

Subzero Temperature Storage to Preserve the Quality Attributes of Veiled Virgin Olive Oil

Anna Díez-Betriu ^{1,2}, Agustí Romero ³, Antonia Ninot ³, Alba Tres ^{1,2}, Stefania Vichi ^{1,2,*} and Francesc Guardiola ^{1,2}

¹ Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Campus de l'Alimentació de Torribera, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, 08921 Santa Coloma de Gramenet, Spain; annadiez@ub.edu (A.D.-B.); atres@ub.edu (A.T.); stefaniavichi@ub.edu; fguardiola@ub.edu (F.G.)

² Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona (UB), 08921 Santa Coloma de Gramenet, Spain

³ Institute of Agrifood Research and Technology (IRTA), Mas Bové Ctra. Reus-El Morell km 3.8, 43120 Constantí, Spain; agusti.romero@irta.cat (A.R.); antonia.ninot@irta.cat (A.N.)

* Correspondence: stefaniavichi@ub.edu

Table S1. Initial characterization (quality indices, fatty acid profile, moisture, oxidative stability index, tocopherols, polar phenolic compounds, volatile compounds and sensory analysis) of the filtered (FO) and unfiltered (UO) oils in the study. Values correspond to mean values of two experimental replicates (all parameters were further determined in analytical duplicate, except for K₂₃₂, K₂₆₈ and ΔK that were determined in triplicate).

	FO	UO
FFA%	0.067	0.073
PV (meq O ₂ /kg)	2.0	2.1
K ₂₃₂	1.37	1.35
K ₂₆₈	0.13	0.12
ΔK	0.008	0.007
Md	0.0	0.8
Mf	6.6	5.1
Palmitic acid (C16:0) (%)	13.3	13.4
Oleic acid (C18:1 n-9) (%)	77.1	76.9
Linoleic acid (C18:2 n-6) (%)	3.81	3.79
Linolenic acid (C18:3 n-3) (%)	0.76	0.76
SFA (%)	16.7	16.9
Oleic/linoleic ratio	20.2	20.3
Moisture and volatile matter (%)	0.036	0.376
OSI (h)	23.3	25.3
<u>Polar and lipophilic phenolic compounds (mg/kg)</u>		
α-Tocopherol	504.0	444.5

	FO	UO
β -Tocopherol	3.32	2.95
γ -Tocopherol	10.6	9.5
Σ Polar phenolic compounds	426.3	370.8
Hydroxytyrosol (3,4-DHPEA)	1.17	7.24
Tyrosol (<i>p</i> -HPEA)	2.61	4.67
3,4-DHPEA-EDA, oxidized form	0.04	0.04
3,4-DHPEA-EDA	56.5	42.4
3,4-DHPEA-EDA, oxidized form	0.04	0.09
Oleuropein	57.2	37.8
3,4-DHPEA-EA, dialdehyde form	69.0	44.3
Tyrosyl acetate	6.29	5.10
<i>p</i> -HPEA-EDA, oxidized form	0.00	0.00
<i>p</i> -HPEA-EDA	70.2	65.7
Pinoresinol + 1-Acetoxypinoresinol	2.53	3.03
<i>p</i> -HPEA-EDA, oxidized form	0.00	0.00
3,4-HPEA-EA, oxidized aldehyde and hydroxylic form	0.42	0.41
3,4-HPEA-EA, aldehyde and hydroxylic form	65.9	64.1
<i>p</i> -HPEA-EA, oxidized aldehyde and hydroxylic form	2.52	5.34
<i>p</i> -HPEA-EDA, aldehyde and hydroxylic form	86.4	83.7
Luteolin	3.68	5.16
Apigenin	1.75	1.82
EFSA CLAIM	409.0	349.8
Σ SEC	405.2	337.9
Σ OL	248.6	188.6
Σ LIG	156.6	149.3
Σ ox SEC	3.02	5.88
<u>Volatile compounds (mg IS/kg oil)</u>		
LOX C₅ compounds		
PD1	0.18	0.18
PD2	0.10	0.10
PD3	0.71	0.70
PD4	0.49	0.54
PD5	0.16	0.17
PD6	0.26	0.27
PD7	0.24	0.29
1-penten-3-ol	0.30	0.23
<i>trans</i> -2-pentenol	0.09	0.05
<i>cis</i> -2-pentenol	0.53	0.40
<i>trans</i> -2-pentenal	0.21	0.09

	FO	UO
1-penten-3-one	1.18	0.28
LOX C₆ aldehydes		
Hexanal	0.57	0.36
<i>cis</i> -3-Hexenal	5.64	1.37
<i>trans</i> -2-Hexenal	3.07	1.31
LOX C₆ alcohols		
1-Hexanol	0.30	0.88
<i>trans</i> -3-Hexenol	0.05	0.21
<i>cis</i> -3-Hexenol	1.37	4.30
<i>trans</i> -2-Hexenol	0.03	0.27
LOX C₆ esters		
Hexyl acetate	0.05	0.06
<i>cis</i> -3-Hexenyl acetate	0.56	0.61
Oxidation products		
Octane	0.04	0.04
1-Octene	0.00	0.00
Pentanal	0.03	0.02
Heptanal	0.00	0.00
1-Pentanol	0.01	0.11
Octanal	0.00	0.00
2-Heptenal	0.00	0.00
Nonanal	0.01	0.01
2,4-Heptadienal	0.00	0.00
Others		
Methyl acetate	0.05	0.22
Ethyl acetate	0.11	1.59
2-Butanone	0.01	0.00
2-Methylbutanal	0.00	0.00
3-Methylbutanal	0.00	0.00
1-Methoxyhexane	0.13	0.12
3-Pentanol	0.01	0.02
3-Pentanone	0.17	0.56
<i>trans</i> -4,8-Dimethyl-1,3,7-nonatriene	0.02	0.02
6-Metil-5-hepten-2-one	0.00	0.00
<i>cis,trans</i> -2,4-Hexadienal	0.37	0.22
<i>trans,trans</i> -2,4-Hexadienal	0.79	0.53
2-ethyl Hexanol	0.01	0.01
α -Copaene	0.01	0.01
Benzaldehyde	0.02	0.02
ΣPD	2.14	2.25
ΣC₅ alcohols	0.92	0.69

	FO	UO
ΣC_5 ketones	1.18	0.28
ΣC_6 aldehydes	9.28	3.04
ΣC_6 alcohols	1.75	5.66
ΣC_6 esters	0.62	0.67
ΣLOX compounds	16.10	12.67
Σox VOC	0.11	0.20
Total volatile compounds	17.92	16.18
<u>Sensory analysis</u>		
Positive attributes¹		
Fruity	6.6	5.1
Bitter	4.9	4.4
Pungent	5.4	4.9
Apple	0.3	0.0
Ripe fruits	0.0	0.0
Green	4.3	3.3
Sweet	3.9	4.1
Astringency	3.0	2.6
Almond	2.7	1.9
Walnut	1.8	0.7
Other positive attributes	3.2	2.6
Global sensory score²	7.8	7.2
Complexity³	6.0	4.7
Secondary sensory attributes⁴		
Green fruity	95.8	79.2
Ripe fruity	8.3	29.2
Ripe banana	8.3	4.2
Ripe kiwi	8.3	4.2
Cut grass	54.2	50.0
Green leaf	29.2	29.2
Defects	0.0	4.0
Tomato leaf	50.0	20.8
Tomato	20.8	16.7
Almond	79.2	67.0
Walnut	66.8	50.3
Fennel	29.2	12.5
Green almond	54.2	29.2
Banana	16.7	4.2
Artichoke	50.0	50.0

	FO	UO
Floral	4.2	0.0
Aromatic	8.3	12.5
Anise	0.0	4.2
Fig tree leaf	0.0	12.5
Mint	16.7	20.8
Vegetables	12.5	4.2

¹ Median of intensity (0-10 scale); ² 0-9 scale, estimated by means of an algorithm based on the panels' outputs, according to Romero [1]. ³ Number of secondary sensory attributes perceived by more than 30% of the panelists; ⁴ Percentage of panelists perceiving the attribute.

Abbreviations: FFA, free fatty acids; PV, peroxide value; Md, Median of defects; Mf, Median of fruity notes; SFA, saturated fatty acids; OSI, oxidative stability index; ND, not detected; 3,4-DHPEA-EDA, dihaldehyde form of decarboxymethyl oleuropein aglycone; 3,4-DHPEA-EA, dialdehyde form of oleuropein aglycone; *p*-HPEA-EDA, dihaldehyde form of decarboxymethyl ligstroside aglycone; *p*-HPEA-EA, dialdehyde form of ligstroside aglycone; EFSA CLAIM, sum of hydroxytyrosol, tyrosol and derivatives; Σ SEC, sum of secoiridoid derivatives; Σ OL, sum of oleuropein derivatives; Σ LIG, sum of ligstroside derivatives; Σ ox SEC, sum of oxidized forms of secoiridoid derivatives; IS, internal standard; LOX, lipoxygenase pathway; PD, pentene dimer; Σ ox VOC, sum of volatile oxidation products (octane, 1-octene, pentanal, heptanal, 1-pentanol, octanal, 2-heptenal, nonanal, 2,4-heptadienal).

	FI	SC	T	FI*SC	SC*T	FI*T	FI*SC*T
6-Methyl-3-hepten-2-one	0.016	<0.001	<0.001	0.082	0.001	0.180	0.788
$\Sigma_{\text{ox VOC}}$	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.064
Sensory assessment							
Fruity	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	<0.001
Bitter	<0.001	<0.001	<0.001	<0.001	<0.001	0.007	<0.001
Pungent	<0.001	<0.001	<0.001	<0.001	<0.001	0.130	<0.001
Green	<0.001	<0.001	<0.001	<0.001	<0.001	0.014	0.001
Astringency	<0.001	<0.001	<0.001	<0.001	0.002	0.007	0.007

Abbreviations: FFA, free fatty acids; PV, peroxide value; OSI, oxidative stability index; Σ_{SEC} , sum of secoiridoid derivatives; Σ_{OL} , sum of oleuropein derivatives; Σ_{LIG} , sum of ligstroside derivatives; OX, oxidation rate of secoiridoid derivatives; $\Sigma_{\text{ox VOC}}$, sum of volatile oxidation products (heptanal, octane, octanal, 1-octene, 2-heptenal, 2,4-heptadienal, compound not identified 1, nonanal).

Table S3. Changes in physicochemical and sensory quality parameters (mean values) assessed in fresh (control) and thawed filtered (FO) oils after 6 months of storage at room temperature. Effect of thawing (TH), storage time (T) and the interaction between them (TH*T) (*p* values) assessed by two-way ANOVA.

	FO Control		FO Thawed		TH ²	T ²	TH*T ²
	CT ₀ ¹	CT ₆ ¹	THT ₀ ¹	THT ₆ ¹			
FFA (% oleic acid)	0.07	0.06	0.07	0.06	0.513	0.087	0.830
PV (meq O ₂ /kg)	2.0	4.5	1.8	4.8	0.358	<0.001	0.031
K ₂₃₂	1.37	1.65	1.38	1.65	0.597	<0.001	0.727
K ₂₆₈	0.13	0.13	0.11	0.13	0.006	0.017	0.005
Positive sensory attributes³							
Fruity	6.6	6.0	5.8	5.9	0.006	0.065	0.016
Bitter	4.9	5.2	4.9	4.9	0.197	0.128	0.109
Pungent	5.4	5.1	4.9	5.1	0.003	0.147	0.003
Green	4.3	3.8	4.0	3.8	0.071	0.010	0.103
Sweet	3.9	3.8	3.8	3.7	0.311	0.208	0.894
Astringency	3.0	2.9	2.8	3.0	0.749	0.716	0.204
Almond	2.7	2.5	2.3	2.5	0.071	0.916	0.151
Global sensory score⁴	7.8	7.5	7.5	7.6	0.021	0.040	0.007
Complexity⁵	7.0	8.0	7.5	9.0	0.251	0.089	0.678

¹ Results from two experimental replicates (all parameters were further determined in analytical duplicate, except for K₂₃₂ and K₂₆₈ that were determined in triplicate). ² *p* values of the main effects (TH, thawing and T, storage at room temperature) and their interaction (TH*T) assessed by two-way ANOVA. ³ Median of intensity (0-10 scale). ⁴ 0-9 scale, estimated by means of an algorithm based on the panels' outputs, according to Romero [1]. ⁵ Number of secondary sensory attributes perceived by more than 30% of the panelists. Abbreviations: CT_x, control oil stored at room temperature; THT_x, oil frozen at -20 °C, stored at -20 °C, thawed and stored at room temperature; T₀: 0 months of storage at room temperature; T₆: 6 months of storage at room temperature; TH, thawing; T, storage time; FFA, free fatty acids; PV, peroxide value.

Table S4. Changes in physicochemical and sensory quality parameters (mean values) assessed in fresh (control) and thawed unfiltered (UO) after 6 months of storage at room temperature. Effect of thawing (TH), storage time (T) and the interaction between them (TH*T) (*p* values) assessed by two-way ANOVA.

	UO Control		UO Thawed		TH ²	T ²	TH*T ²
	CT ₀ ¹	CT ₆ ¹	THT ₀ ¹	THT ₆ ¹			
FFA (% oleic acid)	0.07	0.07	0.06	0.08	0.777	0.103	0.027
PV (meq O ₂ /kg)	2.1	5.5	2.0	5.3	0.297	0.042	0.389
K ₂₃₂	1.35	1.64	1.32	1.57	0.160	0.053	0.546
K ₂₆₈	0.12	0.10	0.10	0.10	0.619	0.183	0.479
Positive sensory attributes³							
Fruity	5.1	2.3	5.0	4.0	0.001	<0.001	0.001
Bitter	4.4	3.6	4.4	4.1	0.280	0.024	0.203
Pungent	4.9	3.9	4.8	4.4	0.037	0.001	0.019
Green	3.3	0.1	3.4	2.7	<0.001	<0.001	<0.001
Sweet	4.1	4.0	4.0	4.1	0.708	0.940	0.474
Astringency	2.6	1.3	2.4	2.1	0.405	0.044	0.150
Almond	1.9	0.0	2.3	0.7	0.065	0.001	0.524
Global sensory score⁴	7.2	4.9	7.2	6.4	<0.001	<0.001	<0.001
Complexity⁵	5.0	2.5	7.0	5.5	0.015	0.031	0.460

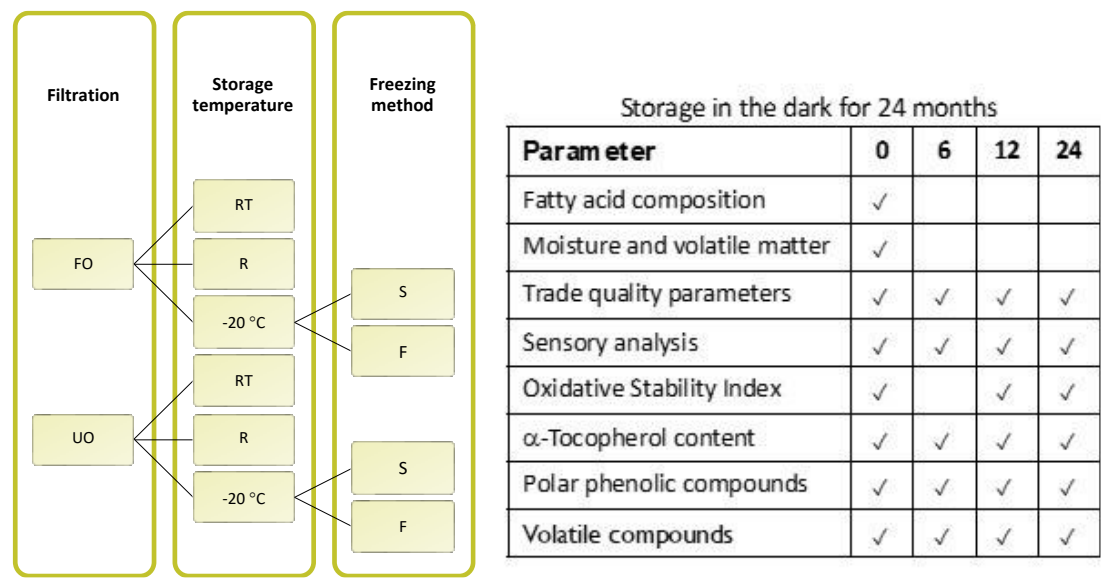
¹ Results from two experimental replicates (all parameters were further determined in analytical duplicate, except for K₂₃₂ and K₂₆₈ that were determined in triplicate). ² *p* values of the main effects (TH, thawing and T, storage at room temperature) and their interaction (TH*T) assessed by two-way ANOVA. ³ Median of intensity (0-10 scale). ⁴ 0-9 scale, estimated by means of an algorithm based on the panels' outputs, according to Romero [1]. ⁵ Number of secondary sensory attributes perceived by more than 30% of the panelists. Abbreviations: CT_x, control oil stored at room temperature; THT_x, oil frozen at -20 °C, stored at -20 °C, thawed and stored at room temperature; T₀: 0 months of storage at room temperature; T₆: 6 months of storage at room temperature; TH, thawing; T, storage time; FFA, free fatty acids; PV, peroxide value.

Reference

1. Romero, A. Caracterización y diferenciación de los aceites vírgenes de oliva de la comarca del Priorat (Tarragona) dentro del mercado global de aceites de la variedad 'Arbequina'. Tesis doctoral, UdL 2011. Available online: <https://tdx.cat/handle/10803/77835#page=70> (accessed on March, 9th 2023).

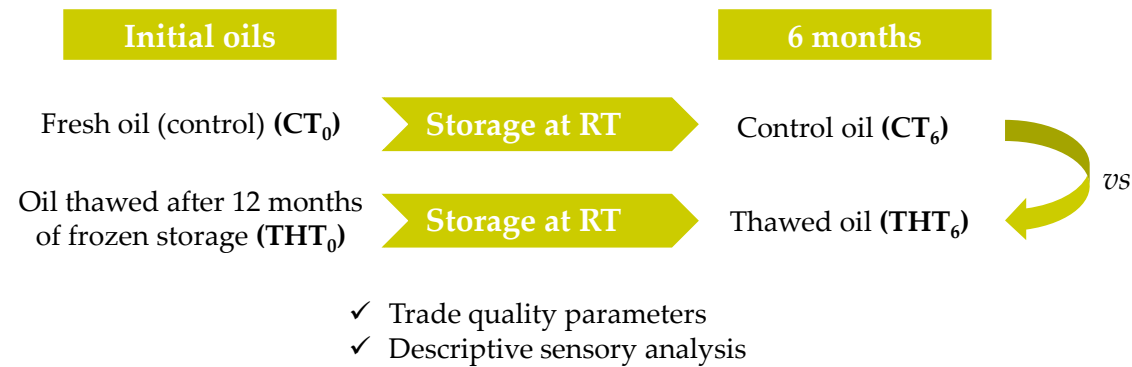
Figure S1. Scheme of the experimental design of (a) the conservation study of filtered oil (FO) and unfiltered oil (UO); (b) the study to evaluate the effect of freezing and thawing on the stability of FO and UO.

a) Experimental design: conservation study



Abbreviations: FO, filtered oil; UO, unfiltered oil; 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.

b) Experimental design: effect of freezing and thawing on the stability of FO and UO.



Abbreviations: RT, room temperature; CT_x, control oil stored at room temperature; THT_x, oil frozen at -20 °C, stored at -20 °C, thawed and stored at room temperature; T₀: 0 months of storage at room temperature; T₆: 6 months of storage at room temperature.

Figure S2. Interaction plot between storage conditions and time for the oleuropein and ligstroside derivatives content (Σ OL and Σ LIG, respectively) of the filtered (FO) and the unfiltered (UO) oils. $p \leq 0.05$ indicates significant interaction between the two factors (storage conditions and storage time). Error bars correspond to the standard deviation.

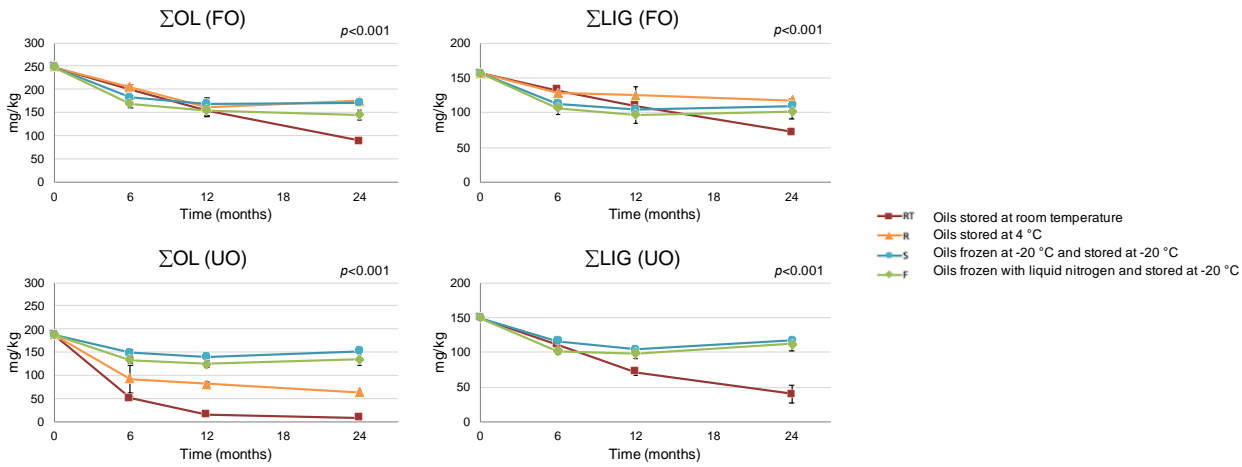


Figure S3. Interaction plot between storage conditions and time for *cis*-2-pentenol, *trans*-2-pentenol, 1-penten-3-ol, 1-hexanol, *trans*-2-hexenol, hexyl acetate, *cis*-3-hexenyl acetate and 6-methyl-5-hepten-2-one content of the filtered (FO) and the unfiltered (UO) oils. $p \leq 0.05$ indicates significant interaction between the two factors (storage conditions and storage time). Error bars correspond to the standard deviation.

