SUPPLEMENTARY MATERIAL

Evolution of monovarietal virgin olive oils as a function of chemical composition and oxidation status

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ABSTRACT: Virgin Olive Oil (VOO) shelf life is determined by the varietal-specific chemical composition and principally by the of phenolic composition. The aim of this study was to investigate the changes in fatty acid profile, phenolic composition, and quality parameters of nine Italian monovarietal VOOs obtained under the same pedoclimatic, agronomic and technological conditions and stored for 12 months at 15 °C in the dark. The varieties with medium-high concentrations of secoiridoids and balanced values between the individual molecules were those with the highest stability. Orthogonal Projections to Latent Structures (OPLS) regression revealed that oleuropein derivatives and phenolic alcohols had the highest antioxidant activity. OPLS discriminant analysis separated well fresh and stored oils. PV, K270, tyrosol, hydroxytyrosol, and oxidated oleacein were the most effective indicators of VOO ageing. Oleacein and oleocanthal decreased after storage, phenolic alcohols, oleacein and ligstroside aglycon increased.

KEYWORDS: Olea europaea; oleocanthal; oleuropein aglycon; OPLS; oxidation stability; shelf life; variety

3. Experimental

3.1. Standards and reagents

Folin Ciocalteu phenol reagent, apigenin (≥95%), hydroxytyrosol (≥98%), luteolin (≥98%), oleuropein (≥98%), p-coumaric acid (≥98%), pinoresinol (≥95%), tyrosol (≥98%), vanillic acid (≥97%), vanillin (≥99%), and methanol (≥99.9%) for high performance liquid chromatography (HPLC) were all acquired from Sigma–Aldrich (Milano, Italy). Acetonitrile and 2-propanol (Chromasolv®) were from ChemLab (Zedelgen, Belgium). Ultrapure water (H2O) was obtained throughout a Milli-Q system (Millipore Corporation, Billerica, MA, USA).2.1.

3.2. Plant material, VOO extraction, and storage

The virgin olive oil samples were obtained from healthy olive fruits of Bosana, Coratina, Frantoio, Leccino, Nera di Oliena, Pizz ‘e Carroga, Semidana, Sivigliana da Olio, and Tonda di Villacidro. Such varieties were selected to obtain a large variability of VOO chemical profile (Deiana, Santona, Dettori, Molinu et al. 2019; Deiana et al. 2021). Olive trees, planted in 1998 with a density of 6x6 m, were grown at same agronomical (mowing of the spontaneous vegetation, drip irrigation, and annual pruning) and pedoclimatic conditions in the University of Sassari’s experimental station sited in Oristano (Italy). The study area is characterized by a typical Mediterranean bioclimate. Annual mean, maximum, and minimum average temperatures are 17.7 °C, 24.2 °C, and 11.3 °C, respectively. July and August are the warmest months, whilst January and February the coldest. Precipitation is concentrated in the autumn and winter seasons, and the annual mean rainfall is 600 mm (Environmental Protection Agency of Sardinia, ARPAS, SM1). Olive fruits, 25 – 30 kg, were handpicked in November 2017 and processed soon after harvest using a small-scale industrial mill “Sintesi 80” Mori TEM (Tavernelle Val di Pesa, Italy) following the extraction procedure described by Deiana, Santona, Dettori, Molinu et al (2019). After 1 month of decantation in 500 ml metal containers, oil samples were stored in 100 ml dark glass bottles at 15 °C, with limited headspace (<1 cm), and protected from light. To select 15°C as storage temperature for this study we based on both previous literature (Krichene et al. 2015; Sinesio et al. 2015; Alvarruiz et al. 2020; Iqdiam et al. 2020, Zullo and Ciafardini 2020) and well-established local producers' experience that indicated storage temperatures between 10 and 20°C those able to reasonably constrain the natural autoxidation process of virgin olive oil within a context of easy-to-manage and not expensive ambient cellar conditions.

Analyses were performed at bottling (Time 1) and repeated after one year (Time 12).

3.3. Quality parameters and fatty acid methyl esters (FAMEs)

Quality parameters: free acidity percentage of oleic acid), peroxide value (PV, meq O_2 kg^{-1}olive oil), K232, K270, and FAMEs, were determined according to the official method for olive oil characteristics described by European Union (EC Reg. No 2568/91) and subsequent amendments (EC Reg. No. 2015/1833).

3.4. Determination of phenolic composition

Phenolic compounds were extracted according to the method described by Deiana, Santona, Dettori, Culeddu et al (2019). Reverse-phase HPLC analysis of phenolic compounds was performed using an Agilent 1100 Liquid Chromatography (LC) system (Agilent Technologies, Palo Alto, CA, USA), equipped with a quaternary pump (G1311A), degasser, column thermostat, auto-sampler (G1313A), and a diode array detector (G1315 B, DAD). Chromatographic separation was achieved with a Luna C18 column (250 x 4.6 mm, 5 µm) from Phenomenex (Torrance, CA, USA) with a security guard cartridge (4 × 2 mm). Chromatographic conditions, data acquisition, identification, and quantification of the phenolic compounds were performed according to the respective standards retention times and regression curves (for apigenin, hydroxytyrosol, luteolin, p-coumaric acid, tyrosol, vanillic acid, and vanillin), whereas for secoiridoid derivatives was used regression curve of oleuropein and regression curve of pinoresinol for 1-acetoxypinoresinol, as described in Deiana, Santona, Dettori, Molinu et al (2019).

3.5. Statistical analysis

The effect of storage and variety on quality parameters, FA and phenolic profile was assessed by the factorial analysis of variance (two-way ANOVA). The η^2 value was calculated to determine the percentage of variance related to each factor and the interaction. OPLS-DA was performed to discriminate between fresh and stored VOOs based on the values of quality parameters, FA, and phenolic profiles at (Time 1 and Time 12). OPLS regression models were performed setting, one at time PV, K232, and K270 as the Y variable. Each of the three quality indexes was evaluated both at time 1 and after storage considering the difference between final and initial values (Δ). The X variables of OPLS models were selected from the initial FA and phenolic compounds. Quality parameters at Time 1, when not set as Y variable, were included within X variables. Initially all those variables were included in the models, then, to improve the model accuracy, the variables with the lowest model relevance (based on the Variable Influence on Projection (VIP) values) were excluded one by one until the achievement of the lowest possible number of latent components with the highest possible R^2Y and Q^2 values. The two parameters indicate respectively the percentage of variation explained by the model and the proportion of variance predictable by the model. For this reason, different number of variables characterized each developed model. To validate the OPLS-DA and OPLS models, a 7-fold cross-validation and permutation tests were performed on the corresponding PLS-DA and PLS models (Deiana, Santona, Dettori, Culeddu et al, 2019). Data were processed with R Studio and SIMCA-P software version 13.0 (Umetrics AB, Umea, Sweden).

References

Alvarruiz A, Pardo JE, Copete ME, de Miguel C, Rabadán A, López E, Álvarez-Ortí M. 2020. Evolution of virgin olive oil during long-term storage. J. Oleo Sci. 69:809-814.

Deiana P, Santona M, Dettori S, Culeddu N, Dore A, Molinu MG. 2019. Multivariate approach to assess the chemical composition of Italian virgin olive oils as a function of variety and harvest period. Food Chem. 300:125243.

Deiana P, Santona M, Dettori S, Molinu MG, Dore A, Culeddu N, Azara E, Naziri E, Tsimidou MZ. 2019. Can all the Sardinian varieties support the PDO "Sardegna" virgin olive oil? Eur J Lipid Sci Technol. 121:1800135.

Deiana P, Filigheddu MR, Dettori S, Culeddu N, Dore A, Molinu MG, Santona M. 2021. The chemical composition of Italian virgin olive oils. In: Preedy VR and Watson RR (ed) Olives and Olive Oil in Health and Disease Prevention (2nd edn), Academic Press, London, UK.

[EC] European Commission Reg. No. 2568/91. 1991. On the characteristics of olive and olive-pomace oils and on their analytical methods. Off J Eur Union. L24:1.

[EC] European Commission Reg. 2015/1833. 2015. Amending Regulation (EEC) No 2568/91 On the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Off J Eur Union. L266:29.

[EC] European Commission Reg. No. 2016/2095. 2016. Amending Regulation (EEC) No 2568/91 On the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Off J Eur Union. L326, 1.

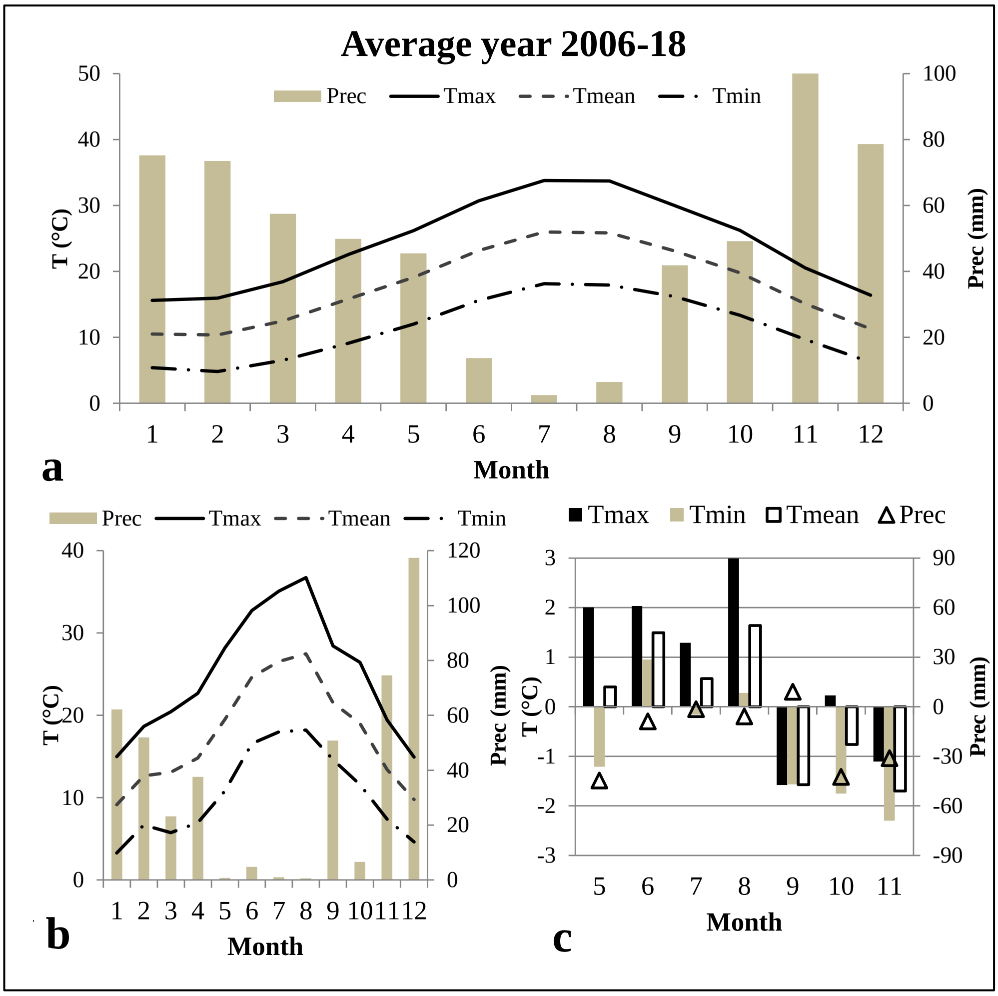
Iqdiam BM, Welt BA, Goodrich-Schneider R, Sims CA, Baker IV GL, Marshall MR. 2020. Influence of headspace oxygen on quality and shelf life of extra virgin olive oil during storage. Food Packag Shelf Life. 23:100433.

Krichene D, Salvador MD, Fregapane G. 2015. Stability of virgin olive oil phenolic compounds during long-term storage (18 months) at temperatures of 5–50 C. J Agric Food Chem. 63:6779-6786.

Sinesio F, Moneta E, Raffo A, Lucchetti S, Peparaio M, D'Aloise A., Pastore G. 2015. Effect of extraction conditions and storage time on the sensory profile of monovarietal extra virgin olive oil (cv Carboncella) and chemical drivers of sensory changes. LWT-Food Sci Tech. 63:281-288.

Zullo BA, Ciafardini G. 2020. Differential microbial composition of monovarietal and blended extra virgin olive oils determines oil quality during storage. Microorganisms. 8:402.

SM1. Average weather conditions (2006 – 2018) in Oristano station (ARPAS). Mateorological conditions (Tmax, Tmin, Tmean, precipitations (Prec)) of 2017 growing season (b) and monthly variations from the average year of the months of olive fruit development (c).



SM2. Significance values (p-value) and eta squared values (**η^2**) for the factorial analysis (two-ways ANOVA) for all the variables analyzed.

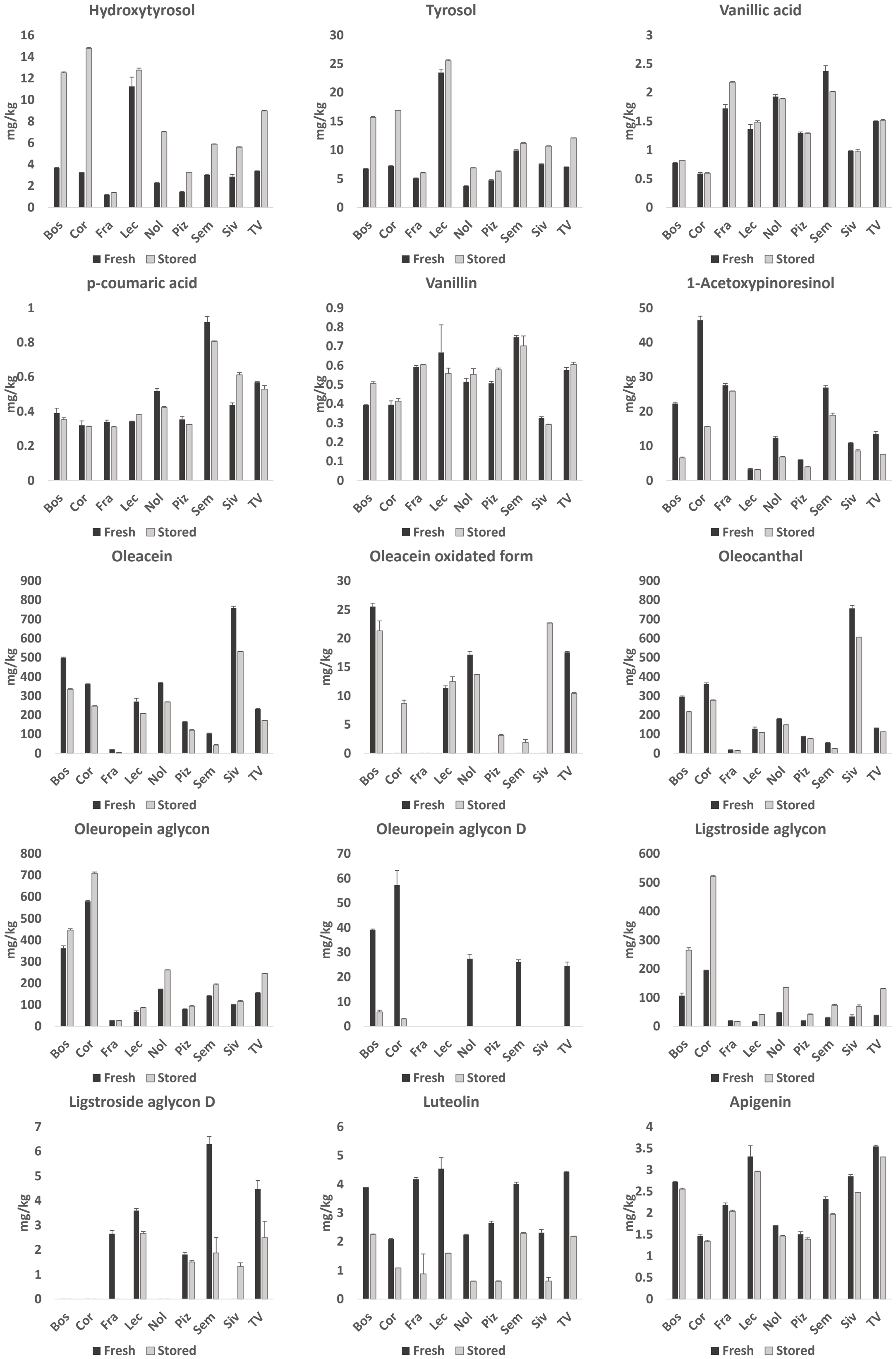
Variable	Variety		Storage		Interaction	
	p-value	η^2	p-value	η^2	p-value	η^2
Acidity	***	0.96	***	0.03	*	0.01
K232	***	0.71	***	0.06	***	0.22
K270	***	0.55	***	0.39	***	0.06
PV	***	0.70	***	0.23	***	0.07
C 16:0	***	1.00	n.s.	0.00	n.s.	0.00
C 16:1	***	0.99	n.s.	0.00	n.s.	0.00
C 18:0	***	0.96	n.s.	0.00	n.s.	0.01
C 18:1	***	0.99	n.s.	0.00	n.s.	0.00
C 18:2	***	0.99	*	0.00	n.s.	0.00
C 18:3	***	0.86	*	0.03	n.s.	0.02
C 20:0	*	0.47	n.s.	0.03	n.s.	0.12
C 20:1	*	0.57	n.s.	0.02	n.s.	0.08
SFA	***	0.99	n.s.	0.00	n.s.	0.00
MUFA	***	0.99	n.s.	0.00	n.s.	0.00
PUFA	***	0.99	*	0.00	n.s.	0.00
Hyd	***	0.54	***	0.28	***	0.18
Tyr	***	0.83	***	0.11	***	0.06
Van ac	***	0.96	n.s.	0.00	***	0.04
Vanil	***	0.89	n.s.	0.00	**	0.07
p-coum ac	***	0.93	n.s.	0.00	***	0.07
Oleac	***	0.92	***	0.05	***	0.03
Oleac OX	***	0.76	***	0.01	***	0.23
Oleoc	***	0.97	***	0.01	***	0.01
Ol Agl	***	0.97	***	0.02	***	0.02
Ol Agl D	***	0.40	***	0.28	***	0.32
Lig Agl	***	0.72	***	0.11	***	0.16
Lig Agl D	***	0.73	***	0.10	***	0.17
Ac Pin	***	0.72	***	0.11	***	0.17
Lut	***	0.75	***	0.23	***	0.02
Api	***	0.99	***	0.01	***	0.00

SM3. Quality parameters and fatty acid[1] composition of VOOs from Bosana (Bos), Coratina (Cor), Frantoio (Fra), Leccino (Lec), Nera di Oliena (Nol), Pizz 'e Carroga (PC), Semidana (Sem), Sivigliana da Olio (Siv), and Tonda di Villacidro (TV) related to the storage period.

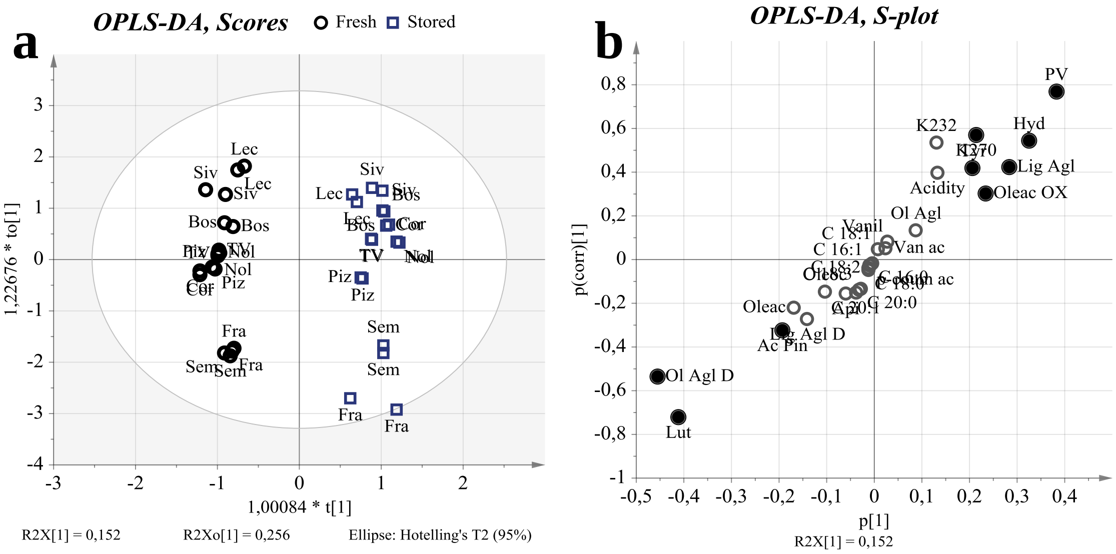
Varables	Time	Bos	Cor	Fra	Lec	Nol	PC	Sem	Siv	TV
Acidity	1	0.32±0.00	0.26±0.01	0.39±0.04	0.25±0.00	0.21±0.00	0.24±0.05	0.24±0.07	0.26±0.04	0.35±0.00
	12	0.44±0.03	0.30±0.02	0.45±0.04	0.32±0.01	0.26±0.02	0.34±0.02	0.25±0.02	0.27±0.03	0.43±0.01
K232	1	2.16±0.00	2.01±0.00	2.02±0.00	2.13±0.00	1.88±0.00	1.86±0.00	1.81±0.00	2.15±0.00	1.92±0.00
	12	2.79±0.02	2.36±0.00	2.51±0.03	2.06±0.03	2.00±0.01	1.91±0.01	2.05±0.01	2.84±0.04	2.08±0.00
K270	1	0.14±0.00	0.15±0.00	0.09±0.00	0.08±0.00	0.09±0.00	0.06±0.00	0.08±0.00	0.14±0.00	0.10±0.00
	12	0.19±0.00	0.20±0.00	0.12±0.00	0.12±0.00	0.12±0.00	0.12±0.00	0.14±0.00	0.17±0.00	0.12±0.00
PV	1	4.78±0.28	2.38±0.01	9.31±0.25	4.92±0.06	4.03±0.07	4.58±0.16	4.03±0.07	3.60±0.11	5.42±0.07
	12	13.26±0.64	6.24±0.06	23.16±0.01	8.55±0.25	8.10±0.99	8.50±0.08	9.14±0.27	10.90±0.06	9.23±0.05
C 16:0	1	11.43±0.07	8.69±0.08	11.57±0.13	12.61±0.24	11.03±0.07	13.88±0.02	13.53±0.33	14.64±0.15	11.99±0.12
	12	11.31±0.01	8.82±0.25	11.69±0.11	12.64±0.14	10.96±0.10	13.76±0.08	13.33±0.00	14.58±0.11	11.96±0.20
C 16:1	1	0.70±0.04	0.36±0.01	1.04±0.08	1.13±0.12	0.85±0.00	1.23±0.03	0.92±0.04	1.43±0.01	1.05±0.01
	12	0.70±0.02	0.37±0.02	1.00±0.02	1.13±0.08	0.84±0.02	1.20±0.02	0.88±0.01	1.40±0.06	1.02±0.04
C 18:0	1	1.86±0.01	1.52±0.01	1.86±0.14	1.50±0.11	1.48±0.03	1.99±0.01	2.11±0.03	1.46±0.02	1.52±0.06
	12	1.82±0.03	1.56±0.04	1.86±0.07	1.45±0.08	1.45±0.03	1.97±0.02	2.02±0.03	1.41±0.03	1.50±0.06
C 18:1	1	75.71±0.31	81.68±0.11	70.75±0.90	73.05±1.34	77.35±0.24	69.12±0.19	68.16±0.70	69.43±0.00	74.25±0.08
	12	76.05±0.27	81.47±0.44	71.00±0.03	73.36±0.68	77.75±0.03	69.36±0.18	68.93±0.23	69.95±0.45	74.54±0.56
C 18:2	1	9.01±0.15	6.49±0.06	13.24±0.39	10.31±0.62	7.90±0.06	12.35±0.09	13.84±0.26	11.73±0.07	9.88±0.00
	12	8.81±0.16	6.50±0.09	13.01±0.07	10.10±0.29	7.71±0.02	12.23±0.02	13.46±0.13	11.43±0.19	9.73±0.21
C 18:3	1	0.67±0.04	0.58±0.01	0.86±0.07	0.76±0.09	0.73±0.01	0.80±0.04	0.77±0.01	0.71±0.01	0.70±0.00
	12	0.65±0.02	0.56±0.01	0.80±0.01	0.73±0.06	0.72±0.01	0.81±0.02	0.75±0.01	0.67±0.04	0.67±0.01
C 20:0	1	0.37±0.01	0.33±0.00	0.36±0.04	0.35±0.08	0.37±0.05	0.38±0.02	0.41±0.00	0.33±0.00	0.34±0.00
	12	0.37±0.00	0.36±0.00	0.35±0.00	0.32±0.04	0.31±0.00	0.40±0.02	0.39±0.04	0.32±0.01	0.33±0.05
C 20:1	1	0.25±0.01	0.35±0.00	0.33±0.06	0.29±0.08	0.29±0.02	0.27±0.02	0.26±0.03	0.28±0.04	0.27±0.00
	12	0.28±0.03	0.36±0.02	0.30±0.02	0.26±0.00	0.27±0.02	0.26±0.00	0.25±0.04	0.24±0.02	0.26±0.01
SFA[2]	1	13.66±0.07	10.54±0.06	13.79±0.30	14.46±0.43	12.88±0.15	16.24±0.01	16.06±0.36	16.42±0.14	13.85±0.06
	12	13.51±0.04	10.74±0.30	13.89±0.05	14.42±0.26	12.72±0.07	16.12±0.12	15.74±0.08	16.31±0.15	13.79±0.30
MUFA[3]	1	76.66±0.27	82.39±0.12	72.11±0.76	74.47±1.15	78.49±0.22	70.62±0.14	69.34±0.63	71.14±0.06	75.57±0.06
	12	77.02±0.22	82.20±0.39	72.31±0.03	74.75±0.61	78.85±0.03	70.83±0.16	70.05±0.20	71.60±0.38	75.82±0.52
PUFA[4]	1	9.68±0.19	7.07±0.06	14.09±0.46	11.08±0.71	8.63±0.07	13.14±0.13	14.61±0.28	12.44±0.08	10.58±0.00
	12	9.47±0.18	7.06±0.10	13.80±0.08	10.83±0.34	8.43±0.04	13.04±0.04	14.21±0.12	12.09±0.23	10.39±0.21

[1]Values are expressed as % of total composition; [2]SFA = sum of saturated fatty acids; [3]MUFA = sum of monounsaturated fatty acids; [4]PUFA = sum of polyunsaturated fatty acids.

SM4. Concentration of phenolic compounds (expressed as mg kg^{-1} of olive oil, vertical axis) in fresh and stored VOOs from Bosana (Bos), Coratina (Cor), Frantoio (Fra), Leccino (Lec), Nera di Oliena (Nol), Pizz ‘e Carroga (Piz), Semidana (Sem), Sivigliana da Olio (Siv), and Tonda di Villacidro (TV)

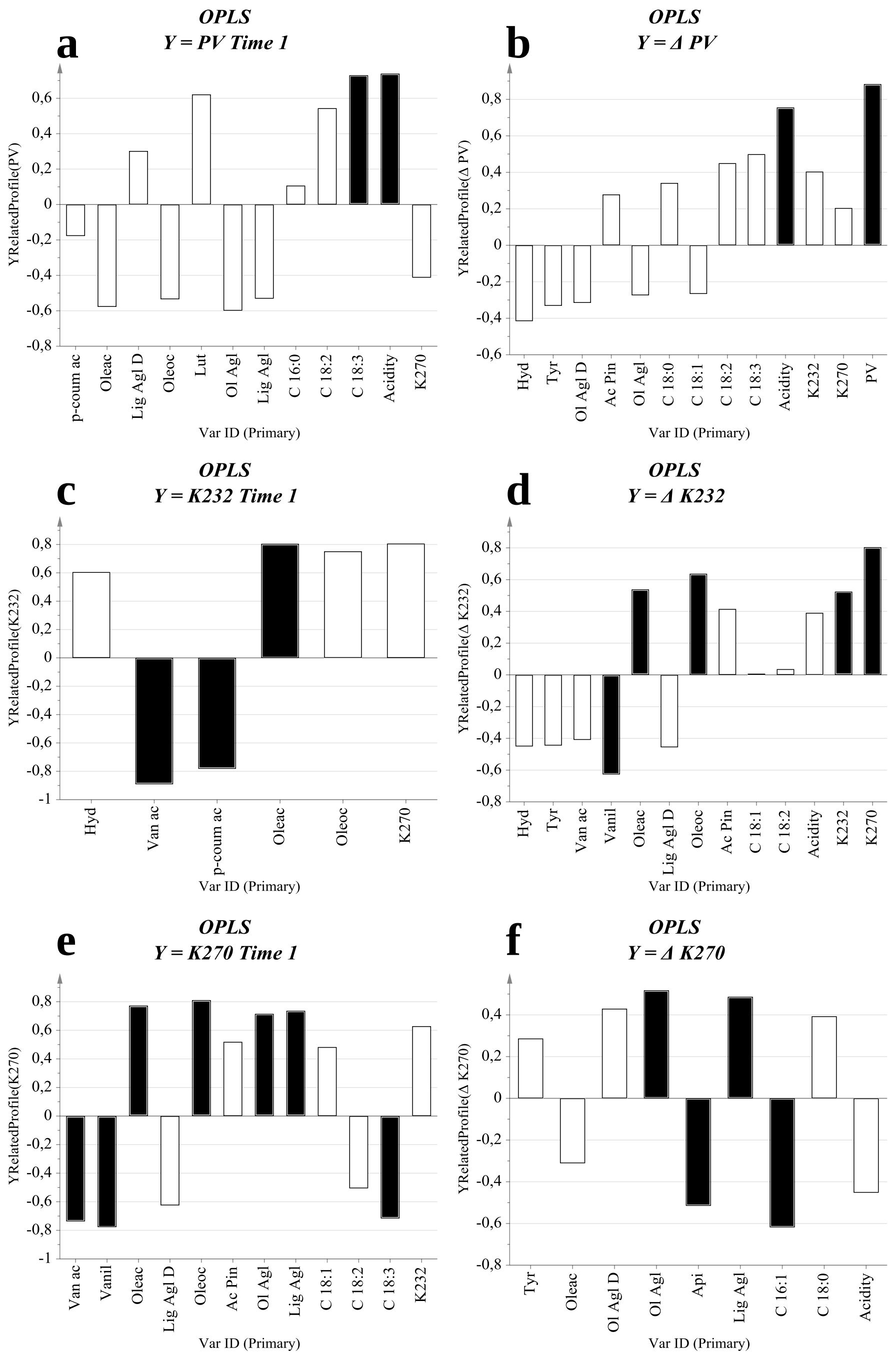


SM5. Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) model, Score plot (a) and S-plot of variables[1,2] (b), discriminating between fresh (1 month) and stored (12 months) VOOs.



[1]Variables abbreviations: PV = peroxide value; Hyd = hydroxytyrosol; Tyr = tyrosol; Van ac = vanillic acid; p-coum ac = p-coumaric acid; Vanil = vanillin; Oleac = oleacein; Oleac OX = oxidated oleacein; Oleoc = oleocanthal; Ol Agl = oleuropein aglycon; Ol Agl D = oleuropein aglycon dialdehydic form; Lig Agl= ligstroside aglycon; Lig Agl D = ligstroside aglycon dialdehydic form; Lut = luteolin; Api = apigenin. [2]Variables highlighted with black dots were those with the highest model relevance (VIP ≥ 1).

SM6. Y-related profile plot representing the predictive components[1,2] of OPLS models related to the VOO quality parameters (initial values and Δ variations[3], respectively): peroxide value (a, b); K232 (c, d); K270 (e, f). The higher is the value, represented by bars, the higher is the correlation between the X variable and the corresponding response variable.

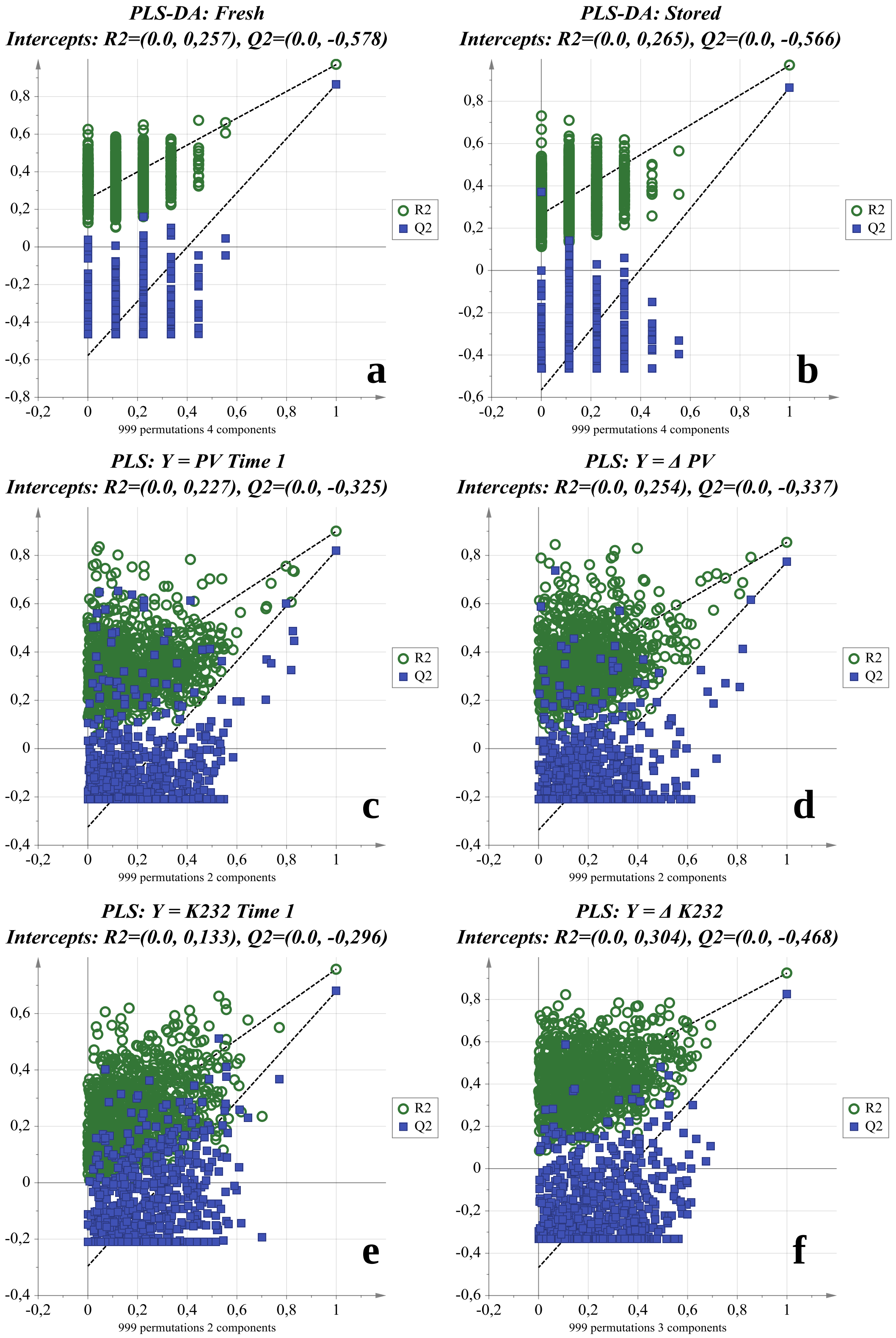


[1]Variables abbreviations: Hyd = hydroxytyrosol; Tyr = tyrosol; Van ac = vanillic acid; p-coum ac = p-coumaric acid; Vanil = vanillin; Oleac = oleacein; Oleoc = oleocanthal; Ol Agl = oleuropein aglycon; Ol Agl D = oleuropein aglycon dialdehydic form; Lig Agl= ligstroside aglycon; Lig Agl D = ligstroside aglycon dialdehydic form; Lut = luteolin; Api = apigenin. [2]Variables highlighted with black columns were those with the highest model relevance (Variable Influence on Projection, VIP ≥ 1). [3] Δ values are Time 12 - Time 1 values.

SM7. Autofit results for the Orthogonal Projections to Latent Structures Discriminant Analysis, (OPLS-DA) model "fresh *vs* stored oils" (with corresponding PLS-DA) and OPLS models (with corresponding PLS) explaining the relationships between quality parameters (PV, K232, and K270, both initial values and Δ variations after the storage period) and further VOO aging indicators (Δ UFA + Δ SFA, Δ hydroxytyrosol + Δ tyrosol, Δ oleacein + Δ oleocanthal) and initial VOO chemical composition.

Model Type	**Components**	**Y variable**	**n. X variables**	**R^2x**	**R^2y**	**Q^2**
OPLS-DA	1+3+0	Fresh-Stored	27	0.737	0.970	0.946
OPLS	1+3+0	PV	12	0.829	0.951	0.868
OPLS	1+1+0	Δ PV	13	0.585	0.854	0.783
OPLS	1+1+0	K232	6	0.765	0.758	0.682
OPLS	1+4+0	Δ K232	13	0.906	0.972	0.946
OPLS	1+3+0	K270	12	0.879	0.971	0.935
OPLS	1+3+0	Δ K270	9	0.806	0.932	0.885
PLS-DA	2	Fresh-Stored	27	0.737	0.970	0.865
PLS	2	PV	12	0.630	0.900	0.820
PLS	2	Δ PV	13	0.585	0.854	0.773
PLS	2	K232	6	0.765	0.758	0.679
PLS	3	Δ K232	13	0.739	0.924	0.825
PLS	3	K270	12	0.848	0.958	0.900
PLS	2	Δ K270	9	0.573	0.852	0.724

SM8. PLS-DA and PLS permutation analysis for the corresponding OPLS-DA (a, b) and OPLS models (c, d, e, f, g, h, i, j, k, l,m,n).



SM8. Continued