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Triacylglycerol Composition of Walnut (*Juglans regia* L.) Cultivars: Characterization by HPLC-ELSD and Chemometrics

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A total of 26 walnut (*Juglans regia* L.) samples from 9 cultivars (Arco, Franquette, Hartley, Lara, Marbot, Mayette, Mellanaise, Parisienne, and Rego) harvested in the 2001, 2002, and 2003 crop years and grown in two geographical origins (Bragança and Coimbra, Portugal) were evaluated with regard to their triacylglycerol composition. The methodology employed was reversed-phase high-performance liquid chromatography coupled to an evaporative light-scattering detector (RP-HPLC-ELSD) after extraction of the lipidic fraction of the nuts. Nine compounds were separated, identified, and quantified. All samples presented an identical qualitative profile composed by LLnLn, LLLn, LLL, OLLn, OLL, PLL, OOL, and PLO (P = palmitoyl; O = oleoyl; L = linoleoyl; Ln = linonenoyl). Trilinolein (LLL) was the major triglyceride, followed by dilinoeoyl-oleoyl-glycerol (OLL) and dilinoleoyl-linolenoyl-glycerol (LLLn), with mean values of 37.7, 18.5, and 18.4%, respectively. Significant differences in composition were found between cultivars, and these differences were also significant when cultivars were grouped by year of production, showing that besides genetic factors, the triacylglycerol composition can be strongly influenced by environmental factors.

KEYWORDS: Triacylglycerols; *Juglans regia* L.; walnut oil; HPLC-ELSD

INTRODUCTION

There is an increasing interest in the lipid composition of vegetable oils as they seem to be an interesting source of functional nutrients. More and more, consumers are demanding food products that combine pleasant flavors with nutritional benefits. Walnuts, as well as other nuts, are being studied in order to evaluate their possible health benefits (*1-4*), especially in what concerns protective effects against cardiovascular and heart diseases. In relation to walnuts, some studies indicate that the frequent consumption of moderate quantities of this nut favorably modifies the lipoprotein profile and decreases serum levels of total cholesterol (*5*, *6*).

Triacylglygerols (TAGs) are the most common lipids in nature. Although fatty acids are certainly important in the characterization of the lipidic fraction, vegetable oils have a characteristic and more or less unique pattern of TAGs. This characteristic can be used to determine oil origin or detect adulterations, and is increasingly used in the food industry to check authenticity (*7*−*11*). The advantage of using TAG analysis compared to fatty acid (FA) profiles is that the stereospecific distribution of FA on the glycerol molecule is genetically controlled and, thus, the information content of intact TAGs is usually higher (*7*, *8*).

Triacylglycerols can be determined either by high-temperature gas−liquid chromatography (GLC) or by high-performance liquid chromatography (HPLC) techniques. HPLC seems to be the most common methodology used in TAG analysis because GLC presents some difficulties due to the low volatility of these compounds (*12*) and also problems associated with the injection system and with the deterioration of the column with temperature (*7*). Nonaqueous eluting solvent mixtures have been used successfully in the reversed phase (RP) HPLC separation of TAGs (*7*, *13*−*19*); this separation is based both on the fatty acid chain length and on the total number of double bonds in the molecule (*14*−*16*, *19*). Several detectors have been used coupled RP-HPLC, such as the ultraviolet (UV), the refractive index (RI), and the evaporative light-scattering (ELSD) detectors and mass spectrometers (MS) (*7*, *12*, *15*). ELSD seems to be a good choice when the disadvantages of the other detectors are taken into consideration: TAGs have weak chromophores, and

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UV is not suitable when using high-UV-absorbing solvents, such as acetone (*12*, *14*−*16*); the RI detector has a poor sensitivity, is susceptible to temperature, and cannot be used in gradient elution (*12*, *14*−*20*); the MS detector presents the advantage of allowing the identification of the compounds, but it is still very expensive and requires particular technical skills (*20*). Besides, the ELSD seems to present some advantages: it allows direct detection of nonvolatile compounds regardless of their chemical structure, it is not sensitive to room temperature fluctuation, it can be used with gradient elution, and it presents a higher sensitivity than the RI detector (*12*, *20*). Nevertheless, the ELSD is generally used only for semiquantitative evaluations, because a nonlinear dependence of the signal with the analyte concentration can be observed (*12*).

Because of the pointed characteristics, the ELSD seems to be an interesting alternative to the above-mentioned detectors and has been already successfully applied to a large range of different kinds of analytes (*12*, *20*, *21*).

Although there are some studies on the lipidic fraction of walnut oil, focusing on fatty acid (*22*), sterol (*22*), tocopherols (*23*), and mineral (*24*) compositions, as far as we know, there are few available data on walnut TAG composition, and only the qualitative profile is given (*25*). This study reports the results obtained for the triacylglycerol composition of nine walnut cultivars, collected during three consecutive years, and some of them in two different regions. Statistical analysis was carried out to check differences between the evaluated cultivars with regard to TAG profiles.

MATERIALS AND METHODS

Samples. A total of nine walnut (*Juglans regia* L.) cultivars [Franquette (Cv1), Lara (Cv2), Marbot (Cv3), Mayette (Cv4), Mellanaise (Cv5), Parisienne (Cv6), Arco (Cv7), Hartley (Cv8), and Rego (Cv9)] were studied. Samples of the cultivars Franquette, Lara, Marbot, Mayette, Mellanaise, and Parisienne were harvested in Bragança, northeastern Portugal (6° 46′ W, 41° 49′ N, 670 m elevation) in three consecutive years (2001, 2002, and 2003). In 2003, samples from another geographical origin were also included in the study: cultivars Franquette, Lara, Mayette, Mellanaise, and Parisienne (in common with the Bragança location), and three others (cultivars Arco, Hartley, and Rego) were harvested in Coimbra (8° 37′ W, 40° 03′ N, 10 m elevation), in central Portugal. All of the samples were collected during September. After the harvesting, walnuts were dried in an oven at 30 °C during at least 3 days, and a final sample of ∼2 kg was randomly taken. The fruits were stored in the shell, closed in plastic bags, and frozen to −20 °C until the analyses.

Sample Preparation. Walnuts were manually cracked and shelled and then chopped in a 643 MX coffee mill (Moulinex, Spain). Crude oil was obtained from finely chopped nuts (.∼15 g with anhydrous sodium sulfate) extracted with light petroleum ether (bp 40−60 °C) in a Universal extraction system B-811 (Büchi, Switzerland); the residual solvent was removed by flushing with nitrogen. A 0.2 g oil sample was dissolved in 4.0 mL of acetone and homogenized by stirring. The mixture was filtered through a 0.22 μm disposable LC filter disk and analyzed by HPLC.

Reagents and Standards. Triacylglycerols 1,2,3-tripalmitoylglycerol (PPP), 1,2,3-tristearoylglycerol (SSS), 1,2,3-trilinolenoylglycerol (LnLnLn), and 1,2,3-tripalmitoleoylglycerol (PoPoPo), of purity >98%, and 1,2,3-trioleoyglycerol (OOO), 1,2,3-trilinoleoyglycerol (LLL), 1,2-dilinoleoyl-3-palmitoyl-*rac*-glycerol (LLP), 1,2-dilinoleoyl-3-oleoyl-*rac*-glycerol (LLO), 1,2-dipalmitoyl-3-oleoyl-*rac*-glycerol (PPO), 1,2-dioleoyl-3-stearoyl-*rac*-glycerol (OOS), 1-palmitoyl-2-oleoyl-3-linoleoylglycerol (POL), and 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (OOP), of ∼99% purity, were purchased from Sigma (St. Louis, MO). The code letters, used as abbreviations for the fatty acids, are as follows: Po, palmitoleic; L, linoleic; Ln, linolenic; M, myristic; O, oleic; P, palmitic; S, stearic. Acetonitrile and acetone were of HPLC grade and obtained from Merck, Darmstadt, Germany.

Triacylglycerol Analysis. The chromatographic analyses were performed with a Jasco (Japan) high-performance liquid chromatograph, equipped with a PU-1580 quaternary pump and a Jasco AS-950 automatic sampler with a 10 μL loop. Detection was performed with an ELSD (model 75-Sedere, France). The chromatographic separation of the compounds was achieved with a Kromasil 100 C_{18} (5 μm; 250 × 4.6 mm) column (Teknokroma, Spain) operating at ambient temperature (∼20 °C). The mobile phase was a mixture of acetone/acetonitrile (70:30, v/v). Elution was performed at a solvent flow rate of 1 mL/min with an isocratic program. The ELSD was programmed with the following settings: evaporator temperature, 40 °C; air pressure, 3.5 bar; and photomultiplier sensitivity, 6. Data were analyzed using Borwin-PDA Controller software (JMBS, France). Taking into account the selectivities (α, relative retention times to LLL), peaks were identified according to the logarithms of α in relation to homogeneous TGA (Sigma). Quantification of the peaks was made by internal normalization, assuming that the detector response was the same for all compounds.

Statistical Analysis. Multivariate analyses of data involved (i) MANOVA to evaluate the hypothesis that "there is at least one group different from the others in at least one triacylglycerol", calculating the Wilks' lambda and the Pillai−Bartlett trace, and checking their significance through the Rao's R and V statistics, respectively; (ii) Hotelling T^2 tests applied to pairs of groups, to evaluate the hypothesis that "the two groups are significantly different in at least one TAG", calculating T^2 values and calculating and tabling the respective F values and corresponding probabilities; (iii) forward stepwise discriminant analysis (DA) to select the most discriminant TAGs; and (iv) canonical variate analysis (CVA) based on all TAGs, or on a subset of the most discriminant TAGs, to further analyze the differences between groups and display those differences in convenient canonical variate plots. (v) When cultivars were represented by only one sample, and Hotelling T^2 tests could not be applied, differences between cultivars were evaluated on the basis of ANOVA applied to each TAG, answering the question, "are the two cultivars significantly different in what concerns this TAG?". All analyses were carried out in the Statistica for Windows statistical package (Statistica for Windows, StatSoft Inc., Tulsa, OK), and comments to statistical results were based on Mardia et al. (*26*).

RESULTS AND DISCUSSION

Chain length and the degree of unsaturation of the fatty acids (FAs) are the main mechanisms for separating TAGs in RP-HPLC. Characterization of TAG molecules and the determination of their relative elution order can be made by means of the partition number (PN = CN − 2DB), where CN is the total number of carbons and DB the total number of double bounds (*14*, *16*). However, difficulties arise in the separation of mixtures of TAGs composed of FAs presenting minimal changes in the chain length or in the degree of unsaturation (that is, compounds with the same PN). Mobile phase composition is an important chromatographic factor affecting the separation of TAGs in RP-HPLC, considering the low variability in the stationary phases employed (*14*). Usually, a mixture of an organic base solvent and an organic modifier is used as mobile phase. The modifier improves TAG solubility in the base solvent and increases peak selectivity. A mixture of acetone/acetonitrile was chosen, because these solvents are reported to be the most widely used as organic modifier and organic base solvent, respectively (*14*, *15*), and have already been used successfully in TAG analysis (*13*, *17*−*19*). The best separation of walnut TAGs was obtained with a mixture of acetone/acetonitrile (70:30, v/v) using an isocratic program. This mobile phase allowed a reduction of retention times of the compounds with higher CN while achieving good peak resolutions.

Three instrumental parameters seemed to be very important in the sensitivity of this method and were optimized: detector

Table 1. Triacylgycerol Contents (Relative Percent) of the Studied Cultivars by Year of Production and Geographical Location

		triacylylycerol								
cultivar	year	LLnLn	LLLn	LLL	OLLn	PLLn	OLL	PLL	OOL	PLO
Bragança	2001									
Franquette		2.09 ± 0.07	19.99 ± 0.17	34.64 ± 0.13	4.64 ± 0.20	3.14 ± 0.17	17.19 ± 0.24	10.62 ± 0.37	3.06 ± 0.11	4.22 ± 0.04
Lara		1.85 ± 0.04	20.79 ± 0.69	39.15 ± 0.58	4.72 ± 0.44	2.10 ± 0.09	16.65 ± 0.28	9.13 ± 0.18	2.54 ± 0.07	2.80 ± 0.20
Marbot		1.74 ± 0.16	18.85 ± 0.22	39.00 ± 0.36	4.43 ± 0.03	2.38 ± 0.11	17.88 ± 0.28	9.58 ± 0.16	2.58 ± 0.01	3.45 ± 0.04
Mayette		2.69 ± 0.04	23.46 ± 0.35	39.24 ± 0.39	4.23 ± 0.35	3.00 ± 0.17	14.11 ± 0.18	8.91 ± 0.27	1.61 ± 0.08	2.56 ± 0.04
Mellanaise		0.86 ± 0.01	13.64 ± 0.51	38.42 ± 0.45	4.60 ± 0.35	1.47 ± 0.04	22.76 ± 0.62	8.25 ± 0.15	5.72 ± 0.16	4.07 ± 0.22
Parisienne		1.24 ± 0.04	16.44 ± 0.36	44.26 ± 0.12	3.74 ± 0.23	1.48 ± 0.07	18.82 ± 0.38	7.80 ± 0.34	2.99 ± 0.07	3.19 ± 0.07
Franquette	2002	2.12 ± 0.15	19.00 ± 0.30	35.37 ± 0.28	5.62 ± 0.37	2.00 ± 0.06	19.81 ± 0.44	7.48 ± 0.07	4.63 ± 0.10	3.27 ± 0.09
Lara		1.00 ± 0.01	16.36 ± 0.49	41.38 ± 0.98	4.42 ± 0.23	1.30 ± 0.10	20.52 ± 0.86	6.82 ± 0.58	4.48 ± 0.03	3.56 ± 0.09
Marbot		0.82 ± 0.11	12.67 ± 0.15	38.46 ± 0.06	3.87 ± 0.22	1.28 ± 0.15	23.35 ± 0.19	7.94 ± 0.01	6.24 ± 0.05	4.32 ± 0.16
Mayette		0.98 ± 0.07	13.65 ± 1.21	38.33 ± 1.04	4.57 ± 0.20	1.09 ± 0.01	22.93 ± 0.93	6.51 ± 0.26	7.59 ± 0.44	3.48 ± 0.31
Mellanaise		0.93 ± 0.02	15.18 ± 0.46	39.62 ± 0.41	4.03 ± 0.12	1.44 ± 0.01	20.91 ± 0.14	7.87 ± 0.20	4.87 ± 0.19	4.47 ± 0.49
Parisienne		0.61 ± 0.03	12.10 ± 0.05	32.15 ± 0.29	5.32 ± 0.12	0.71 ± 0.08	27.57 ± 0.36	4.83 ± 0.10	11.49 ± 0.25	3.61 ± 0.02
Franquette	2003	1.85 ± 0.08	17.32 ± 0.27	33.21 ± 0.55	5.56 ± 0.28	3.18 ± 0.20	18.36 ± 0.17	11.04 ± 0.12	4.24 ± 0.23	4.53 ± 0.31
Lara		2.56 ± 0.05	22.74 ± 0.93	40.85 ± 1.06	3.69 ± 0.24	2.70 ± 0.24	13.73 ± 0.27	8.77 ± 0.60	1.75 ± 0.22	2.96 ± 0.22
Marbot		1.81 ± 0.07	20.49 ± 2.64	37.79 ± 1.91	5.59 ± 0.58	2.40 ± 0.19	16.03 ± 0.58	9.42 ± 0.02	2.61 ± 0.18	3.64 ± 0.30
Mayette		2.44 ± 0.14	20.77 ± 0.14	35.77 ± 0.43	5.24 ± 0.30	3.47 ± 0.12	14.94 ± 0.27	11.00 ± 0.12	2.44 ± 0.12	3.67 ± 0.39
Mellanaise		1.80 ± 0.12	18.14 ± 0.32	36.98 ± 0.58	4.74 ± 0.55	2.54 ± 0.16	18.26 ± 0.07	9.34 ± 0.33	4.29 ± 0.14	3.50 ± 0.29
Parisienne		1.01 ± 0.10	14.00 ± 0.24	34.91 ± 0.35	5.05 ± 0.06	2.01 ± 0.13	21.69 ± 0.37	10.25 ± 0.12	5.78 ± 0.39	4.60 ± 0.14
Coimbra	2003									
Arco		2.60 ± 0.27	22.64 ± 0.69	36.70 ± 0.18	5.33 ± 0.49	2.86 ± 0.26	15.84 ± 0.46	7.98 ± 0.13	2.66 ± 0.16	3.13 ± 0.24
Franquette		1.14 ± 0.03	16.28 ± 0.16	40.43 ± 0.75	5.81 ± 0.30	1.46 ± 0.08	19.89 ± 0.62	7.51 ± 0.70	4.26 ± 0.31	3.20 ± 0.13
Hartley		1.93 ± 0.18	19.77 ± 0.63	34.70 ± 0.99	6.07 ± 0.15	2.60 ± 0.17	17.83 ± 0.43	9.72 ± 0.29	3.33 ± 0.33	3.76 ± 0.28
Lara		2.03 ± 0.08	21.47 ± 0.53	40.23 ± 0.64	4.70 ± 0.24	2.23 ± 0.16	14.76 ± 0.33	9.15 ± 0.52	1.98 ± 0.12	3.31 ± 0.14
Mayette		2.02 ± 0.06	20.20 ± 0.73	37.15 ± 0.67	5.39 ± 0.32	2.66 ± 0.10	16.48 ± 0.32	10.20 ± 0.64	2.53 ± 0.15	3.32 ± 0.27
Mellanaise		1.44 ± 0.05	17.32 ± 0.42	35.83 ± 0.61	5.65 ± 0.13	2.05 ± 0.13	20.02 ± 0.03	9.47 ± 0.23	3.68 ± 0.29	4.31 ± 0.38
Parisienne		1.65 ± 0.12	18.71 ± 0.64	35.81 ± 0.09	5.64 ± 0.26	2.15 ± 0.17	18.60 ± 0.45	9.21 ± 0.57	3.96 ± 0.20	3.96 ± 0.28
Rego		2.71 ± 0.19	23.11 ± 0.63	38.87 ± 0.53	5.35 ± 0.03	1.96 ± 0.14	14.55 ± 0.52	7.42 ± 0.18	2.71 ± 0.18	2.66 ± 0.31

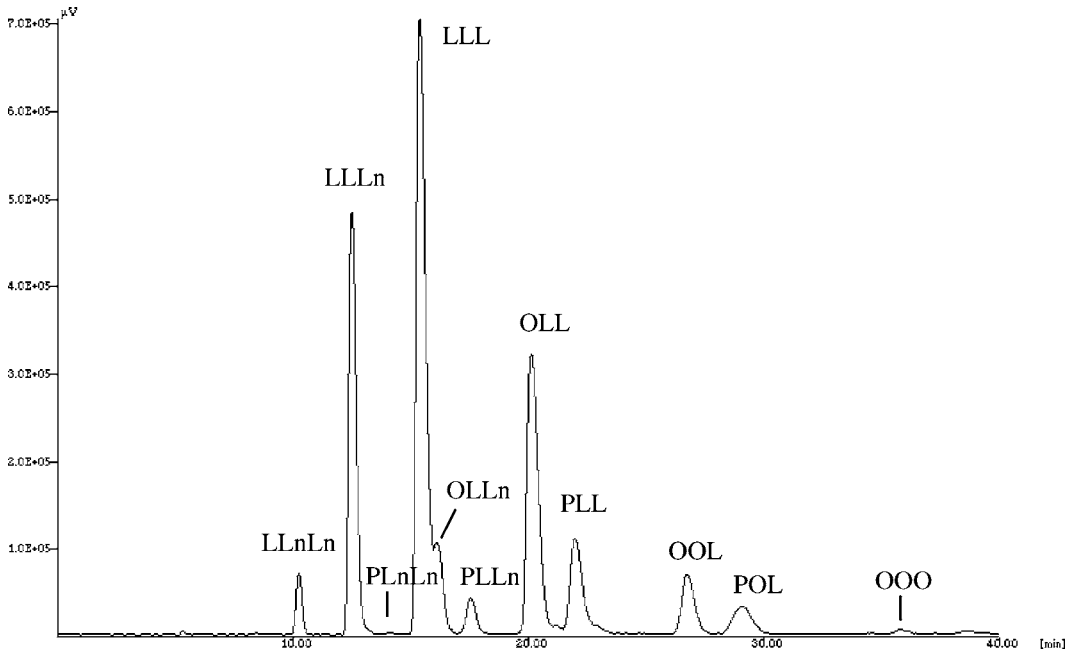

Figure 1. Walnut triacylglycerol profile obtained by HPLC-ELSD.

temperature, photomultiplier gain, and nebulazing gas pressure (*21*). The best conditions were obtained with a temperature of 40 °C, the photomultiplier gain set to 6, and the air flow rate (pressure) of the air compressor set to 3.5 bar.

Nine TAGs were identified and quantified: LLnLn, LLLn, LLL, OLLn, PLLn, OLL, PLL, OOL, and PLO. PLnLn was also present in some samples, but only in vestigial amounts. OOO was also detected in trace amounts in all samples but could not be quantified, not only because of the low quantities observed, but mainly because of peak broadening effects. On the basis of the fatty acid composition previously published for the same cultivars (*22*), SLL can possibly exist. A possible overlap of TAG species could be occurring in the chromatogram, and SLL could be coeluted with POL because they both have identical partition numbers (PN). In a review, Andrikopoulos (*15*) illustrated that, with one exception, authors reported the coelution of the PN critical pair LLL and OLLn. With the conditions used in this work, the two compounds were separated enough to allow quantification. The results here reported for the walnut TAG qualitative profile are consistent with the ones reported by Ayorinde et al. (*25*).

The average values of TAG content for every cultivar, year of production, and geographical origin are shown in **Table 1**. **Figure 1** shows a chromatogram obtained with the experimental

Table 2. MANOVA and Hotelling T^2 Tests for the Determination of the Significance of Observed Between-Year Differences (below Diagonal, *F* Values; above Diagonal, Corresponding Probabilities)

Summary of MANOVA Tests

Wilks' lambda = 0.187619 Pillai–Bartlett trace = 1.084936
Rao's $R_{(18;134)} = 9.7423$ ($p < 0.0000$) $V_{(18;136)} = 8.9582$ ($p < 0.0000$)

Summary of Hotelling T^2 Tests

	2001	2002	2003
2001		$p < 0.00000$	$p < 0.00000$
2002	11.506		$p < 0.00000$
2003	6.6472	17.455	

conditions described. In all the studied samples, the major TAG was trilinolein (LLL) (ranging from 32.2 to 44.3%, 37.7% mean value), followed by dilinoleoyl-oleoyl-glycerol (OLL) (ranging from 13.7 to 27.6%, 18.5% mean value) and dilinoleoyl-linolenoyl-glycerol (LLLn) (ranging from 12.1 to 23.5%, 18.4% mean value). In some samples OLL is the second major TAG and in others it is the third, LLLn being the second major one. Considering the quantified TAGs, all of them contain, at least, one linoleic acid molecule. These data are therefore consistent with the previously published fatty acid composition for the same samples (*22*), in which linoleic acid was on average 60% of the total fatty acid content.

To check if there are significant differences in cultivar composition due to environmental factors, a MANOVA was carried out with the results obtained for the cultivars from the same region (Bragança) and the year of production as the grouping factor. The results (**Table 2**) show that differences exist and are significant, as expressed by significant Wilks' lambda and Rao's *R* statistics and by the Pillai−Bartlett trace and corresponding *V* statistics. Hotelling T^2 tests were subsequently carried out with the year of production as the grouping factor, therefore comparing years in pairs (**Table 2**). Results showed that all groups are statistically different. Univariate analyses of variance and discriminant analysis were subsequently carried out to check for the most important TAGs in the

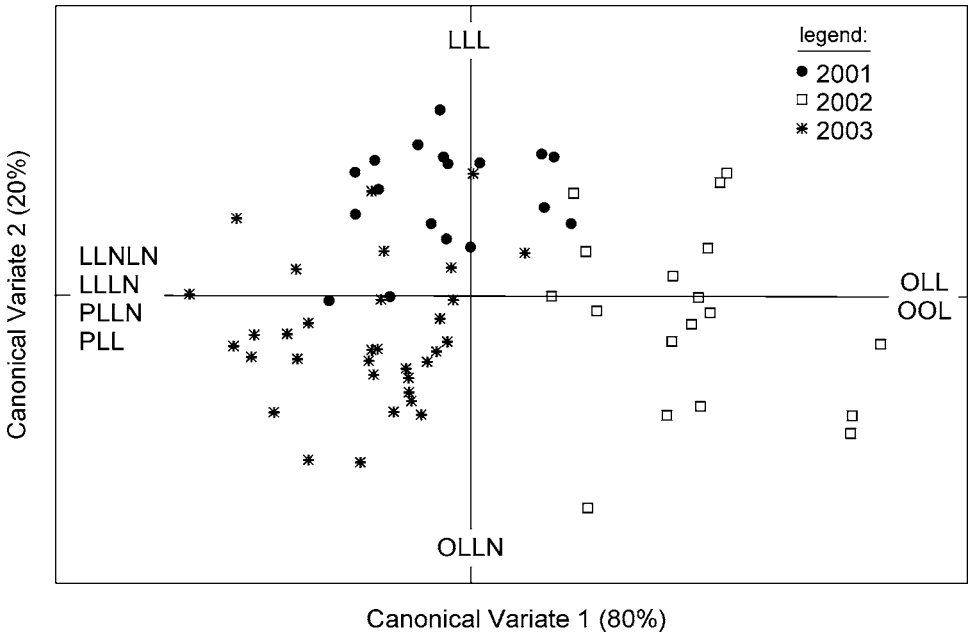

Figure 2. Results from CVA with the year of production as the grouping factor of samples from the Bragança region. Plot of canonical variates 1 vs 2. Triglycerides labeling canonical axes are important for their interpretation. Percentage values refer to the amount of information explained by each canonical dimension.

Table 3. Hotelling T^2 Tests for the Determination of the Significance of Observed Between-Cultivar Differences (below Diagonal, *F* Values; above Diagonal, Corresponding Probabilities; Values for Cv7–9 Were Calculated on the Basis of One Sample Only)

Summary of MANOVA Tests

Wilks' lambda = 071605 Pillai–Bartlett trace = 1.915040
Rao's $R_{(72;378)} = 2.853243$ ($p < 0.0000$) $V_{(72;544)} = 2.377862$ ($p < 0.0000$)

Summary of Hotelling T^2 Tests

	Cv1	Cv2	Cv3	Cv4	Cv5	Cv6	Cv7	Cv8	Cv9
Cv1		0.00048	0.0599	0.0607	0.4425	0.0035	0.0236	0.0299	0.0066
Cv2	7.5820		0.0018	0.0000	0.0053	0.0172	0.0000	0.0005	0.0061
Cv3	2.7275	7.1234		0.0009	0.7440	0.2448	0.0027	0.0187	0.0116
Cv4	2.4959	13.2510	8.3156		0.3219	0.3088	0.0181	0.1109	0.0026
Cv5	1.0636	4.6582	0.6400	1.2921		0.0823	0.0025	0.2778	0.0015
Cv6	1.1102	3.5311	1.5447	1.3214	2.2659		0.0376	0.1225	0.0044
Cv7	6.8650	119.0200	369.2600	7.7596	18.5710	5.4936			
Cv8	6.1341	37.8540	52.8240	3.1305	1.7544	2.9579	*a*		
Cv9	12.1920	12.6640	85.9120	18.3960	23.0190	14.5880	*b*	*c*	

[a] Significant differences (at least $p < 0.05$) in all triacylglycerols but PLLn and OLLn. [b] Significant differences (at least $p < 0.05$) in LLL, PLLn, OLL, and PLL. [c] Significant differences (at least $p < 0.05$) in all triacylglycerols.

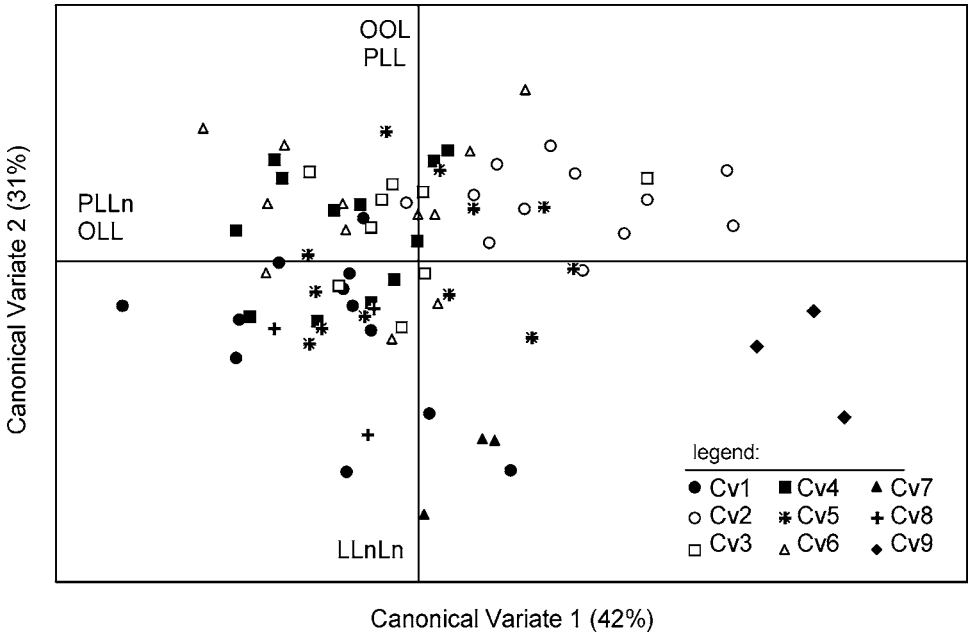

Figure 3. Results from CVA with cultivars as the grouping factor. Plot of canonical variates 1 vs 2. Triacylglycerols labeling canonical axes are important for their interpretation. Percentage values refer to the amount of information explained by each canonical dimension.

discrimination between years, and a canonical variate analysis was developed to enable the visualization of all results. **Figure 2** shows the plot of canonical variates 1 versus 2, where all information from ANOVAs, DA, and CVA is condensed: ~80% of the information in the data is represented in the first dimension, separating year 2002 from the other two years of production and reflecting the fact that 2002 walnuts had higher contents in OLL and OOL and lower contents in LLnLn, LLLn, PLLn, and PLL. The second canonical dimension expresses the fact that walnuts in the year 2001 generally displayed higher levels of LLL and lower levels of OLLn than walnuts in the year 2003. These results suggest that TAG composition can be strongly influenced by climatic conditions because significant differences can be observed among years of production when the same six cultivars growing in the same experimental field under the same agricultural practices are considered.

The statistical work described in the previous paragraph was repeated with reagard to the cultivars as the grouping factor, to check for significant differences in TAG composition among cultivars. Results for the MANOVA and the Hotelling T^2 tests are shown in **Table 3**. Again, MANOVA shows that there are significant differences among cultivars, and the Hotelling T^2 tests show that many cultivars display very distinctive features in what concerns TAG composition, whereas others, mainly Cv4, Cv5, Cv6, and Cv8, seem to share some common patterns. Cultivars Arco (Cv7) and Rego (Cv9) seem to present some differences when compared to the other cultivars (**Table 3**); however, more samples should be studied because these cultivars were studied in only one crop year (2003). ANOVAs, DA, and CVA analyses were performed, and the main conclusions are presented in **Figure 3**. The triacylglycerols labeling the canonical axes represent the main sources of variation when all cultivars are considered together.

In 2003, samples from another location (Coimbra) were included in the study, but more samples should be studied to reach conclusions with regard to differences with respect to geographical origins.

In conclusion, RP-HPLC-ELSD seems to be an appropriate technique for the analysis of TAGs in walnut oil, although the position of fatty acid chains in the glycerol backbone cannot be discriminated. The results herein reported suggest that, besides genetic factors, the TAG composition can be strongly influenced by environmental factors. These results may be considered as reference data for walnut triacylglycerol quantitative composition because, as far as we know, there are few published data on this subject.

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