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Use of DSC for the determination of oxidation temperature of unrefined vegetable oils obtained by cold extraction

Uso de DSC para la determinación de la temperatura de oxidación de aceites vegetales sin refinar obtenidos por extracción en frío

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Technological innovation: Unrefined oils obtained by a physical process are stable at 160 °C for 15 min.

Industrial application area: The food industry, particularly in the frying process and nutritional value of vegetable oils.

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Resumen

El alto valor nutritivo de los aceites vegetales ha contribuido al incremento en su consumo en comparación con los aceites obtenidos de origen animal. Estos aceites se pueden extraer a partir de semillas, frutas y nueces mediante el uso de solventes y métodos físicos. Los aceites vegetales sin refinar son de especial interés debido a que durante su obtención no se utilizan compuestos químicos. Por lo tanto, en este estudio tuvo como objetivo evaluar las propiedades fisicoquímicas [índice de peróxido (PV), acidez libre (AF) y contenido de fenoles totales (TPC)] y la estabilidad a la oxidación mediante calorimetría diferencial de barrido (DSC) de aceites extraídos en frío de pulpa de aguacate, almendra, cacahuate y semillas de calabaza. Los aceites de pulpa de aguacate, almendra y cacahuate se obtuvieron por centrifugación; mientras que los aceites de calabaza por prensado mecánico. Para el PV los valores oscilaron entre 3.56 y 9.06 meq O_2/kg de aceite, mientras que para la AF oscilaron entre 0.55 y 0.81% expresados en ácido oleico. Estos valores se encuentran dentro de los estándares internacionales establecidos. El

mayor TPC determinado fue en aceite de cacahuate (2177.56 ± 2.92 mg GAE/kg aceite), lo cual tiene gran importancia considerando las actividades biológicas asociadas a estos compuestos. Las temperaturas de oxidación más menor y mayor estimadas por DSC fueron 162.84 y 185.15 ºC para los aceites de pulpa de aguacate y pepita de calabaza (*Cucurbita moschata*), respectivamente. Además, los aceites de semilla de calabaza mostraron un mayor tiempo de inducción a la oxidación (> 15 min a 160 ºC). Estos resultados confirman que DSC es una técnica que facilita los análisis de la estabilidad a la oxidación de los aceites, requiriendo muestras pequeñas y análisis de tiempo corto. La alta estabilidad oxidativa de los aceites de calabaza, cacahuate y almendras sugiere que pueden tener aplicaciones prometedoras en el procesamiento de alimentos con calor (incluido el proceso de fritura). Los métodos de centrifugación y prensado mecánico son excelentes alternativas para la obtención de aceites vegetales que cumplan con los estándares internacionales. Además, el uso de este tipo de técnicas para la extracción del aceite contribuye a reducir el uso de compuestos químicos nocivos para la salud humana y el medio ambiente.

Palabras clave: aceites vegetales, estabilidad oxidativa, fenoles totales, índice de peróxido, calorimetría diferencial de barrido.

Abstract

In the last years, the consumption of vegetable oils has increased in comparison with those obtained from animal sources, due to their high nutritional value. These oils can be extracted from seeds, fruits, and nuts by solvent extraction and physical methods. Unrefined vegetable oils are of particular interest because during their obtention chemical compounds are not used. This study aimed the evaluation of the physicochemical properties [peroxide value (PV), free acidity (AF), and total phenols content (TPC)], and oxidation stability by Differential Scanning Calorimetry (DSC) of cold extracted oils from avocado pulp, almond, peanut, and pumpkin seeds. Because of this, oil from avocado pulp, almond, and peanut was obtained by centrifugation; whereas, pumpkin oils were obtained by mechanical pressing. PV and AF values ranged from 3.56 to 9.06 meq O_2/kg oil and 0.55 to 0.81% expressed as oleic acid, respectively. These values were within the established international standards. The higher TPC determined was in peanut oil (2177.56 ± 2.92 mg GAE/kg oil), due to its great important considering the biological activities associated with them. Oxidation temperatures estimated by DSC were 162.84 and 185.15 ºC for avocado pulp and pumpkin seed (*Cucurbita moschata*) oils, respectively. In addition, pumpkin seed oils showed a higher oxidation induction time (> 15 min at 160 ºC). These results confirm that DSC is a technique that facilitates the analyses of the oxidation stability of oils, requiring small samples and short-time analyses. The high oxidative stability of pumpkin, peanut, and almond oils suggested that can have promised for applications in processing foods with heat (including the frying process). Centrifugation and mechanical pressing methods are great alternatives for the obtention of vegetable oils that complies with international standards. Additionally, using mechanical or physical methods to extract oil reduces the need for chemicals that are bad for both human health and the environment.

Keywords: differential scanning calorimetry, oxidative stability, peroxide index, total phenols content, vegetable oils.

1. Introduction

Natural goods called vegetable oils are made from seeds, fruits, or nuts using physical techniques, solvent extraction, or a combination of these (1). Due to the nutritional value associated with their use, these oils have gradually overtaken those originated from animal sources as the primary intake of dietary fat, elevating the importance of oil crops around the world. Solvent extraction is the approach to oil extraction that is most frequently used. This method involves the use of organic compounds such as ether, benzene, and hexane, which can cause harm to the environment, and the residual solvents, in the final product, can turn hazardous to human health (2). Oils obtained by cold-press, ultrasound, and centrifugation without using the refining process have shown pleasant flavor, consistent quality, and a high concentration of bioactive components. For example, the content of oleic acid has been related to the hypotensive effects of the consumption of olive oils (3). These properties are widely appreciated by consumers (4, 5). Similar to different sources and extraction techniques should be assessed to obtain unrefined oils with great physicochemical properties, attractive appearance, a neutral taste, and resistance to oxidation (6).

Environmental factors including light, temperature, oxygen, and others can cause oxidation in edible oils, which is known as autooxidation. This reaction occurs between a free radical chain and oxygen that leads to the oxidative deterioration of oils through the formation of hydroperoxides (HPs). HPs are used to assess the lipid oxidation state of oil samples during oil processing and preservation using the peroxide value (PV) technique. HPs are stable at room temperature. A rotten flavor is produced by high PV, which may even be food poisoning. Making the evaluation of PV in edible oils of utmost relevance (7). Vegetable oils' oxidation stability is influenced by their composition, especially by the amount of antioxidant chemicals like phenols. Phenolic compounds are secondary metabolites produced by the phenylpropanoid and shikimic acid pathways that have several biological functions that protect against disease when consumed through food (8).

Oils' greatest quality is their oxidative stability. Vegetable oils lose their nutritional value and technological capabilities as a result of oxidation of the lipids, which also produces poisonous chemicals and poor taste and flavor. The resistance of oils to reacting in the presence of oxygen is referred to as oxidative stability. Triacylglycerol, fatty acid, and antioxidant concentration in the oil, as well as environmental elements like light and temperature, all have a significant impact on this feature (1). Vegetable oils' oxidative stability is measured by the time prior to an increase in the rate of lipid oxidation, which is known as the "oxidation induction time. Several techniques have been developed to assess oxidation stability under accelerated conditions by exposing the sample to an excess of oxygen and high temperature (9). The traditional technique, also known as the Rancimat method, due to the commercial name of the equipment, is a conductimetric method that consumes a long time and requires a high quantity of samples (1, 9). Several studies have successfully used Differential Scanning Calorimetry (DSC) to assess the oxidative stability of vegetable and animal oils (1, 5, 9). By measuring the temperatures and heat fluxes connected to material transitions as a function of time and temperature, DSC offers an energy profile that makes it easier to detect oil oxidation (9). Hence this study aimed to extract oil from peanuts, almonds, avocado pulp, and pumpkin seeds by cold extraction techniques, evaluate their physicochemical properties (PV, AF, and TPC), and oxidation stability by DSC.

2. Experimental methods

2.1. Raw materials

Leb Trading Products (Guacamodely, S. de R.L. de C.V. Xalisco, Nayarit, Mexico) supplied the little, healthy avocados (*Persea americana* Mill. cv. Hass), which were deemed by-products because of their size and aesthetic flaws. Almonds (*Prunus dulcus* L.) and peanuts (*Arachis hypogaea* L.) were purchased in Tepic, Nayarit, Mexico. Mechanical pressing was used to extract Cushaw pumpkin (*C. argyrosperma* and *C. moschata* Duch.) oils from pumpkin seeds that were purchased at Mercado San Benito in Mérida, Yucatán, Mexico (10). Pumpkin oils were provided by Laboratorio de Instrumentación Analítica, of Instituto Tecnológico de Mérida.

2.2. Oil extraction

2.2.1. Oil extraction of avocado pulp by centrifugation

Avocados were kept at 25 °C until full maturity, accordingly to dry matter content (DMC) of 32.0 ± 1.0% (11, 12). Avocado fruits were washed and disinfected with sodium hypochlorite solution (20 mL/L) for 2 min, then washed with distilled water (12). Avocado pulp was manually gathered and homogenized in a home mixer (NutriBullet® 900 SERIES, Los Angeles, CA, USA) for 1 min. 25 g of fresh avocado pulp were centrifuged (Hermle Z326K, Wehingen, Germany) in 50 mL tubes at 9390 *g* for 10 min at 30 °C to extract the oil. Avocado oil extraction was carried out in triplicate. Oils of each source were pooled and stored in dark conditions at 4 °C until analysis (5).

2.2.2. Oil extraction from almonds and peanuts

Peanuts and almonds were blanched at 80 ± 5 °C for 2 h and grounded in a household mixer. Peanut paste and almonds powder were mixed with distilled water 1:5 ratio (w/v), and pH was adjusted to 9.0 ± 0.2 by using 2 M sodium hydroxide. The sample was collocated into a shaking water bath (Model 290400S, Boekel Scientific, Feasterville, PA, USA) at 60 °C and 70 rpm for 1 h. Then, the oil was obtained by centrifugation at 9390 *g* for 15 min (13). Oil extractions were carried out in triplicate. Oils of each source were pooled and stored in dark conditions at 4 °C until analysis.

2.3. Physicochemical characteristics

Peroxide value (PV, milliequivalent -meq- of O_2/kg oil) was determined according to AOAC Official Method 965.33 (14). Acidity or free fatty acid (AF), expressed as a percentage of oleic acid, was determined according to ISO 660:2020 (15).

2.4. Total phenols content (TPC)

The Folin-Ciocalteu method was used to estimate the TPC of vegetable oils. Briefly, 50 µL vegetable oil were solubilized in ethanol (2 mL, 95% purity, Hycel, Zapopan, Jalisco, Mexico), then deionized water (1.58 mL) was added, followed by 100 µL Folin-Ciocalteu reagent (2 N, Sigma-Aldrich St. Louis, MO, USA). The solution was vortexed and incubated under dark conditions for 5 min, 300 µL sodium carbonate solution (20%, w/v) was added, mixed, and then incubated under dark conditions for 2 h at room temperature. Sample absorbance was measured at 765 nm (Synergy™ HTX Multi-Mode Microplate Reader, BioTek Instruments, Inc., Winooski, USA). TPC was calculated using a standard gallic acid curve and represented as milligrams of gallic acid equivalents per kilogram of oil (mg GAE/kg) (16). Each sample was examined three times.

2.5. Oxidation tests by DSC

2.5.1. Non-isothermal DSC method

Non-isothermal analyses were carried out by DSC (DSC 250 analyzer, TA Instruments, New Castle, USA). 3 mg of each sample, were put inside an aluminum capsule (volume 30 µL). With an airflow rate of 50 mL/min and a heating rate of 10 °C/min, samples were heated to temperatures between 25 and 300 °C. The

software TRIOS 5.0.0.44616 (TA Instruments Universal Analysis, New Castle, USA) was used to calculate the oxidation induction temperature (T_{OX}). DSC instrument was calibrated for temperature and heat flux with high-purity indium standard (T = 156.6 ± 0.6 °C and ΔH = 26.85 J/g). An empty aluminum capsule was used as a reference in each test. Each sample was examined three times.

2.5.1. Isothermal DSC method

Thermal analyses were carried out by DSC (DSC 250 analyzer, TA Instruments, New Castle, USA). A total of 3 mg of each oil sample were placed in an aluminum capsule and heated (almond, peanut, and pumpkin oils, 160 °C; avocado oil,150 ºC) under airflow (50 mL/min) into the DSC cell. The oxidation oil process can be detected on the DSC curve as exothermic heat flows, appearing during starting of the oxidation reaction. Oxidation induction time (O_{IT}, min), was determined from the DSC curve, as the intersection of extrapolated baseline with the tangent line (leading edge) of the exothermic peak. Values were determined with TRIOS 5.0.0.44616 software. Each sample was examined three times.

2.6. Statistical analyses

One-way analysis of variance was used to process the data. Statgraphics Centurion XVI.I software from Statpoint Technologies, Inc., Warrenton, USA, was used to conduct the statistical analysis. For means comparison, the post-hoc least significant difference (LSD) Fisher test (p 0.05) was employed.

3. Discussion of Results

3.1. Physicochemical properties

The primary factors that determine the physicochemical quality of vegetable oils are peroxide value (PV) and free acidity (AF)(17). PV and AF values indicate the oil quality of the five oil samples studied in this work (Table 1). An indicator of the oxidation of unsaturated fatty acids in oil samples is the PV value, which corresponds to the number of hydroperoxides present (18, 19). Oils with high PV values eventually develop secondary oxidation byproducts such as ketones, aldehydes, short hydrocarbon chains, and alcohols, which worsen the rancidity of the final product (19). The highest PV value determined was in the peanut oil sample (Table 1). This outcome was superior to that of the peanut oil that had undergone enzymatic cellulase treatment and ultrasound pre-treatment in *n*-hexane solvent (2.030-4.630 meq O_2/kg oil) (20). The increment in PV values obtained in this work might be due to the temperature (80 ºC) used for heating kernels before oil extraction. However, these values are within the range that the Codex Alimentarius allows for cold-pressed fats and oils and virgin oils (maximum level 15 meq O_2/kg oil) (18). Almond oil PV value (3.50 meq O_2/kg oil) was similar to the reported values of different almond varieties oils obtained by grounded at 100 ºC (1.88-3.18 meq O_2/kg oil) (17). However, the PV values can vary between species (17, 21), In this study, the PV value of *Cucurbita moschata* was higher than the PV value of *Cucurbita argyrosperma* ($p \leq$ 0.05). Species-specific pumpkin oil PV values from Brazil included 14.06 for *Cucurbita pepo*, 2.51 for *Cucurbita maxima*, and 1.00 meq O_2/kg oil for *C. moschata* (21). This may be due to the content variation of carotenoid, chlorophyll, and tocopherol between the species. The high content of carotenoids, particularly lutein, allows pro-oxidant activity in both, dark and light conditions. Lipid oxidation is encouraged by the instability against heat and light (21). PV content determined for avocado oil was similar to the reported for avocado oil obtained by Pérez-Saucedo et al. (5) by using an equal extraction technique (4.12 meq O_2/kg oil). Extraction technique strongly affects PV values, even in oils obtained from the same source, such as PV values of fresh avocado oils obtained by different extraction methods: 11.05

(Soxhlet method), 2.96 (ultrasound and cool extraction method) (5), and 18.5 meq O_2/kg oil (using malaxation at different conditions as pre-treatment before hydraulic press) (22). The extraction method is highly related to the temperature utilized in each extraction technique. The ideal temperature for the lipoxygenase enzyme is 30 ºC (23), this enzyme controls the oxygenation of linoleic and linolenic acids in their C-9 or C-13 forms and the production of hydroperoxides that raise PV values (24). PV values also vary depending on the species, harvesting, and maturity stage, which affect the chemical composition of the species, and the conditions of oil storage (17, 21).

Good oil quality and lack of acylglycerol enzymatic hydrolysis are indicated by low acidity (17). AF remained unvaried (0.50% to 0.59% ($p > 0.05$, Table 1)) in four of the five analyzed oils. The estimated almond oil AF value (0.89%) was greater than the published almond oil AF values of various kinds from Morocco (0.32-0.36%)(17). This AF value, though, was less than the suggested level for unprocessed oils (5%) (18). On the other hand, the avocado oil AF value determined was lower than reported for avocado oil obtained by the same extraction method (1.19%) (5), probably due to the maturity stage of avocado fruits. Higher AF and PV values have been observed in oils extracted from overripe olives (3). AF value can be also affected by the species, cultivars, maturity stage, extraction method, and storing conditions. Because of the increased activity of the lipase enzyme at higher temperatures, the amount of oleic acid in vegetable oil is particularly affected(17, 21, 25). Fatty acids produced from the triacyl glycerides hydrolysis by lipase enzyme increase the AF value (5).

3.2. Total phenols content (TCP)

One of the main categories of dietary additives present in vegetable meals is phenolic compounds These secondary metabolites have a wide variety of chemical structures that includes more than 8,000 compounds. In the last years, the interest in phenols has increased due to their biological activities that comprise antimicrobial, anti-inflammatory, antioxidant, antimutagenic, and anticancer properties (3). Having Gallic acid as a reference, a curve for TPC was defined. The fitted linear regression equation obtained was $y = 723.06x - 184.95$ and the correlation coefficient R^2 = 0.9767. TPC value determined in this work for peanut oil (2.177 mg GAE/g) was lower in comparison to reported for commercial peanut oil (38.02 ± 0.62 GAE mg/g) from China (26). TPC value in vegetable oils, as well as other indexes, depends strongly on the extraction method and the utilized conditions, for instance, phenolic compounds can be increased if ultrasonic time is extended during the extraction process (20). By using organic solvents non-polar and/or amphipathic phenolic compounds are concentrated in the sample, producing higher TPC values (131.96 ± 2.12 mg GAE/g) than other methods. For instance, TPC values of avocado oil obtained by centrifugation (61.10 ± 1.13 mg GAE/g) differ from TPC values of avocado oil obtained by ultrasound-centrifugation (63.12 ± 1.02 mg GAE/g) (5). In contrast, pumpkin oil with TPC levels of 20–40% benefited from mechanical pressing (10). In peanut oil, the increment of the roasting temperature of kernels favors phenols releasing influencing TPC values (2). Besides, the species, cultivar, field environmental conditions, and agricultural practices also play a vital role in TPC value. The TPC value in almond oil varies depending on the cultivar chosen for oil extraction from 18.53 to 28.07 mg GAE/kg (17). The previously listed variables have an impact on both the TCP values and the quantity of each phenolic component in the sample(10, 17).

3.3. Oxidation stability test by DSC

Estimated T_{OX} and O_{IT} values for analyzed oils were obtained by extrapolating the

vectors on the intersection of the tangent straight with the beginning of the exothermal process in the chart of the DSC thermogram (Figure 1). The exothermic oxidation process happens when unsaturated fatty acids react with oxygen (1). The higher T_{OX} value was determined in *C. moschata* Duch oil ($p \leq 0.05$, Table 1). This outcome was better than what Jarret et al. (27) obtained in their pressurized DSC analysis of *C. moschata* oil (150.45 °C). Likewise, the T_{OX} value determined for avocado oil was higher than reported for avocado oil obtained by the Soxhlet method (141.44 °C) and lower than that obtained by using ultrasound (177.33 ºC) (5). This suggests that with alternative extraction methods, such as centrifugation, the T_{OX} values of vegetable oils can be improved. T_{OX} values have been associated with the physicochemical composition of oils and high T_{OX} values are related to the high content of antioxidant compounds such as phenolic acids, flavonoids, and tocopherol (28). A significant amount of α-tocopherol was found in the oils produced by mechanical pressure from *C. moschata* (28.39 ± 1.40 mg/kg of oil) and *C. argyrosperma* (37.45 ± 3.16 mg/kg) (37.45 3.16 mg/kg) (10). The high stability of peanut oil could be associated with α-tocopherol content in peanuts roasted at 80 ºC. When the roasted temperature is increased concentration of α-tocopherol decreases, reducing consequently the T_{OX} value (2). Pumpkin oil flavonoid content, specifically quercetin, and chrysin, supports oxidative stability (10) because the level of fatty acid unsaturation substantially influences the oxidative stability of edible oils. Oleic acid, a crucial component in vegetable oils, is resistant to thermal oxidation both at room temperature and during high-temperature frying and cooking (29). The content of antioxidants in oils improves oxidative stability and prevents oxidative degradation. Antioxidants can slow the propagation phase when reacting with alkoxy and alkyl peroxy radicals or postpone the oxidation reaction when reacting with free radicals (30). Therefore, preserving the oil's natural composition and oxidation stability involves extracting vegetable oil at low temperatures and without the use of organic solvents.

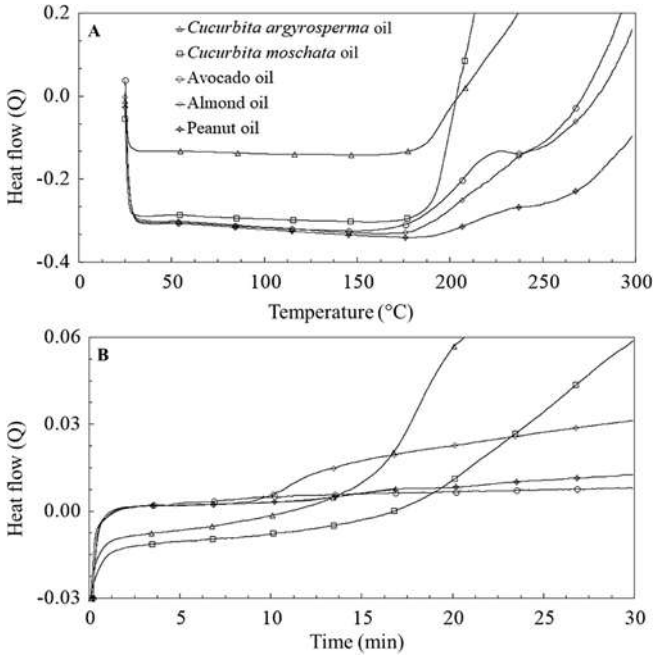


Figure 1. Differential Scanning Calorimetry (DSC) curves for oxidation induction temperature of vegetable oils. Determination of oxidation temperature (A) and oxidation induction time (B) of the analyzed oils. Oxidation induction time was determined for almond, peanut, and pumpkin oils at 160 °C and avocado oil at 150 ºC.

Oxidative stability means oil or fat resistance to oxidation, measured in the time that takes secondary products to arise. When tests are conducted with oxygen present (this process is known as O_{IT}), the rate of lipid oxidation quickly increases (28). Higher OIT values were found in pumpkin oils (Table 1), most likely as a result of the tocopherol concentration (10). Peanut oil O_{IT} value can be influenced by roasted temperature since high temperature affects the content of antioxidant compounds (2). Most studies assess O_{IT} in edible oils at low temperatures. Almoselhy (1) reported the OIT values, analyzed at 140 ºC, for extra virgin olive oil (44.3 min), corn oil (28.5 min), and sunflower oil (19.5 min). However, cooking and frying temperatures are usually quite high (170-180 ºC). Since the extraction method can affect the physicochemical composition of vegetable oils, may also affect the O_{IT} value. The O_{IT} value in avocado oil extracted by ultrasound was higher (11.34 ± 0.19 min) than the O_{IT} value (6.98 ± 0.41 min) of avocado oil extracted by centrifugation at 140 ºC (5). This O_{IT} value was similar to the obtained in this work in avocado oil evaluated at 150 ºC (4.48 min). These results suggest that unrefined oils studied in this work have the potential for being used in food processing at temperatures lower than 150 ºC.

4. Conclusions

Using centrifugation and mechanical pressing as alternatives process for oil extraction in avocado, almond, peanut, and pumpkin seeds was able to keep oils physicochemical properties into the official international standard values. In addition, phenolic content contributed to oxidation stability in obtained oils, agreeing with the phenolic values reported in oils obtained by different extraction methods. The DSC technique was useful to determine T_{OX} values and O_{IT} values with small samples in a short time. High oxidative stability suggests that pumpkin oil peanut and almond oils are promises for applications in processing foods with heat (including the frying process). However, other studies should be carried out on regards the real temperatures for the cooking and frying food process. The frying method uses higher temperatures than assessed in this work. In addition, nanoemulsions development based on unrefined oils can make more stable products, with wide application in the food and pharmaceutical industries.

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