Stable Carbon Isotope Ratios ($^{13}C/^{12}C$) of Olive Oil Components

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A study was carried out relating carbon isotope discrimination (δ ^{13}C) for the whole oil and some of its classes of compounds of four Italian olive varieties collected at six different stages of ripening in the period October–January 1991–1992. The δ ^{13}C value ranges found were as follows: whole oil, −27.18 to −28.57; sterols, −25.18 to −26.21; long-chain alcohols, −26.80 to −28.40; glycerol, −28.07 to −30.33. The major conclusions were that (a) most of the oil components appeared to be strictly of C_3 biosynthetic origin, (b) the ripening stage of the fruit did not affect the δ ^{13}C values, and (c) the δ ^{13}C values of oil samples and the related classes of substances are significantly different. The implication is the δ ^{13}C values could be used in determining the purity and origin of olive oils.

INTRODUCTION

In 1939 Nier and Gulbransen observed that the $^{13}C/^{12}C$ ratio for the two stable carbon isotopes is characteristic in nature for various carbon reservoirs. In particular, atmospheric carbon dioxide contains approximately 1.1% of the heavier carbon isotope ^{13}C and 98.9% of the lighter isotope ^{12}C.

Further detailed investigations on isotopic compositions of carbon in many substrates soon disclosed that the variation in classes of compounds from plants was not random but determined by natural processes (Park and Epstein, 1960; Bender, 1971; Smith, 1972).

Thus, in the processes of photosynthetic CO_2 fixation, a discrimination became apparent in the carbon isotope composition: plants favor the fixation of $^{12}CO_2$ into their tissues, with the result that all plants contain less ^{13}C than the carbon dioxide in the atmosphere and in other environmental domains.

Such an isotopic discrimination, conventionally presented as δ ^{13}C, indicates the difference in per mil 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 $R = {}^{13}C/{}^{12}C$.

In the plant kingdom the isotopic ratio was found to fall mainly in two ranges because two main pathways are operating in plants to fix carbon dioxide into organic substances via photosynthesis (Park and Epstein, 1961; Bender, 1971; Smith and Brown, 1973; Smith and Turner, 1975; Bianchi and Bianchi, 1990).

These photosynthetic processes are known as the C_3, C_4, and CAM pathways, so termed because, during the enzymatic carbon dioxide fixation, the main first metabolites formed are a three-carbon compound (e.g., 3-phosphoglyceric acid) in C_3 plants and a four-carbon acid (e.g., oxaloacetic acid) in C_4 and CAM plants.

In C_3 plants, the δ ^{13}C falls into the range −22 to −33‰; the C_4 plants have $^{13}C/^{12}C$ values in the range −9 to −15‰. For CAM plants that fix CO_2 by both C_3 and C_4 pathways, the δ ^{13}C values found are in the wide range −12 to −30‰ (Park and Epstein, 1961; Bender, 1971; Smith and Brown, 1973; Lerman et al., 1974; O'Leary, 1981).

Theoretical and experimental studies on the physicochemical factors which account for the isotope fractionation accompanying the photosynthetic process were carried out by several research groups (Park and Epstein, 1960; Lerman and Queiroz, 1974; O'Leary, 1981, 1988; Farquhar, 1983; Farquhar et al., 1989). From these studies good pieces of evidence were gained that in C_3 plants ribulosebisphosphate carboxylase plays a key role in carbon isotope discrimination, while diffusion is the limiting factor in C_4 plants.

Most of the δ ^{13}C values reported in the literature are for mixtures of substances obtained from the various plant organs such as leaves, roots, and berries (Bender, 1971; Smith and Turner, 1975; White et al., 1990; Schnyder, 1992; Di Marco et al., 1977). The most studied classes of substances were the ubiquitous carbohydrates and lipids (Galimov, 1985; Gaffney et al., 1979; Rossell, 1991) and also particular metabolites such as aspartate and malate (Di Marco et al., 1977; Deleens et al., 1979; Melzer and O'Leary, 1991). Furthermore, the isotopic intramolecular distribution in some natural compounds was also studied (Rossmann et al., 1991).

Studies on the evaluation of the ratios of the stable isotopes of carbon aimed at solving adulteration problems are now available in literature regarding commodities such as a seed oils (Gaffney et al., 1979; Rossell, 1991), soy–meat mixtures (Gaffney et al., 1979), wine (Martin et al., 1988, 1991), and honey (White and Doner, 1978). As far as edible vegetable oil is concerned, few data are reported in the literature (Gaffney et al., 1979; Rossell, 1991) on $^{13}C/^{12}C$ ratios of sunflower (~27‰), corn (~−12‰), soybean (~−28‰), palm (~−27‰), coconut (~25‰), and peanut oils (~−28‰). To our knowledge, however, no data are available for olive oil.

The latter, which is the main source of lipids for populations of the countries of the Mediterranean basin, is considered peculiar for many physiological and biosynthetic characteristics compared to other vegetable oils.

Olive oil is mainly concentrated in the pulp of the olive fruit, which in contrast to other drupes, stores lipids and sugars as reserve material. The formation and concentration of olive oil in the drupe, a rich reservoir of many classes of substances, is possibly the main cause of the unique flavor and fragrancy of this valuable commodity whose chemical characteristics may be influenced by climate, variety, and ripening degree of the olives (Montedoro and Cantarelli, 1969; Bertuccioli et al., 1978;

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Table I. Carbon Isotope Ratios of Oils from Four Olive Varieties at Six Stages of Maturity[a]

	Dritta		Nebbio		Castiglionese		Leccino	
sampling date	JI	$\delta^{13}C$	JI	$\delta^{13}C$	JI	$\delta^{13}C$	JI	$\delta^{13}C$
Oct 14	a 1.16	−27.18	1.05	−27.86	0.92	−28.28	0.92	−27.79
	b 1.20	−27.67	1.07	−28.00	0.94	−28.44	0.94	−27.87
	c 1.18	−27.24	1.09	−27.71	0.91	−28.29	0.89	−27.78
mean	1.18	−27.36	1.07	−27.86	0.92	−28.34	0.92	−27.81
Oct 28	a 2.28	−27.29	2.08	−27.87	2.04	−27.90	3.41	−27.80
	b 2.09	−27.89	2.25	−27.89	2.21	−28.25	3.33	−27.98
	c 2.07	−27.42	2.13	−28.16	2.17	−27.81	3.27	−28.00
mean	2.15	−27.53	2.15	−27.98	2.14	−27.99	3.34	−27.93
Nov 8	a 2.83	−27.52	3.32	−27.79	4.84	−27.91	4.79	−28.41
	b 2.97	−28.19	3.21	−27.86	5.05	−27.70	4.81	−28.57
	c 2.97	−28.05	3.13	−27.62	5.74	−27.98	4.57	−28.65
mean	2.92	−27.92	3.22	−27.76	5.21	−27.86	4.72	−28.54
Nov 26	a 3.78	−27.71	3.68	−28.08	4.95	−27.79	4.53	−28.30
	b 3.71	−28.02	3.56	−28.05	5.09	−27.85	4.02	−27.99
	c 3.55	−27.73	3.73	−28.27	5.30	−28.18	4.43	−28.06
mean	3.68	−27.82	3.66	−28.13	5.11	−27.94	4.33	−28.12
Dec 12	a 3.26	−27.56	4.29	−28.04	5.28	−28.11	5.05	−28.32
	b 2.95	−27.95	4.41	−28.26	5.57	−28.28	5.24	−28.34
	c 3.03	−27.67	4.16	−28.42	5.34	−28.34	5.40	−28.37
mean	3.08	−27.73	4.29	−28.24	5.40	−28.24	5.23	−28.34
Jan 7	a 3.28	−27.75	4.61	−27.84	4.67	−27.95	4.75	−28.46
	b 3.34	−27.91	3.93	−28.47	4.31	−28.04	4.39	−28.37
	c 3.21	−27.61	4.33	−28.17	4.24	−28.17	4.59	−28.11
mean	3.28	−27.76	4.29	−28.16	4.41	−28.05	4.58	−28.31

[a] JI, ripening degree measured by Jaèn index; a–c, oil samples from olives of three adjacent olive trees.

Hatanaka et al., 1982, 1987; Camera et al., 1975; Camera and Angerosa, 1978; Montedoro and Garofolo, 1984; Solinas et al., 1987, 1988; Cimato et al., 1988; Montedoro et al., 1989; Cimato and Sani, 1990; Mariani et al., 1991; Fiorino and Nizzi Grifi, 1991; Frega et al., 1992; Modi et al., 1992). However, the cited positive properties are such that olive oil commands a higher price than other vegetable oils, and, as a consequence, there is a great temptation to adulterate olive oil with cheaper seed oils.

So we thought that the isotopic composition study of the whole olive oil and of its single-component fractions could represent useful parameters to gain a better knowledge of their biosynthesis and could turn out to be an important means for detecting possible adulterations and for the identification of both variety and geographical origin of this commodity.

Here we present and discuss data on the influence of olive tree variety and ripening on the isotopic composition of olive oil. We determined the isotopic ratios for the whole oil samples, the glycerol moeity, and some components of the unsaponifiable matter, such as sterols and aliphatic alcohols. The oils studied were from some representative Italian varieties, picked at different stages of ripening in the period October 1991 through January 1992.

MATERIALS AND METHODS

The oil samples were extracted by micromill from batches of 1–2 kg of olives of varieties grown in the same orchard area.

The varieties investigated were Leccino, Dritta, Nebbio, and Castiglionese. They were studied during the ripening stages in the season range October 1991–January 1992. The extent of ripening was measured by Jaèn index (JI), based on the pigmentation of olive cuticle and pulp (COI). Each oil sample examined was from olives picked from three adjacent trees (a–c in Table I).

Pure samples of sterols and aliphatic alcohols were isolated according to the IUPAC and CEE methods. The oil sample (5 g) was saponified by an ethanolic potassium hydroxide solution (6 g of KOH in 50 mL of ethyl alcohol 95°), and then the unsaponifiable matter was extracted by diethyl ether and fractionated by preparative thin-layer chromatography. Sterol and aliphatic alcohol bands were identified by comparison with reference cholesterol and 1-eicosanol. Pure fractions were recovered from silica gel following usual workup.

Free glycerol was obtained from 0.5 g of oil by transesterification reaction with anhydrous methanol in the presence of KOH. The esterified fatty acids (methyl esters) were removed by several washings with hexane. Crude glycerol was obtained after complete evaporation of residual solvent.

Isotopic Determination. $^{13}C/^{12}C$ ratios were determined by a method similar to a procedure reported in the literature (Craig, 1957). The system consisted of a Carlo Erba NA 1500 elemental analyzer interfaced with a SIRA II-VG isotopic ratio mass spectrometer. The analyzer had a 50-sample carousel, a combustion tube (at 1020 °C) containing chromium trioxide and silvered cobaltous cobaltic oxide, and a reduction tube (at 650 °C) containing copper wires. Samples in tin capsules are dropped sequentially from the carousel into the combustion tube with helium gas flowing at 80 mL/min. Simultaneously, a pulse of oxygen is introduced in the combustion chamber at 1800 °C, causing flash oxidation of the tin capsule and its content. The combustion products pass through the combustion tube and the reduction tube and are separated by gas chromatography on a Poropak QS column (4 mm i.d. × 2 m) at 50 °C. The water vapor leaving the column is removed by a water trap at −80 °C. Carbon dioxide is condensed in a trap at −180 °C. At the end of the cryogenic trapping (5 min), noncondensable gases remaining in the traps are pumped away. Trapped CO_2 is released by raising

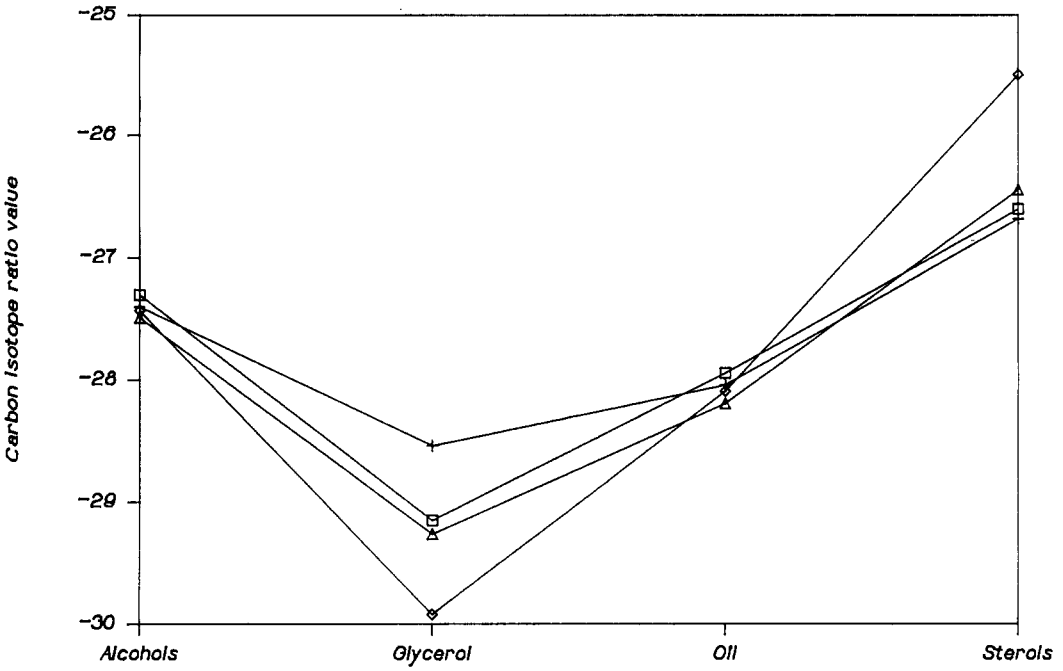

Figure 1. Carbon isotope composition (δ ^{13}C) of whole oils and corresponding components from cultivated Italian olive varieties: Dritta (□), Nebbio (◇), Castiglionese (+), Leccino (△).

the temperature of the trap to 55 °C and is allowed to expand into the mass spectrometer. SIRA II is a dual-inlet triple-collecting (m/z 44, 45, and 46 for $^{13}CO_2/^{12}CO_2$ analysis) instrument. The operation and data acquisition/analysis sequence is controlled automatically by an IBM PS/2 computer system.

The results are expressed in δ ^{13}C‰. The Craig correction was applied to the raw parts per thousand values for the ^{17}O contribution to m/z 45 (Craig, 1957).

The experiments were conducted in duplicate. The precision of the isotopic analysis was better than 0.01‰. The overall reproducibility of the experiments, including the combustion, was within 0.3‰.

RESULTS AND DISCUSSION

The δ ^{13}C values found are shown in Tables I and II and fall well within those characteristic of C_3 plants and are in accordance with the results obtained from olive leaves (Bongi et al., 1987). In the tables are reported both the harvesting data and ripening JI.

The very narrow range of values, −27 through −28 (Table I), is indicative of a strict and constant discrimination in the Calvin biosynthetic process in the olive fruit. The values obtained indicate that the oil is produced in the drupe by biosynthetic processes that are not significantly affected by the ripening stage of the olives. Others have previously reported on the influence of the cited factor for grape juices (Di Marco et al., 1977). Furthermore, as expected, the δ ^{13}C values of oil samples from olives picked up from adjacent trees were found to be very similar. These being the results, it was decided to limit the further study to the oil from just one tree for each variety.

The experiments disclosed the notable homogeneity and constancy of the isotopic ratio values during the ripening, encompassing not only the whole oil samples but also the classes of substances such as sterols, aliphatic alcohols, and glycerol, as evidenced by the low standard deviations (Table II).

Furthermore, regression analysis showed no correlations between the ripening process (Jaèn index) and the δ ^{13}C values as indicated by the very low correlation coefficients (0.035–0.782).

The δ ^{13}C values obtained for sterols, alcohols, and glycerol ranged from −25.2 to −29.9‰ and, at first glance, might appear to be similar. However, when the analysis of variance (ANOVA) with SAS software (SAS Institute, 1988), according to Scheffè test, was applied to the δ ^{13}C values of glycerol, alcohols, sterols, and the whole oil fractions of the varieties studied, significant differences in the data resulted, so that the substances could be described as four separate groups (F = 68.18, α approximating to zero) (Snedecor et al., 1989). We consider this clear distinction worthy of note because the oil and the three classes of substances tested are produced through distinct biosynthetic processes in which sugars are the common primer substrate.

Figure 1 shows that the glycerol moiety of triacylglycerols, with a mean value of about −29‰, appears to be more depleted in ^{13}C than the whole oil, alcohols, and especially sterols. However, the analysis of variance indicated that plant variety did not affect significantly carbon isotope discrimination within the classes of substances considered. Further work on this topic is in progress.

CONCLUSIONS

The present research has evidenced that the δ ^{13}C values for oils and its minor components of some olive varieties are those expected for substances of Calvin (C_3) biosynthetic origin. Harvesting date and ripening degree of olives had no remarkable effects on carbon isotope discrimination.

The δ ^{13}C values found for the whole oil, sterols, aliphatic alcohols, and glycerol, although encompassed in the narrow range −25 to −29‰, were found to be significantly different. The observed differences appeared to be poorly correlated with the plant cultivars. At present, we can only speculate that the δ ^{13}C measurements could represent one of the possible means to substantiate olive oil characterization in relation to both variety and geographical origin.

In conclusion, we believe that the implementation of the ^{13}C method and its application to the study of oils may not solely define the stable isotope characteristics of olive

Table II. Carbon Isotope Ratio Values and Standard Deviations of the Whole Oil Samples and Corresponding Sterols, Aliphatic Alcohols, and Glycerol of the Oils Extracted from Olives of the Examined Varieties at the Indicated Harvesting Date

variety	sampling date	JI[a]	δ ^{13}C oil	sterols	alcohols	glycerol
Dritta	Oct 14	1.20	-27.67	-26.65	-27.30	-29.15
	Oct 28	2.09	-27.89	-26.25	-26.88	-29.24
	Nov 8	2.97	-28.19	-27.21	-27.59	-30.33
	Nov 26	3.71	-28.02	-26.76	-26.82	-29.01
	Dec 12	2.95	-27.95	-26.67	-27.20	-28.92
	Jan 7	3.34	-27.91	-26.06	-27.20	-29.44
mean			-27.94	-26.60	-27.26	-29.35
SD			0.15	0.37	0.35	0.47
Nebbio	Oct 14	1.07	-28.00	-25.66	-27.26	-29.77
	Oct 28	2.25	-27.90	-25.65	-27.48	-29.60
	Nov 8	3.21	-27.86	-25.44	-28.40	-29.76
	Nov 26	3.56	-28.05	-25.47	-26.80	-30.18
	Dec 12	4.41	-28.26	-25.58	-27.03	-30.07
	Jan 7	3.93	-28.47	-25.18	-27.58	-30.11
mean			-28.09	-25.50	-27.43	-29.92
SD			0.21	0.16	0.51	0.21
Castiglionese	Oct 14	0.94	-28.44	-27.05	-27.72	-28.82
	Oct 28	2.21	-28.25	-26.77	-27.41	-28.67
	Nov 8	5.05	-27.70	-26.43	-27.43	-28.07
	Nov 26	5.09	-27.55	-26.88	-27.28	-28.09
	Dec 12	5.57	-28.28	-26.61	-27.35	-28.97
	Jan 7	4.31	-28.04	-26.34	-27.20	-28.60
mean			-28.04	-26.68	-27.40	-28.54
SD			0.32	0.25	0.16	0.34
Leccino	Oct 14	0.94	-27.87	-26.52	-27.92	-28.56
	Oct 28	3.33	-27.98	-26.33	-27.58	-28.89
	Nov 8	4.81	-28.57	-26.98	-27.62	-29.94
	Nov 26	4.02	-27.99	-26.32	-27.16	-28.46
	Dec 12	5.24	-28.34	-26.43	-27.43	-29.89
	Jan 7	4.39	-28.37	-26.03	-27.25	-29.79
mean			-28.19	-26.44	-27.49	-29.26
SD			0.25	0.29	0.25	0.63

[a] Jaèn index.

oil but should be used in combination with other modern analytical approaches to define authenticity and origin of this valuable product.

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