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ORIGINAL PAPER

Effect of freezing, fast-freezing by liquid nitrogen or refrigeration to preserve premium extra virgin olive oil during storage

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Abstract

During storage, premium extra virgin olive oils (PEVOO), which are oils of exceptional sensory quality, may lose the organoleptic characteristics that define them. This study assessed the effect of applying modified atmospheres and low temperatures (refrigeration and freezing) on the quality of 4 PEVOO for 24 months. Also, the effect of two freezing methods was studied (in the freezer at − 20 °C and in a bath of liquid nitrogen), along with the impact of freezing on the quality of the oils after thawing and storing at room temperature. Official quality parameters, organoleptic assessment, phenolic compounds, volatile compounds and oxidative stability index were measured periodically. While no significant effect of headspace composition was found, the oils stored at − 20 °C maintained their initial quality better than the oils stored at room temperature. Physicochemical quality parameters remained unchanged throughout the 24 months at − 20 °C. Polar phenolic and volatile compounds associated with green and fruity aromas were better preserved at − 20 °C, which translated into a minimum change in the sensory profile of the oils. While no significant difference was observed regarding oxidative parameters, freezing at − 20 °C maintained the initial volatile and sensory profile of the oils better than freezing with liquid nitrogen. Lastly, quality of thawed oils showed no significant differences compared to control oils during storage at room temperature. In conclusion, storage at − 20 °C maintains the quality of PEVOO, especially their sensory profile, and does not compromise their quality after thawing.

Keywords Premium EVOO · Storage conditions · Freezing · Sensory quality · Oxidative stability · Secondary sensory attributes

Introduction

Extra virgin olive oils (EVOO) with exceptional sensory quality are currently labeled as "premium" (PEVOO). The high demand for such outstanding product makes that they retail at high prices and currently account for 20% of EVOO market [1]. The decrease of quality during storage is especially critical for PEVOO, since it negatively impacts their distinctive sensory features. Although storage between one harvest to another does not usually cause EVOO to decline to lower

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grades, it may be enough to lose the distinctive characteristics of PEVOO [2], limiting their availability to the first few months after harvest season. A proper control of the storage conditions is crucial to maintain for longer the quality of PEVOO [3] and to enhance their commercialization.

Although several studies have evaluated the effect of light, temperature, and oxygen availability on EVOO quality during storage, most of them aimed to reproduce the actual storage conditions, i.e., room temperature (RT) [4–7]. As the higher value of PEVOO compared to standard EVOO could justify higher storage costs, the benefits of applying conditions that are not common in the olive oil sector, such as controlled atmosphere and cold storage, deserve to be evaluated. A positive effect of cold storage on EVOO quality has been reported at refrigeration [8–10] and freezing [11–14] temperatures, but more research is needed to endorse its suitability for PEVOO quality preservation. For instance, previous studies have reported contradictory information on whether the partial crystallization of oils during the phase transition at low temperatures affects their oxidation rates [15–17]. Assessing freezing methods at different freezing speeds (i.e., speed of phase transition) would allow clarifying this issue. On the other hand, Cerretani et al. [11] hypothesized that freezing and thawing olive oil may induce a partial precipitation of phenols and the consequent reduction of its oxidative resistance, with a negative effect on shelf life. This would question the appropriateness of storing at freezing temperatures, but studies on the evolution of EVOO quality at RT after thawing are necessary to elucidate this fact.

Moreover, previous studies mainly focused on EVOO physicochemical trade parameters [18–22] or on the appearance of sensory defects [4, 7, 8], according to EEC Regulation [23]. Only few reports concern secondary attributes during storage [24, 25]. Since sensory quality is the distinctive trait of PEVOO, the evolution of secondary attributes contributing to the outstanding profile of PEVOO is crucial.

Our aim was to evaluate the effect of oxygen availability, cold storage (4 °C and − 20 °C versus RT), and freezing speed on the overall quality of 4 different PEVOO during 24 months of storage. We monitored the overall sensory profile, physicochemical and compositional data (trade quality parameters, phenolic and volatile compounds, Oxidation Stability Index—OSI), which are essential to guarantee the preservation of PEVOO distinctive features during storage. Moreover, the effect of thawing and storing at RT after 12 months of frozen storage was evaluated.

Materials and methods

Samples

With the aim of including different phenolic, volatile and sensory profiles in the study, four PEVOO produced during 2016/17, two of the Picual cultivar and two of the Arbequina cultivar, were obtained in 25 L bottles from Castillo de Canena (Canena, Jaén, Spain) and La Gramanosa (Avinyonet del Penedès, Barcelona, Spain), respectively. For each cultivar, a sample was produced at the beginning and another at the end of the harvest.

Storage conditions

Even if the four oils were clear and apparently homogeneous, the bottles were vigorously shaken to ensure homogeneity and then aliquots of 100 mL were placed in 130 mL glass bottles (23% headspace) with high density polypropylene caps from Scharlau (Sentmenat, Spain). A factorial design was applied to evaluate headspace composition, storage temperature and freezing method over 24 months of storage in the dark. For each oil, half of the samples were submitted to a nitrogen stream before being closed, obtaining a low oxygen headspace (N), while the headspace of the other half contained air (O). For each headspace condition (O and N), samples were stored at three different temperatures: 20–25 °C (RT), 4 °C (R) and − 20 °C. The samples stored at − 20 °C were frozen by two different methods: a slow-freezing method (S), by putting the samples directly in the freezer at − 20 °C, and a fast-freezing method (F), by immersing the samples in a bath of liquid nitrogen. The samples were analyzed at time 6, 12 and 24 months. Moreover, a characterization of the 4 oils was carried out at the beginning of the study. Altogether, 100 samples were analyzed, that is, 4 at time 0 and 96 during the conservation study: 4 oils × 2 headspace compositions (O, N) × 4 storage temperatures and freezing methods (RT, R, S and F) × 3 storage times (6, 12, 24) (Supplementary information, S1).

In addition, at 12 months, samples of each frozen condition were thawed at room temperature for approximately 10 h (ST or slow-freezing and thawed and FT or fast-freezing and thawed). The samples were analyzed right after thawing and after 6 months of storage at RT and were compared with the same samples analyzed at the beginning of the conservation study and after 6 months of storage at RT (control samples). In total, 24 samples were analyzed at the initial time and after 6 months at RT, corresponding to 4 oils × 2 headspace compositions (O, N) × 3 treatments (control, ST, FT) (Supplementary information, S2).

For each oil, storage condition and point of analysis 8 aliquots were prepared, so that the parameters more susceptible to change once the bottle is open (PV, extinction coefficients, volatile compounds and sensory profile) could be determined from a newly opened bottle. Globally, 992 bottles were prepared.

Analytical methods

Trade quality indices

Determinations of peroxide value (PV), extinction coefficients (K_{232} and K_{268}) and acidity were carried out following the analytical methods described in the EEC Regulation 2568/91 [23]. The sensory analysis was performed according to the same regulation by the Official Tasting Panel of Virgin Olive Oils of Catalunya (panel made of 8–12 panelists). Intensity of sensory defects and positive attributes were assessed and expressed as median of the panelists' scores. Moreover, the presence of secondary sensory attributes was determined by the percent of panelists able to perceive each odor note using an open generic profile [26].

Moisture and volatile matter

Moisture content and volatile matter were determined by the vacuum oven method following the AOCS official method Ca 2d-25 [27].

Oxidative stability

OSI was measured at 120 °C with an air flow rate of 20L/h using a 892 Professional Rancimat (Metrohm, Herisau, Switzerland) and following the AOCS official method Cd 12b-92 [28].

Fatty acids (FA)

For the determination of the FA composition, FA methyl esters were prepared by a double methylation and separated by GC-FID following the method described by Varona et al. [29]. In brief, 1 µL of the FAME extract was injected into an Agilent 4890D gas chromatograph (Agilent Technologies, Santa Clara, CA, USA), equipped with a split–splitless injector and a flame ionization detector (FID). Separation was carried out with a SP-2380 column from Supelco Ltd, Bellefonte, PA, USA (60 m × 0.25 mm and 0.2 µm film thickness). The oven temperature was as follows: 1 min at 150 °C, from 150 to 180 °C at 1.5ºC/min, 0.5 min at 180 °C, from 180 to 220 °C at 14.5 °C/min, 3 min at 220 °C, from 220 to 250 °C at 9.9ºC/min and 9 min at 205 °C. The carrier gas was hydrogen at 25 psi and the split ratio 1:30. Injector temperature was 270 °C and detector 300 °C.

Lipophilic and polar phenolic compounds

Tocopherols were evaluated following the AOCS official method Ce 8-89 [30] with some modifications. The sample (1.5 g) was diluted with hexane in a 10 mL volumetric flask. Then, 20 µL of the solution were injected into an Agilent 1100 series HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled to a Hewlett-Packard 1046A fluorescent detector. Separation was carried out using a 4 × 3.0 mm precolumn (Phenomenex Security Guard Cartridge Silica) and a Luna silica column (150 × 4.6 mm i.d., 3 µm particle size and 100 Å pore size) from Phenomenex (Torrance, CA, USA). Elution was performed using hexane/1,4-dioxane (95/5%, v/v).

Polar phenolic compounds were extracted according to Vichi et al. [31] and analyzed by UHPLC-DAD, adapting the chromatographic conditions of the IOC method COI/T.29/Doc No 29 [32] to an UHPLC system, as described by Nenadis et al. [33]. Briefly, 15 µL of the phenolic extract was injected into an Acquity-UPLC (Waters, Milford, MA, USA) coupled to a PDA 2996 detector (Waters, Milford, MA, USA). Separation was carried by a Halo C18 Fused-Core column (100 × 2.1 mm i.d., 2.7 µm particle size) from Advanced Materials Technology (Wilmington, DE, USA). Elution was performed at a 0.4 mL/min flow rate and 30 °C, using as mobile phase ultrapure water (Milli-Q Millipore Corporation, Billerica, MA, USA)/formic acid (98:2, v/v) (solvent A), and methanol/acetonitrile (50:50, v/v) (solvent B). The solvent gradient changed as follows: from 96% (A)–4% (B), to 20% (B) at 5 min, to 45% (B) at 28 min, to 100% (B) at 30 min, 5 min maintenance until 35 min, then 96% (A)–4% (B) at 36 min, 5 min of equilibration. Detection was performed simultaneously at 335 nm and 280 nm. Identification was carried out according to COI/T.29/Doc No 29 [32] and to Mateos et al. [34], and confirmed by high-resolution mass spectrometry, using a Q-Exactive hybrid Orbitrap (Thermo Fisher Scientific, Bremen, Germany), under the described chromatographic conditions. Ion source and spectrometer conditions were as described by Vichi et al. [31].

The following secoiridoids (SEC) were not quantified by UHPLC-DAD due to coelution: the aldehydic form of ligstroside aglycone and one of the oxidized aldehyde and hydroxylic forms of oleuropein aglycone.

Quantification was made using as internal standards (IS) *o*-coumaric acid (for flavones) and *p*-hydroxyphenylacetic acid (for the rest of the phenolic polar compounds) and the response factors from Mateos et al. [34]. The results were expressed in mg/kg.

Volatile compounds

The extraction of the volatile compounds was carried out by headspace solid-phase microextraction (HS-SPME)

according to Vichi et al. [35] with some modifications: the sample (2 g) was conditioned during 10 min in a silicone bath at 40 °C under magnetic stirring. The analysis was performed by GC–MS on an Agilent GC 6890 N equipped with a split–splitless injector, coupled to a quadrupole mass spectrometer 5973 (Agilent Technology, Palo Alto, USA). The separation of compounds was performed by a column Supelcowax-10 (60 m × 0.25 mm i.d., 0.25 µm film thickness), from Supelco Ltd (Bellefonte, PA, USA). The GC oven temperature was held at 40 °C for 10 min, then increased to 150 °C at 3 °C/min, then to 250 °C at 15 °C/min, holding 5 min at 250 °C. The carrier gas was helium, with a flow rate of 1.5 mL/min. Ion source and transfer line temperatures were 200 °C and 275 °C, respectively. Electron impact mass spectra were recorded at 70 eV in the *m/z* range 35–300, 2 scan/s. Volatile compounds were identified by comparing their mass spectral data with those of the Wiley 6 mass spectra library and by comparison with reference compounds. Semi-quantification was done by spiking the samples with 10 µL of IS (4-methyl-2-pentanol, 0.2 mg in 1 mL sunflower oil). The results were expressed as mg of 4-methyl-2-pentanol (IS) per kg of oil.

Statistical analysis

To assess the effect of the studied storage conditions on the measured parameters, a three-way ANOVA ($n = 96$) was applied. To make analytical data comparable, the values of phenolic compounds, volatile compounds and sensory attributes were percent normalized. Normalization of each parameter was done using as a base value the maximum value among all the samples of each oil. To evaluate the impact of freezing on the quality of the oils after thawing and storing at RT, the evolution of the measured parameters was expressed as the difference between their values at 0 and 6 months and a two-way ANOVA ($n = 24$) was carried out.

In all the cases, SPPS Statistics (v 25, IBM, Armonk, NY, USA) was used. *p*-Values lower or equal to 0.05 were considered significant and Scheffé test was applied to evaluate statistical differences between the mean values when the effect of the main factors was significant.

Results and discussion

The results of the initial characterization of the oils showed that the 4 oils belonged to the EVOO category according to EU regulations (Supplementary material S3). Indeed, the values of the trade quality parameters were notably above (median of positive attributes) or below (acidity, PV and extinction coefficients) the limits established for EVOO, showing that the oils were of the highest quality (premium) and, thus, fit for the research.

As expected, the values of FA composition, OSI, phenolic and volatile compounds, as well as the sensory profile, varied among the oils according to variety and olive maturation, which is in agreement with the previous knowledge [36–41].

Effect of headspace composition

The headspace composition (air, that is O, vs N) showed no influence on any of the studied parameters (Table 1 and Supplementary material S4). This was an unexpected result in disagreement with previous knowledge. As stated in the Materials and Methods section, the free oxygen headspace (N samples) was achieved by streaming nitrogen gas before capping the bottles. A hypothesis that would explain the observed lack of significant differences between the two headspace compositions, especially regarding oxidation parameters, could be that a certain amount of residual oxygen remained in the headspace or dissolved in the oil after applying the stream of nitrogen. This could be enough so that the oxidation rate of the oils would be independent of the oxygen concentration, as reported in the literature [42]. Thus, it would be necessary to evaluate other methods to modify the headspace composition while suitable for their application under the current conditions of industrial production.

Effect of storage temperature

Trade quality indices

Acidity (FFA%) values slightly increased during storage, being significantly higher in the RT oils (Table 1), showing that storage at low temperatures (R, S and F) reduces TAG hydrolysis. These results agree with previous similar studies [12, 13] and can be explained by the low water content of the oils used in this study, which ranged between 0.056 and 0.071 (Supplementary material S3). Low water content limits hydrolysis reactions and makes acidity not a critical parameter during storage of filtered oils.

PV and K_{232} values increased according to the temperature, showing a progressive increment of the primary oxidation. Significant differences were found between the three storage temperatures (Table 1): as expected, RT oils showed the highest values, followed by R oils, and lastly by the oils stored at − 20 °C (S and F oils). At RT, PV and K_{232} values linearly increased, exceeding in less than 24 months the limit fixed by EU legislation for EVOO (Fig. 1).

Storage temperature also affected K_{268} values (Table 1); while storage at low temperatures (R, S, F) maintained the initial values, storage at RT resulted in an increment of K_{268} up to the upper limit for EVOO category (Fig. 1). These results confirm the effect of low storage temperature (R

Table 1 Effect of headspace composition, storage temperature, freezing method and storage time on the quality and composition parameters of 4 premium EVOO

	Fresh[a]	Headspace composition			Temperature and freezing method					Time (months)			
		O[b]	N[b]	s.e.	RT[b]	R[b]	S[b]	F[b]	s.e.	6[b]	12[b]	24[b]	s.e.
FFA%[c]	0.080	0.089	0.087	0.001	0.100[a]	0.085[b]	0.083[b]	0.084[b]	0.002	0.084[b]	0.091[a]	0.090[a]	0.002
PV (meq O_2/kg)	3.6	8.7	8.5	0.470	18.0[a]	7.7[b]	4.3[c]	4.4[c]	0.665	6.1[b]	7.7[b]	12.1[a]	0.576
K_{232}	1.57	2.17	2.15	0.068	3.34[a]	1.99[b]	1.67[b]	1.64[b]	0.096	1.89[b]	2.05[b]	2.54[a]	0.083
K_{268}	0.13	0.14	0.14	0.002	0.18[a]	0.13[b]	0.12[c]	0.12[c]	0.002	0.13[b]	0.13[b]	0.15[a]	0.002
Phenolic compounds													
α-Tocopherol (%)[d]	100.0	53.6	53.6	0.613	48.2[b]	54.4[a]	54.1[a]	56.1[a]	0.867	60.62[a]	50.9[b]	48.1[c]	0.751
∑SEC (%)	99.1	67.1	68.4	1.519	58.8[b]	72.2[a]	70.4[a]	69.7[a]	2.148	86.5[a]	67.0[b]	49.8[c]	1.860
∑OL (%)	99.0	64.1	65.6	1.638	52.9[b]	68.7[a]	70.2[a]	67.6[a]	2.316	84.1[a]	64.1[b]	46.3[c]	2.006
∑LIG (%)	97.6	70.7	71.6	1.377	67.0[b]	76.5[a]	69.5[a,b]	71.9[a,b]	1.948	88.6[a]	70.4[b]	54.5[c]	1.687
SEC hydrolysis (%)	24.6	38.5	37.5	0.935	61.1[a]	34.0[b]	24.5[c]	32.3[c]	1.323	26.6[c]	35.5[b]	51.9[a]	1.146
HTy (%)	74.4	63.3	61.6	1.311	84.0[a]	64.4[b]	41.2[c]	60.1[b]	1.853	62.0[a,b]	65.7[a]	59.5[b]	1.605
Ty (%)	56.5	60.3	59.5	1.442	84.5[a]	57.1[b]	45.3[c]	52.8[b],c	2.039	58.4	59.6	61.7	1.766
SEC oxidation (%)	3.9	17.6	17.3	0.639	49.5[a]	9.4[b]	5.2[c]	5.6[c]	0.903	7.2[c]	12.9[b]	32.2[a]	0.782
OSI (h)	17.8	15.6	15.6	1.002	9.8[b]	15.6[a,b]	18.5[a]	18.5[a]	1.418	-	16.5	14.6	1.002
Volatile compounds													
LOX compounds													
∑PD (%)	73.8	81.6	81.2	0.987	79.2[b]	79.3[b]	78.7[b]	88.3[a]	1.395	78.4[b]	78.3[b]	87.5[a]	1.208
1-Penten-3-ol (%)	74.3	80.8	80.2	1.514	86.6[a]	85.8[a]	74.2[b]	75.3[b]	2.141	81.3[a,b]	83.7[a]	76.5[b]	1.854
trans-2-Pentenol (%)	78.4	81.2	80.5	1.036	77.9[b]	81.9[a,b]	79.0[a,b]	84.5[a]	1.465	76.8[b]	88.0[a]	77.8[b]	1.269
cis-2-Pentenol (%)	75.4	86.9	86.4	0.804	83.1[b]	88.6[a]	86.0[a,b]	89.0[a]	1.137	84.4[b]	89.1[a]	86.5[a,b]	0.984
1-Penten-3-one (%)	81.0	79.6	78.5	0.817	70.4[c]	85.9[a]	77.8[b]	82.2[a,b]	1.156	82.1[b]	87.7[a]	67.3[c]	1.001
Hexanal (%)	31.7	41.6	41.3	1.376	62.2[a]	34.1[b]	31.6[b]	38.0[b]	1.946	33.6[b]	36.0[b]	54.9[a]	1.685
cis-3-Hexenal (%)	91.9	66.3	66.5	1.224	38.6 d	61.8[c]	89.4[a]	75.9[b]	1.731	77.7[a]	65.5[b]	56.1[c]	1.499
trans-2-Hexenal (%)	82.9	83.4	83.0	1.164	76.8[b]	82.7[a,b]	84.8[a]	88.4[a]	1.646	83.1[a,b]	87.5[a]	79.0[b]	1.425
1-Hexanol (%)	74.6	84.6	84.5	1.088	82.7[b]	83.6[b]	81.9[b]	90.0[a]	1.539	81.3[b]	85.7[a,b]	86.8[a]	1.333
cis-3-Hexenol (%)	77.8	85.2	84.9	0.967	81.9[b]	85.3[a,b]	83.3[b]	89.8[a]	1.368	83.9	87.4	83.9	1.185
trans-2-Hexenol (%)	52.9	68.2	67.6	2.529	76.8[a]	64.5[a,b]	61.6[b]	68.7[a,b]	3.577	59.7[b]	67.3[a,b]	76.8[a]	3.098
Hexyl acetate (%)	66.4	76.0	75.1	1.558	71.3[b]	74.0[b]	72.6[b]	84.3[a]	2.203	68.9[b]	74.0[b]	83.8[a]	1.908
cis-3-Hexenyl acetate (%)	67.3	79.1	78.2	1.353	76.1[b]	76.5[b]	75.5[b]	86.5[a]	1.914	72.1[c]	78.2[b]	85.7[a]	1.658
∑OX products (%)	23.4	34.4	34.1	0.628	57.8[a]	27.8[b]	23.2[c]	28.3[b]	0.888	26.0[c]	29.5[b]	47.3[a]	0.769
Sensory analysis													
Positive attributes[e]													
Fruity (%)	98.1	89.5	89.0	0.838	85.8[b]	90.6[a,b]	93.1[a]	87.3[b]	1.186	93.5[a]	88.7[b]	85.4[b]	1.027
Bitter (%)	83.1	86.5	86.9	0.622	81.9[b]	88.4[a]	89.6[a]	86.9[a]	0.880	93.5[a]	84.6[b]	82.0[b]	0.762
Pungent (%)	88.5	90.7	90.5	0.469	88.1[b]	90.9[a]	93.1[a]	90.5[a,b]	0.664	95.9[a]	91.0[b]	85.1[c]	0.575
Green (%)	94.4	85.3	84.4	1.022	81.2[b]	86.6[a,b]	88.1[a]	83.5[a,b]	1.445	90.4[a]	84.6[b]	79.6[c]	1.251
Astringency (%)	83.2	81.5	81.5	1.146	76.1[b]	83.4[a]	85.5[a]	81.1[a,b]	1.621	92.5[a]	79.9[b]	72.2[c]	1.404
Almond (%)	83.4	81.1	82.6	1.489	78.1[b]	81.9[a,b]	86.7[a]	80.8[a,b]	2.106	86.2[a]	80.6[a,b]	78.8[b]	1.823
Secondary sensory attributes[f]													
Green fruity (%)	91.6	89.5	89.6	1.677	82.0[b]	93.0[a,b]	93.5[a]	89.7[a,b]	2.372	94.9[a]	86.9[b]	86.8[b]	2.054
Ripe fruity (%)	41.0	40.2	45.8	3.178	57.3[a]	35.7[b]	32.0[b]	47.0[a,b]	4.494	29.4[b]	48.4[a]	51.2[a]	3.892
Tomato leaf (%)	64.0	55.0	58.7	3.066	45.2[a]	62.6[a]	61.0[a]	58.8[a]	4.335	74.7[a]	40.4[b]	55.5[c]	3.755
Artichoke (%)	67.7	74.9	73.2	2.285	59.1[b]	82.7[a]	80.2[a]	74.1[a]	3.232	73.2	76.1	72.8	2.799
Ripe banana (%)	35.9	43.3	42.6	3.009	37.8[a]	48.9[a]	37.9[a]	47.2[a]	4.255	24.9[b]	49.7[a]	54.3[a]	3.685

s.e. standard error, *O* air, *N* nitrogen, *RT* oils stored at room temperature, *R* oils stored at 4 °C, *S* oils frozen at − 20 °C and stored at − 20 °C, *F* oils frozen with liquid nitrogen and stored at − 20 °C, *FFA* free fatty acids, *PV* peroxide value, *SEC* secoiridoid derivatives, *OL* oleuropein derivatives, *LIG* ligstroside derivatives, *Hty* hydroxytyrosol, *Ty* tyrosol, *OSI* oxidative stability index, *LOX* lipoxygenase pathway, *PD* pentene dimer, ∑OX products, sum of volatile oxidation products (octane, 1-octene, pentanal, hexanal, heptanal, 1-pentanol, octanal, 2-heptenal, nona-

Table 1 (continued)

nal, 2,4-heptadienal, compound not identified 1)

[a]Mean values of the 4 oils at the beginning of the study

[b]Pooled means from three-way ANOVA ($n=96$ corresponding to 2 headspace compositions × 4 temperature and freezing methods × 3 storage times × 4 different oils), except for OSI ($n=64$ corresponding to 2 headspace compositions × 4 temperature and freezing methods × 2 storage times × 4 different oils)

[c]The means within each row for each factor, labeled by different letters, are significantly different ($p \leq 0.05$). The interaction between time and temperature and freezing method is statistically significant ($p \leq 0.05$) in all the studied parameters except for OSI, 1-penten-3-ol, *trans*-2-hexenal, *trans*-2-pentenol, *trans*-2-hexenol, almond, green fruity, tomato leaf and ripe banana

[d]The values of α-tocopherol, polar phenolic compounds, volatile compounds and sensory attributes are percent normalized, using as a base value the maximum value among all the samples of each oil

[e]Measured as the median of intensity (0–10 scale), then percent normalized

[f]Measured as the % of panelists perceiving the attribute, then percent normalized

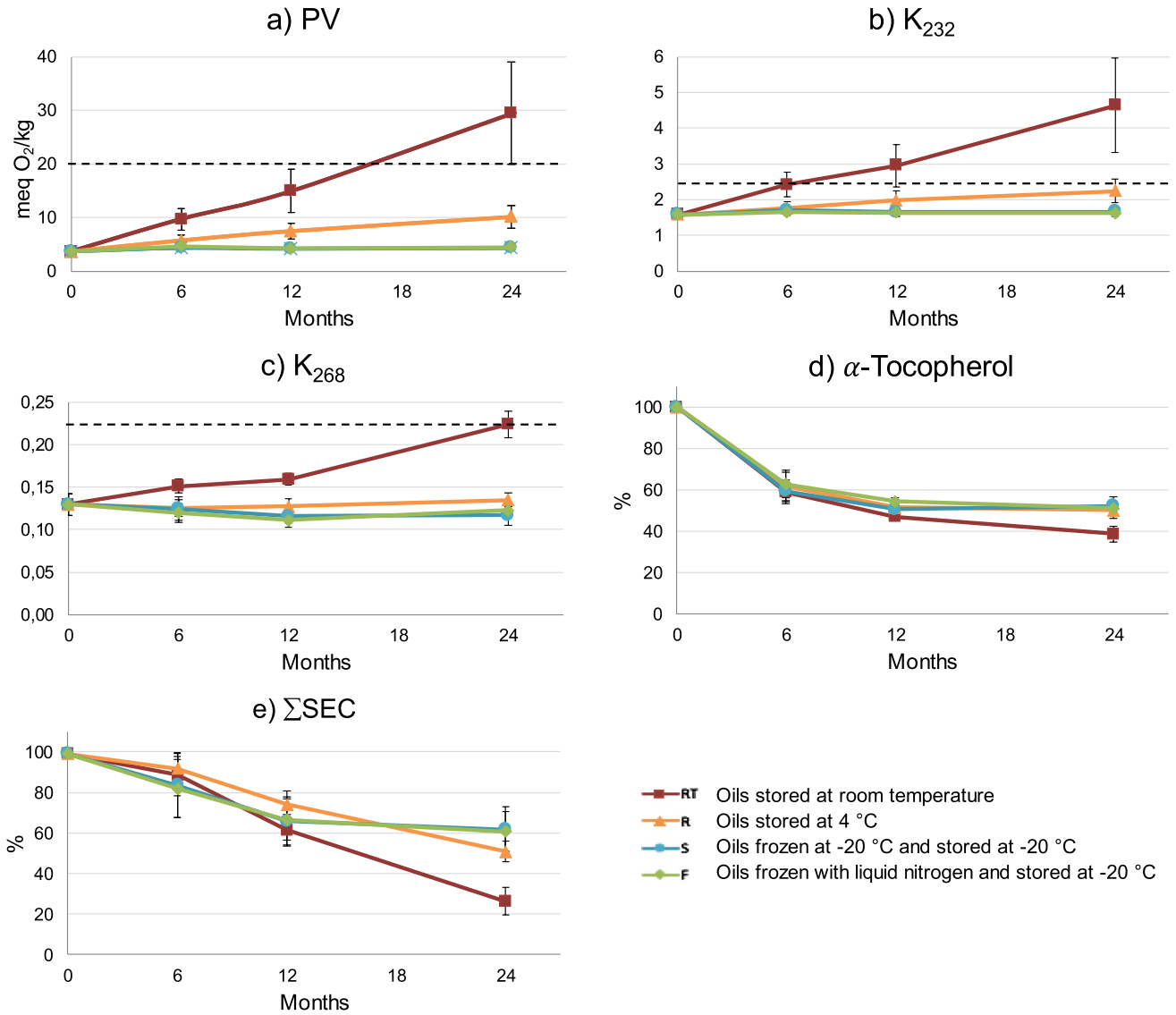

Fig. 1 Interaction plot between the factor time and the factor storage temperature and freezing method for peroxide value (**a**), K_{232} (**b**), K_{268} (**c**), α-tocopherol content (**d**) and secoiridoid derivatives content (∑SEC, **e**). Error bars correspond to the standard deviation. α-Tocopherol content and secoiridoid derivatives content values are percent normalized. The dotted line signals the established limit for the EVOO category according to EC Regulations

and − 20 °C) on the reduction of the lipid oxidation rate, as reported in previous studies [13, 14].

Phenolic compounds

The effect of storage temperature on the main lipophilic (α-tocopherol) and polar (SEC) phenolic compounds was similar, with a significantly higher loss of these compounds in RT oils (Table 1). It is worth noting that their content decreased during the first 12 months regardless of storage temperature (Fig. 1). Then, frozen (S and F) oils maintained their content while the decrease continued in the RT oils and, to a lesser extent, in the R oils. This reduction was more pronounced in SEC than in α-tocopherol (Fig. 1), likely due to the greater contribution of oleuropein derivatives (OL) to oxidative stability of oils compared to α-tocopherol [43, 44]. The decrease of SEC at RT and 4 °C was, to a greater extent, due to a loss of OL, as shown by the lower values of OL in contrast to ligstroside derivatives (LIG) in these two conditions (Table 1). This result highlights the greater antioxidant power of OL compared to LIG [45].

During storage, SEC content may decrease due to both their antioxidant role and hydrolysis reactions, which increase the levels of hydroxytyrosol (HTy) and tyrosol (Ty) [19, 46–48]. SEC hydrolysis (calculated as the % of HTy and Ty on the total SEC, including the oxidized SEC) and oxidation rate (calculated as the % of oxidized forms on the total SEC) were significantly greater in RT oils, followed by R oils, while oils stored at − 20 °C presented the lowest values for both rates (Table 1). In view of these results, it can be concluded that SEC are better preserved under cold storage, particularly at − 20 °C, which slows down oxidation and hydrolytic reactions.

Oxidative stability index (OSI)

The OSI was also affected by storage temperature. RT oils showed the greatest decrease in this parameter (Table 1), which can be explained by the accumulation of peroxides, which are prooxidant, and the decrease of antioxidants (α-tocopherol and SEC) in these oils.

Volatile compounds

Storage temperature influenced all the volatile compounds generated through the lipoxygenase (LOX) pathway (Table 1). These compounds, which represented between 82.2% and 91.7% of the initial volatile fraction of the PEVOO samples (Supplementary material S3), are associated with EVOO positive aroma [49].

An increment of hexanal, 1-penten-3-ol and *trans*-2-hexenol was observed as the storage temperature increased (Table 1). These compounds have already been related to storage temperature: an increment of 1-penten-3-ol has been observed in an accelerated storage study [35], an increase of *trans*-2-hexenol has been reported during storage at RT [20], while it is well known that hexanal can be formed not only through the LOX pathway but also by autoxidation [50]. Volatile oxidation products not related to LOX also increased over time, being significantly influenced by storage temperature (Table 1), as expected. This trend agreed with the results of the oxidation parameters PV, K_{232} and K_{268} (Fig. 1). The behavior of single oxidation compounds is available in Supplementary material S4.

Conversely, the content of *trans*-2-hexenal, 1-penten-3-one and *cis*-3-hexenal decreased as the temperature storage increased (Table 1). These compounds are of special interest because they are considered the main contributors of the positive aroma of EVOO [3, 39]. *trans*-2-Hexenal, which is related to the positive sensory characteristics of almond and green olive fruits [51], was the most abundant LOX compound in the Arbequina oils (Supplementary material S3) and showed a slight decrease over time, which was more marked at RT (Table 1). This result agrees with previous studies [20, 22, 25]. 1-Penten-3-one, a compound related to green attributes and with a very low odor threshold [52, 53], showed a similar trend (Table 1). Its content was maintained throughout cold storage (R, S, F), whereas RT oils experienced a loss of 28.7% at 24 months (Fig. 2). In the case of *cis*-3-hexenal, the effect of storage temperature was more pronounced, as shown by the results of the Scheffé test, which differentiated each storage temperature and even the two freezing methods (Table 1). This compound, related to green attributes, has a great impact on EVOO aroma due to its especially low odor threshold [52, 53]. After 24 months of RT storage, its content was reduced by 67.7%, while only 2.4% was lost by S oils (Fig. 2). This behavior was also observed in the two isomers of 2,4-hexadienal (Supplementary material S4), which are related to green aromas such as artichoke and cut grass [24, 39, 54]. Although these compounds could be originated by both the LOX pathway and autoxidation [55], they have been associated with high-quality EVOO by several studies [56–58].

These results show that the compounds that contribute the most to the positive aroma of olive oils are the most susceptible to storage temperature, being storage at − 20 °C the best condition to preserve their initial levels, hence the sensory quality of PEVOO.

Sensory assessment

Sensory defect was only perceived in 3 samples out of the 96 studied along the conservation assay, corresponding to RT oils stored for 24 months, which presented a certain amount of rancid defect.

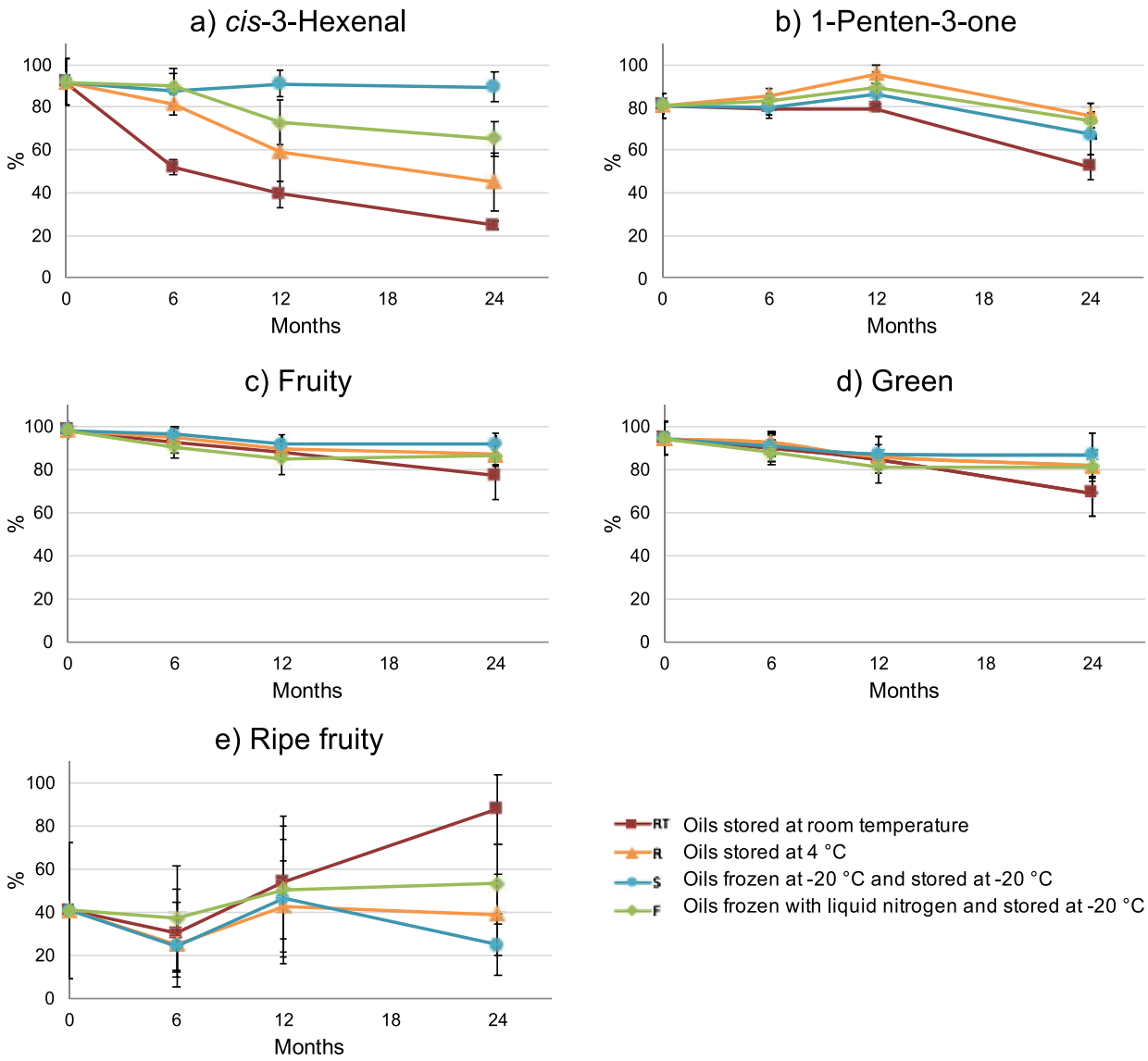

Fig. 2 Interaction plot between the factor time and the factor storage temperature and freezing method for *cis*-3-hexenal (**a**), 1-penten-3-one (**b**), the sensory attributes fruity (**c**) and green (**d**), and the secondary sensory note ripe fruity (**e**). Error bars correspond to the standard deviation. All values are percent normalized

In agreement with the behavior of volatile compounds, most of the positive sensory attributes were affected by storage temperature (Table 1). RT oils showed the biggest decrease in the intensity of these attributes, in agreement with other studies [4, 25], while oils stored at low temperatures (R, S, F) presented a slight decrease, which was almost negligible in the case of S oils. This result suggests that cold storage could effectively maintain the distinctive sensory quality of PEVOO. The decrease of bitterness, astringency and pungency could be ascribed to the observed loss of some polar phenolic compounds (Table 1, Fig. 1), since previous literature has linked their presence to these attributes [59–61]. The reduction of intensity of the attributes fruity and green could be mainly explained by the observed reduction in the content of the volatile compounds *cis*-3-hexenal and 1-penten-3-one (Table 1, Fig. 2), evidencing the important role of these two volatile compounds in the positive aroma of EVOO.

Regarding secondary sensory attributes, from the 21 descriptors used by the panelists in the open generic profile method, only 5 showed an effect of temperature and freezing speed and/or its interaction with time (Table 1). RT oils showed a significant decrease in the perception of green fruity and artichoke, which had been perceived by

most of the panelists at the beginning of the study (Supplementary material S3). Thus, the main green secondary notes that initially characterized the oils were largely lost at RT, while cold storage preserved to a greater extent the original sensory profile. On the other hand, ripe fruity showed a significant increase influenced by time and storage temperature (Table 1). The greatest increase was in the RT oils, especially after 24 months of storage (Fig. 2). This is in agreement with Sinesio et al. [24], who suggested that an increase of ripe fruity could be a consequence of the decrease of other attributes or that it could be pointing the beginning of the oil's deterioration. Besides, the same authors linked this odor note to the presence of C_5 and C_6 alcohols, especially *trans*-2-hexenol and 1-pentanol, two compounds that significantly increased at RT in our study (Table 1, Supplementary material S4).

In the open generic profile method, panelists use their own list of terms for the sensory description of the oils. Since there was not a consensus vocabulary among the panelists, a variability in the use of descriptors related to green and ripe aromas was observed. Thus, to avoid any bias in the statistical analysis and to better visualize the effect of the studied conditions on the secondary sensory profile of the oils, these attributes were classified into two groups: green and ripe notes. Figure 3 shows the full profile of secondary sensory attributes of the Arbequina and Picual oils at the beginning and at the end of the study at RT, R, S and F. A green and a red color palette were used for the green and ripe attributes, respectively. The remaining attributes were assigned different colors. At RT, a decrease of the perception (% of panelists) of the main initial green notes was accompanied by an increase of the ripe notes as well as by the % of panelists perceiving them. This shift from green to ripe aromas was less marked in the Picual oils than in the Arbequina oils. Conversely, a minor change was observed in R and F oils, while S oils presented the least altered sensory profile. In summary, storage at lower temperatures slowed down the shift from green to ripe notes observed at RT, preserving the secondary sensory profile of PEVOO.

Effect of freezing speed

Results showed that the two freezing speeds did not influence most of the quality and oxidative parameters (Table 1). That is, the F oils, in which the phase transition from liquid to solid occurred very rapidly, did not show an improvement in their oxidative quality compared to the S oils, in which the phase transition took place at a slower pace. In this sense, the results are in disagreement with those of Calligaris et al. [16], where an increase of the lipid oxidation rate during the phase transition was observed, explained by an increase of unsaturated FA and a decrease of polyphenols in the liquid phase, generated by the selective crystallization of TAG. Thus, the results of the present study indicate that, at least at the evaluated freezing speeds, the selective crystallization of TAG that takes place during the phase transition does not translate into a higher lipid oxidation rate. This is in agreement with Jansen and Birch [17], who concluded that the

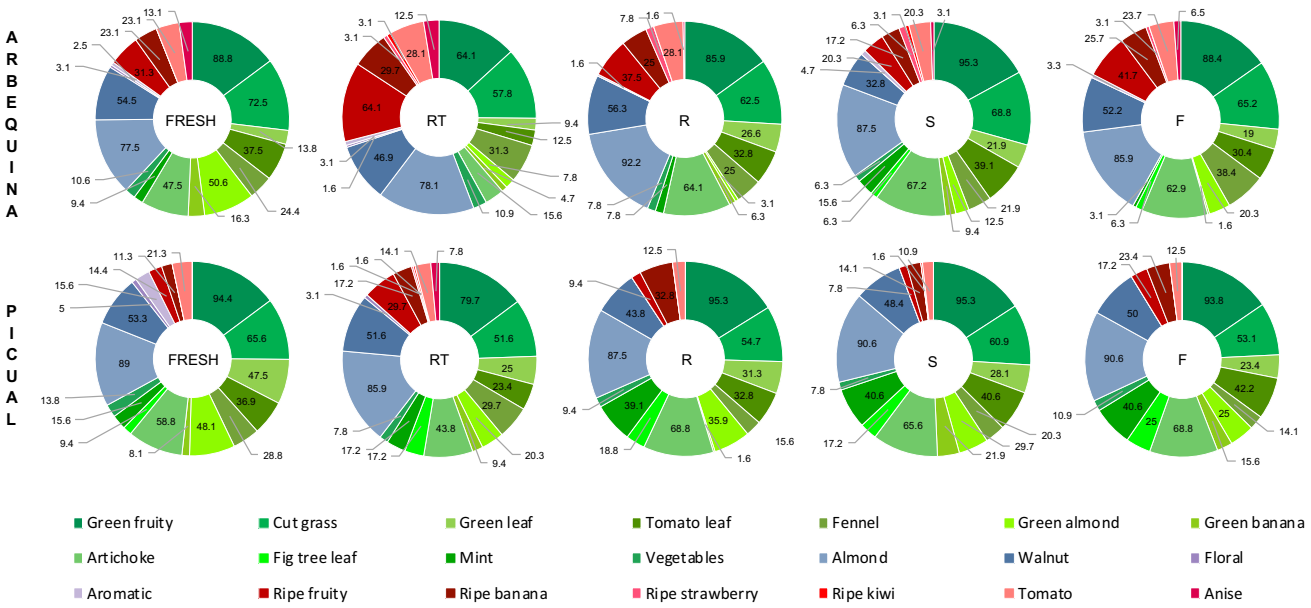

Fig. 3 Full profile of secondary sensory attributes of the Arbequina and Picual oils at the beginning of the study (fresh) and after 24 months at room temperature (RT), 4 °C (R), and − 20 °C (S, frozen at − 20 °C; F, frozen with liquid nitrogen). Secondary sensory attributes are expressed as % of panelists perceiving the attribute. The green and the red color palette correspond to attributes associated with green and ripe notes, respectively

liquid fraction of partially crystallized olive oils is not more susceptible to oxidation.

On the other hand, an effect of freezing speed was observed on some volatiles and sensory attributes. Although F oils experienced a significant increase in some LOX volatiles (Table 1), it did not translate into a better sensory profile than the S oils. In fact, the intensity of some positive attributes, such as fruity, was significantly lower in the F oils (Table 1). Lastly, a great and unexpected increase of 6-methyl-5-hepten-2-one was observed in the F oils (Supplementary material S4). This compound, which is related to fruity and apple aromas [62], has been reported to increase in particular during RT storage [25, 63]. This increase was also observed in the present study in the RT and R oils, but it was greater in the F oils (Supplementary material S4), suggesting that the fast-freezing process played a key role in its formation.

These results indicate that fast-freezing does not improve the preservation of PEVOO quality during storage compared to a slower freezing, but rather worsens the sensory profile. The mechanisms underlying this phenomenon need to be elucidated by further research.

Impact of freezing on the quality of PEVOO after thawing and storing at RT

Results of the ANOVA test comparing the evolution of the main parameters in the thawed (ST, oils frozen at − 20 °C, stored at − 20 °C for 12 months and thawed; FT, oils frozen with liquid nitrogen, stored at − 20 °C for 12 months and thawed) and control oils (oils at the beginning of the study, time 0, not frozen nor thawed) after 6 months of RT storage are shown in Supplementary material S5. There was no significant difference regarding the physicochemical quality parameters. Control oils showed a greater significant loss of α-tocopherol, which can be explained by the fact that this compound decreased around 40% regardless of the storage temperature during the first 6 months of storage and then its level stabilized (Fig. 1). The content of OL and LIG slightly increased in the thawed oils, while it decreased in the control oils (Supplementary material S5). However, the levels of these compounds were similar in all the oils (Supplementary material S6). Moreover, the similar increase in Hty, Ty, and oxidized forms of OL and LIG in both oils indicates that a similar level of hydrolysis and oxidation of these compounds occurred. Also, no significant difference regarding volatile compounds related to oxidation was found. Likewise, the absence of significant differences regarding LOX compounds as well as the main positive sensory attributes denotes that the evolution of the sensory quality was similar in thawed and control oils.

Overall, these findings clearly indicate that thawing PEVOO did not induce any increment in oxidative degradation nor in sensory deterioration during subsequent RT storage.

Conclusions

The application of a nitrogen stream to reduce the oxygen in the headspace did not have a significant effect on the quality of PEVOO during storage. Thus, other methods to create oxygen free conditions need to be assessed. On the other hand, almost all tested parameters were significantly affected by storage temperature. While its effect was almost negligible on FFA%, a decrease of the lipid oxidation rate as the storage temperature decreased occurred, which translated in a maintenance of the quality parameters related to oxidation (PV, K_{232} and K_{268}) in the oils stored at − 20 °C. This lower oxidation rate at low temperatures had an influence on the main antioxidants: at the end of the study, α-tocopherol and SEC content was higher in the oils stored at − 20 °C and 4 °C. Consequently, and also due to their low PV, PEVOO stored at − 20 °C maintained their OSI values throughout storage. Regarding sensory quality, cold storage (4 and − 20 °C) showed a better maintenance of all the positive attributes and the content of some volatile compounds associated with fruity and green sensory notes, such as *cis*-3-hexenal and 1-penten-3-one. This translated into a longer preservation of the distinctive secondary sensory notes of the oils, by slowing down the shift from green to ripe notes observed at RT.

Overall, we can conclude that cold storage significantly improved the maintenance of compositional and sensory characteristics of premium EVOO, offering a valid option for the conservation of EVOO with outstanding features and high value. In particular, storage at − 20 °C maintains the initial quality of PEVOO, especially their sensory profile, which would otherwise degrade at RT. Moreover, we can conclude that fast-freezing with liquid nitrogen does not preserve better the quality of PEVOO than slow-freezing at − 20 °C, and that freezing storage does not compromise PEVOO quality after thawing and storing at RT. Lastly, storage at 4 °C would be a less costly option to maintain the sensory quality of PEVOO, since the difference observed in some parameters compared to storage at − 20 °C did not have a significant effect at the sensory level.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00217-022-04078-9.

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Author contributions Conceptualization: SV; FG; Methodology: SV, FG; Formal analysis and investigation: AD-B, AR, AN, AT; Writing—original draft preparation: AD-B, SV, FG; Writing—review and editing: AR, AT; Funding acquisition resources: FG; Supervision: SV, FG.

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Declarations

Conflict of 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.

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