# Mediterranean Diet vegetable foods protect meat lipids from oxidation during *in vitro* gastrointestinal digestion

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#### 1 Abstract

2 Meat lipids oxidation during digestion gives rise to a post-prandial oxidative stress condition, which 3 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-4 5 digestion of turkey meat with a typical Mediterranean Diet salad containing tomato, onion, black 6 olives, extra-virgin olive oil (EVOO) and basil, dose-dependently reduced lipid peroxidation. Onion 7 and EVOO were more effective in limiting oxidation than the other foods, resulting in negligible 8 concentrations of lipid hydroperoxides after digestion. Specific phenolic classes dominated the 9 phenolic profile of the different foods, such as flavonols and anthocyanins in onion, phenolic acids 10 in tomato and basil, and tyrosol-derivatives in black olives and EVOO. The correlation between lipid 11 peroxidation inhibition, phenolic constituents and antioxidant properties was evaluated by principal 12 component analysis (PCA). Flavonols and anthocyanin were the major contributors to the bioactive 13 response of vegetable foods.

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

#### 16 **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).

21 Meat is peculiarly vulnerable to lipid oxidation due to its content of poly-unsaturated fatty acids 22 such as linoleic, linolenic, arachidonic, and docosahexaenoic acids and high concentrations of iron 23 catalysers (Tirosh, et al. 2015). In fact, high intakes of meat are usually associated to an increased 24 risk of colorectal cancer and cardiovascular diseases (Sasso and Latella 2018; Ferguson, 2010; 25 Micha et al. 2010). It has been supposed that this risk may be not caused by meat per se but a 26 consequence of high-fat intake, generation of carcinogens during meat processing as well as 27 oxidation of poly-unsaturated fatty acids that occurs during cooking and gastro-intestinal digestion 28 (Ferguson, 2010; Gorelik et al. 2013; Kanner and Lapidot 2001; Martini et al. 2018). In addition, 29 lipid peroxidation proceeds promptly when the raw meat structure is broken such as after cooking 30 and mastication (Papuc et al. 2017).

31 The formation of hydroxyl (HO•) and perhydroxy (HOO•) radicals as well as the generation of 32 perferrylmioglobin-containing peptides are considered the main pathways to free radical chain 33 reaction initiation of lipid peroxidation during gastro-intestinal digestion of meat (Carlsen and 34 Skibsted 2004; Oueslati et al. 2016; Tagliazucchi et al. 2010; Martini et al. 2018). Ferrous iron and 35 dissolved oxygen may generate O<sub>2</sub>•, which at low pH such as found in the gastric milieu forms 36 HOO•. Indeed, acidic disproportionation of  $O_2^{\bullet}$  may produce hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and 37 oxygen (Oueslati et al. 2016). The formation of HO• is possible by H<sub>2</sub>O<sub>2</sub> decomposition, catalysed 38 by ferrous iron (Fenton reaction), or by  $H_2O_2$  reaction with  $O_2^{\bullet-}$  (Haber–Weiss reaction) (Papuc et 39 al. 2017). These reactive species are able to initiate lipid peroxidation by subtracting a hydrogen 40 from lipids and generating a fatty acyl radical (L•). The resulting radical may react with dissolved 41 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<sup>2+</sup>), 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

52 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.

62 Therefore, this study was designed to understand if the combined consumption of a typical

63 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-

65 intestinal digestion. Vegetable foods were also characterized for their phenolic profile by LC-ESI-

66 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

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

#### 71 **2. Materials and methods**

#### 72 **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).

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#### 80 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.

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#### 87 2.3. In vitro co-digestion of grilled turkey breast meat with the Mediterranean Diet salad and

#### 88 *determination of lipid hydroperoxides*

89 Turkey breast meat (average size of 10x15x0.4 cm) was grilled at 140°C for 5 min until complete 90 cooking was achieved. After cooking, the meat was cooled on ice and stored at -80°C overnight. 91 Mediterranean Diet salad was prepared by mixing the single fresh vegetables in the proportion 92 reported above. For the digestion, 10 g of frozen meat was homogenized in a laboratory blender 93 together with 26.05 g of the Mediterranean Diet salad. An aliquot of 5 g of the homogenate was 94 then used for the *in vitro* digestion experiments following the protocol previously developed within 95 the COST Action INFOGEST (Minekus et al. 2014). Simulated salivary, gastric, pancreatic and bile 96 fluids were prepared according to Minekus et al. (2014). To simulate the oral phase, 5 g of

97 homogenate were mixed with 5 mL of simulated salivary fluid containing 150 U/mL of porcine α-98 amylase and incubated for 5 min at 37°C in a rotating wheel (10 rpm). The gastric phase was 99 carried out by adding 10 mL of simulated gastric fluid to the bolus. The pH was adjusted to 3.0 with 100 HCl 6 mol/L and supplemented with porcine pepsin (2000 U/mL of digest). The gastric bolus was 101 then incubated for 120 min at 37°C in a rotating wheel (10 rpm). The intestinal digestion was 102 carried out by adding 10 mL of pancreatic fluid (100 U trypsin activity/mL of digest) and 5 mL of 103 bile fluid (10 mmol/L in the total digest) to the gastric bolus and adjusting the pH to 7.0. The chyme 104 was further incubated for 120 min at 37°C in a rotating wheel (10 rpm). 105 At the end of the digestion, lipid hydroperoxides were extracted by 10-fold dilution in methanol 106 HPLC grade containing 4 mmol/L of butyl-hydroxytoluene (BHT) under slow stirring for 60 min 107 (Tagliazucchi et al. 2010). After centrifugation at 3000g for 15 min at 4°C, the hydroperoxides in 108 the supernatants were determined with the FOX assay at 560 nm adapted to a microplate reader 109 (Nourooz-Zadeh 1999; Martini et al. 2018). The FOX reagent contained 250 µmol/L of ammonium 110 ferrous sulphate, 100 µmol/L xylenol orange, 25 mmol/L H<sub>2</sub>SO<sub>4</sub>, and 4 mmol/L BHT in 90% (v/v) 111 methanol HPLC grade. For the assay, 60 µL of extracted sample were added to 140 µL of FOX 112 reagent and incubated for 30 minutes at room temperature. The hydroperoxides content was 113 expressed in nmol H<sub>2</sub>O<sub>2</sub> 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.

119 The dose-response effect of the Mediterranean Diet salad was assessed by homogenising 10 g of

120 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

121 of water). After that, the homogenates were subjected to *in vitro* digestion and lipid hydroperoxides

122 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.

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#### 127 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.

135 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,

 $137 ext{ v/v}$  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

139 evaporator and re-dissolved in 10 mL of water.

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#### 141 2.5. In vitro co-digestion of grilled turkey breast meat with single salad ingredients and

#### 142 vegetables phenolic fractions

143 In these co-digestion experiments, vegetable salad ingredients (tomatoes, onions, black olives,

144 EVOO and fresh basil) were added singularly to the grilled turkey breast meat in the same

- 145 proportions as found in the Mediterranean Diet salad. For the experiments, 10 g of cooked meat
- 146 were homogenized in presence of 20 g of tomato (plus 6.05 g of water) or 2.5 g of onion (plus 23.55

147 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)

148 or 0.05 g of fresh basil (plus 26 g of water). The proportion meat/ingredients were 200% tomato,

149 25% onion or black olives, 10% EVOO and 0.5% basil respect to meat (w/w). After that, the *in*150 *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.

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## 156 2.6. Identification and quantification of phenolic compounds by liquid chromatography 157 electrospray ionization ion trap mass spectrometer (LC-ESI-IT-MS)

158 Phenolic fractions were analyzed on a HPLC Agilent 1200 Series system equipped with a C18 159 column (HxSil C18 Reversed phase, 250×4.6 mm, 5 µm particle size, Hamilton Company, Reno, 160 Nevada, USA) as reported in Martini et al. (2017). The mobile phase consisted of (A) H<sub>2</sub>O/formic acid (99:1, v/v) and (B) acetonitrile/formic acid (99:1, v/v). The gradient started at 4% B for 0.5 161 162 min then linearly ramped up to 30% B in 60 min. The mobile phase composition was raised up to 163 100% B in 1 min and maintained for 5 min in order to wash the column before returning to the 164 initial condition. The flow rate was set at 1 mL/min. After passing through the column, the eluate 165 was split and 0.3 mL/min was directed to an Agilent 6300 ion trap mass spectrometer. Two MS 166 experiments were performed, one in ESI negative ion mode and one using positive ESI ionization 167 (for anthocyanins), under the same chromatographic conditions. Identification of phenolic compounds in all samples was carried out using full scan and data-dependent MS<sup>2</sup> scanning from 168 169 *m*/*z* 100 to 1500.

170 Phenolic compounds were quantified by using representative standards for each identified phenolic

171 class. Flavonols were quantified as quercetin-3-O-glucoside or quercetin-3-O-rutinoside

172 equivalents. Hydroxybenzoic acids were quantified in protocatechuic acid equivalents.

173 Hydroxycinnamic acids were quantified in coumaric or caffeic or ferulic acid equivalents.

174 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<sup>3+</sup> reducing ability, a protocol based on the ferric
- 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
- 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  $\pm$  SD for three

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.

#### 198 **3. Result and discussion**

## 199 3.1. Effect of Mediterranean Diet salad on lipid oxidation during co-digestion with turkey breast 200 meat

201 An eight-fold increase (from  $33.9 \pm 3.1$  to  $277.5 \pm 16.1$  nmol H<sub>2</sub>O<sub>2</sub>/g of meat) in the amount of lipid 202 hydroperoxides was observed after the in vitro gastro-intestinal digestion of turkey breast meat 203 without added vegetables. Whereas numerous studies determined the amount of lipid 204 hydroperoxides after in vitro gastric digestion (Kanner and Lapidot 2001; Gorelik et al. 2018a; 205 Tagliazucchi et al. 2010), very few of them measured their concentration after in vitro intestinal 206 digestion. However, a recent study by our research group showed a sharp increase in the generation 207 of lipid hydroperoxides during intestinal digestion of cooked turkey meat (Martini et al. 2018). This 208 increase could be a consequence of the bile salts emulsification and micellarization of fatty acids 209 present in turkey meat. Berton-Carabin et al. (2014) found that lipid peroxidation occurred much 210 faster in a water/oil system rather than in oil alone or in dispersion without emulsifier. This may be 211 due to several factors. First of all the formation of an interface between the aqueous phase and the 212 fat may favour the contact between the oxidants and the oxygen (dissolved in the aqueous phase) 213 and fatty acids (Berton-Carabin et al. 2014). Secondly, it could be ascribed to the solubilisation of 214 lipid hydroperoxides already formed in the micelles, which in turn may promote oxidation in the 215 micelles themselves (Donnelly et al. 1998). Finally, Sreejayan and von Ritter (1998) suggested that 216 bile salts, in the presence of iron, were able to favour the oxidation of arachidonic acid. The amount 217 of lipid hydroperoxides measured was about 23% lower than that found by Martini et al. (2018) at 218 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.

Several authors demonstrated the correlation between lipid peroxidation during *in vitro* digestion of
meat, with or without phenolic-rich foods, and the concentrations of lipid hydroperoxides and
lipoxidation end-products in the plasma of human volunteers after consumption of the same test
meals (Natella et al. 2011; Kanner et al. 2001; Gorelik et al. 2008a; Sirota et al. 2013). Therefore,
the results of the present *in vitro* digestion study are likely to be relevant for the *in vivo* situation.

## 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).

249 When turkey breast meat was co-digested with the single components of the Mediterranean Diet 250 salad, we observed a differential inhibition in lipid hydroperoxides generation (Figure 2A). Except 251 for fresh basil, the addition of all the vegetable components of the salad during co-digestion with 252 turkey meat resulted in a significantly lower amount of generated lipid hydroperoxides. Among the 253 different vegetables, digests of turkey meat with 25% onion and 10% EVOO had undetectable 254 levels of lipid hydroperoxides (100% inhibition). Tomato, although present in a higher amount (200 255 g per 100 g of meat) respect to onion and EVOO (25 g and 10 g per 100 g of meat, respectively), 256 showed a lower inhibitory effect (P < 0.05) on the generation of lipid hydroperoxides during co-257 digestion with turkey meat (~ 75% of inhibition). The inhibition in lipid hydroperoxides formation 258 was significantly lower (P < 0.05) when 25% of black olives were added to the cooked turkey meat 259 in the digestion system (~ 62% of inhibition). Finally, no significant differences were found in the 260 amount of lipid hydroperoxides in the digests when 0.5% of fresh basil was added to turkey meat 261 (*P*>0.05).

262 Previous studies reported the ability of EVOO and onion to inhibit lipid peroxidation during in vitro 263 digestion of meat (Martini et al. 2018; Kanner et al. 2017; Tirosh et al. 2015). Kanner et al. (2017) 264 also found that tomato inhibited with less effectiveness lipid peroxidation during *in vitro* gastric 265 digestion of turkey meat respect to onion, black olives and EVOO. Differently from our study, they 266 observed a higher effectiveness of black olives respect to onion. However, they measured the lipid 267 peroxidation inhibitory activity at the end of the gastric phase of digestion and not after the 268 intestinal phase. Van Hecke et al. (2017) found that dried basil inhibited lipid peroxidation after in 269 vitro intestinal digestion of beef when added in the amounts of 0.5% or 1% respect to meat. Instead, 270 in this study we found that the addition of 0.5% of fresh basil had no effect on lipid peroxidation 271 during in vitro digestion of turkey meat. This difference was clearly related to the fact that the same 272 amount (0.5%) of dried basil delivered more antioxidant compounds to the digestive system respect 273 to the fresh herb (Henning et al. 2011).

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### 275 3.3. Effect of the phenolic fractions of single components of the Mediterranean Diet salad on

#### 276 *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).

282 Phenolic fractions extracted from onion and EVOO and co-digested with meat at the same 283 concentration as found in 25% onion and 10% EVOO totally inhibited the generation of lipid 284 hydroperoxides without any differences with the data obtained after the co-digestion of meat with 285 whole onion or EVOO (P>0.05). Similarly, co-digestion of turkey breast meat with phenolic 286 fractions extracted from black olives and fresh basil resulted in the same inhibitory potency as the 287 whole foods (P>0.05). However, in the case of tomato phenolic fraction, the effect was less 288 pronounced respect to that observed after co-digestion with whole food (58% vs 75% of inhibition; 289 P < 0.05). Despite phenolic compounds, tomato is rich in other lipophilic antioxidants such as 290 carotenoids and in particular lycopene (Martí et al. 2016). Previous studies described lycopene as an 291 efficient inhibitor of lipid peroxidation both in meat products and in cell cultures (Rohlík et al. 292 2013; Chisté et al. 2014). Lycopene is highly hydrophobic and not extracted in the water/methanol 293 mixture used to prepare the phenolic fraction from tomato. Moreover, it can be released during the 294 intestinal phase of digestion and exert its anti-peroxidative effect (Tagliazucchi et al. 2012).

295

#### 296 3.4. Phenolic profile of vegetables and EVOO

297 The phenolic profile of vegetables and EVOO was investigated using a non-targeted method 298 through LC-ESI-MS/MS experiments. The mass spectrum data along with peak assignments and 299 retention time for the identified phenolic compounds are described in **Table 1**. This approach 300 allowed the tentative identification of 132 compounds (**Table 1**). Seven compounds (compounds 4, 301 23, 26, 40, 53, 95 and 99) were identified by comparison with their respective authentic standards.
302 The remaining compounds were tentatively identified based on the interpretation of the
303 fragmentation patterns obtained from mass spectra and by comparing their mass spectral
304 characteristics with the available literature. The interpretation of the mass spectra fragmentation
305 patterns reported in the literature is not further discussed.

306 The profile of individual phenolic compounds as well as the total phenolic compounds amount for 307 each vegetable and EVOO are reported in **Table 2** and **Figure 3**. The highest phenolic content was 308 found in EVOO > tomato > black olives > onion > fresh basil (P < 0.05). Each single ingredients 309 was characterized for its specific phenolic profile (Figure 3). In tomato, hydroxycinnamic acids 310 were the predominant class of phenolic compounds (94.4%) whereas in fresh basil hydroxybenzoic 311 acids prevailed (63.3%) respect to hydroxycinnamic acids (30.3%). The phenolic profile of onion 312 was mainly characterized by flavonols (58.3%) and anthocyanins (21.9%). EVOO and black olives 313 were characterized for the presence of tyrosol-derivatives. Concerning the individual phenolic 314 profile, sixty-five phenolic compounds were identified in tomato, which was characterized by the 315 presence of relevant amounts of di-hydro-ferulic acid-O-hexoside (47), caffeic acid-O-hexoside 316 (25) and 4- and 5-O-caffeoylquinic acids (56 and 51). Apart from hydroxycinnamic acids, modest 317 amounts of hydroxybenzoic acids and flavonols were detected in tomato. Flavanones were present 318 only in tomato but in very low concentrations. Thirty-five phenolic compounds were identified and 319 quantified in onion (Table 2). Flavonols were the major group of phenolic compounds identified in 320 onion. Quercetin-3-O-hexoside-4'-O-hexoside (82), quercetin-4'-O-hexoside (119) and cyanidin-3-321 O-malonylhexoside (64) were the main individual phenolics. With respect to black olives, a higher 322 prevalence of tyrosol-derivatives was noted (Table 2). Twenty-seven phenolic compounds were 323 quantified in black olives, with oleuropein aglycone (70) and hydroxytyrosol-O-hexoside isomers (5 324 and 8) present in high concentrations. Similar to what was reported for black olives, tyrosol-325 derivatives were the major group of phenolic compounds in EVOO, where oleuropein aglycone 326 (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.

330

#### 331 3.4. Antioxidant properties of vegetable and EVOO phenolic fractions

332 Vegetables and EVOO phenolic fractions were characterized for their ability to scavenge 333 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 334 335 assessed (Table 3). Black olives and onion phenolic compounds showed the highest ABTS and 336 hydroxyl radical scavenging activities. Instead, tomato phenolic compounds displayed the highest 337 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. 338 339 The different antioxidant properties of the phenolic fractions reflect differences in their phenolic 340 compositions (Martini et al. 2019). Onion and black olives were found to be particularly rich in 341 quercetin- and cyanidin-derivatives and hydroxytyrosol-derivatives, respectively. These compounds 342 share a 3',4'-dihydroxy structure in the B-ring (i.e. catechol moiety) which is considered of 343 paramount importance to determine the ABTS and hydroxyl radical scavenging properties (Rice-344 Evans et al. 1999, Ozyürek et al. 2008, Zamora and Hidalgo 2016). Diversely, tomato and fresh 345 basil showed significantly lower ABTS and hydroxyl radical scavenging properties than onion and 346 black olives. Indeed, they were rich in phenolic acids, which showed the lowest hydroxyl radical 347 and ABTS scavenging activities among phenolic compounds (Rice-Evans et al. 1999, Ozyürek et al. 348 2008). Differences between black olives and EVOO hydroxyl radical and ABTS scavenging 349 activities may be related to the presence of additional antioxidant compounds in black olives. The 350 latter contain also non-phenolic compounds such as oleoside and its methyl- and dimethyl-351 derivatives, which showed radical scavenging properties (Wang et al. 2000). Hydroxycinnamic 352 acids seemed to be the best superoxide anions  $(O_2^{\bullet})$  scavenging phenolic compounds since the

353 most active extract against O<sub>2</sub>•<sup>-</sup> was tomato which was mainly consisted of hydroxycinnamic acids 354 (Figure 3). Moreover, hydroxycinnamic acids were also the compounds with the highest ferric 355 reducing properties as suggested by the highest ferric reducing power of tomato and fresh basil 356 phenolic fractions. Previous works indicated that hydroxycinnamic and hydroxybenzoic acids 357 displayed higher ferric reducing activities than flavan-3-ols and glycosylated flavonols (Pulido et al. 358 2000; Martini et al. 2019). Finally, no clear relationship was found between the phenolic composition and the Fe<sup>2+</sup>-chelating ability of the phenolic fractions extracted from vegetables and 359 360 EVOO.

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## 362 3.5. Relationship between the lipid peroxidation inhibitory activity, the phenolic profile and the 363 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).

370 Three principal components explained about 90.5% of total variance. In particular, a bidimensional

371 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

- 374 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

377 PC1, positively correlated to the onion and its typical phenolic classes: anthocyanins and flavonols.

378 This reflects their higher effectiveness in antioxidant and lipid peroxidation inhibitory activities

379 than the other ingredients or phenolic classes. Regarding this, the orthogonal directions of 380 hydroxycinnamic and hydroxybenzoic acids did not suggest any kind of relationship. An inverse 381 relationship between ferric reducing power and the inhibition of the lipid peroxidation is depicted 382 by FRAP loading on PC1. The explanation could lay in the mechanisms of action of the used 383 antioxidant activity assays. According to the chemistry of the ABTS and hydroxyl radical 384 scavenging assays, their mechanisms may involve both the single electron transfer (SET) and 385 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 386 387 optimal conditions to maintain and stimulate the Fenton and Haber-Weiss reactions. Whereas, the 388 HAT mechanism might stop the lipid peroxidation reaction at several levels. Tyrosol- and 389 hydroxytyrosol-derivatives, describing the phenolic profile of black olives and EVOO, had the same 390 negative loadings on PC1 of the lipid peroxidation inhibition, reflecting their possible involvement 391 in the peroxidation phenomena. However, the negative loading vectors on PC2 could reflect their 392 intrinsic and paradoxical behaviour already investigated in Martini et al. (2018) outlining how 393 tyrosol- and hydroxytyrosol-derivatives peroxidation inhibitory activity is strictly related to their 394 final concentration.

#### 395 4. Conclusions

396 This study provides evidence of a protective effect of a typical Mediterranean Diet salad on lipid 397 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 398 399 peroxidation inhibitory effect. With the exception of tomato, there were not significant differences 400 between the inhibitory effect of the whole ingredients and the respective phenolic fractions, 401 implying that phenolic compounds were mainly responsible for the reported effect. Moreover, our 402 data suggested that the inhibitory effect was related to the different phenolic composition of the 403 tested ingredients and that some phenolic compounds, especially that with a B-ring catechol moiety 404 in their structure (i.e. flavonols and anthocyanins), were the most effective in reducing the oxidative 405 phenomena after co-digestion with meat. This effect was ascribed to the highest radical scavenging 406 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 407 408 inhibitory effect. This study gives strong evidence about the structure-activity relationship between 409 phenolic compounds and lipid peroxidation inhibitory activity. Therefore, it is of paramount 410 importance to profile the phenolic composition of antioxidant-rich foods used in this type of study 411 to predict their possible impact on lipid peroxidation during the digestion of meat. Indeed, our study 412 underlines the importance of consuming specific food combinations, in specific amounts to achieve 413 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**

419 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 codigestion 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<sub>2</sub>O<sub>2</sub>/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 tyrosolderivatives 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  $\blacklozenge$  identifies the phenolic classes and the biochemical properties, whereas the symbol  $\blacktriangle$  represents the food ingredients. FRAP: ferric reducing power; ABTS: ABTS radical scavenging activity; HO•: hydroxyl radical scavenging activity; O<sub>2</sub>•<sup>-</sup>: superoxide anion radical scavenging activity; EVOO: extra-virgin olive oil.

	Compound	Rt (min)	[M-H] <sup>-</sup> (m/z)	$MS^2$ ion fragments (m/z)
	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%)
	Caffeoylquinic acid-3- <i>O</i> -hexoside- 4- <i>O</i> -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%)
	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%)
	Caffeoylquinic acid-3- <i>O</i> -hexoside- 4- <i>O</i> -hexoside isomer	14.1	677	515 (100%), 341 (21%)
1.7	Caffeoylquinic acid-O-hexoside isomer	14.9	515	341 (100%), 323 (64%), 179 (57%), 353 (34%)
16	Caffeic acid-3- <i>O</i> -hexoside-4- <i>O</i> -hexoside	15.5	503	341 (100%), 179 (17%)
17	3-O-Caffeoylquinic acid	15.6	353	191 (100%), 179 (24%), 135 (19%)
IA	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%)
//	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%)
/.1	Hydroxybenzoic acid-O-hexoside isomer	17.8	299	137 (100%)
	Di-hydro-coumaric acid- <i>O</i> - hexoside	18.0	327	165 (100%), 121 (4%)
29	Di-hydro-caffeic acid- <i>O</i> -hexoside isomer	18.0	343	181 (100%), 137 (33%)
30	Syringic acid	18.2	197	153 (100%)

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

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- <i>O</i> -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-6a-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- <i>O</i> - hexoside-4- <i>O</i> -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- <i>O</i> -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- <i>O</i> -malonylhexoside	25.2	549 <sup>#</sup>	301 (100%), 463 (6%)
66	Myricetin-di-O-hexoside	25.4	641	479 (100%), 317 (21%)
67	Quercetin-3- <i>O</i> -rutinoside-7- <i>O</i> -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- <i>O</i> -hexoside-4'- <i>O</i> -hexoside	31.3	625	463 (100%), 301 (22%)
81	5-O-Feruloylquinic acid	32.2	367	191 (100%), 173 (4%)
82	Quercetin-3- <i>O</i> -hexoside-4'- <i>O</i> -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- <i>C</i> -hexoside- <i>O</i> - 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	$\beta$ -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 thevegetables. Values represent means  $\pm$  standard deviation of triplicate determination (n.d. means notdetected).

	Compound	Tomato	Onion	Black olives	EVOO	Basil
		Hydroxybe	nzoic acids			
21	Gallic acid	n.d.	< LOQ	n.d.	n.d.	$0.08\pm0.05$
30	Syringic acid	n.d.	< LOQ	n.d.	n.d.	$0.56\pm0.19$
35	Protocatechuic acid-O-pentoside	$0.37\pm0.07$	n.d.	n.d.	n.d.	n.d.
1	Hydroxybenzoic acid-O-hexoside isomer	$0.17\pm0.04$	n.d.	n.d.	n.d.	n.d.
27	Hydroxybenzoic acid-O-hexoside isomer	n.d.	n.d.	n.d.	n.d.	$0.19\pm0.08$
10	Protocatechuic acid-O-hexoside	$0.20\pm0.02$	n.d.	n.d.	n.d.	$0.08\pm0.02$
7	Vanillic acid-4-O-hexoside	$0.13\pm0.02$	n.d.	n.d.	n.d.	$0.13\pm0.04$
36	Syringic acid-4-O-pentoside	n.d.	n.d.	n.d.	n.d.	$10.31 \pm 0.16$
12	Syringic acid-4-O-hexoside	n.d.	n.d.	n.d.	n.d.	$0.14\pm0.01$
43	Syringic acid-4-O-acetylhexoside	n.d.	n.d.	n.d.	n.d.	$0.42\pm0.11$
22	Protocatechuic acid-O-hexoside- O-pentoside	n.d.	n.d.	n.d.	n.d.	$7.17\pm0.27$
2	Hydroxybenzoic acid-dihexoside	$0.32\pm0.07$	n.d.	n.d.	n.d.	n.d.
44	Syringic acid-dihexoside	n.d.	n.d.	n.d.	n.d.	$2.25\pm0.31$
	Total hydroxybenzoic acids	1.19 ± 0.11 (2.4%)	< <i>LOQ</i>	n.d.	n.d.	21.33 ± 0.49 (63.3%)
		Hydroxycini	namic acids			
23	Coumaric acid	$0.13\pm0.01$	n.d.	$0.04\pm0.01$	n.d.	n.d.

23	Coumaric acid	$0.13\pm0.01$	n.d.	$0.04\pm0.01$	n.d.	n.d.
26	Caffeic acid	$0.28\pm0.02$	n.d.	$0.10\pm0.01$	n.d.	n.d.
40	Ferulic acid	$0.73\pm0.18$	n.d.	n.d.	n.d.	n.d.
24	Coumaric acid-O-hexoside isomer	$0.12\pm0.03$	n.d.	n.d.	n.d.	n.d.
45	Coumaric acid-O-hexoside isomer	$0.81\pm0.02$	n.d.	n.d.	n.d.	n.d.
73	Ferulic acid-4-O-pentoside isomer	n.d.	n.d.	n.d.	n.d.	$1.73\pm0.13$
74	Ferulic acid-4-O-pentoside isomer	n.d.	n.d.	n.d.	n.d.	$5.35\pm0.49$
28	Dihydro-coumaric acid- <i>O</i> - hexoside	$0.81\pm0.02$	n.d.	n.d.	n.d.	n.d.
75	4-O-Cumaroylquinic acid	$0.18\pm0.08$	n.d.	n.d.	n.d.	n.d.
77	5-O-Cumaroylquinic acid	$0.53\pm0.06$	n.d.	n.d.	n.d.	n.d.
25	Caffeic acid-O-hexoside isomer	$5.72\pm0.56$	n.d.	$0.67\pm0.01$	n.d.	$0.05\pm0.02$
33	Caffeoyl-6 <sub>β</sub> -hexose	$1.13\pm0.04$	n.d.	n.d.	n.d.	n.d.

46	Caffeoyl-6α-hexose	$2.59\pm0.16$	n.d.	$0.39 \pm 0.04$	n.d.	n.d.
61	Caffeic acid-O-hexoside isomer	$0.17\pm0.05$	n.d.	n.d.	n.d.	n.d.
29	Dihydro-caffeic acid-O-hexoside isomer	$0.55\pm0.05$	n.d.	n.d.	n.d.	n.d.
37	Dihydro-caffeic acid-O-hexoside isomer	$0.69\pm0.12$	n.d.	n.d.	n.d.	n.d.
17	3-O-Caffeoylquinic acid	$0.18\pm0.01$	n.d.	n.d.	n.d.	n.d.
39	4-O-Caffeoylquinic acid cis	$0.38\pm0.17$	n.d.	n.d.	n.d.	n.d.
51	5-O-Caffeoylquinic acid trans	$4.57\pm0.03$	n.d.	n.d.	n.d.	n.d.
56	4-O-Caffeoylquinic acid trans	$4.61\pm0.11$	n.d.	n.d.	n.d.	n.d.
69	5-O-Caffeoylquinic acid cis	$0.90\pm0.01$	n.d.	n.d.	n.d.	n.d.
41	Ferulic acid-4-O-hexoside	$1.56\pm0.46$	n.d.	n.d.	n.d.	n.d.
60	Feruloyl-hexose	$2.95\pm0.85$	n.d.	n.d.	n.d.	$0.05\pm0.01$
47	Dihydro-ferulic acid-O-hexoside	$6.65\pm0.63$	n.d.	n.d.	n.d.	n.d.
20	Rosmarinic acid	n.d.	n.d.	n.d.	n.d.	$0.08\pm0.01$
81	5-O-Feruloylquinic acid	$1.80\pm0.23$	n.d.	n.d.	n.d.	n.d.
52	Sinapic acid-4-O-hexoside	$2.21\pm0.19$	$1.58\pm0.19$	n.d.	n.d.	$2.34\pm0.19$
68	Sinapoyl-hexose	n.d.	$5.75\pm0.33$	n.d.	n.d.	n.d.
71	Caffeic acid-O-malonylhexoside	$0.23\pm0.01$	n.d.	n.d.	n.d.	n.d.
11	Caffeic acid-O-hexoside-O- pentoside isomer	n.d.	n.d.	n.d.	n.d.	$0.16\pm0.05$
31	Caffeic acid-O-hexoside-O- pentoside isomer	n.d.	n.d.	n.d.	n.d.	$0.09\pm0.01$
13	Calceolarioside	n.d.	n.d.	$0.32\pm0.09$	n.d.	n.d.
19	Caffeoyl-coumaroylquinic acid	$0.12\pm0.01$	n.d.	n.d.	n.d.	n.d.
128	Coumaroyl-caffeoylquinic acid	$0.10\pm0.01$	n.d.	n.d.	n.d.	n.d.
16	Caffeic acid-3- <i>O</i> -hexoside-4- <i>O</i> -hexoside	$0.14\pm0.01$	n.d.	n.d.	n.d.	n.d.
49	Dihydro-caffeic acid-3- <i>O</i> - hexoside-4- <i>O</i> -hexoside	$0.19\pm0.02$	n.d.	n.d.	n.d.	n.d.
15	Caffeoylquinic acid- <i>O</i> -hexoside isomer	$0.06\pm0.01$	n.d.	n.d.	n.d.	n.d.
18	Caffeoylquinic acid- <i>O</i> -hexoside isomer	$0.52\pm0.09$	n.d.	n.d.	n.d.	n.d.
38	Caffeoylquinic acid- <i>O</i> -hexoside isomer	$0.38\pm0.17$	n.d.	n.d.	n.d.	n.d.
48	Caffeoylquinic acid- <i>O</i> -hexoside isomer	$0.11\pm0.03$	n.d.	n.d.	n.d.	n.d.
109	4,5-diCaffeoylquinic acid	$1.12\pm0.28$	n.d.	n.d.	n.d.	n.d.
115	3,5-diCaffeoylquinic acid	$0.73\pm0.03$	n.d.	n.d.	n.d.	$0.35\pm0.08$
22						
32	Feruloylquinic acid-O-hexoside isomer	$1.43\pm0.01$	n.d.	n.d.	n.d.	n.d.

6	Caffeoylquinic acid-3- <i>O</i> -hexoside- 4- <i>O</i> -hexoside isomer	$0.33 \pm 0.06$	n.d.	n.d.	n.d.	n.d.
14	Caffeoylquinic acid-3- <i>O</i> -hexoside- 4- <i>O</i> -hexoside isomer	$0.06 \pm 0.01$	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%)
		Flav	onols		-	
131	Quercetin	n.d.	$0.04 \pm 0.01$	n.d.	n.d.	n.d.
114	Kaempferol-3-O-hexoside	n.d.	$0.09\pm0.01$	n.d.	n.d.	n.d.
121	Kaempferol-7-O-hexoside	< LOQ	$0.19\pm0.02$	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	$0.01\pm0.01$	$0.43\pm0.05$	$0.03\pm0.01$	n.d.	n.d.
119	Quercetin-4'-O-hexoside	n.d.	$7.29\pm0.40$	n.d.	n.d.	n.d.
116	Isorhamnetin-3-O-hexoside	n.d.	$0.04\pm0.01$	n.d.	n.d.	n.d.
125	Isorhamnetin-4'-O-hexoside	n.d.	$2.35\pm0.07$	< LOQ	n.d.	n.d.
85	Myricetin-7-O-hexoside	n.d.	$0.01\pm0.01$	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	$0.37\pm0.01$	n.d.	n.d.	n.d.	n.d.
83	Kaempferol-3- <i>O</i> -hexoside-7- <i>O</i> - hexoside	n.d.	$0.21\pm0.01$	n.d.	n.d.	n.d.
62	Quercetin-3-O-hexoside-7-O- hexoside	n.d.	$0.03\pm0.01$	n.d.	n.d.	n.d.
80	Quercetin-7-O-hexoside-4'-O- hexoside	n.d.	$0.15\pm0.01$	n.d.	n.d.	n.d.
82	Quercetin-3-O-hexoside-4'-O- hexoside	< LOQ	$11.13\pm0.18$	$0.03\pm0.01$	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- <i>O</i> -hexoside-4'- <i>O</i> -hexoside	n.d.	$0.38\pm0.01$	n.d.	n.d.	n.d.
66	Myricetin-di-O-hexoside	n.d.	$0.01\pm0.01$	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	$0.17\pm0.01$	< LOQ	n.d.	n.d.	n.d.
67	Quercetin-3-O-rutinoside-7-O- hexoside	$0.01 \pm 0.01$	< LOQ	n.d.	n.d.	n.d.
42	Quercetin-tri-O-hexoside	n.d.	$0.08\pm0.01$	n.d.	n.d.	n.d.
57	Quercetin-3- <i>O</i> -rutinoside- <i>O</i> - hexoside- <i>O</i> -pentoside	$0.01 \pm 0.01$	< 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.	< <i>LOQ</i>
		Antho	cyanins			
53	Cyanidin-3-O-glucoside	n.d.	$1.59\pm0.02$	n.d.	n.d.	n.d.
59	Peonidin-3-O-hexoside	n.d.	$0.45\pm0.01$	n.d.	n.d.	n.d.
64	Cyanidin-3-O-malonylhexoside	n.d.	$4.29\pm0.24$	n.d.	n.d.	n.d.
65	Peonidin-3-O-malonylhexoside	n.d.	$0.84\pm0.04$	n.d.	n.d.	n.d.
54	Cyanidin-di-O-hexoside	n.d.	$1.25\pm0.04$	n.d.	n.d.	n.d.
	Total anthocyanins	n.d.	8.42 ± 0.24 (21.9%)	n.d.	n.d.	n.d.
		Lig	nans			
50	Medioresinol	$0.04\pm0.01$	n.d.	n.d.	n.d.	$1.66\pm0.01$
103	Pinoresinol-O-hexoside	n.d.	n.d.	n.d.	n.d.	$0.07\pm0.01$
90	Lariciresinol-O-hexoside	n.d.	n.d.	n.d.	n.d.	$0.40 \pm 0.04$
86	Secoisolariciresinol-O-hexoside	n.d.	$0.03\pm0.01$	n.d.	n.d.	n.d.
106	Syringaresinol-O-hexoside	n.d.	$0.15\pm0.01$	$0.04\pm0.01$	n.d.	n.d.
	Total lignans	$0.04 \pm 0.01$ (0.1%)	$0.18 \pm 0.15$ (0.5%)	$0.04 \pm 0.01$ (0.1%)	n.d.	$2.14 \pm 0.23$ (6.4%)
		Fla	vones			
55	Apigenin-O-pentoside	$0.03\pm0.01$	n.d.	n.d.	n.d.	n.d.
34	Apigenin-O-hexoside	$0.07\pm0.02$	n.d.	n.d.	n.d.	n.d.
100	Luteolin-O-hexoside	n.d.	n.d.	$0.27\pm0.02$	n.d.	n.d.
72	Amentoflavone	n.d.	n.d.	$0.02\pm0.01$	n.d.	< LOQ
113	Apigenin-O-hexoside-O-pentoside	n.d.	n.d.	< LOQ	n.d.	n.d.
91	Apigenin-C-hexoside-O- rhamnoside	$0.06\pm0.01$	n.d.	n.d.	n.d.	n.d.
112	Apigenin-O-hexoside-O- rhamnoside	n.d.	n.d.	$0.02\pm0.01$	n.d.	n.d.
76	Apigenin-6,8-di-C-hexoside	n.d.	n.d.	$0.01\pm0.01$	n.d.	$0.02\pm0.01$
97	Luteolin-O-rutinoside isomer	n.d.	n.d.	$0.02\pm0.01$	n.d.	n.d.
102	Luteolin-O-rutinoside isomer	n.d.	n.d.	$0.04\pm0.01$	n.d.	n.d.
	Total flavones	0.16 ± 0.02 (0.3%)	n.d.	0.38 ± 0.01 (0.9%)	n.d.	$0.02 \pm 0.01$ (0.1%)
		Flave	anones			
111	Liquiritigenin-7-O-hexoside	$0.02\pm0.01$	n.d.	n.d.	n.d.	n.d.
87	Naringenin-C-hexoside	$0.01\pm0.01$	n.d.	n.d.	n.d.	n.d.

 $0.01\pm0.01$ 

 $0.01\pm0.01$ 

 $0.02\pm0.01$ 

n.d.

117 Naringenin-O-hexoside isomer

Eriodictiol-O-hexoside

129

92

Naringenin-O-hexoside isomer

120	Naringenin- <i>O</i> -hexoside- <i>O</i> - pentoside	$0.01 \pm 0.01$	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 de	erivatives			
4	Hydroxytyrosol isomer	n.d.	n.d.	$0.63\pm0.14$	n.d.	n.d.
9	Hydroxytyrosol isomer	n.d.	n.d.	$0.65\pm0.07$	$1.50\pm0.10$	n.d.
1	Hydroxytyrosol- <i>O</i> -hexoside isomer	n.d.	n.d.	$15.24\pm0.48$	n.d.	n.d.
5	Hydroxytyrosol-O-hexoside isomer	n.d.	n.d.	$2.41 \pm 1.00$	n.d.	n.d.
123	Decarboxymethyl-oleuropein aglycone	n.d.	n.d.	n.d.	$0.77\pm0.01$	n.d.
122	Hydroxy-decarboxymethyl- oleuropein aglycone	n.d.	n.d.	n.d.	$11.31\pm0.27$	n.d.
132	Ligstroside aglycone	n.d.	n.d.	n.d.	$13.66\pm0.54$	n.d.
70	Oleuropein aglycone isomer	n.d.	n.d.	$20.98 \pm 0.67$	n.d.	n.d.
118	Oleuropein aglycone isomer	n.d.	n.d.	n.d.	$46.98 \pm 2.79$	n.d.
126	Di-hydroxy-ligstroside aglycone	n.d.	n.d.	n.d.	$0.07\pm0.03$	n.d.
2	Hydroxytyrosol-di-O-hexoside	n.d.	n.d.	$0.96\pm0.11$	n.d.	n.d.
130	Ligstroside	n.d.	n.d.	$0.11\pm0.02$	n.d.	n.d.
124	Oleuropein	n.d.	n.d.	$0.24\pm0.03$	n.d.	n.d.
107	Verbascoside	n.d.	n.d.	$0.34\pm0.01$	n.d.	n.d.
127	β-methoxylverbascoside	n.d.	n.d.	$0.15\pm0.01$	n.d.	n.d.
104	Nuzhenide	n.d.	n.d.	$0.21\pm0.06$	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.
		Dihydrof	lavonols			
94	Dihydro-quercetin	n.d.	<loq< td=""><td>n.d.</td><td>n.d.</td><td>n.d.</td></loq<>	n.d.	n.d.	n.d.

Dihydroflavonols									
94	Dihydro-quercetin	n.d.	<loq< th=""><th>n.d.</th><th>n.d.</th><th>n.d.</th></loq<>	n.d.	n.d.	n.d.			
63	Taxifolin-O-hexoside	n.d.	$0.02\pm0.01$	n.d.	n.d.	n.d.			
	Total dihydroflavonols	n.d.	$0.02 \pm 0.01 \ (0.1\%)$	n.d.	n.d.	n.d.			
Dihydrochalcones									
105	Phloretin-C-hexoside	n.d.	$0.01\pm0.01$	n.d.	n.d.	n.d.			
98	Phloretin-di-C-hexoside	$0.76\pm0.01$	$0.07\pm0.01$	n.d.	n.d.	n.d.			
	Total dihydrochalcones $0.76 \pm 0.01$ (1.5%) $0.08 \pm 0.01$ (0.2%)n.d.n.d.								
	Total phenolic compounds	50.27 ± 1.47	38.46 ± 0.66	43.93 ± 0.10	74.30 ± 2.85	<i>33.69</i> ± <i>0.77</i>			

	ABTS radical scavenging	Hydroxyl radical scavenging	Superoxide anion scavenging	Fe <sup>3+</sup> reducing properties	Fe <sup>2+</sup> chelating ability
	µmol ascorbic	acid equivalent/n	ng of total phenoli	ic compounds <sup>a</sup>	% chelation <sup>b</sup>
Tomato	$1.60\pm0.05^{\circ}$	$0.89\pm0.05^{\circ}$	$2.77\pm0.45^{\rm a}$	$2.04\pm0.07^{b}$	$4.65\pm1.14^{\rm c}$
Fresh basil	$1.69\pm0.01^{\circ}$	$0.89\pm0.02^{\rm c}$	$0.11 \pm 0.01^{\circ}$	$1.49\pm0.08^{b}$	$54.72\pm6.29^{\rm a}$
Onion	$2.97\pm0.20^{a}$	$1.56\pm0.07^{b}$	$1.04\pm0.05^{\text{b}}$	$1.03\pm0.02^{\rm c}$	$6.90\pm2.98^{\rm c}$
Black olives	$2.70\pm0.04^{\text{b}}$	$1.78\pm0.08^{\rm a}$	$0.14\pm0.04^{\rm c}$	$1.47\pm0.04^{b}$	$43.53 \pm 1.86^{\text{b}}$
EVOO	$1.61\pm0.07^{\rm c}$	$0.87\pm0.07^{\rm c}$	$0.27\pm0.01^{\text{c}}$	$0.63 \pm 0.02^{\text{d}}$	$7.54 \pm 1.61^{\rm c}$

**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.

 $^a\text{data}$  expressed as  $\mu\text{mol}$  ascorbic acid equivalent normalized for the total phenolic content as determined by mass spectrometry experiments

<sup>b</sup>% of chelated Fe<sup>2+</sup> by 100  $\mu$ g of phenolic compounds









