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Fatty Acid and Stable Carbon Isotope Characterization of *Camelina sativa* Oil: Implications for Authentication

ROBERT HRASTAR,[†] MARINKA G. PETRIŠIČ,[‡] NIVES OGRINC,[‡] AND IZTOK JOŽE KOŠIR*,[†]

Slovenian Institute of Hop Research and Brewing, Žalskega Tabora 2, SI-3310 Žalec, Slovenia, and Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia

The importance of authenticity characterization is an increasing and pressing requirement for all foods. Vegetable oil is one of the most studied foods because of its nutritional and medicinal properties in a correct diet. In this study, a total of 53 *Camelina sativa* samples, from all known growing areas, were chemically and isotopically characterized. The fatty acid content of camelina oil was determined by gas chromatography (GC), and the ratios of stable carbon isotopes ($^{13}C/^{12}C$) of individual fatty acids and seed/bulk oil were determined by gas chromatography−combustion-stable isotope ratio mass spectrometry (GC/C/IRMS) and elemental analysis−stable isotope ratio mass spectrometry (IRMS). A total of 17 different fatty acids were detected by GC, with ω3 α-linolenic acid ($C_{18:3n3}$) being the most abundant (29.7−40.0 wt %). Oleic acid ($C_{18:1n9}$), linoleic acid ($C_{18:2n6}$) and eicosenoic acid ($C_{20:1n9}$) all belong to the second group of major fatty acids. The stable carbon isotopic values ($\delta^{13}C$) fell into a range typical for C_3 plants. The use of $\delta^{13}C_{18:2n6}$ vs $\delta^{13}C_{18:3n3}$ correlation could show cases where impurity or adulteration is suspected, whereas principal component analysis clearly separates oil samples from different continents. Preliminary results on the camelina oil authentication procedure provide a basis for the investigation of geographical origin and the further distinction between camelina and camelina refined or other, less expensive oils.

KEYWORDS: Camelina sativa; authenticity; fatty acids; carbon isotope; isotopic characterization

INTRODUCTION

Camelina sativa, an ancient oilseed crop, is a member of the Brassicaceae family, with common names like false flax, gold of pleasure, and leindotter (*1*). Its characterization as a weed in the past caused its cultivation to almost vanish. Recently, interest in *C. sativa* has been renewed in some parts of Central and Northern Europe and in parts of North America because of beneficial nutritional and health effects, suitable use for the biofuel industry and as the most economical crop to produce because of minimal input requirements (*1, 2*). The main product of *C. sativa* is the oil. Traditionally, it is produced by crushing and warm pressing the seeds, which contain 30−40% of oil on a dry weight basis. The high added value of this unique vegetable oil results from a high content of oleic acid $C_{18:1n9}$ (15−20%), linoleic acid $C_{18:2n6}$ (15−20%), ω3 α-linolenic acid $C_{18:3n3}$ (30−40%), eicosenoic acid $C_{20:1n9}$ (15−20%), low content of erucic acid $C_{22:1n9}$ (about 3%), and high content of tocopherols (700 mg/kg) and phenolic compounds (128 mg/kg as chlorogenic acid), making it more stable toward oxidation than highly unsaturated linseed oil (*1, 3*−*5*). The full extent of natural variation of *C. sativa* fatty acids content based on geographical origin is not known or is poorly reported.

High-quality, single source vegetable oils are a target for fraudulent adulteration, i.e., partial or total substitution of minor quality, and hence cheaper oils, for the high quality product. Natural variation in fatty acid composition may mask the adulteration of premium oils by adding small amounts of cheaper varieties or total substitution of geographically characteristic oil with refined ones. Consequently, comparison of the fatty acid composition is not used to distinguish samples of single vegetable oils from partly or fully blended oils, and in these cases other techniques are needed for authentication. Stable carbon isotope analysis is a powerful technique for assessing the authenticity of food products (*6*−*11*). The carbon isotope compositions of plants and their fruits are linked to the processes of photosynthetic atmospheric carbon dioxide (CO_2) fixation. During photosynthetic carbon fixation, plant cells discriminate against the heavier stable carbon isotope ^{13}C compared with ^{12}C, and therefore, natural isotopic fractionation occurs; additionally, factors others than the CO_2-fixation pathway may also have some impact on the isotopic composition of plants (*12*). The most important atmospheric CO_2-fixing reactions are the C_3 or Calvin cycle (rapeseed, soybean) and C_4 or Hatch−Slack

* To whom correspondence should be addressed (telephone +386 3 7121 608; fax +386 3 7121 620; e-mail iztok.kosir@ihps.si.
[†] Slovenian Institute of Hop Research and Brewing.
[‡] JoŽef Stefan Institute.

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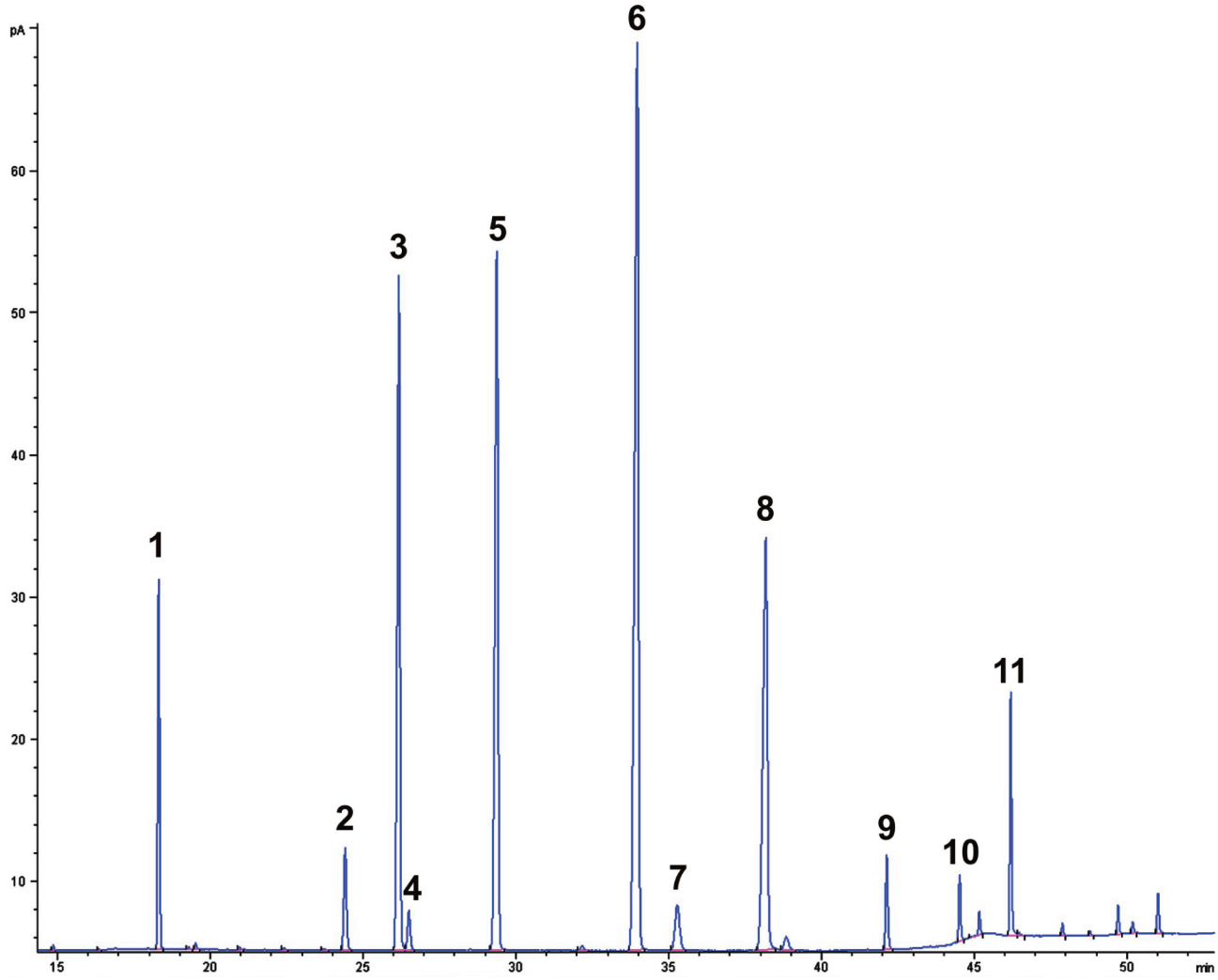

Figure 1. Typical gas chromatogram of camelina oil samples: (1) palmitic acid $C_{16:0}$, (2) stearic acid $C_{18:0}$, (3) oleic acid $C_{18:1n9}$, (4) vaccenic acid $C_{18:1n7}$, (5) linoleic acid $C_{18:2n6}$, (6) linolenic acid $C_{18:3n3}$, (7) eicosic acid $C_{20:0}$, (8) eicosenoic acid $C_{20:1n9}$, (9) eicosdienoic acid $C_{20:2n6}$, (10) eicostrienoic acid $C_{20:3n3}$, (11) erucic acid $C_{22:1n9}$).

cycle (maize, sugar cane), with the $\delta^{13}C$ values in the range of −34 to −22‰ and −23 to −6‰, respectively. These photosynthetic processes are so termed because during the enzymatic CO_2 fixation, the main first metabolite formed is a three-carbon compound (e.g., 3-phosphoglyceric acid) in C_3 plants and a four-carbon compound (e.g., oxaloacetic acid) in C_4 plants (*12*−*14*).

Carbon isotopic composition of single seed and bulk oil may record their source (C_3 or C_4 plant), while the technique of compound-specific isotope analysis of individual lipids by the use of online GC/C/IRMS helps to distinguish between the natural $^{13}C/^{12}C$ variations of genuine C_3 or C_4 oils, geographical origin, and admixtures of oils of different varieties of C_3 plants (*8*−*11, 15, 16*). Royer et al. (*11*) examined olive oil in terms of geographical, temporal, and botanical origin using content and the $\delta^{13}C$ values of individual fatty acids. On the basis of this research, it was possible to differentiate between French and Italian olive oils compared to Greek ones. Woodbury et al. (*9*) determined the $\delta^{13}C$ values of the major fatty acids of more than 150 vegetable oils. However, to our knowledge no isotopic values of seed/bulk oil/fatty acids of *C. sativa* are available.

C. sativa is an alternative oilseed, and total characterization of its beneficial oil is therefore a prerequisite. In our work we collected 53 different seeds from all known countries that grow *C. sativa* at the present. From the nutritional, industrial, and authentication point of view, it is important that the full extent of natural variation of *C. sativa* fatty acids oil is known. Determination of natural fatty acid content and $\delta^{13}C$ ranges and the combination with chemometric analysis of this work should give preliminary results for camelina oil authenticity characterization.

MATERIALS AND METHODS

Samples. Authentic oilseeds, representing all known geographical areas where *C. sativa* is grown, were obtained from suppliers and farmers. Twenty samples were obtained from the U.S. Department of Agriculture. The majority of samples were accompanied by details of origin and year of harvest. In total, 53 *C. sativa* samples were obtained and classified by origin according to the continental region as Central European (Slovenia 14, Austria 8, Germany 2), North European (Denmark 2, Finland 1), and North American (U.S., 26 samples).

Extraction and Methylation. All the solvents and reagents (Fluka, Sigma-Aldrich) were of analytical grade or higher purity. The extraction was performed as described by Lopez et al. (*17*), but some modifications were made as follows. No cooking or preprocessing was performed. After grinding, 1 g of sample was extracted for 2 h with a mixture of 0.17 M NaCl in methanol and heptane (1:3) at 80 °C. An antioxidant, butylated hydroxytoluene (BHT) (0.01% w/v), was also added into the extraction mixture before the start of the extraction procedure. The nonpolar phase was transferred into a glass tube. A second extraction without heating but only with heptane followed. Nonpolar phases were

Table 1. Mean, Standard Deviation, Relative Standard Deviation, Minimum, and Maximum for Fatty Acids Content of Camelina Oil Samples from the Central European (CEU) Region[a]

	mean (%)	±SD (%)	±RSD (%)	min−max (%)
$C_{16:0}$	5.59	0.23	4.14	5.23–6.05
$C_{18:0}$	2.60	0.25	9.69	2.16–3.08
$C_{18:1n9t}$	0.01	<0.01	12.75	<0.01–0.01
$C_{18:1n9}$	14.31	1.18	8.23	12.46–16.72
$C_{18:1n7}$	0.87	0.06	6.97	0.76–1.00
$C_{18:2n6t}$	0.01	0.01	83.14	<0.01–0.02
$C_{18:2n6}$	16.54	1.26	7.60	14.86–20.46
$C_{18:3n3t}$	0.01	<0.01	8.52	0.01–0.02
$C_{18:3n3}$	34.63	1.46	4.22	31.23–38.65
$C_{20:0}$	1.58	0.16	9.95	1.22–1.88
$C_{20:1n9}$	14.45	0.70	4.83	12.24–15.14
$C_{20:2n6}$	1.89	0.17	9.15	1.49–2.24
$C_{20:3n3}$	1.44	0.18	12.74	1.04–1.76
$C_{22:0}$	0.33	0.04	10.70	0.26–0.40
$C_{22:1n9}$	2.90	0.47	16.36	1.66–3.64
$C_{24:0}$	0.16	0.02	11.88	0.13–0.20
$C_{24:1n9}$	0.59	0.05	8.42	0.48–0.69

[a] The relative abundances are given in weight percent.

Table 2. Mean, Standard Deviation, Relative Standard Deviation, Minimum, and Maximum for Fatty Acids Content of Camelina Oil Samples from the North European (NEU) Region[a]

	mean (%)	±SD (%)	±RSD (%)	min−max (%)
$C_{16:0}$	5.43	0.08	1.53	5.33–5.49
$C_{18:0}$	2.74	0.27	9.69	2.46–2.99
$C_{18:1n9t}$	0.01	<0.01	14.52	<0.01–0.01
$C_{18:1n9}$	13.66	2.52	18.48	10.75–15.18
$C_{18:1n7}$	0.77	0.01	1.71	0.75–0.78
$C_{18:2n6t}$	<0.01	<0.01	138.41	ND–0.01
$C_{18:2n6}$	15.71	0.36	2.30	15.34–16.06
$C_{18:3n3t}$	0.01	<0.01	13.22	<0.01–0.01
$C_{18:3n3}$	36.06	0.67	1.87	35.28–36.47
$C_{20:0}$	1.64	0.22	13.20	1.45–1.88
$C_{20:1n9}$	14.13	0.88	6.20	13.36–15.09
$C_{20:2n6}$	1.87	0.25	13.41	1.71–2.16
$C_{20:3n3}$	1.61	0.17	10.77	1.45–1.80
$C_{22:0}$	0.34	0.04	12.94	0.30–0.39
$C_{22:1n9}$	3.01	0.85	28.38	2.51–3.99
$C_{24:0}$	0.16	0.02	9.89	0.15–0.18
$C_{24:1n9}$	0.67	0.06	8.16	0.62–0.73

[a] The relative abundances are given in weight percent. ND: not detected.

combined, and the solvent was removed under a gentle stream of nitrogen resulting in around 300 mg of oil. The oil was frozen at −70 °C until further analysis. The specific extraction procedure was used because of quantitative aspects of some samples.

The oil samples were subjected to two different kinds of methylation. The methylation described by Hamilton and Hamilton (*18*) was performed for methylation of glycerolipids and fatty acids for determination of fatty acid composition of camelina oil. Briefly, 1 mL of 0.5 M sodium hydroxide in methanol was mixed with 100 μL of oil in a screw-capped test tube and refluxed for 5 min at 80 °C. The sample was cooled and acidified with 1 mL of 20% solution of boron trifluoride in methanol, and 0.5 mL of 0.1% hydroquinone in methanol was also added. The mixture was then heated to 80 °C for another 2 min. After the mixture was cooled, 2 mL of 0.73% NaCl solution was added and vortexed for 10 s. Fatty acid methyl esters (FAMEs) were extracted two times with 0.5 mL of hexane. Then 1.0 mL of saturated alkaline NaCl solution was added to the two hexane extracts and mixed, and the water phase was removed. The residual water was removed with anhydrous sodium sulfate. An aliquot was transferred to a GC vial for further analysis.

The second methylation, as described by Annex XB of EEC 2568/91 (*19*), was performed for the GC/C/IRMS technique. Then 200 μL of 2.0 M potassium hydroxide in methanol, 100 μL of oil, and 2 mL of hexane were mixed in a screw-capped test tube for 30 s. An aliquot of

Table 3. Mean, Standard Deviation, Relative Standard Deviation, Minimum. and Maximum for Fatty Acids Content of Camelina Oil Samples from the North American (NAM) Region[a]

	mean (%)	±SD (%)	±RSD (%)	min−max (%)
$C_{16:0}$	5.70	0.52	9.14	4.75–7.00
$C_{18:0}$	2.37	0.31	13.25	1.83–3.05
$C_{18:1n9t}$	0.01	0.01	69.24	ND–0.02
$C_{18:1n9}$	14.01	1.97	14.04	10.57–19.37
$C_{18:1n7}$	0.99	0.08	8.32	0.86–1.14
$C_{18:2n6t}$	0.03	0.01	51.87	<0.01–0.05
$C_{18:2n6}$	18.45	2.36	12.77	15.00–24.53
$C_{18:3n3t}$	0.14	0.06	39.60	0.01–0.19
$C_{18:3n3}$	34.58	3.07	8.88	29.65–40.03
$C_{20:0}$	1.83	0.17	9.42	1.52–2.33
$C_{20:1n9}$	12.71	1.22	9.56	10.56–15.19
$C_{20:2n6}$	1.48	0.24	16.27	1.04–2.02
$C_{20:3n3}$	1.05	0.17	15.80	0.70–1.41
$C_{22:0}$	0.37	0.03	9.24	0.30–0.44
$C_{22:1n9}$	3.24	0.46	14.33	2.39–4.21
$C_{24:0}$	0.20	0.04	20.22	0.13–0.28
$C_{24:1n9}$	0.64	0.08	12.00	0.49–0.79

[a] The relative abundances are given in weight percent. ND not detected.

Table 4. Mean, Standard Deviation, Relative Standard Deviation, Minimum, and Maximum $\delta^{13}C$ Values for Seed and Bulk Oil and Main Individual Fatty Acids of Camelina Oil Samples from CEU Region

	mean (‰)	±SD (‰)	±RSD (%)	min−max (‰)
seed	−29.0	1.2	4.2	−30.8 to −26.1
bulk	−30.8	0.5	1.7	−31.6 to −30.0
$C_{16:0}$	−32.4	0.5	1.6	−33.3 to −31.5
$C_{18:0}$	−33.1	0.6	1.8	−34.3 to −31.9
$C_{18:1n9}$	−31.0	0.6	1.9	−32.2 to −29.8
$C_{18:1n7}$	−31.8	1.1	3.5	−33.9 to −30.0
$C_{18:2n6}$	−30.5	0.5	1.7	−31.4 to −29.6
$C_{18:3n3}$	−30.8	0.6	1.9	−31.8 to −29.5
$C_{20:0}$	−36.3	4.7	13.1	−48.3 to −31.0
$C_{20:1n9}$	−32.1	1.3	3.9	−34.2 to −30.1
$C_{20:3n3}$	−34.1	2.9	8.5	−39.7 to −30.5
$C_{22:1n9}$	−32.5	0.7	2.1	−33.7 to −31.2

Table 5. Mean, Standard Deviation, Relative Standard Deviation, Minimum, and Maximum $\delta^{13}C$ Values for Seed and Bulk Oil and Main Individual Fatty Acids of Camelina Oil Samples from NEU Region

	mean (‰)	±SD (‰)	±RSD (%)	min−max (‰)
seed	−28.9	0.5	1.8	−29.3 to −28.4
bulk	−30.2	0.2	0.5	−30.3 to −30.0
$C_{16:0}$	−31.8	0.2	0.5	−31.9 to −31.6
$C_{18:0}$	−32.5	0.2	0.6	−32.7 to −32.3
$C_{18:1n9}$	−30.7	0.3	1.1	−31.1 to −30.4
$C_{18:1n7}$	−29.6	1.2	3.9	−30.6 to −28.3
$C_{18:2n6}$	−30.0	0.1	0.4	−30.1 to −29.9
$C_{18:3n3}$	−30.4	0.1	0.5	−30.5 to −30.2
$C_{20:0}$	−33.3	0.4	1.1	−33.6 to −32.9
$C_{20:1n9}$	−31.0	0.7	2.2	−31.8 to −30.5
$C_{20:3n3}$	−33.4	2.9	8.8	−36.5 to −30.7
$C_{22:1n9}$	−32.3	0.2	0.7	−32.5 to −32.0

nonpolar phase was transferred to a GC vial for further analysis. The reason we used methylation with KOH is to avoid any additional isotopic fractionation of the target compounds (*20*) with high temperatures and strong acids.

Gas Chromatography. Analysis of FAMESs was performed by using an Agilent model Hewlett-Packard 6890 gas chromatograph (Hoofddorp, The Netherlands) equipped with a flame ionization detector (FID). A 100 m × 0.25 mm i.d., 0.25 μm, Fame Select, highly polar, fused silica column (Varian, Palo Alto, California) was used. Helium was used as the carrier gas, with a flow rate of 1.2 mL/min. The injection volume was 1 μL, and the split ratio was 1:100. The temperatures of the injector and detector were set at 250 °C. After an

Table 6. Mean, Standard Deviation, Relative Standard Deviation, Minimum, and Maximum δ^{13}C Values for Seed and Bulk Oil and Main Individual Fatty Acids of Camelina Oil Samples from NAM Region

	mean (‰)	±SD (‰)	±RSD (%)	min−max (‰)
seed	−28.2	0.9	3.1	−29.4 to −25.6
bulk	−29.9	0.8	2.6	−31.4 to −28.3
$C_{16:0}$	−31.4	0.8	2.6	−33.3 to −29.7
$C_{18:0}$	−32.0	0.8	2.5	−33.4 to −30.3
$C_{18:1n9}$	−29.4	0.8	2.8	−31.4 to −27.8
$C_{18:1n7}$	−30.6	1.6	5.3	−34.4 to −28.5
$C_{18:2n6}$	−29.4	0.7	2.4	−31.0 to −27.9
$C_{18:3n3}$	−29.8	0.7	2.5	−31.3 to −28.1
$C_{20:0}$	−33.3	2.0	5.9	−40.6 to −31.4
$C_{20:1n9}$	−30.5	1.1	3.5	−33.0 to −28.6
$C_{20:3n3}$	−32.0	2.4	7.6	−37.8 to −29.2
$C_{22:1n9}$	−31.6	1.5	4.9	−36.4 to −28.1

initial isothermal period of 12 min at 180 °C, the column was heated to 250 at 2 °C/min followed by an isothermal period of 10 min. Each sample was injected two times. Data were processed with a Hewlett-Packard Chemstation data system. The relative content of the fatty acids was calculated as the mass percentage of total fatty acids (w/w, %),

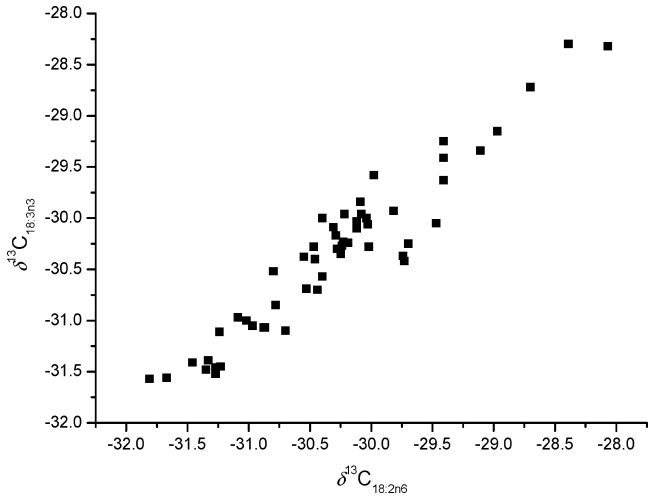

Figure 2. Scatterplot of $\delta^{13}C_{18:2n6}$ vs $\delta^{13}C_{18:3n3}$ of 53 camelina oil samples.

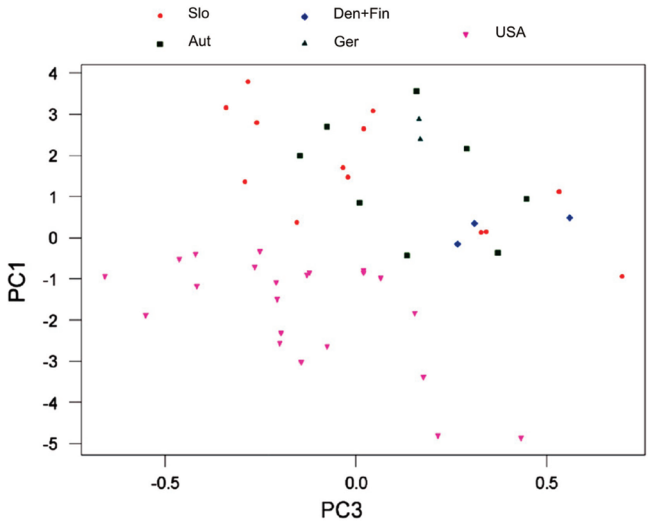

Figure 3. Projection of the 53 objects from Slo (Slovenia), Aut (Austria), Den (Denmark), Fin (Finland), Ger (Germany), and USA (United States of America), represented by the four variables (δ^{13}C values of the $C_{18:1n9}$, $C_{18:2n6}$, $C_{18:3n3}$, and $C_{20:1n9}$ fatty acids and bulk oil), onto the plot defined by the first and the third principal components.

with the correction factor used based on the FID response of the standard fatty acid. The precision of whole method for FAME analysis (oil extraction, methylation, GC) was determined on seven repetitions. The content of fatty acid was precise to ±0.52% for the most abundant fatty acid. The uncertainty of measurements was ±0.23% for the most abundant fatty acid.

***δ*^{13}C of Bulk Oil and Seed.** An amount of 1 mg of Chromosorb was put into a tin capsule followed by 0.5 mL of oil sample and put into the automatic sampler. The seed samples have been homogenized before measurements, and approximately 1 mg was added into a tin capsule. All samples were measured in replicates. The $^{13}C/^{12}C$ measurements in oil and seed were performed using a 20−20 continuous flow IRMS (Europa Scientific, Crew, U.K.) with an ANCA-SL solid−liquid preparation module.

The stable carbon isotopic values are expressed in the usual δ notation as the per mill (‰) deviations of the isotope ratio of a sample relative to that of a standard,

$$\delta^{13}C = [(R_{sam} - R_{std})/R_{std}] \times 10^3$$

where R_{sam} and R_{std} are the $^{13}C/^{12}C$ of the sample and PDB standard, respectively. Analyses were calibrated against international standards: IAEA-NBS22 (oil), IAEA-CH-7, and IAEA-CH-6 with δ^{13}C values of −29.7 ± 0.2‰, −31.8 ± 0.2‰, and −10.4 ± 0.2‰, respectively. The uncertainty of measurements for bulk oil and seed was ±0.2‰.

***δ*^{13}C in Individual Fatty Acids.** The compound-specific carbon isotope analyses of the fatty acids were obtained using an Agilent 6890 GC coupled to an IsoPrime stable IRMS (GV Instruments Ltd., Manchester, U.K.) by a combustion interface (GC/C/IRMS) under a continuous helium flow. The combustion interface consisted of a ceramic furnace with a copper oxide and platinum catalyst at 850 °C. A He-flushed Nafion membrane prevented water from reaching the ion source of the IRMS. The GC was operated with the same type of column and temperature regime as used for the FAME analysis. The performance of the GC/C/IRMS system, including GC and combustion furnace, was evaluated every 10 analyses by injection of a mixture of FAMEs of known δ^{13}C value of −29.8‰. The precision assessed from seven replicate analyses of the samples ranged between ±0.1 and ±0.4‰. The isotopic shift due to the carbon introduced in the fatty acid methylation was corrected by a mass balance equation:

$$\delta^{13}C_{FAME} = [(C_n + 1)\delta^{13}C_{FA} - \delta^{13}C_{MeOH}]/C_n$$

where $\delta^{13}C_{FAME}$, $\delta^{13}C_{FA}$, and $\delta^{13}C_{MeOH}$ are the carbon isotopic values of the FAME, fatty acid, and methanol used for methylation of the fatty acids, respectively, and C_n is the number of C atoms in the fatty acid. The uncertainty of measurements was ±0.2‰.

Statistical Analysis. Statistical analysis was performed using the SCANWIN software (Minitab Inc., USA). The applied chemometric methods were principal component analysis (PCA) and regularized discriminate analysis (RDA). PCA calculates orthogonal linear combinations of a starting set of variables on the basis of a maximum variance criterion. Such linear combinations are called principal components (PC). The coefficients, by which the original variables must be multiplied to obtain the PCs, are referred to as loadings. The numerical value of a given variable loading on a principal component indicates how much the variable has in common with that component. Hence, the loadings can be interpreted as a correlation between the variables and the components (*21*).

RDA obtains discriminant functions calculated to maximize distances between predefined groups (*21*). Its purpose is to calculate class models and boundaries, giving a rule of classification based on a set of known objects (training set). This rule can be applied to define the classification of unknown objects (test set), but it needs to be validated. In this work, the cross-validation approach was applied. For the cross-validation test, one individual at a time is removed from the initial population, and RDA is performed on the remaining individuals. This method gives an indication of the influence of each individual. The coordinates of the removed individual are calculated, and the individual's group assignment is verified. PCA and RDA were successfully applied to different variables determined in food (*6, 10, 22*).

Figure 4. Classification of European and North American clusters on the basis of calculated RDA model and the results of validation model, using the cross-validation test.

RESULTS AND DISCUSSION

On the basis of the geographical origin, 53 seed samples were organized in three different groups: Central European (CEN), North European (NEU), and North American (NAM). To ensure the authenticity of the oils used in this study, oils were extracted from their respective oilseeds.

GC-Fatty Acid Content. A typical gas chromatogram of the major fatty acids of camelina oil is shown in **Figure 1**. The profile of fatty acids is an important characteristic determining the applicability of the oil. The relative compositions of fatty acids exhibit a large variability for the studied camelina oils; in total 17 fatty acids were identified. Values are reported in **Tables 1−3**. Owing to its specific composition, camelina oil appears unique among the common vegetable oils such as rapeseed oil, sunflower oil, soy oil, etc. The profile of fatty acids shows that camelina oil is highly unsaturated. The content of polyunsaturated fatty acids is about 60%, while monounsaturated and saturated fatty acids represent 30% and 10%, respectively. Although there is no significant difference (95% confidence level) between the regions for the content of $\omega 3$ α-linolenic acid ($C_{18:3n3}$), the NAM region indicates higher values for this essential fatty acid. The contents for NEU, CEU, and NAM were 36.1%, 34.6%, and 34.6%, respectively. The NAM region showed the greatest variability of $C_{18:3n3}$ content. The sample with 40.0% of $C_{18:3n3}$ is an absolute maximum and could be a starting material for further breeding. Zubr (*1*) obtained higher values (40.3%) for winter *C. sativa* varieties and values around 34% for the summer varieties. Additionally, Vollman et al. (*23*) reported that low temperatures and high precipitation during the seed filling period may explain high $C_{18:3n3}$ levels in camelina oil. NEU countries are situated in the area with the lowest average year temperatures, which could be a reason for higher $C_{18:3n3}$ values. Oleic acid ($C_{18:1n9}$), linoleic acid ($C_{18:2n6}$), and eicosenoic acid ($C_{20:1n9}$) all belong to the second group of major fatty acids. Variation from 10.6% to 19.4% and from 14.9% to 24.5% for $C_{18:1n9}$ and $C_{18:2n6}$ were determined, respectively. Large variations were also reported elsewhere (*1, 4*). Vaccenic acid ($C_{18:1n7}$) is a $C_{18:1}$ isomer. Because of similar physical properties as $C_{18:1n9}$, the detection is possible only with specific GC columns; however, there have been no reports that this minor fatty acid is present in camelina oil; thus, results in previous papers for $C_{18:1n9}$ could be a sum of $C_{18:1n9}$ and $C_{18:1n7}$. We detected contents from 0.8% to 1.1%. Additionally, camelina oil is interesting for its eicosenoic acid $C_{20:1n9}$. Values ranged from 10.6% to 15.2% of total fatty acids, with the highest average content in CEU region (14.5%), whereas NAM had the lowest content (12.7%), which is similar to NAM results provided by Pilgeram et al. (*2*). Among C_{18} isomers, trans-fatty acids were detected. The NAM group has a content of trans-fatty acids above 0.05 wt % per individual fatty acid, which is the EU regulation value for cold-pressed virgin olive oils (*24*). Extraction, methylation, and GC techniques could be the main reasons for their appearance. The presence of erucic acid ($C_{22:1n9}$) in vegetable oils is a limiting factor for their applicability in human nutrition. The content of $C_{22:1n9}$ among the CEU, NEU, and NAM regions resulted in 2.9, 3.1, and 3.2%, respectively. The one oil sample from NAM, with the maximum 4.2% of $C_{22:1n9}$, is still much below the 5.0%, which is permitted in vegetable oils for human consumption (*25*). Vollmann et al. (*23*) also reported a significant variation in $C_{22:1n9}$ content in different genotypes of *C. sativa* in five growing seasons and that this fatty acid may even be further reduced through targeted selection. Genetically, *C. sativa* is probably the closest crop plant to its well-known and completely sequenced cousin *Arabidopsis thaliana* (*26*). This is of great importance for further research work in breeding of *C. sativa*. The fatty acid composition confirms that camelina oil is a high added value oil source for applications in food and industry because of its high content of $C_{18:3n3}$, which is an essential fatty acid in human nutrition, and monounsaturated fatty acids, an important factor for the use of oil in biodiesel industry.

IRMS-Seed and Bulk δ^{13}C. The δ^{13}C values of the whole seed (ranges from −25.6‰ to −30.8‰) and bulk oil (ranges from −28.3‰ to −31.6‰) are typical of C_3 plants (**Tables 4−6**). The values of seed and bulk oil of North American region are less negative than the values from both European regions. Spangenberg et al. (*27*) also reported less negative results for bulk olive oil from the U.S. than those from different European countries. Variation of the isotopic composition of the camelina oil and seed between North American and European may be due to different climatic and plant growing conditions (*9, 12*). Additionally, seeds are depleted in ^{13}C with more variable δ^{13}C values (RSD = 1.8−4.2%) compared to bulk oil (RSD = 0.5−2.6%). A small correlation ($R = 0.592$) between the δ^{13}C of seed and bulk oil was determined. The outer layer of the seed is mostly composed of protein and fibers, which are rich in the heavy isotope as a result of different fractionation during secondary metabolism and variation in the ratio of diffusional

and carboxylation limitations (*28*). The isotopic difference between bulk oil and seeds could be explained by also considering the different influence of the geoclimatic factors on isotopic composition of proteins and sugars present in the outer layer of the seeds.

GC/C/IRMS-Fatty Acid δ^{13}C and Authenticity Characterization of *C. sativa* Oil. The δ^{13}C values for major and some minor fatty acids of *C. sativa* were determined (**Tables 4−6**). The gas chromatogram (**Figure 1**) displays baseline resolution of components, an essential requirement for the accurate determination of $^{13}C/^{12}C$ data. The isotopic values of the four main fatty acids vary from −27.8‰ to −33.9‰, from −27.9‰ to −31.4‰, from −28.1‰ to −31.8‰, and from −28.6‰ to −34.2‰ for $C_{18:1n9}$, $C_{18:2n6}$, $C_{18:3n3}$, and $C_{20:1n9}$, respectively. All maximum values of these four fatty acids belong to the NAM region and vice versa for the CEU region, which is consistent with bulk and seed δ^{13}C values. Isotopic values of bulk oil and the most abundant fatty acid $C_{18:3n3}$ are in good correlation ($R = 0.954$). Our newly obtained data have revealed that desaturation of the C_{18} carbon chain in the first step ($C_{18:0} > C_{18:1n9}$) has a significant enrichment in ^{13}C. The enzyme desaturase that introduces a double bond to $C_{18:1}$ to produce $C_{18:2}$ and further $C_{18:3n3}$ (*29*) does not affect changes in δ^{13}C values ($C_{18:1n9} \approx C_{18:2n6} \approx C_{18:3n3}$). Therefore, for the highest correlation ($\delta^{13}C_{18:2n6}$ vs $\delta^{13}C_{18:3n3}$, $R = 0.972$), a scatterplot was drawn (**Figure 2**). Spangenberg et al. (*27*) described a similar correlation of $\delta^{13}C_{16:0}$ vs $\delta^{13}C_{18:1n9}$ for cold-pressed olive oil. They explained that substantial separation suggests admixture of cold-pressed virgin olive oils with refined olive oils or other vegetable oils of different $\delta^{13}C_{16:0}$ vs $\delta^{13}C_{18:1n9}$ concentration ratios than the genuine olive oil. Correlation of $\delta^{13}C_{18:2n6}$ vs $\delta^{13}C_{18:3n3}$ could identify cases where impurity or adulteration of camelina oil is suspected.

PCA was applied to the data matrix formed by fatty acid and δ^{13}C values (**Tables 1−6**). Parameters (δ^{13}C values of bulk, $C_{18:1n9}$, $C_{18:2n6}$, $C_{18:3n3}$, and $C_{20:1n9}$) were selected on the basis of the best results on geographical separation of the samples. On the score plot of the first three PCs, which account for 56.7%, 13.8%, and 7.3%, respectively, first and third PCs gave the best visual discrimination (**Figure 3**). A rough clustering of camelina oil samples can be observed according to the different geographical origin. Results of PCA clearly indicate that the European cluster of samples is separated from the NAM cluster. This is not so clearly evident between the samples within the European group. However, two German samples are separated from the Danish samples (Finnish included), within the majority of Austrian and Slovenian samples. The large number of *C. sativa* samples grown at numerous sites around the world and its δ^{13}C values in combination with chemometrics clearly indicate that isotope ^{13}C geographically distinguishes between samples from different continents.

On the basis of the PCA results, RDA was applied and resulted in the formation of two groups, European and NAM (**Figure 4**). This model for classification of the oil according to their geographical origin gave 98.1% correct classification ability, while the cross-validated assignments were also 98.1% correct. One sample was assigned wrongly.

In conclusion, the present work delineates the ranges of natural dispersion exhibited by these two types of analysis. Fatty acid compostion is unique among the common oils, making it suitable for food and industry applications. A study of the geographical ^{13}C profile shows that there is a significant dicrimination between samples results from North American and European region while there is less discrimination of the samples within the European region. Correlation of $\delta^{13}C_{18:2n6}$ vs $\delta^{13}C_{18:3n3}$ provides a basis for impurity or adulteration detection. Preliminary results on the camelina oil authentication procedure give a basis for the investigation of geographical origin and the further distinction between camelina and camelina refined or other, less expensive oils. Further work based on mixing curves (blending) with potential adulterant oils and determination of δ^{18}O isotopic distribution will further improve the efficiency of the camelina oil authenticity characterization.

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