

## Storage Effects on Bioactive Phenols in Calabrian Monovarietal Extra Virgin Olive Oils Based on the EFSA Health Claim

Marialaura Frisina, Sonia Bonacci \*, Manuela Oliverio, Monica Nardi,  
Thomas Patrizio Vatrano and Antonio Procopio

Department of Health Science, University Magna Græcia of Catanzaro, 88100 Catanzaro, Italy

**Figure S1.** Images from <https://www.olimonovarietali.it/en/region-detail/?regione=CALABRIA>.

page 2

**Figure S2.** Full Scan (a) chromatogram obtained by UHPLC-ESI-HRMS for the VN sample and extracted ion chromatograms of detected EVOO phenols hydroxytyrosol (b), oleacein (c), oleocanthal (d) and oleuropein aglycon (e).

page 2

**Table S1.** Evolution of total phenol content ( $\text{mg kg}^{-1}$ ) in Nocellara del Belice (VN) and Dolce di Rossano (VDR) monovarietal EVOO samples.

page 3

**Table S2.** Evolution of total considered biophenols during twelve months of storage expressed as mg of *EVOO phenols* per 20 g in Nocellara di Belice (VN) and Dolce di Rossano (VDR) monovarietal EVOO samples ( $\text{mg/ 20 g}$ ).

page 3

**Figure S3.** Correlation of the peak area obtained by UHPLC-ESI-HRMS and analytical standard concentration of hydroxytyrosol (a); tyrosol (b); verbascoside (c); oleacein (d); oleuropein (e); oleocanthal (f) and oleuropein aglycon (g).

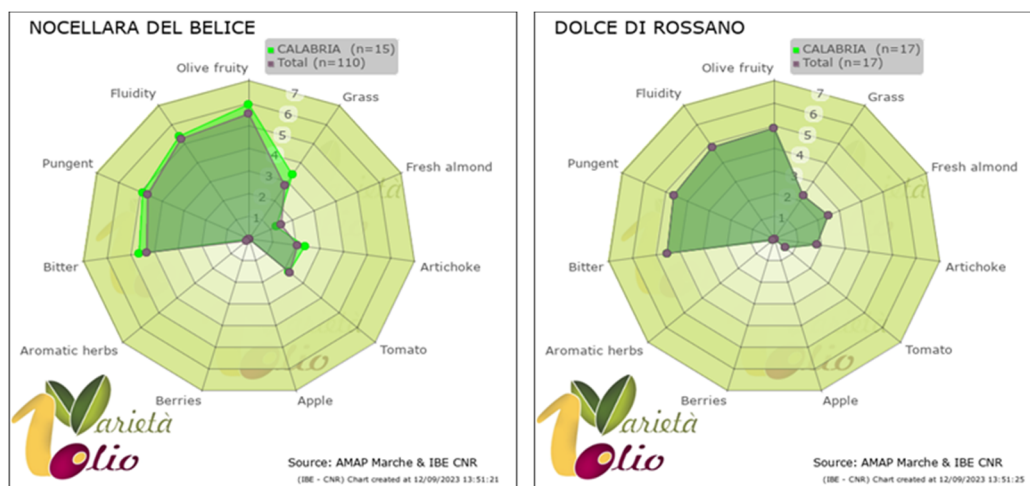
page 4

**Table S3.** Linearity validation results of the analytical method by UHPL-ESI-HRMS.

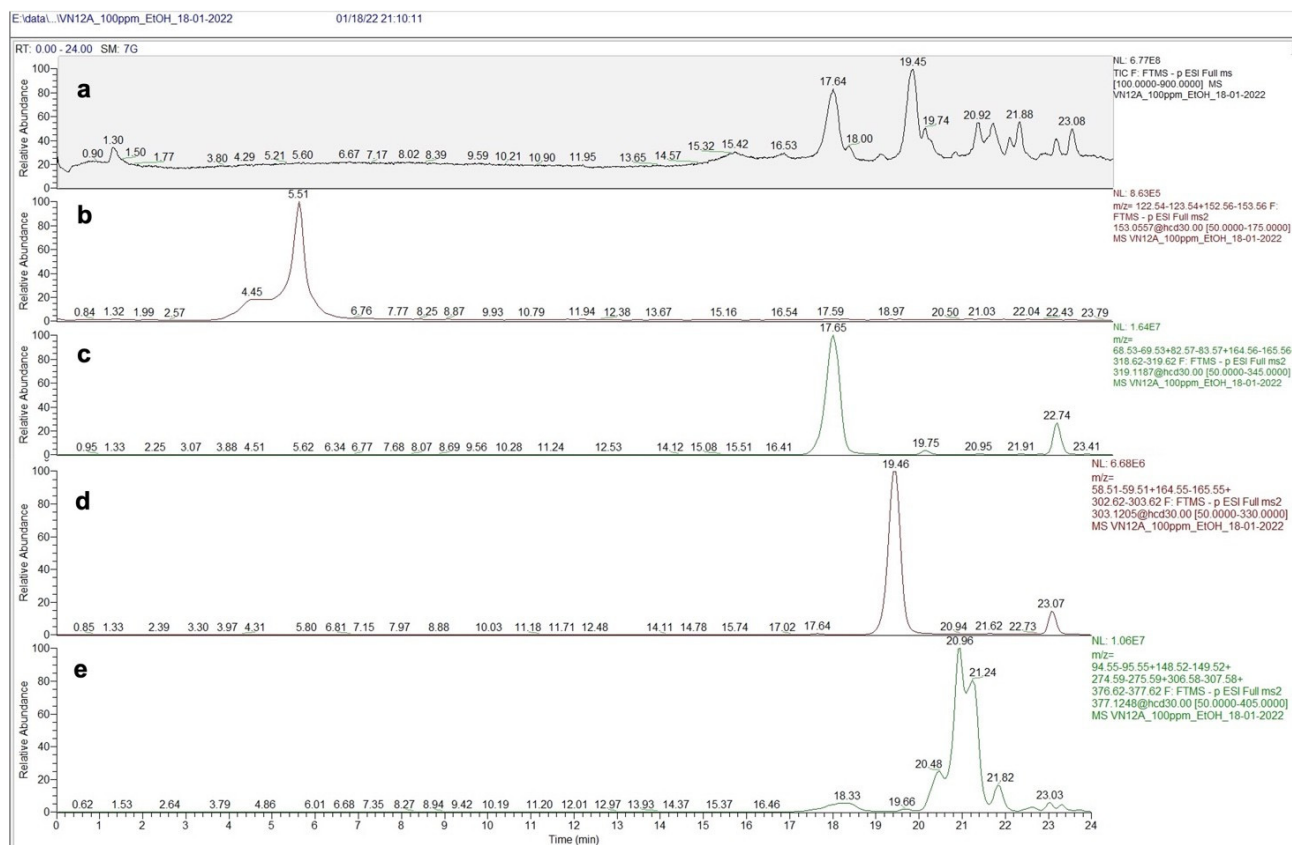
page 5

**Table S4.** Statistical and analytical parameters: variation coefficient ( $\text{CV}_r$ ); limit of repeatability ( $r$ ); percentage recovery ( $R$ ) and extended uncertainty ( $U_e$ ) for each considered concentrations ( $\text{mg g}^{-1}$ ).

page 5



**Figure S1.** Images from <https://www.olimonovarietali.it/en/region-detail/?regione=CALABRIA>.



**Figure S2.** Full Scan (a) chromatogram obtained by UHPLC-ESI-HRMS for the VN sample and extracted ion chromatograms of detected EVOO phenols hydroxytyrosol (b), oleacein (c), oleocanthal (d) and oleuropein aglycon (e).

**Table S1.** Evolution of total phenol content ( $\text{mg kg}^{-1} \pm \text{SD}$ ) in Nocellara del Belice (VN) and Dolce di Rossano (VDR) monovarietal EVOO samples evaluated by spectrophotometric assay (Folin-Ciocalteu) during storage time (months).

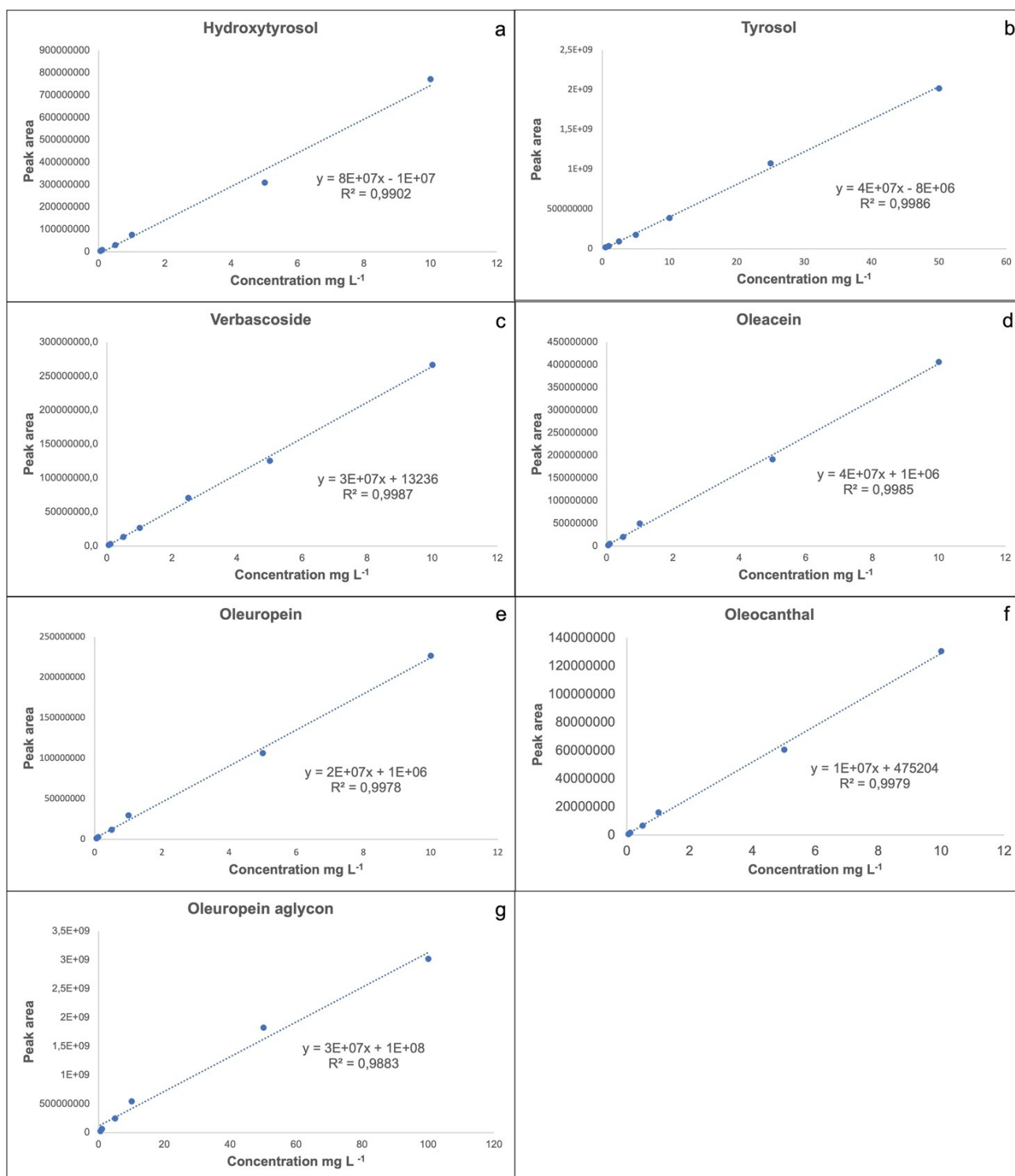
	VN* ( $\text{mg kg}^{-1} \pm \text{SD}$ )	VDR* ( $\text{mg kg}^{-1} \pm \text{SD}$ )
February	472.34 $\pm$ 9.48	566.98 $\pm$ 18.52
March	552.72 $\pm$ 15.76	486.65 $\pm$ 16.46
April	475.40 $\pm$ 15.49	501.22 $\pm$ 22.71
May	486.61 $\pm$ 10.99	486.63 $\pm$ 10.39
June	532.66 $\pm$ 12.46	429.53 $\pm$ 8.47
July	523.85 $\pm$ 13.12	554.22 $\pm$ 15.45
August	506.68 $\pm$ 20.46	569.72 $\pm$ 14.19
September	510.35 $\pm$ 11.24	340.91 $\pm$ 23.15
October	529.31 $\pm$ 12.51	513.36 $\pm$ 15.12
November	511.14 $\pm$ 19.45	624.09 $\pm$ 21.46
December	509.48 $\pm$ 10.82	535.69 $\pm$ 10.24
January	499.54 $\pm$ 17.51	555.11 $\pm$ 14.58

\*Data are expressed as means standard deviations (SD) of tree independent observations.

**Table S2.** Evolution of total considered EVOO phenols during twelve months of storage expressed as mg of EVOO phenols per 20 g in *Nocellara di Belice* (VN) and *Dolce di Rossano* (VDR) monovarietal EVOO samples ( $\text{mg/ 20 g}$ ).

	VN*	VDR*
February	29.58 $\pm$ 1.16	41.43 $\pm$ 2.29
March	29.04 $\pm$ 1.61	40.78 $\pm$ 2.31
April	28.69 $\pm$ 0.78	38.64 $\pm$ 1.50
May	28.00 $\pm$ 1.17	35.83 $\pm$ 2.26
June	27.80 $\pm$ 1.43	35.72 $\pm$ 2.80
July	27.46 $\pm$ 1.17	34.04 $\pm$ 2.51
August	27.04 $\pm$ 1.02	34.35 $\pm$ 2.89
September	26.85 $\pm$ 1.11	34.19 $\pm$ 2.26
October	26.26 $\pm$ 0.91	33.19 $\pm$ 1.27
November	25.82 $\pm$ 0.91	28.04 $\pm$ 1.64
December	25.40 $\pm$ 0.99	28.06 $\pm$ 1.30
January	25.10 $\pm$ 0.95	28.16 $\pm$ 1.52

\*Data are expressed as means and standard deviations (SD) of three independent observations.



**Figure S3.** Correlation of the peak area obtained by UHPLC-ESI-HRMS and analytical standard concentration of hydroxytyrosol (a); tyrosol (b); verbascoside (c); oleacein (d); oleuropein (e); oleocanthal (f) and oleuropein aglycon (g).

**Table S3.** Linearity validation results of the analytical method by UHPL-ESI-HRMS.

Compounds	Concentration range (mg L <sup>-1</sup> )	Calibration curve	R <sup>2</sup>
Hydroxytyrosol	0.05-10	Y=8E+07X-1E+07	0.9902
Tyrosol	0.5- 50	Y=4E+07X-8E+06	0.9986
Verbascoside	0.05-10	Y=3E+07X+13236	0.9987
Oleacein	0.05-10	Y=4E+07X+1E+06	0.9985
Oleuropein	0.05-10	Y=2E+07X+1E+06	0.9978
Oleocanthal	0.05-10	Y=1E+07X+475204	0.9979
Oleuropein aglycone	0.5-100	Y=3E+07X+1E+08	0.9983

**Table S4.** Statistical and analytical parameters: variation coefficients (CV<sub>r</sub> and CV<sub>R</sub>); limit of repeatability (r); percentage recovery (R) and extended uncertainty (U<sub>e</sub>) for each considered concentrations (mg g<sup>-1</sup>).

	mg g <sup>-1</sup>	CV <sub>r</sub> (%)	CV <sub>R</sub> (%)	r (%)	R (%)	U <sub>e</sub> (%)
<b>Hydroxytyrosol</b>	0.033	2.3	2.9	7.2	94	25
	0.037	6.1	7.2	19.4	109	20
	0.047	6.6	7.3	20.8	93	33
<b>Tyrosol</b>	0.37	2.9	3.5	9.8	97	32
	0.58	6.8	7.7	20.7	96	36
	0.75	3.3	3.9	10.5	96	31
<b>Verbascoside</b>	0.003	9.3	9.9	29.0	97	29
	0.007	8.8	9.7	27.8	98	18
	0.015	1.9	2.6	7.3	104	22
<b>Oleacein</b>	0.09	1.7	2.4	5.5	94	33
	0.13	7.5	8.0	24.0	108	31
	0.28	5.7	6.3	18.2	99	29
<b>Oleuropein</b>	0.002	9.7	10.9	32.0	89	38
	0.005	9.0	9.4	28.8	104	20
	0.013	2.1	2.8	6.2	95	23
<b>Oleocanthal</b>	0.16	5.0	5.4	6.9	93	38
	0.23	5.8	6.6	18.7	93	39
	0.53	4.4	5.2	14.2	97	26
<b>Oleuropein aglycon</b>	0.36	3.1	3.9	9.9	98	36
	0.53	6.8	7.2	21.9	95	34
	0.72	3.2	3.7	10.2	96	33