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Fingerprints for recognition of French monovarietal walnut oils making up the Périgord Protected Designation of Origin (PDO)

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ABSTRACT

This study is the first to be carried out for varietal recognition of cold-pressed oils from Périgord walnuts (France). Produced from Corne, Franquette, Grandjean and Marbot varieties, the Périgord oil is currently the sole walnut oil to benefit of a Protected Designation of Origin (PDO, obtained in 2020). The Fernor variety should potentially be added to the PDO. The compositions of fatty acids (FA), triacylglycerols (TAG) and tocopherols of the five walnut varieties were determined from 176 samples over three harvest years. Data of FA and TAG were used to generate graphical representations named "morphotypes" corresponding to each variety. These morphotypes provided rapid visual identification of varieties and their specific markers. Partial Least Square Discriminant Analysis (PLS1-DA) was used to predict the four Périgord walnut varieties with a control chart approach to estimate the degree of reliability of the prediction. Models from FA contents provided the best results with a percentage of samples recognized as belonging to the predicted variety equal to 93%, 81%, 75% and 86% for the varieties Corne, Franquette, Grandjean and Marbot respectively. These approaches offer the advantage to be evolutive and can be supplemented by annually collected data.

1. Introduction

Juglans regia L., commonly known as common walnut, English walnut or Persian walnut, is a tree widely grown across the world, including Asia, Europe, and America. In 2021, world production of inshell walnuts was 3500,173 t and France was the third largest European producer of inshell walnuts (37,740 t) behind Greece (62,810 t) and Romania (54,250 t) (FAOstat, 2021). French walnuts are distinguished by two designations of Origin (RDO) that reflect their production regions: RDO "Noix de Grenoble" for walnuts originating from the Rhône-Alpes region (Journal Officiel de la République Française, 2002a), and RDO "Noix du Périgord" for walnuts grown across the departments of Dordogne, Corrèze and Lot from the Occitanie and Nouvelle Aquitaine regions (Journal Officiel de la République Française, 2002b). Franquette,

Mayette and Parisienne are the main varieties of the "Noix de Grenoble" appellation, while Franquette, Corne, Marbot and Grandjean make up the "Noix du Périgord" appellation. Lara and Fernor are two other varieties widely cultivated in France.

Walnut cultivation generates several by-products including the walnut oil, which is of growing interest due to its nutritional and flavor qualities, cosmetic properties and industrial applications (Song et al., 2022). Walnut oil is obtained through various extraction processes, including mechanical pressing, solvent extraction, supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction, and enzyme-assisted extraction (Masoodi et al., 2022). Currently, only two types of walnut oils benefit of a Protected Designation of Origin (PDO). The first one was the PDO "walnut oil Vaudoise" from Switzerland (Office Fédéral de l'Agriculture Suisse, 2020). The second

Abbreviations: Fatty acids, 14:0, myristic acid (tetradecanoic acid); 15:0, pentadecylic acid (pentadecanoic acid); 16:0, palmitic acid (hexadecanoic acid); 16:1n7, palmitoleic acid 9-(hexadecenoic acid); 17:0, margaric acid (heptadecanoic acid); 17:1n8, margaroleic acid, (9-heptadecenoic acid); 18:0, stearic acid (octadecanoic acid); 18:1n9c, oleic acid (9-octadecenoic acid cis); 18:1n9t, elaidic acid (9-octadecenoic acid trans); 18:1n7, vaccenic acid (11-octadecenoic acid cis); 18:2n6, linoleic acid (9,12-octadecadienoic acid); 18:3n3, linolenic acid (9,12,15-octadecatrienoic acid); 20:0, arachidic acid (eicosanoic acid); 22:0, behenic acid (docosanoic acid); MUFA, Mono-Unsaturated Fatty Acid; PUFA, Poly-Unsaturated Fatty Acid; SFA, Saturated Fatty Acid; OFA, Odd parity Fatty Acid; Triacylglycerols, The triacylglycerols TAG are designated by letters corresponding to abbreviated names of fatty acid carbon chains that are fixed on the glycerol: P, palmitoyl; Po, palmitoleyl; S, stearoyl; O, oleoyl; L, linoleoyl; Ln, linoleolenyl.

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one is the PDO “huile de noix du Périgord” from France (Official Journal of The European Union, 2020). Historically, in Périgord, the main use of walnuts was for oil production. After the exceptional frost in 1956 that devastated walnut orchards, a clear revival began in Périgord as young operators planted new walnut orchards (Laugénie, 1965). The endeavors of the Périgord walnut cultivation sector to uphold connection with its terroir and safeguard traditional know-how were rewarded by the attainment in 2002 of a RDO “Noix du Périgord” (Journal Officiel de la République Française, 2002b) then transformed into PDO by European Union (Official Journal of The European Union, 2004). Afterward, Périgord walnut oil became the first walnut oil to be granted a RDO (Journal Officiel de la République Française, 2018) then, a PDO concerning six departments: Aveyron, Charente, Corrèze, Dordogne, Lot and Lot-et-Garonne. The oil must be obtained from walnut kernels exclusively from Corne, Franquette, Grandjean and Marbot varieties (Official Journal of The European Union, 2020).

High-value-added oils bearing an AOP label may be susceptible to various forms of fraud, including falsifying the varietal or geographical origin, blending with other cheaper vegetable oils, or incorporating undisclosed additives. In Europe, currently no specific standards or regulations for walnut oils exist. The Codex Alimentarius serves as a fundamental benchmark that offers recommendations for vegetable oils intended for human consumption, including walnut oil (Codex Alimentarius Commission, 2022). The World Trade Organization uses the Codex Alimentarius as an international reference for the resolution of conflict concerning food frauds, food safety and consumer protection. However, nothing prevents countries from using scientific studies other than the Codex Alimentarius, when in disagreement. Indeed, the Codex Alimentarius does not differentiate between the geographical or varietal origin of walnut oils. Nevertheless, this issue of discrimination can be a significant concern, especially when ensuring labeling compliance. To address this concern, the profiles of triacylglycerols (TAG) or the profiles of fatty acids (FA) provided information about the varietal-specific and regio-specific composition of walnut oils. Also, Zhang et al. (2023) have developed a promising method for authenticating walnut oils using an orthogonal Partial Least Squares-Discriminant Analysis (PLS-DA) from SFC-QTOF-MS TAG profiles of a wide-ranging and diverse set of walnut oils, including oils blended with other vegetable oils. This method detected adulteration levels down to 5% (w/w). Using stable carbon isotope ratios of FAs analyzed by isotope ratio mass spectrometry, combined with Principal Component Analysis (PCA), has successfully differentiated Chinese walnut oils based on their geographical origins. (Zhang et al., 2021). In another study, FA profiles have been used as valuable authenticity markers for distinguishing between walnut oils from Bulgaria and Greece using PCA and hierarchical clustering (Kalojiouri et al., 2022).

The aim of this study was to highlight discriminant markers that characterize French monovarietal oils obtained from Corne, Franquette, Grandjean and Marbot walnut varieties of “Huile de Noix du Périgord” PDO and from Fernor variety, a potential candidate as a fifth variety. Currently, there is no chemical data in literature to characterize the walnut varieties of Périgord. Therefore, the compositions of FA, TAG and tocopherols have been determined using a comprehensive walnut sampling collected over three harvest years, certified and provided by the Professional Union of Walnuts, Walnut Kernels and Walnut Oil of Périgord. Data were used to build graphical fingerprints (called morphotypes), enabling visual distinction of the varietal origins of walnut oils, thus facilitating authentication. The data were also used to develop predictive models using PLS1-DA combined with control charts, for class assignment, thereby confirming the varietal origin of an oil. These two approaches could be innovatively implemented in the walnut sector to enhance product control and reduce fraudulent practices, thereby ensuring consumer confidence.

2. Material and methods

2.1. Walnut sampling

Sampling was carried out during the harvest years 2019, 2020, and 2021. For this study, a total of one hundred and seventy-six walnut samples from five Périgourdine varieties, were obtained from the Professional Union of Walnuts, Walnut Kernels and Walnut Oil of Périgord (“Syndicat Professionnel de la noix, du cerneau et de l’huile de Noix du Périgord”, Perrical, 46600 Creysse, France). Samples were collected from orchards practicing either organic or conventional agriculture, situated in valleys or on hillsides across 59 municipalities within the six departments identified in the geographic area of the Périgord PDO (1 municipality of Charente department, 5 municipalities of Corrèze department, 43 municipalities of Dordogne department and 10 municipalities of Lot department) (Fig. 1). Overall, the climate is predominantly oceanic, with a temperate character in the Corrèze department, while it tends to become more mountainous in the northern regions of the Lot department. The varieties included Corne (n=41), Fernor (n=30), Franquette (n=48), Grandjean (n= 36) and Marbot (n=21).

Walnut samples were dried from a natural drying on laths or a ventilation drying with hot dry air at a temperature not exceeding 30°C, according to the specifications of the “Périgord walnut oil” designation of origin (Journal Officiel de la République Française, 2018).

2.2. Characteristics of the studied varieties

Currently, four varieties of walnuts are concerned by “Huile de noix du Périgord” PDO (Official Journal of The European Union, 2020): Corne, Franquette, Grandjean, and Marbot. Corne variety is from the canton of Ayen, in Corrèze, with its extension on Hautefort in Dordogne. Marbot is from the area of Meyssac, Beaulieu, Argentat, in Corrèze. Grandjean is from the Sarladais, in Dordogne. Introduced in the 1950’s, Franquette has perfectly adapted into the whole production area and nowadays is widely grown in the four departments concerned by the PDO. Fernor variety, developed by INRAE (Institut National de Recherche pour l’Agriculture, l’Alimentation et l’Environnement) through hybridization of the Franquette and Lara varieties, is widely cultivated in Périgord. It is not presently listed among the approved varieties for the “Noix du Périgord” PDO or the “Huile de noix du Périgord” PDO. Each of these five varieties, depicted in Fig. 1, has distinct characteristics detailed by the French Interprofessional Technical Center for Fruits and Vegetables (Centre Technique Interprofessionnel des Fruits et Légumes, 2023). The main ones are outlined below.

Corne is a robust, highly vigorous, and late maturing variety. The nut has an elliptical to oblong shape with a generally rounded base. The apex is slightly conical rather shouldered, ending in a generally weak mucron. The base is usually pointed, less commonly rounded. The shell is thick, hard, bumpy, deeply grooved, featuring pronounced irregularities along the suture of the half shell. Extracting the kernel is difficult due to the hardness of the shell. The weight of nuts varies from 8.5 to 11 g. The kernel accounts for 35–40% of the total weight of the walnut. It is light blond, offers a pleasant crunch, and has a particularly fine fruity taste.

Franquette is a vigorous variety that matures rather late. The nut has an elliptical to ovoid shape, with an asymmetrical base that sometimes ends in a point. The apex is a slightly conical, little or no shouldered, with a pronounced mucron. The suture of half shell is prominent especially on nearly the two thirds of the fruit. The shell is fairly rough and bumpy, with cracks along the wings. The weight of nuts varies from 9.5 to 12 g. The kernel easy to extract, as the partitions of the fruit are thin. It fills well the shell (about 45% of the total weight of the nut). The kernel is light blond and has a sweet smell. It has an intense walnut taste.

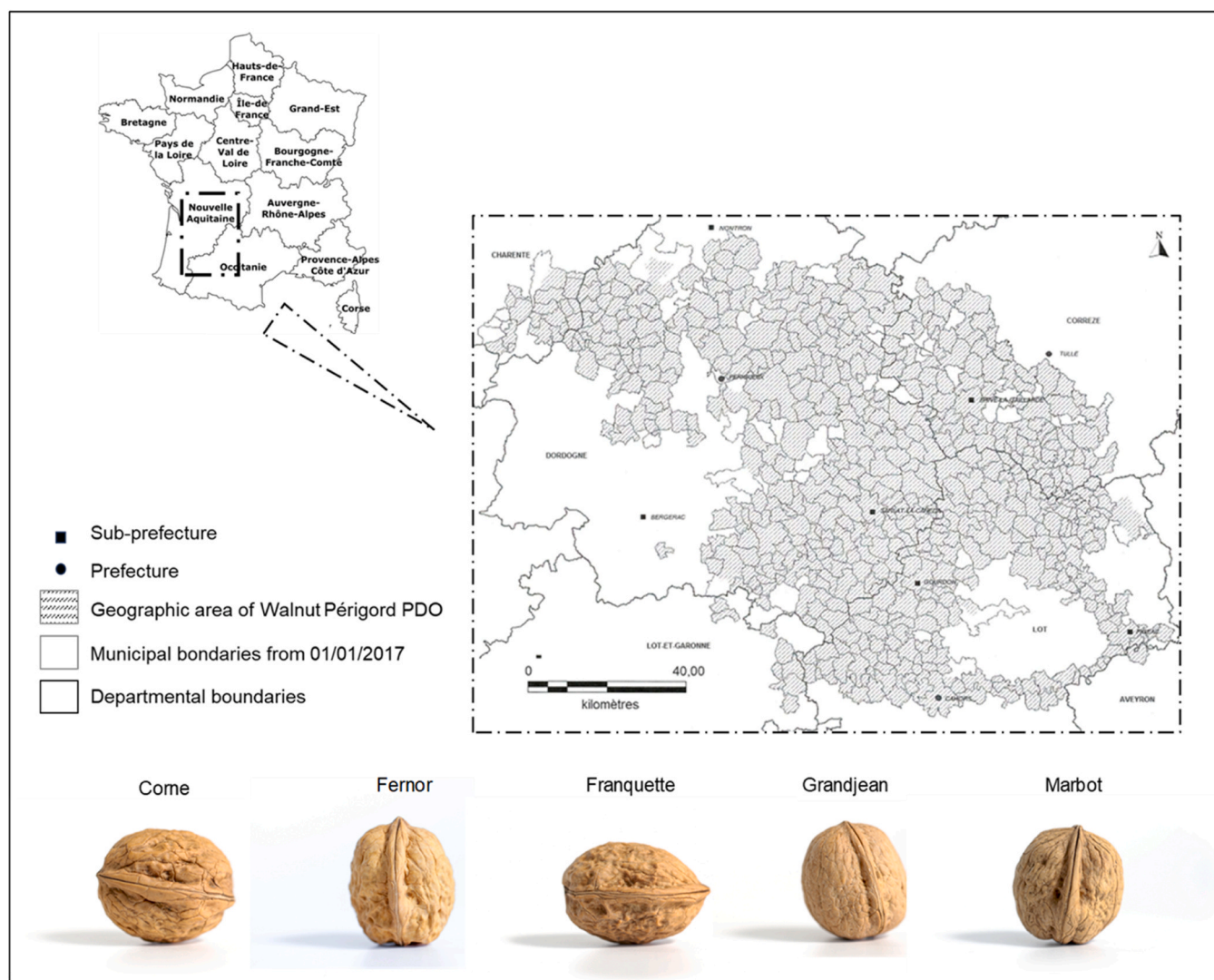


Fig. 1. Geographic area of the walnut Périgord PDO and photographs of Come, Fernor, Franquette, Grandjean and Marbot walnut varieties (source: Syndicat Professionnel de la noix, du cerneau et de l'huile de Noix du Périgord", Perrical, 46600 Creysse, France).

Grandjean is a vigorous, rather late maturing variety. From the front, the nut has an ovoid to short elliptical shape. It has a small mucron and a rounded base. From the side, its shape is usually short oblong. It is generally angular towards the apex, with a very small pistillate tip. The base is slightly rounded, sometimes flat. The shell is thin, not very bumpy, finely furrowed and not very anfractuous along the wings. The weight of nuts varies from 8 to 10 g. The kernel fills the shell well and makes up on average 47% of the total weight of the nut. The nut is easy to break, and the kernel is clear and tasty.

Marbot it a moderately productive variety which matures early. The nut has a short ovoid shape with rounded base and apex and a well-developed mucron. The shell is fairly thin, moderately bumpy, deeply furrowed, and quite anfractuous along the wings. The weight of nuts varies from 9.5 to 12 g. The kernel, which represents on average 43% of the total weight of the nut, does not fill the shell completely. The nut is easy to break. The kernel has a fairly pronounced hazelnut taste.

Fernor has a maturity rather late. The nut is rather elongated like Franquette but with a more humped shell. It is long ovoid to elliptical in shape. In profile, it has an oblong to rectangular shape. The weight of nuts varies from 9 to 12 g. The kernel represents on average 45% of the weight of the nut. The nut is easy to break. Its kernel, very clear, is of excellent quality.

2.3. Screw press extraction of walnut oil

The walnuts were hand-shelled, and the kernels were coarsely crushed into pieces of a few millimeters in size using a kitchen knife. Their moisture content, determined using a classical gravimetric method, ranged from 1.9% to 5.1%, with a mean value of approximately 3.5%, depending on the variety and geographic origin. Given the importance of moisture content in solid-liquid extraction, the crushed walnut kernels were dried in a ventilated oven (heated to 30°C) for 72 hours to optimize the oil yield. Even if 90% of walnut oil producers heat (up to 100°C maximum) the walnut paste, in this study, oils were cold extracted without preheating of the crushed kernels, to preserve the chemical composition of the kernels and minimize potential alterations. It should be noted that these two practices are authorized by the specifications of the Périgord PDO for the walnut oil ([Journal Officiel de la République Française, 2018](#)). Oil extraction was performed using a screw press model Komet Oil Press CA59G (IBG Monforts Oekotec GmbH & Co. KG, Mönchengladbach, Germany). 300 g of dried and crushed walnut kernels were placed in the feed hopper. To guarantee optimal conditions for oil extraction, a 4 mm extrusion nozzle diameter and a rotational speed of 18 rpm were used. A heater ring kept the nozzle at 100°C during oil extraction process. The obtained oil was clarified by centrifugation (SIGMA 2-7 centrifuge, Grosseon) for

10 minutes at 4000 rpm. The oil was kept in amber glass during least 7 days to allow any remaining particles to settle. Clarified oil was preserved in an amber bottle and stored at 4°C until analysis.

2.4. Fatty acid composition

Walnut oils were transmethylated with KOH (2 M) following the previously described method (Laroussi-Mezghani et al., 2015). Fatty acid methyl esters (FAME) were analyzed for 40 min using an Agilent Technology gas chromatograph 7890 A equipped with a split/splitless injector (250°C), a flame ionization detector (250°C), and a capillary column type SGE GC, BPX70 (70% Cyanopropyl Polysilphenylene-siloxane, 60 m × 0.25 mm i.d., 0.25 µm film thickness).

The carrier gas was hydrogen (column flow 1 mLmin⁻¹) and the split ratio was 1:60. The oven temperature program was as follows: 20 min at 210°C, from 210 to 245°C at 6 °C/min, 20 min at 245°C. FA identification was carried out by comparing the retention times of FAME peaks from oil samples with those from commercial FAMES ("Supelco 37 Component FAME Mix", Merck KGaA, Darmstadt, Germany) analyzed under the same conditions. FA percentages were determined through internal standardization without considering mass response factors. The sums of Saturated Fatty Acids (SFA), Mono-Unsaturated Fatty Acids (MUFA), Poly-Unsaturated Fatty Acids (PUFA), and Odd Parity Fatty Acids (OFA) were calculated from the obtained values, following the methodology established in previous work (Guissous et al., 2018). Additionally, the sum of the major FAs (i.e., 16:0+18:1c+18:2+18:3) and the n6/n3 ratio were also determined.

2.5. Triacylglycerol composition

Triacylglycerol analyses were performed with a LC Agilent 1200 series system (Agilent, Santa Clara, California, USA), operated with Chemstation software, equipped with an autosampler, a quaternary pump, a column heater module (28°C), a RID detector (Agilent, Santa Clara, California, USA), and a Merck RP-18 Supersphere 100 column (250 × 4 mm i.d., 4µm) (Merck KGaA, Darmstadt, Germany). The mobile phase was propionitrile (ACROS Organics, Geel, Belgium) with a flow rate linear gradient (0.5–1.0 mLmin⁻¹) for 47 min 10µL of oil in propionitrile (5%, w/v) were injected. Triacylglycerol identification was carried out with the help of literature works on olive and walnut oils (Amaral et al., 2004; Ollivier et al., 2006). Triacylglycerol percentages were determined by internal standardization without considering mass response factors.

2.6. Tocopherol composition

Tocopherol analysis was performed in normal phase HPLC on the previously described LC Agilent 1200 series system, with LiChrospher-Si 60 column (250 × 4 mm i.d., 5µm; Merck, KGaA, Darmstadt, Germany) placed in the column heater module (25°C). A photodiode array (DAD) detector connected in series with a multiwavelength fluorescence detector (FLD) were used for detection. Oil samples (0.7000±001 g) were dissolved in a 5 mL volumetric flask with hexane/2-propanol (99/1, v/v; quality suitable for HPLC, Sigma-Aldrich, Steichein, Germany). Subsequently, 20 µL of these solutions were injected and eluted with a hexane/2-propanol (99/1, v/v) mobile phase at 1 mL min⁻¹ flowrate. Tocopherol identification was performed from a standard mixture (Tocopherols mixed, Sigma-Aldrich). Due to the significant differences in tocopherol concentrations, both UV and fluorescence detections were used. DAD (292 nm wavelength) was employed to quantify γ-tocopherol (the predominant form), whereas α-, β-, and δ-tocopherols were quantified using FLD (higher sensitive detector; at 295 nm excitation and 330 nm emission wavelengths). Quantification was performed via external calibration using α-tocopherol as the standard (Sigma-Aldrich) and applying the relative response factors previously determined for β-,

γ-, and δ-tocopherols. Calibration curve equations and relative response factors are available in supplementary file S1.

2.7. Morphotype

For each walnut oil variety, a modified version of the method outlined by Pinatel et al. (2014) was used to construct morphotypes which are radial graphical representations derived from their FA or TAG contents. They enabled comparison of both the average chemical composition and the range of variation within one variety against data from all the varieties included in the database (176 oil samples). The first step in constructing the morphotypes was to calculate the mean value and standard deviation of each variable (FA or TAG values) of all the samples of the database. Then, for each sample, the centered reduced values (X_i^R) were calculated as follows:

$$X_i^R = \frac{X_i - \bar{X}}{SD} \times 100 \quad (1)$$

where X_i is the value one variable (FA or TAG contents) of the sample i , \bar{X} the average and SD the standard deviation of the considered variable across all the samples.

Then, for each variety, the reduced average M_{25} (i.e., the average after removing 25% of values at the lower and upper ends) was calculated from the X_i^R values, as well as the first and the third quartiles.

2.8. Chemometric analysis

Models were developed to predict the varietal origin of the samples from their FA and TAG contents. Two approaches of Partial Least Square Discriminant Analysis (PLS) exist. PLS1 concerns a single dependent variable (the variety), while PLS2 concerns several dependent variables (the four varieties at the same time). It has been shown that discrimination by PLS1-DA was better (Galtier et al., 2011). PLS-DA was used with a binary coding. Value of 1 indicated that a sample belonged to the modelled variety and value of 0 that it not belonged. A full cross-validation procedure was applied during the calibration of each model to optimize the number of latent variables (LVs). The Root Mean-Square Error of Calibration (RMSEC) and the coefficient of determination R^2 allowed to evaluate the quality of the calibration models. The Root Mean-Square Error of Prediction (RMSEP) and the coefficient of determination Q^2 allowed to evaluate the quality of the predictions. Theoretically, samples were recognized as belonging (or not) to the modelled variety if their predicted value was close to the expected value, i.e., 1 (or 0). To consider the variability of samples, the acceptability thresholds were built as a control chart as done for olive oil samples as previously described (Maléchaux et al., 2019). According to the described processing, warning limits and control limits were established as confidence intervals at 95% and 99% respectively around the Mean Calibration Scores (MCS) for each modelled variety ($MCS \pm 2 \times$ standard deviation and $MCS \pm 3 \times$ standard deviation respectively). Samples were accepted when their predicted values were included within the control limits, i.e., belonging to the predicted variety. Samples with a predicted value lower than the warning limits were rejected as not belonging to the predicted variety. Samples with a predicted value included within the warning zone (between the control and the warning limits) were considered as uncertain.

2.9. Software

The morphotypes were performed with Excel® 2022 (Microsoft Corporation). The chemometric application was performed by the Unscrambler software version 10.3 from CAMO, Norway.

3. Results and discussion

3.1. Fatty acid compositions

Fourteen FAs have been identified in the walnut oils coming from five varieties of walnuts collected at three harvest years. Table 1 gives for each variety, the mean, minimum and maximum value of each identified FA, as well as the SFA, MUFA, PUFA, OFA, the sum of the four main FAs and the n6/n3 values. The 176 analyzed samples had same FAs with variations in abundance among the varieties. However, some overlaps in the abundance of the main FAs across walnut oils were observed. As it is typical in walnut oils, the studied samples featured four main FAs. Linoleic acid (18:2n6) predominated, with a content ranging from 55.30% to 65.38%. Additionally, oleic acid (18:1n9c), linolenic acid (18:3n3), and palmitic acid (16:0) were also abundant, each comprising 12.61–22.02%, 9.61–14.42%, and 6.30–8.66% of the total FA content, respectively. Two other FAs, stearic acid (18:0) and cis-vaccenic acid (18:1n7), were less abundant, accounting for 1.64–2.92% and 0.74–1.03% of the total FA content, respectively. The others were considered minor FAs (< 0.1%). Moreover, within a variety, there were significant variations in content for the same compound. For example, the linoleic acid (18:2n6) content for samples of the Corne variety ranged from 59.50% to 65.38%, representing an absolute

deviation of 2.94% around the average value of 63.18%. These variations reflected the variability due to the harvest year, the dispersion of collection areas, and agricultural practices in different farms. Therefore, conducting studies on a wide variety of samples and over several years to extract the main chemical characteristics, is of great interest.

The total detected FA were approximately distributed as 73% PUFA, 17% MUFA, and 10% SFA. The odd parity fatty acids (OFA) contents were similar and low. The analyzed samples had similar FA compositions but showcasing variations in abundance among the varieties. The Corne oil had the highest PUFA mean value and the Franquette oil had the lowest (75% and 71% respectively). Oils rich in PUFAs including omega-6 and omega-3 FAs are of great interest in human diet because they are essential fatty acids that the body needs but cannot synthesize (Lee et al., 2016). Nevertheless, high PUFA content renders the oil very sensitive to oxidation which requires special attention during extraction and storage (Popovici et al., 2012). According to certain health claims, an imbalance in the human diet in the intake of linoleic acid (“omega-6 FA”) and alpha-linolenic acid (“omega-3 FA”) may induce dysfunction of the classic inflammatory cycle and potentially be co-responsible for many modern diseases (EFSA Panel on dietetic products, nutrition, and allergies (NDA), 2010). Over the last 150 years, omega-6 intake in food has increased, while omega-3 intake has decreased in parallel with the increase in heart disease. Now, some experts use the omega-6 to

Table 1

Relative percentages of fatty acids and triacylglycerols and contents of tocopherols (mg/kg) of 176 oils from the five varieties studied.

	CORNE (n = 41)			FERNOR (n = 30)			FRANQUETTE (n = 48)			GRANDJEAN (n = 36)			MARBOT (n = 21)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
14:0	0.03	0.02	0.04	0.04	0.02	0.05	0.03	0.02	0.03	0.03	0.00	0.04	0.03	0.02	0.04
15:0	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.00	0.02	0.01	0.00	0.02	0.01	0.00	0.02
16:0	7.24	6.38	8.27	7.27	6.60	7.89	7.09	6.30	7.81	7.58	6.67	8.58	7.82	6.96	8.66
16:1n7	0.08	0.06	0.11	0.08	0.06	0.11	0.09	0.07	0.11	0.09	0.07	0.11	0.10	0.08	0.12
17:0	0.06	0.01	0.06	0.06	0.05	0.07	0.05	0.01	0.06	0.05	0.01	0.09	0.05	0.01	0.06
17:1n8	0.02	0.00	0.03	0.03	0.02	0.09	0.03	0.02	0.04	0.03	0.02	0.03	0.03	0.01	0.03
18:0	2.15	1.78	2.66	2.47	1.81	2.92	2.49	1.96	2.90	2.01	1.64	2.26	2.21	1.95	2.44
18:1n9t	0.03	0.01	0.03	0.03	0.02	0.09	0.03	0.01	0.04	0.03	0.01	0.03	0.03	0.02	0.03
18:1n9c	14.41	12.70	18.92	15.32	12.61	18.01	18.10	13.99	22.02	17.20	14.84	19.93	16.06	13.30	19.71
18:1n7	0.83	0.74	1.01	0.81	0.74	0.90	0.85	0.76	0.94	0.91	0.80	1.02	0.94	0.86	1.03
18:2n6	63.18	59.50	65.38	62.35	57.89	64.75	58.96	55.30	62.10	60.26	58.24	63.26	59.25	57.96	63.59
18:3n3	11.80	9.61	13.05	11.40	9.86	14.01	12.11	10.44	13.92	11.64	10.05	12.70	13.27	11.36	14.42
20:0	0.09	0.08	0.11	0.10	0.08	0.11	0.09	0.08	0.11	0.09	0.08	0.10	0.10	0.09	0.11
22:0	0.03	0.02	0.03	0.03	0.02	0.03	0.03	0.02	0.03	0.02	0.01	0.03	0.03	0.03	0.03
SFA	9.60	8.48	10.86	9.97	8.79	11.08	9.79	9.05	10.80	9.80	9.03	11.03	10.26	9.15	11.36
MUFA	15.37	13.75	19.84	16.27	13.48	19.03	19.09	14.93	23.05	18.25	15.82	20.93	17.16	14.31	20.75
PUFA	74.98	70.74	77.28	73.75	71.10	77.21	71.07	67.61	76.02	71.90	69.31	75.11	72.52	69.81	75.30
OFA	0.08	0.01	0.09	0.09	0.08	0.16	0.08	0.03	0.09	0.08	0.03	0.12	0.08	0.04	0.09
Maj	96.63	96.18	97.08	96.35	95.67	96.96	96.26	95.48	96.88	96.68	96.21	97.18	96.40	95.91	96.86
n6/n3	5.35	6.19	5.01	5.47	5.87	4.62	4.87	5.30	4.46	5.18	5.80	4.98	4.47	5.10	4.41
LLnLn	3.51	2.42	4.46	3.30	2.71	4.49	3.52	2.90	4.52	3.35	2.68	3.94	4.15	3.39	4.74
LLLn	16.41	12.96	18.82	15.48	14.00	17.38	15.44	13.76	18.58	15.12	13.19	17.04	16.72	15.41	18.17
PLnLn	0.53	0.38	0.65	0.52	0.36	0.70	0.57	0.43	0.95	0.56	0.42	0.71	0.70	0.54	0.81
LLL	27.73	23.26	30.59	26.57	21.67	28.99	23.5	20.31	29.01	24.59	22.62	27.99	23.14	21.77	27.76
OLLn	5.70	4.95	6.87	5.88	5.21	7.80	6.74	5.37	7.55	6.4	5.52	7.04	6.80	5.40	7.79
PLLn	4.46	3.64	5.27	4.42	3.61	5.13	4.34	3.68	5.13	4.41	3.69	4.94	5.18	4.77	5.74
OLL	15.03	13.76	17.77	15.52	13.65	17.07	16.03	14.03	17.50	16.23	14.99	17.74	14.63	13.84	15.4
OOLn	0.80	0.59	1.23	0.88	0.65	1.37	1.18	0.69	1.65	1.02	0.78	1.27	1.06	0.71	1.46
PLL	11.00	9.29	12.24	11.01	9.45	12.28	9.89	8.57	11.40	10.54	9.50	11.93	10.84	9.07	12.12
POLn	0.64	0.47	0.90	0.70	0.55	0.96	0.80	0.55	1.09	0.77	0.54	0.94	0.90	0.66	1.05
LOO*	4.86	4.05	7.37	5.38	3.94	7.42	6.74	4.62	8.60	6.39	5.06	7.85	5.45	4.36	6.38
PoOO	2.51	2.04	3.19	2.79	1.77	3.60	2.53	1.94	3.03	2.15	1.70	2.65	2.31	1.87	2.95
PLO**	3.86	3.16	4.80	4.09	3.72	4.46	4.43	3.46	5.13	4.54	3.76	5.14	4.50	3.94	5.14
PLP	0.58	0.45	0.71	0.54	0.46	0.61	0.55	0.42	0.62	0.66	0.54	0.77	0.65	0.53	0.77
OOO	0.86	0.61	1.75	1.04	0.61	1.80	1.55	0.78	2.61	1.38	0.93	1.98	1.11	0.74	1.42
SOL	0.98	0.75	1.43	1.21	0.98	1.41	1.35	0.89	1.65	1.06	0.85	1.27	1.09	0.96	1.30
POO	0.54	0.40	0.93	0.66	0.45	0.95	0.86	0.47	1.15	0.85	0.57	1.13	0.78	0.53	1.01
Maj	70.17	59.27	79.42	68.58	58.77	75.72	64.86	56.67	76.49	66.48	60.30	74.70	65.33	51.73	62.79
α-toco	15.64	8.82	34.02	18.28	9.53	37.09	16.50	8.65	29.39	14.25	8.55	20.27	13.60	2.88	18.48
β-toco	0.49	0	1.76	0.84	0	2.43	1.87	0	7.32	0.19	0	1.24	0.40	0	1.25
γ-toco	294.83	225.56	386.39	282.51	194.20	344.47	268.52	176.44	334.96	293.38	246.85	415.47	290.57	205.32	355.68
δ-toco	39.23	26.39	59.63	41.98	31.03	48.97	43.03	24.66	59.98	34.62	23.56	46.82	38.84	29.12	50.61
Total	350.20	266.42	474.45	343.61	245.12	418.32	329.40	213.22	421.76	342.45	282.97	478.84	343.41	248.11	422.21

Maj: major FA (16:0+18:1+18:2+18:3); *: + PLnP; **: + SLL; Maj: major TAG (LLLn+LLL+OLL+PLL)

Table 2
Relative percentages of fatty acids from literature source for Franquette and Marbot varieties.

	Crop year	Country	Storage / drying condition	Ext. type	MC (%)	T Ext (°C)	14:0	15:0	16:0	16:1n7	17:0	17:1n8	18:00	18:1n9t	18:1n9c	18:1n7	18:2n6	18:3n3	20:0	22:0	SFA	MUFA	PUFA	OFA	Maj	n6/n3
Codex Alimentarius							nd	nd	6.0–8.0	nd-0.4	nd-0.1	1.0–3.0	nd	14.0–23.0	nd	54.0–65.0	9.0–15.4	nd-0.3	nd-0.2	7.0–11.6	14.0–23.5	63.0–80.4	nd-0.2	> 83%	4.2–6.0	
Franquette																										
Amaral et al., 2003	2001	Portugal	30°C; 72 h	S	4.4	40–60	0.03	7.48	0.05	0.04		2.43	0.02	16.99	1.53	59.22	11.69	0.07	0.05	10.10	18.59	70.91	0.04	95.38	5.07	
Torres et al., 2005	nd	Argentina	30 ± 2°C; 24 h	HP				7.54				2.12		26.28		52.12	11.93			9.66	26.28	64.05		97.87	4.37	
Martínez et al., 2006	2004	Argentina	30°C; 24 h	HP	nd	23		7.54				2.12		26.28		52.12	11.94			9.66	26.28	64.06		97.88	4.37	
Martínez and Maestri, 2008	2005	Argentina	30°C; 24 h	HP	nd	23		6.59				2.16		28.44		50.23	12.52			8.75	28.44	62.75		97.78	4.01	
Martínez et al., 2008	2008	Argentina	30°C; 24 h	HP	nd	23		7.05				1.89		23.20		53.30	12.50			8.94	23.20	65.80		96.05	4.26	
	2008	Argentina	30 ± 2°C; 24 h	CP	7.50	22		6.98				2.27		28.30		50.10	12.30			9.25	28.30	62.40		97.68	4.07	
				SP	2.50	25		7.10				2.90		26.40		51.90	11.80			10.00	26.40	63.70		97.20	4.40	
					50			8.40				3.10		26.40		50.60	11.30			11.50	26.40	61.90		96.70	4.48	
					70			7.60				2.70		25.60		52.00	12.00			10.30	25.60	64.00		97.20	4.33	
					4.50	25		7.10				3.40		26.60		51.20	11.80			10.50	26.60	63.00		96.70	4.34	
					50			7.00				2.80		27.10		51.60	11.50			9.80	27.10	63.10		97.20	4.49	
					70			7.60				2.70		25.80		51.30	12.50			10.30	25.80	63.80		97.20	4.10	
					7.50	25		7.10				2.80		26.50		51.90	11.70			9.90	26.50	63.60		97.20	4.44	
					50			7.20				2.70		26.50		51.30	12.20			9.90	26.50	63.50		97.20	4.20	
					70			7.30				2.70		26.00		51.60	12.30			10.00	26.00	63.90		97.20	4.20	
Pereira et al., 2008	2006	Portugal	in a stove	S	4.07	40–60	0.03	0.01	6.61	0.10	0.04	0.03	3.07		20.22	55.51	14.01	0.11	0.03	9.89	20.35	69.52	0.07	96.35	3.96	
Bouabdallah et al., 2014	nd	Tunisia	nd	S	4.68	nd		7.98				3.98		16.73		60.47	10.64			11.96	16.73	71.11		95.82	5.68	
Christopoulos and Tsantili, 2015	nd	Greece	bin drier (1500 m ³ h ⁻¹) 36 °C; 24 h then stored at	SP	5.73	40																				
			none					0.04	7.87	0.12		2.62		17.50	0.69	55.52	14.76	0.07		10.83	18.31	70.28		95.88	3.76	
			1°C + air					0.03	7.84	0.10		2.89		16.78	0.61	48.15	13.54	0.07		10.88	17.49	61.69		86.34	3.56	
			20°C + air					0.04	8.25	0.08		2.80		20.88	0.77	47.53	13.23	0.09		10.76	21.73	60.76		89.48	3.59	
			1°C + N ₂					0.04	7.87	0.10		3.18		18.06	0.80	50.28	14.49	0.09		11.56	18.96	64.77		91.08	3.47	
			20°C + N ₂					0.04	8.15	0.09		2.73		18.32	0.79	47.20	14.38	0.07		10.71	19.20	61.58		87.77	3.28	
			1°C + CO ₂					0.03	8.07	0.10		2.91		18.12	0.70	48.93	15.37	0.09		11.18	18.92	64.30		90.57	3.18	
			20°C + CO ₂					0.04	7.87	0.10		3.08		16.87	0.60	48.40	13.86	0.08		11.26	17.57	62.26		87.20	3.49	
Bujdosó et al., 2016	nd	France	Fresh sample	S	nd	nd		7.77				2.59		17.46		59.37	11.35			10.36	17.46	70.72		95.95	5.23	
			Dried sample = in-shell walnuts dried at 36 ± 1°C for 36 or 48 h down to MC = 10%					7.05				2.61		18.92		58.92	11.13			9.66	18.92	70.05		96.02	5.29	
			Stored sample = dried sample stored at 8°C for 2 months					7.00				2.63		17.59		60.63	10.75			9.63	17.59	71.38		95.97	5.64	

(continued on next page)

Table 2 (continued)

Crop year	Country	Storage / drying condition	Ext. type	MC (%)	T Ext (°C)	14:0	15:0	16:0	16:1n7	17:0	17:1m8	18:00	18:1n9t	18:1n9c	18:1n7	18:2n6	18:3n3	20:0	22:0	SFA	MUFA	PUFA	OFA	Maj	n6/n3	
Sena-Moreno et al., 2016	Spain	room temp.	HP	8.00	nd	0.03	7.35	0.15	0.05	0.10	3.00	17.20	60.30	12.00	0.04	0.03	10.50	17.45	72.30	0.15	96.85	5.03				
			SP	8.00	HPT	0.02	8.00	0.20	0.05	3.00	18.00	60.00	12.00	0.04	0.03	11.14	18.20	72.00	0.05	98.00	5.00					
Poggetti et al., 2018	Italy	30°C; 72 h	S	3.50	40-60	9.03	8.51	3.76	17.42	58.03	11.96	17.42	57.05	12.30	12.28	18.06	69.35	69.99	0.08	95.23	4.35					
			Mean*	0.03	0.01	7.58	0.11	0.05	0.07	2.8	0.02	21.76	0.81	53.44	12.44	0.07	0.04	10.43	22.01	65.89	0.04	86.34	3.18			
min				0.02	0.01	6.59	0.05	0.04	0.03	1.89	0.02	16.73	0.6	47.2	10.64	0.04	0.03	8.75	16.73	60.76	0.04	86.34	3.18			
max				0.04	0.01	9.03	0.2	0.05	0.1	3.98	0.02	28.44	1.53	60.63	15.37	0.11	0.05	12.28	28.44	72.3	0.15	98.00	5.68			
Marbot Amaral et al., 2003	Portugal	30°C; 3 days	S	3.6	40-60	0.02	7.14	0.07	0.05	0.02	2.78	16.34	59.66	14.33	0.09	0.03	9.33	16.43	73.99	0.07	96.68	4.16				
Pereira et al., 2008	Portugal	in a stove	S	40-60	0.03	0.01	6.35	0.11	0.05	0.07	2.8	0.02	21.76	0.81	53.44	12.44	0.07	0.04	10.43	22.01	65.89	0.08	95.23	4.35		
Mean**				0.03	0.01	6.75	0.09	0.05	0.05	2.79	0.02	19.05	0.81	56.55	13.39	0.08	0.04	9.88	19.22	69.94	0.08	95.96	4.26			

Ext. type = extraction type; S= soxhlet; HP = hydraulic press; SP = screw press; CP = cold press; MC = moisture content; nd = non detected; Maj: major FA (16:0+18:1+18:2+18:3).
 Ext. type = extraction type; S= soxhlet; HP = hydraulic press; SP = screw press; CP = cold press; MC = moisture content; nd = non detected; Maj: major FA (16:0+18:1+18:2+18:3); Mean* = mean values for data from Franquette variety; min = minimum value; max = maximum value; Mean** = mean values for data from Marbot variety.

omega-3 ratio (n6/n3) as an indicator of dietary excess or deficiency. The n6/n3 ratio of walnut oils analyzed was near of the 'ideal' ratio, equal to 5, which is an indicator of good nutrition (ANSES, 2011, 2008; Simopoulos, 2008).

Fatty acid contents fell within the content intervals specified in the Codex Alimentarius for the composition of walnut oil (Codex Alimentarius Commission, 2022). They were in accordance with the literature data for the Franquette and Marbot varieties (the only ones available), compiled in Table 2. It was reported that the FA contents varied based on experimental conditions to obtain oils. The six main FAs were often the only ones to be detected, but some authors, however, managed to detect FAs present in very low content. Higher molecular weight FAs (22:1n9, 23:0 and 24:0) have been also detected in one study (Pereira et al., 2008).

Unlike the oils examined in this study, which were obtained under strictly the same conditions over three harvest years and in a limited geographical area, the literature presents a wide diversity of experimental conditions, making difficulties for meaningful comparisons. Indeed, the compiled literature have demonstrated that various factors, other than the variety, could affect the chemical composition of walnut oils, including the geographic origin, harvest year, storage conditions of walnut kernels or extraction process (Table 2). The content of FAs and the PUFA/MUFA ratio as well as the content of some minor unsaponifiable lipid components are mainly dependent of walnut variety (Amaral et al., 2003; Martínez et al., 2006; Martínez and Maestri, 2008; Torres et al., 2005). Additionally, an interannual variability in oil composition has been observed for the Franquette variety (Poggetti et al., 2018). This trend has also been observed among the samples in this study but was not presented. The oils, extracted from walnut kernels pressed under varying temperature conditions and with different seed moisture contents, showed minor differences in fatty acid composition (Martínez et al., 2008). Losses in both total and individual MUFA and PUFA were observed in cold-pressed oils obtained from walnuts stored for 12 months under various storage conditions. Indeed, storing walnuts in air at 20°C was associated with advanced deterioration in the quality and nutritional value of the oils. However, losses were significantly reduced when walnuts were stored at low temperature and with low O₂ availability. Nevertheless, storing walnuts at 20°C under N₂ or CO₂ atmosphere proved to be equally effective in maintaining the high quality of oil as storing them in air at 1°C, which is too expensive for prolonged periods (Christopoulos and Tsantili, 2015). Significant differences in FA composition were also observed when kernels were stored for different durations (Bujdosó et al., 2016). Finally, some studies suggested that the pressing extraction impact (screw pressing or hydraulic press) was negligible on the chemical composition of walnut oils (Martínez et al., 2008; Sena-Moreno et al., 2016). Despite the influence of these parameters, the literature data, although not abundant, appear to indicate significantly different fatty acid compositions based on varieties. Specifically, the distribution of FAs in Franquette oil averaged approximately 61–72% PUFA, 17–28% MUFA, and 9–11% SFA, while Marbot oil exhibited an average composition ranging from 66% to 74% PUFA, 16–22% MUFA, and 9–10% SFA (Table 2).

As discerning differences or similarities between the five studied walnut varieties wasn't directly accessible from the raw chemical data obtained, the morphotype representation was used. This approach has previously proven effective as a visual aid in characterizing oils from various olive varieties (Guissous et al., 2018; Laroussi-Mezghani et al., 2015; Pinatel et al., 2014). Morphotypes are radial graphical representations. The advantage of this representation is to use standardized centered values to make them comparable, rather than the initial values that may have mean and standard deviation of different magnitudes. This approach ensured that each factor analysis (FA) was equally considered, regardless of whether it was predominant or not. On each axis, the reduced average M25 (i.e. the average after removing 25% of the extreme values) of the standardized centered values of a FA was projected, framed by the values of the first and third quartiles of the

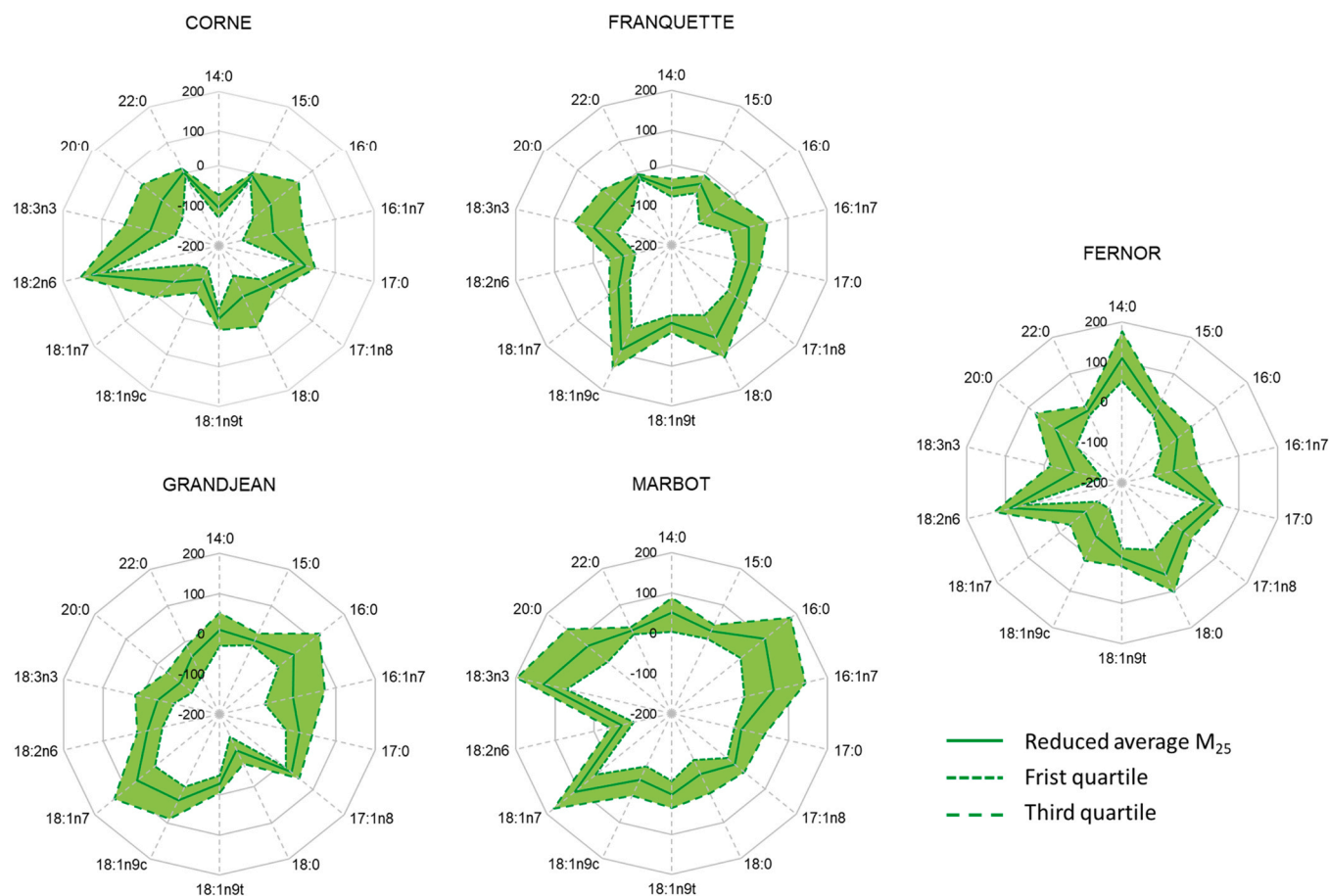


Fig. 2. Fatty acid morphotypes of oils from Corne, Fernor, Franquette, Grandjean and Marbot walnut varieties.

variety. The reduced average M25 has been chosen instead of the average to exclude the atypical samples that could create bias and distort the shape. Indeed, the morphotypes of each of the five studied varieties exhibited characteristic shapes (Fig. 2), showing areas of greater or lesser variations depending on the FA. Moreover, the specificity of the obtained shape revealed specific markers for the variety. The Corne variety has a star-shaped morphotype with higher 18:2n6 content, and a lower 14:0 and 18:1n9c contents than the other varieties. The Franquette variety has the highest 18:1n9c content, and Fernor variety the highest 14:0 and 18:2n6 contents. Both varieties have similar 18:0 content, which is not surprising since Fernor is a hybrid variety developed from the Franquette variety crossed with Lara variety. The Grandjean variety is characterized by a very low 18:0 content and a high 18:1n7 content. The Marbot variety has a high content of 18:1n7, 18:3n3 and 16:0, although the latter two have larger ranges of variation. Except for the Franquette and Fernor varieties, the range of variation of 16:0 and 16:1n7 was very large. Thus, these compounds cannot be considered as a specific marker of any variety.

3.2. Triacylglycerol compositions

Table 1 reported the mean, minimum and maximum values of seventeen triacylglycerol (TAG) variables (corresponding in fact to nineteen TAGs because of co-elutions of LOO+PLnP and PLO+SLL) for each of the five varieties studied. Walnuts oils contained four main TAGs (each typically contributing over 10% of the total TAG content) collectively accounting for 65–70% of the total TAG content, depending on the variety. Trilinoleyl glycerol (LLL), consistently most abundant, ranged from 20.3% to 30.6%, 1,2-dilinoleoyl-3-linolenoyl-glycerol (LLLn) from 13.0% to 18.8%, 1,2-dilinoleoyl-3-oleoyl-glycerol (OLL) from 13.7% to

17.8%, and 1–2-dilinoleoyl-3-palmitoleoyl-glycerol (PLL) from 8.6% to 12.3%. These main TAGs are made up of at least one linoleoyl chain, which agrees with the high percentage of linoleic acid (mean value of 60.8%) found in the oils. Six other TAGs, less abundant (each ranging from 1% to 10%) collectively accounted for 25–28% of the total TAG content.

Today poor data is available in the literature on TAG content for walnut varieties approved for Périgord PDO oil production. Fourteen TAGs were identified and quantified (Table 3). The data available to date is solely derived from Soxhlet extracted oils of Franquette and Marbot walnut varieties grown in Portugal and Tunisia (Amaral et al., 2004; Bouabdallah et al., 2014). As highlighted in the study by Amaral et al. (2004), beyond the discernible distinctions among six walnut varieties, including Franquette and Marbot, the TAG contents in walnut oils exhibit variability linked to geographical origins (Bragança and Coimbra, Portugal, and Bizerte, Tunisia) and harvest years, spanning three consecutive years in September.

Just as for the FA contents, the morphotypes of the TAGs of the five varieties were built to highlight the potential markers for distinguishing the different varieties (Fig. 3). The Corne and Fernor varieties showed similarities with the highest LLL, PLL and PoOO contents and the lowest OLLn content compared to the other varieties. The Franquette variety morphotype highlighted four characteristic TAGs: PLL in smaller content, and OOLn, OOO, and SOL in higher contents than in other oil varieties. These three last TAGs contain at least one oleoyl (O) group, confirming that oleic acid is a marker for the Franquette variety. The Grandjean variety showed off a regular shaped morphotype with an absence of specific markers, except may be PoOO and SOL which have to be guardedly highlighted because they represented only a small proportion of the total TAGs (respectively around 2.15% and 1.06%,

Table 3
Relative percentages of triacylglycerols from literature source for Franquette and Marbot varieties.

	Crop year	Country	Storage / drying condition	Ext. type	MC (%)	LlLn	LLn	OLLn	PLLn	OLL	PLL	OOL	PLO	POO	SOS	OOO	SLL	SOL		
Franquette	2001	Portugal (Bragança)	Dried in an oven at 30 °C during at least 3 days	S		2.09	34.64	4.64	3.14	17.19	10.62	3.06	4.22							
	2002					19.99	35.37	5.62	2.00	19.81	7.48	4.63	3.27							
	2003					17.32	33.21	5.56	3.18	18.36	11.04	4.24	4.53							
	2003	Portugal (Coimbra)				1.14	16.28	40.43	5.81	1.46	19.89	7.51	4.26	3.2						
	ND	Tunisia		S	4.68	0.99	30.38	1.03	3.59	17.36	12.02	5.66	4.59	0.85	0.70	1.48	5.7	2.44		
						16.86	34.81	4.53	2.67	18.52	9.73	4.37	3.96							
						0.99	30.38	1.03	1.46	17.19	7.48	3.06	3.20							
						2.12	40.43	5.81	3.59	19.89	12.02	5.66	4.59							
						1.74	39.00	4.43	2.38	17.88	9.58	2.58	3.45							
						0.82	38.46	3.87	1.28	23.35	6.51	6.24	4.32							
						1.81	37.79	5.59	2.4	16.03	9.42	2.61	3.64							
						1.46	38.42	4.63	2.02	19.09	8.50	3.81	3.80							
						0.82	37.79	3.87	1.28	16.03	6.51	2.58	3.45							
						1.81	39.00	5.59	2.4	23.35	9.58	6.24	4.32							

Ext. type = extraction type; S= soxhlet; MC = moisture content; * mean for all data from Franquette variety; min = minimum value; max = maximum value

Table 1). The Marbot variety morphotype highlighted four characteristic TAGs (LLnLn, PLnLn, PLLn and POLn) in higher contents than in other oil varieties. They were all made up of linolenic acid (Ln), already considered as a characteristic FA of this variety, as well the palmitic acid (P).

3.3. Tocopherol compositions

Four tocopherols (α , β , γ and δ -tocopherols) were commonly present in vegetable oils in varying contents in vegetable oils depending on their botanical origin. These are antioxidants that protect oils from oxidation (Seppanen et al., 2010). Table 1 reported the mean, minimum, and maximum values of these four isomers, as well as the total tocopherol content for each oil variety. The total content showed few differences across the varieties and range from 329 to 350 mg/kg of oils (mean values, for Franquette and Corne respectively). The predominant compounds were γ - and δ -tocopherols, constituting 82–86% and 10–13% of the total tocopherols, respectively, depending on the variety (i.e., 269–295 mg/kg and 35–43 mg/kg, respectively). Alpha tocopherol, recognized as the most antioxidant potent (Lavedrine et al., 1997), was present in relatively small amounts, ranging from 13 to 18 mg/kg. Beta tocopherol was detected in trace amounts, with mean values ranging from 0.19 to 1.87 mg/kg, varying to the varieties.

The obtained results fell within the tocopherol content ranges specified by the Codex Alimentarius for walnut oil. They were comparable to literature findings regarding oils from Franquette and Marbot varieties (Table 4). However, the obtained total tocopherol contents were higher: 329 and 343 mg/kg, in contrast to 279 and 265 mg/kg for Franquette and Marbot varieties, respectively. It has been shown that the tocopherol contents depend on walnut variety (Cittadini et al., 2020; Martínez and Maestri, 2008) Furthermore, the published tocopherol contents showed minor differences among the studied varieties, including Franquette and Marbot, from three consecutive harvest years and two distinct locations. These variations were influenced by both the harvest year and geographical origin (Amaral et al., 2005). Lavedrine et al. (1997) reported a substantial reduction in tocopherol contents (approximately 30%) in Franquette variety after the three-month refrigerated storage of walnuts at 4°C, occurring between the harvest and the subsequent oil analysis. Similar results were reported for walnuts from Chandler and Howard varieties stored for four months at 5°C and 23°C (Ampofo et al., 2022). Given the minimal variation in tocopherol content among the five varieties and considering the reported breakdown of these compounds during the walnut drying process (Rébufa et al., 2022), which was also applied to the samples under study, the tocopherol data have been excluded from the subsequent chemometric analyses.

3.4. Prediction of the PDO varietal origin by partial least square discriminant analysis

Since the morphotypes established on FA and TAG contents highlighted differences between varieties, these chemical data were used to create PLS1-DA models able to distinguish the different oil varieties. A calibration set of 97 oil samples from the four PDO varieties, randomly chosen ($n_{COR}=27$, $n_{FQT}=32$, $n_{GDJ}=24$, $n_{MAR}=14$), was used to build PLS1-DA models to predict one variety against all the others with a binary codification attributing the value of 1 if a sample belonged to the modelled variety and the value of 0 if not belonged. A validation set composed with the 49 remaining oil samples not considering in the calibration set ($n_{COR}=14$, $n_{FQT}=16$, $n_{GDJ}=12$, $n_{MAR}=7$) allowed evaluating the predictive capability of the model. Unbalanced classes have been used in both the calibration and validation sets, primarily due to the scarcity of Marbot samples. Indeed, there are fewer orchards of the Marbot variety, which is highly sensitive to handling during harvesting and transport hence the limited number of samples. It was obvious that a balanced model will give better results in terms of predictive ability for different reasons exposed in different works (Lindström et al., 2011).

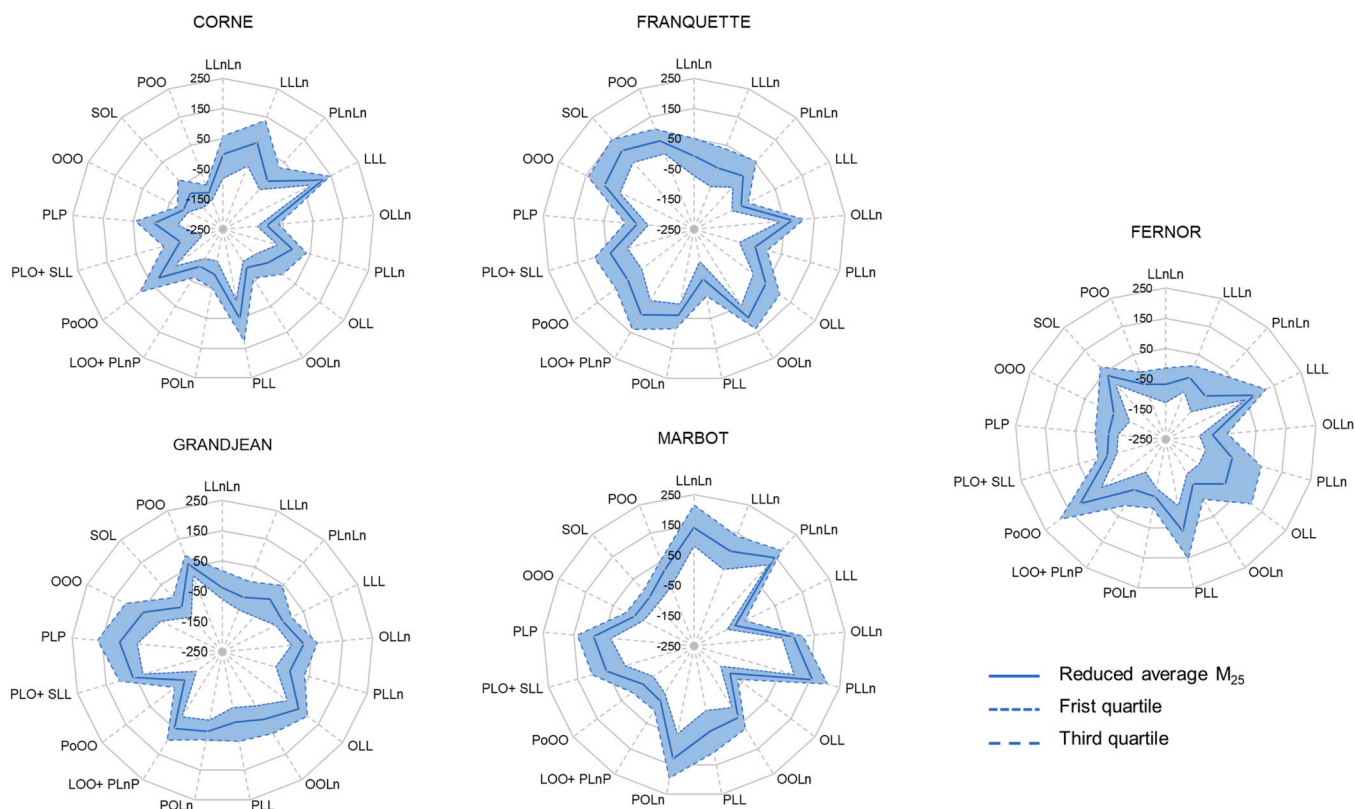


Fig. 3. Triacylglycerol morphotypes of oils from Corne, Fernor, Franquette, Grandjean and Marbot walnut varieties.

Table 4

Concentration (mg/kg oil) of α -, β -, γ - and δ -tocopherols from literature source for Franquette and Marbot varieties.

	Crop year	Country	Storage / drying condition	Ext. type	Analyse date	α	β	γ	δ	Σ
Codex Alimentarius						nd-170	nd-110	120–400	nd-60	
Franquette										
	Amaral et al., 2005	2001	Portugal	30°C; 72 h	vortex extracted with hexane of finely chopped walnuts	14.36	1.46	261.60	16.91	294.33
		2002	(Bragança)			12.50	1.08	240.89	13.20	267.67
		2003				8.96	0.88	189.85	9.43	209.12
		2003	Portugal (Coimbra)			10.16	0.73	191.01	8.68	210.58
	Lavedrine et al., 1997	1993	France	unshelled walnuts; 4°C	Soxhlet with hexane	Dec. 1993		311.20*	41.10	363.80
						Fev. 1994		223.40*	28.80	260.40
						Nov. 1994		233.00*	32.60	276.10
		1994	France			Nov. 1994		236.80*	40.70	296.20
		1994	USA			Nov. 1994		217.90*	35.20	267.10
	Martínez et al., 2006	2004	Argentina	30°C; 24 h	hydraulic press; 23°C	Tr		86.89%	13.11%	
		2005				2.55%		83.72%	13.74%	
	Martínez and Maestri, 2008	2008	Argentina	30°C; 24 h	hydraulic press; 23°C					318.00
	Cittadini et al. 2020	2019	Argentina			10.68	nd	304.46	24.55	339.68
	Mean					11.96	1.04	241.01	25.12	278.50
	min					8.20	0.73	189.85	8.68	209.12
	max					18.70	1.46	311.20	41.10	363.80
Marbot										
	Amaral et al., 2005	2001	Portugal	30°C; 72 h	vortex extracted with hexane of finely chopped walnuts	12.65	0.90	262.01	13.05	291.31
		2002	(Bragança)			10.77	0.89	255.01	13.62	283.46
		2003				10.36	0.83	197.24	8.24	219.51
	Mean					11.26	0.87	238.09	11.64	264.76
	min					10.36	0.83	197.24	8.24	219.51
	max					12.65	0.90	262.01	13.62	291.31

Ext. type = extraction type; min = minimum value; max = maximum value; * = $\beta + \gamma$ (not separated during analytic process); Tr = trace; nd = non determined.

Table 5

Statistical parameters for PLS1-DA calibration and validation models based on fatty acid data, triacylglycerol data or fatty acid and triacylglycerol data for each modelled variety.

Modelled variety	Fatty acids					Triacylglycerols					Fatty acids + triacylglycerols				
	Calibration (n = 97)		Validation (n = 49)			Calibration (n = 97)		Validation (n = 49)			Calibration (n = 97)		Validation (n = 49)		
	LV	R ²	RMSEC	Q ²	RMSEP	LV	R ²	RMSEC	Q ²	RMSEP	LV	R ²	RMSEC	Q ²	RMSEP
COR	4	0.86	0.24	0.86	0.24	2	0.76	0.29	0.76	0.27	3	0.84	0.25	0.84	0.24
FQT	8	0.94	0.15	0.91	0.21	3	0.86	0.24	0.81	0.28	7	0.94	0.16	0.87	0.21
GDJ	2	0.81	0.26	0.77	0.27	3	0.77	0.28	0.71	0.31	3	0.82	0.24	0.78	0.27
MAR	3	0.76	0.23	0.74	0.24	1	0.68	0.26	0.67	0.26	2	0.73	0.24	0.73	0.24

COR: Corne, FQT: Franquette, GDJ: Grandjean, MAR: Marbot; LV: number of latent variables; R²: coefficient of determination for the calibration; RMSEC: root mean square error of calibration; Q²: coefficient of determination for the prediction; RMSEP: root mean square error of prediction; n: number of samples.

Table 6

Confusion matrices of the PLS1-DA models based on fatty acid data predicting the varietal origin of validation samples from the five varieties.

Modelled cultivar	Real Class	Predicted class			Control limits	Warning limits
		Accepted	Rejected	Uncertain		
COR	COR (n=14)	13	1	0	1.15; 0.55	1.30; 0.40
	Others (n=35)	1	34	0		
	FER (n = 30)	10	8	12		
FQT	FQT(n=16)	13	1	2	1.20; 0.66	1.33; 0.52
	Others (n=33)	0	32	1		
	FER (n = 30)	6	18	6		
GDJ	GDJ (n=12)	9	1	2	1.17; 0.44	1.36; 0.25
	Others (n=37)	0	36	1		
	FER (n = 30)	3	21	6		
MAR	MAR (n=7)	6	1	0	0.81; 0.56	0.87; 0.50
	Others (n=42)	0	41	1		
	FER (n = 30)	1	29	0		

n: number of samples, COR: Corne, FER: Fernor, FQT: Franquette, GDJ: Grandjean, MAR: Marbot

Although reducing the number of members of the larger class to achieve balanced classes would have been preferable, all available walnut data were used. The aim of Périgord walnut sector is to uphold old varieties within the PDO, even if the promising Fernor variety should be integrated into the PDO. In PLS-DA, the class membership was based on comparison of the predicted value with target values 0 or 1. However, predicted values can range from 0 to 1, and even extend beyond and below these limits. For this reason, a decision rule is required to properly assign predicted value to meaningful class membership.

In the literature, samples were generally classified either by employing a scalar threshold, commonly set arbitrarily at 0.5 as the decision limit, or, less frequently, determined using the Bayes theorem (with a possible no-classification of samples) or by relying on class probabilities (assigning all samples to a class) (Jiménez-Carvelo et al., 2021; Ballabio and Consonni, 2013). Another decision rule for PLS-DA modelling, adopted here, involved the implementation of control charts, a common and essential tool in the field of quality management in many sectors (Maléchaux et al., 2019). This approach here was used to establish an acceptability range for the predicted values centered around a target value specific to each variety. The target value was set at the Mean Calibration Scores (MCS) value of each modelled variety. Furthermore, on both sides of the MCS value, upper and lower acceptability limits, varying among varieties, were set to consider data variance across harvest years and the geographical extent of the PDO area.

PLS1-DA models were built from FA or TAG or both FA and TAG contents to see which data set lead to best models. Statistical parameters for each PLS1-DA calibration and validation models are shown in Table 5. Best quality parameters were obtained with FA set for all varieties. Franquette and Corne were the best predicted varieties (Q² equal to 0.91 and 0.86 respectively). Results were reported in Table 6 given samples distribution (accepted, rejected or uncertain) according to the warning limits and control limits. The percentage of samples recognized as belonging to the predicted variety is 93%, 81%, 75% and 86% for the varieties Corne, Franquette, Grandjean and Marbot respectively. Very

few samples were wrongly rejected: one for each variety. Franquette and Grandjean models predicted two samples belonging to the modeled variety as uncertain. Only one sample from one of the three other varieties was wrongly accepted as belonging to Corne variety.

To evaluate the models' capacity to identify samples of unknown origin and to exclude those not matching the modeled varieties, the prediction was expanded to include thirty samples from the Fernor variety. Results were included in Table 6. Fernor samples were not clearly distinguishable from other varieties. Indeed, ten Fernor samples were mistakenly identified as Corne samples, six as Franquette samples, three as Grandjean samples, and one as Marbot sample. It should be noted that some of these samples may be mistakenly recognized as belonging to multiple varieties. Ideally, if the models were strong and reliable, they would accurately reject all Fernor samples with 100% certainty. However, the reality is different. The Corne model only rejected 27% of Fernor samples, while the Franquette and Grandjean models rejected 60% and 70%, respectively. Only the Marbot model demonstrated robustness, with 97% of Fernor samples being correctly rejected. To enhance the robustness of the other models, it would be beneficial to enrich the database with samples from future harvest years. This approach would allow for a more refined specificity of each model. Nevertheless, they offer insights into how the unknown variety is assessed in terms of compliance with Périgord PDO standards.

4. Conclusions

The contents of fatty acids, triacylglycerols and tocopherols allowed to characterize cold pressed monovarietal walnut oils from Périgord PDO varieties (Corne, Franquette, Grandjean and Marbot). The same chemical determinations have been performed for Fernor variety to bring necessary features to candidate as potential fifth walnut variety authorized for making Périgord PDO oil. The graphic representations (morphotypes) of the fatty acid and triacylglycerol compositions of the five varieties allowed rapid visual identification of the varieties and the

highlighting of specific markers. PLS1-DA chemometric models based on fatty acid content appeared as a second efficient way for the prediction of oil varietal origin. Using a control chart approach to estimate the reliability of the PLS1-DA prediction allowed to consider the data variability due to the year harvest and the extent of the Périgord PDO geographical area. The results obtained constitute a basis for ensuring the purity control of walnut oils labeled Périgord PDO and thus defending this regional production against fraudulent practices.

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CRedit authorship contribution statement

Jacques Artaud: Writing – review & editing, Conceptualization. **Carmen Vilhena de Castro:** Resources. **Catherine Rébufa:** Writing – review & editing, Conceptualization. **Yveline Le Dréau:** Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jfca.2024.106189](https://doi.org/10.1016/j.jfca.2024.106189).

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