Hindawi
Journal of Chemistry
Volume 2017, Article ID 2609549, 11 pages
https://doi.org/10.1155/2017/2609549

Research Article

Chemical Characterization of Major and Minor Compounds of Nut Oils: Almond, Hazelnut, and Pecan Nut

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Received 5 February 2017; Accepted 6 April 2017; Published 2 May 2017

Academic Editor: Maria B. P. P. Oliveira

The aim of this work was to characterize the major and minor compounds of laboratory-extracted and commercial oils from sweet almond, hazelnut, and pecan nut. Oils from sweet almond, hazelnut, and pecan nut were obtained by means of an expeller system, while the corresponding commercial oils were provided from Vital Âtman (BR). The contents of triacylglycerols, fatty acids, aliphatic and terpenic alcohols, desmethyl-, methyl-, and dimethylsterols, squalene, and tocopherols were determined. Oleic, palmitic, and linoleic acids were the main fatty acids. Desmethylsterols were the principal minor compounds with β-sitosterol being the most abundant component. Low amounts of aliphatic and terpenic alcohols were also found. The major tocopherol in hazelnut and sweet almond oils was α-tocopherol, whereas γ-tocopherol prevailed in pecan nut oil. Principal component analysis made it possible for us to differentiate among samples, as well as to distinguish between commercial and laboratory-extracted oils. Heatmap highlighted the main variables featuring each sample. Globally, these results have brought a new approach on nut oil characterization.

1. Introduction

Nuts belong to various plant families, although they have special common features such as high oil content and large seed size when compared to other oilseed species. Almonds (*Prunus dulcis*, family Rosaceae), hazelnuts (*Corylus avellana*, family Betulaceae), and pecan nuts (*Carya illinoinensis*, family Juglandaceae) are part of the main group of tree nuts and nut oil sources. In many parts of the world such as the Mediterranean countries and North America, tree nuts are not only an important oil crop but also an essential dietary component, acting as energy and functional compound sources. Actually, nut oils have been widely enjoyed for food applications, mainly due to their particular flavor and, more recently, because of their relationship with health-promoting effects. Besides, tree nut oils are also widely used in the cosmetic industry [1, 2].

The almond group is composed of two species, namely, *Prunus dulcis* (sweet almonds) and *Prunus amara* (bitter almond). Almond oil is extracted mainly from sweet almonds, which contain around 50% oil. This extraction is commercially conducted by the cold press and/or solvent extraction [3]. According to FAO [4], USA is the main almond producer in the world (~62% of the total production) followed by Spain and Australia (~5% each). Chemically speaking, sweet almond oil has been described as an unsaturated oil, with oleic acid (O, C18:1) being the main fatty acid (~65%) [5], with β-sitosterol as the most representative sterol and α-tocopherol as the major tocopherol [6, 7]. Table 1 shows the detailed composition of almond oil based on bibliographic research [1–3, 5–7].

Hazelnut *(Corylus avellana)* is a nut included in the Mediterranean diet, whose main worldwide producer is Turkey, with just about 63% of the total production

in 2012 [4]. Hazelnut kernels contain around 60% oil, which is obtained by cold press and/or solvent extraction. Hazelnut oil has been frequently compared to olive oil due to their similar compositions: oleic acid as the main fatty acid and β-sitosterol as the main minor compound (Table 1) [8, 9]. Actually, hazelnut oil is commonly used in the cosmetic industry, although its current prominence as health-promoting oil has increased its uses as food and even gourmet oil.

Pecan nut *(Carya illinoinensis)* originates from the USA but is well adapted in several countries including Australia, South Africa, and different parts of South America. Nowadays, more than 80% of the total world production is produced in the USA. This nut contains 70% oil, which is easily extracted by means of an expeller press. Also here, oleic acid is the predominant fatty acid (~60%), and, curiously, γ-tocopherol has been reported as the main tocopherol, as can be seen in Table 1 [10–12].

All these nuts are perennial crops; thus, there is a particular concern about the sustainability of these crops. Most of these crops are related to some specific regions in the world, especially USA Midwest and Mediterranean east. In this way, they are very important to support the commercial balance of these regions/countries, enabling the economic development of some communities. Additionally, from the environmental point of view, nut orchards have been considered an excellent option for native reforestation with commercial exploitation capacity [2, 13].

Nut oils are getting an outstanding position as gourmet and health-promoting oils, for both their sensory and their nutritional characteristics. Actually, as far as the nutritional features are concerned, the high amounts of oleic acid, as well as those of phytosterols, allow frequent comparisons of these oils with olive oil. However, nut oils have also been indicated as inducers of allergic reactions in consumers [14].

The aim of this work is to characterize the major and minor compounds of laboratory-extracted and commercial nut oils from sweet almond, hazelnut, and pecan nut in order to increase the knowledge about the chemical composition of nut oils as well as to establish, based on a statistical approach, the main compounds which would allow for distinguishing among oil samples and their origin.

2. Materials and Methods

2.1. Chemicals. Acetone, diethyl ether, hexane, propionitrile, and tetrahydrofuran (THF) were supplied by VWR International (West Chester, PA, USA). Silica solid phase extraction (Si-SPE) cartridges were from Varian (EA Middelburg, The Netherlands). Potassium hydroxide (KOH) was from Panreac (Montcada i Reixac, Barcelona, Spain). Hexamethyldisilazane, pyridine, trimethylchlorosilane, and standards of α-, γ-, β-, and δ-tocopherol were from Merck (Merck Group, Darmstadt, Germany). Standards of 5α-cholestan-3β-ol, squalane, and n-eicosanol were from Sigma-Aldrich Co. (St. Louis, MO, USA). All chemical reagents were at least of analytical grade.

2.2. Samples. Nuts from sweet almonds, hazelnuts, and pecan nuts were obtained in local grocery stores in Brazil. One individual sample of each commercial nut oil from sweet almonds, hazelnut, and pecan nut were provided by Vital Âtman (Uchoa, SP, Brazil). Both nuts and nut oils were properly stored at 4°C until extraction and analysis. Each chemical characterization was performed in triplicate.

2.3. Extraction of Nut Oils. For each extraction, 1000 g nuts were milled in a knife mill and the obtained products were taken to an expeller press system, Komet Oil Press (IBG Monforts Oekotec GmbH & Co.KG., Germany), and then filtered through filter paper to remove any solid material. Each laboratory sample was a pooled sample from the whole extraction process, which was performed separately for each nut. The oil obtained was stored at 4°C until analysis.

2.4. Chemical Characterization

2.4.1. Fatty Acid Composition. The fatty acid composition was determined according to IUPAC Standard Methods [15, 16], as the composition of fatty acid methyl esters (FAME) by GC. Transesterification of the oils was carried out with KOH in methanol at a concentration of 2 mol/L. The chromatographic analysis was done using an Agilent 5890 GC system (Agilent Technologies, Santa Clara, California) equipped with an automated liquid sampler (1 μL injections), split injector (1 : 50 split ratio), polar capillary column (SPTM-2380, 100 m × 0.25 mm internal diameter (i.d.) × 0.20 μm film thickness), and flame ionization detector (FID). Hydrogen was used as carrier gas at a flow rate of 1.0 mL/min. The initial oven temperature was 180°C, and the temperature gradient was from 180°C to 220°C at 3°C/min. The detector and injector temperatures were 225°C and 250°C, respectively. Peak identification was carried out by means of comparison with a standard chromatogram. Data were described as fatty acid profiles by peak area normalization.

2.4.2. Triacylglycerol Composition. This determination was done following the procedure established by Moreda et al. [17]. For oil purification, a Si-SPE cartridge was washed without vacuum with 6 mL hexane. After that, a solution of the oil (0.12 g) in 0.5 mL hexane was added. The solution was pulled through the cartridge and then eluted with 10 mL hexane-diethyl ether (87 : 13 v/v) solution. The eluted solvents were evaporated to dryness under reduced pressure at room temperature. The residue was dissolved in 2 mL acetone. For triacylglycerol (TAG) analysis, 10 μL of this solution was injected directly, using the autosampler (508 system), in a RP-HPLC system. The separation was done on a Merck LiChrospher 100 RP-18 column (250 mm × 4 mm i.d. × 4 μm particle size) thermostated at 20°C. The liquid chromatograph (Beckman Coulter, Fullerton, CA, USA) was equipped with a pumping unit (118 solvent module) and propionitrile was used as mobile phase at a flow rate of 0.6 mL/min. Detection was done with a PerkinElmer 200 RI detector. Identification of TAG peaks was done by comparison with the chromatograms established by the authors' method, as well as the Supelco 37 Component FAME Mix (47885-U SUPELCO). The data were processed by peak area normalization and expressed as TAG percentage.

Table 1: Bibliographic information on the chemical composition of almond, hazelnut, and pecan nut oils. References [1–3, 5–8, 10, 21–29].

Nut oil		Almond	Hazelnut	Pecan nut
Oil amount (g.100 g^{-1})		25.1–60.7	8.10–67	58-74
Fatty acid composition (% area)	Myristic acid - 14:0	0–0.07	0–0.1	0.05–0.09
	Palmitic acid - 16:0	4.7–15.8	4.5–6.5	6.4–7.6
	Palmitoleic acid - 16:1	0.1–2.5	0.1–0.3	0.1–0.2
	Stearic acid - 18:0	0.3–2.5	0.4–3.8	2.2–2.8
	Oleic acid - 18:1	50.4–81.2	76.3–86.5	49.6–62.1
	Linoleic acid - 18:2	6.21–37.1	6.5–15.6	27.2–37.7
	Linolenic acid - 18:3	0–11.1	0.1–1.9	1.4–1.9
	Arachidic acid - 20:0	0.04–0.2	0–0.2	0.34
Triacylglycerol composition (% area)	LLLn		0.1	—
	LLL	8.7	3.7	—
	OLLn	0.1	0.5	—
	OLLn	27.6	12.3	—
	OLnO	—	0.7	—
	LLP	4.8	1.6	—
	OLO	28.0	28.2	—
	LOP	11.3	5.2	—
	PLP	0.5	0.2	—
	OOO	13.3	36.5	—
	SLO	1.8	1.4	—
	OOP	2.7	6.1	—
	SOO	0.6	2.8	—
Total sterols mg·kg^{-1}		2178–2777	1096–6031	1899
Sterol composition (% area)	Cholesterol	—	0.8–2.3	—
	24-Methylene-cholesterol	—	0–0.1	—
	Campesterol	2.5	4.8–7.4	2.7
	Campestanol	—	0–0.2	—
	Stigmasterol	2.5	1.3–2.1	17.9
	Δ7-Campesterol	—	0–0.4	—
	Δ5,23-Stigmastadienol + Clerosterol	—	0.9–1.3	—
	β-Sitosterol	55.9–95.1	78.1–90.4	82.8
	Sitostanol	—	1.8- 3.6	—
	Δ5-Avenasterol	8.5–28.2	1.3–5.2	—
	Δ5,24-Stigmastadienol	—	0.3–1.1	—
	Δ7-Stigamastenol	—	0.3–2.3	—
	Δ7-Avenasterol	—	0.5–1.9	—
Squalene mg·kg^{-1}		95.0	186.0–371.0	152.0
Total tocopherol mg·kg^{-1}		451.0	25.8–690.8	180.0
Tocopherol and tocotrienol composition (% of total content)	α-Tocopherol	97.3	53.8–90.6	12.0
	β-Tocopherol	—	2.1–4.2	—
	γ-Tocopherol	2.8	3.1–41.9	168.0
	δ-Tocopherol	—	—	—
	α-Tocotrienol	—	0–7.1	—

—: not determined and/or evaluated by the authors.
P: palmitic acid; S: stearic acid; O: oleic acid; L: linoleic acid; Ln: linolenic acid.

2.4.3. Sterol Composition and Aliphatic Alcohols. Sterols and aliphatic alcohols are components of the unsaponifiable fraction. Therefore, removing the saponifiable compounds previously to their determination is essential. In this line, the samples were analyzed according to the methodology proposed by the International Olive Council [18, 19]. To summarize, 5 g of oil was saponified with 50 mL ethanolic KOH solution, at a concentration of 2 mol/L, during 1 h under reflux. The unsaponifiable compounds were then extracted with diethyl ether (3 × 80 mL) and the organic phase was washed with

distilled water until complete neutralization. After drying, the unsaponifiable matter was fractionated by thin layer chromatography (TLC) using silica plates impregnated with KOH. Each plate was developed twice with a mixture of petroleum ether : diethyl ether (87 : 13, v/v). After separation, four bands could be observed, corresponding to desmethylsterols, methylsterols, aliphatic alcohols, and dimethylsterols. Each band was then scratched off and extracted with hot chloroform and diethyl ether. The solutions were evaporated to dryness, derivatized with 500 μL of a 1 : 3 : 9 (v/v/v) trimethylchlorosilane : hexamethyldisilazane : pyridine solution, and analyzed by GC. The gas chromatograph (Agilent 6890N) was equipped with an automated liquid sampler (1 μL injections), split injector (1 : 50 split ratio), a fused silica low-polarity capillary column (DB-5HT, 30 m × 0.25 mm i.d. × 0.25 μm film thickness, Agilent Technologies, Santa Clara, California), and FID. The oven program for the determination of desmethylsterols was set isothermally at 260°C. Hydrogen was used as carrier gas at a flow rate of 1 mL/min. For the analysis of the other fractions, a temperature gradient was applied: starting at 220°C (2 min) until 295°C at 2°C/min. The temperatures of injector and detector were 300°C. The quantitative determinations were done using internal standards: α-cholestanol for desmethylsterols and n-eicosanol for aliphatic alcohols, methyl- and dimethylsterols. Data were always expressed as the total (mg/kg oil) of each compound class, and the profile of each class was described as the percentage of the area of each compound within the class, according to the method recommendation. Peak assignments were carried out by relative retention time calculation and comparison with reference chromatograms as described in each method.

2.4.4. Squalene. This procedure derives from that published previously [20]. Oil samples (0.04 g) together with 40 μL internal standard (squalane 5 mg/mL) were dissolved in 1 mL hexane and saponified at room temperature with 200 μL methanolic KOH at a concentration of 2 mol/L. After separation (by gravity), the upper phase was washed with 3 × 400 μL ethanol : water 1 : 1, v/v, and 1 μL of the supernatant was analyzed by GC. GC analyses were carried out with an Agilent 6890N Gas Chromatograph (Agilent Technologies, Santa Clara, California) equipped with an automatic liquid sampler, split injection (20 : 1 ratio), and a FID. The conditions for the GC assays were DB5-HT column; 30 m × 0.25 mm i.d. × 0.10 μm film (Agilent Technologies, Santa Clara, California); hydrogen carrier gas at 0.8 mL/min. The oven worked isothermally at 250°C for 10 minutes. The injector and detector temperatures were 300°C and 345°C, respectively. Peak identification was conducted by relative retention time calculation, based on the internal standard. The quantitative evaluation was carried out using squalane as an internal standard, and the data was expressed in mg/kg oil.

2.4.5. Tocopherols. Tocopherols were determined following IUPAC Standard Method 2432 [30], according to which 10 mg oil was diluted with 1 mL hexane and directly injected into a liquid chromatograph with an Si-column (250 mm × 4 mm i.d. × 4 μm particle size). The elution solvent was a mixture of hexane : 2-propanol (99 : 1, v/v) at a flow rate of 1 mL/min. Detection was done by means of a RF-10AXL Shimadzu fluorescence detector, setting excitation at λ = 290 nm and emission at λ = 330 nm. The analytical curve for quantitative and qualitative determinations was performed by means of injections of tocopherol standards at concentrations of 4–6 μg/mL in hexane. Results were expressed in mg/kg for each tocopherol compound.

2.4.6. Stigmastadienes. In order to verify the presence of refined oils, stigmastadienes were determined only in commercial samples. The method described in COI/T.20/Doc. number 11 was followed for this determination [31]. Oil samples (20 g) together with 1 mL internal standard solution (3,5-cholestadiene, 20 μg/mL) were saponified with 75 mL alcoholic KOH (10%) during 30 min under reflux. The unsaponifiable compounds were then extracted with hexane (2 × 100 mL), and the organic phase neutralized washing it with an ethanol-water (1 : 1 v/v) solution. The solvent was then evaporated to dryness in a rotary evaporator at 30°C. After this preparation, the obtained unsaponifiable matter was fractionated by silica gel column chromatography using hexane as mobile phase. The first 30 mL eluate were discarded and the following 40 mL were collected, dried, and injected into the chromatograph. The GC system was an Agilent 6890N Gas Chromatograph (Agilent Technologies, Santa Clara, California) equipped with an automatic liquid sampler, split injection (15 : 1 ratio), and a FID. The parameters for the GC assays were DB5-HT column; 30 m × 0.25 mm i.d. × 0.10 μm film (Agilent Technologies, Santa Clara, California); 1.0 μL injection volume and hydrogen as carrier gas at 1 mL/min. The injector and detector temperatures were 300°C and 320°C, respectively. The oven temperature program was 235°C for 6 minutes, rising at 2°C/min up to 285°C. For peak identification, the retention time along with a comparison with the standard chromatogram described in the method was evaluated. For quantitative evaluation, 3,5-cholestadiene was used as internal standard. Data were expressed in mg/kg.

2.5. Statistical Analysis. In order to verify significant differences ($p < 0.05$) among samples, for each feature, an ANOVA test was accomplished in Metaboanalyst 3.0 web-based tool [32]. After that, a multivariate statistical analysis was performed with full information of the chemical characterization; data files were saved as.csv format and uploaded into the Metaboanalyst 3.0 web-based tool. A principal component analysis (PCA) was performed considering all features and their relationship. To make the features more comparable, a range scaling (mean-centered and divided by the value range of each variable) was applied.

Additionally, cluster hierarchical analysis was also performed, using a word clustering algorithm and a Euclidean distance measure. The cluster was then plotted with a heatmap composed of the 15 most important characteristics of the samples. These characteristics were selected by random forest analysis using random features selection from a bootstrap sample until the best grouping was reached.

3. Results and Discussion

3.1. Major Component, Fatty Acid, and Triacylglycerol Profiles. Fatty acids composition is the most common feature for fat and oil characterization. It is related to oxidative stability as well as to some nutritional characteristics. The fatty acid profile has been widely described for almonds, hazelnuts, and pecan nuts, as Table 1 shows. However, in this work, it was possible to bring some new information about this feature (Table 2), as it is the case of the description of some isomers present in each sample. According to ANOVA analysis, the amount of ω-9 palmitoleic acid (C16:1ω-9), ω-7 and ω-11 oleic acids (C18:1ω-7, C18:1ω-11), behenic acid (C22:0), and lignoceric acid (C24:0) did not differ statistically among the samples ($p > 0.05$), whereas for all other fatty acids a statistical difference was found ($p \leq 0.05$) for this set of samples.

For all the samples, ω-9 oleic acid (C18:1ω-9) was the main fatty acid, with its concentration being around 80% in hazelnut, 70% in pecan nut, and 60% in sweet almond. Other isomers of oleic acid like C18:1ω-7 and C18:1ω-11 were also found, although their sum never reached 2% of the total fatty acids. In the case of palmitoleic acid isomers, their sums did not exceed 1% in each sample, and C16:1ω-7 was the most abundant one. Linoleic acid (C18:2ω-6) was the second most abundant fatty acid, with the highest presence in sweet almond, almost 30%, followed by pecan nut with around 20% and hazelnut with approximately 10%.

Table 2 provides the complete fatty acid composition for both sweet almond and hazelnut. These results are in general within the ranges shown in Table 1. Exceptions can be found regarding pecan nut, where lower amounts of palmitic (C16:0), linoleic (C18:2ω-6), and linolenic (C18:3ω-3) acids were found, as well as higher concentrations of stearic (C18:0) and oleic (C18:1) acids. Although statistically different, the fatty acid compositions of the three nut oils are quite similar, calling to mind the similarities between olive and avocado oils, both of them fruit oils [33, 34].

Although the fatty acid composition is quite similar, the distributions of the fatty acids in the triacylglycerol molecules are very different (Table 2). While in hazelnut and pecan nut oil samples there is a clear predominance of PLP + OOO + PoPP, in sweet almond samples, this predominance is equally shared between PLP + OOO + PoPP and OOL + LnPP. Another particularity of the sweet almond is the amount of OLL, near 18%, whereas it does not reach 5% and 10% in hazelnut and pecan nut, respectively.

The presence of SOL (around 12%) is a particularity of hazelnut and it does not exceed 3% in sweet almond and pecan nut, in contrast to POO, which does not surpass 1% in hazelnut and is around 7 and 8% in sweet almond and pecan nut, respectively. Fatty acid and TAG profiles are complementary since TAG profile shows how fatty acids are grouped.

3.2. Minor Compounds and Unsaponifiable Matter. Minor components are commonly known as the fingerprint of some vegetable oils. In this way, they have been widely related to oil identity in many regulations [33]. Sterols are the most abundant class of compounds in the unsaponifiable matter [20]. In this work, desmethyl-, methyl-, and dimethylsterols have been analyzed. Desmethylsterols are the most commonly analyzed group. The total amount of desmethylsterols was higher for both commercial and extracted sweet almond oils. As expected, β-sitosterol was the main desmethylsterol, followed by Δ5-avenasterol, which was higher in pecan nut oil (around 15%) than in sweet almond and hazelnut oils, around 9% and 5%, respectively (Table 3). Among all samples, cholesterol and stigmasterol concentrations did not show significant statistical differences ($p > 0.05$).

In the case of methylsterols (Table 3), citrostadienol is the main species in all samples. Sweet almond oil shows higher obtusifoliol presence (reaching 28%) than hazelnut and pecan nut oils, whose concentrations do not exceed 20% and 10%, respectively. The total amount of methylsterols is at least three times higher in pecan nut oil samples than in the other samples. Regarding dimethylsterols, the total amount in pecan nut oil was at least six times higher than that in the other samples, reaching 200 $mg \cdot kg^{-1}$, while in sweet almond and hazelnut oils it was around 30 $mg \cdot kg^{-1}$. For sweet almond and hazelnut oils, the main dimethylsterol was 24-methylencycloartanol, followed by butyrospermol in hazelnut oil, while in sweet almond oil the profile changed between commercial and extracted samples. However, in pecan nut oil samples, it is possible to see a very particular behavior, since in pecan nut oil samples the main dimethylsterol was cycloartenol, reaching 70% of the total dimethylsterols.

Squalene is a terpenic hydrocarbon, a precursor of sterols, which has been highlighted due to its health benefits [20]. In general, the total amount of squalene in commercial oils was always higher than in laboratory-extracted samples (Table 3).

Terpenic alcohols' presence in sweet almond oil was below 10 $mg \cdot kg^{-1}$, whereas it went beyond 20 $mg \cdot kg^{-1}$ in hazelnut oil and 30 $mg \cdot kg^{-1}$ in pecan nut oil. Actually, it was higher than 60 $mg \cdot kg^{-1}$ in the extracted samples of pecan nut. In the case of aliphatic alcohols in sweet almond samples, the total amount did not exceed 7 $mg \cdot kg^{-1}$, while in hazelnut and pecan nut oils the total amount was above 12 $mg \cdot kg^{-1}$, with the content in the extracted samples being higher than in commercial oils (Table 3). Differences can be noted in the profile of both terpenic and aliphatic alcohols and seem to be related to sample processing and origin, although there is no concrete evidence to prove it. Tocotrienols were not present in any of the samples; however, the total amount of tocopherols exceeded 200 $mg \cdot kg^{-1}$ in all cases, with no statistical difference ($p > 0.05$). The amount of δ-tocopherol was not statistically different as well. When it comes to sweet almond and hazelnut oils, the main compound was α-tocopherol (Table 3), the same as in most vegetable oils. Regarding pecan nut samples, the main tocopherol was γ-tocopherol, which is also present in walnut and corn oils [20].

3.3. Statistical Grouping Analysis. Principal component analysis (PCA) was performed in order to establish a statistical relationship among samples. Figure 1 shows results plotted by

Table 2: Fatty acid and triacylglycerol composition of sweet almond, hazelnut, and pecan nut oil laboratory and commercial samples.

Sample		Ext. sweet almond oil		Com. sweet almond oil		Ext. hazelnut oil		Com. hazelnut oil		Extr. pecan nut oil		Com. pecan nut oil	
Fatty acid composition % area													
Lauric - 12:0		**0.02**	0.00	**ND**	—	**ND**	—	**ND**	—	**ND**	—	**ND**	—
Miristic - 14:0		**0.04**	0.00	**0.04**	0.00	**0.03**	0.00	**0.03**	0.00	**0.04**	0.00	**0.03**	0.00
Pentadecanoic - 15:0		**0.01**	0.00	**0.01**	0.00	**0.01**	0.00	**0.01**	0.00	**0.01**	0.00	**0.01**	0.00
Palmitic - 16:0		**6.60**	0.08	**6.62**	0.02	**5.57**	0.04	**5.55**	0.01	**5.61**	0.07	**5.57**	0.02
Palmitoleic ω9 - 16:1 ω9		**0.03**	0.00	**0.03**	0.00	**0.03**	0.00	**0.03**	0.00	**0.03**	0.00	**0.02**	0.00
Palmitoleic ω7 - 16:1 ω7		**0.43**	0.00	**0.43**	0.00	**0.19**	0.00	**0.23**	0.00	**0.07**	0.00	**0.08**	0.00
Palmitoleic ω11 - 16:1 ω11		**0.02**	0.00	**0.02**	0.00	**0.01**	0.00	**0.01**	0.00	**ND**	—	**ND**	—
Margaric - 17:0		**0.05**	0.00	**0.05**	0.00	**0.05**	0.00	**0.03**	0.00	**0.07**	0.00	**0.07**	0.00
Margaroleic - 17:1		**0.12**	0.00	**0.12**	0.00	**0.08**	0.00	**0.07**	0.00	**0.06**	0.00	**0.06**	0.00
Stearic - 18:0		**1.34**	0.02	**1.33**	0.00	**2.48**	0.01	**1.85**	0.02	**2.87**	0.02	**2.58**	0.01
Oleic ω9 - 18:1 ω9		**60.22**	0.59	**59.70**	0.20	**80.64**	0.12	**78.34**	0.10	**67.55**	0.22	**70.96**	0.01
Oleic ω7 - 18:1 ω7		**1.17**	0.86	**1.69**	0.06	**1.44**	0.06	**1.80**	0.10	**1.31**	0.08	**1.22**	0.02
Oleic ω11 - 18:1 ω11		**0.16**	0.03	**0.20**	0.02	**0.21**	0.02	**0.13**	0.04	**0.27**	0.10	**0.16**	0.04
Linoleic trans - 18:2 t		**0.02**	0.00	**0.02**	0.00	**0.01**	0.00	**0.01**	0.00	**0.01**	0.00	**0.01**	0.00
Linoleic - 18:2 ω6		**29.58**	0.13	**29.54**	0.14	**8.76**	0.02	**11.53**	0.06	**20.67**	0.06	**17.97**	0.08
Linoleic isomer - 18:2		**ND**	—	**ND**	—	**0.05**	0.00	**0.04**	0.01	**0.19**	0.01	**0.12**	0.01
Linolenic - 18:3 ω3		**0.04**	0.00	**0.04**	0.00	**0.11**	0.01	**0.09**	0.00	**0.80**	0.01	**0.70**	0.01
Arachidic - 20:0		**0.07**	0.00	**0.07**	0.00	**0.13**	0.00	**0.10**	0.00	**0.12**	0.00	**0.12**	0.00
Gondoic - 20:1		**0.07**	0.00	**0.06**	0.00	**0.17**	0.00	**0.15**	0.00	**0.29**	0.00	**0.28**	0.00
Behenic - 22:0		**0.02**	0.00	**0.02**	0.00	**0.03**	0.00	**0.02**	0.00	**0.02**	0.00	**0.02**	0.00
Lignoceric - 24:0		**ND**	—	**0.01**	0.00	**ND**	—	**ND**	—	**ND**	—	**0.01**	0.00
Triacylglycerol composition % area													
LLL		**2.88**	0.07	**2.93**	0.04	**0.90**	0.02	**0.81**	0.00	**2.63**	0.03	**2.19**	0.08
OLLn + PoLL	ECN 42	**0.25**	0.03	**0.29**	0.03	**0.08**	0.03	**0.08**	0.00	**0.68**	0.01	**0.58**	0.04
PLLn		**0.11**	0.01	**0.10**	0.02	**ND**	—	**ND**	—	**0.14**	0.04	**0.17**	0.03
OLL		**18.05**	0.07	**18.04**	0.45	**3.42**	0.65	**4.55**	0.03	**9.71**	0.20	**8.33**	0.05
OOLn + PoOL	ECN 44	**0.49**	0.03	**0.38**	0.13	**0.57**	0.12	**0.32**	0.05	**0.99**	0.05	**1.02**	0.21
PLL + PoPoO		**2.49**	0.01	**2.29**	0.23	**0.63**	0.20	**0.55**	0.01	**1.74**	0.09	**1.67**	0.26
POLn + PPoPo + PPoL		**0.38**	0.08	**0.27**	0.03	**ND**	0.00	**0.05**	0.01	**0.27**	0.07	**0.17**	0.01
OOL + LnPP		**26.34**	0.17	**26.76**	0.16	**14.04**	0.79	**18.89**	0.33	**23.24**	0.28	**21.11**	0.26
PoOO + SLL + PLO	ECN 46	**10.75**	0.17	**10.78**	0.20	**3.55**	0.10	**3.60**	0.26	**6.03**	0.16	**5.99**	0.40
PoOP + SPoL + POLn + SPoPo		**0.34**	0.06	**0.34**	0.01	**0.42**	0.17	**0.57**	0.06	**0.16**	0.03	**0.38**	0.26
PLP + OOO + PoPP		**27.39**	0.29	**27.21**	0.26	**55.68**	2.22	**53.63**	0.04	**38.75**	0.11	**42.89**	0.69
SOL	ECN 48	**1.96**	0.05	**1.89**	0.26	**13.41**	0.32	**12.05**	0.12	**2.60**	0.14	**2.19**	0.19
POO		**7.00**	0.29	**6.96**	0.14	**ND**	—	**0.33**	0.00	**8.12**	0.16	**8.51**	0.20
POP	ECN 50	**0.17**	0.00	**0.23**	0.02	**1.08**	0.54	**0.57**	0.01	**0.50**	0.05	**1.12**	0.07
SOO		**1.40**	0.05	**1.53**	0.17	**5.68**	0.88	**3.66**	0.02	**4.07**	0.27	**3.35**	0.10
POS + SSL	ECN 52	**ND**	—	**ND**	0.00	**0.53**	0.08	**0.32**	0.04	**0.36**	0.04	**0.32**	0.03

Bold numbers: mean; normal numbers: standard deviation; ND: not detected; Ext.: extracted; Com.: commercial; P: palmitic acid; Po: palmitoleic acid; S: stearic acid; O: oleic acid; L: linoleic acid; Ln: linolenic acid.

Table 3: Minor compounds of sweet almond, hazelnut, and pecan nut oil samples: desmethyl-, methyl-, and dimethylsterols, terpenic alcohols, squalene, aliphatic alcohols, and tocopherols.

Sample	Ext. sweet almond oil		Com. sweet almond oil		Ext. hazelnut oil		Com. hazelnut oil		Extr. pecan nut oil		Com. pecan nut oil	
Desmethylsterols % area												
Cholesterol	**0.25**	0.03	**0.13**	0.02	**0.22**	0.01	**0.18**	0.01	**0.35**	0.06	**0.16**	0.01
Brassicasterol	**ND**	—	**0.06**	0.03	**ND**	—	**ND**	—	**ND**	—	**ND**	—
24-Methylene-cholesterol	**0.04**	0.00	**0.03**	0.00	**ND**	—	**ND**	—	**0.29**	0.00	**0.30**	0.01
Campesterol	**2.46**	0.02	**2.53**	0.02	**5.21**	0.04	**4.85**	0.02	**4.45**	0.00	**4.16**	0.03
Campestanol	**0.13**	0.04	**0.09**	0.01	**0.39**	0.01	**0.26**	0.01	**0.09**	0.00	**0.12**	0.06
Stigmasterol	**0.91**	0.00	**0.86**	0.01	**0.91**	0.01	**0.88**	0.03	**0.90**	0.01	**0.86**	0.01
Δ7-Campesterol	**0.78**	0.47	**0.83**	0.00	**ND**	—	**ND**	—	**ND**	—	**ND**	—
Δ5,23-Stigmastadienol + Clerosterol	**1.40**	0.05	**1.37**	0.00	**0.96**	0.02	**0.81**	0.03	**0.89**	0.04	**1.06**	0.00
β-Sitosterol	**77.42**	0.37	**77.28**	0.13	**81.39**	0.03	**81.79**	0.03	**75.51**	0.09	**74.86**	0.01
Sitostanol	**1.91**	0.09	**1.52**	0.04	**3.54**	0.09	**2.55**	0.03	**0.57**	0.05	**0.36**	0.01
Δ5-Avenasterol	**9.89**	0.03	**9.93**	0.04	**4.37**	0.04	**5.44**	0.03	**14.94**	0.03	**15.81**	0.09
Δ5,24-Stigmastadienol	**1.48**	0.02	**1.64**	0.04	**0.83**	0.02	**1.02**	0.02	**1.07**	0.00	**1.27**	0.03
Δ7-Stigamasterol	**1.94**	0.04	**2.30**	0.00	**1.45**	0.04	**1.32**	0.04	**0.24**	0.01	**0.40**	0.00
Δ7-Avenasterol	**1.39**	0.03	**1.44**	0.01	**0.73**	0.01	**0.89**	0.01	**0.69**	0.01	**0.65**	0.00
Total mg·kg^{-1}	**2870.48**	29.24	**2804.31**	21.85	**1787.82**	2.94	**1807.72**	5.28	**1791.44**	14.58	**1746.01**	3.21
Methylsterols % area												
Obtusifoliol	**26.57**	0.35	**28.81**	0.17	**18.53**	0.30	**14.39**	0.67	**6.82**	0.17	**6.48**	0.15
Gramisterol	**13.46**	0.16	**12.18**	0.58	**6.72**	1.28	**5.83**	0.24	**13.88**	0.25	**8.98**	0.02
Citrostadienol	**59.96**	0.00	**59.02**	1.22	**74.75**	1.11	**79.78**	1.85	**79.31**	0.15	**84.54**	0.56
Total mg·kg^{-1}	**29.73**	0.57	**33.86**	0.65	**43.09**	0.10	**39.85**	0.40	**110.78**	2.76	**111.09**	2.81
Dimethylsterols % area												
Dammaradienol	**7.44**	0.66	**6.37**	0.68	**2.05**	0.32	**3.04**	0.55	**0.17**	0.02	**0.56**	0.01
Taraxerol	**4.55**	0.30	**3.90**	0.62	**9.38**	0.48	**9.02**	0.46	**2.15**	0.24	**3.06**	0.11
$\alpha + \beta$ Amyrin	**24.97**	1.01	**6.89**	1.22	**5.13**	0.39	**5.61**	0.10	**3.76**	0.17	**3.62**	0.07
Butyrospermol	**ND**	—	**ND**	—	**29.37**	0.20	**28.21**	0.68	**6.15**	0.02	**1.55**	0.04
Cycloartenol	**11.91**	0.24	**18.67**	1.48	**7.49**	0.41	**8.19**	0.12	**78.69**	0.03	**75.58**	0.31
24-Methylencycloartanol	**51.13**	0.42	**64.18**	1.41	**46.58**	0.45	**45.94**	0.81	**9.08**	0.05	**15.64**	0.08
Total mg·kg^{-1}	**28.35**	4.16	**32.57**	2.55	**29.20**	0.45	**26.11**	1.43	**214.69**	11.51	**192.51**	1.28
Squalene mg·kg^{-1}	**96.43**	0.59	**113.11**	5.37	**340.05**	1.39	**431.72**	5.34	**298.83**	5.10	**320.81**	1.63
Terpenic alcohols % area												
Phytol	**71.65**	0.09	**73.00**	1.26	**49.58**	7.04	**55.66**	1.04	**70.68**	1.01	**44.60**	1.15
Geranylgeraniol	**28.35**	0.09	**27.00**	1.26	**50.42**	7.04	**44.34**	1.04	**29.32**	1.01	**55.40**	1.15
Total mg·kg^{-1}	**9.74**	0.36	**9.61**	0.63	**26.54**	4.06	**20.37**	2.42	**66.57**	0.65	**36.64**	0.12

Table 3: Continued.

Sample	Ext. sweet almond oil		Com. sweet almond oil		Ext. hazelnut oil		Com. hazelnut oil		Extr. pecan nut oil		Com. pecan nut oil	
Aliphatic alcohols % area												
C22-OH	**23.13**	0.62	**11.29**	2.05	**3.71**	0.57	**8.30**	0.67	**8.56**	0.31	**15.91**	0.60
C23-OH	**2.56**	0.24	**1.46**	0.34	**ND**	—	**ND**	—	**ND**	—	**ND**	—
C24-OH	**29.66**	0.90	**19.29**	6.26	**20.21**	0.48	**16.43**	1.58	**21.30**	0.24	**39.60**	2.87
C25-OH	**7.70**	0.24	**7.37**	3.11	**4.47**	0.05	**0.00**	0.00	**3.36**	0.17	**6.25**	0.76
C26-OH	**40.31**	1.83	**30.81**	18.03	**55.10**	1.91	**33.93**	2.64	**13.16**	0.04	**24.47**	1.75
C27-OH	**ND**	—	**ND**	—	**9.38**	0.22	**41.31**	1.00	**ND**	—	**ND**	—
C28-OH	**ND**	—	**ND**	—	**3.96**	0.05	**3.42**	0.50	**55.22**	2.29	**11.37**	0.29
Total mg·kg^{-1}	**5.55**	0.27	**6.05**	1.69	**31.70**	0.20	**12.40**	0.33	**24.88**	0.95	**12.85**	0.03
Tocopherols mg·kg^{-1}												
α-Tocopherol	**233.40**	0.40	**236.06**	4.71	**227.95**	9.81	**205.35**	13.22	**5.08**	0.00	**5.07**	0.13
β-Tocopherol	**8.45**	0.45	**8.52**	0.30	**9.97**	0.16	**7.12**	0.70	**ND**	—	**ND**	—
γ-Tocopherol	**10.10**	0.10	**10.12**	0.37	**102.00**	0.61	**8.26**	0.42	**306.34**	2.83	**197.09**	2.08
δ-Tocopherol	**0.87**	0.07	**0.91**	0.15	**5.31**	0.13	**0.78**	0.08	**2.38**	0.14	**1.57**	0.18
Total mg·kg^{-1}	**252.83**	1.03	**255.60**	5.53	**345.22**	9.23	**221.51**	14.42	**313.80**	2.70	**203.73**	1.77

Bold numbers: mean; normal numbers: standard deviation; ND: not detected; Ext.: extracted; Com.: commercial.

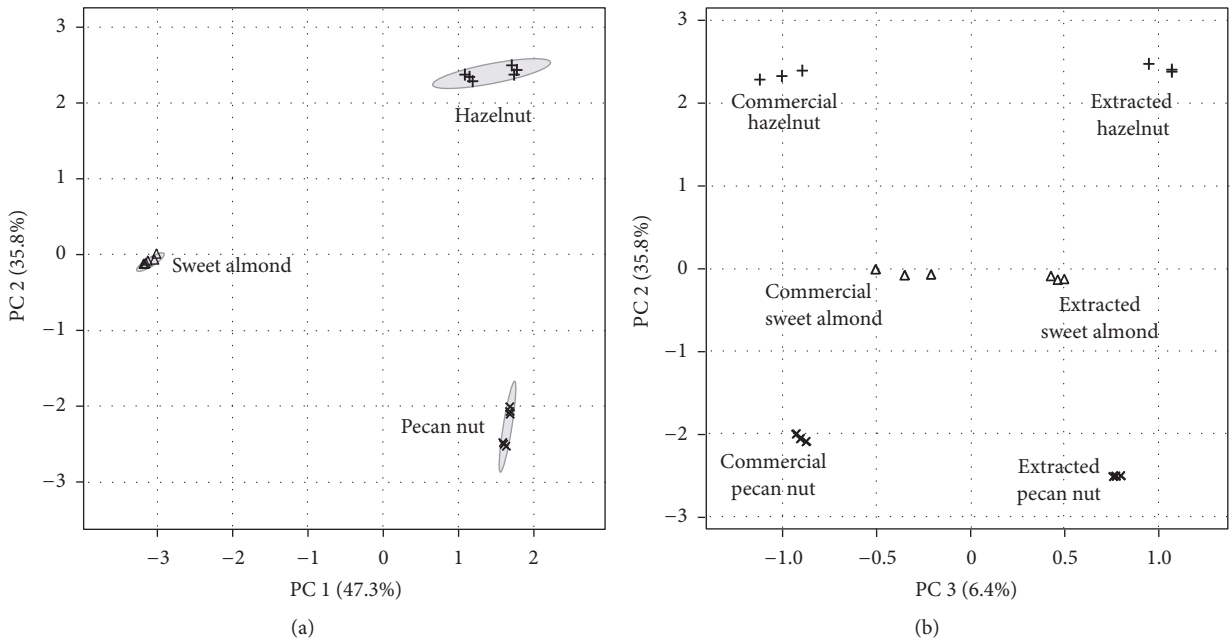

Figure 1: Principal component analysis score plot for all analyzed samples. PC1 × PC2 (a); PC2 × PC3 (b).

means of principal components PC1 versus PC2 (Figure 1(a)) and PC2 versus PC3 (Figure 1(b)). PC1 explains 47.3% of the sample variances while PC2 and PC3 explain 35.8% and 6.4%, respectively. According to the results in Figure 1(a), PC1 clearly isolates sweet almond samples (PC1 < 0 zone) from hazelnut and pecan nut oils (PC1 > 0 zone), while PC2 separates hazelnut (PC2 > 0 zone) from pecan nut samples (PC2 < 0 zone). Figure 1(b) reflects the important influence of PC3, which disconnects the extracted (PC3 > 0 zone) from the commercial (PC3 < 0 zone) samples.

Cluster hierarchical analysis results (Figure 2) reaffirm the close relationship between hazelnut and pecan nut oils, while sweet almond oil is clearly isolated. Observing the heatmap in Figure 2, there is a reflex of previous results and discussion, once it highlights the 15 main compounds responsible for the distinction of the samples, considering the relationship of all samples and features. In this way, for sweet almond samples, obtusifoliol, damaradienol, C16:1ω-7 acid, OLL + LnPP, C18:2t acid, and Δ5,24-stigmastadienol were the main features selected through random forest analysis. For pecan nut oil, C20:1 acid, cycloartenol, Δ5-avenasterol, OLL + PoLL, PLLn, and C17:0 acid were the most important characteristics, whereas hazelnut oil, β-sitosterol, C18:1ω-9 acid, and campesterol were the main ones.

4. Conclusion

Oils from sweet almond, hazelnut, and pecan nut are monounsaturated fats whose fatty acid composition is dominated by oleic acid. For the first time, the presence of ω-7, ω-9, and ω-11 isomers of palmitoleic and oleic acids has been described in these kinds of oils. In general, when TAG are formed, those with oleic acids prevail, even though a distinctive profile could be defined about sweet almond oil, in a way that this may help to differentiate it from hazelnut and pecan nut oils.

β-Sitosterol (desmethylsterols) was the main minor compound found, and the amount of Δ5-avenasterol was one of the most distinguishing features of pecan nut oil. The high concentrations of total methyl- and dimethylsterols were important particularities of pecan nut oils, as well as the higher quantity of γ-tocopherol since in hazelnut and sweet almond oils the main tocopherol was α-tocopherol.

This work also describes for the first time the presence in nut oils of other minor compounds like terpenic and aliphatic alcohols.

Using multivariate statistical analysis, it was possible to establish relationships among samples and carry out sample grouping. In this sense, hazelnut oils resulted to be chemically closer to pecan nut oils than to sweet almond oils. From these data analyses, it was also possible to differentiate commercial from extracted oils. Heatmap highlighted the components that are more important for the distinction of the samples considering the relationship among all samples and features.

The full characterization of the samples was the main novelty of this work, which brings a new approach to the characterization of nut oils. In this way, it performs a complete characterization of minor and major identity parameters, and establishes the relationship among samples and features.

All this information may be taken into account for giving regulatory recommendations and laws.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

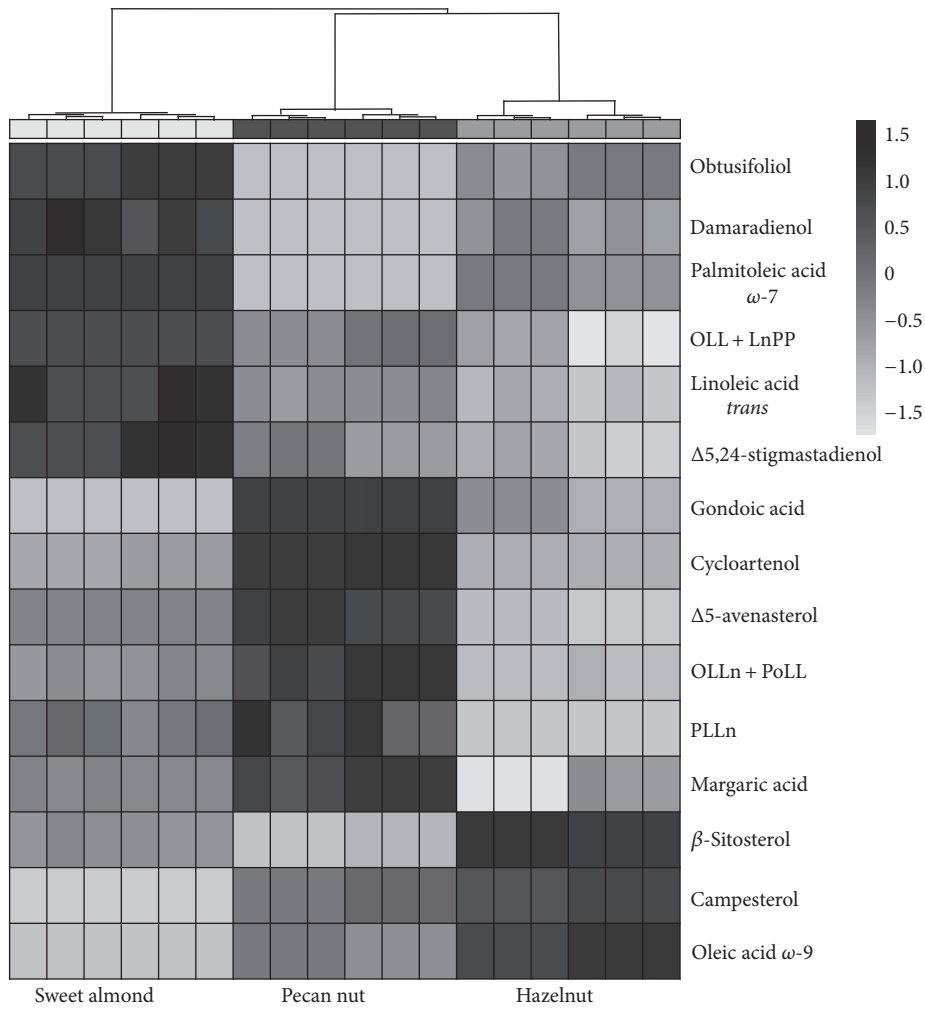

Figure 2: Cluster hierarchical analysis and heatmap of the fifteen more important features for sample grouping, selected by random forest statistical tool.

Acknowledgments

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil) and the National Council for Scientific and Technological Development (CNPq, Brazil) for Fernandes' scholarship and financial assistance.

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