



Application of chemometric tools to compare Algerian olive oils produced in different locations

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Abstract

The aim of this study was to determine whether the chemical characteristics of olive oils collected from five areas in Algeria (El Milia, Constantine, Tizi Ouzou, Setif and Skikda) could be used to discriminate their geographical origin. Little recent data is available concerning the composition of Algerian olive oils. These compositional parameters were statistically analysed using three chemometrical methods, Principal Components Analysis (PCA), Canonical Discriminant Analysis (CDA) and Direct Orthogonalisation (DO). The first Principal Component (PC1) was dominated by the following variables: acidity, α - and γ -tocopherols, extinction coefficient at 232 nm (K_{232}) as well as the iodine index and the saponification index. The second Principal Component (PC2) reflects essentially the opposition between the extinction coefficient at 270 nm (K_{270}), the difference in the absorption coefficients at wavelengths around 270 nm (Delta K), peroxide index and the iodine and saponification indexes. CDA shows that a good separation of the groups of samples is to be had in the plane of the first two Canonical Discriminant Functions. Region 2 (Constantine) is characterised by higher levels of K_{270} and Delta K; Region 3 (Tizi Ouzou) by high peroxide index and Region 4 (Setif) by high γ -tocopherol, peroxide and iodine indexes. The separation of the groups after subtracting one orthogonal component by DO, did not improve the results obtained with the CDA. The results obtained show that it is possible to discriminate different Algerian oils, using easily performed chemical and chemometric techniques. It is therefore feasible to proceed with the large-scale collection of compositional data on olive oils in order to establish a databank to serve to characterise their quality and geographical authenticity.

Key words: Olive oil, geographical origin, chemical parameters, Principal Component Analysis, Canonical Discriminant Analysis, Direct Orthogonalisation (DO).

Introduction

Olive oil is an important element of the Mediterranean diet. Very present in the food of Mediterranean countries and recommended by many dieticians, it has been the subject of much nutritional research which has shown its medicinal and cosmetic properties¹. Because of its nutritional and organoleptic properties, olive oil is very different from other vegetable oils and is well appreciated throughout the world. Much data is available on olive properties and olive oil characterization²⁻⁶. In 2005 the International Olive Oil Council estimated the world production of olive oil for the 2004/2005 crop year at more than three million tons (3.001.000 t). The European Union (UE) is by far the largest producer with 78%, of which Spain (41.7%), Italy (37.4%), Greece (18.5%) and Portugal (2%) together assure 99.6% of European production⁷.

In Algeria, the area reserved for olive-tree cultivation represents 165,260 hectares, that is to say 36.2% of the total agricultural area. Olive oil production rose to 33.5 thousand tons in the 2004/2005 campaign, while consumption reached 35 thousand tons for the same season. Olive oil is still extracted following old fashioned cottage industry techniques (press system) in several geographical regions within the country, especially in the mountainous zones of Kabylie. Nevertheless, the super-press

trituration system and the continuous chain system, also called three phase centrifugation system, are starting to be used. Many studies have shown that oils obtained by centrifugation systems are of good quality⁸⁻¹¹. At the same time, Chimi¹² estimates that the olive production and transformation circuits of the traditional process generate many losses, both at the quantitative and qualitative levels.

Several studies were carried out to correlate the chemical composition of the olive oil with the geographical origin¹³⁻¹⁹ and chemometric methods have been applied to several chemical components for the classification of the olive oils. The geographical origin denominations are of interest for high-grade olive oils. Differences between oils are usually related to changes in content of minor constituents (phenolic acids, waxes, sterols, hydrocarbons) as well as subtle differences in the concentration of major components that are influenced by climate, soil, predominant variety and the technology used in each particular geographical region²⁰. In previous studies on oils from other countries, it was reported that the extraction method, geographical origin and climatic factors influenced chemical characteristics of oils²¹.

The aim of this study was to evaluate the characteristics of olive oils from many geographical origins in Algeria. Determinations were done on several physicochemical parameters. In this work, these measurements were analysed by chemometric techniques to characterise olive oils as a function of their geographical origin.

Materials and Methods

Sampling: The olive oil samples were collected directly from several extraction units located in different regions (Table 1). These oils were classified according to their geographical origin and the extraction process. This study was carried out on 16 samples collected during the 2004 season and distributed as follows: 8 samples from the district of El Milia, department of Jijel (1), 1 sample from the department of Constantine (2), 2 samples from the department of Tizi Ouzou (3), 1 sample from the department of Setif (4) and 4 samples from the department of Skikda (5) (Fig.1). The distribution of samples among regions not being well balanced, in some cases it is not possible to draw irrefutable conclusions concerning the regional characteristics of the samples.

Each sample intended for laboratory analysis was taken from the total extracted olive oil after homogenisation and appropriate weight reduction so as to be representative of the batch. The olive oil samples were put into clean, dry dark-glass bottles of a minimal size of 250 ml provided with stopper and refrigerated according to the standard AFNOR methods²². Each bottle was labelled indicating the geographical origin, the sample number and the extraction process.

Analytical methods: The physicochemical characteristics were determined according to standard AFNOR methods²².

Acidity expresses the free fatty acid content (expressed in oleic acid) as a percentage present in olive oil and is a very significant parameter in its quality evaluation. The quantification of acidity is based on the neutralisation of the free fatty acids by titration with

alcoholic sodium hydroxide of known normality.

The peroxide index is the quantity of peroxide present in the sample, expressed as active oxygen milliequivalents contained in one kilogramme of product, determined by oxidising potassium iodide with release of iodine. The peroxide index enables an evaluation of the oil freshness. The principle is based on the titration of the iodine released by addition of a sodium thiosulfate solution, Na_2SO_3 .

The iodine index evaluates the degree of unsaturation of a fat as the number of grams of iodine which can be fixed by the double bonds in 100 grams of the oil. This quantification is based on the back-titration of the excess of Wijs reagent transformed.

The saponification index is the number of milligrams of KOH necessary to transform into soap the free fatty acids and glycerides contained in one gram of fat. The fat triglyceride solution is treated with an excess of alcoholic potassium hydroxide which is then titrated by a hydrochloric acid solution.

UV spectrophotometric analysis: Conjugated dienes in olive oil were determined using the standard method of the International Olive Oil Council²³. This spectrophotometric measurement can provide an indication of the fat quality. Conjugated dienes absorb at 232 nm and trienes around 270 nm. The extinction coefficients K_{232} and K_{270} were calculated from absorption at 232 and 270 nm, respectively, with an UV spectrophotometer (Cary 50 Probe) for a 1% solution of oil in cyclohexane and a path length of 1 cm.

The absorption coefficients at wavelengths around 270 nm are also evaluated by calculating the DeltaK value: $\text{DeltaK} = K_m - (K_{m-4} + K_{m+4})/2$ where K_m is the specific extinction at wavelength m , which is the wavelength around 270 nm with the maximum absorbance. We use this index as an indicator of all oxidation products of triacylglycerol.

Tocopherols were analysed according to the method described by Birlouez-Aragon *et al.*²⁴ using a HPLC 560 (Kontron, France) with reverse phase column C18 Equisil OD 5 μm , 250 mm x 4.6 mm (Cluzeau, France), equipped with a fluorimetric detector (FL 3000, Thermo Separation Products, France) with excitation and emission wavelengths of 290 and 330 nm, respectively. Elution conditions were 1.2 ml/min flow-rate with an injection volume of 20 μl ; eluent acetonitrile (342 ml)/methanol (34 ml) and ammonium acetate 1 g/1000 ml (14 ml). The oil samples were diluted 30 times in propanol before analysis.

Tocopherol quantification was done by optical density (OD) measurement of standards (Merck, Darmstadt, Germany) at the specific wavelength for which the extinction coefficient was known in g/100 ml of methanol ($\epsilon^{1\%} \alpha$ -tocopherol (292 nm) = 76, OD = 0.52; $\epsilon^{1\%} \gamma$ -tocopherol (298 nm) = 91, OD = 0.638). The tocopherol concentrations were expressed as mg/100 g of oil. All analyses were done in triplicate.

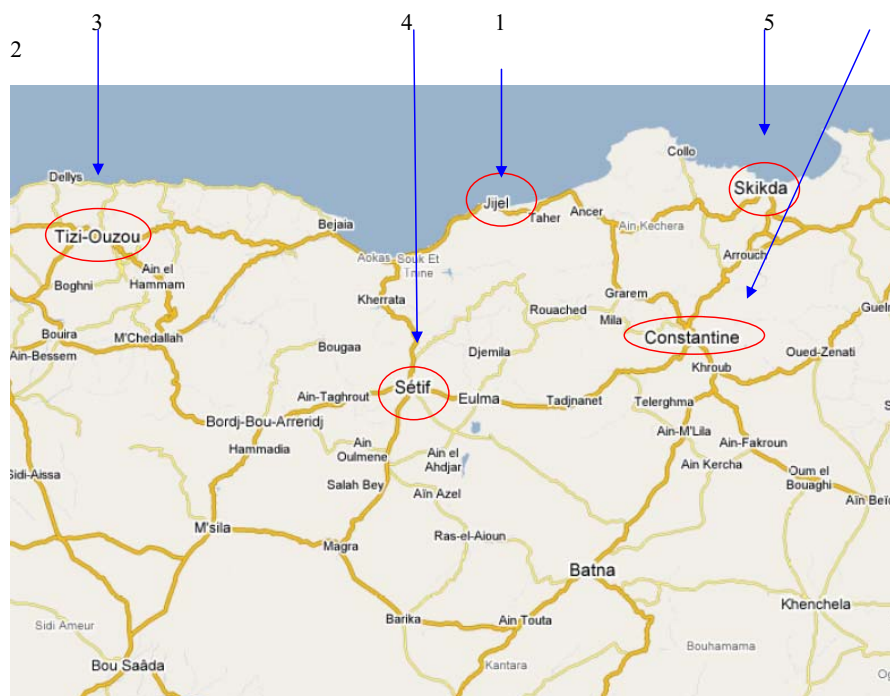


Figure 1. Localization of regions which participated in the study.

Table 1. Olive oil sample characteristics.

Regions (1-5)	Locality (a-p)	Extraction System (S/P)	Sample name
El Milia (Jijel)	Béni Belaid	Centrifugation	1a/S
El Milia (Jijel)	Béni Hbib	Centrifugation	1b/S
El Milia (Jijel)	Ouled yahia	Centrifugation	1c/S
El Milia (Jijel)	Setara	Centrifugation	1d/S
El Milia (Jijel)	Bordj Ali	Semi automatic	1e/S
El Milia (Jijel)	Bordj Ali	Press	1f/P
El Milia (Jijel)	Sidi Maarouf	Centrifugation	1g/S
El Milia (Jijel)	Sidi Maarouf	Centrifugation	1h/S
Constantine	Hamma Bouziane	Centrifugation	2i/S
Tizi Ouzou	Béni Yéni	Centrifugation	3j/S
Tizi Ouzou	Béni Yéni	Press	3k/P
Setif	Maoklène	Press	4l/P
Skikda	Tamalous	Press	5m/P
Skikda	Ain Tabia	Press	5n/P
Skikda	Collo	Centrifugation	5o/S
Skikda	Zitouna	Centrifugation	5p/S

Statistical analysis: The chemical and spectroscopic data were statistically tested with a one-way analysis of variance (ANOVA) to identify those variables which differed significantly for samples from different production regions. The null hypothesis was rejected if the calculated F value exceeded the critical F at the $\alpha = 0.05$ significance level. Principal Component Analysis (PCA) and Canonical Discriminant Analysis (CDA) were carried out with the software Biplot 1.1²⁵ while Direct Orthogonalisation (DO)²⁶ was done using Matlab²⁷.

PCA is a commonly used unsupervised multivariate technique. The purpose of PCA is to express the main information contained in the initial variables in a smaller number of principal components which describe the main variations in the data. It is based on a linear transformation of the initial variables to give a new coordinate system for the data set such that the greatest dispersion

of the samples lies on the first axis (called the first Principal Component), the second greatest dispersion on the second axis, and so on. The dispersion of the samples may be calculated using the correlation matrix of the dataset. Each component of a PCA model is characterized by three complementary sets of attributes: variances associated with each PC, loadings describing the PC in terms of contributions of the initial variables, and scores describing the position of the samples on the PCs.

The interpretation of the results of a Principal Component Analysis is usually carried out by visualization of the component scores and loadings. As no prior information concerning the characteristics of the samples (geographical origin, extraction system ...) is used in the calculation of the Principal Components, if the samples are clustered along the PCs as a function of one or more of these characteristics, one may conclude that the measurements actually do vary systematically as a function of those characteristics.

Whereas the unsupervised PCA method creates a new set of variables that maximizes the dispersion of the *samples*, Canonical Discriminant Analysis is a supervised method that creates a new set of variables that maximizes the dispersion of the predefined *groups* of samples. Each new variable is a linear function (canonical discriminant function) of the original variables, and this discriminant function may be used to calculate a set of discriminant scores. The number of discriminant functions generated is the smaller of $T-1$ or N , where T is the number groups and N is the number of variables.

For any one discriminant function, the correlations between the n observations for each of the N original variables and the n discriminant scores (often termed the canonical structure) can be used to interpret the features of the data set described by a particular discriminant function. In CDA, the matrix algebraic equations which provide the canonical discriminant functions are constrained so that the successive discriminant functions define the maximum possible difference between the predefined groups²⁸.

Direct Orthogonalization²⁶ is a multivariate pre-treatment method that has been proposed to remove systematic variations in the data set that are not related to the phenomenon being studied. The method has been suggested for improving regression by pre-treating the \mathbf{X} -matrix before proceeding with a multivariate regression onto a \mathbf{y} vector; or for improving discrimination by eliminating variations in \mathbf{X} not related to the \mathbf{Y} -matrix of predefined groups.

The principle of Direct Orthogonalization (DO) is to establish a model with scores independent of the variable (\mathbf{y} or \mathbf{Y}) being modelled. The contribution of this orthogonal model is removed from the initial \mathbf{X} -matrix, and a conventional regression or discrimination model is then calculated for this corrected data set. DO ensures that information in \mathbf{X} which correlates to \mathbf{y} is not removed during pretreatment.

Results and Discussion

The analytical quality parameters of olive oil of the different geographical origins and extraction systems are listed in Table 2. As can be seen in Table 2, the percent acidity of oils from Regions 1 and 5 often exceeds the limits established by the International Olive Oil Council²⁹ (between 1 and 3.3%) and were highest for 1a/S and 5n/P. These two oils were obtained from the centrifugation and press systems respectively, and the fruit of

Table 2. Mean values and standard deviations of measured variables of different groups of olive oil.

Sample	Acidity (%)	Peroxide index (meq O ₂ /kg)	K ₂₃₂	K ₂₇₀	DeltaK	Iodine index	Saponification index	α-Tocopherol (mg/100g)	γ-Tocopherol (mg/100g)
1a/S	9.26 0.19	6.49 0.01	0.76 0.33	0.11 0.010	0.024 0.009	86.71 1.83	182.36 0.01	9.48 0.07	0.28 0.03
1b/S	3.60 0.34	0.01 0.02	0.77 0.13	0.08 0.001	0.014 0.008	84.06 2.74	196.37 0.01	15.30 0.69	0.34 0.02
1c/S	1.20 0.05	0.02 0.03	2.30 0.07	0.10 0.02	0.005 0.004	77.18 0.90	201.05 4.04	15.21 0.36	0.40 0.015
1d/S	4.76 0.15	6.19 0.01	1.50 0.17	0.159 0.007	0.028 0.004	78.86 1.28	195.21 2.02	25.12 0.34	1.02 0.005
1e/S	7.40 0.19	7.49 0.005	0.93 0.13	0.12 0.02	0.007 0.007	79.07 0.34	201.05 4.04	10.51 0.19	0.46 0.01
1f/P	7.41 0.31	8.03 0.05	1.06 0.06	0.11 0.008	0.003 0.001	80.38 1.81	192.05 3.78	14.64 0.37	0.56 0.01
1g/S	0.77 0.06	0.02 0.04	0.79 0.34	0.05 0.02	0.0005 0.00001	80.36 0.91	200.99 3.99	15.26 0.66	0.36 0.03
1h/S	3.25 0.07	11.48 0.02	2.20 0.30	0.16 0.003	0.023 0.014	80.61 1.65	196.71 5.80	14.78 0.26	0.76 0.03
2i/S	2.57 0.01	6.96 0.05	1.40 0.31	0.94 0.26	0.43 0.17	84.61 0.90	192.88 0.01	15.06 0.23	0.53 0.01
3j/S	1.75 0.005	32.83 0.57	1.77 0.43	0.17 0.01	0.029 0.019	85.61 0.05	191.74 4.01	10.30 0.07	1.23 0.04
3k/P	2.58 0.20	19.66 0.28	1.73 0.55	0.17 0.03	0.008 0.001	87.02 0.19	199.87 0.02	11.74 0.07	1.14 0.08
4l/P	1.74 0.01	7.48 0.02	0.27 0.04	0.07 0.004	0.008 0.001	85.12 0.90	194.03 2.03	17.77 0.20	1.3 0.03
5m/P	4.53 0.14	7.93 0.11	0.81 0.01	0.07 0.001	0.02 0.001	87.24 0.005	185.12 3.73	20.54 0.23	1.14 0.023
5n/P	9.23 0.06	2.03 0.05	0.96 0.13	0.19 0.04	0.07 0.002	81.74 0.18	183.03 5.57	28.00 0.22	1.31 0.01
5o/S	4.18 0.06	2.48 0.02	1.35 0.08	0.10 0.01	0.004 0.002	87.26 0.03	198.72 4.04	28.03 0.55	1.07 0.03
5p/S	3.21 0.06	13.7 0.26	1.80 0.67	0.08 0.009	0.0048 0.004	83.02 1.84	189.35 6.06	16.16 0.12	0.89 0.03

the olive tree has not undergone any treatment other than washing, decantation, centrifugation and filtration. In addition, the sample 5m/P has an acidity slightly greater than that from 5o/S and the difference was found to be significant at the 0.05 level. These oils come from the same region but were extracted using different processes.

Olive oil samples of El Milia (1) and Skikda (5) presented peroxide indexes in the ranges of 0.01-11.48 and 2.03-13.7 meq/kg, respectively. Furthermore, the one-way ANOVA based on their extraction systems shows that statistically significant differences were found for peroxide index in the 3j/S and 3k/P samples which contained respectively 32.83 and 19.66 meq O₂/kg. In other studies, it was demonstrated that there were no such differences between olive oils obtained by press and centrifugation systems^{10,30,31}.

Our peroxide values are higher than those of Salvador *et al.*³¹ who obtained values which varied between 7.8 and 12.9 meq O₂/kg in olive oils from different geographical origins located in central Spain. Also, our samples contained more α-tocopherol than γ-tocopherol, in the ranges of 9.48-28.03 and 0.28-1.31 mg/100 g respectively. Nevertheless, some significant differences were noticed among all samples. On average, the amount present in the oil is about 12-25 mg/100 g, as reported by Psomiadou *et al.*³². Our results are in agreement with those reported by Gutierrez *et al.*³³ who found even higher values of 24-43 mg/100 g. Obviously, the amount present in the oil depends on various factors; it appears that the cultivar, the ripeness of the fruit as well as the conditions and the duration of storage are of particular

importance^{32,34}. According to Alasalvar *et al.*³⁵, the proportion of tocopherols varies with the nature of the oil and also with other factors such as the cultivation region and weather. Results of absorbance measurements at 232 and 270 nm did not exceed the established limits of ≤2.60 and ≤0.25 respectively, except for 2i/S with a value of 0.94 at 270 nm. As for Delta K, we observed that there is variability among the samples but the values were comparable to those established by the International Olive Oil Council²⁹. However, the Delta K of 2i/S was significantly higher (P=0.0220).

Principal Component Analysis (PCA): The interpretation of the results of the Principal Component Analysis (PCA) was based on an examination of the scores and loading. Our data constituted a matrix with 16 x 3 individuals and 9 variables which were column-

Table 3. Percentage of variance for each principal component.

PC	% Variance	% Total
1	22.69	22.69
2	21.54	44.23
3	19.86	64.10
4	14.34	78.44
5	10.87	89.31
6	4.70	94.01

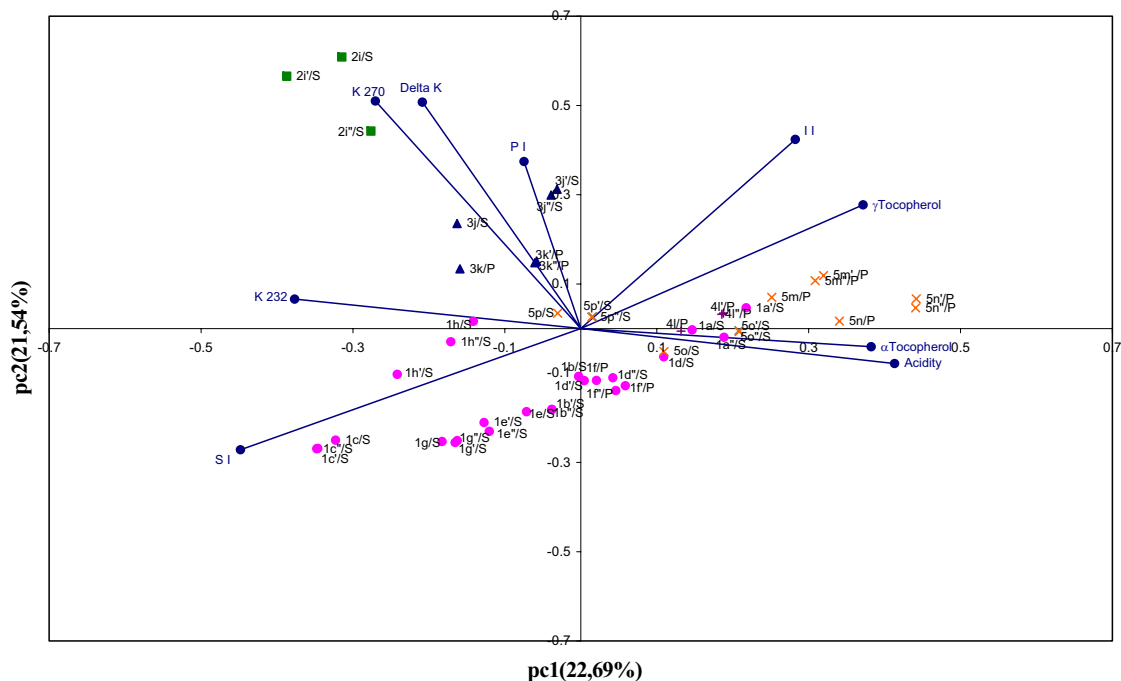


Figure 2. Scores and loadings biplot of PC1 vs PC2 for all regions.

centred and -standardised before PCA. As shown in Table 3, the first three Principal Components (PC) contain about 64% of the original variance of the samples from the five regions. Only the first 3 Principal Components were retained as no new information about the oil groups was to be found in the following PCs.

Fig. 2 presents the projections of the different samples onto the first two Principal Components along with the contributions of the original variables to PC1 and PC2. This figure reveals 4 different groups of variables. The first group contains the peroxide index (PI), the specific extinction at 270 nm (K_{270}) and DeltaK; the second group the acidity and α -tocopherol; the third one, the iodine index (I I) and γ -tocopherol; and the forth group, K_{232} and saponification index (SI). PC1, containing 22.69% of the total variability, was dominated by the variables: acidity, α - and γ -tocopherols as well as the iodine index (positive values), and by SI, K_{232} (negative values). PC2 accounts for 21.54% of total variability and reflects essentially the opposition between K_{270} , DeltaK, PI and I I (positive) and SI (negative).

Fig. 2 shows that the large number of samples from the region of El Milia (1) have mainly negative PC2 scores and are spread along PC1. These oils were extracted for the most part by the centrifugation system, except for the oil 1f/P which, although it was obtained by the traditional system of press, is not separated from the others. The scores of samples 5 tend to be positive on PC1 and close to zero on PC2; the samples of Region 3 were positive on PC2 and negative on PC1. In addition, it can be seen that the samples 2i/S are well separated on the first two principal components and are associated with the variables K_{270} and DeltaK.

In the biplot on PC1-PC3 (Fig. 3), it can be seen that the samples 5n/P are separated from the other oils of Region 5, probably as a result of their higher acidity value. The situation is similar for the 1a/S samples. The 2i/S samples are, as in the PC1-PC2 plot, closely associated with the variables K_{270} and DeltaK, while the samples of Region 3 are well separated and strongly related to the variable

PI. This is in agreement with their higher values observed for these variables. Fig. 3 shows that the projection PC1-PC3, resulting from the unsupervised PCA, clearly separates the geographical origins. No significant separation was observed between the two extraction systems. Consequently, the following supervised Canonical Discriminant Analysis was done based on the regions only.

Canonical Discriminant Analysis (CDA): The PCA having shown that the data contain information on the geographical origin of the samples, a multivariate discriminant analysis of the oils was done by Canonical Discriminant Analysis (CDA) ³⁶ again using Biplot. This method was used to discriminate the olive oils from different areas and to position the samples with respect to the vectors which maximise the differences between these predefined groups.

The biplot of the samples and original variables projected onto CD1-CD2 (Fig. 4) shows that a good separation of the groups of samples is to be had in the plane of the first two Canonical Discriminant functions. Indeed, we observe that the oils of Region 2 are well discriminated along CD1, which is explained by variables K_{270} and Delta K. CD2 shows a discrimination of oils from Regions 3 and 4 on the one hand, and the Region 1 on the other. This axis is positively influenced by peroxide index, γ -tocopherol and iodine index, and negatively by acidity.

The plane of the Canonical Discriminant functions 2 and 3 (Fig. 5 and 6) reveals an almost perfect separation of all regions except Region 2 which was already separated from the others by CD1. From these two figures, it is clear that Region 2 is characterised by higher levels of K_{270} and DeltaK; Region 3 by high PI and Region 4 by high γ -tocopherol. For the other regions, the separation is based much more on a combination of variables and not just on one particular constituent in greater quantity.

The first two components explain respectively 81.4 and 14.3% of the variance, whereas the third component explains 2.67%.

Direct Orthogonalisation (DO): After having applied the Direct Orthogonalisation (DO) pretreatment, the results obtained show negligible change in **X** after eliminating the part orthogonal to **Y**. The first two factors explain respectively approx. 81.7 and 14% of the variance of the orthogonalized matrix (instead of 81.4 and 14.3%). The separation of the groups after elimination of one orthogonal component from **X** by Direct Orthogonalisation (Fig. 7) has not significantly improved on the results obtained with the original data (Fig. 4). Subtracting more orthogonal components did not improve the separation.

Conclusions

The results of the present study showed that the chemical composition of olive oils from different regions in Algeria is quite variable. Thus the acidity was significantly higher in pressure-extracted oils from Skikda (5) and in centrifugation-extracted oils from El Milia (1). At the same time, one oil from El Milia (1h/S) had higher K_{232} value than all other oils. Principal Component Analysis was used for an initial exploratory analysis of the data. A subsequent Canonical Discriminant Analysis showed that the variables with the greatest discriminating power were K_{270} and DeltaK for oil samples of Region 2, peroxide index and γ -tocopherol for oil samples of Regions 3 and 4, respectively. No significant discrimination between the two systems of extraction S and P was observed using PCA, and this was confirmed by CDA. Direct Orthogonalisation (DO) showed that there was no systematic variability in the data unrelated to the geographical origin. The results of this study show that these easily determined chemical parameters of olive oils contain sufficient information to distinguish the regions of the production of these Algerian oils.

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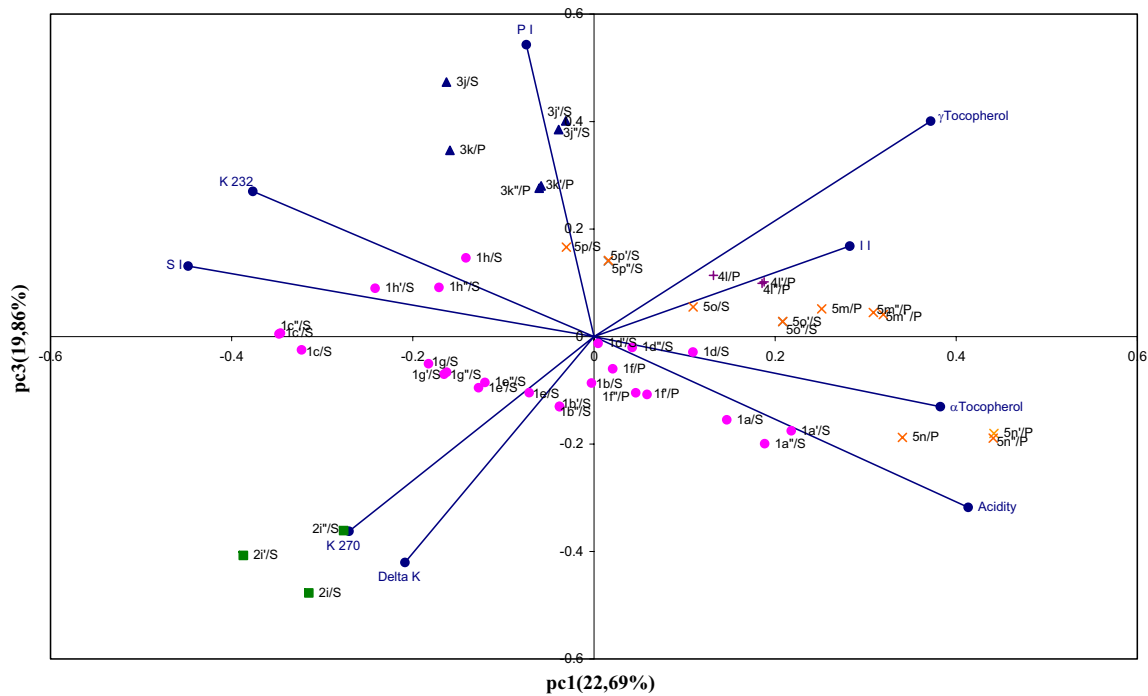


Figure 3. Scores and loadings biplot of PC1 vs PC3 for all regions.

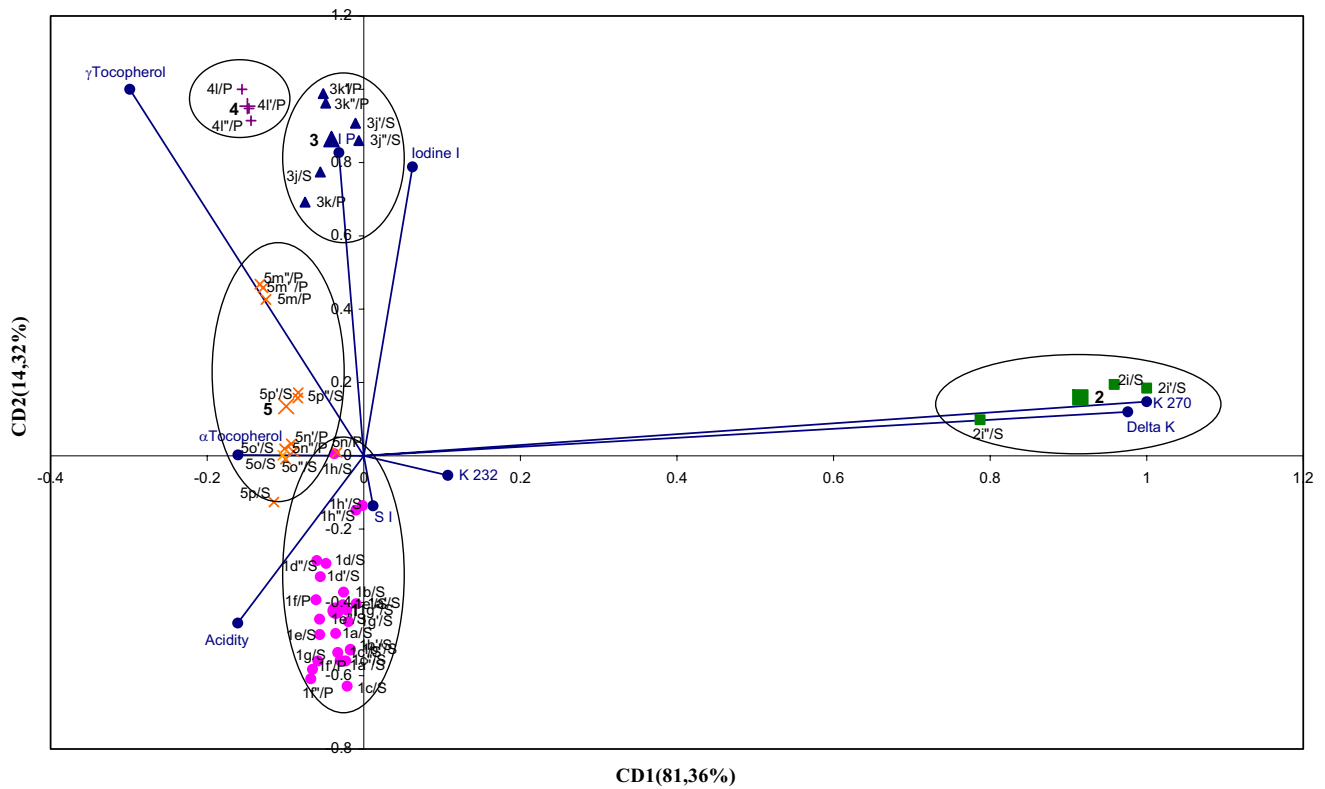


Figure 4. Biplot showing discrimination of clusters for all regions on CD1 vs CD2.

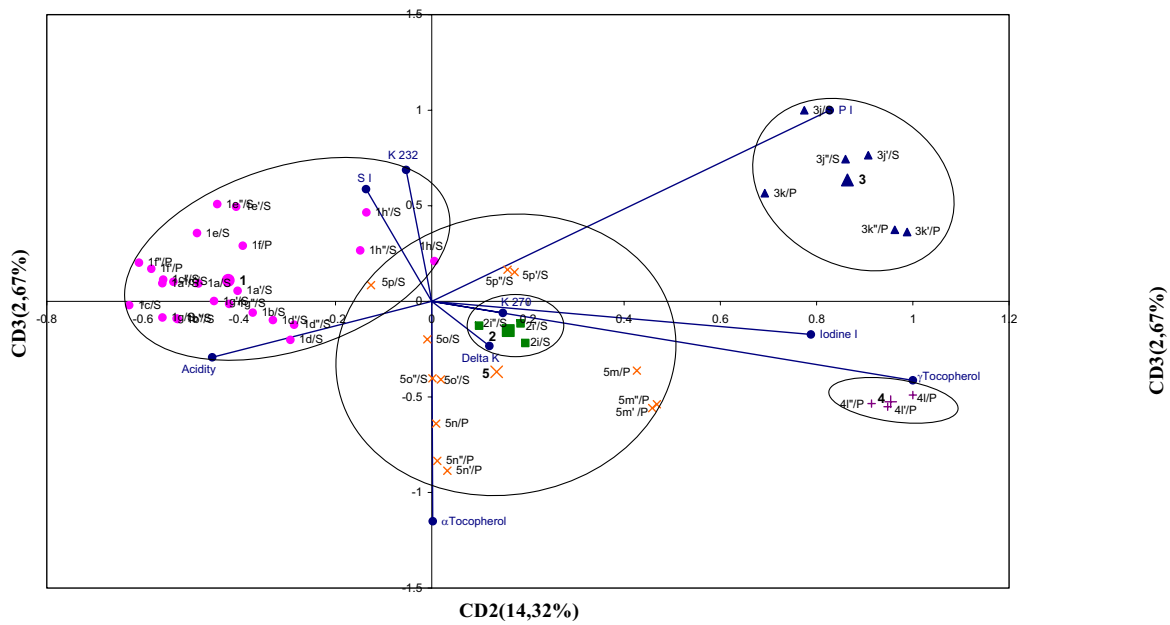


Figure 5. Biplot showing discrimination of clusters for all regions on CD2 vs CD3.

