the extraction and industrialization of its oil; however, future research is required regarding the technique for extracting and refining this oil in order to obtain better oxidative stability.

1. Introduction

Some nuts and seeds have protective effects on human health, since there is evidence that some may reduce cholesterol, blood pressure and diabetes (Moodley et al., 2007). Additionally, oily seeds are important sources of oils used in nutrition and/or in food preparation. The characteristics of these oils depend mainly on their composition (Mohammed and Jorf-Thomas, 2003). Oils from seeds deteriorate when they are inadequately processed, with oxidation as the principal deteriorative reaction that propitiates the formation of hydroperoxides and several secondary products of oxidation such as aldehydes, peroxides and ketones (Nzikou et al., 2010), which are compounds used to determine the quality of oils. It is important to study these constituents in order to determine their extraction and use in food products.

The seed of *Oecopetalum mexicanum* may be considered as a non-conventional source because of its high lipid content; so far, this plant has been unexploited. *O. mexicanum* is a tree that belongs to the Icacinaceae family, originally from south eastern Mexico. It blooms from June to September and is harvested from April to June; its fruit is a green and brown drape (when ripe) with an intensely bitter taste and a size of 2–3 cm long by 1–2 cm wide (Lascurain et al., 2007). It is also known as cacaté, jamacuquiaca and cachichin; this last name is of Mayan origin and consists of "kaj", meaning bitter and "ichim", which means corn (Peredo-Fernandez et al., 1993). The fruit is picked up from the ground when it is light brown in color, then it is dried in the shade, by which it can be preserved for as much as one year (Gabina, 2003). The seed is consumed raw, boiled and toasted or as an appetizer in some regional dishes like enfrijoladas or enmoladas. Nevertheless, as far as we know, there is no information concerning the physicochemical properties of the seed and the oil composition of the same. Therefore, the aim of this work was to evaluate the physicochemical properties of the fruit and the composition of the oil extracted from *O. mexicanum*. This is essential for evaluating the possibility of using the oil for different industrial purposes.

2. Material and methods

2.1. Collection and preparation of samples

The samples of *O. mexicanum* were gathered at "El Olmo" ranch, located in Cruz Blanca, Buenavista, Municipality of Misantla, situated at 19°50′36.5″ north latitude and 96°53′30.4″ west longitude, at an elevation of 734 m above mean sea level. The taxonomic identification of the tree was confirmed by biologist Luis Herman

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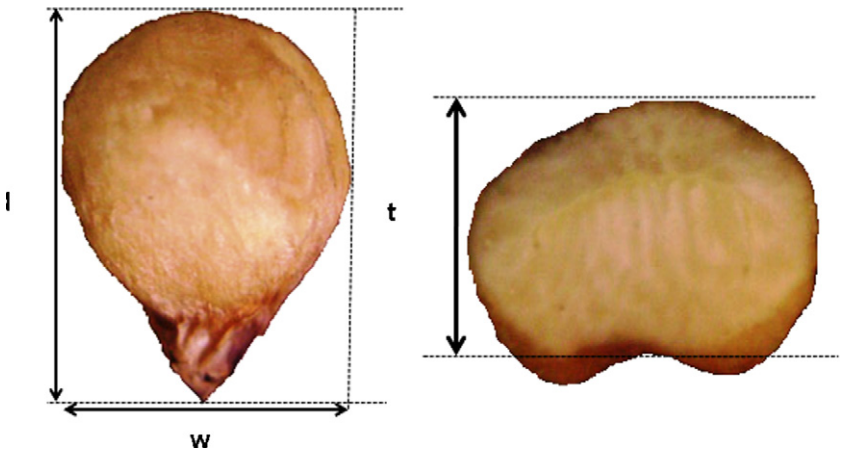

Fig. 1. Image of the crude seed and the principal measurements. (*l*) Length, (*w*) width and (*t*) thickness.

Bojorquez, assigning the registration numbers B. Hernández V.1 (CIB 9095) and B. Hernández V.2 (CIB 9096). These specimens can be found in the Herbarium of the Institute of Biological Research of Veracruz University. The collected sample (10 kg) was dried in the shade for a period of two weeks. Subsequently, the sample were ground, sifted and placed in vacuum-packed polyethylene bags, then frozen at -40 °C for later analysis and extraction of the oil.

2.2. Analysis of the physicochemical properties of the seeds

2.2.1. Analysis of the physical properties

A representative sample of 100 seeds was selected; these were evaluated in terms of weight, color and dimensions by means of a Mitotuyo digital vernier (number 293–721 Japan). The color was assessed with a Hunter-Lab CX115 colorimeter using CIE L^*, a^* and b^*, as initial parameters of color.

2.2.2. Determining the chemical properties

The determinations of moisture content, protein, crude fiber, crude fat, titratable acidity and ash were carried out using AOAC methods (1995). The determination of moisture in the seeds was accomplished by the elimination of moisture until a constant weight of a known weight in a vacuum stove was obtained.

2.2.3. Preliminary phytochemical analysis

O. mexicanum raw seeds (428.0 g) were macerated and placed in continuous extraction with solvents of different polarities: hexane, chloroform, ethanol and water. Several tests were performed on each of these extracts for the preliminary identification of alkaloids, flavonoids, quinones, coumarins, saponins, lignans and sterols, using the techniques described by Dominguez (1982).

2.3. Oil extraction

Oil extraction was performed with a Soxhlet extractor using hexane as the solvent for 5 h. The hexane was removed with a rotary vacuum evaporator in a water bath at 40 °C. An analysis of moisture was applied to the oil, according to the methodology proposed by Cenzano (1994), using a completely dry and sterilized porcelain capsule. Ten grams of oil were weighed and heated 105 °C for 30 min; then, they were cooled and weighed until a constant weight was attained. The peroxide, iodine saponification and unsaponifiable indices were evaluated in accordance with AOCS guidelines (1998). The percentage of acidity (% of free fatty acids) was determined according to a standard method (ISO, 1996). The refraction index was determined with an Abbe refractometer at 40 °C, and the viscosity was measured with an Anton Paar viscosimeter at 25 °C. The triglycerides determination was made follow the method reported by Bucolo et al. (1973), the assay was carried out in 50 μl of sample, and the enzymatic hydrolysis is considered specific for triglycerides. All analyses were done in triplicate.

2.4. Profile of fatty acids

Fatty acids were determined by converting the oil into methyl esters through the addition of BF_3, in accordance with the methods of Lopez et al. (2001). The sample was stirred at 80 °C for 10 min, then the supernatant layer was extracted with hexane HPLC, dried with anhydrous sodium sulfate and filtered for subsequent injection into the gas chromatograph. The hexane extract obtained from the esterification process was analyzed in a GCD Plus gas chromatograph, Hewlett-Packard model 1800 B, with the following parameters: the initial temperature of both the injector and the detector was 250 °C; the temperature was adjusted as follows: initial temperature of 80 °C for 5 min, then later elevated by 30 °C/min reaching to 250 °C. A Carbowax column with a length of 30 m, a diameter of 0.25 mm and a film thickness of 0.25 μm was used, with helium as the carrier gas at a flow of 1 mL/min. The mass spectra were obtained by means of ionization through electronic impact at 70 eV. For identification, the mass spectra obtained for each compound were compared with a database (HP Chemstation-NIST 05 Mass Spectral search program, version 2.0d), in addition to their comparison to a standard (FAME mix, C8:C22, Catalogue No. 18920-1AMP, Sigma–Aldrich) analyzed under the same conditions.

2.5. Analysis of volatile compounds

The seed and oil were analyzed for volatile compounds. The seeds were analyzed after collection and oil immediately after extraction. The seeds were stored during three and six months and oil sample during three months at 25 °C for posterior analysis. This analysis was carried out in an Agilent Technologies Head-space model 7694E. For this process, 3.0 g of oil were placed in a vial; this was sealed with a PTFE/Teflon cap and heated to 100 °C for 20 min. A gas chromatograph 6890 GCPLUS by Hewlett-Packard, model 1800B, was used for the analysis of volatiles. The temperature of the split mode injector was 250 °C, adjusted as follows: initial temperature of 40 °C for 5 min, subsequently increased to 280 °C with a final hold time of 3 min, thus obtaining a final run time of 32 min. A DB-5 capillary column (5% phenyl methyl polysiloxane) was employed (J & W Scientific) with a length of 60 m, a diameter of 25 mm and a film thickness of 0.25 mm. The carrier gas was helium with a flow speed of 1 mL/min.