

Supplementary Materials

Table S1: Olive oil mill plant set-up: Conventional facilities.

| | | |
|-----------------------|---|--|
| Hammer Crusher |  |  |
| Malaxer |  |  |

Extraction (3-phase Decanter)**Extraction (Vertical Centrifuge)**



Figure S1: 2021 4xUS-PEF assisted process.

Table S2: Analysis of olive oils produced by classical oil mill (CONTROL) and by the application of non-conventional techniques (4xUS, 4xUS-PEF and PEF) from half ripening **Coratina variety**.

| Analysis | Method | Compound or test (Meas. Unit) | Half ripening Coratina | | | | |
|----------|--------|----------------------------------|------------------------|------|----------|------|-----|
| | | | Control | 4xUS | 4xUS-PEF | PEFM | PEF |
| | | α -tocopherol (mg/kg) | 224 | 244 | 258 | 246 | 235 |

| | | | | | | | |
|-------------------------------|------------------------------------|--|-----|-----|-----|-----|-----|
| Tocopherols and tocotrienols* | ISO 9936:2016 (E) | β -tocopherol (mg/kg) | 2 | 2 | 2 | 2 | 4 |
| | | γ -tocopherol (mg/kg) | 10 | 11 | 11 | 11 | 11 |
| | | δ -tocopherol (mg/kg) | <1 | <1 | <1 | <1 | <1 |
| | | Total tocopherols (mg/kg) | 236 | 257 | 271 | 259 | 250 |
| | | α -tocotrienol (mg/kg) | 10 | 18 | 14 | 18 | 20 |
| | | β -tocotrienol (mg/kg) | 5 | 6 | 6 | 6 | 6 |
| | | γ -tocotrienol (mg/kg) | 19 | 32 | 25 | 30 | 31 |
| | | δ -tocotrienol (mg/kg) | 1 | 1 | 1 | 2 | 2 |
| | | Total tocotrienols (mg/kg) | 35 | 57 | 46 | 56 | 59 |
| | | Total tocopherols and tocotrienols (mg/kg $\pm U$) [§] | 271 | 314 | 317 | 315 | 309 |
| | | Vitamin E (D- α -tocopherol) (mg/100 g) | 22 | 25 | 25 | 26 | 25 |
| | | Vitamin E (D,L- α -tocopherol) (mg/100 g) | 17 | 18 | 19 | 19 | 18 |
| Polyphenols* | COL/T.20/ Doc. No 29/Rev.1/2017 | Total Biophenols (mg/kg $\pm U$) [§] (RRF (5.2)) | 506 | 502 | 487 | 492 | 521 |
| | | Tot natural biophenols (mg/kg) | 492 | 487 | 470 | 479 | 502 |
| | | Total aromatic alcohols (mg/kg) | 8 | 7 | 7 | 6 | 7 |
| | | Hydroxytyrosol (mg/kg) | 5 | 5 | 5 | 4 | 5 |
| | | Tyrosol (mg/kg) | 3 | 2 | 2 | 2 | 2 |
| | | Oleuropein (mg/kg) | 4 | 4 | 6 | 3 | 4 |
| | | Oleuropein derivatives (mg/kg) | 241 | 236 | 211 | 230 | 245 |

| | | | | | |
|---|------|------|------|------|------|
| Ligstroside derivatives (mg/kg) | 180 | 185 | 173 | 185 | 184 |
| Oleocanthal (mg/kg) | 106 | 115 | 94 | 116 | 104 |
| Total lignans (Pinoresinol and acetoxyphenol resinol) (mg/kg) | 54 | 50 | 60 | 47 | 58 |
| Total phenolic acids (mg/kg) | 1 | 1 | 1 | 1 | 1 |
| Total flavonoids (mg/kg) | 16 | 15 | 17 | 16 | 15 |
| Luteolin (mg/kg) | 9 | 8 | 10 | 9 | 9 |
| Apigenin (mg/kg) | 7 | 7 | 7 | 7 | 6 |
| Total secoiridoid acids (mg/kg) | 15 | 11 | 16 | 14 | 15 |
| Decarboxymethyl elenolic acid (mg/kg) | <1 | <1 | <1 | <1 | <1 |
| Elenolic acid (mg/kg) | 15 | 11 | 16 | 14 | 15 |
| Total oxidized biophenols (mg/kg) | 14 | 15 | 17 | 13 | 19 |
| Oxidation ratio % (Total oxidized biophenols/Total biophenols) | 2.8 | 3.0 | 3.5 | 2.6 | 3.6 |
| Hydrolysis ratio % (Total aromatic alcohols/Total biophenols) | 1.6 | 1.4 | 1.4 | 1.2 | 1.3 |
| Total oxidized secoiridoid acids (mg/kg) | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Oxidized decarboxymethyl elenolic acid (mg/kg) | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Oxidized elenolic acid (mg/kg) | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Hydroxytyrosol and derivatives | 435 | 436 | 409 | 428 | 447 |

(#) REG. CE 2568/91 Annex 1 and Doc. COI/T.15/NC No 3/Rev. 14-2019. (§) U = Expanded measurement uncertainty with a coverage factor k = 2 and a confidence level of 95%.

(*) Test not accredited by ACCREDIA.

Table S3: Analyses of EVOOs produced by classical oil mill (CONTROL) and by the application of non-conventional techniques (ULTRASOUND) from mature and green **Taggiasca variety**.

| Analysis | Method | Compound or test (Meas. Unit) | EVOO Specification # | Taggiasca variety | | | |
|--|--|---|----------------------------|---------------------|----------------------|----------------------|----------------------|
| | | | | Mature | | Green | |
| | | | | Control | US | Control | US |
| Free fatty acids (expressed as oleic acid) | COI/T.20/Doc. No 34/Rev.1 2017 | % ±U \$ | ≤ 0.80 | 0.30±0.07 | 0.29±0.07 | 0.37±0.07 | 0.43±0.07 |
| Peroxide value | COI/T.20/Doc. No 35/Rev.1 2017 | meq O ₂ /kg ±U \$ | ≤ 20.0 | 7.1±1.1 | 8.5±2.0 | 9.9±2.0 | 10.2±2.0 |
| Insoluble impurities | ISO 663:2017 | w/w % ±U \$ | ≤ 0.10 | 0.01±0.03 | 0.03±0.03 | 0.03±0.03 | 0.01±0.03 |
| Moisture and volatile matter (method B) | ISO 662:2016 | w/w % ±U \$ | ≤ 0.20 | 0.12±0.04 | 0.16±0.05 | 0.16±0.05 | 0.18±0.05 |
| UV spectrophotometric analysis | COI/T.20/Doc. No 19/Rev.5 2019 | K232 (CL 0.95; SE 0.033) ^a | ≤ 2.50 | 0.74 (0.62÷0.85) | 1.62 (1.50÷1.73) | 1.39 (1.27÷1.50) | 1.57 (1.45÷1.68) |
| | | K268 (CL 0.95; SE 0.0062) ^b | ≤ 0.22 | 0.09 (0.07÷0.10) | 0.09 (0.07÷0.11) | 0.10 (0.09÷0.12) | 0.11 (0.09÷0.12) |
| | | ΔK (CL 0.95; SE 0.0019) ^b | ≤ 0.01 | 0.00 (0.00÷0.01) | 0.00 (-0.01÷0.00) | 0.00 (-0.01÷0.00) | 0.00 (-0.01÷0.00) |
| | | | | | | | |
| Fatty acids composition | Reg CEE 2568/1991, GU CEE L248 Annex X, Reg UE 1833/2015, GU UE L266/29 Annex IV | Myristic acid (% ±U) \$ | < 0.03 | 0.01±0.01 | 0.01±0.01 | 0.01±0.01 | 0.01±0.01 |
| | | Pentadecanoic acid (% ±U) \$ | - | 0.01 | 0.01 | < 0.01 | 0.01 |
| | | Palmitic acid (% ±U) \$ | 7.50-20.00 | 12.08±0.71 | 12.11±0.71 | 12.13±0.71 | 11.97±0.71 |
| | | Palmitoleic acid (% ±U) \$ | 0.30-3.50 | 0.93±0.07 | 0.93±0.07 | 0.79±0.07 | 0.76±0.07 |
| | | Heptadecanoic acid (% ±U) \$ | ≤ 0.40 | 0.05±0.02 | 0.04±0.02 | 0.05±0.02 | 0.05±0.02 |
| | | | | | | | |

| | | | | | | | |
|--|--|--|-------------|------------|-------------|-------------|-------------|
| | | Heptadecenoic acid (% ±U) [§] | ≤ 0.60 | 0.10±0.02 | 0.09±0.02 | 0.10±0.02 | 0.10±0.02 |
| | | Stearic acid (% ±U) [§] | 0.50-5.00 | 2.12±0.14 | 2.12±0.14 | 2.01±0.14 | 2.00±0.14 |
| | | Oleic acid (% ±U) [§] | 55.00-83.00 | 75.04±0.71 | 75.05±0.71 | 75.40±0.71 | 75.72±0.71 |
| | | Linoleic acid (% ±U) [§] | 2.50-21.00 | 8.05±0.35 | 8.03±0.35 | 7.85±0.35 | 7.66±0.35 |
| | | Arachidic acid (% ±U) [§] | ≤ 0.60 | 0.37±0.07 | 0.37±0.07 | 0.39±0.07 | 0.39±0.07 |
| | | Eicosenoic acid (% ±U) [§] | ≤ 0.50 | 0.33±0.07 | 0.33±0.07 | 0.36±0.07 | 0.37±0.07 |
| | | Linolenic acid (% ±U) [§] | ≤ 1.00 | 0.74±0.07 | 0.74±0.07 | 0.72±0.07 | 0.76±0.07 |
| | | Behenic acid (% ±U) [§] | ≤ 0.20 | 0.12±0.07 | 0.12±0.07 | 0.13±0.07 | 0.13±0.07 |
| | | Erucic acid (% ±U) [§] | - | <0.01 | <0.01 | <0.01 | <0.01 |
| | | Lignoceric acid (% ±U) [§] | ≤ 0.20 | 0.05±0.03 | 0.05±0.03 | 0.06±0.03 | 0.06±0.03 |
| Fatty acids <i>trans</i> -isomers | Reg CEE 2568/1991, GU CEE L248 Annex X, Reg UE 1833/2015, GU UE L266/29 Annex IV | Octadecenoic acids (% ±U) [§] | ≤ 0.05 | 0.01±0.01 | < 0.01±0.01 | < 0.01±0.01 | < 0.01±0.01 |
| | | Octadecadienoic + octadecatrienoic acids (% ±U) [§] | ≤ 0.05 | 0.01±0.01 | 0.01±0.01 | 0.01±0.01 | 0.01±0.01 |
| Sterol composition and content and alcoholic compounds | Reg CEE 2568/1991, GU CEE L248 Annex XIX, Reg UE 1604/2019, GU UE L250 Annex VII | Cholesterol (% ±U) [§] | ≤ 0.5 | < 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.2±0.1 |
| | | tR Brassicasterol (% ±U) [§] | ≤ 0.1 | < 0.1±0.1 | < 0.1±0.1 | < 0.1±0.1 | < 0.1±0.1 |
| | | 24-Metilencolesterol (% ±U) [§] | - | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 |

| | | | | | | | |
|------------------------------|--|---|-----------|------------------|------------------|---------------|----------------|
| | Campesterol (% ±U) [§] | ≤ 4.0 | 3.1±0.2 | 3.1±0.2 | 3.2±0.2 | 3.4±0.2 | |
| | Campestanol (% ±U) [§] | - | 0.2±0.1 | 0.2±0.1 | 0.1±0.1 | 0.2±0.1 | |
| | Stigmasterol (% ±U) [§] | < campesterol | 0.9±0.1 | 0.9±0.1 | 1.3±0.1 | 1.6±0.1 | |
| | Δ-7-campesterol (% ±U) [§] | - | < 0.1±0.1 | < 0.1±0.1 | < 0.1±0.1 | < 0.1±0.1 | |
| | Δ-5,23-stigmastadienol (% ±U) [§] | - | < 0.1±0.1 | < 0.1±0.1 | < 0.1±0.1 | < 0.1±0.1 | |
| | Chlerosterol (% ±U) [§] | - | 0.9±0.1 | 0.9±0.1 | 0.9±0.1 | 0.9±0.1 | |
| | β-sitosterol (% ±U) [§] | - | 81.6±0.7 | 81.8±0.7 | 82.8±0.7 | 82.5±0.7 | |
| | Sitostanol (% ±U) [§] | - | 1.2±0.2 | 1.2±0.2 | 1.2±0.2 | 1.4±0.2 | |
| | Δ-5-avenasterol (% ±U) [§] | - | 10.2±0.1 | 9.8±0.2 | 9.0±0.2 | 8.4±0.2 | |
| | Δ-5,24-stigmastadienol (% ±U) [§] | - | 1.1±0.1 | 1.0±0.1 | 0.7±0.1 | 0.6±0.1 | |
| | Δ-7-stigmastenol (% ±U) [§] | ≤ 0.5 | 0.3±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 | |
| | Δ-7-avenasterol (% ±U) [§] | - | 0.7±0.1 | 0.7±0.1 | 0.5±0.1 | 0.5±0.1 | |
| | Apparent β-sitosterol (% ±U) [§] | ≥ 93.0 | 94.7±0.5 | 94.7±0.5 | 94.4±0.5 | 93.9±0.5 | |
| | Total sterols (mg/kg) | ≥ 1000 | 1559±123 | 1552±123 | 1355±123 | 1375±123 | |
| | Erythrodiol + uvaol (% ±U) [§] | ≤ 4.5 | 1.0±0.6 | 1.2±0.6 | 1.1±0.6 | 0.9±0.6 | |
| Tocopherols and tocotrienols | IUPAC 1992 | α-tocopherol (the only detectable) (mg/kg) (CL 0.95; SE 7.55) ^b | - | 144 (124÷166) | 139 (118÷160) | 74 (53÷95) | 80 (60÷101) |

| | | | | | | | |
|-------------|---------------------------------|--|---|------------------|------------------|------------------|------------------|
| Polyphenols | COI/T.20/ Doc. No 29/Rev.1/2017 | mg/kg (CL 0.95; SE 3.62) ^a (RRF 5.5) | - | 152 (139÷165) | 174 (161÷186) | 289 (276÷302) | 292 (279÷304) |
|-------------|---------------------------------|--|---|------------------|------------------|------------------|------------------|

(#) REG. CE 2568/91 Annex 1 and Doc. COI/T.15/NC No 3/Rev. 14-2019.

(§) U = Expanded measurement uncertainty with a coverage factor k = 2 and a confidence level of 95%.

(a) CL = Confidence level used: 0.95, SE = standard error. Conf-level adjustment: Bonferroni method for 2 estimates, significance level used: $\alpha = 0.05$.

(b) CL = Confidence level used: 0.95, SE = standard error. P value adjustment: Tukey method for comparing a family of 4 estimates, significance level used: $\alpha = 0.05$.

Table S4: Determination of tocopherols and tocotrienols and polyphenols content in EVOOs produced by classical oil mill (CONTROL) and by the application of non-conventional techniques (ULTRASOUND) from mature and green Taggiasca varieties.

| Analysis | Method | Compound or test (Meas. Unit) | Taggiasca | | | |
|---|-------------------|----------------------------------|----------------|--------------|---------------|--------------|
| | | | Mature control | US | Green control | US |
| Tocopherols and tocotrienols * | ISO 9936:2016 (E) | Tocopherols (mg/kg) | 160 | 171 | 108 | 118 |
| | | α -tocopherol (mg/kg) | 155 | 168 | 105 | 114 |
| | | β -tocopherol (mg/kg) | 2 | 2 | 1 | 1 |
| | | γ -tocopherol (mg/kg) | 3 | 3 | 2 | 2 |
| | | δ -tocopherol (mg/kg) | < 1 | < 1 | < 1 | < 1 |
| | | Tocotrienols(mg/kg) | 4 | 6 | < 1 | < 1 |
| | | α -tocotrienol (mg/kg) | < 1 | < 1 | < 1 | < 1 |
| | | β -tocotrienol (mg/kg) | 2 | 2 | < 1 | < 1 |
| | | γ -tocotrienol (mg/kg) | 1 | 2 | < 1 | < 1 |
| | | δ -tocotrienol (mg/kg) | 1 | 1 | < 1 | < 1 |
| Total tocopherols and tocotrienols (mg/kg \pm U) [§] | | | 164 \pm 27 | 248 \pm 32 | 108 \pm 27 | 176 \pm 34 |

| | | | | | | |
|--|-----------------------------------|---|--------|--------|--------|--------|
| Biophenols determina- tion in olive oils by HPLC * | COI/T.20/Doc. No 29/Rev.1 2017 | Total Biophenols (mg/kg ±U) (RRF 5.3) | 110±41 | 105±38 | 207±53 | 205±52 |
| | | Tot natural biophenols (mg/kg) | 100 | 93 | 190 | 188 |
| | | Total aromatic alcohols (mg/kg) | 1 | 1 | 3 | 3 |
| | | Hydroxytyrosol (mg/kg) | < 1 | < 1 | 1 | 1 |
| | | Tyrosol (mg/kg) | 1 | 1 | 2 | 1 |
| | | Oleuropein (mg/kg) | < 1 | < 1 | < 1 | < 1 |
| | | Oleuropein derivatives (mg/kg) | 5 | 4 | 43 | 42 |
| | | Ligstroside derivatives (mg/kg) | 39 | 38 | 60 | 62 |
| | | Oleocanthal (mg/kg) | 29 | 30 | 34 | 37 |
| | | Total lignans (Pinoresinol and acetoxypinoresinol) (mg/kg) | 46 | 40 | 72 | 67 |
| | | Total phenolic acids (mg/kg) | 3 | 2 | 4 | 4 |
| | | Total flavonoids (mg/kg) | 7 | 6 | 11 | 10 |
| | | Luteolin (mg/kg) | 5 | 4 | 7 | 6 |
| | | Apigenin (mg/kg) | 2 | 2 | 4 | 4 |
| | | Total secoiridoid acids (mg/kg) | 1 | 1 | 17 | 12 |
| | | Decarboxymethyl elenolic acid (mg/kg) | < 1 | < 1 | 1 | 1 |
| | | Elenolic acid (mg/kg) | 1 | 1 | 16 | 12 |
| | | Total oxidized biophenols (mg/kg) | 10 | 13 | 17 | 18 |
| | | Oxidation ratio % (Total oxidized biophenols/Total biophenols) | 9.1 | 10.2 | 8.2 | 9.6 |
| | | Hydrolysis ratio % (Total aromatic alcohols/Total biophenols) | 0.9 | 1.1 | 1.5 | 1.5 |
| | | Total oxidized secoiridoid acids (mg/kg) | 0.1 | 0.1 | 0.3 | 0.3 |

| | | | | |
|---|-------|-------|-------|-------|
| Oxidized decarboxymethyl elenolic acid (mg/kg) | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| Oxidized elenolic acid (mg/kg) | 0.1 | 0.1 | 0.3 | 0.3 |

(§) U = Expanded measurement uncertainty with a coverage factor k = 2 and a confidence level of 95%.

(*) Test not accredited by ACCREDIA.