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Conventional and Ultra-fast Analysis Exposing the Harvest Date Impact on Lebanese Olive Oil: *The Soury Variety*

Omar H. Dib[1, 2], Christophe B. Y. Cordella[1, 4], Rita Yaacoub[2], Hussein Dib[2], Nathalie Locquet[1], Luc Eveleigh[3] & Ali Bassal[2]

[1] UMR PNCA, AgroParisTech, INRA, Université Paris-Saclay, Paris, 75005, France

[2] Department of Food Science and Technology, Lebanese University, Beirut, Dekweneh, 1003, Lebanon

[3] Ingénierie Procédés Aliments, INRA, AgroParisTech, Université Paris-Saclay, Massy, 91300, France

[4] C[2]B research group – Chimiométrie pour la Caractérisation de Biomarqueurs, France

Correspondence: Christophe B. Y. Cordella, UMR PNCA, AgroParisTech, INRA, Université Paris-Saclay, Paris, 75005, France. Tel: 33-166-641-6900. E-mail: christophe.cordella@inrae.fr

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Abstract

The impact of harvest period on the quality parameters, polyphenols, fatty acids, sterols, and volatile compounds of Lebanese olive oil from the Soury variety was investigated in this study. Two groups of olive oil were compared, each with a specific harvest date. HD1 was harvested in October, whereas HD2 was picked in November. The analysis of both olive oil categories showed that HD2 witnessed a significant increase in all quality parameters except K270 and a decrease in total polyphenol content from 138 mg/mL to 44 mg/mL. Oleic and linoleic acids had an inverse relation, where the former decreased and the latter increased with the harvest date's advancement. Palmitic acid in both groups was higher than the standards set for extra virgin olive oil. The relative amount of β-Sitosterol was mainly found to decrease, while those of stigmasterol, $\Delta^{5,24}$-stigmastadienol, Δ^{7}-stigmastenol, and Δ^{7}-avenasterol increased with delaying harvest time. As for the volatile compounds, principle component analysis was used on the flash GC data to differentiate HD1 from HD2. Ethanol was found mostly characterizing HD2, whereas HD1 was influenced by 1-hexanol and (E,E)-2,4-decadienal. It can be concluded that the Soury variety should be harvested early, and a delay would result in the declassification of Lebanese olive oil quality from extra virgin to virgin olive oil.

Keywords: chemical characterization, harvest date, Lebanese olive oil, soury variety

1. Introduction

Lebanon is home to the oldest olive trees dating back centuries (at least 1,500 years). It has been distinguished for its trade-in crop products, including olive and olive oil along the Mediterranean Basin (Thalman, 2000; Beayno et al., 2002; Mahfoud, 2007). Lebanon produces around 24,000 tons of olive oil. For the last six years, the production ranged between 16,500 t and 25,000t (International Olive Council [IOC], 2018). This fluctuation in production can be induced by several agronomical (El Antari, Hilal et al., 2000, Stefanoudaki, Chartzoulakis et al., 2001) and technological factors (García et al., 1996; Koutsaftakis et al., 1999). Among these factors, an agronomical factor that is recognized as the one having the most detrimental effect on olive oil chemical composition is the harvest date (De La Torre et al., 1985; Fiorino & Nizzi, 1991; Koutsaftakis et al., 2000).

As olive fruit matures, the color of the fruit shifts from green at the start of the harvest period to small reddish-green spots to purple and lastly to black at the end of the harvest period (Motilva & Romero, 2010). Each stage imparts its chemical variations on the level of macro and minor components of olive oil, such as triglycerides, fatty acids, polyphenols, sterols, and chlorophylls, which in turn have an impact on olive oil quality (Gargouri et al., 2016). Besides, these variations influence not only the quality but also the nutritional, the organoleptic characteristics, and the oxidative stability of olive oil (Maaitah et al., 2009). However, the variation based on fruit maturity depends also on the cultivar in the study.

For instance, Issaoui et al., (2010) related the increase of polyunsaturated and the decrease of monounsaturated fatty acid to fruit maturation. In other cases, the level of monounsaturated fatty acids increased, along with the level of saturated and polyunsaturated fatty acids, as in Barnea variety. As for the Soury variety, Lodolini et al.,

(2017) showed that the delay of harvest has a detrimental effect on olive oil quality. As it ripens, oleic content declines, and linoleic increases, quality indices, mostly free fatty acids, are negatively affected, while polyphenol levels and oxidative stability drop sharply. Also, it has been reported that the sterol content decreases sharply from 2850 g/kg to 1644 g/kg (Noorali et al., 2014). Moreover, fruit maturation has also been shown to affect olive oil quality parameters such as free fatty acids, peroxide value, specific UV absorbances, and volatile compounds (Famiani et al., 2002; Lazzez et al., 2008; Mailer et al., 2010; Varzakas et al., 2010; Dag et al., 2011; Gomez et al., 2011).

Because the macro and minor compounds of the Lebanese olive oil have not been studied extensively, we are interested in identifying some minor and macro components characterizing the Soury variety and studying the harvest date's impact on their evolution. Also, this study stresses the volatiles as a research gap in Lebanese olive oil.

2. Methodology

2.1 Sampling

Sixty-three olive oil samples of Soury variety were used in this study. The samples were divided into two groups (HD1 and HD2) according to their harvest date.

HD1 includes 21 extra virgin olive oil samples (EVOO), each extracted from 5 kg of healthy Soury olive fruits picked from mid of October till the first week of November. The color of the olive fruits of this group was green with purple dots. Oil samples were obtained by cold extraction using an Abencor analyzer (Mc2 Ingenieria y Sistemas, Seville, Spain) with a hammer mill (5.5 mm sieve), a mixer (50 rpm for 30 mins at 28 ℃), and a centrifuge (3500 rpm for 2 mins). All oil samples were stored in glass bottles at 4 ℃ without headspace till analysis.

HD2 is composed of 42 virgin olive oil (VOO) samples (500 mL) collected from Lebanese olive farmers. The olive fruits were harvested from mid of November till the end of November. The color of the olive fruits of this group was purple to black.

2.2 Quality Parameters

The determination of olive oil quality indices, such as acidity (calculated as oleic acid), peroxide value (PV), and UV spectrophotometric indices (K232, K270), was carried out according to the European Union Commission Regulation EEC No 2568/91 (European Union Commission [EEC], 2013).

2.3 Total Polyphenols

The analysis of total polyphenol content was carried out based on Montedoro et al., (1992). The phenols were isolated from oil in hexane by double extraction with methanol-water (60:40, v/v). Total polyphenols were determined by a UV spectrophotometer (Hitachi U-2900, Spectrophotometer, Japan) at 765 nm using a Folin-Ciocalteu reagent. Caffeic acid standard solutions were used as a means of method calibration (R^2 = 0.9995).

2.3 Fatty Acids

The fatty acid profile of olive oil was determined by gas chromatography (GC) after methyl esterification of the acids, according to the International Olive Council (IOC) method COI/T.20/Doc. No 33 (IOC, 2017). Chromatographic analysis was performed using Shimadzu GC-2025 (Kyoto, Japan) equipped with a capillary column SP-2380 (30 m × 0.32 mm i.d. x 0.20 μm film thickness; Supelco, Bellefonte, PA, USA) and an FID detector. Injector and detector temperatures were held at 230 ℃ and 240 ℃, respectively. The injection volume into a split GC port was 1μL, and a split injection mode (1/100) was used. Helium was employed as the carrier gas at a flow rate of 1 mL/min. The following oven temperature program was used: initial temperature of 165 ℃ held for 10 min, ramped at 1.5 ℃ min^{-1} up to 200 ℃. Fatty acids were identified by comparing the retention time of experimental peaks with those obtained by the standard external mixture.

2.4 Sterols

The sterols of olive oil were extracted according to the International Olive Council (IOC, 2013). 5g of olive oil were introduced to a 250 mL flask, already containing α-cholestanol solution (internal standard solution), and saponified with 2 N ethanolic 2M potassium hydroxide solution. After boiling the solution, 100 mL of distilled water was added, and three extractions of the same unsaponifiable fraction were carried out using diethyl ether (80ml, 70ml, 70 ml). The extracts were then washed with water (50 mL) until the wash water no longer gives a pink color upon the addition of phenolphthalein solution. The extracts were filtered on anhydrous sodium sulfate, and the filtered solvent was evaporated by distillation in a rotary evaporator at 30 ^{o}C under vacuum.

Separation of the sterol and triterpene dialcohols fraction (erythrodiol + uvaol). 5% solution of the unsaponifiable was prepared in chloroform and using the 100 µL microsyringe, 0.3 mL of the solution was disposed on a narrow and uniform streak on the lower end (2 cm) of the TLC plate. The plate was then placed in the prepared developing chamber and allowed eluting until the solvent reaches approximately 1 cm from the plate's upper edge. The plate was then sprayed with 0.2% 2,7-dichlorofluorescein to identify the sterol area. The sterol band was then identified using UV light, and the silica gel on the marked area was scraped off using a metal spatula, dissolved in chloroform and diethyl ether, and evaporated to dryness. The obtained sterols and triterpene dialcohols were transformed into trimethylsilyl ethers by adding a 9:3:1 (v/v/v) mixture of pyridine/hexamethyldisilazane/trimethylchlorosilane (in the ratio of 50 µL for every milligram of sterols).

GC-MS Analysis. The mixture was analyzed using ITQ 900 GC-MS (Trace 1310 GC, Thermo Scientific, USA) system with a quadrupole ion trap mass analyzer supplied with split/splitless injection autosampler (Thermo Scientific AI/AS 1310). A DB-5 type 5% phenyl–95% methyl polysiloxane fused- silica capillary column, DB-5MS (30 m x 0.25 mm x 0.25 um, Agilent Technologies, USA), non-polar, low bleed, and with high-temperature limits (up to 350 ^{o}C), was chosen for testing sterol samples. The helium carrier gas was used at a flow rate of 1.2 mL/min. The oven temperature was initially set at 100 ^{o}C for 2 mins, then gradually raised to 267 ^{o}C at 40 ^{o}C /min rate and held for 40 mins. The injection was spitless at 280 ^{o}C. The ion source temperature was set at 230 ^{o}C, and the transfer line was at 290 ^{o}C. No calibration ranges were needed, as all samples have an internal standard (α-cholestanol). Sterols were identified by comparing the retention time of the obtained peaks with the retention time of IOC reference peaks (similar experimental conditions were executed).

Sterol relative amounts (%) were expressed with respect to the internal standard and proportions of total sterols. The apparent β-Sitosterol was calculated as the sum of β-sitosterol, Δ^5-avenasterol, clerosterol, sitostanol, and $\Delta^{5,24}$-stigmastadienol.

2.5 Volatile Compounds

Volatile compounds of olive oil samples were analyzed using FGC E-nose Heracles II (AlphaMos, Toulouse, France), equipped with two columns: a non-polar column (MXT5: 5% diphenyl, 95% methylpolysiloxane, 10 m length and 180 mm diameter) and a slightly polar column (MXT1701: 14% cyanopropylphenyl, 86% methylpolysiloxane, 10 m length and 180 mm diameter).

500 µL of olive oil was placed in a 20 mL vial and sealed with a cap. The vial present in Heracles' auto-samples was then transferred to a shaker oven for 15 min at 80 ^{o}C, shaken at 500 rpm. Next, a syringe pierced the cap's silicone septum and sampled 5 mL of the headspace, which were then adsorbed on a CARBOWAX trap, maintained at 30 ^{o}C for 36 s, while the carrier gas (H_2) flowed through it to concentrate the analytes by removing excess air and moisture. The analytes were then desorbed by increasing the trap's temperature to 240 ^{o}C in 90 s, and the sample was injected. The thermal program started at 40 ^{o}C (held for 2 s) and increased to 280 ℃ at 4 ^{o}C /s. The final temperature was held for 21 s. The total separation time was 100 s. At the end of each column, an FID detector was placed, and the acquired signal was digitalized every 0.01 s.

For calibration, an alkane solution (from n-hexane to n-hexadecane) was used to convert retention time in Kovats indices and identify the volatile compounds using specific software (AroChemBase, Alpha MOS, France).

2.6 Statistical analysis

Statistical analysis was performed using Matlab version R2015b (The Mathworks Inc., MA, USA) using the SAISIR package (Cordella & Bertrand, 2013), and the ANOVA results are presented as means ± standard deviation. Tukey's test was used to establish the significance of difference, at a 5 % level among means, for each parameter taking harvest date into account. A model based on principal component analysis (PCA) was developed to study the harvest period's influence on volatile compounds.

3. Results and Discussion

3.1 Quality Parameters and Total Polyphenol Content

The results illustrated in Table 1 show the fundamental differences in the analyzed physicochemical parameters between olive fruits harvested in October and those in November. The quality parameters of olive oils (acidity and peroxide value) were found to be significantly different during the two notable harvest dates in Lebanon. On average, acidity values were 0.31% and 1.21 % for HD1 and HD2, respectively, exceeding the limit established by the International Olive Council (IOC) for oils to be considered extra virgin (0.8 %) for the latter. The observed increase in November corroborates with other authors (Youssef et al., 2010; Fuentes et al., 2013). It can be explained by the increase in lipolytic activity as the olives become more sensitive to mechanical damage and pathogenic infections (Anastasopoulos et al., 2011).

Peroxide value of olive oils harvested in November showed a significant increase of about 9.34 meq O_2/kg compared to those picked in October. However, the delay in harvesting olive fruits should show a lower peroxide level due to the decrease in lipoxygenase activity (Salvador et al., 2001; Alowaiesh et al., 2016). The significant increase observed in HD2 was unexpected and required further examination. Although an increase was noticed, all olive oils presented peroxide values that did not exceed the maximum acceptable limit for their classification as extra virgin olive oils (< 20mEq O_2/kg).

K232 and K270 are the markers of olive oil alteration. K232 is in direct relation with poly-unsaturated fatty acids and peroxide value, whereas K270 relates to aldehyde and ketone substances. As the harvest date is delayed, the value of K232 increases, while K270 decreases (Hamidoghli et al., 2008). However, a study by Bengana et al., (2013) showed an inverse pattern as olive fruit ripens, while in other studies, both indicators decreased with maturity and depended on olive cultivar and harvest date. In our case, a decrease in K232 and an increase in K270 were observed for the studied Soury variety shown in Table 1. All studied samples had K232 and K270 values consistent with IOC's limit for extra virgin olive oil.

Phenolic compounds are of great organoleptic and nutritional interest. They are associated with the taste and the oil's stability due to their antioxidant properties (Maga, 1978; Nergiz & Ünal, 1991). As the harvest time is delayed, oleuropein, the main bitterness-producing component in olives, progressively decreases (Amiot et al., 1986; Amiot et al., 1990). This was confirmed by our results (Table 1), as the total polyphenol content observed for HD1 was significantly higher than that for HD2. These findings suggest a decline in the oil's oxidative stability as harvesting is delayed (Gutiérrez et al., 1999; Trentacoste et al., 2020). The observed decline as ripening advances may be attributed to the polyphenol oxidase that dominates the final stage of the ripening process and to the fusion of the phenolic acids into the cell walls as a fruit defensive mechanism against pathogens (Amira et al., 2012; Rodríguez et al., 2016).

Table 1. Quality parameters and total polyphenol content for both groups (HD1 and HD2)

	Acidity † (g/100g)	PV ‡ (meq O_2/kg)	K232	K270	Total polyphenols (mg/mL)
HD1	0.31 ± 0.06b	3.10 ± 1.06b	1.81 ± 0.22b	0.17 ± 0.04a	138.31 ± 43.44a
HD2	1.21 ± 0.37a	12.44 ± 4.54a	2.04 ± 0.30a	0.15 ± 0.04b	44.08 ± 21.34b
EVOO*	≤0.8	≤20	≤2.5	≤0.22	-

All determinations were carried out in duplicate. Different letters within the same column show significant differences (Tukey's test, $p \leq 0.05$). † Calculated as oleic acid; ‡ Peroxide value; *Standards of IOC for extra virgin olive oil (EVOO)

3.2 Fatty Acid Composition

Fatty acids are the major constituents of olive oil and are an essential factor in determining the authenticity and the quality of olive oil (Essiari et al., 2014). Changes in the fatty acid composition are presented in Table2. The major fatty acids included in this study are palmitic, oleic, and linoleic acid. The minor fatty acids are palmitoleic, stearic, linolenic.

Among fatty acids, oleic acid was high in HD1 and showed a slight decrease in HD2. On the contrary, linoleic acid concentration significantly increased from 9.09 % to 11.09 % (El Qarnifa et al., 2019). This increase may be related to the activity of oleate desaturase. Oleate desaturase is an enzyme responsible for the desaturation of Oleoyl-ACP (precursor of longer-chain unsaturated fatty acids) into linoleate-ACP, which might be the reason behind the notable difference in the oleic and linoleic concentrations between these two groups. This enzyme has been extensively discussed in seed oils; however, the information regarding the enzyme activity in olive oil is still limited (Hernandez et al., 2011). Another factor for the existing difference between HD1 and HD2 is the continuing biosynthesis of triglycerides as the harvesting date is delayed (Flamini, 2010; Guiterrez, 1999). Lodolini et al. (2017) have also reported that the Soury variety should be harvested early. The postponement of olive fruit harvest has a detrimental effect on olive oil's fatty acid composition, mainly oleic and linoleic content.

All fatty acids were within the acceptable limit for their classification as extra virgin olive oils except for palmitic acid in both HD1 and HD2, which may be a characteristic of the Soury variety and, further investigation is required.

Table 2. Mean values ± standard deviation of fatty acids (%) composition of Lebanese olive oil at two harvest dates (October and November)

	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic
HD1	10.71 ± 1.37a	0.50 ± 0.17a	3.70 ± 0.71a	73.79 ± 2.20a	9.09 ± 1.53b	0.59 ± 0.07a
HD2	11.28 ± 1.3a	0.58 ± 0.17a	4.01 ± 0.5a	70.86 ± 2.34b	11.09 ± 1.45a	0.63 ± 0.08a
EVOO*	2.0-7.5	0.3-3.5	0.5-5	55-83	3.5-21	≤ 1

All determinations were carried out in duplicate. Different letters within the same column show significant differences (Tukey's test, $p \leq 0.05$). *Standards of IOC for extra virgin olive oil (EVOO).

3.3 Sterol Content

The sterol content of olive oil samples for the two harvesting periods is recorded in Table3. The sterols with the highest amounts were β-sitosterol, Δ^5-avenasterol, and campesterol representing more than 90% of total sterol content, whereas other sterols like stigmasterol, clerosterol, sitostanol, $\Delta^{5,24}$-stigmastadienol, Δ^7-stigmastenol, Δ^7-avenasterol and two triterpene dialcohols (erythrodiol and uvaol) were within small amounts.

The total sterol content of HD2 exceeded the 1000 mg/kg threshold for HD2, as shown in Table 3. This result is in agreement with Lukić et al., (2013), who stated that the levels of sterols continue to increase until the olive fruit is ripened. Apparent β-Sitosterol of both groups was higher than 93% as determined by the EU regulations.

The mean relative amount of β-sitosterol was mainly found to decrease, while those of stigmasterol, $\Delta^{5,24}$-stigmastadienol, Δ^7-stigmastenol, and Δ^7-avenasterol increased as per previous studies (Camera et al., 1975; Koutsaftakis et al., 1999; Salvador et al., 2001; Vekiari et al., 2010). The percentage of clerosterol was relatively stable, which agrees with the findings of Lazzez et al., (2008). The same was observed for campesterol, sitostanol, Δ^5-avenasterol, and erythrodiol + uvaol.

Concerning the amount of β-Sitosterol, representing the most copious compound in the sterolic fraction, the maximum relative amount (about 91.49%) was observed for HD1, whereas the lowest content was about 89.67% for HD2. The Δ5-avenasterol, the second most abundant sterolic compound, reached 4.47% during the maturity process (HD2). Several authors had found a negative correlation between β-sitosterol content, Δ^5-avenasterol and Δ^7-avenasterol in different varieties (Koutsaftakis et al., 1999; Fernández-Cuesta et al., 2013; Lukić et al., 2013; Yorulmaz et al., 2013; Noorali et al., 2014). They stated that β-Sitosterol is minimum and Δ^5-avenasterol and Δ^7-avenasterol is maximum when olives are obtained at their optimum maturation stage. In this study, a decrease in β-Sitosterol content, and an increase in Δ^7-avenasterol was observed. This complements the results obtained by the latter authors. However, Δ^5-avenasterol content showed no significant differences between HD1 and HD2 in disagreement with the same research.

The third sterolic compound, the campesterol, reached the highest of 2.10% for HD2. Our samples presented low levels of this sterol (less than the 4% limit value), and no significant difference was observed between HD1 and HD2. As for the stigmasterol, the authors found its high content to be an indicator for lower olive oil/olive fruit quality (JM et al., 1996; Koutsaftakis et al., 1999; Gutiérrez and Fernández, 2002; Temime et al., 2008). This is in agreement with the results shown in Table 1 and Table 3. HD2 (VOO) has a lower quality than HD1 (EVOO) and thus higher stigmasterol content. The campesterol/stigmasterol ratio, another parameter classified as a quality index of olive oils (Koutsaftakis et al., 1999), also decreased as the harvest date is delayed. The Δ^7-Stigmastenol and $\Delta^{5,24}$-stigmastadienol witnessed a significant increase in HD2. The primary factor of this increase is the delay in the harvest. Erythrodiol + uvaol content in all the oil samples studied was below the limit of 4.5% and was not affected by the postponement of harvest time.

Table 3. Relative amounts (%) of sterols and triterpene diols determined in olive oils at two harvest dates (October and November)

	HD1	HD2	IOC standards
Relative amount (%)			
Cholesterol	0.05a ±0.02a	0.04 ±0.02a	≤0.5
Campesterol	2.06 ±0.83a	2.10 ±0.74a	≤4
Stigmasterol	0.44 ±0.29b	0.72 ±0.55a	<campesterol
Campesterol/Stigmasterol ratio	5.20 ±1.29a	3.56 ±1.52b	
Clerosterol	0.69±0.24a	0.71 ±0.20a	
β-Sitosterol	91.49 ±3.51a	89.67 ±3.33b	
Sitostanol	0.15 ±0.09a	0.15 ±0.13a	
Δ^5-Avenasterol	3.99 ±2.04a	4.47 ±2.21a	
$\Delta^{5,24}$-Stigmastadienol	0.35 ±0.20b	0.50 ±0.25a	
Δ^7-Stigmastenol	0.35 ±0.20b	0.69 ±0.38a	≤0.5
Δ^7-Avenasterol	0.49 ±0.37b	0.71 ±0.40a	
Apparent β-Sitosterol	96.59 ±1.48a	95.52 ±1.69a	≥93
Erythrodiol + Uvaol	2.55 ±1.21a	2.18 ±1.07a	4.5%
Total sterols (mg/Kg)	894.40 ±211.84b	1077.48 ±327.7a	≥1000

Results are denoted as mean value ±standard deviation of two replicates. Different letters in the same row show significant differences (Tukey's test, $p \leq 0.05$).

3.4 Volatile Compounds

FGC was carried out to detect the volatile compounds that may arise due to the olive fruit harvest delay, particularly the Soury variety.

A PCA model with 21 olive oil samples composing the HD1 group and 42 olive oil samples, belonging to the group HD2 and 10201 data points (constituting the flash GC chromatogram), i.e., an initial X data matrix of 63 x 10201 was conditioned. The initial chromatograms were corrected for misalignments before any further analysis using correlation optimized warping (COW) (Nielsen et al., 1998). Data were then normalized using standard normal variate (SNV) (Zeaiter & Rutledge, 2009).

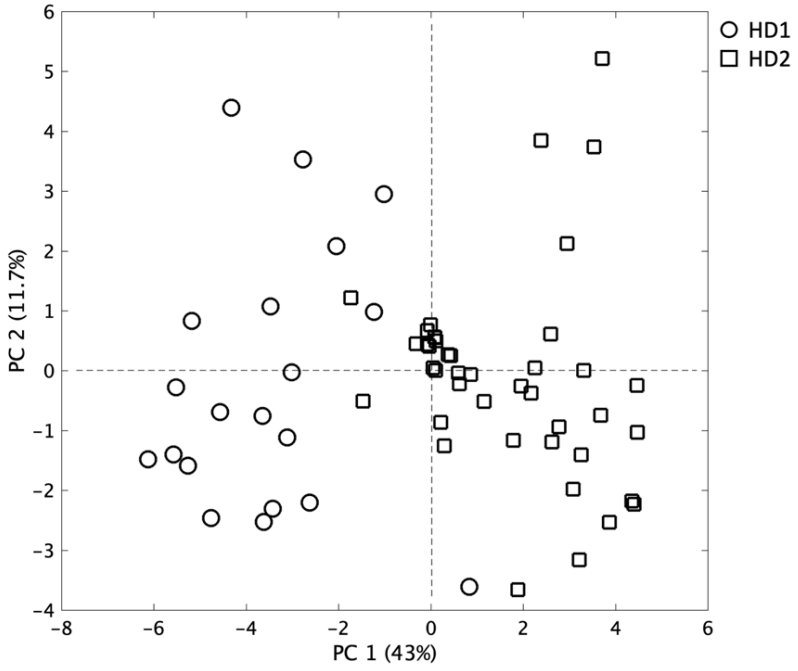

Figure 1. PCA scatter plots of the two groups (HD1 and HD2) obtained by analyzing the flash-GC chromatogram

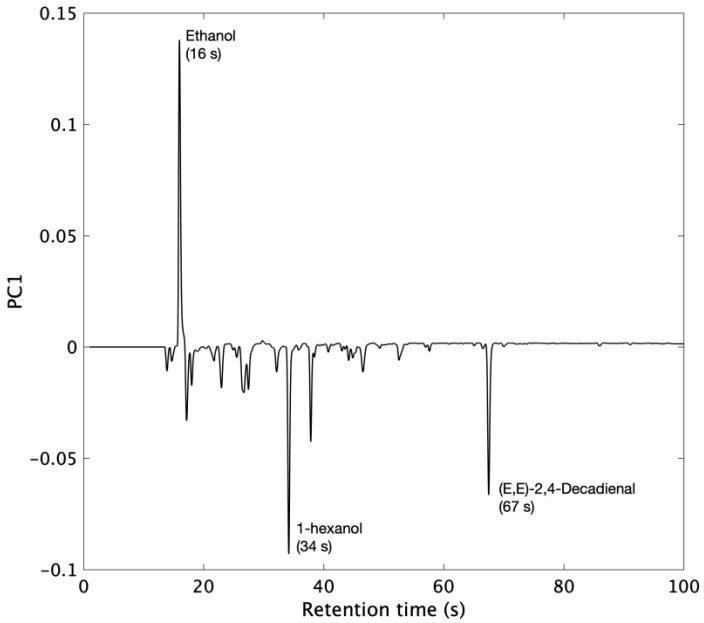

Figure 2. Loading plot showing the peaks differentiating HD1 from HD2

Six principal components (PCs) were extracted, covering 86.5 % of the total variance, where the first two components accounted for 54.7 % of the variability in the data set. The first two PCs were plotted, showing a good differentiation between HD1 and HD2 (Figure 1). PC1 clearly shows the discrimination between HD1 and HD2. One-way ANOVA was also conducted on PC1 scores as an extra measure of certainty for the PCA results. A significant difference was also noted between HD1 and HD2 ($p \leq 0.05$), as shown in Figure 3. In comparing HD1 and HD2, three peaks are identified as the determinant factor in separating the two latter groups (Figure 2). The first peak is ethanol, belonging to one of the major volatile olive oil compounds, i.e., alcohol. The Kovats indices (KI) for this peak were identified through AroChembase software and compared with KI available in the literature (Table 4).

Table 4. Olive oil volatile compounds based on Kovats indices (KI) calculated on retention time (RT) for two columns

RT DB5 (sec)	RT DB1701 (sec)	KI (DB5)	KI (DB1701)	Possible chemical candidate	Reference
16	39	440	883	Ethanol	(Ivanova-Petropulos et al., 2015) (Silva et al., 2012)
34	59	835	1340	1-hexanol	(Silva et al.. 2012) (Brkić Bubola et al., 2012) (Ben Mansour et al., 2017) (Pouliarekou et al., 2011)
67	92	1320	1730	(E,E)-2,4 -Decadienal	(Silva et al.. 2012) (Kesen et al., 2013) (Reiners and Grosch, 1998)

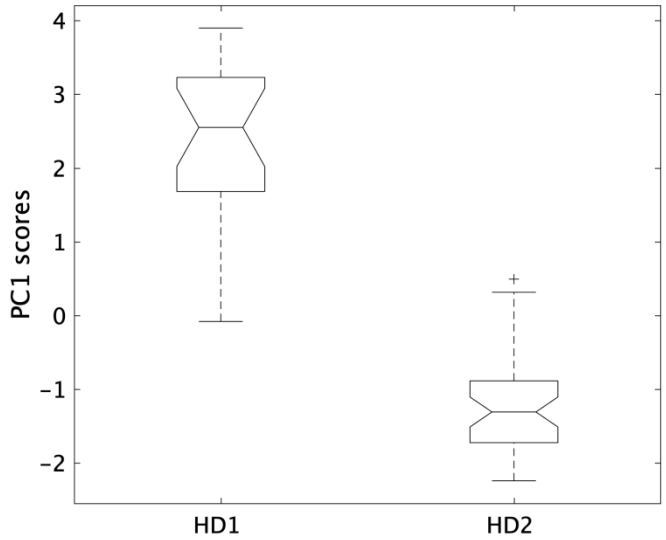

Figure 3. Boxplot showing the discrimination of both groups HD1 and HD2 ($p \leq 0.05$)

Ethanol is a major component, which is related to the fermentation activity occurring before olive oil extraction (Beltrán et al., 2015), and it is responsible for the sensory descriptor "Alcohol" (Morales et al., 2005). The possible source of ethanol can be from the olive fruit itself. Beltrán et al., (2015) showed that ethanol content increase during the ripening process. This increase is directed by alcohol dehydrogenase activity, where its levels were high due to advanced stages of maturation. The remaining two high-intensity peaks, mostly characterizing HD1, are 1-hexanol and (E, E)-2,4-decadienal. 1-hexanol appears in HD1 as a sign of unripe olives (Aparicio and Morales, 1998). It is known that at a later harvest date (HD2), the aromatics, like 1-hexanol, describing the term "fruity-grassy," are lower while other negative aromatic notes increase like ethanol (Salvador et al., 2001). As for (E, E)-2,4-decadienal, its presence in HD1 may be due to the long incubation period of olive oil just before injection into the FGC, which led to such a volatile compound. This peak is also present in HD2; however, its presence is hidden by the ethanol high-intensity peak (Figure 4).

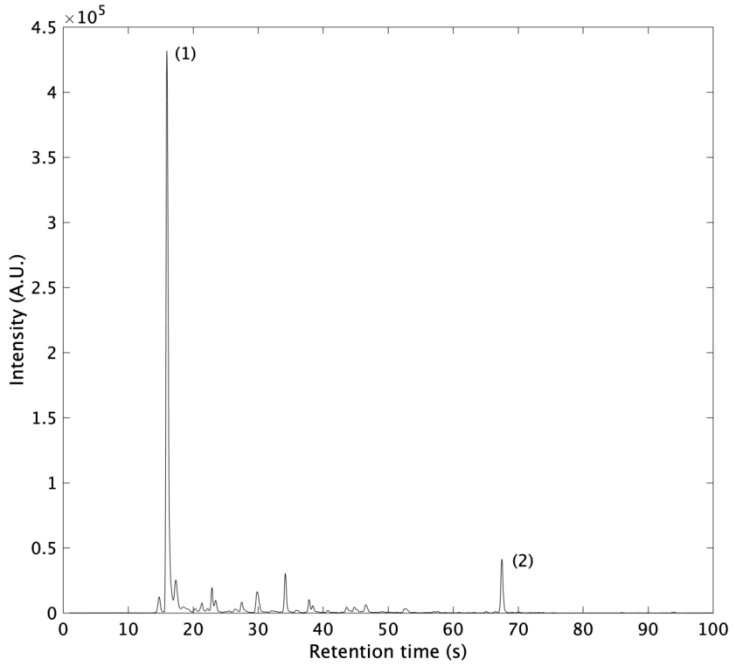

Figure 4. Flash GC chromatogram of olive oil sample belonging to HD2. (1): Ethanol; (2): (E, E)-2,4-decadienal

This is backed up by several studies whose results indicate the presence of (E, E)-2,4-decadienal in seed oil after applying heat (Andrikopoulos et al., 2004; Boskou et al., 2006).

4. Conclusion

The harvest date appears to have a decisive role in the qualitative characteristics, saponifiable and non-saponifiable fraction of Lebanese olive oil. All results obtained showed that the harvest date strongly influenced acidity and total polyphenols. Besides, a change in the fatty acid profile characterized by a higher linoleic and lower oleic content, an increase in Δ^7-stigmastenol exceeding the limit set by the IOC standards, and a dominating aromatic compound (ethanol) was noticed for olive fruits picked at a later date

Generally, it can be concluded that the harvest from mid-October to the first of November could be a criterion to obtain olive oil of the highest quality grade. Harvesting at this period is preferred for the Soury variety, as it ripens most of the macro and minor components exceed the limits set by the international olive council for extra virgin olive oil. Nonetheless, palmitic acid was the only compound to exceed the limit regardless of the harvest date, and further investigations are required to determine the possible cause of such high content.

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