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New derivatives of phenolic compounds as index of olive oil quality

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ABSTRACT

The purpose of this study was to carried out identify of some bioactive derivatives of phenolic compounds as evidence of the quality of olive oil. Olive oil extracted from olive fruits (Coratina and Picual varieties) during season 2017/2018 at two stage ripening (mid. October and mid. December). Moisture and oil contents (%) in olive fruits were determined. Some Physicochemical properties (refractive index, color index free fatty acids, peroxide value, UV absorbance at 232 and 270 nm and ΔK were determined. Identify of fatty acids composition by GC were determined. Oxidative stability, total polyphenols, tocopherols, organoleptic evaluation and same bioactive derivative by NMR of olive oil extracted from all samples were studied. All results indicated that there were a wide variation in the chemical and characteristics of all olive oils samples. Also, the results showed clear differences in phenolic content, oxidative stability and phenolic compounds between olive oil samples. Using the NMR was identified of some bioactive compounds in different in olive oil samples.

Keywords: Olive oil, quality indices, bioactive components, oxidative stability, NMR.

1. INTRODUCTION

The olive tree (*Oleaeuropaea L.)* is known the oldest cultivated tree in the world (Ozbek,1975) and it has been widely cultivated in Southern Europe and played a significant role in the early civilizations of Egypt and Greece (Zamora, *et al.*,2001). Olive trees are distributed all continents, 98% of the world production of olive is concentrated in the Mediterranean basin countries (Mora, *et al*., 2007). Its antioxidant capacity is stable due to its high monounsaturated fatty acid content with low polyunsaturated fatty acid content andthe presence of natural antioxidants such as phenols, tocopherols and carotenoids. The fatty acid composition, especially the MUFA (monounsaturated fatty acid) content, and the natural antioxidants provides advantages for health Boskou, (1996); Kiritsakis, (1998); Diraman, and Dibeklioglu (2009). The phenolic compounds in olive oil are secondary metabolites that arise through the conversion of complex substances produced by olive trees, and they can be classified as lignans, flavonoids and secoiridoids. Virgin olive oil contains atleast 30 different phenolic compounds (Bendini *et al.,* 2007). The most common lignans in olive oil are pinoresinol, acetoxypinoresinol and hydroxyl pinoresinol (Owen *et al.*, 2000) and the most common flavonoids are luteolin and apigenin Pinelli, *et al*., (2003). While lignans and flavonoids are also in other foods, such as wine, secoiridoids are specific for oliveoil (Ryan*et al*., 2002 and Montedoro *et al*., 1993). The two main secoiridoids in olive oil are ligstroside and oleuropein, and their conversion products give olive oil its unique aroma and taste. During the olive-pressing process or if the drupes are injured, ligstroside and oleuropein in the fresh drupes can enter different transformation-reaction pathways, such as their enzymatic and chemical transformation to aldehyde or hydroxy forms (Rovellini and Cortesi 2002). Some studies have suggested that secoiridoid derivatives of hydroxytyrosol are the main contributors to olive oil bitterness (Bendini *et al*., 2007). Caponio, *et al*., (2001) showed that the bitter to pungent taste can be ascribable to oleuropeinaglycon. Furthermore, oleuropein and its aglycon decrease as the ripening of olives progresses. Rotondi, *et al.,*(2004) confirmed the relationship between the decrease in bitterness and pungency and the reduction in total phenols and diphenol levels. In particular a positive correlation between the content of oleuropein and ligstroside derivatives and the bitterness and pungency was shown. Frank,*et al.,* (2001) reported that when an isomer (or isomers) of oleuropeinaglycon was prepared by β- glucosidase hydrolysis of oleuropein isolated from olives and evaluated by assessors, it was defined as bitter. Using the same evaluation technique, no bitterness was observed for hydroxytyrosol or elenolic acid according to Andrewes, *et al.,* (2003). The dialdehyde form of decarboxymethylligstrosideaglycone (p-HPEA-EDA) is the key source of the pungent sensation found in olive oil, while 3,4-DHPEA-EDA produces very little burning sensation. Moreover, Beauchamp *et al*., (2005) assessed the pungent intensity of p-HPEA-EDA isolated from different virgin olive oils, and confirmed that p-HPEA-EDA isthe principal agent responsible for throat irritation. Gutierrez-Rosales, *et al*., (2003) concluded that the chromatographic peaks corresponding to 3,4-DHPEA-EDA, oleuropein-aglycone mono-aldehyde (3,4-DHPEA-EA) and p-HPEA-EDA aremainly responsible for the bitter taste of virgin olive oil. Overall, some phenols mainly define the bitterness of olive oil, while others define the perception of pungency, and these might be related to the olive variety. The aim from recent study is to determination of quality indices and quality attributes of olive oil extracted from Coratina and Picual varieties at two ripening stage. And also identification of new bioactive components in olive oil by using NMR device.

2. MATERIAL AND METHODS

Materials

Source of olive fruits: Two varieties of olive fruits, i.e., Coratina, and Picual were obtained from a private farm at El-Khtatba, Giza Governorate, Egypt. All varieties were collected by hand at the mid. of October and December during the crop season 2017/2018. Only healthy fruits, without any kind of infection or physical damage were processed.

Reagents, solvents and standards: All solvents in this study were purified and distilled before use. Folin-Ciocalteau reagent was obtained from Gerbsaure Chemical Co. Ltd., Germany.

Methods

Oil extraction: After harvest, fresh olives (1.5-2.0 kg) were washed and deleafed, crushed with mill and pressed using hydraulic laboratory (Carver) press. Oil produced from each extraction was 200-250 ml/kg, filtered then transferred into dark glass bottles and stored in the dark at 4˚C until analysis.

Quality parameters: Acidity, peroxide value and UV absorption characteristics, K232nm (conjugated dienes) and K270nm (conjugated trienes) and ΔK [ΔK = k270 – (k266-4) + (k274+4)/2] were carried out following the analytical methods described by A. O. A. C. (2012) and EEC 2568/ (1991).

Oil stability: Oxidative stability was evaluated by the Rancimat method (Gutierrez and Dobarganes, (1988). Stability was expressed as the oxidation induction time (h), measured with the Rancimat 679 apparatus (Metrohm Co., Herisou, Switzerland), using 5.00 g oil heated to 100˚C ± 2˚C with an air flow of 20 l/hr^{-1}.

Total phenolic content: Total phenol content was calorimetrically quantified (Ranalli, *et al*., 1999). Phenolic compounds were isolated by triple extraction of a solution of oil (10 g) in hexane (20 ml) with 30 ml of a methanol-water mixture (60:40, v/v). The Folin-Ciocalteau reagent was added to a suitable aliquot of the combined extracts, and the absorption of the solution at 725nm was measured. Values are given as milligrams of gallic acid per kilogram of oil (Gutfinger, 1981).

Fatty acid composition: The fatty acid methyl esters were prepared as described in the International Olive Council (IOC, 2018). Methyl esters were prepared from olive oil, after saponification and analyzed by gas chromatography (Pye-Unicam model 104) equipped with flame ionization detector and glass coiled column (1.6 m X 4 mm) supported on chromosorb (W-AW 100-200 mesh), was used. The samples (µl) were injected into the column using a Hamilton microsiringe. The gas chromatographic conditions for isothermal analysis were: temperatures: column 170˚C detector 300˚C and injector 250˚C, flow rates: hydrogen 33 ml/ min., nitrogen 30 ml/ min and air 330 ml/ min. Peak areas were measured using a spectra physics chronjet integrator.

Organoleptic test: The organoleptic test was determined for the extracted oil according to the International Olive Council (IOC, 2018). The oil samples (15 ml) were presented in covered blue glasses (diameter, 70 mm, capacity, 130 ml) at 28˚C ± 2˚C. The glass warmed and after removing the cover, the samples were smelled then tested by the panelist to judge its flavour. The different attributes of the oils were assessed and their intensities were evaluated as a mean value of the panelists score.

NMR Spectral Analysis: The olive oil samples was dissolved in CDCl3 (750 µL) and an accurately measured volume of the solution (550 µL) was transferred to a 5 mm NMR tube. 1H NMR spectra were recorded at Varian400spectrometer with advanced capillary tube system. Typically, 50 scans were collected into 32K data points over a spectral width of 0−16 ppm with a relaxation delay of 1 s and anacquisition time of 1.7 s. Prior to Fourier transformation (FT), anexponential weighting factor corresponding to a line broadening of 0.3Hz was applied. The spectra were phased corrected and integrated automatically. When necessary, accurate integration was performed manually for the peaks of interest.

3. RESULTS AND DISCUSSION

Chemical composition of olive fruits

Table 1 show that the chemical composition of Coratina and Picual olive fruits during two stages of ripening. All varieties contained more than 50% moisture content. The Picual fruits contained 63.17% moisture at Mid-December, but the Picual fruits at Mid-October had low moisture content (60.11%). On the other hand, the Coratina fruits were recorded highest value in moisture content (56.01%) at Mid-December than at Mid-October (51.00%). Generally, the Picual fruits recorded highest value in moisture content than of the Coratina fruits during two stages ripening. These results are in agreement with those reported by Yorulmaz, *et al.,* (2013). On contrast, the oil content in Coratina fruits were Highest level at Mid-October (30.44%), when the oil content of Picual fruits were recorded high level (38.81%) at Mid-December than at Mid-October. These results are in agreement with those reported by Keceli, (2013).

Table 1 Moisture and oil content (%) of Coratina and Picual olive fruit varieties

Ingredients	Coratina		Picual	
	Mid. October	Mid. December	Mid October	Mid December
Moisture content (%)	51.00	56.01	60.11	63.17
Oil content (%)	30.44	34.65	28.57	38.81

Some Physical and chemical properties of olive oils

Table 2 shows that the olive oil quality indices extracted from Picual and Coratina fruits during two stages of ripening. The color of all samples found that yellow (35) but the red color found that (2.4, 2.00) of Coratina, (2.00 and 1.00) of Picual and (2.8) of local market and the blue color found that (1.1, 0.6) of Coratina, (1.00, 0.5) of Picual and (4.4) of local market respectively. Refractive index of virgin olive oils ranges from 1.4677 to 1.4705 according to Codex (2001). Therefore, our results for refractive index were in good agreement with the fact that olive oil used in this present study should be considered as virgin olive oil. Refractive index for olive oils obtained from the two studied olive varieties, Coratina and Picual during two stages of ripening and local market were (1.4668, 1.4676) , (1.4672, 1.4671) and (1.4679) at 25°C, respectively. Table (2) shows that free fatty acid content (%as oleic acid) found that (0.14, 0.16), (0.13, 0.17) and (1.00) for Coratina and Picual during two stages of ripening and local market, respectively. The Peroxide values were (3.98, 5.19 meq/kg), (4.00, 6.81 meq/kg) and (7.11meq/kg) respectively. On the other hand, the absorbance at K232nm, K270nm, and ΔK values found that (1.37, 1.55, 1.42, 1.77 and 2.81), (0.032, 0.078, 0.039, 0.088 and 0.56) and (-0.0051, -0.0031, -0.0029, -0.0013 and 0.205) for all olive oil samples. Table (2) show very low values for the classical physico-chemical parameters (acidity ≤ 0.80; peroxide value ≤ 20.00 meq.O2/kg; K232 ≤ 2.50; K270 ≤ 0.22 and ΔK ≤ 0.01) and the values were falling within the "extra virgin" category, as stated by IOC (2018) except local market is classified as virgin olive oil.

Table 2 Physical and chemical properties of olive oils during ripening stages

Properties		Coratina Mid October	Coratina Mid December	Picual Mid October	Picual Mid December	Commercial sample
Color index	Yellow	35	35	35	35	35
	Red	2.4	2.00	2.00	1.00	2.8
	Blue	1.1	0.6	1.00	0.5	4.4
Refractive index at 25°C		1.4668	1.4676	1.4672	1.4671	1.4679
Acidity (% as oleic acid)		0.14	0.16	0.13	0.17	0.38
Peroxide value (meq.O_2/kg oil)		3.98	5.19	4.00	6.81	7.11
UV Absorbance at K232		1.37	0.22	1.42	1.77	1.81
UV Absorbance at K270		0.032	0.078	0.030	0.088	0.56
ΔK		-0.0051	-0.0031	-0.0029	-0.0013	0.205

Fatty Acid Composition

The tabulated data in Table (3) noted that there were remarkable differences among the studied samples. It could be observed that the highest level of total saturated fatty acids was recorded in Coratina samples at Mid-October (19.71%) of the total fatty acids, followed by Picual sample at Mid-October (18.74%), while, the lowest level of total saturated fatty acids was recorded in Coratina sample at Mid-December (14.81%). In the same context, the highest level of total monounsaturated of fatty acids was found in Picual sample at Mid-December (75.29%), followed by commercial sample (73.80%) and the lowest level of total monounsaturated fatty acids was found in Coratina samples at Mid-December (71.39%. On the other hand, the highest level of total polyunsaturated fatty acids was found in Coratina sample at Mid-December (13.80%) followed by commercial sample (11.10%) and the lowest valued of total polyunsaturated fatty acids was found in Picual sample at Mid-October (5.61%). From the results in Table (3) the main saturated fatty acids in all olive oil samples under study was palmitic acid. The highest levels of unsaturated fatty acids were oleic acid in all olive oil samples.

As shown in Table (3), 11 fatty acids were detected in the studied extra virgin olive oils. In general, the distribution of most of the fatty acid composition covered the normal ranges indicated by IOC, (2018). Regulations with a minor exception that could have been due to the harvest year or genetic factors Cicerale, *et al.,* (2010). Palmitic, oleic, and linoleic acids were predominant in the studied olive oils; the other fatty acids occurred in small amounts. The distribution of fatty acids, from all olive oil samples extracted from Coratina and Picual fruits during two ripening stages, As shown in Table (3), palmitic acid(15.23,12.43, 16.99,14.26 and 12.22, respectively) and oleic acid (72.65, 70.39, 72.86,70.54 and 69.00 respectively) and linoleic acid (5.45,12.91, 4.59,6.94 and 11.85, respectively). These results are similar with those reported by Keceli (2013) and Yorulmaz, *et al.,* (2013).

Table 3 The relative percentage of fatty acids of olive oils during ripening stages

Name of Fatty acids	Coratina		Picual		Commercial samples
	Mid October	Mid December	Mid October	Mid December	
C16:0	16:23	12.43	16.63	14.26	12.22
C16:1	2.80	0.39	2.00	2.43	0.57
C17:0	0.05	0.04	0.04	0.05	0.05

C17:1	0.07	0.07	0.08	0.11	0.07
C18:0	3.11	1.80	1.92	3.72	2.19
C18:1	69:00	70.39	72.86	70.54	72.65
C18:2	7.10	12.91	4.59	6.94	10.20
C18:3	1.10	0.89	1.02	1.10	0.90
C20:0	0.34	0.42	0.04	0.50	0.50
C20:1	0.20	0.54	0.35	0.23	0.51
C22:0	0.00	0.12	0.11	0.12	0.14
Σ SFA	19.73	14.81	18.74	18.65	15.10
Σ MUFA	72.07	71.39	75.29	73.31	73.80
Σ PUFA	8.20	13.80	5.61	8.04	11.10

Organoleptic test

Sensory analysis of oil, meaning the official organoleptic assessment of olive oil respectively the panel test (PT), relies on the standards of the International Olive Council (IOC), furthermore as on the Regulation of the European Commission (EC). These regulations lead to the classification of oil as extra virgin (EVOO), virgin (VOO) or Lampante, that but isn't comfortable to obviously discriminate between totally different quality levels inside the grade EVOO. Sensory evaluation of olive oil extracted from Coratina, Picual fruits during two ripening stages (mid. October and mid. December) and commercial olive oil samples were evaluated by 10 panelists (Table 4). From a sensory point of view all the samples examined are belong to the extra virgin olive oil grade. The direct observation of the intensities of attributes detected by tasters showed that the oils studied were mainly characterized by high intensities of fruity, bitter, and pungent. Data in table (4) shown that the sensory attributes in Coratina and Picual samples at Mid-October had high levels more than Coratina and Picual samples at mid. December. These results are similar with those reported by Arafat and Ahmed (2011), Lazzez*et al.,* (2011) and Sousa *et al.,* (2014).

Table 4 The organoleptic characteristics of olive oils extracted from Coratina and Picual varieties at two ripening stages.

Varieties	Ripening stages	Perception positive attributes		
		Fruity	Bitter	Pungent
Coratina	Mid October	6.9	7.40	5.60
	Mid December	3.8	3.00	3.50
Picual	Mid October	5.2	4.10	3.70
	Mid December	0.4	0.50	0.50
Commercial sample		7.3	7.20	5.40

Oxidative stability and phenolic content

Phenolic compounds are very important for the stability of olive oil. The total phenolic content for extracted oil from Coratina and Picual fruits at two ripening stages and commercial samples are shown in Table (5). The phenolic content decreased with olive ripeness. The high level in phenolic content was found in commercial samples 410.00 mg/kg, followed by Coratina sample at Mid-October (400.11 mg/kg). On the other hand, the lowest level in phenolic content was found in Picual sample at Mid-December (121.66 mg/kg). It can be observed that the phenolic content showed a correlation with the degree of ripening. The extra virgin olive oil contains a considerable amount from phenolic compounds that are responsible for its peculiar taste for its high stability.

Oxidative stability is an important for olive oil. Data in Table (5) shown that the commercial sample had the highest oxidative stability (26,3hr). On the context, the oxidative stability of Coratina sample was (17.40hr) at Mid-December. On the other hand, the oxidative stability of Picual samples at Mid-December is lowest levels (12.80hr). From the previous results it was found that there was a relationship between phenol content and oxidative stability of all samples. Studies show that oxidative stability increases with the increase of phenol content in oil.

Table 5 Oxidative stability (hr) and phenolic content (mg/kg) of olive oil extracted from Coratina and Picual varieties at two ripening stages.

varieties	Ripening stages	Oxidative stability (hr)	Phenolic content mg/kg
Coratina	mid October	17.4	400.11
	mid December	15.4	205.01
Picual	mid October	16.2	215.33
	mid December	12.8	121.66

Commercial sample	26.3	410.00

Phenolic compounds

Phenolic compounds play important role in the quality of olive oil and affect its stability, taste and flavor. High-performance liquid chromatography (HPLC) was used for the identification and quantitative analysis of polyphenolic compounds of Coratina, Picual olive oil samples during two stage of repining and commercial samples. Table (6) presents the composition of polyphenolic compounds of olive oil extracted from Coratina and Picual olives and commercial olive oil sample. The polyphenolic of olive oil samples under study were fractionated into 21 different components by HPLC. The results in Table (8) showed that the major phenolic components in Coratina olive oil at Mid-October was pyrogallol which was found in 428.71µg/g and the other major compounds was Ellagic (248.8171µg/g), Catechein (240.1271µg/g) and followed by Salysilic acid (205.7971µg/g). Also, the major phenolic compounds in Coratina olive oil sample at Mid-December was Salysilicacid (207.6371µg/g) followed by pyrogallol (193.5171µg/g and Benzoic acid (139.0871µg/g). on the other hand, the major phenolic compound in Picual olive oil at Mid-October was Salysilic acid (180.07µg/g) followed by Pyrogallol (115.78µg/g) and (93.34µg/g), while the major phenolic compound in Picual olive oil at Mid December was Benzoic acid (299.06µg/g) followed by Salysilic acid (172.92µg/g) and Pyrogallol (139.10 µg/g). Also found was the major phenolic compound in commercial olive oil sample was Benzoic acid (165.19µg/g) followed by Salysilic acid (159.90µg/g) and Pyrogallol (105.72µg/g). On the other hand, some phenolic compound such as Oleuropen, 3-hydroxy tyrosol, gallic acid, catechein, coffeic acid Vanillic acid was found in all samples under study at different levels. Generally, extra virgin olive oil contains considerable amounts of phenolic components, e.g., hydroxyterosol and oleuropein which are responsible for its peculiar taste, flavor and high stability.

The differences in phenolic compounds between the olive oil samples included in the study were due to different cultivars and repining degree.These results are similar with those reported by Bengana, *et al.,*(2013) and Jimenez *et al.,* (2013).

Table 6 Phenolic compounds fraction (µg/g) of olive oil extracted from Coratina and Picual varieties at two ripening stages.

Phenolic compounds	Coratina		Picual		Commercial samples
	Mid. October	Mid. December	Mid. October	mid December	
Gallic	47.39	19.12	19.46	15.71	28.91
Pyrogallol	428.71	193.51	115.78	139.10	105.72
4-aminobenzoic	25.07	11.78	5.07	9.40	6.41
Protocatchoic	88.48	34.86	26.91	12.64	12.07
Catechein	240.12	86.08	54.41	50.54	50.42
Catechol	150.23	30.30	38.17	21.95	16.65
Caffeine	76.56	13.78	10.64	16.67	15.72
p-OH-benzoic	42.71	20.08	12.53	22.83	9.73
Caffeic	5.01	7.74	2.45	2.14	2.99
Vanillic	7.68	3.70	8.55	1.89	4.37
p-coumaric	4.32	1.36	1.16	1.92	1.95
Ferulic	12.47	1.59	1.67	1.47	1.49
Iso- Ferulic	7.23	2.14	1.61	1.76	1.85
Ellagic	248.81	49.87	15.04	95.63	74.30
Alpha-coumaric	9.36	1.58	1.43	2.35	2.39
Benzoic	101.87	139.08	93.34	299.06	165.19
Salycillic	205.97	207.63	180.07	172.92	159.97
Coumarin	29.04	11.00	4.04	9.06	5.52
Cinnamic	49.33	20.38	2.73	30.10	30.22
3-Hydroxy tyrosol	162.77	76.52	61.03	41.65	14.73
oleuropen	121	42.66	16.13	50.33	66.13

NMR Spectral Analysis of some phenolic compound in Olive Oil samples.

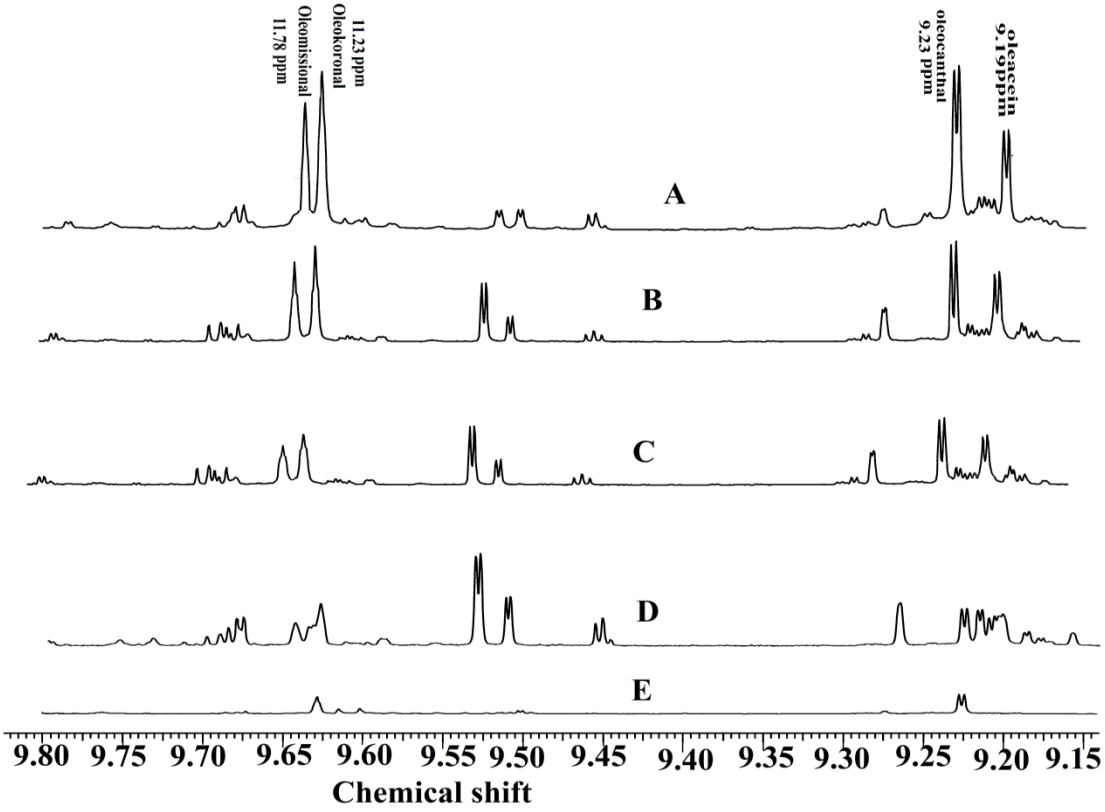

Figure 1 Each variety presents a unique profile in the 9.15−12.80 ppm region in the 1H NMR spectrum: (A) Coratina at Mid. October (B) Picualat Mid. December (C) Coratina at Mid. October (D) Picual at Mid. December (E) Commercial sample

Table 8 Different concentrations of different component of olive oil by NMR

Sample	oleocanthal integration	oleocanthal (mg/kg)	oleacein integration	oleacein mg/kg	Oleokoronal integration	Oleokoronal (mg/kg)	Oleomissional integration	Oleomissional (mg/kg)
A	1.30	350.4	1.02	293.1	1.08	278.1	0.98	272.8
B	1.02	283.20	0.90	255.6	0.97	246.6	0.85	238.5
C	0.77	210.6	0.75	212.5	0.66	205.5	0.59	192.3
D	0.72	192.5	0.69	185.2	0.55	172.4	0.47	163.8
E	0.66	181.5	0.51	145.1	0.45	133.1	0.42	121.6

Each variety presents a unique profile in the 9.15−12.80 ppm region in the 1H NMR spectrum: (A) Coratina at Mid. October (B) Picualat Mid. December (C) Coratina at Mid. October (D) Picual at Mid. December (E) Commercial sample

NMR studies the reaction of oleocanthal and oleacein with different source. On the past the quantify oleocanthal, oleacein, oleokoronal and oleomissional in olive oil extracts was performed using HPLC-UV Di Donna,*et al.*,(2011) and Impellizzeri, *et al.*, (2006) with reversed phase columns and aqueous mobile phase, and in that case we observed that pure oleocanthal, oleacein, Oleokoronal and oleomissional did not give a single sharp peak. This problem, which had been previously observed Christophoridou and Dais (2009) prompted us to investigate in more detail the reaction of oleocanthal, oleacein, Oleokoronal and oleomissional with water, methanol, acetonitrile, DMSO, or their mixtures. The study was performed by NMR using deuterated solvents and monitoring

in situ the formation of the corresponding derivatives. We found that compounds react spontaneously with water or methanol to give mixtures of hemiacetals or acetals that were characterized using 1D and 2DNMR spectra. The percent of concentrations was determined by integration of thealdehyde protons of the dialdehyde form in comparison with the integration of the hemiacetal or acetal proton of the produced monoaldehydes. Interestingly, oleocanthal, oleacein, oleokoronal and oleomissional gave a NMR spectrum corresponding each to a single molecule only in the case of pure CDCl3, d-ACN, and DMSO. The above findings confirm that the classic chromatographic measurement of these compounds in aqueous media is problematic and that many of the previous measurement reported in the literature are more or less questionable. For example, as shown in Table 1, the proportion between the aldehydic and the hydrated form in water/acetonitrile mixtures is time and solvent ratio dependent, making very difficult the accurate measurement. To override the above-described problem, we applied a method for olive oil extraction without the use of any reacting solvent and developed a method for direct measurement of theoleocanthal, oleacein, Oleokoronal and oleomissional levels by quantitative 1H NMR inCDCl3 at 400 MHz.

NMR Spectral Analysis of oleocanthal, oleacein, Oleokoronal and oleomissional in Olive Oil

1H NMR spectroscopy was envisaged asa simple and reliable alternative methodology for monitoring envisaged in olive oil and has been recently applied for the quantification of other olive oil phenolic Christophoridou and Dais (2009) as well as in chemometric studies (D'Imperio, *et al*., (2010), Alonso-Salces, *et al.,* (2010) and Cicerale, *et al*., (2012)). The method was based on the observation that the 1H NMR spectrum of olive oil when recorded inCDCl3 and in magnetic fields of 400 MHz with capillary tube system presented a very well resolved set of peaks corresponding to the aldehydicprotons of the studied compounds between 9.23 and 9.19 ppm which corresponding to oleocanthal and oleacein, while at 11.23 and 11.78 corresponding to Oleokoronal and oleomissional (Figure 1). This spectrum region in all of the studied samples was clearly resolved, making feasible the integration of the Corresponding. As we see from figure 1; The first curve which corresponding to Coratina olive oil at start of season (A), we notice that the highly intensity orconcentration, integration of the peaks of oleocanthal (350.4 mg/Kg), oleacein (293.1mg/Kg), oleokoronal (278.1mg/Kg) and oleomissional (272.8mg/Kg) which causes highly organoleptic and pungency properties, and also natural anti-inflammatory drug due to inhibition activity, also it has therapeutic properties of olive oil Evangelia *et al*., (2012) and Panagiotis *et al.,* (2015). The intensity of these peaks will decrease in concentrations due to different olive oil samples and seasons of collection as we see from Figure1 and Table 1 there are five charts, first chart (A)belong Coratina at start of season, second chart; (B) belong Picual at start of seasonwe notice that the highly intensity of concentration, integration of the peaks of oleocanthal (283.20 mg/Kg), oleacein (255.6 mg/Kg), oleokoronal (246.6 mg/Kg) and oleomissional (238.5 mg/Kg); (C) belong Coratina at finish of season while concentration, integration of the peaks of oleocanthal (210.6 mg/Kg), oleacein (212.5 mg/Kg), oleokoronal (205.5 mg/Kg) and oleomissional (192.3 mg/Kg) Evangelia *et al*.,(2012) and Panagiotis, *et al.,*(2015); (D) belong Picual at finish of season while concentration, integration of the peaks of oleocanthal (192.5 mg/Kg), oleacein (185.2mg/Kg), oleokoronal (172.4mg/Kg) and oleomissional (163.8mg/Kg), and finally (E) corresponding of commercial olive oil while concentration, integration of the peaks of oleocanthal (181.5 mg/Kg), oleacein (145.1mg/Kg), oleokoronal (133.1mg/Kg) and oleomissional (121.6mg/Kg) Evangelia,*et al*.,(2012) and Panagiotis, *et al.,*(2015). As we notice at start of season (chart A) it's clear that the highest intensity of the peaks corresponding to oleocanthal, oleacein as seen in Figure 1, Oleokoronal and oleomissional this leads to highest organoleptic and pungency properties and this intensity, integration and properties will decrease by time till we reached the final of season either to Coratina or Picual, also these integration of signal and this properties decrease in commercial sample rather than at finished of the season Table 1. We notice also there are signals at (9.5-9.55 ppm), we suggested this signals corresponding to the olepicuanal, the height and intensity of this signals increase at mid of December in picuanal.

Selection of NMR solvent

The selection of CDCl3 as solvent for the NMR measurement was based on the observation that it was one of the three common solvents that does not react with the studied compounds, in contrast to methanol or water. The advantage of CDCl3 when compared with d-ACN or d6-DMSO is that in the two latter solvents the aldehydic protons of the studied compounds overlap and cannot be integrated Christophoridou and Dais (2009) and Cicerale *et al*., (2012)., whereas in the case of CDCl3 all of the measured peaks could be clearly observed. There was a highly significant variation concerning the concentrations of oleocanthal and oleacein among the studied between different type of olive as: The highest concentrations of oleocanthal and oleacein among the studied samples.

4. CONCLUSION

All results indicated that there were a wide variation in the chemical and characteristics of all olive oils samples. Also, the results showed clear differences in phenolic content, oxidative stability and phenolic compounds between olive oil samples. Using the NMR

was identified of some bioactive compounds (oleocanthal, oleokoronal and oleokoronal) in different in olive oil samples and also we suggested that the name of some signals at9.5-9.55 ppm as oleopicuanal.

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