Major Fatty Acid Composition of 19 Almond Cultivars of Different Origins. A Chemometric Approach

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Multivariate chemometric techniques have been applied to the fatty acid composition of gas chromatographic data from 19 almond cultivars. Analysis of variance indicates the important contribution of all major fatty acids to the typification of almond cultivars. Principal component analysis applied to all of the individual values of fatty acids of the different cultivars leads to three new variables which accumulate about 90% of the total variance. The projection of different cultivars in the reduced space allows the visualization of some different cultivar groups. Cluster analysis classifies the studied almond cultivars into three groups. Within a large group many cultivars of the Mediterranean area and the American cultivar Non Pareil are found. The second group includes some Italian and American cultivars. A third group covers one American and one Australian cultivar, in association with two Spanish ones. Discriminant analysis applied to the groups previously established by cluster analysis and principal component analysis brings out two discriminant functions, which explain 68.53% and 31.47% of the total variance, respectively. Application of these discriminant functions verified a correct assignment (100%) of each member to its group.

Keywords: *Fatty acid composition; almond cultivars; chromatographic analysis; multivariate techniques*

INTRODUCTION

The origin and specifity of agricultural foods are often used to define their quality and commercial value. This is the case for almonds, of which many cultivars are known, although they are not equally appreciated. It is likely that fat composition of this oleaginous seed can provide information valuable for its characterization.

Fat content has been reported by Holland et al. (1992), Romojaro et al. (1977), and Mateos and Castañer (1993). Other authors have reported the fatty acid composition of reduced groups of some almond cultivar from different geographic origins: Italian (Salvo et al., 1986), American (Fourie and Basson, 1990; Bathi et al., 1986), and Spanish (Saura et al., 1988; Sánchez, 1990; Esteban, 1985).

All of these data are of limited value if the cultivars are not strictly defined, and a lot of information is lost unless more elaborate statistical methods are applied. Multivariate methods are powerful tools to characterize products, and often have been applied in assessment of the genuineness of diverse foods (Parolari et al., 1992; Bicchi et al., 1993) and beverages (Scarponi et al., 1982; Shimoda et al., 1993).

This paper provides information about the application of different multivariate methods of analysis to chromatographic data of major fatty acid composition of a very well defined set of almond cultivars, to establish chemical parameters useful for their classification or characterization. This information could be very useful for the food, pharmaceutical, and cosmetic industries and for fraudulent adulteration detection.

EXPERIMENTAL PROCEDURES

Samples. The set studied consists of seven Spanish cultivars (Malagueña, Peraleja, Atocha, Del Cid, Desmayo-Largueta, Ramillete, and Marcona), three Italian cultivars (Genco, Tuono, and Cristomorto), one Australian cultivar (Chellaston), four Americans cultivars (Texas, Non Pareil, Titan, and Wawona), one Tunisian cultivar (Achaak), one cultivar from Caucasian country (Primorskyi), one French cultivar (Ferragnes), and a hybrid obtained at CEBAS (Centro de Edafologia y Biología Aplicada del Segura, Murcia, Spain). They were all cultivated under the same conditions in 1993 in experimental fields of CEBAS.

After the coats were removed, the seeds were triturated, and the fraction passing through a 1 mm light sieve was dried at 60 °C, stored in a desiccator, and finally submitted to fat extraction and analysis.

Chromatography. About 3 g (to the nearest 1 mg) of each sample was defatted with 30 mL of ethyl ether/hexane (2:1) for 90 min in a high-efficiency modified Soxhlet extractor, using an electrically controlled heating mantle kept at 100 °C. Fatty acid methyl esters were prepared by methylation of the lipids according to the AOCS (1969) method. All solvents and chemicals were of analytical grade, except ethyl ether and hexane that were of capillary GC Grade (Baker). Standards of fatty acid methyl esters were purchased from Sigma.

Experimental data were obtained by using a Carlo Erba Series 8000 gas chromatograph, equipped with a split/splitless injector and a flame ionization detector and interfaced to a computer provided with the analytical program Chrom-Card for data acquisition and processing. The chromatographic column used was a DB-23 (J&W Scientific), 30 m × 0.32 mm i.d. Samples of 1 μL were injected into the split injector at 1:10 ratio. Helium at a flow rate of 2 mL min^{-1} was used as carrier gas. The injector and detector temperatures were set at 275 and 300 °C, respectively. Oven temperatures were held at 115 °C for 3 min, ramped from 115 to 200 °C at 49 °C min^{-1}, and finally held at 200 °C for 6 min. Methyl ester nonanoic acid was used as internal standard.

Data Processing. Experimental data were processed with the aid of the SPSS statistical package (SPSS, 1994).

Analysis of variance (ANOVA) was applied to the mean values of fatty acids, according to the homogeneity test of Tukey (Morrison, 1976), which uses the Studentized statistic range to make all of the pairwise comparisons between groups. This test assumes that the experimental error is the error in collecting rate of all pairwise comparisons. Data relative to

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Table 1. Mean Concentrations and Standard Deviations of Total Fat and Fatty Acid Methyl Esters Relative to Almond Cultivars

			g of fatty acid methyl esters/100 g of oil				
sample	abbrev	total fat, %	palmitic C16:0	palmitoleic C16:1	stearic C18:0	oleic C18:1	linoleic C18:2
Achaak	AC	58.47	7.50 ± 0.27	0.368 ± 0.011	1.70 ± 0.15	73.1 ± 5.8	19.5 ± 1.7
Atocha	AT	56.62	7.33 ± 0.15	0.503 ± 0.010	1.87 ± 1.8	72.6 ± 1.8	18.8 ± 0.5
Chellaston	CH	57.90	6.21 ± 0.42	0.347 ± 0.022	2.39 ± 0.24	57.5 ± 5.4	21.3 ± 2.0
Clon Cebas	CE	58.10	6.30 ± 0.13	0.447 ± 0.002	1.51 ± 0.02	64.9 ± 0.8	17.6 ± 0.2
Cristomorto	CR	58.62	5.47 ± 0.28	0.524 ± 0.030	1.71 ± 0.11	70.4 ± 4.1	15.1 ± 0.8
Del Cid	DC	56.60	7.08 ± 0.20	0.452 ± 0.011	2.24 ± 0.10	69.0 ± 2.7	18.5 ± 0.7
Desmayo Largueta	DL	59.02	7.68 ± 0.14	0.477 ± 0.008	1.69 ± 0.05	68.9 ± 0.7	23.5 ± 0.4
Ferragnes	FE	61.70	5.89 ± 0.08	0.425 ± 0.005	1.90 ± 0.12	71.2 ± 4.0	14.7 ± 0.7
Genco	GE	60.02	4.43 ± 0.32	0.405 ± 0.024	1.62 ± 0.20	63.7 ± 7.0	8.0± 1.2
Malagueña	MA	54.89	7.60 ± 0.22	0.420 ± 0.010	2.58 ± 0.16	60.7 ± 2.9	23.6 ± 1.0
Marcona	MR	58.10	8.12 ± 0.06	0.583 ± 0.005	2.64 ± 0.03	61.5 ± 0.6	24.6 ± 0.2
Non Pareil	NP	53.10	6.79 ± 0.10	0.436 ± 0.006	2.03 ± 0.03	71.8 ± 1.1	20.9 ± 0.3
Peraleja	PE	56.34	7.03 ± 0.46	0.423 ± 0.029	2.70 ± 0.27	62.4 ± 6.1	21.9 ± 2.0
Primorskyi	PR	58.62	6.24 ± 0.37	0.344 ± 0.008	1.34 ± 0.05	74.8 ± 3.7	20.1 ± 1.6
Ramillete	RA	60.60	8.09 ± 0.17	0.604 ± 0.011	1.49 ± 0.05	74.2 ± 2.5	18.5 ± 0.6
Texas	TE	55.60	5.92 ± 0.14	0.362 ± 0.008	2.60 ± 0.18	56.4 ± 3.6	28.8 ± 1.7
Titan	TI	56.10	5.20 ± 0.10	0.319 ± 0.006	1.13 ± 0.03	58.8 ±2.1	16.8 ± 1.0
Tuono	TU	54.80	5.19 ± 0.02	0.275 ± 0.002	1.97 ± 0.05	59.5± 1.0	12.5 ± 0.2
Wawona	WA	55.87	5.14 ± 0.16	0.358 ± 0.011	1.12 ± 0.03	64.0 ± 1.3	15.2 ± 0.3

fatty acid composition from all almond cultivars were previously autoscaled and then used for the principal component analysis. The appropiate principal component number was selected by application of several criteria: scree test (Cattel, 1966), mean eigenvalue (Cela, 1994) and indicator function (Malinowski, 1990). For cluster analysis, we used a matrix consisting of the squared Euclidean distances between objects and a hierarchical agglomeration method: the average linkage method (Afifi and Clark, 1990). From all individual data of almond cultivars the discriminant functions were derived by direct method (Tabachnick and Fidell, 1992) with a probability 0.333 for each group.

RESULTS AND DISCUSSION

Total fat content of all almond cultivars studied ranged between 53% and 62% (referred to dried samples), which means that the fat is the major fraction of the seed. Riquelme (1982) and Romojaro et al. (1977) report similar results. Non Pareil almonds, originally from America, have the lowest fat content (53%), followed by Texas, which is also an American almond, and the Italian Tuono. The fattiest almonds are the Spanish Ramillete and the French Ferragnes, the fat contents of which reached 60% and 62%, respectively. Due to the rather high level of fat in all almonds in general, it is not likely that this parameter can be useful in almond characterization.

The peaks for all fatty acid methyl esters are completely resolved in the chromatographic conditions stated.

Analytical calibration functions are linear ($r > 0.999$) in general up to 100 μg of fatty acid/g of total fat. Precision of analysis is better than 5% within-day and is between 5% and 10% from day to day.

Mean values of major fatty acid methyl ester contents, from data obtained for three samples independently extracted, derivatized, and analyzed, are given in Table 1. After the conversion of data of triglycerides, it is found that the total fatty acid composition in no cultivar differs significantly from 95% (which can be considered the total content of triglycerides in almond), even at a probability level of $p = 0.1$. Clearly, two unsaturated acids (C18:1 and C18:2) account for more than 90% of the total fatty acids. Unsaturated acids do not contribute to cholesterol in the blood (Mata, 1994), which may be the reason for the interest in almonds and other nuts in the human diet (Sabate et al., 1993).

A preliminary test for the possibilities of differentiation and/or association of different cultivars is made by analysis of variance. Comparison of all possible pairs of individual values of each fatty acid in every cultivar allows us to establish associations based on homogeneity of variance, at a probability level of $p = 0.05$. The number and size of these associations depend on the fatty acid considered. Figure 1 shows eight associations formed among cultivars with comparable mean C16:0 and C18:1 values. Cultivars are ordered according to the increasing fatty acid content from top to bottom. The greater the ability of a defined fatty acid to differenciate cultivars or groups of cultivars is, the less overlapped the bars are. It can be seen that C16:0 and C18:1 bring about eight associations, although they have very different significances. Thus, as to C16:0 the groups 6−8, considered either as individuals or as a whole, appear to be clearly different from the groups 1−3 (note that the former larger group covers practically all of the Spanish cultivars studied). In contrast, the groups formed on the basis of C18:1 fatty acid content show clearly a higher overlapping. Similar graphs have been obtained for the other fatty acids. From a comparison of them, we can state that the relative capacity of fatty acids to reveal differences and/or similarities among cultivars follows the order C16:0 $\approx$ C16:1 $\approx$ C18:2 $\approx$ C18.0 $>$ C18:1. Since all fatty acids contribute to different extents to differentiate and/or associate cultivars, multivariate techniques are likely to be the most appropiate to this purpose.

By means of principal component analysis three new variables are obtained. It is found that the variance explained by the first three principal components was 48.2%, 29.4%, and 11.3%, respectively, so that the first three principal components accumulate 89% of the total variance. The communality values, which are judged as an index of explanation of original variables in the new ones, are over 0.81 for all of the fatty acids, which compares well enough with the accepted value as a sufficiently good explanation of 0.3. Table 2 shows loadings or weighted contributions of different fatty acids to the first three principal components. It appears that C16:0 is the fatty acid predominant in the first component, followed by C18:2, C16:1, and C18:0. The second principal component is mainly determined by C18:1 and C18:0. The third principal component is

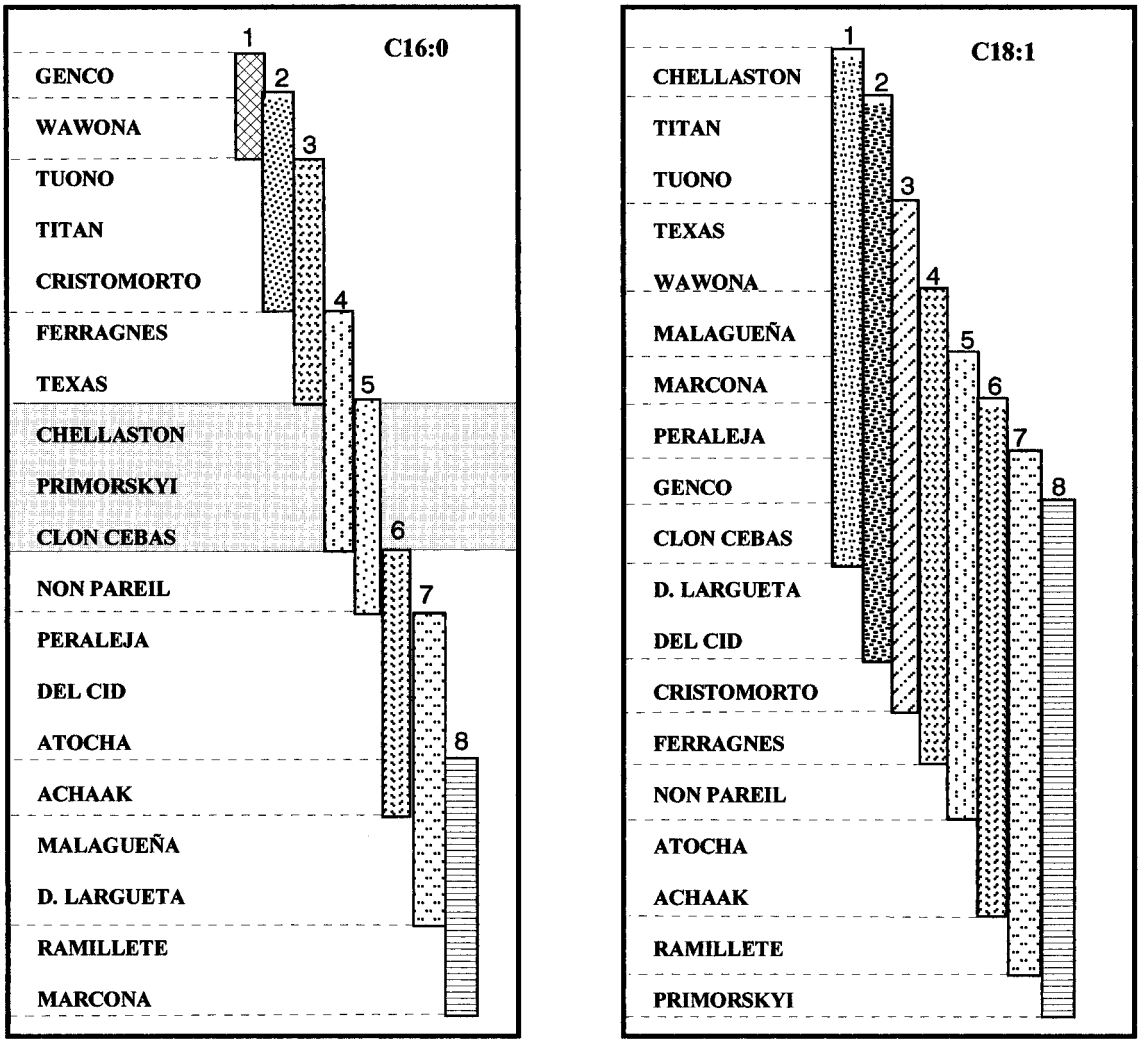

Figure 1. Plot for associations, in bar form, based on homogeneity of variance for fatty acids C16:0 and C18:1. The shaded band separates two completely diffferent associations, one formed by groups 1−3 and another one formed by groups 6−8. Cultivars Chellaston, Primorskyi, and Clon Cebas should be considered intermediate as to C16:0 fatty acid.

Table 2. Loadings of the First Three Principal Components

	principal component		
variable	PC1	PC2	PC3
C16:0	0.926	0.111	0.085
C16:1	0.690	0.461	−0.523
C18:0	0.593	−0.648	−0.208
C18:1	0.376	0.796	0.326
C18:2	0.764	−0.440	0.370

determined to a great extent by C16:1. Figure 2 shows a projection of different cultivars of almonds in the reduced space determined by the three principal components. There are some apparent associations, but we also find peculiar relative isolations of some cultivars apart from others with a common geographic origin, such as Marcona and Ramillete.

Cluster analysis allows a classification of the studied almond cultivars in the groups previously suggested by principal component analysis. According to this algorithm, three groups of different size can be established at dissimilarity over 15, as the dendogram in Figure 3 reveals. The first group includes the cultivars Titan, Wawona, Tuono, and Genco. A second group consists of Chellaston, Texas, Peraleja, Malagueña, and Marcona. The third and largest group is formed by Atocha, Del Cid, Desmayo-Largueta, Non Pareil, Achaak, Clon CEBAS, Ferragnes, Cristomorto, Primorskyi, and

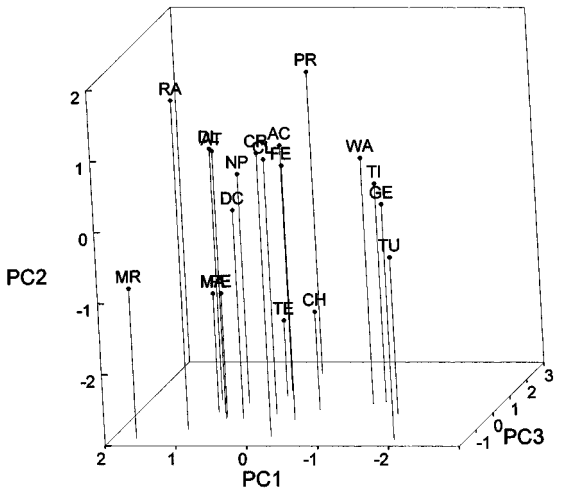

Figure 2. Plot of the mean scores of almond cultivars projected on the reduced space of the three principal components (PC1, PC2, and PC3).

Ramillete. It can be seen, however, that Spanish cultivars Marcona and Ramillete appear somehow differentiated from the rest of almond cultivars of their groups, so that they can be considered as two new singular groups at a dissimilarity under 13.

Discriminant analysis of all individual data within every previously established group, except Marcona and

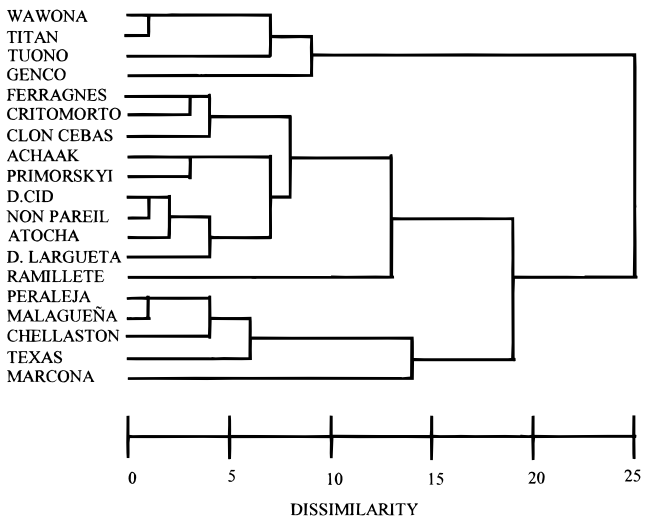

Figure 3. Dendogram showing the results of cluster analysis.

Table 3. Standardized Canonical Discriminant Function Coefficients

	discriminant function coefficient	
variable	function 1	function 2
C16:0	−0.245	0.331
C16:1	−0.045	0.532
C18:0	0.900	0.034
C18:1	−0.618	0.507
C18:2	−0.751	−0.364

Ramillete, leads to two discriminant functions, which account for 68.53% and 31.47% of total variance, respectively.

Matrix of standardized canonical discriminant function coefficients is given in Table 3. The first discriminant function is mainly influenced by the C18 fatty acids; the second discriminant function is determined by the C18 and C16 fatty acids with a similar global contributions. Application of the calculated discriminant functions gave entirely correct (100%) assignment of each member to its group.

Figure 4 is a plot of individual data on the plane determined by the two discriminant functions, wherein cultivars appear associated in a similar way as it is concluded from cluster analysis, suggesting once again three main groups of almonds. This consistency of two different algorithms, cluster and discriminant analysis, may be considered as a mutual validation of the above postulated associations.

The meaning of these associations is open to discussion and further research. Clearly the largest group includes many almond cultivars encountered in the Mediterranean area. This area is often considered as the near origin of almond cultivation and until recent years was the main source of this seed in the world. It is no wonder that almonds from this area, where they have been propagated through seeds in a traditional way, exhibit little differences as to major components of their main fraction, namely the fatty fraction. Cultivars introduced first by Europeans in America and Australia are often carefully selected, and therefore more differentiated, although they remain related with the Mediterranean ones, as some associations indicate. Tuono and Genco, both autocompatible and from the same geographic origin, are strongly associated with Titan and Wawona, constituting a characteristic group. Peraleja and Malagueña, also from the same geographic region, on the other hand, are associated with Texas

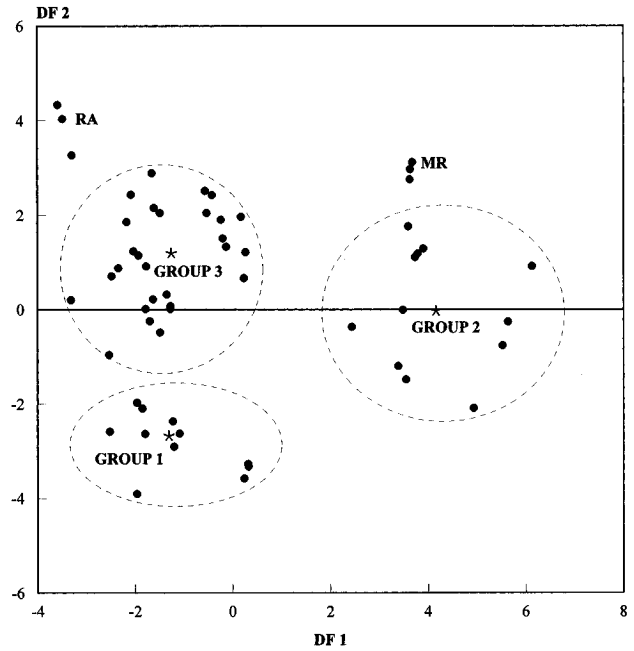

Figure 4. Plot of the mean scores of almond cultivars projected on the reduced space of the two discriminant functions (DF1 and DF2). Group 1, TI, WA, TU, GE; group 2, CH, TE, PE, MA; group 3, AT, DC, DL, NP, AC, CE, FE, CR, PR; ungrouped, RA and MR.

(also known as Mission, likely suggesting its method of introduction into America), and Texas is associated with Chellaston, probably indicating a traceable common origin. The Ferragnes and Cristomorto cultivars are grouped at a dissimility level under five from cluster analysis; the former is known genetically as Cristomorto × Aï.

On the contrary, the American cultivar Non Pareil is classified within the group labeled here as Mediterranean, together with the Spanish almonds, due to its similar fatty acid composition. Non Pareil is commercialized in Spain and used frequently instead of or mixed with other Spanish cultivars traditionally employed in the nugat industry. The similarity of these almonds was also demonstrated elsewhere taking into account the free amino acid composition (Prats and Berenguer, 1994). Non Pareil should be considered as less differentiated from the Mediterranean almonds.

Finally, it is worth noting that Spanish Ramillete and Marcona show such peculiar fatty acid compositions that it is difficult to associate them with any other cultivar in this respect.

CONCLUSION

Profile of the fatty acid components of almond cultivars is likely to be a useful base to classify the almond cultivars in three groups. It can be expected that minor fatty acid composition will provide subsidiary confirmation criteria. Work in this direction is now in progress.

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