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Nutritional, Fatty Acid and Triacylglycerol Profiles of *Castanea sativa* Mill. Cultivars: A Compositional and Chemometric Approach

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Four Portuguese chestnut cultivars from the "Castanha da Terra Fria" protected designation of origin were selected: Aveleira, Boa Ventura, Judia and Longal. The nutritional parameters (moisture, fat, protein, carbohydrates, ash and energy) as well as fibers (neutral detergent fiber, acid detergent fiber, acid detergent lignin and cellulose) were characterized. Moisture was the major component followed by carbohydrates, protein and fat, resulting in an energetic value lower than 195 kcal/100 g of fresh fruit. In order to find significant differences among cultivars, the lipidic fraction was studied in detail. Fatty acids (FA) were determined by gas−liquid chromatography with flame ionization detection, revealing a clear prevalence of C18:1 and C18:2, two FA very well-known due to their beneficial effects on human health, e.g., in the prevention of cardiovascular diseases. A triacylglycerols (TAG) profile was obtained by reversed-phase high-performance liquid chromatography with evaporative light-scattering detection. TAG analysis is very important because it furnishes highly specific information due to genetic control of the stereospecific distribution of FA on the glycerol molecule. OLL, PLL, OOL and POL were the major compounds. As far as we know this is the first complete characterization of TAG in chestnut. The obtained data were screened through an analysis of variance (to evaluate the accuracy of the method as well as the uniformity of results for each cultivar) and a discriminant analysis (DA), which gave good results, once that, in some cases, the four cultivars were clustered in four individual groups, obtained through the definition of two DA dimensions.

KEYWORDS: Chestnut; nutritional composition; fatty acids; triacylglycerols; discriminant analysis

1. INTRODUCTION

Castanea sativa Miller belongs to the *Fagaceae* family, which includes several ecologically and economically important species (*1*). Chestnuts are found in three major geographical areas: Asia (with predominance of *C. crenata*, *C. molissima*, *C. seguinii*, *C. davidii* and *C. henryi*), North America (where *C. dentata*, *C. pumila*, *C. floridana*, *C. ashei*, *C. alnifolia* and *C. paucispina*, thrive) and Europe where *C. sativa* is predominant (*2*). Among the 12 world chestnuts species, *C. sativa* is the most consumed. This species is predominant in Portugal, where it has a relevant place at the socioeconomic level, reaching an annual fruit production of 20 000 tons (*3*). The main production area is located in the Trás-os-Montes region (Northeast), with importance at the economic level, contributing with 84% of the total of Portuguese production. In order to preserve chestnut biological material and improve its cultivation, three protected designations of origin (PDO) called "Castanha da Terra Fria", "Castanha dos Soutos da Lapa" and "Castanha da Padrela" were defined (*4*). The PDO "Castanha da Terra Fria" was created in 1994, in the normative decree 44/94 from February 3rd (*5*), where it is defined as the fruit obtained from *C. sativa*, including the varieties Longal, Judia, Amarelal, Lamela, Aveleira, Boa Ventura, Trigueira, Martainha and Negral. The best development conditions are found at altitudes higher than 500 m and winter low temperatures, as in the Trás-os-Montes region in which 12 500 ha are used for chestnut cultivation, especially Longal and Judia cultivars, representing one of the few regions with a largely positive trade balance (*6*, *7*).

From a nutritional point of view, chestnut has interesting characteristics. Chestnuts are among the main sources of starch (up to 70%), presenting minerals and vitamins and appreciable levels of fiber, but low amounts of protein (2−4%) and, unlike typical nuts, fat (2−5%) (*8*). Nevertheless, this fruit is an interesting

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source of essential fatty acids (FA), mainly linoleic acid, which play an important role in preventing cardiovascular diseases in adults and promoting the development of the brain and retina of infants (*7*, *9*−*11*). Consumers have been revealing an increased interest in chestnut fruits because of their nutritional qualities and potential beneficial health effects (*7*, *9*). The substitution of phytotherapeutics by natural products with bioactive compounds in many industrial formulations can provide very consistent advantages, first of all at the biocompatibility level and also for preservation of the environment (*12*). Hence, research focusing on the nutritional quality and corresponding health benefits of chestnut should be more detailed in order to increase our knowledge and enhance chestnut commercial value.

The lipidic fraction of a natural product has a characteristic and almost unique pattern of triacylglygerols (TAG). The different TAGs are 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 species. Although with some exceptions, in vegetable oils, saturated FA occupy the *sn*-1 and *sn*-3 positions, while unsaturated ones are generally present in the *sn*-2 position (*13*, *14*). Despite the utility and importance of FA analysis in the characterization of a determined lipidic fraction, the TAG profile should always be warily studied. The advantage of TAG analysis compared to FA 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 (*15*, *16*). However, the information concerning TAG in chestnut lipidic fraction is rather scarce, and, as far as we know, this is the first study applied to *C. sativa*.

Although some studies (*4*, *6*, *16*, *17*) have already been conducted on single chestnut components, we thought that its chemical and nutritional compositions remain an interesting field of study, especially when considered from a global point of view. We consider that the exhaustive characterization of the more productive cultivars may reveal some distinctive features that could be important for the improvement of their industrial development, contributing for new industrial products or applications. Nutritional and chemical characterizations can also be used by producers and breeders, once it provides a useful reference about the quality of each cultivar and might stimulate new chestnut orchard cultivation.

The characterization of chestnut cultivars is important in different fields from the nutritional point of view, technological processes and applications. FA and TAGs were used as discriminator factors of different kinds of fat, and could be used as authenticity parameters. In order to analyze the variability among different cultivars, two different statistical analyses were conducted. First, the results were submitted to an analysis of variance to evaluate the accuracy of the applied methods and the consistency of the obtained results. Afterward, the results were evaluated through discriminant analysis considering different sets of the assayed parameters, in order to find which one discriminates better chestnut cultivars.

2. MATERIALS AND METHODS

Standards and Reagents. All reagents were of analytical grade purity: methanol and diethyl ether were supplied by Laboratory-Scan (Lisbon, Portugal); toluene from Riedel-de-Haen (Seelze, Germany); sulfuric acid from Fluka (Madrid, Spain). The fatty acids methyl ester (FAME) reference standard mixture (37 fatty acids from C4 to C24) was from Supelco (Bellefonte, PA) as also other individual fatty acid isomers.

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; O, oleic; P, palmitic; S, stearic. Acetonitrile and acetone were of HPLC grade and obtained from Merck (Darmstadt, Germany). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, Brea, CA). All other reagents were of analytical grade.

Samples and Sample Preparation. A sample of fruits was haphazardly collected from each one of the five trees per cultivar. The assays were carried out in duplicate, obtaining ten results per cultivar. The results are expressed as mean values ± standard deviation (SD). Fruits were obtained in orchards located in the geographical region of Vinhais (Trás-os-Montes), in the Northeast of Portugal: Aveleira, 41°49′N, 7°01′O; Boa Ventura, 41°51′N, 7°01′O; Judia, 41°50′N, 7°01′O; Longal, 41°50′N, 7°00′O), from cultivars belonging to the PDO "Castanha da Terra Fria". Longal was used during method development and validation procedures. Five trees were selected in each orchard, according with the tree phenological cycle (Aveleira has the earliest production cycle, and the remaining cultivars have similar production cycles) during the crop year of 2006.

Chestnut fruits were kept at −20 °C and protected from light until further use. Immediately before the extraction procedure, each sample was manually peeled off (inner and outer skins).

Extraction Procedure. After being manually peeled off, chestnuts were chopped in a 643 MX mill (Moulinex, Barcelona, Spain). Crude lipidic fraction was obtained from finely chopped chestnuts (≈50 g with anhydrous sodium sulfate) extracted with light petroleum ether (bp 40−60 °C) during 1.5 h (for the determination of total fat content the extraction time was 24 h) in a Universal extraction system B-811 (Büchi, Flawil, Switzerland); the residual solvent was removed by flushing with nitrogen.

Proximate Analysis. Chestnut samples were analyzed for chemical composition (moisture, protein, fat, ash, fiber) using the AOAC procedures (*19*). The crude protein content of the samples was estimated by the macroKjeldahl method (*20*); the crude fat was determined by extracting a known weight of powdered chestnut sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 550 ± 15 °C until whitish ash appear. Neutral detergent fiber (NDF), including cellulose, hemicelluloses and lignin,; acid detergent fiber (ADF), including cellulose and lignin less digestible and woody fibers; and acid detergent lignin (ADL) were determined by the Robertson and Van Soest method (1981) (*21*) with minor changes. Total carbohydrates were calculated by difference: Total carbohydrates = 100 − (g of moisture + g of protein + g of fat + g of ash + g of fiber). Total energy was calculated according to the following equation: Energy (kcal) = 4 × (g of protein + g of carbohydrate) + 9 × (g of lipid) (*22*).

Fatty Acid Composition. Fatty acid methyl esters (FAMEs) were prepared by hydrolysis with a 2 M methanolic potassium hydroxide solution, and extraction with *n*-heptane, in accordance with ISO 5509 method (*23*). The fatty acid profile was analyzed with a Chrompack CP 9001 chromatograph (Chrompack, Middelburg, The Netherlands) equipped with a split-splitless injector, a flame ionization detection (FID), and a Chrompack CP-9050 autosampler. The temperatures of the injector and detector were 230 and 270 °C, respectively. Separation was achieved on a 50 m × 0.25 mm i.d. fused silica capillary column coated with a 0.19 μm film of CP-Sil 88 (Chrompack). Helium was used as carrier gas at an internal pressure of 120 kPa. The column temperature was 140 °C, for a 5 min hold, and then programmed to increase to 220 °C at a rate of 5 °C/min and then held for 10 min. The split ratio was 1:50, and the injected volume was 1.2 μL. The results are expressed in relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards.

Table 1. Proximate Composition (g/100 g Fresh Weight) of the Assayed Chestnut Cultivars[a] and Correspondent Energy

	Aveleira	Boa Ventura	Judia	Longal
moisture	52.1 ± 1.3 b[b]	54.6 ± 1.0 a	53.3 ± 1.8 ab	51.9 ± 1.0 b
crude fat	0.84 ± 0.06 a	0.78 ± 0.07 a	0.81 ± 0.08 a	0.79 ± 0.07 a
crude protein	3.13 ± 0.48 a	2.29 ± 0.19 b	2.96 ± 0.25 a	2.47 ± 0.19 b
carbohydrates	43.2 ± 1.0 ab	41.6 ± 0.84c	42.1 ± 1.5 bc	44.1 ± 0.89 a
total ash	0.73 ± 0.03 c	0.68 ± 0.03 c	0.90 ± 0.04 a	0.79 ± 0.06 b
energy (kcal)	192.7 ± 5.4 a	182.6 ± 4.5 b	187.4 ± 7.4 ab	193.2 ± 4.4 a
fibers				
NDF	1.8 ± 0.21 a	1.5 ± 0.12 b	1.6 ± 0.10 b	1.3 ± 0.11 c
ADF	0.28 ± 0.03 a	0.25 ± 0.02 ab	0.26 ± 0.02 a	0.23 ± 0.02 b
ADL	0.01 ± 0.00 a	0.01 ± 0.01 a	0.01 ± 0.00 a	0.01 ± 0.00 a
cellulose	0.27 ± 0.02 a	0.24 ± 0.02 ab	0.26 ± 0.02 a	0.22 ± 0.02 b

[a] Results are presented as mean ± standard deviation. [b] Different letters mean significant differences ($p < 0.05$).

Table 2. Fatty Acid Composition (%) of the Four Selected Cultivars[a]

	Aveleira	Boa Ventura	Judia	Longal
C14:0	0.14 ± 0.03 ab[b]	0.11 ± 0.01 b	0.13 ± 0.02 b	0.16 ± 0.04 a
C15:0	0.09 ± 0.02 b	0.10 ± 0.04 ab	0.10 ± 0.03 ab	0.13 ± 0.03 a
C16:0	17.3 ± 0.59 a	14.6 ± 0.27 bc	14.2 ± 0.36 c	14.8 ± 0.28 b
C16:1	0.34 ± 0.09 a	0.32 ± 0.06 a	0.30 ± 0.06 a	0.28 ± 0.09 a
C17:0	0.13 ± 0.05 b	0.21 ± 0.13 ab	0.25 ± 0.16 ab	0.33 ± 0.17 a
C17:1	0.16 ± 0.02 a	0.12 ± 0.02 a	0.17 ± 0.08 a	0.14 ± 0.06 a
C18:0	0.92 ± 0.05 a	0.94 ± 0.03 a	0.86 ± 0.07 a	0.95 ± 0.26 a
C18:1	37.4 ± 0.80 a	35.7 ± 2.3 a	32.2 ± 2.4 b	29.6 ± 0.83 c
C18:2	37.9 ± 0.90 c	40.3 ± 1.6 b	44.3 ± 1.5 a	45.5 ± 0.70 a
C18:3	4.0 ± 0.22 b	6.2 ± 0.49 a	6.0 ± 0.48 a	6.4 ± 0.46 a
C20:0	0.40 ± 0.04 a	0.34 ± 0.03 b	0.31 ± 0.01 b	0.39 ± 0.04 a
C20:1	0.74 ± 0.10 b	0.73 ± 0.03 b	0.81 ± 0.08 ab	0.83 ± 0.05 a
C22:0	0.30 ± 0.01 a	0.27 ± 0.08 ab	0.23 ± 0.02 b	0.33 ± 0.04 a
C24:0	0.11 ± 0.03 a	0.07 ± 0.02 a	0.12 ± 0.08 a	0.12 ± 0.02 a
SFA	19.4 ± 0.56 a	16.7 ± 0.27 c	16.2 ± 0.43 c	17.2 ± 0.42 b
MUFA	38.7 ± 0.78 a	36.8 ± 2.3 a	33.5 ± 2.3 b	30.9 ± 0.85 c
PUFA	42.0 ± 0.93 c	46.5 ± 2.1 b	50.3 ± 2.0 a	51.9 ± 1.0 a

[a] Results are presented as mean ± standard deviation. [b] Different letters mean significant differences ($p < 0.05$).

Table 3. Triacylglycerol Composition (%) of the Four Selected Cultivars[a]

	Aveleira	Boa Ventura	Judia	Longal
LLnLn	0.20 ± 0.16 a[b]	0.26 ± 0.14 a	0.26 ± 0.09 a	0.23 ± 0.06 a
LLLn	0.69 ± 0.16 c	2.0 ± 0.46 b	2.9 ± 0.79 ab	3.7 ± 1.6 a
LLL	3.8 ± 0.79 c	5.4 ± 0.90 b	9.5 ± 2.1 a	9.8 ± 0.89 a
OLLn	1.6 ± 0.10 b	3.8 ± 0.37 a	3.4 ± 0.46 a	3.9 ± 1.0 a
PLLn	1.3 ± 0.26 b	2.3 ± 0.47 a	2.0 ± 0.41 a	2.7 ± 0.92 a
OLL	18.9 ± 2.4 b	23.5 ± 1.1 a	24.2 ± 3.5 a	24.5 ± 2.7 a
LLP	15.0 ± 1.9 bc	14.4 ± 1.1 c	16.1 ± 2.9 ab	17.4 ± 0.72 a
OLO	17.5 ± 1.4 a	18.4 ± 1.2 a	14.1 ± 2.1 b	13.2 ± 2.6 b
LOP	20.5 ± 0.64 a	13.3 ± 1.9 b	11.5 ± 0.89 b	12.1 ± 1.4 b
PLP	1.8 ± 0.36 a	0.85 ± 0.15 b	0.79 ± 0.11 b	1.20 ± 0.45 b
OOO	10.9 ± 2.0 a	10.5 ± 1.6 a	6.9 ± 2.1 b	4.54 ± 0.85 c
OOP	8.1 ± 1.2 a	5.1 ± 1.0 b	3.4 ± 0.71 c	3.0 ± 0.48 c
POP	0.36 ± 0.21 a	0.08 ± 0.02 b	0.08 ± 0.04 b	0.11 ± 0.08 b

[a] Results are presented as mean ± standard deviation. [b] Different letters mean significant differences ($p < 0.05$).

Triacylglycerol Analysis. The chromatographic analyses were performed with a Jasco (Tokyo, Japan) high-pressure 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 evaporative light-scattering detector (ELSD) (model 75-Sedere, Alfortville, France). The chromatographic separation of the compounds was achieved with a Kromasil 100 C_{18} (5 μm; 250 × 4.6 mm) column (Teknokroma, Barcelona, 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, Carnoux en Provence, France). Taking into account the selectivities (*R*, relative retention times to LLL), peaks were identified according to the logarithms of *R* in relation to homogeneous TAG (Sigma). Quantification of the peaks was made by internal normalization, assuming that the detector response was the same for all compounds.

Statistical Analysis. The influence of the cultivars over nutritional and chemical composition was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with $\alpha = 0.05$, coupled with Welch's statistic.

Discriminant analysis (DA) was done to determine which variables discriminate between the four naturally occurring groups. In stepwise DA, the model of discrimination is built step by step. At each step, all variables are reconsidered to find which one will better contribute to the discrimination between groups. That variable will then be included in the model, restarting the process. The values of *F* to enter and *F* to remove are the guidelines of the stepwise procedure. The *F* value for a variable indicates its statistical significance in the discrimination between groups. Discriminant analysis defines a combination of varieties

Table 4. The Most Important Parameters Defined for Discrimination between Cultivar Groups Considering Nutritional Parameters, Triacylglycerols or Fatty Acids

	Wilks' lambda	*F*-remove	*p*-level	tolerance	1 − tolerance (R^2)
		Nutritional Parameters			
total ashes	0.0577	37.13	<0.001	0.7755	0.2245
crude protein	0.0228	8.204	<0.001	0.6537	0.3463
carbohydrates	0.0394	21.99	<0.001	0.4951	0.5049
NDF	0.0321	15.87	<0.001	0.4207	0.5793
ADL	0.0186	4.708	<0.001	0.5047	0.4953
		Triacylglycerols			
LLL	0.0087	6.763	<0.001	0.3576	0.6424
OLLn	0.0112	11.69	<0.001	0.1558	0.8442
LLLn	0.0076	4.576	<0.001	0.0629	0.9371
LLnLn	0.0095	8.294	<0.001	0.4665	0.5335
PLP	0.0089	7.209	<0.001	0.1686	0.8314
OOO	0.0081	5.581	<0.001	0.5211	0.4789
		Fatty Acids			
16:0	0.0060	67.31	<0.001	0.4551	0.5449
18:2	0.0087	102.2	<0.001	0.2604	0.7396
18:3	0.0093	111.0	<0.001	0.1601	0.8399
20:0	0.0017	11.50	<0.001	0.8723	0.1277
18:0	0.0012	4.687	<0.001	0.7083	0.2917

Table 5. The Nine Most Important Parameters Defined for Discrimination between Cultivar Groups Considering All the Parameters Together

	Wilks' lambda	*F*-remove	*p*-level	tolerance	1 − tolerance (R^2)
ash	0.0008	9.399	<0.001	0.7337	0.2663
MUFA	0.0005	2.871	<0.001	0.3381	0.6619
carbohydrates	0.0012	18.37	<0.001	0.0220	0.9780
energy	0.0010	12.45	<0.001	0.0240	0.9760
LLnLn	0.0010	13.50	<0.001	0.3338	0.6662
LLL	0.0013	19.30	<0.001	0.2141	0.7859
LLP	0.0006	3.394	<0.001	0.3044	0.6956
PLP	0.0010	12.21	<0.001	0.5859	0.4141
OLLn	0.0016	26.05	<0.001	0.3784	0.6216

in a way that the first function furnishes the most general discrimination between groups, the second provides the second most, and so on (*24*).

These treatments were carried out using the SPSS v. 16.0 program.

3. RESULTS AND DISCUSSION

Proximate Analysis. Table 1 shows the results obtained for proximate composition. Moisture was the major component, revealing some statistical differences among cultivars. Carbohydrates are the major nutrient, due to the high starch content, and Longal showed the highest carbohydrates levels. The higher carbohydrate content with the lower moisture values, in Longal, may be related with its sweeter taste, as it is often described by consumers. Crude fat lay below 1% for all cultivars. It should be pointed out that these values are expressed as g/g fresh weight and not as g/g dry matter as in other works. Regarding protein content, the determined amounts lay around 3%, with Aveleira and Judia showing higher values than Boa Ventura and Longal. Ash content is very low and ranged between 0.68% and 0.90%. The obtained results are in agreement with previous works (*4*, *17*, *18*, *25*, *26*).

Dietary fiber was revealed to be very similar among all the cultivars, with the exception of Aveleira, which showed a significantly higher value. The cultivars demonstrated significant differences regarding their NDF contents, while ADF and ADL were very similar. Aveleira and Judia showed higher cellulose values. NDF, or cell wall, consists of hemicellulose, cellulose and lignin; ADF consists mainly of cellulose and lignin; ADL is measured by further treating ADF with strong acid, which dissolves cellulose, or with permanganate, which oxidizes (removes) the lignin. Either approach allows calculation of the amount of lignin. These relatively high fiber amounts can also explain the beneficial effects of chestnut consumption on human health, once fibers had already been reported as an important cardiovascular disease risk reduction factor, having also a preventive role in certain cancers and the ability of lowering plasma cholesterol (*27*).

Fatty Acids. The results for fatty acid composition are presented in **Table 2**. These results enlighten that chestnut lipidic fraction is mainly constituted by three fatty acids: linoleic, oleic and palmitic acids accounting for more than 88% of the total FA content, a value slightly higher when compared with the results obtained by other research groups (*7*). Besides these three main fatty acids, 11 more were identified and quantified.

With respect to the differences observed among cultivars, Aveleira has significantly higher amounts of SFA. It is known that SFA provide stability, but these are not beneficial to the cardiovascular system (*28*). Palmitic acid is clearly the most important SFA with values ranging from 14.3% and 17.3%.

Considering total MUFA content, the studied cultivars showed significant differences varying from 30.9% to 38.7%. The total PUFA content varied significantly within cultivars. Aveleira has the lowest PUFA content while Longal contained the highest, being also the cultivar with the highest value of linoleic and linolenic (C18:3) acids. It is well accepted that PUFA play an important role in preventing cardiovascular diseases, and consumers are very interested and sensitive to this kind of compound (*29*, *30*), but PUFA decrease the shelf life of the product.

Triacylglycerols. For a complete characterization of the chestnut lipidic fraction, TAGs were also analyzed. As far as

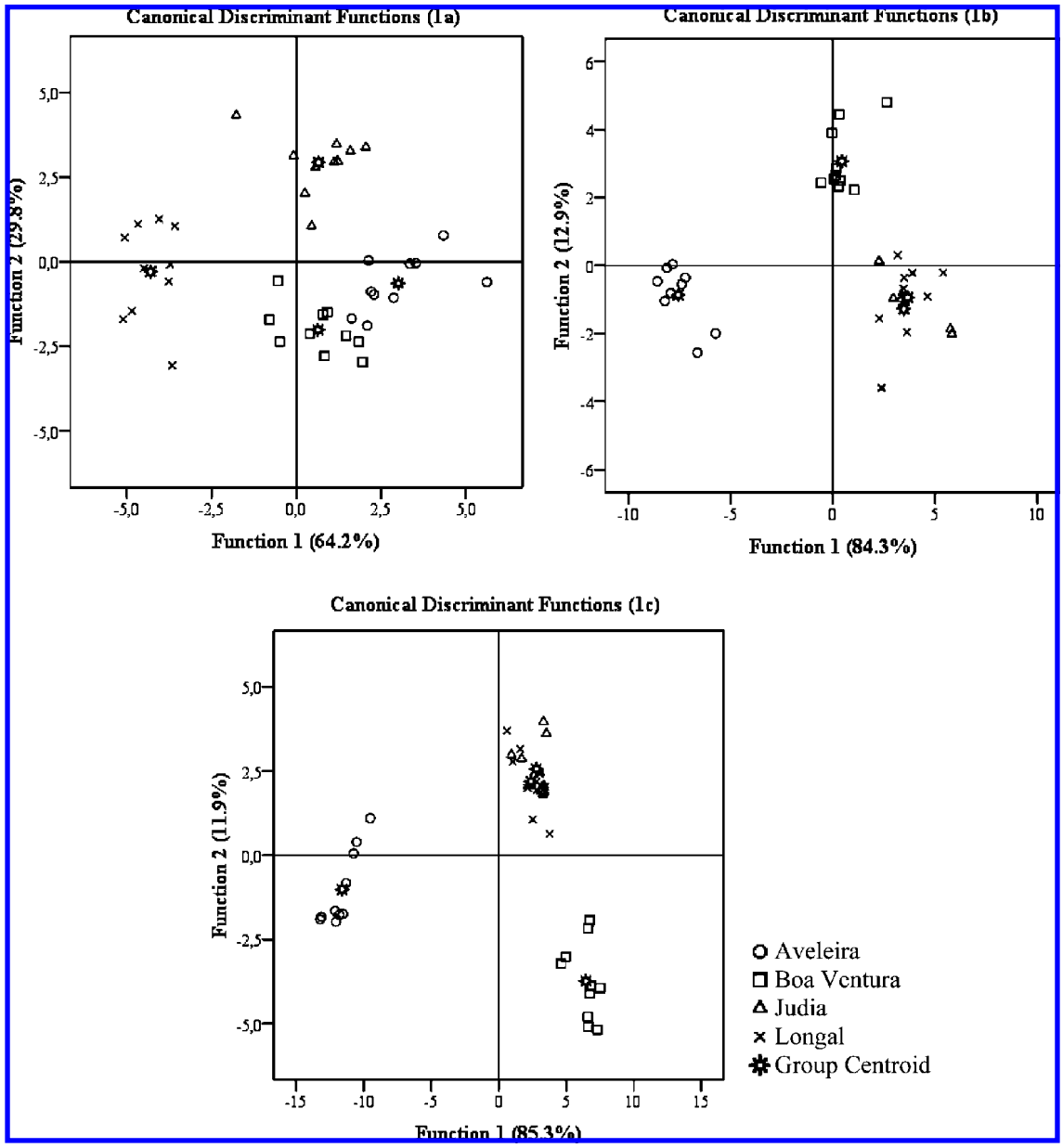

Figure 1. Canonical analysis of chestnut varieties based on nutritional parameters (**a**), triacylglycerols (**b**) and fatty acids (**c**).

we know this is the first work reporting TAG profile in chestnut. The analyses were conducted in high pressure liquid chromatography (HPLC) using nonaqueous eluting solvent mixtures and an evaporative light-scattering detector (ELSD) (*31*).

Thirteen compounds (**Table 3**) were determined in chestnuts: LLnLn, LLLn, LLL, OLLn, PLLn, OLL, LLP, OLO, LOP, PLP, OOO, OOP and POP (L, linoleoyl; Ln, linolenoyl, P, palmitoyl; O, oleoyl). OLL was the main component in Boa Ventura (23.5%), Judia (24.2%) and Longal (24.5%). In Aveleira, the predominant TAG was LOP (20.5%), reflecting the high contents of linoleic acid. There were four major TAGs (OLL, LLP, OLO and LOP), with contents superior to 10%, in all chestnut samples. OOO and LLL were also present in significant amounts, once more reflecting the fatty acids profile. Actually, considering the quantified TAGs, all of them contain, at least, one linoleic acid or one oleic acid molecule. LLnLn and POP were present in minute amounts (inferior to 1%). Although some differences exist, Judia and Longal presented a similar qualitative and quantitative profile, defining a chemical fingerprint that may be useful for evaluating the identity and quality of chestnut fruits (**Table 3**).

The specificity of TAG profile is being used more and more in the food industry as a tool to assess quality and authenticity of vegetable oils, determining its origin or detecting adulterations (*32*−*34*).

Statistical Analysis. In the discriminant analysis (DA) several combinations of the obtained results were used (**Tables 4** and **5**). As it can be seen in **Figures 1** and **2**, only the first two functions defined in each one of the DA studies were plotted and represented 94−97% of the information.

Regarding nutritional parameters, the DA defined three functions, with 94.0% of the observed variance explained by the first two (**Figure 1a**). The first function separates primarily Longal from the other cultivars (means of the canonical variance (MCV): Aveleira = 3.004, Boa Ventura = 0.637, Judia = 0.656 and Longal = −4.297), and revealed to be more powerfully correlated with NDF, cellulose and ADF. The second dimension permitted the separation of Boa Ventura and Judia (MCV: Boa Ventura = −2.010 and Judia = 2.939) and showed to be more correlated with ash content, protein and ADL. Neither function 1 (3.004) nor function 2 (−0.636) separated clearly Aveleira from the other cultivars.

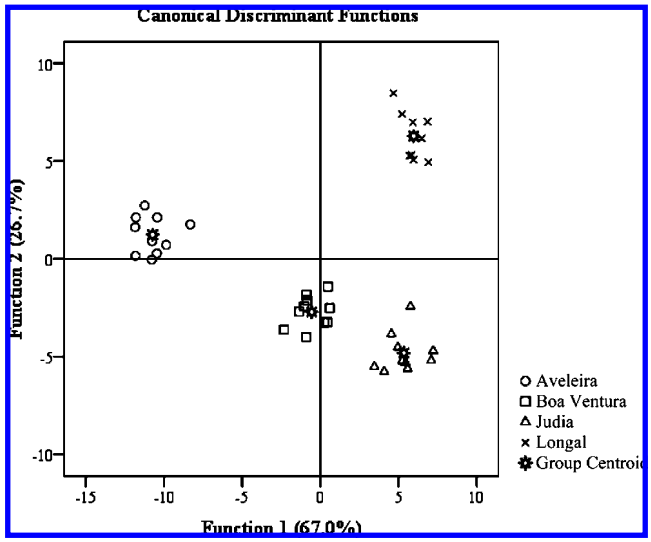

Figure 2. Canonical analysis of chestnut varieties based on all the parameters together.

Concerning TAG profile, DA defined also three dimensions, being 97.2% of the observed variance explained by the first two (**Figure 1b**). The first function separates primarily Aveleira and Boa Ventura from the other cultivars (MCV: Aveleira = −7.556, Boa Ventura = 0.438, Judia = 3.651 and Longal = 3.467), and revealed to be more effectively correlated with LOP, LLL and OLLn. The second function separates only Boa Ventura from the other cultivars (MCV: Aveleira: −0.859, Boa Ventura = 3.068, Judia = −0.934 and Longal = −1.275) and showed to be more correlated with LLL, OLO, OOO and LLP. Neither function 1 nor function 2 separated clearly Judia and Longal from each other.

From the three functions defined when the FA profiles were used, the first two explained 97.2% of the observed variance (**Figure 1c**). The first function separates mostly Aveleira and Boa Ventura from the other cultivars (MCV: Aveleira = −11.608, Boa Ventura = 6.457, Judia = 2.563 and Longal = 2.190), and revealed to be more strongly correlated with 16:0, 18:3 and 20:1. The second function confirmed the separation of Aveleira and Boa Ventura, but no separation was observed among Judia and Longal (MCV: Aveleira: −1.017, Boa Ventura = −3.736, Judia = 2.563 and Longal = 2.190) and showed to be more correlated with 18:2, 18:1 and 16:0.

The best results were obtained when the parameters were applied all together and considering MUFA, PUFA and SFA as groups, and not the individual FA. The analysis defined again three functions, 93.7% of the observed variance being explained by the first two (**Figure 2**). The first dimension permitted the clear separation of all the cultivars except Judia and Longal (MCV: Aveleira = −10.732, Boa Ventura = −0.559, Judia = 5.333 and Longal = 5.959), and revealed to be more strongly correlated with OOO, OOP and PUFA. The second function was efficient in the separation of Judia and Longal (MCV: Judia = 1.239 and Longal = 6.283) and showed to be more correlated with SFA, carbohydrates and ADF.

The fact that all the samples' marks cluster together in the respective groups signifies that there are not great differences among duplicates, as well as between the fruits collected from each tree. This fact is also reflected in the small residual errors revealed by the analysis of variance (**Tables 1−3**).

Conclusion. The results obtained for the proximate analysis are in agreement with the reference values for chestnut, and it is not expectable that the differences among the assayed parameters could be explained by edaphoclimatic conditions, because all the chestnuts were collected from orchards with very similar geographical localization. Hence, the significant differences observed might be explained by the influence of a specific cultivar. Due to the low fat contents, chestnuts have low caloric value, proving their importance in a healthy diet. All the cultivars presented energy values below 195 kcal/100 g of fresh weight. The relative high fiber amounts can also increase the beneficial effects of chestnut consumption on human health.

The FA profiles were analogous, with the isomers *cis*-9-octadecenoate (C18:1ω9), *cis*-9,12-octadecadienoate (C18:2ω6) and palmitic acid (C16:0) as major compounds. From this study, we can conclude that Longal is the better health-promoting cultivar, due to its PUFA, and especially linoleic and linolenic acid contents.

The main components in the TAG profile were OLL, LLP, OLO and LOP, reflecting the high content of oleic and linoleic acids. The results herein reported suggest that, besides genetic factors, TAG composition can be somehow influenced by cultivars. These results may be considered as reference data for chestnut TAG quantitative composition because, as far as we know, there are no published data on this subject.

Univariate analyses of variance and discriminant analysis were carried out to check for the most important components in the discrimination between cultivars, and a canonical variate analysis was developed to enable the visualization of all results. The four cultivars were clustered in four individual groups obtained through the definition of two DA dimensions, especially when the algorithm was applied for selecting variables according with all the parameters together. This result might be used as an authenticity indicator, conveying economical benefits.

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