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Chemical characterization of oil from four Avocado varieties cultivated in Morocco☆

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Abstract – The notable growth in the use of avocado oil in the nutritional and cosmetic field was the main objective to valorize the oil production of important varieties of avocados existing in Morocco by analyzing its chemical composition in fatty acids, sterols, tocopherols and its physico-chemical properties. Oleic acid is the main fatty acid in the oil; they constitute between 50 and 65% of the total fatty acids. The study of the unsaponifiable fraction revealed that avocado oil contains 3259.9–5378.8 mg/kg sterols and 113.13–332.17 mg/kg tocopherols. Chemo-metric tools were employed in manner optimization, such as principal component analysis, agglomerative hierarchical clustering, analysis of variance, and classification trees using Chi-squared Automatic Interaction Detector. Chemo-metric tools revealed a difference in the composition of fatty acid, sterols, and tocopherol of avocado oil samples. This difference resulted from a variety of avocado fruits. Agglomerative Hierarchical Clustering (AHC) method was efficient distinguishing avocado oil samples based on fruit variety using fatty acids, tocopherols, sterol compositions and total sterol. Principal component analysis (PCA) method allowed the distinction the set avocado oil dataset based on fruit varieties, supplied a correct discrimination rate of 95.44% for avocado fruit varieties using the fatty acid. Chi-squared Automatic Interaction Detector (CHAID) carried out using the same variables, also provided an acceptable classification rate of 50% for avocado fruit varieties using the total tocopherol content. Besides, a comparative study of the physico-chemical properties in terms of acidity index, saponification index, iodine index, chlorophylls, carotenoids, and methyl and ethyl esters was performed.

Keywords: Avocado oil / chemical composition / chemo-metric tools / varieties / oleic acid

Résumé – Caractérisation chimique de l'huile de quatre variétés d'Avocat cultivées au Maroc. La croissance notable de l'utilisation de l'huile d'avocat dans le domaine nutritionnel et cosmétique était l'objectif principal pour valoriser la production d'huile d'importantes variétés d'avocats existant au Maroc en analysant sa composition chimique en acides gras, stérols, tocophérols et ses propriétés physico-chimiques. L'acide oléique est le principal acide gras de l'huile ; ils constituent entre 50 et 65 % des acides gras totaux. L'étude de la fraction insaponifiable a révélé que l'huile d'avocat contient 3259,9–5378,8 mg/kg de stérols et 113,13–332,17 mg/kg de tocophérols. Des outils chimiométriques ont été utilisés pour l'optimisation des méthodes, tels que l'analyse en composantes principales (ACP), La classification ascendante hiérarchique (CAH), l'analyse de variance (ANOVA) et les arbres de classification utilisant le détecteur automatique d'interaction du chi carré (CHAID). Les outils chimiométriques ont révélé une différence dans la composition des acides gras, des stérols et du tocophérol des échantillons d'huile d'avocat. Cette différence est due à une variété d'avocats. La méthode CAH s'est avérée efficace pour distinguer les échantillons d'huile d'avocat en fonction de la variété de fruit, en utilisant les acides gras, les tocophérols, les compositions de stérols et le stérol total. La méthode d'analyse en composantes principales

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a permis de distinguer l'ensemble des données sur l'huile d'avocat en fonction des variétés de fruits, et a fourni un taux de discrimination correct de 95,44 % pour les variétés de fruits d'avocat utilisant la composition en acides gras. La méthode Chi-squared Automatic Interaction Detector, réalisée à l'aide des mêmes variables, a également fourni un taux de classification acceptable de 50 % pour les variétés d'avocats utilisant la teneur totale en tocophérols. En outre, une étude comparative des propriétés physico-chimiques en termes d'acidité, d'indice de saponification, d'indice d'iode, de chlorophylles, de caroténoïdes et d'esters méthyliques et éthyliques a été réalisée.

Mots clés : Huile d'avocat / composition chimique / outils chimiométriques / acide oléique / variétés

1 Introduction

The Avocado (*Persea Americana* Mill.) is from the Lauraceae family (Krumreich *et al.*, 2018). Avocado is one of the most important crops in the tropical and subtropical countries of the world (Ojewole *et al.*, 2007; Indriyani *et al.*, 2016). In Morocco, most of the crops are located in the northwestern coastal strip of the country, between the south of Rabat and Tangier (Denis, 2008). It's mainly consumed in the form of fresh fruit for its good nutritional value (Hurtado-Fernandez *et al.*, 2018).

Avocado is a major market worldwide, alongside its use in the cosmetics, edible oil and food processing industries (Swisher, 1988; Athar and Nasir, 2005; Indriyani *et al.*, 2016). The pulp of this fruit is known for its high lipid content similar to that of olive oil, containing mainly fatty acid (Tango *et al.*, 2004) as well as minerals such as iron, magnesium, phosphorus, and potassium (Goff and Klee, 2006). Compared to other sources of vegetable oil, the major fatty acid of avocado oil is oleic and linoleic acid. Oleic acid is supposed to present modulatory effects in a extensive physiological functions, while some studies also suggest a beneficial effect on cancer, autoimmune and inflammatory diseases, besides its ability to facilitate wound healing (Sales-Campos *et al.*, 2013). Linolenic acid is an essential fatty acid needed for human health. it has been reported to have cardiovascular-protective, anti-cancer, neuro-protective, anti-osteoporotic, anti-inflammatory, and antioxidative effects (Kim *et al.*, 2014). These fats are believed to have incredible health benefits as they help increase levels of HDL-cholesterol (the good cholesterol) and decreasing levels of LDL-cholesterol (the bad cholesterol), both of which significantly reduce the risk of cardiovascular disease (Lunn and Theobald, 2006).

Avocado is also rich in unsaponifiable compounds such as sterols, mainly β-sitosterol, vitamins, carotenoids, tocopherols, and the phenolic compounds are of significant interest, for their antioxidant and anti-inflammatory properties (Kosińska *et al.*, 2012; Zhang *et al.*, 2013).

In addition to its high content of good fats, avocado pulp also contains several hundred phytochemical molecules that may play a role in cancer prevention (Lu *et al.*, 2005). In addition to certain molecules that are widespread in the plant world such as flavonoids (quercetin, luteolin, apigenin, etc.) or coumarins (scopoletine), avocados have the particularity of containing alkanols, a class of fat-soluble molecules that show a great inhibitory activity on cancer cells (Lu *et al.*, 2005). For example, several studies have shown that avocado extracts containing some of these molecules (persine, isopersine, persenone) stopped the growth of cancer cells from several different types of cancer, including those of the mouth, breast, prostate, and lung (Lu *et al.*, 2005).

This work aims at evaluating the effects of different avocado varieties on oil yield, chemical composition and physico-chemical characteristics, using chemo-metric tools. In order to identify the most promising varieties for fresh sale or transformation.

2 Material and methods

2.1 Plant material

The four avocado fruit varieties (*Ettinger*, *Fuerte*, *Hass* and *Reed*) was harvested in the region of Rabat-Salé-Kenitra. The sampling was done in January 2019. The avocado pulp was sorted and then dried in the oven overnight at 45 °C. The plant material, once dried, it is reduced to powder in a blender and stored in bags.

50 g of ground dried avocado pulp were extracted in a Soxhlet extractor for 8 h using 250 mL of n-hexane (analytical grade). This solvent was removed at 50 °C under reduced pressure using a rotary evaporator. The extracted oils were subsequently placed in brown glass bottles and stored at 4 °C.

2.2 Physical and chemical analysis of crude oil

The oil yield was measured according to ISO 659 (2009), Official Methods of the American Oil Chemists' Society were used for the determination of iodine value (method Cd 1-25), acid value (method Ca 5a-40), and saponification value (method Cd 3-25) (AOCS, 1998). Acid value (AV) was determined by titration of a solution of oil in ethanol with ethanolic KOH and is expressed as (mg KOH/g oil). Iodine value was expressed as (g of I_2/100 g of oil), It was experimentally determined by treatment with Wijs reagent followed by titration of the iodine excess with $Na_2S_2O_3$ and Saponification value was determined by titration of a solution of oil in 0.1N ethanolic KOH with 0.5M HCl, it was expressed as (mg KOH/g of oil)

Carotenoid and chlorophyll compounds were determined in cyclohexane at 470 and 670 nm, respectively, using the specific extinction coefficients, according to the method of Mínguez-Mosquera *et al.* (1991) et Gharby *et al.* (2018).

Fatty acids alkyl esters were determined by gas chromatography (GC-FID) according to the method reported by Rabeh *et al.* (2018). 500 mg of avocado oil was weighed and added with 0.225 mL of a 0.02% w/v of a methyl heptadecanoate

Table 1. Physical characteristics of four varieties of avocado fruit.

	Shape	Average weight (g)	Skin	Pulp color	Seed	Size (cm)
Ettinger	Pear, longer and broad neck	150–300	Smooth, medium gloss and green	Green	Medium to large, conical with pointed apex	12–15
Fuerte	Pear	250–400	Green and slightly pebbled	Pale green	Medium	10–12
Hass	Ovale	120–200	Dark green and bumpy	Pale green	Small	6.5–9
Reed	Round	300–400	Medium to thick and easy peeling	Green	Medium to large	10–11

solution in n-heptane, used as internal standard. The obtained solution was transferred into the chromatographic column (internal diameter 15 mm, lenght 40 cm, fitted with a suitable stopcock). Using a mixture of n-hexane/ethyl ether (99:1) For elution. The collected fraction, containing alkyl esters, was transferred into a rotary evaporator to remove solvents. The residue was redissolved in 2 mL of n-heptane. GC-FID analyses were performed on a 7890 GC (Agilent), equipped with a HP-5 column (15 m × 0.32 mm i.d. × 0.25 μm).

2.3 Chemical composition of crude oil

For the fatty acid composition determination, the methyl esters were analyzed according to ISO 5508 (1990), a CP-Wax 52CB column (30 m × 0.25 mm i.d.) was used, the carrior gas used was helium (flow rate 1 mL/mn). Initial oven temperature was set at 170 °C; injector temperature 200 °C; detector temperature 230 °C. Injected quantity was 1 uL. Sterol composition was determined according to ISO 6799 (1991), after trimethylsilylation of the crude sterol fraction using a Varian 3800 instrument equipped with a VF-1 ms column (30 m and 0.25 mm i.d.) and using helium (flow rate 1.6 mL/mn) as carrier gas. Column temperature was isothermal at 270 °C, injector and detector temperature was 300 °C. Injected quantity was 1 uL. while the tocopherols was separated and quantified by HPLC, according to ISO 9936 (2006). The HPLC used for this study was Shimadzu CR8A instruments (Champ sur Marne, France) equipped with a C18-Varian column (25 cm × 4 mm; Varian Inc., Middelburg, The Netherlands). Detection was performed using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330 nm). Eluent used was a 99:1 isooctane/isopropanol (V/V) mixture, flow rate of 1.2 ml/min. Chromatographic peaks were identified and quantified using α-tocopherol standards.

2.4 Statistical analysis

Analysis of variance (ANOVA) was performed by the software IBM SPSS Statistics 21, for the checking of the statistical significance by Tukey tests at a confidence level of 95.0%, as well as, and the results were presented as means ± standard error of the mean. Multivariate statistical treatments, hierarchical agglomerative clustering, principal component analysis, and discrimination trees were carried out by using the XLSTAT version 2019.

3 Results and discussion

3.1 Physical characteristics

As for the physical characteristics to identify the different varieties of the avocado fruit, each is popular for its shape, color, skin, and size (Salunkhe and Kadam, 1995). Table 1 presents the characteristics of the four selected varieties.

The results, as summarized in Table 1, are very useful to recall the main characteristics of the fruits of each briefly. The color of the skin varies from light green to dark green. The variety of *Hass* becomes purplish-black when ripe (Ojewole and Amabeoku, 2006).

The *Ettinger* and *Fuerte* varieties are mainly pear-shaped, while the *Hass* is oval, and the *Reed* is round. The four varieties of Avocado have different skin structures. Some varieties have smooth skin (*Ettinger*), while others are grainy or granular skin (*Fuerte* and Hass).

3.2 Physical and chemical parameters

Avocado oil extracted from all the pulp samples had a yellowish-green color and a characteristic hazelnut smell. The investigated avocado fruit contained relatively high amounts of oil in comparison with other vegetable oils, like pumpkin seed oil (Boujemaa *et al.*, 2020) or cactus oil (Gharby *et al.*, 2020). The "*Ettinger*" fruit, in particular, contains the highest oil level (72.54%). These amounts were relatively high in comparison to maximal values reported from Chile (25.8%) (Olaeta *et al.*, 1986); South Africa (40.0%) (Pearson, 1975) and Brazil (25.5%) (Tango *et al.*, 1969).

However, the oil content of the other avocado varieties was lower than that of *Ettinger* (*Fuerte*, *Hass* and *Reed* 44.69%, 55.71% and 56.5% respectively) (Tab. 2).

Table 2 shows the data on some physicochemical characteristics of pulp oils from the four varieties.The free fatty acids in a sample are measured using the acid value assay; in this case, from the pulp oil of the four varieties studied. The results have demonstrated a significant difference ($p < 0.05$) between the varieties. The values were 1.4, 2.6, 1.9, and 2.4 mg KOH/g oil for *Ettinger*, *Fuerte*, *Hass*, and *Reed* varieties, respectively. These values are higher than those reported previously for the *Fuerte* variety (1.23 mg/g) (Bora *et al.*, 2001), and avocados grown in Mexico (1.46 mg/g) (Moreno *et al.*, 2003). Also, the saponification value of the *Hass* variety (231.41 mg KOH/g oil) was higher than the values reported previously for avocado oil as it reached 178 (Bora *et al.*, 2001),

Table 2. Physico-chemical parameters of oil from four avocado varieties.

	Ettinger	*Fuerte*	Hass	*Reed*	Standard
Oil content (%)	72.54 ± 0.28^{a}	44.69 ± 0.68^{b}	55.71 ± 0.47^{c}	56.25 ± 0.84^{c}	
Acid value (mg KOH/g oil)	1.4 ± 0.01^{a}	2.6 ± 0.01^{b}	1.9 ± 0.006^{c}	2.4 ± 0.006^{d}	MAX 5.0
Iodine value (g of I_2/100g of oil)	90.45 ± 0.02^{a}	94.04 ± 0.006^{b}	84.76 ± 0.006^{c}	83.22 ± 0.06^{d}	MIN 82.0
Saponification value (mg KOH/g of oil)	172.50 ± 0.006^{a}	186.53 ± 0.01^{b}	231.41 ± 0.003^{c}	189.34 ± 0.006^{d}	177–198
Ethyl Esters	72.55 ± 1.5^{a}	15.77 ± 1.8^{b}	17.87 ± 1.2^{b}	96.97 ± 2.5^{c}	
Methyl Esters	48.34 ± 2.3^{a}	3.47 ± 0.25^{b}	33.01 ± 1.4^{c}	18.56 ± 0.8^{d}	
Alkyl Esters	120.89	19.24	50.88	115.53	35 mg/kg

Means of three determinations ± standard deviation. Values followed by the same small letters in a row are not significantly different at $p < 0.05$ according to ANOVA. Tukey HSD test.

Table 3. Determination of chlorophyll and carotenoid in oil from four avocado varieties.

	Ettinger	*Fuerte*	Hass	*Reed*
Chlorophyll (mg /kg)	0.54 ± 0.006^{a}	1.63 ± 0.003^{b}	1.04 ± 0.003^{c}	0.83 ± 0.009^{d}
Carotenoid (mg/kg)	0.77 ± 0.05^{a}	0.40 ± 0.03^{b}	0.56 ± 0.03^{c}	0.35 ± 0.01^{d}

Means of three determinations ± standard deviation. Values followed by the same small letters in a row are not significantly different at $p < 0.05$ according to ANOVA. Tukey HSD test.

175–190 (Medina 1980), and 178 mg KOH/g oil (Soares *et al.*, 1992).

The iodine values of pulp oil of the four varieties varied from 83.22 to 94.04 g of I_2/100g of oil, and these values are higher to those reported by Bora *et al.* (2001) and Medina (1980). Still, they are inside the range of 82–95 g of I_2/100g of oil reported by Moreno *et al.* (2003).

Ethyl esters assay was imposed by the International Olive Council as a quality criterion in oil investigations. As a result, a standard was set for different types of oils to be respected by manufacturers (Beltran *et al.*, 2015). Currently, the European commission regulation suggested a limit for the ethyl esters content of 35 mg/kg (oil) (COI, 2012). Like olives, avocados contain a certain amount of sugars that transform into alcohol through fermentation. An important indicator of oil quality is free fatty acids; these free fatty acids react with the methyl and ethyl alcohols (R'-OH) to form fatty acid ethyl esters (FAEE) and fatty acid methyl esters (FAME) (Rabeh *et al.*, 2018).

The lowest content of fatty acid alkyl esters FAAEs (FAEEs + FAMEs) was observed in *Fuerte* (19.24 mg/kg) and the highest in *Ettinger* (120.89 mg/kg). In general, studies about alkyl esters in avocado oil are still an unfamiliar world. Three essential factors could affect the content of such studies, ripening stage, post-harvest storage conditions and oil filtration (Rabeh *et al.*, 2018).

3.3 Determination of oil pigments

Table 3 shows that the *Fuerte* has the highest content of chlorophyll (1.63 mg/kg), followed by *Hass* and *Reed* which contain doses of 1.04 mg/kg and 0.83 mg/kg respectively, while *Ettinger* is the variety with the lowest chlorophyll value (0.54 mg/kg). The chlorophyll content of the pulp of the avocados of the four varieties was higher than that reported by Krumreich *et al.* (2018) (0.18 $mg.kg^{-1}$). However, it was lower than that reported by Wang *et al.* (2010) (28.7 mg/kg). Chlorophyll does not contribute to oil stability but it can act as a sensitizer for photo-oxidation to occur. Therefore, it is important to store the oil away from light (Rukmini and Raharjo, 2010).

The consumption of carotenoids may bring health benefits because lutein helps reduce macular degeneration due to aging (Koh *et al.*, 2004). In the market, this type of oil has high benefits, especially among people interested in the field of cuisine. In this case, the concentration and nature of the oils are important determinants of quality and, therefore, marketing. Avocados contain important amounts of carotenoids in their oil that are believed to be potential anti-carcinogenic agents (Mooz *et al.*, 2012). *Ettinger* variety has the highest content of Carotenoids (0.77 mg/kg), followed by *Hass* and *Fuerte*, which contain 0.56 mg/kg and 0.40 mg/kg of carotenoids respectively, while *Reed* present the lowest carotenoids value (0.35 mg/kg). Our values were lower than those found by Mardigan *et al.* (2019) for other varieties cultivated in Brazil (2.7 mg/kg).

According to Ashton *et al.* (2006) all carotenoids are present in unripe avocados, but as they ripen, all carotenoids other than lutein decrease to near-zero. The fruit used in this study were mature; therefore, many of the minor carotenoids would be unlikely to be extracted into the oils. In cold-pressed olive oil (similarly to the extraction of avocado oil), the lutein concentrations ranged from 0.2 to 3.9 $\mu g.g^{-10}$(Psomiadou and Tsimidou, 2001).

3.4 Fatty acid composition

Several studies have reported the fatty acid composition of avocado oil, where oleic acid was reported to be the major acid.

Table 4. Fatty acid composition of oil from four avocado varieties.

	Ettinger (%)	*Fuerte* (%)	Hass (%)	*Reed* (%)	Standard
Palmitic Acid C16:0	15.23 ± 0.05^{a}	15.63 ± 0.05^{b}	20.91 ± 0.03^{c}	18.43 ± 0.003^{d}	13.0–22.0
Palmitoleic Acid C16:1	8.64 ± 0.06^{a}	2.06 ± 0.003^{b}	9.82 ± 0.02^{c}	7.55 ± 0.003^{d}	4.0–10.0
Stearic Acid C18:0	0.46 ± 0.08^{a}	0.83 ± 0.03^{b}	0.49 ± 0.07^{a}	0.49 ± 0.04^{a}	0.35–1.0
Oleic Acid C18:1	60.79 ± 0.09^{a}	57.50 ± 0.03^{b}	54.53 ± 0.05^{c}	61.18 ± 0.04^{d}	55.0–68.0
Linoleic Acid C18:2	13.31 ± 0.09^{a}	19.84 ± 0.03^{b}	12.93 ± 0.03^{c}	10.60 ± 0.01^{d}	9.0–15.0
Linolenic Acid C18:3	1.10 ± 0.05^{a}	1.57 ± 0.06^{b}	0.90 ± 0.03^{c}	0.83 ± 0.03^{c}	0–2.0
Gadoleic Acid C20:1	0.24 ± 0.01^{ab}	0.28 ± 0.03^{a}	0.17 ± 0.003^{b}	0.23 ± 0.003^{ab}	0–0.2
SFA	15.69	16.46	21.4	18.92	
USFA	84.08	81.25	78.35	80.39	
PSFA	14.41	20.74	13.1	11.43	
P/S	0.91	1.26	0.61	0.60	
U/S	5.35	4.93	3.66	4.24	

Means of three determinations ± standard deviation. Values followed by the same small letters in a row are not significantly different at $p < 0.05$ according to ANOVA. Tukey HSD test.
SFA = saturated fatty acids; USFA = unsaturated fatty acids; PUFA = polyunsaturated fatty acids; P/S = Polyunsaturated fatty acid/Saturate fatty acid ratio; U/S = Unsaturated fatty acid/Saturate fatty acid ratio.

This fact supports the consumption of this oil for its beneficial health effect as oleic acid is related to the reduction of diabetes, oxidative stress and to relieve cardiovascular disease effects (Ortiz-Avila *et al.*, 2013; Carvajal-Zarrabal *et al.*, 2014). Table 4 shows the results obtained.

The avocado oil is rich in oleic, palmitic, linoleic, and palmitoleic acids, while stearic acid is present in tiny amounts. The *Reed* variety reported the highest amount of oleic acid (61.18%) amongst all studied varieties. On the other hand, the *Hass* variety recorded the lowest oleic acid amount (54.53%) which differs from the findings of Lozano *et al.* (1985), where oleic acid accounted for 65–80% in Mediterranean avocado oil. Palmitic and linoleic acid are in second and third places with different proportions depending on the variety. The fatty acid composition found is comparable to those of palm and olive oils as reported in previous studies (Gharby *et al.*, 2013, 2014). When it comes to unsaturated fatty acids, it is the *Ettinger* that has the highest proportion with a value of 84.04%.

The *Fuerte* in our case reported values of 57.5% for oleic acid, followed by linoleic and palmitic acids, with values of 19.84% and 15.63%, respectively. These findings are similar to those previously reported for avocado oil in other countries, where oleic acid was found to be at about 45.9–54.5%, followed by palmitic acid with 19.7–20.0%, and linoleic acid with 5.7–12.5% (Bora *et al.*, 2001; Azizi and Najafzadeh, 2008). A high ratio of PUFA/SFA (recommended minimum value of 0.4) is linked to beneficial health effects especially for cardiovascular diseases (Wood *et al.*, 2004). Those values were 0.91, 1.26, 0.61, and 0.60 for *Ettinger*, *Fuerte* Hass, and *Reed*, respectively, values higher than 0.4 show the nutritional value of avocado oil. The Table 4 shows also the ratio of unsaturated to saturated fatty acids (PUFA/SFA). The values of this ratio were 5.35, 4.93, 3.66, and 4.24 for *Ettinger*, *Fuerte* Hass, and *Reed*, respectively. These values were close to those found by Vekiari *et al.*, (2004) for the same varieties from Greece. For avocado oil the amount of PUFA is about five folds of SFA, which is also true for Greece's avocado oil, as reported by Vekiari *et al.* (2004).

3.5 Sterol composition

Sterols are among the essential components of the unsaponifiable fraction. They are antioxidant compounds widely distributed in plants that give the oils a great nutritional value (Youssef *et al.*, 2010).

The results presented in Table 5 allowed us to determine the total sterol contents present in our oils quantitatively. Sterol identification assay is one of the most reliable ways for adulteration and authenticity detection for most vegetal oils. In all the studied varieties, the major sterols found are β-sitosterol, Δ5-avenasterol, and campesterol, while cholesterol, stigmasterol are present in small amounts (Tab. 5).

The Codex Alimentarius standard stipulates that total sterols for Avocado oils must be above 2437 $mg.kg^{-1}$ (Codex Alimentarius, 2019). In this study, all varieties reported a total sterol concentration above the previously cited limit, with 3259.9 $mg.kg^{-1}$ being the lowest value found for the Ettinger. In contrast, Fuerte reported the highest content with

Table 5. Sterol composition of oil from four avocado varieties.

	β-sitosterol (mg/kg)	Δ5-avenasterol (mg/kg)	Campesterol (mg/kg)	Cholesterol (mg/kg)	Stigmasterol (mg/kg)	Total sterol (mg/kg)
Ettinger	2686.81 ± 0.03^{a}	265.68 ± 0.03^{a}	155.82 ± 0.01^{a}	17.28 ± 0.03^{a}	19.23 ± 0.05^{a}	3259.90 ± 0.04^{a}
Fuerte	4499.90 ± 0.06^{b}	327.03 ± 0.04^{b}	306.05 ± 0.05^{b}	6.45 ± 0.008^{b}	43.03 ± 0.06^{b}	5378.80 ± 0.07^{b}
Hass	3650.01 ± 0.03^{b}	311.56 ± 0.06^{c}	245.14 ± 0.01^{b}	5.68 ± 0.03^{b}	27.09 ± 0.03^{ab}	4369.7 ± 0.05^{c}
Reed	2998.13 ± 0.03^{c}	144.48 ± 0.05^{d}	263.28 ± 0.04^{c}	10.35 ± 0.04^{c}	3.21 ± 0.003^{c}	3567.5 ± 0.09^{d}
Standard	1998–5580	73–372	121–496	0–31	0–124	2437–6200

Means of three determinations ± standard deviation. Values followed by the same small letters in a column are not significantly different at $p < 0.05$ according to ANOVA. Tukey HSD test.

5378.8 mg.kg^{-1} (not exceed the limit of Codex of 6200 mg.kg^{-1}) (Codex Alimentarius, 2019).

Those values are higher than those of olive oil (Gharby *et al.*, 2012). Its antioxidant activity and health-promoting effects have already been mentioned (Moreau *et al.*, 2002; Yoshida and Niki, 2003); the Fuerte oil seems to have better quality when it comes to sterols.

β-sitosterol is the most abundant sterol in avocado oil, the same sterol was found to be the major sterol for olive oil (Gharby *et al.*, 2012). Fuerte recording the highest amount with 4499.90 mg.kg^{-1}, and the lowest concentration was Ettinger with 2686.81 mg.kg^{-1}. Previous studies have also reported that β-sitosterol represented more than 75% of the overall total sterols content (Boskou, 2006; Plaza *et al.*, 2009). The obtained results are also similar to the findings reported previously (Phillips *et al.*, 2005). Awad *et al.* (2003) discussed the health benefits of β-sitosterol, it reduce plasma cholesterol and prevent different types of cancer.

Regarding the fact that all avocado fruits were harvested at the same stage of maturity, the same region and the observed differences might be due to variety, climate, or soil type.

The Δ5-avenasterol content for the *Fuerte* was the highest (327.03 mg.kg^{-1}), while the Reed recorded the lowest value (144.48 mg.kg^{-1}). These values are lower than those reported by Berasategi *et al.* (2012), and in agreement with the results obtained by Flores *et al.* (2019). In fact, Δ5-avenasterol can exhibit an important antioxidant ability and can improve the oxidative stability of olive oils at high temperature or even under frying conditions (Wang *et al.*, 2002).

3.6 Tocopherol composition

Tocopherols are the main lipophilic compounds with antioxidant activity in avocado oil. The antioxidant activity of these compounds is due to the possibility of giving their phenolic hydrogen to free lipid radicals, thus delaying propagation reactions ((Burton and Ingold, 1981; Seppanen *et al.*, 2010). Although α-tocopherol is recognized as an important antioxidant (Jiang *et al.*, 2001), Table 6 presented the tocopherol composition of studied avocado oil varieties.

The analysis of the tocopherol fraction by liquid chromatography (HPLC) shows the variability of this fraction from one variety to another. The highest content of total tocopherols is very important in the *Fuerte* 332.17 mg/kg, followed by the *Hass* 252.92 mg/kg. On the other hand, tocopherol contents are low in the *Reed* and *Ettinger* varieties with values of 186.14 and 113.13 mg/kg, respectively. The variation of tocopherol composition in avocado oils might depend mainly on the geographical conditions and harvest session of the fruits (Flores *et al.*, 2019).

Table 6. α-Tocopherol and total Tocopherols of oil from four avocado varieties.

	α-tocopherol (mg/kg)	Total tocopherols (mg/kg)
Ettinger	46.82 ± 0.02^{a}	113.13 ± 0.03^{a}
Fuerte	177.90 ± 0.008^{b}	332.17 ± 0.02^{b}
Hass	159.80 ± 0.05^{c}	252.92 ± 0.03^{c}
Reed	79.07 ± 0.01^{d}	186.14 ± 0.03^{d}

Means of three determinations ± standard deviation. Values followed by the same small letters in a column are not significantly different at $p < 0.05$ according to ANOVA. Tukey HSD test.

These results are lower than ones reported for olive oil, as the total tocopherol content in olive oils ranges from a few mg to 450 mg/kg of oil (Boskou, 2006; Gharby *et al.*, 2013).

The vitamin capacity of tocopherols is related to the content of α-tocopherol (vitamin E). This tocopherol is the most common in nature and the most biologically active (Leger, 2000). Indeed, the highest percentage is observed in the *Fuerte* (177.9 mg/kg). This value is intermediate compared to those reported for olive oil (96 mg.kg^{-1}) (Kamal-Eldin and Appelqvist, 1996), 120 mg.kg^{-1} (Kim *et al.*, 2008) and 392 mg.kg^{-1} (De Leonardis and Macciola, 2012). On the other hand, the *Fuerte* has a high content of this tocopherol.

4 Chemometric

In the present study, the discrimination of avocado oil samples according to the chemical composition of its four varieties. Thus, the distinction between avocado oils extracted from different varieties avocado fruit has been based on variances of the measured contents of fatty acid, sterol, and tocopherol. In other words, chemical composition has been used as a chemical expression in the statistical manners in

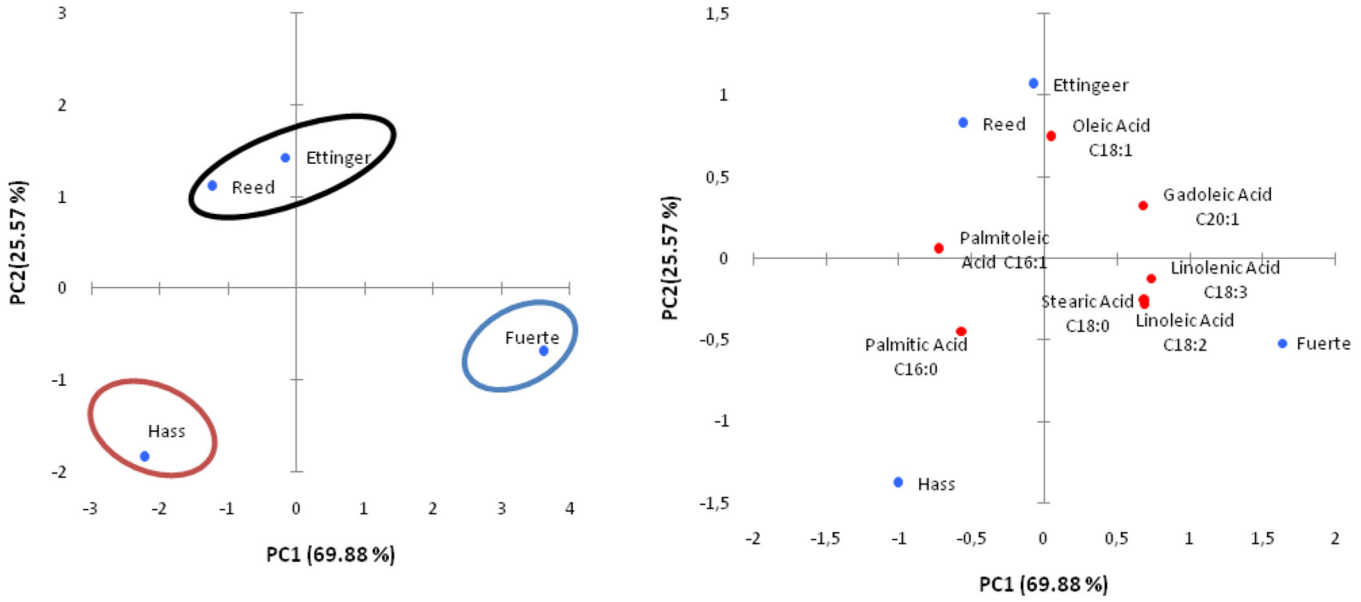

Fig. 1. PCA score plot of the first two principal components (PC1 and PC2; 95.44% of avocado oil samples from four varieties.

Table 7. Discrimination table for PCA models constructed with fatty acid profile of avocado oil samples from four varieties that explain 95.44% of total inertia.

	Eigenvalue	% Inertia	% Cumulative inertia
PC1	4.891	69.875	69.875
PC2	1.79	25.566	95.441
PC3	0.319	4.559	100

order to determine differences between avocado oil samples to discriminate quality oil. PCA, AHC, and CHAID were used for chemometric treatment, data found were treated in order to establish the variance between avocado oil samples.

4.1 Principal Component Analysis (PCA)

To determine correlations between variety and the fatty acid composition of avocado oil, analytical data were undergone to normalize PCA as a statistical manner employing four variables. Two principal components (PC1, PC2) explain 95.44% of total inertia (Fig. 1). PC1 presents 69.88% of the total variance of the data, PC2 exhibits 25.57% of the total variance of the data (Tab. 7).

Left Figure 1 shows three groups, the first one is avocado oil of Ettinger and Reed variety, the second one contains avocado oil of Fuerte variety, while the last one is composed by avocado oil of Hass variety. Right Figure 1 presents the difference in terms of the fatty acid composition of avocado oil of different varieties. The oleic and gadoleic acid content in the avocado oil of Ettinger and Reed variety are close to each other, different from The oleic acid C18:1 and gadoleic acid C20:1 content in the avocado oil of Fuerte and Hass variety. Right Figure 1 also shows stearic, and linoleic acid of avocado oil of Fuerte variety is very different from the other samples.

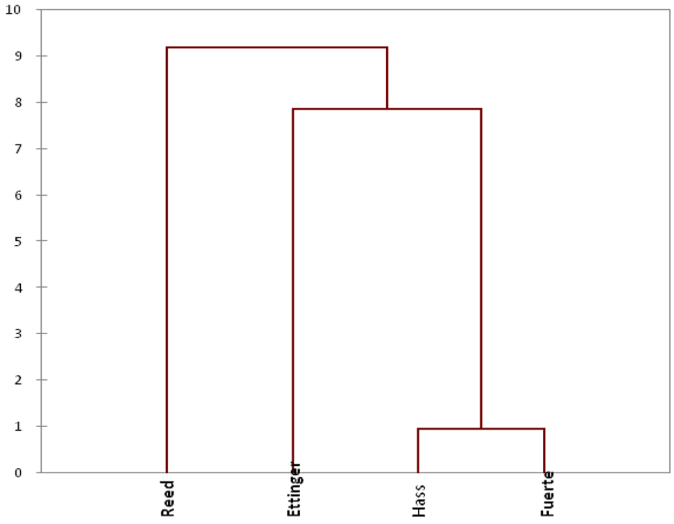

Fig. 2. Dendrograph classification of the avocado oil using the sterol composition, based on four varieties of avocado fruits.

PCA regression model showed successful discrimination avocado oil extracted of different varieties at 95.44% accuracy level.

4.2 Agglomerative Hierarchical Clustering (AHC)

AHC is a strong multivariate exploratory manner popularly employed to identify the (dis)similarity between the N variables (Mohammed *et al.*, 2019). We tentatively used the sterol composition in avocado oil to distinguish four varieties of avocado fruits. Figure 2 presents the dendrogram of sterol compositions in avocado oil obtained from the 4 evaluated varieties. Two sets were determined. The first set (Fig. 2, left)

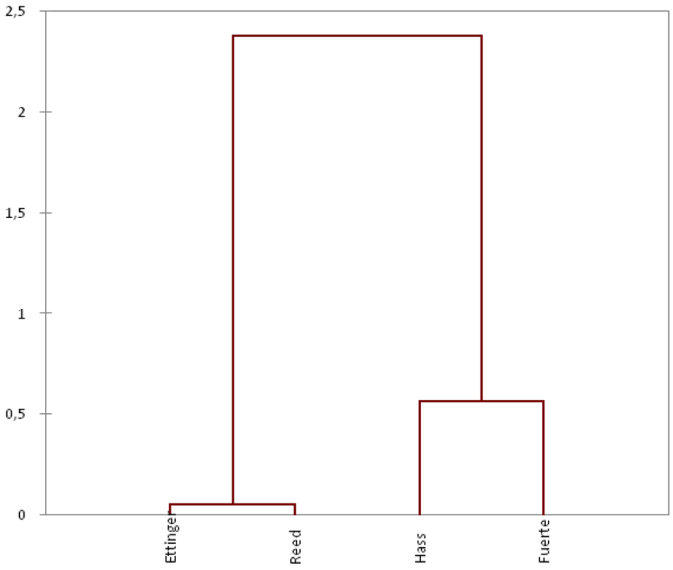

Fig. 3. Dendrograph classification of the avocado oil using the total sterol, based on four varieties of avocado fruits.

comprises the avocado oil of Reed variety. The second set includes two sub-groups, one with avocado oil from Ettinger variety, while the second sub-group contains avocado oil from Hass and Fuerte variety. Particularly, Agglomerative Hierarchical Clustering shows that the sterol content in the avocado oil of Hass and Fuerte variety was close to each other and different compared to the oil of Ettinger and Reed variety.

Figure 3 shows the dendrogram of total sterol content in avocado oil obtained from the 4 varieties. The dating dendrogram (Fig. 3) presented two main sets. One set includes the avocado oil of Ettinger and Reed variety. This set shows less uptake compared to other varieties. The second set is comprised of avocado oil of Hass and Fuerte varieties. This last displays the uptake of total sterol on a higher side. This study reported that AHC analytical was more efficient in unambiguously distinct avocado oils extracted from four different varieties using sterol composition.

4.3 Classification trees

Chi-squared Automatic Interaction Detector (CHAID) is a most useful technique at discovering which variables are the most helpful for separating studied samples into known sets. Here, the CHAID manner was used to the 4 varieties avocado fruits using total tocopherol content, providing a model to discriminate between avocado oils extracted from a of avocado fruits. The correct classification rate achieved by CHAID for avocado oil of different fruit varieties is 50% using total tocopherol content (Tab. 8). The method employed a basic algorithm to built non-binary trees, which depends on the Chi-square test to establish the best split. Figure 4 shows the tree derived from the CHAID model. The small frames contain the node numbers indicate the domain of the variable employed for the split. Regarding avocado oil of Reed and Ettinger varieties, it should be highlighted that the correct distinction rate achieved is 100%, characterized by low values of total

Table 8. Confusion matrix for the classification avocado oil extracted of different avocado fruit varieties.

From\To	*Ettinger*	*Reed*	Hass	*Fuerte*	Total	% correct
Ettinger	1	0	0	0	1	100.00
Reed	0	1	0	0	1	100.00
Hass	1	0	0	0	1	0.00
Fuerte	1	0	0	0	1	0.00
Total	3	1	0	0	4	50.00

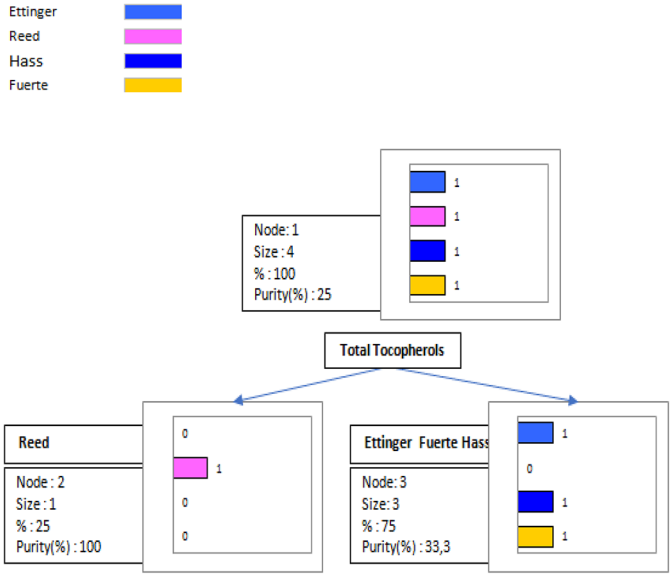

Fig. 4. Classification tree obtained from CHAID for avocado oil samples extracted from different avocado varieties. Inset classification of samples based on total tocopherol content.

tocopherol compared with total tocopherol of avocado oil extracted from Hass, and Fuerte varieties. Avocado oil of Hass variety is characterized by a high content of total tocopherol compared to the avocado oil of of Reed and Ettinger varieties. At last, avocado oil of Fuerte variety is characterized by the highest content values of total tocopherol than other samples (Fig. 4). The big interest of CHAID analysis is owing to the fact that a good classification was realized, although the employ of only a few variables. It is possible to make sure the avocado fruits variety (Reed, Ettinger, Hass, and Fuerte variety), as shown in Figure 4.

5 Conclusion

The chemical composition of fatty acids, total sterol and tocopherol varies considerably from one variety to another. Oleic acid, β-sitosterol, and α-tocopherol are the majority of compounds in the four varieties of Avocado studied. A higher percentage of oleic acid (61.18%) in oil was obtained for the *Reed*; besides, a higher content of sterol (5378.80 mg/kg) and tocopherol (332.17 mg/kg) was obtained for the *Fuerte*. This

reflects the high nutritional value of this oil. it may have important applications as an edible oil for human nutrition and different industrial applications. On the other hand, discrimination avocado fruit varieties carried out using chemometric tools that allowed detaching the set avocado oil dataset, getting accurate discrimination results.

Disclosure of interests

The authors declare that they have no conflicts of interest related to any portion of the study or the preparation of the manuscript.

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