

Analytical Methods

Characterization of various chestnut cultivars by means of chemometrics approach

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Received 10 October 2006; received in revised form 22 June 2007; accepted 8 August 2007

Abstract

Chestnuts like other foodstuffs may be characterized by their chemical composition. The chemical composition of ascorbic acid, total acidity, pH, starch, refraction index (°Brix), moisture, ashes, insoluble fibre, soluble fibre, total dietary fibre, total proteins, cations (Na, K, Ca, Fe, Cu, Zn, Mn, Mg) and phosphorous was determined in 19 local chestnut varieties from three areas of production in the island of Tenerife. Significant differences were found between the mean values of moisture, starch, total phenols contents, total soluble and non-soluble fibre, Ca, Cu, K, Mg and Zn, obtained according to the area of production. Distribution patterns of the samples were established for correlating the chemical composition of the chestnuts and varieties, using different chemometrics tools such as cluster analysis, principal component analysis, factor analysis and linear discriminant analysis (LDA). Initially, cluster analysis made it possible to establish a primary relationship between the variables. When applying stepwise LDA the chestnut samples were well classified within their area of production. Also, when the stepwise LDA was used on the chestnut samples from area of production 2, 100% of the chestnut samples were also correctly classified within their variety.

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Keywords: Chestnuts; *Castanea* sp.; Chemical composition; Multivariate analysis; Pattern recognition

1. Introduction

“Agricultural biodiversity of all food species is a vital sub-set of general biodiversity, highly threatened by globalisation of food markets and tastes, intellectual property systems and the spread of unsustainable industrial food production” (<http://www.ukabc.org/ukabc3.htm#c>). The project “Germobanco Agrícola de la Macaronesia” within Interreg III-B European program in collaboration with the Cabildo Insular of Tenerife (Tenerife, Spain) have begun studies on the characterization of local cultivars of different vegetal species to conserve and recuperate the biodiversity (http://www.fao.org/biodiversity/crops_en.asp; Francisco-Ortega, Santos-Guerra, Kim, & Crawford, 2002; González

Rodríguez & Hernández Suárez, 2003) of such cultivations in the Macaronesic region.

Chestnut trees are included among these cultivations. The chestnut (*Castanea sativa* Miller) belongs to the Fagaceae family, Castaneoideae Subfamily, Orden Fagales. There are 12 species of chestnut, *C. mollissima*, *C. crenata*, *C. henry*, *C. segunii*, *C. davidii*, *C. dentata*, *C. ozarkensis*, *C. ashei*, *C. paucispina*, *C. pumila*, *C. floridiana* and *C. alni-folia*, which are produced in several regions in the world (Berrocal et al., 1988). According to Food and Agricultural Organization (FAO) the global production of chestnuts in 2004 was around 1042.743 metric tonnes (MT). Chestnuts are an important food crop in southern Europe, southwestern and eastern Asia, and before the chestnut blight they were also important in north-eastern America (Chung et al., 2004). China is by far the largest producer country, followed by Korea, Italy, Turkey, Bolivia, Portugal and

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Japan. European chestnut production (*Castanea sativa*) was 119,123 MT in 2004 (FAO) and they are grown in 75,620 Ha. Spain is the fifth largest producer country in Europe, with a total of 9510 MT (FAO) and a cultivated surface of 6254 Ha. Most of the production in Spain is found in Galicia (9361 MT) (MAPA).

Chestnut trees were brought to the Canary Islands by Spanish and Portuguese settlers. Nowadays, thirty-eight varieties of chestnuts have been identified in the Canary Islands (Pereira-Lorenzo, Ríos, González-Pérez, Cubas, Perdomo, Calzadilla, 2001; Pereira-Lorenzo, Ramos-Cabrer, Díaz-Hernández, Ciordia-Ara, & Rios-Mesa, 2006). The main area of chestnut cultivation is on the island of Tenerife Island, where there are two clearly differentiated zones: the Northern zone between La Orotava and La Esperanza municipalities and the Southern zone, which includes the Arafo municipality.

The nutritional characterization of chestnuts as a food-stuff is important, since this food has a high nutritional value and great cultural interest. In the past, chestnut consumption was mainly found in rural areas and was considered as food for the poor (De la Montaña Míguez, Míguez Bernárdez, & García Queijeiro, 2004). Nowadays, the use of chestnuts as ingredients in cooking is not only much more common, but also highly sophisticated. They are used to complement vegetables, fish or meat. Besides which, their industrial use was commented on by Mottin Demiate, Oettere, and Wosiacki (2001), and their importance in traditional Chinese medicine was referred to by Xu (2005). In the last few years, the consumption of fresh, or previously transformed, chestnuts has considerably increased. Chestnuts have a relatively short shelf-life due to their sugar content and high water activity. There are many conservation processes and ways to prepare chestnuts in the Canary Islands and Spain, such as steaming, drying in the sun or roasting in an oven (Florez Serrano, Santín Fernández, Sánchez Rodríguez, Del Pino Gutiérrez, & Melcón Martínez, 2001). However, few studies have dealt with the chemical composition and evaluation of their nutritional characteristics.

The application of chemometrics tools (Alonso-Salces et al., 2006; Arvanitoyannis, Katsota, Psarro, Soufleros, & Kallithraka, 1999; Brito, Novotná, Peña-Méndez, Díaz, & García, 2004; Casañas, González, Rodríguez, Marrero, & Díaz, 2002; Chia-Hui & Zhi-Kai, 2005; Forina, Armanino, & Raggio, 2004; Hernández, Rodríguez, & Díaz, 2005; Kamimura, Biciato, Shimizu, Alford, & Stephanopoulos, 2000) to research into the characterization, determination of the geographic origin and/or quality control of food products has recently become a very active area. The aim of this paper is to apply pattern recognition tools (cluster analysis, principal component analysis, factor analysis and linear discriminant analysis) to physico-chemical parameters determined in 19 local chestnut varieties from Tenerife (Canary Islands, Spain) to differentiate the chestnut samples according to the variety and/or the region of production. This aim is developed under the

necessity of characterizing chestnuts by varieties and areas of production which will result in the maintenance of the biodiversity of such cultivations in Canary Islands and in the Macaronesia.

2. Materials and methods

2.1. Samples

One hundred and five commercial chestnuts (*Castanea sativa*) Mill. from 19 different cultivars directly harvested by chestnut producers in Tenerife (Spain) were used. The varieties of chestnuts were authenticated and characterized by technicians from the Insular Authorities (Cabildo Insular de Tenerife, Tenerife, Spain). Their varietal denomination, type and production are described in Table 1. According to the regional area of production (AP), the chestnuts samples were divided into three groups, namely: 1 (La Orotava municipality), 2 (La Matanza, La Victoria and Sauzal municipalities), and 3 (Arafo municipality). Tenerife has a steep relief, formed by the presence of the mountain Teide (3718 m), which splits the island into two slopes (South and North side) that are significantly different in terms of their climatic regime. The geographical situation of areas of production was also considered: north (N) and south (S).

Table 1
Description of chestnut varieties, areas and zones of production

Variety	Municipality	Area of Production	Zone
Castaña grande	El Sauzal	2	North
	La Victoria de Acentejo	2	North
	La Matanza de Acentejo	2	North
Redondo	El Sauzal	2	North
	La Victoria de Acentejo	2	North
	La Matanza de Acentejo	2	North
De sala	El Sauzal	2	North
	La Matanza de Acentejo	2	North
	Arafo	1	South
Mulato	El Sauzal	2	North
	Arafo	1	South
	La Matanza de Acentejo	2	North
	La Victoria de Acentejo	2	North
De pata	La Orotava	3	North
	El Sauzal	2	North
	La Matanza de Acentejo	2	North
Manso	Arafo	1	South
	La Victoria de Acentejo	2	North
Grande	La Victoria de Acentejo	2	North
Del Haya	La Victoria de Acentejo	2	North
Picudo	La Victoria de Acentejo	2	North
Polegre	La Victoria de Acentejo	2	North
Matancera	La Victoria de Acentejo	2	North
Donosa	La Orotava	3	North
Pico claro	La Orotava	3	North
Arafero	La Orotava	3	North
Corujero	La Orotava	3	North
Temprano	La Orotava	3	North
Piñero	La Orotava	3	North
Culo chico	La Victoria de Acentejo	2	North
Negro	La Victoria de Acentejo	2	North
Siete pernadas	La Orotava	3	North

2.2. Analytical procedures

The seeds were manually removed from the spiny fruits and peeled. The analytical procedures for the analysis of the samples have been described in previous papers (Pérez et al., 2006; Díaz Gómez et al., 2006) following analytical parameters were determined: ascorbic acid, total acidity, pH, starch, (°Brix), total phenols, moisture, ashes, insoluble fibre, soluble fibre, total dietary fibre, total proteins and minerals such as P, Na, K, Ca, Fe, Cu, Zn, Mn, Mg. The methods used were AOAC methods (AOAC, 1990) with the exceptions of total phenols content and minerals. All chemicals were reagent grade. Briefly, total fibre and non-soluble fibre were determined by the enzymatic-gravimetric methodology described by AOAC (1990). The protein content was determined by Kjeldhal method using as factor 5.3 given for chestnuts by McCarthy and Meredith (1988). Ascorbic acid was determined by extracting the ascorbic acid, followed by redox titration of the ascorbic acid using DCP (2,6-dichloroindophenol). The content of total phenolics was determined according to a modification of the Folin–Ciocalteu method and expressed as gallic acid equivalents (GAE), described by Kujala, Loponen, Klika, and Pihlaja (2000). Total acidity was determined by acid–base titration with NaOH and the results are expressing as milligrams of citric acid by 100 g of sample. Phosphorous was measured by colorimetric method, which uses Vanadate–Molybdate reagent (BOE, 1995). The content of the rest of the minerals was determined by atomic absorption spectrophotometry following nitric digestion of the samples. The procedure previously described by us (Forster, Rodríguez Rodríguez, Darias Martín, & Díaz Romero, 2002a; Forster, Rodríguez Rodríguez, & Díaz Romero, 2002b) was then applied.

2.3. Data analysis

The data set consisted of 105 samples \times 21 variables, in which the rows represented chestnuts samples and the columns showed the contents of ascorbic acid, total acidity, pH, starch, refraction index (°Brix), moisture, ashes, insoluble fibre, soluble fibre, total dietary fibre, total proteins, Na, K, Ca, Fe, Cu, Zn, Mn, Mg and phosphorous. A data pretreatment was performed to prevent differences due to the measurement units, each datum was standardized according to $z_{ij} = (x_{ij} - \text{average}_j) / (\text{standard deviation})_j$. Univariate characterization of the chestnuts by area of production was carried out based on Fischer's weight (F) by means of analysis of variance (one-way ANOVA) applied to compare the mean values obtained among the different categories.

Cluster analysis (CA) was applied as a preliminary item to discover possible natural groupings among samples characterized by the set of analysed variables. CA is one of the most useful information chemometrics tools for discovering groups and localizing (identifying) interesting distributions and patterns in the underlying information

contained in the data. There are several clustering algorithms, for our purposes Ward's method was selected as the linkage method and with euclidean distance being the measure of similarity.

Principal Component analysis (PCA): in order to explore the physico-chemical data, principal components (PCs) were extracted from the multidimensional (105 \times 21) data matrix, with the aim of narrowing the focus of our attention to the main factors. The information contained in the original variables is projected onto a smaller number of underlying variables called PC. PCA results are discussed in detail according to the selected variables to characterize the samples.

The presence of classes within the chestnuts samples was investigated by linear discriminant analysis (LDA). A basic problem in LDA is deciding which variables should be included in the analysis. This may be achieved with a step-wise LDA using Wilk's lambda as the selection criterion, and an F-statistic to determine the significance of the changes in lambda when a new variable is tested. Validation of these results was performed by using leave-one-out cross-validation.

All calculations were performed using STATGRAPHICS® Plus V.5 (INST, CA, USA) and SPSS V. 12.1 (SPSS Inc., Chicago, USA).

3. Results and discussion

Chestnuts analysis are summarised in Table 2 for all of the chestnut samples and areas of production. In order to establish the discriminant capacity of each variable, a one-way ANOVA was performed, using the area of production as a discrimination factor. Significant differences ($p < 0.05$) were found between the mean values for moisture, starch, total phenols content, total soluble and non-soluble fibre, Ca, Cu, K, Mg and Zn. The chestnuts sampled in the area of production number 2 located in the north of the island had the highest content levels of starch, total acidity, Ca, and the three fractions of fibre analysed. The highest content of total phenols and Zn are present in the area of production 3. On the other hand, the chestnuts produced in the area 1 from the north of the island, corresponding to the municipality of La Orotava presented higher moisture content, K, Mg and Cu than those samples from the areas of production 2 and 3 (El Sauzal and Arafo municipalities, respectively).

3.1. Cluster analysis

Cluster analysis (CA) was applied in order to discover possible similarities or affinities amongst the chestnut samples. No tendency to natural grouping of the samples, based on common characteristics, was observed (figure is not shown here due to its size). Since no natural groups of samples were observed, CA was performed to follow random similarities in the behaviour of the variables under study (Gonçalves, Esteves da Silva, & Apendurada, 2006).

Table 2
Descriptive statistics

Variable	Area of production 1	Area of production 2	Area of production 3
Ascorbic acid	30.8 ± 9.01	36.3 ± 10.4	31.8 ± 8.68
Total acidity	21.2 ± 21.2	74.8 ± 16.2	31.8 ± 8.68
Starch	24.1 ± 2.63	29.8 ± 1.91	25.2 ± 3.16
Brix	8.83 ± 1.82	5.71 ± 1.41	9.51 ± 2.11
Total phenols	117 ± 23.5	100.7 ± 14.8	150.2 ± 29.0
Non-soluble fibre	5.45 ± 0.88	7.12 ± 0.51	5.61 ± 0.83
Soluble fibre	0.997 ± 0.30	1.56 ± 0.31	1.11 ± 0.37
Total dietary fibre	6.45 ± 0.87	8.68 ± 0.72	6.72 ± 0.90
Moisture	57.2 ± 3.55	53.06 ± 1.91	55.03 ± 2.38
pH	7.08 ± 0.15	7.04 ± 0.1	7.04 ± 0.1
Total proteins	3.37 ± 0.48	2.72 ± 0.22	3.54 ± 1.23
Ashes	1.03 ± 0.10	0.94 ± 0.11	1.05 ± 0.08
P	611.4 ± 85.4	547.0 ± 36.72	605.0 ± 149.5
Ca	156.0 ± 46.8	219.4 ± 12.43	167.5 ± 50.62
K	5142 ± 722.4	4613 ± 220.6	4751 ± 614
Na	32.5 ± 17.3	17.53 ± 4.70	28.35 ± 11.77
Mg	352.7 ± 40.45	313.02 ± 13.44	338.2 ± 57.3
Mn	4.36 ± 2.21	4.32 ± 0.71	4.30 ± 2.0
Zn	3.94 ± 0.74	3.03 ± 0.26	4.70 ± 1.50
Cu	2.34 ± 0.41	1.34 ± 0.15	1.81 ± 0.78
Fe	5.24 ± 1.42	3.10 ± 0.32	5.62 ± 1.52

Results expressed as average ± standard deviation of the analyzed parameters according to the area of production. Metal concentration expressed as mg/Kg wet weight. Ascorbic acid, total acidity and total phenol content expressed as mg/100 g wet weight Other variables: content expressed in percentages.

Fig. 1 presents the dendrogram obtained when clustering variables using the Ward's linkage method (euclidean distance). A group of variables (*A*) composed of ascorbic acid, starch, soluble fibre, insoluble fibre and total dietary fibre. These variables are associated with the polysaccharide components (starch and fibre compounds) and ascorbic acid. A second cluster (*B*) is divided in two sub-clusters (*C* and *D*). Sub-cluster *C* includes relatively heterogeneous variables such as moisture, total acidity, ashes, pH, total phenol content, refraction index ($^{\circ}$ Brix), and metals such as Ca, Na and Mn. Whilst sub-cluster *D* associates variables like total proteins, phosphorous, K, Fe, Mg and trace elements such as Cu and Zn. Sub-cluster *C* included vari-

ables related to the water content. Whilst sub-cluster *D* mainly comprises variables related with total proteins and enzymatic components such as Fe, Cu and Zn and some major minerals as K, Mg and P. The association between the protein and phosphorous variables suggests that an important protein fraction present in the chestnuts is linked to P.

3.2. Principal component analysis

PCA is a well-known statistical method for reducing the dimensionality of data sets. In order to visualize response patterns in the feature space of principal components

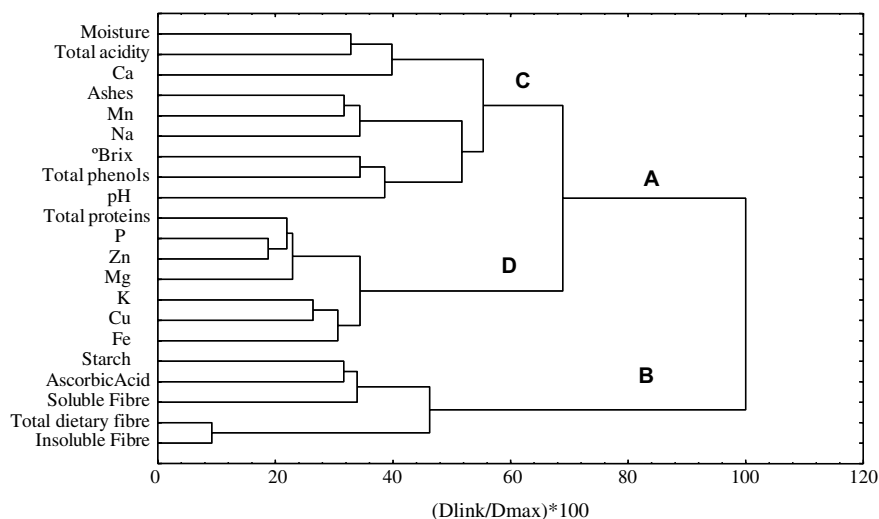


Fig. 1. Dendrogram obtained by cluster analysis of variables using Ward's method of linkage.

(PCs), the experimental data were examined by PCA. The data were previously transformed as described above. The first seven PCs accounted for 82.8% of the variance (eigenvalue ≥ 1). Fig. 2a shows the PCA results of variables used for the characterization of chestnut(s) data projected onto their first two PCs. It is evident in the loading plot that the ascorbic acid, soluble fibre, insoluble fibre, total dietary fibre and starch load on the (+)PC1; while the P, Mg, Cu, Zn and total proteins load on (-) PC1. Moreover, moisture is the value with the largest contribution on the PC2. The macroelements Ca and K, and ash content do not reveal an important contribution to the total variance. An association between Mg, Cu and Zn microelements and the total dietary protein and phosphorous content was found. However, the Mn contribution is

shown to be irrelevant. The control of moisture content in chestnuts is highly important to prevent problems during storage and delivery, which has been observed by other authors (Attanasio, Cinquanta, Albanese, & Di Matteo, 2004).

When the score plot is displayed (Fig. 2b) the PCA results agree with those obtained by CA, since a clear natural clustering trend in the chestnut data is not observed according to the variety of the chestnut. These results are in agreement with those of Pereira et al. (2001). The authors studied 47 cultivars of chestnuts from different Spanish production regions using the main nutrient components finding variability between cultivars and regions of production due to the high genetic diversity. However, when the location of the area of production is considered as belonging to the southern or northern part of Tenerife, a grouping tendency is observed (samples marked with * in Fig. 2b). Three samples considered as belonging to area of production 3 (Arafo) were erroneously grouped with samples located in the northern part. This can be explained because the chestnut production areas are close to each other from a geographical point of view.

After performing PCA analysis, the eigenvalues analysis (eigenvalue ≥ 1) indicates that 74.8% of the variance could be explained by six main components. A Varimax rotated solution of principal components highlighted the most important variables in each factor. The rotated FA matrix obtained is shown in Table 3. The first factor (F1) has a high load of proteins, phosphorous, Mg, Cu and Zn. This

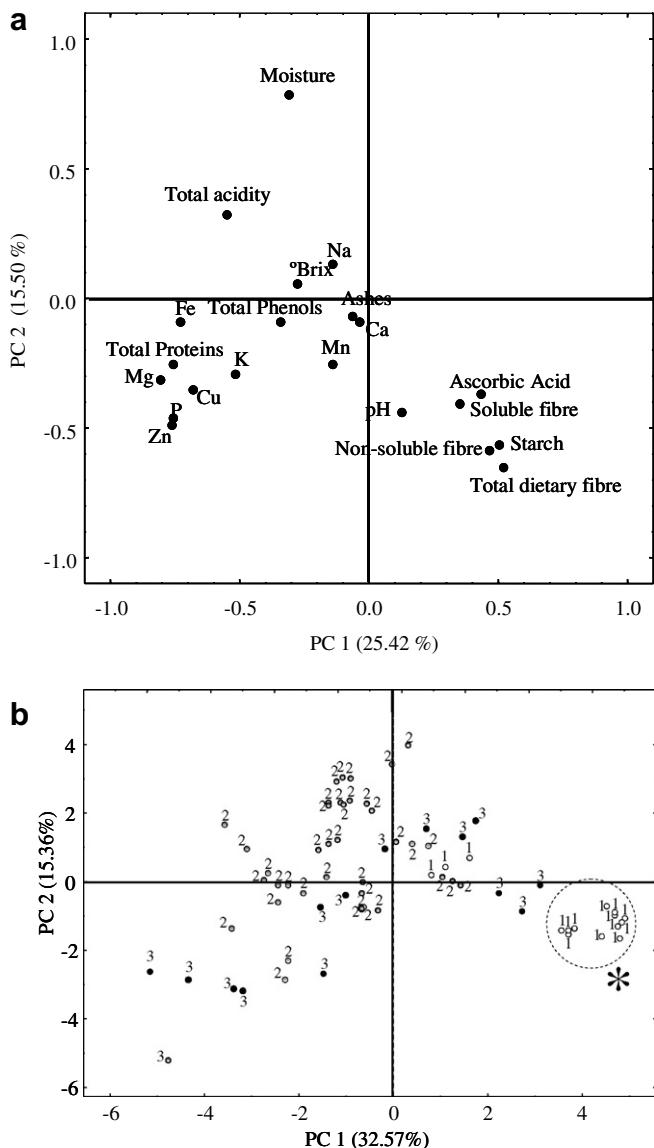


Fig. 2. Results of principal components analysis. (a) Loading plot projected on the space of PC1 vs. PC2. (b) Scores plot of the chestnuts samples projected on the space of PC1 vs. PC2.

Table 3
Results of factor analysis after a Varimax rotation

Variables	Rotated Factors					
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Moisture	-0.0844	0.3778	-0.1924	0.0013	0.1337	-0.8158
Ashes	-0.0220	0.2232	0.1725	0.7023	-0.1390	0.3738
Starch	-0.1717	-0.2940	-0.1968	-0.0218	0.0956	0.7983
Total Proteins	0.7552	0.0281	0.0077	-0.0141	-0.4107	-0.1358
Total dietary fibre	-0.0816	-0.9280	-0.0368	-0.1095	0.0229	0.2672
Soluble fibre	-0.0715	-0.2555	-0.2215	-0.4385	0.0206	0.4776
Insoluble fibre	-0.0672	-0.9618	0.0422	0.0398	0.0186	0.1253
°Brix	0.1590	0.3179	0.6138	0.0925	-0.2166	-0.0081
Ascorbic acid	-0.2024	-0.0681	0.3823	0.0131	0.2158	0.5950
Total phenols	0.2529	0.0612	0.1271	-0.0570	-0.7962	0.0609
pH	0.1500	-0.3160	0.7359	-0.1067	0.2372	0.1183
Total acidity	0.2629	0.3303	-0.3946	0.0564	-0.4822	-0.2899
P	0.9104	-0.0263	0.0619	-0.0303	-0.0915	0.0090
Na	0.0179	-0.0995	0.0292	0.8281	0.1257	-0.2553
K	0.6211	0.2601	-0.0029	0.1892	0.5223	0.1457
Ca	0.1248	-0.1147	-0.7873	-0.1928	0.1944	0.0431
Mg	0.8672	0.1037	-0.1411	0.0043	0.0090	-0.0824
Fe	0.6614	0.1940	-0.1829	0.2136	-0.0667	-0.1538
Cu	0.8182	0.0115	0.0820	-0.0146	0.2950	-0.1436
Zn	0.8497	0.0479	0.0581	0.0309	-0.3315	0.1169
Mn	0.2010	0.0945	-0.4024	0.5224	0.1778	0.4293

F1 groups together variables related directly to the proteins and minerals associated with the protein fraction, which could be influenced by their genetic and/or environmental characteristics. The second factor (F2) has an important loading of total fibre and insoluble fibre. The third (F3) and fourth factors (F4) highlight the combination of the pH and Ca in F3, and Na in F4, respectively. Both factors have a high prevalence among the soil and water qualities (ground and/or well waters). The total phenols content load highly on factor 5 (F5), while moisture and starch load on factor 6 (F6). The last two factors F5 and F6 seem to be logically related to the ripening degree of the chestnuts (Pérez et al., 2006; Díaz Gómez et al., 2006). These observations from the results of FA may be explained by the fact that the variables profiles of the chestnuts depend on agronomic, climatological factors and soil, which can change between regions and/or seasons.

3.3. Linear discriminant analysis

LDA, a supervised pattern recognition method, is applied to distinguish among the chestnut samples. The method supplies a number of linear discriminant functions in order to provide a method for predicting the group into which a new case will most likely fall. A stepwise LDA using the forward selection approach was performed and, in order to assess its discriminating capacity, the Wilk's lambda as selection criterion, and an F-statistic to determine the significance of the changes in lambda when a new variable is tested were applied. Using area of production as a classification variable, the variables selected were ashes, total phenols content, °Brix, total dietary fibre, moisture, Ca, Cu, Fe, and Zn, all related to soil composition and/or ripening degree. LDA achieved (Fig. 3a) high recognition percentages for the classification of the chest-

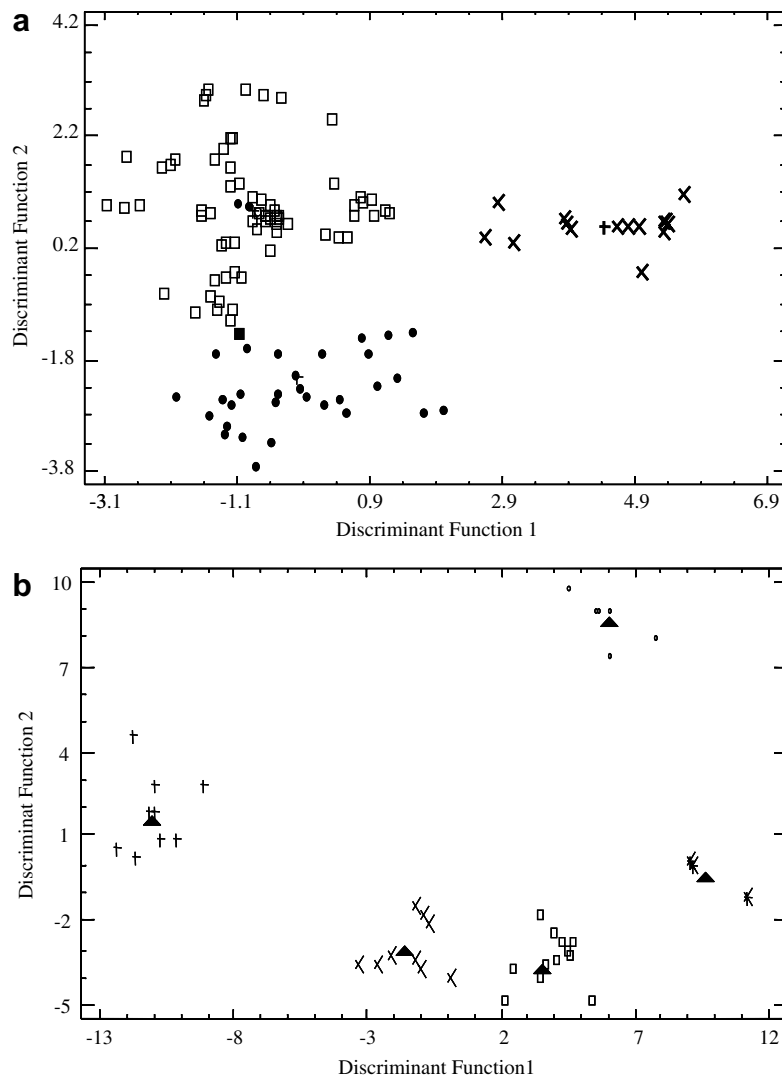


Fig. 3. Scattered plot of the samples using for variables: (a) ashes, total phenols content, °Brix, total dietary fibre, moisture, Ca, Cu, Fe, and Zn, projected in the plane defined by the discriminant functions according to area of production. Codification of area of productions: (X) 1 (□) 2 (●) 3. (b) Ascorbic acid, total phenols content, soluble fibre, moisture, K, Mg, Mn, Na, and proteins, projected in the plane defined by the two discriminant functions according to variety as classification variable. Samples belong to area of production 2. Varieties: (□) Castaña grande (X) Redonda (○) De sala (†) Mulata (*/) Manso.

nut(s) samples according to area of production 1 (La Orotava) and 3 (Arafo), reaching a recognition of 100% and 92.75% respectively. However, prediction ability was not so high, especially, for samples belonging to the area of production 2 (El Sauzal, La Matanza and La Victoria), obtaining a classification of 87.8%. Two samples belonging to area of production 2 were erroneously classified as belonging to the area of production 1. It is important to emphasize that such samples were sampling near the border between both areas of production 2 and 3.

When LDA was applied to differentiate the chestnuts according to variety, low percentages of classification were obtained. This result is explained by in base of heterogenicity of the origin of the chestnut samples and the low number of samples per variety. Due to this fact, we selected the area of production which presents more samples per variety. Thus, a new data set was created in order to evaluate the differentiation between different varieties. This new data set includes samples belonging to the following varieties: Castaña grande (Cg), Redonda (R), De sala (Ds), Mulata (M), and Manso (Ma). All the varieties are produced by chestnut trees located in the area of production 2 (North of the Island). A data matrix, 36×21, was studied by chemometrics tools. LDA was applied and the following variables were selected: ascorbic acid, total phenols content, soluble fibre, moisture, K, Mg, Mn, Na, and proteins. Selected variables are related to soil composition, ripening degree and marine aerosol (Frías, Pérez-Trujillo, Peña, & Conde, 2001). Fig. 3b shows the LDA results where differentiation of the samples according to the variety is observed.

4. Conclusions

The area of production has a higher influence on the physicochemical variables than the variety, particularly on the mineral composition. The main physicochemical variables (factors) characterizing classification according to the area of production were identified. Three groups of variables were established: (a) polysaccharide components and ascorbic acid; (b) moisture and parameters related with the ripening and water content; and (c) proteins, enzymatic components and some major minerals. PCA was applied to follow the trends in chestnuts samples. Samples tend to be classified according to the zone (North, South) of the Island where the area of production is located. LDA applied on all the chestnut samples enables one to distinguish the area of production. When the chestnut samples from an area of production were previously selected, the application of LDA was successful in differentiating between the chestnut samples according to their variety. Thus, the combination between the physicochemical characterization and the multivariate analysis allows the differentiation of the chestnuts according to variety and the area of production. The results are useful in the recognition of the varieties of chestnuts and to follow the influence of different factors such as soil and environment in their characterization, and, therefore in

the maintenance and preservation of the biodiversity of such cultivations in Canary Islands.

Acknowledgements

This work was supported by Grant PI042004/030 of the Canarian Government. The authors wish to acknowledge the Excelentísimo Cabildo Insular of Tenerife and Germobanco Agrícola de la Macaronesia within the Interreg III-B European program for providing chestnut samples. The English in this article has been kindly revised by Patrick Dennis.

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