

Content of selected polyphenolic substances in parts of grapevine

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REVIEW

Abstract

This review provides an overview of the variety of occurrences, content, extraction and health effects of selected polyphenolic compounds associated with different parts of grapevine (seeds, peel, pulp and stems). The review provides a brief characterisation of grape parts, the content of polyphenolic compounds and their extraction together with their graphical forms of presentation and diversity as determined by different studies. The content of individual polyphenolic compounds differed with studies. Effects of different factors were evident in both growing style and geographical location of vineyards as well as extraction methods and analytical conditions.

Keywords: grapevine, peel, polyphenolic compound, seed, skin, stem

Introduction

The aim of the review was to summarize the occurrence, distribution and determination of polyphenolic compounds in different parts of grapes. The review also demonstrated variations in the values of polyphenolic compounds with respect to the methods used as well as variations within varieties.

The polyphenolic compounds were selected based on the literature. Among the most studied polyphenolic compounds in grapes are catechin, epicatechin gallate, quercetin, resveratrol and gallic acid (GA). Owing to its simple structure among other factors, GA represents the most commonly used standard for determining total polyphenolic compounds. The aim of creating a comparative table was to focus on the current literature. The analytical methods used were listed for each compound. However, in the case of some less usual analytes, older

sources (from 2003 onwards) were also used. For the purpose of clarity and to emphasise the diversity of individual results, all mass concentrations were converted to a common unit of $\mu\text{g/g}$. For determining concentration in solution, the units were changed to $\mu\text{g/mL}$. Moreover, this review considered polyphenols in accordance to their distribution in grapes reflecting their health benefits.

Polyphenolic compounds found in grapes, their distribution and health benefits

Polyphenols are a class of compounds comprising one or more phenolic hydroxyl groups bonded to at least one aromatic ring (Di Lorenzo *et al.*, 2021). The majority of polyphenolic compounds are generated from phenylpropanoid and phenylpropanoid acetate pathway and represent 40% of organic carbon in plants. Classification of phenolic compounds is based on different approaches according

to the functional group bound to phenol or the number of phenolic units found in the molecule, and this differs mainly according to studies. The easiest division of phenolic compounds is into flavonoids, the most studied group, and non-flavonoids. Nowadays, phenolic compounds are classified into groups and subgroups based on the number of phenolic rings and structural elements attached to the rings (Butterfield, Castegna, Lauderback, & Drake *et al.*, 2002). According to this approach, the major classes represent phenolics, flavonoids, stilbenes and lignans (Pietta *et al.*, 1998). The group of flavonoids, as the most abundant class in grapes or wine, can be represented by flavanols, flavones, flavanonols and anthocyanins (Nollet and Gutierrez-Urbe, 2018; Teixeira *et al.*, 2018; Tsimogiannis and Oreopoulou, 2019).

Depending upon products (Nollet and Gutierrez-Urbe, 2018; Teixeira *et al.*, 2018; Tsimogiannis and Oreopoulou, 2019), flavanols, flavones, flavanonols and anthocyanins belong to a group of flavonoids, and are the most abundant polyphenols present in grapes or wine and related products. Other explanation divided polyphenolic into the following sub-classes: coumarins, furanocoumarins

stilbenes, tannins, lignans, lignins, monolignols, anthocyanins, isoflavones, chalcones, naphthoquinones and anthraquinones, and diarylheptanoids. As observed, phenol sorting is not dogmatic, and it depends, in particular, on the purpose of dividing them (Brenes *et al.*, 2016).

The distribution of polyphenols within a grape bunch is different as shown in Figures 1–4.

The content, abundance and distribution of polyphenolic compounds in grapevines are highly dependent on the geographical and climatic conditions, grape variety, cultivation processes and the stage of ripeness. However, there is no doubt that *Vitis vinifera* is one of the most important sources of polyphenolic substances, especially wine industry wastes (grape skin, stems and seeds), which represent 20% of the weight of processed grapes (Teixeira *et al.*, 2018) and therefore are a matter of growing interest and emphasis for farmers.

As mentioned above, the concentration of polyphenols varies not only between plant species but also between plant parts. Thus, in the following sections, seeds, peels,

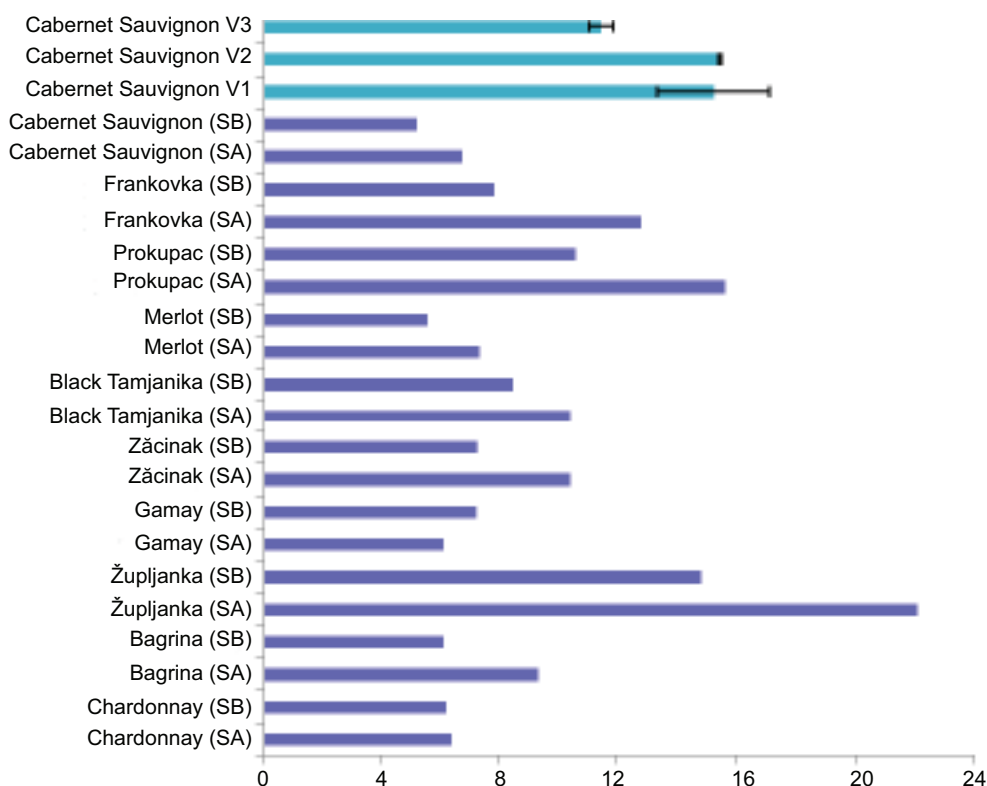


Figure 1. Graph shows the values of total polyphenols by using Folin’s reagent in seeds. Values are expressed in milligram of GA equivalent to per gram of seeds, except for Silva *et al.* (2018), where the equivalent is epicatechin gallate. The same colour shows the measurements of one research group. Next to each bar is the description indicating the variety used for measurements. SA: solvent A; AcEtOH: acidified aqueous ethanol; SB: solvent B; ChCit: choline chloride: citric acid; I: irrigation; LR: leaf removal. Light blue (Castro-Lopez *et al.*, 2019), purple (Dabetić *et al.*, 2020), green (Chorti *et al.*, 2016), yellow (Dinis *et al.*, 2020), grey (Radovanović *et al.*, 2019), orange (Silva *et al.*, 2018) and dark blue (Pantelić *et al.*, 2016).

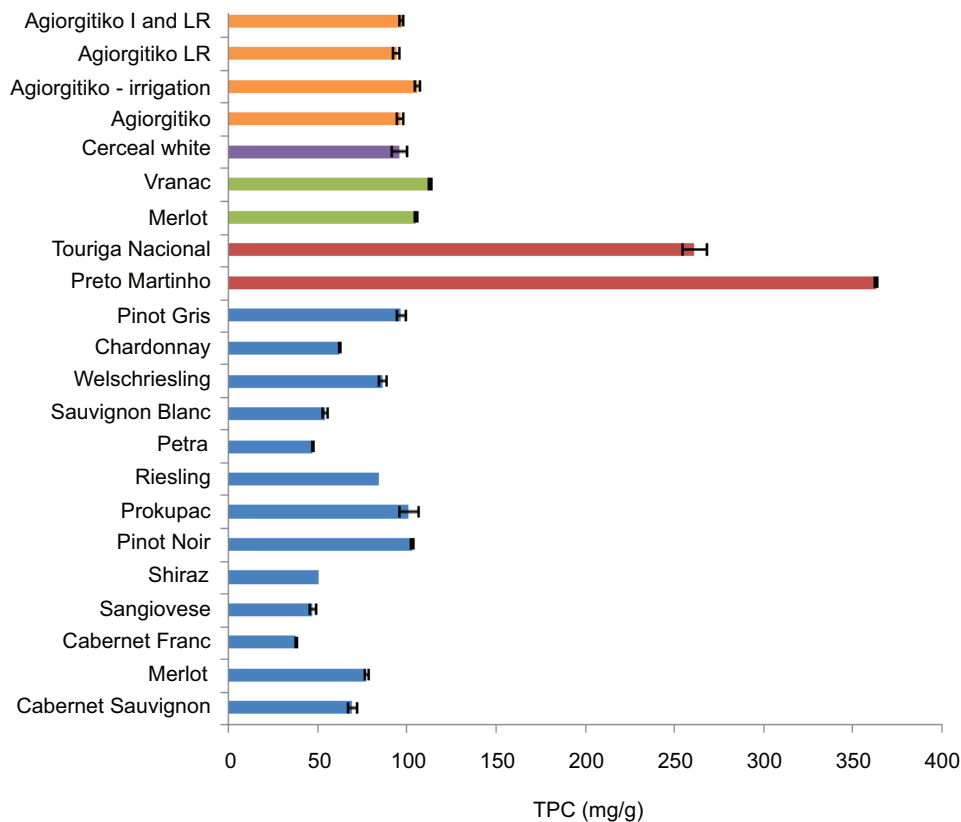


Figure 2. Graph shows the values of total polyphenols by using Folin's reagent in grape peel. Values are expressed in milligram of GA equivalent to per gram of peel. The same colour shows the measurements of one research group. Below each bar is the description indicating the variety used for measurements. SA: solvent A; AcEtOH: acidified aqueous ethanol; SB: solvent B; ChCit: choline chloride: citric acid; P1-10: samples from Piranshahr city; S1-10: samples from Sardasht city. Purple (Dabetić *et al.*, 2020), light blue (Castro-Lopez *et al.*, 2019), pink (Singha and Das, 2015), black (Ni *et al.*, 2017), dark blue (Radovanović *et al.*, 2019), light green (Khoshamad *et al.*, 2020), dark green (Chorti *et al.*, 2016), yellow (Dinis *et al.*, 2020), grey (Radovanović *et al.*, 2019), red (Baiano and Terracone, 2012) and orange (Silva *et al.*, 2018)

pulp and stems are described separately. The most abundant group of polyphenols found in grapes are flavanols, represented by simple monomers of catechin and epicatechin gallate, oligomeric proanthocyanidins (OPCs; 2–5 units), and condensed tannins (polymers of more than five phenolic units), mainly present in the pulp (Crozier *et al.*, 2008). The most abundant form of flavanol group is catechin, mainly found in grape skin and seeds, and traces of monomers or dimers are discovered in grape pulp.

The phenolic hydroxyl group is relatively acidic compared with other hydroxyl groups because of its bond to the aromatic ring, causing deprotonation of the oxygen substituent and stabilisation of the complex. It causes reactivity and determines phenols as the building blocks of polymers, such as lignins or suberins, as well as their involvement in the production of wide spectra of compounds in plants. Owing to their chemical variety, polyphenols have different functions in plants. Many of them are involved in providing defence against herbivores and

pathogens. Other phenols have a mechanical function and are pollinator attractants, adsorb ultraviolet (UV) rays and reduce the growth of surrounding competitive plants.

Polyphenols could have a negative allelopathic effect if released from the leaves, roots or decomposing plant tissues. To compete with surrounding plants for water, sunlight and minerals, plants release phenols that may inhibit the growth of adjacent plants. This effect could possibly be utilised in the production of genetically modified plants that produce compounds with allelopathic effects to eradicate weeds (Crozier *et al.*, 2008; Novak *et al.*, 2008; Taiz *et al.*, 2015). Polyphenolics played an important role in overcoming the challenges of water-to-land transition. As an example, the evolution of phenylpropanoid pathway in primitive terrestrial plants and algae helped them to adapt to exposition to UV radiation even before transition to land. This pathway leads to the production of more than 5,000 compounds called flavonoids. The land ecosystem means decline of water

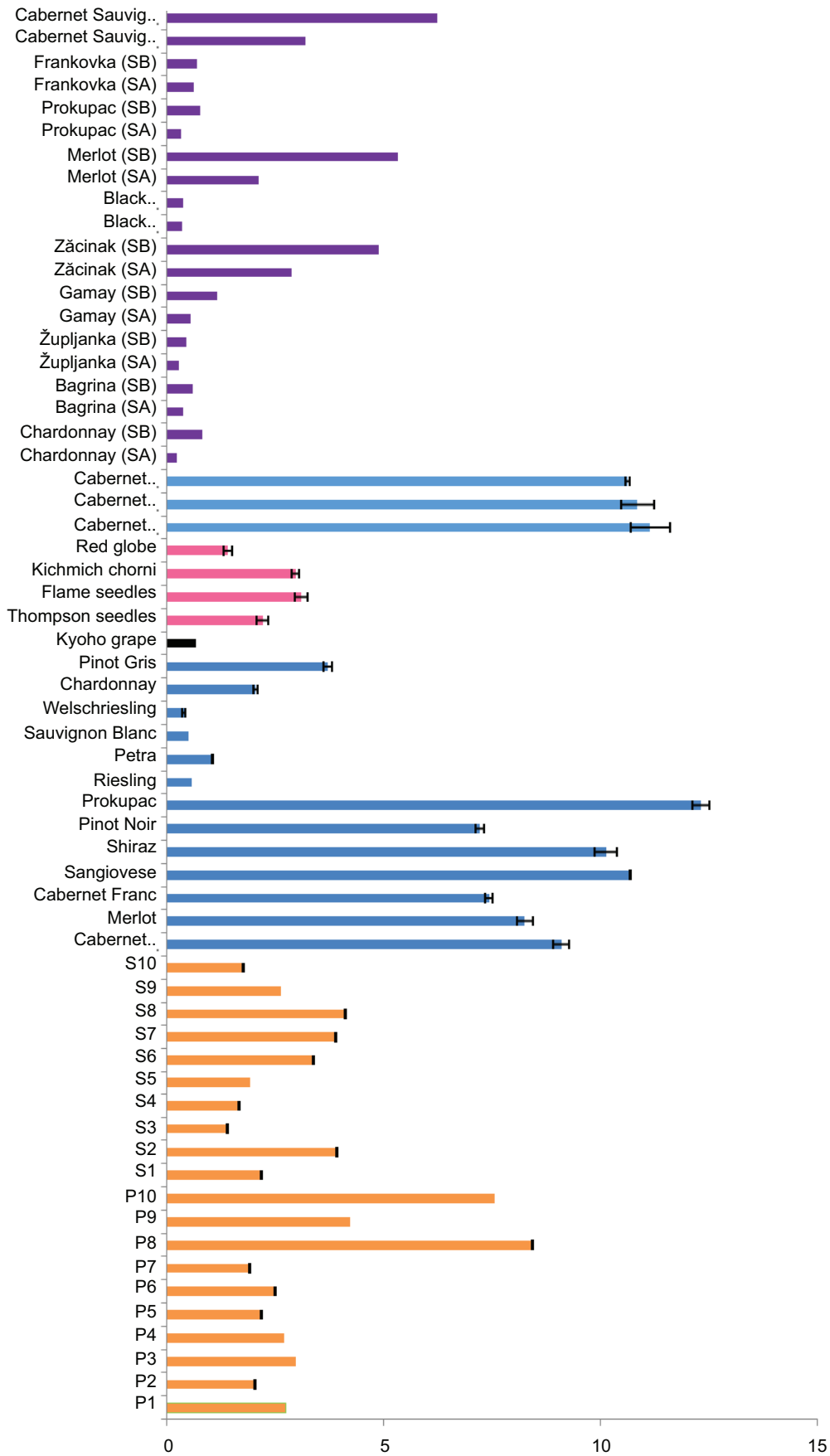


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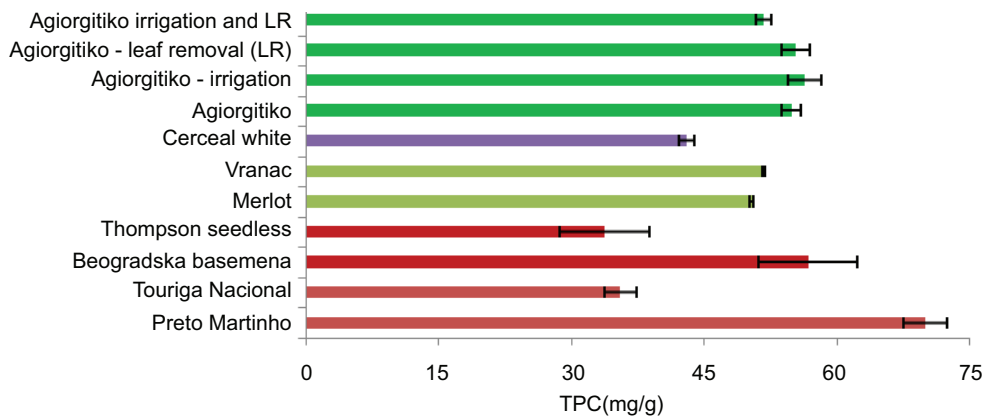


Figure 2. Continued.

sources, resulting in the formation of a group of substances called suberin. These are polymers of phenolic (hydrophilic) and aliphatic (hydrophobic) groups important in forming periderm in the root and bark because they provide a hydrophobic barrier and prevent water loss. Suberin is the main component of the cork and provides a physical barrier against pests. Lignin is a trimer from monolignol monomers that toughens cellulose fibres in specialised cell wall structures in tracheids and vessels. These structures allow the carrying of plant's weight on land, which is not required in the aquatic environment, and transportation of water and minerals from the roots to all plant tissues. Lignin formation helped plants to overcome the pull of gravity and compete for sunlight with other plants (Novak *et al.*, 2008; Taiz *et al.*, 2015).

Last but not least, in addition to many changes, challenges and problems, plants were exposed to a new spectrum of pathogens and herbivores. To deal with this challenge, different subgroups of phenolics that mediate chemical defence, and thus have antiherbivorous, insecticidal and allelopathic (interaction) effects and prevent the spread of fungi and bacteria, were evolved. Owing to their toxicity, phenolic metabolites do not occur in the form of free aglycones but are conjugated with cell wall components or generate conjugated glycosides (GL). In addition to the toxic effects, phenols are responsible for the colour, aroma, taste and antioxidative properties of plant organs (War *et al.*, 2012).

Grapes and their products have displayed a wide range of utilisation. Grape by-products have been used for feeding agriculturally important animals with different results. The feeding of chickens by grape pomace and seed extracts led to improvement in growth in a study conducted by Liu *et al.* (2014). On the other hand, no significant increase in the growth was observed in chickens fed with grape seed extracts (GSE) and grape pomace (Brenes *et al.*, 2016; Chamorro *et al.*, 2015).

A positive boost in growth could be explained due to various reasons. GSE inhibits growth of the pathogen causing coccidiosis oxidative stress (Wang *et al.*, 2008), reduces meat lipid oxidation (Brenes *et al.*, 2016; Iqbal *et al.*, 2015) and increases the abundance of polyunsaturated fatty acids in poultry meat (Chamorro *et al.*, 2015). Other studies showed a significant effect on pigs' metabolism after the feeding of grape marc meal or grape pomace. Intake of grape by-products improved nitrogen metabolism and growth, modified fatty acid patterns in subcutaneous fat (Yan and Kim, 2011), and increased feed conversion ratio and antimicrobial effect on *Escherichia Coli* in the faeces of weaned pigs after feeding with grape by-product (Fiesel *et al.*, 2014).

In contrast, no effect on the growth of rabbits was observed (Nicodemus *et al.*, 2007; Tortuero *et al.*, 1994). Moreover, in the study conducted by Ferreira *et al.* (1996), feeding rabbits with grape pomace led to a decrease in feed conversion ratio. No change in the production or composition of milk was observed in case of dairy cows fed with grape pomace or marc (Eleonora *et al.*, 2014; Hansen and Nielsen, 2004). However, Moate *et al.* (2014) described an increase of monosaturated fatty acids, polysaturated fatty acids and linoleic acid in milk after feeding cows with ensiled or dried grape marc. Feeding with grape marc decreased weight gain in beef cattle (Manterola *et al.*, 1997), and the presence of lignin, tannin and fibre decreased nutrients' digestibility in fattening lambs after feeding them with grape pomace (Eleonora *et al.*, 2014). In contrast, Abarghuei *et al.* (2010) showed a positive effect on retained nitrogen and ruminal parameter in sheep fed with grape pomace. Numerous experiments confirmed the use of grape by-products as a nutrient for farm animals. Different effects of feeding grape by-products are caused due to different production processes used in wineries, affecting the quality of grape by-products, and also variations among cultivars (Gierus *et al.*, 2020; Milder *et al.*, 2005).

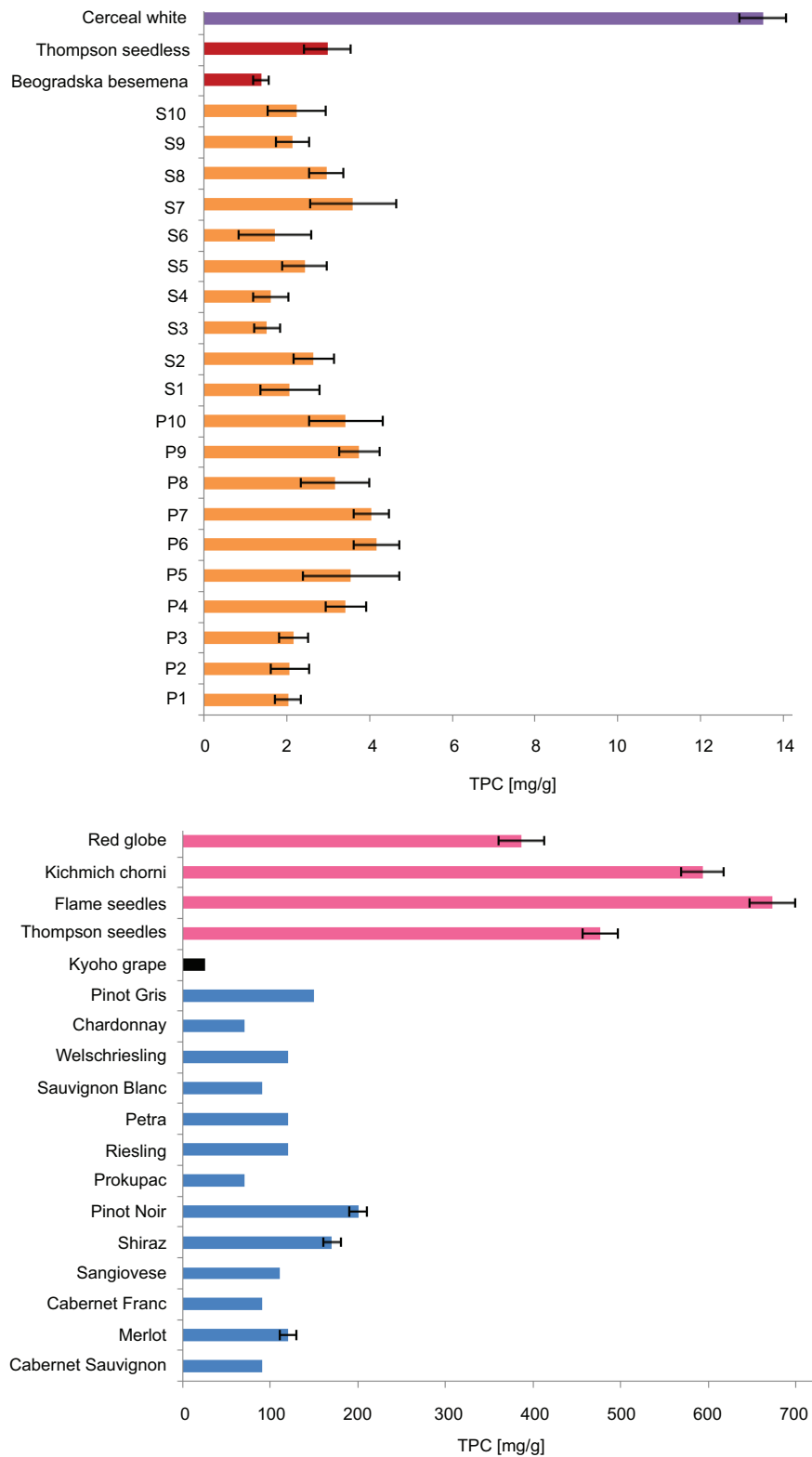


Figure 3. Graph shows the values of total polyphenols by using Folin’s reagent in grape pulp. Values are expressed in milli-gram or microgram of GA equivalent to per gram of pulp. The same colour shows the measurements of one research group. Below each bar is the description indicating the variety used for measurements. P1-10: samples from Piranshahr city; S1-10: samples from Sardasht city. Yellow (Dinis *et al.*, 2020), red (Baiano and Terracone, 2012), green (Khoshamad *et al.*, 2020), pink (Singha and Das, 2015), black (Ni *et al.*, 2017) and blue (Pantelic *et al.*, 2016).

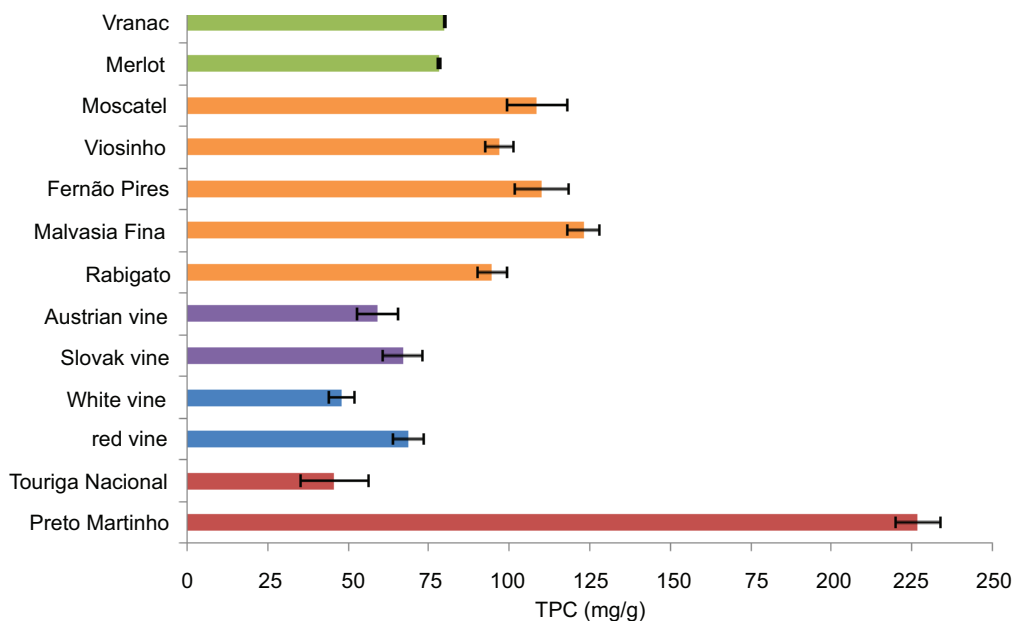


Figure 4. Graph shows the values of total polyphenols by using Folin's reagent in grape stems. Values are expressed in milligram of GA equivalent to per gram of stems, except for Silva *et al.* (2018), where the equivalent is epicatechin gallate. The same colour shows the measurements of one research group. Below each bar is a description indicating the variety used for measurements. Grey (Radovanović *et al.*, 2019), green (Leal *et al.*, 2020), yellow (Hanusovský *et al.*, 2020), blue (Dominguez-Perles *et al.*, 2014) and orange (Silva *et al.*, 2018).

Polyphenolic compounds of grape seeds, distribution, extraction and health potential

Grape seeds are pear shaped with a trigone transverse section. The seeds are composed of a cuticle, an epidermis and two integuments going around the albumen and the embryo (Cadot *et al.*, 2006). Development of the seed and fruit are related. Their maximum fresh weight (fw) occurs during colouring of berries, while maximum dry seed weight coincides with maximum berry weight (Ristic and Iland, 2005). Colour of seeds varies from green to dark brown during maturation (Kennedy *et al.*, 2000). Change of colour, along with hardening, is concomitant with the oxidation of phenolics and maturing of the bunch (Cadot *et al.*, 2006).

Grape seeds are composed of fibre (40%), volatile oils (16%), protein (11%), polyphenolic compounds (7%) and other substances (sugars, minerals etc.; de Campos *et al.*, 2008). Within the grapes, soluble phenols are distributed unevenly, with dominant representation in seeds (70%), 28–35% found in skins and, in spite of their large volume, the least presence of 10% is found in pulp. Supported by various studies (Cheng *et al.*, 2012; Makris *et al.*, 2007), total polyphenols are maximum in seeds among different analysed grape components in different vine varieties.

Catechin and epicatechin gallate are the most abundant phenolic compounds present in the seeds and stems.

Within flavonols, the main representative phenol is rutin (Cheng *et al.*, 2012; Makris *et al.*, 2007). Presence of different phenols is affected by genetic diversity between varieties, regions, light intensity, soil compositions, climatic and agronomic conditions, ripening stages, processes of extraction and storage conditions (de la Cerda-Carrasco *et al.*, 2015; Jordão *et al.*, 2001; Nassiri-Asl and Hosseinzadeh, 2009).

Presence of the most abundant oligomers is different between plant compositions. The major oligomer found in the seeds, according to different studies, is procyanidin B2. On the other hand, procyanidin B1 is reported to be the dominant oligomer in the skin and bunch stems (Dwyer *et al.*, 2014; Topalovic and Mikulic-Petkovsek, 2010; Vujasinović *et al.*, 2021). Despite differences, the studies showed the highest content of polyphenols in the following order: the highest concentration was in the seeds followed by the berry skins and must (mustum or young wine) (Boso *et al.*, 2019).

Antioxidant activity of grapes was confirmed by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, where the highest was found in seed extracts, followed by stem and skin extracts. The higher antioxidant capacity of the seeds may correlate with its higher total phenolic content (TPC), supported by previous results that phenols, dominantly found in the seeds, possess antioxidant activity.

Surprisingly, a negative correlation between the content of phenolics and EC50 describing antioxidant activity was proven by Wen *et al.* (2016), suggested that among phenolics, antioxidant activity is the combined effect of phenolic compounds, sterols and vitamin E. On the other hand, a negative relationship was observed between the quantity of tocols and the content in total phenol (Vujasinović *et al.*, 2021).

Extraction of grape polyphenols in the seeds is dependant upon two conditions, dissolution of concrete polyphenolic compounds in the plant material matrix, and their diffusion in the external solvent medium. Ethanol as an extracting solvent was shown to be an efficient method of extracting polyphenol. Nawaz *et al.* (2006) studied different conditions of extraction of polyphenols from grape seeds using 50% ethanol and 50% water as solvents. When compared with a GA standard, extraction of grape seed polyphenols with a 0.2-g/mL solid to liquid ratio, double-stage extraction, and 0.22- μ m pore size membrane seem to be the most optimal conditions (polyphenols represents 11.4% of the total seeds weight). In respect of concrete polyphenols, it mostly depends on the applied method.

As an example, monomeric procyanidins are found in large amounts in grape seeds, but the quantity of extraction is low due to their low water solubility. Therefore, methods utilising solvents with lower polarity (Soxhlet, supercritical fluid extraction (SFE) and ethanol-assisted extraction) are preferred to increase extractability (Colibaba *et al.*, 2015). In their findings, the choice of solvent used for extraction and the effect of extraction were supported by the Teixeira group, who tested three different solvents and three different methods for extraction. Specifically, Soxhlet, ultrasound extraction, and maceration were performed, and methanol, ethanol and, acetone with different polarities were used as solvents, with the highest TPC using 70% acetone, followed by 70% ethanol extract and 70% methanol extract. However, comparable results were obtained with different extraction methods (Teixeira *et al.*, 2014).

Extraction efficiency does not only depend on the selection of the solvent but also on the extracted phenolic compound. The utilisation of ethyl acetate for extraction showed a large recovery effect on flavonols, whereas methanol was the preferred solvent for flavan-3-ol (catechin, epicatechin gallate and epigallocatechin) extraction. Owing to the low permeability of tissues to non-polar aprotic solvents, addition of water to the solvent increases process efficiency. As an example, the efficiency of proanthocyanidin extraction was significantly increased by adding water to acetone or ethyl acetate. Moreover, the type of analysed material influences measured concentrations because of the relation between the number and weight of seeds, and weight

and stage of berry ripening. For example, the highest content of catechin is at grape veraison; then the concentrations decrease until near maturity (Downey *et al.*, 2003; Freitas and Glories, 1999; Kennedy *et al.*, 2000). Also, decline of monomeric flavanols is more rapid than oligomers because of increasing polymerisation during maturity.

Many positive effects of polyphenolic compounds extracted from seeds have been described for human health, especially their ability to decrease the occurrence of heart disease. Polyphenols mostly contribute to lower the oxidation levels of low-density lipoprotein cholesterol and blood pressure, enhance the functioning of endothelium, reduce inflammation and platelet aggregation, and decrease cell senescence by inhibiting novel proteins activating this process (Dohadwala and Vita, 2009; Moreno *et al.*, 2003; Shi *et al.*, 2003). Grape seed extracts, through their inhibition of enzymes lipoprotein lipase and pancreatic lipase involved in fat metabolism, could be utilised as a dietary supplement to limit fat absorption and fat accumulation in tissues (Bagchi *et al.*, 2000). Intake of grape extracts by mice resulted in reducing myocardial injury and myocardial ischemia-reperfusion and decrease of superoxide anion production as well as platelet adhesion and aggregation (Bagchi *et al.*, 2000; Karthikeyan *et al.*, 2007; Olas *et al.*, 2008). In addition, procyanidins from GSEs have demonstrated inhibition of thrombus formation in mice after oral and intravenous administration (Sano *et al.*, 2005).

Moreover, Anastasiadi *et al.* (2012) proved that extracts having abundance of flavonoids and its derivatives from grape seeds, along with the occurrence of phenolic acids, stilbenes and flavonoids from grape stems, possess antimicrobial properties. The antimicrobial activities of several non-flavonoid phenolic compounds from wine were tested, and vanillic and GA exhibited inhibitory effects towards *K. pneumoniae* and *E. coli* (Vaquero *et al.*, 2007). It has been demonstrated that extracts from red grape seeds inhibit the growth of important human pathogens, such as *E. coli*, *Candida albicans*, *Listeria monocytogenes* and *Salmonella typhimurium*. Moreover, proanthocyanidins are indicated as dominant components in the protective prevention of inflammation mediated through the reduction of *Faecalibacterium prausnitzii* in the intestinal lumen, leading to the blockade of inflammatory response cascade in the gut.

In the central nervous system of mice, the beneficial effects of GSE to modulate lipid peroxidation and oxidative damage of DNA bases were observed (Balu *et al.*, 2006; Feng *et al.*, 2005; Teixeira *et al.*, 2014).

For the above-mentioned reasons, a growing interest is observed in the use of grape seeds, and because the

Table 1. Comparison of concentrations of selected major polyphenolic compounds presented in grapevine seeds according to studies.

Analyte	Concentration (µg/g or % w/w*)	Analytical method	Concentration in	Studies
Gallic acid	10,580–105,000	HPLC/DAD	Dry weight	Cotea <i>et al.</i> (2018)
	300–6,700	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	3,130–3,210	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	745–2,450	HPLC/DAD	Dry weight	Dabetić <i>et al.</i> (2020)
	310–270	HPLC/DAD	Extract	Aybastier <i>et al.</i> 2018
	0.02–2.06 [†]	HPLC/DAD	Extract	Nakamura <i>et al.</i> 2003
	210–1,250	HPLC/PDA	Dry weight	Bucić-Kojić <i>et al.</i> (2009)
	4,000	HPLC/DAD/QMS	Extract	Chamorro <i>et al.</i> (2012)
Catechin	820	HPLC/DAD	Dry weight	Cotea <i>et al.</i> (2018)
	7,700–17,200	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	456.66–823.90	HPLC/MS/QTOF	Dry weight	Boso <i>et al.</i> (2019)
	7,620–8,080	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	2,911–15,587	HPLC/DAD	Dry weight	Dabetić <i>et al.</i> (2020)
	1,360	HPLC/DAD	Extract	Aybastier <i>et al.</i> (2018)
	1.03–4.93 [†]	HPLC/DAD	Extract	Nakamura <i>et al.</i> (2003)
	1,790–6,640	HPLC/PDA	Dry weight	Bucić-Kojić <i>et al.</i> (2009)
	8,000	HPLC/DAD/QMS	Extract	Chamorro <i>et al.</i> (2012)
	674–1,418	HPLC/DAD	Dry weight	Iacopini <i>et al.</i> (2008)
Epicatechin gallate	11,200–25,500	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	429.86–445.20	HPLC/MS/QTOF	Dry weight	Boso <i>et al.</i> (2019)
	10,340–10,600	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	948–6,269	HPLC/DAD	Dry weight	Dabetić <i>et al.</i> (2020)
	790–6,200	HPLC/PDA	Dry weight	Bucić-Kojić <i>et al.</i> (2009)
	8,000	HPLC/DAD/QMS	Extract	Chamorro <i>et al.</i> (2012)
	472–2,057	HPLC/DAD	Dry weight	Iacopini <i>et al.</i> (2008)
Trans-resveratrol	3,940–4,990	HPLC/DAD	Dry weight	Cotea <i>et al.</i> (2018)
	7.11–37.93	HPLC/DAD	Dry weight	Silva <i>et al.</i> (2018)
Ferulic acid	11,210–16,290	HPLC/DAD	Dry weight	Cotea <i>et al.</i> (2018)
	3,100–11,800	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
Procyanidin B1	420–1,410	HPLC/DAD	Dry weight	Cotea <i>et al.</i> (2018)
	97.75–106.27	HPLC/MS/QTOF	Dry weight	Boso <i>et al.</i> (2019)
	0.7–1.73 [†]	HPLC/DAD	Extract	Nakamura <i>et al.</i> (2003)
	6,000	HPLC/DAD/QMS	Extract	Chamorro <i>et al.</i> (2012)
Procyanidin B2	310–670	HPLC/DAD	Dry weight	Cotea <i>et al.</i> (2018)
	141.92–149.00	HPLC/MS/QTOF	Dry weight	Boso <i>et al.</i> (2019)
	7,600–7,860	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	0.66–1.54 [†]	HPLC/DAD	Extract	Nakamura <i>et al.</i> (2003)
	450–5,670	HPLC/PDA	Dry weight	Bucić-Kojić <i>et al.</i> (2009)
Vanillic acid	5,000	HPLC/DAD/QMS	Extract	Chamorro <i>et al.</i> (2012)
	5,360–13,020	HPLC/DAD	Dry weight	Cotea <i>et al.</i> (2018)
	500–1,500	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)

(continues)

Table 1. Continued.

Analyte	Concentration ($\mu\text{g/g}$ or % w/w*)	Analytical method	Concentration in	Studies
Epicatechin gallate	13,880–15,500	HPLC-DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	20–80	HPLC/PDA	Dry weight	Bucić-Kojić <i>et al.</i> (2009)
	2,000	HPLC/DAD/QMS	Extract	Chamorro <i>et al.</i> (2012)
Rutin	6.74–9.37	HPLC/DAD	Dry weight	Szabó <i>et al.</i> (2021)

HPLC: high-performance liquid chromatography; DAD: diode-array detection; PDA: photodiode array; QMS: quadrupole mass spectrometry; MS: mass spectrometry; QTOF: quadrupole time of flight.

highest phenolic content is found in seeds, production of grape seed oil (GSO) is increasing. Consumption of GSO is reported to be beneficial to health, especially because of its high content of phenolic compounds, unsaturated fatty acids, pigments, tocopherols and low cholesterol. In spite of relatively small presence of the mentioned compounds, GSO has been determined as a food supplement with considerably positive health effects. GSO and its components show health protective effects from antiradical, antioxidant and vitamin activity to a high metabolic value in the body (Assumpção *et al.*, 2016; Fernandes *et al.*, 2013).

Bioactive anticancer, antimutagenic and anti-lipid effects and reduction in the risk of cardiovascular diseases qualify GSO to be used as a supplement in food and pharmaceutical industries (Garavaglia *et al.*, 2016; Shinagawa *et al.*, 2015). Comparing GSO of red and white grapes, on average, higher content of phenolic compounds was found in GSO from red varieties, with the highest content found in the Hamburg variety ($336.3 \pm 4.8 \mu\text{g/g}$) in accordance with the study conducted by Vujasinović *et al.* (2021), declaring higher content of phenols in GSE.

Figure 1 shows that in spite of using the same analytical method (values of total polyphenols), the results of individual studies vary widely.

Table 1 shows the content of individual polyphenolic components monitored in grape seeds, depending on the analytical method used.

The results of individual studies are different. These differences are due to the choice of the solvent, the standard used, different laboratory conditions, different maturity conditions, and characteristics of different varieties from different locations.

An overview of the most abundant polyphenolic compounds in grape seeds—their content and extraction

Gallic acid

Gallic acid, as one of the most representative phenolic compounds, was analysed by Cotea *et al.* (2018) using

different extraction methods, attaining more than the 10th of dry weight (dw) in the Fetească neagră variety. The content of GA varies from 10.58 mg/g of dw using Soxhlet extraction to 105 mg/g of dw using the subcritical water extraction method. The use of 75% ethanol for extraction is more effective in comparison to water. Additionally, higher pressure (15 bar) was significantly more effective than using 3-bar pressure. Bucić-Kojić *et al.* (2009), Chamorro *et al.* (2012), Dabetić *et al.* (2020) and Radovanović *et al.* (2019) measured significantly lower concentrations of GA related to dry weight. Radovanović *et al.* (2019) reported concentrations of 3.21 and 3.13 mg of GA in g of dw extracted ultrasonically for 1 h with 40 mL of solvent system consisting of methanol:acetone:water:acetic acid mixture in the ratio of 30:42:27.5:0.5. Dabetić *et al.* (2020) described the impact of using different solvents for extraction—acidified aqueous ethanol (AAE), green solvent (GS) and choline chloride: citric acid (ChCit). Aybastier *et al.* (2018) investigated the effect of addition of 10 M HCl in a methanol:water extraction composition with no significant differences. Nakamura *et al.* (2003) compared concentrations of GA in different health foods containing GSE, and obtained different concentrations of GA. Bucić-Kojić *et al.* (2009) discovered that 50% ethanol was more efficient for GA extraction than 70% or 96% strength.

Catechin

A high range of concentration, that is, 7,700–17,200 $\mu\text{g/g}$ in residue was found by Silva *et al.* (2018). Dabetić *et al.* (2020) tested extraction with GS and compared it with AAE. It was observed that for each variety, the extraction reagents were effective in different manners. The lowest concentrations were found for the Gammay variety, where it was 3,020 $\mu\text{g/g}$ for GS and 2,911 $\mu\text{g/g}$ for AAE, with lower but statistically insignificant difference. On the other hand, higher concentrations were obtained for the Zupljanka cultivar using AAE solvent (15,587 $\mu\text{g/g}$) compared with GS (10,197 $\mu\text{g/g}$). These values were statistically significant. Radovanović *et al.* (2019), who used ultrasonic extraction (1 h, 40 mL of solvent consisting of methanol:acetone:water:acetic acid in a ratio of 30:42:27.5:0.5), discovered the concentration of 7,620 $\mu\text{g/g}$ and 8,080 $\mu\text{g/g}$ for the Merlot and Vranac varieties.

Table 2. Comparison of concentrations of selected analytes in grapevine peel according to studies.

Analyte	Concentration (µg/g or % w/w**)	Analytical method	Concentration in	Studies
Resveratrol	6–255	HPLC/DAD	Dry weight	Iacopini <i>et al.</i> (2008)
	4,700–8,400	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	9.7	HPLC/PDA	Fresh weight	Ni <i>et al.</i> (2017)
	21.5–174	HPLC/DAD	Dry weight	Chafer <i>et al.</i> (2005)
	9.2–29.8	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
Rutin	5.64–13.42	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
	403–1,690	HPLC/DAD	Dry weight	Iacopini <i>et al.</i> (2008)
	140–150	HPLC/DAD	Dry weight	adovanović <i>et al.</i> (2019)
	9,800–27,000	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	15.1–54.4	HPLC/DAD	Dry weight	Chafer <i>et al.</i> (2005)
Quercetin	208–298	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	0.88–38.97	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
	2.9–10.07	HPLC/DAD	Dry weight	Iacopini <i>et al.</i> (2008)
	40–50	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	72.1–254.7	HPLC/DAD	Dry weight	Chafer <i>et al.</i> (2005)
Gallic acid	306–405	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	0.57–121.94	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
	600–800	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	1,360–1,400	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	</=1,200	HPLC/DAD	Dry weight	Teixeira <i>et al.</i> (2014)
Epicatechin gallate	122–319	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	2.34–8.76	UHPLC/DAD/MSMS	Dry weight	Pantelić <i>et al.</i> (2016)
	12,000–23,500	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	ND	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	28–263	HPLC/PDA/MSMS	Fresh weight	Rusjan and Mikulic-Petkovsek (2017)
Protocatechic acid	1.26–4	HPLC/MS/QTOF	Dry weight	Boso <i>et al.</i> (2019)
	91.5–233.6	HPLC/DAD	Dry weight	Chafer <i>et al.</i> (2005)
	232–482	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	2.95–3.56	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
	32–5,219	HPLC/DAD	Dry weight	Dabetić <i>et al.</i> (2020)
Catechin	2,300–7,200	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	1.5–2.4	HPLC/FD	Fresh weight	Teixeira <i>et al.</i> (2014)
	0.4–0.55	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
	65–1,663	HPLC/DAD	Dry weight	Dabetić <i>et al.</i> (2020)
	4.83–19.65	HPLC-MS-QTOF	Dry weight	Boso <i>et al.</i> (2019)
Catechin	48–178	HPLC/PDA/MSMS	Fresh weight	Rusjan and Mikulic-Petkovsek (2017)
	1,890–2,020	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	29,800–55,800	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	536–897	HPLC/DAD	Dry weight	Chafer <i>et al.</i> (2005)
	567–945	HPLC/PDA	Dry weight	Liu <i>et al.</i> (2018)

(continues)

Table 2. Continued.

Analyte	Concentration ($\mu\text{g/g}$ or % w/w**)	Analytical method	Concentration in	Studies
Myricetin GL	3,27–7,47	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
	17–307	HPLC/DAD	Dry weight	Dabetić <i>et al.</i> (2020)
	80–90	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	7.7–46.64	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
Epigallocatechin gallate	1.9–2.42	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)

HPLC: high-performance liquid chromatography; UHPLC: ultra high-performance liquid chromatography; DAD: diode-array detection; PDA: photodiode array; QMS: quadrupole mass spectrometry; MS: mass spec-trometry; QTOF: quadrupole time of flight; FD: fluorescence detection; GL: glucoside.

Bucić-Kojić *et al.* (2009) measured catechin concentration for the Frankovka variety. The authors applied three different concentrations of EtOH as an extraction solvent and obtained results in the range of 1,790–6,640 $\mu\text{g/g}$, with the 50% EtOH in water being the most effective concentration. Aybastier *et al.* (2018) measured catechin in GSE (1,360 $\mu\text{g/g}$) and acid-hydrolysed GSE, where it was not observed. Iacopini *et al.* (2008), who studied more than seven varieties and clones, measured catechin in the range of 674–1,418 $\mu\text{g/g}$ dw. Chamorro *et al.* (2012) used double extraction method with methanol–water and acetone–water combination, and obtained a concentration of around 8,000 $\mu\text{g/g}$ of GSE. Different double extraction method was used by Boso *et al.* (2019) for the Mencía (457.66 $\mu\text{g/g}$) and Albariño (823.9 $\mu\text{g/g}$) varieties. A similar concentration of 820 $\mu\text{g/g}$ dw was obtained through SFE by Cotea *et al.* (2018). Nakamura *et al.* (2003) obtained 1.03–4.93% w/w of catechin in GSE.

Epicatechin gallate

Silva *et al.* (2018) measured concentration of epicatechin gallate in extract residue of the Preto Martinho and Touriga Nacional varieties (25.5 mg/g and 11.2 mg/g, respectively). Comparing abundance of epicatechin gallate in dry weight, Radovanović *et al.* (2019) reported relatively high concentrations in the Merlot (10.34 mg/g) and Vranac (10.60 mg/g) varieties using ultrasonic extraction. Contrarily, Boso *et al.* (2019) reported concentrations of 0.445 mg/g in Albariño and 0.430 mg/g in Mencía varieties using double extraction method with methanol/water/formic acid (ratio: 50:49:1, v/v/v) and 20 mL acetone/water (ratio: 70:30, v/v). In the study conducted by Dabetić *et al.* (2020), usage of AAE for extracting epicatechin gallate led to higher efficiency, compared with the application of GS ChCit. Bucić-Kojić *et al.* (2009) compared different concentrations of ethanol for extracting epicatechin gallate with the highest efficiency by using 50% ethanol at 80°C (0.62 mg/g of dw). Iacopini *et al.* (2008) reported variance in concentration of epicatechin

gallate among different varieties, with the highest concentration found in the Canaiolo variety. Chamorro *et al.* (2012) measured concentration, reaching 8 mg/g of dw and described the effect of heat treatment on antioxidant activity and polyphenolic content of grape pomace and GSE. Silva *et al.* (2018) reported relatively high concentrations of epicatechin gallate in the GSE residue of the Preto Martinho (25.5 mg/g) and Touriga Nacional (11.2 mg/g) varieties.

Epicatechin gallate

Relatively high concentrations of epicatechin gallate were measured after extraction with methanol:acetone:water:acetic acid concoction (ratio: 30:42:27.5:0.5) in the Merlot and Vranac varieties (Radovanović *et al.*, 2019). Contrarily, use of different strengths of ethanol solution (96%, 70%, 50%) reported a low effective extraction concentration of 2 mg/g of dw of epicatechin gallate in the Frankovka variety (Bucić-Kojić *et al.*, 2009; Chamorro *et al.*, 2012) using a mixture of methanol/water (50:50, v/v, pH = 2), followed by acetone/water (70:30, v/v) extraction.

Procyanidin B1

Chamorro *et al.* (2012) measured 6,000 $\mu\text{g/g}$ of procyanidin B1 in GSE. Cotea *et al.* (2018) tested subcritical water extraction with different solvents and pressures using Soxhlet extraction and supercritical fluid extraction. The last was found to be the most effective method and provided a result of 1,410 $\mu\text{g/g}$ dw. Results of other extraction methods were from 420 to 640 $\mu\text{g/g}$ dw. Boso *et al.* (2019) studied concentration of Procyanidin B1 in both Albariño and Mencía varieties, with respective results of 97.75 $\mu\text{g/g}$ and 106.27 $\mu\text{g/g}$ of dw. Nakamura *et al.* (2003) measured the concentration of 0.7–1.73% by volume in GSE.

Procyanidin B2

Compared with other studies, which related concentration to dry weight, Radovanović *et al.* (2019) measured the

highest concentration of 7,860 µg/g. Cotea *et al.* (2018), who tested various extraction methods, obtained results in the range of 310–730 µg/g dw in the Fetească neagră variety. Bucić-Kojić *et al.* (2009) also optimised the extraction process and used three levels of ethanol concentration to obtain results from 450 to 5,670 µg/g dw, reaching the highest concentration when 50% ethanol was used. A similar high result was obtained by Chamorro *et al.* (2012), who determined procyanidin B2 in approximately 5,000 µg/mg of extract. Nakamura *et al.* (2003) reported a concentration of 0.66–1.54 in volume percentage of GSE. Boso *et al.* (2019) carried out double extraction method of seeds for both Albariño and Mencía varieties and obtained the results of 141.92 µg/g and 149.00 µg/g dw.

As mentioned above, Cotea *et al.* (2018) tested different types of extraction methods and, in addition to the already described analytes, studied resveratrol (3,940–4,990 µg/g dw), ferrulic acid (11,210–16,290 µg/g dw) and vanilic acid (5,360–11,220 µg/g dw). Resveratrol has been also studied by Szabó *et al.* (2021) in the Pinot Noir, Cabernet Sauvignon, Syrah and Blue Portugal varieties, arriving at concentrations ranging from 7.11 µg/g to 37.93 µg/g. In this wide range of analytes, the authors also studied rutin, typical of grapevine seeds, for which the obtained concentration was 6.74–9.37 µg/g. Silva *et al.* (2018) measured the concentration of vanillic acid in the residue seeds of the Preto Martinho (500 µg/g of residue) and Touriga Nacional (1,500 µg/g of residue) varieties. The concentration of Preto Martinho was close to the results reported by Cotea *et al.* (2018).

Polyphenolic compounds in peels and pulp of grapes—distribution, extraction and effects on health

Content of polyphenols in grape peels and pulp is generally lower than in seeds and bunch stems. Consequently, the abundance of analytes has been less investigated. Resveratrol, catechin and epicatechin gallate, procyanidin derivatives, hydroxycinnamic acids, flavonols, stilbenes, anthocyanins and GA are the most abundant polyphenols found in grape peels and pulp. As in the case of seeds, the content of these compounds with the ability to neutralise free radicals is highly influenced by the cultivation and genotype of the grape variety (Boso *et al.*, 2019).

Pantelić *et al.* (2016) determined hydroxybenzoic acid to be the most abundant polyphenolic compounds present in grape pulp; unequally minor quantities of the flavan-3-ols from peels of red and white varieties were also obtained. From the flavanol group, epigallocatechin gallate was only found in grape pulp.

As demonstrated by different studies, flavanols are the most abundant group of phenols found in grapes peel

(Di Lecce *et al.*, 2014; Georgiev *et al.*, 2014; Pantelić *et al.*, 2016). From this group, rutin and quercetin were found in all peel samples in the study conducted by Pantelić *et al.* (2016). In analysing the peels of white varieties, quercetin was the only flavonol with the highest concentration, except in the Chardonnay and Pinot Gris varieties, where concentration of rutin was predominant. Additionally, quercetin had the highest concentration in peels of red varieties, with the exclusion of Cabernet Franc and Pinot Noir, where concentration of myricetin (MYR) was predominant. These findings align with the results reported by Castillo-Muñoz *et al.* (2007), who reported myricetin as a typical flavanol of red grapes and wines. Comparing white and red varieties, the contents of flavan-3-ols in peels were of the same order between the groups, supported by the literature (Montealegre *et al.*, 2006; Pantelić *et al.*, 2016; Peña-Neira *et al.*, 2004).

In contrast, Cook and Samman (1996) reported that, within the flavonoids, flavan-3-ols were most abundantly found in peels and seeds and in similar concentrations, and anthocyanins were the predominant group of phenols in grape peels.

Khoshamad *et al.* (2020) showed significant differences in total polyphenols, flavonoids and anthocyanins content in grape pulp and peels of 20 wild grape varieties. In different studies, the TPC of grape berries varied widely (Figures 1–4) because of environmental and genetic factors influencing the composition of grapes.

Hassanpour *et al.* (2011) also proved that TPC, as well as total anthocyanins and flavonoids, was affected by climate status in all studied varieties of grapes. The flavonoid content was higher in the varieties grown in the area with a high average annual temperature and low average annual humidity. In addition to the effect of climate, variation in TPC and anthocyanins within the varieties depended on soil composition and genetic factors. Antioxidant DPPH assay and fluorescence recovery after photo bleaching method proved that antioxidant activity was higher in peels than in pulp, correlating with its TPC and results of the study conducted by Guo *et al.* (2003).

Correlation between TPC and radical-scavenging activities (RSA) was confirmed by analysing grape seeds and skins and was different between red and white varieties. On the other hand, no correlation was discovered between TPC and RSA in pulp extracts (Pantelić *et al.*, 2016).

Numerous extraction techniques are applied for the recovery of polyphenolic compounds from grape pulp and skin, including solid-liquid extraction, ultrasound-, microwave-, and enzyme-assisted extractions, etc. (Tomaz *et al.*, 2019). Solid-liquid extraction is the most utilized method for the recovery of phenols from grape pulp. Methanol (MeOH),

Table 3. Comparison of concentrations of selected polyphenolic compounds in grapevine pulp according to studies.

Analyte	Concentration (µg/g or µg/mL*)	Analytical method	Concentration in	Studies
Resveratrol	<0.1	HPLC/PDA	Fresh weight	Ni <i>et al.</i> (2017)
	9.2–28.7	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	nd	UHPLC/DAD/MSMS	Dry weight	Pantelić <i>et al.</i> (2016)
	Traces	HPLC/DAD		Palomino <i>et al.</i> (2000)
	0.00–4.29	HPLC/DAD	Dry weight	Marshall <i>et al.</i> (2012)
	nd	HPLC/DAD	Dry weight	Wongnarat and Srihanam (2017)
	11.14–39.75	HPLC/PDA	Dry weight	Özcan <i>et al.</i> (2017)
Gallic acid	109–192	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	0.38–0.74	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
	0.219–2.262	HPLC/DAD	Fresh weight	Liu <i>et al.</i> (2018)
	0.07–0.17	HPLC/DAD	Fresh weight	Topalovic and Mikulic-Petkovsek (2010)
	370–440	HPLC/DAD	Dry weight	Wongnarat and Srihanam (2017)
	125.54–567.47	HPLC/PDA	Dry weight	Özcan <i>et al.</i> (2017)
Catechin	364–514	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	nd	UHPLC/DAD/MSMS	Dry weight	Pantelić <i>et al.</i> (2016)
	2.39–3.74	HPLC/DAD	Fresh weight	Liu <i>et al.</i> (2018)
	70–80	HPLC/DAD	Dry weight	Topalovic and Mikulic-Petkovsek (2010)
	0.05–0.15 ¹	UV/VIS		Ivanova <i>et al.</i> (2010)
Epicatechin gallate	31.39–760.081	HPLC/PDA	Dry weight	Özcan <i>et al.</i> (2017)
	149–234	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	nd	UHPLC/DAD/MSMS	Dry weight	Pantelić <i>et al.</i> (2016)
	0.630–2.464	HPLC/DAD	Fresh weight	Liu <i>et al.</i> (2018)
	1.02–6.17	HPLC/DAD	Fresh weight	Topalovic and Mikulic-Petkovsek (2010)
	<50–350	HPLC/DAD	Fresh weight	Nile <i>et al.</i> (2013)
Caffeic acid	50	HPLC/DAD	Dry weight	Wongnarat and Srihanam (2017)
	nd	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	0.301–1.488	HPLC/DAD	Fresh weight	Liu <i>et al.</i> (2018)
	100<<500	HPLC/DAD	Fresh weight	Nile <i>et al.</i> (2013)
	30–40	HPLC/DAD	Dry weight	Wongnarat and Srihanam (2017)
Rutin	7.72–162.78	HPLC/PDA	Dry weight	Özcan <i>et al.</i> (2017)
	77–178	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	0.11–0.13	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
	1.267–8.074	HPLC/DAD	Fresh weight	Liu <i>et al.</i> (2018)
	Traces	HPLC/DAD		Palomino <i>et al.</i> (2000)
Quercetin	2–10	HPLC/DAD	Dry weight	Wongnarat and Srihanam (2017)
	9.46–169.26	HPLC/PDA	Dry weight	Özcan <i>et al.</i> (2017)
	87–198	HPLC/PDA	Dry weight	Farhadi <i>et al.</i> (2016)
	nd	HPLC/DAD	Dry weight	Pantelić <i>et al.</i> (2016)
	nd	HPLC/DAD		Palomino <i>et al.</i> (2000)
	0.00	HPLC/DAD	Dry weight	Marshall <i>et al.</i> (2012)
	<50–450	HPLC/DAD	Fresh weight	Nile <i>et al.</i> (2013)

(continues)

Table 3. Continued.

Analyte	Concentration (µg/g or µg/mL*)	Analytical method	Concentration in	Studies
Protocatechuic acid	4	HPLC/DAD	Dry weight	Wongnarat and Srihanam (2017)
	35.65–131.37	HPLC/PDA	Dry weight	Özcan <i>et al.</i> (2017)
Epigallocatechin gallate	0.08–0.12	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
	0.143–0.371	HPLC/DAD	Fresh weight	Liu <i>et al.</i> (2018)
Myricetin	0.38–0.46	UHPLC/DAD/MSMS	Frozen sample	Pantelić <i>et al.</i> (2016)
	0.00	HPLC/DAD	Dry weight	Marshall <i>et al.</i> (2012)
	10	HPLC/DAD	Dry weight	Wongnarat and Srihanam (2017)

*Determination of total catechin content.

HPLC: high-performance liquid chromatography; UHPLC: ultra high-performance liquid chromatography; DAD: diode-array detection; PDA: photodiode array; QMS: quadrupole mass spectrometry; MS: mass spectrometry; QTOF: quadrupole time of flight; FD: fluorescence detection.

ethanol (EtOH), acetone (ACE), ethyl acetate (EtAc) and their aqueous solutions are the most frequently used extraction solvents for the recovery of polyphenolics from grapes (Rusjan and Mikulic-Petkovsek, 2017; Yilmaz and Toledo, 2004). Enzyme-assisted extraction (EAE) has been mostly recommended for the recovery of polyphenolic compounds from grape skin. In recent years, enzyme-assisted extraction has become popular because of its low cost, excellent efficiency, and environment-friendly approach (Tomaz *et al.*, 2019).

In Figures 2 and 3, we observe, as in Figure 1, large variability in the values of total polyphenols in both pulp and peels. This variability is probably due to, as in the case of total polyphenols in seeds, interference of the Folin method, different laboratory conditions and the the variety of grapes examined. However, we generally observe lower concentrations in pulp and peels than in seeds.

The most abundant polyphenols in peels and pulp and their extraction

Resveratrol

Silva *et al.* (2018) found high concentration of resveratrol in Preto Martinho and Touriga Nacional peel residues (4,700–8,400 µg/g). Charef *et al.* (2005) used supercritical fluid extraction for the Tinto Mazueleo, Cabernet, Boval, Merlot and Tempranillo varieties and obtained results in the range of 21.5–174 µg/g dw, with Tempranillo being the most concentrated sample. Iacopini *et al.* (2008) also examined different varieties and observed a similar range of concentration (6–255 µg/g dw). Farhadi *et al.* (2016) applied ultrasonic extraction with HCl and MeOH for the six varieties grown in West Azerbaijan, and determined concentrations of 9.2–29.8 µg/g dw. Pantelić *et al.* (2016) studied 13 grape varieties for the concentration of resveratrol. They obtained results in the range of 5.64–13.42 µg/g in frozen samples, and Procupac was

the variety with the highest resveratrol content. Ni *et al.* (2017) gauged a 9.7 µg/g fw concentration from the skin of Kyoho grapes.

Rutin

Iacopini *et al.* (2008) reported relatively high concentration of rutin after a 4-h ethanol:water:HCl 0.12 M solution (ratio: 70:29:1, v/v/v) extraction from the Merlot, Sangiovese, Cabernet Sauvignon, Canaiolo, Colorino, Foglia Tonda and Montepulciano varieties (0.403–1.69 mg/g of dw). Farhadi *et al.* (2016) reported lower concentrations of rutin in dried grape seeds after ultrasonic extraction with methanol–HCl (99:1, v/v). Radovanović *et al.* (2019) reported even lower concentrations of rutin in the Merlot and Vranac varieties, also using ultrasonic extraction but with a different mixture. Supercritical fluid extraction is not effective for the extraction of rutin in the Tinto mozueleo, Cabernet, Boval, Merlot and Tempranillo varieties (Chafer *et al.*, 2005). Pantelić *et al.* (2016) reported significantly different concentrations of rutin in the skins of 13 Serbian varieties (in the range of 0.88–38.97 µg/g of fw), with the highest concentration observed in the Sangiovese variety. Silva *et al.* (2018) reported concentration of rutin in the extract residues of the Preto Martinho (9.8 mg/g) and Touriga Nacional (27 mg/g) varieties.

Quercetin

Farhadi *et al.* (2016) studied concentration of quercetin in six different varieties, ranging from 306 to 405 µg/g dw, finding no major differences between them. Chafer *et al.* (2005) studied the Tinto mazueleo, Cabernet, Boval, Merlot and Tempranillo varieties, and discovered the highest concentration of quercetin in Merlot (254.7 µg/g dw) and the lowest in Cabernet (72.1 µg/g dw). Pantelić *et al.* (2016) found the highest concentration in Shiraz (121.94 µg/g fw) and the lowest in Chardonnay (0.57 µg/g fw), but overall there was no trend towards higher quercetin concentration in blue varieties. Samples

of Merlot and Vranac skins were ultrasonically extracted for 1 h with 40 mL of solvent consisting of methanol:acetone:water:acetic acid (ratio: 30:42:27.5:0.5), with the concentration of 40 µg/g and 50 µg/g dw (Radovanović *et al.*, 2019). Iacopini *et al.* (2008) studied polyphenols in red grapes and found quercetin concentration in the range of 2.9–10.07 µg/g dw.

Gallic acid

Radovanović *et al.* (2019) discovered similar concentrations of GA in the Merlot (1.36 mg/g dw) and Vranac (1.40 mg/g dw) varieties. Farhadi *et al.* (2016) reported GA concentration of 1.2 mg/g dw in different white varieties. Use of methanol:HCl solution (ratio: 99:1, v/v) for the extraction of GA did not lead to high concentrations of GA (0.122–0.319 mg/g dw) in different varieties (Muscat, Hosseini, Graha shira, Ag shani, Graha shani and Ghara ghandome). Moreover, Silva *et al.* (2018) observed relatively low concentrations of GA from extract residue in comparison with other analytes in Preto Martinho and Touriga Nacional. Pantelić *et al.* (2016) used methanol containing 0.1% HCl for extraction, reporting the concentration of GA in the range of 2.34–8.76 mg/kg of frozen samples, with the highest concentration discovered in the Cabernet Franc variety.

Epicatechin gallate

The highest concentration of epicatechin gallate was discovered in skin residue (12–23.5 mg/g). Water:ethanol solution (ratio: 50:50) was used for extraction (Silva *et al.*, 2018). Dabetić *et al.* (2020) found a wide range of concentrations in 10 different varieties, from 32 to 5,219 µg/g dw, with the highest being in Cabernet Sauvignon. Despite the fact that Rusjan and Mikulic-Petkovsek (2017) calculated the concentration of the fresh weight of Merlot trigger, the authors arrived at relatively high values compared with other varieties, that is, 28–263 µg/g. Chafer *et al.* (2005) applied supercritical fluid extraction method to the Tinto Mazuleo, Cabernet, Boval, Merlot and Tempranillo varieties, and obtained results in the range of 91.5–233.6 µg/g dw, with Merlot having the highest concentration of epicatechin gallate, with the same as quercetin. Farhadi *et al.* (2016) also studied a larger number of samples for extraction; their results indicated that the lowest measured concentration corresponded to the highest measured concentration of the previous author, that is, 232 µg/g dw; their highest measured concentration was 482 µg/g dw. However, these results could be compared superficially only, because the sample treatment procedures differed. In their research, Boso *et al.* (2019) examined two Portuguese varieties, Albariño and Mencía, and measured concentrations of 1.26 µg/g and 4 µg/g dw by double extraction method. Pantelić *et al.* (2016) had a set of peels of 13 different varieties and obtained the results in the range of 2.95–3.65 µg/g (on a

frozen weight basis), with a concentration of 3.65 µg/g fw for the Petra variety.

Catechin

Silva *et al.* (2018) extracted grape skin with a water:ethanol solution (ratio: 50:50) and determined concentration of catechin per 29.8–55.8 mg/g of residue. Radovanović *et al.* (2019) studied the Merlot and Vranac varieties and found concentrations of 1,890 µg/g and 2,020 µg/g dw by using ultrasonic extraction method. This type of extraction was also used by Farhadi *et al.* (2016), who assessed concentrations between 567–945 µg/g dw in five native grape cultivars of West Azerbaijan province. A comparable scope of concentrations was obtained by measurements made by Chafer *et al.* (2005; 536–897 µg/g dw), who used supercritical fluid extraction process. Dabetić *et al.* (2020) studied 10 grape varieties and found the highest concentration of catechin (307 µg/g dw) in the skin of Frankovka. Rusjan and Mikulic-Petkovsek (2017) studied double reasonable maturation and differences in grape composition, compared with the control, and found catechin concentration in the range of 48–178 µg/g fw. Pantelić *et al.* (2016) used extraction with methanol acidified with 0.1% HCl and obtained a result of 3.27–7.47 µg/g in frozen mass. Of the 13 varieties studied, Merlot had the highest catechin content. Boso *et al.* (2019) studied the Albariño and Mencía varieties using double extraction method (methanol/water/formic acid and acetone/water), and found concentrations of 4.83 µg/g and 19.65 µg/g dw.

Protocatechic acid

Teixeira *et al.* (2014) reported concentrations of protocatechic acid (1.5–2.4 µg/g fw) in the skins of red varieties of *Vitis vinifera* L. Pantelić *et al.* (2016) observed lower concentrations in the range of 0.4–0.55 µg/g fw, with the highest concentration in Sauvignon Blanc. Concentrations of protocatechic acid in the study conducted by Dabetić *et al.* (2020) were in a wide range of 65–1,663 µg/g dw, with the highest significant concentration found in the Začinak variety with ChCit extraction. Silva *et al.* (2018) reported a concentration of 7.2 mg/g in Preto Martinho and 2.3 mg/g in Touriga Nacional grape skin extract residues.

Myricetin

Radovanović *et al.* (2019) discovered myricetin GL in grape skin only in the concentration of 80–90 µg/g dw, but it was not observed in other grape wastes. Of the 13 varieties studied, myricetin was not discovered in the Chardonnay variety. Contrarily, the highest concentration was reported in the Sangiovese variety (46.64 µg/g fw) (Pantelić *et al.*, 2016).

Epigallocatechin gallate was discovered in lower concentration in the range of 1.9–2.42 µg/g fw in 13 varieties studied (Pantelić *et al.*, 2016).

Gallic acid

Compared with grape stems, skins and seed pulp are characterised by a small amount of polyphenols, often under the detection limit. Wongnarat and Srihanam (2017) used triple methanol extraction and discovered slightly higher concentration in white grape pulps. Özcan *et al.* (2017) observed GA as one of the most abundant polyphenols in grape pulps of the Razaki, Müşküle and Cardinal varieties. Farhadi *et al.* (2016) performed ultrasonic extraction of pulp polyphenols using hydrochloric acid in methanol as an extraction solvent, and discovered GA concentration ranging from 109 µg/g fw in the Muscat variety to 192 µg/g fw in the Ghara shani variety. Of the 30 analysed varieties, GA was found to be the most abundant polyphenolic compound in grape pulp, with the highest concentration discovered in the Pearl Black grape variety (2.262 µg/g fw) (Liu *et al.*, 2018). Topalovic and Mikulic-Petkovsek (2010) analysed concentrations of GA in pulps using the maturation process, with peak concentration found in the earlier stages and constant values in the following maturation stages. Pantelić *et al.* (2016) confirmed the high levels of GA in the pulp of different grape varieties, with the highest concentration in the Pinot Noir variety (0.74 µg/g fw).

Resveratrol

As reported by different studies, the concentration of resveratrol in grape pulp is a compound balanced around discovered limit values. Resveratrol was not found in the selected white and blue varieties (Wongnarat and Srihanam, 2017). Palomino *et al.* (2000) discovered only traces of this compound, and Ni *et al.* (2017) noticed a concentration of <0.1 µg/g fw in the Kyoho grape variety. Marshall *et al.* (2012) discovered a concentration of 4.29 µg/g dw in the Janet variety, but resveratrol was not found in the majority of varieties examined. Farhadi *et al.* (2016) measured concentrations in the range of 9.2–28.7 µg/g dw using ultrasonic extraction, and Özcan *et al.* (2017) reported concentrations between 11.14–39.75 µg/g dw, with the highest concentration in the Razaki variety.

Catechin

As in the case of other analytes, high concentrations of catechin were observed after ultrasonic extraction, the highest in the Ghara shani variety (514 µg/g dw) (Farhadi *et al.*, 2016). Özcan *et al.* (2017) observed significantly different concentrations of catechin in grape pulp differing in variety and harvest time. Wongnarat and Srihanam (2017) found similar concentrations between white and blue varieties in the range of 70–80 µg/g dw. Topalovic and Mikulic-Petkovsek (2010) observed concentrations in the range of 2.39–3.74 µg/g fw during different stages of ripening. Ivanova *et al.* (2010) reported concentrations of total catechin using the spectrometric

method, extracting twice for 15 min with 10-mL acetone: water solution (ratio: 80:20, v/v) containing HCl (ratio: 0.1:10, v/v).

Epicatechin gallate

Nile *et al.* (2013) studied epicatechin gallate in 20 grape cultivars, and the range of concentration determined was around 50–350 µg/g in the lyophilised sample. Of the six examined varieties, epicatechin gallate was less abundant than catechin, with the highest concentration in the Ghara shani variety (234 µg/g dw; Farhadi *et al.*, 2016). Wongnarat and Srihanam (2017) discovered the same concentration of 50 µg/g dw in both white and blue varieties using the triple methanol extraction method. Of the 30 varieties examined, the highest concentration of epicatechin gallate was found in the black grape variety, that is, 2.464 µg/g fw (Liu *et al.*, 2018). Topalovic and Mikulic-Petkovsek (2010) also found micrograms of epicatechin gallate in 1 g of fresh sample and observed significantly increased synthesis of epicatechin gallate in samples collected in the early stages of ripening. Pantelić *et al.* (2016) did not discover epicatechin gallate in the pulp because either it was not present or was at a concentration below the detection limit.

Rutin

Liu *et al.* (2018) used ultrasonication to gain pulp extracts and measure rutin in six varieties, with results in the range of 77–178 µg/g dw. Özcan *et al.* (2017) extracted rutin with a mixture of methanol, water and formic acid from three varieties, and discovered its concentration between 9.46 µg/g and 169.26 µg/g dw, with the highest concentration in the Razaki variety. Surprisingly, Wongnarat and Srihanam (2017), and Liu *et al.* (2018) discovered similar concentrations. Wongnarat and Srihanam (2017), who studied one white and one blue variety and used triple extraction method, discovered a concentration of 2–10 µg/g dw. Liu *et al.* (2018) converted the concentration of this analyte to frozen weight and accessed a concentration of 1.267–8.074 µg/g, also using three extractions. Pantelić *et al.* (2016) assessed a narrow range of concentrations of 0.11–0.13 µg/g fw in 13 varieties (Cabernet Sauvignon, Merlot, Cabernet Franc, Sangiovese, Shiraz, Pinot Noir, Prokupac, Riesling, Petra, Sauvignon Blanc, Welschriesling, Chardonnay and Pinot Gris). Trace amount of rutin in grape flesh was found by Palomino *et al.* (2000).

Quercetin

Nile *et al.* (2013) studied 20 grape cultivars for quercetin concentration, and the range was less than 50–450 µg/g of lyophilised sample. Farhadi *et al.* (2016) measured quercetin in the pulp of five native West Azerbaijan varieties and one international variety (Muscat Alexandra), with the results ranging from 87 to 198 µg/g dw. The highest value was found in the Ghara Shira variety. As mentioned

above, the authors used ultrasonic extraction. Özcan *et al.* (2017) studied the effect of harvest time on physicochemical properties and bioactive compounds, and in the case of rutin discovered the range of 35.65–131.37 µg/g dw in the pulp of three different varieties. The concentration of 4 µg/g dw was found in the white variety by Wongnarat and Srihanam (2017). The authors used the triple extraction of pulp from one red and one white variety. Quercetin was not observed in the pulp of the red variety because it was either not present in this variety or its concentration was under the detection limit. The same reasons could have led to the results reported by other studies (Marshall *et al.*, 2012; Palomino *et al.*, 2000; Pantelic *et al.*, 2016).

Liu *et al.* (2018) studied 30 different grape varieties and discovered protocatechuic acid in 11 samples in the range of 0.143–0.371 µg/g fw. The authors used six-fold extraction. The first two extractions were carried out with tetrahydrofuran. The residue was mixed with acidified methanol for two times. The residue was then hydrolysed, and fatty acids were removed with n-hexane. The remainder was extracted twice with a 5-mL mixture of diethyl ether and ethyl acetate.

Pantelić *et al.* (2016) used a slightly simpler extraction method, a triple extraction with methanol acidified with HCl. Their results appeared to be lower than the results of Liu *et al.* (2018), but this was not stated with certainty because they related the concentration to the weight of the frozen sample. In 13 varieties studied, Pantelić *et al.* (2016) measured protocatechuic acid in the range of 0.08–0.12 µg/g. The highest value belonged to Cabernet Franc. The authors also measured epigallocatechin gallate (0.38–0.46 µg/g in the frozen sample), with the highest value being for the Petra variety; myricetin was not detected in any of the studied varieties. Marshall *et al.* (2012) also did not find myricetin in several clones of muscadines. Wongnarat and Srihanam (2017), who used triple methanol extraction, observed 10 µg/g dw of myricetin.

Polyphenols in stems—characteristics, distribution and effect on health

Grape stems represent 1.4–7.0% of the raw material and 25% of the winemaking by-products, and polyphenols represent 5.8% of dry weight. The grape stem is defined by the abundance of crude fibre and protein, nitrogen-free extracts and antioxidant properties.

Water content in grape stems varies in the range of 55–80%, depending on the grape variety, and the dry matter contains 71% of alcohol-insoluble residues. No differences were reported between red and white varieties (González-Centeno *et al.*, 2012; Prusova *et al.*, 2020).

Polyphenols content was studied in grape seeds, skins, must and wines. Polyphenols are stored in every part, including stems, except the berry. However, many studies have proved that stem extract contains lower amount of polyphenols and showed lower antioxidant activity, compared with the other parts of the vine (Castillo-Muñoz *et al.*, 2007; Gonzalez-Centeno *et al.*, 2012; Nassiri-Asl and Hosseinzadeh, 2016).

Wenzel *et al.* (2015) observed higher TPC, expressed as the amount of GA per dry sample, and related antioxidant activity in seed extracts in comparison with stem and peel extracts, compared with the study conducted by Hanušovský *et al.* (2020). Hanušovský *et al.* (2020) studied samples of three varieties—Zweigelt red-skinned, Pinot Blanc white-skinned and Green Veltliner white-skinned, obtained from six different locations (Nitra and Vienna wine regions). It was discovered that stem TPC was higher than that of other parts of a bunch or the grape pomace in samples from both regions. Also, grape stem extracts possessed the highest antioxidant activity. In addition, a higher abundance of lignin increased this property.

The steam also confirmed the influence of different grape varieties as well as extraction methods on TPC along with the effect of temperature on extraction (Hanušovský *et al.*, 2020; Wenzel *et al.*, 2015). The study conducted on flavanol content in the Albariño variety showed the greatest abundance of flavanols in bunch stems, with lower quantity in grape peels, must and wine. In the stem bunch, analyses confirmed catechin as the dominant flavanol and high concentration of procyanidin B1 and its derivatives (Boso *et al.*, 2019). This observation correlates with the results of Püssa *et al.* (2006), who observed a significantly higher content of polyphenols in bunch stems of red grapes, compared with white varieties. Moreover, the analysis also determined higher content in anthocyanins in the bunch stems of red varieties.

On the other hand, a study conducted by Gonzalez-Centeno *et al.* (2012) on the bunch stems of red (Cabernet Sauvignon, Callet, Manto Negro, Syrah and Tempranillo) and white (Chardonnay, Macabeu, Parellada and Prebsal Blanc) varieties did not show typical differences in flavanol content. Therefore, it could be argued that TPC is affected more by other factors than the classification of grapes being white or red. This needs to be supported by the studies observing different cultivating factors on the same varieties (Boso *et al.*, 2019). The study on the composition of polyphenols in grape stems showed high levels of flavan-3-ols, hydroxycinnamic acids, flavonols (monomeric and oligomeric) and stilbenes (Cao and Ito, 2003; Karvela *et al.*, 2009). (*An Investigation on Factors Affecting Recovery of Antioxidant Phenolics and Anthocyanins from Red Grape*). Several studies confirmed

Table 4. Comparison of concentrations of selected analytes in grapevine stems according to studies.

Analyte	Concentration ($\mu\text{g/g}$ or $\mu\text{g/mL}^*$)	Analytical method	Concentration in	Studies
Quercetin	>70	HPLC/UV-VIS	Extract	Esparza <i>et al.</i> (2020)
	8–38	HPLC/DAD	Dry weight	Jiménez-Moreno <i>et al.</i> (2019)
	2–21	HPLC/MS/MS	Dry weight	Anastasiadi <i>et al.</i> (2012)
	13–108	HPLC/DAD	Extract	Esparza <i>et al.</i> (2020)
	120–140	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	0.041–0.215*	HPLC/DAD	Extract	Prusova <i>et al.</i> (2020)
Gallic acid	>150	HPLC/UV-VIS	Extract	Esparza <i>et al.</i> (2020)
	43–310	HPLC/DAD	Dry weight	Jiménez-Moreno <i>et al.</i> (2019)
	70–469	HPLC/MS/MS	Dry weight	Anastasiadi <i>et al.</i> (2012)
	10,500–11,500	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	32,960	HPLC/DAD	Dry extract	Apostolou <i>et al.</i> (2013)
	1,430–1,580	HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	120–1,290	HPLC/DAD	Extract	Esparza <i>et al.</i> (2020)
	0.822–4.015	HPLC/DAD	Extract	Prusova <i>et al.</i> (2020)
Catechin	225–710	HPLC/DAD	Dry weight	Jiménez-Moreno <i>et al.</i> (2019)
	385–1,858	HPLC/MS/MS	Dry weight	Anastasiadi <i>et al.</i> (2012)
	900–3,500	HPLC/DAD	Extract	Esparza <i>et al.</i> (2020)
	29,300–38,700	HPLC/UV-VIS	Residue	Silva <i>et al.</i> (2018)
	157.57–1201.00	HPLC/MS-QTOF	Dry weight	Boso <i>et al.</i> (2019)
	2,310–2,550	HPLC-DAD	Dry weight	Radovanović <i>et al.</i> (2019)
	18.398–78.930*	HPLC/DAD	Extract	Prusova <i>et al.</i> (2020)
	Epicatechin gallate	12.3–189	HPLC/MS/MS	Dry weight
1.742–33.589 [†]		HPLC/DAD	Extract	Prusova <i>et al.</i> (2020)
7.04		HPLC/MS-QTOF	Dry weight	Boso <i>et al.</i> (2019)
2,460–2,600		HPLC/DAD	Dry weight	Radovanović <i>et al.</i> (2019)
15,500		HPLC/UV-VIS	Of residue	Silva <i>et al.</i> (2018)
Resveratrol	10–370	HPLC/DAD	Extract	Esparza <i>et al.</i> (2020)
	74–266	HPLC/MS/MS	Dry weight	Anastasiadi <i>et al.</i> (2012)
	>250	HPLC/UV-VIS	Extract	Esparza <i>et al.</i> (2020)
	21–162	HPLC/DAD	Dry weight	Jiménez-Moreno <i>et al.</i> (2019)
	2,150–25,410	HPLC/UV-VIS	Dried extract	Sahpazidou <i>et al.</i> (2014)
Quercetin-3-glucoside	240–1,500	HPLC/DAD	Extract	Esparza <i>et al.</i> (2021)
	54.1–137	HPLC/MS/MS	Dry weight	Anastasiadi <i>et al.</i> (2012)
	>800	HPLC/UV-VIS	Extract	Esparza <i>et al.</i> (2020)
	96–485	HPLC/DAD	Dry weight	Jiménez-Moreno <i>et al.</i> (2019)
	1.783–11.158 [†]	HPLC/DAD	Extract	Prusova <i>et al.</i> (2020)
E-viniferin	150–690	HPLC/DAD	Extract	Esparza <i>et al.</i> (2021)
	>500	HPLC/UV-VIS	Extract	Esparza <i>et al.</i> (2020)
	91–310	HPLC/DAD	Dry weight	Jiménez-Moreno <i>et al.</i> (2019)
	167–499	HPLC/MS/MS	Dry weight	Anastasiadi <i>et al.</i> (2012)
	170–760	HPLC/DAD	Dry weight	Leal <i>et al.</i> (2020)

HPLC: high-performance liquid chromatography; UHPLC: ultra high-performance liquid chromatography; DAD: diode-array detection; PDA: photodiode array; QMS: quadrupole mass spectrometry; MS: mass spec-trometry; QTOF: quadrupole time of flight; FD: fluorescence detection.

trans-caftaric acid as the phenol with highest concentration in both white and red varieties (Anastasiadi *et al.*, 2012; Apostolou *et al.*, 2013).

The analysis of flavanols in the stems of red varieties showed the presence of diverse flavonols, with a high abundance of quercetin derivatives and the highest content of quercetin-3-O-glucuronide (Negro *et al.*, 2003; Souquet *et al.*, 2000). In addition, quercetin-3-O-glucoside, quercetin-3-O-galactoside and quercetin-3-O-rutinoside were the most abundantly discovered flavanols (Apostolou *et al.*, 2013; Souquet *et al.*, 2000). Comparison of red and white variety stems showed similar characterisation of flavanols, but with much higher flavanol content in red varieties.

Catechin is the most abundant of all the flavan-3-ols, in both white and red varieties, with the highest concentration in grape stems, followed by skins and seeds. Within white varieties, the analysis also showed that catechins are the most concentrated flavan-3-ols in all tissues, with the highest quantity found in grape stems.

Analysis of stilbenes in grape residues from red varieties confirmed the presence of their derivatives in stems, seeds, pomaces and leaves. Contrarily, stilbenes have been found only in skins and stems in white varieties (Anastasiadi *et al.*, 2012; Apostolou *et al.*, 2013; Di Lecce *et al.*, 2014; Rockenbach *et al.*, 2011).

Nowadays, grape stems as a winemaking by-product often represent undervalued material and a waste, a problem for the environment. The rich content of bioactive compounds potentiates grape stems as a prospective material for the introduction of added-value products.

Grape stems are widely used for the production of alcoholic beverages, dietary fibre, plant protein supplements, animal feed and fertilisers (Arvanitoyannis *et al.*, 2006), but bioactive compositions remain poorly defined. The transformation of agro-food wastes into products with added value has caught the attention of the food and pharmaceutical sectors (Martins *et al.*, 2011). Owing to the presence of proanthocyanidins, grape stems and grape clusters are a source of compounds causing excessive astringent taste and influencing organoleptic properties of wine. Therefore, they are removed before the vinification process, but the usage of this waste is being discussed intensively.

The usage of grape waste as a material source for food production could lead to a replacement of intake of synthetic antioxidants with adverse effects. However, bioactive compounds contained in the vine and their impact on the human and animal health has to be investigated in detail.

The chemical composition of grape stems, along with grape variety and growing conditions, strongly influence extraction processes. Domínguez-Perles *et al.* (2014) compared the conditions to increase the effectiveness of phenol extraction as determined by response surface methodology. Performing experiments on grape stems of Greek varieties, lower extraction temperature led to a 34% increase in extracted phenols.

Compared with pomace and the whole bunch, ABTS showed significantly higher ($p < 0.01$) antioxidant activity in grape stems. On the other hand, in Slovak samples, DPPH assay did not show significant differences in the antioxidant activities of grape by-products.

In Slovakia and Austria, grape stems had significantly fewer ($p < 0.01$) proteins in comparison with grape pomace and bunch. Compared with grape pomace and bunch, TPC analysis showed significantly ($p < 0.01$) higher content of grape stem in the samples of both countries. The comparison of Slovak and Austrian wine by-products was characterised by similar nutrition content, condensed tannins and TPC as well as antioxidant activity.

Gouvinhas *et al.* (2020) described the effect of climate and altitude on the production of phenols in grape stems. The authors discovered increased numbers of phenols, orthodiphenols and flavonoids in the grape stems cultivated in low altitude areas (Lower Corgo sub-region). This region is characterised by stressful vine conditions and represented by heavy rains caused by the Atlantic Ocean and thermal stress. Plants respond to stress by synthesising secondary metabolites, including phenols. The impact of thermal stress on these metabolites in the vine was evident during the 2017 and 2018 seasons. The results demonstrated that altitude was a determining factor for the content of polyphenolics.

Though fluctuating levels of phenols in stems were observed, this by-product is a potential source of phenols. Moreover, as in grape seed extracts, antimicrobial activity was observed in grape stem extracts against gastrointestinal tract bacteria *S. aureus* and *E. faecalis*. Additionally, Anastasiadi *et al.* (2012) described the antimicrobial activity of grape extracts caused by the high abundance of flavonoids, phenolic acids and stilbenes in stems along with flavonoids and their derivatives in seeds.

This graph visually shows the least heterogeneous results in terms of percentage values. The rationale could be that the amount of phenolic compounds in the trefoil is least burdened by grapevine species. However, as mentioned above, the Folin method is highly burdened by interference, so we cannot prove this claim. Moreover, there are still noticeable differences over 100 mg/g.

The most abundant polyphenols in grape stems and their effect on health

Gallic acid

The highest amount of GA in grape stems was reported by Apostolou *et al.* (2013), that is, 32,960 µg/g of dry extract. The Mazuelo variety was studied by Jiménez-Moreno *et al.* (2019), whose results were in the range of 43–310 µg/g dw. They used solvent extraction with five levels of ethanol concentration, two ratios of solid and solvent, and two levels of extraction temperature. This could be the reason for the high scatter of results. GA in Mazuelo stem extracts was also studied by Esparza *et al.* (2020). The authors showed the result of a measured concentration higher than 150 µg/mg, which matched their results of 120–1,290 µg/g of extract from different Spanish varieties, including Mazuelo (Esparza *et al.*, 2020). Ordinarily, the same results were measured in Mandilaria, Mavrotragano, Voidomatis, Asyrtiko, Athiri and Aidani in the range 70–469 µg/g dw. Quite higher results were discovered by Radovanović *et al.* (2019) using extraction with MeOH/H₂O/HCl (1,430–1,580 µg/g dw) and Anastasiadi *et al.* (2012) (10.5–11.5 mg/g of residue).

The lowest concentration of 0.822–4.005 µg/mL of extract was measured by Prusova *et al.* (2020). The reason for different results could be the extraction method. Moreover, Prusova *et al.* (2020) worked with fresh material. As mentioned above, stems contain up to 80% water. Low concentration of GA in the range of 0.013–0.024 µg/g dw in grape stems was discovered by Teixeira *et al.* (2018).

Esparza *et al.* (2020) reported a quercetin concentration of >0.07 µg/mL of extract from dried powdered stems, and observed the impact of light and temperature on the stability of phenolic compounds during storage. Prusova *et al.* (2020) measured a concentration of 0.041–0.215 µg/mL of quercetin extract from fresh stems of 10–12-year-old vines. Relatively small concentrations were due to its calculation of analyte in diluted stem extract. Contrarily, Radovanović *et al.* (2019) Anastasiadi *et al.* (2012) and Jiménez-Moreno *et al.* (2019) reported quercetin concentrations of 8–38 µg/g, 2–21 µg/g and 120–140 µg/g of dw, respectively, and the highest concentrations were observed in the Merlot and Vranac varieties. Vines were cultured in Serbia, and dried, milled, and phenols were extracted using ultrasound-assisted extraction with a mixture of methanol:acetone:water:acetic acid (ratio: 30:42:27.5:0.5) (Radovanović *et al.*, 2019).

Catechin

The highest catechin concentration was measured by Silva *et al.* (2018) in dry extract residue (29.3–38.7 mg/g).

Catechin concentration was measured by Anastasiadi *et al.* (2012; 385–1,858 µg/g), Karvela *et al.* (2009; 900–3,500 µg/g), Radovanović (2019; 2,310–2,550 µg/g) and Boso *et al.* (2019; 1,201 µg/g). The extraction method and solvent used differed depending on the study. For example, a mixture of methanol, water and some organic acid was applied, or a mixture of ethanol and water. Boso *et al.* (2019) used a two-step extraction method, but their results were still comparable with others (1,201 µg/g of catechin in stems from red variety). However, Boso *et al.* (2019) had measured a concentration of 157.57 µg/g dw in the stems of the white variety. Close to this value were the results of Jiménez-Moreno *et al.* (2019), who used different ethanol concentrations for extraction and found results in the range from 225–710 µg/g dw. Considering these results, we concluded that the extraction method was not a strong factor in the comparison of results. The lowest concentration was found by Prusova *et al.* (2020) in fresh weight.

Epicatechin gallate

The highest concentration of epicatechin gallate was determined by Silva *et al.* (2018; 15.5 mg/g), possibly because of assessing extract residue. This was followed by an epicatechin gallate concentration of 2.6 mg/g by Radovanović *et al.* (2019); these authors used ultrasonic extraction with a solvent consisting of methanol, acetone, water and acetic acid. The respective concentration of epicatechin gallate discovered by Boso *et al.* (2019), Prusova *et al.* (2020), and Anastasiadi *et al.* (2012) was 12.3–189 µg/g dw, 1.742–33.589 mg/L of extract and 7.04 µg/g dw.

Resveratrol

Anastasiadi *et al.* (2012) and Jiménez-Moreno *et al.* (2019) reported the concentration of resveratrol as 74–266 µg/g and 21–162 µg/g dw, respectively. Jiménez-Moreno *et al.* (2019) investigated the influence of three process parameters on extraction: ethanol concentration, extraction temperature and solid/solvent ratio, observing a wide variety of results of all analytes. The most effective extraction used 50% ethanol as solvent, a temperature of 40°C and a 1:50 solid/solvent ratio. Esparza *et al.* (2020, 2021) analysed the concentration of resveratrol directly from grape stem extract with comparable results. Comparing the Assyrtiko, Mavrotragano, Voidomato and Muscat varieties, Sahnazidou *et al.* (2014) reported the lowest concentration in the white variety Assyrtiko (2,150 µg/g of extract), and the highest abundance in the red variety Voidomato (25,410 µg/g of extract).

E-viniferin

In contrast with other analytes, the value of E-viniferin was found to be the same throughout different studies (Esparza *et al.*, 2020, 2021; Jiménez-Moreno *et al.*, 2019). Analysing dry weight, the abundance of E-viniferin was in the range of 91 µg/g dw of stem powder of the Mazuelo

variety to 310 µg/g dw using different extraction methods (Jiménez-Moreno *et al.*, 2019). Esparza *et al.* (2020) analysed the abundance of E-viniferin in stem extract, which showed a relatively high concentration of viniferin and its susceptibility to light exposition and higher temperatures (25°C and 40°C). However, according to Esparza *et al.* (2021), the concentration of viniferin was in the range of 150 µg/g for the Tempranillo variety (vintage 2018) extract to 690 µg/g for the Mazuleo variety.

Quercetin-3-glucoside

Among stem extracts, the highest concentration of quercetin-3-glucoside was observed in a vintage 2018 Mazuleo variety (1.5 mg/g) by Esparza *et al.* (2021). The high abundance was in correlation with their earlier study (Esparza *et al.*, 2020), where they found the concentration of >800 µg/g. The most effective extraction of quercetin-3-glucoside was achieved using 50% ethanol as a solvent, an extraction temperature of 40°C and a solid/solvent ratio (w/v) of 1:50 (Esparza *et al.*, 2020).

Conclusion

Phenolic compounds are secondary metabolites of plants; accordingly, these are not necessary for plant growth and development but are essential for plant survival. Phenolic compounds play an important role in photosynthesis, respiration, and plant defence, among others. In recent years, interest in the detailed study of phenolic compounds has increased, mainly because of their positive effects on human health. Their presence in grapes has already been proved by many studies. However, the reported concentrations of total and individual polyphenols vary widely. It depends on the way the sample is treated, the method used, the detector used, and the calculation to dry or fresh matter.

By comparing the determined values of selected analytes from different parts of grapevine, a large variability in individual results of different studies was observed. Therefore, it was concluded that the concentration determined not only depended on the choice of variety and the site of growing habitat of the plant but also on the choice of extraction conditions, analytical methods and laboratory conditions. The most commonly used technique is the extraction method with mixtures of organic solvents and water. The mixtures used are adjusted according to the desired polarity. The most common analytical method used is HPLC, or its variations with mass and spectrophotometric detectors or combinations thereof.

We were not able to determine the phenolic substance that was concentrated in each part of the plant, because individual studies provided these concentrations based on their measurements. However, we were able to

determine the most studied analytes. We observed variations in the determination of total polyphenolic compounds in Figures 1–4. These variations could be due to the use of different solvents or the strong influence of interferences, such as carbohydrates, in the method used. However, it is clear that the higher proportion of phenolic compounds is found in seeds and stems, compared with lower proportions in the fruit peel. In general, the pulp of the berry contains the least proportion of phenolic compounds.

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