European Food Research and Technology (2019) 245:1811–1824
https://doi.org/10.1007/s00217-019-03303-2

ORIGINAL PAPER

Assessment of Tunisian virgin olive oils via synchronized analysis of sterols, phenolic acids, and fatty acids in combination with multivariate chemometrics

Karim Ennouri[1,2] · Hajer Ben Hlima[3] · Rayda Ben Ayed[4] · Olfa Ben Braïek[5] · Maura Mazzarello[6] · Ennio Ottaviani[6] · Lotfi Mallouli[2] · Slim Smaoui[2]

Received: 19 March 2019 / Revised: 8 May 2019 / Accepted: 11 May 2019 / Published online: 24 May 2019

Abstract

Olive oil composition and connection between effective physicochemical factors characterizing accessions from different Tunisian farming sites viz. Chemlali Sfax, Chemalali Medenine, and Zalmati Medenine, located in the centre and the south of Tunisia, was probed in this study. The relationship between olive oil composition and physicochemical characteristics from different Tunisian cultivars, namely, Chemlali Sfax, Chemlali Medenine, and Zalmati Medenine, located in the centre and the south of Tunisia, was investigated using multivariate statistical analysis. Multiple linear regressions (MLR) and artificial neural network (ANN) methodologies were employed to expose hidden relationships between oxidative stability and olive oil components such as fatty acids, phenolic acids, and sterol contents. Obtained results showed not only that the selected components are dependent on geographical location and varietal origin of olive oils, but also that fatty acids (C16:1, C17:1, C18:0, C18:1, and C18:2), specific phenols (p-hydroxyphenylacetic, o-coumaric, and gallic acids), and sterols (campestanol, stigmasterol, and sitostanol) are directly implied in the oxidative stability variation. However, ANN analysis allowed to obtain more accurate models with higher robustness ($R^2 > 98\%$). The combined analytical approaches, MLR and ANNs, could be considered as an adequate experimental model to restrain the influence of olive oil components in characterization of olive oil quality. Here, we have addressed the aim of using different analytical instruments in this field and the application of chemometrics for sterols, phenolic acids, and fatty acid analysis.

Keywords Olive oil · Fatty acids · Phenolic acids · Sterols · Multiple linear regressions · Artificial neural networks

Introduction

Olive (*Olea europaea* sp. *europaea* var. *sativa*) is a historically capital crop in the Mediterranean Basin [19]. Using automated or concrete methods, virgin olive oil is the merely edible oil of great production in the worldwide [44]. Chemically, olive oil has several constituents with antioxidant characteristics, especially polyunsaturated fatty acids, carotenoids, tocopherols, chlorophylls, and other phenolic complexes [61]. The most significant categories of phenolic compounds present in olive fruits comprise phenolic alcohols (hydroxytyrosol and tyrosol), flavonoids (luteolin, apigenin, and methyl luteolin), sterols (e.g., stigmasterol, β-sitosterol, and sitostanol), and phenolic acids (e.g., o- and p-coumaric acid, ferulic acid and cinnamic acid, and dihydroxybenzoic derivatives such as vanillic acid and vanillin) [51, 64].

✉ Karim Ennouri
1karimennouri1@gmail.com

1 Digital Research Centre of Sfax, Technopark of Sfax, Sfax, Tunisia

2 Laboratory of Microorganisms and Biomolecules of the Center of Biotechnology of Sfax-Tunisia, Road of Sidi mansour, km 6, PB 1177, 3018 Sfax, Tunisia

3 Unité de Biotechnologie des Algues, Biological Engineering Department, National School of Engineers of Sfax, University of Sfax, Sfax, Tunisia

4 Molecular and Cellular Screening Process Laboratory, Center of Biotechnology of Sfax, PB 1177, 3018 Sfax, Tunisia

5 Laboratory of Transmissible Diseases and Biologically Active Substances, University of Monastir, PB 56, 5000 Monastir, Tunisia

6 On Air s.r.l, via Carlo Barabino 26/4B, Genoa, Italy

Olive oil has been extensively studied for its benefits on coronary heart diseases, principally for its aptitude to decrease low-density lipoprotein (LDL) cholesterol levels and blood pressure [74]. Other health advantages of olive oil consumption described in numerous studies comprise decreasing probability of developing several diseases including inflammatory ones such as atherosclerosis [58] and asthma [79], cardiovascular diseases and hypertension [57] as well as metabolic syndromes such as diabetes [32] and obesity [11] of olive oil. Moreover, many works have shown that olive oil compounds are characterized by the ability to fight cancer cells [40, 60]. The antioxidants present in olive oil can diminish oxidative damage due to free radicals, which is supposed to be one of the leading causes of cancer [54]. Studies suggest that olive oil can fight several age-related diseases such as Alzheimer's disease [2] and Parkinson's disease [50]. Furthermore, olive oil can help treat rheumatoid arthritis, olive oil supplementation shows to increase inflammatory markers and decrease oxidative stress in individuals having rheumatoid arthritis [43].

Olive crop growing, extensive throughout the Mediterranean Basin area, is essential for the local tradition, rural market, and environment [80]. In North Africa and especially Tunisia, the olive oil segment plays a considerable role in the economy, offering employment and also exports profits [16]. Olive trees cover a wide region of 1,611,200 ha with an annual oil production of 170,000 tons [33].

In Tunisia, the oleoculture depends principally on two varieties: Chetoui and Chemlali [10]. In addition, there are also other secondary local varieties specific to minor regions such as "Sayali" in the northern regions, "Oueslati" in Kairouan, "Zalmati", and "Zarrazi" in Zarzis [8].

In Tunisia, olive orchards are characterized by the cultivar population Chemlali planted in the north, the Sahel, the centre and the south covering approximately 80% of the Tunisian olive grove [10]. Similarly, Chemlali cultivar endures an important lack of rainfall unhelpful for growth and reproductive expansion in cultivated dry areas [16]. The presence of different factors makes difficult to characterize olive oils using a small number of chemical compounds or a simple data manipulation. Therefore, samples should be identified for their chemical profiles such as: major components (fatty acid), minor components, and/or sensory descriptors, and data should be analyzed by statistical techniques and artificial intelligence algorithms.

The bootstrap is a computational method that employs accurate resampling with replacement, in the aim to reduce uncertainty [22]. In fact, the bootstrap approach is constructed on statistical resampling procedure, to generate varied training sets that are used to train the members making a group [22]. This practice is replicated and every network member is produced with a different arbitrary sampling of the original training set. It is imperative to mention that when resorting to bootstrap sampling, mainly on a relative little data size [42], it is essential that all combinations of the networks be well trained, to avoid that some biased network components of the bootstrap ensemble noticeably influence the estimate values [6, 23, 26]. In the bootstrap method of uncertainty examination, the total example set is splitted into two various sets: validation sets and training sets. In addition, evaluating these data is difficult with univariate statistical method, such as one-way ANOVA and *t* test, multivariate statistical methods are applied to numerous variables to obtain significant interpretation [47]. These statistical techniques, such as multiple linear regressions (MLR) and artificial neural network (ANN), could be applied to the classification of olive oils with respect to their different characteristics such as geographical origin or olive cultivar using various spectroscopic or chemical parameters. Eventually, this study examines the effect of growing areas on numerous quality characteristics of Chemlali and Zalmati olive oil cultivars based on different parameters such as oxidative stability, fatty acids and on minor components such as phenolic acids and sterols. Ultimately, this study allowed us to establish many relationships between oxidative stability and fatty acids, phenolic acids, and sterols by investigative statistical techniques.

Materials and methods

Olive oil samples

Olive fruits were collected during three successive harvests (from 2014 to 2016), from entirely ripened olive trees belonging to several Tunisian cultivars: Chemlali Sfax (N: 34.444°, E: 10.451°), Chemlali Medenine and Zalmati Medenine (N: 33.524°, E: 10.056°). After harvesting, olive fruits were immediately transported to laboratory, where oil was extracted within 30 h by extraction equipment (oleodoseur). The extracted oil was conserved into dark glass bottles and saved at 4 °C to later analyses.

Quality parameter: oxidative stability

Oxidative stability was estimated in term of hour by Rancimat (Metrohm, Switzerland). The temperature was diversified between 50 and 220 °C with temperature stability less than 0.1 °C. Five grams of experiment were put into the glass reaction vessel for the analysis. For equipment columns, the reaction temperature and air-flow phases were kept constant at 100 °C in air flow of 20 l/h as defined by Uncu and Ozen [76]. All measurements were performed in triplicate.

Fatty acid methyl ester analysis

Fatty acid methyl ester (FAMEs) was prepared as defined by the European Union standard method [29]. FAMEs had been prepared by melting vigorously a 0.2 g of studied oil in 3 ml of hexane and 0.4 mL of methanolic potassium hydroxide at 2 N, and analyzed by gas chromatography (Shimadzu) with a fused silica column made with 50% cyanopropylmethyl and 50% phenylmethyl-polysiloxane. 1 µl was injected using nitrogen as carrier gas with a flow rate of 1 ml/min. The injector and detector temperatures were anchored at 220 °C, while the oven temperature was held to 180 °C. Ten fatty acids including palmitic acid (C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), margaroleic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), and eicosenoic acid (C20:1) were recognized from the retention intervals. Each sample was analyzed in triplicate.

Phenolic profiles of olive oils

Phenolic extraction

Fourteen grams of each olive experiment were extracted by employing 4 × 14 ml of methanol/water (80:20 v/v). Then, the methanol was discarted, and 15 ml of acetonitrile were included to the residue and rinsed with hexane (3 × 20 ml). The resulting acetonitrile solution was evaporated, flushed with nitrogen, and dissolved in 1 ml of methanol/water [63]. Final extract was refined through a 0.45 µm pore-size membrane filter (MF-Millipore®, Merck). 20 µl of extract was instantly filled to HPLC and the gallic acid (Sigma Chemical Company, St Louis, MO) was employed as internal reference [53]. Sampling was done in triplicate.

HPLC analysis of phenolic acids

To investigate phenolic acids, an HPLC system (JASCO) was employed. Preliminary concentrations of the dynamic phases were 90% for water/acetic acid and 10% for MeOH and the concentrations had been adjusted according to the following procedure: in the beginning, the concentration of MeOH was increased to 30% in 10 min and kept for 20 min. Next, MeOH% was enhanced to 40% in 10 min, and kept for another 5 min. Followed by increasing it up to 50% in 5 min, and preserving it at this concentration for another 5 min, MeOH was enhanced to 60, 70 and 100% in 5 min. At last, primary conditions were achieved at the end of 85 min. Chromatograms were attained at 280 and 320 nm and diverse phenolic acids were identified by comparing their retention time with those of reference. Phenolic acids had been evaluated by employing their respective five-point calibration curve and the results were determined in term of mg/kg. All samples were performed in triplicate.

Sterol determination

The essay of sterolic parts extracted from olive oil was characterized by a modified method used by Abood et al. [1] and in agreement with the European Union Commission Regulation EEC 2568/91 [53]. Five grams of olive oil from each variety was initially joined to α-cholestanol advised as internal standard and then saponified with potassium hydroxide solution in ethanol at 2 M. After 1 h of swelling, 100 ml of water was joined and the extraction of unsaponifiable was carried-out by 200 ml of ethyl ether. Subsequently, 20 mg of unsaponifiable fraction was melted in 0.5 ml of chloroform and again eliminated by silica gel plate chromatography. The elution was completed by a blend of hexane and ether (65/35, v/v); then, the plate was crushed by 2,7-dichlorofluorescein (0.2% in the ethanol). The band corresponded to sterols was afterwards fragmented. Sterols recovered from the plate were dissolved in chloroform and purified. The solvent was vanished under N_2 and the sterols were adapted into trimethylsilyl ethers by the accumulation of pyridine–hexamethyldisilzane–trimethylchlorosilane (9:3:1, v/v/v), left for 15 min and subsequently centrifuged. The assortment was then analyzed with the aid of gas chromatography via a gas chromatograph system (model 7890A, Agilent Technologies) with flame ionization detector. The employed column was a capillary HP-5 (5% phenyl: 95% dimethylpolysiloxane), and the operating circumstances for the analysis were as follows: the oven temperature was isothermal and constant at 260 °C, while injector and detector temperatures were maintained at 280 °C and 290 °C, respectively. Helium was used as carrier gas, with a flow rate through the column of 1 ml/min and 1:50 Split ratio. The injection amount was set to 5 µl and samples were tested in triplicate.

Statistical analysis

All factors were evaluated in triplicate for each trial. Analysis of variance (ANOVA) was done by Statistical Package for the Social Sciences (SPSS) software (Version 19.0 SPSS Ltd). The significance of differences at 5% level between means was established by one-way ANOVA, using Tukey's test. Analysis of variance (ANOVA) was employed to assess the potency of growing region conditions on Tunisian olive oil cultivars.

Linear regression model

The general purpose of multiple regressions is to learn more about the relationship between several independent or

predicted variables and a dependent or criterion variable [28, 48]. The general form of the regression equations is

$$Y = A_0 + A_1X_1 + A_2X_2 + \cdots + A_nX_n,$$

where Y is the dependent variable, A_0 is the intercept, $A_1 \ldots A_n$ are regression coefficients, and X_1–X_n are independent variables referring to measured indices. Determination coefficient (R^2) was used as predictive success criteria for regression model [26, 28]. To establish prediction equations, the multiple regression analyses were conducted (Minitab Inc., State College, PA).

Neural networks

The artificial neural network (ANN) can be defined as a machine-learning forecast algorithm that is similar to human nervous system. ANN adapted from a theoretical way to the widely employed knowledge with useful appliances to diverse problems [24, 70, 77]. The most important of these appliances are applied to the prediction and ANN are frequently employed as non-linear estimators that reveal an elevated tolerance to inaccuracy and achieve well despite noisy situations [49]. Those advantages also make ANN suitable for biotechnological problems, and principally for food science [13]. The most employed representation in ANN uses is the multilayer perceptron (MLP) because of a high ability to estimate the functional construction in the non-linear structures. MLP is composed of an input layer, output layer, and hidden layers [55]. Variables that were related to the oxidative stability were selected as candidates for input into the final ANN model. For statistical analysis, an exploratory three-layer multiplayer perceptron (MLP) ANN model with a back-propagation algorithm was assembled [5, 27]. The multilayer perceptron (MLP) algorithm is an influential structure of an artificial neural network that is usually employed for regression, and also classification. The MLP algorithm is an excellent algorithm to apply for the mapping and regression. It can be employed to transform an N-dimensional input indicator to an M-dimensional output indicator; this transformation can as well be non-linear. The validation data are used only when the predictor model is organized and are dissimilar from those used for training data. In general, 80% of all experimental data are reserved for training, and the remaining 20% are used for validation. The ANN model is tested on the bootstrapped training set with unchanged initial weights; the remaining samples in the training set independently from bootstrapped training set are used for split test validation so as to avoid any over-fitting. Only one node was used in the output layer, corresponding to the oxidative stability. The hidden layer consisted of several nodes ranged from 5 to 20. The feed-forward topology was implemented in all organizations. In this study, K-fold cross-validation procedure was used for the validation and testing of ANN model, consisting on dividing the original data into K subsets. In turn, each of the K sets is used to validate the model fit on the rest of the data, fitting a total of K models. Cross validation is a statistical technique employed to test the ability of machine-learning designs. It is generally employed in applied machine learning to evaluate and choose a model for a specified predictive modeling complication for the reason that it is simple to understand, simple to execute, and results in ability evaluates that usually have a minor bias when compared with other techniques [81]. Besides, cross validation is known as a resampling procedure employed to calculate machine-learning prototypes. The procedure has a unique factor called k that is attributed to the groups that a specified data example is to be contained into [7]. Then, the procedure is frequently named k-fold cross validation [78]. Cross validation is a very powerful device. It aids us better employ the data, and it provides us much more details about the algorithm performance. Values of R^2 from training and validation data were used as selection criteria for the best predictor model. The neural module from the Statistical Analysis System (SAS) package (version 9; SAS Institute Inc., Cary, NC) was used for training and validation of the networks.

Results

Oxidative stability

The oxidative stability is a major element in the characterization of vegetable oils [12]. In fact, this parameter is a quality index to confirm the preservation status of the oils as well as their predictive resistance to oxidative phenomena [12]. Oxidative stability of all olive oils tested is presented in Table 1.

It was evident that olive oils belonging to Zalmati Medenine cultivar have the highest average with 10.14 h. Statistically, olive oils from Chemlali Sfax had lower ($P < 0.05$) oxidative stability in comparison with samples of Chemlali Medenine and Zalmati Medenine (Table 1). In general, the oxidative stability provides precious information on oil's hypothetical shelf life; similarly, the resistance to oxidative deterioration is usually endorsed to the lipid composition and the antioxidant content [39, 52].

Fatty acid composition

The specific fatty acid composition in olive oil varies depending on various factors such as olive cultivar, latitude, climate, and maturity stage [45, 67]. On the other hand, the composition of fatty acids influences the stability, nutritional value and is characteristic of olive oils [20, 36]. In this study, fatty acid estimation was besides performed

Table 1 Oxidative stability and fatty acids composition of Chemlali Sfax, Chemlali Medenine, and Zalmati Medenine virgin olive oils

Variety	Chemlali Sfax	Chemlali Medenine	Zalmati Medenine
Oxidative stability (h)	4.27 ± 0.2^{a}	8.47 ± 0.4^{b}	10.14 ± 0.33^{c}
Fatty acids (%)			
C16:0	15.81 ± 0.6^{a}	17.44 ± 0.82^{b}	17 ± 1.01^{b}
C16:1	3.61 ± 0.15^{c}	1.34 ± 0.2^{a}	1.93 ± 0.38^{b}
C17:0	0.03 ± 0.002^{a}	0.03 ± 0.007^{a}	0.03 ± 0.05^{a}
C17:1	0.06 ± 0.005^{a}	0.04 ± 0.003^{a}	0.06 ± 0.001^{a}
C18:0	1.77 ± 0.08^{a}	2.73 ± 0.09^{c}	2.06 ± 0.01^{b}
C18:1	61.92 ± 2.7^{a}	70.76 ± 3.6^{c}	63.43 ± 2.31^{b}
C18:2	15.77 ± 1.02^{b}	6.68 ± 0.7^{a}	14.47 ± 0.6^{b}
C18:3	0.56 ± 0.03^{b}	0.48 ± 0.04^{a}	0.51 ± 0.08^{ab}
C20:0	0.29 ± 0.06^{a}	0.34 ± 0.01^{b}	0.32 ± 0.02^{ab}
C20:1	0.13 ± 0.08^{b}	0.10 ± 0.008^{a}	0.14 ± 0.01^{b}

Values are expressed as mean ± SD

Values with different superscript letters (a–c) within a row are significantly different ($P < 0.05$) by Tukey's test multiple comparison post hoc test

on the olive oils, following the common product analyses. Table 1 reports the identified fatty acids: palmitic (C16:0), palmitoleic (C16:1), margaric (C17:0), margaroleic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), and eicosenoic acid (C20:1). In the entire experiments, the oleic acid is continually the most abundant. In addition, a considerable difference ($P < 0.05$) was discovered among oleic acid in Chemlali Sfax, Chemlali Medenine, and Zalmati Medenine olive oils. Similarly, the oleic acid mean values for the Chemlali Medenine cultivar had the best average (Table 1). This result is consistent with the finding of Servili et al. [68] who found that fatty acid composition could be related to the geographical location, the characteristics of the olive grove zones, and the salinity of soil. Likewise, our results were similar with those of Temime et al. [73] who suggested the importance of the environment parameter in defining the oil quality of the Tunisian Chetoui cultivar.

Phenolic composition

Total phenol content

As shown in Table 2, the results of the colorimetric determination of total phenol content in oil extracts were ranged between 187.86 ± 2.98 mg of gallic acid equivalent per kg (GAE/kg) for Chemlali Medenine and 266.68 ± 5.76 mg of gallic acid equivalent per kg (GAE/kg) for Chemlali Sfax. Moreover, no significant difference ($P > 0.05$) was detected in total phenol content between Chemlali Medenine and Zalmati Medenine olive oils (Table 2). These results are in agreement with many previous reports demonstrating that quantities of phenolic compounds in olive oil ranged from 50 to 1000 mg GAE/kg in function of geographical zone, cultivar origin, and oil extraction technique [9].

Phenolic acids profile

The foremost phenolic acids ($P < 0.05$) for olive oils collected from the Chemlali and Zalmati cultivars are: phenylacetic acid, p-hydroxyphenylacetic acid, protocatechuic

Table 2 Total phenols content and phenolic acids of Chemlali Sfax, Chemlali Medenine, and Zalmati Medenine virgin olive oils

Variety	Chemlali Sfax	Chemlali Medenine	Zalmati Medenine
Phenolic acids (ppm)			
Total phenol content	266.68 ± 5.76^{b}	187.86 ± 2.98^{a}	189.63 ± 4.67^{a}
Phenylacetic acid	5.51 ± 0.01^{a}	15.85 ± 0.37^{c}	8.21 ± 0.32^{b}
p-Hydroxyphenylacetic acid	9.91 ± 0.26^{b}	17.82 ± 2.3^{c}	3.03 ± 0.05^{a}
Protocatechuic acid	4.42 ± 0.25^{a}	7.52 ± 0.66^{b}	3.21 ± 0.02^{a}
o-Coumaric acid	2.32 ± 0.11^{b}	0.00 ± 0.00^{a}	2.11 ± 0.14^{b}
Ferulic acid	4.51 ± 0.19^{c}	0.00 ± 0.00^{a}	2.31 ± 0.31^{b}
4-Hydroxy cinnamic acid	5.61 ± 0.15^{b}	7.11 ± 1.02^{c}	0.00 ± 0.00^{a}
4-Hydroxy-3methoxy benzoic acid	0.00 ± 0.00^{a}	8.9 ± 0.71^{b}	0.00 ± 0.00^{a}
4-Hydroxy benzoic acid	3.21 ± 0.09^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Gallic acid	4.02 ± 0.22^{b}	8.71 ± 0.25^{c}	2.57 ± 0.03^{a}
Syringic acid	5.55 ± 0.31^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Vanillic acid	4.53 ± 0.15^{b}	8.18 ± 0.48^{c}	0.00 ± 0.00^{a}
Trans-cinnamic acid	4.11 ± 0.23^{ab}	7.33 ± 0.24^{b}	3.00 ± 0.037^{a}

Values are expressed as mean ± SD

Values with different superscript letters (a–c) within a row are significantly different ($P < 0.05$) by Tukey's test multiple comparison post hoc test

acid, gallic acid, and trans-cinnamic acid (Table 2). Interestingly, Chemlali Medenine had essentially the most elevated intensities ($P < 0.05$) of phenylacetic acid (15.85 ± 0.37 ppm), p-hydroxyphenylacetic acid (17.82 ± 2.3 ppm), protocatechuic acid (7.52 ± 0.66 ppm), gallic acid (8.71 ± 0.25 ppm), and trans-cinnamic acid (7.33 ± 0.24 ppm).

Sterol composition

The sterol compositions of the Chemlali Sfax, Chemlali Medenine, and Zalmati Medenine oils are shown in Table 3.

All experiments displayed high amounts of campesterol, with percentages ranging from 2.4 to 3.26%, which were in conformity among the limit (4%) established by European Union Regulations [18]. Another remarkable attribute of Chemlali and Zalmati varieties was their high Δ-5-avenasterol content, which ranged between 7.85 and 11.5% [75]. In fact, countless studies in the literature have connected this compound with antioxidant action 75]. Likewise, all olive oil experiments were distinguished to comprise greater than 1200 ppm of total sterols, which is the minimum value established by European Union Regulations with indication to the extra virgin olive oil class. All experiments had been additionally noted to exhibit expanded levels of β-sitosterol (84% of total sterols).

Linear regression analysis

In this section, the multiple linear regression analysis using the oxidative stability parameter as dependent variable was performed. Fatty acids values (C16:1, C17:1, C18:1, and C18:2), phenolic acids (hydroxyphenylacetic acid, o-coumaric acid, and gallic acid), sterols (campestanol, stigmasterol, and sitostanol) were considered as variables. The multiple linear regression analysis (Table 4A, B, C) revealed a high correlation ($R^2 > 62.7$) between overall acceptability and all parameters.

Relationship between oxidative stability and fatty acids

Concerning the regression model for oxidative stability determination of Chemlali Sfax, the studied fatty acids have positive effect, meaning an increase in the phenol contents, and lead to improve the oxidative stability (Table 4A). On the basis of these obtained results, we remark that margaroleic acid (C17:1) has the higher effect on oxidative stability, followed by stearic acid (C18:0), palmitoleic (C16:1), and linoleic (C18:2) acids with similar effects. Oleic acid (C18:1) has very low consequence on oxidative stability. On the other hand, the regression model for determination of oxidative stability of Chemlali Medenine olive oil is represented in the following equivalence in Table 4A. We observe that C17:1 has the higher effect on oxidative stability, followed by C16:1 and C18:0 acids with similar effects and finally by C18:2. C18:1 has a very low role on oxidative stability variation (Table 4A).

Relationship between oxidative stability and phenolic acids

The regression models for determination of oxidative stability were illustrated in the mentioned equality (Table 4B). The obtained results showed that Chemlali Medenine has the highest amount of p-hydroxyphenylacetic acid and the lowest amount of o-coumaric acid. In addition, p-hydroxyphenylacetic and gallic acids have a positive effect on improvement of oxidative stability. Moreover, these results describe that Zalmati Medenine has the lowest amount of gallic and p-hydroxyphenylacetic acids. All parameters have an active contribution on enhancement of oxidative stability.

Table 3 Sterol composition of Chemlali Sfax, Chemlali Medenine, and Zalmati Medenine virgin olive oils

Variety	Chemlali Sfax	Chemlali Medenine	Zalmati Medenine
Sterols (ppm)			
Campesterol	56.62 ± 3.02^{c}	39.87 ± 2.2^{a}	44.02 ± 1.88^{b}
Campestanol	3.45 ± 0.17^{b}	3.20 ± 0.3^{b}	1.86 ± 0.3^{a}
Stigmasterol	4.35 ± 0.2^{a}	26.36 ± 0.6^{b}	5.76 ± 0.72^{a}
β-Sitosterol	1957.92 ± 7.54^{c}	1025.13 ± 8.55^{a}	1313.18 ± 5.79^{b}
Sitostanol	4.90 ± 0.38^{a}	10.26 ± 1.02^{b}	3.81 ± 0.24^{a}
Delta-5-avenasterol	266.3 ± 9.4^{c}	95.9 ± 3.8^{a}	156.19 ± 10^{b}
Delta-5,2,4-stigmastadienol	5.31 ± 0.27^{a}	4.72 ± 1.4^{a}	3.95 ± 0.05^{a}
Delta-7-stigmastenol	5.63 ± 0.1^{a}	8.14 ± 2.3^{b}	10.64 ± 0.88^{c}
Delta-7-avenasterol	12.02 ± 0.4^{c}	7.72 ± 0.66^{a}	10.47 ± 0.27^{b}

Values are expressed as mean ± SD

Values with different superscript letters (a–c) within a row are significantly different ($P < 0.05$) by Tukey's test multiple comparison post hoc test

Table 4 Multiple linear regression equations of oxidative stability versus measured parameters

	Accession	Oxidative stability		
		Chemlali Sfax	Chemlali Medenine	Zalmati Medenine
A	Fatty acids	19.4 + 2.72 × C16:1 + 57 × C17:1 + 12.5 × C18:0 − 0.38 5 × C18:1 + 2.24 × C18:2 (R^2 = 79.4%)	17.0 + 6.38 × C16:1 + 75 × C17:1 + 7.08 × C18:0 − 0.29 5 × C18:1 + 4.62 × C18:2 (R^2 = 88%)	4.2 + 1.11 × C16:1 + 13.1 × C17:1 + 2.34 × C18:0 − 0.082 0 × C18:1 + 0.532 × C18:2 (R^2 = 88.6%)
B	Phenolic acids	− 17.9 + 1.96 × p-hydroxyphenylacetic acid + 9 × o-coumaric acid + 14 × gallic acid (R^2 = 74.1%)	− 7.9 + 0.708 × p-hydroxyphenylacetic acid + 7.06 × gallic acid (R^2 = 66.6%)	− 3.909 + 0.833 × p-hydroxyphenylacetic acid + 2.161 × o-coumaric acid + 4.757 × gallic acid (R^2 = 74%)
C	Sterols	− 24.6 + 4.53 × campestanol + 6 × stigmasterol + 8.81 × sitostanol (R^2 = 66.3%)	− 28.04 + 8 × campestanol + 1.30 × stigmasterol + 2.94 × sitostanol (R^2 = 70.9%)	− 5.97 + 3.3 × campestanol + 1.25 × stigmasterol + 2.11 × sitostanol (R^2 = 62.7%)

It is obvious that the effect of p-hydroxyphenylacetic acid is more important in the increase of oxidative stability of Chemlali Sfax olive oil, and this influence is less significant for Zalmati Medenine and Chemlali Medenine. Moreover, o-coumaric acid has more important effect in the oxidative stability of Chemlali Sfax when compared to Zalmati Medenine. Nevertheless, this phenol has no effect on oxidative stability of Chemlali Medenine. Finally, gallic acid has an essential role in the oxidative stability of Chemlali Sfax. This role is less intensive for oxidative stability of Chemlali Medenine and Zalmati Medenine.

Relationship between oxidative stability and sterols content

It can be seen, as shown in Table 4C, that the studied sterols have positive consequence, which signifies that an increase in the sterol contents leads to improve oxidative stability. In fact, Eussen et al. [30] reported that major phytosterols found usually in plants comprise sitosterol, stigmasterol, and campestanol. They have much stand out due to their nutritional value as natural components of regular diet. The regression model for determination of oxidative stability of Chemlali Medenine olive oil is shown in Table 4C. On the basis of regression model proposed for estimation of oxidative stability of Zalmati Medenine olive oil (Table 4C), it is obvious that all parameters have an active contribution on enhancement of oxidative stability.

ANN modeling of oxidative stability Analyses

Table 5 illustrates the coefficient of determination (R^2) percentages for training and validation of established artificial neural network models. Two main quantitative measures were used to evaluate the modeling performance: R^2 and cross validation R^2. The cross validation R^2 indicates the predictive ability of the model. The predictor model should be able to correctly estimate the other values, different from those used during adjustment. Data analysis of oxidative stability demonstrated that obtained ANN could be classified as a back-propagation feed-forward multilayer-perception neural net type. This neural network is considered by a set of non-linear equations that predict output variables (y) from input variables (x) in a flexible way using layers of linear regressions and S-shaped functions. From Table 5, Chemlali Sfax model (Model 1) had a better training performance $R^2 = 99.962\%$ and validation performance $R^2 = 98.734\%$. Training data refer to the data set used to effectively adjust the predictor model to experimental data, whereas validation data refer to another set of data previously reserved and is not used to adjust the predictor model but to check if the model is properly

Table 5 Coefficient of determination (R^2) percentages for training and cross validation (CV R^2) of established artificial neural network models

Hidden nodes	Chemlali Sfax		Chemlali Medenine		Zalmati Medenine	
	R^2 (%)	CV R^2 (%)	R^2 (%)	CV R^2 (%)	R^2 (%)	CV R^2 (%)
2	97.287	88.497	98.884	97.854	96.18	89.655
3	99.773	92.443	99.473	98.053	99.781	95.948
4	99.864	98.183	99.628	98.675	99.927	99.77
5	99.962	98.734	99.708	98.876	99.97	99.621
6	99.981	95.878	99.753	98.667	99.968	97.533
7	99.975	97.679	99.796	98.948	99.986	99.01
8	99.969	97.704	99.819	98.717	99.972	99.695
9	99.972	98.247	99.808	98.562	99.972	99.537
10	99.984	97.348	99.809	98.52	99.983	98.108

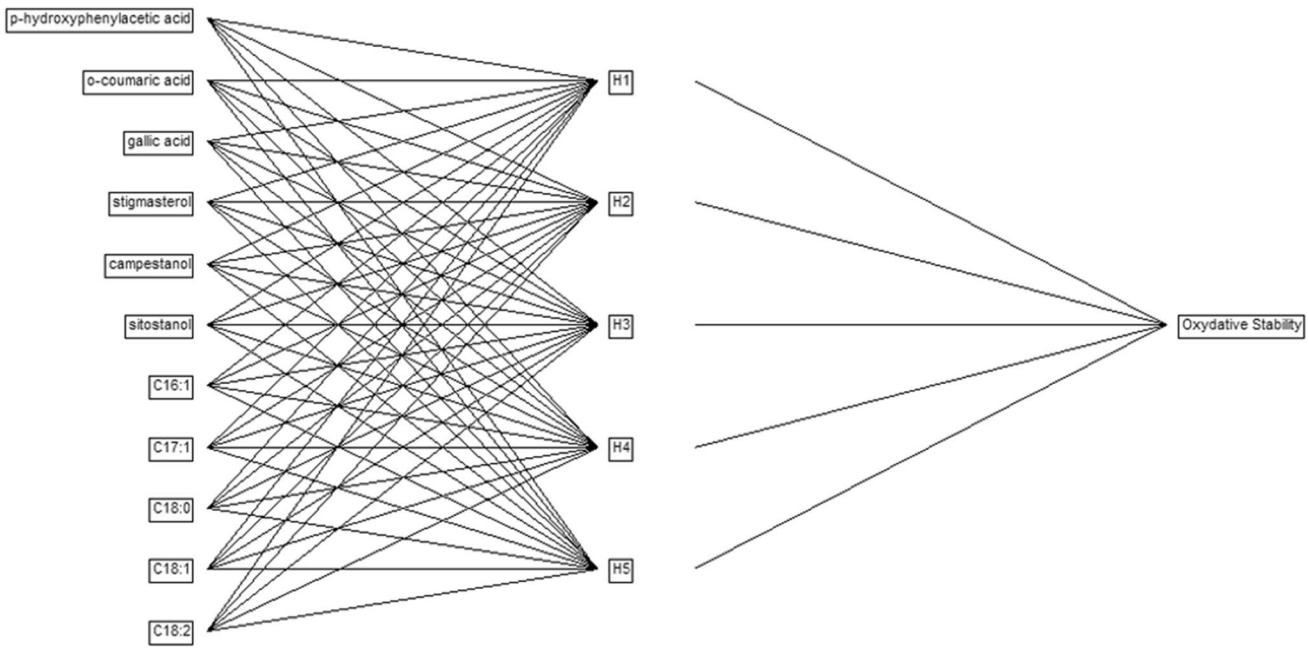

Fig. 1 Structure of artificial neural network (11-5-1) for predicting oxidative stability of Chemlali Sfax olive oil

tuned. In this model, configuration with five hidden nodes is illustrated in Fig. 1 and yielded the highest score.

Moreover, Chemlali Medenine model (Model 2) had a better training performance $R^2 = 99.796\%$ and validation performance $R^2 = 98.948\%$. Configuration with seven hidden nodes is represented in Fig. 2 and yielded the highest score.

Thus, this model was considered the best one for prediction of oxidative stability based on olive oil components. Furthermore, Zalmati Medenine model (Model 3) had a better training performance $R^2 = 99.927\%$ and validation performance $R^2 = 99.77\%$. Configuration with 4 hidden nodes illustrated in Fig. 3 and yielded the highest score. Thus, this model was considered the best predictor particularly for Zalmati Medenine.

Discussion

Fatty acid composition

The differentiations detected between areas for the fatty acid composition might be proven by the variation in altitude and temperature between the chosen zones. In fact, region of Sfax is known as a coastal central province and Medenine is known as a south coastal province. This result is concordant with the finding of Pinelli et al. [59] who observed that fatty acid composition could be associated with geographical locality, the kind of the olive grove zones, and the salinity of soil. Similarly, obtained results were analogous with those of Kotti et al. [41] who proposed the effect of environment factor in

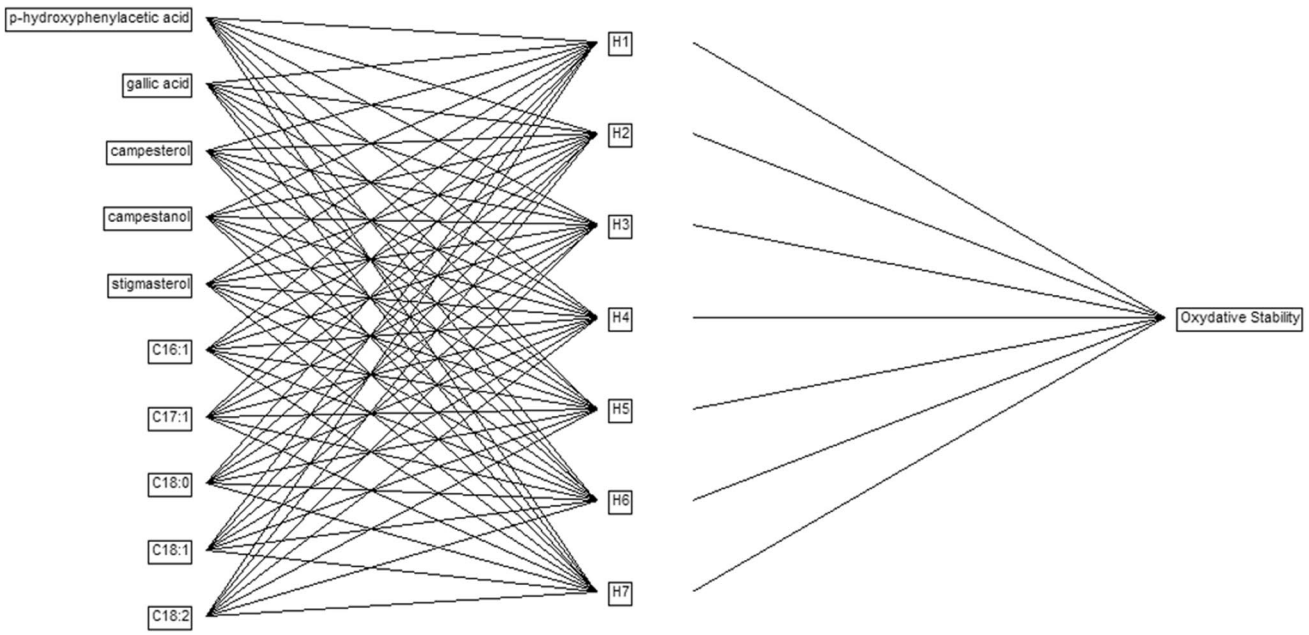

Fig. 2 Structure of artificial neural network (10-7-1) for predicting oxidative stability of Chemlali Medenine olive oil

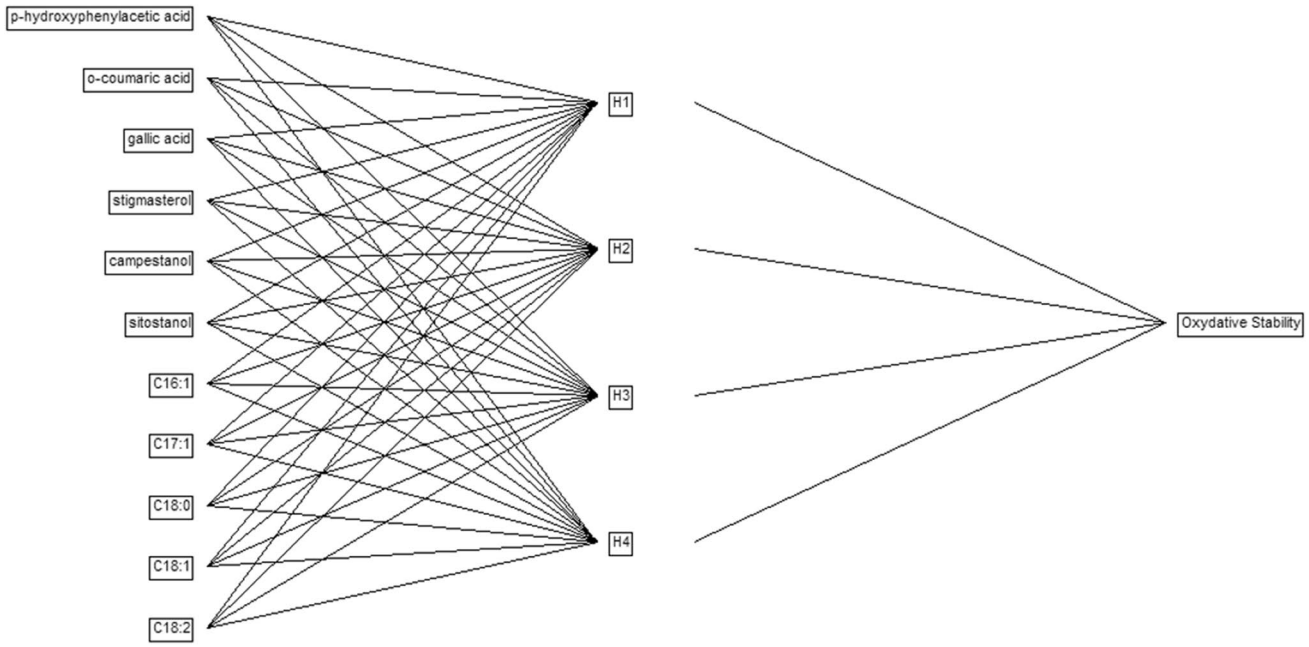

Fig. 3 Structure of artificial neural network (11-4-1) for predicting oxidative stability of Zalmati Medenine olive oil

defining the oil quality of Tunisian Chemlali cultivar. Truly, they showcase that virgin olive oils produced by Chemlali olive cultivar had been in most cases high in oleic acid.

Sterol composition

In the present study, the sterolic composition of three varieties of virgin olive oils had been compared: namely, Chemlali Sfax and Chemlali Medenine, which showed elevated

oxidative stabilities, and Zalmati Medenine, which showed instable factors. Sterols have most commonly been suggested to compose essential factors entailed within the selection of olive oil quality and are frequently applied in olive oil authenticity assessments [62]. Actually, virgin olive oil is well recognized for its composition of minor contents that have health-promoting traits [56].

Relationship between oxidative stability and fatty acids

From established equations, we deduced that palmitoleic acid (C16:1), margaroleic acid (C17:1), and linoleic acid (C18:2) of Chemlali Medenine variety have the most important consequence on oxidative stability variation compared to Chemlali Sfax and Zalmati Medenine. Stearic acid (C18:0) of Chemlali Sfax variety is considered has the most important effect on oxidative stability when compared to two studied olive cultivars. It is noted that the geographical location enhances this difference with a preference for the south of Tunisia for major fatty acid contents. These differences could be explained by the diverse fatty acid composition of varieties grown in regions far apart. In fact, the geographical location had an influence on fatty acid composition despite the same Chemlali variety origin. The fatty acid values of olive oils present an extensive range of variability with genetic factors explaining from 70 to 80% of the differences between olive tree accessions [17]. These observations validate the obtained results with a significant difference ($P < 0.05$) between fatty acid composition of two different olive tree cultivars (Chemlali Medenine and Zalmati Medenine) grown in a common geographical area.

Relationship between oxidative stability and phenolic acids

It was demonstrated that coumaric acid has several biological actions such as antioxidant and radical scavenging activities, anti-inflammatory, and neuroprotective actions [38]. In this study, gallic acid was shown to have an essential role in the oxidative stability of Chemlali Sfax. This role is less important for oxidative stability of Chemlali Medenine and Zalmati Medenine. In agreement with our results, the previous studies reported that geographical location is tightly related to the variation of phenolic composition [3] and oxidative stability of olive oils [65]. It has been shown that phenolic compounds are more efficient than tocopherols in enhancing the stability of olive oil toward oxidation [65].

Relationship between oxidative stability and sterols content

On the basis of obtained results, the campestanol, stigmasterol, and sitostanol have a positive effect on improvement of oxidative stability. Virgin oil shows a very acceptable correlation between stability and sterols concentration [37]. On the basis of regression model proposed for estimation of oxidative stability of Zalmati Medenine olive oil (Table 4C), it is obvious that all parameters have an active contribution on enhancement of oxidative stability. The effect of campestanol appears to be more important in the oxidative stability enhancement of Chemlali Sfax and Chemlali Medenine olive oils when compared to Zalmati Medenine.

Furthermore, stigmasterol and sitostanol appear to be more effective in the oxidative stability of Chemlali Sfax compared to Zalmati Medenine and Chemlali Medenine. It should be observed that stigmasterol and sitostanol contribution in variation of oxidative stability is linked with geographical location. In fact, it appears that the center of Tunisia is more adequate for enhancement of stigmasterol and sitostanol. Ryan et al. [66] have shown that climatic factors such as temperature and precipitation are closely related with geographical location and have notable effect on plant physiology, and thus on vegetative components. Besides, the quality of this product can be established from geographical and varietal origins, which are immediately linked with olive oil distinctive characteristics [25].

ANN modeling of oxidative stability analyses

The model illustrated in Fig. 1 was characterized by the best ANN architecture and a more adequate structure. It presented high values of R^2 for both training and validation data, as shown in Table 5. In fact, several studies deduced that a perfect model would result in a cross-validation R^2 value of 1 [25, 69].

As shown in Table 5, all selected values presented high R^2 (more than 98%) for both training and validation data despite the variation in the ANN architecture. This difference is located in the number of hidden nodes. The MLP architecture is a method supported on back-propagation algorithm, which is an extremely flexible and adjustable in designing and simulation [72]. It can be considered as a standardized algorithm for any supervised-learning pattern recognition process. However, it uses "black box" for classification that is very difficult to explain the performance of results in some cases [31].

The high coefficients of determination (R^2) for the three established models at the training stage indicate a strong relationship between the output (oxidative stability) and the inputs (sterols, phenols, and fatty acids). Deciding the number of neurons in the hidden layer is a very important part of deciding the overall neural network architecture [46]. Hidden layers do not directly interact with the external environment, but still have a tremendous influence on the final output. This shows that both models have good predictive abilities and are suitable for predicting the oxidative stability for new predictions. On the other side, it is obvious that bootstrap technique has proved to be significantly useful and appropriate for predictors such as ANNs. In fact, Carney and Cunningham [14] studied bootstrap aggregation in the perspective of ANNs and deduced that model generalization aptitude can be considerably developed. It is the simplest approach, since it does not entail the complex calculation of derivatives and Hessian-matrix inversion implicated in linear techniques or the Monte Carlo solutions of the integrals concerned in the Bayesian method [21].

The MLR is an algorithmic usual model generally employed in biological sciences and technology [4, 71]. In comparison with other artificial intelligence approaches, MLR modeling offers a more clear vision to forecast [15]. ANNs are a category of non-linear numerical models that are distinguished by a complex construction of computational and interconnected components, the neurons. In comparison with MLR models, ANN provides a computational approach of deciding a non-linear connection between inputs and one or more outputs. ANN has employed for modeling, recognition, and forecast of complicated organizations [35]. In our study, ANNs as an alternative are proposed for predicting oil oxidative stability.

Our results indicated that one potential application of ANN may be evaluating the quality of MLR prediction models due to of their inherent flexibility [34], and ANN may perform better than MLR in many cases as shown in this study. In fact, the R^2 values estimated from ANN method are higher than those from multiple linear regression method despite the number of parameters simultaneously implied in each analysis. Therefore, ANN technique may be a more appropriate tool when emphasis is put on the prediction itself and not on the underlying relations between independent and dependent variables.

Conclusion

Oxidative stability as well as fatty acid composition, phenolic acid, and sterols content of different Tunisian olive oils were investigated to study the differences among oils from different geographical locations. On the basis of the combination of ANOVA analysis and multiple linear regressions (MLR), different approaches were investigated to improve the geographical classification. This study demonstrates that fatty acid, phenolic acid, and sterol compositions were significantly ($P < 0.05$) influenced by both olive cultivar and geographical region. The findings showed also that phenolic acids content and sterols content displayed significant qualitative and quantitative differences among the cultivar and their geographical origins.

The models based on multiple linear regressions cannot predict oxidative stability of studied olive oils with similar accuracy as the obtained for the selected neural networks. Besides, the modeling based on artificial neural networks brings more advantages, because opens the possibility of the use of networks for prediction of more complex parameters. This approach gets easy doing artificial neural network models by connections between different variables and interpretation of obtained results by highlighting various effects of related explanatory variables.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication

Compliance with ethics requirements The research does not include any human subjects and animal experiments.

References

1. Abood AA, Al-Ansari AM, Migdadi HM, Okla AM, Assaed AM, Hegazy AK, Alshameri AM, Khan MA (2017) Molecular and phytochemical analysis of wild type and olive cultivars grown under Saudi Arabian environment. 3 Biotech 7:289. https://doi.org/10.1007/s13205-017-0920-5
2. Abuznait AH, Qosa H, Busnena BA, ElSayed KA, Kaddoumi A (2013) Olive-oil-derived oleocanthal enhances β-amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: in vitro and in vivo studies. ACS Chem Neurosci 4:973–982
3. Alkan D, Tokatli F, Ozen B (2012) Phenolic characterization and geographical classification of commercial extra virgin olive oils produced in Turkey. J Am Oil Chem Soc 89:261–268
4. Andreu-Sevilla A, Hartmann A, Sayas E, Burló-Carbonell F, Delgado-Estrella P, Valverde JM, Carbonell-Barrachina ÁA (2008) Mathematical quantification of total carotenoids in Sioma® oil using color coordinates and multiple linear regression during deep-frying simulations. Eur Food Res Technol 226(6):1283–1291
5. Asnaashari E, Asnaashari M, Ehtiati A, Farahmandfar R (2015) Comparison of adaptive neuro-fuzzy inference system and artificial neural networks (MLP and RBF) for estimation of oxidation parameters of soybean oil added with curcumin. J Food Meas Charact 9(2):215–224
6. Austin PC, Small DS (2014) The use of bootstrapping when using propensity-score matching without replacement: a simulation study. Stat Med 33(24):4306–4319

7. Barrow DK, Crone SF (2016) Cross-validation aggregation for combining autoregressive neural network forecasts. Int J Forecast 32(4):1120–1137
8. Bedbabis S, Ben Rouina B, Mazzeo A, Ferrara G (2017) Irrigation with treated wastewater affected the minor components of virgin olive oil from cv. Chemlali in Tunisia. Eur Food Res Technol 243:1887–1894
9. Ben Hlima H, Ben Ayed R, Ennouri K, Smaoui S (2017) Geographical discrimination of virgin olive oils from the Tunisian coasts by combining fatty acids and phenolic acids profiles within a multivariate analysis. J Oleo Sci 66:963–971
10. Ben Mansour A, Porter EA, Kite GC, Simmonds MS, Abdelhedi R, Bouaziz M (2015) Phenolic profile characterization of Chemlali olive stones by liquid chromatography-ion trap mass spectrometry. J Agric Food Chem 63:1990–1995
11. Bes-Rastrollo M, Sanchez-Villegas A, de la Fuente C, de Irala J, Martinez JA, Martinez-Gonzalez MA (2006) Olive oil consumption and weight change: the SUN prospective cohort study. Lipids 41:249–256
12. Bilušić T, Žanetić M, Ljubenkov I, Generalic Mekinic I, Stambuk S, Bojovic V, Soldo B, Magiatis P (2018) Molecular characterization of Dalmatian cultivars and the influence of the olive fruit harvest period on chemical profile, sensory characteristics and oil oxidative stability. Eur Food Res Technol 244:281–289
13. Borsato D, Pina MVR, Spacino KR, dos Santos Scholz MB, Androcioli Filho A (2011) Application of artificial neural networks in the geographical identification of coffee samples. Eur Food Res Technol 233(3):533
14. Carney JG, Cunningham P (1999) Tuning diversity in bagged neural network ensembles. Trinity College Dublin Technical Report, 44
15. Chakraborty S, Basu S (2014) Mechanistic insight into the radical scavenging activity of polyphenols and its application in virtual screening of phytochemical library: an in silico approach. Eur Food Res Technol 239(5):885–893
16. Chtourou M, Gargouri B, Jaber H, Abdelhedi R, Bouaziz M (2013) Comparative study of olive oil quality from Chemlali Sfax versus Arbequina cultivated in Tunisia. Eur J Lipid Sci Technol 115:631–640
17. Cicatelli A, Fortunati T, De Feis I, Castiglione S (2013) Oil composition and genetic biodiversity of ancient and new olive (*Olea europea* L.) varieties and accessions of southern Italy. Plant Sci 210:82–92
18. Del Alamo RR, Fregapane G, Aranda F, Gomez-Alonso S, Salvador MD (2004) Sterol and alcohol composition of Cornicabra virgin olive oil: the campesterol content exceeds the upper limit of 4% established by EU regulations. Food Chem 84:533–537
19. Diez CM, Trujillo I, Martinez-Urdiroz N, Barranco D, Rallo L, Marfil P, Gaut BS (2015) Olive domestication and diversification in the Mediterranean Basin. New Phytol 206:436–447
20. Dıraman H, Saygı H, Hışıl Y (2011) Classification of three Turkish olive cultivars from Aegean region based on their fatty acid composition. Eur Food Res Technol 233(3):403
21. Dybowski R, Roberts SJ (2000) Confidence and prediction intervals for feed-forward neural networks. In: Dybowski R, Gant V (eds) Clinical applications of artificial neural networks. Cambridge Univ Press, New York, pp 298–326
22. Efron B, Tibshirani TJ (1993) An introduction to the bootstrap. Chapman and Hall, New York
23. Ennouri K, Ayed RB, Ercisli S, Smaoui S, Gouiaa M, Triki MA (2018) Variability assessment in *Phoenix dactylifera* L. accessions based on morphological parameters and analytical methods. Acta Physiol Plant 40(1):5
24. Ennouri K, Ayed RB, Smaoui S (2018) Opportunities for computational biology uses in enhancement of bioproduct quality: lessons from olive oil. Emir J Food Agric. 30(7):532–538
25. Ennouri K, Ben Ayed R, Ercisli S, Ben Amar F, Triki MA (2017) Evaluation of variability in tunisian *Olea europaea* L. accessions using morphological characters and computational approaches. Not Bot Horti Agrobot Cluj-Napoca 45:262–269
26. Ennouri K, Ben Ayed R, Mazzarello M, Ottaviani E, Hertelli F, Azouz H (2016) Classical and Bayesian predictions applied to *Bacillus* toxin production. 3 Biotech. https://doi.org/10.1007/s13205-016-0527-2
27. Ennouri K, Ben Ayed R, Triki MA, Ottaviani E, Mazzarello M, Hertelli F, Zouari N (2017) Multiple linear regression and artificial neural networks for delta-endotoxin and protease yields modelling of *Bacillus thuringiensis*. 3 Biotech. https://doi.org/10.1007/s13205-017-0799
28. Ennouri K, Ben Hassen H, Zouari N (2013) Optimization of bioinsecticides overproduction by *Bacillus thuringiensis* subp. *kurstaki* using linear regression. Pol J Microbiol 62:287–293
29. European Commission Regulation EEC/2568/ 91 of 11 July (1991) On the characteristics of olive and olive pomace oils and their analytical methods. Off J Eur Communities L 248:1–82
30. Eussen SR, Feenstra TL, Toxopeus IB, Hoekstra J, Klungel OH, Verhagen H, Van Kranen HJ, Rompelberg CJ (2011) Costs and health effects of adding functional foods containing phytosterols/-stanols to statin therapy in the prevention of cardiovascular disease. Eur J Pharmacol 668:S91–S100
31. Foley AM, Leahy PG, Marvuglia A, McKeogh EJ (2012) Current methods and advances in forecasting of wind power generation. Renew Energ 37:1–8
32. Garg A (1998) High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. Am J Clin Nutr 67:577S–582S
33. Gargouri B, Ammar S, Zribi A, Ben Mansour A, Bouaziz M (2013) Effect of growing region on quality characteristics and phenolic compounds of Chemlali extra-virgin olive oils. Acta Physiol Plant 35:2801–2812
34. Ghamali M, Chtita S, Ousaa A, Elidrisse B, Bouachrine M, Lakhlifi T (2017) QSAR analysis of the toxicity of phenols and thiophenols using MLR and ANN. J Taibah Univ Sci 11:1–10
35. Gomes JFS, Leta FR (2012) Applications of computer vision techniques in the agriculture and food industry: a review. Eur Food Res Technol 235(6):989–1000
36. Gurdeniz G, Ozen B, Tokatli F (2008) Classification of Turkish olive oils with respect to cultivar, geographic origin and harvest year, using fatty acid profile and mid-IR spectroscopy. Eur Food Res Technol 227(4):1275–1281
37. Gutiérrez F, Jimenez B, Ruiz A, Ali MA (1999) Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. J Agric Food Chem 47:121–127
38. Guven M, Aras AB, Akman T, Murat Sen H, Ozkan A, Salis O, Sehitoglu I, Kalkan Y et al (2015) Neuroprotective effect of p-coumaric acid in rat model of embolic cerebral ischemia. Iran J Basic Med Sci 18:356–363
39. Jimenez B, Sánchez Ortiz A, Lorenzo ML, Rivas A (2014) Effect of organic cultivation of Picual and Hojiblanca olive varieties on the quality of virgin olive oil at four ripening stages. Eur J Lipid Sci Technol 116:1634–1646
40. Juan ME, Wenzel U, Ruiz-Gutiérrez V, Daniel H, Planas JM (2006) Olive fruit extracts inhibit proliferation and induce apoptosis in HT-29 human colon cancer cells. J Nutr 136:2553–2557
41. Kotti F, Chiavaro E, Cerretani L, Barnaba C, Gargouri M, Bendini A (2009) Chemical and thermal characterization of Tunisian extra virgin olive oil from Chetoui and Chemlali cultivars and different geographical origin. Eur Food Res Technol 228:735–742
42. Kreiss JP, Paparoditis E (2011) Bootstrap methods for dependent data: a review. J Korean Stat Soc 40(4):357–378

43. Kremer JM, Lawrence DA, Jubiz W, DiGiacomo R, Ryns R, Bartholomew LE et al (1990) Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis clinical and immunologic effects. Arthritis Rheum 33:810–820
44. Laincer F, Iaccarino N, Amato J, Pagano B, Pagano A, Tenore G et al (2016) Characterization of monovarietal extra virgin olive oils from the province of Béjaïa (Algeria). Food Res Int 89:1123–1133
45. Laroussi-Mezghani S, Le Dréau Y, Molinet J, Hammami M, Grati-Kamoun N, Artaud J (2016) Biodiversity of Tunisian virgin olive oils: varietal origin classification according to their minor compounds. Eur Food Res Technol 242(7):1087–1099
46. Lee S, Choeh JY (2014) Predicting the helpfulness of online reviews using multilayer perceptron neural networks. Expert Syst Appl 41:3041–3046
47. Lubes G, Goodarzi M (2017) Analysis of volatile compounds by advanced analytical techniques and multivariate chemometrics. Chem Rev 117:6399–6422
48. Ma J, Yu G, Wang D, Sun Y, Lu J, Cao H, Lin F (2017) Assessment of triglyceride and cholesterol in overweight people based on multiple linear regression and artificial intelligence model. Lipids Health Dis 16:42. https://doi.org/10.1186/s12944-017-0434-5
49. Mirjalili S (2019) Evolutionary algorithms and neural networks. Springer, Cham
50. Miyake Y, Sasaki S, Tanaka K, Fukushima W, Kiyohara C, Tsuboi Y, Yamada T, Oeda T et al (2010) Dietary fat intake and risk of Parkinson's disease: a case–control study in Japan. J Neurol Sci 288:117–122
51. Monasterio RP, Olmo-García L, Bajoub A, Fernandez-Gutierrez A, Carraco-Pancorbo A (2017) Phenolic compounds profiling of Virgin olive oils from different varieties cultivated in Mendoza, Argentina, by using liquid chromatography-mass spectrometry. J Agric Food Chem 65:8184–8195
52. Nieto LM, Hodaifa G, Lozano Peña JL (2010) Changes in phenolic compounds and Rancimat stability of olive oils from varieties of olives at different stages of ripeness. J Sci Food Agric 90:2393–2398
53. Ocakoglu D, Tokatli F, Ozen B, Korel F (2009) Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for 2 harvest years. Food Chem 113:401–410
54. Owen RW, Haubner R, Wurtele G, Hull E, Spiegelhalder B, Cartsch H (2004) Olives and olive oil in cancer prevention. Eur J Cancer Prev 13:319–326
55. Pentoś K, Łuczycka D, Kapłon T (2015) The identification of relationships between selected honey parameters by extracting the contribution of independent variables in a neural network model. Eur Food Res Technol 241(6):793–801
56. Pérez-Jiménez F, Ruano J, Perez-Martinez P, Lopez-Segura F, Lopez-Miranda J (2007) The influence of olive oil on human health: not a question of fat alone. Mol Nutr Food Res 51(10):1199–1208
57. Pérez-López FR, Chedraui P, Haya J, Cuadros JL (2009) Effects of the Mediterranean diet on longevity and age-related morbid conditions. Maturitas 64:67–79
58. Perona JS, Cabello-Moruno R, Ruiz-Gutiérrez V (2006) The role of virgin olive oil components in the modulation of endothelial function. J Nutr Biochem 17:429–445
59. Pinelli P, Galardi C, Mulinacci N, Vincieri FF, Cimato A, Romani A (2003) Minor polar compound and fatty acid analyses in monocultivar virgin olive oils from Tuscany. Food Chem 80:331–336
60. Psaltopoulou T, Kosti RI, Haidopoulos D, Dimopoulos M, Panagiotakos DB (2011) Olive oil intake is inversely related to cancer prevalence: a systematic review and a meta-analysis of 13,800 patients and 23,340 controls in 19 observational studies. Lipids Health Dis 10(1):127
61. Puértolas E, De Marañón IM (2015) Olive oil pilot-production assisted by pulsed electric field: impact on extraction yield, chemical parameters and sensory properties. Food Chem 167:497–502
62. Reboredo-Rodríguez P, Figueiredo-González M, González-Barreiro C, Simal-Gandara J, Desamparados M, Cancho-Grande B, Fregapane G (2017) State of the art on functional virgin olive oils enriched with bioactive compounds and their properties. Int J Mol Sci 18:668. https://doi.org/10.3390/ijms18030668
63. Rigane G, Salem RB, Sayadi S, Bouaziz M (2011) Phenolic composition, isolation, and structure of a new deoxyloganic acid derivative from Dhokar and Gemri-Dhokar olive cultivars. J Food Sci 76(7):C965–C973
64. Rodrigues N, Casal S, Pinho T, Peres AM, Bento A, Baptista P, Pereira JA (2019) Ancient olive trees as a source of olive oils rich in phenolic compounds. Food Chem 276:231–239
65. Romero N, Saavedra J, Tapia F, Sepulveda B, Aparicio R (2016) Influence of agroclimatic parameters on phenolic and volatile compounds of Chilean virgin olive oils and characterization based on geographical origin, cultivar and ripening stage. J Sci Food Agric 96:583–592
66. Ryan D, Robards K, Lavee S (1998) Evolution de la qualité de l'huile d'olive. Olivae 72:23–41
67. Serrano L, Cruz A, Sousa S, Morais Z (2016) Alterations in monovarietal, blended and aromatized Portuguese virgin olive oils under four storage conditions for 12 months. Eur Food Res Technol 242(7):1041–1055
68. Servili M, Selvaggini R, Esposto S, Taticchi A, Montedoro G, Morozzi G (2004) Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. J Chromatogr A 1054(1–2):113–127
69. Shamseldin AY (2010) Artificial neural network model for river flow forecasting in a developing country. J Hydroinform 12:22–35
70. Smaoui S, Ennouri K, Chakchouk-Mtibaa A, Sellem I, Bouchaala K, Karray-Rebai I, Mellouli L (2018) Statistical versus artificial intelligence-based modeling for the optimization of antifungal activity against *Fusarium oxysporum* using *Streptomyces* sp. Strain TN71. J Mycol Med 28(3):551–560
71. Smaoui S, Hsouna AB, Lahmar A, Ennouri K, Mtibaa-Chakchouk A, Sellem I et al (2016) Bio-preservative effect of the essential oil of the endemic *Mentha piperita* used alone and in combination with BacTN635 in stored minced beef meat. Meat Sci 117:196–204
72. Tang J, Deng C, Huang GB (2016) Extreme learning machine for multilayer perceptron. IEEE Trans Neural Netw Learn Syst. 27:809–821
73. Temime SB, Manai H, Methenni K, Baccouri B, Abaza L, Daoud D et al (2008) Sterolic composition of Chétoui virgin olive oil: influence of geographical origin. Food Chem 110(2):368–374
74. Tsartsou E, Proutsos N, Castanas E, Kampa M (2019) Network meta-analysis of metabolic effects of olive-oil in humans shows the importance of olive oil consumption with moderate polyphenol levels as part of the Mediterranean diet. Front Nutr 6:6
75. Uğurlu M, Kula I (2007) The removal of colour, carotene and acidity from crude olive oil by using sepiolite. Int J Food Sci Technol 42:359–365
76. Uncu O, Ozen B (2016) Geographical differentiation of a monovarietal olive oil using various chemical parameters and mid-infrared spectroscopy. Anal Methods 8:4872–4880
77. Wang ZW, Duan HW, Hu CY, Wu YM (2010) Development and comparison of multivariate respiration models for fresh papaya (*Carica papaya* L.) based on regression method and artificial neural network. Eur Food Res Technol 231(5):691–699
78. Wong TT (2015) Performance evaluation of classification algorithms by k-fold and leave-one-out cross validation. Pattern Recognit 48(9):2839–2846
79. Wood LG, Hazlewood LC, Foster PS, Hansbro PM (2010) Lyprinol reduces inflammation and improves lung function in a mouse model of allergic airway disease. Clin Exp Allergy 40:1785–1793

80. Zegane O, Keciri S, Louaileche H (2015) Physicochemical characteristics and pigment content of algerian olive oils: effect of olive cultivar and geographical origin. Int J Chem Biomol Sci 1:153–157
81. Zhang Y, Yang Y (2015) Cross-validation for selecting a model selection procedure. J Econom 187(1):95–112