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Influence of Cultivar and Environmental Conditions on the Triacylglycerol Profile of Hazelnut (*Corylus avellana* L.)

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The oil of several hazelnut (*Corylus avellana* L.) samples was extracted and evaluated for their triacylglycerol (TAG) composition. Trials were conducted in two Portuguese localities (Vila Real and Felgueiras) during three consecutive years and involved a total of 19 cultivars. The samples were analyzed by reversed-phase high-performance liquid chromatography with evaporative light-scattering detection. Sample preparation was fast and simple, consisting only of the dissolution of the oil in acetone, homogenization, and filtration, allowing this technique to be suitable for routine analyses. All samples presented a similar qualitative profile composed of eleven compounds: LLL, OLL, PLL, OOL, POL, PPL, OOO, POO, PPO, SOO and PSO (P, palmitoyl; S, stearoyl; O, oleoyl; and L, linoleoyl). The main components were OOO, LOO, and POO, reflecting the high content of oleic acid in hazelnut oils. A total of 79 different samples were studied, and the obtained data were statistically analyzed. Significant differences were verified in canonical variate plots when cultivars were grouped by country of origin. In general, the American cultivars were richer in TAGs with saturated fatty acids moieties, and the group of French, German, and English cultivars was richer in TAGs containing linoleic acid moieties. Differences were also significant when cultivars were grouped by year of production, showing that besides genetic factors, the TAG composition can be influenced by environmental factors.

KEYWORDS: Triacylglycerols; *Corylus avellana* L.; hazelnut oil; HPLC/ELSD; chemometrics

INTRODUCTION

Triacylglycerols (TAGs) are a group of nonpolar lipids representing the major components of vegetable oils and animal fats. In foods, lipids provide texture, enhance palatability, contribute to a feeling of satiety, are sources of essential fatty acids (FAs), and aid in the absorption of fat vitamins in the intestine (*1*).

The existence of different TAGs is determined by the FA composition and their distribution on the glycerol backbone. This distribution is not random but is more or less characteristic for each vegetable oil. Although with some exceptions, in vegetable oils, saturated FAs occupy the *sn*-1 and *sn*-3 positions, while unsaturated ones are generally present in the *sn*-2 position (*2*−*4*). Given the TAGs specificity, its profile is increasingly used in the food industry as a tool to assess quality and authenticity of vegetable oils (*3*−*6*). Several authors have reported that TAGs, together with other parameters, can be helpful in the assessment of the adulteration of olive oil with hazelnut oil (*7*−*9*).

The FA composition of vegetable oils, and thus the composition of TAGs, can also present a slight, but natural, variability when the same species is considered. It has been described that factors such as cultivar, growing conditions, climate, soil type, and plant maturity can affect the composition of vegetable oils (*10*). Although there are already reports on hazelnut TAG compositions (*9*, *11*−*17*), the work herein aimed to study the influence of several factors on the TAG compositions of hazelnuts.

In this work, samples from 19 different cultivars, with several origins, growing in two different localities (Vila Real and Felgueiras, Portugal), collected among three consecutive crop years, in a total of 79 different samples, were analyzed by high-performance liquid chromatography/evaporative light-scattering

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detection (HPLC/ELSD). Statistical analysis was carried out in order to evaluate the differences related to slight climate changes along the 3 years, to different origins of the cultivars, and growing locality.

MATERIALS AND METHODS

Samples. A total of 19 hazelnut (*Corylus avellana* L.) cultivars (Butler, Campanica, Cosford, Couplat, Daviana, Ennis, Fertille de Coutard, Grossal, Gunslebert, Lansing, Longa d'Espanha, Merveille de Bollwiller, Morell, Negreta, Pauetet, Round du Piemont, Santa Maria de Jesus, Segorbe, and Tonda de Giffoni) were studied. Samples of all cultivars (with the exception of cv. Daviana that was not obtained in 2003, due to its low productivity in that year) were collected in an experimental orchard at Vila Real, in the north inland region of Portugal (district of Vila Real, 41° 19′N, 7° 44′ W, 470 m asl) during three consecutive years (2001, 2002, and 2003). In 2002 and 2003, samples from another geographical location (Felgueiras, district of Oporto, 41° 22′N, 8° 11′ O, 50 km from the Atlantic Ocean, 320 m asl) were also included in this study: In 2002, 10 cultivars were studied (Butler, Campanica, Cosford, Couplat, Ennis, Fertille de Coutard, Merveille de Bollwiller, Morell, Pauetet, and Tonda di Giffoni), and in 2003, three more were added (cvs. Longa d'Espanha, Negreta, and Segorbe). Care was taken in choosing cultivars common to those from Vila Real in order to evaluate the influence of the geographical origin.

After they were harvested in September, as they fell into the ground, hazelnuts were sun-dried and a final sample of about 2 kg was randomly taken. The nuts were stored in shell, closed in plastic bags, flushed with nitrogen, and frozen to −20 °C until the analyses were performed.

Sample Preparation. Hazelnuts were manually cracked and shelled and then chopped in a 643 MX coffee mill (Moulinex, Spain). Crude oil was obtained from finely chopped nuts extracted with light petroleum ether (bp 40−60 °C) in an Universal Extraction System B-811 (Büchi, Switzerland); the residual solvent was removed by a stream of 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. TAGs 1,2,3-tripalmitoylglycerol (PPP), 1,2,3-tristearoylglycerol (SSS), 1,2,3-trilinolenoylglycerol (LnLnLn), and 1,2,3-tripalmitoleoylglycerol (PoPoPo) of purity greater than 98%, and 1,2,3-trioleoylglycerol (OOO), 1,2,3-trilinoleoylglycerol (LLL), 1,2-dilinoleoyl-3-palmitoyl-*rac*-glycerol (PLL), 1,2-dilinoleoyl-3-oleoyl-*rac*-glycerol (OLL), 1,2-dipalmitoyl-3-oleoyl-*rac*-glycerol (PPO), 1,2-dioleoyl-3-stearoyl-*rac*-glycerol (SOO), 1-palmitoyl-2-oleoyl-3-linoleoylglycerol (POL), and 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (POO) with a purity of approximately 99% were purchased from Sigma (St. Louis, MO). Acetonitrile and acetone were of HPLC grade and obtained from Merck (Darmstadt, Germany).

TAG Analysis. The chromatographic analyses were performed with a Jasco (Tokyo, Japan) high-performance liquid chromatograph, equipped with PU-1580 quaternary pump and a Jasco AS-950 automatic sampler with a 10 μL loop. Detection was performed with a model 75 ELSD (Sedere, Alfortville, France). The chromatographic separation of the compounds was achieved with a Kromasil 100 C_{18} (5 μm; 250 mm × 4.6 mm) column (Teknokroma, Barcelona, Spain) operating at ambient temperature (∼20 °C). The mobile phase used was a mixture of acetone/acetonitrile (70:30, v/v) (A) and acetone/acetonitrile (80:20, v/v) (B). Elution was performed at a solvent flow rate of 1 mL/min with a two step gradient, starting with 0% B, changing to 100% B at 35 min, keeping these conditions during 15 min, and then returning to the initial conditions. The ELSD was programmed with the following settings: evaporator temperature, 40 °C; air pressure, 3.5 bar; and photomultiplier sensitivity, 5. Data were analyzed using the Borwin-PDA Controller Software (JMBS, France). Peaks were identified taking into account relative retention times to OOO. Peak identification was also supported by literature data on hazelnut oil, based on matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (*11*) and atmospheric pressure chemical ionization (APCI) mass spectrometry (*9*, *11*). Quantification of the peaks was made by internal normalization, assuming that the detector response was similar for all compounds (*18*).

Statistical Analysis. Multivariate analyses of data involved a forward stepwise discriminant analysis to select the most discriminant TAGs and a canonical variate analysis (CVA) based on a subset of the most discriminant TAGs, to further analyze the differences between groups and display those differences in convenient canonical variate plots. 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 literature (*19*−*21*).

RESULTS AND DISCUSSION

Various chromatographic techniques have been used for TAG analyses, mainly including capillary GLC and normal or reversed phase HPLC. This last technique has the advantage of analyzing TAGs at ambient or slightly higher temperatures, thereby avoiding thermal stress on the thermolabile polyunsaturated TAGs (*4*). RP-HPLC using nonaqueous solvent mixtures has been successfully used in TAG analyses (*22*−*24*). As the objective of this work was to analyze a high number of samples, a simple and fast method that could be applied to routine analyses was needed. Sample preparation involved only the dilution of the sample in acetone, homogenization, and filtration. On the basis of literature data, a mixture of acetone/acetonitrile was chosen as the mobile phase (*22*, *23*); a two step gradient was established in order to simultaneously allow a good peak resolution and an easier elution of saturated and higher molecular mass TAGs from the column, thus decreasing analysis time. The detection was performed by ELSD since this detector presented some advantages over other systems, such as UV, RI, and MS detectors (*23*−*26*). The whole analysis, from weighing the sample to obtaining the results, takes about 1 h, making this method appropriate for routine analyses.

Eleven compounds were determined in hazelnuts: LLL, OLL, PLL, OOL, POL, PPL, OOO, POO, PPO, SOO, and PSO. In all samples, the main component was OOO (ranging from 53.6 to 73.5%, with 65.3% as a mean value), followed by OOL (ranging from 9.4 to 25.1%, with 15.4% as a mean value) and POO (ranging from 7.4 to 15.9%, with 11.4% as mean value), reflecting the high contents of oleic acid of hazelnut oils. There were five TAGs (LLL, PLL, PPL, PPO, and PSO) whose contents were always less than 1%. Although some differences exist, all samples presented an identical qualitative and quantitative profile, defining a chemical fingerprint that may be suitable for assessing identity and quality of hazelnut oils (**Figure 1**). The average values of TAG content for every cultivar, year of production, and locality are shown in **Table 1**. **Figure 2** shows a chromatogram obtained with the experimental conditions described.

Considering the FA composition previously obtained for those same samples regarding the first year crop (*27*), a higher content of TAGs containing stearic acid moieties and linoleic acid moieties was expected. Ayorinde et al. (*11*) have analyzed hazelnut oil by MALDI/TOF mass spectrometry and pointed out that minor quantities of SLL and SOL could be coeluted with OOL and with OOO, respectively. Likewise, Holcapek et al. (*12*) also referred that minor amounts of SLO could exist in hazelnut oil. Because in RP-HPLC TAGs are separated by chain length and the degree of unsaturation of the FAs, TAGs with the same partition number (PN = CN − 2DB, were CN is the total number of carbons and DB the total number of double bonds) (*22*, *23*) are difficult to resolve and can coelute. In the analyzed samples, minor amounts of SLL and SOL can exist but are undetectable by the used methodology, which can explain the small differences regarding the previously reported FA composition data. Nevertheless, when the same samples

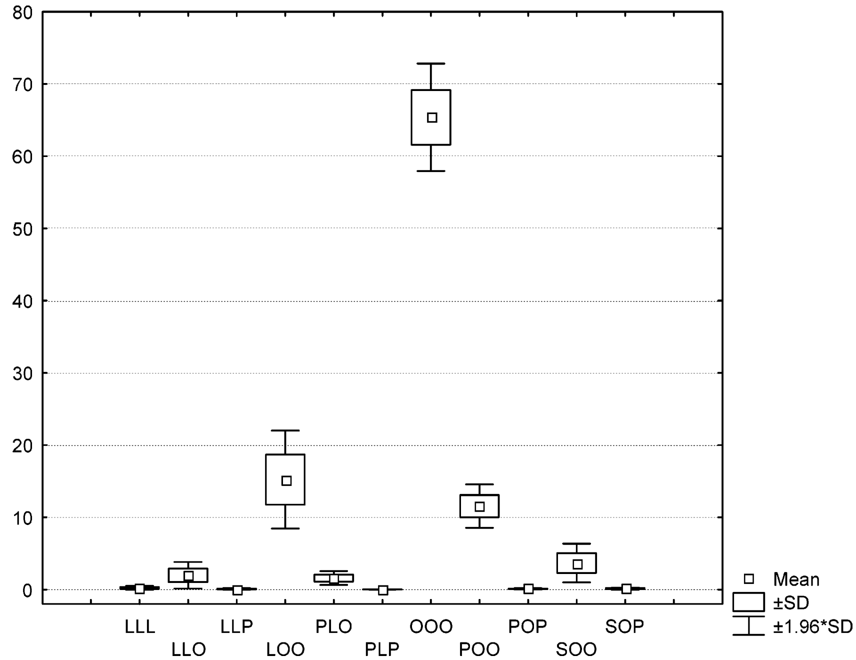

Figure 1. Box and whisker plot for all TAGs analyzed in hazelnuts (P, palmitoyl; S, stearoyl; O, oleoyl; and L, linoleoyl).

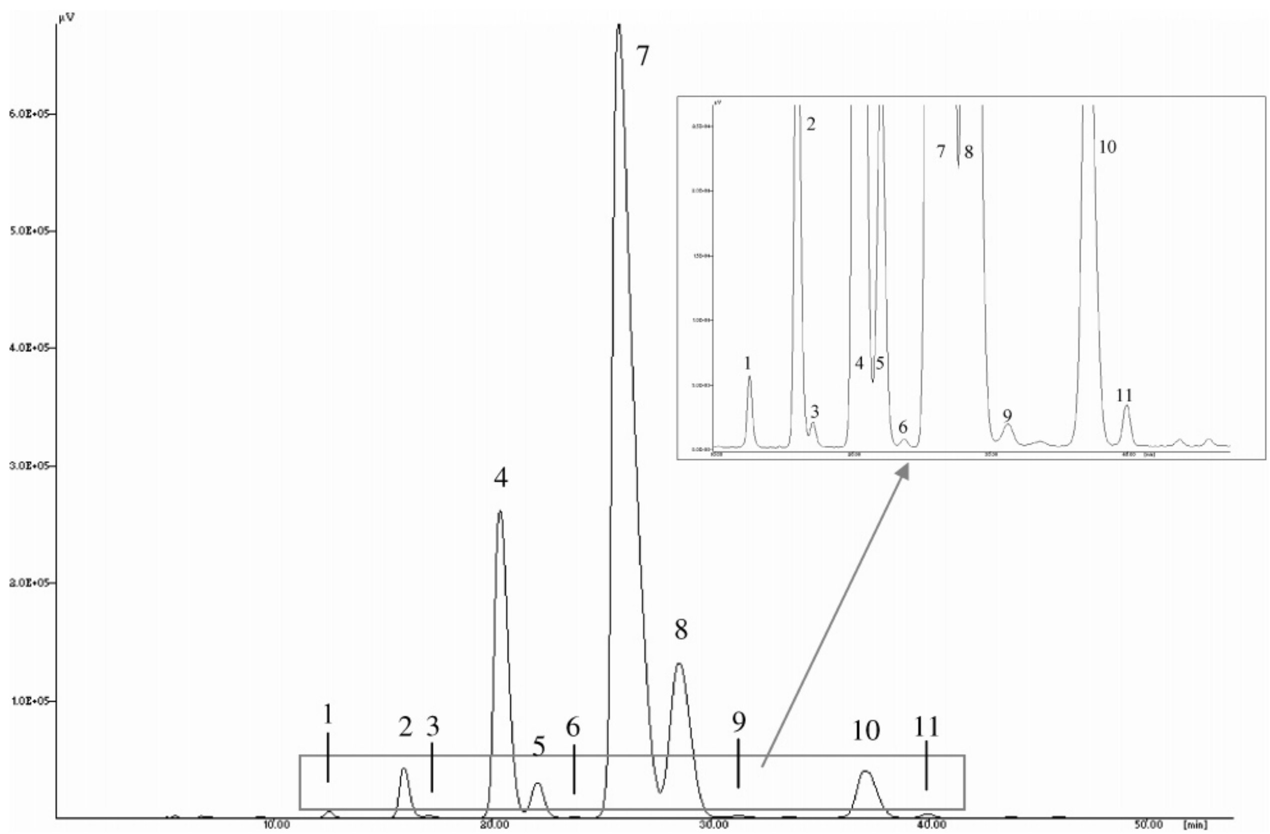

Figure 2. Hazelnut TAG profile obtained by HPLC/ELSD. Peaks: 1, LLL; 2, OLL; 3, PLL; 4, OOL; 5, POL; 6, PPL; 7, OOO; 8, POO; 9, PPO; 10, SOO; and 11, PSO (P, palmitoyl; S, stearoyl; O, oleoyl; and L, linoleoyl).

from 2001 year crop are compared, there is a good agreement between the FA (*27*) and the TAGs composition, herein reported.

Other works reporting TAGs composition (%) of hazelnut oil are available. Parcerisa and co-workers, using a RI detector (*13*, *14*) and a MS detector (*9*), have reported higher values for LLL (0.5−3.6%), LLO (1.8−10.8%), PLO (2.7−6.5%), and POP (0.7−2.4%), and much lower values for OOO (32.2−57.0%) than the ones herein reported. Nevertheless, our data are in fairly good agreement with the results reported by the same authors when studying TAGs composition during hazelnut development using an ELSD detector (*15*) and with the data reported by Bernardo-Gil et al. (*16*).

Parcerisa et al. have already studied the influence of the cultivar (study with 10 cultivars) (*13*), the year crop, and localities (four cultivars, during 3 years, in two localities) (*14*) on TAGs composition of hazelnut oil. The authors found significant differences in the majority of TAGs related to year crop and localities and significant differences in a lower number

Table 1. TAG Contents of the Studied Cultivars by Year of Production and Locality[a]

Year Crop 2001

cultivar	LLL	OLL	PLL	OOL	POL	PPL	OOO	POO	PPO	SOO	PSO
	TAGs (relative %)										
Locality: Vila Real											
Butler	0.24 ± 0.00	2.03 ± 0.02	0.09 ± 0.00	14.92 ± 0.15	1.67 ± 0.02	0.03 ± 0.00	63.99 ± 0.06	12.05 ± 0.04	0.11 ± 0.01	4.76 ± 0.07	0.11 ± 0.00
Camponica	0.22 ± 0.03	1.15 ± 0.10	0.08 ± 0.01	11.24 ± 0.32	1.17 ± 0.14	0.03 ± 0.00	71.37 ± 1.26 1	0.93 ± 0.35	0.06 ± 0.01	3.67 ± 0.29	0.09 ± 0.00
Cosford	0.16 ± 0.02	1.44 ± 0.10	0.06 ± 0.01	13.78 ± 0.09	1.41 ± 0.09	0.03 ± 0.00	67.41 ± 0.70	11.94 ± 0.24	0.13 ± 0.00	3.54 ± 0.20	0.11 ± 0.01
Couplat	0.12 ± 0.00	1.11 ± 0.04	0.05 ± 0.00	11.46 ± 0.14	1.22 ± 0.01	0.02 ± 0.00	67.99 ± 0.10	12.76 ± 0.11	0.14 ± 0.00	4.98 ± 0.08	0.14 ± 0.00
Daviana	0.19 ± 0.00	1.49 ± 0.05	0.05 ± 0.00	14.60 ± 0.08	1.16 ± 0.01	0.02 ± 0.00	69.09 ± 0.05	10.45 ± 0.08	0.06 ± 0.00	2.77 ± 0.01	0.09 ± 0.01
Ennis	0.30 ± 0.02	2.38 ± 0.21	0.14 ± 0.02	16.60 ± 0.38	2.08 ± 0.09	0.04 ± 0.00	63.27 ± 0.50	12.70 ± 0.17	0.15 ± 0.00	2.28 ± 0.07	0.06 ± 0.00
F. de Coutard	0.15 ± 0.00	2.15 ± 0.06	0.06 ± 0.00	18.85 ± 0.20	1.43 ± 0.02	0.01 ± 0.00	64.78 ± 0.05	8.51 ± 0.11	0.03 ± 0.00	3.90 ± 0.14	0.12 ± 0.00
Grossal	0.11 ± 0.00	0.98 ± 0.03	0.04 ± 0.00	11.63 ± 0.22	1.18 ± 0.04	0.02 ± 0.00	70.13 ± 0.05	12.48 ± 0.06	0.12 ± 0.00	3.25 ± 0.15	0.07 ± 0.00
Gunslebert	0.70 ± 0.00	3.31 ± 0.00	0.24 ± 0.01	17.15 ± 0.08	2.37 ± 0.03	0.04 ± 0.00	57.56 ± 0.23	12.89 ± 0.06	0.20 ± 0.00	5.13 ± 0.01	0.30 ± 0.03
Lansing	0.12 ± 0.01	1.06 ± 0.01	0.04 ± 0.00	12.28 ± 0.29	1.15 ± 0.05	0.01 ± 0.00	65.66 ± 0.29	12.30 ± 0.11	0.09 ± 0.00	6.91 ± 0.11	0.36 ± 0.01
L. Espanha	0.19 ± 0.01	1.68 ± 0.04	0.05 ± 0.00	14.32 ± 0.41	0.97 ± 0.02	0.01 ± 0.00	70.15 ± 0.36	8.36 ± 0.22	0.03 ± 0.00	4.15 ± 0.11	0.10 ± 0.00
M. Bollwiller	0.28 ± 0.00	2.68 ± 0.02	0.08 ± 0.00	17.20 ± 0.15	1.51 ± 0.02	0.02 ± 0.00	66.23 ± 0.16	9.66 ± 0.08	0.06 ± 0.01	2.23 ± 0.04	0.03 ± 0.00
Morell	0.24 ± 0.00	1.92 ± 0.03	0.08 ± 0.00	14.98 ± 0.23	1.54 ± 0.02	0.05 ± 0.00	65.80 ± 0.69	10.75 ± 0.10	0.13 ± 0.00	4.01 ± 0.01	0.11 ± 0.01
Negreta	0.31 ± 0.01	2.00 ± 0.03	0.08 ± 0.01	15.42 ± 0.12	1.39 ± 0.01	0.03 ± 0.00	66.85 ± 0.32	10.80 ± 0.12	0.09 ± 0.01	2.98 ± 0.05	0.07 ± 0.01
Pauetet	0.11 ± 0.01	1.45 ± 0.06	0.06 ± 0.00	14.28 ± 0.05	1.69 ± 0.02	0.03 ± 0.00	66.27 ± 0.22	13.08 ± 0.04	0.13 ± 0.01	2.83 ± 0.09	0.07 ± 0.00
R. Piemont	0.17 ± 0.00	1.72 ± 0.02	0.09 ± 0.01	14.98 ± 0.10	1.82 ± 0.04	0.03 ± 0.00	63.68 ± 0.26	13.02 ± 0.26	0.16 ± 0.00	3.80 ± 0.04	0.31 ± 0.01
Segorbe	0.14 ± 0.01	1.49 ± 0.04	0.05 ± 0.00	13.85 ± 0.08	1.31 ± 0.03	0.02 ± 0.00	68.84 ± 0.10	10.95 ± 0.11	0.08 ± 0.00	3.20 ± 0.05	0.06 ± 0.00
St. Maria Jesus	0.10 ± 0.00	0.97 ± 0.09	0.04 ± 0.00	11.58 ± 0.19	1.06 ± 0.11	0.02 ± 0.00	70.54 ± 1.15	11.75 ± 0.43	0.10 ± 0.01	3.77 ± 0.28	0.08 ± 0.00
T. de Giffoni	0.26 ± 0.00	2.40 ± 0.17	0.09 ± 0.00	16.63 ± 0.02	1.54 ± 0.05	0.02 ± 0.00	65.96 ± 0.41	10.12 ± 0.20	0.06 ± 0.01	2.94 ± 0.38	0.07 ± 0.01
mean	0.22	1.76	0.08	14.51	1.46	0.03	66.61	11.34	0.10	3.74	0.12
range	0.10–0.70	0.97–3.31	0.04–0.24	11.24–18.85	0.97–2.37	0.01–0.05	8.36–13.08	57.56–71.37	0.03–0.20	2.23–6.91	0.03–0.36

Year Crop 2002

cultivar	LLL	OLL	PLL	OOL	POL	PPL	OOO	POO	PPO	SOO	PSO
	TAGs (relative %)										
Locality: Vila Real											
Butler	0.12 ± 0.01	1.39 ± 0.04	0.05 ± 0.00	13.42 ± 0.08	1.32 ± 0.03	0.02 ± 0.00	67.93 ± 0.32	12.30 ± 0.18	0.10 ± 0.01	3.27 ± 0.05	0.08 ± 0.01
Camponica	0.07 ± 0.01	0.85 ± 0.06	0.04 ± 0.00	11.08 ± 0.24	0.96 ± 0.06	0.02 ± 0.00	73.49 ± 0.54	10.67 ± 0.15	0.07 ± 0.01	2.72 ± 0.07	0.04 ± 0.00
Cosford	0.28 ± 0.03	2.90 ± 0.19	0.12 ± 0.01	20.34 ± 0.20	1.98 ± 0.14	0.03 ± 0.00	62.31 ± 0.59	9.79 ± 0.23	0.05 ± 0.00	2.15 ± 0.20	0.05 ± 0.00
Couplat	0.13 ± 0.00	1.49 ± 0.02	0.08 ± 0.00	14.14 ± 0.14	1.76 ± 0.02	0.03 ± 0.00	64.92 ± 0.34	14.05 ± 0.22	0.17 ± 0.00	3.13 ± 0.05	0.10 ± 0.00
Daviana	0.25 ± 0.01	2.09 ± 0.03	0.09 ± 0.00	15.77 ± 0.17	1.64 ± 0.03	0.03 ± 0.00	64.68 ± 0.12	11.50 ± 0.09	0.12 ± 0.01	3.77 ± 0.06	0.10 ± 0.01
Ennis	0.40 ± 0.03	3.25 ± 0.13	0.19 ± 0.01	19.39 ± 0.22	2.77 ± 0.07	0.06 ± 0.00	58.29 ± 0.47	13.02 ± 0.08	0.19 ± 0.01	2.35 ± 0.03	0.08 ± 0.00
F. de Coutard	0.19 ± 0.01	2.25 ± 0.18	0.10 ± 0.01	16.90 ± 1.00	2.08 ± 0.11	0.04 ± 0.00	63.67 ± 1.28	11.67 ± 0.09	0.12 ± 0.00	2.89 ± 0.22	0.09 ± 0.00
Grossal	0.10 ± 0.00	1.23 ± 0.03	0.05 ± 0.00	13.49 ± 0.11	1.37 ± 0.02	0.02 ± 0.00	69.08 ± 0.41	12.26 ± 0.33	0.11 ± 0.00	2.25 ± 0.05	0.04 ± 0.00
Gunslebert	0.20 ± 0.00	1.84 ± 0.00	0.08 ± 0.00	15.76 ± 0.08	1.74 ± 0.00	0.03 ± 0.00	64.15 ± 0.10	12.27 ± 0.16	0.14 ± 0.00	3.61 ± 0.03	0.17 ± 0.00
Lansing	0.18 ± 0.02	2.21 ± 0.15	0.12 ± 0.01	16.74 ± 0.38	2.34 ± 0.09	0.04 ± 0.00	59.86 ± 0.51	13.66 ± 0.65	0.20 ± 0.02	4.34 ± 0.42	0.26 ± 0.02
L. Espanha	0.23 ± 0.03	2.71 ± 0.15	0.10 ± 0.01	19.56 ± 0.18	1.98 ± 0.12	0.03 ± 0.00	63.20 ± 0.55	10.15 ± 0.27	0.07 ± 0.01	1.90 ± 0.14	0.06 ± 0.01
M. Bollwiller	0.35 ± 0.02	3.24 ± 0.10	0.14 ± 0.01	20.06 ± 0.09	2.27 ± 0.06	0.04 ± 0.00	60.88 ± 0.35	10.54 ± 0.06	0.09 ± 0.01	2.28 ± 0.02	0.12 ± 0.00
Morell	0.18 ± 0.00	2.14 ± 0.02	0.09 ± 0.00	16.78 ± 0.01	2.05 ± 0.03	0.04 ± 0.00	63.02 ± 0.16	12.10 ± 0.12	0.14 ± 0.00	3.35 ± 0.03	0.12 ± 0.01
Negreta	0.43 ± 0.03	3.40 ± 0.32	0.20 ± 0.02	20.08 ± 0.38	2.48 ± 0.28	0.02 ± 0.00	59.81 ± 1.36	10.99 ± 0.18	0.10 ± 0.01	2.20 ± 0.07	0.19 ± 0.01
Pauetet	0.09 ± 0.00	1.47 ± 0.01	0.06 ± 0.00	14.98 ± 0.17	1.68 ± 0.03	0.02 ± 0.00	66.07 ± 0.01	13.00 ± 0.13	0.12 ± 0.01	2.36 ± 0.10	0.15 ± 0.02
R. Piemont	0.08 ± 0.00	0.81 ± 0.02	0.04 ± 0.00	10.85 ± 0.20	1.22 ± 0.06	0.02 ± 0.00	69.63 ± 0.38	14.13 ± 0.03	0.17 ± 0.01	2.97 ± 0.11	0.08 ± 0.01
Segorbe	0.25 ± 0.01	2.12 ± 0.05	0.12 ± 0.00	16.32 ± 0.09	1.89 ± 0.00	0.03 ± 0.00	64.14 ± 0.31	11.71 ± 0.20	0.13 ± 0.00	3.21 ± 0.06	0.09 ± 0.00
St. Maria Jesus	0.16 ± 0.01	1.49 ± 0.07	0.07 ± 0.00	13.62 ± 0.11	1.47 ± 0.06	0.03 ± 0.00	67.51 ± 0.72	12.36 ± 0.29	0.12 ± 0.01	3.10 ± 0.16	0.06 ± 0.01
T. de Giffoni	0.17 ± 0.00	1.60 ± 0.05	0.07 ± 0.00	13.36 ± 0.25	1.44 ± 0.05	0.03 ± 0.00	67.89 ± 0.36	11.69 ± 0.08	0.12 ± 0.00	3.56 ± 0.07	0.09 ± 0.00
mean	0.20	2.02	0.09	15.93	1.81	0.03	64.76	11.99	0.12	2.92	0.10
range	0.07–0.43	0.81–3.40	0.04–0.20	18.85–20.34	0.96–2.77	0.02–0.06	58.29–73.49	9,79–14.13	0.05–0.20	1.90–4.34	0.04–0.26
Locality: Felgueiras											
Butler	0.28 ± 0.01	2.18 ± 0.07	0.10 ± 0.00	15.33 ± 0.22	1.59 ± 0.03	0.02 ± 0.00	62.41 ± 0.20	11.53 ± 0.14	0.08 ± 0.01	6.17 ± 0.12	0.31 ± 0.01
Camponica	0.26 ± 0.01	1.75 ± 0.01	0.07 ± 0.00	18.91 ± 0.08	1.05 ± 0.02	0.01 ± 0.00	66.82 ± 0.12	9.32 ± 0.11	0.05 ± 0.00	2.15 ± 0.04	0.06 ± 0.00
Cosford	0.17 ± 0.00	2.00 ± 0.01	0.07 ± 0.01	16.83 ± 0.24	1.76 ± 0.02	0.03 ± 0.00	62.78 ± 0.24	11.43 ± 0.06	0.10 ± 0.01	4.66 ± 0.08	0.18 ± 0.00
Couplat	0.15 ± 0.00	1.56 ± 0.03	0.06 ± 0.00	13.52 ± 0.14	1.37 ± 0.01	0.03 ± 0.00	67.69 ± 0.23	11.65 ± 0.03	0.10 ± 0.00	3.77 ± 0.10	0.10 ± 0.00
Ennis	0.22 ± 0.01	1.63 ± 0.02	0.05 ± 0.00	16.20 ± 0.10	1.33 ± 0.02	0.03 ± 0.00	63.25 ± 0.71	12.81 ± 0.11	0.09 ± 0.00	4.23 ± 0.02	0.22 ± 0.00
F. de Coutard	0.72 ± 0.01	2.05 ± 0.03	0.26 ± 0.00	14.44 ± 0.05	1.99 ± 0.01	0.04 ± 0.00	64.48 ± 0.38	12.41 ± 0.16	0.15 ± 0.01	3.17 ± 0.06	0.14 ± 0.00
M. Bollwiller	0.37 ± 0.01	3.21 ± 0.04	0.13 ± 0.00	20.69 ± 0.31	2.00 ± 0.02	0.03 ± 0.00	62.77 ± 0.28	9.10 ± 0.06	0.06 ± 0.00	1.60 ± 0.14	0.04 ± 0.00
Morell	0.81 ± 0.01	5.83 ± 0.05	0.35 ± 0.01	25.09 ± 0.19	3.27 ± 0.03	0.05 ± 0.00	53.57 ± 0.12	9.58 ± 0.01	0.10 ± 0.00	1.30 ± 0.01	0.06 ± 0.00
Pauetet	0.27 ± 0.01	2.09 ± 0.02	0.11 ± 0.01	15.73 ± 0.26	1.74 ± 0.04	0.03 ± 0.00	65.51 ± 0.25	11.44 ± 0.04	0.10 ± 0.01	2.89 ± 0.17	0.10 ± 0.00
T. de Giffoni	0.19 ± 0.01	2.10 ± 0.03	0.07 ± 0.00	15.83 ± 0.28	1.51 ± 0.02	0.03 ± 0.00	66.64 ± 0.40	10.53 ± 0.06	0.07 ± 0.00	2.95 ± 0.06	0.07 ± 0.01
mean	0.34	2.44	0.13	17.26	1.76	0.03	63.59	10.98	0.09	3.29	0.13
range	0.15–0.81	1.56–5.83	0.05–0.35	13.52–25.09	1.05–3.27	0.01–0.05	53.57–67.6	9 9.10–12.81	0.05–0.15	1.30–6.17	0.04–0.31

Table 1 (Continued)

Year Crop 2003

cultivar	TAGs (relative %) LLL	OLL	PLL	OOL	POL	PPL	OOO	POO	PPO	SOO	PSO
					Locality: Vila Real						
Butler	0.15 ± 0.00	1.31 ± 0.01	0.05 ± 0.00	11.02 ± 0.13	1.02 ± 0.01	0.01 ± 0.00	67.62 ± 0.31	12.30 ± 0.13	0.11 ± 0.01	6.21 ± 0.06	0.21 ± 0.00
Camponica	0.28 ± 0.03	3.81 ± 0.20	0.11 ± 0.01	24.40 ± 0.02	1.80 ± 0.16	0.01 ± 0.00	61.18 ± 0.61	7.44 ± 0.31	0.02 ± 0.00	0.80 ± 0.10	0.02 ± 0.00
Cosford	0.19 ± 0.01	1.44 ± 0.01	0.07 ± 0.00	12.72 ± 0.05	1.31 ± 0.02	0.02 ± 0.00	67.49 ± 0.16	12.94 ± 0.08	0.12 ± 0.01	3.59 ± 0.08	0.10 ± 0.00
Couplat	0.18 ± 0.00	1.04 ± 0.02	0.06 ± 0.00	9.84 ± 0.10	1.03 ± 0.03	0.02 ± 0.00	68.83 ± 0.38	13.75 ± 0.22	0.18 ± 0.00	4.92 ± 0.05	0.15 ± 0.01
Ennis	0.31 ± 0.01	2.10 ± 0.05	0.12 ± 0.01	14.17 ± 0.24	1.72 ± 0.06	0.03 ± 0.00	63.54 ± 0.69	13.99 ± 0.17	0.20 ± 0.01	3.66 ± 0.13	0.14 ± 0.00
F. de Coutard	0.17 ± 0.01	1.95 ± 0.15	0.17 ± 0.01	20.04 ± 0.19	1.85 ± 0.07	0.04 ± 0.00	60.74 ± 0.72	11.54 ± 0.07	0.15 ± 0.02	3.27 ± 0.02	0.14 ± 0.00
Grossal	0.07 ± 0.00	0.83 ± 0.01	0.03 ± 0.00	10.84 ± 0.16	1.11 ± 0.04	0.02 ± 0.00	69.26 ± 0.11	13.00 ± 0.23	0.12 ± 0.00	4.58 ± 0.38	0.15 ± 0.01
Gunslebert	0.57 ± 0.02	4.83 ± 0.03	0.24 ± 0.01	23.61 ± 0.16	2.76 ± 0.02	0.02 ± 0.00	55.89 ± 0.17	10.27 ± 0.09	0.08 ± 0.00	1.65 ± 0.06	0.07 ± 0.00
Lansing	0.19 ± 0.00	1.51 ± 0.02	0.10 ± 0.00	13.67 ± 0.12	1.90 ± 0.03	0.02 ± 0.00	62.84 ± 0.21	15.89 ± 0.12	0.24 ± 0.00	3.50 ± 0.02	0.14 ± 0.00
L. Espanha	0.17 ± 0.00	1.31 ± 0.01	0.07 ± 0.00	11.80 ± 0.08	1.24 ± 0.02	0.02 ± 0.00	68.68 ± 0.04	11.93 ± 0.08	0.13 ± 0.01	4.52 ± 0.03	0.13 ± 0.01
M. Bollwiller	0.43 ± 0.01	4.38 ± 0.01	0.15 ± 0.01	22.18 ± 0.28	2.09 ± 0.01	0.02 ± 0.00	58.03 ± 0.07	9.37 ± 0.07	0.07 ± 0.01	3.17 ± 0.31	0.11 ± 0.00
Morell	0.12 ± 0.00	1.38 ± 0.11	0.05 ± 0.00	12.77 ± 0.21	1.30 ± 0.10	0.02 ± 0.00	68.16 ± 0.93	12.71 ± 0.34	0.15 ± 0.01	3.21 ± 0.42	0.10 ± 0.01
Negreta	0.30 ± 0.01	3.00 ± 0.11	0.13 ± 0.01	18.98 ± 0.48	2.21 ± 0.04	0.03 ± 0.00	61.45 ± 0.75	11.21 ± 0.18	0.11 ± 0.01	2.53 ± 0.02	0.06 ± 0.00
Pauetet	0.16 ± 0.01	1.51 ± 0.04	0.06 ± 0.00	13.67 ± 0.22	1.37 ± 0.02	0.02 ± 0.00	67.98 ± 0.31	11.57 ± 0.09	0.11 ± 0.00	3.48 ± 0.05	0.07 ± 0.00
R. Piemont	0.10 ± 0.00	1.12 ± 0.02	0.04 ± 0.00	11.53 ± 0.08	1.24 ± 0.02	0.02 ± 0.00	67.71 ± 0.02	13.41 ± 0.20	0.15 ± 0.01	4.53 ± 0.19	0.17 ± 0.01
Segorbe	0.15 ± 0.00	1.39 ± 0.02	0.07 ± 0.00	12.59 ± 0.17	1.36 ± 0.02	0.02 ± 0.00	68.40 ± 0.31	11.97 ± 0.08	0.12 ± 0.00	3.84 ± 0.04	0.09 ± 0.01
St. Maria Jesus	0.17 ± 0.00	1.35 ± 0.02	0.06 ± 0.00	11.60 ± 0.13	1.12 ± 0.03	0.02 ± 0.00	68.43 ± 0.23	11.29 ± 0.15	0.10 ± 0.00	5.69 ± 0.04	0.15 ± 0.00
T. de Giffoni	0.05 ± 0.01	0.85 ± 0.02	0.03 ± 0.00	11.18 ± 0.36	0.99 ± 0.05	0.01 ± 0.00	69.97 ± 0.69	11.78 ± 0.42	0.08 ± 0.01	4.14 ± 0.28	0.32 ± 0.01
mean	0.21	1.95	0.09	14.81	1.52	0.02	65.34	12.02	0.12	3.74	0.13
range	0.05–0.57	0.83–4.83	0.03–0.24	9.84–24.40	0.99–2.76	0.01–0.04	55.89–69.97	7.44–15.89	0.02–0.24	0.80–6.21	0.02–0.32
					Locality: Felgueiras						
Butler	0.12 ± 0.01	1.09 ± 0.02	0.03 ± 0.00	10.22 ± 0.04	0.83 ± 0.01	0.01 ± 0.00	69.52 ± 0.22	11.04 ± 0.15	0.07 ± 0.00	6.82 ± 0.06	0.26 ± 0.00
Camponica	0.20 ± 0.00	1.70 ± 0.01	0.07 ± 0.00	13.80 ± 0.08	1.44 ± 0.03	0.03 ± 0.00	66.04 ± 0.16	11.43 ± 0.02	0.09 ± 0.00	5.02 ± 0.09	0.17 ± 0.01
Cosford	0.26 ± 0.01	2.10 ± 0.09	0.08 ± 0.01	12.92 ± 0.16	1.27 ± 0.03	0.02 ± 0.00	63.84 ± 0.37	12.54 ± 0.13	0.13 ± 0.00	6.56 ± 0.45	0.29 ± 0.02
Couplat	0.08 ± 0.00	0.67 ± 0.01	0.02 ± 0.00	9.43 ± 0.07	0.61 ± 0.00	0.01 ± 0.00	70.08 ± 0.07	10.18 ± 0.05	0.05 ± 0.00	8.56 ± 0.06	0.31 ± 0.01
Ennis	0.17 ± 0.00	1.27 ± 0.01	0.03 ± 0.00	10.93 ± 0.25	0.89 ± 0.09	0.01 ± 0.00	69.04 ± 0.44	12.16 ± 0.20	0.08 ± 0.01	5.22 ± 0.06	0.20 ± 0.00
F. de Coutard	0.34 ± 0.03	2.31 ± 0.07	0.11 ± 0.01	15.53 ± 0.14	1.49 ± 0.03	0.03 ± 0.00	66.91 ± 0.49	10.24 ± 0.16	0.08 ± 0.00	2.89 ± 0.21	0.08 ± 0.01
L. Espanha	0.25 ± 0.01	2.45 ± 0.03	0.09 ± 0.00	17.73 ± 0.05	1.65 ± 0.01	0.03 ± 0.00	65.34 ± 0.03	9.72 ± 0.04	0.07 ± 0.00	2.59 ± 0.05	0.08 ± 0.00
M. Bollwiller	0.31 ± 0.01	3.27 ± 0.01	0.07 ± 0.00	21.12 ± 0.30	1.52 ± 0.03	0.01 ± 0.00	61.35 ± 0.24	7.85 ± 0.11	0.02 ± 0.00	4.36 ± 0.11	0.13 ± 0.01
Morell	0.18 ± 0.02	2.05 ± 0.08	0.09 ± 0.01	15.84 ± 0.06	1.93 ± 0.08	0.04 ± 0.01	63.66 ± 0.62	12.42 ± 0.24	0.16 ± 0.02	3.49 ± 0.17	0.14 ± 0.02
Negreta	0.17 ± 0.00	1.65 ± 0.04	0.06 ± 0.00	14.14 ± 0.23	1.18 ± 0.03	0.02 ± 0.00	66.77 ± 0.60	10.37 ± 0.20	0.07 ± 0.01	5.84 ± 0.29	0.27 ± 0.01
Pauetet	0.13 ± 0.00	1.93 ± 0.05	0.09 ± 0.01	16.73 ± 0.22	2.09 ± 0.05	0.03 ± 0.00	62.20 ± 0.76	12.87 ± 0.13	0.14 ± 0.01	3.51 ± 0.30	0.27 ± 0.01
Segorbe	0.16 ± 0.01	1.38 ± 0.06	0.05 ± 0.00	12.56 ± 0.44	1.06 ± 0.04	0.02 ± 0.00	68.31 ± 0.15	10.80 ± 0.68	0.07 ± 0.01	5.43 ± 0.08	0.16 ± 0.00
T. de Giffoni	0.13 ± 0.01	1.61 ± 0.05	0.04 ± 0.00	15.02 ± 0.22	1.13 ± 0.03	0.01 ± 0.00	68.82 ± 0.24	9.08 ± 0.08	0.03 ± 0.00	4.02 ± 0.09	0.10 ± 0.00
mean	0.19	1.81	0.06	14.31	1.31	0.02	66.30	10.82	0.08	4.95	0.19
range	0.08–0.34	0.67–3.27	0.02–0.11	9.43–21.12	0.61–2.09	0.01–0.04	61.35–70.08	7.85–12.87	0.02–0.16	2.59–8.56	0.08–0.31 51

[a] Mean ± SD of three determinations. P, palmitoyl; S, stearoyl; O, oleoyl; and L, linoleoyl.

of TAGs among several of the studied cultivars. In both studies (*13*, *14*), the statistical approach to evaluate the significance of statistical differences involved the performance of two-way analyses of variance. Recent works published in the area of statistics have shown that these approaches can lead to overoptimistic results because they do not take into consideration the undesirable effects of collinearity (*19*−*21*). In the work herein reported, to check possible differences in what concerns TAGs' profiles, multivariate analyses of data were performed, aiming to obtain a global picture of major differences between cultivars and to analyze the influence of production year. A forward stepwise discriminant analysis was applied to data from samples grouped by year of production, allowing the selection of seven TAGs as the most discriminant ones. This step ensures that only TAGs with relevant information will be taken into consideration for the evaluation of the significance of observed differences. A CVA was performed based on the selected TAGs, and the differences between groups were displayed in canonical variate plots. **Figure 3** shows the results of an exploratory canonical analysis carried out with all available data, expressed as a plot of variate 1 vs 2. The first dimension represents 69.5% of the information in the data, separating year 2003 from the other two years of production and reflecting the fact that in 2003 hazelnuts had higher contents of SOO and less contents of POL. The second canonical dimension describes the differences between 2001 and 2002 year crops, since in 2002 hazelnuts generally displayed higher levels of OOL, PLL, and POO. The pointed differences can possibly be related to the climatic differences among the years. In Portugal, the year of 2003 was characterized by a very hot summer, the second hottest since 1931 (*28*). According to the definition of heat wave of the World Meteorological Organization (WCDMP No. 47, WMO-TD No. 1071), Portugal was under a heat wave from July 29th until August 15th (*28*). In Vila Real, the month of August was very hot, with minimum and maximum temperatures higher than the ones in 2001 and 2002; as compared with 2001 and 2002, the air relative humidity was also higher in July of 2003. Although climatic differences between 2001 and 2002 exist, they were not so clear. The main differences were related to the air relative humidity (which was higher in August 2002), to the maximum temperatures (lower in May 2002), and to the minimum temperatures (lower from January to June and August) (data

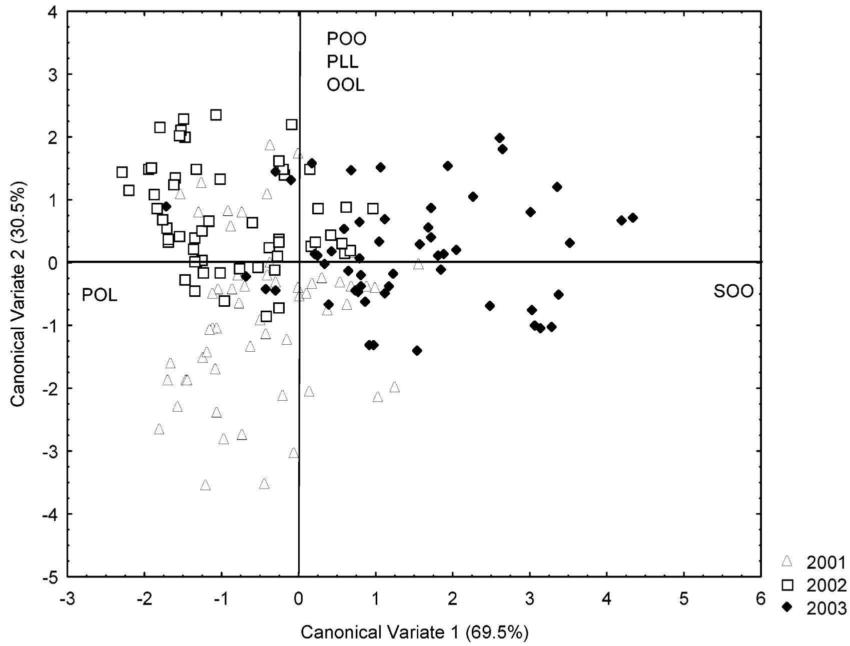

Figure 3. Results from CVA, with the year of production as the grouping factor, of samples from Vila Real locality. Plot of canonical variates 1 vs 2. Triglycerides labeling canonical axes are important for their interpretation. Percentage values refer to the amount of information explained by each canonical dimension.

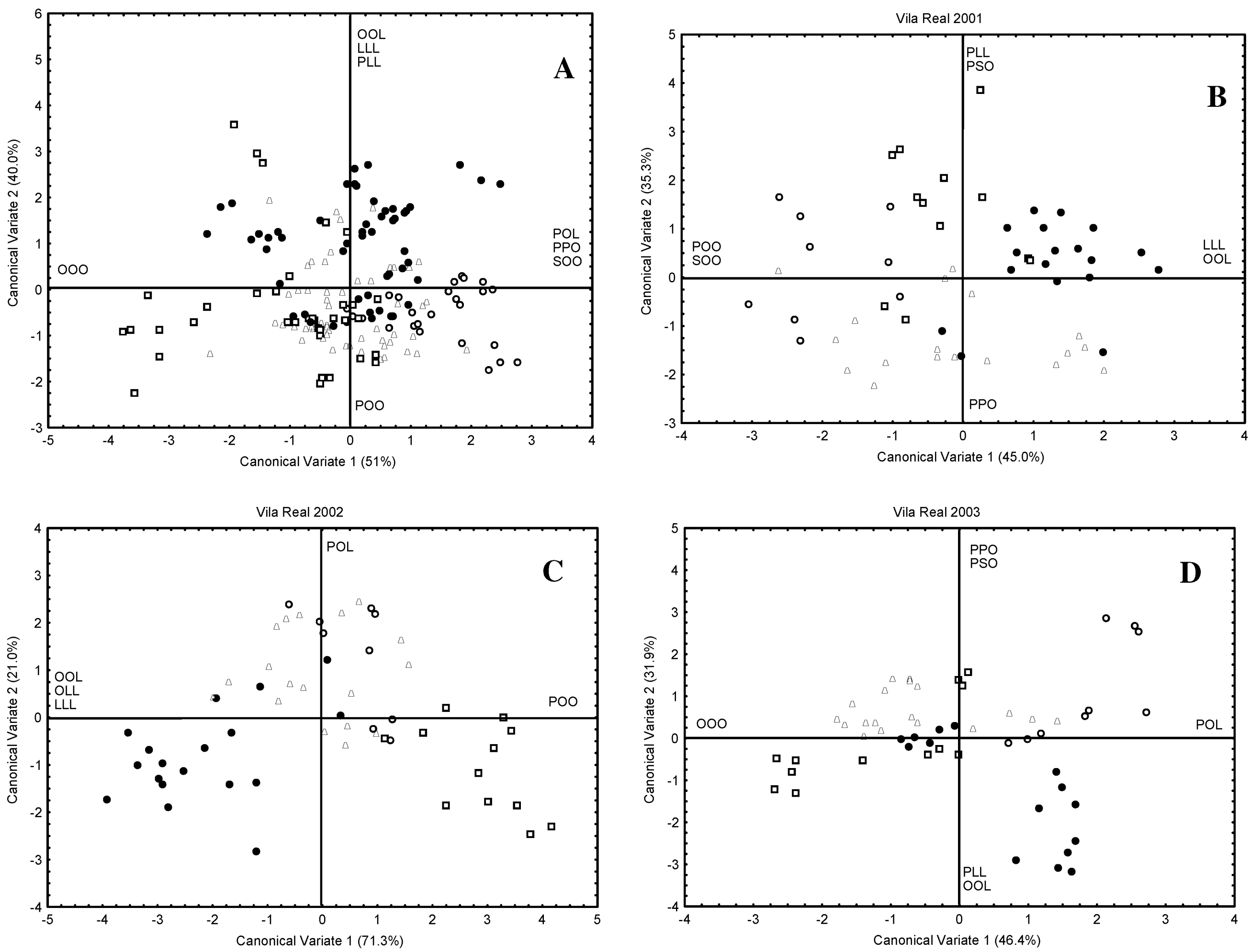

Figure 4. Results from CVA with cultivars' origins as the grouping factor, performed with data from Vila Real locality. Plots of canonical variates 1 vs 2. (**A**) Considering data of 3 year crops; (**B**) considering only data of the 2001 year crop; (**C**) considering only data of the 2002 year crop; and (**D**) considering only data of the 2003 year crop. Percentage values refer to the amount of information explained by each canonical dimension. TAGs labeling canonical axes are important for their interpretation. USA, ○; Italy, □; Spain, △; and others, ●.

not shown). These results suggest that TAG composition can be influenced by climatic conditions because the differences observed between years of production were based on the same cultivars (grown in the same experimental field and under the same agricultural practices).

In a previous work, reporting the FA composition of several hazelnut cultivars grown in the same orchard in Oregon, Parcerisa et al. (*17*) referred that hazelnut cultivars were grouped according to their origin when performing a discriminant analysis using FA content as variables. In this work, a similar approach was performed using TAGs as variables. Four groups were made according to the origin of hazelnut's cultivars (*29*, *30*): United States (Butler, Ennis, and Lansing), Italy (Campanica, Round de Piemont, Santa Maria de Jesus, and Tonda de Giffoni), Spain (Couplat, Grossal, Morell, Negreta, Pauetet, and Segorbe), and "others" (comprising the French cultivar Fertille de Coutard; the British cultivars Cosford, Daviana, and Longue d'Espanha; and the German cultivars Gunslebert and Merveille de Bollwiller). These cultivars were gathered in only one group (others) having in mind the geographical proximity and the climatic similarities of countries of origin. A CVA preceded by a forward stepwise analysis was performed using the results obtained in the 3 year crop study from Vila Real locality, and the differences between groups were displayed in a plot condensing 91% of all information (**Figure 4A**). Although some differences regarding the origin of the cultivars are evident in the plot, a detailed analysis of the differences between cultivars is difficult because the variations due to year crop within cultivars enlarge the within-groups variability and consequently shadow the between-groups differences. Therefore, a CVA, based on TAGs selected by forward stepwise discriminant analysis, was performed for each year crop (**Figure 4B–D**), therefore separating the year effect from the within-cultivars variations. In general, although the Italian cultivars presented a higher dispersion of results and the Spanish cultivars were sometimes close to the other groups, more evident differences were seen among the groups. In the 2001 year crop, the "others" group presented higher amounts of LLL and OOL and lower amounts of POO and SOO, while the opposite was observed for the U.S. group. In the 2002 year crop, the "others" group was higher in OOL, OLL, and LLL and lower in POO and POL; the Italian group presented higher contents of POO, and in general, the United States and Spanish cultivars were higher in POL. In the 2003 year crop, the "others" group generally presented more PLL and OOL; the U.S. group presented higher amounts of POL, and some of the cultivars also presented higher amounts of PPO and PSO; the Spanish group, lying in the plot's central part, was seen to display intermediate characteristics; the Italian cultivars were more disperse, i.e., less homogeneous, some presenting higher amounts of OOO, while some were higher in PPO and PSO. In general, it seems that the "others" group, concerning cultivars of French, English, and German origins, was higher in TAGs that contain linoleic acid. These cultivars, although showing some differences among each other, seemed to be similar in global terms. It also seems that, in general, the U.S. cultivars presented higher amounts of TAGs with a saturated FA moiety. Identical results were attained when the same statistical approach was applied to the results obtained with the samples of the 2002 and 2003 year crops from Felgueiras locality (data not shown).

Hazelnuts can be consumed raw, and thus are considered table hazelnuts, or can be used after industrial processing. The studied Italian and Spanish cultivars are generally used after processing while the remaining cultivars are generally consumed raw (*30*). Industrial processing generally includes a roasting step, which improves palatability and extends the range of aromas and taste. Consequently, a possible taste coming from rancidity phenomena can be masked. By the contrary, table hazelnuts are intended to be consumed raw, and any rancid taste is more easily detected. Having in mind that FA composition as well as the FA distribution on TAGs can affect their lipolitic and oxidative stability (*1*), it seems that the U.S. origin cultivars, generally richer in TAGs with saturated FA moieties and presenting less TAGs with the polyunsaturated linoleic acid, could be less prone to rancidity and, consequently, more suited to be consumed as table hazelnuts, which is in good agreement with their general use. By the contrary, the TAGs composition of the cultivars considered in the "others" group, which can also be consumed as table hazelnuts, seems to point to a higher susceptibility to rancidity. The data herein reported seem to indicate that some caution should be taken when these cultivars are intended to be consumed raw.

In conclusion, the results herein reported suggest that TAG compositions can be strongly influenced by genetic factors; besides, it seems that it can also be influenced by environmental factors. Also, data seem to indicate that considering the different cultivar's origins, U.S. cultivars are probably less prone to rancidity, and therefore more suited to be consumed raw, since in general these cultivars presented higher levels of TAGs with saturated FAs and lower levels of TAGs with linolenic acid in their moieties.

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