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Mediterranean Diet vegetable foods protect meat lipids from oxidation during *in vitro* gastro-intestinal digestion

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Abstract

Meat lipids oxidation during digestion gives rise to a post-prandial oxidative stress condition, which negatively affects human health. Mediterranean Diet vegetables contain high amount of phenolic compounds, which potentially may reduce the oxidative phenomena during digestion. *In vitro* co-digestion of turkey meat with a typical Mediterranean Diet salad containing tomato, onion, black olives, extra-virgin olive oil (EVOO) and basil, dose-dependently reduced lipid peroxidation. Onion and EVOO were more effective in limiting oxidation than the other foods, resulting in negligible concentrations of lipid hydroperoxides after digestion. Specific phenolic classes dominated the phenolic profile of the different foods, such as flavonols and anthocyanins in onion, phenolic acids in tomato and basil, and tyrosol-derivatives in black olives and EVOO. The correlation between lipid peroxidation inhibition, phenolic constituents and antioxidant properties was evaluated by principal component analysis (PCA). Flavonols and anthocyanin were the major contributors to the bioactive response of vegetable foods.

Keywords: onion, mass spectrometry, lipid hydroperoxides, flavonols, anthocyanins, antioxidant activity

1. Introduction

Poly-unsaturated fatty acids peroxidation during gastro-intestinal digestion of foods is an oxidative phenomenon, which may result in the generation of toxic compounds such as lipid hydroperoxides and lipid oxidation end-products that might adversely affect human health (Nogueira et al. 2016; Perše, 2013; Sies et al., 2005).

Meat is peculiarly vulnerable to lipid oxidation due to its content of poly-unsaturated fatty acids such as linoleic, linolenic, arachidonic, and docosahexaenoic acids and high concentrations of iron catalysers (Tirosh, et al. 2015). In fact, high intakes of meat are usually associated to an increased risk of colorectal cancer and cardiovascular diseases (Sasso and Latella 2018; Ferguson, 2010; Micha et al. 2010). It has been supposed that this risk may be not caused by meat per se but a consequence of high-fat intake, generation of carcinogens during meat processing as well as oxidation of poly-unsaturated fatty acids that occurs during cooking and gastro-intestinal digestion (Ferguson, 2010; Gorelik et al. 2013; Kanner and Lapidot 2001; Martini et al. 2018). In addition, lipid peroxidation proceeds promptly when the raw meat structure is broken such as after cooking and mastication (Papuc et al. 2017).

The formation of hydroxyl (HO•) and perhydroxy (HOO•) radicals as well as the generation of perferrylmioglobin-containing peptides are considered the main pathways to free radical chain reaction initiation of lipid peroxidation during gastro-intestinal digestion of meat (Carlsen and Skibsted 2004; Oueslati et al. 2016; Tagliazucchi et al. 2010; Martini et al. 2018). Ferrous iron and dissolved oxygen may generate $O_2^{\bullet -}$, which at low pH such as found in the gastric milieu forms HOO•. Indeed, acidic disproportionation of $O_2^{\bullet -}$ may produce hydrogen peroxide (H_2O_2) and oxygen (Oueslati et al. 2016). The formation of HO• is possible by H_2O_2 decomposition, catalysed by ferrous iron (Fenton reaction), or by H_2O_2 reaction with $O_2^{\bullet -}$ (Haber–Weiss reaction) (Papuc et al. 2017). These reactive species are able to initiate lipid peroxidation by subtracting a hydrogen from lipids and generating a fatty acyl radical (L•). The resulting radical may react with dissolved oxygen to form a hydroperoxyl radical (LOO•), which can further abstract a hydrogen atom from

another unsaturated fatty acyl group (LH) producing a new fatty acyl radical (L•) and a lipid hydroperoxide (LOOH). In the presence of ferrous iron (Fe^{2+}), lipid hydroperoxide can decompose giving rise to a vast range of volatile and non-volatile compounds, collectively known as advanced lipoxidation end-products (Papuc et al. 2017).

Since meat is considered the best dietary source of essential amino acids and contains an array of important micronutrients such as iron, zinc, selenium, potassium and a range of B-vitamins, it is essential for optimal health throughout the lifecycle. Due to this, the elimination of meat from the diet does not seem to be a nutritionally concrete strategy (Binnie et al. 2014). A recent proposed strategy suggests consuming meat with foods rich in antioxidant compounds typical of the Mediterranean Diet to mitigate the production of lipid oxidation toxic compounds during meat digestion (Gorelik et al. 2013; Kanner et al. 2017).

Recently, *in vitro* and *in vivo* studies have demonstrated that oxidation during digestion of various type of meat can be reduced when is combined with Mediterranean Diet antioxidant-rich foods such as red wine, herbs, spices and extra-virgin olive oil (Gorelik et al. 2008a; Gorelik et al. 2008b; Van Hecke et al. 2017; Martini et al.,2018).

Although Mediterranean Diet pattern is often described as being low in meat intake, in the last twenty years a general increased consumption of meat (especially in pork and poultry) has been observed in Mediterranean countries (Leone et al. 2017; Chamorro et al. 2012). Nevertheless, in the typical Mediterranean cuisine meat is consumed in combination with antioxidant-rich vegetable foods such as tomatoes, onions, herbs and extra-virgin olive oil.

Therefore, this study was designed to understand if the combined consumption of a typical Mediterranean Diet salad (containing tomatoes, onions, black olives, fresh basil and extra-virgin olive oil) with grilled turkey meat could affect the oxidative phenomena during *in vitro* gastro-intestinal digestion. Vegetable foods were also characterized for their phenolic profile by LC-ESI-IT-MS/MS and for their antioxidant properties. Moreover, to gain more information about the role of phenolic compounds, co-digestions between grilled turkey meat and extracted phenolic fractions

were carried out. Finally, multivariate analysis was applied to investigate the relationships between the phenolic composition, the antioxidant properties and the lipid peroxidation inhibitory activity of tested vegetable foods.

2. Materials and methods

2.1. Materials

All of the digestive enzymes (α-amylase from porcine pancreas, pepsin from porcine gastric mucosa and pancreatin from porcine pancreas), phenolic standards and reagents for analytical determination were obtained from Sigma-Aldrich (Milan, Italy). The mass spectrometry reagents and solvents for phenolic compounds extraction were obtained from BioRad (Hercules, CA, USA). Turkey breast meat (*pectoralis major*) and vegetables were purchased in a local supermarket (Reggio Emilia, Italy).

2.2. Preparation of the Mediterranean Diet salad

The salad was prepared following the typical recipe from South Italy. The Mediterranean Diet salad contained 200 g of tomato, 25 g of pink onion, 25 g of black olives, 10 g of extra-virgin olive oil (EVOO) and 0.5 g of fresh basil. The above quantities were intended as a salad dish consumed with 100 g of cooked turkey meat. **Figure S1** shows a visual impact of the proportion of the single vegetables in the Mediterranean Diet salad and of the salad/meat proportion.

2.3. In vitro co-digestion of grilled turkey breast meat with the Mediterranean Diet salad and determination of lipid hydroperoxides

Turkey breast meat (average size of 10x15x0.4 cm) was grilled at 140°C for 5 min until complete cooking was achieved. After cooking, the meat was cooled on ice and stored at -80°C overnight. Mediterranean Diet salad was prepared by mixing the single fresh vegetables in the proportion reported above. For the digestion, 10 g of frozen meat was homogenized in a laboratory blender together with 26.05 g of the Mediterranean Diet salad. An aliquot of 5 g of the homogenate was then used for the *in vitro* digestion experiments following the protocol previously developed within the COST Action INFOGEST (Minekus et al. 2014). Simulated salivary, gastric, pancreatic and bile fluids were prepared according to Minekus et al. (2014). To simulate the oral phase, 5 g of

homogenate were mixed with 5 mL of simulated salivary fluid containing 150 U/mL of porcine α-amylase and incubated for 5 min at 37°C in a rotating wheel (10 rpm). The gastric phase was carried out by adding 10 mL of simulated gastric fluid to the bolus. The pH was adjusted to 3.0 with HCl 6 mol/L and supplemented with porcine pepsin (2000 U/mL of digest). The gastric bolus was then incubated for 120 min at 37°C in a rotating wheel (10 rpm). The intestinal digestion was carried out by adding 10 mL of pancreatic fluid (100 U trypsin activity/mL of digest) and 5 mL of bile fluid (10 mmol/L in the total digest) to the gastric bolus and adjusting the pH to 7.0. The chyme was further incubated for 120 min at 37°C in a rotating wheel (10 rpm).

At the end of the digestion, lipid hydroperoxides were extracted by 10-fold dilution in methanol HPLC grade containing 4 mmol/L of butyl-hydroxytoluene (BHT) under slow stirring for 60 min (Tagliazucchi et al. 2010). After centrifugation at 3000*g* for 15 min at 4°C, the hydroperoxides in the supernatants were determined with the FOX assay at 560 nm adapted to a microplate reader (Nourooz-Zadeh 1999; Martini et al. 2018). The FOX reagent contained 250 μmol/L of ammonium ferrous sulphate, 100 μmol/L xylenol orange, 25 mmol/L H_2SO_4, and 4 mmol/L BHT in 90% (v/v) methanol HPLC grade. For the assay, 60 μL of extracted sample were added to 140 μL of FOX reagent and incubated for 30 minutes at room temperature. The hydroperoxides content was expressed in nmol H_2O_2 equivalents per g of meat.

In the control digestion, 10 g of cooked meat were mixed with 26.05 g of distilled water (in place of the salad) and homogenized as reported above. The *in vitro* digestion was carried out exactly as reported above. At the end of the digestion lipid hydroperoxides were extracted and quantified, representing the amount of lipid hydroperoxides generated during the digestion of meat without vegetables.

The dose-response effect of the Mediterranean Diet salad was assessed by homogenising 10 g of cooked meat with 13.025 g of salad (plus 13.025 g of water) and with 6.51 g of salad (plus 19.54 g of water). After that, the homogenates were subjected to *in vitro* digestion and lipid hydroperoxides quantification.

Finally, a blank digestion, which included only the gastro-intestinal juices and enzymes and water in place of meat and salad, was carried out to consider the possible impact of the digestive enzymes and fluids in the subsequent analysis.

2.4. Extraction of phenolic compounds from vegetables and extra-virgin olive oil

Phenolic compounds from extra-virgin olive oil (EVOO) were extracted following the procedure reported in Martini et al. (2018). Briefly, 15 grams of EVOO were mixed with 15 mL of a solution of methanol/water (70:30, v/v) and incubated for 120 minutes at 30°C in a rotary wheel. After incubation, the mixture was centrifuged at 3000*g* for 30 minutes at 4°C. When extraction was completed, the samples were stored on freezer shelves at −20°C and allowed to stand overnight for lipid precipitation and separation. The liquid supernatant containing phenolics was withdrawn and stored at -20°C until analysis.

Phenolic compounds from vegetables were extracted adapting the procedure reported in Martini et al. (2017). Vegetables (10 g) were homogenized with 20 mL of methanol/water solution (70:30, v/v) and incubated for 30 min at 37°C. Homogenates were then centrifuged (6000*g*, 20 min, 4°C) and the collected supernatant filtered on paper. The filtrates were concentrated by a rotary evaporator and re-dissolved in 10 mL of water.

2.5. In vitro co-digestion of grilled turkey breast meat with single salad ingredients and vegetables phenolic fractions

In these co-digestion experiments, vegetable salad ingredients (tomatoes, onions, black olives, EVOO and fresh basil) were added singularly to the grilled turkey breast meat in the same proportions as found in the Mediterranean Diet salad. For the experiments, 10 g of cooked meat were homogenized in presence of 20 g of tomato (plus 6.05 g of water) or 2.5 g of onion (plus 23.55 g of water) or 2.5 g of black olives (plus 23.55 g of water) or 1 g of EVOO (plus 25.05 g of water) or 0.05 g of fresh basil (plus 26 g of water). The proportion meat/ingredients were 200% tomato,

25% onion or black olives, 10% EVOO and 0.5% basil respect to meat (w/w). After that, the *in vitro* digestions were carried out as reported above.

Further experiments were carried out to gain more information about the effect of vegetables and EVOO phenolic compounds on the oxidative phenomena during *in vitro* co-digestion with meat. These co-digestions were carried out as reported above but replacing the vegetable foods or EVOO with the corresponding amount of phenolic fraction.

2.6. Identification and quantification of phenolic compounds by liquid chromatography electrospray ionization ion trap mass spectrometer (LC-ESI-IT-MS)

Phenolic fractions were analyzed on a HPLC Agilent 1200 Series system equipped with a C18 column (HxSil C18 Reversed phase, 250×4.6 mm, 5 μm particle size, Hamilton Company, Reno, Nevada, USA) as reported in Martini et al. (2017). The mobile phase consisted of (A) H_2O/formic acid (99:1, v/v) and (B) acetonitrile/formic acid (99:1, v/v). The gradient started at 4% B for 0.5 min then linearly ramped up to 30% B in 60 min. The mobile phase composition was raised up to 100% B in 1 min and maintained for 5 min in order to wash the column before returning to the initial condition. The flow rate was set at 1 mL/min. After passing through the column, the eluate was split and 0.3 mL/min was directed to an Agilent 6300 ion trap mass spectrometer. Two MS experiments were performed, one in ESI negative ion mode and one using positive ESI ionization (for anthocyanins), under the same chromatographic conditions. Identification of phenolic compounds in all samples was carried out using full scan and data-dependent MS^2 scanning from *m/z* 100 to 1500.

Phenolic compounds were quantified by using representative standards for each identified phenolic class. Flavonols were quantified as quercetin-3-*O*-glucoside or quercetin-3-*O*-rutinoside equivalents. Hydroxybenzoic acids were quantified in protocatechuic acid equivalents. Hydroxycinnamic acids were quantified in coumaric or caffeic or ferulic acid equivalents. Flavanones and flavones were quantified as naringenin-7-*O*-rutinoside equivalents. Tyrosol-

175 derivative were quantified in hydroxytyrosol equivalents. Anthocyanins were quantified as
176 cyanidin-3-*O*-glucoside equivalents.
177 ESI-MS parameters, limits of detection (LOD) and limits of quantification (LOQ) for the different
178 standards were the same as reported in Martini et al. (2017).
179 Quantitative results were expressed as mg of compounds per 100 g of vegetable or EVOO.
180

181 ***2.7. Antioxidant activity determination in vegetables and extra-virgin olive oil phenolic-rich***
182 ***fraction***

183 The total antioxidant properties of phenolic fractions were analyzed by using five different assays.
184 The radical scavenging ability was assayed by using the ABTS assay according to Re et al. (1999).
185 For the determination of the Fe^{3+} reducing ability, a protocol based on the ferric
186 reducing/antioxidant power (FRAP) assay was utilized (Benzie and Strain 1999). The capacity to
187 scavenge hydroxyl radical and superoxide anion were evaluated according to the methods reported
188 by Martini et al. (2017). The results were expressed as μmol of ascorbic acid equivalent/mg of
189 phenolic compounds. The Fe^{2+}-chelation ability of phenolic–rich fractions was evaluated by the
190 ferrozine assay (Karama and Pegg 2009).
191

192 ***2.8. Statistics***

193 All the digestions were carried out in triplicate and data are presented as mean ± SD for three
194 analytical replicates for each prepared sample. Univariate analysis of variance (ANOVA) with
195 Tukey's post-hoc test was applied using Graph Pad prism 6.0 (GraphPad Software, San Diego, CA,
196 U.S.A.) when multiple comparisons were performed. The differences were considered significant
197 with $P < 0.05$.

3. Result and discussion

3.1. Effect of Mediterranean Diet salad on lipid oxidation during co-digestion with turkey breast meat

An eight-fold increase (from 33.9 ± 3.1 to 277.5 ± 16.1 nmol H_2O_2/g of meat) in the amount of lipid hydroperoxides was observed after the *in vitro* gastro-intestinal digestion of turkey breast meat without added vegetables. Whereas numerous studies determined the amount of lipid hydroperoxides after *in vitro* gastric digestion (Kanner and Lapidot 2001; Gorelik et al. 2018a; Tagliazucchi et al. 2010), very few of them measured their concentration after *in vitro* intestinal digestion. However, a recent study by our research group showed a sharp increase in the generation of lipid hydroperoxides during intestinal digestion of cooked turkey meat (Martini et al. 2018). This increase could be a consequence of the bile salts emulsification and micellarization of fatty acids present in turkey meat. Berton-Carabin et al. (2014) found that lipid peroxidation occurred much faster in a water/oil system rather than in oil alone or in dispersion without emulsifier. This may be due to several factors. First of all the formation of an interface between the aqueous phase and the fat may favour the contact between the oxidants and the oxygen (dissolved in the aqueous phase) and fatty acids (Berton-Carabin et al. 2014). Secondly, it could be ascribed to the solubilisation of lipid hydroperoxides already formed in the micelles, which in turn may promote oxidation in the micelles themselves (Donnelly et al. 1998). Finally, Sreejayan and von Ritter (1998) suggested that bile salts, in the presence of iron, were able to favour the oxidation of arachidonic acid. The amount of lipid hydroperoxides measured was about 23% lower than that found by Martini et al. (2018) at the end of the intestinal digestion.

Data in **Figure 1** show that lipid hydroperoxides production from turkey meat after gastro-intestinal digestion was greatly reduced by including increasing amounts of the Mediterranean Diet salad mixture and was totally inhibited when meat and salad were co-digested in the original proportion (260.5 g of salad/100 g of meat). Halving the amount of the Mediterranean Diet salad (130.3 g of

223 salad/100 g of meat) also resulted in a complete inhibition in the formation of lipid hydroperoxides
224 whereas further halving (65.2 g of salad/100 g of meat) produced an inhibition of 49.5% (**Figure 1**).
225 No previous data were found in literature about the inhibitory activity of food combination (e.g.
226 salads) on the generation of lipid hydroperoxides after gastro-intestinal digestion of meat. However,
227 in agreement with our results, Kanner and co-worker (2017) reported an inhibition of about 90% in
228 the formation of malondialdheyde after *in vitro* gastric digestion of meat with a Greek salad (274 g
229 of salad/200 g of meat) composed of tomato, cucumber, red pepper, green-cabbage, onion and black
230 olives.
231 Several authors demonstrated the correlation between lipid peroxidation during *in vitro* digestion of
232 meat, with or without phenolic-rich foods, and the concentrations of lipid hydroperoxides and
233 lipoxidation end-products in the plasma of human volunteers after consumption of the same test
234 meals (Natella et al. 2011; Kanner et al. 2001; Gorelik et al. 2008a; Sirota et al. 2013). Therefore,
235 the results of the present *in vitro* digestion study are likely to be relevant for the *in vivo* situation.
236
237 ***3.2. Effect of the single components of the Mediterranean Diet salad on lipid oxidation during***
238 ***co-digestion with turkey breast meat***
239 To understand which component of the Mediterranean Diet salad was mainly responsible for the
240 observed inhibitory effect, we carried out co-digestion with turkey meat and each single
241 components of the salad in the same proportion as found in the Mediterranean Diet salad itself. As
242 reported in section 2.2, the Mediterranean Diet salad, related to 100 g of meat, consisted of 200 g of
243 tomato, 25 g of pink onion, 25 g of black olives, 10 g of extra-virgin olive oil (EVOO) and 0.5 g of
244 fresh basil. This means that, for example, in the co-digestion between turkey meat and tomato, the
245 proportion between meat and tomato was 100 g of meat and 200 g of tomato (200% of tomato
246 respect to meat; w/w). Basing on the same rationale, onion or black olives were added in the
247 proportion of 25% respect meat (w/w), EVOO in the proportion of 10% respect to meat (w/w) and
248 basil in the proportion of 0.5% respect to meat (w/w).

When turkey breast meat was co-digested with the single components of the Mediterranean Diet salad, we observed a differential inhibition in lipid hydroperoxides generation (**Figure 2A**). Except for fresh basil, the addition of all the vegetable components of the salad during co-digestion with turkey meat resulted in a significantly lower amount of generated lipid hydroperoxides. Among the different vegetables, digests of turkey meat with 25% onion and 10% EVOO had undetectable levels of lipid hydroperoxides (100% inhibition). Tomato, although present in a higher amount (200 g per 100 g of meat) respect to onion and EVOO (25 g and 10 g per 100 g of meat, respectively), showed a lower inhibitory effect ($P<0.05$) on the generation of lipid hydroperoxides during co-digestion with turkey meat (~ 75% of inhibition). The inhibition in lipid hydroperoxides formation was significantly lower ($P<0.05$) when 25% of black olives were added to the cooked turkey meat in the digestion system (~ 62% of inhibition). Finally, no significant differences were found in the amount of lipid hydroperoxides in the digests when 0.5% of fresh basil was added to turkey meat ($P>0.05$).

Previous studies reported the ability of EVOO and onion to inhibit lipid peroxidation during *in vitro* digestion of meat (Martini et al. 2018; Kanner et al. 2017; Tirosh et al. 2015). Kanner et al. (2017) also found that tomato inhibited with less effectiveness lipid peroxidation during *in vitro* gastric digestion of turkey meat respect to onion, black olives and EVOO. Differently from our study, they observed a higher effectiveness of black olives respect to onion. However, they measured the lipid peroxidation inhibitory activity at the end of the gastric phase of digestion and not after the intestinal phase. Van Hecke et al. (2017) found that dried basil inhibited lipid peroxidation after *in vitro* intestinal digestion of beef when added in the amounts of 0.5% or 1% respect to meat. Instead, in this study we found that the addition of 0.5% of fresh basil had no effect on lipid peroxidation during *in vitro* digestion of turkey meat. This difference was clearly related to the fact that the same amount (0.5%) of dried basil delivered more antioxidant compounds to the digestive system respect to the fresh herb (Henning et al. 2011).

3.3. Effect of the phenolic fractions of single components of the Mediterranean Diet salad on lipid oxidation during co-digestion with turkey breast meat

Previous studies described a strong correlation between the concentration of total phenolic compounds in fruit, beverages, vegetables and spices and the reduction in the lipid peroxidation during *in vitro* digestion of meat (Kanner et al. 2017; Van Hecke et al. 2017; Martini et al. 2018). Therefore, the phenolic fractions extracted from the different vegetables were co-digested with turkey breast meat (**Figure 2B**).

Phenolic fractions extracted from onion and EVOO and co-digested with meat at the same concentration as found in 25% onion and 10% EVOO totally inhibited the generation of lipid hydroperoxides without any differences with the data obtained after the co-digestion of meat with whole onion or EVOO ($P>0.05$). Similarly, co-digestion of turkey breast meat with phenolic fractions extracted from black olives and fresh basil resulted in the same inhibitory potency as the whole foods ($P>0.05$). However, in the case of tomato phenolic fraction, the effect was less pronounced respect to that observed after co-digestion with whole food (58% vs 75% of inhibition; $P<0.05$). Despite phenolic compounds, tomato is rich in other lipophilic antioxidants such as carotenoids and in particular lycopene (Martí et al. 2016). Previous studies described lycopene as an efficient inhibitor of lipid peroxidation both in meat products and in cell cultures (Rohlík et al. 2013; Chisté et al. 2014). Lycopene is highly hydrophobic and not extracted in the water/methanol mixture used to prepare the phenolic fraction from tomato. Moreover, it can be released during the intestinal phase of digestion and exert its anti-peroxidative effect (Tagliazucchi et al. 2012).

3.4. Phenolic profile of vegetables and EVOO

The phenolic profile of vegetables and EVOO was investigated using a non-targeted method through LC-ESI-MS/MS experiments. The mass spectrum data along with peak assignments and retention time for the identified phenolic compounds are described in **Table 1**. This approach allowed the tentative identification of 132 compounds (**Table 1**). Seven compounds (compounds **4**,

23, **26**, **40**, **53**, **95** and **99**) were identified by comparison with their respective authentic standards. The remaining compounds were tentatively identified based on the interpretation of the fragmentation patterns obtained from mass spectra and by comparing their mass spectral characteristics with the available literature. The interpretation of the mass spectra fragmentation patterns reported in the literature is not further discussed.

The profile of individual phenolic compounds as well as the total phenolic compounds amount for each vegetable and EVOO are reported in **Table 2** and **Figure 3**. The highest phenolic content was found in EVOO > tomato > black olives > onion > fresh basil ($P < 0.05$). Each single ingredients was characterized for its specific phenolic profile (**Figure 3**). In tomato, hydroxycinnamic acids were the predominant class of phenolic compounds (94.4%) whereas in fresh basil hydroxybenzoic acids prevailed (63.3%) respect to hydroxycinnamic acids (30.3%). The phenolic profile of onion was mainly characterized by flavonols (58.3%) and anthocyanins (21.9%). EVOO and black olives were characterized for the presence of tyrosol-derivatives. Concerning the individual phenolic profile, sixty-five phenolic compounds were identified in tomato, which was characterized by the presence of relevant amounts of di-hydro-ferulic acid-*O*-hexoside (**47**), caffeic acid-*O*-hexoside (**25**) and 4- and 5-*O*-caffeoylquinic acids (**56** and **51**). Apart from hydroxycinnamic acids, modest amounts of hydroxybenzoic acids and flavonols were detected in tomato. Flavanones were present only in tomato but in very low concentrations. Thirty-five phenolic compounds were identified and quantified in onion (**Table 2**). Flavonols were the major group of phenolic compounds identified in onion. Quercetin-3-*O*-hexoside-4'-*O*-hexoside (**82**), quercetin-4'-*O*-hexoside (**119**) and cyanidin-3-*O*-malonylhexoside (**64**) were the main individual phenolics. With respect to black olives, a higher prevalence of tyrosol-derivatives was noted (**Table 2**). Twenty-seven phenolic compounds were quantified in black olives, with oleuropein aglycone (**70**) and hydroxytyrosol-*O*-hexoside isomers (**5** and **8**) present in high concentrations. Similar to what was reported for black olives, tyrosol-derivatives were the major group of phenolic compounds in EVOO, where oleuropein aglycone (**118**) and ligstroside aglycone (**64**) were the main individual phenolics (**Table 2**). Finally, twenty-

six individual phenolic compounds were identified and quantified in fresh basil with a prevalence of phenolic acids. Syringic acid-4-*O*-pentoside (**36**), protocatechuic acid-*O*-hexoside-*O*-pentoside (**22**) and ferulic acid-4-*O*-pentoside isomers (**73** and **74**) were the main phenolic acids.

3.4. Antioxidant properties of vegetable and EVOO phenolic fractions

Vegetables and EVOO phenolic fractions were characterized for their ability to scavenge superoxide anions and hydroxyl radicals as well as for their total radical scavenging capacity (ABTS assay). Moreover, their ability to chelate Fe^{2+} and their ferric reducing properties were assessed (**Table 3**). Black olives and onion phenolic compounds showed the highest ABTS and hydroxyl radical scavenging activities. Instead, tomato phenolic compounds displayed the highest ability to scavenge superoxide anions and the highest ferric reducing ability. With respect to the Fe^{2+}-chelating ability, black olives and fresh basil phenolic compounds were the most active.

The different antioxidant properties of the phenolic fractions reflect differences in their phenolic compositions (Martini et al. 2019). Onion and black olives were found to be particularly rich in quercetin- and cyanidin-derivatives and hydroxytyrosol-derivatives, respectively. These compounds share a 3',4'-dihydroxy structure in the B-ring (i.e. catechol moiety) which is considered of paramount importance to determine the ABTS and hydroxyl radical scavenging properties (Rice-Evans et al. 1999, Ozyürek et al. 2008, Zamora and Hidalgo 2016). Diversely, tomato and fresh basil showed significantly lower ABTS and hydroxyl radical scavenging properties than onion and black olives. Indeed, they were rich in phenolic acids, which showed the lowest hydroxyl radical and ABTS scavenging activities among phenolic compounds (Rice-Evans et al. 1999, Ozyürek et al. 2008). Differences between black olives and EVOO hydroxyl radical and ABTS scavenging activities may be related to the presence of additional antioxidant compounds in black olives. The latter contain also non-phenolic compounds such as oleoside and its methyl- and dimethyl-derivatives, which showed radical scavenging properties (Wang et al. 2000). Hydroxycinnamic acids seemed to be the best superoxide anions ($O_2^{\bullet -}$) scavenging phenolic compounds since the

most active extract against $O_2^{\bullet -}$ was tomato which was mainly consisted of hydroxycinnamic acids (**Figure 3**). Moreover, hydroxycinnamic acids were also the compounds with the highest ferric reducing properties as suggested by the highest ferric reducing power of tomato and fresh basil phenolic fractions. Previous works indicated that hydroxycinnamic and hydroxybenzoic acids displayed higher ferric reducing activities than flavan-3-ols and glycosylated flavonols (Pulido et al. 2000; Martini et al. 2019). Finally, no clear relationship was found between the phenolic composition and the Fe^{2+}-chelating ability of the phenolic fractions extracted from vegetables and EVOO.

3.5. Relationship between the lipid peroxidation inhibitory activity, the phenolic profile and the antioxidant properties of phenolic fractions extracted from vegetables and EVOO

Principal component analysis (PCA) was performed as exploratory analysis allowing data comprehension, clusters association and a quick network identification between phenolic compounds determined by LC-MS/MS, the antioxidant properties and the lipid peroxidation inhibitory activity of vegetables and EVOO. This approach can help to describe the variance (information) in a set of multivariate data where the original variables (here: phenolic classes) may be expressed as linear combination of orthogonal principal components (PCs).

Three principal components explained about 90.5% of total variance. In particular, a bidimensional plot (PC1xPC2 biplot) was reported (**Figure 4**), recording the 63.3% cumulative percentage of the total variance. **Figure 4** shows a clear separation of the phenolic-rich food ingredients, described by the respective and representative phenolic classes. In fact, aiming to fully understand the causative variables for the obtained distribution and the correlation between phenolic classes and bioactivities, they were added to the bidimensional plot. ABTS and hydroxyl radical scavenging activities and the inhibition of the lipid peroxidation displayed the same negative loading vectors on PC1, positively correlated to the onion and its typical phenolic classes: anthocyanins and flavonols. This reflects their higher effectiveness in antioxidant and lipid peroxidation inhibitory activities

than the other ingredients or phenolic classes. Regarding this, the orthogonal directions of hydroxycinnamic and hydroxybenzoic acids did not suggest any kind of relationship. An inverse relationship between ferric reducing power and the inhibition of the lipid peroxidation is depicted by FRAP loading on PC1. The explanation could lay in the mechanisms of action of the used antioxidant activity assays. According to the chemistry of the ABTS and hydroxyl radical scavenging assays, their mechanisms may involve both the single electron transfer (SET) and hydrogen atom transfer (HAT) (Prior et al. 2005); whereas FRAP assay is only characterized by single electron transfer mechanism. Indeed, the capacity to reduce Fe^{3+} to Fe^{2+} may retain the optimal conditions to maintain and stimulate the Fenton and Haber-Weiss reactions. Whereas, the HAT mechanism might stop the lipid peroxidation reaction at several levels. Tyrosol- and hydroxytyrosol-derivatives, describing the phenolic profile of black olives and EVOO, had the same negative loadings on PC1 of the lipid peroxidation inhibition, reflecting their possible involvement in the peroxidation phenomena. However, the negative loading vectors on PC2 could reflect their intrinsic and paradoxical behaviour already investigated in Martini et al. (2018) outlining how tyrosol- and hydroxytyrosol-derivatives peroxidation inhibitory activity is strictly related to their final concentration.

4. Conclusions

This study provides evidence of a protective effect of a typical Mediterranean Diet salad on lipid peroxidation during co-digestion of turkey breast meat. The co-digestion carried out with the single ingredients and phenolic extracts of the Mediterranean Diet salad displayed differences in the lipid peroxidation inhibitory effect. With the exception of tomato, there were not significant differences between the inhibitory effect of the whole ingredients and the respective phenolic fractions, implying that phenolic compounds were mainly responsible for the reported effect. Moreover, our data suggested that the inhibitory effect was related to the different phenolic composition of the tested ingredients and that some phenolic compounds, especially that with a B-ring catechol moiety in their structure (i.e. flavonols and anthocyanins), were the most effective in reducing the oxidative phenomena after co-digestion with meat. This effect was ascribed to the highest radical scavenging and hydroxyl radical scavenging activities of these compounds. On the contrary, phenolic acids, which showed the highest ability to reduce Fe^{3+} to Fe^{2+}, exhibited the lowest lipid peroxidation inhibitory effect. This study gives strong evidence about the structure-activity relationship between phenolic compounds and lipid peroxidation inhibitory activity. Therefore, it is of paramount importance to profile the phenolic composition of antioxidant-rich foods used in this type of study to predict their possible impact on lipid peroxidation during the digestion of meat. Indeed, our study underlines the importance of consuming specific food combinations, in specific amounts to achieve significant biological effects.

Lipid peroxidation inhibitory properties of phenolic compounds in the gastro-intestinal tract, during a meal, may play a key role in the health effect of the Mediterranean Diet. The maintenance of the right redox balance in the gastro-intestinal tract by phenolic-rich foods seems to be a concrete nutritional strategy for healthy living.

Disclosure Statement

The authors report no conflict of interest.

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Figure captions

Figure 1. Turkey breast meat lipid peroxidation as affected by Mediterranean Diet salad after *in vitro* gastro-intestinal digestion. A portion of Mediterranean Diet salad contained 200 g of tomato, 25 g of pink onion, 25 g of black olives, 10 g of extra-virgin olive oil and 0.5 g of fresh basil. The above quantities were intended as a salad dish consumed with 100 g of cooked turkey meat. Lipid hydroperoxides were expressed as nmol H_2O_2/g of meat. Different letters indicate that the values are significantly different ($P<0.05$). n.d. means not detected.

Figure 2. Effect of the Mediterranean Diet salad ingredients and their phenolic-rich fractions on the amount of lipid hydroperoxides measured at the end of the gastro-intestinal digestion of turkey breast meat. Black column represents meat alone. Light grey columns represent the co-digestion of meat with the different food ingredients. Dark grey columns represent the co-digestion of meat with the phenolic fractions extracted from the different food ingredients. The tested ingredients were tomato (200g/100g of meat), onion (25 g/100 g meat), black olives (25 g /100 g of meat), EVOO (10 g /100 g of meat) and fresh basil (0.5 g/100 g of meat). Lipid hydroperoxides were expressed as nmol H_2O_2/g of meat. EVOO: extra-virgin olive oil. Different letters indicate that the values are significantly different ($P < 0.05$). n.d. means not detected.

Figure 3. Occurrence of phenolic classes in the tested vegetables and EVOO. (A) Incidence of phenolic classes in tomato, onion, fresh basil and black olives. (B) Incidence of individual tyrosol-derivatives in black olives and EVOO. EVOO: extra-virgin olive oil.

Figure 4. Principal component analysis of vegetable and EVOO phenolic-rich fractions activities, phenolic classes and lipid peroxidation inhibitory activity. Graph of the biplot of PC1 versus PC2. The symbol ♦ identifies the phenolic classes and the biochemical properties, whereas the symbol ▲ represents the food ingredients. FRAP: ferric reducing power; ABTS: ABTS radical scavenging activity; HO•: hydroxyl radical scavenging activity; $O_2^{\bullet -}$: superoxide anion radical scavenging activity; EVOO: extra-virgin olive oil.

Table 1. Mass spectral characteristics of phenolic compounds identified in the studied vegetables

	Compound	*Rt (min)*	*$[M-H]^-$ (m/z)*	*MS^2 ion fragments (m/z)*
1	Hydroxybenzoic acid-*O*-hexoside isomer	8.7	299	137 (100%)
2	Hydroxybenzoic acid-dihexoside	9.9	461	137 (100%), 299 (62%)
3	Hydroxytyrosol-di-*O*-hexoside	10.7	477	153 (100%), 315 (53%), 123 (10%)
4	Hydroxytyrosol isomer*	11.2	153	123 (100%)
5	Hydroxytyrosol-*O*-hexoside isomer	11.3	315	153 (100%), 123 (17%)
6	Caffeoylquinic acid-3-*O*-hexoside-4-*O*-hexoside isomer	11.6	677	515 (100%), 353 (23%), 191 (5%)
7	Vanillic acid-4-*O*-hexoside	11.8	329	167 (100%), 152 (9%)
8	Hydroxytyrosol-*O*-hexoside isomer	12.0	315	153 (100%), 123 (25%)
9	Hydroxytyrosol isomer	12.1	153	123 (100%)
10	Protocatechuic acid-*O*-hexoside	12.8	315	153 (100%)
11	Caffeic acid-*O*-hexoside-*O*-pentoside isomer	13.7	473	341 (100%), 179 (48%), 135 (7%)
12	Syringic acid-4-*O*-hexoside	14.1	359	197 (100%), 182 (8%), 167 (5%)
13	Calceolarioside	14.1	477	323 (100%), 315 (90%), 161 (16%)
14	Caffeoylquinic acid-3-*O*-hexoside-4-*O*-hexoside isomer	14.1	677	515 (100%), 341 (21%)
15	Caffeoylquinic acid-*O*-hexoside isomer	14.9	515	341 (100%), 323 (64%), 179 (57%), 353 (34%)
16	Caffeic acid-3-*O*-hexoside-4-*O*-hexoside	15.5	503	341 (100%), 179 (17%)
17	3-*O*-Caffeoylquinic acid	15.6	353	191 (100%), 179 (24%), 135 (19%)
18	Caffeoylquinic acid-*O*-hexoside isomer	15.6	515	353 (100%), 191 (82%), 179 (12%) 323 (8%)
19	Caffeoyl-coumaroylquinic acid	15.7	499	337 (100%), 173 (36%), 191 (34%)
20	Rosmarinic acid	15.8	359	197 (100%), 161 (6%), 153 (8%)
21	Gallic acid	15.9	169	125 (100%)
22	Protocatechuic acid-*O*-hexoside-*O*-pentoside	16.7	447	315 (100%), 271 (43%), 153 (15%)
23	Coumaric acid*	16.7	163	119 (100%)
24	Coumaric acid-*O*-hexoside isomer	16.9	325	163 (100%), 119 (24%)
25	Caffeic acid-*O*-hexoside isomer	17.0	341	179 (100%), 135 (36%)
26	Caffeic acid*	17.1	179	135 (100%)
27	Hydroxybenzoic acid-*O*-hexoside isomer	17.8	299	137 (100%)
28	Di-hydro-coumaric acid-*O*-hexoside	18.0	327	165 (100%), 121 (4%)
29	Di-hydro-caffeic acid-*O*-hexoside isomer	18.0	343	181 (100%), 137 (33%)
30	Syringic acid	18.2	197	153 (100%)

31	Caffeic acid-*O*-hexoside-*O*-pentoside isomer	18.2	473	341 (100%), 179 (6%), 135 (4%)
32	Feruloylquinic acid-*O*-hexoside isomer	18.6	529	367 (100%), 191 (17%)
33	Caffeoyl-6β-hexose	18.7	341	281 (100%), 179 (83%), 251 (42%), 135 (14%), 323 (12%), 221 (9%)
34	Apigenin-*O*-hexoside	18.8	431	269 (100%)
35	Protocatechuic acid-*O*-pentoside	19.3	285	153 (100%), 109 (20%)
36	Syringic acid-4-*O*-pentoside	19.3	329	197 (100%), 182 (6%), 153 (2%)
37	Di-hydro-caffeic acid-*O*-hexoside isomer	19.5	343	181 (100%), 137 (9%)
38	Caffeoylquinic acid-*O*-hexoside isomer	19.8	515	323 (100%), 353 (18%), 191 (14%), 341 (5%)
39	4-*O*-Caffeoylquinic acid *cis*	19.9	353	173 (100%), 191 (38%)
40	Ferulic acid*	20.4	193	149 (100%), 134 (82%), 178 (36%)
41	Ferulic acid-4-*O*-hexoside	20.5	355	193 (100%)
42	Quercetin-tri-*O*-hexoside	20.6	787	625 (100%), 463 (52%)
43	Syringic acid-4-*O*-acetylhexoside	21.0	401	197 (100%)
44	Syringic acid-dihexoside	21.0	521	197 (100%), 167 (6%), 183 (2%)
45	Coumaric acid-*O*-hexoside isomer	21.0	325	163 (100%), 119 (24%)
46	Caffeoyl-6α-hexose	21.0	341	179 (100%), 135 (41%), 281 (21%), 221 (11%), 323 (8%), 251 (5%)
47	Di-hydro-ferulic acid-*O*-hexoside	21.2	357	195 (100%), 177 (8%), 151 (8%), 136 (6%), 119 (2%)
48	Caffeoylquinic acid-*O*-hexoside isomer	21.4	515	353 (100%), 341 (74%), 191 (64%), 179 (19%)
49	Di-hydro-caffeic acid-3-*O*-hexoside-4-*O*-hexoside	22.0	505	343 (100%), 181 (9%)
50	Medioresinol	22.3	387	207 (100%), 369 (53%), 163 (35%)
51	5-*O*-Caffeoylquinic acid *trans*	22.9	353	191 (100%)
52	Sinapic acid-4-*O*-hexoside	22.9	385	223 (100%), 208 (6%)
53	Cyanidin-3-*O*-glucoside*	23.0	449[#]	287 (100%)
54	Cyanidin-di-*O*-hexoside	23.2	611[#]	449 (100%), 287 (21%)
55	Apigenin-*O*-pentoside	23.2	401	269 (100%)
56	4-*O*-Caffeoylquinic acid *trans*	23.4	353	173 (100%)
57	Quercetin-3-*O*-rutinoside-*O*-hexoside-*O*-pentoside	23.5	903	741 (100%), 609 (5%), 301 (2%)
58	Feruloylquinic acid-*O*-hexoside isomer	24.1	529	367 (100%), 191 (60%)
59	Peonidin-3-*O*-hexoside	24.4	463[#]	301 (100%)
60	Feruloyl-hexose	24.6	355	193 (100%), 235 (30%), 295 (4%)
61	Caffeic acid-*O*-hexoside isomer	24.8	341	179 (100%), 135 (36%)
62	Quercetin-3-*O*-hexoside-7-*O*-hexoside	24.9	625	463 (100%), 301 (12%), 271 (7%)
63	Taxifolin-*O*-hexoside	24.9	465	303 (100%)

64	Cyanidin-3-*O*-malonylhexoside	25.2	535[#]	287 (100%), 449 (5%)
65	Peonidin-3-*O*-malonylhexoside	25.2	549[#]	301 (100%), 463 (6%)
66	Myricetin-di-*O*-hexoside	25.4	641	479 (100%), 317 (21%)
67	Quercetin-3-*O*-rutinoside-7-*O*-hexoside	25.4	771	609 (100%)
68	Sinapoyl-hexose	25.8	385	223 (100%), 208 (2%), 265 (6%), 325 (1%)
69	5-*O*-Caffeoylquinic acid *cis*	26.5	353	191 (100%)
70	Oleuropein aglycone isomer	26.5	377	197 (100%), 153 (61%)
71	Caffeic acid-*O*-malonylhexoside	27.5	457	341 (100%), 179 (14%)
72	Amentoflavone	27.5	537	375 (100%), 179 (14%)
73	Ferulic acid-4-*O*-pentoside isomer	28.0	325	193 (100%), 149 (36%), 134 (3%)
74	Ferulic acid-4-*O*-pentoside isomer	28.6	325	193 (100%), 149 (36%), 134 (3%)
75	4-*O*-Cumaroylquinic acid	28.9	337	173 (100%), 163 (17%)
76	Apigenin-6,8-di-*C*-hexoside	29.1	593	473 (100%), 353 (49%), 383 (33%)
77	5-*O*-Cumaroylquinic acid	29.3	337	191 (100%), 173 (5%) 163 (3%)
78	Isorhamnetin-di-*O*-hexoside isomer	29.5	639	477 (100%), 315 (5%)
79	Kaempferol-3-*O*-acetylhexoside	30.0	489	285 (100%), 255 (7%)
80	Quercetin-7-*O*-hexoside-4'-*O*-hexoside	31.3	625	463 (100%), 301 (22%)
81	5-*O*-Feruloylquinic acid	32.2	367	191 (100%), 173 (4%)
82	Quercetin-3-*O*-hexoside-4'-*O*-hexoside	33.6	625	463 (100%), 301 (31%), 179 (4%)
83	Kaempferol-3-*O*-hexoside-7-*O*-hexoside	33.8	609	285 (100%), 447 (73%), 255 (7%)
84	Isorhamnetin-di-*O*-hexoside isomer	34.1	639	477 (100%)
85	Myricetin-7-*O*-hexoside	34.4	479	317 (100%), 289 (65%)
86	Secoisolariciresinol-*O*-hexoside	35.0	523	361 (100%)
87	Naringenin-*C*-hexoside	35.1	433	313 (100%)
88	Isorhamnetin-3-*O*-hexoside-4'-*O*-hexoside	35.4	639	315 (100%), 477 (63%), 301 (17%), 271 (6%)
89	Quercetin-3-*O*-rutinoside-7-*O*-pentoside	36.5	741	609 (100%), 300 (80%)
90	Lariciresinol-*O*-hexoside	37.3	521	329 (100%), 359 (15%)
91	Apigenin-*C*-hexoside-*O*-rhamnoside	37.9	577	341 (100%), 413 (50%), 311 (15%)
92	Eriodictiol-*O*-hexoside	38.5	449	287 (100%), 151 (42%)
93	Quercetin-3-*O*-hexoside isomer	39.6	463	301 (100%), 151 (5%), 179 (3%)
94	Di-hydro-quercetin	39.8	303	285 (100%), 267 (54%), 257 (41%)
95	Quercetin-3-*O*-rutinoside*	39.9	609	301 (100%), 343 (46%), 273 (28%), 243 (13%)
96	Kaempferol-3-*O*-rutinoside-7-*O*-pentoside	40.1	725	593 (100%), 285 (30%), 255 (7%), 257 (3%)
97	Luteolin-*O*-rutinoside isomer	40.1	593	285 (100%), 447 (2%)

98	Phloretin-di-*C*-hexoside	40.1	597	357 (100%), 387 (91%), 477 (81%)
99	Quercetin-3-*O*-glucoside*	41.0	463	301 (100%), 151 (23%), 179 (2%)
100	Luteolin-*O*-hexoside	41.5	447	285 (100%)
101	Naringenin-di-*O*-hexoside	41.6	595	271 (100%)
102	Luteolin-*O*-rutinoside isomer	41.7	593	285 (100%), 447 (67%)
103	Pinoresinol-*O*-hexoside	42.0	519	359 (100%), 151 (2%)
104	Nuzhenide	42.4	685	523 (100%), 453 (93%), 421 (32%), 299 (3%)
105	Phloretin-*C*-hexoside	42.9	435	315 (100%), 345 (5%)
106	Syringaresinol-*O*-hexoside	43.3	579	417 (100%), 181 (8%)
107	Verbascoside	43.5	623	461 (100%), 315 (2%)
108	Kaempferol-3-*O*-rutinoside	44.4	593	285 (100%)
109	4,5-diCaffeoylquinic acid	44.5	515	353 (100%), 179 (18%), 335 (15%), 191 (14%)
110	Quercetin-3-*O*-acetylhexoside	44.9	505	301 (100%), 463 (67%), 179 (35%)
111	Liquiritigenin-7-*O*-hexoside	45.0	417	255 (100%)
112	Apigenin-*O*-hexoside-*O*-rhamnoside	45.1	577	269 (100%)
113	Apigenin-*O*-hexoside-*O*-pentoside	45.3	563	269 (100%), 431 (23%)
114	Kaempferol-3-*O*-hexoside	45.9	447	284 (100%), 255 (70%), 285 (51%)
115	3,5-diCaffeoylquinic acid	46.0	515	353 (100%), 191 (4%)
116	Isorhamnetin-3-*O*-hexoside	46.8	477	315 (100%), 300 (12%)
117	Naringenin-*O*-hexoside isomer	46.8	433	271 (100%)
118	Oleuropein aglycone isomer	47.2	377	307 (100%), 333 (65%), 275 (55%), 139 (12%), 345 (7%)
119	Quercetin-4'-*O*-hexoside	47.6	463	301 (100%), 179 (12%), 151 (4%)
120	Naringenin-*O*-hexoside-*O*-pentoside	47.6	565	271 (100%), 403 (11%)
121	Kaempferol-7-*O*-hexoside	48.8	447	285 (100%), 257 (11%)
122	Hydroxy-decarboxymethyl-oleuropein aglycone	49.1	335	199 (100%), 181 (29%)
123	Decarboxymethyl-oleuropein aglycone	49.6	319	195 (100%), 165 (18%)
124	Oleuropein	49.8	539	377 (100%), 307 (66%), 275 (32%), 345 (14%)
125	Isorhamnetin-4'-*O*-hexoside	50.6	477	315 (100%), 299 (15%), 300 (12%)
126	Di-hydroxy-ligstroside aglycone	50.6	393	361 (100%), 257 (79%), 323 (27%), 195 (19%), 151 (16%)
127	β-methoxylverbascoside	51.1	653	491 (100%), 635 (93%)
128	Coumaroyl-caffeoylquinic acid	52.2	499	353 (100%), 191 (10%), 173 (7%)
129	Naringenin-*O*-hexoside isomer	52.9	433	271 (100%)
130	Ligstroside	56.8	523	361 (100%), 259 (19%)
131	Quercetin	60.8	301	151 (100%), 179 (71%)
132	Ligstroside aglycone	64.0	361	291 (100%), 259 (31%), 223 (4%)

*identified by comparison with authentic standards

#Indicates $[M+H]^+$ rather than $[M-H]^-$

Table 2. Quantitative results (mg/100 g fresh food) for phenolic compounds identified in the vegetables. Values represent means ± standard deviation of triplicate determination (n.d. means not detected).

	Compound	*Tomato*	*Onion*	*Black olives*	*EVOO*	*Basil*
			Hydroxybenzoic acids			
21	Gallic acid	n.d.	< LOQ	n.d.	n.d.	0.08 ± 0.05
30	Syringic acid	n.d.	< LOQ	n.d.	n.d.	0.56 ± 0.19
35	Protocatechuic acid-*O*-pentoside	0.37 ± 0.07	n.d.	n.d.	n.d.	n.d.
1	Hydroxybenzoic acid-*O*-hexoside isomer	0.17 ± 0.04	n.d.	n.d.	n.d.	n.d.
27	Hydroxybenzoic acid-*O*-hexoside isomer	n.d.	n.d.	n.d.	n.d.	0.19 ± 0.08
10	Protocatechuic acid-*O*-hexoside	0.20 ± 0.02	n.d.	n.d.	n.d.	0.08 ± 0.02
7	Vanillic acid-4-*O*-hexoside	0.13 ± 0.02	n.d.	n.d.	n.d.	0.13 ± 0.04
36	Syringic acid-4-*O*-pentoside	n.d.	n.d.	n.d.	n.d.	10.31 ± 0.16
12	Syringic acid-4-*O*-hexoside	n.d.	n.d.	n.d.	n.d.	0.14 ± 0.01
43	Syringic acid-4-*O*-acetylhexoside	n.d.	n.d.	n.d.	n.d.	0.42 ± 0.11
22	Protocatechuic acid-*O*-hexoside-*O*-pentoside	n.d.	n.d.	n.d.	n.d.	7.17 ± 0.27
2	Hydroxybenzoic acid-dihexoside	0.32 ± 0.07	n.d.	n.d.	n.d.	n.d.
44	Syringic acid-dihexoside	n.d.	n.d.	n.d.	n.d.	2.25 ± 0.31
	Total hydroxybenzoic acids	***1.19 ± 0.11 (2.4%)***	***< LOQ***	***n.d.***	***n.d.***	***21.33 ± 0.49 (63.3%)***
			Hydroxycinnamic acids			
23	Coumaric acid	0.13 ± 0.01	n.d.	0.04 ± 0.01	n.d.	n.d.
26	Caffeic acid	0.28 ± 0.02	n.d.	0.10 ± 0.01	n.d.	n.d.
40	Ferulic acid	0.73 ± 0.18	n.d.	n.d.	n.d.	n.d.
24	Coumaric acid-*O*-hexoside isomer	0.12 ± 0.03	n.d.	n.d.	n.d.	n.d.
45	Coumaric acid-*O*-hexoside isomer	0.81 ± 0.02	n.d.	n.d.	n.d.	n.d.
73	Ferulic acid-4-*O*-pentoside isomer	n.d.	n.d.	n.d.	n.d.	1.73 ± 0.13
74	Ferulic acid-4-*O*-pentoside isomer	n.d.	n.d.	n.d.	n.d.	5.35 ± 0.49
28	Dihydro-coumaric acid-*O*-hexoside	0.81 ± 0.02	n.d.	n.d.	n.d.	n.d.
75	4-*O*-Cumaroylquinic acid	0.18 ± 0.08	n.d.	n.d.	n.d.	n.d.
77	5-*O*-Cumaroylquinic acid	0.53 ± 0.06	n.d.	n.d.	n.d.	n.d.
25	Caffeic acid-*O*-hexoside isomer	5.72 ± 0.56	n.d.	0.67 ± 0.01	n.d.	0.05 ± 0.02
33	Caffeoyl-6β-hexose	1.13 ± 0.04	n.d.	n.d.	n.d.	n.d.

46	Caffeoyl-6α-hexose	2.59 ± 0.16	n.d.	0.39 ± 0.04	n.d.	n.d.
61	Caffeic acid-*O*-hexoside isomer	0.17 ± 0.05	n.d.	n.d.	n.d.	n.d.
29	Dihydro-caffeic acid-*O*-hexoside isomer	0.55 ± 0.05	n.d.	n.d.	n.d.	n.d.
37	Dihydro-caffeic acid-*O*-hexoside isomer	0.69 ± 0.12	n.d.	n.d.	n.d.	n.d.
17	3-*O*-Caffeoylquinic acid	0.18 ± 0.01	n.d.	n.d.	n.d.	n.d.
39	4-*O*-Caffeoylquinic acid *cis*	0.38 ± 0.17	n.d.	n.d.	n.d.	n.d.
51	5-*O*-Caffeoylquinic acid *trans*	4.57 ± 0.03	n.d.	n.d.	n.d.	n.d.
56	4-*O*-Caffeoylquinic acid *trans*	4.61 ± 0.11	n.d.	n.d.	n.d.	n.d.
69	5-*O*-Caffeoylquinic acid *cis*	0.90 ± 0.01	n.d.	n.d.	n.d.	n.d.
41	Ferulic acid-4-*O*-hexoside	1.56 ± 0.46	n.d.	n.d.	n.d.	n.d.
60	Feruloyl-hexose	2.95 ± 0.85	n.d.	n.d.	n.d.	0.05 ± 0.01
47	Dihydro-ferulic acid-*O*-hexoside	6.65 ± 0.63	n.d.	n.d.	n.d.	n.d.
20	Rosmarinic acid	n.d.	n.d.	n.d.	n.d.	0.08 ± 0.01
81	5-*O*-Feruloylquinic acid	1.80 ± 0.23	n.d.	n.d.	n.d.	n.d.
52	Sinapic acid-4-*O*-hexoside	2.21 ± 0.19	1.58 ± 0.19	n.d.	n.d.	2.34 ± 0.19
68	Sinapoyl-hexose	n.d.	5.75 ± 0.33	n.d.	n.d.	n.d.
71	Caffeic acid-*O*-malonylhexoside	0.23 ± 0.01	n.d.	n.d.	n.d.	n.d.
11	Caffeic acid-*O*-hexoside-*O*-pentoside isomer	n.d.	n.d.	n.d.	n.d.	0.16 ± 0.05
31	Caffeic acid-*O*-hexoside-*O*-pentoside isomer	n.d.	n.d.	n.d.	n.d.	0.09 ± 0.01
13	Calceolarioside	n.d.	n.d.	0.32 ± 0.09	n.d.	n.d.
19	Caffeoyl-coumaroylquinic acid	0.12 ± 0.01	n.d.	n.d.	n.d.	n.d.
128	Coumaroyl-caffeoylquinic acid	0.10 ± 0.01	n.d.	n.d.	n.d.	n.d.
16	Caffeic acid-3-*O*-hexoside-4-*O*-hexoside	0.14 ± 0.01	n.d.	n.d.	n.d.	n.d.
49	Dihydro-caffeic acid-3-*O*-hexoside-4-*O*-hexoside	0.19 ± 0.02	n.d.	n.d.	n.d.	n.d.
15	Caffeoylquinic acid-*O*-hexoside isomer	0.06 ± 0.01	n.d.	n.d.	n.d.	n.d.
18	Caffeoylquinic acid-*O*-hexoside isomer	0.52 ± 0.09	n.d.	n.d.	n.d.	n.d.
38	Caffeoylquinic acid-*O*-hexoside isomer	0.38 ± 0.17	n.d.	n.d.	n.d.	n.d.
48	Caffeoylquinic acid-*O*-hexoside isomer	0.11 ± 0.03	n.d.	n.d.	n.d.	n.d.
109	4,5-diCaffeoylquinic acid	1.12 ± 0.28	n.d.	n.d.	n.d.	n.d.
115	3,5-diCaffeoylquinic acid	0.73 ± 0.03	n.d.	n.d.	n.d.	0.35 ± 0.08
32	Feruloylquinic acid-*O*-hexoside isomer	1.43 ± 0.01	n.d.	n.d.	n.d.	n.d.
58	Feruloylquinic acid-*O*-hexoside isomer	1.70 ± 0.42	n.d.	n.d.	n.d.	n.d.

6	Caffeoylquinic acid-3-*O*-hexoside-4-*O*-hexoside isomer	0.33 ± 0.06	n.d.	n.d.	n.d.	n.d.
14	Caffeoylquinic acid-3-*O*-hexoside-4-*O*-hexoside isomer	0.06 ± 0.01	n.d.	n.d.	n.d.	n.d.
	Total hydroxycinnamic acids	***47.48 ± 1.46 (94.4%)***	***7.32 ± 0.39 (19.0%)***	***1.52 ± 0.09 (3.5%)***	***n.d.***	***10.20 ± 0.55 (30.3%)***
			Flavonols			
131	Quercetin	n.d.	0.04 ± 0.01	n.d.	n.d.	n.d.
114	Kaempferol-3-*O*-hexoside	n.d.	0.09 ± 0.01	n.d.	n.d.	n.d.
121	Kaempferol-7-*O*-hexoside	< LOQ	0.19 ± 0.02	n.d.	n.d.	n.d.
93	Quercetin-3-*O*-hexoside isomer	n.d.	< LOQ	n.d.	n.d.	n.d.
99	Quercetin-3-*O*-glucoside	0.01 ± 0.01	0.43 ± 0.05	0.03 ± 0.01	n.d.	n.d.
119	Quercetin-4'-*O*-hexoside	n.d.	7.29 ± 0.40	n.d.	n.d.	n.d.
116	Isorhamnetin-3-*O*-hexoside	n.d.	0.04 ± 0.01	n.d.	n.d.	n.d.
125	Isorhamnetin-4'-*O*-hexoside	n.d.	2.35 ± 0.07	< LOQ	n.d.	n.d.
85	Myricetin-7-*O*-hexoside	n.d.	0.01 ± 0.01	n.d.	n.d.	n.d.
79	Kaempferol-3-*O*-acetylhexoside	n.d.	< LOQ	n.d.	n.d.	n.d.
110	Quercetin-3-*O*-acetylhexoside	n.d.	< LOQ	n.d.	n.d.	< LOQ
108	Kaempferol-3-*O*-rutinoside	< LOQ	n.d.	n.d.	n.d.	n.d.
95	Quercetin-3-*O*-rutinoside	0.37 ± 0.01	n.d.	n.d.	n.d.	n.d.
83	Kaempferol-3-*O*-hexoside-7-*O*-hexoside	n.d.	0.21 ± 0.01	n.d.	n.d.	n.d.
62	Quercetin-3-*O*-hexoside-7-*O*-hexoside	n.d.	0.03 ± 0.01	n.d.	n.d.	n.d.
80	Quercetin-7-*O*-hexoside-4'-*O*-hexoside	n.d.	0.15 ± 0.01	n.d.	n.d.	n.d.
82	Quercetin-3-*O*-hexoside-4'-*O*-hexoside	< LOQ	11.13 ± 0.18	0.03 ± 0.01	n.d.	< LOQ
78	Isorhamnetin-di-*O*-hexoside isomer	n.d.	< LOQ	n.d.	n.d.	n.d.
84	Isorhamnetin-di-*O*-hexoside isomer	n.d.	< LOQ	n.d.	n.d.	n.d.
88	Isorhamnetin-3-*O*-hexoside-4'-*O*-hexoside	n.d.	0.38 ± 0.01	n.d.	n.d.	n.d.
66	Myricetin-di-*O*-hexoside	n.d.	0.01 ± 0.01	n.d.	n.d.	n.d.
96	Kaempferol-3-*O*-rutinoside-7-*O*-pentoside	< LOQ	< LOQ	n.d.	n.d.	n.d.
89	Quercetin-3-*O*-rutinoside-7-*O*-pentoside	0.17 ± 0.01	< LOQ	n.d.	n.d.	n.d.
67	Quercetin-3-*O*-rutinoside-7-*O*-hexoside	0.01 ± 0.01	< LOQ	n.d.	n.d.	n.d.
42	Quercetin-tri-*O*-hexoside	n.d.	0.08 ± 0.01	n.d.	n.d.	n.d.
57	Quercetin-3-*O*-rutinoside-*O*-hexoside-*O*-pentoside	0.01 ± 0.01	< LOQ	n.d.	n.d.	n.d.

	Total flavonols	***0.56 ± 0.01 (1.1%)***	***22.44 ± 0.45 (58.3%)***	***0.06 ± 0.01 (0.1%)***	***n.d.***	***< LOQ***
			Anthocyanins			
53	Cyanidin-3-*O*-glucoside	n.d.	1.59 ± 0.02	n.d.	n.d.	n.d.
59	Peonidin-3-*O*-hexoside	n.d.	0.45 ± 0.01	n.d.	n.d.	n.d.
64	Cyanidin-3-*O*-malonylhexoside	n.d.	4.29 ± 0.24	n.d.	n.d.	n.d.
65	Peonidin-3-*O*-malonylhexoside	n.d.	0.84 ± 0.04	n.d.	n.d.	n.d.
54	Cyanidin-di-*O*-hexoside	n.d.	1.25 ± 0.04	n.d.	n.d.	n.d.
	Total anthocyanins	***n.d.***	***8.42 ± 0.24 (21.9%)***	***n.d.***	***n.d.***	***n.d.***
			Lignans			
50	Medioresinol	0.04 ± 0.01	n.d.	n.d.	n.d.	1.66 ± 0.01
103	Pinoresinol-*O*-hexoside	n.d.	n.d.	n.d.	n.d.	0.07 ± 0.01
90	Lariciresinol-*O*-hexoside	n.d.	n.d.	n.d.	n.d.	0.40 ± 0.04
86	Secoisolariciresinol-*O*-hexoside	n.d.	0.03 ± 0.01	n.d.	n.d.	n.d.
106	Syringaresinol-*O*-hexoside	n.d.	0.15 ± 0.01	0.04 ± 0.01	n.d.	n.d.
	Total lignans	***0.04 ± 0.01 (0.1%)***	***0.18 ± 0.15 (0.5%)***	***0.04 ± 0.01 (0.1%)***	***n.d.***	***2.14 ± 0.23 (6.4%)***
			Flavones			
55	Apigenin-*O*-pentoside	0.03 ± 0.01	n.d.	n.d.	n.d.	n.d.
34	Apigenin-*O*-hexoside	0.07 ± 0.02	n.d.	n.d.	n.d.	n.d.
100	Luteolin-*O*-hexoside	n.d.	n.d.	0.27 ± 0.02	n.d.	n.d.
72	Amentoflavone	n.d.	n.d.	0.02 ± 0.01	n.d.	< LOQ
113	Apigenin-*O*-hexoside-*O*-pentoside	n.d.	n.d.	< LOQ	n.d.	n.d.
91	Apigenin-*C*-hexoside-*O*-rhamnoside	0.06 ± 0.01	n.d.	n.d.	n.d.	n.d.
112	Apigenin-*O*-hexoside-*O*-rhamnoside	n.d.	n.d.	0.02 ± 0.01	n.d.	n.d.
76	Apigenin-6,8-di-*C*-hexoside	n.d.	n.d.	0.01 ± 0.01	n.d.	0.02 ± 0.01
97	Luteolin-*O*-rutinoside isomer	n.d.	n.d.	0.02 ± 0.01	n.d.	n.d.
102	Luteolin-*O*-rutinoside isomer	n.d.	n.d.	0.04 ± 0.01	n.d.	n.d.
	Total flavones	***0.16 ± 0.02 (0.3%)***	***n.d.***	***0.38 ± 0.01 (0.9%)***	***n.d.***	***0.02 ± 0.01 (0.1%)***
			Flavanones			
111	Liquiritigenin-7-*O*-hexoside	0.02 ± 0.01	n.d.	n.d.	n.d.	n.d.
87	Naringenin-*C*-hexoside	0.01 ± 0.01	n.d.	n.d.	n.d.	n.d.
117	Naringenin-*O*-hexoside isomer	0.01 ± 0.01	n.d.	n.d.	n.d.	n.d.
129	Naringenin-*O*-hexoside isomer	0.01 ± 0.01	n.d.	n.d.	n.d.	n.d.
92	Eriodictiol-*O*-hexoside	0.02 ± 0.01	n.d.	n.d.	n.d.	n.d.

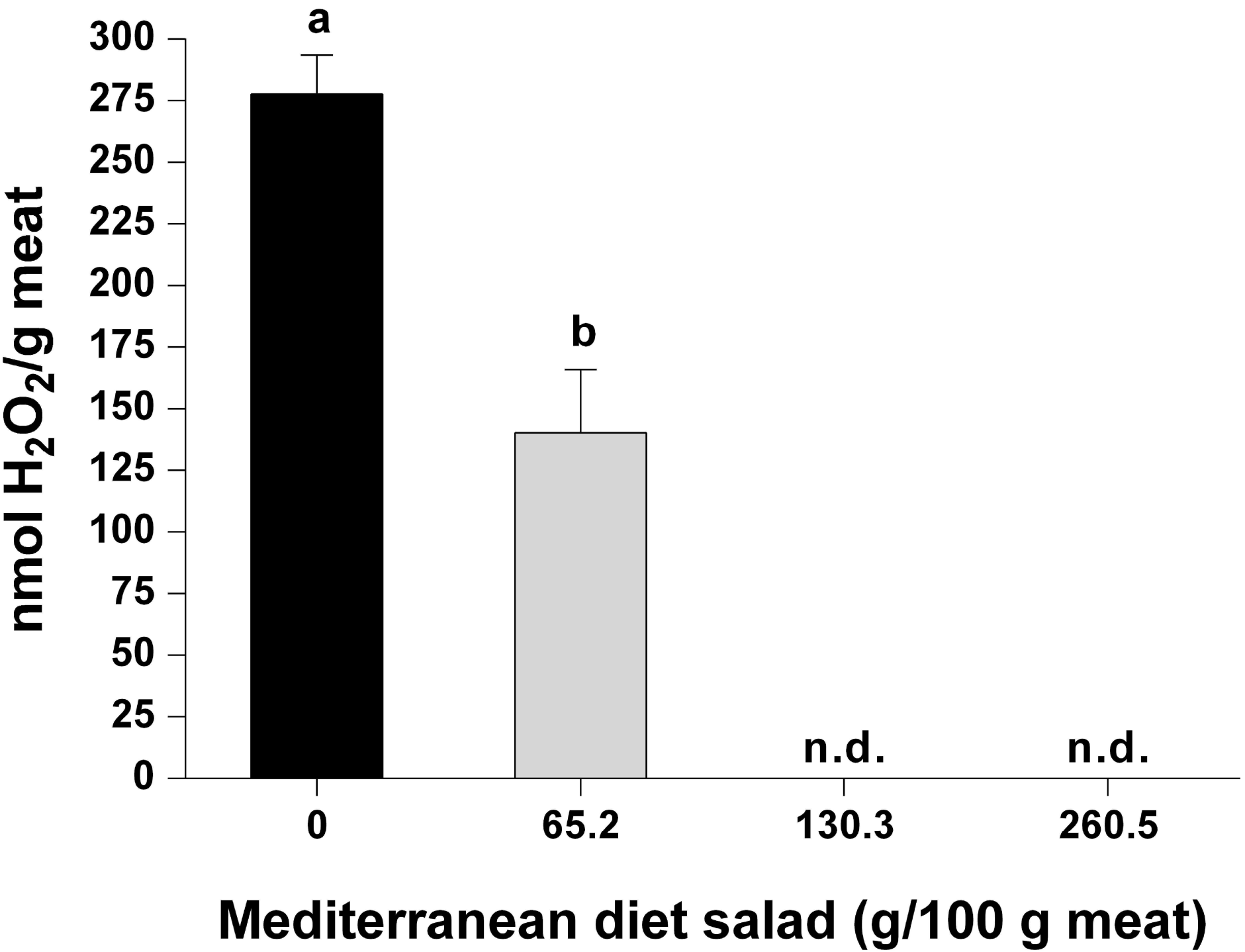
120	Naringenin-*O*-hexoside-*O*-pentoside	0.01 ± 0.01	n.d.	n.d.	n.d.	n.d.
101	Naringenin-di-*O*-hexoside	< LOQ	n.d.	n.d.	n.d.	n.d.
	Total flavonones	***0.09 ± 0.01 (0.2%)***	***n.d.***	***n.d.***	***n.d.***	***n.d.***
			Tyrosol derivatives			
4	Hydroxytyrosol isomer	n.d.	n.d.	0.63 ± 0.14	n.d.	n.d.
9	Hydroxytyrosol isomer	n.d.	n.d.	0.65 ± 0.07	1.50 ± 0.10	n.d.
1	Hydroxytyrosol-*O*-hexoside isomer	n.d.	n.d.	15.24 ± 0.48	n.d.	n.d.
5	Hydroxytyrosol-*O*-hexoside isomer	n.d.	n.d.	2.41 ± 1.00	n.d.	n.d.
123	Decarboxymethyl-oleuropein aglycone	n.d.	n.d.	n.d.	0.77 ± 0.01	n.d.
122	Hydroxy-decarboxymethyl-oleuropein aglycone	n.d.	n.d.	n.d.	11.31 ± 0.27	n.d.
132	Ligstroside aglycone	n.d.	n.d.	n.d.	13.66 ± 0.54	n.d.
70	Oleuropein aglycone isomer	n.d.	n.d.	20.98 ± 0.67	n.d.	n.d.
118	Oleuropein aglycone isomer	n.d.	n.d.	n.d.	46.98 ± 2.79	n.d.
126	Di-hydroxy-ligstroside aglycone	n.d.	n.d.	n.d.	0.07 ± 0.03	n.d.
2	Hydroxytyrosol-di-*O*-hexoside	n.d.	n.d.	0.96 ± 0.11	n.d.	n.d.
130	Ligstroside	n.d.	n.d.	0.11 ± 0.02	n.d.	n.d.
124	Oleuropein	n.d.	n.d.	0.24 ± 0.03	n.d.	n.d.
107	Verbascoside	n.d.	n.d.	0.34 ± 0.01	n.d.	n.d.
127	β-methoxylverbascoside	n.d.	n.d.	0.15 ± 0.01	n.d.	n.d.
104	Nuzhenide	n.d.	n.d.	0.21 ± 0.06	n.d.	n.d.
	Total tyrosol derivatives	***n.d.***	***n.d.***	***41.93 ± 1.13 (95.4%)***	***74.30 ± 2.85 (100.0%)***	***n.d.***
			Dihydroflavonols			
94	Dihydro-quercetin	n.d.	<LOQ	n.d.	n.d.	n.d.
63	Taxifolin-*O*-hexoside	n.d.	0.02 ± 0.01	n.d.	n.d.	n.d.
	Total dihydroflavonols	***n.d.***	***0.02 ± 0.01 (0.1%)***	***n.d.***	***n.d.***	***n.d.***
			Dihydrochalcones			
105	Phloretin-*C*-hexoside	n.d.	0.01 ± 0.01	n.d.	n.d.	n.d.
98	Phloretin-di-*C*-hexoside	0.76 ± 0.01	0.07 ± 0.01	n.d.	n.d.	n.d.
	Total dihydrochalcones	***0.76 ± 0.01 (1.5%)***	***0.08 ± 0.01 (0.2%)***	***n.d.***	***n.d.***	***n.d.***
	Total phenolic compounds	***50.27 ± 1.47***	***38.46 ± 0.66***	***43.93 ± 0.10***	***74.30 ± 2.85***	***33.69 ± 0.77***

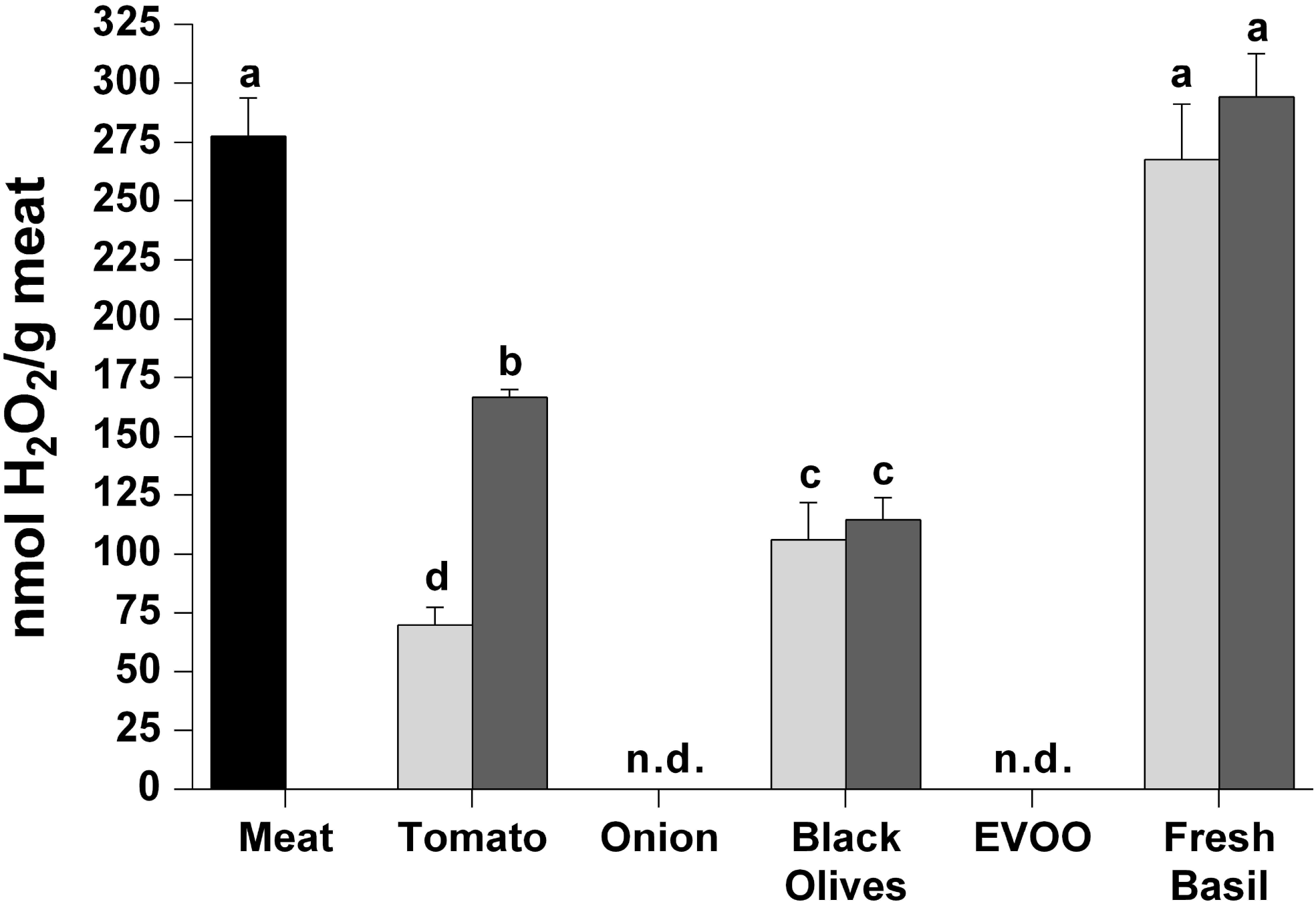
Table 3. Radical scavenging properties, ferrous ions chelating ability and ferric ions reducing properties of phenolic fractions from vegetable foods and extra-virgin olive oil.

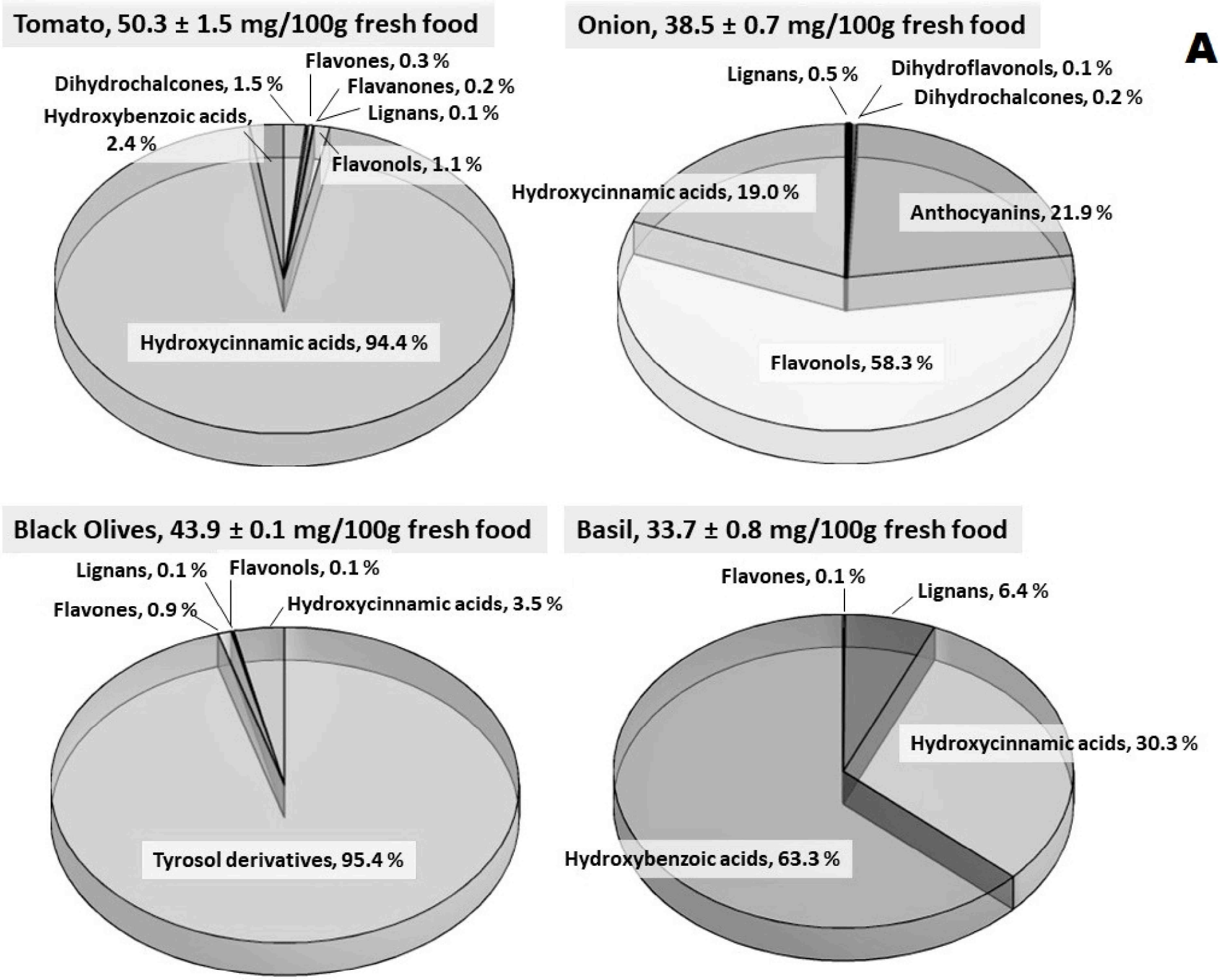
	ABTS radical scavenging	*Hydroxyl radical scavenging*	*Superoxide anion scavenging*	*Fe^{3+} reducing properties*	*Fe^{2+} chelating ability*
	μmol ascorbic acid equivalent/mg of total phenolic compounds[a]				*% chelation*[b]
Tomato	1.60 ± 0.05[c]	0.89 ± 0.05[c]	2.77 ± 0.45[a]	2.04 ± 0.07[b]	4.65 ± 1.14[c]
Fresh basil	1.69 ± 0.01[c]	0.89 ± 0.02[c]	0.11 ± 0.01[c]	1.49 ± 0.08[b]	54.72 ± 6.29[a]
Onion	2.97 ± 0.20[a]	1.56 ± 0.07[b]	1.04 ± 0.05[b]	1.03 ± 0.02[c]	6.90 ± 2.98[c]
Black olives	2.70 ± 0.04[b]	1.78 ± 0.08[a]	0.14 ± 0.04[c]	1.47 ± 0.04[b]	43.53 ± 1.86[b]
EVOO	1.61 ± 0.07[c]	0.87 ± 0.07[c]	0.27 ± 0.01[c]	0.63 ± 0.02[d]	7.54 ± 1.61[c]

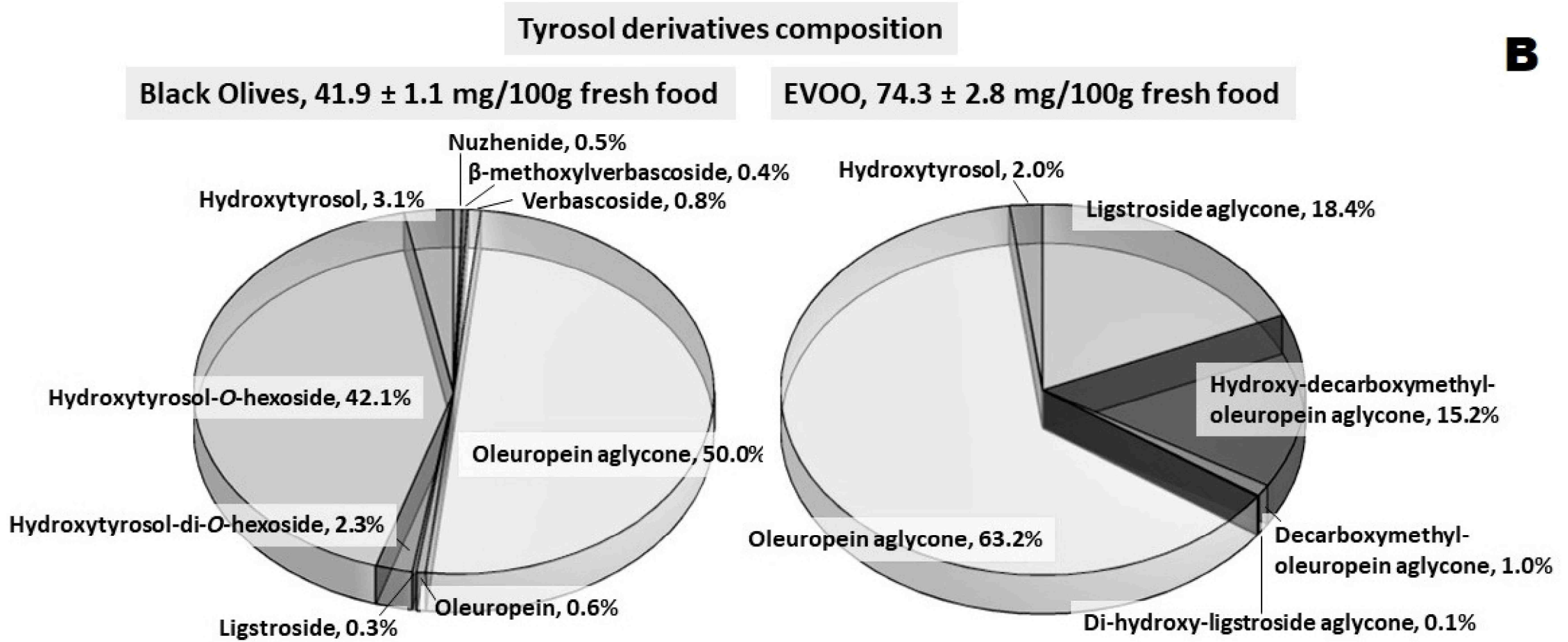
[a]data expressed as μmol ascorbic acid equivalent normalized for the total phenolic content as determined by mass spectrometry experiments

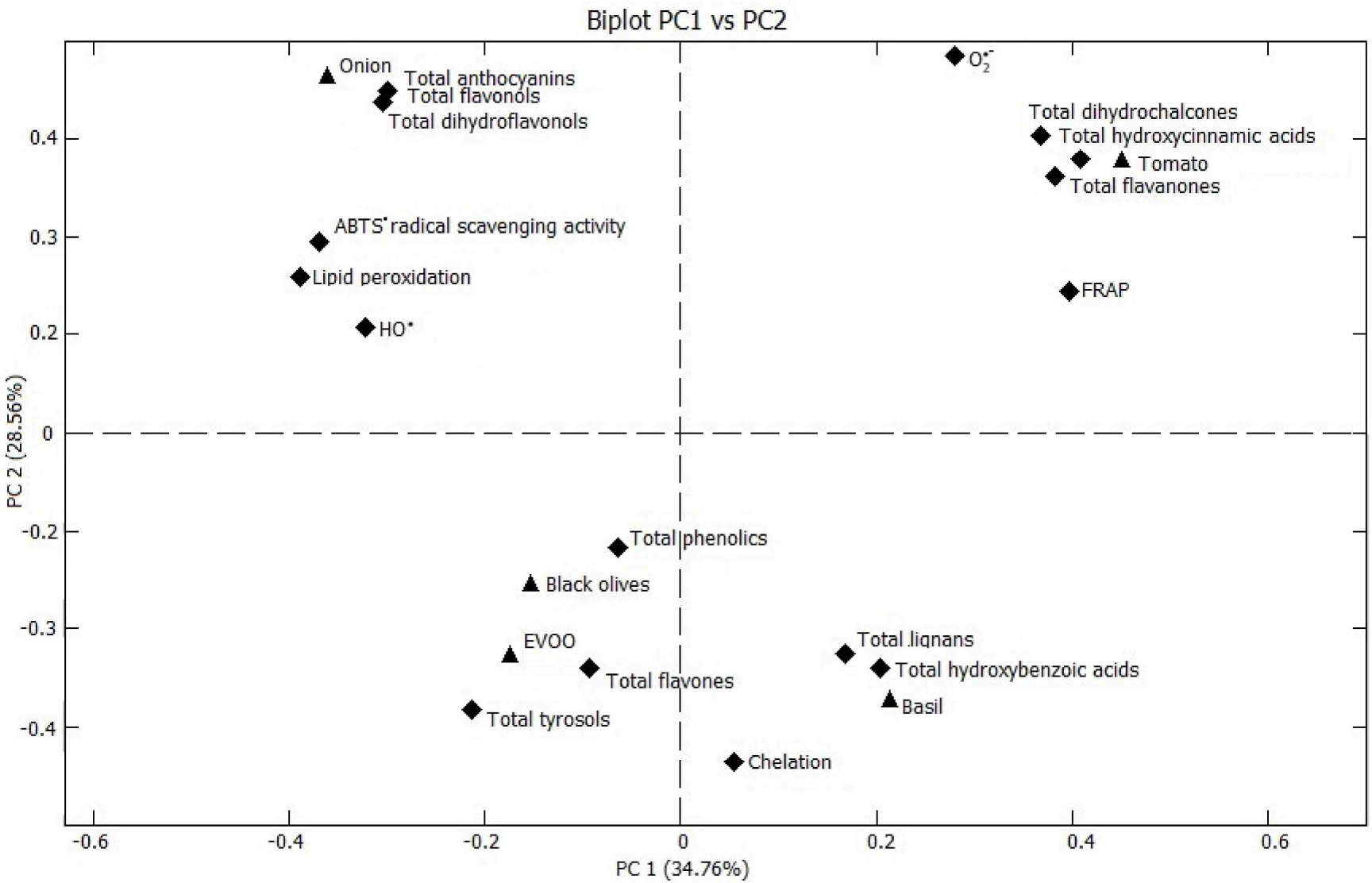
[b]% of chelated Fe^{2+} by 100 μg of phenolic compounds


a
b
n.d.
n.d.
300
275
250
225
200
175
150
125
100
75
50
25
0
nmol H2O2/g meat
0
65.2
130.3
260.5
Mediterranean diet salad (g/100 g meat)


nmol H_2O_2/g meat
325
300
275
250
225
200
175
150
125
100
75
50
25
0
a
d
b
n.d.
c
c
n.d.
a
a
Meat
Tomato
Onion
Black Olives
EVOO
Fresh Basil


A
Tomato, 50.3 ± 1.5 mg/100g fresh food
Flavones, 0.3 %
Dihydrochalcones, 1.5 %
Flavanones, 0.2 %
Hydroxybenzoic acids, 2.4 %
Lignans, 0.1 %
Flavonols, 1.1 %
Hydroxycinnamic acids, 94.4 %
Onion, 38.5 ± 0.7 mg/100g fresh food
Lignans, 0.5 %
Dihydroflavonols, 0.1 %
Dihydrochalcones, 0.2 %
Hydroxycinnamic acids, 19.0 %
Anthocyanins, 21.9 %
Flavonols, 58.3 %
Black Olives, 43.9 ± 0.1 mg/100g fresh food
Lignans, 0.1 %
Flavonols, 0.1 %
Flavones, 0.9 %
Hydroxycinnamic acids, 3.5 %
Tyrosol derivatives, 95.4 %
Basil, 33.7 ± 0.8 mg/100g fresh food
Flavones, 0.1 %
Lignans, 6.4 %
Hydroxycinnamic acids, 30.3 %
Hydroxybenzoic acids, 63.3 %


Tyrosol derivatives composition
B
Black Olives, 41.9 ± 1.1 mg/100g fresh food
EVOO, 74.3 ± 2.8 mg/100g fresh food
Nuzhenide, 0.5%
β-methoxylverbascoside, 0.4%
Hydroxytyrosol, 3.1%
Verbascoside, 0.8%
Hydroxytyrosol-O-hexoside, 42.1%
Oleuropein aglycone, 50.0%
Hydroxytyrosol-di-O-hexoside, 2.3%
Oleuropein, 0.6%
Ligstroside, 0.3%
Hydroxytyrosol, 2.0%
Ligstroside aglycone, 18.4%
Hydroxy-decarboxymethyl-
oleuropein aglycone, 15.2%
Oleuropein aglycone, 63.2%
Decarboxymethyl-
oleuropein aglycone, 1.0%
Di-hydroxy-ligstroside aglycone, 0.1%


Biplot PC1 vs PC2
Onion
Total anthocyanins
Total flavonols
Total dihydroflavonols
$O_2^{\bullet-}$
Total dihydrochalcones
Total hydroxycinnamic acids
Tomato
Total flavanones
ABTS• radical scavenging activity
Lipid peroxidation
HO•
FRAP
Total phenolics
Black olives
EVOO
Total flavones
Total tyrosols
Total lignans
Total hydroxybenzoic acids
Basil
Chelation
PC 2 (28.56%)
PC 1 (34.76%)
0.4
0.3
0.2
0
-0.2
-0.3
-0.4
-0.6
-0.4
-0.2
0
0.2
0.4
0.6