CARBON ISOTOPE ANALYSIS OF VEGETABLE LIPIDS AS TRACER OF ENVIRONMENTAL CHANGES?

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Summary

The carbon isotope ratios of the individual lipids from different vegetable oils were analyzed by gas chromatography - combustion - isotope ratio mass spectrometry (GC/C/IRMS). This approach provides further insight into the purity and geographical origin of the oils. The $\delta^{13}C$ of the fatty acids appear to be preserved where lipids distribution have been altered by degradation or burial (diagenesis). The results indicate that GC/C/IRMS of plants lipids may be a potential tracer of the global changes recorded in the terrestrial carbon cycle.

Introduction

The effects of environmental or physical factors on the carbon isotope composition of plant and trees have revealed long-term trends in global environmental changes, particularly the increase in atmospheric carbon dioxide concentrations due to anthropogenic activities (e.g., Freyer and Belay 1983; Walcroft *et al.* 1997). Products derived from plants having a close link between CO_2 assimilation and yield may record these changes. Understanding how the natural atmospheric CO_2/plant/soil biomass system works now and has done in the past will help in predicting the effects of human-induced global-scale changes. The aim of this work was to explore the use of the composition of vegetable lipids as a tool for tracking the changes in the carbon cycle.

Food chemists and archaeologists are increasingly using the novel instrumentation of GC/C/IRMS for carbon isotope analysis of the individual lipids as a tool for assessing their preservation/degradation and mixing processes. Presently, the vast majority of edible vegetable oils are removed from the source oilseed or nut by solvent extraction methods. The resulting crude oil is then subjected to a series of rigorous physical and chemical refinement processes including degumation, refining, bleaching, and hot-steam deodorization. All these processes may entail molecular, structural, and isotopic effects. Thus, the isotopic composition of the bulk oil and its individual lipids serve to distinguish oils from a definite oilseed having undergone these refination-processes from those extracted mechanically (cold-pressed) and/or purposely left unrefined to enhance the flavor characteristics of the oil. These cold-pressed (CP) "gourmet" oils, such as olive, maize or sunflower oil, are highly esteemed, owing to its delicate flavor and nutritional and health benefits. Vegetable oils, particularly olive oil, were extracted traditionally from the fruits around the Mediterranean and Adriatic seas only by physical methods for thousands of years ago. Blending of these high-cost edible oils with refined oil or other vegetable oils of lower cost may lead to substantial economic profit. Stable carbon isotope analyses have proven to be a powerful tool for characterizing vegetable products from plants of different photosynthetic pathways (e.g., Doner 1991; Rossell 1994). In fact, isotopic discrimination against the heavier carbon isotope (^{13}C) occurs during photosynthesis, and is reflected in the isotopic compositions of the plant tissues and products. The most important atmospheric carbon dioxide-fixing reactions are the C_3 (Calvin) and C_4 (Hatch-Stack) photosynthetic pathways. All trees operate with C_3 pathway, and their carbon isotope compositions fall into the range -22 to -34‰ $\delta^{13}C$. Most plants in the tropic are C_4 plants, including tropical grasses, sedge, maize, sugar cane and salt marsh plants, and are isotopically heavier (-6 to -23 ‰). Clear isotopic distinctions are therefore observed between C_3 or C_4 plant products. The stable carbon isotopic data are expressed in the usual delta (δ) notation as the per mil (‰) deviation of the isotope ratio of a sample relative to that of a standard (Peedee belemnite limestone, PDB).

Studies evaluating or reporting the application of the novel instrumentation of GC/C/IRMS for carbon isotope analysis of the individual lipids as a tool for assessing adulteration (mixing and or refinement) of vegetable oils are Woodbury *et al.* (1995), Kelly *et al.* (1997), and Spangenberg *et al.* (1998). We report here the chemical and isotopic compositions of the fatty acids of the CP olive oils from the main producer countries of the Mediterranean and Adriatic region, and compare them with lower grade olive oils and other vegetable oils. The preservation of the isotopic signature of the individual fatty acids during early diagenesis is assessed in a plant-soil system. The isotopic results (1) enable a distinction between genuine cold -pressed vegetable oils and the refined oils, (2) help to gain insight into the origin of the lipids, and (3) provide information on the $\delta^{13}C$ values of atmospheric carbon dioxide.

Materials and Methods

Forty-five samples of CP *extra virgin olive oil* were obtained from the major oil-producing regions of Spain (7), Italy (18), Greece (4), France (3), Slovenia (6), and Croatia (7). All the samples are from the 1996-1997 and 1997-1998 olive oil seasons. These oils were compared with CP olive oils from Australia (2) and South Africa (1), refined olive and pomace oils from Turkey (1), Tunisia (2), and Morocco (1), and other vegetable oils, including maize (France: 1, Slovenia: 1, Australia: 1), sunflower (France: 1, Switzerland: 1, Slovenia: 2, Australia: 1, South Africa: 1), walnut (France: 1), rape (Switzerland: 1, Slovenia: 1), "canola" -which is a genetically modified rapeseed- (Canada: 3), hazelnut (France: 1), pumpkin (Slovenia: 4), sesame (Peru: 2), avocado (South Africa: 1), soy (geographical origin unknown: 1), and grape seed (Italy: 1). The fatty acids from the oil samples were separated by alkaline hydrolysis and derivatized to methyl esters for their GC analyses. The analytical approach combined chemical characterization of the fatty acids methyl esters by capillary column gas chromatography - mass spectrometry (GC/MS), and isotope analysis of individual fatty acids by the use of GC/C/IRMS. The carbon isotope composition of the bulk oil samples were determined using the on-line elemental analyzer (EA) -continuous flow - isotope ration mass spectrometer (IRMS). The EA/IRMS system consist of a Carlo Erba 1108 elemental analyzer coupled by a Finnigan MAT continuous

flow interface (ConFlo II) to the Delta S IRMS. The compound specific isotope analyses of the fatty acids were obtained by the use of a HP 6890 GC coupled to the Delta S IRMS by a combustion (C) interface III (GC/C/IRMS).

Results and discussion

Bulk isotopic composition

The non-maize vegetable oils have isotopic compositions (-26.5 to -35.5‰) typical of C_3 plants. The scatter of the $\delta^{13}C$ values of the C_3-vegetable oils may be attributed to variation of the isotope effect during fixation of carbon dioxide by different plants varieties, different climate and growth condition. This isotopic shift is at least partially explained by factors affecting the chemical distribution of the fatty acids, and particularly by the physiological processes and enzymatic reactions occurring in the plants cells (Rossell 1994; Vogel 1993; O'Leary 1988). The $\delta^{13}C$ differences may be enhanced by the different $\delta^{13}C$ values of atmospheric carbon dioxide in different geographical regions. Additionally, the chemical changes (transmerization and oxidation) during refining of the vegetable oil may cause a further isotopic discrimination. In fact, the preferential cleavage of the ^{13}C-^{12}C single or double C-C bonds and loss of light ^{12}C moieties occurs during degradation and structural transformation of the fatty acids.

Molecular isotope data and implications for adulteration

The $\delta^{13}C$ values of the virgin olive oil fatty acids vary between -26.5 to -35.5 ‰ (Fig. 1). The values for oil of the different C_3 source plants and distinct quality fall in to this broad range. The isotopic composition of the major fatty acids from oils of different origin, particularly maize, olive, groundnut, sunflower, rape, and pumpkin are similar within the analytical uncertainty (Figs. 1 and 2). To explain this relationship, one has to consider fatty acid synthesis in plant cells (e.g., Cherif *et al.* 1979; Jaworski *et al.* 1990).

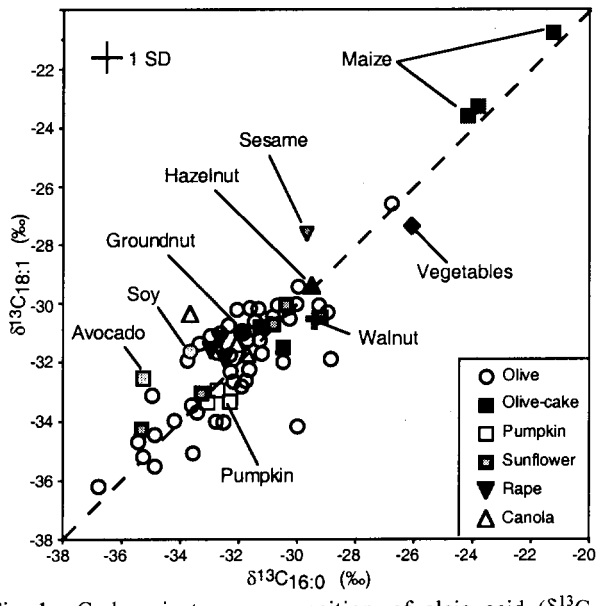

Fig. 1. Carbon isotope composition of oleic acid ($\delta^{13}C_{18:1}$) versus palmitic acid ($\delta^{13}C_{16:0}$) of the vegetable oils.

The biosynthetic reactions of fatty acids are essentially the same in all plants. The fundamental reaction sequence by which the longer chains of fatty acids are assembled is catalyzed by a multienzyme complex. Elongation of carbon chains occurs in a fashion similar to that of synthesis, but differ in the enzymes which catalyze these reactions. The product formed by addition of one acetyl group to palmitic acid (16:0) is stearate (18:0). At the same site of the plant tissue (the endoplasmic reticulum) unsaturated oxidative reactions catalyzed by fatty acyl-coenzyme A desaturase introduces the unsaturation to the fatty acids. The enzyme that introduces further double bonds, to produce the 18:2, and 18:3 acids do not act on free fatty acids but on a phospholipid, containing at least one oleate (18:1) linked to the glycerol moiety. One can safely assume that the isotopic discrimination between the first biosynthesized fatty acid (16:0) and the first elongation and unsaturated product will be less than the analytical error (±0.5‰) of the GC/C/IRMS measurement (Fig. 1).

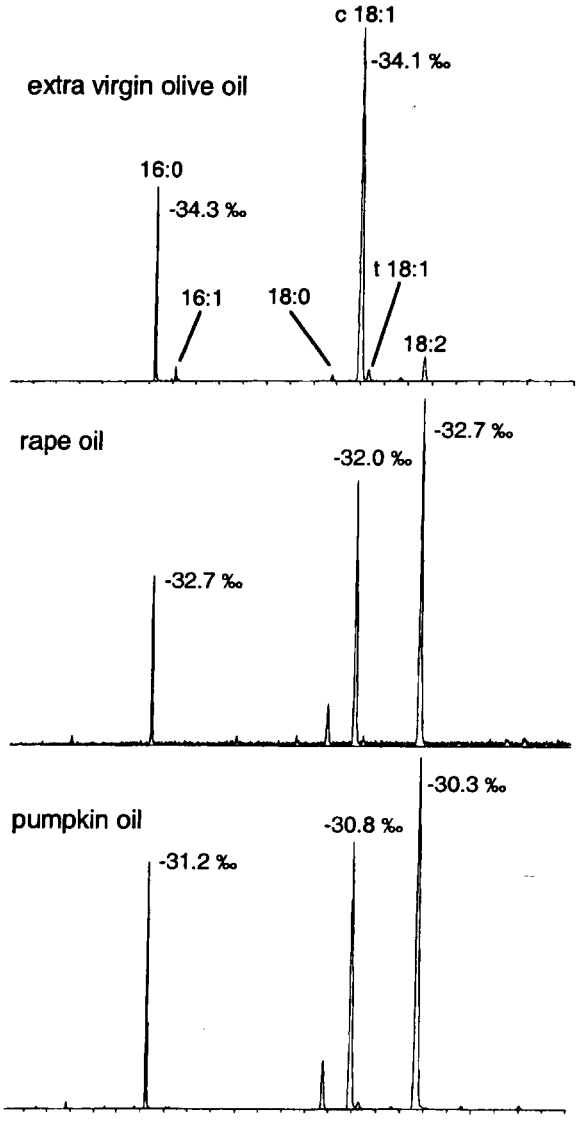

Fig. 2. GC/MS chromatograms of the fatty acid methyl esters of CP extra virgin olive oil, rapeseed oil, and pumpkin oil from Slovenia. Fatty acids: palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), elaidic (t18:1), linoleic (18:2). The $\delta^{13}C$ values of the major fatty acids is given.

Therefore, substantial separation of the oils from the 1:1 line in the $\delta^{13}C_{16:0}$ versus $\delta^{13}C_{18:1}$ diagram suggest admixture of a CP vegetable oil with refined oils or other vegetable oils of different 18:1/16:0 concentration-ratios than the genuine cold pressed vegetable oil. This helps to explain the depletion in ^{13}C of the known blend oils, as the olive pomace and "vegetable" oil, and strongly suggests the blending or inappropriate processing of many vegetable oils. Furthermore, some of the variations of the isotopic composition of the individual lipids of the oil samples may be due to differences in the metabolism of different plants variety, climatic and plant growth conditions, including concentration of atmospheric carbon dioxide and cultivation practices. These factors may affect the isotopic composition of the main fatty acids in a similar way and, consecuently, the oils-samples would move along the 1:1 line in the $\delta^{13}C_{16:0}$ versus $\delta^{13}C_{18:1}$ diagram.

Preservation of the isotopic composition of oil fatty acids

Although the composition of vegetable lipids in contemporary material and plant lipids is well established, the effects of degradation, e.g. chemical, microbiological or physical, on these individual lipids and their distributions during plant decay and burial (diagenesis) is still poorly understood (Dudd *et al.* 1998). Ongoing research on the early diagenesis of these lipids in soils, fresh-water or recent-sediment columns, and archaeological residues will improve our understanding of the high-frequency recycling of organic carbon in the carbon cycle. One example of our work in this arae is the $\delta^{13}C$ analysis of fatty acids from an olive tree leaf - olive oil - soil system (Fig. 3).

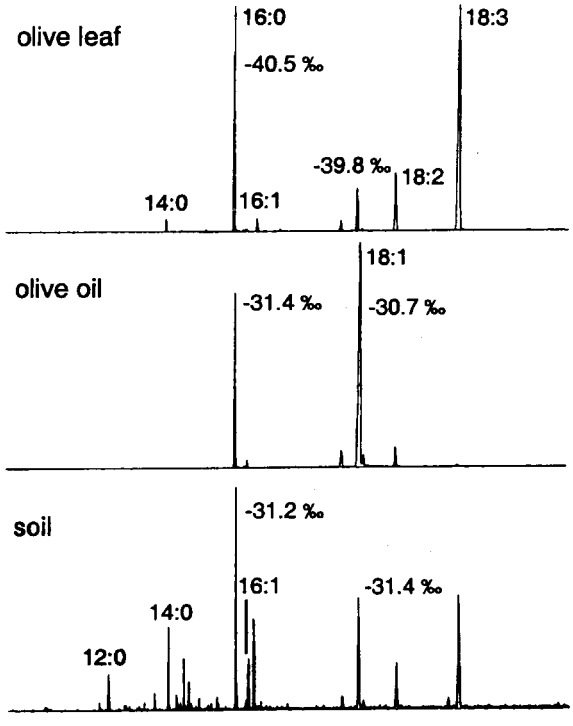

Fig. 3. GC/MS chromatograms of the fatty acid methyl esters from olive tree leaf, olive oil, and soil from a Slovenian farm. $\delta^{13}C$ values of the individual major fatty acids are given.

The preservation of the isotopic composition in the oil-soil system suggest that the isotopic composition of the individual lipids from plants products (Fig. 3), combined with molecular ratios (e.g., 18:0/16:0, 18:1/16:0) may be utilize to link the origin, pathway, and final fate of the lipids. Further work is needed for an evaluation of the preservation of the isotopic composition of individual fatty acids in different matrices (plants, soils, sediments, water, aerosols, archaeological residues) along the environmental pathway.

$\delta^{13}C$ of individual fatty acids as tracer of environmental changes

We believe that the carbon isotope composition of individual fatty acids in genuine olive oil may be a sensitive molecular trace of paleoclimatic changes in the Mediterranean and Adriatic basins. This assumption is based on the following facts: (1) olive trees have been cultivated around the Mediterranean and Adriatic Seas since the late Holocene (2000 B.C.); (2) these trees are highly productive, with a closer link between photosynthesis and yield than in most deciduous trees; (3) the $\delta^{13}C$ of bulk oil and individual lipids (glycerols, sterols, alcohols) are slightly affected by the olive variety, harvesting date, and olive ripening degree (Bianchi *et al.* 1993); and (4) the results of archaeological studies and our own research suggest that the isotopic composition of the individual fatty acids is preserved during early diagenesis. Furthermore, the measured differences in the isotopic composition of bulk oil and individual fatty acids from C_3 (olive, sunflower) and C_4 (maize) vegetable oils from distinct hemispheres may be explained by the different $\delta^{13}C$ values of the atmospheric carbon dioxide in each hemisphere (Table 1).

Table 1. Isotope ratios of the bulk and major fatty acids of vegetable oils from each hemisphere.
Med/Adr = Mediterranean and Adriatic countries.

	$\delta^{13}C$ bulk oil	$\delta^{13}C$ 16:0	$\delta^{13}C$ 18:1
Maize oil			
Australia	-17.6	-23.9	-23.2
France	-16.5	-21.1	-20.9
Olive oil			
Australia	-30.0	-32.1	-31.8
South Africa	-28.6	-32.4	-30.8
Med/Adr (54)	-29.7±1.1	-32.1±1.8	-31.9±1.9
Sunflower			
Australia	-30.2	-35.4	-34.3
Med/Adr (4)	-30.0±0.6	-31.4±1.8	-31.3±1.3

The Mediterranean/Adriatic region is highly affected by population increases, industrial expansion, and anthropogenic or accidental biomass burning (e.g., forest fires at Southern France and Spain). Thus, an important increase in the amount of atmospheric carbon dioxide was produced during the last century, and historical changes are well documented. The subject of a current project is to trace the paleoclimatic changes in the late Holocene (600 B.C. to the present time) in this region by using carbon isotope composition of the major fatty acids in genuine olive oil extracted from archaeological vessel residues or from barrels transporting olive oil found in sunken ships in the Mediterranean Sea.

Fatty acids and in general all the oleochemicals from plants are of environmental importance (Richtler and Knaut 1991). They are readily biodegradable, they can replace fossil fuels as bio-diesel and lubricants, and they may serve to reduce the

amount of atmospheric carbon dioxide. This last point merit a further explanation. Many tree-plant cultivars (oil-palm, sunflower, rape) are productive systems with positive net CO_2 fixation (they assimilate more CO_2 than they release by decay), and may be utilize as alternative to fossil fuel products, thus decreasing the anthropogenic contribution to atmospheric CO_2 due to fuel burning. Thus, the use of oleochemicals as "energy-saver" will utilize the rapidly recycled early biomass energy of the small carbon cycle, and avoid the exploitation of stored energy as fossil biomass in the geosphere.

CONCLUSIONS

The $\delta^{13}C$ values of the bulk oil and its individual fatty acids can be used for the identification of the sources of a particular vegetable oil. The use of $\delta^{13}C_{16:0}$ versus $\delta^{13}C_{18:1}$ covariations holds great promise for assessing cases where impurity or adulteration is suspected. Blending of olive oil with edible oils with slightly different fatty acids composition (olive pomace, sunflower, hazelnut) may be detected using this approach combined with molecular information and carbon isotope composition of the bulk oil. This approach will also serve to track mixing processes and preferential degradation during early diagenesis of plant lipids. The results demonstrate the importance of elucidating the metabolism and biosynthesis (chain elongation and unsaturation) of the fatty acids which cause the $^{13}C/^{12}C$ discriminations observed in the individual lipids of the vegetable oils, in order to better utilize the $\delta^{13}C$ values as indicators of their origin. Our first results show that the $\delta^{13}C$ values of individual fatty acids appear unaffected by degradation during shallow burial. The preliminary results suggest that the carbon isotope composition of individual fatty acids in genuine olive oil may be a sensitive molecular trace of paleoclimatic changes in the Mediterranean/Adriatic basin.

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