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ARTICLE

Characterization of Rapeseed (*Brassica napus*) Oils by Bulk C, O, H, and Fatty Acid C Stable Isotope Analyses

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Rapeseed (*Brassica napus*) oils differing in cultivar, sites of growth, and harvest year were characterized by fatty acid concentrations and carbon, hydrogen, and oxygen stable isotope analyses of bulk oils ($\delta^{13}C_{bulk}$, $\delta^{2}H_{bulk}$, $\delta^{18}O_{bulk}$ values) and individual fatty acids ($\delta^{13}C_{FA}$). The $\delta^{13}C_{bulk}$, $\delta^{2}H_{bulk}$, and $\delta^{18}O_{bulk}$ values were determined by continuous flow combustion and high-temperature conversion elemental analyzer−isotope ratio mass spectrometry (EA/IRMS, TC-EA/IRMS). The $\delta^{13}C_{FA}$ values were determined using gas chromatography−-combustion−isotope ratio mass spectrometry (GC/C/IRMS). For comparison, other C_3 vegetable oils rich in linolenic acid (flax and false flax oils) and rich in linoleic acid (poppy, sunflower, and safflower oils) were submitted to the same chemical and isotopic analyses. The bulk and molecular $\delta^{13}C$ values were typical for C_3 plants. The $\delta^{13}C$ value of palmitic acid ($\delta^{13}C_{16:0}$) and *n*-3 α-linolenic acid ($\delta^{13}C_{18:3n\text{-}3}$) differed ($p < 0.001$) between rape, flax, and poppy oils. Also within species, significant differences of $\delta^{13}C_{FA}$ were observed ($p < 0.01$). The hydrogen and oxygen isotope compositions of rape oil differed between cultivars ($p < 0.05$). Major differences in the individual $\delta^{13}C_{FA}$ values were found. A plant-specific carbon isotope fractionation occurs during the biosynthesis of the fatty acids and particularly during desaturation of C_{18} acids in rape and flax. Bulk oil and specific fatty acid stable isotope analysis might be useful in tracing dietary lipids differing in their origin.

KEYWORDS: ***Brassica napus*; rapeseed oil; fatty acid composition; carbon stable isotope; oxygen isotope; hydrogen isotope; GC/FID; GC/MS; EA/IRMS; TC-EA/IRMS; GC/C/IRMS**

INTRODUCTION

Rape crops, *Brassica napus*, *Brassica rapa*, and other *Brassica* species (Cruciferae, also known as the mustard family), are believed to be among the oldest plants cultivated by man (*1*). Rapeseed production in India and China started at least 1500 years ago. *B. napus* probably developed in Europe during the Middle Ages, first in the Mediterranean area, and by the 15th century, rapeseed was being cultivated in the Rhineland (*2*). It is not clear when rapeseed oil became an important component of the human diet as food oil in addition to its use as fuel for lamp lighting, soap, and candle production (*2*, *3*). The optimal growing temperature of rape is around 20 °C, and the highest seed oil content is attained when seeds mature between 10 and 15 °C (*4*). Therefore, it has become an important oilseed crop in various countries in cool temperate northern climates where most other oilseed crops do not grow.

Rapeseed oil is the third most important vegetable oil after palm oil and soybean oil. Rape is now grown and traded for the production of rapeseed as animal feed, vegetable oil for human consumption, biodiesel, lubricants, and hydraulic liquids. More than 30 countries on five continents cultivate several rapeseed species, and the worldwide rapeseed production in the 2008−2009 season was about 21 million metric tons (*5*). The leading producers are Canada, China, European Union (EU) countries (Denmark, France, Germany, Italy, the United Kingdom, Spain), and India. Winter type *B. napus* is the main rapeseed crop in most of Europe, and one of the leading oilseed plants grown in Switzerland, with a production of about 45 million kilograms per year (*6*). The rising rapeseed demand in EU countries for both the feed industry and biodiesel manufacturers, triggered by the temperature drop in European winters, has strongly motivated the improvements in agronomic techniques, processing methods, client-oriented breeding, genetic manipulations, and seed variety production. Breeding developments led to the production of single-low (low in erucic acid, 22:1*n*-9) and double-low (low in 22:1*n*-9 in the oil and low in glucosinolates in the meal) rape cultivars (*7*). In Canada, all double-low rape varieties are known as canola, and in Europe the term rapeseed includes double-low and other quality varieties, such as the high 22:1*n*-9 acid rapeseed (industrial rapeseed).

The main fatty acid composition varies strongly between rape varieties and their genetic modifications, having as end members high-erucic rapeseed oils (45% 22:1) and high-oleic rapeseed oils (80% 18:1). High-erucic rapeseed oils are also rich in eicosenoic

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Table 1. Fatty Acid Composition of the Studied Vegetable Oil Samples

					fatty acid content[b] (%)					
sample	plant species	cultivar	growing site[a]	year of harvest	palmitic (16:0)	stearic (18:0)	oleic (18:1*n*-9)	linoleic (18:2)	linolenic (18:3)	other fatty acids[c]
CHOIL-342	*Brassica napus*	Bioraps	district of Aargau (Aargau, CH)	2007	4.2	1.8	62.6	17.7	7.7	5.9, 2.7 (18:1*n*-7), 1.2 (20:1*n*-9)
CHOIL-350	*Brassica napus*	Bioraps	district of Aargau (Aargau, CH)	2007	4.2	1.9	62.8	17.7	7.6	5.9, 2.4 (18:1*n*-7), 1.2 (20:1*n*-9)
CHOIL-345	*Brassica napus*	Cabriolet	Reckenholz (Zürich, CH)	2007	4.0	1.5	70.7	9.0	9.1	5.7, 3.0 (18:1*n*-7), 1.2 (20:1*n*-9)
CHOIL-352	*Brassica napus*	Cabriolet	Feldbach (Zürich, CH)	2008	4.0	1.5	70.4	9.6	9.0	5.6, 3.0 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-327	*Brassica napus*	Expert	Reuenthal (Aargau, CH)	2007	4.7	1.5	57.7	21.7	8.8	5.6, 3.0 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-334	*Brassica napus*	Expert	Reckenholz (Zürich, CH)	2007	4.9	1.5	57.2	21.7	8.8	6.0, 3.2 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-353	*Brassica napus*	Expert	Feldbach (Zürich, CH)	2008	4.7	1.9	58.9	20.4	8.8	5.1, 2.7 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-349	*Brassica napus*	Oase	unknown site (DE)	2006	4.2	1.9	63.3	17.8	7.5	5.3, 2.4 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-329	*Brassica napus*	Oase	Reuenthal (Aargau, CH)	2007	4.4	1.9	62.2	17.9	8.4	5.3, 2.7 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-332	*Brassica napus*	Oase	Reckenholz (Zürich, CH)	2007	4.3	2.2	62.6	17.6	8.1	5.3, 2.6 (18:1*n*-7), 1.0 (20:1*n*-9)
CHOIL-354	*Brassica napus*	Oase	Feldbach (Zürich, CH)	2008	4.3	1.7	63.5	16.9	8.3	5.5, 2.9 (18:1*n*-7), 1.0 (20:1*n*-9)
CHOIL-328	*Brassica napus*	Robust	Reckenholz (Zürich, CH)	2007	4.8	1.8	59.5	18.5	9.4	6.0, 3.2 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-356	*Brassica napus*	Robust	Feldbach (Zürich, CH)	2008	5.0	1.5	59.1	17.7	10.1	5.2, 3.6 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-337	*Brassica napus*	Remy	Reuenthal (Aargau, CH)	2007	4.7	1.8	59.7	18.9	9.2	5.6, 3.0 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-347	*Brassica napus*	Remy	Reuenthal (Aargau, CH)	2007	4.7	1.8	59.6	19.4	9.0	5.6, 2.9 (18:1*n*-7), 1.0 (20:1*n*-9)
CHOIL-348	*Brassica napus*	Remy	Reuenthal (Aargau, CH)	2007	4.7	1.8	59.8	19.5	8.8	5.5, 2.9 (18:1*n*-7), 1.0 (20:1*n*-9)
CHOIL-346	*Brassica napus*	Remy	Reckenholz (Zürich, CH)	2007	4.9	1.9	59.5	19.2	8.9	5.5, 2.9 (18:1*n*-7), 1.0 (20:1*n*-9)
CHOIL-355	*Brassica napus*	Remy	Feldbach (Zürich, CH)	2008	4.8	1.7	59.5	19.1	9.3	6.4; 3.6 (18:1*n*-7), 1.0 (20:1*n*-9)
CHOIL-326	*Brassica napus*	Viking	Reuenthal (Aargau, CH)	2007	4.5	1.5	57.5	22.1	8.9	5.4, 2.8 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-331	*Brassica napus*	Viking	Reckenholz (Zürich, CH)	2007	4.6	1.5	56.8	22.5	9.1	5.5, 2.9 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-351	*Brassica napus*	Viking	Feldbach (Zürich, CH)	2008	4.4	1.5	58.7	21.0	9.2	5.9, 3.0 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-116	*Brassica napus*	unknown	Albertschwil (St.Gallen, CH)	2006	4.1	1.8	62.7	17.3	7.5	6.4, 2.9 (18:1*n*-7), 1.3 (20:1*n*-9)
CHOIL-117	*Brassica napus*	unknown	Albertschwil (St. Gallen, CH)	2008	4.1	1.8	62.8	17.3	7.5	6.3, 2.8 (18:1*n*-7), 1.3 (20:1*n*-9)
CHOIL-118	*Brassica napus*	unknown	Albertschwil (St. Gallen, CH)	2008	4.2	1.7	62.1	17.2	8.8	6.0, 3.0 (18:1*n*-7), 0.6 (20:1*n*-9)
CHOIL-121	*Brassica napus*	unknown	Neunkirch (Schaffhausen, CH)	2007	4.6	1.8	60.1	19.1	8.8	5.6, 2.8 (18:1*n*-7), 1.1 (20:1*n*-9)
CHOIL-113	*Linum usitatissimum*		St. Otmar (St.Gallen, CH)	2006	4.7	6.3	24.4	14.9	47.3	2.5, 0.6 (18:1*n*-7), 0.2 (22:0)
CHOIL-114	*Linum usitatissimum*		St. Otmar (St. Gallen, CH)	2007	4.4	6.3	23.6	14.8	48.5	2.3, 0.5 (18:1*n*-7), 0.2 (22:0)
CHOIL-115	*Linum usitatissimum*		St. Otmar (St. Gallen, CH)	2008	4.5	6.1	21.7	15.2	50.2	2.3; 0.5 (18:1*n*-7), 0.2 (22:0)
CHOIL-122	*Camelina sativa*		Neunkirch (Schaffhausen, CH)	2007	5.4	2.4	15.6	16.7	36.5	23.5, 13.5 (20:1*n*-9), 2.5 (22:1*n*-9)
CHOIL-110	*Papaver somniferum*		Albertschwil (St. Gallen, CH)	2006	8.6	2.2	18.0	68.4	0.8	1.9, 1.1 (18:1*n*-7), 0.1 (20:0)
CHOIL-111	*Papaver somniferum*		Albertschwil (St. Gallen, CH)	2007	8.8	2.1	17.1	69.4	0.7	1.8, 1.0 (18:1*n*-7), 0.1 (20:0)
CHOIL-112	*Papaver somniferum*		Uzwil (St. Gallen, CH)	2007	8.8	2.3	15.0	71.2	0.7	1.9, 1.0 (18:1*n*-7), 0.1 (20:0)
CHOIL-120	*Papaver somniferum*		Neunkirch (Schaffhausen, CH)	2007	9.2	1.8	11.6	75.0	0.7	1.7, 1.1 (18:1*n*-7), 0.1 (20:0)
CHOIL-119	*Helianthus annuus*		Neunkirch (Schaffhausen, CH)	2007	5.8	5.0	17.1	69.1	0.1	2.9, 0.5 (18:1*n*-7), 0.7 (20:0)
CHOIL-357	*Carthamus tinctorius*		Dettighofen (Thurgau, CH)	2004	5.6	1.8	6.5	83.6	0.2	2.3, 0.6 (18:1*n*-7), 0.3 (22:0)

[a] CH, Switzerland; DE, Germany. [b] The relative abundances are given in weight percent. [c] 18:1*n*-7, vaccenic acid; 20:0, arachidic acid; 20:1*n*-9, eicosenoic acid; 22:0, behenic acid; 22:1*n*-9, erucic acid.

acid (6% 20:1) and contain significant levels of linolenic acid (10% 18:3*n*-3) and linoleic acid (14% 18:2*n*-6) (*4*). The high-oleic rapeseed oils have a lower content of 18:2*n*-6 (7%) and 18:3*n*-3 (5%) (*4*). Edible rapeseed oil is characterized by a relatively low level of saturated acids (6%) and is high in oleic acid (50−65% 18:1) compared to other vegetable oils. The main polyunsaturated fatty acids are linoleic acid (20−30% 18:2*n*-6) and linolenic acid (6−14% 18:3*n*-3), occurring in a favorable omega-6 (*n*-6) to omega-3 (*n*-3) ratio (*4*, *7*). The most abundant *n*-3 fatty acid in rape oil is α-linolenic acid (18:3*n*-3), which has been found to be beneficial in the primary prevention of cardiovascular diseases and is of high importance for brain development (*8*−*10*). Rape oil, with an *n*-6/*n*-3 ratio of much lower than 4, is considered to be a very healthy edible oil (*8*, *10*, *11*). The fatty acid composition of vegetable oils originated from naturally (organic) bred plants varies with environmental conditions, mainly temperature and water availability, which may be related to some extent with the geographical origin (*12*).

To ensure the purity of edible vegetable oils and fats, and to protect high-quality varieties against adulteration and incorrect processing and labeling of the trading goods, the combined fatty acid composition and bulk and individual fatty acids' carbon isotope composition has been proven to be a powerful tracer of potential frauds in authenticity (*13*−*19*). The carbon isotope composition (reported as δ^{13}C values, where δ value in ‰ = $(R_{sample} - R_{standard})/R_{standard} \times 1000$ and $R = {}^{13}C/{}^{12}C$) of plants and their products is mainly controlled by the isotopic composition of the fixed CO_2, the isotope fractionation accompanying the photosynthetic CO_2 uptake, and the isotopic composition and amount of the respired CO_2 (*20*). The most important atmospheric CO_2-fixing reaction pathways are C_3 and C_4 (*20*). C_3 plants (plants adapted to temperate ecosystems, including most vegetables, fruits, and temperate grasses) use the Calvin−Benson cycle for CO_2 fixation, and the δ^{13}C values fall into the range from −34 to −22‰. The C_4 plants use the Hatch−Slack cycle and have lower isotopic fractionation compared to the C_3 plants. C_4 plants are plants adapted to hot, arid environments, comprising most plants in the tropics, including maize, sugar cane, and savanna grasses, and the δ^{13}C values are generally between −16 and −9‰. Rape and all of the members of the Cruciferae are C_3 plants. Lipids are the major form of carbon storage in seeds of several plant species (*21*), and carbon discrimination during their synthesis might differ between individual fatty acids (*14*, *16*). Other factors, including plant variety, water availability, cultivation practices, local atmospheric CO_2 concentration, temperature, and air humidity, can induce further δ^{13}C variability in the photosynthetic products (*22*). The variations in the hydrogen and oxygen isotope composition (reported as δ^2H and δ^{18}O values,

where in the calculation of the δ value $R = {}^2H/{}^1H$ or ${}^{18}O/{}^{16}O$) of vegetable oils and fats depend mainly on the composition of their source (e.g., hydrogen mainly from water, oxygen from fixed CO_2), and soil−water−plant physico- and biochemical (e.g., metabolic) processes before and after incorporation in the plant tissues. Therefore, the combination of bulk carbon, oxygen, and hydrogen stable isotope ratios may provide some information on the geographical origin of vegetable oils (*13*, *23*−*25*).

In this study, we investigated the chemical and carbon isotope composition of the fatty acids ($\delta^{13}C_{FA}$) and the bulk carbon, oxygen, and hydrogen ($\delta^{13}C_{bulk}$, δ^2H_{bulk}, and $\delta^{18}O_{bulk}$) isotopes of rapeseed oils from different rape cultivars, sites of growth in Switzerland and harvest year. This sample set helped to analyze the importance of factors such as cultivar, site location, local climate, and planting time on the fatty acid and stable isotope composition of rapeseed oils. For comparison, other C_3 vegetable oils rich in linolenic acid, such as flax (*Linum usitatissimum*) oil and false flax (*Camelina sativa*) oil, and rich in linoleic acid, such as poppy (*Papaver somniferum*), sunflower (*Helianthus annuus*), and safflower (*Carthamus tinctorius*), were submitted to the same chemical and isotopic analyses. The results of this study provide insights into plant-specific carbon isotope fractionation during biosynthesis of individual fatty acids (e.g., 18:0, 18:1, 18:2, and 18:3).

MATERIALS AND METHODS

Samples. Cold-pressed edible seed oils from six different C_3 plant species were collected from local organic farmers in northwestern Switzerland and Germany (34 Swiss and 1 German samples). The samples come from six harvesting sites situated within a radius of 60 km with similar geographical and climatic (moderate humid) conditions, including five Swiss sites in the cantons of Zurich (Feldbach and Reckenholz), Aargau (Reuenthal), and St. Gallen (Albertschwil) and one German site (**Table 1**).

Twenty-one rapeseed oils came from the same organic breeding company (P. Kunz S.A., canton Zurich, Switzerland) and were derived from seven different cultivars, including Bioraps ($n = 2$), Cabriolet ($n = 2$), Expert ($n = 3$), Oase ($n = 4$), Robust ($n = 2$), Remy ($n = 5$), and Viking ($n=3$). The rapeseed oils were compared with other vegetable oils produced at the same sites, including flax ($n=3$), false flax ($n=1$), poppy ($n = 4$), sunflower ($n = 1$), and safflower ($n = 1$). All oil samples, except the safflower oil sample from the 2004−2005 harvest season, are from the harvest seasons between 2006 and 2008. The climate was quite similar during the main growing seasons of April−August 2006, 2007, and 2008, with average temperatures of 15.6, 15.5, and 14.8 °C and mean precipitations of 121, 126, and 128 mm, respectively (*26*). All samples were stored in 10 mL glass vials with PTFE-lined screw caps at 4 °C in the dark prior to analysis.

Sample Preparation. All samples were saponified with methanolic sodium hydroxide prior to conversion of fatty acid to fatty acid methyl ester (FAME) with methanolic BF_3. After boiling of approximately 80 mg of pure fat with 2 mL of NaOH (0.5 M) for 3 min, 3 mL of methanolic boron trifluoride (1.3 M) was added and the mixture was heated again for 4 min. The reaction was stopped by adding 7 mL of NaCl (0.34 M) and 4 mL of hexane. The tubes were shaken for 30 s and centrifuged at 4000 rpm before the upper layer was transferred to 2 mL vials for gas chromatographic analysis.

Fatty Acid Analysis by GC/FID and GC/MS. The fatty acid composition was determined in duplicate by separating the FAME on an Agilent model 6890 gas chromatograph (GC; Wilmington, DE) equipped with a 30 m × 320 μm, 0.25 μm, Supelcowax-10 fused silica column (Sigma Aldrich, Bellefonte, PA) and a flame ionization detector (GC/FID). Hydrogen was used as carrier gas with a flow of 2.2 mL/min. The sample was injected at a temperature of 240 °C and a split of 30:1. The oven temperature program was 1 min at 160 °C, raised at 20 °C/min to 190 °C, raised at 7 °C/min to 230 °C, held for 5 min at 230 °C, and finally raised at 20 °C/min to 250 °C for 12 min; the total time was 26 min. The identification of individual FAME was performed by comparison of retention times with those of a standard FAME mixture (Supelco, Bellefonte, PA), after characterization of the single fatty acids by gas chromatography−mass spectrometry (GC/MS, Thermo Fisher, Argenteuil, France) equipped with a Supelcowax-10 fused silica column. Chromatograms were evaluated by using HP ChemStation software (Hewlett-Packard, Palo Alto, CA). The proportion of the different FAME was calculated using the ratio of the peak area of the respective FAME to the sum of total FAME peak areas.

C, O, and H Isotope Analyses of Bulk Oils by EA/IRMS and TC-EA/IRMS. All stable isotope analyses were performed in the laboratories of the Institute of Mineralogy and Geochemistry at the University of Lausanne. The carbon isotope ratio (${}^{13}C/{}^{12}C$) was determined by flash combustion on a Carlo Erba 1108 (Milan, Italy) elemental analyzer (EA) connected to a Thermo Fisher Delta V (Bremen, Germany) isotope ratio mass spectrometer (IRMS) that was operated in the continuous helium flow mode via a Conflo II split interface (EA/IRMS). An aliquot of the oil sample (100−500 μg) was wrapped in a tin capsule and combusted in the EA under a stream of helium and oxygen by flash combustion in a quartz reactor at 1020 °C packed with Cr_2O_3 and $(Co_3O_4)Ag$ to form CO_2, N_2, NO_x, and H_2O. The gases were then passed through a reduction reactor containing elemental copper and copper oxide at 640 °C. Water was subsequently removed by anhydrous $Mg(ClO_4)_2$, CO_2 was separated in a gas chromatograph with a 5 m × $^1/_4$ in. i.d., Pora-PLOT Q packed column (Varian, Palo Alto, CA) at 70 °C and analyzed for its isotopic composition on the IRMS. Reference CO_2 gas was inserted in the He carrier flow as pulses of pure standard gas. The oxygen and hydrogen isotope ratios (${}^{18}O/{}^{16}O$, ${}^2H/H$) were measured with a Thermo Fisher high-temperature conversion elemental analyzer (TC-EA) coupled to a Delta Plus XL IRMS via a Conflo II split interface (TC-EA/IRMS). For O and H isotope analyses, separate pyrolysis of aliquots of the oil samples was done in Ag capsules and in a reactor containing a glassy carbon tube filled with glassy carbon grains at 1250 °C. The produced CO and H_2 gases were separated in a GC and analyzed in the IRMS. Reference CO and H_2 gases were inserted in the He carrier flow as pulses of pure standard gases.

The stable isotope composition of carbon, oxygen, and hydrogen are reported in delta (δ) notation as the per mil (‰) deviations of the isotope ratio relative to known standards:

$$\delta = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$$

R is the ratio of the heavy to light isotopes (i.e., ${}^{13}C/{}^{12}C$, ${}^{18}O/{}^{16}O$, ${}^2H/H$). For carbon the standard is Vienna Pee Dee Belemnite limestone (VPDB), and for oxygen and hydrogen it is Vienna Standard Mean Ocean Water (VSMOW). Each analytical sequence consisted of two sets of calibration standards, to test the precision and accuracy of the unknown samples. The repeatability and intermediate precision of the EA/IRMS and TC-EA/IRMS method, defined as the observed variability from separately replicate analyses of laboratory standard materials (glycine, $\delta^{13}C$ = −26.1‰; urea, $\delta^{13}C$ = −43.1‰) (*27*) and vegetable oil samples, were better than 0.1‰ (1 SD) for $\delta^{13}C$ and 0.3‰ for both $\delta^{18}O$ and δ^2H values. The accuracy of the analyses was checked periodically by analyses of the international reference materials USGS-24 graphite (−15.9‰ $\delta^{13}C$), IAEA-PEF1 polyethylene foil (−31.8‰ $\delta^{13}C$), and NBS-22 oil (−29.7‰ $\delta^{13}C$). The δ^2H analyses were calibrated with the international standards IAEA-PEF1 (δ^2H = −100.3), NBS-22 oil (δ^2H = −120.0), IAEA-CH3 (δ^2H = −43.5), and IAEA-CH6 (δ^2H = −11.7). The calibration standards used for oxygen were IAEA-601 ($\delta^{18}O$ = 23.3), IAEA-602 ($\delta^{18}O$ = 71.4), IAEA-CH3 ($\delta^{18}O$ = 32.6), IAEA-CH6 ($\delta^{18}O$ = 36.4), and an in-house standard UNIL-TP5 ($\delta^{18}O$ = 29.8) as described by Spangenberg (*27*). The accuracy of the isotopic analyses was checked sporadically by analyses of international standards.

Isotopic Analysis of Individual Fatty Acids by GC/C/IRMS. The compound-specific stable carbon isotope analyses ($\delta^{13}C$ values) of the fatty acids were obtained by the use of an Agilent 6890 GC coupled to a Thermo Fischer Delta V IRMS by a combustion (C) interface III (GC/C/IRMS) under a continuous helium flow. The combustion interface consists of two ceramic furnaces: an oxidation reactor with CuO/NiO/Pt wires at 940 °C and a reduction reactor with Cu wires at 600 °C. Water was removed from the effluent gas by passing a Nafion tube (Perma Pure, Toms River, NJ) with an annular back-flow of He. The GC was operated with the same type of column (Supelco-Wax 10 column) and temperature program used for GC/FID analyses. The background subtraction and

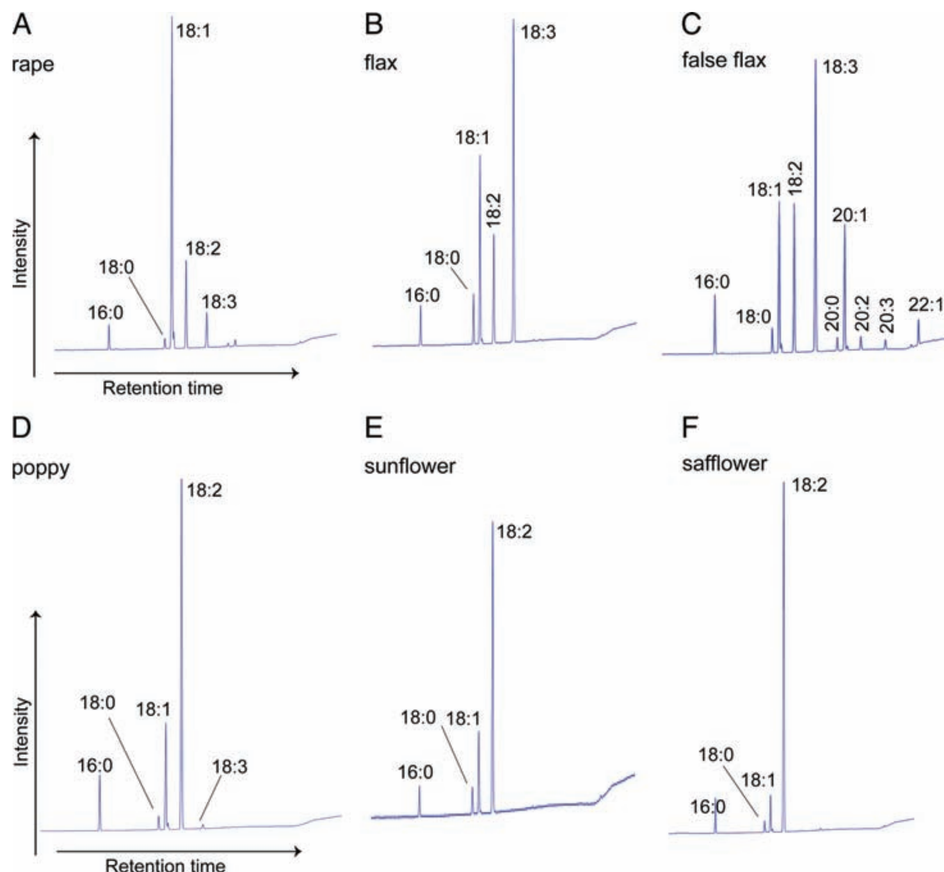

Figure 1. GC/FID chromatograms of the fatty acid methyl esters of rape (**A**), flax (**B**), false flax (**C**), poppy (**D**), sunflower (**E**), and safflower (**F**) oils.

δ^{13}C values were calculated using ISODAT 7.2 software. The repeatability and intermediate precision of the GC/C/IRMS procedure and the performance of the GC and combustion interface were evaluated by injection of an in-house mixture of *n*-alkanoic acids (UNIL-FAME-MIX), of known isotopic composition, and at least three replicate analyses of the oil samples. The standard deviations for repeatability ranged between 0.05 and 0.4‰ for the main FAME and for intermediate precision between 0.3 and 1.1‰. The accuracy of the GC/C/IRMS analyses was checked every 10 analyses by injection of a 20:0 methyl ester isotope standard prepared by A. Schimmelman from the Biogeochemical Laboratories at Indiana University, Bloomington, IN. The isotopic shift due to the carbon introduced in the fatty acid methylation was corrected by the mass balance equation (*16*)

$$\delta^{13}C_{\mathrm{FAME}} = f_{\mathrm{FA}}\delta^{13}C_{\mathrm{FA}} + f_{\mathrm{MeOH}}\delta^{13}C_{\mathrm{MeOH}}$$

where $\delta^{13}C_{\mathrm{FAME}}$, $\delta^{13}C_{\mathrm{FA}}$, and $\delta^{13}C_{\mathrm{MeOH}}$ are the carbon isotope compositions of the fatty acid methyl ester, the fatty acid, and the methanol used for methylation of the fatty acid, respectively, and f_{FA} and f_{MeOH} are the carbon fractions in the fatty acid methyl ester due to the alkanoic chain and methanol, respectively. The variability introduced by this correction was determined by GC/C/RMS measurements of replicate derivatized aliquots of palmitic (16:0) and stearic (18:0) acids of known isotopic composition (*28*). The differences of the measured and calculated $\delta^{13}C_{\mathrm{FA}}$ values are much smaller than the standard deviation for repeatability of GC/C/RMS analyses of FAME from similar C-chain length (*28*).

Statistical Evaluation. Statistical analysis was performed using SAS software (version 9.1, SAS Institute Inc., Cary, NC). Average isotopic values of bulk oils and individual fatty acids were subjected to analysis of variance using the general linear model (GLM procedure), considering separately the different plant species and rape cultivars. Multiple comparisons among means were performed with Tukey's method. Principal component analysis (PCA) was performed using SPSS software (version 17.0, 2008, SPSS Inc., Chicago, IL) to cluster the isotopic measurements within a limited number of independent variables (principal components).

RESULTS AND DISCUSSION

Fatty Acid Contents. The fatty acid composition of the oil samples differed as expected for the different vegetable oils (**Table 1**; **Figure 1**). The concentrations of saturated fatty acids (mainly 16:0) were low in all samples, but important differences in mono- and polyunsaturated fatty acid contents were observed. The highest relative concentrations in oleic acid were observed in rapeseed oils ($61.2 \pm 3.5\%$). Flax and false flax oils differed from the other by their high 18:3 content (48.7 ± 1.5 and 36.5%, respectively), whereas poppy, sunflower, and safflower oils were rich in 18:2 (71.0 ± 2.9, 69.1, and 83.6%, respectively), but poor in 18:3. Relatively high 18:3 concentrations were also found in rape oil ($8.7 \pm 0.7\%$). Differences in the concentrations of mono- and polyunsaturated fatty acids in the oil samples from different rape cultivars were observed. The lowest concentration of polyunsaturated fatty acids was found for Cabriolet rape (18.4%) and the highest for Viking rape (30.9%).

Stable Isotope Composition of Bulk Oils. The δ^{13}C values of all the analyzed bulk oils (−30.8 to −27.6‰) were typical for oils from C_3 plants (*14*−*19*). The differences between the average δ^{13}C values of rape, poppy, and flax oils were small but still significant ($p < 0.001$; **Table 2**; **Figure 2**). Rape and flax oils had lower values (-30.2 ± 0.3 and -29.9 ± 0.1‰, respectively) than poppy oil (-28.8 ± 0.9‰). The δ^{18}O values of all oil samples studied varied within a narrow range (19.0−27.5‰). One rape oil sample had the highest δ^{18}O value and false flax the lowest. No significant differences were observed between rape, poppy, and flax oils (**Table 2**). The δ^{2}H values of all oils varied between −188 and −153‰. The average δ^{2}H value (−175‰) of rapeseed oils is statistically different ($p < 0.01$) from that for flax (−167‰) and poppy (−161‰). Sunflower oil had the lowest value (−188‰). Angerosa et al. (*13*) showed that the combination of δ^{18}O and

Table 2. Ranges, Means, and Standard Deviation (1 SD) for Fatty Acid Content (%) and Isotopic Ratios of Bulk Oil ($\delta^{13}C_{bulk}$ in ‰ VPDB, $\delta^{18}O_{bulk}$ and $\delta^{2}H_{bulk}$ in ‰ VSMOW) and Individual Fatty Acids ($\delta^{13}C_{FA}$)[a]

	flax (n = 3)	poppy (n = 4)	rape (n = 25)	false flax (n = 1)	sunflower (n = 1)	safflower (n = 1)	p value plant species
FA content							
16:0	4.4 to 4.7 (4.6a ± 0.1)	8.6 to 9.2 (8.8b ± 0.2)	4.0 to 5.0 (4.5a ± 0.3)	5.4	5.8	5.6	<0.001
18:1	21.7 to 24.3 (23.3a ± 1.4)	11.5 to 18.0 (15.4b ± 2.9)	56.8 to 70.7 (61.2c ± 3.5)	15.6	17.1	6.5	<0.001
18:2	14.8 to 15.2 (15.0a ± 0.2)	68.4 to 75.0 (71.0b ± 2.9)	9.0 to 22.5 (18.3a ± 3.2)	16.7	69.1	83.6	<0.001
18:3	47.3 to 50.2 (48.7a ± 1.5)	0.6 to 0.8 (0.7b ± 0.1)	7.5 to 10.1 (8.7c ± 0.7)	36.5	0.1	0.2	<0.001
isotopic composition of bulk oil							
$\delta^{13}C_{bulk}$	−30.0 to −29.8 (−29.9ab ± 0.1)	−29.6 to −27.6 (−28.8c ± 0.9)	−30.8 to −29.6 (−30.2b ± 0.3)	−28.8	−29.1	−30.8	<0.001
$\delta^{18}O_{bulk}$	20.2 to 22.5 (22.0 ± 1.6)	20.5 to 25.6 (22.8 ± 2.5)	20.3 to 27.5 (22.7 ± 1.5)	19.0	19.8	26.0	<0.769
$\delta^{2}H_{bulk}$	−170 to −164 (−167ab ± 3.6)	−165 to −153 (−161a ± 5.6)	−188 to −156 (−175b ± 9.5)	−163	−185	−153	<0.013
$\delta^{13}C$ of individual fatty acids							
$\delta^{13}C_{16:0}$	−32.7 to −32.1 (−32.4a,z ± 0.3)	−33.1 to −30.5 (−31.3b,x ± 0.9)	−33.6 to −31.5 (−32.6a,y ± 0.5)	−33.6	−31.7	−31.8	<0.001
$\delta^{13}C_{18:1}$	−31.0 to −30.4 (−30.6x ± 0.4)	−32.2 to 29.3 (−30.3x ± 1.1)	−32.5 to −29.8 (−31.0x ± 0.6)	−32.5	−32.1	−31.4	<0.103
$\delta^{13}C_{18:2}$	−31.4 to −30.7 (−31.0xy ± 0.4)	−32.8 to −30.5 (−31.1x ± 0.6)	−32.3 to −29.2 (−31.1x ± 0.8)	−31.8	−30.9	−30.6	<0.986
$\delta^{13}C_{18:3}$	−32.8 to −31.8 (−32.0a,yz ± 0.8)	−27.2 to −25.1 (−26.1b,y ± 1.1)	−36.4 to −32.1 (−34.0c,z ± 1.1)	−32.8	−31.1		<0.001
p value FA content or $\delta^{13}C$ value	0.007	0.002	<0.001				

[a] The relative abundances are given in weight percent. Mean values within the same row (flax, poppy, rape) followed by different letters (a−c) are significantly different at the given p value ($p < 0.05$). Mean values within the same column (16:0, 18:1, 18:2, 18:3) followed by different letters (x−z) are significantly different ($p < 0.05$).

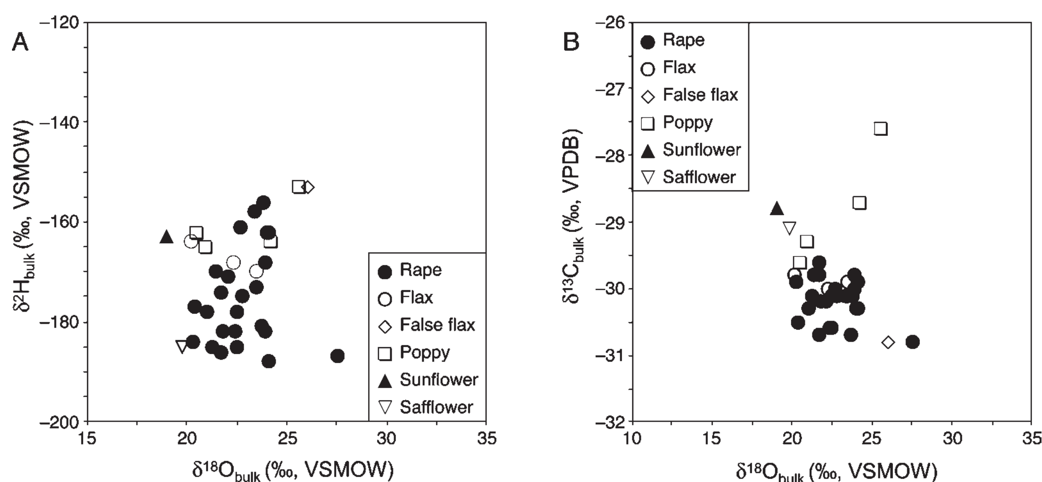

Figure 2. Hydrogen isotope composition ($\delta^{2}H_{bulk}$) versus oxygen isotope composition ($\delta^{18}O_{bulk}$) (**A**) and carbon isotope composition ($\delta^{13}C_{bulk}$) versus oxygen isotope composition ($\delta^{18}O_{bulkl}$) (**B**) of studied C_3 vegetable oils.

$\delta^{13}C$ data provides information about geographical origin of olive oils from different Mediterranean countries. Bontempo et al. (*23*) and Camin et al. (*24*) explored the use of $\delta^{2}H$ values from bulk extra virgin olive oils from Italy as geographic discriminator. The analyzed bulk vegetable oils were depleted in ^{18}O and ^{2}H by up to 8.4 and 22‰, respectively, compared to Italian extra virgin

Table 3. Range, Mean, and Standard Deviation (1 SD) from δ^2H, δ^{18}O, and δ^{13}C Values of Bulk Oils and δ^{13}C of Individual Fatty Acids from Different Rape Cultivars

	δ^{13}C (‰, VPDB)					δ^{18}O bulk	δ^2H bulk
cultivar	bulk	palmitic	oleic	linoleic	linolenic	(‰, VSMOW)	(‰, VSMOW)
Bioraps (n = 2)	−30.5 to −30.0 (−30.2 ± 0.2)	−33.2 to −32.2 (−32.8 ± 0.7)	−30.9 to −30.7 (−30.8 ± 0.1)	−31.7 to −31.2 (−31.4 ± 0.4)	−34.0 to −33.8 (−33.9 ± 0.1)	22.1 to 23.5 (22.8 ± 1.0)	−173.1 to −170.9 (−172.0 ± 1.6)
Cabriolet (n = 2)	−30.6 to −29.8 (−30.2 ± 0.5)	−32.7 to −32.2 (−32.4 ± 0.3)	−31.4 to −30.4 (−30.9 ± 0.7)	−30.3 to −29.2 (−29.7 ± 0.8)	−33.8 to −32.7 (−33.2 ± 0.7)	22.5 to 23.9 (23.2 ± 1.0)	−185.0 to −181.9 (−183.5 ± 2.2)
Expert (n = 3)	−30.6 to −29.9 (−30.2 ± 0.4)	−32.9 to −32.3 (−32.7 ± 0.3)	−31.8 to −30.7 −31.1 ± 0.6)	−32.3 to −30.0 (−31.3 ± 1.2)	−36.2 to −32.1 (−33.9 ± 2.1)	20.3 to 22.8 (21.8 ± 1.3)	−184.3 to −174.5 (−180.4 ± 5.2)
Oase (n = 4)	−30.7 to −29.8 (−30.1 ± 0.5)	−33.6 to −31.5 (−32.4 ± 1.0)	−31.8 to −29.8 −30.8 ± 0.9)	−31.9 to −30.4 (−31.0 ± 0.7)	−34.8 to −33.5 (−34.1 ± 0.6)	21.4 to 24.1 (22.2 ± 1.3)	−185.3 to −161.8 (−172.8 ± 9.7)
Robust (n = 2)	−30.4 to −29.6 (−30.0 ± 0.5)	−32.8 to −32.7 (−32.7 ± 0.1)	−31.8 to −30.9 −31.3 ± 0.6)	−31.8 to −31.2 (−31.5 ± 0.4)	−34.3 to −33.8 (−34.1 ± 0.3)	21.7 to 23.7 (22.2 ± 1.3)	−181.3 to −173.9 (−177.6 ± 5.3)
Remy (n = 5)	−30.8 to −30.1 (−30.3 ± 0.2)	−33.4 to −32.4 (−32.8 ± 0.4)	−32.5 to −31.0 −31.4 ± 0.6)	−32.3 to −30.6 (−31.6 ± 0.6)	−36.4 to −34.0 (−34.8 ± 1.0)	21.0 to 27.5 (23.1 ± 2.7)	−187.8 to −177.9 (−183.9 ± 4.0)
Viking (n = 3)	−30.3 to −30.0 (−30.1 ± 0.1)	−32.6 to −31.9 (−32.3 ± 0.4)	−30.7 to −30.4 −30.6 ± 0.2)	−30.8 to −30.0 (−30.4 ± 0.4)	−35.0 to −32.9 (−33.9 ± 1.1)	22.7 to 24.0 (23.3 ± 0.7)	−162.5 to −158.5 (−160.7 ± 2.0)
undefined (n = 4)	−30.5 to −30.0 (−30.2 ± 0.2)	−32.6 to −32.2 (−32.5 ± 0.2)	−31.6 to −30.6 −31.0 ± 0.5)	−31.4 to −30.8 (−31.2 ± 0.3)	−34.7 to −32.1 (−33.4 ± 1.2)	20.4 to 23.9 (22.7 ± 1.6)	−178.1 to −155.7 (−169.7 ± 10.4)

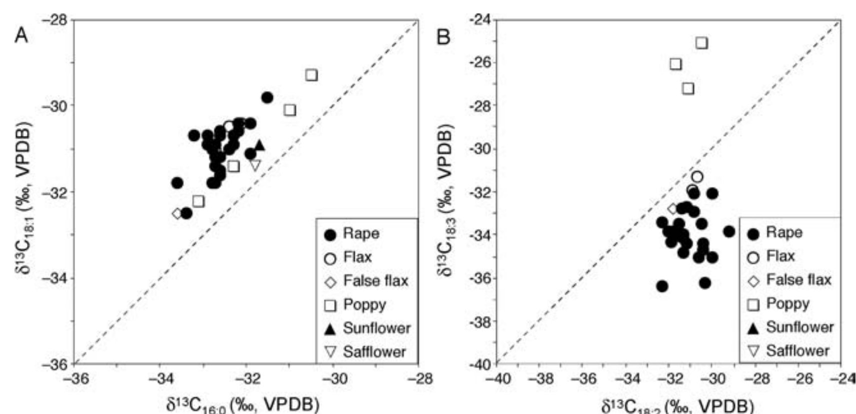

Figure 3. Carbon isotope composition of oleic acid ($\delta^{13}C_{18:1}$) versus palmitic acid ($\delta^{13}C_{16:0}$) (**A**) and α-linolenic acid ($\delta^{13}C_{18:3}$) versus linoleic acid ($\delta^{13}C_{18:2}$) (**B**) of the studied C_3 vegetable oils.

olive oils [19.1−26.8‰ δ^{18}O and −165 to −137‰ δ^2H (*24*)]. These differences nicely reflect the isotopically lighter soil−water and precipitation in Switzerland compared with those from warmer Italian regions.

There were significant differences in δ^{13}C ($p < 0.001$) and δ^2H ($p < 0.05$) in the rape bulk oil harvested in 2006, 2007, and 2008 (data not shown). These differences are most probably due to variations in temperature, water availability, atmospheric CO_2 concentration, and other environmental (abiotic) factors affecting the photosynthetic shield.

Small but still significant differences in δ^2H ($p < 0.05$) and δ^{18}O values ($p < 0.01$) from rapeseed oils from different cultivars were detected (**Table 3**). The oils from the cultivar Viking had the highest δ^2H values (−160.7 ± 2.0‰) and those from cultivar Remy the lowest (−183.9 ± 4.0‰).

The δ^{18}O values of the rapeseed oils from the cultivars Cabriolet (23.2 ± 1.0‰) and Viking (23.3 ± 0.7‰) were significantly ($p < 0.05$) higher than those from the cultivar Expert (21.8 ± 2.0‰). Similarly, Aramendia et al. (*25*) reported differences in the oxygen isotope composition of bulk olive oils from two different cultivars. Our results also indicate that the effect of cultivars (plant variety) may obliterate the effect of geographical origin on the oxygen and hydrogen isotope composition of vegetable oils.

Variations in δ^{13}C of Individual Fatty Acids. The δ^{13}C values of the individual fatty acids for all vegetable oils studied range between −36.4 and −26.1‰ (−31.9 ± 1.6‰; **Table 2**). Significant differences in the δ^{13}C values of individual fatty acids were observed within plant species (**Figure 3**). Differences in the δ^{13}C values of the main fatty acids were observed in all species, those for rape being most prominent ($p < 0.001$). Differences ($p < 0.001$) between oils from poppy, flax, and rape were observed for 16:0 and 18:3 (**Figure 3**). In poppy and rape oils, the δ^{13}C values of 18:3 were different ($p < 0.05$) from those of all other fatty acids. In all plant species, oleic acid and linoleic acid were depleted in ^{13}C by up to 2.5 and 3‰, respectively, compared to palmitic acid. Linolenic acid in rape oil (−34.0 ± 1.1‰) and flax oil (−32.0 ± 0.8‰) had similar δ^{13}C values, which were significantly lower than those measured in poppy oil (−26.1 ± 1.1‰; **Table 2**; **Figure 3**). This suggests that a plant-specific carbon isotope

Table 4. Principal Component Analysis Performed on Fatty Acid Composition and Carbon Isotope Ratios of Bulk Oil and Main Individual Fatty Acids of Rape Samples

	principal component			
	1	2	3	4
variance proportion (%)	25.4	19.7	17.4	15.1
rotated loadings				
16:0	0.78	−0.20	−0.49	−0.15
18:1	−0.97	0.09	0.10	−0.04
18:2	0.94	−0.05	0.09	0.08
18:3	0.94	−0.05	0.09	0.08
$\delta^{13}C_{bulk}$	0.10	0.31	0.71	−0.44
$\delta^{18}O_{bulk}$	−0.08	0.03	−0.14	0.87
$\delta^{2}H_{bulk}$	0.23	0.24	0.43	0.69
$\delta^{13}C_{16:0}$	0.04	0.73	0.20	0.24
$\delta^{13}C_{18:1}$	0.07	0.79	0.38	0.11
$\delta^{13}C_{18:2}$	−0.29	0.64	0.08	−0.33
$\delta^{13}C_{18:3}$	−0.34	0.63	−0.23	0.04

fractionation occurs during the biosynthesis of the fatty acids and, particularly, during desaturation of C_{18} acids in rape and flax. The lipid biosynthesis in seeds and leaves of oilseed plants is a very complex genetically determined process (*12*). The basic reaction of the fatty acid biosynthesis is the same in all plants and seeds. The synthesis of 16:0 and 18:0 acids takes place in specific organelles in plant cells. Palmitic acid and small amounts of stearic acids are the final products of de novo synthesis in the chloroplasts (*29*). The C_2 elongation of the 16:0 liberated from the fatty acid synthase system to produce stearate takes place in a different site of the cell (endoplasmic reticulum and the mitochondria). The first desaturation of 18:0 via soluble Δ^9-desaturase produces 18:1 in the chloroplast (plastide). These biosynthetic pathways explain why the carbon isotope fractionation between 16:0, 18:0, and 18:1 is very low and much lower than the analytical uncertainty of the GC/C/IRMS measurements. Therefore, the relationship of $\delta^{13}C_{16:0}$ versus $\delta^{13}C_{18:1}$ was used to trace mixing of vegetable oils and fats (*16*, *17*, *19*). For further desaturations to 18:2 ($\Delta^{9,12}$) and 18:3 ($\Delta^{9,12,15}$) by membrane-bound Δ^{12}- and Δ^{15}-desaturases, the intermediates are transported to the fluid that fills the inside of the cell (cytosol) after being incorporated into phosphatidylcholine by analogy of leaf chloroplasts (*29*, *30*). The differences between the measured $\delta^{13}C_{18:1}$ and $\delta^{13}C_{18:2}$ values ($\Delta^{13}C_{18:1-18:2} = \delta^{13}C_{18:1} - \delta^{13}C_{18:2}$) for rape flax and false flax range between −1.2 and 1.5‰, the $\Delta^{13}C_{18:1-18:3}$ between 0.5 and 4.9‰, and the $\Delta^{13}C_{18:2-18:3}$ between 0.6 and 5.1‰. The small $\Delta^{13}C_{18:1-18:2}$ differences (average ± 1 SD, 0.1 ± 0.6‰) can be explained by environmental factors affecting the isotopic composition of primary and secondary metabolites combined with the analytical uncertainty (better than ±0.3‰). However, the high $\Delta^{13}C_{18:1-18:3}$ (2.8 ± 1.1‰) and $\Delta^{13}C_{18:2-18:3}$ values (2.7 ± 1.1‰) reflect a more important isotopic fractionation in the biosynthesis of 18:3 than 18:0, 18:1, and 18:2. For poppy, the $\Delta^{13}C_{18:1-18:2}$ values ranged between −5.4 and −2.9 (−4.2 ± 1.3‰), $\Delta^{13}C_{18:1-18:3}$ ranged between 0.3 and 1.1‰ (0.8 ± 0.5‰), and $\Delta^{13}C_{18:2-18:3}$ ranged between −5.6 and −3.9‰ (5.0 ± 0.9‰). These are exactly the opposite trends from those observed for rape, flax, and false flax. This needs an explanation. The isotopic fractionations during the second and third desaturation steps seem to be species specific. Linoleic and linolenic acids were most probably synthesized via different pathways in rape, flax, and false flax compared with poppy. For instance, 18:3 may have been formed by elongation of 16:3 in chloroplast, as shown by studies with ^{14}C-labeled spinach (*31*). However, studies of the variation of fatty acids in opium poppy genotypes by Singh et al. (*32*, *33*) showed that shorter chain

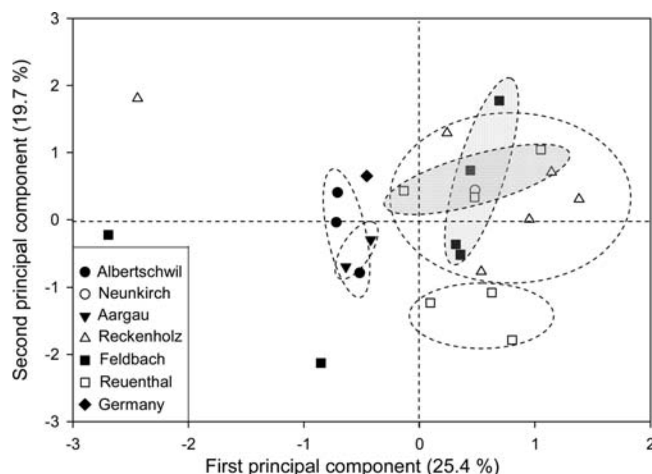

Figure 4. Scatterplot of the scores from the first two principal components describing harvesting site and using stable isotope ratios (δ^2H_{bulk}, $\delta^{18}O_{bulk}$, $\delta^{13}C_{bulk}$, $\delta^{13}C_{16:0}$, $\delta^{13}C_{18:1n\text{-}9}$, $\delta^{13}C_{18:2n\text{-}6}$, and $\delta^{13}C_{18:3n\text{-}3}$) and fatty acid concentrations ($C_{16:0}$, $C_{18:1n\text{-}9}$, $C_{18:2n\text{-}6}$, and $C_{18:3n\text{-}3}$).

trienoic acids as precursor are not produced in response to reduction in 18:3 content in the opium poppy. Therefore, chain elongation is probably not involved in the synthesis of 18:3 in poppy oil, which could explain the difference from the other studied oils.

The $\delta^{13}C$ values of the main fatty acids of rape oil from different cultivars and four samples of unknown cultivars were compared, and no significant differences were found (**Table 4**). PCA was used to facilitate statistical differentiation of geographic origin of the 25 rapeseed oils analyzed in the present study (**Figure 4**). The concentrations of 18:1, 18:2, 18:3, and 16:0 characterize the high loaded PC1, whereas PC2 is mostly explained by $\delta^{13}C$ variations of individual fatty acids ($\delta^{13}C_{16:0}$, $\delta^{13}C_{18:1}$, $\delta^{13}C_{18:2}$, $\delta^{13}C_{18:3}$). Six clusters can be distinguished in the PC1 versus PC2 space. Samples from different harvest sites are not completely differentiated and sometimes overlap, most probably due to the close geographical distribution and similar climate conditions.

The results of this study show that compound-specific isotope analysis (CSIA) of individual fatty acids is a good tool to distinguish between different C_3 plants species. Carbon isotope values for the individual fatty acids 16:0 and 18:3 differed between rape, flax, and poppy oil significantly ($p < 0.001$). Some overlaps in the $\delta^{13}C$ fields of seed oils of C_3 species indicate that an unequivocal classification or detection of fraud in claiming a certain origin would be difficult by using only CSIA. The combination with concentrations and bulk oil $\delta^{13}C$, $\delta^{18}O$, and δ^2H values significantly improves the discriminatory potential of $\delta^{13}C$ values of individual fatty acids, as shown for rape cultivars.

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