Biophenols from table olive cv Bella di Cerignola: chemical characterization, biaccessibility and intestinal absorption

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Biophenols from table olive cv *Bella di Cerignola*: chemical characterization, biaccessibility and intestinal absorption

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ABSTRACT

In this study, the naturally debittered table olives cv *Bella di Cerignola*, were studied in order to: i) characterize their phenolic composition; ii) evaluate the polyphenols bioaccessibility; iii) assess their absorption and transport, across Caco2/TC7. LC-MS/MS analysis has highlighted the presence of caffeoyl-6-secologanoside and comselogoside, two new compounds not yet identified in table olives. *In vitro* bioaccessibility ranged from 7% of luteolin to 100% of tyrosol, highlighting the flavonoids sensitivity to the digestive conditions. The Caco2/TC7 polyphenols accumulation was rapid (60 min) with an efficiency of 0.89%; the overall bioavailability was 1.86% (120 min), with hydroxytyrosol and tyrosol the highest bioavailables, followed by verbascoside and luteolin. In the cells and basolateral side, caffeic and coumaric acids metabolites, probably derived from esterase activities, were detected. In conclusion, the naturally debittered table olives cv *Bella di Cerignola* can be considered as a source of bioaccessible, absorbable and bioavailable polyphenols that, for their potential health promoting effect, permit to include table olives as a functional foods suitable for a balanced diet.

KEYWORDS: *Polyphenols, Naturally debittered table olives, Bioavailability, Simulated digestion, Caco2 TC/7 intestinal cells*

INTRODUCTION

Table olives production is considered one of the major agronomic practices of Mediterranean countries such as Italy, Greece and Spain that together supply almost 30% of world olive annual production.[1] Moreover, table olives are an important component of the Mediterranean diet with potential beneficial effects on human health due to biological properties of phenolic compounds.[2] Olive drupes have high concentrations of phenolic compounds (≈ 1-2 % of fresh weight)[3] important for their antioxidant, anti-inflammatory and antitumoral properties, with beneficial effect on human health.

The most important classes of phenolic compounds in olive fruit include phenolic acids, phenolic alcohols, flavonoids and secoiridoids.[4-6] In particular, hydroxytyrosol and tyrosol are the most abundant and they represent derivative products from the desterification of oleuropein and lingstroside, the most significant oleosides in olive fruit. In addition, verbascoside is the main hydroxycinnamic derivative and a number of simple phenols are also present, these include flavonol glycosides such as quercetin-3-rutinoside (rutin), luteolin-7-glucoside, apigenin-7-glucoside and luteolin.[7]

Polyphenols content and composition in table olives can be affected by several factors such as cultivars, climate, fruits ripeness, and mainly to the processing methods. In fact, table olives processing can follow three main procedures: green or Spanish-style olives, black ripe or Californian style olives, and turning color or naturally fermented olives (Greek-style).[8] Spanish-type protocol expected that olives were treated with 2-3% NaOH aqueous solution to reduce their bitterness under alkaline conditions by which oleuropein is hydrolyzed into hydroxytyrosol [9] and elenolic acid glucoside.[10] The further lactic acid fermentation step does not modify the *o*-diphenol composition.[11] For Californian style process, the table olives were preserved in brine and darken with air under alkaline conditions, where oleuropein undergoes to hydrolysis reducing the bitterness of fruits. Further, ferrous gluconate was added in order to maintain the black color.[12] In the Greek-style production system, the fruits are placed directly into brine with a salt concentration of about

6–10% (w/v), thus allowing spontaneous fermentation to take place. [12] This treatment is the most time-consuming because debittering is achieved by in-brine treatment only, without the help of preliminary alkaline hydrolysis. The elimination of bitterness is due to diffusion of a portion of the phenolic compounds into the brine, and equilibrium is reached in 8-12 months;[13] the final product generally retains a slight bitter taste.[12] Many studies have been carried out about the influence of different processing methods of table olives that, affecting oleuropein levels, significantly modify the phenolics profile.[8,14,15] It is known that California-style processing method gives the lowest concentrations of phenolic compounds, especially hydroxytyrosol; instead Greek- and Spanish style processing methods result in the higher retention of phenolic compounds in table olives. [15]

The main objective of the present study was the characterization of phenolic compounds from naturally fermented cv *Bella di Cerignola*. This cultivar is one of the most important in Apulia (Southern Italy), that received the "Protected Denomination of Origin" (DOP) in 2000 by EU. It has attracted the attention of researcher for the particular size and it has mainly studied for its microbiological aspect,[16-18] although less is known regarding its specific phenolic compositions.[19]

The nutraceutical aspect of table olives is very important mainly for the phenolic compositions that results higher respect olive oil (about 5 times).[20] Further, the bioaccessibility evaluation of the identified polyphenols, meant as the amount of polyphenols released from the matrix after gastro-intestinal digestion, was performed. Moreover, in order to assess the bioavailability of table olive polyphenols, the absorption and transport were investigated by using human intestinal cell line (Caco2/TC7).

MATERIAL AND METHODS

Chemicals.

Methanol (MeOH), glacial acetic acid (AcOH), , were HPLC certified. Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate-buffered saline (PBS), 200 mM L-glutamine, antibiotic and antimycotic solution, non-essential amino acid solution, Cohn V fraction fatty acid depleted bovine serum albumin (BSA), ethanol (EtOH), and ethyl acetate (EtOAc) were purchased

from Sigma Aldrich (Milan, Italy). Caco2 intestinal cell line, clone TC7, were kindly supplied by Dr. Rousset, INSERM U505, Paris, (France). Foetal bovine serum (FBS) was purchased from Gibco (Milan, Italy). The enzymes, α-amylase (from Bacillus species; A-6814), pepsin (from pig; P-7000), pancreatin (from pig; P-1750), mucin (from pig; M-2378), lipase (from pig; L-3126), and bovine bile extract (B-3883), used in vitro digestion were obtained from Sigma Aldrich (St Louis, MO, USA). The polyphenol standards used in this study were supplied by PhytoLab GmbH & Co. KG (Dutendorfer Str. 5-7, 91487 Vestenbergsgreuth, Germany).

Table olives sampling and polyphenols extraction.

Natural debittered marketable table olives, cv *Bella di Cerignola* (BdC), were provided from the local farm (Cooperativa "La Bella di Cerignola S.C.A." Foggia – Italy). The olives are processed with the method named "Untreated", that consists in leaving the olives in brine for at least eight months, during which they lose much of the bitterness and acquire the characteristic color ranging from mustard yellow to brown. In particular, the main processing steps are: size grading; spontaneous fermentation in brine (4% NaCl); desalination and pasteurization heat treatment. Further, 15 olives were sampled from three different glass jars and approximately 10 g of each, were homogenized and refluxed with 100 mL of boiling methanol/H_2O (50:50) twice for 1 hr. After filtration through a Whatman 1 paper filter, methanolic extracts were concentrated to dryness under vacuum to obtain a residue that was suspended and brought to the final volume of 50 mL with methanol/water (1:1 v/v). The phenolic concentration of the extracts, after filtration at 0.22 μm, was determined by HPLC-DAD according to Lattanzio.[21]

Identification and quantification of phenolic compounds.

HPLC-DAD analysis.

HPLC was performed employed Agilent 1260 infinity system equipped with a 1260 binary pump, 1260 HiP Degasser, 1260 TCC Thermostat, 1260 Diode Array Detector and Agilent Open Lab Chem Station Rev C.01.05(35) software.

The UV–visible absorption chromatogram was detected at 325 nm, 280 nm and 360 nm. Separation was performed by gradient elution on a 4.6 × 250 mm reversed phase Luna C-18 (5 μm) column (Phenomenex Torrance, California, USA). The elution was performed using methanol (eluent A) and water/acetic acid 95:5 (eluent B). The gradient profile was: 85–60% B (0–25 min), 60% B (25–30 min), 60–37% B (30–45 min), 37% B (45–47 min), 37–0% B (47–52 min). The flow rate was 1 mL/min and the injection volume was 25 μL. Phenolics were identified by the retention time and spectra of the pure standard supplied by PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany).

LC-MS/MS analysis.

LC-MS/MS analyses were performed by a QTrap® MS/MS system, from Applied Biosystems (Foster City, CA, USA) equipped with an electrospray ionization (ESI) interface and a 1100 Series micro-LC system. The analytical column was a Synergi Hydro® (150 × 2 mm, 4 μm particles) (Phenomenex, Torrance, CA, USA), preceded by an Aqua C18 guard column (4 x 2 mm, 10 μm particles) (Phenomenex, Torrance, CA, USA). The flow rate of the mobile phase was 200 μL/min, while the injection volume was 20 μL. The column temperature was set at 40°C and the autosampler was 20°C. A gradient elution was performed using water (eluent A) and methanol (eluent B), both containing 2% acetic acid. The elution was performed by changing the mobile phase composition as follows. The eluent B was increased from 15 to 40% in 25 min and kept constant for 5 min. Then it was increased to 63% in 15 min and kept constant for 2 min, finally increased to 100% in 4 min and kept constant for 4 min. For column re-equilibration, eluent B was decreased to 15% in 1 min and kept constant for 8min. For MS analyses, the ESI interface was used with the following settings: temperature (TEM) 300 °C, curtain gas (CUR), nitrogen, 30 psi; nebulizer gas (GS1), air, 10 psi; auxiliary gas (GS2), air, 30 psi; ionspray voltage - 4500V, negative ion mode. For metabolite identification and characterization, the mass spectrometer operated in full scan and product ion scan mode. Full scan chromatograms were acquired in the range 50–800a.m.u. Product ion chromatograms were acquired with collision energy of 30 eV.

***In vitro* digestion process to assess the bioaccessibility of table olives polyphenols.**

Table olives BdC were subjected to simulated oral, gastric and small intestinal digestion in sequence as previously described by Moser et al.[22] with minor modifications. Homogenized flesh olives (6g) were transferred to a centrifuge tube and 6 mL of simulated saliva fluid, containing 10.6 g α-amylase/g of olives, 5% mucin (w/v), 3% uric acid (w/v) and 40% urea (w/v). The reaction tube was vortexed, blanketed with nitrogen, and rotated head-over-heels (55 rpm at 37 °C) (Rotator Type L2, Labinco BV, Netherlands) for 10 min. Samples were removed from the rotation system, placed immediately on ice and saline solution (0.9% NaCL) was added until 50 mL. The gastric phase was initiated by the addition of 2 mL porcine pepsin solution (19 mg/mL in 0.1 M HCl) and adjustment of the pH to 3.0 ± 0.1 with 1.0 M HCl. After the addition of 0.9% saline solution to 40 mL total volume, samples were blanketed with nitrogen and rotated head-over-heels (55 rpm at 37 °C) for 1 h. The small intestinal phase was initiated by the addition of 1.0 M $NaHCO_3$ to adjust the pH of gastric digesta to 6.5 ± 0.1. A cocktail of small intestinal enzymes (2 mL, 30 mg/mL pancreatin, 15 mg/mL lipase in 0.1 M $NaHCO_3$) and porcine bile salts (3 mL, 40 mg/mL bile extract in 0.1 M $NaHCO_3$) were added to the solution and the pH was adjusted, if necessary, to pH. 6.5 ±0.5. Samples were then standardized to 50 mL with saline solution, blanketed with nitrogen and rotated head-over-heels (55 rpm at 37 °C) for 2 h. After completion of the small intestinal phase, aliquots of digesta (15 mL) were centrifuged (10,400 ×g, 4 °C, 1 h). Aliquots centrifuged aqueous small intestinal digesta (DG) were collected, acidified with 2% aqueous acetic acid (1:1) and stored frozen at −80 °C until analysis by HPLC-DAD for assessment of polyphenols bioaccessibility. Each experiment was carried out in triplicate.

The bio-accessibility of polyphenols is defined as the fraction of external dose released from its matrix in the gastrointestinal (GI) tract.

$$\text{Bioaccessibility (\%)} = CF/CI \times 100$$

Where CF is the amount of polyphenols present in the digesta (chyme) and CI is the initial amount of polyphenols in undigested flesh table olives.

Intestinal bioavailability of polyphenols by using Caco2/TC7 human cell line.

To assess the potential intestinal absorption of table olives polyphenols, experiments were carried out using the Caco2/TC7 human intestinal cell line, following the method described by Failla et al.[23] with some modifications. Briefly, Caco2/TC7 cells were seeded at 1.2×10^6 cells mL^{-1} in cell culture inserts for 6 well plates with polyethylene terephthalate (PET) track-etched membranes (pore size 0.4 μm, growth area 4.2 cm^2, Falcon, BD), pretreated with poly-L-lysine (50 μg mL^{-1}), in complete DMEM, with 4.5 g L^{-1} glucose supplemented with 10% foetal bovine serum, 1% L-glutamine, 1% antibiotic and antimycotic solution, 1% non-essential amino acid solution at 37 °C under a humidified atmosphere containing 5% CO_2. The basolateral compartment was filled with 3 mL of complete DMEM. Cell monolayers were cultured for 21 days in order to obtain fully differentiated cells and media from apical and basolateral compartments were replaced twice a week. The integrity of the cell monolayer was evaluated by transepithelial electrical resistance (TEER) measurements using a volt-ohm meter (Millicel ERS-2, Millipore, Italy). TEER values were expressed as Ω cm^2. Only Caco2/TC7 monolayers showing TEER values higher than 700 Ω cm^2 were used for in vitro experiments. The absorption experiments were performed following the protocol described by Neilson et al.[24] with some modifications. Briefly, monolayers were first washed with 2 mL PBS (pH 5.5), and then 2 mL of DMEM phenol red free, containing the table olive hydroalcoholic extract at a final polyphenol concentration of 65 μg mL^{-1}, was applied to each well. Cells were then incubated at 37 °C for 30, 60, 90 and 120 min. Following incubation, media of apical and basolateral compartments were aspirated and stored at −80 °C before HPLC-DAD analysis.

Accumulation and transport of polyphenols by Caco2/TC7 cells.

For the experiments related to the polyphenol Caco2/TC7 accumulation, the monolayers were washed first with PBS (pH 5.5), then with 2 mL of 0.1% fatty acid free bovine albumin (w/v) in PBS (pH 5.5) and, finally, cells were scraped from the plate into 1 mL of cold PBS (pH 5.5), collected and stored at −80 °C under N_2 until analysis. Protein values for cell monolayers were

186 determined by the Bio-Rad protein assay method.[25] Table olives polyphenols from sonicated
187 Caco2/TC7 cells and basolateral solutions were extracted with 3 mL of EtOAc (0.01% BHT).
188 Extraction was repeated a total of 3 times and EtOAc layers were pooled, dried under vacuum, and
189 resolubilized in a 200 µL mobile phase for HPLC-DAD analysis.

190 **Permeability coefficient calculation.**

191 The permeability coefficient (P_{app}), that represents the apical to-basolateral transport rate of BdC
192 table olives polyphenols, across the Caco2/TC7 monolayer, was calculated using the following
193 equation:

194 $$P_{app} = Q/(A \times C \times t)$$

195 were Q is the total amount (µg) of polyphenols in basolateral side, A is the diffusion area (4.2 cm^2),
196 C is the initial concentration in the donor compartment (µg mL^{-1}) and t is the time in which the
197 maximum absorption is reached (s).

198 **RESULTS AND DISCUSSION**

199 **Phenolic profiles in table olive cv *Bella di Cerignola*.**

200 Table olive polyphenols are very important in human diet in the prevention of chronic diseases,
201 cardiovascular diseases, and some cancers, for their antioxidant and free radical scavenger
202 activities. Furthermore, table olive polyphenols could be used as sources of potentially safe natural
203 antioxidants for the food industry.[26] The phenolic fraction in table olives can range significantly in
204 term of quality and quantity; it is depending upon the processing method, the cultivar, the irrigation
205 practices, and the degree of maturation.[12] In the present study the phenolic fraction of naturally
206 debittered table olives BdC was analyzed and characterized before *in vitro* GI digestion. The
207 HPLC-DAD analysis showed the presence of six major peaks attributable to the main phenolic
208 compounds identified in table olives (Figure 1), in particular: hydroxytyrosol (HT), tyrosol (Tyr),
209 verbascoside (VB), isoverbascoside isoVB), luteolin (LUT), apigenin (API). were identified by
210 their UV spectra and/or by comparison with authentic standards.

In addition, to confirm the identity of the six identified phenolic compounds and to investigate on the presence of further compounds, the LC-MS and LC-MS/MS analysis were performed with the same sample set. A summary of HPLC-DAD and LC-MS(MS) data is reported Table 1. Besides structure confirmation of the six major phenolic compounds, LC-MS and LC-MS/MS analysis highlighted the presence of hydroxytyrosol acetate (HTAc), caffeoyl-6-secologanoside (SEC) and comselogoside (COM) (Figure 1 and Table 1).

In Table 2 are reported the polyphenols concentrations expressed as μg/g FW. The highest relative abundance of HT found in this study (65%) was in agreement with findings by others authors, although a difference related to the cultivars cannot be excluded even considering the same debittering process.[8,14] The production of HT can be attributed to the hydrolysis of oleuropein, the main phenolic compound recovered in the olive leaves, seed, pulp and peel of unripe olives (up to 14% of the dry weight), [27] and responsible of characteristic bitter taste disappearing during the table olive processing.[8,11] Tyrosol, a degradation product of ligstroside,[28] resulted the second most abundant compound, followed by HTAc and VB, with a relative abundance of about 8%, for both. Verbascoside is the major hydroxycynnamic acid derivative in olive pulp and its presence is related to the cultivar and harvest time.[15] In some studies it was reported that the Italian olive cultivars (*Carolea, Coratina,* and *Cassanese* olives) showed the highest VB concentration [29] with respect to Portuguese ones.[30] In addition, VB was demonstrated to be stable after brine storage.[31] Furthermore, VB is also important for its biological activity, having antioxidant protective effects on phospholipid membranes, on the Low density Lipoprotein (LDL) oxidation inhibition, and ability to modulate plasma antioxidant capacity *in vivo*.[32-35] Moreover HTAc already identified in olive oil and probably derived from a hydrolysis of more complex compounds like, oleuropein, and VB, it is also recognized for biological activity. In fact, HTAc acts as inhibitor of platelet aggregation in rats, with similar effect of acetyl salicylic acid.[36] The low concentration of flavonoids identified in BdC, LUT and API (3% and 0.8 %, respectively) was in agreement with those reported by other authors on table olives, that recognized the influence of maturation index, nature of the cultivar and

geographical origin, on the flavonoids amount.[30,37] The absence of glycosilated flavonoids, generally recovered in the fresh fruits, can be attributable to the effect of brine storage process, as reported by Brenes et al.[31] Futhermore, HTAc, SEC and COM were quantified as HT, caffeic acid and coumaric acid equivalents, respectively. Interesting to underline the identification of the two secoiridoids compounds, COM and SEC, already identified in olive mill waste waters and olive fruits, that exhibit antioxidant activity comparable to other bioactive compounds such as HT and oleuropein.[28,38,39] To the best of our knowledge, this is the first time in which these two compounds are recovered in processed table olives.

Finally, according to other author,[12] our results on the bioactive compound profiles of the naturally debittered BdC, were similar to the fresh olives. In addition, the polyphenols content resulted higher (30%) respect to those recovered in BdC table olives debittered by Spanish-type process (data not shown).

***In vitro* bioaccessibility of table olives polyphenols after simulated digestion process.**

In order to evaluate the bioaccessibility of polyphenols from BdC table olives, the *in vitro* GI digestion process was performed. This model applies physiologically based conditions, i.e. chemical composition of digestive fluids, pH, and residence time typical of each compartment, of the GI tract in humans. The procedure followed the three-steps of digestive process (mouth, stomach and small intestine). The influence of GI digestion on total phenolics is reported, as percentage of bioaccessibility, in Table 2.

After *in vitro* GI digestion, the phenolic profile of table olives remained qualitatively unchanged, although the recovered phenolics showed a different behavior at the GI condition. In particular, HT and Tyr were highly bioaccessibles (86 and 100%, respectively), according to other authors.[40,41] This effect could be related to the suitable extraction and to the higher solubility of table olive polyphenols in the aqueous medium, such as digesta fluids, respect to the oil phase. The higher HT bioaccessibility at the end of GI process, could be due also, to the release from its precursors (VB and HTAc) during the digestion.[40] Verbascoside, instead, showed a bioaccessibility of 56%

resulting more stable of its isomer isoVB (33%). These two compounds were slightly more bioaccessibles respect to the previously results obtained by this research group.[42] These differences could be ascribed to the presence of matrix that develops a protective effect toward the phenolics in the GI environment.[43] The two secoiridoids identified in BdC, SEC and COM, were very stable and highly bioaccessibles (90%, for both) at the GI conditions. Studies performed by other authors[44,45,40] on olive oil digestion, showed a very low bioaccessibility of secoiridoids derivatives. Actually, it is no possible to compare our results with other publications, because, at our best knowledge, these compounds, SEC and COM, are not studied in relation to their recovery after *in vitro* GI digestion.

In addition, the two flavonoids, LUT and API, were unstable to the enzymes activity, pH and temperature characteristic of GI tract. In fact, API was not detected in the digesta and LUT showed a very low bioaccessibility of about 7%. This very poor stability and bioaccessibility probably is due to their hydrophobicity and to their low resistance at the acidic gastric conditions.[44]

Intestinal accumulation, bioavailability and P_{app} of polyphenols on Caco2/TC7 cells.

The bioavailability of polyphenols could be influenced by several potentially affecting factors, such as food matrix[46] and biotransformation and conjugation occurring during the absorption.[40,44] In this study, the BdC polyphenols bioavailability was performed using human colon adenocarcinoma cell line, clone TC7 (Caco2/TC7), that corresponds to a spontaneously differentiating clone derived from the original Caco2 cell population. These cells have the main advantage to be more stable compared to the parental Caco2 cell line, when they grown as monolayers on transwell inserts.[47] Preliminary experiments were carried out, following the protocol described by Minervini et al.,[48] in order to assess the highest non-cytotoxic concentration of the hydroalcoholic extract, by using the MTT test after 24 h exposure on Caco2/TC7 cells. The cells were incubated with olives hydroalcoholic extract, at the concentration of 65 μg mL^{-1} (total polyphenols quantified by HPLC-DAD analysis, Figure 1), for different incubation period (30, 60, 90 and 120 min). Figure 2 shows the concentration of the main identified polyphenols and their metabolites, in cells and in culture media (apical and basolateral). The amount of phenolics recovered in the apical side has given some

information on polyphenols stability in the culture medium, in relation to the incubation time. Instead, the phenolics concentration recovered in the basolateral side has given time related information on polyphenols/metabolites transport.

After 30 and 60 min of incubation, the percentage of polyphenols recovered in the apical side, was about 50% respect to the initial amount. A slightly decrease was occurred after 90 and 120 min of incubation (40%). Except for flavonoids LUT and API, which showed the highest susceptibility to the medium condition, all other phenolic compounds were quite stable (Fig. 2a).

The polyphenols absorbed by the Caco2/TC7 monolayer were only HT, VB and LUT, with a maximum of uptake after 60 min. In addition, others compounds, not detected in the apical compartment, were identified. In particular, five caffeic acid derivatives and coumaric acid, identified on the basis of their characteristic absorption spectra, were recovered at a very low amount at every times of incubation (Fig. 2b). The presence of these derivatives compounds could be attributable to a metabolism activity occurred on the intestinal epithelium. In fact, some studies have demonstrated that enterocyte-like differentiated Caco2/TC7 cells have extra- and intracellular esterases able to de-esterify hydroxycinnamate and diferulate esters that could be responsible of the metabolism.[49,50] Our data allow us to hypothesize an intracellular esterase activity toward VB, isoVB, COM and SEC, which presenting an ester bond, can release metabolites such as derivatives of caffeic acid and coumaric acid. Interesting to underline is the absence of one of the most abundant compound, Tyr, followed by others minors, such as isoVB, SEC, COM and API (Fig 2b). The absence of Tyr in cells monolayer could be attributed to its poor uptake and/or metabolism as was reported by other author.[51] Instead, the presence of HT could be derived from hydrolytic activity on VB and isoVB, by enzymes recognized in Caco2/TC7 monolayer.[52] The efficiency of cellular accumulation was about 0.89% (60 min, respect to the initial amount), showing that the polyphenols from table olives were poorly accumulated in the cell system. This low percentage of accumulation by Caco2/TC7 cells was similar to the accumulation for other polyphenols such as catechins and caffeoylquinic acids,[53-56] and could be linked to the influence of several factors

affecting polyphenol *in vivo* absorption, such as the affinity for transporters [57,58] present in intestinal epithelial cells[56], and the metabolism by the intestinal cells/tissue, as well as microbiome.[59] In addition, our data on the VB accumulation were about 10 times higher respect the data obtained from our previous studies on VB in olive mill waste water purified fraction.[42] This difference can be attributable to external factors such as food matrix, that could influence also the bioavailability, as reported by D'Archivio et al.[46]

The basolateral side, that simulated the blood plasma compartment, was analyzed with the aim to have insights on the polyphenol transport and bioavailability. Contrary to what observed in the cellular accumulation step, the main identified phenolics of BdC such as HT, Tyr, HTAc, VB, LUT and API, were recovered in the basolateral side and their bioavailability was positively related to the incubation time. The highest concentration of the olive polyphenols was reached at 120 min, unless VB and LUT that showed their higher bioavailability at 60 min. In the basolateral compartment were also detected coumaric acid, and caffeic acid derivatives, that were not detected in the apical side and probably derived from a metabolism activity of cellular monolayer (Fig 2c).[52] Moreover, the presence of HT, Tyr, and HTAc, in the basolateral side was in agreement with other authors,[44,60] that have also demonstrated a passive diffusion mechanism of these polyphenols on Caco2 cells/TC7. Our results showed that HT and Tyr were the highest bioavailable compounds, with percentages, respect to the initial amount, of 2.02% and 2.25%, respectively. Finally, the total bioavailability of the polyphenols identified in BdC was of about 1.86% with a maximum absorption at 120 min.

The data related to the transport across the Caco2/TC7 monolayer, have been used for the permeability coefficient calculation (P_{app}) in the aim to predict the small intestinal table olives polyphenols absorption. As shown in Table 3, the highest P_{app} value was obtained for HT and Tyr (1.33 and 1.49 x 10^{-6} cm s^{-1}, respectively) confirming their potential bioavailability. Moreover, VB, HTAc and LUT showed a low P_{app} coefficient, probably for their low concentration in the BdC extract used for the experiments (Table 3). Finally, the poor absorption and the low bioavailability

recovered, typical of dietary polyphenols, could be attributable to several aspects that can influence the absorption, such as glucuronation and sulphatation of the free hydroxyl groups present in their molecule;[58] and efflux mechanism into the apical compartment that provokes the excretion of metabolites back into the intestinal lumen.[61] In addition, the accumulation of VB and LUT in the Caco 2/TC7, permits to hypothesize their localized protective effect, toward the small intestinal epithelia, contrary to the others polyphenols recovered in the basolateral side (mainly HT and Tyr) that could have a biological systemic effects.

In conclusion, this study provides information on the healthy promoting compounds present in the naturally debittered table olives BdC. The results obtained give novel evidence on the chemical composition, bioaccessibility and bioavailability of the main phenolics compounds identified in the matrix. The LC-MS/MS analysis, has highlighted the presence of caffeoyl-6-secologanoside and comselogoside, never identified in processed table olives. The identified polyphenols, after *in vitro* GI digestion, were on average bioaccessible with a percentage ranging from 7% of LUT to 100% of TYR. The highest bioavailable polyphenols were HT and Tyr; and it was very interesting to note the presence in cellular compartment and basolateral side, of caffeic and coumaric metabolites derived from intracellular esterase activities toward the main phenolic compounds present in the apical side. Finally, the results obtained suggest that the naturally debittered table olives BdC can be considered as a source of bioaccessible, absorbable and bioavailable phenolics compounds. Considering the potential health promoting effect of polyphenols, the table olives BdC could be considered suitable for a balanced diet and considered as a functional foods.

CONTRIBUTORS

AC and ID provided the conception and the design of the research, study. FM, AG, ID, VL conducted experimental procedures of the study and collaborated in analyzing data. VMTL and BC performed LC-MS analysis. VL and ID performed HPLC-DAD analysis. FM, AG performed cell line experiments. AC, ID, FM, AG, VMTL, VL, BC performed drafting the manuscript. AFL performed critical reading of the manuscript. AC, ID, FM, AG, VMTL, VL, BC, AFL read and approved the manuscript.

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ABBREVIATIONS

MeOH, Methanol; AcOH, glacial acetic acid; EtOH, ethanol; EtOAc, ethyl acetate; DMEM, Dulbecco's modified Eagle's medium; PBS, Dulbecco's phosphate-buffered saline; BSA, bovine serum albumin; FBS, Foetal bovine serum; BdC, cv. *Bella di Cerignola*; DG, small intestinal digesta; GI, gastrointestinal; PET, polyethylene terephthalate; TEER, transepithelial electrical resistance; P_{app}, permeability coefficient; HT, hydroxytyrosol ; Tyr, tyrosol; VB, verbascoside; isoVB, isoverbascoside; LUT, luteolin; API, apigenin; HTAc, hydroxytyrosol acetate; SEC, caffeoyl-6-secologanoside; COM, comselogoside; Caco2/TC7, human colon adenocarcinoma cell line clone TC7.

References

1. International Olive Council (IOC). (2014). *World and European Table Olive Figures*. Available On line at:http://www.internationaloliveoil.org/ (AccessedMay2014).
2. Corsetti, A.; Perpetuini, G.; Schirone, M.; Tofalo, R.; Suzzi, G.. Application of starter cultures to table olive fermentation: an overview on the experimental studies. *Front. Microbiol*. **2012,** *3*, 248.
3. Romero, C.; Brenes, M.; García, P.; Garrido, A.. Hydroxytyrosol 4-β-glucoside an important phenolic compounds in olive fruit and derived product. *J. Agric. Food Chem.* **2002,** *50*, 3835-3839.

4. Macheix, J. J.; Fleuriet, A.; Billot, J. (1990). Fruit phenolics (pp**.** 1–126). Boca Raton, FL: CRC Press.
5. Ryan, D.; Robards, K. Phenolic compounds in olives. *Analyst*. **1998,** *123*, 31R–44R.
6. Soler-Rivas, C.; Espìn, J. C.; Wichers, H. J. Oleuropein and related compounds. *J. Sci. Food Agr*. **2000**, *80*, 1013–1023.
7. Motilva, M. J.; Tovar, M. J.; Romero, M. P.; Alegre, A.; Girona, J.). Influence of regulated deficit irrigation strategies applied to olive trees (Arbequina cultivar) on oil yield and oil composition during the fruit ripening. *J. Agric. Food Chem*. **2000**, *80*, 2037–2043.
8. Pereira, J. A.; Pereira, A. P.; Ferreira, I. C.; Valentão, P.; Andrade, P. B.; Seabra, R.; Bento, A. Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity. *J Agric Food Chem.* **2006**, *54*, 8425-8431.
9. Amiot, M. J.; Tacchini, M.; Fleuriet, A.; Macheix, J. J. The technological debittering process of olives: characterization of fruits before and during alkaline treatment. *Sci. Aliments*. **1990**, *10*, 619-63.
10. Brenes, M.; De Castro, A. Transformation of oleuropein and its hydrolysis products during Spanish-style green olive processing. *J. Sci. Food Agric*. **1998**, *77*, 353-358.
11. Brenes, M.; Rejano, L.; Garcìa, P.; Sànchez, A. H.; Garrido, A. Biochemical changes in phenolic compounds during Spanish style green olives processing. *J. Agric. Food Chem*. **1995**, *43*, 2702-2706.
12. Romero, C.; Brenes, M.; Yousfi, K.; Garciä, P.; Garciä, A.; Garrido, A. Effect of Cultivar and Processing Method on the Contents of Polyphenols in Table Olives. *J. Agric. Food Chem*. **2004,** *52*, 479-484.
13. Sánchez, A. H.; García, P.; Rejano, L. Trends in table olive production. Elaboration of table olives. *Grasas Aceites*. **2006**, *57*, 86-94.
14. Blekas, G.; Vassilakis, C.; Harizanis, C.; Tsimidou, M.; Boskou, D. G. Biophenols in table olives. *J. Agric. Food Chem*. **2002**, *50*, 3688-3692.
15. Charoenprasert, S.; Mitchell, A. Factors influencing phenolic compounds in table olives (Olea europaea). *J. Agric. Food Chem*. **2012,** *60*, 7081-7095.
16. Perricone, M.; Bevilacqua, A.; Corbo, M. R.; Sinigaglia, M.. Use of Lactobacillus plantarum and glucose to control the fermentation of "*Bella di Cerignola*" Table Olives, a traditional variety of Apulian region (Southern Italy). *J Food Sci.* **2010***, 75*, M430-M436.
17. Bevilacqua, A.; Altieri, C.; Corbo, M. R,; Sinigaglia, M.; Ouoba, L. I. I. Characterization of Lactic Acid Bacteria Isolated from Italian Bella di Cerignola Table Olives: Selection of Potential Multifunctional Starter Cultures. *J. Food Sci,* **2010**, *75*, M536-M544.
18. Pasqualone, A.; Nasti, R.; Montemurro, C.; Gomes, T. Effect of natural-style processing on the oxidative and hydrolytic degradation of the lipid fraction of table olives. *Food Control.* **2014,** *37*, 99-103
19. De Angelis, M.; Campanella, D.; Cosmai, L.; Summo, C.; Rizzello, C. G.; Caponio, F. Microbiota and metabolome of un-started and started Greek-type fermentation of *Bella di Cerignola* table olives. *Food Microbiol.* **2015,** *52*, 18-30.
20. Phenol Explorer database (http://www.phenol-explorer.eu) (January 15 2016)
21. Lattanzio, V.. High performance reversed phase liquid chromatography of free and bound phenolic acids in eggplant (Solanum melongena L.). *J Chromatogr.* **1982**, 250,143–148.
22. Moser, S.; Chegeni, M.; Jones, O G.; Liceaga, A; Ferruzzi, M. G. The effect of milk proteins on the bioaccessibility of green tea flavan-3-ols *Food Res Int.* **2014,** *66,* 297–305.
23. Failla, M. L.; Chitchumronchokchai, C.; Ferruzzi, M. G.; Goltz, S. R.; Campbell W. W. Unsaturated fatty acids promote bioaccessibility and basolateral secretion of carotenoids and α-tocopherol by Caco-2 cells. *Food Funct*. **2014,** *5*, 1101–1112.
24. Neilson, A. P.; Song, B. J.; Sapper, T. N.; Bomser, J. A.; Ferruzzi M. G. Tea catechin auto-oxidation dimers are accumulated and retained by Caco-2 human intestinal cells**.** *Nutr. Res*. **2010,** *30*, 327–40.

25. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. **1976**, *72*, 248-254.
26. Uylaşer, V.; Yildiz, G. The historical development and nutritional importance of olive and olive oil constituted an important part of the Mediterranean diet. *Crit Rev Food Sci Nutr.* **2014**, *54*, 1092-1101.
27. Ghanbari, R.; Anwar, F.; Alkharfy, K.M.; Gilani, A.H.; Saari, N. Valuable nutrients and functional bioactives in different parts of olive (Olea europaea L.)-A review. *Int. J. Mol. Sci*. **2012**, *13*, 3291–3340.
28. Kanakis, P.; Termentzi, A.; Michel, T.; Gikas, E.; Halabalaki, M.; Skaltsounis, A. L.. From Olive Drupes to Olive Oil. An HPLC-Orbitrap-based Qualitative and Quantitative Exploration of Olive Key Metabolites. *Planta med.* **2013**, 79, 1576-1587.
29. Sivakumar, G.; Bati, C. B.; Uccella, N. HPLC-MS screeing of the antioxidant profile of Italian olive cultivars. *Chem. Nat. Compd*. **2005**, *41*, 588−591.
30. Vinha, A. F.; Ferreres, F.; Silva, B. M.; Valentao, P.; Goncalves, A.; Pereira, J. A.; Oliveira, M. B.; Seabra, R. M.; Andrade, P. B. Phenolic profiles of Portuguese olive fruits (Olea europaea L.): influences of cultivar and geographical origin. *Food Chem*. **2005**, *89*, 561−568.
31. Brenes, M.; Garcia, P.; Duran, M. C.; Garrido, A. Concentration of phenolic compounds change in storage brines of ripe olives. *J. Food Sci*. **1993**, *58*, 347−350.
32. Funes, L.; Laporta, O.; Cerdán-Calero, M.; Micol, V. Effects of verbascoside, a phenylpropanoid glucoside from lemon verbena, on phospholipid model membranes. *Chem Phys Lipids*. **2010,** *163*, 190–199.
33. Wu, Y.T.; Lin, L.C.; Sung, J.S.; Tsai, T.H. Determination of acteoside in Cistanche deserticola and Boschniakia rossica and its pharmacokinetics in freely-moving rats using LC-MS/MS. *J Chromatogr B.* **2006**, *844*, 89–95.
34. Cardinali, A.; Pati, S.; Minervini, F.; D'Antuono, I.; Linsalata, V.; Lattanzio, V.. Verbascoside, Isoverbascoside, and Their Derivatives Recovered from Olive Mill Wastewater as Possible Food Antioxidants. *J. Agric. Food Chem*. **2012,** *60*, 1822-1829.
35. Funes, L.; Fernández-Arroyo, S.; Laporta, O.; Pons, A.; Roche, E.; Segura-Carretero, A.; Fernández-Gutiérrez, A.; Micol V.. Correlation between plasma antioxidant capacity and verbascoside levels in rats after oral administration of lemon verbena extract. *Food Chem* **2009,** *117*, 589–98.
36. González-Correa, J.A.; Navas, M.D.; Muñoz-Marín, J.; Trujillo, M.; Fernández-Bolaños, J.; De La Cruz, J.P. Effects of hydroxytyrosol and hydroxytyrosol acetate administration to rats on platelet function compared to acetylsalicylicacid. *J AgricFoodChem.* **2008**, *56*, 7872–7876.
37. Ryan, D.; Robards, K.; Lavee, S. Determination of phenolic compounds in olives by reversed-phase chromatography and mass spectrometry. *J. Chromato., A,* **1999**, *832*, 87−96.
38. Obied, H. K.; Karuso, P.; Prenzler, P. D.; Robards, K. Novel Secoiridoids with Antioxidant Activity from Australian Olive Mill Waste. *J. Agric. Food Chem*. **2007a,** *55*, 2848-2853.
39. Obied, H. K.; Bedgood Jr, D. R.; Prenzler, P. D.; Robards, K. Chemical screening of olive biophenol extracts by hyphenated liquid chromatography. *Anal Chim Acta.* **2007b**, 603, 176-189.
40. Rubió, L.; Macià, A.; Castell-Auví, A.; Pinent, M.; Blay, M. T.; Ardévol, A.; Romero, M.P.; Motilva, M. J. Effect of the co-occurring olive oil and thyme extracts on the phenolic bioaccesibility and bioavailability assessed by in vitro digestion and cell models. *Food Chem*. **2014**, *149*, 277-284.
41. Dinnella, C.; Minichino, P.; D'Andrea, A.M.; Monteleone E. Bioaccessibility and antioxidant activity stability of phenolic compounds from extra-virgin olive oils during *in vitro* digestion. *J. Agric. Food Chem.* **2007,** *55,* 8423–8429.

42. Cardinali, A.; Linsalata, V.; Lattanzio, V.; Ferruzzi, M.G.. Verbascosides from Olive Mill Waste Water: Assessment of their Bioaccessibility and Intestinal Uptake using an in vitro Digestion/Caco-2 Model System. *J Food Sci*. **2011,** *76*, H48-H54.
43. Ahmad-Qasem, M. H.; Cánovas, J.; Barrajón-Catalán, E.; Carreres, J. E.; Micol V.; García-Pérez, J. V. Influence of Olive Leaf Processing on the Bioaccessibility of Bioactive Polyphenols. *J. Agric. Food Chem.* **2014**, *62*, 6190–6198.
44. Soler, A.; Romero, M.P.; Macià, A.; Saha, S.; Furniss, C.S.M.; Kroon, P. A.; Motilva, M. J. Digestion stability and evaluation of the metabolism and transport of olive oil phenols in the human small-intestinal epithelial Caco-2/TC7 cell line. *Food Chem.* **2010**, *119* 703–714.
45. Pinto, J.; Paiva-Martins, F.; Corona, G.; Debnam, E. S.; Jose Oruna-Concha, M.; Vauzour, D., et al. Absorption and metabolism of olive oil secoiridoids in the small intestine. *Br J Nutr.* **2011**, *105*, 1607–1618.
46. D'Archivio, M.; Filesi, C.; Varì, R.; Scazzocchio, B.; Masella, R. Bioavailability of the Polyphenols: Status and Controversies. *Int. J. Mol. Sci*. **2010**, *11*, 1321–1342.
47. Chantret, I.; Rodolosse, A.; Barbat, A.; Dussaulx, E.; Brot-Laroche, E.; Zweibaum, A.;et al.. Differential expression of sucrase-isomaltase in clones isolated from early and late passages of the cell line Caco-2: Evidence for glucose dependent negative regulation. *J Cell Sci*. **1994,** *107*, 213–225.
48. Minervini, F.; Garbetta, A.; D'Antuono, I.; Cardinali, A.; Martino, N.A.; Debellis, L.; Visconti, A. Toxic mechanisms induced by Fumonisin B1 mycotoxin on human intestinal cell line. *Arch. Environ. Con. Tox.* **2014**, *67*, 115-123.
49. Kern, S. M.; Bennett, R. N.; Needs, P. W.; Mellon, F. A.; Kroon, P. A.; Garcia-Conesa, M. T., Characterization of Metabolites of Hydroxycinnamates in the in Vitro Model of Human Small Intestinal Epithelium Caco-2 Cells. *J. Agric. Food Chem*. **2003**, 51, 7884–7891.
50. Andreasen, M. F.; Kroon, P. A.; Williamson, G.; Garcia-Conesa, M. T. Esterase Activity Able To Hydrolyze Dietary Antioxidant Hydroxycinnamates Is Distributed along the Intestine of Mammals. *J. Agric. Food Chem*. **2001**, *49*, 5679–5684.
51. Mateos, R.; Goya, L.; Bravo, L. Metabolism of the olive oil phenols hydroxytyrosol, tyrosol, and hydroxytyrosyl acetate by human hepatoma HepG2 cells. *J. Agric. Food Chem*. **2005**, *53*, 9897-9905.
52. Imai, T.; Imoto, M.; Sakamoto, H.; Hashimoto, M. Identification of esterases expressed in Caco-2 cells and effects of their hydrolyzing activity in predicting human intestinal absorption. *Drug Metab Dispos*, **2005,** *33*, 1185-1190.
53. Neilson, A.P.; George, J.C.; Janle, E.M.; Mattes, R.D.; Rudolph, R.; Matusheski, N.V.; Ferruzzi, M.G. Influence of food matrix on cocoa flavan-3-ol bioaccessibility *in vitro* and bioavailability in humans. *J Agric Food Chem.* **2009**, *57*, 9418–9426.
54. Peters, C.M.; Green, R.J.; Janle, E.M.; Ferruzzi, M.G. Formulation with ascorbic acid and sucrose modulates catechin bioavailability. *Food Res Int*. **2010**, 43, 95–102.
55. Vaidyanathan, J.B.; Walle, T. Cellular uptake and efflux of the tea flavonoid (-)-epicatechin-3-gallate in the human intestinal cell line Caco-2. *J Pharmacol Exp Ther.* **2003**, *307*, 745–752.
56. D'Antuono, I.; Garbetta, A.; Linsalata, V.; Minervini, F.; Cardinali, A. Polyphenols from artichoke heads (Cynara cardunculus (L.) subsp. scolymus Hayek): in vitro bio-accessibility, intestinal uptake and bioavailability. *Food Funct.* **2015,** *6*, 1268-1277.
57. Di Pietro, A.; Conseil, G.; Pérez-Victoria, J. M.; Dayan, G.; Baubichon-Cortaya; H.; Trompiera, D.; et al. Modulation by flavonoids of cell multidrug resistance mediated by P-glycoprotein and related ABC transporters. *Cell. Mol. Life Sci*. **2002,** *59*, 307–322.
58. Teng, Z.; Yuan, C.; Zhang, F.; Huan, M.; Cao, W.; Li, K.; Yang, J.; Cao, D.; Zhou, S.; Mei, Q. Intestinal Absorption and First-Pass Metabolism of Polyphenol Compounds in Rat and Their Transport Dynamics in Caco-2 Cells. *PLoS One*. **2012**, *7*, e29.

59. van Duynhoven, J.; Vaughan, E. E.; Jacobs, D. M.; Kemperman, R. A.; van Velzena, E. J. J.; Grossa, G.; et al. Metabolic fate of polyphenols in the human superorganism. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 4531–4538.
60. Manna, C.; Galletti, P.; Maisto, G.; Cucciolla, V.; D'Angelo, S.; Zappia, V. Transport Mechanism and Metabolism of Olive Oil Hydroxytyrosol in Caco-2 Cells. *FEBS Lett.* **2000**, *470* ,341-344.
61. Carbonell-Capella, J. M.; Buniowska, M.; Barba, F. J.; Esteve, M. J.; Frígola, A. Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: A review. *Compr Rev Food Sci Food Saf*. **2014**, *13*, 155-171.

Table 1. Chemical characterizion (HPLC-DAD and LC-MS/MS) of main polyphenolic compounds in cv *Bella di Cerignola* extract.

Compounds	Retention time	UV λmax	$[M-H]^-$	MS/MS fragments
Hydroxytyrosol	5.4	280	153	123, 95
Tyrosol	8.0	275	137	119
Hydroxytyrosol acetate	17.2	280	195	153, 135, 123
Verbascoside	17.9	330	623	461, 161
Isoverbascoside	21.2	328	623	461, 161
Caffeoyl-6-secologanoside	25.4	328	551	507, 489, 393, 323, 161
Comselogoside	30.0	315	535	491, 265, 161
Luteolin	34.7	255-350	285	267, 241, 175, 151, 133
Apigenin	40.6	267-338	269	225, 201, 181,149

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Table 2. Polyphenols concentration of table olive cv *Bella di Cerignola* extract and their bioaccessibility after *in vitro* digestion

	Extract	Bioaccessibility
	(μg/g FW)	*(%)*
Hydroxytyrosol	356.6 ± 24.8	86.3 ± 5.2
Tyrosol	85.3 ± 5.7	99.0 ± 6.1
Hydroxytyrosol acetate	28.2 ± 2.3	nd ± --
Verbascoside	27.9 ± 6.1	55.5 ± 9.9
Isoverbascoside	8.3 ± 1.3	32.9 ± 11.5
Caffeoyl-6-secologanoside	9.1 ± 2.8	95.7 ± 11.5
Comselogoside	10.2 ± 2.4	91.1 ± 14.8
Luteolin	18.1 ± 0.5	7.0 ± 0.6
Apigenin	4.8 ± 0.5	nd ± --
Total	548.5 ± 31.5	84.0 ± 5.9

Results are presented as means ± SD of triplicate independent experiments.

nd = not detected

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Table 3 Apparent permeability coefficient P_{app} (apical to basolateral) for the main olive table polyphenols in Caco2/TC7 monolayers (n= 3)

Polyphenols	**P_{app} x 10^{-6} (cm s^{-1})**
Hydroxytyrosol	1.33 ± 0.01
Tyrosol	1.49 ± 0.07
Hydroxytyrosol acetate	0.62 ± 0.04
Verbascoside	0.58 ± 0.02
Luteolin	0.91 ± 0.03

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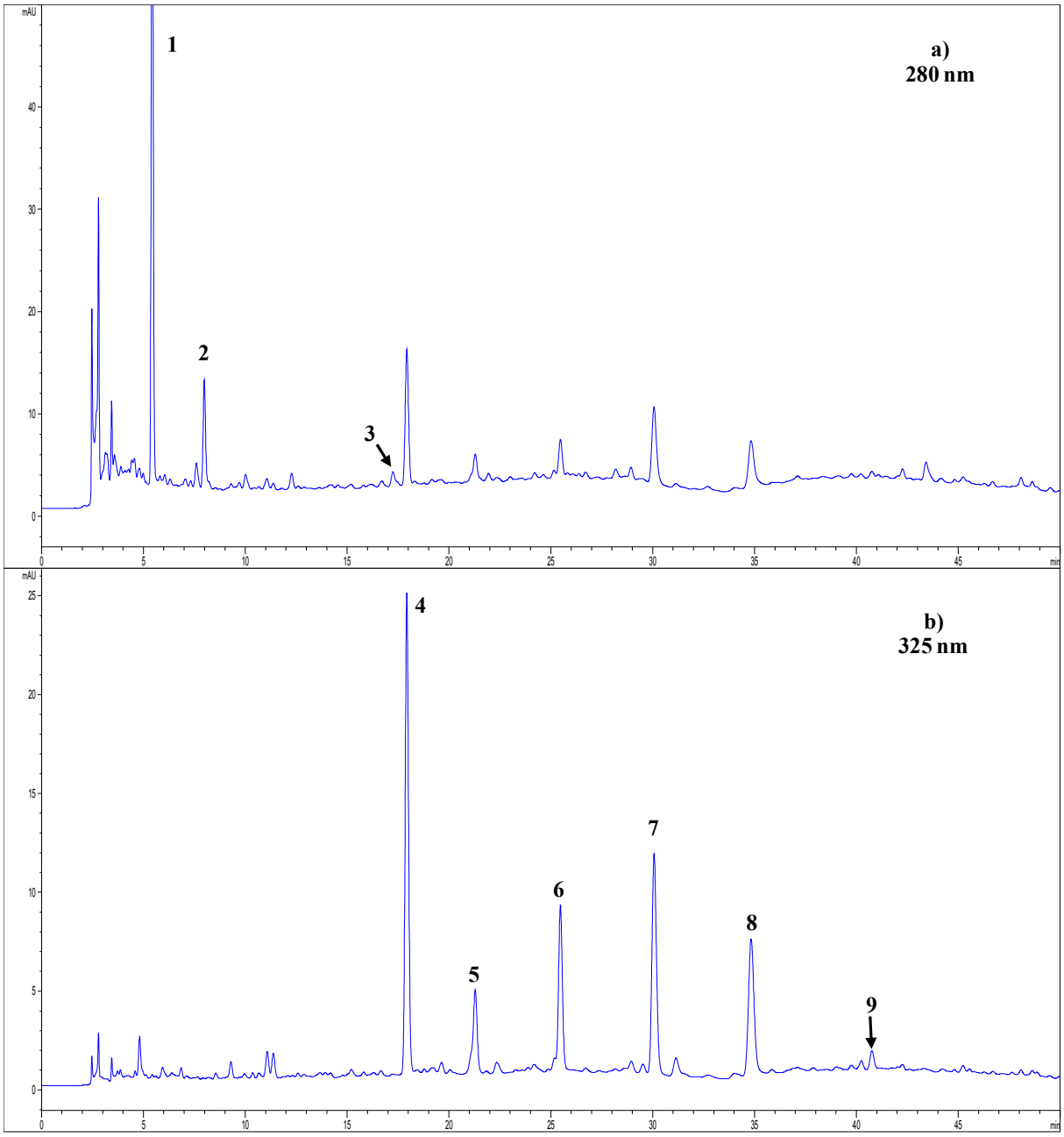

Figure 1. HPLC-DAD chromatograms of hydroalcoholic extract of table olives cv *Bella di Cerignola*: (**a**) 280 nm trace, and (**b**) 325 nm traces. Hydroxytyrosol, (1); Tyrosol (2); Hydroxytyrosol acetate (3); Verbascoside (4); Isoverbascoside (5); Caffeoyl-6-secologanoside (6); Comselogoside (7); Luteolin (8); Apigenin (9).

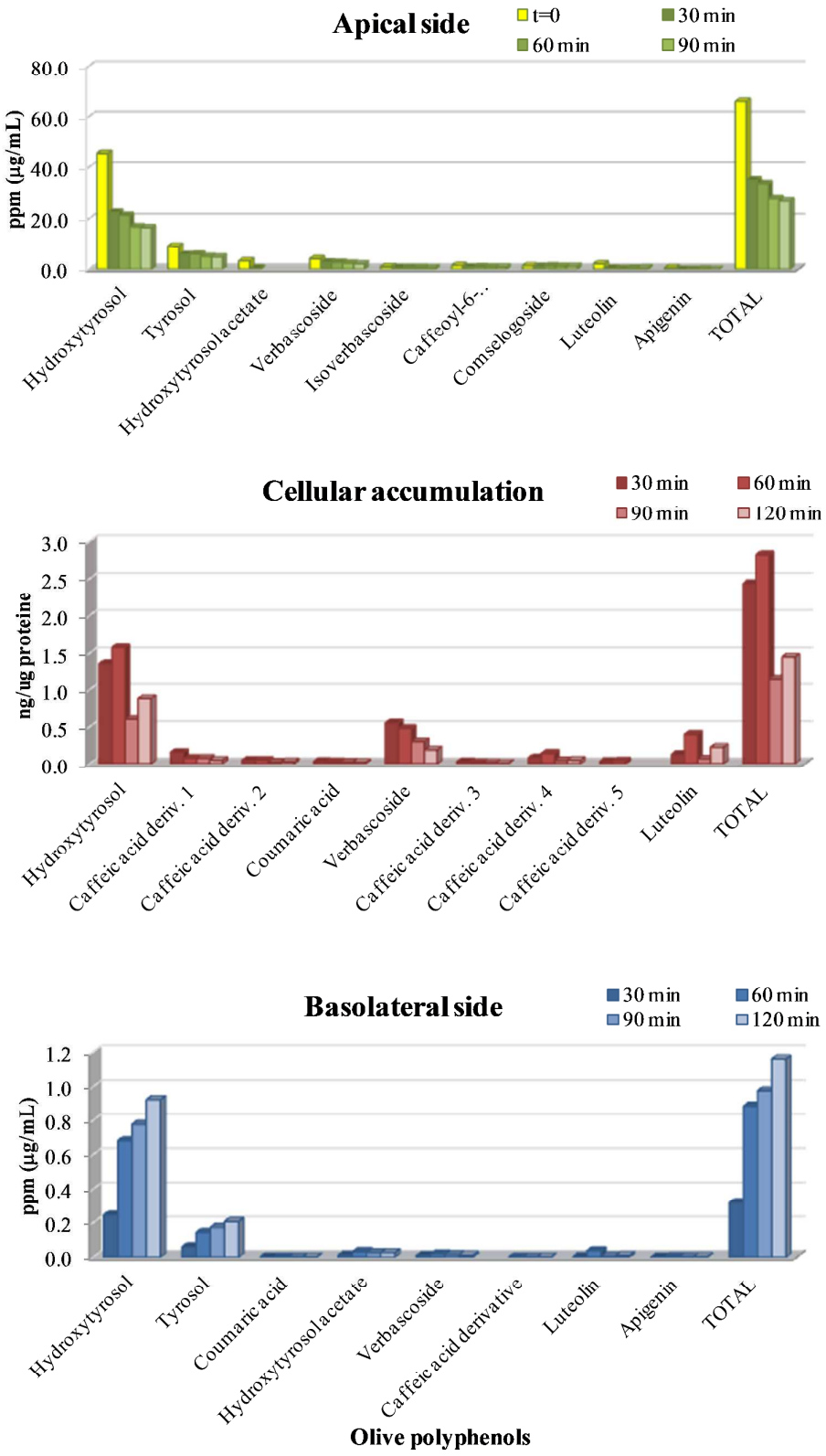

Figure 2. Main identified polyphenols and their metabolites in apical (a), cells (b) and basolateral (c) sides.

580 **TOC Graphic**

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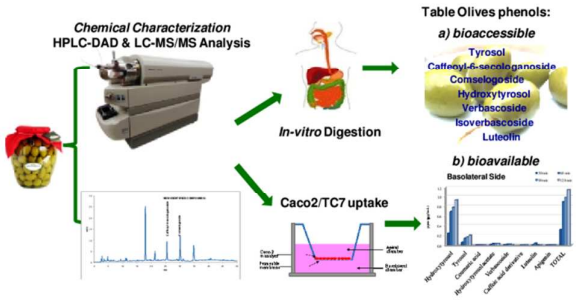

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