Carbon Stable Isotopes and Olive Oil Adulteration with Pomace Oil

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Adulteration of olive oils can be detected by comparison of the $^{13}C/^{12}C$ ratios as obtained subsequently by high-resolution gas chromatography (HRGC) and isotope ratio mass spectrometry (IRMS). In $\delta^{13}C$ values determined for the aliphatic alcoholic fraction of pomace oil, the adulterant was significantly more negative than those of virgin and refined olive oils. Quantitative analysis of isoprenoids and methylsterols isolated from each grade of olive oil supports the decreasingly negative values of $^{13}C/^{12}C$ ratios for the better grades of olive oil. These results have turned out to be a good tool for detecting adulteration of olive oil with the cheaper pomace olive oil, even down to a 5% level.

Keywords: *Carbon stable isotopes; olive oil; adulteration; aliphatic alcohols; waxes*

INTRODUCTION

Two main commodities are obtained by mechanical extraction of *Olea europaea* L. fruits: virgin olive oil, which can be directly consumed, and a lower grade oil known as olive pomace oil. Olive pomace residue contains quite a large quantity of oil (2−5% by weight), which can be recovered by extraction with a solvent such as hexane. The oil obtained after removal of solvent, called crude olive pomace oil, shows high free acidity and has roughly double the amount of unsaponifiable matter with respect to the oil extracted by simple pressure. It can be consumed only after a refining process composed of three steps: neutralization, decolorization, and deodorization.

Furthermore, there is another lower grade olive oil, the refined olive oil, obtained by refining processes of oils mechanically extracted from damaged olive fruits or from olives stored in unsuitable conditions. This oil (called "olive oil") is sold and consumed when mixed with virgin olive oil.

Virgin olive oil and olive oil command a higher price than the olive pomace oil. As a consequence, there is a great temptation to adulterate the best olive oil with cheaper products.

The adulteration of virgin olive oil with pomace oil can be detected in several ways; however, the UE official methods are based on the quantitative determination of waxes developped by Mariani and Fedeli (1986) and of their saponification derivatives: long-chain C22−C28 aliphatic alcohols studied by several researchers (Karleskind, 1968; Wolff, 1968; Tiscornia et al., 1985; Camera, 1991).

In fact, free and esterified alcohols in waxes are considerably more abundant in pomace oils than in virgin or refined oils, since solvents are able to solubilize waxes concentrated on the drupe skin and not extracted by the oil.

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However, some virgin olive oils obtained by pression show contents of waxes and alcohols higher than the averaged expected levels and could be erroneously assessed as being adulterated with pomace oils.

On the other hand, the wax content can be dramatically reduced when the oil is treated according to the conditions in the winterization process (Favini et al., 1971; Cucurachi, 1971).

Another possible method for detection of olive oil adulteration by pomace oil addition is based on the determination of the triterpenic dialcohols, erythrodiol and uvaol, whose concentrations are considerably higher in pomace oils than in virgin and refined olive oils (Karleskind, 1968; Cucurachi, 1971; Dimoulas, 1969; Minguzzi and Capella, 1972; Ghimenti et al., 1973). However, this methodology may fail, as it is possible to reduce the concentration of triterpenic dialcohols of pomace oils to the virgin or refined olive oil levels, by using suitable oxidizing substances that eliminate the two pentacyclic dialcohols (Di Giovacchino et al., 1987).

For all the above-mentioned reasons, the considered analytical methods are not safe enough to verify the purity of this commodity.

The evaluation of the stable carbon isotope ratios has proven to be a good tool for solving adulteration problems of commodities such as seed oils (Gaffney et al., 1979; Rossell, 1991), soy-meat mixtures (Gaffney et al., 1979), wine (Martin et al., 1988, 1991), honey (White and Doner, 1978), and fruit juices (Koziet et al., 1993).

Data are available in the literature on $^{13}C/^{12}C$ ratios of sunflower, corn, soybean, palm, coconut, and peanut oils. In recent years, isotopic ratio values were measured for virgin olive oil and some of its components such as sterols, aliphatic alcohols, and glycerol of some olive varieties (Bianchi et al., 1993). This research has shown that the harvesting date and the degree of ripening of olives have no remarkable effects on $\delta^{13}C$ carbon isotope discrimination.

In this paper, we demonstrate that the isotopic composition of the whole oil and some its fractions could be a useful means for detecting possible adulteration of olive oil with cheaper refined pomace oil.

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MATERIALS AND METHODS

Materials. The following olive oil samples, differing in varieties and geographical origin, were used for experiments: 16 virgin olive oils; 31 samples of both crude and refined pomace oil (4 samples were extracted from pure olive pomace by Soxhelet, and the remaining were from industrial plants); 10 samples of unadulterated refined olive oil; and 1 sample of crude pomace oil submitted to winterization treatment that eliminated 78% of the original wax content.

All solvents, for organic residual analysis, were purchased from J. T. Baker (Deventer, Holland); KOH, $AgNO_3$, 2′,7′-dichlorofluorescein, and all analytical grade reagents were from Carlo Erba (Milano, Italy). Silica gel G 60 for thin layer chromatography was from E. Merck (Schuchardt, Germany). 1-Eicosanol and cholesterol were purchased from Aldrich (Steinheim, Germany), and lauryl arachidonate was from Larodan Fine Chemical AB (Malmo, Sweeden).

Methods. Fatty acid methyl esters were obtained according to UE methods (UE Regulation no. 2568/91/UE). The GC analysis of fatty acid methyl esters was carried out with a Carlo Erba Mega Series 5160 apparatus fitted with a Supelco (Bellefonte, PA) SP 2380 silica capillary column (60 m length; 0.32 mm i.d.; 0.20 μm film thickness) and equipped with an on-column injection system and a flame ionization detector (FID). The gas chromatographic conditions were in agreement with UE methods.

Extraction and Analysis of Sterols and Aliphatic Alcohols. Sterolic and aliphatic alcoholic fractions were isolated according to UE methods (UE Regulation no. 2568/91UE).

The oil sample (5 g) was saponified by an ethanolic potassium hydroxide solution (6 g of KOH in 50 mL of ethanol 95°), and then the unsaponifiable matter was extracted by diethyl ether and fractionated by preparative thin layer chromatography. Sterol and aliphatic alcohol bands, identified by comparison with reference cholesterol and 1-eicosanol standards, were recovered from silica gel by the usual workup.

Their purity was tested by gas chromatography, according to the UE method, carrying out the GC analyses with a Carlo Erba Mega Series 5160 apparatus fitted with a Nordion (Helsinki, Finland) fused silica capillary SE 54 column (25 m length; 0.32 mm i.d.; 0.20 μm film thickness), equipped with a split injection system and a FID. The splitting ratio was 1/20, and the split injection system was held at 280 °C. The gas chromatographic conditions were in agreement with UE methods.

The samples were then submitted to isotopic ratio measurement as described below.

The gas chromatographic analysis showed that the sterolic fraction was homogeneous, while the so-called alcoholic fraction was made up of linear aliphatic alcohols (50%), the remaining 50% being citrostadienol, phytol, and geranyl geraniol.

In order to purify the aliphatic alcoholic fraction, the sample was separated in its components by silver nitrate thin layer chromatography on 20% $AgNO_3$ silica gel plates with $CHCl_3$ as the developing solvent. The band corresponding to aliphatic alcohols was located by UV after spraying with a 2% 2′,7′-dichlorofluorescein ethanolic solution and identified by comparison with reference 1-eicosanol. The purity of this fraction was tested by HRGC.

Winterization. In order to lower the wax content of the pomace olive oil, the oil was diluted 1/5 (v/v) with acetone and then the mixture was frozen at −20 °C for 2 h (Ghimenti et al., 1973). The solid fraction was removed by filtration on a filter paper (Carlo Erba no. 0892.00115) under vacuum.

The winterization treatment reduced the oil wax content from 4.496 to 1.000g/(kg of oil).

Wax Extraction and Analysis. For the extraction of waxes, 0.5 mg of lauryl arachidonate was added as an internal standard to 500 mg of oil. The mixture was dissolved in hexane and subjected to silica gel column chromatography (15 g of silica gel 60; Merck, Darmstadt, Germany) according to UE methods. The ester fractions were eluted with 160 mL of a 99/1 mixture of hexane/diethyl ether. The solvent was evaporated under reduced pressure in a rotary evaporator (Büchi, Switzerland) to give a crude residue, which was dissolved in 2 mL of heptane. A suitable aliquot of this solution was used for GC analysis with a Carlo Erba Mega Series 5160 apparatus fitted with a Nordion fused silica capillary SE 54 column (15 m length; 0.32 mm i.d.; 0.10 μm film thickness), equipped with an on-column injection system and a FID. The gas chromatographic conditions were in agreement with the UE method.

Table 1. δ^{13}C Values and Standard Deviations of the Virgin Whole Oil Samples and Corresponding Sterols and Aliphatic Alcohols

	δ^{13}C [(‰)$_{PDB}$]			
virgin olive oil cultivar	oil (*A*)	sterols	aliphatic alcohols (*B*)	*B* − *A*
(1) several varieties	−28.97	−27.54	−28.61	+0.36
(2) Dritta	−28.35	−26.40	−28.18	+0.17
(3) Coratina	−29.26	−26.93	−28.79	+0.47
(4) several varieties	−28.44	−25.11	−28.09	+0.35
(5) Maurino	−28.33	−25.79	−27.91	+0.42
(6) Coratina	−29.40	−26.43	−28.48	+0.92
(7) more varieties	−28.94	−26.45	−28.78	+0.16
(8) Dritta	−28.19	−27.21	−27.59	+0.60
(9) Castiglionese	−28.44	−27.05	−27.72	+0.72
(10) Leccino	−27.98	−26.33	−27.58	+0.40
(11) Leccino	−28.57	−26.98	−27.62	+0.95
(12) Frantoio	−28.80	−26.31	−27.26	+1.54
(13) Caroleo	−29.32	−27.70	−28.58	+0.74
(14) Coratina	−29.40	−26.46	−28.48	+0.92
(15) Leccino	−30.54	−27.58	−29.65	+0.89
(16) Leccino	−30.30	−27.15	−29.32	+0.98
mean value	−28.95	−26.73	−28.29	+0.66
SD	0.70	0.67	0.65	0.35

Isotopic Determination. $^{13}C/^{12}C$ ratios of oils and fractions were determined with a Finnigan MAT delta-S isotope ratio mass spectrometer (IRMS), interfaced on-line with a Fisons NA 1108 elemental analyzer for the quantitative conversion of samples into CO_2. Samples placed in tin containers were submitted to a flesh combustion in a stream of helium enriched with pure oxygen. Masses 44, 45, and 46 were simultaneously detected and their intensities integrated. $^{13}C/^{12}C$ values were calculated by taking into account the contribution of the 170 isotope to the peak mass 45 (Craig, 1957). Each sample was measured in triplicate. The reproducibility of measurements was within 0.2‰.

Carbon isotope ratios are expressed in δ^{13}C scale (percent), indicating the difference of the $^{13}C/^{12}C$ ratio of the sample relative to an international standard, i.e., CO_2 obtained from Pee Dee Belemnite (PDB) limestone of South Carolina. The δ^{13}C value is calculated according to the equation

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 10^3$$

where $R = {}^{13}C/{}^{12}C$.

$^{13}C/^{12}C$ measurements on single compounds were performed with a chromatograph coupled via a combustion furnace (Isochrom I-VG system) to a VG SIRA 24 IRMS. The reproducibility of measurements was within 0.3‰.

HRGC was carried out with a 5160 Carlo Erba gas chromatograph equipped with an on-column injection system and a FID, on a silica capillary SE-54 column (25 m length; 0.32 mm i.d.; 0.25 μm film thickness). The oven temperature program was as follows: from 80 to 170 °C at 25 °C/min, from 170 to 260 °C at 4 °C/min, 10 min at 260 °C, from 260 to 270 °C at 3 °C/min, and 20 min at 270 °C. The detector temperature was held at 315 °C. The carrier gas was H_2, and the pressure on the head of the column was 35 kPa.

RESULTS AND DISCUSSION

The δ^{13}C values found for the whole oil, sterols, and aliphatic alcohols of the 16 virgin olive oil samples are shown in Table 1. They are in agreement with the results obtained in previous research (Bianchi et al., 1993) and prove again the notable homogeneity and

Table 2. $\delta^{13}C$ Values and Standard Deviations of the Refined Olive Whole Oil Samples and Corresponding Sterols and Aliphatic Alcohols

refined olive oil	^{13}C [(‰)$_{PDB}$]			
	oil (*A*)	sterols	aliphatic alcohols (*B*)	*B* − *A*
1	−29.01	−26.87	−28.61	+0.40
2	−29.12	−27.02	−28.64	+0.48
3	−29.51	−27.23	−28.90	+0.61
4	−30.26	−27.53	−29.18	+1.08
5	−30.43	−27.63	−29.40	+1.03
6	−30.06	−27.98	−29.67	+0.39
7	−29.76	−27.29	−29.11	+0.65
8	−29.47	−27.19	−29.05	+0.42
9	−29.42	−27.31	−29.10	+0.32
10	−29.49	−27.31	−29.01	+0.48
mean value	−29.65	−27.34	−29.07	+0.59
SD	0.47	0.31	0.32	0.28

constancy of the $\delta^{13}C$ values for each class of investigated substances.

The $\delta^{13}C$ values were also measured on the fatty acids obtained by hydrolysis of different oils. As expected, they were the same of those as the whole oils (unpublished results). Therefore, we thought it was easier to measure $^{13}C/^{12}C$ ratios on the whole oils without carrying out the triacylglycerol hydrolysis.

Aliphatic alcohols always had $\delta^{13}C$ values more negative than those of sterols, similar to those of the whole oil. This means a lower content of the ^{13}C isotope. On the contrary, the algebraic difference between the $\delta^{13}C$ of aliphatic alcohols and that of the corresponding whole oils was positive in all the examined samples.

The whole unadulterated refined olive oils, sterols, and aliphatic alcohols submitted to the measurement of $\delta^{13}C$ gave the values shown in Table 2. They were very similar to those of virgin olive oil because the refining process, as expected, does not influence $^{13}C/^{12}C$ values. Also, the algebraic difference of isotopic ratios between aliphatic alcohols and the corresponding whole oil was always positive as seen for virgin olive oils.

Finally, we analyzed the isotopic content of 31 samples of crude and refined pomace oil.

The whole oils and sterols of crude and refined pomace oils (Table 3) showed $^{13}C/^{12}C$ ratios in agreement with data recorded for virgin olive oils, but in all examined samples, the aliphatic alcohols provided $\delta^{13}C$ values significantly more negative according to the Scheffé test (Snedecor and Cochran, 1989) than those of virgin olive oils. Consequently, the algebraic difference between the isotopic ratios of aliphatic alcohols and the corresponding whole oil was always negative.

The fact that more negative $\delta^{13}C$ values were found for aliphatic alcohols from pomace oils than from those of virgin or refined olive oils could be explained by the heterogeneous composition of this fraction. The methodology adopted for the isolation of the alcoholic fractions is the UE official method, whose procedure does not allow separation of all the components, that is aliphatic alcohols, phytol, geranyl geraniol (GEGE), and methyl sterols (mainly citrostadienol, obtusifoliol, and gramisterol), as shown in Figure 1A. The varying averaged levels of these components in olive oils and pomace oils are shown in Table 4. These data show that aliphatic alcohols are 48% of the whole fraction isolated from virgin or refined olive oil, while they rise to 90% in the case of pomace oil.

The fact that $\delta^{13}C$ values of pomace oil aliphatic alcohols were more negative than those of virgin and refined oils suggests that they are more representative of the real isotopic content of the aliphatic alcoholic fraction.

Table 3. $\delta^{13}C$ Values and Standard Deviations of the Pomace Whole Oil Samples and Corresponding Sterols and Aliphatic Alcohols

crude and refined pomace oil	$\delta^{13}C$ [(‰)$_{PDB}$]			
	oil (*A*)	sterols	aliphatic alcohols (*B*)	*B* − *A*
1	−28.33	−27.43	−29.98	−1.65
2	−28.85	−27.50	−31.02	−2.17
3	−28.93	−27.83	−30.38	−1.45
4	−28.78	−26.90	−30.25	−1.47
5	−28.93	−27.43	−30.23	−1.30
6	−28.91	−27.17	−30.49	−1.58
7	−29.70	−27.70	−31.06	−1.36
8	−28.83	−27.47	−31.49	−2.66
9	−29.02	−27.60	−31.52	−2.50
10	−28.70	−27.09	−30.72	−2.02
11	−28.77	−27.84	−30.73	−1.96
12	−28.95	−27.18	−31.02	−2.07
13	−28.83	−27.15	−30.35	−1.52
14[a]	−28.60	−27.97	−30.49	−1.89
15[a]	−29.06	−27.24	−30.04	−0.98
16[a]	−28.94	−27.97	−30.58	−1.64
17[a]	−27.63	−26.25	−29.57	−1.94
18[a]	−28.50	−27.70	−29.96	−1.46
19	−27.95	−26.53	−30.93	−2.98
20	−28.03	−26.75	−30.29	−2.26
21	−28.52	−27.05	−31.15	−2.63
22	−28.30	−26.75	−30.72	−2.42
23	−29.15	−28.30	−29.50	−0.35
24	−28.29	−27.02	−29.81	−1.52
25	28.27	−27.16	−29.91	−1.64
26	−28.72	−28.23	−30.64	−1.92
27	−28.54	−26.97	−30.62	−2.08
28	−28.88	−27.17	−31.59	−2.71
29	−28.63	−28.11	−31.31	−2.68
30	−28.36	−27.50	−31.04	−2.68
31	−28.75	−27.62	−30.29	−1.54
mean value	−28.67	−27.37	−30.57	−1.90
SD	0.40	0.49	0.56	0.58

[a] Refined pomace oil.

This means that the more positive $\delta^{13}C$ values found for virgin and refined olive oils are to be attributed to the presence of isoprenoids and methyl sterols, which therefore should show $\delta^{13}C$ values similar to those of the sterol fraction as expected for classes of compounds having a common biosynthesis.

Therefore, attributing to aliphatic alcohols the mean $\delta^{13}C$ value measured in pomace oils (−30.57) and to phytol, geranyl geraniol, and methyl sterols those of sterols (−26.73 for virgin and −27.34 for refined oil), and taking into account the purity percentages of aliphatic alcohols reported above, we calculated the $\delta^{13}C$ values for the aliphatic alcoholic fraction of virgin and refined olive oil, isolated as previously described. This resulted in values of −28.60 for virgin olive oil and −28.91 for refined olive oil. These results are in good agreement with those experimentally measured [−28.29 for virgin (Table 1) and −29.07 for refined oils (Table 2)].

Furthermore, let us consider the alcoholic fraction (Figure 1A,B) of virgin oil number 1 of Table 1, which showed before purification a $\delta^{13}C$ of −28.61. After purification by $AgNO_3$ thin layer chromatography, the $\delta^{13}C$ value of the alcoholic fraction was −30.24, a typical value of aliphatic alcohols of pomace oil.

In confirmation of these pieces of evidence, GC-isotopic ratio mass spectrometry was applied and measurements of $^{13}C/^{12}C$ ratios were carried out on a sample of a crude aliphatic fraction of a virgin olive oil.

The isotopic analysis results, summarized in Table 5, confirmed, as expected, the fact that the acyclic isoprenoids and methyl sterols had the same $\delta^{13}C$ values

Table 4. Average Levels, Expressed as Parts per Million 1-Eicosanol, of Aliphatic Alcohols, Isoprenoids, and Citrostadienol of Refined Olive and Pomace Oils in the Aliphatic Alcoholic Fraction Isolated According to the UE Method

oil	phytol (ppm)	GEGE (ppm)	aliphatic alcohols (ppm)	citrostadienol (ppm)	total amount of the aliphatic fraction (ppm)	% aliphatic alcohols
refined olive oil	172 ± 10	21 ± 1	235 ± 14	58 ± 3	486	48.6
refined pomace oil	119 ± 7	16 ± 1	2612 ± 150	171 ± 9	2918	89.5

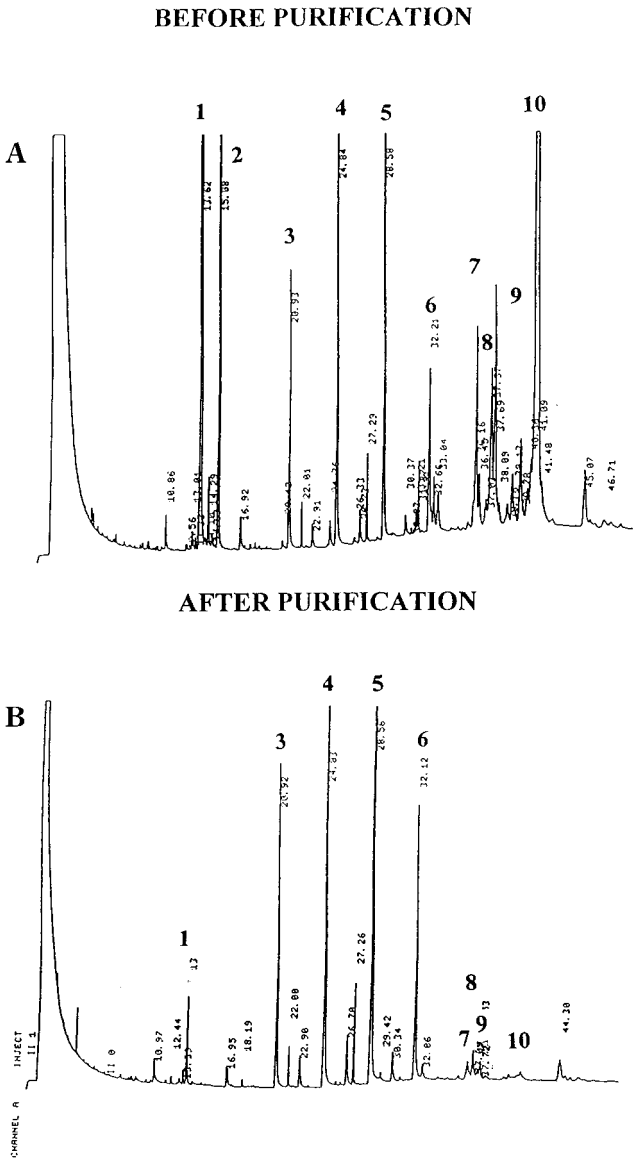

Figure 1. (A) Gas chromatogram of the aliphatic alcoholic fraction isolated according to the UE method and (B) gas chromatogram of a pure fraction alcoholic fraction: (1) phytol, (2) geranyl geraniol, (3) docosanol, (4) tetracosanol, (5) hexacosanol, (6) octocosanol, (7) obtusifoliol, (8) gramisterol, (9) ciclobranol, and (10) citrostadienol.

of sterols, while aliphatic alcohols showed $\delta^{13}C$ values significantly more negative.

We consider all these findings good evidence that the carbon isotopic content of pomace virgin and refined oils could be helpful in detecting adulterations of olive oils with pomace oils.

In order to verify the limit of detection of additions of pomace oil to olive oil, samples with different percentages from 3 to 15% of pomace oil in refined olive oil were prepared.

The values measured for the refined olive oil, the pomace oil, and their mixtures are shown in Table 6.

Table 5. $\delta^{13}C$ Values of Isoprenoids, Citrostadienol, and Aliphatic Alcohols Measured by GC-Isotopic Ratio Mass Spectrometry

compounds	$\delta^{13}C$
phytol	−25.03
geranyl geraniol	−25.84
citrostadienol	−25.24
docosanol (C_{22})	−30.06
tetracosanol (C_{24})	−29.77
hexacosanol (C_{26})	−29.58

Table 6. $\delta^{13}C$ Values of Whole Oils and Aliphatic Alcoholic Fraction Measured for Mixtures of an Olive Oil with a Pomace Oil

	$\delta^{13}C$ [(‰)$_{PDB}$]		
oil	oil (*A*)	aliphatic alcohols (*B*)	*B* − *A*
refined olive oil (ROO)	−28.44	−28.09	+0.35
refined pomace oil (RPO)	−29.02	−31.52	−2.50
97% ROO + 3% RPO	−28.52	−28.98	−0.46
95% ROO + 5% RPO	−28.49	−29.26	−0.77
93% ROO + 7% RPO	−28.49	−29.57	−1.08
90% ROO + 10% RPO	−28.43	−29.97	−1.54
85% ROO + 15% RPO	−28.52	−30.41	−1.89

Table 7. $\delta^{13}C$ Values of Whole Oils and Aliphatic Alcoholic Fraction Measured for Mixtures of a Refined Olive Oil with a Dewaxed Pomace Oil

	$\delta^{13C\ [‰]}_{PDB}$		
oil	oil (*A*)	aliphatic alcohols (*B*)	*B* − *A*
refined olive oil (ROO)	−29.26	−28.79	+0.47
dewaxed pomace oil (DOP)	−28.30	−29.77	−1.47
97% ROO + 3% DOP	−28.90	−29.10	−0.20
95% ROO + 5% DOP	−28.86	−29.15	−0.29
93% ROO + 7% DOP	−28.94	−29.21	−0.27
90% ROO + 10% DOP	−28.89	−29.30	−0.41
85% ROO + 15% DOP	−28.72	−29.36	−0.64

The $\delta^{13}C$ values of aliphatic alcohols of the mixtures were always more negative than those of refined olive oil and showed a significant linear correlation with the percentage of added pomace oil ($R = -0.983$). Therefore, $\delta^{13}C$ values measured for the aliphatic alcohols of olive oils could allow for detection of possible adulterations of olive oil with pomace oils at the low levels indicated.

It is well known that the wax fraction concentration can be dramatically reduced by winterization. This operation sometimes is performed in very drastic conditions for illegal purposes so that the addition of pomace oil cannot always be revealed by the wax quantitative determination.

In order to verify if $\delta^{13}C$ measurement could be a good tool for detecting this type of adulteration, we prepared a dewaxed pomace oil, with a wax content reduced by 78%. The oil thus obtained was than employed to prepare mixtures with refined olive oil. The isotopic ratios measured for the refined olive oil, dewaxed pomace oil, and their mixtures are shown in Table 7, in which is also reported the difference between $\delta^{13}C$ values of aliphatic alcohols and the corresponding whole oil samples.

Table 8. Contents of Waxes and Aliphatic Alcohols of a Refined Olive Oil, a Dewaxed Pomace Oil, and a Mixture of the Two

oil	waxes (ppm)	aliphatic alcohols (ppm)
refined olive oil (ROO)	241	234
dewaxed pomace oil (DOP)	1000	910
10% DOP + 90% ROO	317	302

We carried out a preliminary gas chromatographic quantitation of aliphatic alcohol and wax contents of the oil obtained mixing a refined olive oil with 10% dewaxed pomace oil. The analysis failed to discover this sort of adulteration. In fact, the contents of waxes and alcohols were 317 and 302 ppm, respectively (Table 8), both values lower than the maximum concentration allowed by UE regulations.

The isotopic analysis was instead successful. The $\delta^{13}C$ values of the alcoholic fraction of mixtures (Table 7) were always slightly more negative than that of refined olive oil used to prepare mixtures according to a significant linear correlation ($R = -0.975$). However, the regression straight line showed a slope (−0.036) lower than that (−0.121) recorded for the mixtures obtained by the addition of pomace oil to the refined olive oil (Table 6). Therefore, by considering the $\delta^{13}C$ value, it is possible to detect adulteration at the 10−15% level, taking into account the fact that the error on the measurement is within 0.2‰.

On the other hand, the difference between the $\delta^{13}C$ value of aliphatic alcohols and the corresponding $\delta^{13}C$ value of whole oils was always negative for all the mixtures, providing evidence of the adulteration at a level as low as 3%.

CONCLUSIONS

This research has shown that the $^{13}C/^{12}C$ ratios measured on the aliphatic alcoholic fraction of pomace oils are more negative than values recorded for virgin and refined oils.

These findings can be explained by the fact that the UE methodology adopted for the isolation of the mentioned fraction does not allow for separation of aliphatic alcohols from isoprenoids (phytol and geranyl geraniol) and methyl sterols (mainly citrostadienol), especially abundant in virgin and refined oils.

These results have been proven by measuring $\delta^{13}C$ values of aliphatic alcohols after their purification by $AgNO_3$ thin layer chromatography and recording $^{13}C/^{12}C$ ratios by GC-isotopic ratio mass spectrometry.

The different isotopic content of pomace oil aliphatic alcoholic fractions from virgin and refined olive oils turned out to be helpful in detecting adulterations of olive oil with pomace oils at low levels, and also in those cases where the usual official methodologies failed.

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