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CHARACTERIZATION OF OLIVE OILS FROM SLOVENIA AND CROATIA BY COMPOUND SPECIFIC ISOTOPE ANALYSIS

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ABSTRACT

The fatty acids of olive oils from Slovenia and Croatia were chemically and isotopically characterized. The analytical approach utilized combined gas chromatography - mass spectrometry (GC-MS) and the novel technique of compound specific isotope analysis (CSIA) through gas chromatography coupled to a stable isotope ratio mass spectrometer (IRMS) via a combustion (C) interface (GC-C-IRMS). This approach provides further insights into the control of the purity and the geographical origin of oils. The differences in the $\delta^{13}C$ values of palmitic and oleic acids are discussed as the differences in biosynthesis of these acids in the plant tissue, admixing of distinct vegetable oils, and degradation of the lipids during oil extraction and refinement.

Key words: *olive oil, vegetable lipids, carbon isotope, CSIA, adulteration, geographical origin*

INTRODUCTION

Stable carbon isotope analyses have proven to be a powerful tool for assessing the authenticity of vegetable food products from plants of different photosynthetic pathways (Doner, 1991). During photosynthetic fixation of carbon dioxide into plant biomass, plant cells discriminate against the heavier stable carbon isotope ^{13}C. The most important atmospheric CO_2-fixing reactions are the C_3 and C_4 pathways (Farquhar *et al.*, 1989; O'Leary, 1988, 1993). C_3 plants use the Calvin cycle for CO_2 fixation, while the C_4 plants use the Hatch-Stack cycle. All trees operate with the C_3 pathway, and their carbon isotope compositions fall into the range of -34 to -22‰. C_4 plants comprise most plants in the tropics, including tropical grasses, sedge, maize, sugar cane and salt marsh plants, and are isotopically heavier (-23 to -6‰). Factors other than the CO_2-fixation, however, may also have a less important impact on the isotopic composition of plants and their products. These include plant growth rate, local atmospheric CO_2 concentration, nutritional status of the cells, water availability, and cultivation practices (O'Leary, 1993). Therefore, the carbon isotopic composition of bulk oil and individual lipids may record the source and geographical origin of a plant product. Food chemists are increasingly using on-line gas chromatography - combustion - stable isotope ratio mass spectrometry (GC-C-IRMS) for carbon isotope analysis of the individual lipids as a tool for assessing adulteration of vegetable oils (Woodbury *et al.*, 1995; Kelly, 1997; Remaud, 1997). Previous work focusing on the chemical and isotopic composition of the major fatty acids (palmitic, stearic, oleic, and linoleic acids) of cold pressed (CP) olive oils from the main producing countries of the Mediterranean region were reported (Spangenberg *et al.*, 1998). We present herewith the isotopic compositions of fatty acids of the CP olive oils from Slovenia and Croatia.

MATERIALS AND METHODS

Thirteen samples of extra virgin olive oil were obtained from Slovenia (Koper region, n=6) and Croatia (Istra, Dalmatia, n=7). All the samples were from the 1997-1998 olive season. The analytical approach combined chemical characterization of the fatty acid methyl esters (FAMES) by gas chromatography - mass spectrometry (GC-MS), and carbon isotope analyses of individual fatty acids by GC-C-IRMS. The bulk oils were analyzed for carbon isotope composition by combustion isotope ratio mass spectrometry using an on-line Carlo Erba 1108 elemental analyzer (EA) connected to a Finnigan MAT Delta S IRMS via a Conflo II split interface (EA-IRMS). All the analyses were performed at the Department of Earth Sciences of the University of Lausanne. The stable carbon isotope ratios are reported in the delta (δ) notation as the per mil (‰) deviations relative to the Pee Dee Belemnite limestone (PDB). The reproducibility of the EA-IRMS analyses was better than 0.1‰ (1 SD). Three to five replicate GC-C-IRMS runs were performed for each sample. The reproducibility ranged between ±0.1 and ±0.4‰ (1 SD). The accuracy of the GC-C-IRMS analyses was monitored by co-injection of a FAME laboratory standard of the known isotopic composition. The isotopic shift due to the carbon introduced in the fatty acid methylation was corrected by a mass balance equation (Spangenberg *et al.*, 1998).

RESULTS AND DISCUSSION

Fatty acids composition

The scatter of the compositions of fatty acids for the virgin olive oils probably reflects the variation in variety, climatic conditions of the area, water-use efficiency in cultivars, salinity, temperature and pH of the irrigation water, olive-ripening stage and other factors (results not shown for brevity).

Bulk isotopic composition

The $\delta^{13}C$ of the bulk olive oils (-27.7 to -30.6‰ show isotopic compositions typical of C_3 plants (Tab. 1). The scatter of the $\delta^{13}C$ values of the oils (2.9‰ may be attributed to factors affecting the chemical distribution of the fatty acids, and particularly by the physiological processes and enzymatic reactions occurring in the plant cells. Additionally, the chemical changes (transmerization of oleic acid and oxidation) during thermal degradation (natural or induced during steam washing or other refining procedures) of the olive oil, or blending of CP oil with refined olive oil or other vegetable oil may cause a further isotopic discrimination.

Tab. 1: Carbon isotope composition of bulk oil and main individual fatty acids in olive oil samples from Slovenia and Croatia.
Tab. 1: Izotopska sestava celokupnega ogljika in ogljika posameznih maščobnih kislin v vzorcih oljčnih olj iz Slovenije in Hrvaške.

		$\delta^{13}C$ (‰ PDB)			
Sample	**Country**	**bulk oil**	**palmitic (16:0)**	**stearic (18:0)**	**oleic (18:1)**
COIL-58	Slovenia	-30.1	-34.3	-33.3	-34.1
COIL-59	Slovenia	-28.4	-31.4	-31.3	-30.1
COIL-60	Slovenia	-29.4	-32.1	-32.2	-30.1
COIL-75	Slovenia	-29.1	-32.8	-32.2	-31.6
COIL-76	Slovenia	-30.0	-33.5	-	-33.6
COIL-79	Slovenia	-29.1	-32.4	-32.8	-31.9
COIL-65	Croatia	-29.6	-32.9	-32.4	-31.0
COIL-66	Croatia	-29.4	-31.8	-32.0	-30.3
COIL-67	Croatia	-29.1	-30.6	-31.5	-30.7
COIL-68	Croatia	-29.8	-33.3	-32.7	-31.3
COIL-77	Croatia	-30.6	-35.0	-	-33.1
COIL-78	Croatia	-27.7	-31.8	-	-32.6
COIL-80	Croatia	-28.0	-35.4	-31.2	-32.3

- = not analysed

Isotopic composition of individual fatty acids

The $\delta^{13}C$ values of the virgin olive oil fatty acids vary between -34.1 to -28.5‰ (Table 1). A substantial separation of the oils from the 1:1 line in the $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:1}$ diagram (16:0 = palmitic acid, 18:1 = oleic acid) suggests admixing of cold pressed virgin olive oil with refined olive oils or other vegetable oils of different 18:1/16:0 concentration-ratios than the genuine olive oil. The distribution of the samples in the $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:1}$ diagram strongly suggests the adulteration or inappropriate processing of some CP olive oils (Fig. 1). Virgin CP olive oils are separated from the lower grade olive oils by dedicated principal component analysis performed combining the fatty acid composition and the bulk and molecular carbon isotope ratios (results not shown for brevity).

CONCLUSIONS

The $\delta^{13}C$ values of the bulk oil and individual fatty acids can be used for identification of the sources of olive oil and control of their authenticity. The use of $\delta^{13}C_{16:0}$ vs. $\delta^{13}C_{18:1}$ covariations serves to assess cases where impurity or adulteration is suspected. Blending of olive oil with edible oils with slightly different fatty acid compositions *(e.g.,* olive pomace, sunflower, hazelnut) may be detected using this approach combined with molecular information (GC-MS) and the carbon isotope composition of the bulk oil.

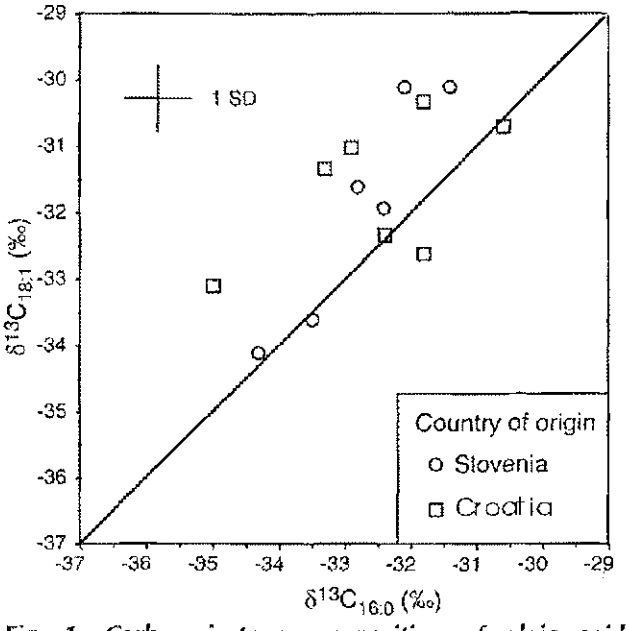

Fig. 1: Carbon isotope composition of oleic acid ($\delta^{13}C_{18:1}$) vs. palmitic acid ($\delta^{13}C_{16:0}$) of olive oils from Slovenia and Croatia.
Sl. 1: Izotopska sestava ogljika oleinske kisline ($\delta^{13}C_{18:1}$) v odvisnosti od izotopske sestave ogljika palmitinske kisline ($\delta^{13}C_{16:0}$) v vzorcih oljčnih olj iz Slovenije in Hrvaške.

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UPORABA STABILNIH IZOTOPOV OGLJIKA PRI KARAKTERIZACIJI OLJČNEGA OLJA IZ SLOVENIJE IN HRVAŠKE

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POVZETEK

Meritve izotopske sestave ogljika so se izkazale kot izvrstno naravno sledilo za spremljanje različnih procesov, zato jih s pridom izkoriščamo tudi v živilski industriji pri določanju kakovosti in pristnosti (avtentičnosti) različnih živil - vin, sadnih sokov, medu, olj. Poleg tega se stabilni izotopi uporabljajo tudi pri določitvi geografskega porekla.

V prispevku, ki je nastal v sodelovanju z Laboratorijem za stabilne izotope, Inštituta za mineralogijo in petrologijo Univerze v Luzani v Švici, smo predstavili uporabo stabilnih izotopov ogljika pri določitvi avtentičnosti in geografskega porekla oljčnega olja. Določili smo kemijsko in izotopsko sestavo maščobnih kislin v oljčnih oljih iz Slovenije in Hrvaške. Koncentracije maščobnih kislin smo določili s plinskim kromatografom s kapilarno kolono (GC-MS), njihovo izotopsko sestavo ($\delta^{13}C$) pa z masnim spektrometrom za stabilne izotope GC-C-IRMS. Iz korelacije med izotopsko sestavo ogljika palmitinske kisline, $\delta^{13}C_{16:0}$, od izotopske sestave ogljika oleinske kisline, $\delta^{13}C_{18:1}$, lahko ugotovimo možne potvorbe in nepravilno predelavo oljčnega olja. Drugi možni vzroki, ki privedejo do razlik med vrednostmi $\delta^{13}C_{16:0}$ in $\delta^{13}C_{18:1}$, so še: različna biosinteza teh kislin v rastlinskih tkivih ter razgraditev maščob pri ekstrakciji olj in nadaljnjem čiščenju ekstrakta.

Ključne besede: oljčno olje, rastlinske maščobe, izotopi ogljika, CSIA, potvorjenost, geografsko poreklo

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