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REVIEW

Traceability Markers to the Botanical Origin in Olive Oils

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This review provides an overview of traceability studies performed to date (April 2009) for olive oils. Special emphasis has been made on the botanical origin because high-quality monovarietal olive oils have been recently introduced on the markets and their quality control requires the development of new and powerful analytical tools as well as new regulations to avoid fraud to consumers. Several parameters with discriminant power have been used for olive oil traceability according to the olive variety used in the production of the oil. They have been considered as traceability markers to the botanical origin and classified, in this work, as compositional and genetical markers.

KEYWORDS: Traceability; olive oil; botanical origin; marker

INTRODUCTION

Food traceability implies the control of the entire chain of food production and marketing, allowing the food to be traced through every step of its production back to its origin. The verification of food traceability is necessary for the prevention of deliberate or accidental mislabeling, which is very important in the assurance of public health. As a consequence, food traceability is required by consumers and government organizations because it is a significant component of food safety. Thus, Regulation 178/2002 (*1*) provides the basis for the assurance of a high level of protection of human health and consumers' interest in relation to food. In the case of olive oils, the increase in the demand for high-quality olive oils has led to the appearance in the market of olive oils elaborated with specific characteristics. They include oils of certain regions possessing well-known characteristics, that is, olive oils with a denomination of origin, or with specific olive variety composition, that is, coupage or monovarietal olive oils.

The appearance of denominations and protected indications of origin has promoted the existence of oils labeled according to these criteria. Regulation 2081/92 (*2*) created the systems known as Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) to promote and protect food products. Nowadays, these denominations are regulated by Regulation EC 510/2006 (*3*). These two above-mentioned protected denominations are compared in **Table 1**. For example, an olive oil with a PDO denomination requires that it meet precise definitions of several parameters such as cultivar, geographical origin, agronomic practice, production technology, and organoleptic qualities (*4*), and all of these parameters have to be investigated to study its traceability and to certify its quality. Among the above-mentioned factors, the two first are the most important. Additionally, a Database of Origin and Registration (DOOR) was created to support these denominations (*5*).

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Moreover, there are olive oils obtained from one genetic variety of olive (monovarietal) or from several different varieties (coupage). Monovarietal olive oils have certain specific characteristics related to the olive variety from which they are elaborated. Coupage olive oils are obtained from several olive varieties to achieve a special flavor or aroma. However, there are great variations in the prices of these oils. This fact can lead consumers to doubt the quality of commercialized olive oils. Since 2002, a Commission Regulation (1019/2002) (*6*) has controlled the marketing standards for olive oils. This regulation highlights the necessity to reach an obligatory regimen of origin designation in extra virgin and virgin olive oils, but recognizes the absence of control systems applicable for this purpose. Nevertheless, the mixture of olive oils of different botanical origins is not mentioned in this regulation.

The works dealing with olive oil traceability are usually focused on investigating the botanical or geographical origin. However, the concept of geographical traceability, in which the objective is the geographical location of the olive tree, is slightly different from the concept of botanical traceability, in which the olive used for the olive oil production is the aim. In both cases, the selection of the markers (compounds with discriminating power) to be studied is complicated because the composition of extra virgin olive oils is the result of complex interactions among olive variety, environmental conditions, fruit ripening, and oil extraction technology (*7*). Therefore, a careful definition of the geographical or botanical origin of an olive oil based on its chemical composition requires that many factors be taken into account, it being very difficult to find an appropriate marker. As a consequence, the aim of this review was to provide an overview of the traceability studies performed to date for olive oils. From the two main factors studied to control the traceability of olive oils (geographical and botanical origin), special emphasis will be put on the botanical origin. In fact, virgin olive oils made from a certain variety (monovarietal olive oils) or a mixture of several varieties (coupage olive oils) are being increasingly introduced in the markets, and their quality control requires the development of

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Table 1. General Regimen for Food and Certain Other Agricultural Products Based on Regulation 510/2006 (*3*)

general regimen	origin	characteristics	restriction
Protected Designation of Origin (PDO)	In that region, specific place, or country	Quality essentially or exclusively due to a particular geographical area	Produced, processed and prepared in a given geographical area
Protected Geographical Indication (PGI)	In that region, specific place, or country	Slightly less strict; food reputation of a product from a given region is sufficient	One of the stages of production, processing, or preparation takes place in the area

new and powerful analytical tools as well as new regulations to avoid fraud to the consumers.

TRACEABILITY TO THE BOTANICAL ORIGIN

The verification of the cultivars employed to produce an olive oil sample may contribute to certify the oil origin. This fact may have commercial interest in the case of monovarietal olive oils or olive oils with PDO (which involve a specific olive variety composition) because these high-quality olive oils may be adulterated by other oils of lower quality using anonymous or less costly cultivars (*8*). However, as the quality of an olive oil depends on the olive variety from which it is elaborated, the production of olive oils from certain varieties has increased (*9*). The olive variety selection has been made according to its adaptation to different climatic conditions and soils. In addition, whereas some cultivars are characteristic of a given zone, others can be found in several countries (*10*). As a consequence, one olive variety can be cultivated and named in a different way (a variety may adopt several synonyms) in distinct geographical locations, making the differentiation of olive varieties in olive oils quite complex. Traditionally, differentiation among olive cultivars has been supported by numerous morphological (study of the form or shape) and pomological (the development, cultivation, and physiological studies of fruit trees) traits. Unfortunately, morphological traits have been difficult to evaluate, are affected by subjective interpretations, and are severely influenced by the environment and plant developmental stage (*9*). Nowadays, different efforts have been focused on the investigation of one or several compounds present in olive oils with capability to differentiate among olive varieties. They have been considered as traceability markers. According to their own identification potential, they have been classified in this work as compositional and genetical markers.

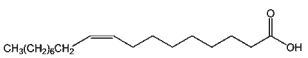
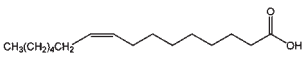
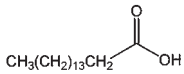
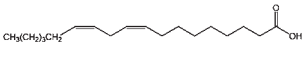
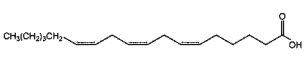
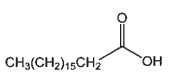
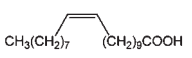
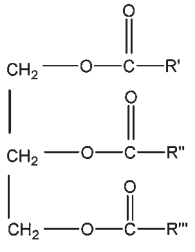
Compositional Markers. When the botanical origin or the olive variety of an olive oil is investigated, it is important to realize that the composition or the amount of the compounds studied is influenced by several factors (*11*), making difficult the interpretation of the results obtained. For this reason, up to now, there are several and varied parameters with discriminant power used for olive oil traceability according to the variety of olive used in the production of the oil (*12*). In this work, those compounds that take part of the composition of olive oils are considered to be compositional markers. They have been differentiated in major components (fatty acids and triglycerides) and minor components (sterols, phenolic compounds, volatile compounds, pigments, hydrocarbons, and tocopherols) according to their presence in olive oils. The major components used up to now as traceability markers to the botanical origin of olive oils have been grouped in **Table 2**. This table shows the compounds mainly studied, their chemical structure, the analytical and chemometric technique used, the number of varieties analyzed, and their geographical origin as well as the most important information obtained on the discriminating capability of olive varieties in olive oils.

Fatty Acids. Fatty acids are simple structures made up of long chains of various numbers of carbon atoms, with a carboxylic acid group at one end. The main fatty acids present in olive oils are grouped in **Table 2**. High amounts of monosaturated fatty acids are present in olive oils, which confer to them a high nutritional value. Moreover, oil characteristics are influenced by the proportions of fatty acids present. As can be seen in **Table 2**, the studies on the composition of fatty acids in olive oils were usually performed by gas chromatography (GC) coupled with flame ionization detection (FID) (*13*−*26*). In most papers collected in **Table 2**, the use of chemometric tools was required to perform an olive oil differentiation according to the fatty acid composition. The content in fatty acids was used by some researchers to differentiate olive varieties (*15*, *16*, *19*). For example, D'Imperio et al. (*15*) established a qualitative similarity among olive oils of different cultivars and showed that these varieties were grouped together by discriminant analysis. This fact revealed that the fatty acid composition of olive oils was strongly influenced by several factors such as cultivar, maturation stage of fruit, and zone of origin (*15*, *19*). To relate the fatty acid composition of olive oils with the cultivar influence, Mannina et al. (*17*) studied olive oil in a well-limited geographical region, neglecting the pedoclimatic factor (soil characteristics such as temperature and humidity) and finding a relationship between the fatty acid composition and some specific cultivars. However, Di Bella et al. (*13*) maintained that although the effect of the cultivar was significant in an olive oil classification based on the fatty acid composition, a predominant and well-defined geographical effect was also present. It is important to consider that chemometric tools can be used not only to describe the characteristics of oils and classify them but also to select the best variables to obtain satisfactory results (*18*, *20*−*22*, *27*). For example, variability in fatty acid and triglyceride concentrations among olive oil samples was used by some authors (*14*, *21*) applying chemometric methods. Moreover, in comparison with other markers, the fatty acid and triglyceride composition allowed a better differentiation of olive oils than the sterol composition (*26*).

Triglycerides. Triacylglycerols or triglycerides are the main components of olive oils. They are formed from a single molecule of glycerol combined with three fatty acids. As can be seen in **Table 2**, HPLC equipped with a refraction index detector (RID) was the most widely used technique for the analysis of triglycerides in olive oils (*14*, *21*, *23*, *25*, *26*, *28*−*31*), and it was usually combined with chemometric tools to investigate different cultivars (*14*, *21*, *23*, *24*, *26*, *29*−*32*). There are several studies focused on the analysis of triglycerides in olive oils, being the triglycerides analyzed very different. For this reason, only the triglycerides mainly studied in olive oils are included in **Table 2**. Individual contents of some triglycerides allowed differentiation among olive cultivars using chemometric tools (*29*, *30*). Triglyceride composition has been shown to be a powerful discrimination tool for olive oil differentiation (*21*, *28*), and it has been reported to be more useful than other compositional markers such as sterols (*26*, *31*, *32*). Moreover, triglyceride and fatty acid composition have been used to make a database that allows the differentiation of French olive oils (*14*).

Major components of olive oils may provide basic information on olive cultivars. However, minor components can provide more useful information and have been more widely used to differentiate the botanical origin of olive oils. **Table 3** shows the minor components of olive oils more useful for olive oil differentiation.

Table 2. Compositional Markers of Olive Variety Present in Olive Oils, Considering the Major Components[a]

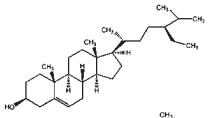
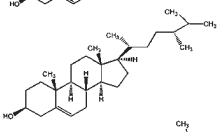
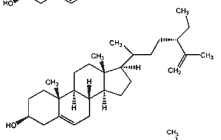
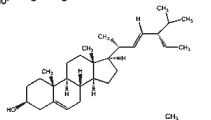
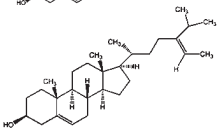
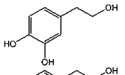
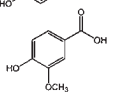
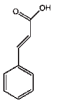
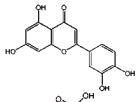
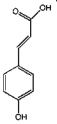
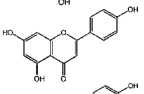
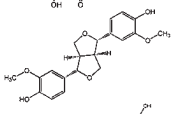
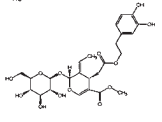
Marker	Compounds mainly studied	Chemical structure	Analytical technique	Chemometric treatment	Number of varieties analyzed	Geographical area of origin	Information obtained	Reference
Fatty acids	**Oleic acid, O (C18:1)**		GC-FID	PCA, DA, and LDA	4	Maghrebian and Peloritana areas (Sicily, Italy)	Fatty acid composition is capable to differentiate olive oils according to botanical origin and geographical area	(*13*)
			GC-FID	SLDA	5	PDO Nyons and PDO Vallée des Baux (France)	Construction of a data bank using fatty acid and triglyceride composition of French virgin olive oils to identify their origin	(*14*)
	Palmitoleic acid, Po (C16:1)		GC-FID	PCA, ANOVA, and LDA	22	Different areas of Sicily (Italy)	Qualitative similarity exists on fatty acid composition among olive oils of different olive variety	(*15*)
			GC-FID	DA	7	Different areas of Extremadura (Spain)	Fatty acid contents vary among monovarietal olive oils	(*16*)
	Palmitic acid, P (C16:0)		GC-FID	MANOVA, PCA, HCA, MSA, and LDA	4	Sicily (Italy)	Fatty acid composition is related with some cultivars grown in a well-limited geographical region	(*17*)
			GC-FID	LDA, PCA, and ANN	5	Sabina, Lazio (Italy)	Olive oil differentiation according to their olive variety is achieved using some fatty acids and sterols analyzed	(*18*)
			GC-FID	ANOVA	2	Five different areas of Chania region (Crete)	Fatty acid composition shows significant potential for olive oil classification according to botanical origin and location	(*19*)
	Linoleic acid, L (C18:2)		GC-FID	MANOVA, PCA, and HCA	3	Tras-os-Montes PDO (Portugal)	Fatty acid composition allows differentiation of three olive varieties of a specific PDO oil	(*20*)
			GC-FID	ANOVA, PCA, and DA	4	Montes de Toledo PDO (Castilla-La Mancha) and others (not specified)	Total fatty acids and several combinations of triglycerides are selected for satisfactory classification of four Spanish virgin olive oil varieties studied	(*21*)
	Linolenic acid, Ln (C18:3)		GC-FID	LDA	3	Beira Baixa Region (Portugal)	Stearic acid, campesterol, total sterol and oxidative stability are the most discriminating variables in an olive oil	(*22*)
	Stearic acid, S (C18:0)		GC-FID	PCA and ANOVA	7	Sardinia (Italy) and Corsica (France)	The ratio between oleic and linoleic acid shows the most variation among the different monovarietal olive oils	(*23*)
			GC-FID	PCA and ANOVA	5	Tunisia and Sicily (Italy)	Fatty acid composition change according to the olive variety and the ripening degree of the olives	(*24*)
	Eicosenoic acid, E (C20:1)		GC-FID	Not used	14	Sfax (Tunisia)	Fatty acid composition are useful for distinguish the monovarietal olive oils belonging to particular cultivars	(*25*)
			GC-FID and ^{13}C-NMR	PCA, HCA, DA, and ANOVA	4	Apulia region (Italy)	Fatty acid and triglyceride composition allow to distinguish olive varieties in olive oils better than sterols composition	(*26*)
			MS	LDA	3	Different regions of Spain	Fatty acid and phenolic composition variables allow to predict the olive variety in olive oils	(*27*)
Triglycerides	PLnL		HPLC-RID	SLDA	5	PDO Nyons and PDO Vallée des Baux (France)	Construction of a data bank using fatty acid and triglyceride composition of French virgin olive oils to identify their botanical origin	(*14*)
			HPLC-RID	ANOVA, PCA, and DA	4	Montes de Toledo PDO (Spain) and others (not specified)	Several combinations of triglycerides and total fatty acids variables are selected for a satisfactory classification of four Spanish monovarietal olive oils studied	(*21*)
	POP		HPLC-RID	PCA and ANOVA	7	Sardinia (Italy) and Corsica (France)	Triglyceride composition is particularly useful in discriminating monovarietal olive oils	(*23*)
	POO		HPLC-RID	Not used	14	Sfax (Tunisia)	Triglyceride profile helps to classify and characterize monovarietal olive oils. Triglyceride composition shows variations among samples from different monovarietal olive oils.	(*25*)
	PPoO		HPLC-RID	Not used	4	Tataounie region (Tunisia)	Triglyceride composition is an useful parameter to discriminate between olive varieties in olive oils	(*28*)
	OPL		HPLC-RID	ANOVA and LDA	7	Different areas of Extremadura (Spain)	Some triglyceride contents allow the differentiation among olive varieties in olive oils	(*29*)
	POO		HPLC-RID	ANOVA, PCA and CDA	2	Different areas of Chania region (Crete)	Triglyceride composition allows the differentiation of the two olive varieties examined	(*30*)
	OLnL		HPLC-RID	PCA and SIMCA	2	Cáceres (Spain)	Triglyceride composition, better than sterol composition, makes possible the characterization of olive oils obtained from a specific type of olives	(*31*)
	OLnO		HPLC-RID and ^{13}C-NMR	PCA, HCA, DA, and ANOVA	4	Apulia region (Italy)	Fatty acid and triglyceride composition allows to distinguish olive varieties in olive oils better than the sterol composition	(*26*)
	OLO		HPLC-ELSD	PCA and ANOVA	5	Tunisia and Sicily (Italy)	Triglyceride content shows variations among monovarietal olive oils from different cultivars	(*24*)
	OOO		HPLC-MS	PCA, DA, and LDA	3	Sicily (Italy), Umbria (Italy), Toscana (Italy), and Greece	Triglyceride and sterol composition allows to predict/classify olive oils with different botanical origin	(*32*)
	LOO							
	OLL							
	LLL							

[a] Abbreviations: PCA, principal component analysis; HCA, hierarchical clustering analysis; DA, discriminant function analysis; LDA, linear discriminant analysis; MANOVA, multivariate analysis of variance; SLDA, stepwise linear discriminant analysis; PLS-DA, partial least squares-discriminant analysis; MDS, multidimensional scaling; CDA, canonical discrimination analysis; SIMCA, soft independent modeling class analogy; ANN, artificial neural network; MSA, multivariate statistical analyses. Triglyceride abbreviations are shown in fatty acid columns. Bold letters indicate the most relevant components for establishing the botanical origin of olive oils (those more useful or more used).

It details the compounds mainly studied, their chemical structures, the analytical and chemometric techniques employed, the number of varieties analyzed, and their geographical areas of origin as well as the most important information obtained on the discriminating capability of olive varieties in olive oils for minor components. The most studied minor components have been sterols, phenolic compounds, volatile compounds, pigments, hydrocarbons, and tocopherols.

Sterols. Sterols are the major constituents of the unsaponifiable fraction (around 20%), and they play an important role in the stability of olive oils because they act as inhibitors of polymerization reactions (free radicals among them or with fatty acids

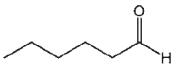
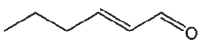
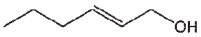
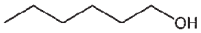
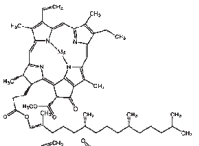
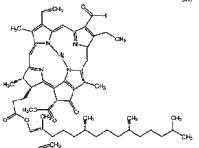
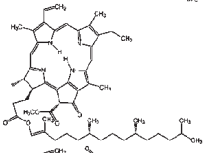
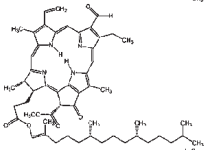
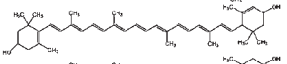
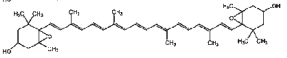
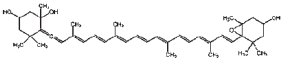
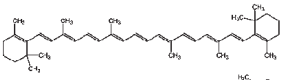
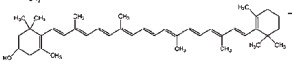
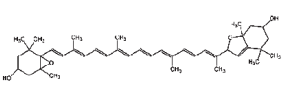
Table 3. Compositional Markers of Olive Variety Present in Olive Oils, Considering the Minor Components[a]

Marker	Compounds mainly studied	Chemical structure	Analytical technique	Chemometric treatment	Number of varieties analyzed	Geographical area of origin	Information obtained	Reference
Sterols	β-Sitosterol		GC-FID	LDA, PCA, and ANN	5	Sabina, Lazio (Italy)	Olive oil differentiation according to its olive variety is achieved using some fatty acids and sterols analyzed	(*18*)
	Campesterol		GC-FID	MANOVA, PCA, and HCA	3	Tras-os-Montes PDO (Portugal)	Sterol composition allows a significant differentiation of olive varieties of a specific PDO oil although is worse than with other chemical parameters such as fatty acids	(*20*)
			GC-FID	LDA	3	Beira Baixa Region (Portugal)	Stearic acid, campesterol, total sterol and oxidative stability are the most discriminating variables in an olive oil	(*22*)
	Clerosterol		GC-FID	PCA, HCA, DA, and ANOVA	4	Apulia region (Italy)	Sterol composition is worse than triglyceride and fatty acid composition to differentiate olive varieties in olive oils	(*26*)
	Stigmasterol		GC-FID	PCA and SIMCA	2	Cáceres (Spain)	Sterol composition is worse than triglyceride composition to differentiate olive oils obtained from specific olives	(*31*)
			GC-FID	PCA, ANOVA, MANOVA, and CDA	4	Portugal	Sterol composition allows to discriminate among different monovarietal olive oils	(*32*)
	Δ^5-Avenasterol		HPLC-MS	PCA, DA, and LDA	3	Sicily (Italy), Umbria (Italy), Toscana (Italy), and Greece	Triglyceride and sterol composition allows to predict/classify olive oils with different botanical origin	(*33*)
Phenolic compounds	Hydroxytyrosol		HPLC-DAD	LDA	4	Different regions of Spain	Some phenolic compounds contents allow to classify monovarietal Spanish virgin olive oils	(*35*)
	Tyrosol							
	Vanillic acid							
	Cinnamic acid		HPLC-DAD	LDA	4	Toledo and Ciudad Real (Spain)	Concentrations of many phenolic compounds differed significantly among the main Spanish virgin olive oil varieties	(*36*)
	Luteolin		HPLC-DAD	ANOVA, PCA, SIMCA, and PLS-DA	6	Izmir (Turkey)	Distribution of phenols allow to differentiate olive cultivars when the harvest season is well established	(*37*)
			HPLC-DAD and HPLC-MS	PCA and ANOVA	7	Sardinia (Italy) and Corsica (France)	Monovarietal olive oils are not differentiated by minor components such as tocopherols, phenolic compounds, and pigments.	(*23*)
	p-Coumaric acid		HPLC-DAD and HPLC-MS	PCA and ANOVA	5	Tunisia and Sicily (Italy)	Variation in the concentration of phenolic compounds in monovarietal olive oils could be a consequence of different irrigation conditions	(*24*)
	Apigenin		-	Not used	14	Sfax (Tunisia)	Triglycerides, fatty acids, phenolic compounds, and tocopherol composition allow the differentiation among olive oils from different varieties cultivated in the same pedoclimatic conditions	(*25*)
	Pinoresinol		HPLC-DAD and HPLC-DAD-MS/MS	Not used	18	Different areas of Portugal	Phenolic profiles are characteristic of some olive varieties in olive oils, when olive fruits are examined, even with different maturation degree and geographical origin	(*38*)
	Oleuropein		MS	LDA	3	Different regions of Spain	The fatty acid and phenolic composition variables allow to predict the olive variety in olive oils	(*27*)

polymerized, increasing the oil viscosity and the formation of foam) occurring at high temperature. **Table 3** shows that the determination of the sterol fraction in olive oils was mainly made using GC-FID (*18*, *20*, *22*, *26*, *31*, *33*). The application of different chemometric treatments revealed that some sterols present in olive oils can discriminate among olive varieties in olive oils (*18*, *20*). Thus, Matos et al. (*20*) carried out a chemometric characterization of three varietal olive oils showing that cultivars presented significant differences in some sterol compounds. Moreover, it is important to emphasize that the sterol composition was used in several cases together with other compositional markers such as fatty acids (*18*, *26*) or triglycerides (*26*, *31*, *33*) and other parameters (*22*) as it was shown to be less useful to differentiate olive oils. In fact, the use of the total sterol content with some variables such as stearic acid, campesterol, and oxidative stability enabled the classification of olive oils according to olive variety (*22*).

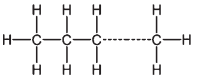
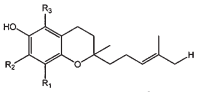
Phenolic Compounds. The main antioxidants present in olive oils are carotenoids and phenolic compounds, which have both lipophilic and hydrophilic properties. In this group, phenolic compounds having hydrophilic properties are collected. The prevalent classes of hydrophilic phenols found in virgin olive oils

Table 3. Continued

Marker	Compounds mainly studied	Chemical structure	Analytical technique	Chemometric treatment	Number of varieties analyzed	Geographical area of origin	Information obtained	Reference
Volatile compounds			GC-FID	Not used	7	Spain and Greece	Oils from some varieties cultivated in Spain and Greece show contents of volatiles very similar to those detected in the oils of the same varieties cultivated in Italy	(*39*)
	Hexanal		GC-FID	SLDA and PCA	2	Different regions of Tunisia	Hexyl acetate, hexanal, and (*E*)-hex-2-enal concentrations and the total concentration of ketones allow the correct classification of olive oils according to their olive variety	(*40*)
	(*E*)-Hex-2-enal		GC-FID	Test Kolmogorov–Smirnov and HCA	18	Garda lake (Italy)	Olive oils with genetic similarity present similar volatile profile	(*41*)
			GC-FID	SLDA and HCA	9	Different producers countries	Volatile compounds cluster varieties native to the same country but this is not enough to qualify all the oils from a country	(*42*)
	(E)-Hex-2-enol		GC-FID and GC-MS	ANOVA and PCA	4	Tataounie region (Tunisia)	The level of (*E*)-hex-2-enal shows variability from one variety to another grown in the same environmental conditions	(*28*)
	Hexanol		GC-FID and GC-MS	ANOVA and PCA	6	Tunisia, Greece, Spain, Italy, and Algeria varieties planted in Sfax (Tunisia)	Monovarietal olive oils tested show different volatile profiles	(*43*)
			GC-FID and GC-MS	PCA	11	Tunisia and France	The percentage of volatile compounds from oils of different varieties is different and genetic factors and the geographic region also influence the volatile production	(*44*)
			GCxGC-MS	ANOVA, PCA, and statistical image treatment	3	Portugal	Volatile composition allows the identification of olive varieties as well as the extraction technologies used to produce olive oils	(*45*)
Pigments	Chlorophyll a		HPLC-DAD	ANOVA, MSA, and AD	5	Different regions of Spain	Pigment composition allows classification of oils according to their monovarietal origin, the degree of ripeness, and the oil storage	(*46*)
	Chlorophyll b		HPLC-DAD	Not used	3	Sicily (Italy)	The ratio of lutein/β-carotene allows differentiation of monovarietal olive oils	(*47*)
	Pheophytin a		HPLC-DAD	Not used	2	Catalonia (Spain)	The pigment content and pigment retention is different with respect to the olive variety. All the pigments in the fruit are transferred to the oils	(*48*)
	Pheophytin b							
	Lutein		HPLC-DAD	ANOVA and PCA	6	Different regions of Italy	The composition of the pigment fractions shows significant quantitative differences in different monovarietal olive oils	(*49*)
	Violaxanthin							
	Neoxanthin							
Pigments	**β-Carotene**		HPLC-UV and HPLC-LIF	PCA and HCA	12	Different regions, not specified	Pigment composition allows the distinction of olive varieties in olive oils	(*50*)
	β-Cryptoxanthin							
	Luteoxanthin		UV-Vis spectrophotometry	PCA and ANOVA	7	Sardinia (Italy) and Corsica (France)	Carotenoid and chlorophyll content determined by spectrophotometry does not contribute to discriminate oils produced from different olive varieties	(*23*)

are lignans and secoiridoids (*34*). **Table 3** includes phenolic compounds widely studied to date in olive oils. This table shows that HPLC with diode array detection (DAD) was the main analytical technique used for olive oil differentiation according to

Table 3. Continued

Marker	Compounds mainly studied	Chemical structure	Analytical technique	Chemometric treatment	Number of varieties analyzed	Geographical area of origin	Information obtained	Reference
Hydrocarbons	n-Alkanes: C21 – C35 **(C30, C28, C25, C22)**		CG-FID	PCA and ANOVA	5	Tunisia and Sicily (Italy)	Squalene is the major olive oil hydrocarbon and its content depends on the olive variety	(*24*)
			GC-FID	MANOVA and DA	7	Different regions of Extremadura (Spain)	Some hydrocarbon contents (n-alkanes, n-alkenes, and sesquiterpenes) are capable of differentiate olive varieties in olive oils	(*51*)
			GC-FID	LDA and ANOVA	3	Istria (Croatia)	The n-alkane profile allows olive variety identification in olive oils depending on the year of production	(*52*)
Tocopherols	α-, β-, γ-, Tocopherol	 α- : $R_1 = R_2 = R_3 = CH_3$ β- : $R_1 = R_3 = CH_3$, $R_2 = H$ γ- : $R_1 = R_2 = CH_3$, $R_3 = H$	HPLC-DAD and HPLC-FLD	MANOVA, PCA, and HCA	3	Tras-os-Montes PDO (Portugal)	The total tocopherol content and the α- and γ- tocopherol individual content allow the differentiation of the varieties present in the olive oils	(*20*)
			HPLC-FLD	PCA and ANOVA	7	Sardinia (Italy) and Corsica (France)	α-Tocopherol content does not appear to be dependent on the varietal origin of oils	(*23*)
			HPLC-DAD	PCA and ANOVA	5	Tunisia and Sicily (Italy)	Five monovarietal olive oils analyzed behave differently according to the variety, irrigation time, and olive ripening	(*24*)
			HPLC-DAD	Not used	14	Sfax (Tunisia)	Triglycerides, fatty acids, phenolic compounds, and tocopherol composition allow the differentiation among olive oils from different varieties cultivated in the same pedoclimatic conditions	(*25*)
			HPLC-LIF	LDA	4	Different regions of Spain	The α-tocopherol content is very similar for all the olive varieties studied, except for Arbequina variety	(*35*)

[a] Abbreviations as in **Table 2**. Bold letters indicate the most relevant components for establishing the botanical origin of olive oils (those more useful or more used).

cultivar (*23*, *24*, *35*−*38*). The use of chemometric treatments, as in the case of other compositional markers, is widespread (*23*, *24*, *27*, *35*−*37*). It was found that phenolic compounds present in olive oils were different from those present in olive fruits because different chemical and enzymatic reactions may take place during ripening and processing (*36*). In addition, these compounds depend on the time of harvesting of the olive and the geographical origin (*37*). Maintaining some of these variables, the phenolic profiles obtained allowed the discrimination of different olive varieties (*36*, *37*). Vinha et al. (*38*) found that two olive oils from the same variety but with different maturation stage of fruit and geographical origin showed the same phenolic profile for the main compounds. However, the olive variety of one olive oil has been predicted using the phenolic composition and the fatty acid composition of the olive oil (*27*).

Volatile Compounds. Virgin olive oil is characterized by a unique flavor, which represents one of the most important qualitative aspects of this vegetable oil. Although a full description of the organoleptic characteristics of the oil is obtainable only through sensory analysis, the quali-quantitative determination of the volatile compounds can provide very useful information on product quality (*44*). The determination of these volatile compounds was carried out using mainly GC-FID (*28*, *39*−*44*) or GC-MS (*28*, *43*−*45*). The volatile fraction in olive oils consists of a complex mixture of more than 100 compounds, but the most important substances useful for olive cultivar differentiation are the products of the lipoxygenase pathway (LOX), generally the C6 compounds collected in **Table 3**. Not all of the volatile compounds have the same ability to distinguish olive varieties, but there can be a subset of compounds that, combined among them, provide valuable information. Angerosa et al. (*39*) showed that oils from two varieties cultivated in different countries had very similar volatile contents, proving a low influence of climatic conditions. For Tena et al. (*40*) three volatile compounds [hexyl acetate, hexanal, and (*E*)-hex-2-enal] and the total concentration of ketones were the variables of the canonical equations that were able to distinguish the olive varieties analyzed in olive oils. Oueslati et al. (*28*) found that the level of (*E*)-hex-2-enal in the analyzed samples showed variability from one variety to another, suggesting an influence of genetic factors on the biosynthesis of this compound. In fact, genetic (*41*, *44*) and geographic (*44*) factors influence the volatile compound production of the olive fruits and affect the differentiation of olive oils according to their olive variety. However, the volatile compound contents allowed differentiation among monovarietal olive oils (*43*) and even have identified the olive variety and the technique used for olive oil production (*45*).

Pigments. The color of a virgin olive oil is due to the solubilization of the lipophilic chlorophyll and carotenoid pigments present in the fruit. The green-yellowish color is due to various pigments, that is, chlorophylls, pheophytins, and carotenoids (*50*). Chlorophyll *a* is the major chlorophyll pigment, followed by chlorophyll *b*. The carotenoid fraction is composed by lutein, violaxanthin, neoxanthin, β-carotene, β-cryptoxanthin, and luteoxanthin. The chemical structures of these compounds are also shown in **Table 3**. As can be seen in this table, HPLC-DAD is the most widely used technique for pigment analysis (*46*−*50*). Several researchers reported the same qualitative composition in chlorophyll and carotenoid pigments, independent of the olive variety and the time of picking (*46*, *47*). However, differences in pigment content and pigment retention with the olive variety were observed at a quantitative level (*46*, *50*). Cerretani et al. (*23*) showed that the carotenoid and chlorophyll content determined using UV−vis spectrophotometry was not useful to discriminate oils produced from different olive varieties. Guifridda et al. (*47*) differentiated oils from a single cultivar according to the lutein/β-carotene ratio. Moreover, the presence of a specific pigment profile in olive oils was presented as useful to guarantee the genuineness and typicality of the product (*49*).

Hydrocarbons. In the hydrocarbon fraction of an olive oil, squalene is the major component. However, it was shown that the presence of other compounds, such as alkenes and sesquiterpenes, has a major differentiating power in a hydrocarbon analysis, especially for single-variety virgin olive oils (*51*). Baccouri et al. (*24*) found that squalene was the major olive oil hydrocarbon, and its content depended on the olive variety. The *n*-alkane profile was used by Koprivnjak et al. (*52*) for olive oil variety differentiation, showing a dependence on the year of oil production because the *n*-alkane content in olive oils varied according to the climatic conditions of one specific year. Moreover, *n*-alkane, *n*-alkene, and sesquiterpene contents were capable of differentiating olive varieties studied in olive oils (*51*).

Tocopherols. Four tocopherols (α, β, γ, and δ) and four tocotrienols (α, β, γ, and δ) make up vitamin E. They are phenolic compounds having lipophilic properties. They seem to be involved in a diversity of physiological and biochemical functions, mainly due to their antioxidant action, but also by their action as a membrane stabilizer. In olive oil, the compounds usually described are those that were also detected by Matos et al. (*20*): α-, β-, and γ-tocopherols. The individual α- and γ-tocopherol contents and the total tocopherol content were analyzed by HPLC-DAD and HPLC-FLD to study their influence in the olive variety differentiation of olive oils (*20*). As did Cerretani et al. (*23*), García et al. (*35*) also found very similar values in the α-tocopherol concentration of olive oils for four different varieties. However, Baccouri et al. (*24*) analyzed five monovarietal olive oils, finding that the tocopherol content was affected by irrigation time, olive ripening, and olive variety. In the same pedoclimatic conditions, Issaoui et al. (*25*) concluded that biochemical characterization (including tocopherols, fatty acids, triglycerides, and phenolic compounds) of monovarietal olive oils was variety dependent.

Because a unique compositional marker does not allow differentiation of olive oils according to their botanical origin, the most relevant compositional markers (components more useful and more used to date) for establishing the botanical origin of olive oils are fatty acids such as oleic (O), palmitic (P), and linoleic (L) acids; triglycerides such as POO, OOO, and OLL; sterols such as sitosterol and avenasterol; phenolic compounds such as tyrosol and hydroxytyrosol; volatile compounds such as (*E*)-hex-2-enal; pigments such as lutein and β-carotene; C22, C25, C28, and C30 hydrocarbons; and tocopherols such as α-tocopherol. These compositional markers have shown different discriminant potentials. Thus, fatty acid, triglyceride, and sterol components enabled the differentiation of olive oils when several of these parameters were analyzed by means of chemometrical tools. Volatile compounds, pigments, or phenolic compounds have been shown to be more useful individually for the differentiation of olive oils according to variety. Tocopherols and hydrocarbons have been the compositional markers less studied to date to differentiate olive oils as a function of their variety. However, an important common aspect is that the content and composition of these markers are very affected by the environmental conditions, the fruit ripening, and the extraction technology. For this reason, in most cases, several parameters were used to achieve the discrimination among olive oils according to the variety. Different chemometrical tools such as principal component analysis (PCA), Hierarchical clustering analysis (HCA), discriminant function analysis (DA), linear discriminant analysis (LDA), multivariate analysis of variance (MANOVA), stepwise linear discriminant analysis (SLDA), partial least squares-discriminant analysis (PLS-DA), multidimensional scaling (MDS), canonical discrimination analysis (CDA), soft independent modeling class analogy (SIMCA), artificial neural networks (ANN), and multivariate statistical analyses (MSA) were used to select the most useful parameters enabling the differentiation of olive oils according to their botanical origin.

In addition to the compositional markers described, other studies were performed to analyze olive oils because their complete chemical characteristics were capable of discriminating among olive varieties (*18*, *53*, *54*). Thus, Stefanoudaki et al. (*54*) carried out a sensory evaluation of the olive oils to study the effect of the variety as botanical factor. Differential scanning calorimetry (DSC) permitted differentiation among extra virgin olive oil cultivars of different geographic origins (*55*, *56*). FT-IR (*57*) and the determination of $\delta^{18}O$ isotopic abundance (*58*) were also employed to analyze directly olive oils and to characterize them according to olive cultivar.

Genetic Markers. DNA markers have been used to identify olive cultivars, and they are increasingly being applied to solve traceability and provenance issues (*59*). Food DNA analysis may represent an attractive and alternative choice to the more classical analytical methods, because DNA, relative to other macromolecules and metabolites, is less influenced by environmental and processing conditions and provides an opportunity for direct comparison of different genetic material (*60*−*67*). However, Claros et al. (*60*) gave evidence that the soil and climate (indirectly related with the geographic origin) had significant influence on cultivar differentiation during the years. In addition, Breton et al. (*8*) found that the genetic diversity of olive cultivars was strongly dependent on the region and country of origin. Thus, Gemas et al. (*68*) evaluated the genetic relationships among the most used Portuguese cultivars and their association according to the fruit employed and the agro-ecological origin.

Since the discovery of amplifiable DNA from olive oil, different genetic markers have been used to target DNA in an attempt to recognize the cultivar employed for the production. The genetic markers are amplified fragments of DNA, and the most useful are random amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), and microsatellites or simple sequence repeat (SSR). The usefulness of these genetic markers for variety identification or olive oil differentiation according to olive variety is shown in **Table 4**. As can be seen in this table, the most important uses of these genetic markers can be summarized in three aspects. First, genetic markers can be used to distinguish olive tree cultivars, extracting the DNA from the olive leaves (*9*, *60*−*62*, *68*, *69*). Furthermore, it usually includes a clustering or discriminant analysis to perform a classification of the olive varieties studied. Moreover, in some discriminant analyses, the geographical influence of the growing location of the olive trees was shown (*60*, *68*). Second, genetic markers can be used to distinguish olive varieties when DNA is extracted directly from the olive oils (*8*, *65*, *66*, *70*). This procedure has some limitations because, as mentioned above, oil DNA is highly degraded and many interferences exist. In this sense, microsatellites, which consist of a specific sequence of DNA bases or nucleotides that contains mono, di, tri, or tetra tandem repeats, were used by Breton et al. (*8*), allowing the main cultivar used for an elaborated olive oil to be distinguished. Finally, the traceability from olive leaves to olive oils was the aim of some researchers (*63*, *64*, *67*, *69*−*71*). Thus, a high degree of concordance between DNA extracted from olive leaves and the DNA extracted from olive oils was achieved when the DNA extraction procedure for both samples was well established. However, DNA extracted from olive oil was highly degraded and contaminated with inhibitors of PCR reactions, which limited the applicability of DNA genetic markers to internal traceability olive/oil (*64*, *69*, *70*). Two reasons were the main factors affecting the reproducibility of this traceability. On the one hand, the amount of DNA extracted from leaf, flesh, embryo, and paste samples was variable because more DNA was recovered from leaves than from any other material (*71*). On the other hand, the quantity and quality of DNA extracted from the olive leaves or the olive oils had a great influence on the PCR conditions and provided low reproducibility for the genetical markers used (*60*). For this reason, the methods used for DNA extraction in olive leaves or olive oils are varied. The cetyltrimethylammonium bromide (CTAB) method (*72*, *73*) has been the most used method for DNA extraction. CTAB is a cationic detergent, which solubilizes membranes and forms DNA

Table 4. Genetic Markers and Their Usefulness for Studying the Traceability of Olive Oils to the Botanical Origin

genetic marker	informative samples	method for DNA extraction	number and type of primers applied	usefulness for studying the traceability to the botanical origin	ref
RAPD	leaf	CTAB method[a] modified	12; random primers	differentiation of olive tree cultivars by a clustering analysis, showing geographical influences	60
	leaf	CTAB method[a] modified	7; random primers	study of intercultivar diversity with RAPD and ISSR markers; detection of intracultivar variability according to the geographical location of one Portuguese olive variety	68
	oil	DNeasy Plant mini kit[b], Nucleo-Spin Plant kit[c], Nucleo-Spin Food kit[d], CTAB method[a], and hexane method[e]	11; random primers	combination of RAPD, ISSR, and SSR data in a PCA analysis allowing the olive variety differentiation in olive oils	65
SCAR	leaf → oil	leaf: CTAB method[a] modified oil: Palmieri method[f] modified	2; specific primers	detection of some SCAR markers in oil DNA with similar intensity in leaf DNA, showing possible traceability from oil to olive	64
AFLP	leaf	leaf: CTAB method[a]	2; AFLP primer−enzyme combinations	observation of intracultivar similarity and olive variety classification by cluster analysis of AFLP markers	9
	leaf → oil	leaf: CTAB method[a] oil: CTAB method[a]; Palmieri method[f]; CTAB method[a] modified	2; AFLP primer−enzyme combinations	observation of a high correspondence between oil and plant AFLP markers (~70%), oil DNA extraction being the critical step	63
	leaf → oil	leaf: Gene Elute Plant kit[g] oil: Gene Elute Plant kit[g]	2; AFLP primer−enzyme combinations	use of AFLP markers to identify olive variety in the derived olive oil differentiation of the AFLP electrophoretic patterns obtained from leaves and oils	70
ISSR	leaf	CTAB method[a] modified	3; specific primers	study of intercultivar diversity with RAPD and ISSR markers detection of intracultivar variability according to the geographical location of one Portuguese olive variety	68
	oil	DNeasy Plant mini kit[b], Nucleo-Spin Plant kit[c], Nucleo-Spin Food kit[d], CTAB method[a], and hexane method[e]	8; specific primers	combination of RAPD, ISSR, and SSR data in a PCA allowing olive variety differentiation in olive oils	65
microsatellite or SSR	leaf	CTAB method[a] modified	14; specific primers	finding microsatellite markers such as a powerful tool to discriminate among olive varieties	61
	leaf	Dellaporta method[h] modified	20; specific primers	use of chemometric analysis from microsatellite data and establishment of synonymous for istrian olive varieties	62
	oil	Wizard Magnetic DNA Purification System for Food,[i] silica,[j] and hydroxyapatite biogel[k]	10; specific primers	differentiation of the main olive variety used to elaborate an olive oil, not revealing secondary cultivars	8
	oil	DNeasy Plant mini kit,[b] Nucleo-Spin Plant kit,[c] Nucleo-Spin Food kit,[d] CTAB method,[a] and hexane method[e]	4; specific primers	combination of RAPD, ISSR, and SSR data in a PCA analysis allowing olive variety differentiation in olive oils	65
	oil	Gene Elute Plant kit[g] with modifications	7; specific primers	examination of a limited number of DNA microsatellites to identify one olive variety in a PDO olive oil	66
	leaf → oil	leaf: DNeasy Plant Mini kit[b] oil: QIAamp DNA Stool[l]	6; specific primers	correspondence between purified oil DNA and leaf DNA of the same cultivar	67
	leaf → oil	leaf: Gene Elute Plant kit[g]; Dellaporta method[h], phenol-chloroform method[m] oil: Gene Elute Plant kit[g] modified; phenol-chloroform method[m] modified	1; specific primers	trying to set up a DNA bank using microsatellite markers of olive varieties from different countries; identification of a high degree of similarity between oil DNA and leaf DNA	69
	leaf → oil	leaf: DNeasy Plant mini kit[b] oil: official Swiss method for lecithin and oil DNA extraction[n]	7; specific primers	finding a high degree of nonconcordance between a commercial monovarietal olive oil profile and the profile of the reference leaf	71

[a] CTAB method proposed by Doyle et al. (*72*) and Murray et al. (*73*). [b] DNeasy Plant mini kit: Qiagen, Hilden, Germany (http://www1.qiagen.com/). [c] Nucleo-Spin Plant kit: Macherey Nagel, Duren, Germany (http://www.macherey-nagel.com/). [d] Nucleo-Spin Food kit: Macherey Nagel, Duren, Germany (http://www.macherey-nagel.com/). [e] Hexane method proposed by Consolandi et al. (*53*). [f] Method proposed by Palmieri (*74*). [g] Gene Elute Plant kit: Sigma, St. Louis, MO (http://www.sigmaaldrich.com/sigma-aldrich/home.html). [h] Method proposed by Dellaporta et al. (*75*). [i] Wizard Magnetic DNA Purification System for Food: Promega, Madison, WI (www.promega.com). [j] Silica: Sigma, Louis, MO (http://www.sigmaaldrich.com/sigma-aldrich/home.html). [k] Hydroxyapatite biogel: Bio-Rad Laboratories, Hercules, CA (www.bio-rad.com). [l] QIAamp DNA Stool: Qiagen, Hilden, Germany (http://www1.qiagen.com/). [m] Phenol−chloroform method proposed by Sambrook et al. (*76*). [n] Official Swiss method for lecithin and oil DNA extraction (*77*).

complexes. The method using sodium dodecyl sulfate (SDS) developed by Dellaporta et al. (*75*) has also been widely used. However, the use of commercial kits for DNA extraction has, in general, the advantages of be less time-consuming and easily adapted for routine analysis. All DNA extraction methods used to obtain the genetic markers for studying the traceability of olive oils to the botanical origin are shown in **Table 4**. However, the comparison of DNA extracted from leaves and oils is difficult. For example, Montemurro et al. (*70*) used AFLP markers, DNA sequences that allow the simultaneous screening of a large number of specific locations (loci) without any preliminary sequence knowledge, to obtain the AFLP electrophoretic patterns for leaf and oil DNAs. Because patterns showed differences in the band intensity between the two agarosa gels, the authors

suggested that results obtained depended on the different quality of DNA extracted in the studied samples. Doveri et al. (*71*) found a high degree of nonconcordance between commercial monovarietal olive oil DNA and DNA from a reference leaf. They concluded that the commercial monovarietal olive oils could contain 5−10% of oil deriving from other cultivars.

In conclusion, olive oil traceability is gaining importance due to the development of olive oils with specific characteristics. Consumers demand high-quality olive oils, including olive oils with PDO labels or coupage/monovarietal olive oils with certain properties characteristic of the olive variety from which they are elaborated. This review is focused on the olive oil traceability to the botanical origin, specifically to the study of the olive varieties from which olive oils are produced. To solve this problem, it is necessary to investigate certain compounds present in oils with potential to discriminate the olive variety. They have been designated traceability markers to the botanical origin. Because several and varied compounds have been studied to date, they have been classified into two groups: compositional and genetic markers. Compositional markers are substances that take part of the composition of the olive oils, but they are influenced by the environmental or technical conditions. For this reason, it is usual to study several compositional markers at the same time and to use chemometric tools to achieve the differentiation among olive oils according to the olive variety from which they are elaborated. Genetic markers are focused on DNA analysis and have better discriminatory power; however, they are more difficult to extract from the oils in sufficient quantity and with high quality. As a consequence, it is necessary to find new traceability markers less influenced by the environmental conditions, fruit ripening, and extraction technology than compositional markers and more easily obtainable than DNA markers. The investigation of other biomacromolecular components present in olive oils as novel traceability markers could be promising for establishing the botanical origin of olive oils because they may present a high differentiation potential due to their own molecular complexity.

ABBREVIATIONS USED

PDO, protected designation of origin; PGI, protected geographical indication; DOOR, database of origin and registration; O, oleic acid; Po, palmitoleic acid; P, palmitic acid; L, linoleic acid; Ln, linolenic acid; S, stearic acid; E, eicosenoic acid; GD-FID, gas chromatography−flame ionization detector; ^{13}C NMR, carbon-13 nuclear magnetic resonance spectroscopy; MS, mass spectrometer; HPLC-RID, high-performance liquid chromatography−refractive index detection; HPLC-ELSD, high-performance liquid chromatography−evaporative light scattering detection; PCA, principal component analysis; HCA, hierarchical clustering analysis; DA, discriminant function analysis; LDA, linear discriminant analysis; MANOVA, multivariate analysis of variance; SLDA, stepwise linear discriminant analysis; PLS-DA, partial least-squares-discriminant analysis; MDS, multidimensional scaling; CDA, canonical discrimination analysis; SIMCA, soft independent modeling class analogy; ANN, artificial neural network; MSA, multivariate statistical analyses; HPLC-MS, high-performance liquid chromatography−mass spectrometry detector; HPLC-DAD, high-performance liquid chromatography−diode array detector; HPLC-DAD-MS/MS, high-performance liquid chromatography−tandem mass spectrometry detector; GC-MS, gas chromatography−mass spectrometry detector; GC×GC-MS, two-dimensional gas chromatography−mass spectrometry detector; HPLC-UV, high-performance liquid chromatography−ultraviolet detector; HPLC-LIF, high-performance liquid chromatography−laser-induced fluorescence detection; HPLC-FLD, high-performance liquid chromatography−fluorescence detector; LOX, lipoxygenase pathway; DSC, differential scanning calorimetry; FT-IR, Fourier transform infrared; RAPD, random amplified polymorphic DNA; SCAR, sequence-characterized amplified region; AFLP, amplified fragment length polymorphism; ISSR, inter simple sequence repeat; SSR, simple sequence repeat; CTAB, cetyltrimethylammonium bromide; PCR, Polymerase Chain Reaction; SDS, sodium dodecyl sulfate.

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