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Chemometric Classification of the Fat Residues from the Conditioning Operations of Table Olive Processing, Based on their Minor Components

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ABSTRACT: The work characterizes the unsaponifiable matter of fats released in pitting green (GP) and ripe (RP) olives, in pitting/stuffing green olives with vegetable (GPSV) and animal (GPSA) products and in the fat settled at the end of the factory sewer system (W). The unsaponifiable matter ranged from 1.94% (RP) to 5.91% (GPSA); total sterols from 1319 mg/kg fat (GPSV) to 2002 mg/kg fat (RP), with β-sitosterol as the most abundant. Fatty alcohols ranged from 242 mg/kg (GP) to 556 mg/kg (W), with C22 as the most abundant. Triterpene diols were found only in GPSV (erythrodiol + uvaol, 0.80%). Wax was not present in the fats from GP and RP but increased with the general fat degradation in the order GPSV (128 mg/kg), GPSA (171 mg/kg) and W (263 mg/kg). Chemometric analysis was able to detect differences among the diverse fats; sterols + fatty alcohols cluster analysis was useful for fat grouping.

KEYWORDS: chemometric analysis, conditioning process, cluster analysis, discriminant analysis, fatty alcohols, oil composition, principal component analysis, processing, sterols, table olives, triterpene diols, wax

INTRODUCTION

The world production of table olives was 2,082,500 tons in the 2008/2009 season.[1] Spain is the main producer with about 25% of the total.[1] There are several styles, but only a few of them have reached commercial relevance.[2] Currently, presentations derived from green (Spanish style) and ripe olives (Californian style) are the most accepted by consumers and international markets. Green olives are a very stable fermented product which, due to its color, shape and texture, permits multiple mechanical operations (pitting, slicing, pitting/stuffing, etc.).[2] Ripe olives are also becoming very popular due to the contrast of their black color with the green of vegetable salads, but their number of presentations is reduced (usually, pitted and sliced).

Conditioning operations include separation of damaged or unmarketable fruits, grading by size, and pitting or pitting/stuffing, but only in the last two operations a certain proportion of fat is released from the olives. Pitting is a common operation for both green and ripe styles while pitting/stuffing is particularly applied in green olives. In table olive factories, stuffed olives are processed in two separate lines according to the stuffing material, one that is dedicated to vegetal sources (red pepper paste, natural red pepper, etc.) and another one that is dedicated to animal sources (anchovy paste, salmon paste, cheese, etc). Fat is emulsified in the solutions used for the separation of olives from pieces of stone or improperly pitted or pitted/stuffed fruits and accumulates in the respective tanks. Usually, the fat is removed at these places to cut down on pollution and prevent obstructions in the pipelines; however, inevitably, some residual fat goes into the sewers and is deposited at the end of the sewer system. Potential revenues from marketing these fats, which may reach about 1% of the olive weight, depend on the quality and destination of such byproduct. Due to their origin, fats from the conditioning operations cannot be considered as olive oils, but good characterization could nevertheless help to promote commercialization opportunities, particularly if its composition could be assimilated to any class of olive oil. Fatty acid composition of these products has been published;[3] however, minor components are also important in olive oil[4] and, possibly, in these fats, but no information on sterols, fatty alcohols, triterpene diols and waxes in them are yet available.

Usually, sterols are among the compounds studied for the classification of olive oils.[5] The contents and relationships of specific sterols in the diverse categories of extra virgin, virgin and lampante olive oils have been included in the EC Regulations[6,7] and IOOC standards.[8] Detailed information on the sterols, fatty alcohols, and triterpene diols in diverse commercial presentations of table olives have been published recently,[9] and the overall composition was similar to that found in olive oil.[4] A study of the changes in unsaponifiable matter, sterols and alcohols during the diverse steps of ripe olive processing has shown that these compounds may suffer significant changes in processing, particularly in the storage phase.[10] The sterol and alcohol content in Cornicabra cv. showed that the proportion of campesterol was above the 4% limit set by the EC Regulations[6,7] and IOOC standards,[8] indicating that the variability of these compounds could be higher than that originally considered when establishing the legislation.[11] The free sterol composition has been used for the detection of hazelnut oil in virgin olive oil.[12] The methodology has been extended to the detection of other refined vegetable oils in olive oil adulteration (proportions above 5%) based on the contents in campesterol and stigmasterol.[13]

Chemometric techniques are usual in chemical characterization/differentiation of products when the number of variables under study is high. Principal component analysis and soft

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Table 1. Types of Fat Residues Produced during the Conditioning Processes of Table Olives

acronym	description of the fat residue
GP	fat released in the pitting/slicing conditioning of green table olives
RP	fat released in the pitting/slicing conditioning of ripe table olives
GPSV	fat released in the pitting/stuffing conditioning line of green table olives with vegetable origin stuffing material
GPSA	fat released in the pitting/stuffing conditioning line of green table olives with animal origin stuffing material
W	fat from the end of the sewer system

independent modeling class analogy (SIMCA) based on triacylglycerols and sterols have been used for the typification of a variety of olive oils belonging to Spanish origin denominations.[14] A new approach to the geographical characterization of virgin olive oils based on the NMR fingerprint of the unsaponifiable fraction of virgin olive oils has been recently proposed for authentication purposes.[15] The sterol profiles in extra virgin olive oils from *La Comunitat Valenciana*, obtained by high performance chromatography with mass spectrometry detection, have been used for their classification according to genetic variety.[16]

The aim of this work was to study the unsaponifiable matter and its components (sterols, alcohols, and waxes) in the fats released during pitting and pitting/stuffing operations of table olive processing and in the fat settled at the end of the waste pipelines of these industries. A general linear model (GLM) as well as unsupervised (hierarchical clustering and principal component analysis) and supervised (discriminant analysis) chemometric methods were used for their grouping, characterization and detection of differences.

MATERIALS AND METHODS

Cultivar and Maturation Degree. Fat used in this work belonged to Manzanilla cv., which is the most popular Spanish cultivar for preparing green table olives, and is also used for ripe olives. The green maturation degree fruits followed the normal green (Spanish style) or ripe olive (Californian style) processing before being subjected to the conditioning operations.

Conditioning Operations and Samples. Two different conditioning operations were considered: pitting (green and ripe olives) and pitting/stuffing (lines using products from vegetal, mainly red pepper paste, and animal origin, anchovy paste). So, the number of fats coming from these conditioning operations was four. However, not all the fat released during these processes can be separated and recovered; a certain proportion continues emulsified in the liquid residues and is progressively separated at the end of the waste pipes or in the homogenization tank. It constitutes the fifth type of fat studied. This fat is related to the previous ones but cannot be accurately identified due to the complex wastewater system and diverse operational conditions of the factory. The acronyms used for their identification are shown in Table 1. Oil samples were obtained from the centrifuges used for the fat separation in the conditioning facilities (GP, RP and GPSV) while that from GPSA and W were obtained with a skimmer and ladle, respectively. Several samples from pitting and pitting and stuffing processes were taken along the working day and combined. Sample W was composed of diverse subsamples from the fat layer of the homogenization tank. Fats were warmed slightly, when necessary, and then filtered. An aliquot of the filtered fat was used for analysis. Sampling was repeated twice. In this way, samples mimic the oil accumulated in the industrial conditioning process which is a mixture of fats from different batches and working days.

Determination of the Unsaponifiable Fraction. The unsaponifiable matter was determined, according to UNE 55004 standard method,[17] by saponification of the oil with potassium hydroxide in an ethanolic solution, followed by extraction with diethyl ether.

Determination of Sterols and Triterpene Diols. This analysis was performed according to the method described by the Official Journal of the European Communities.[18] The lipid with added α-cholestanol and betulin as internal standards was saponified and the unsaponifiable matter was extracted as mentioned above. The bands corresponding to the sterol and triterpene diol fractions were separated from the extract by TLC on a basic silica gel plate. The bands corresponding to sterols and uvaol + erythrodiol were identified, under UV light, by comparison of the sample chromatogram with that of a solution of α-cholestanol and betulin run in parallel. Both were scraped together. The sterols and erythrodiol and uvaol recovered from the plate were transformed into trimethylsilyl ethers, and the mixture was analyzed by GC using an HP 5890 series II gas chromatograph equipped with a flame ionization detector and a 30 m × 0.32 mm i.d. Tracsil TRB-5 (95% dimethylpolysiloxane−5% diphenyl, film thickness 0.25 μm) capillary column (Teknokroma, Barcelona, Spain). The chromatographic conditions were as follows: injector 300 °C, isothermal column 275 °C, and detector 300 °C. The split ratio was 1:50. Hydrogen carrier gas was used at 1.0 mL/min.

Determination of Fatty Alcohols. This analysis was performed according to the method described by the Official Journal of the European Communities.[19] The fatty substance, with 1-eicosanol added as internal standard, was treated as mentioned in Determination of the Unsaponifiable Fraction. The alcohol fraction was separated from the unsaponifiable matter by chromatography on a basic silica gel plate. The band was identified, under UV light, by comparison of the sample chromatogram with that of a solution of 1-eicosanol run in parallel. The alcohols recovered from the silica gel were transformed into trimethylsilyl ethers and analyzed using capillary gas chromatography. The chromatographic conditions were the same as those mentioned above for sterols and triterpene diols, except that oven temperature was as follows: 215 °C (5 min); 3 °C/min increase to 290 °C and held for 2 min. All analyses were performed in duplicate.

Determination of Wax Content. This analysis was performed according to the method described by the Official Journal of the European Communities.[20] In short, the method consists of the addition of lauryl arachidate internal standard to the fat, then fractionation by chromatography on a hydrated silica gel column. The fraction eluted first (the polarity of which is less than that of the triacylglycerols) was recovered under the test conditions and then subjected to direct analysis by capillary column gas chromatography. Wax esters were quantified using 1.2 response factor to account for the increased response of the internal standard due to acylation. Peaks corresponding to ester C_{36} and C_{38} were discarded while esters C_{40}−C_{46} were added and the value expressed as wax.

Chemicals. All reagents were of analytical grade and chromatographic grade, according to the step.

Statistical Analysis. Data were arranged in a matrix array, where rows were cases (types of fats × replicates) and columns were variables (unsaponifiable matter, sterols, fatty alcohols, triterpene diols, and waxes). The concentration of not detected compounds was set to 0 for statistical analysis when appropriate. The different groups were first subjected to GLM (one-way ANOVA) to estimate the means and

Table 2. Proportion of Unsaponifiable Matter and Their Main Groups of Components, According to the Type of Fat Residue Produced during the Conditioning Operation of Table Olives[a]

types of fat	unsaponifiable (%, w/w)	total sterols (mg/kg)	total fatty alcohols (mg/kg)	erythrodiol + uvaol (mg/kg)	total wax (mg/kg)
GP	3.42 (0.07) a	1489 (66) a	242 (37) a	nd	nd
RP	1.94 (0.07) b	2002 (66) b	306 (37) a	nd	nd
GPSV	4.46 (0.07) c	1319 (66) a	314 (37) a	15 (1)	128 (7)
GPSA	5.91 (0.07) d	1736 (66) c	446 (37) ab	nd	171 (7)
W	3.54 (0.07) a	1366 (66) a	567 (37) b	nd	263 (7)

[a] See Table 1 for acronym meanings. Data are the average ($n = 2$) of replicates; pooled standard error for type of compound (column) in parentheses; nd, not detected. Data with different superscripts are different at $p < 0.05$

standard error of the diverse variables as well as to detect significant differences among types of fat. Effects of the five conditioning operations also include the variability derived from fermentation/storage and possible different maturation degrees.

Data were first studied by multiple analyses of variance (MANOVA) to test overall differences between groups across the different variables. Then, data were standardized using the auto scale procedure, which is the most commonly used scaling technique. The procedure standardizes a variable m according to

$$y_{mj} = \frac{(x_{mj} - \bar{x}_m)}{s_m}$$

where y_{mj} is the value j for the variable m after scaling, x_{mj} is the value j of the variable m before scaling, $\bar{x}_m$ is the mean of the variable m and s_m is the standard deviation for the variable m. The result is a variable with zero mean and a unit standard deviation.

Then, standardized data were subjected to hierarchical cluster analysis, principal component analysis (PCA) and discriminant analysis (DA). PCA was carried out using a varimax rotation to detect the data structure, to determine the relationships among the different components, to derive common dimensions in order to classify the samples and to map the different population groups into these dimensions. For the selection of the number of principal components (PCs), the Kaiser criterion[21] was followed and only factors with eigenvalues higher than 1.00 were retained. Then, the loadings of the original variables were projected onto the plane formed by the first and second components or in 3D (three components).

The selection of variables containing the most powerful information for the correct classification of the fat samples from the conditioning operations was carried out using the backward stepwise analysis option, which first includes all the variables in the model and then, at each step, eliminates the variable that least contributes to membership prediction. The process continues until only the important variables that contribute most to discrimination between groups are in the model. The values of probability to enter or to remove were fixed at 0.05 and 0.10, respectively. The number of steps was fixed at 100, the minimum tolerance at 0.001, and no variable was forced to enter into any model.

DA classification was achieved by means of the corresponding classification functions. For k groups, k linear combinations of variables are constructed, called classification functions. The calculation of the values of these functions for each sample makes it possible to allocate this sample to the group for which the probability of belonging is the highest. Prior probabilities were the same for each group.

A leaving-one-out cross validation procedure was performed for assessing the performance of the classification rule. In this step, the sample data minus one observation was used for the estimation of the classification functions and then the omitted variable was classified from them. The procedure was repeated for all samples. Consequently, each sample was classified by classification functions which were estimated without its contribution.[22] However, the objective of this analysis was always more related to the detection of differences than to application to future classification. Later, a canonical analysis of data was achieved. The scores of fat samples were plotted on the canonical axes (discriminant coordinates, called functions). These axes were determined in such a way that the rate of the variance between groups compared to the variance within groups was maximized.[22]

The different statistical techniques used in this work were implemented using STATISTICA, release 6.0 (GLM, PCA and clustering analysis), and SYSTAT, release 10.2 (DA analysis).

RESULTS AND DISCUSSION

The statistically lowest ($p < 0.05$) proportion of unsaponifiable matter (Table 2) was obtained in the ripe olive fat (RP) while the highest was found in GPSA, coming from anchovy stuffed olives. Apparently, the trend observed was not related to the conditioning operations. The concentration of unsaponifiable matter was lower in W than in the fats released in the pitting/stuffing conditioning lines; this may indicate that part of the substances that contributed to the unsaponifiable matter may have been degraded or partially dissolved as the fat moved forward in the sewer system. The unsaponifiable matter in the fat from the animal origin pitting/stuffing line was lower than in the GPSV fat. Overall, the unsaponifiable matter in the fats from the conditioning processes was within the range found in the oils from table olives (~9%, ripe, and 2%, directly brined).[9] However, the total unsaponifiable matter found in the diverse steps of ripe olive processing was never above 1.5%,[10] approximately of the same order as the proportion in RP (Table 1). The similarity between the values in ripe olive fat and in the fat released after pitting shows that it is unlikely that pitting might affect the unsaponifiable fraction. The unsaponifiable matter in the fat byproduct studied in this work was higher than in olive oil, in which the percentage is usually around 1%;[4] however, this level cannot affect the fat byproduct classification because there is no limit for this parameter in the EC Regulations[6,7] and IOOC Standards[8] for olive oils.

Total sterols in GP, GPSV and W fats were similar (at $p < 0.05$) and showed the lowest concentrations while the highest value was observed in RP (Table 3). The values were above the limit of 1000 mg/kg established in the EC Regulations[6,7] and IOOC Standards,[8] and, as result, the oils released during the conditioning do not have any limitation with respect to sterols. The concentrations found here are higher than those in the oils from the diverse steps of Manzanilla and Hojiblanca ripe olive processing, although the last cultivar had marked total sterols (2543 mg/kg) after sterilization,[10] which is higher than any value obtained in this study but fairly close to that from ripe olive conditioning fat (RP). However, the high content in RP can hardly be attributed to processing only because, in this case, the olives, apart from being from another cultivar, were not sterilized.

Table 3. Average Content of Specific Sterol in the Fats Released in the Table Olive Conditioning Operations, According to Treatments, and Univariate ANOVA Results for Comparisons within Each Component[a]

	type of fat					comparison among treatments[b]	
sterols (mg/kg)	GP	RP	GPSV	GPSA	W	*F* value	*P* value
cholesterol	11.7 (3.8)	18.3 (3.8)	18.4 (3.8)	216.6 (3.8)	33.2 (3.8)	547.68	0.000
24-methylenecholesterol	nd	12.0 (0.7)	5.9 (0.7)	6.4 (0.7)	nd	47.28	0.000
campesterol	39.4 (2.5)	52.4 (2.5)	34.5 (2.5)	41.9 (2.5)	34.9 (2.5)	8.82	0.017
campestanol	9.7 (0.8)	11.6 (0.8)	10.7 (0.8)	9.3 (0.8)	10.1 (0.8)	1.16	0.427
stigmasterol	17.0 (0.7)	12.5 (0.7)	21.2 (0.7)	25.2 (0.7)	21.8 (0.7)	47.29	0.000
clerosterol	14.3 (2.8)	18.6 (2.8)	16.2 (2.8)	14.4 (2.8)	35.8 (2.8)	10.47	0.012
β-sitosterol	1327.5 (58.7)	1773.1 (58.7)	1128.7 (58.7)	1321.3 (58.7)	1176.5 (58.7)	18.84	0.003
sitostanol	13.5 (2.3)	23.2 (2.3)	11.7 (2.3)	4.7 (2.3)	9.8 (2.3)	8.96	0.017
Δ^5-avenasterol	39.5 (2.5)	58.6 (2.5)	51.9 (2.5)	59.7 (2.5)	39.2 (2.5)	15.35	0.005
$\Delta^{5,24}$-stigmastadienol	7.5 (0.7)	9.8 (0.7)	10.9 (0.7)	10.0 (0.7)	nd	45.76	0.000
Δ^7-stigmastenol	6.0 (0.7)	9.3 (0.7)	5.7 (0.7)	21.8 (0.7)	4.2 (0.7)	120.65	0.000
Δ^7-avenasterol	4.1 (1.4)	3.0 (1.4)	4.3 (1.4)	4.8 (1.4)	nd	1.97	0.238

[a] Data are the average ($n = 2$) of replicates; standard error in parentheses; nd, not detected. Degree of freedom for comparison: 4 and 3 (for rows with not detected compounds). [b] Except between samples with not detected compounds.

In oils from table olives, however, the concentrations of total sterols were always slightly above the levels found in any fat from the conditioning operations.[9]

The total fatty alcohols in GP, RP, GPSV, and GPSA were relatively low and statistically similar while the greatest content ($p < 0.05$, except with respect to GPSA) was detected in W (Table 2), probably due to the marked degradation suffered by this fat. The concentrations of fatty alcohols in oils from green, directly brined and ripe table olives[9] were higher than those found in the conditioning operations. In the ripe olive processing, fatty alcohol contents in the fats from Hojiblanca were low (minimum of 136 mg/kg) but fairly high in that from Manzanilla (854 mg/kg).[10] Overall, the levels found in the fats released from the conditioning processes have, approximately, values of total fatty alcohols which could be expected in the fat from Manzanilla and Hojiblanca cv. olives. No limits for total fatty alcohols are mentioned in the EC Regulations[6,7] and the IOOC Standards.[8]

The presence of wax was detected in only three of the conditioning fats, which were GPSV, GPSA and W in order of increasing proportions (Table 1). The limits for wax in olive oil in EC Regulations[6,7] and IOOC standards[8] is 250 mg/kg while that for lampante is 300 mg/kg. So, according to this parameter, GPSV and GPSA fats can be classified as olive oil but W fat should be considered as lampante olive oil.

The GLM analysis of the individual sterol components (Table 3) indicated significant differences among the diverse conditioning fats except in campestanol and Δ^7-avenasterol. In addition, 24-methylenecholesterol was not detected in some fats (GP and W); its absence in W may indicate an apparent degradation (or partition between the aqueous and fat phases) of sterols along the sewer system. The highest individual sterol content in all fats was observed for β-sitosterol (Table 3). Cholesterol was present in low concentrations in all fats and its levels increased in the order GP < RP < GPSV (Table 3). However, the effect of stuffing material was apparent because cholesterol reached a marked high level in GPSA (Table 3). The W fat had an intermediate value, possibly because its composition was the result of the mixture of all released fats (thoroughly mixed in the homogenization tank) or because of an eventual degradation or partition between the aqueous and fat phases. Low concentration of cholesterol in table olive fat was also found in a survey of commercial presentations[9] and during the diverse steps of ripe olive processing.[10] This cholesterol may come from the microbial populations that always grow during olive brining regardless of the style. The levels of other sterols may be found in Table 3.

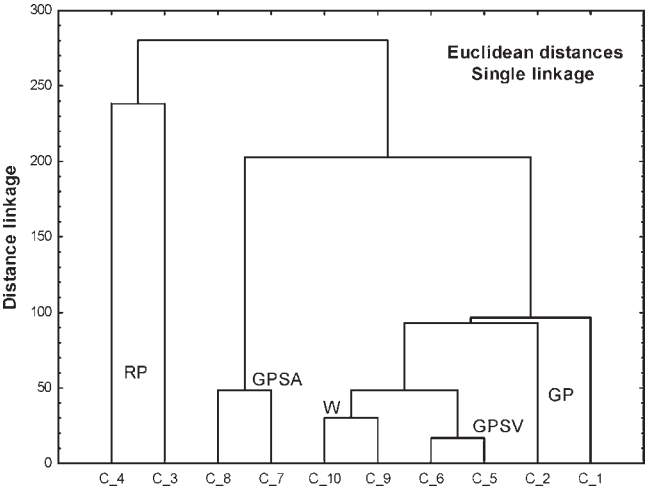

Figure 1. Tree diagram of case linkage according to the Euclidean distances, based on sterol concentration (mg/kg fat).

The previous GLM analysis and the appropriate MANOVA showed that there were significant differences among samples and that they could be submitted to a chemometric analysis. A first approach may be a hierarchical clustering analysis (Figure 1). Most of the cases were correctly assigned except for cases 1 and 2, from GP, which were not linked to the same group; C2 was linked to the higher order group formed by W and GPSV, and C1 was linked to the group formed with C2, W, and GPSV. The relatively high linkage distance (~200) of this subgroup with sample GPSA is apparent. The distance between the higher level subgroup with RP was also relevant (~280 Euclidean distance).

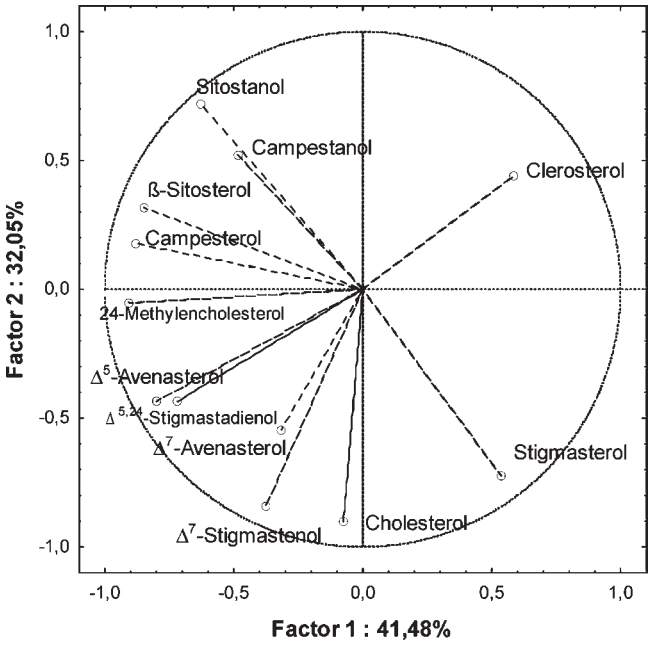

Figure 2. Projections (loadings) of the sterol components in the first two principal components (PCs).

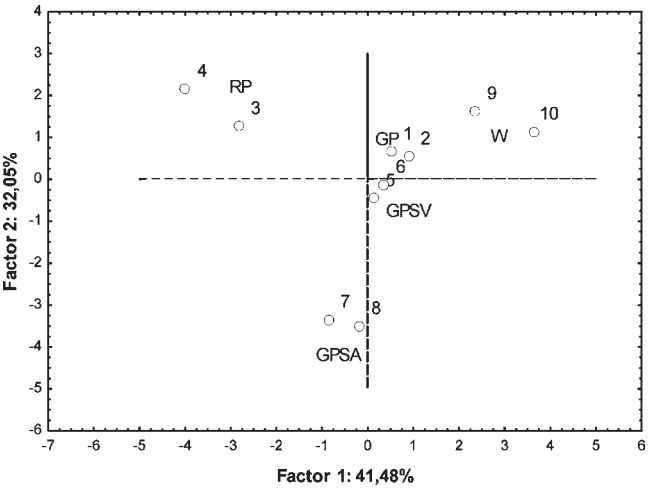

Figure 3. Plots of the treatment scores as a function of the first two PCs.

The application of PC analysis showed that there were 3 eigenvalues higher than 1 which accounted for 87.35% of the total variance. The three factors were defined as a function of the variables; however, reduction of variables was not evident. The relationships among variables (sterols) and between these with the PCs can also be obtained from the polar graph of variables on the plane of the first two PCs (Figure 2). The sterols more negatively related with function 1 were 24-methylenecholesterol, campesterol, Δ^5-avenasterol, β-sitosterol and $\Delta^{5,24}$-stigmastadienol while clerosterol was positively associated with this PC. Those negatively related to function 2 were cholesterol, Δ^7-stigmastenol, stigmasterol and Δ^7-avenastenol, but sitostanol or campestanol had a positive relationship. The angle between sterols may indicate their relationships; there was no link between clerosterol and stigmasterol (they form an angle of about 90° with a very low cosine). Similarly, β-sitosterol was scarcely related to Δ^7-stigmastenol. On the contrary, β-sitosterol was closely related to campesterol or 24-methylenecholesterol, Δ^5-avenasterol and $\Delta^{5,24}$-stigmastadienol.

The projection of the sample scores on the plane of the first two PCs may indicate the discrimination ability of this analysis (Figure 3). There is good separation among samples RP, GPSA and W; however, samples belonging to GP and GPSV were fairly close. This low discrimination capacity of PCs for the last two fat types is in agreement with their origins and with results described in the previous dendogram (Figure 1), in which difficulties for linking samples from GP were observed.

A DA, which is a more specialized tool for describing differences, was then applied to check the possibilities of a better discrimination between the fat groups not well separated by PC analysis. The variables retained for discrimination were cholesterol, 24-methylenecholesterol, campesterol, campestanol, and stigmasterol (Table 4). The discriminant functions obtained led to a 100% correct assignation of cases although the ability for future assignations was fairly lower (Table 5). However, the predictive DA analysis was able to find differences among the samples.

The loads of the different variables with respect to discrimination were obtained from the standardized canonical functions (Table 4). Cholesterol, campestanol and stigmasterol were the main variables contributing to function 1; 24-methylenecholesterol to function 2, and campesterol to function 3. A graphical presentation of the discriminant ability of canonical functions may be obtained by plotting the case loads onto the plane of the two functions. The analysis correctly grouped the samples from the diverse types of fat but those corresponding to GP, GPSV and W were moderately close (data not shown). Further refinement was achieved by using the first three canonical functions (Figure 4). In this way, a perfect separation of the different fats in 3D was obtained. Results from both PCs and DA show that, overall, there are sterol differences among the samples (expressed in mg/kg) which are moderately low, although detectable, in 2D but quite clear in 3D.

Usually, the composition of sterols is given as a percentage of the total sterols because the EC Regulations[6,7] and IOOC Standards[8] use proportions when setting the limits for these compounds. In this study, the highest proportion corresponded to β-sitosterol in GPSA and GP samples while the lowest level was observed in GPSA, due to the high proportion of cholesterol in this fat (Table 6). However, the legislation does not refer to β-sitosterol alone but to the so-called apparent β-sitosterol (last row of Table 6), which is the sum of Δ^{5-23}-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ^5-avenasterol, and $\Delta^{5,24}$-stigmastadienol and must be higher than 93% regardless of the olive oil class. According to apparent β-sitosterol values only GP and RP fats could be considered olive oil. On the contrary, GPSV, W, and GPSA have values below the limit, due to the relatively high proportion of cholesterol, and cannot be classified as olive oil.

With respect to other sterols, cholesterol was above the limits in all fat types, particularly in GPSA. The increase in cholesterol in the oil from table olives has already been observed in a previous work,[9] particularly in Manzanilla olives stuffed with anchovies (3.4 mg/100 g edible portion), salmon (2.6 mg/100 g e.p.), ham (1.7 mg/100 g e.p.) or tuna (1.1 mg/100 g e.p.) when expressed as percentage of oil. Ripe olive processing also increased the cholesterol content with respect to the raw material; but, in this case, the levels in the final products were always lower[10] than those found in this study (Table 6). Limits for campesterol, stigmasterol, and Δ^7-stigmastenol (except for GPSA) always

Table 4. Discriminant Analysis Based on Sterol Contents: Retained Variables and Canonical Discriminate Functions

sterols	*F* to remove	tolerance	function 1	function 2	function 3	function 4
cholesterol	128.674	0.123	2.844	0.057	0.163	0.044
24-methylenecholesterol	11.098	0.290	0.044	1.457	−1.143	0.110
campesterol	0.943	0.285	0.745	−0.377	1.458	−0.588
campestanol	1.606	0.111	2.732	0.556	0.197	−0.479
stigmasterol	9.563	0.126	−2.330	−1.342	−0.728	−0.287

Table 5. Discriminant Analysis Based on Sterols: Classification Matrix (Cases in Row Categories Classified into Columns)[a]

	GP	RP	GPSV	GPSA	W	% correct assignation
GP	2 (1)	0 (0)	0 (0)	0 (1)	0 (0)	100% (50%)
RP	0 (0)	2 (0)	0 (0)	0 (2)	0 (0)	100% (0%)
GPSV	0 (1)	0 (0)	2 (0)	0 (1)	0 (0)	100% (0%)
GPSA	0 (0)	0 (1)	0 (0)	2 (1)	0 (0)	100% (50%)
W	0 (0)	0 (1)	0 (0)	0 (0)	2 (1)	100% (50%)
Totals	2 (2)	2 (2)	2 (0)	2 (5)	2 (1)	100% (30%)

[a] Jackknifed classification matrix in parentheses.

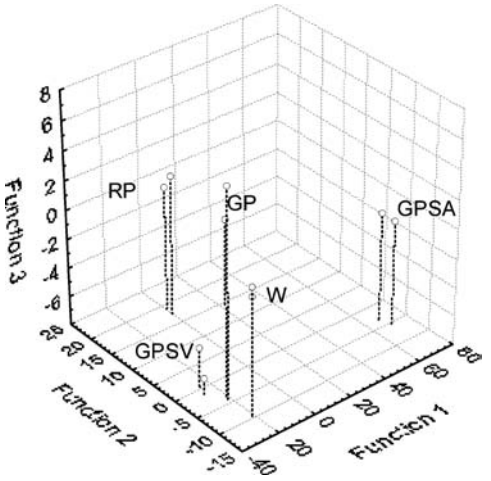

Figure 4. Plot of the samples as a function of the first three canonical discriminant functions, according to fat type.

were fulfilled. Overall, the sterol contents in the fats released from the conditioning operations were not in agreement with the EC Regulations[6,7] nor with the IOOC Standards,[8] mainly because of the cholesterol percentage which also decreased the apparent β-sitosterol in most of them. Therefore, proportions of sterols prevent these fats from being considered as olive oil.

However, these limits are currently being questioned because, as the number of studies increases, more results above the values originally established are reported. For example, it has been observed that the percentages of campesterol in carefully obtained extra virgin olive oils from Cornicabra cultivar were higher than the 4% legal maximum currently in force for this parameter.[11]

The chemometric analysis for the sterol content expressed as proportion was also carried out. Results from PC analysis and DA were similar to those obtained using the concentrations as mg/kg, but there was an interesting improvement when the hierarchical analysis was carried out (data not shown) because the grouping of cases and types of fats was apparently more realistic (each case was grouped within its fat, and there was not any wrong assignation). Furthermore, the linkage of GP and RP was fairly close. Since the total fatty alcohol contents have been commented on above, only the individual concentrations will be discussed (Table 7). The most abundant was docosanol, particularly in GP and GPSA samples. Hexacosanol showed high levels in all the pitting/stuffing processes and also had a remarkable level in W while the lowest content was detected in RP. The presence of tetracosanol followed the same trend as hexacosanol but with lower concentrations. Octacosanol had the lowest content in RP and highest in W (Table 7). Thus, degradation increased all the fatty alcohols proportionally.

The concentrations of total fatty alcohols found in this work were within the range of values reported for the fats from the different steps of ripe olive processing (136 mg/kg oil to 854 mg/kg oil);[10] GP, RP and GPSV data were of the same order as in the fat from raw material and the diverse processing steps of Hojiblanca cv. or sterilized Manzanilla while Manzanilla olives at the end of storage had higher values (854 mg/kg oil) than any of the fats included in this study.[10] In any case, the concentration of fatty alcohols in table olive fats followed a different order since the most abundant was octacosanol, followed by hexacosanol.[9] No limits with respect to fatty alcohols are set in the EC Regulations[6,7] or IOOC Standards.[8]

Triterpene diols (erythrodiol and uvaol) were detected only in sample GPSV (Table 8). However, erythrodiol was always present in all fats from the different steps of Manzanilla ripe olive processing, and was particularly high in the end product (~86.61 mg/kg). The levels in Hojiblanca were lower. However, uvaol was not detected in some fats and its content was lower.[10] In table olives, on the contrary, both triterpene diols were present in all commercial presentations.[9] The proportion of erythrodiol + uvaol in all fats from the conditioning operations was always far below the limit (<4.5%) established for this parameter in EC Regulations[6,7] or IOOC Standards;[8] therefore, such fats, when attending only this characteristic, may be included in any olive oil class.

Wax was not detected in GP and RP (only pitting) fats but had diverse concentrations in the other cases (Figure 5). No data related to the wax in fats from table olives or ripe olive processing is available. Its content must be ≤250 mg/kg for extra virgin or virgin olive oils and ≤300 mg/kg for lampante, according to the EC Regulations[6,7] and the IOOC Standards.[8] All fats considered in this study had wax values below the limits set in regulations; so, bearing in mind only this parameter, all of them can be included in any olive oil class.

An overall cluster analysis based on sterol and fatty alcohol contents was performed to study the general differences among fats. As shown in Figure 6, a correct assignation of cases was always found. The closest samples were those from GPSV fat,

Table 6. Average Percentage of Specific Sterols in the Fats Released in the Table Olive Conditioning Operations, According to Treatments[a]

	type of fat					
sterols (%)	GP	RP	GPSV	GPSA	W	EC and IOOC limits[b]
cholesterol	0.79 (0.23)	0.92 (0.23)	1.39 (0.23)	12.48 (0.23)	2.44 (0.23)	≤0.5; ≤0.5; ≤0.5
24-methylenecholesterol	nd	0.60 (0.02)	0.45 (0.02)	0.37 (0.02)	nd	
campesterol	2.64 (0.07)	2.62 (0.07)	2.61 (0.07)	2.41 (0.07)	2.55 (0.07)	≤4.0; ≤4.0; ≤4.0
campestanol	0.66 (0.06)	0.58 (0.06)	0.80 (0.06)	0.53 (0.06)	0.74 (0.06)	
stigmasterol	1.14 (0.04)	0.63 (0.04)	1.61 (0.04)	1.45 (0.04)	1.60 (0.04)	<campesterol
clerosterol	0.96 (0.20)	0.93 (0.20)	1.23 (0.20)	0.83 (0.20)	2.61 (0.20)	
β-sitosterol	89.06 (0.37)	88.54 (0.37)	85.51(0.37)	76.12 (0.37)	86.17 (0.37)	
sitostanol	0.91 (0.13)	1.16 (0.13)	0.89 (0.13)	0.27 (0.13)	0.72 (0.13)	
Δ^5-avenasterol	2.65 (0.08)	2.92 (0.08)	3.93 (0.08)	3.44 (0.08)	2.87 (0.08)	
$\Delta^{5,24}$-stigmastadienol	0.51 (0.05)	0.49 (0.05)	0.83 (0.05)	0.58 (0.05)	nd	
Δ^7-stigmastenol	0.41 (0.03)	0.46 (0.03)	0.43 (0.03)	1.26 (0.03)	0.31(0.03)	≤0.5; ≤0.5; ≤0.5
Δ^7-avenasterol	0.28 (0.07)	0.16 (0.07)	0.32 (0.07)	0.27 (0.07)	nd	
apparent β-sitosterol[c]	94.06	94.07	92.39	81.21	92.37	≥93.0; ≥93.0; ≥93.0

[a] EC Regulation[6,7] and IOOC Standard limits[8] are also included. Data are the average (n = 2) of replicates; standard error in parentheses; nd, not detected. [b] According to the EC Regulations[6,7] and IOOC Standards[8] for extra virgin, virgin and lampante olive oil, respectively. [c] Sum of $\Delta^{5,23}$-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ^5-avenasterol, and $\Delta^{5,24}$-stigmastadienol.

Table 7. Average Content in Fatty Alcohols (mg/kg fat), According to the Type of Conditioning Process[a]

	conditioning process					comparison among treatments	
fatty alcohols	GP	RP	GPSV	GPSA	W	*F* value	valor *p*
docosanol (C22)	208 (27)	286 (4)	215 (5)	370 (36)	335 (2)	970	<0.001
tetracosanol (C24)	11 (3)	7 (2)	36 (2)	27 (3)	60 (8)	242	<0.001
hexacosanol (C26)	18 (6)	10 (<1)	51 (8)	37 (9)	137 (41)	35	0.002
octacosanol (C28)	5 (2)	2 (2)	11 (2)	11 (2)	35 (9)	43	0.001
total alcohols	242 (39)	306 (5)	314 (7)	446 (49)	556 (55)	504	<0.001

[a] Data are the average (n = 2) of replicates; standard error in parentheses. Degrees of freedom for comparison: 4.

Table 8. Average Content in Triterpene Diols, According to the Type of Conditioning Process[a]

	conditioning process		
triterpene diols	erythrodiol (%)	erythrodiol + uvaol (mg/kg)	erythrodiol + uvaol (%)
GP	nd	nd	nd
RP	nd	nd	nd
GPSV	0.80 (0.05)	15.13 (1.14)	0.80 (0.05)
GPSA	nd	nd	nd
W	nd	nd	nd

[a] Data are the average (n = 2) of replicates; standard error in parentheses.

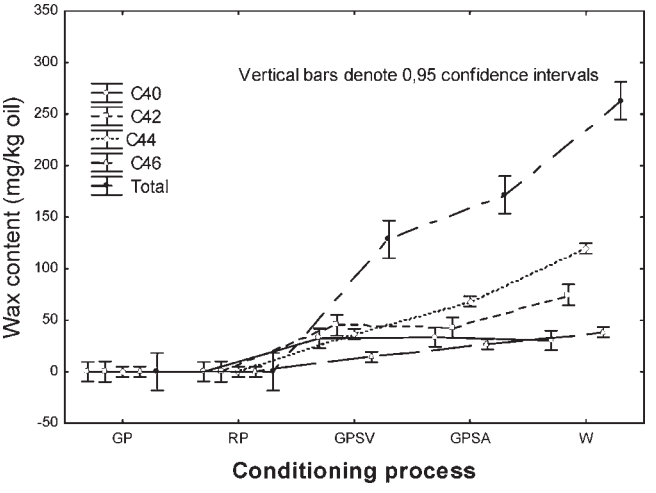

Figure 5. Wax content in the diverse fats released from the conditioning process of table olives. See Table 1 for acronyms.

which joined at almost a similar distance with GP and W. Larger distances were necessary for the linkage with GPSA and, mainly, with RP. The graph points out that, possibly, the fat residues from the conditioning process of table olives could be gathered into about three different groups with respect to their sterol and fatty alcohols: the first will be constituted by GP, GPSV and W; the second by GPSA; and the third by RP.

In conclusion, this work characterizes the diverse fats released from the conditioning processes of table olives. The concentrations of unsaponifiable matter, total sterols, fatty alcohols and wax were studied. Among sterols, β-sitosterol was the most abundant individual sterol, as usual in olive oil, followed by Δ^5-avenasterol,

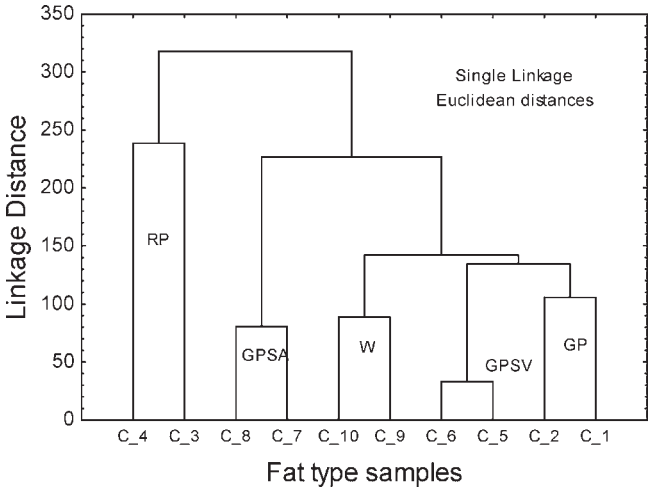

Figure 6. Tree diagram of linkage of cases according to the Euclidean distances, based on sterol and fatty alcohol concentrations (mg/kg fat).

campesterol, and stigmasterol in decreasing levels. Cholesterol content was low in most of the fats but reached a high level in GPSA. Proportions of apparent β-sitosterol were below the 93% limit established in the EC Regulations[6,7] or IOOC Standards[8] for this parameter, in 3 out of the 5 samples. Overall, the sterol content makes all these fats similar to lampante olive oil. The lowest C22 fatty alcohols was observed in GP and the highest in GPSA, which was also close to W. Triterpene diols were found only in GPSV and in a low proportion. Waxes were absent in fats from only pitting but were quantified in the other types; the most abundant compound was C44, followed by C40, and C46; apparently, waxes were always higher in W (more degraded fat). Overall minor component contents of these oils can hardly qualify them as edible oils, although final uses should also consider the refining effect. Alternative nonedible applications of these oils could be production of biolubricants, biodiesel, or soap making.

The chemometric analysis based on sterols was able to detect differences among the diverse fats; the cluster analysis based on sterol composition (in mg/kg) led to erroneous linkage with the samples from GP fat but assigned the samples correctly when expressed as percentage or when the analysis was based on sterol plus fatty alcohols. PCs and DA also pointed out clear differences among samples; especially clear was predictive DA, which led to a 100% assignment in the first discrimination (but not in cross validation). Graphically, both PCs and 3D DA led to good segregation among samples, although some of them were relatively close. The characterization of these byproducts will be of direct practical repercussion with respect to the consideration that they will receive in the national, currently under revision, and international legislations.

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