Classification and characterization of olive oils by UV-Vis absorption spectrometry and sensorial analysis

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Summary

A number of 193 olive oil samples of five different olive oil types and three different locations of origin have been characterized by their UV-Vis spectra (absorbances at 2001 wavelengths) as well as by sensorial evaluation using a nine-point scale. Four methods of discriminant analysis and artificial neural networks were used for chemometrical data processing in order to accomplish the classification of the oil. The applied approach did not depend on chemical standards, required less laboratory work but demanded more calculation efforts. The technique of K-th nearest neighbour was the best for oil classification by variety since 98.7% of the samples were correctly classified. Linear discriminant analysis was the best for oil classification by sensorial quality since 89.0% of the samples were correctly classified. The latter method was also very successful at classification by origin since 98.4% of the samples were correctly classified.

Keywords

olive oil; electronic spectra; sensorial evaluation; classification; discriminant analysis; artificial neural networks

Olive oil is an important food component, which enjoys special and increasing popularity in many countries not only due to its delicate taste but also because of its nutritive value. Depending on regional conditions, a variety of olive oils is produced in different qualities. Olive oil has several favourable health effects related to reducing the content of adversely acting blood LDL cholesterol and the risk of cardiovascular diseases, a decrease in blood pressure, glucose content in blood and an increase in the absorption of vitamins A, D, E, and K. The beneficial health effects of olive oil are caused mainly by high contents of monounsaturated fatty acids and antioxidative substances.

Chemical analysis of edible oils is cumbersome since they consist of a complex mixture of chemical compounds and also due to a strong matrix effect [1]. However, characterization and classification of olive oils has been described using various analytical methods and chemometrical techniques [1–26]. Authentification of olive oils as an important problem has also been studied [1–11]. In most cases, spectral [1–3, 10, 12–19] and chromatographic properties [9, 19–22] were used for description of oil samples. Various variants of electronic noses employ further utilizable descriptors of olive oils [22–26]. The most often employed classification techniques were various kinds of discriminant analysis [1–3, 9, 10, 13–18, 21, 26], mostly the linear discriminant analysis, various artificial neural networks [1–3, 9, 10, 12, 17, 22–26] and the partial least square regression [10, 16, 19].

In this study, olive oil samples of different oil types were characterized by measuring absorbances in their UV-Vis spectra and performing their sensorial assessment. The spectral data were used without attempts to assign the absorbing compounds. The applied approach did not require analytical standards and was based on selecting the most informative wavelengths in the absorption spectra, which characterized the chosen classes of oils. Years ago, this approach was successfully uti-

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Quality category	Acidity [%]	Peroxide value [mekv O _{2.} kg ⁻¹]	K232	K270	Sensory score
Extra virgin olive oil	< 1.0	< 20	< 2.50	< 0.20	> 6.5
Virgin olive oil	< 2.0	< 20	< 2.60	< 0.25	< 6.5
Lampante virgin olive oil	> 3.3	> 20	< 3.70	> 0.25	< 6.5
Refined olive oil	< 0.5	< 5	< 3.40	< 1.20	< 6.5
Olive oil	< 1.5	< 15	< 3.30	< 1.00	< 6.5

Tab. 1. Traditional characteristics of olive oils of different quality.

lized for chemometrical classification of pumpkin seed oils using UV–Vis, NIR and FTIR spectra [1, 2].

MATERIALS AND METHODS

Olive oil samples

Altogether 193 olive oil samples of Greek origin from four olive campaigns were studied, which belonged to five different oil types, namely, type M (31 samples), type K (37 samples), type E (13 samples), type N (94 samples), and type T (18 samples). The oil samples were marked only by codes, as demanded by the donators. Sensorial assessment of the samples was made in a nine-category scale. Spectral data were obtained in the form of absorbances at 2001 wavelengths in the range from 200 to 700 nm. In addition, four traditional oil characteristics were measured, namely, acidity and oxidation indicators – the peroxide value (PV), as well as absorbances K232 a K270, which are traditionally connected to the oil quality (Tab. 1) and reflect the concentration of conjugated dienes and trienes, respectively.

Instrumentation and analytical procedures

The molecular absorption UV-Vis spectra of 193 olive oil samples were recorded and the absorbances were measured at 2001 wavelengths. A computer-controlled spectrophotometer Cary 50 Conc (Varian, Victoria, Australia) was used with a quartz cuvette of a 1 cm optical path. The software package Cary Win UV (Varian) was used for data acquisition and processing. Absorption spectra of the diluted (1 : 300, v/v) solution of olive oil in isooctane (spectroscopy grade; Merck, Darmstadt, Germany) were measured in the region from 200 to 700 nm. The spectra were digitized using, on average, a step of 0.25 nm and absorbances at 2001 wavelengths were finally used as the spectral variables.

Categories of olive oils

Sensory assessment of the selected type of olive oils, performed by the experienced panel of 12 experts, represented another sort of olive oil descriptors, added to the spectral and chemical descriptors. Sensorial quality of olive oils is described in Tab. 2.

The panel of experts rated several properties of the olive oils: smell, taste and the visual character. According to sensorial characteristics, categorization into three basic classes was made: the highest quality oils with scores between 9.0 and 6.5 points (further denoted as "best"), the medium quality samples with scores between 6.4 and 3.5 (denoted "good") and the unacceptable quality samples with scores between 3.4 and 1.0 points ("worst"). The three mentioned olive oil categories were differentiated by the adopted sensorial categorical variable Sens.

Two further categorization principles were applied. One of them concerned the olive oil type, according to which five categories M, K, E, N, and T were differentiated using the adopted five-class categorical variable Variety. Another categoriza-

Disadvantages	Description	Score
		9
None	Fruity flavour of olive and other fresh fruits	8
		7
Just noticeable	iceable Any disappeared fruit taste	
Noticeable	Fruit taste minimal, bad odour and taste not regular	5
Acceptance threshold	Totally defective, unpleasant odour and taste	4
		3
Serious; clearly noticeable	Odour and taste unaccept- able for consuming	2
		1

Tab. 2. Hedonic scale for sensorial evaluation.

tion principle reflected the geographical locality, according to which the samples were assigned to three Greece regions – Peloponnese, Central Greece and Crete. The introduced three-class categorical variable Location was used for description of geographical origin of the selected olive oil samples.

Chemometrical processing

Basic chemometric characterization of the olive oil samples was made by principal component analysis (PCA). It is an unsupervised technique, which depicts natural grouping of the studied objects as well as the variables (descriptors) in the multidimensional space without forcing the objects or variables to be organized according to some classification principle. For classification purposes, the relevant categorical variable was created (Sens, Variety or Location) and four multidimensional discriminant techniques [27,28] were utilized, namely, linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), logistic regression (LR) and the nonparametric K-th nearest neighbour method (KNN). Artificial neural networks (ANN) were used as the fifth classification technique. This technique was applied in the form of a three-layer perceptron, which provided nonlinear classification calculations. The classification performance of the used techniques was evaluated for two basic sample sets: (1) the training set used for computing the classification model, (2) the test set used for validation and created by the individual samples excluded in a stepwise mode from the training set according to the "leave-one-out" principle [29]. All chemometric calculations were realized using commercial software packages SPSS ver. 15 (SPSS, Chicago, Illinois, USA), SAS ver. 9.1.3 and SAS JMP ver. 6.0.2 (SAS Institute, Cary, North Carolina, USA), and Trajan, ver. 6.0 (Trajan Software, Durham, UK).

RESULTS AND DISCUSSION

Optimal region of wavelengths

The representative electronic spectra of five types of the investigated olive oils were reconstructed in the UV and Vis spectral region using the median values of each type. The calculated medians were supposed to be the best characteristics of five oil classes. They should have revealed the mutual differences among them and could be optimally utilized for their discrimination. It was found that the sufficiently observable differences were limited only to the region from 200 to 261 nm; the longer wavelengths were not suitable for further chemometrical processing since no significant spectral differences were observed there. This spectral feature was observed regardless of the class to which the oil samples belonged (the classes were created by the oil type or its sensorial quality or the place of origin). The approach based on the use of UV-Vis spectra was entirely different compared to traditional ways of the olive oil characterization, in which the chromatographic analysis of selected compounds is employed [30]. An exam-



Fig. 1. UV spectra of five types of olive oils (varieties E, K, M, N and T) represented by their median values.

ple of the spectra corresponding to five oil classes organized by the oil type is in Fig. 1, where an uncommon absorbance scale 0–10 was applied. It was caused by vast differences in absorbance values in the examined parts of the spectra and its use was justified by the purpose of only qualitative characterization of the oil types.

When inspecting the median spectra in detail, several conclusions could be specified about the separation of the oil types at the given wavelength. The wavelength of 206 nm separated the oil type T (with a high absorbance) from M, K, E and N (low absorbances). Both T and N (high absorbance) were separated from E, K and M at 208 nm (low absorbances). Situation at 210, 214 or 220 nm was similar, where absorbances were high for T and N, medium for E, and low for K and M. A low absorbance for M separates this oil type from all others at 228 nm with larger absorbance. Absorbance at 234 and 244 nm separated N with a high value from other oil types, which exhibited a lower absorbance.

Different sensorial quality of the investigated olive oils was reflected by the differences in the absorbance at following wavelengths. The best oil quality was characterized by the highest absorbance at 212 nm, the worst quality oil exhibited the lowest absorbance and the absorbance of the medium quality oils was in between. The worst quality oils exhibited the highest absorbance at 216 nm and the lowest at 224, 234 and 244 nm. The most pronounced differences in absorbance with respect to the olive oil quality were observed at 216 and 244 nm. The geographical origin of olive oils was manifested by different absorbance at the following selected wavelengths (the geographical region was marked by G for Central Greece, C for Crete and P for Peloponnese). For individual wavelengths, the order of absorbances was G > C > P for 204, 212 and 216 nm, G = P > C for 234 and 244 nm, and G > C = P for 238 nm. At 214 nm and in the region of 220–232 nm, the oil samples had very similar absorbances and no separation by the geographical origin was possible. On the other hand, the most pronounced differences in absorbance were observed at 212 and 216 nm.

Classification of olive oils – assessment of the applied multivariate techniques

Principal component analysis (PCA) displayed natural grouping of the studied objects, olive oil samples, in the plane or 3D space of the most important principal component, which were created by an uncorrelated linear combination of all original variables (absorbances at selected wavelengths) optimized with respect to preservation, as much as possible, of the total variance of the data. It is obvious from Fig. 2 that natural grouping of olive oils was influenced by the oil type only in part. The varieties T and M were partly separated from other varieties except for some outlying samples. Nevertheless, even less noticeable was the natural separation of the olive oil samples by sensorial quality or by the geographical origin. Discrimination of the oil samples according to the mentioned principles was not manifested in PCA, however, this was not necessarily expected since the object clas-



Varietv -10⊽ E ▲ K Μ 5 DF2 (16.3%) N Т 0 5 10 105 20 DF1 (79.7%) 0 DF3 (3.2%) 0 10 -10 -5

Fig. 2. Principal component analysis showing the natural interposition of various types of olive oils in the 3D space of the principal components PC3 - PC1 - PC4.

Fig. 3. Linear discriminant analysis of the olive oil samples categorized by five oil types in the plane of three most important discriminant functions DF2 – DF1 – DF3.

sification was actually not the task of PCA, but was the main task of several classification methods like various kinds of discriminant analysis or artificial neural networks.

Linear discriminant analysis (LDA) is the basic discriminant technique, which, as it is common also for other classification techniques, requires the reduction of the number of original variables to an optimum size. It also requires exclusion of all unimportant variables, which do not contain useful information. In the studied problem, such exclusion concerned those wavelengths, for which the ratio of the useful information to the noise was small. The reduction in the variables was performed using stepwise selection, which is generally considered the most effective and combines the principles of forward and backward selection. In this way, 60 optimal wavelengths were selected for the categorization by the oil variety and 37 best wavelengths were selected for the categorization of oil samples by sensorial quality. Considering the geographical origin of the oil samples, 60 wavelengths were selected but they were different compared to those used for the categorization by variety. The same three sets of wavelengths were employed also when other classification techniques were applied.

Fig. 3 depicts the discrimination of oil samples by the type (variety) as the 3D display of the first three, the most important discriminant functions. Analogous 2D graphs, exhibiting the oils discrimination by sensorial quality and geographical origin, are shown in Fig. 4 and Fig. 5, respectively. Compared to the PCA display, the use of discriminant analysis markedly improved the separation of olive oil samples using all chosen classification principles.

Quadratic discriminant analysis (QDA) as well as the K-th nearest neighbour method (KNN), logistic regression (LR) and artificial neural networks (ANN) were also successfully used for classification of the olive oil samples. They all facilitated a very good separation of the samples by the selected criterion. However, a disadvantage of the KNN and LR techniques was the absence of some essential graphical output.

Success in classification is expressed by the ratio of the number of successfully categorized objects to the number of all objects. This and the success expressed in per cent are summarized in Tab. 3 for two classification criteria expressed by the target categorical variables Variety and Sens. If the type of the olive oil is concerned (Variety), the classification success was close to 100% when the classification was made by KNN. It was approved best in this case if the optimal value of K = 5 (in-



Fig. 4. Linear discriminant analysis of the olive oil samples categorized into three classes by sensorial quality in the plane of two most important discriminant functions DF2 – DF1.



Fig. 5. Linear discriminant analysis of the olive oil samples categorized by three locations in Greece (illustrated in the side map) in the plane of two most important discriminant functions DF2 – DF1.

Method	Success	Criterion: Variety		Criterion: Sens		Software
		Training set	Leave-one-out	Training set	Leave-one-out	
LDA	Number	186	177	87	81	SPSS
	[%]	99.5	94.7	95.6	89.0	
QDA	Number	187	131	91	60	SAS
	[%]	100.0	70.1	100.0	65.9	
KNN	Number	186	185	85	80	SAS
	[%]	99.5	98.7	93.4	87.9	
LR	Number	187	-	91	-	SPSS
	[%]	100.0	-	100.0	_	
ANN	Number	_	_	91	_	JMP
	[%]	-	-	100.0	_	

Tab. 3. Success in classification of olive oil samples by variety (column Criterion: Variety) and sensorial quality (column Criterion: Sens) calculated by different classification methods and software.

Note: A number of 187 olive oil samples were classified by variety using absorbances at 60 optimally selected wavelengths; 91 olive oil samples of the variety N were categorized by sensorial quality using absorbances at 37 optimally selected wavelengths. Number denotes the number of correctly classified olive oil samples. K = 3, 5 and 7 were used in the KNN method, K=5 was found optimal.

dicating the number of neighbour samples examined with respect to its class) was used. When the sensorial quality is concerned (Sens), LDA was the best technique with the classification success of 89%. Both mentioned results concern predicting the category of an unknown, not yet classified olive oil sample. By leave-one-out cross-validation technique; the classification success of the olive oil samples included in the training set was even higher, close to 100% for most of the applied techniques. Cross-validation results for logistic regression and ANN are absent because it was impossible to carry out the leave-one-out procedure using the accessible software packages in an automatic way. Manual calculation would be too laborious, e.g. classification by Variety using just one classification method should be repeated 187 times (since n = 187).

With regard to the origin of the oil sample, classification by LDA was so successful that further chemometrical techniques were not used. Only one sample was incorrectly classified out of 62 samples of type N, which means a 98.4% classification success when the training set is concerned. Four samples out of 62 were incorrectly classified in cross-validation by the leave-one-out technique, which means a 93.5% classification success. All incorrectly classified samples belonged either to Peloponnese or Central Greece region and were mutually interchanged. It means that the similarity of these samples was larger compared to the samples from Crete, which were classified in all cases by a 100% success.

The choice of best descriptors depended on

the purpose of classification but in all cases absorbances at wavelengths between 206 and 228 nm were the most useful. Sensorial quality was mostly reflected by absorbances at 212, 216 and 244 nm, the origin of oil samples was mostly characterized by absorbances at 212 and 216 nm. Several wavelengths were important for discrimination of oil varieties, in particular 206, 208, 210, 214, 220, 228, 234 and 244 nm. It was impractical to set the rank of best wavelengths for the given classification criterion since the corresponding absorbances were not independent and, after elimination of absorbance at one wavelength, the importance of absorbances at remaining wavelengths was changed.

CONCLUSIONS

Classification of Greek olive oils was performed with a success close to 100% using various classification criteria, namely, the type of olive oil (5 classes created by the olive variety), sensorial quality (3 classes based on the result in the ninepoint scale) and geographical origin (3 classes by the region of Greece). Several techniques of discriminant analysis and artificial neural networks were well applicable for these classifications. In the applied chemometrical procedure, absorbances at pre-selected optimal wavelengths were used. A stepwise selection procedure in linear discriminant analysis was suitable for the choice of wavelengths most sensitive to a change in the olive oil type, sensorial quality and geographical origin, respectively. The applied procedure did

not require determination of any standards representing important components of the olive oil matrix, which saved time otherwise needed for the complex quantitative determination of the olive oil components and thus made the olive oil classification as well as prediction of the properties for unknown samples less expensive. The developed classification approach can be applied for differentiation of olive oils to basic categories according to EC Council Regulation [31]. For this purpose, representative olive oil samples of all considered categories should be thoroughly picked up by a competent institution and used for elaboration of the classification model, its validation and category prediction.

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