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Phenolic profile of foods from the Mediterranean basin, included in a Mediterranean diet intervention aimed at tackling youth obesity

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ABSTRACT

Obesity, characterized by excessive adipose tissue accumulation, has reached epidemic proportions globally, and strongly correlates with various diseases, including cardiovascular disease, diabetes, and cancer. This burden is increasingly affecting adolescents. The Mediterranean diet, known for its health benefits, is being increasingly studied for its potential in preventing metabolic diseases like obesity. This study aims to characterize and quantify the phenolic compounds in typical Mediterranean diet products, such as sourdough bread, chickpeas, pomegranate juice, nuts, and olive oil, which can be part of personalized dietary interventions targeting youth obesity thanks to their nutritional profile and phytochemical content. Using UHPLC-MS^n, a total of 93 phenolic compounds across 12 subclasses were identified, with notable differences in both the number and concentration of compounds among food products. The diversity of phenolic compounds suggests potential synergistic effects, which may contribute to the bioactive profile of the Mediterranean diet and its association with health-promoting outcomes. This work provides a comprehensive reference for the phenolic composition of Mediterranean diet foods, supporting their selection in nutritional interventions.

1. Introduction

Obesity is defined as a disproportionate body weight for height, with an excessive accumulation of adipose tissue usually accompanied by mild chronic systemic inflammation. Abdominal fat accumulation, characteristic of obesity, is strongly associated with cardiovascular disease, diabetes, hypertension, and cancer [1]. The statistics about overweight and obesity levels and trends are overwhelming [2]: adult prevalence has increased by 138% for obesity and 51% for overweight since 1975, and almost 60% of adults were living with overweight or obesity in Europe in 2022. Similarly, overweight and obesity are increasing among children, with rates of overweight (including obesity) varying from 7.9% in children under 5years old up to 29.5% in the age group 5-9 years, with 24.9% in the age group 10-19 years. For the age groups 5-9 and 10-19 years, overweight and obesity were more prevalent in males and in the countries of the Mediterranean basin [2]. Tackling obesity in youth is required as it may persist into adulthood, leading to more serious health consequences at both individual and population levels [2–5].

Global awareness of the relationship between dietary habits, health status, and life expectancy has increased due to numerous nutrition studies investigating the effects of different dietary patterns on multiple health outcomes, like obesity [6–9]. The Mediterranean diet is among the most extensively studied dietary patterns. The term Mediterranean

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diet was coined by Ancel Keys in 1960 and refers to the traditional eating habits in countries bordering the Mediterranean Sea [10]. The main characteristics of this dietary pattern are a greater intake of extra virgin olive oil, whole cereal grains, fruits, vegetables, legumes, and nuts; a low to moderate intake of dairy products, red meat, and red wine; as well as a low intake of sweets and eggs [11]. The Mediterranean diet is considered one of the healthiest diets in the world, with demonstrated large beneficial properties towards the prevention of obesity, cardiovascular diseases, and type II diabetes [10], due to its balanced nutritional profile and high intake of dietary fibre and phytochemicals, mainly phenolic compounds (aka (poly)phenols) [11].

A wide range of (poly)phenolic compounds are found across the main food groups of the Mediterranean diet. These include flavonoids (such as flavan-3-ol monomers, flavonols, anthocyanins, and flavanones) and phenolic acids, which exert diverse biological activities, including, among others, analgesic, anti-inflammatory and gut microbiota-modulating effects, and may contribute to the Mediterranean diet's protective role against metabolic disorders, including obesity [12–15]. There is a considerable body of knowledge indicating that dietary (poly)phenols, such as flavonoids, phenolic acids, tannins, stilbenes or lignans, can be effective in managing obesity in adulthood by regulating lipid metabolism and adipogenesis, among others [16].

Beyond the individual biological activities of distinct (poly)phenolic subclasses, an emerging body of evidence indicates that combinations of phenolic compounds can interact synergistically to enhance their functional properties. A comprehensive review by Mitra et al. [17] demonstrated that (poly)phenols exhibit synergistic and additive effects across multiple bioactivities, including modulation of cellular pathways related to inflammation and metabolic regulation, when present in complex mixtures commonly found in foods, rather than as isolated compounds [17]. Although the review only included *in vitro* studies, preclinical *in vivo* evidence has also shown synergistic interactions between anthocyanin and flavanones, resulting in enhanced antinociceptive effects in a murine model [18]. Such findings support the concept that the chemical diversity inherent to Mediterranean diet foods may enhance their biological efficacy compared to isolated compounds.

Differently, there is a lack of solid evidence about the health effects of the Mediterranean diet in the younger population (< 18 years). A few observational studies reported an association between high adherence to the Mediterranean diet and decreased risk of experiencing overweight, obesity, and metabolic syndrome in adolescents living in Mediterranean countries [19,20], but nutritional interventions assessing the effect of the Mediterranean diet on obesity and cardiometabolic risk factors in adolescents from different Mediterranean countries are missing.

The MED4Youth study [21], a multicentric intervention targeting adolescents (11–17 years) with obesity/overweight from Spain, Italy, and Portugal, aimed to assess whether an energy-restricted Mediterranean diet including healthy products from the Mediterranean basin (extra virgin olive oil, mixed nuts, pomegranate, chickpeas, and multigrain sourdough bread) is more effective against youth obesity than a conventional low-fat diet [22].

Despite the large body of evidence linking (poly)phenol intake to health outcomes, most studies rely on estimates derived from food composition databases. These databases cannot fully capture the variability introduced by cultivar differences, processing methods, or the specific formulations used in dietary interventions [23], introducing errors or biases in exact intake estimates. To address this limitation, the present study provides, for the first time, a direct UHPLC-MS^n characterization of phenolic compounds in the foods supplied within the MED4Youth clinical trial. This characterization ensures accurate quantification of the (poly)phenol profile that participants will consume, accounting for geographical origin, processing, and formulation. By generating analytical data that reflects the real composition of these products, the study goes beyond existing food composition resources and creates the basis for a more precise evaluation of the potential contribution of (poly)phenols to the intervention's outcomes.

Beyond its relevance to the MED4Youth trial, the generation of a comprehensive and analytically verified phenolic dataset has broader implications for nutritional science. First, it enhances the reproducibility and transparency of dietary interventions by providing detailed compositional information on the exact foods administered, thereby facilitating comparison across studies. Second, it contributes to more accurate exposure assessments, reducing uncertainty associated with database-derived estimates. Third, such extensive compositional data may support the refinement and updating of food composition databases.

In this context, we hypothesize that the accurate characterization and quantification of phenolic compounds in key Mediterranean foods, such as extra virgin olive oil, nuts, pomegranate, chickpeas, and multigrain sourdough bread, will enable the establishment of meaningful dose–response relationships between (poly)phenol intake and improvements in anthropometric measurements and cardiometabolic risk factors in adolescents.

This study therefore aimed to perform a detailed UHPLC-MS^n characterization and quantification of the phenolic compounds present in the healthy products included in the Mediterranean diet intervention of MED4Youth. By directly analysing the intervention foods, this work represents one of the most comprehensive initiatives to date in assessing the (poly)phenol composition of Mediterranean diet products within a clinical trial in adolescents with obesity, thereby providing novel insights beyond existing composition databases.

2. Materials and methods

2.1. Chemicals and solvents

All chemicals and solvents used in this study were of analytical grade. Sodium hydroxide was purchased from Alfa Aesar Chemicals (Thermo Fisher Scientific Inc., MA, USA) and citric acid from Sigma-Aldrich (Merck KGaA, DA, Germany). HPLC-grade solvents and reagents were purchased from VWR International (Radnor, PA, USA). Gallic acid, syringic acid, ferulic acid, 3-hydroxybenzoic acid, daidzein, secoisolariciresinol, ellagic acid, catechin, epicatechin, epigallocatechin-3-*O*-gallate, quercetin, quercetin-3-*O*-rutinoside, luteolin, apigenin, naringin, naringenin, delphinidin-3-*O*-glucoside, and pelargonidin-3-*O*-glucoside were purchased from Sigma-Aldrich (St. Louis, MO, USA). Punicalin (A + B mixture), punicalagin (A + B mixture), procyanidin B2, epigallocatechin, and epicatechin-3-*O*-gallate were acquired from Phytolab phyproof, reselled by Sigma-Aldrich (Heidelberg, Germany). Vanillic acid was purchased from Alfa Aesar Chemicals and *p*-coumaric acid from Honeywell Fluka™ Chemicals (Thermo Fisher Scientific Inc., MA, USA). Cyanidin-3-*O*-glucoside was acquired from Extrasynthese (Genay, France), oleuropein aglycone and hydroxytyrosol from Toronto Research Chemicals Inc. (ON, Canada).

2.2. Foods from the Mediterranean basin

The multigrain rebola sourdough bread was manufactured and distributed by NOVAPAN (La Puebla de Alfindén, Zaragoza, Spain). Canned chickpeas and pomegranate juice were produced and distributed by Shikma Field Crops (Kibbutz, Mishmar HaNegev, Israel). Raw walnuts, almonds, hazelnuts, and extra virgin olive oil were provided by COSELVA (La Selva del Camp, Tarragona, Spain). It must be highlighted that almonds and hazelnuts were provided with skin, which contains a large amount of phenolic compounds.

2.3. Sample processing

Extraction protocols were optimized for each food matrix based on their physicochemical characteristics and validated literature methodologies. Consequently, quantitative phenolic values are intended for matrix-specific characterization and dietary exposure estimation rather

than for direct analytical comparison across different food categories.

2.3.1. Sourdough bread and chickpea

The preparation of sourdough bread and chickpea samples was performed following the method reported by Dall'Asta et al. with the following modifications [24]. Briefly, 1.5 g of sample (sourdough bread or chickpea) was finely ground using a mixer. The powder of each sample was diluted with 10 mL of a 70/30 methanol/water solution (v/v) acidified with 1% of formic acid, and then vortexed for 30 sec, sonicated for 10 min, vortexed again for 30 sec, and finally centrifuged at 15900 x *g* for 5 min. After centrifugation, the supernatant was collected, and the remaining pellet was reextracted, revortexed, and recentrifuged as above. The resulting supernatant was added to the previous one. About 1 mL of extract was dried on a speed vacuum (Savant SPD121P, Thermo Fisher Scientific Inc.) overnight, reconstituted in 0.5 mL of a 70/30 methanol/water solution (v/v) acidified with 1% of formic acid, and then filtered through a 0.45μm PVDF filter prior to UHPLC-MS^n analysis. All extractions were performed in triplicate, with each replicate consisting of sample taken from a different package.

For the extraction of phenolic compounds bound to the fibre fraction in the sample, the method of Dall'Asta et al. was followed [25]. Briefly, the residual pellet was further hydrolysed with 1.5 mL of a sodium hydroxide solution 2 M. Following the addition of the sodium hydroxide solution, the mixture was vortexed for 30 sec and left at room temperature for 1 h. After alkaline hydrolysis, the mixture was vortexed again for 30 sec, and then its pH was adjusted to 3 by adding 1.35 mL of a citric acid solution 3 M. Bound phenolics were extracted with 6 mL of ethyl acetate. Then, 1 mL of ethyl acetate extract was dried on a speed-vacuum overnight and reconstituted in 0.5 mL of a 70/30 methanol/water solution (v/v) acidified with 1% of formic acid, and then filtered through a 0.45μm PVDF filter prior to UHPLC-MS^n analysis. All extractions were performed in triplicate.

2.3.2. Nuts

Sample preparation was adapted from previously published procedures for nut phenolic extraction with minor modifications [26,27]. In brief, 2.5 g of each nut (almond, hazelnut, and walnut) were finely ground using a pestle and mortar. Nut powders were first diluted with 2.5 mL of water for one night to improve the extraction. Then, the solution was defatted by adding 5 mL of hexane, vortexing for 30 sec, sonicating for 10 min, centrifuging at 15900 x *g* for 5 min, and eliminating the hexane supernatant. This process has been repeated 3 times. Thus, the defatted powders were extracted with 5 mL of methanol, sonicated for 10 min, and centrifuged at 15900 x *g* for 5 min. Then, 1.5 mL of supernatant was collected, dried in a speed-vacuum and reconstituted in 1 mL a 70/30 methanol/water solution (v/v) acidified with 1% of formic acid, and then filtered through a 0.45 μm PVDF filter prior to UHPLC-MS^n analysis. The extraction of bound phenolic compounds has been performed as described in the previous section. All extractions were performed in triplicate, with each replicate consisting of sample taken from a different package.

2.3.3. Pomegranate juice

Pomegranate juice samples were centrifuged at 15900 x *g* for 5 min, diluted 20 times with a 70/30 methanol/water solution (v/v) acidified with 1% of formic acid and then filtered through a 0.45 μm PVDF filter prior to injection in the UHPLC-MS system, according to previous methodologies [28,29]. All extractions were performed in triplicate, with each replicate consisting of sample taken from a different package.

2.3.4. Extra virgin olive oil

For extra virgin olive oil samples, the extraction method developed by Lozano-Castellon and colleagues was applied, with some modifications [30]. Briefly, 1 mL of olive oil was diluted with 1 mL of hexane and then extracted with 2 mL of a 70/30 methanol/water solution (v/v) acidified with 1% formic acid. Samples were vortexed vigorously and centrifuged at 15900 x *g* for 5 min. The methanolic phase was collected, and the remaining hexane phase was re-extracted with 2 mL of the same methanolic solution, vortexed and centrifuged as before. The hexane phase was discarded, and the two methanolic phases were combined and reextracted (cleaned) with 1 mL of hexane to remove any lipophilic residues. After vortexing and centrifugation, 1.5 mL of the resulting methanolic phase was collected and dried in the speed-vacuum overnight. Samples were reconstituted in 0.1 mL of a 70/30 methanol/water solution (v/v) acidified with 1% formic acid prior to UHPLC-MS^n analysis. All extractions were performed in triplicate, with each replicate consisting of sample taken from a different package.

2.4. Qualitative and quantitative analysis through UHPLC-MS^n

The extracts were analysed by ultra-high-performance liquid chromatography (UHPLC) coupled with mass spectrometry (MS), following the method previously reported by Mena et al. with some modifications [28]. Briefly, an Accela UHPLC 1250 apparatus equipped with a linear ion trap mass spectrometer (LIT-MS) (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-ESI (H-ESI-II) probe was used. Separation was carried out by means of an Acquity HSS T3 column (100 × 2.1mm; 1.8μm particle size; Waters, Milford, MA, USA) installed with its precolumn cartridge and maintained at 40°C. The mobile phase, pumped at a flow rate of 0.4 mL/min, consisted of a mixture of acidified acetonitrile (0.1% formic acid) (solvent A) and acidified water (0.1% formic acid) (solvent B). Following 0.5 min of 1% solvent A in B, the proportion of A was increased linearly to 80% over 9 min and maintained for 1.5 min, and the starting conditions were re-established in 0.5 min and maintained for 2 min to re-equilibrate the column, for a total run time of 13 min.

Samples were analysed in both negative ionization mode to identify most (poly)phenols and positive ionization mode for anthocyanins. In negative ionization mode, the MS worked with a capillary temperature of 275°C and a source heater temperature of 200°C. The sheath gas (N_2) flow rate was set at 40 (arbitrary units) and the auxiliary gas (N_2) flow rate at 5. The source voltage was 4kV, the capillary voltage was -42 V, and tube lens voltage was -118 V. Analyses were carried out using full scan, data-dependent MS^2 and MS^3 scanning from *m/z* 100–2000, with collision induced dissociation (CID) equal to 35 (arbitrary units). Pure helium gas was used for CID. To detect anthocyanins, the capillary temperature was set at 275°C and the source heater temperature at 300°C. The sheath gas flow rate was set at 40, and the auxiliary gas flow rate at 5. The source voltage was 4.5 kV, and the capillary and tube lens voltage were 20 and 95 V, respectively. Analyses were carried out using full scan, data-dependent MS^2 and MS^3 scanning from *m/z* 100–2000, with collision induced dissociation (CID) equal to 35 (arbitrary units). The extensive profiling was conducted based on previous literature for each food item [28,31–36], targeting all predefined compounds, which were subsequently grouped into 12 chemical subclasses for clarity. For compounds without authentic standards, quantification was performed using calibration curves of structurally related standards from the same phenolic subclass (semi-quantification), based on full MS peak areas. Lignan and isoflavone derivatives were quantified with secoisolariciresinol and daidzein, respectively. Additional phenolic compounds that were analysed but found to be below the limit of detection (LOD) or limit of quantification (LOQ) were not included in the reported results. Results were expressed as mg per 100 g of fresh weight for sourdough bread, chickpeas and nuts, and mg per 100 mL for pomegranate juice and extra virgin olive oil.

3. Results and discussion

3.1. Qualitative analysis of phenolic compounds

The characterization of the phenolic profile of the different foods led

to the identification of 93 compounds. Ellagitannins accounted for the largest number of identified compounds, followed by hydroxycinnamic acids, hydroxybenzoic acids, and procyanidins. Flavonoids (anthocyanins, flavan-3-ol monomers, flavanones, flavones, flavonols, isoflavones), lignans, and tyrosols were also found (Fig. 1).

Up to 27 phenolic compounds were identified by comparison with authentic standards. The remaining 66 compounds were tentatively identified interpreting the information on their retention time (RT), molecular ions, and fragmentation patterns obtained from MS^2 and MS^3 experiments, by comparison with an in-house database. RT and mass spectrum data, along with peak assignments for identified compounds, are described in Table 1.

3.2. Quantitative analysis of phenolic compounds

3.2.1. Sourdough bread

Sourdough bread is one of the most important baked goods derived from cereal fermentation. In Mediterranean countries (Italy, Greece, Spain, and Portugal), the annual per capita intake of bread is about 100kg, and sourdough bread is a highly appreciated product [37]. The analysis of sourdough bread enabled identification and quantification of (poly)phenolic compounds from flavan-3-ol monomer, flavonol, hydroxybenzoic acid, hydroxycinnamic acid, ellagitannin, and lignan subclasses (Table 2).

The total amount of phenolic compounds in sourdough bread was 80.2mg/100 g, within the range proposed by Palla et al. [38], 68 to 108 mg/100 g, after assessing five different cereal wholegrain flours during sourdough fermentation with various yeast strains. In this sense, it provides approximately 40.1 mg of phenolic compounds per serving (serving size: 50 g). It should be noted that the cited study quantified total phenolics using the Folin–Ciocalteu method, whereas our study reports chromatographic quantification of individual phenolic compounds. Therefore, while the values are roughly comparable, differences in analytical approach, including the potential contribution of non-phenolic reducing substances in the Folin assay, may influence the comparability of absolute concentrations.

The semi-quantitative approach used for some compounds assumes comparable ionization efficiency and detector response between the target compound and the reference standard, which may not fully reflect compound-specific behaviour in the mass spectrometer. Consequently, concentrations obtained for these compounds should be interpreted as approximate estimates rather than absolute values. While this may introduce a degree of uncertainty in absolute intake calculations, the approach remains suitable for comparative profiling and relative exposure estimation within the context of this analytical study.

Moreover, it is well established that the different cereal varieties and yeast result in significant variations in phenolic amount [38,39]. The most representative (poly)phenol subclass was hydroxycinnamic acids (75% of the total amount), mainly due to ferulic acid and its derivates. Ferulic acid is a phenolic acid with some potential health benefits, mostly due to its preventive potential against several non-communicable disorders, such as cardiovascular diseases [40–42]. Moreover, at the pre-clinical level, Wang et al. have demonstrated the ability of ferulic acid consumption, combined with exercise, to manage dietary-induced obesity in high-fat diet-fed mice, as it reduced body weight and regulated the lipid contents in serum, liver, and skeletal muscle [43]. In addition, the multigrain sourdough bread analysed herein has been demonstrated, in rats, to decrease the circulating glucose levels postprandially and the blood concentrations of the pro-inflammatory marker monocyte chemoattractant protein-1 (MCP-1) in fasting conditions, in comparison to a refined wheat bread with no sourdough content [44].

3.2.2. Chickpea

Chickpeas are among the most consumed legumes, especially in the Mediterranean area, with a daily consumption between 8 and 23 g per capita [45]. These legumes are an excellent source of energy, plant-derived proteins, minerals, vitamins, fibre, and contain phytochemicals. Their consumption has been associated with potential health benefits, including the reduction of cardiovascular, diabetic and cancer risk [46,47]. The analysis of canned chickpeas allowed the identification and quantification of (poly)phenolic compounds belonging to isoflavone and hydroxybenzoic acid subclasses (Table 3).

The concentration of phenolic compounds in chickpeas was 22.4 mg/100 g cooked weight, in line with the literature and despite the influence of the variety of chickpea on the phenolic content [48,49]. In this sense, it provides approximately 22.4 mg of phenolic compounds per serving (serving size: 100 g). This concentration was represented mainly by hydroxybenzoic acids (85%), in accordance with previous studies that identified these as the predominant phenolic compounds [34]. Several studies have reported the potential of hydroxybenzoic acids and their metabolites against obesity and cardiopathies due to their ability to reduce total cholesterol, triglycerides, free fatty acids, and phospholipids [50,51]. Interestingly, isoflavones, rarely present in European diets as consumed mainly through soy products, also contributed to the phenolic profile of chickpeas [52].

3.2.3. Nuts

Nuts are an important component of the Mediterranean diet,

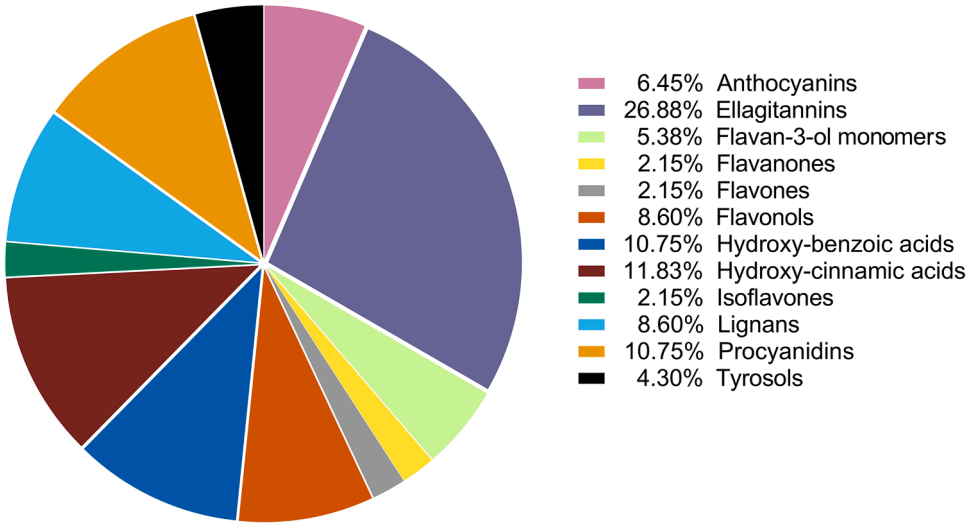


Fig. 1. Distribution of identified phenolic compounds per subclass in the food products.

Table 1
Tentative and confirmed identification of phenolic compounds by UHPLC-MS^n.

Compound*	RT (min)	[M-H]$^-$ or [M]$^+$ (*m/z*)	MS^2 ion fragments (*m/z*)	MS^3 ion fragments (*m/z*)
Flavan-3-ol monomers				
Epigallocatechin	3.79	305	221, 219, 179	
Catechin	3.95	289	245, 205, 179	
Epicatechin	4.21	289	245, 205, 179	
Epigallocatechin-3-*O*-gallate	4.26	457	331, 305, 169	125
Epicatechin-3-*O*-gallate	4.77	441	331, 289, 169	245, 205, 179
Flavonols				
Hydroxykaempferol-diglucoside	4.12	625	463, 301	301, 255, 139
Dihydroxykaempferol-glucoside	4.22	449	431, 287, 269, 259	269, 259, 243
Isorhamnetin-diglucoside	4.32	639	621, 529, 459	487,469, 459, 277, 179
Quercetin-3-*O*-rutinoside	4.56	609	301, 300, 271, 179	179, 151, 273, 193, 257, 283
Kaempferol-3-*O*-rutinoside	4.81	593	547, 285	267, 257, 241
Kaempferol-glucoside	4.98	447	327, 285, 255	267, 257, 241
Quercetin-rhamnoside	5.03	447	301, 300	271, 255, 243
Quercetin	5.96	301	273, 257, 179, 151, 107	
Flavones				
Luteolin	5.95	285	241, 217, 199, 175	199, 145
Apigenin	6.42	269	225, 201, 149	
Flavanones				
Naringin	4.98	579	271	151, 125
Naringenin	6.37	271	151, 125, 107	107
Isoflavones				
Methyl-isoflavone isomer I	6.11	283	267, 239	239, 223
Methyl-isoflavone isomer II	6.94	283	267, 239	239, 223
Anthocyanins				
Delphinidin-3,5-*O*-diglucoside	3.17	627	465, 303	303
Cyanidin-3,5-*O*-diglucoside	3.34	611	449, 287	287
Delphinidin-3-*O*-glucoside	3.53	465	303	257, 229
Pelargonidin-3,5-diglucoside	3.57	595	433, 271	271
Cyanidin-3-*O*-glucoside	3.72	449	287	223, 205
Pelargonidin-3-*O*-glucoside	3.93	433	271	
Hydroxycinnamic acids				
Caffeic acid-glucoside	3.51	341	179	135
Ferulic acid-glucoside	3.71	355	193, 177, 133	177, 149, 133
Coumaric acid-glucoside	3.90	325	163, 119	119, 93
Rosmarinic acid-diglucoside	4.17	683	521, 359	359
***p*-coumaric acid**	4.52	163	119, 93	
Ferulic acid	4.76	193	133, 119	
Dihydroferulic acid-glucoside	4.93	357	195, 151, 135	151, 135
Dimeric ferulic acid isomer I	5.54	385	341, 193	
Dimeric ferulic acid isomer II	5.86	385	341, 313, 193	
Trimeric ferulic acid isomer I	5.92	577	533, 355, 311, 193	
Trimeric ferulic acid isomer II	6.27	577	489, 311, 193	
Hydroxybenzoic acids				
Gallic acid	2.94	169	141, 125, 81	125, 97, 81
Vanillic acid-hexoside-pentoside	3.11	461	329, 167	152
Dihydroxybenzoic acid-glucoside	3.20	315	153, 109	109
Hydroxybenzoic acid-hexoside- pentoside	3.26	431	299, 137, 93	137, 93
Dihydroxybenzoic acid-hexoside-deoxyhexoside	3.29	461	315, 153	
Vanillic acid	3.33	167	152, 123, 108	123, 108
Dihydroxybenzoic acid-hexoside-pentoside	3.43	447	315, 271, 207, 163	225, 153, 108
Methyl-gallate	4.07	183	169, 151	139, 125
Syringic acid	4.33	197	153, 137	
3-hydroxybenzoic acid	4.79	137	93	
Ellagitannins				
Punicalagin like	0.70	1083	781, 721, 601, 575	299, 271, 243
HHDP-glucose isomer I	1.38	481	421, 301, 275	257, 229, 185
HHDP-glucose isomer II	2.07	481	301, 275	257, 229, 185
Monogalloyl-glucose isomer I	2.88	331	271, 169	125
Digalloyl-HHDP-glucose isomer I	2.98	483	331, 313, 169	271, 193, 169
Punicalin	2.98	781	601	299, 271
Monogalloyl-glucose isomer II	3.23	331	271, 169	125
DiHHDP-glucose isomer I	3.25	783	481, 301	283, 257, 229, 185
Digalloyl-HHDP-glucose isomer II	3.26	483	331, 313, 169	271, 193, 169
Punicalagin A	3.44	1083	781, 721, 601, 575	299, 271, 243, 187
DiHHDP-glucose isomer II	3.57	783	481, 301	283, 257, 229
Galloyl-HHDP-glucose isomer I	3.59	633	301, 275	257, 229, 185
Trigalloyl-HHDP-glucose isomer I	3.60	951	907, 783, 481	783, 481, 301
Punicalagin B	3.63	1083	781, 721, 601, 575	601, 299, 271
Punigluconin	3.74	799	781, 479, 301	
DiHHDP-glucose isomer III	3.77	783	481, 301	283, 257, 229, 185
Trigalloyl-HHDP-glucose isomer II	3.77	951	907, 783, 481	783, 481, 301
Galloyl-HHDP-glucose isomer II	3.96	633	301, 275	257, 229, 185
Trigalloyl-HHDP-glucose isomer III	4.00	951	907, 783, 481	783, 481, 301
Tetragalloyl-glucose isomer I	4.04	787	635, 617, 465	465, 169
Ellagic acid-glucoside	4.11	463	301, 273	257, 229, 185, 173
Trigalloyl-glucoside	4.23	635	483, 465, 313, 169	
Ellagic acid-arabinoside	4.42	433	301, 273	257, 185, 173
Tetragalloyl-glucose isomer II	4.54	787	635, 617	465, 169
Ellagic acid	4.74	301	257, 229, 185, 173	
Lignans				
Guaiacyl-(8-*O*-4)-ferulate-hexoside	4.10	551	389, 341, 193	341, 193
Secoisolariciresinol-diglucoside isomer I	4.35	685	523, 361	361
Secoisolariciresinol-diglucoside isomer II	4.46	685	523, 361	361
Dimethyl-secoisolariciresinol-glucoside	4.70	535	373, 329, 193	329, 193
Secoisolariciresinol-glucoside	4.74	523	361	345, 313, 165

(*continued on next page*)

Table 1 (*continued*)

Compound*	RT (min)	[M-H]$^-$ or [M]$^+$ (*m/z*)	MS^2 ion fragments (*m/z*)	MS^3 ion fragments (*m/z*)
Hydroxysecoisolariciresinol	5.23	373	355, 213, 179, 113	
Pinoresinol	5.50	357	341, 325, 313	299
Hinokinin	5.77	353	337, 293	323, 293, 279
Tyrosols				
Hydroxytyrosol	3.44	153	123, 109	123, 95
Oleacin	6.04	319	301, 251, 195, 165, 123, 121	
Oleuropein aglycone	6.87	377	345, 307, 275, 195	275, 179, 139
Hydroxytyrosol acetate	6.89	195	153, 151, 135	123, 93
Procyanidins				
Procyanidin dimer B-type	3.72	577	559, 451, 425, 407, 289	289
Procyanidin trimer	3.92	865	577, 449, 407	407, 289
Procyanidin trimer A-type	3.95	863	711, 693, 573	451, 289
Procyanidin dimer gallate B-type	4.13	729	577, 559, 451, 289	289, 245
Procyanidin dimer isomer I	4.25	561	543, 435, 289	289
Procyanidin tetramer B-type	4.37	1153	1135, 865, 577	289
Procyanidin pentamer	4.71	1441	1315, 1153, 577	577, 289
Prodelphinidin trimer	4.79	881	467, 451, 433, 289	289
Procyanidin dimer isomer II	4.92	593	467, 441, 305	289, 245
Procyanidin dimer A-type	5.15	575	449, 423, 407, 289	407, 289

*HHDP, Hexahydroxydiphenoyl. Compounds in bold were identified using authentic standards. Lignan and isoflavone derivatives were quantified with secoisolariciresinol and daidzein, respectively. Anthocyanins were analysed in positive mode.

considered nutrient-dense foods containing health-promoting compounds, including unsaturated fatty acids, proteins, fibre, fat-soluble vitamins, minerals, phytosterols, and (poly)phenols [53]. The anti-obesity and cardioprotective properties of nut consumption have been extensively studied. In this sense, a recent observational study has demonstrated an inverse relationship between regular nut consumption and the prevalence of abdominal obesity and metabolic syndrome [54]. Moreover, Larsson et al. demonstrated the association between nut consumption, reduced atrial fibrillation risk,and possible heart failure [55,56].

Regarding almonds, diverse phenolic compounds belonging to flavan-3-ol monomer, flavanone, flavonol, hydroxybenzoic acid and procyanidin subclasses were identified and quantified (Table 4).

The total amount of phenolic compounds quantified in almonds was 21.0 mg/100 g. Thereby, a serving of it provides approximately 5.9 mg of phenolic compounds (serving size: 28 g). These values align with those obtained in previous characterizations of phenolics in the same nut type [36]. Differences in the concentration of single compounds among studies could be related to variety and harvest season [57]. Hydroxybenzoic acids and flavan-3-ol monomers were the most representative phenolic subclasses, with abundances of 47% and 36% of the total, respectively. As the health-promoting properties of hydroxybenzoic acids have been previously discussed, those of flavan-3-ols will be addressed in this section. For instance, a study conducted in mice fed a high-fat diet showed that epigallocatechin-gallate and catechin could

Table 2
Phenolic composition of sourdough bread.

Compound	Content (mg/100 g; Mean ± SD; n=3)
Flavan-3-ol monomers	
Catechin	0.35 ± 0.01
Total flavan-3-ol monomers	0.35 ± 0.01
Flavonols	
Quercetin-3-*O*-rutinoside	0.44 ± 0.02
Kaempferol-glucoside	0.03 ± 0.00
Dihydroxykaempferol-glucoside	0.06 ± 0.01
Quercetin	0.15 ± 0.02
Quercetin-rhamnoside	0.44 ± 0.05
Total flavonols	1.14 ± 0.01
Hydroxybenzoic acids	
Vanillic acid	6.00 ± 0.34
Syringic acid	6.29 ± 0.48
Total hydroxybenzoic acids	12.29 ± 0.82
Hydroxycinnamic acids	
Coumaric acid-glucoside	0.10 ± 0.00
Caffeic acid-glucoside	0.29 ± 0.00
Ferulic acid	9.87 ± 0.17
Dimeric ferulic acid isomer I	11.68 ± 0.16
Dimeric ferulic acid isomer II	12.83 ± 0.08
Trimeric ferulic acid isomer I	7.33 ± 0.26
Trimeric ferulic acid isomer II	6.67 ± 0.13
Ferulic acid-glucoside	10.75 ± 0.71
Rosmarinic acid-diglucoside	0.04 ± 0.00
Dihydroferulic acid-glucoside	0.22 ± 0.02
Total hydroxycinnamic acids	59.80 ± 0.23
Ellagitannins	
Ellagic acid	0.43 ± 0.02
Total ellagitannins	0.43 ± 0.02
Lignans	
Secoisolariciresinol-diglucoside isomer I	4.59 ± 0.76
Secoisolariciresinol-diglucoside isomer II	0.22 ± 0.04
Secoisolariciresinol-glucoside	0.07 ± 0.01
Dimethyl-secoisolariciresinol-glucoside	0.20 ± 0.00
Hydroxysecoisolariciresinol	0.25 ± 0.03
Guaiacyl-(8-*O*-4)-ferulate-hexoside	0.7 ± 0.16
Hinokinin	0.18 ± 0.01
Total lignans	6.22 ± 0.99

Table 3
Phenolic composition of chickpea.

Compound	Content (mg/100 g; Mean ± SD; n=3)
Isoflavones	
Methyl-isoflavone isomer I	3.13 ± 0.14
Methyl-isoflavone isomer II	0.24 ± 0.02
Total isoflavones	3.37 ± 0.16
Hydroxybenzoic acids	
Dihydroxybenzoic acid-hexoside-pentoside	1.72 ± 0.07
Dihydroxybenzoic acid-hexoside-deoxyhexoside	0.78 ± 0.04
Hydroxybenzoic acid-hexoside-pentoside	13.61 ± 0.13
Vanillic acid-hexoside-pentoside	0.28 ± 0.00
Vanillic acid	2.61 ± 0.01
Total hydroxybenzoic acids	19.00 ± 0.18

modulate the gut microbiota and liver metabolome to prevent obesity [58]. Moreover, flavan-3-ols consumption may mediate vascular protective effects by modulating gene expression and DNA methylation [59].

Hazelnuts contained flavan-3-ol monomers, flavanones, flavonols, hydroxybenzoic acids and procyanidins (Table 5).

The total amount of phenolic compounds quantified in hazelnut was 17.6 mg/100 g. In this sense, a serving of it provides approximately 4.8 mg of phenolic compounds (serving size: 28 g). Although there is considerable diversity in the content of these compounds among cultivars, the values obtained were similar to the ones obtained previously by other researchers [36,60]. Procyanidins were the most representative compounds, with 44% of the total amount. These compounds are

Table 4
Phenolic composition of almond.

Compound	Content (mg/100 g; Mean ± SD; n=3)
Flavan-3-ol monomers	
Epicatechin	1.68 ± 0.18
Catechin	5.28 ± 0.08
Epigallocatechin-3-*O*-gallate	0.56 ± 0.02
Total flavan-3-ol monomers	7.52 ± 0.07
Flavanones	
Naringenin	0.04 ± 0.00
Total flavanones	0.04 ± 0.00
Flavonols	
Kaempferol-3-*O*-rutinoside	0.57 ± 0.04
Kaempferol-glucoside	0.01 ± 0.00
Isorhamnetin-diglucoside	0.04 ± 0.00
Total flavonols	0.62 ± 0.03
Hydroxybenzoic acids	
Gallic acid	0.87 ± 0.05
Dihydroxybenzoic acid-glucoside	0.12 ± 0.00
Vanillic acid	8.86 ± 0.04
Total hydroxybenzoic acids	9.84 ± 0.01
Procyanidins	
Procyanidin dimer isomer I	0.21 ± 0.03
Procyanidin dimer A-type	0.40 ± 0.00
Procyanidin dimer B-type	0.51 ± 0.07
Procyanidin dimer gallate B-type	0.22 ± 0.01
Procyanidin trimer A-type	0.20 ± 0.01
Procyanidin trimer	0.34 ± 0.02
Prodelphinidin trimer	0.18 ± 0.00
Procyanidin tetramer B-type	0.45 ± 0.03
Procyanidin pentamer	0.46 ± 0.01
Total procyanidins	2.97 ± 0.05

Table 5
Phenolic composition of hazelnut.

Compound	Content (mg/100 g; Mean ± SD; n=3)
Flavan-3-ol monomers	
Epigallocatechin	2.01 ± 0.08
Catechin	0.64 ± 0.01
Epigallocatechin-3-*O*-gallate	0.61 ± 0.03
Epicatechin-3-*O*-gallate	1.03 ± 0.01
Total flavan-3-ol monomers	4.30 ± 0.05
Flavanones	
Naringenin	0.08 ± 0.00
Total flavanones	0.08 ± 0.00
Flavonols	
Kaempferol-glucoside	0.18 ± 0.02
Quercetin-rhamnoside	0.38 ± 0.01
Total flavonols	0.56 ± 0.02
Hydroxybenzoic acids	
Gallic acid	1.24 ± 0.02
Methyl-gallate	0.12 ± 0.03
Dihydroxybenzoic acid-glucoside	1.20 ± 0.01
Vanillic acid	2.48 ± 0.20
Total hydroxybenzoic acids	5.04 ± 0.20
Procyanidins	
Procyanidin dimer isomer I	0.67 ± 0.03
Procyanidin dimer A-type	1.03 ± 0.06
Procyanidin dimer B-type	1.62 ± 0.01
Procyanidin dimer isomer II	1.20 ± 0.02
Procyanidin dimer gallate B-type	0.55 ± 0.03
Procyanidin trimer A-type	0.31 ± 0.01
Procyanidin trimer	0.93 ± 0.03
Prodelphinidin trimer	0.35 ± 0.01
Procyanidin tetramer B-type	0.66 ± 0.12
Procyanidin pentamer	0.49 ± 0.02
Total procyanidins	7.82 ± 0.17

characterized by their cardioprotective activity. For instance, Zhang et al. demonstrated the ability of grape seed proanthocyanidin extracts to attenuate inflammation of vasculature and endothelial dysfunction [61]. Nevertheless, the health effects of procyanidins could be more related to their microbial metabolites than to their parent structures, as they are poorly absorbed due to their high molecular weight [62].

Lastly, regarding walnuts, flavan-3-ol monomers, flavanones, flavonols, hydroxybenzoic acids, ellagitannins, and procyanidins represented the main subclasses behind their (poly)phenol profile (Table 6).

The sum of the concentration of individual compounds in walnuts was 227.5 mg/100 g. Thereby, a serving of it provides approximately 63.7 mg of phenolic compounds (serving size: 28 g). This concentration was in accordance with the concentrations reported by Slatnar et al. for walnut kernels of different cultivars [35]. Ellagitannins were the most representative compounds for this nut, with 60% of the total amount. Recently, Micucci et al. showed the cardioprotective effects of an ellagitannin-rich *Castanea sativa* extract. Using fat-induced obese rats, the study demonstrated a reduction in food intake and weight gain, a reduction of pro-inflammatory and augment of anti-inflammatory cytokines in plasma, a reduction of cholesterol and triglycerides, and normalization of gastric and intestinal contractility and cardiovascular parameters, among others [63].

3.2.4. Pomegranate juice

Pomegranate is a fruit rich in bioactive compounds, such as (poly) phenols and organic acids, which have been associated with potential health benefits, including glycaemic control and anti-inflammatory

Table 6
Phenolic composition of walnut.

Compound	Content (mg/100 g; Mean ± SD; n=3)
Flavan-3-ol monomers	
Epicatechin	14.90 ± 0.02
Epigallocatechin	18.40 ± 0.17
Catechin	2.14 ± 0.20
Total flavan-3-ol monomers	35.44 ± 0.14
Flavanones	
Naringenin	0.10 ± 0.00
Total flavanones	0.10 ± 0.00
Flavonols	
Kaempferol-3-*O*-rutinoside	2.64 ± 0.06
Total flavonols	2.64 ± 0.06
Hydroxybenzoic acids	
Gallic acid	16.36 ± 0.40
Methyl-gallate	15.89 ± 0.41
Vanillic acid	5.79 ± 0.07
Total hydroxybenzoic acids	38.03 ± 0.88
Procyanidins	
Procyanidin dimer isomer I	0.67 ± 0.00
Procyanidin dimer A-type	1.44 ± 0.04
Procyanidin dimer B-type isomer I	1.88 ± 0.04
Procyanidin dimer B-type isomer II	7.14 ± 0.16
Procyanidin dimer gallate B-type	0.75 ± 0.05
Procyanidin trimer A-type	0.23 ± 0.01
Procyanidin trimer	0.76 ± 0.03
Prodelphinidin trimer	0.27 ± 0.00
Procyanidin tetramer B-type	1.07 ± 0.05
Procyanidin pentamer	0.70 ± 0.01
Total procyanidins	14.90 ± 0.15
Ellagitannins	
Ellagic acid	8.53 ± 0.34
Ellagic acid-glucoside	0.62 ± 0.07
Ellagic acid-arabinoside	6.46 ± 0.79
HHDP-glucose isomer I	16.17 ± 0.21
HHDP-glucose isomer II	15.65 ± 0.41
Galloyl-HHDP-glucose isomer I	21.19 ± 0.43
Galloyl-HHDP-glucose isomer II	22.69 ± 0.27
Digalloyl-HHDP-glucose isomer I	6.75 ± 0.81
Digalloyl-HHDP-glucose isomer II	1.81 ± 0.05
Monogalloyl-glucose isomer I	0.83 ± 0.04
Monogalloyl-glucose isomer II	0.52 ± 0.01
Trigalloyl-HHDP-glucose isomer I	9.36 ± 0.55
Trigalloyl-HHDP-glucose isomer II	9.49 ± 0.35
Trigalloyl-HHDP-glucose isomer III	10.53 ± 0.22
Trigalloyl-glucose	6.03 ± 0.14
Tetragalloyl-glucose isomer I	1.18 ± 0.05
Tetragalloyl-glucose isomer II	1.58 ± 0.01
Total ellagitannins	139.38 ± 2.45

effects [64]. Most of the harvested fruits are intended for fresh consumption, while a smaller proportion is processed by the food industry, mainly for juice production. Regarding the phenolic composition of the studied pomegranate juice, anthocyanins, flavan-3-ol monomers, flavanones, flavonols, hydroxycinnamic acids and ellagitannins were identified (Table 7).

The total amount of phenolic compounds in pomegranate juice was 436.5 mg/100 mL, being in the range of commercial pomegranate juices [65] and in line with Wonderful cultivars, the most representative one [29]. A serving of this juice provides approximately 873.0 mg of phenolic compounds (serving size: 200 mL). Ellagitannins were the most representative family, with 85% of the total amount, followed by anthocyanins (8% of the total). Pomegranate anthocyanins have been recently proposed to prevent insulin resistance, obesity, and associated metabolic disorders, by targeting microbiota and lipid metabolism [66], but further research is needed.

3.2.5. *Extra virgin olive oil*

Extra virgin olive oil is a fundamental component of the Mediterranean diet, used for both dressing and cooking. It has been associated with different health benefits attributed to the abundance of valuable nutrients and phytochemicals, such as phenolic compounds, squalene, and terpenoids [67]. The European Union is the leading producer, consumer and exporter of olive oil, and Italy and Spain are the largest consumers of olive oil in the EU, with an annual consumption of around 500000tonnes each [68]. Regarding the phenolic composition of the extra virgin olive oil under study, flavones, hydroxybenzoic acids, lignans and tyrosols were identified (Table 8).

The concentration of phenolic compounds in this extra virgin olive oil was 14.1 mg/100 mL. In this sense, it provides approximately 1.41 mg of phenolic compounds per serving (serving size: 10 mL, ~2 teaspoons). It was relatively low in comparison to other studies, but the importance of the cultivar and ripening status on the final content must be highlighted [69–71]. The concentration found was represented mainly by tyrosols (76% of total phenolics), compounds able to reduce lipid levels in blood [72]. For example, supplementing hydroxytyrosol in its aglycone form from a standardized olive fruit extract improved endothelial function, blood pressure and circulating oxidized LDL levels in adults, showing anti-atherosclerotic properties [73].

Table 7
Phenolic composition of pomegranate juice.

Compound	Content (mg/100 mL; Mean ± SD; n=3)
Anthocyanins	
Cyanidin-3-*O*-glucoside	5.35 ± 0.05
Cyanidin-3,5-*O*-diglucoside	13.58 ± 0.05
Delphinidin-3-*O*-glucoside	2.28 ± 0.15
Delphinidin-3,5-*O*-diglucoside	3.01 ± 0.12
Pelargonidin-3-*O*-glucoside	3.71 ± 0.08
Pelargonidin-3,5-*O*-diglucoside	5.15 ± 0.16
Total anthocyanins	33.08 ± 0.09
Flavan-3-ol monomers	
Epicatechin-3-*O*-gallate	0.38 ± 0.00
Total flavan-3-ol monomers	0.38 ± 0.00
Flavanones	
Naringin	1.31 ± 0.15
Total flavanones	1.31 ± 0.15
Flavonols	
Quercetin-3-*O*-rutinoside	0.02 ± 0.00
Total flavonols	0.02 ± 0.00
Hydroxycinnamic acids	
p-coumaric acid	30.30 ± 1.27
Coumaric acid-glucoside	1.01 ± 0.05
Total hydroxycinnamic acids	31.31 ± 0.87
Ellagitannins	
DiHHDP-glucose isomer I	21.62 ± 1.36
DiHHDP-glucose isomer II	77.47 ± 0.92
DiHHDP-glucose isomer III	29.71 ± 2.95
Ellagic acid-arabinoside	2.64 ± 0.25
Ellagic acid	82.26 ± 4.43
Galloyl-HHDP-glucose isomer I	2.52 ± 0.37
Galloyl-HHDP-glucose isomer II	4.84 ± 0.53
Punicalagin A	41.54 ± 6.28
Punicalagin B	39.66 ± 3.76
Punicalagin like	47.80 ± 5.15
Punigluconin	10.26 ± 1.54
Punicalin	2.36 ± 0.19
Trigalloyl-glucoside	7.69 ± 0.87
Total ellagitannins	370.37 ± 14.59

Table 8
Phenolic composition of extra virgin olive oil.

Compound	Content (mg/100 mL; Mean ± SD; n=3)
Flavones	
Apigenin	0.63 ± 0.06
Luteolin	2.55 ± 0.20
Total flavones	3.17 ± 0.26
Hydroxybenzoic acids	
3-Hydroxybenzoic acid	0.08 ± 0.01
Total hydroxybenzoic acids	0.08 ± 0.01
Lignans	
Pinoresinol	0.12 ± 0.01
Total lignans	0.12 ± 0.01
Tyrosols	
Hydroxytyrosol	1.08 ± 0.05
Hydroxytyrosol acetate	1.86 ± 0.11
Oleuropein aglycone	5.34 ± 0.31
Oleacin	2.48 ± 0.12
Total tyrosols	10.76 ± 0.36

4. Conclusions

Comprehensive phenolic profiling of sourdough bread, almonds, hazelnuts, walnuts, chickpeas, pomegranate, and extra virgin olive through UHPLC-MS^n allowed the identification of 93 compounds across multiple subclasses, including anthocyanins, flavan-3-ol monomers, flavanones, flavones, isoflavones, hydroxybenzoic acids, hydroxycinnamic acids, ellagitannins, procyanidins, lignans, and tyrosols. The concentration ranges of these compounds were comparable to those reported in previous studies, confirming that the analysed foods are a rich source of bioactive phenolics. The diversity of phenolic compounds present in these foods may suggests potential additional/synergistic interactions, whereby the combined presence of multiple subclasses may enhance the overall bioactive profile available in the Mediterranean diet.

This study is analytical in scope and does not aim to establish clinical dose–response relationships. Instead, it provides the quantitative basis for estimating individual phenolic exposure in the MED4Youth trial using dietary intake data. In addition, it is important to consider that the biological effects of these compounds in adolescents depend on how well they are absorbed and transformed in the body. Many dietary (poly) phenols are extensively metabolized by human phase II enzymes at the intestinal and hepatic levels, while those that are not absorbed are further broken down by the gut microbiota into smaller compounds that can also enter circulation. These metabolites are likely the forms that actually exert biological effects. Because gut microbiota functionality, absorption efficiency, and metabolic capacity may vary between individuals, the health outcomes of the MED4Youth intervention should be interpreted with these factors in mind, rather than solely on the original compound content.

These results provide a solid basis for future studies on the metabolism, bioavailability, and functional effects of these compounds within the context of the Mediterranean diet.

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Declaration of competing 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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