SUPPLEMENTARY MATERIAL

EVALUATING THE RELIABILITY OF SPECIFIC AND GLOBAL METHODS TO ASSESS THE PHENOLIC CONTENT OF VIRGIN OLIVE OIL: DO THEY DRIVE TO EQUIVALENT RESULTS?

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Fig. 1 SM. (I, II, III) Correlations between the sum of concentrations found for each determined phenolic compound by the LC-MS profiling method and total phenolic content determined by the three different non-specific methods for the 50 EVOO samples under evaluation (results expressed in molar basis). (IV, V, IV) Same correlations as above, excluding elenolic acid and derivatives from the sum of individual compounds determined by LC-MS (results expressed in mg/kg).

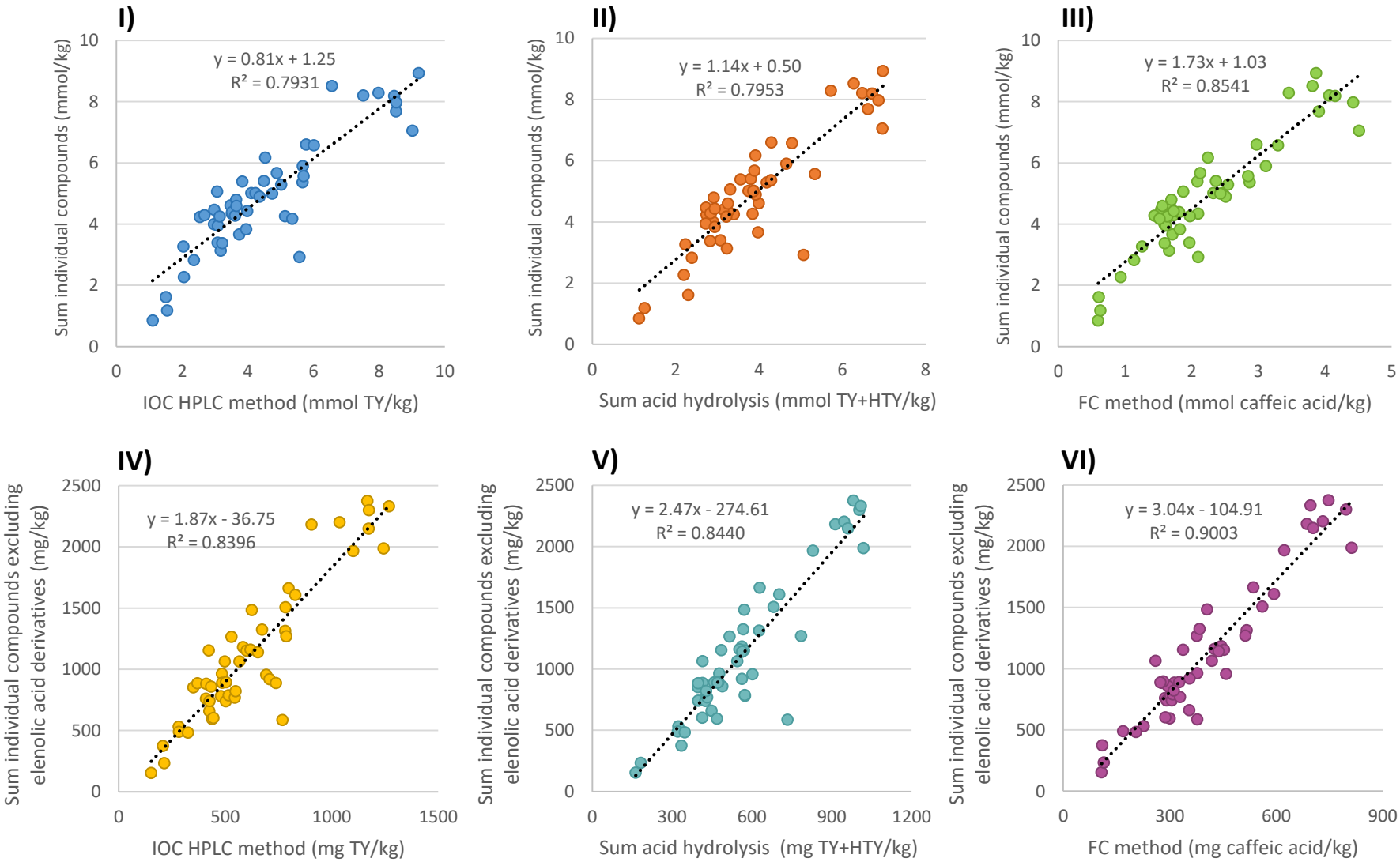

Fig. 2 SM. Correlations between the total phenolic content determined by the three different non-specific methods for the 50 EVOO samples under evaluation. Results expressed in molar basis.

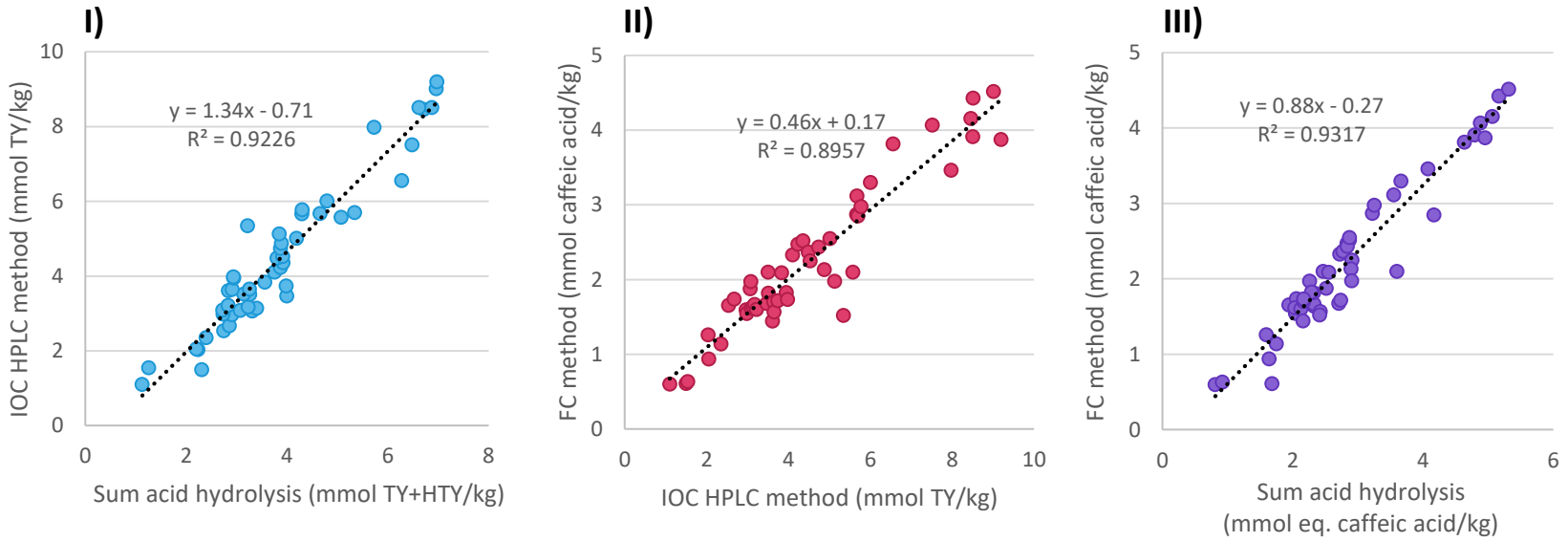

Fig. 3 SM. Correlations between HTY (I) and TY (II) related compounds determined after acid hydrolysis and by the LC-MS method, expressed in terms of the corresponding phenolic alcohol. Correlation between the theoretically calculated and the actual molar concentration of HTY (III) and TY (IV) after hydrolysis.

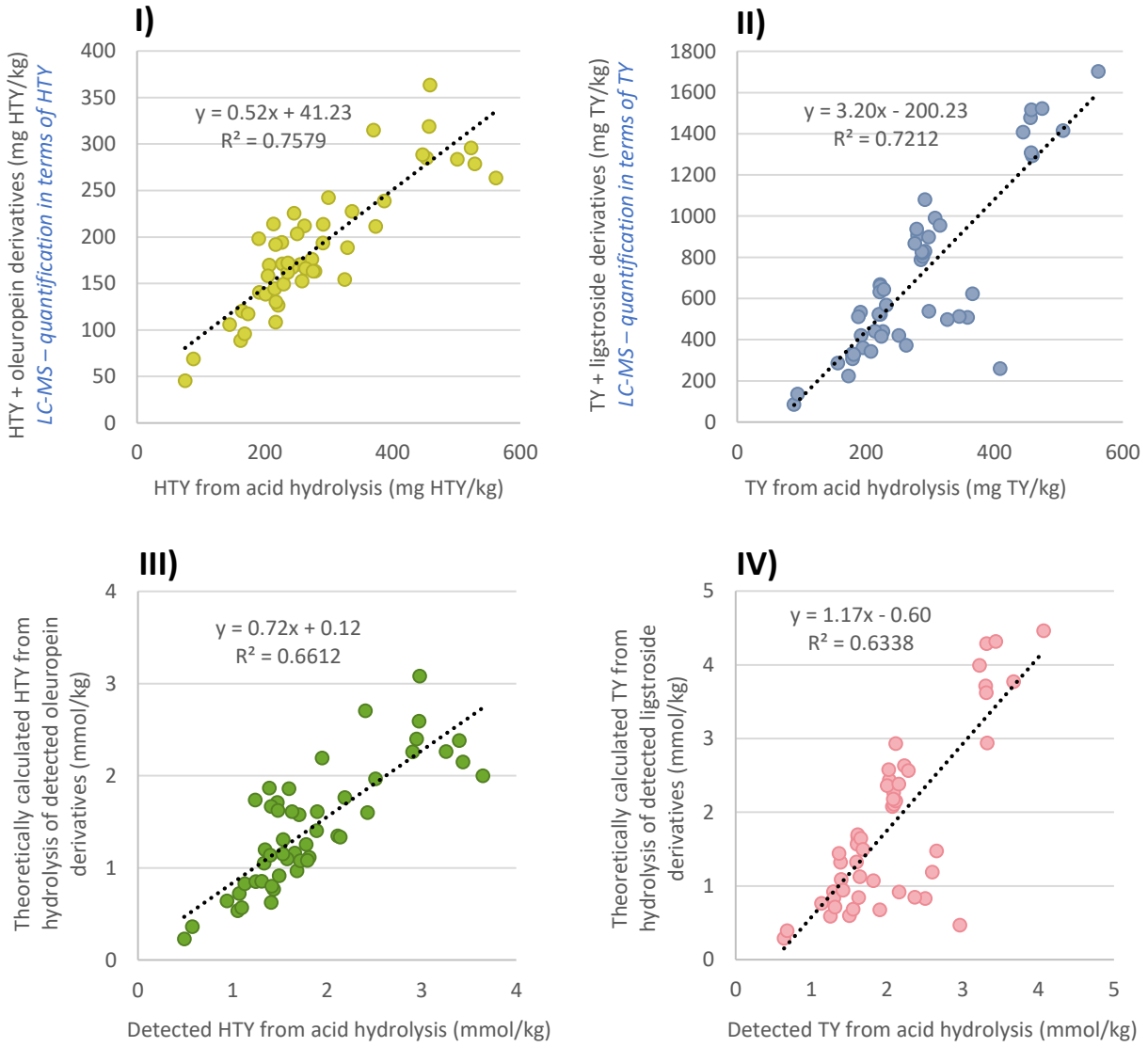

Table 1SM. Advantages and drawbacks of the evaluated methodologies.

	Advantages	***Drawbacks***	***Green aspects***
FC colorimetric assay	- Global index - Fast - Cheap instrumentation - Equivalent result to the hydrolysis approach (appropriate for the health claim requirements)	- Low selectivity - Reducing substances may cause interferences - Results differ depending on the used standard - Cannot give information about the phenolic profile	- *Sample size*: 10 g oil - *Sample prep:* 6 mL solvent (3.6 mL MeOH + 2.4 mL water)/g oil + 0.25 mL FC reagent
IOC HPLC method	- Global index - Could allow individual quantification of non-coeluting compounds - Easy to apply - Affordable instrumentation	- Overlapped peaks - Considers equal response factor for all the analytes - Omit EA and derivatives - Long chromatographic run time	- *Sample size*: 2 g oil - *Sample prep:* 2.5 mL solvent (2 mL MeOH + 0.5 mL water)/g oil; 15 min ultrasounds - *Chromatographic separation:* 82 mL solvent (ACN/MeOH/acidified water) per sample
Hydrolysis approach	- Secoiridoids indirect global measurement (appropriate for the health claim requirements) - Allows differentiation among TY-related and HTY-related substances - Chromatographic run time could be shortened - Easy to carry out - Affordable instrumentation	- Chemical reaction required (6 h) - Does not consider other relevant families of phenolic compounds - The efficiency of the hydrolysis reaction should be validated for VOOs with very high phenolic content	- *Sample size*: 1.5 g oil - *Sample prep:* 20 mL solvent (2.5 mL HCl + 17.5 mL water)/g oil; 6 h orbital shaker - *Chromatographic separation:* 20 mL solvent (ACN/acidified water) per sample
LC-MS profiling	- Information about 24 individual phenolic compounds - Allows the individual determination of the compounds to which the health claim refers	- Questionable artificial sum to generate a global index - Expensive instrumentation - High cost of commercial standards - Difficult isolation of non commercially available standards - Short linear dynamic ranges for quantification	- *Sample size*: 2 g oil - *Sample prep:* 3 mL solvent (1.8 mL MeOH + 1.2 mL water)/g oil; 6 min vortex - *Chromatographic separation:* 20 mL solvent (ACN/acidified water) per sample