This is the peer reviewed version of the following article:

Mediterranean diet vegetable foods protect meat lipids from oxidation during in vitro gastro-intestinal digestion / Martini, S.; Conte, A.; Bottazzi, S.; Tagliazucchi, D.. - In: INTERNATIONAL JOURNAL OF FOOD SCIENCES AND NUTRITION. - ISSN 0963-7486. - 71:4(2020), pp. 424-439. [10.1080/09637486.2019.1677570]

Terms of use:
The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher’s website.

22/10/2023 16:07

(Article begins on next page)
Mediterranean Diet vegetable foods protect meat lipids from oxidation during \textit{in vitro} gastro-intestinal digestion

Serena Martini, Angela Conte, Silvia Bottazzi, Davide Tagliazucchi*

Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42100 Reggio Emilia, Italy

* Corresponding author. Tel.: +39-0522-522060; fax: +39-0522-522053

\textit{E-mail address}: davide.tagliazucchi@unimore.it (D. Tagliazucchi)
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 O₂•⁻, which at low pH such as found in the gastric milieu forms HOO•. Indeed, acidic disproportionation of O₂•⁻ may produce hydrogen peroxide (H₂O₂) and oxygen (Oueslati et al. 2016). The formation of HO• is possible by H₂O₂ decomposition, catalysed by ferrous iron (Fenton reaction), or by H₂O₂ reaction with O₂•⁻ (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, \textit{in vitro} and \textit{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 \textit{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 3000g 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 H₂SO₄, 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 H₂O₂ 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 \textit{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 \textit{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. \textit{Identification and quantification of phenolic compounds by liquid chromatography}

\textit{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$_2$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 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-
derivative were quantified in hydroxytyrosol equivalents. Anthocyanins were quantified as
cyanidin-3-O-glucoside equivalents.

ESI-MS parameters, limits of detection (LOD) and limits of quantification (LOQ) for the different
standards were the same as reported in Martini et al. (2017).

Quantitative results were expressed as mg of compounds per 100 g of vegetable or EVOO.

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

The total antioxidant properties of phenolic fractions were analyzed by using five different assays.
The radical scavenging ability was assayed by using the ABTS assay according to Re et al. (1999).
For the determination of the Fe$^{3+}$ reducing ability, a protocol based on the ferric
reducing/antioxidant power (FRAP) assay was utilized (Benzie and Strain 1999). The capacity to
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
phenolic compounds. The Fe$^{2+}$-chelation ability of phenolic–rich fractions was evaluated by the
ferrozine assay (Karama and Pegg 2009).

2.8. Statistics

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

10
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$_2$O$_2$/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
salad/100 g of meat) also resulted in a complete inhibition in the formation of lipid hydroperoxides whereas further halving (65.2 g of salad/100 g of meat) produced an inhibition of 49.5% (Figure 1).

No previous data were found in literature about the inhibitory activity of food combination (e.g. salads) on the generation of lipid hydroperoxides after gastro-intestinal digestion of meat. However, in agreement with our results, Kanner and co-worker (2017) reported an inhibition of about 90% in the formation of malondialdehyde after in vitro gastric digestion of meat with a Greek salad (274 g of salad/200 g of meat) composed of tomato, cucumber, red pepper, green-cabbage, onion and black 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.

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

To understand which component of the Mediterranean Diet salad was mainly responsible for the observed inhibitory effect, we carried out co-digestion with turkey meat and each single component of the salad in the same proportion as found in the Mediterranean Diet salad itself. As reported in section 2.2, the Mediterranean Diet salad, related to 100 g of meat, consisted of 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. This means that, for example, in the co-digestion between turkey meat and tomato, the proportion between meat and tomato was 100 g of meat and 200 g of tomato (200% of tomato respect to meat; w/w). Basing on the same rationale, onion or black olives were added in the proportion of 25% respect meat (w/w), EVOO in the proportion of 10% respect to meat (w/w) and 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 \textit{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 \textit{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 \textit{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 \textit{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 \textit{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,
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 \(\text{Fe}^{2+}\) 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 \(\text{Fe}^{2+}\)-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 (\(O_{2}^{•}\)) scavenging phenolic compounds since the
most active extract against $O_2^{•−}$ 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.
Financial support

This work was supported by a grant from Department of Life Sciences, University of Modena and Reggio Emilia (research project FAR2016 “Dieta Mediterranea e salute: riduzione dei fenomeni ossidativi durante la digestione della carne”). The authors acknowledge the Fondazione Cassa di Risparmio di Modena for funding the HPLC-ESI-IT system at the Centro Interdipartimentale Grandi Strumenti (CIGS).
References

Benzie IFF, Strain JJ. 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 299:15-27.

Berton-Carabin CC, Ropers MH, Genot C. 2014. Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. Compr Rev Food Sci Food Saf. 13:945-977.

Binnie MA, Barlow K, Johnson V, Harrison C. 2014. Red meats: time for a paradigm shift in dietary advice. Meat Sci. 98:445-451.

Carlsen CU, Skibsted LH. 2004. Myoglobin species with enhanced prooxidative activity is formed during mild proteolysis by pepsin. J Agric Food Chem. 52:1675-1781.

Chamorro A, Miranda FJ, Rubio S, Valero V. 2012. Innovations and trends in meat consumption: An application of the Delphi method in Spain. Meat Sci. 92:816-822.

Chisté RC, Freitas M, Mercadante AZ, Fernandes E. 2014. Carotenoids inhibit lipid peroxidation and hemoglobin oxidation, but not the depletion of glutathione induced by ROS in human erythrocytes. Life Sci. 99:52-60.

Donnelly JL, Decker EA, McClements DJ. 1998. Iron-catalyzed oxidation of Menhaden oil as affected by emulsifiers. J Food Sci. 63:997-1000.

Ferguson LR. 2010. Meat and cancer. Meat Sci. 84:308-313.

Gorelik S, Ligumsky M, Kohen R, Kanner J. 2008a. The stomach as a “bioreactor”: when red meat meets red wine. J Agric Food Chem. 56:5002-5007.

Gorelik S, Ligumsky M, Kohen R, Kanner J. 2008b. A novel function of red wine polyphenols in humans: prevention of absorption of cytotoxic lipid peroxidation products. FASEB J. 22:41-46.

Gorelik S, Kanner J, Schurr D, Kohen R. 2013. A rational approach to prevent postprandial modification of LDL by dietary polyphenols. J Funct Foods. 5:163-169.
Henning SM, Zhang Y, Seeram NP, Lee RP, Wang P, Bowerman S, Heber D. 2011. Antioxidant capacity and phytochemical content of herbs and spices in dry, fresh and blended herb paste form. Int J Food Sci Nutr. 62:219-225.

Kanner J, Lapidot T. 2001. The stomach as a bioreactor: Dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. Free Radic Biol Med. 31:1388-1395.

Kanner J, Selhub J, Shpaizer A, Rabkin B, Schacham I, Tiros O. 2017. Redox homeostasis in stomach medium by foods: The Postprandial Oxidative Stress Index (POSI) for balancing nutrition and human health. Redox Biol. 12:926-936.

Karama M, Pegg RB. 2009. Limitations of the tetramethylmurexide assay for investigating the Fe(II) chelation activity of phenolic compounds. J Agric Food Chem. 57:6425-6431.

Leone A, Battezzati A, De Amicis R, De Carlo G, Bertoli S. 2017. Trends of adherence to the Mediterranean dietary pattern in Northern Italy from 2010 to 2016. Nutrients. 11:E734.

Martí R, Roselló S, Cebolla-Cornejo J. 2016. Tomato as a source of carotenoids and polyphenols targeted to cancer prevention. Cancers. 20:E58.

Martini S, Conte A, Tagliazucchi D. 2017. Phenolic compounds profile and antioxidant properties of six sweet cherry (Prunus avium) cultivar. Food Res Int. 97:15-26.

Martini S, Cavalchi M, Conte A, Tagliazucchi D. 2018. The paradoxical effect of extra-virgin olive oil on oxidative phenomena during in vitro co-digestion with meat. Food Res Int. 109:82-90.

Martini S, Conte A, Tagliazucchi D. 2019. Bioactivity and cell metabolism of in vitro digested sweet cherry (Prunus avium) phenolic compounds. Int J Food Sci Nutr. 70:335-348.

Micha R, Wallace SK, Mozaffarian D. 2010. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus. Circulation. 121:2271-2283.

Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, et al. 2014. A standardised static in vitro digestion method suitable for food – an international consensus. Food Funct. 5:1113-1124.
Natella F, Macone A, Ramberti A, Forte M, Mattivi F, Matarese RM, Scaccini C. 2011. Red wine prevents the postprandial increase in plasma cholesterol oxidation products: A pilot study. Br J Nutr. 105:1718-1723.

Nogueira MS, Kessuane MC, Lobo Ladd AA, Lobo Ladd FV, Cogliati B, Castro IA. 2016. Effect of long-term ingestion of weakly oxidised flaxseed oil on biomarkers of oxidative stress in LDL-receptor knockout mice. Br J Nutr. 116: 258-269.

Nourooz-Zadeh J. 1999. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxides in plasma. Methods Enzymol. 300:58-62.

Oueslati K, de La Pomélie D, Santé-Lhouelliger V, Gatellier P. 2016. Impact of Fenton process in meat digestion as assessed using an in vitro gastro-intestinal model. Food Chem. 209:43-49.

Ozyürek M, Bektaşoğlu B, Güçlü K, Apak R. 2008. Hydroxyl radical scavenging assay of phenolics and flavonoids with a modified cupric reducing antioxidant capacity (CUPRAC) method using catalase for hydrogen peroxide degradation. Anal Chim Acta. 616:196–206.

Papuc C, Goran GV, Predescu CN, Nicorescu V. 2016. Mechanisms of oxidative processes in meat and toxicity induced by postprandial degradation products: A Review. Compr Rev Food Sci Food Saf. 16:96-123.

Perše M. 2013. Oxidative stress in the pathogenesis of colorectal cancer: Cause or consequence? BioMed res Int. 725710.

Prior RL, Wu X, Schaich K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements J Agric Food Chem. 53:4290-4302.

Pulido R, Bravo L, Saura-Calixto F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J Agric Food Chem. 48:3396–3402.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 26:1231-1237.
Rice-Evans CA, Miller NJ, Paganga G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 20:933–956.

Rohlík BA, Pipek P, Pánek J. 2013. The effect of natural antioxidants on the colour and lipid stability of paprika salami. Czech J Food Sci. 31:307-312.

Sasso A, Latella G. 2018. Dietary components that counteract the increased risk of colorectal cancer related to red meat consumption. Int J Food Sci Nutr. 69:536-548.

Sies H, Stahl W, Sevanian A. 2005. Nutritional, dietary and postprandial oxidative stress. J Nutr. 135:969-972.

Sirota R, Gorelik S, Harris R, Kohen R, Kanner J. 2013. Coffee polyphenols protect human plasma from postprandial carbonyl modifications. Mol Nutr Food Res. 57:916-919.

Sreejayan N, von Ritter C. 1998. Effect of bile acids on lipid peroxidation: The role of iron. Free Radic Biol Med. 25:50-56.

Tagliazucchi D, Verzelloni E, Conte A. 2010. Effect of dietary melanoidins on lipid peroxidation during simulated gastric digestion: Their possible role in the prevention of oxidative damage. J Agric Food Chem. 58:2513-2519.

Tagliazucchi D, Verzelloni E, Conte A. 2012. The first tract of alimentary canal as an extractor. Release of phytochemicals from solid food matrices during simulated digestion. J Food Biochem. 36:555-568.

Tirosh O, Shpaizer A, Kanner J. 2015. Lipid peroxidation in a stomach medium is affected by dietary oils (Olive/Fish) and antioxidants: The Mediterranean versus Western diet. J. Agric. Food Chem. 63:7016-7023.

Van Hecke T, Ho PL, Goethals S, De Smet S. 2017. The potential of herbs and spices to reduce lipid oxidation during heating and gastrointestinal digestion of a beef product. Food Res Int. 102:785-792.

Wang H, Gan D, Zhang X, Pan Y. 2010. Antioxidant capacity of the extracts from pulp of *Osmanthus fragans* and its components. LWT Food Sci Technol. 43:319-325.
Zamora R, Hidalgo FJ. 2016. The triple defensive barrier of phenolic compounds against lipid oxidation-induced damage in food products. Trends Food Sci Technol. 54:165-174.
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$_2$O$_2$/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$_2$O$_2$/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$_2$•: 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) |
|--------------------------------------------------|----------|----------------|--------------------------|
| Hydroxybenzoic acid-O-hexoside isomer            | 8.7      | 299            | 137 (100%)               |
| Hydroxybenzoic acid-dihexoside                   | 9.9      | 461            | 137 (100%), 299 (62%)    |
| Hydroxytyrosol-di-O-hexoside                     | 10.7     | 477            | 153 (100%), 315 (53%), 123 (10%) |
| Hydroxytyrosol isomer*                           | 11.2     | 153            | 123 (100%)               |
| Hydroxytyrosol-O-hexoside isomer                 | 11.3     | 315            | 153 (100%), 123 (17%)    |
| Caffeoylquinic acid-3-O-hexoside-4-O-hexoside isomer | 11.6     | 677            | 515 (100%), 353 (23%), 191 (5%)   |
| Vanillic acid-4-O-hexoside                       | 11.8     | 329            | 167 (100%), 152 (9%)     |
| Hydroxytyrosol-O-hexoside isomer                 | 12.0     | 315            | 153 (100%), 123 (25%)    |
| Hydroxytyrosol isomer                            | 12.1     | 153            | 123 (100%)               |
| 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%)   |
| Syringic acid-4-O-hexoside                       | 14.1     | 359            | 197 (100%), 182 (8%), 167 (5%)    |
| Calceolarioside                                  | 14.1     | 477            | 323 (100%), 315 (90%), 161 (16%) |
| Caffeoylquinic acid-3-O-hexoside-4-O-hexoside isomer | 14.1     | 677            | 515 (100%), 341 (21%)    |
| Caffeoylquinic acid-O-hexoside isomer            | 14.9     | 515            | 341 (100%), 323 (64%), 179 (57%), 353 (34%)   |
| Caffeic acid-3-O-hexoside-4-O-hexoside           | 15.5     | 503            | 341 (100%), 179 (17%)    |
| 3-O-Caffeoylquinic acid                          | 15.6     | 353            | 191 (100%), 179 (24%), 135 (19%) |
| Caffeoylquinic acid-O-hexoside isomer            | 15.6     | 515            | 353 (100%), 191 (82%), 179 (12%) 323 (8%)  |
| Caffeoyl-coumaroylquinic acid                    | 15.7     | 499            | 337 (100%), 173 (36%), 191 (34%) |
| Rosmarinic acid                                  | 15.8     | 359            | 197 (100%), 161 (6%), 153 (8%)    |
| Gallic acid                                      | 15.9     | 169            | 125 (100%)               |
| Protocatechuic acid-O-hexoside-O-pentoside       | 16.7     | 447            | 315 (100%), 271 (43%), 153 (15%)   |
| Coumaric acid*                                   | 16.7     | 163            | 119 (100%)               |
| Coumaric acid-O-hexoside isomer                  | 16.9     | 325            | 163 (100%), 119 (24%)    |
| Caffeic acid-O-hexoside isomer                   | 17.0     | 341            | 179 (100%), 135 (36%)    |
| Caffeic acid*                                    | 17.1     | 179            | 135 (100%)               |
| Hydroxybenzoic acid-O-hexoside isomer            | 17.8     | 299            | 137 (100%)               |
| Di-hydro-coumaric acid-O-hexoside                | 18.0     | 327            | 165 (100%), 121 (4%)     |
| Di-hydro-caffeic acid-O-hexoside isomer          | 18.0     | 343            | 181 (100%), 137 (33%)    |
| Syringic acid                                    | 18.2     | 197            | 153 (100%)               |
| No. | Compound                                      | Retention Time | M/z | Percentages                        |
|-----|----------------------------------------------|----------------|-----|-----------------------------------|
| 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-cafeic 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-cafeic 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-4-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%)                        |
| Compound                                      | R | m/z | Parent Mass (100%) | Fragment Mass (%) |
|-----------------------------------------------|---|-----|-------------------|-------------------|
| Cyanidin-3-O-malonylhexoside                  | 25.2 | 535* | 287 (100%), 449 (5%) |
| Peonidin-3-O-malonylhexoside                  | 25.2 | 549* | 301 (100%), 463 (6%) |
| Myricetin-di-O-hexoside                       | 25.4 | 641  | 479 (100%), 317 (21%) |
| Quercetin-3-O-rutinoside-7-O-hexoside         | 25.4 | 771  | 609 (100%)         |
| Sinapoyl-hexose                               | 25.8 | 385  | 223 (100%), 208 (2%), 265 (6%), 325 (1%) |
| 5-O-Caffeoylquinic acid cis                   | 26.5 | 353  | 191 (100%)         |
| Oleuropein aglycone isomer                   | 26.5 | 377  | 197 (100%), 153 (61%) |
| Caffeic acid-O-malonylhexoside                | 27.5 | 457  | 341 (100%), 179 (14%) |
| Amentoflavone                                 | 27.5 | 537  | 375 (100%), 179 (14%) |
| Ferulic acid-4-O-pentoside isomer            | 28.0 | 325  | 193 (100%), 149 (36%), 134 (3%) |
| 4-O-Cumaroylquinic acid                      | 28.9 | 337  | 173 (100%), 163 (17%) |
| Apigenin-6,8-di-C-hexoside                   | 29.1 | 593  | 473 (100%), 353 (49%), 383 (33%) |
| 5-O-Cumaroylquinic acid                      | 29.3 | 337  | 191 (100%), 173 (5%) 163 (3%) |
| Isorhamnetin-di-O-hexoside isomer            | 29.5 | 639  | 477 (100%), 315 (5%) |
| Kaempferol-3-O-acetylhexoside                | 30.0 | 489  | 285 (100%), 255 (7%) |
| Quercetin-7-O-hexoside-4'-O-hexoside         | 31.3 | 625  | 463 (100%), 301 (22%) |
| 5-O-Feruloylquinic acid                      | 32.2 | 367  | 191 (100%), 173 (4%)  |
| Quercetin-3-O-hexoside-4'-O-hexoside         | 33.6 | 625  | 463 (100%), 301 (31%), 179 (4%) |
| Kaempferol-3-O-hexoside-7-O-hexoside         | 33.8 | 609  | 285 (100%), 447 (73%), 255 (7%) |
| Isorhamnetin-di-O-hexoside isomer            | 34.1 | 639  | 477 (100%)         |
| Myricetin-7-O-hexoside                        | 34.4 | 479  | 317 (100%), 289 (65%) |
| Secoisolariciresinol-O-hexoside               | 35.0 | 523  | 361 (100%)         |
| Naringenin-C-hexoside                         | 35.1 | 433  | 313 (100%)         |
| Isorhamnetin-3-O-hexoside-4'-O-hexoside      | 35.4 | 639  | 315 (100%), 477 (63%), 301 (17%), 271 (6%) |
| Quercetin-3-O-rutinoside-7-O-pentoside        | 36.5 | 741  | 609 (100%), 300 (80%) |
| Lariresinol-O-hexoside                        | 37.3 | 521  | 329 (100%), 359 (15%) |
| Apigenin-C-hexoside-O-rhamnoside             | 37.9 | 577  | 341 (100%), 413 (50%), 311 (15%) |
| Eriodictiol-O-hexoside                        | 38.5 | 449  | 287 (100%), 151 (42%) |
| Quercetin-3-O-hexoside isomer                | 39.6 | 463  | 301 (100%), 151 (5%), 179 (3%) |
| Di-hydro-quercetin                            | 39.8 | 303  | 285 (100%), 267 (54%), 257 (41%) |
| Quercetin-3-O-rutinoside*                     | 39.9 | 609  | 301 (100%), 343 (46%), 273 (28%), 243 (13%) |
| Kaempferol-3-O-rutinoside-7-O-pentoside       | 40.1 | 725  | 593 (100%), 285 (30%), 255 (7%), 257 (3%) |
| Luteolin-O-rutinoside isomer                 | 40.1 | 593  | 285 (100%), 447 (2%) |
| Substance                                      | m/z | m/z | Relative Abundance |
|-----------------------------------------------|-----|-----|--------------------|
| Phloretin-di-C-hexoside                       | 40.1| 597 | 357 (100%), 387 (91%), 477 (81%) |
| Quercetin-3-O-glucoside*                      | 41.0| 463 | 301 (100%), 151 (23%), 179 (2%) |
| Luteolin-O-hexoside                           | 41.5| 447 | 285 (100%)         |
| Naringenin-di-O-hexoside                      | 41.6| 595 | 271 (100%)         |
| Luteolin-O-rutinoside isomer                  | 41.7| 593 | 285 (100%), 447 (67%) |
| Pinocerinol-O-hexoside                        | 42.0| 519 | 359 (100%), 151 (2%) |
| Nuzhenide                                      | 42.4| 685 | 523 (100%), 453 (93%), 421 (32%), 299 (3%) |
| Phloretin-C-hexoside                          | 42.9| 435 | 315 (100%), 345 (5%) |
| Syringaresinol-O-hexoside                     | 43.3| 579 | 417 (100%), 181 (8%) |
| Verbascoside                                  | 43.5| 623 | 461 (100%), 315 (2%) |
| Kaempferol-3-O-rutinoside                     | 44.4| 593 | 285 (100%)         |
| 4,5-diCaffeoylquinic acid                     | 44.5| 515 | 353 (100%), 179 (18%), 335 (15%), 191 (14%) |
| Quercetin-3-O-acetylhexoside                  | 44.9| 505 | 301 (100%), 463 (67%), 179 (35%) |
| Liquiritigenin-7-O-hexoside                   | 45.0| 417 | 255 (100%)         |
| Apigenin-O-hexoside-O-rhamnoside              | 45.1| 577 | 269 (100%)         |
| Apigenin-O-hexoside-O-pentoside               | 45.3| 563 | 269 (100%), 431 (23%) |
| Kaempferol-3-O-hexoside                       | 45.9| 447 | 284 (100%), 255 (70%), 285 (51%) |
| 3,5-diCaffeoylquinic acid                     | 46.0| 515 | 353 (100%), 191 (4%) |
| Isorhamnetin-3-O-hexoside                     | 46.8| 477 | 315 (100%), 300 (12%) |
| Naringenin-O-hexoside isomer                  | 46.8| 433 | 271 (100%)         |
| Oleuropein aglycone isomer                    | 47.2| 377 | 307 (100%), 333 (65%), 275 (55%), 139 (12%), 345 (7%) |
| Quercetin-4’-O-hexoside                       | 47.6| 463 | 301 (100%), 179 (12%), 151 (4%) |
| Naringenin-O-hexoside-O-pentoside             | 47.6| 565 | 271 (100%), 403 (11%) |
| Kaempferol-7-O-hexoside                       | 48.8| 447 | 285 (100%), 257 (11%) |
| Hydroxy-decarboxymethyl-oleuropein aglycone   | 49.1| 335 | 199 (100%), 181 (29%) |
| Decarboxymethyl-oleuropein aglycone           | 49.6| 319 | 195 (100%), 165 (18%) |
| Oleuropein                                    | 49.8| 539 | 377 (100%), 307 (66%), 275 (32%), 345 (14%) |
| Isorhamnetin-4’-O-hexoside                    | 50.6| 477 | 315 (100%), 299 (15%), 300 (12%) |
| Di-hydroxy-ligstroside aglycone               | 50.6| 393 | 361 (100%), 257 (79%), 323 (27%), 195 (19%), 151 (16%) |
| β-methoxylverbascoside                        | 51.1| 653 | 491 (100%), 635 (93%) |
| Coumaroyl-caffeoylquinic acid                 | 52.2| 499 | 353 (100%), 191 (10%), 173 (7%) |
| Naringenin-O-hexoside isomer                  | 52.9| 433 | 271 (100%)         |
| Ligstroside                                   | 56.8| 523 | 361 (100%), 259 (19%) |
| Quercetin                                     | 60.8| 301 | 151 (100%), 179 (71%) |
| 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. |
|   | Compound                                    | Concentration      |   |   |   |
|---|--------------------------------------------|--------------------|---|---|---|
| 46| Caffeoyl-6α-hexose                         | 2.59 ± 0.16        |   | 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-cafeic acid-O-hexoside isomer     | 0.55 ± 0.05        | n.d. | n.d. | n.d. | n.d. |
| 37| Dihydro-cafeic 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. | 0.05 ± 0.01 | n.d. |
| 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. | 0.08 ± 0.01 | n.d. |
| 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. | 2.34 ± 0.19 | n.d. |
| 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. | 0.16 ± 0.05 | n.d. |
| 31| Caffeic acid-O-hexoside-O-pentoside isomer| n.d.               | n.d. | n.d. | 0.09 ± 0.01 | n.d. |
| 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-cafeic 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. | 0.35 ± 0.08 | n.d. |
| 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. |
| Substance                                      | 6 | 14 | 0.33 ± 0.06 | n.d. | n.d. | n.d. | n.d. |
|------------------------------------------------|---|----|-------------|------|------|------|------|
| Caffeoylquinic acid-3-O-hexoside-4-O-hexoside  | 6 | 14 | 0.06 ± 0.01 | n.d. | n.d. | n.d. | n.d. |
| isomer                                         |    |    |             |      |      |      |      |
| **Total hydroxycinnamic acids**                |   |    | **47.48 ± 1.46** | **7.32 ± 0.39** | **1.52 ± 0.09** | n.d. | **10.20 ± 0.55** |
|                                                |   |    | (94.4%)     | (19.0%) | (3.5%) |     | (30.3%) |
| **Flavonols**                                  |   |    |             |      |      |      |      |
| Quercetin                                      | 131|    | n.d.        | 0.04 ± 0.01 | n.d. | n.d. | n.d. |
| Kaempferol-3-O-hexoside                        | 114|    | n.d.        | 0.09 ± 0.01 | n.d. | n.d. | n.d. |
| Kaempferol-7-O-hexoside                        | 121|    | < LOQ       | 0.19 ± 0.02 | n.d. | n.d. | n.d. |
| Quercetin-3-O-hexoside isomer                  | 93 |    | n.d.        | < LOQ   | n.d. | n.d. | n.d. |
| Quercetin-3-O-glucoside                        | 99 |    | 0.01 ± 0.01  | 0.43 ± 0.05 | 0.03 ± 0.01 | n.d. | n.d. |
| Quercetin-4’-O-hexoside                        | 119|    | n.d.        | 7.29 ± 0.40 | n.d. | n.d. | n.d. |
| Isorhamnetin-3-O-hexoside                      | 116|    | n.d.        | 0.04 ± 0.01 | n.d. | n.d. | n.d. |
| Isorhamnetin-4’-O-hexoside                     | 125|    | n.d.        | 2.35 ± 0.07 | < LOQ | n.d. | n.d. |
| Myricetin-7-O-hexoside                         | 85 |    | n.d.        | 0.01 ± 0.01 | n.d. | n.d. | n.d. |
| Kaempferol-3-O-acetylhexoside                  | 79 |    | n.d.        | < LOQ   | n.d. | n.d. | n.d. |
| Quercetin-3-O-acetylhexoside                   | 110|    | n.d.        | < LOQ   | n.d. | n.d. | < LOQ |
| Kaempferol-3-O-rutinoside                      | 108|    | < LOQ       | n.d.    | n.d. | n.d. | n.d. |
| Quercetin-3-O-rutinoside                       | 95 |    | 0.37 ± 0.01  | n.d.    | n.d. | n.d. | n.d. |
| Kaempferol-3-O-hexoside-7-O-hexoside           | 83 |    | n.d.        | 0.21 ± 0.01 | n.d. | n.d. | n.d. |
| Quercetin-3-O-hexoside-7-O-hexoside            | 62 |    | n.d.        | 0.03 ± 0.01 | n.d. | n.d. | n.d. |
| Quercetin-7-O-hexoside-4’-O-hexoside           | 80 |    | n.d.        | 0.15 ± 0.01 | n.d. | n.d. | n.d. |
| Quercetin-3-O-hexoside-4’-O-hexoside           | 82 |    | < LOQ       | 11.13 ± 0.18 | 0.03 ± 0.01 | n.d. | < LOQ |
| Isorhamnetin-di-O-hexoside isomer              | 78 |    | n.d.        | < LOQ   | n.d. | n.d. | n.d. |
| Isorhamnetin-di-O-hexoside isomer              | 84 |    | n.d.        | < LOQ   | n.d. | n.d. | n.d. |
| Isorhamnetin-3-O-hexoside-4’-O-hexoside        | 88 |    | n.d.        | 0.38 ± 0.01 | n.d. | n.d. | n.d. |
| Myricetin-di-O-hexoside                        | 66 |    | n.d.        | 0.01 ± 0.01 | n.d. | n.d. | n.d. |
| Kaempferol-3-O-rutinoside-7-O-pentoside        | 96 |    | < LOQ       | < LOQ   | n.d. | n.d. | n.d. |
| Quercetin-3-O-rutinoside-7-O-pentoside         | 89 |    | 0.17 ± 0.01  | < LOQ   | n.d. | n.d. | n.d. |
| Quercetin-3-O-rutinoside-7-O-hexoside          | 67 |    | 0.01 ± 0.01  | < LOQ   | n.d. | n.d. | n.d. |
| Quercetin-tri-O-hexoside                       | 42 |    | n.d.        | 0.08 ± 0.01 | n.d. | n.d. | n.d. |
| Quercetin-3-O-rutinoside-O-hexoside-O-pentoside| 57 |    | 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. |
|   | Compound                                | Concentration    | 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**  | n.d. | n.d. | n.d. | n.d. |

### Tyrosol derivatives

|   | Compound                                | Concentration    | n.d. | n.d. | n.d. | n.d. |
|---|-----------------------------------------|------------------|------|------|------|------|
| 4 | Hydroxytyrosol isomer                   | n.d.             | 0.63 ± 0.14 | n.d. | n.d. | n.d. |
| 9 | Hydroxytyrosol isomer                   | n.d.             | 0.65 ± 0.07 | 1.50 ± 0.10 | n.d. | n.d. |
| 1 | Hydroxytyrosol-O-hexoside isomer        | n.d.             | 15.24 ± 0.48 | n.d. | n.d. | n.d. |
| 5 | Hydroxytyrosol-O-hexoside isomer        | n.d.             | 2.41 ± 1.00 | n.d. | n.d. | n.d. |
| 123| Decarboxymethyl-oleuropein aglycone     | n.d.             | n.d. | 0.77 ± 0.01 | n.d. | n.d. |
| 122| Hydroxy-decarboxymethyl-oleuropein aglycone | n.d.     | n.d. | 11.31 ± 0.27 | n.d. | n.d. |
| 132| Ligstroside aglycone                    | n.d.             | n.d. | 13.66 ± 0.54 | n.d. | 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. | 46.98 ± 2.79 | n.d. | n.d. |
| 126| Di-hydroxy-ligstroside aglycone         | n.d.             | n.d. | 0.07 ± 0.03 | n.d. | 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-methoxyverbasoside                    | 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**

|   | Concentration    | n.d. | n.d. | **41.93 ± 1.13** (95.4%) | **74.30 ± 2.85** (100%) | n.d. |
|---|------------------|------|------|--------------------------|--------------------------|------|

### Dihydroflavonoids

|   | Compound                                | Concentration    | n.d. | <LOQ | n.d. | n.d. | n.d. |
|---|-----------------------------------------|------------------|------|------|------|------|------|
| 94 | Dihydro-quercetin                        | n.d.             | n.d. | n.d. | n.d. | n.d. | n.d. |
| 63 | Taxifolin-O-hexoside                     | n.d.             | 0.02 ± 0.01 | n.d. | n.d. | n.d. | n.d. |

**Total dihydroflavonoids**

|   | Concentration    | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
|---|------------------|------|------|------|------|------|------|

### Dihydrochalcones

|   | Compound                                | Concentration    | n.d. | n.d. | n.d. | n.d. | n.d. |
|---|-----------------------------------------|------------------|------|------|------|------|------|
| 105| Phloretin-C-hexoside                    | n.d.             | 0.01 ± 0.01 | n.d. | 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. | n.d. |

**Total dihydrochalcones**

|   | Concentration    | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
|---|------------------|------|------|------|------|------|------|

**Total phenolic compounds**

|   | Concentration    | 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<sup>3+</sup> reducing properties | Fe<sup>2+</sup> chelating ability |
|------------------|--------------------------|-----------------------------|-----------------------------|------------------------------------|---------------------------------|
|                  | μmol ascorbic acid equivalent/mg of total phenolic compounds<sup>a</sup> | % chelation<sup>b</sup>     |                             |                                    |                                 |
| Tomato           | 1.60 ± 0.05<sup>c</sup>  | 0.89 ± 0.05<sup>c</sup>     | 2.77 ± 0.45<sup>a</sup>     | 2.04 ± 0.07<sup>b</sup>            | 4.65 ± 1.14<sup>c</sup>         |
| Fresh basil      | 1.69 ± 0.01<sup>c</sup>  | 0.89 ± 0.02<sup>c</sup>     | 0.11 ± 0.01<sup>c</sup>     | 1.49 ± 0.08<sup>b</sup>            | 54.72 ± 6.29<sup>a</sup>        |
| Onion            | 2.97 ± 0.20<sup>a</sup>  | 1.56 ± 0.07<sup>b</sup>     | 1.04 ± 0.05<sup>b</sup>     | 1.03 ± 0.02<sup>c</sup>            | 6.90 ± 2.98<sup>c</sup>         |
| Black olives     | 2.70 ± 0.04<sup>b</sup>  | 1.78 ± 0.08<sup>a</sup>     | 0.14 ± 0.04<sup>c</sup>     | 1.47 ± 0.04<sup>b</sup>            | 43.53 ± 1.86<sup>b</sup>        |
| EVOO             | 1.61 ± 0.07<sup>c</sup>  | 0.87 ± 0.07<sup>c</sup>     | 0.27 ± 0.01<sup>c</sup>     | 0.63 ± 0.02<sup>d</sup>            | 7.54 ± 1.61<sup>c</sup>         |

<sup>a</sup>data expressed as μmol 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 μg of phenolic compounds
Tomato, 50.3 ± 1.5 mg/100g fresh food

- Dihydrochalcones, 1.5%
- Hydroxybenzoic acids, 2.4%
- Flavones, 0.3%
- Flavanones, 0.2%
- 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

Black Olives, 41.9 ± 1.1 mg/100g fresh food
- Hydroxytyrosol, 3.1%
- Hydroxytyrosol-O-hexoside, 42.1%
- Hydroxytyrosol-di-O-hexoside, 2.3%
- Ligstroside, 0.3%
- Oleuropein aglycone, 50.0%
- Oleuropein, 0.6%

- Nuzhenide, 0.5%
- β-methoxyverbascoside, 0.4%
- Verbascoside, 0.8%

EVOO, 74.3 ± 2.8 mg/100g fresh food
- Hydroxytyrosol, 2.0%
- Ligstroside aglycone, 18.4%
- Oleuropein aglycone, 63.2%
- Oleuropein, 0.6%
- Hydroxy-decarboxymethyl-oleuropein aglycone, 15.2%
- Decarboxymethyl-oleuropein aglycone, 1.0%
- Di-hydroxy-ligstroside aglycone, 0.1%
