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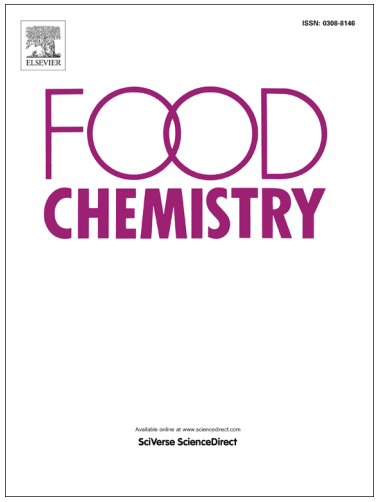

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In vitro digestion of meat- and cereal-based food matrix enriched with grape extracts: How are polyphenol composition, bioaccessibility and antioxidant activity affected?

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Running title: *In vitro* digestion of complex food matrix with grape extracts

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Abstract

The aim of this study was to evaluate the effect of enriching a complex food matrix (FM) with grape extracts on polyphenol content, composition, bioaccessibility and antioxidant activity during digestion. The grape extracts and FM were separately tested under the same conditions as controls. The FM by itself contains a significant amount of phenolic acids and flavonols, influencing the final recovery of polyphenols from grape extracts. The FM significantly increased the total recovery of polyphenols after digestion of grape seed extracts compared to those digested without the FM; however, a low recovery of proantocyanidins and total flavonoids was observed. Digestive fluids and FM compounds significantly increased the total polyphenol content of grape digests and significantly contributed to their ABTS•+ scavenging activity and ferrous-ion-chelating capacity. The present study suggested that enrichment of meat- and cereal-based products with grape polyphenol extracts could be a good strategy to formulate a healthier diet.

Keywords: *in vitro* digestion, grape extracts, bioaccessibility, polyphenols, food matrix, antioxidant activity

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1. Introduction

The wine-making process generates considerable quantities of by-products that can be used as rich sources of phenolic compounds that possess a broad range of biological activities such as antioxidant, antibacterial, anticancer, anti-inflammatory and antidiabetic activities, as well as hepatoprotective, cardioprotective and neuroprotective effects (Gülçin, 2010; Nassiri-Asl & Hosseinzadeh, 2016). Grape skin and seed extracts are very promising food ingredients that have drawn the attention of food scientists in the past decade. Grape seeds contain a considerable amount of flavan-3-ols and phenolic acids, whereas flavonols and anthocyanins are dominant in grape skin (Pantelić, et al., 2016).

An essential parameter that limits the biological activity of polyphenols is their bioaccessibility, which determines the proportion of polyphenols released from the food matrix during digestion. Polyphenols are further solubilized in digestive fluids and can be subsequently, absorbed and metabolized; thus, the bioaccessibility of polyphenols is a key factor for the expression of their health-promoting features. The importance of the interaction between the food matrix and phenolic compounds during digestion has been extensively studied in the past decade and has been well-reviewed (Jakobek, 2015). The majority of polyphenols (with a few exceptions) are considered as moderately to highly soluble in water. This property indicates that their bioaccessibility does not rely on micellization but rather on release from the matrix and their solubilization in the aqueous phase, because certain polyphenols may be complexed with food proteins or digestion enzymes and minerals. The recovery of polyphenols can be affected by almost all major food components such as proteins, carbohydrates, lipids (Jakobek, 2015; Ozdal, Capanoglu, & Altay, 2013) or fibres (González-Aguilar, Blancas-Benítez, & Sáyago-Ayerdi, 2017). It has been determined that the structure, composition of food matrix and the effect of co-

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digestion of polyphenols with different food components may affect their bioaccessibility, digestibility or antioxidant activity (Pineda-Vadillo, et al., 2016; Wang, Amigo-Benavent, Mateos, Bravo, & Sarriá, 2017).

Investigations on *in vitro* digestion of polyphenols have been mainly performed in naturally enriched food matrices such as fruits and vegetables (Dufour, et al., 2018) or powdered polyphenol-rich extracts (Wang, Amigo-Benavent, Mateos, Bravo, & Sarriá, 2017). Only few studies address non-naturally enriched food matrices such as dairy, egg products and bakery products (Karakaya, et al., 2016; Pineda-Vadillo, et al., 2016). Interestingly, according to our knowledge, the fortification of complex food matrices including meat and carbohydrates has been scarcely studied till date (Stanisavljević, et al., 2015). However, it has been documented that meat lipids are very susceptible to peroxidation during meat cooking and gastrointestinal digestion; this results in the formation of lipid oxidation products, which could be toxic to the human body (Vieira, Zhang, & Decker, 2017). It has been proposed that the incidence of cancer and vascular diseases associated with high meat consumption may be reduced by the addition of antioxidants in the diet, especially, at the same time as meat preparation or meat consumption or by modification of food preparation methods (Vieira, Zhang, & Decker, 2017). Thus, the fortification of meat-based meals with plant polyphenols can be a good strategy to mitigate this health risk.

A healthy diet is very important in infants and young children to prevent infection and chronic diseases in the short term and during the life time (Jackson, 2015); taking this into account, the aim of the present study was to examine the effect of a complex food matrix on grape-polyphenol bioaccessibility and antioxidant activity during *in vitro* digestion. For this purpose, an infant puree composed of turkey meat, potato, corn and rice was enriched with grape skin and

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seed extracts and was subjected to standardized static *in vitro* digestion using previously accepted methods (Minekus et al. 2014). In many *in vitro* studies, the bioaccessibility of phenolic compounds during digestion has been assessed by determining the total phenolic content and the content of major classes of polyphenols, which does not yield information on the recovery of specific phenolic compounds; considering this limitation, structural analysis of polyphenol profiles during digestion was performed. The obtained results could be helpful to estimate the bioactive potential of grape skin and seed extracts in the fortification of complex food matrices that are based on meat and carbohydrates such as food products for infants and young children.

2. Material and methods

2.1. Grape extracts and food matrix

The indigenous red grape variety "Prokupac", was obtained from a vinery located in Aleksandrovac, which is at the centre of the Župa district of Serbia. The grapes were pressed, and the residual grapes, skin and seeds were immediately dried in a drying oven (Thermo Scientific Haraeus, MA, USA) at 60 °C for 72 h. Next, the skin was manually separated from the seed, and both were ground in a coffee grinder (Bosch MKM 6003 UC, BSH Hausgeräte GmbH, Munich, Germany). The obtained powder was sieved (model Analysette 3 pro, Fritsch, Idar-Oberstein, Germany), selecting a particle size between 0.6 mm and 1.12 mm. The material was maintained at -20 °C in vacuum-packed plastic containers until further analysis. Aqueous extracts of the grape skin and seeds were prepared according to the method described by Pantelić, et al. (2016). Briefly, the skin (about 2 g) and the seed (about 1 g) were extracted three times with 20 mL of methanol containing 0.1% HCl (skin) or 10 mL methanol/water (80/20) containing 0.1% HCl (seed). The acidified methanol was used to prevent oxidation of the

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phenolics and to increase the efficiency of the phenolic extractions since the phenol-phenolate equilibrium shift towards the less polar phenol form (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014). Furthermore, the acidic solvents denature the membranes od cell tissue and simultaneously dissolve and stabilize pigments such as anthocyanins (Rodriguez-Saona & Wrolstad, 2001). It is known that phenolics present in the grape by-products can be found in the soluble (free and esterified) and insoluble-bound forms. Depending on the extraction procedures, analytical methods, grape variety and selected by-products, the different amount of phenolics in the insoluble form was found: 19.88-56.75% (Lutterodt, Slavin, Whent, Turner, & Yu, 2011) and 63-79% of the total amount (de Camargo, Regitano-D'Arce, Biasoto, & Shahidi, 2014) contributing to the antioxidative properties of total phenolics in different extent: from negligible (Tang, et al., 2018) to significant (de Camargo, Regitano-D'Arce, Biasoto, & Shahidi, 2014).

The extractions were carried out by stirring mixtures for 1 h at room temperature on a mechanical shaker (Thys 2, MLW Labortechnik GmbH, Seelbach, Germany). After shaking, the extracts were placed in the fridge at 5 ˚C for 22 h. Thereafter, the extracts were filtered through Whatman No.42 filter paper and were collected. The extracts obtained after repeated extractions were combined and were evaporated to dryness by rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany) under reduced pressure at 40 ˚C. The residues after evaporation were dissolved in 15 mL of milliQ water and these solutions were used for further analysis. The extracts were filtered with 0.45 μm filters (Syringe Filter, PTFE, Supelco) before further analysis. Infant puree (Juvitana, Swisslion Product d.o.o. Indjija, Serbia) was prepared from boiled turkey meat (20%), boiled potato paste (10%), boiled corn paste (25%), rice flour (5%), 0.1% NaCl and water (39.9%). The mixture contained 3% protein, 10% carbohydrate and 1% of total fat.

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2.2. *Simulated in vitro gastrointestinal digestion (GID)*

In vitro GID was conducted following the standardized static *in vitro* digestion protocol (Minekus, et al., 2014). Briefly, 2 mL of aqueous grape extract was mixed with 3 g of infant puree and was digested according to the standardized static digestion protocol (Minekus, et al., 2014). These samples were labelled as DSK/FM (digested grape skin extract with food matrix) and DSE/FM (digested grape seed extract with food matrix). To examine the digestion products of the matrix under the same conditions, 3 g of infant formula was mixed with 2 mL of distilled water, and this sample was labelled as DFM (digested food matrix). To establish the effect of digestion without the food matrix, an additional control was performed by mixing 2 mL of extract with 3 mL of distilled water, which was labelled as DSK (digested grape skin extract) or DSE (digested grape seed extract). The samples were subjected to the oral phase of digestion by addition of 3.5 mL of the simulated salivary fluid (SSF), 0.5 mL of salivary α-amylase solution (1500 U/mL), 25 µL of 0.3 M $CaCl_2$ and 975 µL of water, shaken vigorously and incubated 2 min at 37 °C. During the following gastric phase of digestion the whole amount of oral bolus (~10 mL) was mixed with 7.5 mL of simulated gastric fluid (SGF), 1.6 mL of pepsin solution (25000 U/mL), prepared in SGF, 5 µL of 0.3 M $CaCl_2$ and pH was adjusted to 3.0 using 1M HCl. The volume of the mixture was then adjusted to 20 mL using distilled water and incubated at 37 °C for 2 h, using orbital shaker (Lab-Shaker SMX 1300, Adolf Kühner, Basel, Switzerland), adjusted to 300 rpm. For the final intestinal phase, 20 mL of obtained gastric chyme was mixed with 11 mL of simulated intestinal fluid (SIF), 5 mL of pancreatin solution 800 U/mL (trypsin activity) prepared in SIF, 2.5 mL of 160 mM bile salt solution and 40 µL of 0.3 M $CaCl_2$. The pH value of 7.0 was adjusted using 1M NaOH and the mixture was supplemented with distilled water to reach final volume of 40 mL. Additional incubation was

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performed with constant shaking at 300 rpm during 2 h at 37 °C. After completed digestion supernatants were separated by centrifugation at 4500*g* (Centrifuge 5804R, Eppendorf, Hamburg, Germany) for 10 min at 4°C, filtrated through 45 µm syringe filters (Filtropur S 0.45, 83.1826, Sarstedt, Germany) then immediately frozen in liquid nitrogen and kept at -80 °C for further analysis in the next few days. To prepare controls that represent the contribution of digestive fluids, the extract of grape skin and seeds with and without the food matrix and the food matrix alone were immediately mixed on ice with all the components and enzymes in the same ratio as during the simulated digestion; the pH value was adjusted to a final value of 7.0. These samples were labelled SK/FMC, SE/FMC, SKC, SEC, and FMC, respectively. To allow the comparison of the digested specimens with the initial extracts and food matrix, adequate amounts of grape skin and seed extracts, as well as infant puree, were diluted with distilled water to achieve the total volume of 40 mL that was obtained after complete digestion of the analysed samples. These samples were labelled as SK, SE and FM, respectively.

2.3. Total phenolic, total flavonoid and proanthocyanidin content

The total phenolic content (TPC) in the samples was determined using Folin-Ciocalteu's reagent, following the method described by Singleton, Orthofer, and Lamuela-Raventós (1998). Briefly, an aliquot of sample (70 µL) was mixed with 300 µL of Folin-Ciocalteu reagent and 230 µL of 7.5% Na_2CO_3, followed by incubation for 1 h 30 min at room temperature. The total flavonoid content (TFC) was determined using a colorimetric assay with aluminium chloride, as previously described by Ribeiro, Ribani, Francisco, Soares, Pontarolo, and Haminiuk (2015). Briefly, an aliquot of sample (125 µL) was mixed with 625 µL of milliQ water and 37.5 µL of 5% $NaNO_2$. After 6 min, 75 µL of 10% $AlCl_3$ was added to form a flavonoid-aluminium complex. An aliquot of 1 M NaOH (250 µL) was added in the mixture 5 min later. The content of extractable

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proantocyanidins (PC) in the samples were determined using the butanol-HCl assay, as described by Deng, Penner, and Zhao (2011). Briefly, an aliquot of sample (0.5 mL) was mixed with 3 mL of buthanol-HCl (95:5) and 0.1 mL of iron reagent (2% $FeNH_4(SO_4)_2$ in 2 M HCl) and vigorously vortexed. After that, the mixture was incubated at 95 ˚C for 40 min. All three methods are based on spectrophotometric measurements (765 nm for TPC, 510 nm for TFC and 540 nm for PC) which were performed using a Shimadzu spectrophotometer (UV-1800, Shimadzu USA Manufacturing Inc, UR, USA). TPC was expressed as milligrams of gallic acid equivalents per 100 ml of sample (mg GAE/100 ml), while TFC was expressed as milligrams of catechin equivalents per 100 ml of sample (mg CE/100 ml). The proanthocyanidin content was expressed as milligrams per 100 ml and was calculated using 0.1736 (mg/ml) as the conversion factor (Lavelli, Sri Harsha, Torri, & Zeppa, 2014) with the following equation:

$$PAC = (A_s - A_c) \times 0.1736 \times DF$$

where A_c represents the absorbance of blank, A_s is the absorbance of samples, and DF is the dilution factor.

2.4. UHPLC–DAD MS/MS analysis of non-flavan-3-ols

The separation, determination, and quantification of the components of interest were performed using a Dionex Ultimate 3000 UHPLC system equipped with a diode array detector (DAD) and TSQ Quantum Access Max triple-quadrupole mass spectrometer (ThermoFisher Scientific, Basel, Switzerland) according to the method previously described by Gašić, et al. (2015). The elution was performed at 40 ºC on a Syncronis C18 column (100 × 2.1 mm, 1.7 µm particle size). The mobile phase consisted of (A) water + 0.1% formic acid (v/v), and (B) 100% acetonitrile (MS grade), which were applied in the following gradient elution: 5% B in the first 2.0 min, 2.0–14.0 min 5–95% B, 14.0–14.2 min from 95% to 5% B, and 5% B until the 20 min. The flow rate

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208 was set to 0.3 mL/min and the detection wavelengths to 254 and 280 nm. The injection volume
209 was 5 µL.

210 A TSQ Quantum Access Max triple-quadrupole mass spectrometer equipped with an heated
211 electrospray ionization (HESI) source was used with the vaporizer temperature kept at 200 °C,
212 and the ion source settings as follows: spray voltage 5000 V, sheet gas (N2) pressure 40 AU, ion
213 sweep gas pressure 1 AU and auxiliary gas (N2) pressure 8 AU, capillary temperature 300 °C,
214 and skimmer offset 0 V. The mass spectrometry data was acquired in the negative mode, in the
215 *m/z* range from 100 to 1000. Multiple mass spectrometric scanning modes, including full
216 scanning (FS), and product ion scanning (PIS), were conducted for the qualitative analysis of the
217 targeted compounds. The collision-induced fragmentation experiments were performed using
218 argon as the collision gas, and the collision energy was varied depending on the compound. The
219 time-selected reaction monitoring (tSRM) experiments for quantitative analysis were performed
220 using two MS^2 fragments for each compound that were previously defined as dominant in the
221 PIS experiments. Xcalibur software (version 2.2) was used for instrument control. The phenolics
222 were identified via direct comparison with commercial standards. The total amounts of each
223 compound were evaluated via calculation of the peak areas, and they are expressed as µg/L.

224 *2.5. Bioaccessibility*

225 To analyse the effect of *in vitro* GID on TPC, TFC, PC and phenolic compounds, the
226 bioaccessibility of polyphenols was presented as the percent recovery (R). This percent recovery
227 allows calculation of the amount of total phenolics, flavonoids, proantocyanidins and each
228 phenolic compound recuperated after GID treatment, through comparison with the total amount
229 in the initial samples.

230 $$Recovery\ (\%) = \frac{PC_{DS}}{PC_{IS}}$$

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where PC_{DS} is the TPC, TFC, PC, the content of each polyphenols and total phenolics in the digested samples and PC_{IS} is the TPC, TFC, PC the content of each polyphenols and total phenolics in the initial samples (SK, SE and FM).

Further, the total percent recovery (TR) of the total phenolic acids, flavonols and phenolics in the digested samples with the food matrix were calculated because the food matrix polyphenols contributed to the initial amount of polyphenols that were subjected to digestion with grape extracts. This value was calculated through comparison of the amount of the specific group of phenolic compounds in the digested samples with the sum of the amount of the specific group of phenolic compounds in the initial grape extracts and initial food matrix.

$$Total\ recovery\ (\%) = \frac{TPC_{DS}}{TPC_{IS} + TPC_{FM}}$$

where TPC_{DS} is the total phenolic acids, total flavonols and total phenolics in the digested samples; TPC_{IS} is the total phenolic acids, total flavonols and total phenolics in the initial samples (SK, SE); and TPC_{FM} is their amount in the initial food matrix (FM).

2.6. UHPLC-MS/MS Orbitrap qualitative analysis of flavan-3-ols and anthocyanins

Separation of anthocyanins, as well as flavan-3-ols, were performed using an ultrahigh-performance liquid chromatography (UHPLC) system that consisted of a quaternary Accela 600 pump and Accela autosampler (ThermoFisher Scientific, Bremen, Germany). The UHPLC system was coupled to a linear ion trap - Orbitrap mass spectrometer (LTQ OrbiTrap MS) that was equipped with a heated electrospray ionization probe (HESI-II, ThermoFisher Scientific, Bremen, Germany) in negative mode for flavan-3-ol analysis and in the positive mode for anthocyanin analysis. The protocol used for anthocyanin analysis has been previously described (Pantelić, et al., 2016). A Syncronis C18 column (100 × 2.1 mm, 1.7 μm particle size) at 40°C

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was used for flavan-3-ol separation, the flow rate was set at 0.250 mL/min, and the mobile phase consisted of (A) water + 0.1% formic acid and (B) acetonitrile. The injection volumes were 5 µl, and the linear gradient program was as follows: 0.0-1.0 min 5% B, 1.0-14.0 min from 5% to 95% (B), 14.0-14.1 min from 95% to 5% (B), and 5% (B) for 6 min.

Parameters of the ion source were as previously described in the literature (Božunović, et al., 2018). The MS spectra were acquired through full-range acquisition covering 100-1000 *m/z*. The resolution was set to 30,000 for full-scan analysis. The data-dependent MS/MS events were always performed on the most intense ions detected in the full-scan MS. The ions of interest were isolated in the ion trap with an isolation width of 5 ppm and were activated with 35% collision energy levels. The settings for dynamic exclusion were as previously described in Pantelić, et al. (2016). The Xcalibur software (version 2.1) was used for the instrument control, data acquisition and data analysis. Flavan-3-ol derivatives were identified on the basis of their monoisotopic mass and MS^4 fragmentation, and confirmed using previously reported MS fragmentation data found in literature (Rockenbach, et al., 2012).

Due to the lack of specific standards, the quantities of the individual flavan-3-ols were expressed as micrograms of catechin equivalents per liter of sample (µg CE/L). The bioaccessibility of flavan-3-ols was presented as the percent recovery as already described, whereas the total flavan-3-ols recovery in samples was calculated as a present of total detected flavan-3-ols in the digested sample to their content in the initial grape seed sample.

2.7. Antioxidant properties

To assess antioxidant activity, three methods were used:

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Ferric reducing power assay (FRP): This assay was conducted using the method described by Medouni-Adrar, et al. (2015). Briefly, an aliquot of diluted samples (250 µL) was mixed with 250 µL of 0.2 M phosphate buffer, pH 6.6 and 250 µL of 1% potassium ferricyanide solution and incubated for 20 min at 50 ˚C. After that, 250 µL of 10% TCA was added, and the mixture was centrifuged at 17000*g* (Sigma 201M Centrifuge, Osterode am Harz, Germany) for 5 min. Then, 500 µL of supernatant were combined with 500 µL of milliQ water and 100 µL of 0.1% ferric chloride. After 10 min, absorbance at 700 nm was measured. Higher absorbance of the reaction mixture indicates a stronger reducing power (Gülçin, Güngör Şat, Beydemir, Elmastaş, & İrfan Küfrevioğlu, 2004).

Ferrous-ion-chelating capacity assay (FCC): The ferrous-ion-chelating capacity was determined according to a previously described method by Stanisavljević, et al. (2015). Briefly, an aliquot of sample (200 µL) was mixed with 740 µL of milliQ water and 20 µL of 2 mM $FeSO_4$. After standing for 30 min at room temperature, 200 µL of 5 mM ferrozine was added in the reaction mixture and 10 min later absorbance was recorded at 562 nm against blank containing milliQ water instead of sample aliquot. The FCC was calculated as follows:

$$Fe^{2+}\ chelating\ capacity\ (\%) = \left\{(A_c - A_s)/A_c\right\} \times 100$$

where A_c represents the absorbance of the blank, and A_s is absorbance of samples.

ABTS radical scavenging activity assay: Evaluation of free-radical scavenging activity was analysed using a previously published method (Arnao, Cano, & Acosta, 2001). The stock solution (7 mM aqueous solution of ABTS (2,2-azino-bis/3-ethil-benothiazoline-6-sulphonic acid) with 2.45 mM potassium persulfate) was allowed to stand in a dark place for 16 h. The working solution of $ABTS^{\cdot+}$ was prepared by diluting the stock solution with methanol to obtain an absorbance between 0.7-0.8 at 734 nm. Thereafter, 10 µL of sample was mixed with 1 mL of

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298 ABTS•+ working solution. After 7 min the absorbance measurement was performed at 734 nm.
299 Percentage of quenched radicals for standard and samples were calculated as:

300 $$ABTS^{\cdot+}\ scavenging\ activity\ (\%) = (Ac - As)/Ac \times 100$$

301 where *Ac* is the absorbance of ABTS•+ working solution, *As* is the absorbance of sample or
302 standard solution mixed with ABTS•+ working solution.
303 Ascorbic acid solutions ranging from 10 to 100 µg/mL were used to create a calibration curve.
304 The free-radical scavenging activity was expressed as the ascorbic-acid equivalent in
305 micrograms of ascorbic acid per mL of sample (µg AAE/ mL).

306 *2.8. Statistical analysis*

307 Statistical analysis was performed using the Statistica software ver 8.0 (StatSoft Co., Tulsa, OK,
308 USA). All experiments were performed at least in duplicate. The results were presented as the
309 mean values ± standard deviation (SD). Student's t-test was used to determine the significance of
310 differences between the means at $p < 0.05$. The correlation analysis between the various
311 antioxidant assays and the phenolic composition and content were performed by calculating
312 Pearson's correlation coefficient (r). Correlations at $p < 0.05$ were considered significant.
313 The limits of detection (LOD) and quantification (LOQ), for LC/MS analysis of non-flavan-3-ols
314 and anthocyanins, were calculated using standard deviations of the responses (SD) and the slopes
315 of the calibration curves (S) according to the formulas: LOD = 3(SD/S) and LOQ = 10(SD/S).
316 The values of standard deviations and slopes were obtained from the calibration curves created in
317 MS Excel. The LOD and LOQ, together with correlation coefficients are presented in
318 Supplementary material (Table S1).
319

320 **3. Results and Discussion**

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3.1. Polyphenol composition of the initial grape skin and seed extracts

The major polyphenols in the initial grape skin extract were phenolic acids (24.4%) and flavonols (65.9%), composing 90.3% of the total polyphenol content (Table 1). A low quantity of anthocyanins (approximately 6%) in SK was not surprising considering that the Prokupac grape variety contains moderate amounts of anthocyanins compared to other red grape varieties (Mitić, Souquet, Obradović, & Mitić, 2012), which is further reduced due to degradation during the drying process (Karasu, et al., 2016). Malvidin-3-*O*-glucoside was the most abundant anthocyanin in grape skin extracts, followed by peonidin-3-*O*-glucoside, which is consistent with previous results (Pantelić, et al., 2016).

In grape seed extract, the dominant non-flavan-3-ol class of polyphenols was phenolic acids (93%), among which ellagic acid was the most abundant, followed by gallic acid (Table 2). The high content of ellagic and gallic acids in the grape seed extract of the Prokupac variety compared to the grape seed extract of other grape varieties was previously observed (Pantelić, et al., 2016). Ellagic acid was also the major phenolic acid in the extract of grape skin, whereas gallic acid was not detected. Among flavonols, quercetin and isorhamnetin were the most abundant in both grape extracts, whereas in the grape skin extract, glycosides of quercetin and isorhamnetin were also detected in significant amount. This is in agreement with published data for several other grape by-product extracts (Pantelić, et al., 2016; Wang, Amigo-Benavent, Mateos, Bravo, & Sarriá, 2017).

UHPLC-Orbitrap MS characterization of flavan-3-ols and masses of the molecular ions ($[M-H]^-$) and MS^2, MS^3 and MS^4 fragment ions of each detected compound are listed in Table 3, together with their retention times. The identified compounds represented four structurally distinct groups: 1) monomeric flavan-3-ols (3 compounds), 2) procyanidin isomers A type (2

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compounds), 3) procyanidin isomers B type (17 compounds) and 4) procyanidin gallate isomers (11 compounds). The content of each detected flavan-3-ol expressed as μg CE/L is presented in Table 4. The procyanidin oligomers, dimers to tetramers, showed higher relative amount (approximately 70%) than the monomers. The most abundant oligomers were procyanidin isomers B type (approximately 50%). The domination of procyanidin isomers B type and oligomers in grape seed extracts was previously observed (Ivanova, Stefova, et al., 2011; Pineda-Vadillo, et al., 2016; Serra, et al., 2009). Although flavan-3-ols have been reported in grape skin extracts (Pantelić, et al., 2016), their presence in the extract of grape skin, as analysed in this study, has not been observed.

It is important to note that the food matrix contains a significant amount of phenolic acid and flavonols, which originated from the constituents of the analysed infant puree: potato, maize and rice (Blessington, Nzaramba, Scheuring, Hale, Reddivari, & Miller Jr, 2010; Thakur, Singh, Kaur, & Singh, 2017; Zaupa, Calani, Del Rio, Brighenti, & Pellegrini, 2015). The most abundant phenolic acids were ellagic, 5-*O*-caffeoylquinic and caffeic acids, whereas quercetin and isorhamnetin were the dominant flavonols (Table 1 and 2). The total relative content of phenolic acids and flavonols was approximately 76% and 9%, respectively, compared to those detected in grape skin and seed extracts. The presence of procyanidin gallate isomers was also registered; however, their relative amount compared to the total amount of flavan-3-ols observed in the grape seed extract was negligible, 0.04% (Table 4).

3.2. Polyphenol composition of digested grape skin and seed extracts without food matrix

Mixing the extracts with digestive fluids and digestion itself differently affected the concentration of major phenolics. Their content in the skin extract increased (protocatehuic acid), decreased (quercetin, quercetin-3-*O*-galactoside, isorhamnetin, malvidin-3-*O*-glucoside and

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taxifolin) or remained unchanged (5-*O*-caffeoylquinic acid, ellagic acid, ferulic acid, rutin and isorhamnetin-3-*O*-glucoside). Similar changes were observed in the content of polyphenols extracted from grape seed; however, recovery of the major class of polyphenols significantly differs compared to grape skin extract, especially for phenolic acids (133.5% against 43.5% recovery from grape skin and seed, respectively). This resulted in almost two times lower recovery of all detected non-flavan-3-ol polyphenols that were extracted from grape seed compared to that for grape skin (44.7% against 84.6%, respectively). The main reason was the liberation of significant amount of *p*-coumaric and caffeic acids upon the mixture and digestion of grape skin polyphenol extract with digestive cocktails. It is known that these two acids are the most abundant phenolic acids, which form anthocyanin derivatives in grapes (Ivanova, Stefova, et al., 2011; Mitić, Souquet, Obradović, & Mitić, 2012; Pantelić, et al., 2016) from which the liberation of phenolic acid probably occurs. It was found that peonidin-3-*p*-coumaroylmonoglucoside and malvidin-3-*p*-coumaroylmonoglucoside were the most abundant among *p*-coumaroylmonoglucosides in the wine grape variety Prokupac (Mitić, Souquet, Obradović, & Mitić, 2012). The malvidin-3-*O*-caffeoylhexoside was also found in Prokupac (Pantelić, et al., 2016), whereas peonidin-3-*O*-caffeoylglucoside and malvidin-3-*O*-coumaroylglucoside-5-*O*-glucoside were registered in grapes (He, et al., 2010). These acylated anthocyanins were not detected in the initial grape skin polyphenol extract probably due to their reduced polarity compared to monoglucosides (Ivanova, Dörnyei, et al., 2011). Namely, due to the weaker solubility in milliQ water, these compounds were removed from the initial skin polyphenol extract by the filtration through 0.45μm filter before UHPLC-MS/MS analysis. On the other hand, in digestion study, the whole extract was used for the experiment, thus the acylated anthocyanins were present in the reaction mixture. After the addition of digestive

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cocktails, composed of different substances (digestive enzymes, salts, HCl), to the skin polyphenol extract, the deacylation of anthocyanidin mono- and diglucosides occurred resulting in the appearance of *p*-coumaric and caffeic acids as well as malvidin-3,5-di-*O*-glucoside in the DSKC and DSK samples. It was reported that the acetylated anthocyanins is more prone to degradation than anthocyanidin glycosides (Howard, Brownmiller, Mauromoustakos, & Prior, 2016). However, the amount of monoglucosides was not increased in these samples due to their instability in such environment (Sharma, Gupta, Singh, Bansal, & Singh, 2016). The liberation of minor phenolics: luteolin, genistein and pterostilben upon the mixture and digestion of grape skin polyphenol extract with digestive cocktails was also observed. Cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and cyanidin-3,5-di-*O*-glucoside were not detected in the initial grape skin extract. Digestion significantly reduced the content of total anthocyanins to approximately 29% recovery. Low anthocyanin stability after *in vitro* digestion was also observed by other researchers (Pineda-Vadillo, et al., 2016; Wang, Amigo-Benavent, Mateos, Bravo, & Sarriá, 2017). Digestion of the food matrix resulted in 73.2% recovery of phenolic acids, following a similar behaviour as during the digestion of phenolic acids from grape skin extracts.

It was found that flavonols, rutin and quercetin were stable, while isorhamnetin was not recovered after *in vitro* digestion of grape extracts and food matrix; however, it is known that the digestive stability of isorhamnetin-3-*O*-glucoside and quercetin-3-*O*-galactoside was dependent on origin. Recovery from grape skin extract was 100.1% and 79.7%, respectively, whereas recovery of those flavonols from grape seed extract was not observed (Table 2). Antunes-Ricardo, Rodríguez-Rodríguez, Gutiérrez-Uribe, Cepeda-Cañedo, and Serna-Saldívar (2017) have previously reported that the recoveries of isorhamnetin after oral and gastric digestion of

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413 *Opuntia ficus-indica* extract, was lower compared with that for its glycosides. However, Wang,
414 Amigo-Benavent, Mateos, Bravo, and Sarriá (2017) obtained similar recoveries for isorhamnetin
415 aglycone and isorhamnetin-3-*O*-glucoside after digestion of grape pomace extract. It appears that
416 the recovery of flavonoid glycosides after digestion depends on other polyphenols present in the
417 analysed extracts, which could have protective effects on them. The total flavonol recovery from
418 grape skin extract after GID was 69%, which is in accordance with the results obtained by Wang,
419 Amigo-Benavent, Mateos, Bravo, and Sarriá (2017) who estimated 65% flavonol recovery from
420 red grape pomace after GID.
421 The addition of digestive fluids exhibited the most significant effect on total flavan-3-ol recovery
422 of grape seed extract. Only 0.31% of the total detected flavan-3-ols were recovered after addition
423 of digestive fluids, and digestion further decreased their recovery to 0.23% (Table 4). It is
424 obvious that the strong binding capacity of flavan-3-ols to digestive enzymes has been
425 established. A considerable loss of flavan-3-ols was also observed by other authors due to their
426 interaction with proteins/enzymes (Pineda-Vadillo, et al., 2016; Serra, et al., 2009).
427 *3.3. Polyphenol composition of digested grape skin and seed extracts with food matrix*
428 The addition of the food matrix to the grape skin extract did not significantly affect the recovery
429 of the major grape skin polyphenols, compared to their digestion without food matrix (Table 1).
430 However, recovery was calculated as the ratio of their content in DSK/FM to their content in SK,
431 without taking into account the amount of polyphenols added by the food matrix. In that case, the
432 total recovery of the major classes was significantly lower (68.1% instead of 125.2% for
433 phenolic acids and 36.7% instead of 62.9% for flavonols). A significant effect of FM was also
434 observed on anthocyanin recovery. FM additionally decreased the stability of anthocyanins,
435 which resulted in the disappearance of malvidin-3-*O*-glucoside and the release of malvidin-3,5-

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436 di-*O*-glucoside to a lower extent in the final digest compared to that in DSK, implicated to be
437 10.2% recovery of anthocyanins. Similar results have been reported upon addition of complex
438 FM consisting of meat proteins, carbohydrates and lipids to chokeberry juice, in which reduction
439 of the anthocyanin content was up to 91% (Stanisavljević, et al., 2015). According to the
440 investigation performed by Pineda-Vadillo, et al. (2016) and Karakaya, et al. (2016)
441 bioaccessibility of anthocyanins was significantly influenced by the FM type (39-60%);
442 however, in all formulations (which does not contain meat) the presence of the food matrix had a
443 protective effect on anthocyanin degradation, especially in egg-based products. These results
444 suggested that the significant decrease of anthocyanin content in grape skin extract upon addition
445 of the food matrix in this study was probably a result of interactions with meat proteins or other
446 meat constituents. It has been previously described that polyphenols interact with milk and meat
447 proteins, modulating their content, bioaccessibility and functional properties (Jakobek, 2015;
448 Ozdal, Capanoglu, & Altay, 2013). The final polyphenol recovery of the grape skin extract was
449 44.1% instead of 74.8%, as calculated without FM polyphenols. These data highlighted the
450 importance of determining the polyphenol composition of non-naturally polyphenol-rich food
451 matrices, which could significantly influence the calculation of the bioaccessibility of
452 polyphenols that are present in the samples before digestion.
453 The influence of food matrix on the recovery of phenolic acids present in grape seed extract was
454 less pronounced than that for grape skin extract due to the significantly lower total phenolic-acid
455 content in the FM than in the SE; however, in relation to specific phenolic acids, the FM
456 increased the recovery of gallic acid. The flavonols almost disappeared after addition of the FM,
457 and recovery was reduced to less than 1% in DSE/FM due to the total reduction of quercetin in
458 the final digest, which is completely different from DSK/FM (quercetin total recovery was

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459 49.5%) or its recovery after digestion without the FM (DSE, 99.7%). Interestingly, the
460 disappearance of quercetin occurred before digestion after addition of the FM and digestive
461 fluids. These facts indicated that the difference in stability of quercetin in the presence of a
462 complex food matrix depended on the other polyphenol compounds present in the extracts. The
463 major difference between grape seed and skin extracts was in the content of flavan-3-ol and
464 ellagic acid. The flavan-3-ol content was reduced significantly after addition of digestive fluids
465 in both cases with or without the FM; however, the reduction in ellagic acid content in the
466 presence of the FM was approximately ten folds of the initial content in SE, whereas without the
467 FM it was 1.4-fold. A possible reason could be that ellagic acid probably interacted with FM
468 compounds under this condition; thus, the capability of ellagic acid or/and FM compounds to
469 protect quercetin during digestion was reduced. It has been demonstrated that quercetin is less
470 stable at a higher pH, and its recovery depended on the food matrix: onion, 52.5% (Boyer,
471 Brown, & Liu, 2005), persimmon flours, 0% (Lucas-González, Viuda-Martos, Pérez Álvarez, &
472 Fernández-López, 2018), red grape pomace, 54.5% (Wang, Amigo-Benavent, Mateos, Bravo, &
473 Sarriá, 2017). The final non-flavan-3-ol recovery was 50.9% (calculated with FM polyphenols)
474 or 55.5% (calculated without FM polyphenols).
475 On other hand, the FM protected flavan-3-ols during digestion and increased their recovery
476 approximately twenty times compared to DSE, which increased the recovery from 0.23% to
477 4.71%. Poor bioaccesibility of flavan-3-ols is expected because it is known that
478 proanthocyanidins strongly interact with digestive enzymes and food components (protein,
479 carbohydrates and lipids), and the interactions enhanced the increased degree of polymerization
480 (Jakobek, 2015; Sugiyama, et al., 2007). According to Pineda-Vadillo, et al. (2016), their
481 bioaccessibility could increase with digestion depending on the food matrix; however, in most

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cases, most proanthocyanidins remained insoluble at the end of digestion. Similar results were obtained by Serra, et al. (2009), who found that less than 1% of soluble proantocyanidins were present after digestion of grape seed procyanidin extract without and with carbohydrate-rich food.

3.4 Total polyphenol content

Some of the previous *in vitro* studies have investigated the total polyphenol recovery following *in vitro* digestion of grapes and grape extracts using the Folin-Ciocalteu method (Wang, Amigo-Benavent, Mateos, Bravo, & Sarriá, 2017). Keeping in mind the limitations of the Folin-Ciocalteu (FC) assay, the obtained data should be always interpreted with great caution, especially in situations where aside from the studied extract or compound, the system contains a complex food matrix. It has been determined previously that the FC reagent can be non-specifically reduced by ascorbate, reducing sugars, aromatic amines, organic acids, fatty acids and Fe^{2+} ions, as well as by proteins and small peptides that are formed during digestion of food proteins (Prior, Wu, & Schaich, 2005). Comparing the results of TPC obtained in the present study (Fig. 1a) for SK, 131.7 mg GAE/100 mL, with SKC (contains grape skin extract and whole digestive cocktail at zero time of digestion), 230.8 mg GAE/100 ml, it can be concluded that digestive enzymes and fluids contribute to the total polyphenol content with approximately 100 mg GAE/100 mL. A similar situation was observed in the case of the food matrix (FM/FMC), where the joint contribution of the digestive cocktail was even more pronounced (approximately 150 mg GAE/100 mL). It is evident that the digestive cocktail itself contained a considerable amount of the FC reagent reactive substance, which resulted in the elevation of TPC. On the other hand, a totally opposite behaviour was recorded upon mixing the grape seed extract with digestive fluids, where a 40% decrease in TPC was observed, reaching the similar value of TPC

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that was obtained for the control sample for the grape skin extract (SKC). This could be explained by the difference in composition of the grape seed extract, which contains a considerable amount of flavan-3-ols that are at the same time strongly reactive towards the components in the digestive cocktail; thus, the flavan-3-ols and the FC reagent reactive substances of the digestive cocktails could not be oxidized by a mixture of tungstate and molybdate, which resulted in decreased TPC. The TPC values of grape skin and seed extracts remained unchanged after digestion. On the other hand, digestion of the food matrix alone resulted in an increase of TPC by almost 35% (FMC compared to DFM); however, the phenolic composition did not significantly change. This indicated that FC reactive substances were released during digestion of the food matrix such as small peptides, amino acids, reducing sugars and fatty acids.

The addition of the FM in the digestive cocktail at zero time together with grape extract did not change the TPC values, indicating far lower contribution of food matrix than the digestive cocktail to the overall phenolic content; this effect might potentially be of great importance, because the contribution of digestive fluids to TPC in these types of studies is usually overlooked. However, combined digestions of the matrix and extracts have led to 39% and 30% increase of TPC in the grape skin and seed samples, respectively, compared to that in the control samples and digested samples of skin, seed and FM alone. A major consequence of polyphenol-digestive enzyme interactions is the inhibition of their activity (Cirkovic Velickovic & Stanic-Vucinic, 2018); considering this, it could be expected that digested grape skin/seed extracts with FM samples contained, besides amino acids, reducing sugars and fatty acids, the products of partial hydrolysis of macromolecules such as peptides and oligosaccharides, which could act as reducing agents. Furthermore, liberation of polyphenols that were naturally present in the food

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matrix and grape samples after digestion, as well as grape seed polyphenols that were initially captured by macromolecules of the FM and digestive enzymes, released and additionally contributed to the increase of TPC. These phenomena implied a 275% and 75% recovery of the initial TPC in SK and SE, respectively. Numerous studies performed on the effect of protein-polyphenol interactions on the total polyphenol content of foods outlined the significantly reduced recovery of polyphenols (Ozdal, Capanoglu, & Altay, 2013), especially, when the main polyphenols are flavan-3-ols, for which the TPC recovery observed was 25% in the soluble phase or even less (Pineda-Vadillo, et al., 2016). Higher polyphenol recovery from grape skin and seed extracts after digestion with FM in this study, compared to the literature data, could be attributed to the significant reducing properties of food matrix constituents, probably meat proteins and their hydrolysates, which have been previously estimated to possess reductive power (Elias, Kellerby, & Decker, 2008; Serpen, Gökmen, & Fogliano, 2012). These data suggested that TPC reflected rather total reducing activity of analysed samples, which is frequently considered as antioxidant activity, than the total polyphenol content.

3.5. Total flavonoid content

The spectrophotometric assay based on aluminium-chloride complex formation is one of the most commonly applied analytical procedures for determination of the total flavonoid content in food and medical plant samples, which is well reviewed by Pękal and Pyrzynska (2014). There are two commonly applied procedures (with or without $NaNO_2$); however, both procedures are dependent on the type of flavonoid present in the sample and are specific only for a limited number of flavonoid compounds (Pękal & Pyrzynska, 2014). The procedure in the presence of $NaNO_2$ in alkaline medium is widely used for the estimation of total flavonoid content in grape and grape by-products (Ivanova, Stefova, et al., 2011; Ribeiro, Ribani, Francisco, Soares,

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Pontarolo, & Haminiuk, 2015) using catechin as standard. Considering that this procedure is specific for catechins, rutin and luteolin among flavonoids and that the phenolic acids can also exhibit considerable absorbance at 510 nm (Pękal & Pyrzynska, 2014), the obtained results should be commented from this point of view.

For the food matrix and grape skin extract the total flavonoids were detected in a low quantity in all analysed samples (Fig. 1b). The main reason was that the most abundant flavonoid quercetin did not contribute to the total absorbance at 510 nm. Rutin and phenolic acids probably mostly participated in the determination of TFC, which showed good stability during digestion experiments, but significant correlations were not found among them at $p < 0.05$.

On the other hand, the TFC of the grape seed extract was detected in a high amount (121.8 ± 0.71 mg/100 ml), indicating the presence of catechins in a significant quantity. After addition of digestive cocktails and digestion with or without the FM, this content reduced significantly following changes in flavan-3-ol composition and recoveries before and after digestion ($r = 0.98$, for total flavan-3-ols, monomeric flavan-3-ols, procyanidin isomers type B and procyanidin gallate isomers). The final recovery of TFC from grape seed after digestion with FM, 33.1%, was higher than expected, reflecting the influence of phenolic acids on the overall determination of TFC via the applied method.

3.6. Proanthocyanidin content

As shown in Fig. 1c, a small amount of proantocyanidins was detected in the grape skin extract; however, upon mixing with the digestive cocktail their content was not detected, neither before nor after digestion with or without FM. In the food matrix alone, their presence was not detected; however, UHPLC-Orbitrap MS analysis determined procyanidin gallate isomers. It is possible that their content was under the limit of detection for the applied spectrophotometric method.

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574 The PC content of the initial grape seed extract was 261.27±8.59 mg/100 ml, which was
575 significantly reduced to 26% recovery in the DSE sample. After mixing with the food matrix, the
576 reduction of their content was even more pronounced, declining to only 10% recovery. The
577 obtained results were in good agreement with the UHPLC-Orbitrap MS analysis of flavan-3-ols
578 ($r = 0.96$, for total flavan-3-ols, monomeric flavan-3-ols, procyanidin isomers type B and
579 procyanidin gallate isomers). These results confirmed the strong capacity of flavan-3-ols to
580 interact with enzymes and food matrix compounds. It was also observed by other researchers that
581 proantocyanidins are more prone, than other polyphenol classes, to bind with food components,
582 which results in the formation of insoluble aggregates during digestion (Pineda-Vadillo, et al.,
583 2016)
584
585 *3.7. Antioxidant properties*
586 The second part of the study focused on the evaluation of the effect of the food matrix on the
587 antioxidant capacity of grape skin and seed extracts during *in vitro* digestion. Antioxidant
588 properties of natural antioxidants cannot be evaluated through only a single method due to their
589 multi-functionality (Gülçin, Elİas, Gepdİremen, Taoubİ, & Köksal, 2009). Several antioxidant
590 assays are required to evaluate different aspects of their functionality, because each assay
591 involves different chemical mechanisms of action (Serpen, Gökmen, & Fogliano, 2012). The
592 methods for measuring antioxidant capacity are basically classified into two groups, depending
593 on the reaction mechanism: methods based on the antioxidant ability to quench free radicals via
594 hydrogen donation (HAT-based methods) and based on single-electron-transfer mechanism
595 (SET-based methods) (Prior, Wu, & Schaich, 2005). A widely used method based on electron
596 transfer is the ferric-reducing-power method, whereas quencher ability is commonly measured

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by ABTS radical scavenging activity based on both HAT and SET mechanisms (Prior, Wu, & Schaich, 2005). Further, it is known that metal ions, such as ferrous, can act as pro-oxidants for lipid oxidation; thus, the ability of substances to chelate iron can be valuable for estimating their antioxidant activity.

3.7.1. Ferric reducing power

Ferric reducing power is based on the antioxidant ability to reduce the Fe^{3+}/ferricyanide complex to the ferrous form by donating an electron. The Fe^{2+} ion was than monitored by measuring absorbance of Perl's Prussian blue complex at 700 nm (Gülçin, Bursal, Şehitoğlu, Bilsel, & Gören, 2010). The highest and similar values of FRP were recorded for grape skin and seed extracts, whereas the food matrix exhibited approximately a 50% lower value (Fig. 2a). Addition of digestive fluids with or without FM to grape extracts demonstrated a similar outcome, a decrease in FRP of approximately 25%. The digestion of both extracts alone showed no significant changes in FRP; however, digestion in the presence of the FM exhibited a different impact on FRP. FRP was slightly elevated for DSK/FM, reaching 85% of the initial FRP value for skin extract; no significant changes were observed for the FRP of DSE/FM, and the remaining FRP was 75% of the initial SE value. Similar results were observed by Stanisavljević, et al. (2015) who reported that FRP of chokeberry juice significantly decreased after mixing the juice with digestive fluids and food matrix and slightly increased after digestion reaching about 61% of initial FRP.

Correlation between the total flavonoid content and FRP of the grape skin and seed extracts was very high ($r = 0.94$, for skin $r = 0.98$ for seed). Correlation analysis also revealed a significant positive correlation between the FRP of the grape seed extract and PC content ($r = 0.96$), total flavan-3-ols ($r = 0.95$), monomeric flavan-3-ols ($r = 0.95$) procyanidin isomers type B ($r = 0.96$)

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and procyanidin gallate isomers ($r = 0.95$). Considering that the total flavonoid content of the skin extract actually reflected the content of rutin and phenolic acids, the obtained data indicated that those compounds were the major antioxidants with ferric-reducing ability in the skin extract. In the grape seed extract, flavan-3-ols exerted this ability, showing similar patterns of behaviour. Although the content of phenolic acids and FRP of the grape seed extract was not significantly correlated, their contribution to FRP should also be taking into account due to their high content in the seed extract and digested samples.

The reducing ability of grape by-products before and upon GID was also observed by other authors, but direct comparison is not possible due to different measure units and methods used (Pineda-Vadillo, et al., 2016; Wang, Amigo-Benavent, Mateos, Bravo, & Sarriá, 2017). In these studies the ferric reducing antioxidant power (FRAP) was applied to measure reducing ability of samples. However, the reduction or similar FRAP values to the initial ones were observed upon GID.

3.7.2. ABTS radical-scavenging activity

As presented in Fig. 2b, the ABTS radical-scavenging activity of the skin extract was minor and that for the food matrix was under the quantification limit, while the grape seed extract showed a considerable activity of 61.56 µg AAE/mL. The addition of digestive fluids yielded a significant increase of $ABTS^{\bullet+}$ scavenging activity (approximately five times for skin extract and 1.3 times for seed extract) and similar results for the digestion control samples, without a significant difference between them. After digestion of both grape extracts without food matrix, the $ABTS^{\bullet+}$ scavenging activity was not changed. This could indicate that 1) digestive fluids liberated compounds from the grape extracts with quencher ability of ABTS radical; 2) digestive cocktail contained substances with $ABTS^{\bullet+}$ scavenging activity. The liberated phenolic acids such as *p*-

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coumaric, caffeic and protocatechuic acids from the grape skin extract and caffeic acid from the grape seed extract were probably mostly contributed to their antioxidant activity. Concerning food matrix, it has been reported that meat, zein and potato proteins have important ability to scavenge ABTS radical which can be increased by their partial denaturation after addition of digestive cocktails (Elias, Kellerby, & Decker, 2008; Serpen, Gökmen, & Fogliano, 2012). Enzymes probably mostly contributed to $ABTS^{\bullet+}$ scavenging activity of digestive cocktails. On the other hand, digestion significantly elevated the scavenging activity of the food matrix and both grape extracts in the presence of the food matrix. All three digested samples showed a high ability to quench the ABTS radical (102.85 ± 0.55 µg AAE/ mL for food matrix, 98.29 ± 8.91 µg AAE/ mL for grape skin extract, 99.84 ± 0.14 µg AAE/ mL for grape seed extract). The obtained results clearly indicated that, only in the presence of the food matrix during digestion, the radical scavenging capacity could be increased; however, increase in the scavenging activity was not the same among the samples. The highest increase was recorded in the grape skin extract (34%), than that in the food matrix (25%), and the lowest increase was determined in the grape seed extract (13%). It appears that the release of food matrix components had a major influence on the $ABTS^{\bullet+}$ radical scavenging, which was improved by grape skin extract polyphenols (flavonols and phenolic acids) or reduced by grape seed extract polyphenols (flavan-3-ols). This observation was confirmed via correlation analysis. A high positive correlation was found between the total polyphenol content and $ABTS^{\bullet+}$ scavenging activity ($r = 0.92$) for grape skin extract, whereas the $ABTS^{\bullet+}$ scavenging activity of the grape seed extract showed a highly negative correlation with the total PC ($r = -0.94$), total flavan-3-ols ($r = -0.91$), monomeric flavan-3-ols ($r = -0.91$) procyanidin isomers type B ($r = -0.92$) and procyanidin gallate isomers ($r = -0.90$).

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Different components of the food matrix could be responsible for the increased ABTS radical-scavenging activity of the samples: 1) liberated peptides of digested meat, zein and potato proteins, which had been demonstrated to have increased antioxidative capacity compared to the initial proteins (Elias, Kellerby, & Decker, 2008); 2) liberation of histidine-containing dipeptide such as carnosine, which have high antioxidant activity, as well as solubilization of tocopherols, which are powerful antioxidants present in meat (Chan, Decker, & Feustman, 1994). Synergistic effects of the grape skin extract with FM compounds could be probably attributed to the liberation of hydroxycinnamic acids (caffeic and *p*-coumaric) from the grape skin extract during digestion, for which a high total antioxidant activity has been established (Rice-Evans, Miller, & Paganga, 1996). The high binding capacity of flavan-3-ols with food matrix components in the final digest evidently reduced their $ABTS^{\bullet+}$ scavenging capacity. However, the $ABTS^{\bullet+}$ scavenging activity of the digested grape skin and seed extracts increased for 36% and 10% in the presence of food matrix. Other studies provided information supporting the radical scavenging activity of polyphenols determined with different methods and measure units used (DPPH, $ABTS^{\bullet+}$); however, the significant decrease of radical quenching ability upon GID was observed (Lucas-González, Viuda-Martos, Pérez Álvarez, & Fernández-López, 2018; Stanisavljević, et al., 2015; Wang, Amigo-Benavent, Mateos, Bravo, & Sarriá, 2017).

3.7.3. Ferrous-ion-chelating capacity

Fe^{2+} induces lipid peroxidation mainly through the Fenton reaction or by accelerating the transformation of lipid hydroperoxides to the respective radicals (Gülçin, 2012). In meat, iron can be liberated during cooking and digestion (Vieira, Zhang, & Decker, 2017). Thus, the presence of chelating agents in the grape skin and seed extracts is substantial for the reduction of free transitional metals and their protective effect against oxidative stress of biomolecules. The

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capacity of samples to chelate ferrous ion is presented in Fig. 2c. Considering the results obtained, it can be concluded that neither the grape skin extract nor the grape seed extract are potent Fe^{2+} chelators. The food matrix alone, on the other hand, possessed a considerable FCC of 32.4%. It is evident that the majority of the chelating capacity originates from digestive fluids and the food matrix; however, surprisingly, the mixture of grape skin extract, seed extract and FM with digestive fluids exhibited very similar values (94.6%, 90.1%, and 90.6%, respectively). Digestion did not exhibit any significant effect on FCC; however, the samples of grape skin extract digested with or without the matrix demonstrated a higher ability to bind Fe^{2+} than those of seed extracts. The low chelating capacity and significant increase after addition of digestive fluids and food matrix to chokeberry juice before digestion was also observed by Stanisavljević, et al. (2015); however after digestion mildly increase of this value was observed.

The correlation analysis revealed that the FCC of the skin extract demonstrated a strong positive correlation with the ABTS radical-scavenging capacity ($r = 0.93$) and a strong negative correlation with the total flavonoid content ($r = -0.91$) and ferric-reducing power ($r = -0.94$). On the other hand, the FCC of the grape seed extract was strongly affected by the presence of total flavan-3-ols and their composition. The increase in procyanidin content ($r = -0.96$), total flavan-3-ols ($r = -1$), monomers ($r = -0.99$), procyanidin isomers type B ($r = -1$) and gallate isomers ($r = -0.99$) resulted in a decrease in FCC. The negative correlation was also observed between FCC and FRP ($r = -0.96$), TPC ($r = -0.91$) and TFC ($r = -0.98$). In contrast, the ABTS radical-scavenging activity and FCC exhibited a strong positive correlation ($r = 0.92$).

It can be concluded that the chelating capacity is considerably more than simple sum of matrix and digestive fluid capacities and is considerably affected by the presence of grape extracts, which tends to reduce it. Procyanidins of the grape seed extract exerted greater inhibition effects

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on FCC of digestive cocktails and FM compounds than phenolic acids and flavonoids of the grape skin extract. The inhibition effects were probably a result of complex protein-phenolic interactions, which were more pronounced with catechins than phenolic acids and flavonols. Further, considering that polyphenols were able to reduce Fe^{3+} to Fe^{2+}, they can increase the amount of Fe^{2+} in the reaction mixture upon digestion, resulting in an additional decrease in FCC following digestion. Thus, strong metal chelators from the food matrix such as peptides and carnosine (Chan, Decker, & Feustman, 1994; Elias, Kellerby, & Decker, 2008), which also scavenge free radicals, probably contributed the most to the FCC of the digested samples.

Decreased, increased or unchanged antioxidant activity of the digested polyphenols with or without food matrix was measured via different methods that were reported in the literature (Ozdal, Capanoglu, & Altay, 2013; Pineda-Vadillo, et al., 2016; Wang, Amigo-Benavent, Mateos, Bravo, & Sarriá, 2017); the results indicated that the type of polyphenols and the food matrix subjected to digestion strongly influenced the final antioxidant properties.

Conclusions

In summary, under conditions of simulated gastrointestinal digestion, grape skin and seed extracts exhibited different patterns of behaviour, which is most likely conditioned by the different composition of the polyphenolic compounds present in the extracts. The major polyphenols in the initial grape skin and seed extracts were phenolic acids and flavonols, and phenolic acids and flavan-3-ols, respectively. Recovery of total non-flavan-3-ol phenolics extracted from grape seed was almost two times lower after digestion compared to that for grape skin extract, which could be explained by the release of hydroxycinnamic acids (caffeic and *p*-coumaric) from the grape skin extract. A considerable loss of flavan-3-ols to only 0.23% total recovery was recorded upon digestion of the grape seed extract due to their strong binding

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735 capacity to digestive cocktail compounds which was also confirmed by the significant reduction
736 of proanthocyanidin content. The addition of the food matrix to the grape skin extract did not
737 significantly affect the recovery of the major grape skin polyphenols, compared to their digestion
738 without food matrix, but the total recovery of the major classes was significantly lower due to the
739 significant contribution of the food matrix polyphenols to the total amount of phenolics in the
740 mixture before digestion. On the other hand, the addition of FM to the grape seed extract
741 increased both, the total recovery of flavan-3-ols and non-flavan-3-ol polyphenols compared to
742 their digestion without food matrix which was in accordance with the TFC recovery. *In vitro*
743 digestion significantly elevated total phenolic content of the grape extracts after digestion with
744 FM compared to the digestion control, which could be attributed aside from the liberated
745 phenolic compounds, reducing sugars and amino acids, to the significant reducing properties of
746 other food matrix constituents such as meat proteins hydrolysates. The significant influence of
747 food matrix compounds to antioxidant properties of the grape extracts was also observed: the
748 ferric reducing power decreased, while ferrous chelating capacity and ABTS quenching ability
749 significantly increased. The meat protein hydrolysates and carnosine probably mostly attributed
750 to the improved $ABTS^{\bullet+}$ radical scavenging activity and ferrous-ion chelating capacity. The
751 increase of antioxidant activities of digested grape extracts in the presence of food matrix was
752 more pronounced in the grape skin extract than in the grape seed extract. The main reason was
753 the high binding capacity of flavan-3-ols with food matrix and digestive fluids components
754 which possess antioxidant activities.
755 An important feature observed in the present study, which should not be ignored, is the
756 significant contribution of digestive fluids and food matrix compounds to overall polyphenolic
757 content and antioxidant activities. This result should be extensively studied in the future because

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in vitro models are being more extensively exploited in investigating the fate of specific food components. The present study suggests that enrichment of meat- and cereal-based products with grape polyphenol extracts could be a good strategy to formulate a healthier diet. However, further elaborative studies are needed, especially, to elucidate how this approach reflects on the formation of lipid hydroperoxides, advanced lipoxidation end-products during GID and digestion of food components. The balance between the inhibitory effects of polyphenols on the digestion of food components and their beneficial antioxidant effects should be considered in developing new functional food products.

Acknowledgement

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941 **Figure captions**

942 **Fig. 1**. Total polyphenol content (a), total flavonoid content (b) and proanthocyanidin content (c)
943 of analysed samples. Bars followed by the same lower case letters are not significantly different
944 ($p > 0.05$). Abbreviations: **FM-** diluted food matrix; **FMC-** food matrix control; **DFM-** digested
945 food matrix; **SK-** diluted grape skin extract; **SKC-** grape skin digestion control; **DSK-** digested
946 grape skin extract; **SK/FMC-** skin extract with food matrix control; **DSK/FM-** digested skin
947 extract with food matrix; **SE-** diluted grape seed extract; **SEC-** grape seed extract control; **DSE-**
948 digested grape seed extract; **SE/FMC-** grape seed extract with food matrix control; **DSE/FM-**
949 digested grape seed extract with food matrix.

950 **Fig. 2**. Ferric-reducing power (a), ABTS radical-scavenging activity (b), Ferrous-ion-chelating
951 capacity (c) of analysed samples. Bars followed by the same lower case letters are not
952 significantly different ($p > 0.05$). Abbreviations: **FM-** diluted food matrix; **FMC-** food matrix
953 control; **DFM-** digested food matrix; **SK-** diluted grape skin extract; **SKC-** grape skin digestion
954 control; **DSK-** digested grape skin extract; **SK/FMC-** skin extract with food matrix control;
955 **DSK/FM-** digested skin extract with food matrix; **SE-** diluted grape seed extract; **SEC-** grape
956 seed extract control; **DSE-** digested grape seed extract; **SE/FMC-** grape seed extract with food
957 matrix control; **DSE/FM-** digested grape seed extract with food matrix.

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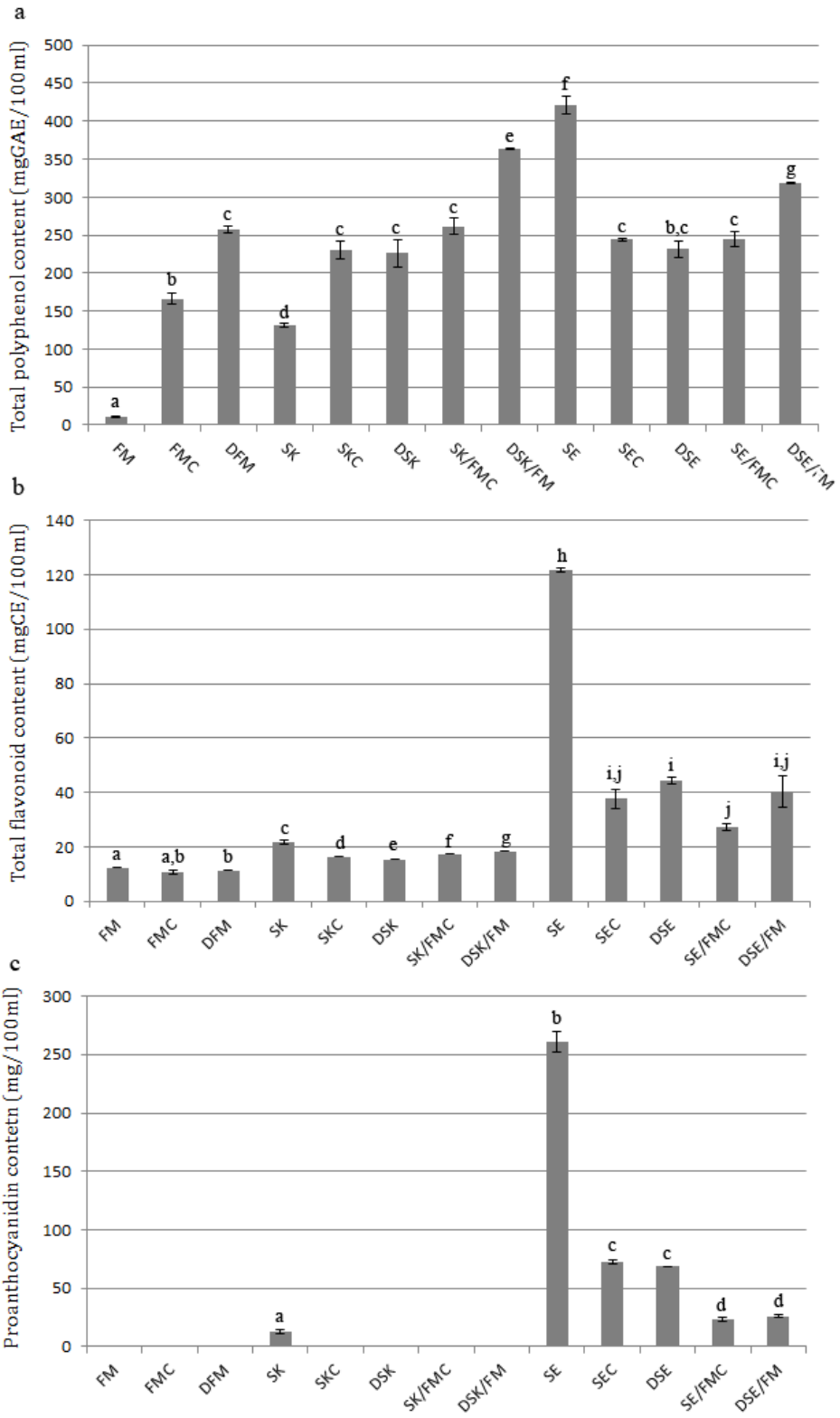

Figure 1.

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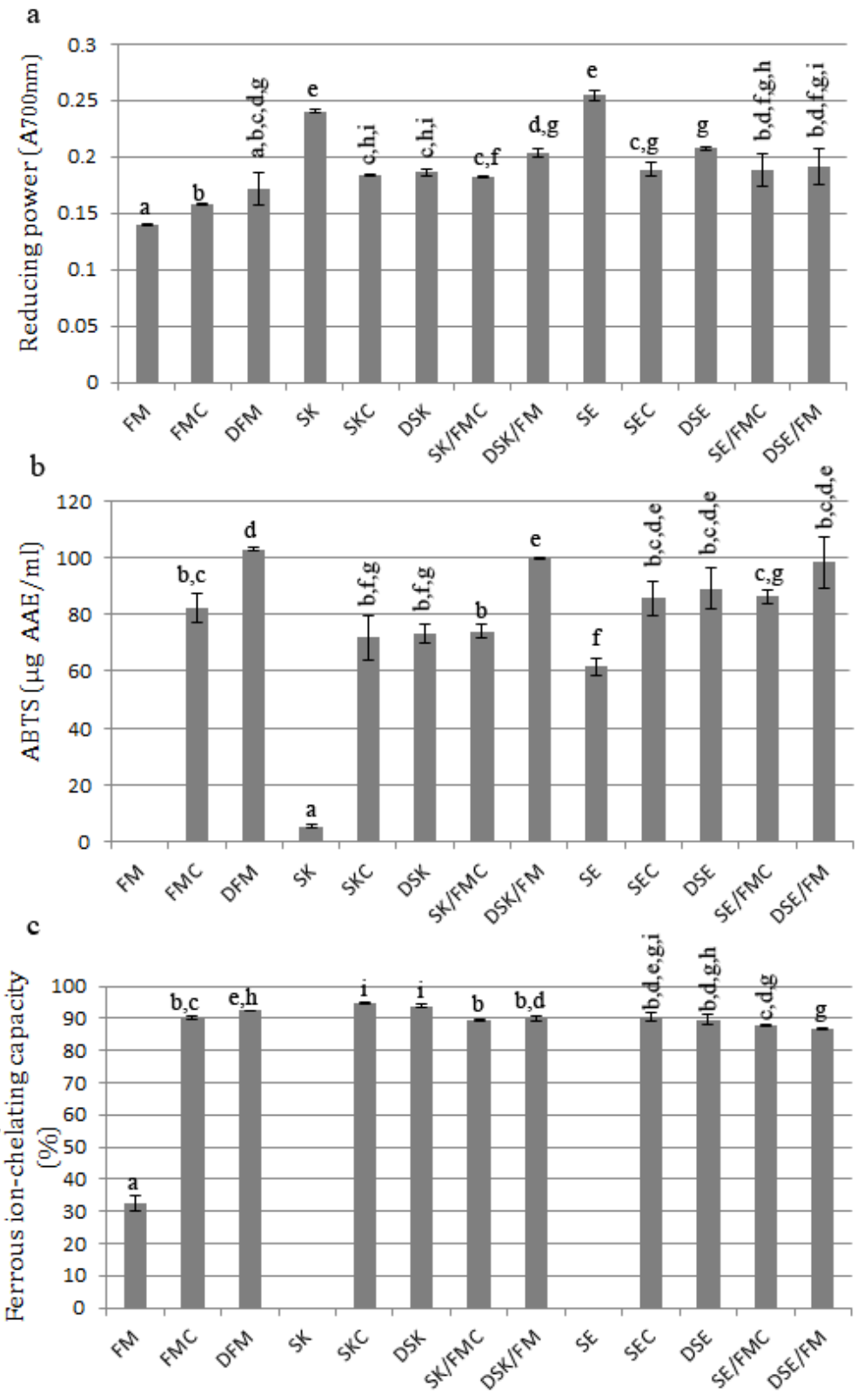

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962 **Figure 2.**

963

964 **Table 1**.

965 The content of polyphenols in the undigested and digested samples of the grape skin (results are

966 expresed as μg/L)*.

Phenolic compounds	**SK**	**SKC**	**DSK**	**DSKR, %**	**FM**	**FMC**	**DFM**	**DFMR, %**	**SK/FMC**	**DSK/FM**	**DSK/FMR,% (DSK/FMTR,%)**
Phenolic acids											
Protocatechuic acid	45.85±0.44[a]	43.46±4.06[a]	56.40±0.69[b]	123.0	n.d.	22.28±0.22[c]	n.d.	-	47.66±4.40[ab]	n.d.	0
Ellagic acid	532±5[ad]	491±76[acd]	442±5[abc]	83.0	408±48[abc]	284±70[bc]	354±1[c]	86.7	617±17[d]	401±42[ac]	75.4
5-*O*-caffeoylquinic acid	48.23±1.12[a]	n.d.	56.34±8.74[abc]	116.8	79.82±14.67[abc]	50.05±1.92[ab]	52.53±0.32[b]	65.8	59.18±0.50[c]	49.30±0.25[a]	102.2
Ferulic acid	91.44±0.46[a]	70.23±0.29[b]	91.54±22.75[abc]	100.0	n.d.	n.d.	n.d.	-	138.26±21.58[ac]	129.13±6.89[c]	141.2
Caffeic acid	n.d.	107.07±10.97[abd]	120.75±0.8[a]	-	82.47±0.35[b]	n.d.	n.d.	0	148.60±7.10[c]	129.8±0.22[cd]	-
p-Coumaric acid	n.d.	141.04±23.1[ae]	191.15±25.8[be]	-	30.87±4.49[cd]	42.24±0.04[c]	33.58±0.07[d]	108.8	165.30±0.22[ab]	189.10±0.95[e]	-
Total phenolic acids	**717.5 (24.4)**	**852.3 (38.6)**	**958.2 (38.5)**	**133.5**	**600.6 (29.3)**	**398.1 (28.8)**	**439.6 (31.5)**	**73.2**	**1176.0**	**898.3 (40.7)**	**125.2 (68.1)**
Flavonols											
Rutin	31.14±0.	28.01±0.7	32.40±2.	104.	22.06±0.	n.d.	22.92±0	103.	29.60±0.	28.90±0.	92.8

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	19[a]	6[b]	01[ab]	0	75[c]		.05[c]	9	60[ab]	04[b]	
Quercetin	851.97±0.24[a]	833.63±0.68[bc]	834.90±0.20[b]	97.9	833.58±0.43[bc]	833.58±0.19[c]	833.60±0.38[c]	100.0	834.20±1.15[bc]	834.70±0.53[bc]	98.0 (49.5)
Quercetin-3-*O*-galactoside	246.60±15.08[a]	141.68±26.7[bc]	196.50±6.59[b]	79.7	n.d.	n.d.	n.d.	-	117.67±4.70[c]	130.30±4.36[c]	52.8
Isorhamnetin	535.98±1.58[a]	n.d.	n.d.	0	533.75±0.19[a]	n.d.	n.d.	0	533.40±0.23[a]	n.d.	0
Isorhamnetin-3-*O*-glucoside	275.20±18.97[a]	192.90±34.2[ab]	275.45±20.5[a]	100.1	n.d.	n.d.	n.d.	-	189.50±0.03[b]	228.00±8.28[a]	82.8
Total flavonols	**1940.9 (65.9)**	**1196.2 (54.2)**	**1339.3 (53.8)**	**69.0**	**1389.4 (67.8)**	**833.6 (60.3)**	**856.5 (61.3)**	**61.6**	**1704.4**	**1222 (55.4)**	**62.9 (36.7)**
Anthocyanins											
Malvidin-3,5-di-*O*-glucoside	n.d.	23.47±0.31[a]	25.40±0.53[c]	-	n.d.	n.d.	n.d.	-	n.d.	16.77±0.14[b]	-
Peonidin-3-*O*-glucoside	64.70±0.41[a]	35.83±0.08[b]	n.d.	0	n.d.	n.d.	n.d.	-	26.25±0.26[c]	n.d.	0
Malvidin-3-*O*-glucoside	100.80±15.01[a]	19.50±0.36[b]	22.68±0.27[c]	22.3	n.d.	n.d.	n.d.	-	n.d.	n.d.	0
Total anthocyanins	**165.5 (5.6)**	**78.8 (3.6)**	**48(1.92)**	**29.0**	**-**	**-**	**-**	**-**	**26.2**	**16.8**	**10.2**
Other phenolics											
Aesculin	34.12±2.95	n.d.	n.d.	0	n.d.	n.d.	n.d.	-	n.d.	n.d.	0

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Luteolin	n.d.	n.d.	51.65±0.32	-	n.d.	n.d.	n.d.	-	n.d.	n.d.	-
Genistein	n.d.	n.d.	13.36±1.88^{abc}	-	n.d.	11.30±0.32^{a}	9.44±0.38^{b}	-	n.d.	13.85±0.11^{c}	-
Phloretin	32.64±0.14	n.d.	n.d.	0	n.d.	n.d.	n.d.	-	n.d.	n.d.	0
Taxifolin	55.46±0.21^{a}	52.71±0.21^{b}	53.96±0.1^{c}	97.3	n.d.	52.51±0.32^{b}	n.d.	-	56.26±0.22^{a}	n.d.	0
Pterostilbene	n.d.	28.03±6.57^{ab}	25.70±0.11^{a}	-	n.d.	25.42±0.78^{a}	32.89±0.08^{b}	-	52.00±2.93^{c}	52.55±0.83^{c}	-
Eriodictyol	n.d.	n.d.	n.d.	-	58.83±0.07^{a}	58.86±0.05^{a}	58.73±0.25^{a}	99.8	n.d.	n.d.	-
Total	**2944.0**	**2208.0**	**2490.2**	**84.6**	**2048.8**	**1379.7**	**1397.2**	**68.2**	**3014.9**	**2203.5**	**74.8 (44.1)**

*value in parenthesis represent relative amount of phenolic class in the sample. Different letters in the same row denote a significant difference according to *t*-test, p<0,05. **'**n.d.**'-**stands for not detected; Abbreviations: **SK-** diluted grape skin extract; **SKC** – grape skin digestion control; **DSK**-digested grape skin extract; **FM**-diluted food matrix; **FMC**-food matrix control; **DFM**-digested food matrix; **SK/FMC** –skin extract with food matrix control; **DSK/FM** digested skin extract with food matrix; **DSKR –** digested skin extract recovery; **DFMR** – digested food matrix recovery; **DSK/FMR** - digested skin extract with food matrix recovery; **DSK/FMTR -** digested skin extract with food matrix total recovery**.**

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973 **Table 2**.

974 The content of non-flavan-3ol polyphenols in the undigested and digested samples of the grape

975 seed extracts (results are expresed as μg/L).*

Phenolic compounds	SE	SEC	DSE	SER, %	FM	FMC	DFM	FMR, %	SE/FM
Phenolic acids									
Gallic acid	1224.70±31.70^{a}	n.d.	772.30±1.01^{b}	63.1	n.d.	n.d.	n.d.	-	852.40
Protocatechuic acid	35.59±0.23^{a}	30.78±1.41^{bd}	32.20±0.46^{b}	90.5	n.d.	22.30±0.29^{c}	n.d.	-	27.60±
Ellagic acid	19457±882^{a}	13800±2135^{b}	8093±313^{c}	41.6	408±48^{d}	284±70^{d}	354±1^{d}	86.7	1793±
5-*O*-caffeoylquinic acid	48.55±1.45^{a}	n.d.	58.30±13.97^{abc}	120.1	79.82±14.67^{abc}	50.05±1.90^{a}	52.58±0.25^{a}	65.8	61.82±
Caffeic acid	n.d.	84.09±1.12^{a}	83.93±0.70^{a}	-	82.47±0.34^{a}	n.d.	n.d.	0	93.94±
p-Coumaric acid	n.d.	n.d.	n.d.	-	30.87±4.49^{ab}	42.30±0.11^{a}	33.67±0.20^{b}	109.1	37.69±
Total phenolic acids	**20765.8 (93.0)**	**13915.0 (93.3)**	**9039.7 (90.5)**	**43.5**	**600.7 (29.3)**	**398.1**	**439.7 (31.5)**	**73.2**	**2866.5**
Flavonols									
Rutin	23.32±0.11^{a}	23.30±0.21^{ab}	27.73±4.09^{abc}	118.9	22.06±0.75^{abc}	n.d.	22.90±0.05^{b}	103.8	21.85±
Quercetin	836.73±0.71^{a}	833.74±0.19^{b}	834.50±0.27^{ab}	99.7	833.57±0.42^{b}	833.70±0.38^{b}	833.55±0.31^{b}	100.0	n.d.
Quercetin-3-*O*-galactoside	12.83±0.02	n.d.	n.d.	0	n.d.	n.d.	n.d.	-	n.d.
Isorhamnetin	533.65±0.31^{a}	n.d.	n.d.	0	533.70±0.12^{a}	n.d.	n.d.	0	n.d.
Isorhamnetin-3-*O*-glucoside	10.88±1.41	n.d.	n.d.	0	n.d.	n.d.	n.d.	-	n.d.
Kaempferol	38.79±0.13	n.d.	n.d.	0	n.d.	n.d.	n.d.	-	n.d.
Total flavonols	**1456.2 (6.5)**	**857.0 (5.7)**	**862.2 (8.6)**	**59.2**	**1389.3 (67.8)**	**833.7**	**856.4 (61.3)**	**61.6**	**21.9**
Other phenolics									
Luteolin	n.d.	51.48±0.58	n.d.	-	n.d.	n.d.	n.d.	-	n.d.
Genistein	n.d.	n.d.	14.53±2.04^{ab}	-	n.d.	11.43±0.50^{a}	9.76±0.07^{b}	-	11.05±
Phloretin	33.29±0.03	n.d.	n.d.	0	n.d.	n.d.	n.d.	-	n.d.
Taxifolin	n.d.	n.d.	n.d.	-	n.d.	52.30±0.09^{a}	n.d.	-	57.65±
Pterostilbene	8.87±0.08^{a}	22.64±3.37^{bcd}	14.42±0.45^{b}	162.6	n.d.	25.40±0.71^{c}	32.80±0.14^{d}	-	116.30
Eriodictyol	60.02±0.2^{ac}	58.94±0.09^{bc}	59.01±0.25^{bc}	98.3	58.83±0.07^{b}	58.90±0.05^{bc}	58.50±0.51^{abc}	99.4	59.19±
Total	**22324.2**	**14905.0**	**9989.8**	**44.7**	**2048.8**	**1379.9**	**1397.3**	**68.2**	**3132.5**

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976 *value in parenthesis represent relative amount of phenolic class in the sample. Different letters in the same row denote a significant difference
977 according to *t*-test, p<0,05. **'**n.d.**'-**stands for not detected; Abbreviations: **SK-** diluted grape skin extract; **SKC** – grape skin digestion control;
978 **DSK**-digested grape skin extract; **FM**-diluted food matrix; **FMC**-food matrix control; **DFM**-digested food matrix; **SK/FMC** –skin extract with
979 food matrix control; **DSK/FM** digested skin extract with food matrix; **DSKR** – digested skin extract recovery; **DFMR** – digested food matrix
980 recovery; **DSK/FMR** - digested skin extract with food matrix recovery; **DSK/FMTR -** digested skin extract with food matrix total recovery.
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Table 3.

Characterisation of flavan-3-ols in the initial grape seed extract using UHPLC-MS/MS Orbitrap. Target compounds, mean expected retention time (t_R), molecular formula, calculated mass, exact mass and MS^4 fragments are presented.

t_R, min	Compound name	Molecular formula, $[M–H]^–$	Calculated mass, $[M–H]^–$	Exact mass, $[M–H]^–$	Δ ppm	MS^2 Fragments, (% Base Peak)	MS^3 Fragme Peak)
Monomeric flavan-3-ols							
5.34	**Catechin**	$C_{15}H_{13}O_6^-$	289.07176	289.07156	0.69	271(5), **245***(100), 205(40), 179(15), 125(5)	227(30), **203**(175(10), 161(
5.83	**Epicatechin**	$C_{15}H_{13}O_6^-$	289.07176	289.07188	-0.42	271(5), **245**(100), 205(40), 179(15), 125(5)	227(35), **203**(175(15), 161(
4.93	**(epi)Catechin hexoside**	$C_{21}H_{23}O_{11}^-$	451.12404	451.12463	-1.31	361(30), **331**(100), 289(70), 247(10)	**313**(100), 287
Procyanidin isomers A type							
4.90	**Procyanidin dimer A type isomer 1**	$C_{30}H_{23}O_{12}^-$	575.11950	575.11993	-0.75	559(20), 421(50), **425**(100), 407(90), 289(50), 287(10)	**407**(100)
5.53	**Procyanidin dimer A type isomer 2**	$C_{30}H_{23}O_{12}^-$	575.11950	575.11938	0.21	559(20), 421(60), **425**(100), 407(90), 289(60), 287(10)	**407**(100)
Procyanidin isomers B type							
4.85	**Procyanidin dimer B type isomer 1**	$C_{30}H_{25}O_{12}^-$	577.13515	577.13513	0.03	559(10), 451(30), **425**(100), 407(50), 289(25), 287(10)	**407**(100), 381
5.10	**Procyanidin dimer B type isomer 2**	$C_{30}H_{25}O_{12}^-$	577.13515	577.13525	-0.17	425(25), 407(10), 329(10), **289**(100), 287(80)	**245**(100), 205
5.50	**Procyanidin dimer B type isomer 3**	$C_{30}H_{25}O_{12}^-$	577.13515	577.13531	-0.28	559(10), 451(30), **425**(100), 407(50), 289(25), 287(10)	**407**(100), 381
6.01	**Procyanidin dimer B type isomer 4**	$C_{30}H_{25}O_{12}^-$	577.13515	577.13519	-0.07	559(10), 451(30), **425**(100), 407(50), 289(25), 287(10)	**407**(100), 381
6.53	**Procyanidin dimer B type isomer 5**	$C_{30}H_{25}O_{12}^-$	577.13515	577.13489	0.45	559(10), 451(30), **425**(100), 407(50), 289(25), 287(10)	**407**(100), 381
4.66	**Procyanidin dimer B type hexoside isomer 1**	$C_{36}H_{35}O_{17}^-$	739.18797	739.18768	0.39	649(90), **619**(100), 587(80), 449(60), 407(15), 289(10)	601(30), 577(449(70), **289**(
5.24	**Procyanidin dimer B type hexoside isomer 2**	$C_{36}H_{35}O_{17}^-$	739.18797	739.18805	-0.11	649(20), 619(40), **587**(100),	**569**(100), 509

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						449(60), 407(30), 289(20)	407(90), 289(
3.58	**Procyanidin trimer B type isomer 1**	$C_{45}H_{37}O_{18}^-$	865.19854	865.19849	0.06	**695**(100), 577(60), 425(30), 407(30), 287(30)	**543**(100), 451
4.21	**Procyanidin trimer B type isomer 2**	$C_{45}H_{37}O_{18}^-$	865.19854	865.19739	1.33	**695**(100), 577(80), 425(30), 407(40), 287(35)	**543**(100), 451
5.26	**Procyanidin trimer B type isomer 3**	$C_{45}H_{37}O_{18}^-$	865.19854	865.19910	-0.65	**695**(100), 577(70), 425(30), 407(40), 287(30)	**543**(100), 451
5.85	**Procyanidin trimer B type isomer 4**	$C_{45}H_{37}O_{18}^-$	865.19854	865.19958	-1.20	**695**(100), 577(80), 425(35), 407(35), 287(30)	**543**(100), 451
4.75	**Procyanidin tetramer B type isomer 1**	$C_{60}H_{49}O_{24}^-$	1153.26193	1153.26062	1.14	1027(70), **983**(100), 865(80), 739(50), 575(90), 407(50)	**947**(100), 445
5.02	**Procyanidin tetramer B type isomer 2**	$C_{60}H_{49}O_{24}^-$	1153.26193	1153.26001	1.66	1027(50), 983(80), **863**(100), 739(40), 575(50), 407(20)	827(30), 737(575(90), 405(
5.35	**Procyanidin tetramer B type isomer 3**	$C_{60}H_{49}O_{24}^-$	1153.26193	1153.26086	0.93	1027(70), **983**(100), 865(80), 739(50), 575(90), 407(50)	932(40), 819(573(90), 423(
5.60	**Procyanidin tetramer B type isomer 4**	$C_{60}H_{49}O_{24}^-$	1153.26193	1153.26123	0.61	1027(40), 983(80), 863(70), 739(40), **575**(100), 407(30)	577(20), 449(**287**(100)
5.97	**Procyanidin tetramer B type isomer 5**	$C_{60}H_{49}O_{24}^-$	1153.26193	1153.26160	0.29	1027(50), 983(80), **863**(100), 739(40), 575(50), 407(30)	737(40), 693(413(40)
6.30	**Procyanidin tetramer B type isomer 6**	$C_{60}H_{49}O_{24}^-$	1153.26193	1153.26062	1.14	1027(40), 983(60), 863(80), 739(30), **575**(100), 407(30)	533(30), 449(413(40), **287**(
Procyanidins gallate isomers							
6.69	**(epi)Catechin gallate**	$C_{22}H_{17}O_{10}^-$	441.08272	441.08282	-0.23	331(10), **289**(100), 271(10), 169(25)	271(5), **245**(1 179(20)
5.78	**Procyanidin dimer B type gallate isomer 1**	$C_{37}H_{29}O_{16}^-$	729.14611	729.14685	-1.01	**577**(100), 559(50), 425(30), 407(50), 289(5), 287(5)	559(50), 421(**407**(100), 289
6.04	**Procyanidin dimer B type gallate isomer 2**	$C_{37}H_{29}O_{16}^-$	729.14611	729.14679	-0.93	711(10), 603(30), 577(90), 559(90), **407**(100), 289(25)	389(25), 297(255(20), 243(
6.41	**Procyanidin dimer B type digallate**	$C_{44}H_{33}O_{20}^-$	881.15707	881.15698	0.10	**729**(100), 711(30), 577(10), 559(20), 541(10), 407(30)	711(20), 603(559(30), **407**(
5.08	**Procyanidin trimer B type gallate isomer 1**	$C_{52}H_{41}O_{22}^-$	1017.20950	1017.20850	0.98	**965**(100), 847(10), 727(50), 695(40), 575(30), 557(30)	**847**(100), 695
5.51	**Procyanidin trimer B type gallate isomer 2**	$C_{52}H_{41}O_{22}^-$	1017.20950	1017.20892	0.57	999(25), 865(30), **729**(100), 595(25), 575(30), 407(20)	603(20), **577**(425(25), 407(
5.75	**Procyanidin trimer B type gallate isomer 3**	$C_{52}H_{41}O_{22}^-$	1017.20950	1017.20911	0.38	999(50), 865(60), **847**(100),	829(40), 803(

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						729(80), 575(30), 407(30)	**677**(100), 363
6.06	**Procyanidin trimer B type gallate isomer 4**	$C_{52}H_{41}O_{22}^-$	1017.20950	1017.20905	0.44	999(30), **865**(100), 847(60), 729(70), 575(30), 407(20)	847(80), **739**(407(30), 287(
6.34	**Procyanidin trimer B type gallate isomer 5**	$C_{52}H_{41}O_{22}^-$	1017.20950	1017.20850	0.98	999(60), 865(80), **847**(100), 729(80), 577(40), 407(40)	847(90), 739(407(20), 289(
6.54	**Procyanidin trimer B type gallate isomer 6**	$C_{52}H_{41}O_{22}^-$	1017.20950	1017.20984	-0.33	999(30), 865(30), 847(60), **729**(100), 577(20), 407(30)	711(40), 603(407(90), 289(
6.89	**Procyanidin trimer B type gallate isomer 7**	$C_{52}H_{41}O_{22}^-$	1017.20950	1017.20837	1.11	999(30), 891(70), 847(60), **729**(100), 577(40)	603(50), 577(**407**(100), 287

986 '–'-stands for not detected fragments; *Peaks further fragmentated in MS^3 and MS^4 experiment are **bold**.

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Table 4.

Composition of flavan-3-ols (expressed as catechin equivalents, μg CE/L) and flavan-3-ol recovery in the grape seed extracts before and after digestion*.

Compound name	SE*	SEC	DSE	SER, %	FM*	FMC	DFM	FMR, %	SE/FMC	DSE/FM	DSE/FMR
Monomeric flavan-3-ols											
Catechin	3830.50±0.39[a]	n.d.	n.d.	-	n.d.	n.d.	n.d.	0	n.d.	207.23±21.38[b]	5.4
(epi)Catechin hexoside	246.00±15.60[a]	12.18±0.77[b]	9.40±0.59[b]	3.8	n.d.	n.d.	n.d.	0	11.71±0.74[b]	18.35±1.16[c]	7.5
Epicatechin	2634.00±198.00[a]	10.54±0.79[b]	7.38±0.55[c]	0.3	n.d.	n.d.	n.d.	0	126.17±9.48[d]	159.09±11.96[d]	6.0
Total monomeric flavan-3-ols	**6710.5 (27.3)**	**22.7**	**16.8**	**0.3**	**0**	**0**	**0**	**0**	**137.9**	**384.7**	**5.7**
Procyanidin isomers A type											
Procyanidin dimer A type isomer 1	81.50±6.40[a]	10.44±0.82[b]	4.32±0.34[c]	5.3	n.d.	n.d.	n.d.	0	67.91±5.30[a]	6.50±0.51[d]	8.0
Procyanidin dimer A type isomer 2	64.50±6.40	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Total procyanidin isomers A type	**146.0 (0.6)**	**10.4**	**4.3**	**3.0**	**0**	**0**	**0**	**0**	**67.9**	**6.5**	**4.5**
Procyanidin isomers B type											
Procyanidin dimer B type isomer 1	3295±208[a]	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	169.03±10.66b	5.1
Procyanidin dimer B type isomer 2	644±8.50[a]	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	14.00±1.41[b]	2.2
Procyanidin dimer B type isomer 3	2626±248[a]	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	51.50±0.71[b]	2.0
Procyanidin dimer B type isomer 4	146.00±7.10	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin dimer B type isomer 5	97.50±6.40	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0

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Procyanidin dimer B type hexoside isomer 1	327.50±19.10[a]	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	3.11±0.18[b]	11.17±0.65[c]	3.4
Procyanidin dimer B type hexoside isomer 2	245.50±14.80[a]	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	4.04±0.06[b]	1.6
Procyanidin trimer B type isomer 1	1543.50±163.30[a]	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	55.41±5.86[b]	3.6
Procyanidin trimer B type isomer 2	69.50±3.50	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin trimer B type isomer 3	1042.00±8.50[a]	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	22.00±2.83[b]	2.1
Procyanidin trimer B type isomer 4	1061.00±52.30	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin tetramer B type isomer 1	37.50±2.10	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Pro1.cyanidin tetramer B type isomer 2	292.50±29.00[a]	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	4.98±0.04[b]	1.7
Procyanidin tetramer B type isomer 3	180.50±7.80[a]	14.57±0.63[b]	9.60±0.41[c]	5.3	n.d.	n.d.	n.d.	0	198.04±8.53[a]	n.d.	0
Procyanidin tetramer B type isomer 4	144.00±1.40	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin tetramer B type isomer 5	419.50±3.50	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin tetramer B type isomer 6	196.00±12.70	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Total procyanidin isomers B type	**12367.0 (50.3)**	**14.6**	**9.6**	**0.1**	**0**	**0**	**0**	**0**	**201.2**	**332.1**	**2.7**
Procyanidin gallate isomers											
(epi)Catechin gallate	1071.50±119.50[a]	12.75±1.42[b]	21.86±2.44[c]	2.0	2.20±0.14[d]	1.02±0.07[e]	n.d.	0	15.43±1.72[bc]	188.48±21.02f	17.6
Procyanidin dimer B type gallate isomer 1	940.50±87.00[a]	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	17.21±1.59[b]	98.66±9.12[c]	10.5
Procyanidin dimer B type gallate isomer 2	1804.00±87.70[a]	n.d.	n.d.	0	4.45±0.07[b]	n.d.	n.d.	0	33.37±1.62[c]	145.94±7.09[d]	8.1
Procyanidin dimer B type digallate	205.50±17.70[a]	15.25±1.31[b]	2.90±0.25[c]	1.4	1.65±0.07[d]	n.d.	n.d.	0	n.d.	n.d.	0

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Procyanidin trimer B type gallate isomer 1	151.00±2.80	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin trimer B type gallate isomer 2	213.00±15.60	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin trimer B type gallate isomer 3	196.00±12.70	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin trimer B type gallate isomer 4	382.00±24.00	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin trimer B type gallate isomer 5	106.50±3.50	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin trimer B type gallate isomer 6	205.00±26.90[a]	0.78±0.10[b]	n.d.	0	1.25±0.07[b]	n.d.	n.d.	0	n.d.	n.d.	0
Procyanidin trimer B type gallate isomer 7	78.50.±3.50	n.d.	n.d.	0	n.d.	n.d.	n.d.	0	n.d.	n.d.	0
Total procyanidin gallate isomers	**5353.5 (21.8)**	**28.8**	**24.8**	**0.5**	**9.6 (100)**	**1.0**	**0**	**0**	**66.0**	**433.1**	**8.1**
Total flavan-3-ols	**24577.0**	**76.5**	**55.5**	**0.2**	**9.6**	**1.0**	**0**	**0**	**473.00**	**1156.4**	**4.7****

* value in parenthesis represents a relative amount of specific flavan-3-ol group in the sample. ** total flavan-3-ols recovery, a percent of total detected flavan-3-ols in the initial grape seed extract. Different letters in the same row denote a significant difference according to *t*-test, $p<0.05$; „n.d.“ stands for not detected; Abbreviations: **SE**-diluted grape seed extract; **SEC**- grape seed extract control; **DSE**-digested grape seed extract; **FM**-diluted food matrix; **FMC**-food matrix control; **DFM**-digested food matrix; **SE/FMC** - grape seed extract with food matrix control; **DSE/FM** - digested grape seed extract with food matrix; **SER** – digested seed extract recovery; **FMR** – digested food matrix recovery; **DSE/FMR** - digested seed extract with food matrix recovery.

Highlights

- Food matrix (FM) contains significant amount of phenolic acids and flavonols.
- Total recovery of polyphenols of grape extracts was significantly influenced by FM.
- Low recovery of proantocyanidins and total flavonoids of seed extracts was observed.
- FM and digestive fluids increased total polyphenol content of grape digests.
- FM and digestive fluids contributed to ABTS•+ scavenging activity of grape digests.