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Analysis of the Infrared Spectrum of Oil Samples of 5 Pecan nut Varieties (Carya Illinoensis K) and their Comparison with Other Vegetable oils

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ABSTRACT
The objective of the present work was to evaluate the stability of pecan nut oil (*Carya Illinoensis K*) from 5 different varieties, by chemical analysis of peroxides and saponification index, as well as an analysis of infrared spectroscopy in order to compare the spectra of the oils, and determine the degree of similarity they have with other vegetable oils rich in oleic and linolenic acid. The oil samples had a good stability over 3 months and no significant differences were found in the composition of the same, and they were very similar to other oils of vegetable origin.

Keywords: Infrared, spectroscopy, nut, oil, pecan -

I. INTRODUCTION

Pecan (*Carya Illinoensis K*) is a tree native to the southeastern of United States and Mexico, which stands out mainly for you fruit the walnut [1], [2]. This walnut, is a highly nutritive food, since per 100 g of edible portion, contains 70% lipids, 14% carbohydrates, 9% protein, 3% water, 3% fiber, vitamins A, B and C, calcium, iron, potassium, phosphorus and magnesium, with an energy value of about 700 kcal [3], [4]. Pecan nut consumption has been associated with improved cardiovascular performance due to its high content of mono and polyunsaturated fatty acids, which are highlighted by lowering the levels of cholesterol (LDL) and triglycerides in the blood [5], [6].Tree nut oils are primarily composed of triacylglycerols, but also contain diacylglycerols, monoacylglycerols, free fatty acids and other minor components, including natural antioxidants and fat-soluble vitamins [7], [8]. Generally, tree nut oils are rich in monounsaturated fatty acids, predominantly oleic acid, but contain much lower amounts of polyunsaturated fatty acids, predominantly linoleic acid, and small amounts of saturated lipids [4], [7], [8].

Oleic acid (omega-9) lowers the low density lipoprotein (LDL), responsibles of the cardio cardiovascular diseases and increases the high density lipoprotein (HDL) [5], [6]; has one vasodilatory action and, therefore, low the blood pressure; they also present an anti-inflammatory and protective action on various cancers, especially breast cancer. Oleic acid promotes the migration of neurons and this in turn facilitates the formation of synapses, the contact between these cells that allows the transmission of nerve impulses [9]. The omega-6 long-chain polyunsaturated fatty acids as arachidonic acid (AA), and omega-3 (ω-3) as docosahexaenoic acid (DHA), are fundamental in the formation of structure and in the functionality of the nervous and visual system of humans. Both fatty acids constitute more than 30% of the lipid structure of the brain and the cones and rods of the retina [10]. These acids are produced from the intake of linoleic and linolenic acids present in vegetable oils such as olive and walnut oil. In addition, they have demonstrated physiological benefits in blood pressure, heart beat, decreased levels of triglyceride endothelial function and cardiac diastolic function among others [10]. A technique that can be used to know qualitatively the chemical structure of vegetable oils is infrared (IR) spectroscopy, which is mainly used in the elucidation of molecular structures, although it is also used for quantitative purposes [11], [12], [13]. This technique is based on the different absorptions of infrared radiation that manifest the different functional groups present in a molecule. The infrared region of the spectrum includes radiation with a wave length between 12800 and 10 cm-1 which corresponds to wavelengths of 0.78 to 1000 μm, divided into three regions called near, mid and far infrared [11], [14].The region between 4000 to 400 cm -1 (from 2.5 to 25 μm) is used in organic chemistry for the structural study of molecules [11]. Small changes in the molecular structure of a compound often cause significant changes in absorption peaks [13].

In general, the average infrared spectrum of an organic compound provides a unique spectrum, with characteristics that easily distinguish it from the rest of the compounds, only the optical isomers absorb exactly the same form [11]. In addition, the measurements in the infrared are also finding an increasing use in quantitative analysis, adulteration assays [11], [13] and to have oils of better quality, [15] Its high precision makes possible the quantification of a substance in a complex mixture, not being necessary a previous separation [11].IR spectroscopy is, in contrast to most other analytical (e.g. gas and high performance liquid chromatography) and conventional chemical (e.g. Kjeldahl, Soxhlet) methods, rapid, chemical-free, easy to use (once calibrations have been developed) and non-destructive [14]. Although the accuracy of the IR method depends to a great extent on the accuracy and precision of the reference method, IR measurements and predictions are considered more reproducible [15]. In the present study, oil was obtained from 5 varieties of pecan (Carya Illinoensis K), and it was evaluated your stability over 3 months by chemical analysis of peroxides and saponification index, as well as an analysis of infrared spectroscopy in order to compare the spectra of the oils and determine the degree of similarity they have with other vegetable oils rich in oleic and linolenic acid.

II. MATERIAL AND METHODS

2.1 Obtaining samples

Samples of oil of 5 varieties of pecan (*Carya Illinoensis K var Pawnee, Carya Illinoensis K, Carya Illinoensis K var Wichita, Carya Illinoensis K var Cheyenne, Carya Illinoensis K var Sioux, Carya Illinoensis K var Western*) were obtained from farms of Zaragoza Coahuila, Mexico, using the Soxhlet extraction technique using ethyl ether as solvent [16]. They were stored at 5 ±1 ° C until you use.

2.2 Determination of the Saponification Value (S.V.)

An ethanolic-aqueous solution of sodium hydroxide (NaOH) was prepared by dissolving one (1) g of NaOH in 5 mL of distilled water, and adding 20 mL of ethanol. One (1) g of oil was weighed into a flask and a 10 mL aliquot of the ethanolic-aqueous solution was added with a volumetric pipet and stirred. The mixture was then heated for 30 min, the flask was capped with a watch glass to prevent leakage. It was removed from the heat source and added 5 drops of phenolphthalein indicator. It was titrated when it was still hot with the 0.2 N hydrochloric acid solution. In another 10 mL aliquot of ethanolic-aqueous solution in a 125 mL erlenmeyer flask (blank), one or two drops of phenolphthalein was added, stirred and titrated using the standard solution of HCl 0.2 N. The test was repeated every month for three months to determine changes in the saponification of the samples.
The difference between the volumes of HCl solution used in the titrations represented the amount of alkali consumed in the saponification.

Calculation was made to obtain the saponification value (S.V.) with the formula.

$$S.V = \frac{N\ x\ MW\ x\ (V_2 - V_1)}{m}$$

Where:
N = normality of the HCl solution
MW= molecular weight of the NaOH
V_1 = volume (L) of HCl used in the titration of the NaOH remaining of saponification
V_2 = volume (L) of HCl used in the titration of the blank
m = mass of oil (g)

2.3 Detection of Oxidative Rancidity in lipids for Peroxide Value

For this assay 1 g of oil was weighed into an Erlenmeyer flask and 30 mL of the glacial acetic acid / benzene solution (3: 2) was added and were shaken. And 0.5 mL of the saturated Potassium Iodide (KI) solution (1.5 g KI in 10 mL distilled water) was added. The mixture turned yellow on standing for one minute. 30 ml of distilled water and 0.5 mL o of starch indicator was added and a blue color appeared. It was titrated with 0.01 N sodium thiosulfate ($Na_2S_2O_2$) dropping dropwise, while stirring vigorously, until the disappearance of the blue color and remaining "milky". The test was repeated every month for three months to determine the stability of the oil.

Calculations
The peroxide index was calculated by expressing the milliequivalents (meq.) of peroxide contained in one kilogram of oil by the following formula:

$$P.V = \frac{(V - V_1)(N)(1000)}{m}$$

V = mL of sodium thiosulfate solution spent on sample titration
V_1 = mL of sodium thiosulfate solution spent on titration of the target
N = Normality of the sodium thiosulphate solution
m = mass of the sample in g

2.4 Analysis by Infrared Spectroscopy

The oil samples were analyzed in an Infrared Spotlight 400 FT-IR equipment Perkin-Elmer trademark. 0.1 g of sample was used and a

sweep was performed in the infrared region of the spectrum between 650 and 4000 cm-1, the spectrophotometer cell was maintained at 25 ± 1 ° C. The results were compared with those of different vegetable oils standards.

2.5 Design of experiments

For the statistical analysis of the data, a completely randomized design with the same number of repetitions was used, with a sensitivity of p = 0.5. The Tukey HSD multiple comparison test was also used with a p = 0.5. The results were analyzed using the statistical package Statgraphics Centurion Version XV.

III. RESULTS AND DISCUSSION

3.1 Determination of the Saponification Value

The evolution of the saponification value was determined during 3 months with the aim of observing degradation of the pecan nut oil over time and observed stability of the same. The saponification value of the samples of pecan oil proved to be very high a, 0.616 g of NaOH per g of oil, compared to other oils of vegetable origin such as olive oil which has a saponification index of 0.180 g of NaOH per g of oil [17]. However, this index had a great deterioration (p = 0.05) over 3 months, the *Western* variety had the highest reduction of the saponification value with 85% in 3 months, followed by the *Pawnee* variety with 82%, and The *Wichita* 75% while the *Sioux* variety was the most stable with only 19% change (Figure 1).

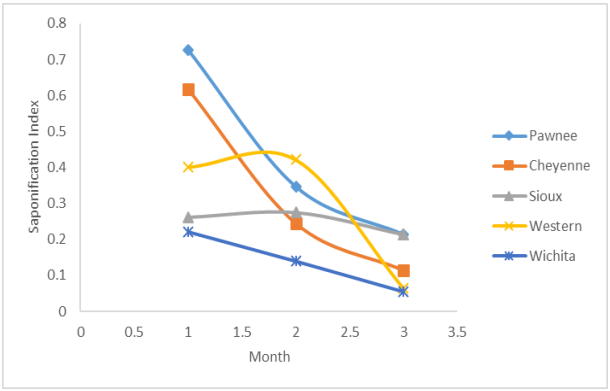

Figure 1. Variation of the saponification value of five samples of oil of different varieties of pecan nut.

3.2 Determination of Peroxide value

Some international standards such as the Official Mexican Standard [18] indicate that pecan nut oil must have a value equal to zero milliequivalents of peroxide/kg of oil. Otherwise it will be considered with a degree of rancidity not acceptable. In the present study, however samples had more than two milliequivalents of peroxide/kg of oil after being stored in refrigeration for one month (figure 2). The values fluctuated in all samples between 2 and 4.5 milliequivalents during the storage time, which theoretically indicates that the oil has an unacceptable degree of rancidity. However, the observed changes were not significant between the duration of the experiment. In addition, the storage results coincide with those reported by Oro et al, 2008 [19] who stored the peroxide value of pecan nut oils in nylon-polyethylene films in vacuum and in plastic polypropylene containers for 150 days. The peroxide (PV) values ranged from 1.04 to 2.66 meq O_2 / kg and 1.14 and 4.67 meq O2 / kg at the beginning and after 150 days of storage, for walnuts on film Nylon-polyethylene under vacuum and in plastic polypropylene containers, respectively [19].

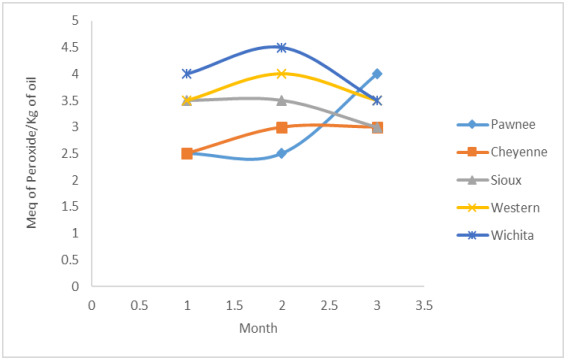

Figure 2. Variation of the peroxide value of five samples of oil of different varieties of pecan nut

3.3 Infrared Analysis of Pecan Walnut Oil Samples

Table 1, shows the regions in which peaks in the IR spectrum of the pecan oil of the Cheyenne variety were found, which as shown in Figure 3, are the same for the 5 oil samples of the different varieties used in the present study. The first peak observed in the infrared spectrum is in the 722 cm^{-1} within the region known as the "fingerprint" which lies between 700 and 1200 cm^{-1}, this being the most characteristic zone that differentiates the individual compounds with each other. This peak indicates flexion vibrations of (CH_2), which are part of the linear chain of all fatty acids [11], [20]

Table 1. List of Infrared Spectrum peaks found in Cheyenne nut variety

Peak Number	X (cm^{-1})	Y (%T)
1	3007.73	94.61
2	2923.34	63.2
3	2853.98	72.93
4	1744.27	58.02
5	1463.89	84.97
6	1377.71	90.7
7	1236.9	85.45
8	1160.23	70.51
9	1119.54	81.86
10	1096.61	82.13
11	771.47	91.58
12	722.2	81.65

The first peak that is observed in the infrared spectrum is in the 722 cm^{-1} within the region known as the "fingerprint" which lies between 700 and 1200 cm^{-1}, this being the most characteristic zone that differentiates the individual compounds with each other. This peak indicates flexural vibrations of (CH_2), which are part of the linear chain of fatty acids [11], [20].

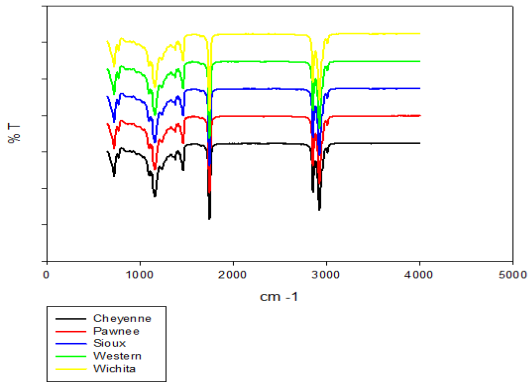

Figure 3. Comparison of the infrared spectra of oils of five pecan nut (*Carya Illinoensis K*) varieties

It is also observed a peak at 1119 cm^{-1}, in which O-CH_2 tension vibrations are visualized, this is very similar to a peak in the 1114 cm^{-1} observed in palm oil [11]. In addition, a band in the 1160 cm^{-1}, characteristic of the vibrations of tension C-O, is also shown in 1165 cm^{-1} in palm oil [11]. In the region between 1463 and 1237 cm-1 a broad band with several peaks is observed, this range is associated with the presence of C-H flexion vibrations in CH_2 and CH_3, which are characteristic of the linear chain of fatty acids [11] In 1744 cm^{-1} a peak associated with the extension movement of the C = O bond typical of the triglyceride esters is observed, this peak has been found in olive oil and palm [11], [16]. Two peaks at 2923 and 2853 cm^{-1} are also associated, respectively, with symmetric C-H tension vibration and asymmetric C-H tension at CH_2 [11]. Finally, a signal corresponding to the stress C = CH in 3002 cm^{-1} present in the unsaturated fatty acids of the sample under study is observed, the which appears in different vegetable oils [11], [16].A comparison with other oils of plant origin and a liquid standard of monounsaturated and polyunsaturated fatty acids were also done to see the degree of agreement of the results. The fatty acid content of the pecan nut (*Carya Illinoensis K*) was very similar to walnut oil (*Junglans regia*) (p = 0.93) and to cottonseed oil (*Gossypium hirsutum*) (p = 0.93). Although it was more Similar to the sample of pure liquid fatty acid standards (p = 0.94) (Table 2).

Table 2. Reference samples with which the comparison of the samples of pecan nut oil

Search Score	Search Reference Spectrum
0.940944	Fatty Acid Ester, Neet Liquid, -Ref:FLTIII-
0.935457	Walnut Oil
0.93174	Cottonseed Oil
0.930625	Linseed Oil
0.928274	Cottonseed Oil
0.926624	Peanut Oil; Ground Nut Oil
0.926386	Mustard Seed Oil
0.925276	Soybean Oil
0.916243	Peanut Oil; Ground Nut Oil

IV. CONCLUSIONS

Walnut oil samples showed good stability for three months of storage at 5 ° C. Infrared analyzes revealed that the variety does not influence the type of fatty acids present in the oil. The nut oil pecan (*Carya Illinoensis K*) is very similar to other oils of vegetal origin in its composition.

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