Original scientific paper
DOI: /10.5513/JCEA01/22.2.3135
Journal of Central European Agriculture, 2021, 22(2), p.429-442

Effect of different drying methods on the content of polyphenolic compounds of red grape skins

Utjecaj različitih metoda sušenja na sadržaj polifenolnih spojeva u kožicama crvenih sorata grožđa

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Received: December 27, 2020; accepted: February 12, 2021

ABSTRACT

The aim of this work was to determine the effect of different drying treatments: freeze-drying, room-drying, and oven-drying on the grape phenolic composition of the 'Regent' and 'Cabernet Sauvignon' varieties. After drying, the samples were grounded, submerged to ultrasound-assisted extraction, and analyzed with high-performance liquid chromatography (HPLC). This study demonstrated that the use of different drying methods significantly affects the content of polyphenols in grape skins extracts. The greatest content of anthocyanins-diglucoside was preserved using freeze-drying, i.e. 15706.86 mg/kg for 'Regent', while the content of anthocyanins-monoglucoside was best preserved by room-drying, i.e. 216778.68 and 9220.30 mg/kg for 'Regent' and 'Cabernet Sauvignon', respectively. The highest content of flavonol glycosides (2583.04 and 1429.64 mg/kg for 'Regent' and 'Cabernet Sauvignon', respectively), hydroxycinnamic acids (1303.31 and 544.88 mg/kg for 'Regent' and 'Cabernet Sauvignon', respectively), and stilbene (2321.52 and 79.36 mg/kg for 'Regent' and 'Cabernet Sauvignon', respectively) was observed in the oven-dried samples. By applying freeze-drying the most optimal content of flavan-3-ol was preserved. Contents of polyphenolic compounds in oven-dried samples after 6 months of storage were almost identical to those in the samples analyzed immediately after drying. The greatest rate of degradation was observed in the room-dried samples while it was moderate in the freeze-dried ones. The results of this experiment demonstrate that it is necessary to dry samples in different ways to obtain the highest content of a certain polyphenolic group of compounds. The application of a drying method is determined by the goal of the final dried product in terms of content and composition of different polyphenolic compounds; thus, the obtained results could have an application in scientific research and for commercial purposes as well.

Keywords: grape skins, polyphenols, drying methods, stability, 'Regent', 'Cabernet Sauvignon', preservation

SAŽETAK

Cilj ovog rada bio je utvrditi učinak različitih postupaka sušenja: liofilizacije, sušenja u sobi i sušenja u pećnici na fenolni sastav grožđa sorata 'Regent' i 'Cabernet Sauvignon'. Nakon sušenja uzorci su usitnjeni, podvrgnuti ultrazvukom potpomognutoj ekstrakciji te potom analizirani primjenom tekućinske kromatografije visoke djelotvornosti. Ovo je istraživanje jasno pokazalo da uporaba različitih metoda sušenja značajno utječe na sadržaj polifenola u ekstraktima kožice grožđa. Najveći sadržaj antocijan-diglukozida sačuvan je liofilizacijom, 15706.86 mg/kg u slučaju 'Regenta', dok je sadržaj antocijan-monoglukozida najbolje očuvan sušenjem pri sobnoj temperaturi, 216778.68 i 9220.30 mg/

kg u slučaju 'Regenta' odnosno 'Cabernet Sauvignona'. Najveći sadržaj flavonol-glikozida (2583.04 i 1429.64 mg/kg u slučaju 'Regenta' odnosno 'Cabernet Sauvignona'), hidroksicimetnih kiselina (1303.31 i 544.88 mg/kg u slučaju 'Regenta' odnosno 'Cabernet Sauvignona') i stilbena (2321.52 i 79.36 mg/kg u slučaju 'Regenta' odnosno 'Cabernet Sauvignona') zabilježen je u uzorcima sušenim u pećnici. Primjenom liofilizacije očuvan je najoptimalniji sadržaj flavan-3-ola. Sadržaj polifenolskih spojeva u uzorcima sušenim u pećnici, nakon razdoblja od 6 mjeseci skladištenja, bio je gotovo identičan sadržaju u uzorcima analiziranim neposredno nakon sušenja. Najveća razgradnja zabilježena je u uzorcima sušenim u sobi, dok je u liofiliziranim uzorcima bila umjerena. Rezultati ovog eksperimenta pokazuju da je uzorke potrebno sušiti na različite načine kako bi se dobio najveći sadržaj određenih skupina fenolnih spojeva. Primjena metode sušenja određena je konačnim ciljem osušenog proizvoda s obzirom na sadržaj i sastav različitih polifenolskih spojeva; stoga bi dobiveni rezultati mogli imati primjenu u znanstvenim istraživanjima, ali i u komercijalne svrhe.

Ključne riječi: kožice grožđa, polifenoli, metode sušenja, stabilnost, 'Regent', 'Carbernet Sauvignon', očuvanje

INTRODUCTION

Over the past few decades, there has been an increasing interest in polyphenolic compounds. They play key roles in plant physiology such as in growth, reproduction, pigmentation, and resistance to pathogens and predators. These compounds are very important components of the human diet because they have cardio-protective, anti-cancer, anti-diabetic, anti-aging, and neuroprotective effects. Some polyphenols have antifungal, antibacterial, and anti-inflammatory activities (Cheynier, 2012). Due to these characteristics, phenolics are widely used as dietary supplements (Myburgh, 2014), active ingredients for cosmetic products (Zillich et al., 2015), and food additives especially for fish and fish products (Maqsood et al., 2013). Many plant species contain large amounts of different polyphenols and grapes are one of them. Most polyphenols are in the grape skin and grape seed. Grape skin contains a large amount of flavonoids, hydroxybenzoic and hydroxycinnamic acids as well as stilbenes. Grape skins have a water content in a range of 75 to 80% thus the conditions for growth of microorganisms are suitable (de Torres et al., 2010). These facts could favour degradation of some chemicals especially degradation of polyphenolic compounds thus it is important to find methods which could maintain these compounds. The drying of fresh plant material has long been used to obtain stabile products which can be stored for a prolonged time period. Dehydration has many advantages as follow: prevention of growing microorganisms and degradation of some compounds; obtaining a sample with very small particles which could enhance extraction by increasing of surface contact between solid and extraction solvent. The drying process could change the material's structure by changing the porosity of matrices (Garcia-Perez et al., 2010). Dehydration is the necessary process prior to further operation, such as extract of phenolic compounds, flavonoids, and anthocyanin pigments. During drying, temperature is the key influence parameter for maintaining bioactive components. Thus, freeze-drying has been widely studied and indicated ideal drying conditions. However, large-scale commercial production and the high cost of freeze-drying should be considered (Wang et al., 2016).

It is expected that different drying methods could have a different impact on polyphenolic compounds. Different research groups (de Torres et al., 2010; Larrauri et al., 1998; Tseng and Zhao, 2012) used various drying methods to dehydrate grape skins among which oven-drying at different temperatures in the various periods and freeze-drying were the most used. Freeze-drying is a very expensive and time-consuming method, and it has limited usage in commercial applications.

From a quantitative point of view, results obtained by different drying methods could not be compared between each other, because different methods would have a diverse effects on polyphenolics. De Torres et al. (2015) found both phenolic compounds, anthocyanins, and flavonols in fresh and dehydrated samples, thus concluding that the freeze-drying method was less aggressive than oven-drying method. According to

Adiletta et al. (2016), the best drying temperature for total phenolic content was 50 °C, for both untreated and pre-treated grape samples. Coklar and Akbulut (2017) investigated the effect of sun, oven and freeze-drying on anthocyanins and phenolic compounds of grapes. Significant decrease in anthocyanins was found in grapes after sun and oven drying, while no loss of anthocyanins was found in freeze-dried grapes. Losses in total phenolic content were the greatest in sun-dried grapes (46.79%), followed by oven-drying (20.26%). Freeze-drying showed just 1.89% loss in total phenolic content in grapes, with the highest levels of procyanidin B1, (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, *trans*-resveratrol and rutin compared to other drying methods. Qin et al. (2020) showed that contents of total phenolic and total flavonoid in room drying were higher compared to sun drying and shielding film drying methods in the production of raisins. Recently, Silva et al. (2020) compared two drying methods, i.e. oven-drying and freeze-drying, on the polyphenols content in grape skin of the new hybrid grape variety 'BRS Magna'. Total phenolic content in grape skin had a reduction of 69.3% and 69.7%, but an increase in total anthocyanin content of 20.7% and 108.3% in comparison with the residues of fresh peel (control) in conventional drying and freeze-drying, respectively. The best drying method for preserving the content of hidroxycinnamic acids: caftaric, caffeic, ferulic and p-coumaric acid in grape skin was oven-drying, which was also shown in our research. The highest content of gallic acid was obtained in oven-dried samples compared to freeze-drying. The content of trans-resveratrol showed a decrease of 33.3% and 83.3% due to conventional oven drying and freeze-drying, respectively, compared to fresh peel on a dry basis.

The main purpose of grape drying is its preservation, and thus raisins production. Fresh grapes generally contain 80% water by weight. In the process of making raisins, water content was traditionally reduced/removed by sun drying, especially in developing countries due to its low initial and running costs (Jairaj et al., 2009). In recent years, and due to the considerable degradation of quality (Pangavhane and Sawhney, 2002; Ruiz et al., 2014), sun drying has been widely replaced by mechanical drying, most often in the forced air convection oven. However, the quality of the final product is strongly affected by drying conditions and grapes characteristics (Role et al., 2011; Xiao et al., 2010; Caglar et al., 2009). The drying process demands high energy to increase drying efficiency, which requires optimization of drying temperature and duration (Sinhg et al., 2012; Bennet et al., 2011).

Production of sweet wines usually requires a long postharvest drying process. The technology of grape drying for raisins production is not completely useful for wine production. The aim of grape drying for the wine industry is to maintain the aromatic and phenolic potential of fresh grapes. Wine grapes undergo lower temperatures (27-30 °C), which extends the dehydration process (Doneche, 1993). Chen et al. (2016) argue that grapes destemmed from bunches without pedicel showed the highest dehydration effectiveness and the lowest activation energy, followed by whole grape bunches and fruits detached from bunches with a 2 mm pedicel.

Lately, a lot of effort was put into the study about the impact of different drying methods on polyphenols content from different plant species (Katsube et al., 2009; Madrau et al., 2009; Piga et al., 2003) but still little is known about the effect of different drying methods on individual grape polyphenolics. In this context, the aim of this study was to investigate the effect of different drying methods, including freeze-drying, 25 °C room-drying and 60 °C conventional oven-drying on polyphenolic content and composition of grape skins as well as to evaluate the stability of individual phenolic compounds in dried grape skins samples for 6 months storage at 25 °C.

MATERIALS AND METHODS

Sample preparation

Grape samples ('Regent' and 'Cabernet Sauvignon') were obtained in 2018 from the vineyard located at the Experimental station Jazbina, Faculty of Agriculture, University of Zagreb, Croatia. Grapes were harvested in a state of full ripeness with the amount of total reducing sugars of 180 g/L and immediately separated from the

stalk. To obtain homogenous samples of the berries at a similar level of ripeness (sugar and flavonoid content), a simple flotation method was used with sucrose water solutions of different densities. 'Regent' grape berries with a density range of 1.088 to 1.099 g/cm^3 and 'Cabernet Sauvignon' grape berries with a range of 1.094 to 1.099 g/cm^3 were selected for further analysis. The berry skins were manually removed from the pulp and seeds and dried by different drying methods. Dry skins were ground, and the powder obtained was immediately extracted and analyzed as well as stored for six months (25 °C) in a glass container equipped with polytetrafluoroethylene (PTFE) cap in a dark and dry place. Grape skins from two quite different red grapevine cultivars, 'Regent' and 'Cabernet Sauvignon', were evaluated.

Drying methods

Fresh samples of grape skins obtained from 'Regent'and 'Cabernet Sauvignon' were dried by applying three different methods as follow. Oven-drying was conducted by applying convection laboratory oven ST60L (InkoLab, Croatia) without mechanical agitation at 60 °C for 18 hours. Room-drying was performed in a room equipped with professional air-conditioning system (Daikin, Japan) at 25 °C for 7 days. Freeze-drying was performed in Alpha 1-2 LDplus (Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The frozen grape skins were placed in a freeze-dryer. Primary drying was conducted at 0.1 mbar for 48 hours while for the secondary drying following conditions were applied: 0.01 mbar and 24 hours. In all drying tests, 100 g of fresh samples were weighed before drying. All drying experiments were performed in triplicates and all samples were dried until constant mass.

After drying, samples were weighed again to determine their drying yields according to the equation:

Drying yield = (mass of dried sample)/(mass of fresh sample) × 100 (1)

The drying yield was expressed as grams of dried sample per 100 g of the fresh sample.

The total dry matter of fresh samples and residual moisture of dried samples were determined according to the Association of Official Analytical Chemists (AOAC 1998) official methods of analysis using the hot-air drying methods at 135 °C for 2 h. The total dry matter was expressed as grams of dried sample per 100 g of the fresh sample while the residual moisture was expressed in %.

Total dry matter = (mass of dried sample)/(mass of fresh sample) × 100 (2)

Residual moisture = total dry matter – the mass of dried sample (3)

Dry skins were ground (CryoMill, Retsch, Germany) and the powder obtained was immediately extracted and analyzed as well as stored for six months (25 °C) in a glass container equipped with polytetrafluoroethylene (PTFE) cap in a dark and dry place.

Extraction method

Extraction of grape skin polyphenols was done by applying the ultrasound-assisted method described by Tomaz et al. (2016). In brief, 125 mg of grape skins powder was extracted by 10 mL of 25% aqueous acetonitrile containing 1% formic acid for 15 min at 50 °C in an ultrasonic bath (Sonorex Super RK 100H, Bandelin Electronic, Berlin, Germany). The extract was centrifuged in an LC-321 centrifuge (Tehtnica, Železnik, Slovenia) for 20 min at 3200 rpm at room temperature. The supernatant was brought to the final volume of 10 mL with eluent A (water: phosphoric acid, 99.5:0.5, v/v). The extract was filtered with Phenex-PTFE 0.20 μm syringe filter (Phenomenex, Torrance, USA) and analyzed by HPLC.

Liquid chromatography analysis

The separation, identification, and quantification of polyphenols from grape skin extracts were performed according to the method described by Tomaz and Maslov (2016) on an Agilent 1100 Series system (Agilent, Germany). The separation was performed with a reversed-phase column Luna Phenyl-Hexyl (4.6×250 mm; 5 μm particle (Phenomenex, Torrance, USA)). The solvents were water:phosphoric acid (99.5:0.5, *v/v*, eluent A) and acetonitrile:water:phosphoric acid; 50:49.5:0.5, *v/v/v*, eluent B). Using DAD, flavonol glycosides were detected at

360 nm, anthocyanins at 518 nm, hydroxycinnamic acids at 320 nm, stilbenes at 308 nm, and hydroxybenzoic acids at 280 nm. Using FLD, flavan-3-ols were detected at λ_{ex} = 225 nm and λ_{em} = 320 nm. Quantification of individual flavonoid peaks was completed by using a calibration curve of the corresponding standard compound. The results are expressed in mg/kg of dry weight (d.w.) of grape skin. For peak assignment, grape extracts were analyzed with an Agilent 1200 Series system (Agilent, Germany) coupled in-line to an Agilent model 6410 mass spectrometer fitted with an ESI source.

Statistical analysis

The mean values and significant differences between data were calculated and reported using SAS System Software, v.9.0 (SAS Institute Inc., Cary, NC, USA, 2004). The results were analyzed using one-way ANOVA and the differences between means were evaluated by Duncan's posthoc test at a confidence level of 95% ($P<0.05$). The data reported in all the tables were the average of triplicate observation.

RESULTS AND DISCUSSION

The effect of the drying method on drying yield and residual moisture

A good method for drying grape skin samples must meet several important criteria, such as the retention of high polyphenols content, ease of use, cost-effectiveness as well as low energy consumption. By applying the AOAC method, a total dry matter of grape skins obtained from cultivars 'Regent' and 'Cabernet Sauvignon' were determined (equation 2) and these values were 22.80 and 20.38 g of dry sample/100 g of fresh weight, respectively. Different drying methods had a significant effect on drying yield and residual moisture of the samples. An important factor affecting the efficiency of the method is drying yield which was calculated using equation 1.

Drying yield depends on the drying method, as well as a grape cultivar. The highest drying yields for both examined cultivars were obtained using freeze-drying, and their values were 28.14 and 26.16 g of dry sample/100 g of fresh weight for 'Regent' and 'Cabernet Sauvignon', respectively. The lowest drying yield was obtained by drying samples at room temperature (Table 1). The drying yield depends upon temperature and relative humidity. The differences in the obtained drying yields for various drying methods could be explained by the factors mentioned above. During the freeze-drying, the process of drying takes place under a vacuum at low temperature with extremely low relative humidity, while during the drying at room temperature the sample was exposed to relatively high humidity (greater than 50%).

The residual moisture was calculated according to equation 3. The values obtained for the 'Regent' ranging from 5.34 to 6.26%, and for 'Cabernet Sauvignon' from 5.76 up to 6.36% (Table 1). There was no statistically significant difference between room-drying and oven-drying. The obtained values were consistent with those obtained in earlier studies (de Torres et al., 2010; Tseng and Zhao, 2012). Tseng and Zhao (2012) obtained the lowest value for the freeze-dried samples of 'Pinot Noir' skins (5.60%), slightly greater for the samples dried in an oven at 40 °C (5.92%), and the greatest for the samples dried in air (6.95%).

The effect of the drying method on the content of individual phenolics

Grape skins extract contains 6 groups of polyphenols and more than 25 different individual compounds. Even though all of these compounds belong to the same large group of compounds, e.g. polyphenols, their physicochemical properties are quite different (Tomaz et al., 2016). In previous researches, in which the influence of different drying methods on the polyphenol content was studied, usually, the total content of polyphenols, anthocyanins, flavan-3-ol and antioxidant capacity was determined using various spectrophotometric methods (Larrauri et al, 1998; Tseng and Zhao, 2012; Vashisth et al, 2011). Spectrophotometric methods get insight into the total content of polyphenols, or different sub-groups, but do not give insight into the content and composition of the individual compounds. Since it is sometimes necessary to isolate only a specific class of compounds or

Table 1. Effect of different drying methods on drying yield and residual moisture

Drying Method	Regent		Cabernet Sauvignon	
	Drying Yield (g dry sample/100 g fresh sample)	Residual moisture (%)	Drying Yield (g dry sample/100 g fresh sample)	Residual moisture (%)
Freeze-drying	28.14[a]	5.34[a]	26.14[a]	5.76
Room-drying	29.06[b]	6.26[b]	26.74[b]	6.36
Oven-drying	28.90[b]	6.10[b]	26.50[b]	6.12

Means with different superscript letters, for each variety separately, in the same row differ significantly (P≤0.05)

any of the individual compounds, it is important to know the influence of drying methods on each compound of interest, therefore, in this study HPLC method was used. This method provides more reliable and accurate results in this type of study.

Table 2 shows the contents of individual polyphenolic compounds in grape skins extracts and the effect of the different drying methods on the final content in the extracts. The results clearly indicate that the sample drying method and examined cultivar had a great influence on the content of individual polyphenols.

Anthocyanins and hydroxybenzoic acids

Anthocyanins are the most abundant group of polyphenols in red grape skins. The highest content of anthocyanins was retained in the freeze-dried samples ('Regent') and room-dried samples ('Cabernet Sauvignon'). The observed differences could be attributed to the different anthocyanin's composition of the grape skins among investigated cultivars. Considering only the monoglucosides of 'Regent', the best drying method would be room-drying, but this cultivar contains more than 40% of diglucosides for which freeze-drying was a better drying method. Differences between the contents of anthocyanins in grape skins, dried by these methods, for both analyzed cultivars, were less than 5%. The minimum contents of anthocyanins were obtained in the oven-dried samples (Figure 1). It is well known that these molecules are extremely thermally unstable. Heating anthocyanins at temperatures higher than 50 °C could lead to their degradation (Cemeroglu et al., 1994; Tomaz et al., 2016). By comparison of the contents of individual monoglucosides relative to corresponding diglucosides, it was shown that diglucosides were significantly more thermally stable than the corresponding monoglucosides, This observation was in an agreement with numerous other studies (García-Viguera and Bridle, 1999; Martí et al., 2002). For example, loss of malvidin-3-*O*-glucoside content, during the oven-drying was 40% in relation to the freeze-dried ones, while the loss of malvidin-3,5-*O*-diglucoside was only 15%. Higher values were obtained in the anthocyanin content of the extracts of dried skins on the air or by freeze-drying in comparison to extracts of the fresh skins, which may be due to changes in the structure of cell walls and membranes which were occurred during drying. The freeze-drying process leads to the formation of ice crystals in the plant tissue that could cause perforation of the cell structure and cell walls, which in turn could significantly facilitate the release of compounds from inside the cell in the bulk extraction solvent (Vashisth et al., 2011). Generally, the drying process leads to the loss of the selective permeability and denaturation of the membranes and to an increase of the porosity of the plant tissues which could also improve the extraction of the compounds contained in the interior of the cells (Garcia-Perez et al., 2010; Raynal at al., 1989).

The highest contents of gallic acid and protocatechuic acid were obtained in oven-dried samples, but this value must be taken with extreme caution. The very high content of protocatechuic acid may be due to the thermal decomposition of cyanidin-3-*O*-glucoside. Some research had shown that one of the degradation products of cyanidin-3-*O*-glucoside is protocatechuic acid (de Ferrars et al., 2014; Seeram et al., 2001).

Table 2. Effect of different sample pretreatments on composition and content of grape skins obtained from 'Regent' and 'Cabernet Sauvignon'. Results are expressed in mg/kg of d.w. skins

Compound	'Regent'				'Cabernet Sauvignon'			
	Fresh	Freeze-drying method	Room-drying method	Oven-drying method	Fresh	Freeze-drying method	Room-drying method	Oven-drying method
Delphinidin-3,5-*O*-diglucoside	1440.15^{b}	1537.79^{a}	1560.97^{a}	1516.59^{a,b}	n.d.	n.d.	n.d.	n.d.
Cyanidin-3,5- *O*-diglucoside	1061.13^{a}	1032.65^{b}	1026.35^{b,c}	1015.54^{c}	n.d.	n.d.	n.d.	n.d.
Delphinidin-3- *O*-glucoside	9751.65^{b}	10976.59^{a}	10981.34^{a}	7082.74^{c}	6561.12^{b}	7258.92^{a}	7182.92^{a,b}	1874.80^{c}
Peonidin-3,5- *O*-diglucoside	1284.36^{b}	1337.09^{a}	1283.19^{c}	953.56^{c}	n.d.	n.d.	n.d.	n.d.
Malvidin-3,5- *O*-diglucoside	10039.09^{c}	11799.73^{a}	11295.17^{b}	10024.87^{c}	n.d.	n.d.	n.d.	n.d.
Cyanidin-3- *O*-glucoside	2198.79^{b}	2376.41^{a}	2356.17^{a}	1332.65^{a}	605.67^{b}	661.24^{a}	651.00^{a,b}	136.99^{c}
Petunidin-3- *O*-glucoside	n.d.	n.d.	n.d.	n.d.	2509.66^{b}	2811.30^{a}	2834.12^{a}	687.39^{c}
Peonidin-3- *O*-glucoside	548.62^{b}	601.73^{a}	590.76^{a}	287.11^{c}	536.72^{b}	557.30^{a,b}	579.71^{a}	119.18^{c}
Malvidin -3- *O*-glucoside	7053.46^{b}	7783.57^{a}	7749.41^{a}	4652.20^{c}	8416.71^{b}	8220.02^{c}	9220.30^{a}	2264.16^{d}
Myricetin-3- *O*-glucoside	667.22^{b}	625.60^{c}	624.16^{c}	741.55^{a}	287.53^{b}	313.98^{a}	293.95^{c}	326.47^{d}
Rutin	150.34^{a}	145.14^{a}	143.33^{a}	175.08^{a}	53.50^{b}	56.87^{a}	58.96^{c}	55.34^{c}
Quercetin-3-*O*-glucuronide	118.68^{a}	80.97^{d}	87.51^{c}	114.43^{b}	67.32^{c}	95.02^{a}	94.29^{b}	65.67^{c}
Quercetin-3- *O*-glucoside	1353.12^{a}	1225.31^{b}	1218.99^{b}	1340.80^{a}	597.96^{d}	799.42^{a}	755.01^{b}	898.75^{c}
Kaempferol-3-*O*-glucuronide	44.63^{b}	35.12^{c}	31.59^{c}	54.80^{a}	37.97^{c}	53.46^{a}	47.37^{b}	35.16^{c}
Isorhamnetin-3- *O*-glucoside	70.43^{d}	102.13^{c}	109.88^{b}	156.38^{a}	38.83^{c}	49.03^{c}	72.59^{c}	48.24^{b}
Gallocatechin	55.85^{c}	70.38^{a,b}	66.30^{a,b}	70.94^{a}	12.65^{a}	13.25^{a}	12.05^{a}	12.85^{a}
Procyanidin B1	70.61^{b}	83.72^{a}	57.82^{c}	18.73^{d}	0.00^{c}	30.07^{a}	31.57^{a}	11.45^{b}
Epigallocatechin	34.03^{a}	36.51^{a}	37.38^{a}	35.90^{a}	134.06^{a}	138.34^{a}	113.42^{b}	37.44^{c}
Catechin	52.50^{a}	51.98^{a}	38.43^{c}	38.65^{c}	92.92^{a}	87.84^{a}	73.28^{b}	37.48^{c}
Procyanidin B2	11.58^{b}	54.45^{a}	30.57^{a,b}	31.30^{a,b}	0.00^{b}	25.29^{a}	24.23^{a}	20.64^{a}
Epicatechin	35.83^{a}	32.05^{a}	21.58^{b}	19.84^{b}	34.21^{a}	31.43^{a}	28.74^{a}	19.11^{b}
Caftaric acid	255.61^{d}	417.35^{b}	389.90^{c}	595.22^{a}	40.77^{c}	157.94^{b}	158.03^{b}	190.03^{a}
Caffeic acid	54.46^{a}	42.55^{c}	43.64^{c}	48.28^{b}	34.56^{d}	68.08^{b}	49.17^{c}	91.11^{a}
Coutaric acid	204.85^{d}	382.32^{b}	375.48^{c}	591.89^{a}	72.73^{c}	168.21^{b}	166.00^{b}	198.70^{a}
Coumaric acid	23.92^{a}	24.89^{a}	23.49^{a}	17.70^{b}	0.00^{d}	14.38^{b}	12.22^{c}	19.95^{a}
Fertaric acid	31.58^{b,c}	38.38^{a}	34.03^{b}	29.09^{c}	0.00^{b}	7.02^{a}	6.60^{a}	6.15^{a}
Sinapic acid	24.65^{a}	20.00^{b}	17.27^{c}	21.14^{b}	35.03^{a}	0.00^{b}	0.00^{b}	38.94^{a}
Gallic acid	0.00^{c}	11.91^{b}	12.92^{b}	58.06^{a}	15.55^{a}	15.04^{a}	10.51^{a}	23.68^{a}
Protocatechuic acid	40.81^{c}	78.50^{b}	25.96^{c}	851.57^{a}	0.00^{c}	39.06^{b}	25.90^{b}	207.22^{a}
trans-piceid	168.92^{c}	219.91^{b}	221.64^{a,b}	231.52^{a}	48.27^{c}	68.34^{c}	74.33^{a}	79.36^{a}

Contents expressed as mean ± standard deviation (n = 3). Means with different superscript letters, for each variety separately, in the same row differ significantly (P≤0.05)

Considering the above explanation, the most appropriate method for drying grape skin with maximum retention of hydroxybenzoic acids was freeze-drying (Figure 1).

Flavonol glycosides and stilbenes

Flavonols are the second most abundant group of polyphenols contained in the grape skins. Contents of individual flavonol glycosides and stilbenes in grape skin samples subjected to different drying methods were determined (Table 2). The best drying method for grape skins obtained from both cultivars was oven-drying. It is presumed that this result may be attributed to the relatively short period of drying at a higher temperature (60 °C), which significantly reduces the possibility of oxidation and thus degradation of flavonol glycosides. Grapes contain enzymes that belong to a large group of *β*-glycosidases. These enzymes can be present as part of the genome of the grape or may arise from wild yeasts naturally present on grape berries (Belancic et al., 2003;

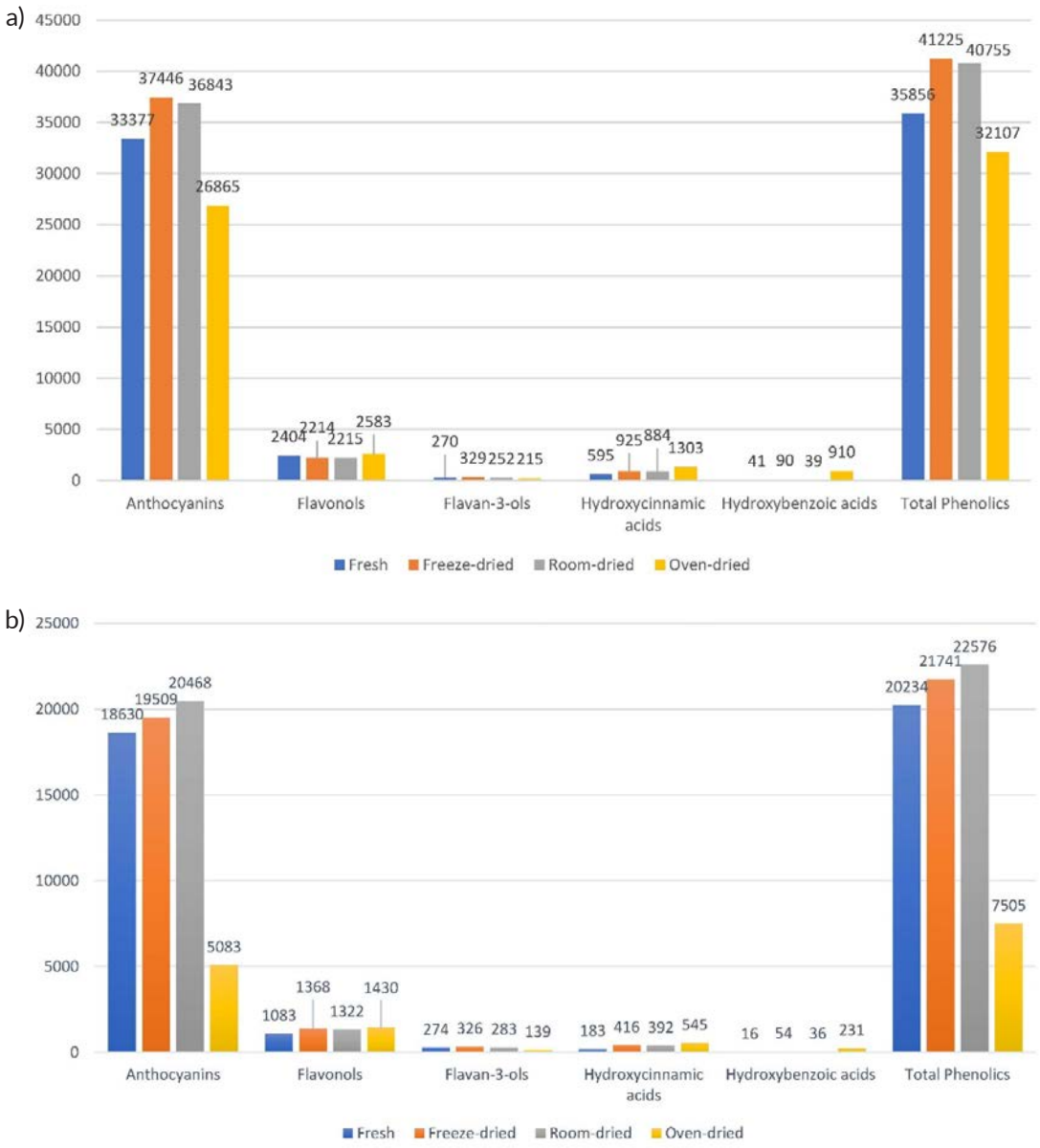

Figure 1. Content of total anthocyanins, flavonol, flavan-3-ols, phenolic acids and phenolicis in a) 'Regent' and b) 'Cabernet Sauvignon'. Results are expressed in mg/kg of d.w. skins

Ümit Ünal et al., 2014). They cleave *β*-glycoside bonds thus they can cleave sugar moiety linked to the flavonol aglycon. Denaturation and complete inactivation of most enzymes occur at temperatures above 50 °C. Contents of individual and total flavonols after room-drying and freeze-drying were not statistically significant. Among stilbenes, grape skins contain *trans*-piceid (trans-resveratrol-glucoside). Room-drying, as well as oven-drying, retained the largest amount of this compound. A somewhat smaller content was observed in the freeze-dried samples.

Flavan-3-ols

The content of all individual flavan-3-ols was the highest in freeze-dried grape skins samples, while the lowest content was determined in the oven-dried ones (Table 2). Using the oven-drying method, extremely low content of procyanidin B1 was obtained. By comparing fresh samples with room-dried ones, no statistically significant differences in the total content of flavan-3-ols were observed. Significant differences were observed only in the case of catechin. The content of catechin in 'Regent' room-dried skins in comparison with its content in the fresh or the freeze-dried samples was lower by approximately 26%. In the case of 'Cabernet Sauvignon', these differences were somewhat smaller (approximately 20%). Such observation could be attributed to the activity of the enzyme polyphenol oxidase (PPO). This enzyme has the ability to oxidize mono- and di-phenols, and thus the catechin (Lee et al., 1983). The properties of PPO greatly depend on the cultivar from which it was isolated (Lee et al., 1983; Rapeanu et al., 2006; Ünal and Şener, 2006; Valero et al., 1988). According to our results, it appears that PPO from 'Regent' had a greater affinity for the catechin than the PPO from 'Cabernet Sauvignon'.

Hydroxycinnamic acids

Table 3 shows the contents of individual hydroxycinnamic acids after subjecting grape skins to various drying treatments. The obtained results demonstrate that the best drying method was oven-drying, followed by freeze-drying, while a smaller content of these compounds was determined in the room-dried samples. Enzyme PPO, as mentioned above, is naturally contained in the grape skins. This enzyme causes enzymatic oxidation of many polyphenolic compounds and is considered to have the greatest affinity toward the hydroxycinnamic acids (Lee et al., 1983; Rapeanu et al., 2006; Ünal and Şener, 2006). As with most other enzymes, its optimum activity is at temperatures below 40 °C, while the higher temperatures lead to its denaturation and complete inactivation. Consequently, the obtained results could be explained by the activity of PPO. Oven-drying was carried out at a temperature of 60 °C for 18 hours, which led to enzyme denaturation and thus to its irreversible inactivation. In the case of room-drying in a period of seven days, this enzyme had optimum activity and therefore the lowest content of these acids was obtained in these samples. During the freeze-drying process, the low temperature set at the beginning of the process causes temporary deactivation of this enzyme, but subsequent temperature increase led to the reactivation of PPO, and it was again able to oxidize the analyzed compounds. Especially low contents were obtained for caftaric and coutaric acid which are the most favorable substrates for PPO. Besides the activity of PPO, the low contents of these acids in freeze-dried samples could be attributed to the formation of ice crystals in the cell walls structure. Some of the previously conducted studies suggested that hydroxycinnamic acids could be bound to the cell wall structures, thus during the formation of ice crystals, their degradation could occur due to the breakdown of cellular constitutes and covalent bonds (Acosta-Estrada et al., 2014).

Total Phenolics

The total polyphenols content was expressed as the sum of the contents of the individual compounds. Considering all analyzed compounds and ignoring the effect of drying methods on the individual compounds, in the case of 'Regent' the most appropriate drying-method was freeze-drying. The differences in the contents of the freeze-dried and the room-dried samples were not large (less than 2%). The preferred drying method in the

case of 'Cabernet Sauvignon' was room-drying, followed by freeze-drying. The differences in the polyphenol contents of room-dried and freeze-dried samples were approximately 4%. The observed differences between cultivars may be attributed to the fact that 'Regent' contains a large amount of anthocyanins-diglucoside.

Stability of dry grape skins

After being dried and milled, grape skin powders were stored in amber glass vials equipped with PTFE caps. Vials with the samples were left in a dark place at a temperature of 25 °C for 6 months. The main purposes of these experiments were to establish stability during storage of grape skins submitted to the different drying methods. For almost all biological samples, including raisins, dry cranberries, dry pineapple etc. the most appropriate storage conditions are in dry and dark places. Because plastic containers can release some compounds such as phthalates as a contaminant to the samples, glass containers equipped with extremely stabile PTFE were chosen.

Tables 3 and 4 show the contents of the individual polyphenol compounds evaluated after 6 months of storage. The percentage remaining after storage was determined by the dividing content determined after 6 months of storage with those evaluated immediately after drying. The storage period had a significant effect on the content of individual polyphenols in the skins dried by different methods. Among the three different drying methods, the oven-dried samples were the most stable. In these samples, the contents of individual anthocyanins of 'Regent' remained almost unchanged. The largest decrease was observed in the content of peonidin-3-O-glucoside and it was less than 3%. The content of anthocyanins in the oven-dried skins of 'Cabernet Sauvignon' remained virtually unchanged. The total content of flavonol-glycosides in the oven-dried skins of both varieties also remained virtually unchanged. The most significant decrease of the content of the oven-dried samples was observed in the case of flavan-3-ol of 'Cabernet Sauvignon' and especially, in the case of catechin, and procyanidin B2.

The maximum decrease in the content of individual polyphenols was observed in room-dried samples. The greatest decrease in the content was observed for anthocyanins. When comparing the reduction of the corresponding monoglucoside with those of the corresponding diglucoside, a decrease of diglucoside was smaller, and this confirms that they are more stable than monoglucosides. During room-drying there was no denaturation of the enzyme PPO and it was able to continue the oxidation of suitable polyphenols. This enzyme does not directly act on the glycosylated forms of polyphenols. Anthocyanins could have been degraded by the mechanism of coupled oxidation (Yokotsuka and Singleton, 1997). Enzyme *β*-glycosidase could lead to cleavage of glycoside bonds and formation of aglycons, which are significantly less stable than anthocyanins. Among anthocyanins, in the room-dried samples, peonidin-3-*O*-glucoside was the least stable. The remaining content of this anthocyanin was 44.85 and 26.24% for 'Regent' and 'Cabernet Sauvignon', respectively. Among the analyzed compounds, the greatest stability in room-dried samples was exhibited by flavonol glycosides. A significant reduction of hydroxycinnamic acids could be attributed to the activity of the enzyme PPO. Depending on the cultivar from which it was isolated, this enzyme had the maximum activity at the temperatures of around 15 °C, but it retained much of its activity at 25 °C. A significant increase in the content of protocatechuic acid was observed. This observation could be attributed to the degradation of cyanidin-3-*O*-glucoside. The decrease in the content of trans-piceid may be due to the activity of the enzyme *β*-glycosidase. In these samples, an increase in the content of trans-resveratrol was observed (data not shown).

The freeze-dried samples of grape skins showed moderate stability. The remaining content of anthocyanin after 6 months of storage was in the range from 92.84 to 86.90% for 'Regent' and 'Cabernet Sauvignon', respectively. As in the case of room-drying, decreases of diglucosides were less than that of corresponding monoglucosides. The results showed the smallest change in the content of flavonol glycoside for both tested

Table 3. Stability of polyphenolic compounds from 'Regent' grape skins during 6 months of storage. Results are expressed in mg/kg d.w. of grape skins

Compound	Oven		Ambient Air		Freeze dry	
	Content	Remaining %	Content	Remaining %	Content	Remaining %
Delphinidin-3,5-*O*-diglucoside	1475.04	97.26	1036.81	66.42	1390.93	90.45
Cyanidin-3,5- *O*-diglucoside	1003.56	98.82	760.84	74.13	920.19	89.11
Delphinidin-3- *O*-glucoside	7008.37	98.95	7070.93	64.39	9240.09	84.18
Peonidin-3,5- *O*-diglucoside	942.59	98.85	660.76	51.49	1232.03	92.14
Malvidin-3,5- *O*-diglucoside	10000.81	99.76	7748.00	68.60	10240.53	86.79
Cyanidin-3- *O*-glucoside	1309.99	98.30	1399.55	59.40	1892.00	80.30
Peonidin-3- *O*-glucoside	278.64	97.05	251.50	44.85	483.71	80.39
Malvidin -3- *O*-glucoside	4641.97	99.78	3985.71	51.43	6272.43	80.59
TOTAL ANTHOCYANINS	**26660.97**	**99.24**	**22914.10**	**62.24**	**31671.81**	**92.84**
Myricetin-3- *O*-glucoside	736.38	99.30	623.89	99.96	619.42	99.01
Rutin	174.73	99.80	129.89	90.62	144.37	99.47
Quercetin-3-*O*-glucuronide	113.87	99.51	86.65	99.02	80.50	99.41
Quercetin-3- *O*-glucoside	1331.81	99.33	1165.91	95.65	1160.47	94.71
Kaempferol-3-*O*-glucuronide	54.37	99.22	31.15	98.62	29.31	83.45
Isorhamnetin-3- *O*-glucoside	156.14	99.85	104.57	95.17	93.39	91.45
TOTAL FLAVONOLS	**2571.31**	**99.55**	**2142.06**	**96.69**	**2133.46**	**96.35**
Gallocatechin	70.46	99.32	59.34	89.51	69.92	99.35
Procyanidin B1	18.16	96.94	45.75	79.13	57.25	68.38
Epigallocatechin	35.37	98.52	35.02	93.70	36.12	98.92
Catechin	38.48	99.57	23.81	61.96	41.25	79.99
Procyanidin B2	29.72	94.94	27.06	88.52	28.21	51.81
Epicatechin	17.95	90.44	9.36	43.36	25.18	78.57
TOTAL FLAVAN-3-OLS	**215.14**	**99.89**	**200.35**	**79.48**	**257.94**	**78.46**
Caftaric acid	595.21	100.00	311.02	79.77	286.83	68.73
Caffeic acid	47.50	98.38	29.90	68.53	24.55	57.67
Coutaric acid	591.82	99.99	244.19	59.66	228.83	59.85
Coumaric acid	17.45	98.59	15.63	66.53	14.18	57.15
Fertaric acid	28.77	98.92	18.93	55.60	16.61	43.27
Sinapic acid	20.38	96.39	9.42	54.54	11.24	56.22
TOTAL HYDROXYCINNAMIC ACIDS	**1301.13**	**99.83**	**629.10**	**71.18**	**582.25**	**62.91**
Gallic acid	40.19	69.22	14.54	112.51	17.75	148.99
Protocatechuic acid	863.91	101.45	29.28	112.80	106.83	136.07
TOTAL HYDROXYBENZOIC ACIDS	**904.09**	**99.39**	**43.82**	**112.71**	**124.58**	**137.77**
trans-piceid	231.23	99.88	125.06	55.52	165.83	74.82
TOTAL PHENOLICS	**31884**	**99.31**	**26052**	**63.97**	**35899**	**87.08**

Table 4. Stability of polyphenolic compounds from 'Cabernet Sauvignon' grape skins during 6 months of storage. Results are expressed in mg/kg d.w. of grape skins

Compound	Oven		Ambient Air		Freeze dry	
	Content	Remaining %	Content	Remaining %	Content	Remaining %
Delphinidin-3-O-glucoside	1823.70	97.27	4008.93	55.81	6061.50	83.50
Cyanidin-3-O-glucoside	134.57	98.23	303.31	46.59	613.41	92.77
Petunidin -3-O-glucoside	668.96	97.32	1596.66	56.34	2384.87	84.83
Peonidin-3-O-glucoside	117.11	98.27	152.10	26.24	465.82	83.58
Malvidin -3-O-glucoside	2353.44	99.53	3849.04	41.75	7428.26	90.37
TOTAL ANTHOCYANINS	**5097.78**	**99.91**	**9910.02**	**48.42**	**16953.86**	**86.90**
Myricetin-3-O-glucoside	208.33	91.99	216.66	79.09	233.25	74.29
Rutin	34.79	98.44	45.45	90.99	42.50	74.74
Quercetin-3-O-glucuronide	69.63	98.41	77.77	92.26	88.14	92.76
Quercetin-3-O-glucoside	682.88	97.73	761.35	99.91	709.65	88.77
Kaempferol-3-O-glucuronide	44.25	97.41	43.02	90.83	42.36	79.24
Isorhamnetin-3-O-glucoside	49.26	99.83	46.08	63.48	39.07	79.67
TOTAL FLAVONOLS	**1089.14**	**98.15**	**1174.59**	**92.33**	**1154.96**	**84.44**
Gallocatechin	12.96	99.25	10.75	89.26	15.93	97.58
Procyanidin B1	11.59	99.42	31.30	99.16	7.32	72.75
Epigallocatechin	32.27	86.21	49.59	59.45	55.72	56.66
Catechin	30.79	82.14	36.68	35.51	83.18	94.69
Procyanidin B2	17.27	83.68	20.41	84.22	22.72	89.84
Epicatechin	17.61	92.15	6.81	23.68	14.09	65.73
TOTAL FLAVAN-3-OLS	**122.48**	**88.13**	**155.54**	**54.90**	**198.96**	**77.65**
Caftaric acid	186.57	98.19	114.88	72.70	104.83	66.37
Caffeic acid	90.37	99.19	31.51	64.07	24.96	36.67
Coutaric acid	197.23	99.26	66.35	39.97	75.00	44.59
Coumaric acid	18.87	94.57	0.00	0.00	0.00	0.00
Fertaric acid	6.01	97.65	0.00	0.00	0.00	0.00
Sinapic acid	38.44	98.71	0.00	0.00	0.00	0.00
TOTAL HYDROXYCINNAMIC ACIDS	**537.49**	**98.64**	**212.74**	**54.27**	**204.79**	**49.27**
Gallic acid	26.60	112.35	15.32	145.72	2.43	16.18
Protocatechuic acid	1240.07	102.72	29.01	111.98	40.20	102.92
TOTAL HYDROXYBENZOIC ACIDS	**1266.67**	**102.91**	**44.33**	**121.72**	**42.64**	**78.80**
trans-piceid	75.05	99.20	35.67	47.98	55.96	81.89
TOTAL PHENOLICS	**8199**	**100**	**11532**	**51.20**	**18595**	**85.81**

varieties. Compared with the room-dried samples, the content of hydroxycinnamic acid in the freeze-dried samples decreased to a greater extent. The observed increase in the content of protocatechuic acid was correlated with a decrease in the content of cyanidin-3-*O*-glucoside. Generally, in the case of freeze-dried samples, the highest contents of total polyphenols were retained.

CONCLUSION

This study demonstrates that the use of different drying methods significantly affects the content of polyphenols in grape skins extracts. The effects of various drying methods on individual polyphenols were observed, which could be attributed to their different physicochemical properties and their position within the cell of grape skins. The highest content of anthocyanins-diglucoside was preserved using freeze-drying, while the highest content of anthocyanins-monoglucoside was preserved by room-drying. The highest content of flavonol glycosides, hydroxycinnamic acids, and stilbene was determined in the oven-dried samples. The highest content of flavan-3-ols was preserved in freeze-dried samples. Considering all polyphenolic compounds, the most appropriate drying method for 'Regent' was freeze-drying, while for 'Cabernet Sauvignon' room-drying was more appropriate. Differences between polyphenol contents of dried grape skins obtained by these two methods are not significant and it can be concluded that room-drying could be a good drying method. In comparison with freeze-drying, room-drying is significantly less expensive and could be used for commercial application of drying large quantities of grape skins. The stability during storage of dry grape skins greatly depended on the applied drying method. The greatest stability was observed in the oven-dried samples. The content of polyphenolic compounds in the oven-dried samples after 6 months of storage was almost the same as those in the samples analyzed immediately after drying. The greatest rate of degradation was observed in room-dried samples, while it was moderate in the freeze-dried samples. After 6 months of storage in the freeze-dried ones of both cultivars, the highest contents of nearly all analyzed polyphenolic compounds were observed, thus this observation could give a minimal durability of the dry grape skins of about 6 months. Future research should consider the evaluation of examined drying methods for both raisins and sweet wine production. There are many different grape varieties that require adjustment of different drying methods and protocols for obtaining high-quality products. Overall, the preservation of polyphenolic compounds during the drying is important as these compounds are strong antioxidants with many other health benefits.

ACKNOWLEDGEMENT

This research has been supported by the project KK.01.1.1.01.0005 Biodiversity and Molecular Plant Breeding, Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia.

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