Journal of Food Composition and Analysis 62 (2017) 211–216

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original research article

Differentiation between Croatian dessert wine Prošek and dry wines based on phenolic composition

Irena Budić-Leto[a,*], Goran Zdunić[a], Jasenka Gajdoš-Kljusurić[b], Ana Mucalo[a], Urška Vrhovšek[c]

[a] Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, 21 000 Split, Croatia
[b] University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia
[c] Research and Innovation Center, Edmund Mach (FEM), Food Quality and Nutrition Department, via E. Mach, 1, 38010 San Michele all'Adige, Italy

ARTICLE INFO

Keywords:
Food composition
Food analysis
Vitis vinifera
Grape drying
Dessert wine
Prošek
Phenolic composition
UPLC/MS/MS
PCA

ABSTRACT

The phenolic composition of the Croatian dessert wine Prošek and dry wines Plavac mali and Pošip produced from the same autochthonous cultivars was investigated to determine which phenolic compounds best discriminate between these wine types. The wines were analyzed by the targeted metabolomic method using UPLC/QqQ-MS/MS. Forty-five (45) phenolic compounds were identified and classified into five groups based on chemical structure: benzoic acid derivatives, cinnamic acid derivatives, flavan-3-ols, stilbenes and flavonols. ANOVA indicated that the grape-drying process heavily influences the complex phenolic composition of Prošek dessert wine, which differs significantly from dry wines produced from the same cultivars. The data was grouped by principal component analysis and linear discriminant analysis to derive a classification function that distinguished dry and dessert wines with 98% accuracy. Principal component analysis separated the samples and showed that 23 phenolic compounds depending to phenolic acids, phenolic aldehydes, flavan-3-ols and flavonols were the compounds that best differentiated the Prošek from the dry wines.

1. Introduction

Recent research has focused on plant bioactive compounds with potential beneficial effects on human health. Phenolic compounds may have the potential to naturally prevent some major diseases such as cancer, cardiovascular and neurodegenerative diseases like Parkinson's and Alzheimer's (Aguilera et al., 2016; Rangel-Huerta et al., 2015; Rodriguez-Mateos et al., 2014; Stefani and Rigacci, 2014). Wine is a rich source of dietary phenols, particularly red wine. Wine contains different classes of flavonoid and non-flavonoid phenolic compounds originating from grapes (Mattivi et al., 2006). The phenolic composition of a wine depends on grape variety, ripeness, cultivation system, sunlight exposure and UV radiation, winemaking process and phenolic reactivity during winemaking and ageing (Lorrain et al., 2011; Bindon et al., 2013; Fulcrand et al., 2006; Song et al., 2015).

Traditional sweet dessert wines produced in winemaking regions where grapes are dried to naturally concentrate sugars are rich in phenolic compounds and demonstrate interesting antioxidant activity (Moreno et al., 2008; Loizzo et al., 2013). The dessert wine Prošek is produced exclusively on the Mediterranean coast of Croatia (Dalmatia) and can be made only from autochthonous cultivars. An important step in Prošek production is the dehydration of grapes prior to vinification by sun drying or under controlled conditions with the temperature below 40 °C. Dehydration increases the sugar concentration and substantially modifies the chemical composition of phenolics due to changes in concentration, chemical modification or degradation (López de Lerma et al., 2014; Sarratosa et al., 2008; De Torres et al., 2010). Water evaporation can cause grape skin deterioration that allows phenolic compounds to migrate from the skin to the pulp, increasing the pulp concentration (Panceri et al., 2013). However, phenols present in grape juice can participate in reactions of enzymatic oxidation and non-enzymatic browning, reducing their concentration (Serratosa et al., 2008).

Recently, attention in Croatia has focused on enological and overall evaluation of wines from native cultivars. Phenolic compounds play an important role in understanding the unique qualities of wines produced from native cultivars (Tamborra et al., 2003; Letaief et al., 2007). Phenolic characterization has highlighted the contribution of these compounds to the structure and overall quality of wine. Total phenolic content has been studied in several commercial Croatian wine samples (Piljac et al., 2005), and several studies have examined flavonoids, phenolic acids, anthocyanins and resveratrol (Herjavec et al., 2007; Rastija et al., 2009; Vinković Vrček et al., 2011). However, none of these studies provided a detailed phenolic profile of Prošek dessert

* Corresponding author.
E-mail address: irena.budic-leto@krs.hr (I. Budić-Leto).

http://dx.doi.org/10.1016/j.jfca.2017.05.015
Received 18 March 2016; Received in revised form 5 July 2016; Accepted 30 May 2017
Available online 15 June 2017

Table 1
List of the wine samples produced in three biological replicates.

Variety	Wine type	Vintage	Wine color	Maceration
Plavac mali	dessert	2007	red	+
Plavac mali	dessert	2008	red	+
Pošip	dessert	2008	white	–
Plavac mali	dry	2007	red	+
Pošip	dry	2010	white	–
Plavac mali	dry	2011	red	+

wines. The objectives of this work were (i) to identify and quantify multiple classes of phenolics in Prošek sweet wines using targeted metabolomics methodology (UPLC/QqQ-MS/MS); and (ii) to differentiate the dessert wine Prošek from dry wines made from the same autochthonous cultivars based on their phenolic composition (Villiers et al., 2005). To our knowledge, this in the first comprehensive study on the polyphenol composition of Prošek dessert wines encompassing ANOVA and PCA to classify wine types based on the phenolic composition.

2. Material and methods

2.1. Wine samples

Experimental dry wines Plavac mali of vintages 2007 and 2011 and Pošip (2010) (Table 1) from the principal Croatian autochthonous cultivars, red Plavac mali and white Pošip, were selected. All wines were made from grape samples from the germplasm collection at the Institute for Adriatic Crops and Karst Reclamation in Split in the wine-producing area of Croatia, Dalmatia. The wines were produced using micro-scale vinification in three biological replicates as part of an evaluation of the enological properties of autochthonous cultivars maintained in the germplasm collection at IAC. The original technology for making Prošek wine involves drying the grapes, but commercial wines produced by different methods are currently on the Croatian market. To equalize the production conditions of samples and facilitate direct comparison, we produced (in IAC) experimental Prošek dessert wines from both Plavac mali and Pošip grape samples from the wine-producing area of Dalmatia (Pelješac and Korčula, respectively; more details about grape sampling and experimental winemaking are included in online Supplementary information 1).

2.2. Experimental winemaking

Table 1 presents the list of wine samples produced for this study; the process is described below.

2.2.1. Prošek dessert wines

Approximately 400 kg of grapes were dried in a greenhouse equipped with a system for temperature control and ventilation (maximum daily temperature 40 °C) until the berries reached ~ 32 Brix. The dried fruit was destemmed, crushed, sulfited and divided into three containers for alcoholic fermentation using the commercial yeast strain EC1118 (*Saccharomyces bayanus*, Lallemand Inc, Montreal, Quebec, Canada). Fermentation of Plavac mali was carried out with five days of skin contact, while fermentation of Pošip was done without skin contact. The pomace was pressed in a hydraulic press at < 2 bar. The must was put in 25 L glass vessels, where alcoholic fermentation continued. The first racking was done at ~ 30 days and the second six months after the beginning of fermentation. After the second racking, wines were sulfited to 50 mg/L free sulfur dioxide (SO_2) and bottled. The wines were stored at the experimental winery until analysis.

2.2.2. Dry wines

Approximately 50 kg of grapes was destemmed, crushed, sulfited to 25 mg/L free sulfur dioxide (SO_2) and stored in a 10 L stainless steel vat. Micro-scaled fermentations were carried out using the commercial strain EC1118 in three replicates. Red pomace from Plavac mali was punched down twice daily until it remained submerged during the six-day maceration. The wine was then pressed and transferred to a stainless tank at 20 °C. Fermentation of white Pošip grapes was done without skin contact. At the completion of fermentation, the wine was racked and sulfited to 50 mg/L (SO_2). Malolactic fermentation was not performed.

2.3. Ultra high-performance liquid chromatography

Samples were analyzed by targeted metabolomics using ultra high-performance liquid chromatography coupled with triple-quadrupole mass spectrometers (UPLC/QqQ-MS/MS). Methods for identifying and quantifying non-colored phenolic compounds were as described (Vrhovšek et al., 2012; Arapitsas et al., 2012). Wine was filtered with a 0.2 µm PTFE filter prior liquid chromatography with a Waters Acquity UPLC system (Miliford, MA, USA) coupled to a Waters Xevo TQMS in ESI ionization mode. The separation of the phenolic compounds was achieved on a Waters Acquity HSS T3 column 1.8 µm, 100 mm × 2.1 mm (Miliford, MA, USA) kept at 40 °C. In the end, 2 µL were injected in the instrument by an auto-sampler at the temperature of 6 °C. Data were processed by the Waters MassLynx 4.1 and Target Lynux software.

Basic chemical parameters were determined according to reference OIV methods for wine analysis (OIV, 2005).

2.4. Chemicals and reagents

Methanol and acetonitrile were of LC–MS grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid was also purchased from Sigma-Aldrich. Milli-Q water was used for the chromatography. The chemical standards were obtained from different suppliers or isolated as described in Vrhovšek et al., 2012.

2.5. Data analysis

Statistical analysis used average values of triplicate determinations. The data matrix was constructed from the analytical data obtained for wines, with rows representing wine samples (two varieties, two different wine types and four different vintage years – objects) and columns representing chemical measurements (45 phenolic compounds – variables). Autoscaling was used to produce variables with zero means and unit standard deviation (De Villiers et al., 2005). To establish which compounds differed significantly among wines, univariate characterization based on Fischer's weight (F) was conducted using one-way ANOVA. For multivariate analysis, factor analysis (FA) and principle component analysis (PCA) were used. FA and PCA present unsupervised pattern recognition techniques that seek to summarize and explain key feature of the data. Factorial analysis was used to reduce the initial set of 45 phenolic parameters to 23. Varimax rotation was performed on the reduced dataset to obtain maximum information from the extracted PCs. Linear discriminant analysis (LDA) was used to evaluate the efficiency of wine separation based on type. LDA is a supervised pattern recognition technique whit the task of inferring a function from labeled training data. The training data were wines types, including only the significant phenolic components, and the validation set was the starting data set of wines with all observed phenolic compounds included (see Supplementary material, Table S1 for all 45 phenolic parameters). All statistical data analysis was performed using STATISTICA, version 8.1 (Statsoft Inc., Tulsa, OK, USA).

Table 2
Significant differences and standard deviations between means for basic physical-chemical parameters of wines.

	Relative density	Alcohol	Total dry extract	Reducing sugars	Total acidity	Volatile acidity	pH	Ash
	(20/20 °C)	(% vol)	(g/L)	(g/L)	g/L (as tartaric acid)	g/L (as acetic acid)		(g/L)
dessert	1.0385 ± 0.038	15.3 ± 1.2	151 ± 94	110 ± 93	5.7 ± 1.0	1.0 ± 0.4	3.9 ± 0.28	4.2 ± 1.0
dry	0.9941 ± 0.002	13.2 ± 2.2	29.1 ± 8.9	3.1 ± 1.6	5.5 ± 0.9	0.5 ± 0.1	3.6 ± 0.39	3.3 ± 0.9
	ns	ns	*	*	ns	*	ns	ns

* – significant differences ($p < 0.05$); ns – not significant.

3. Results and discussion

3.1. Chemical composition of wines

The standard physical-chemical parameters of the wines were examined using ANOVA (Table 2). Dessert wines showed statistically greater total dry extract (152 ± 95 g/L), reducing sugars, (110 ± 93 g/L) and volatile acidity (1.0 ± 0.4 g/L) than the dry wines. The values for Prošek dessert wine are similar to Passito wines, where the alcohol concentrations were 14.2 ± 0.6 vol%, reducing sugars were 116 ± 4.5 g/L, total dry extracts were 151 ± 2.8 g/L and volatile acidities were 0.9 ± 0.1 g/L (Loizzo et al., 2013). These parameters vary according to the origin and processing technology of sweet wines, especially to the degree of dehydration of grape. Climate conditions (temperature, humidity) and the date of harvest are crucial factor for determine the sugar content and acidity level of grapes. Usually, a higher sugar content in dehydrate grape is always associated with a higher volatile acidity in sweet wine, as it was the case of the Prošek wine.

3.2. Phenolic composition of Prošek dessert wine and dry wines

Forty-five non-colored phenolic compounds from Prošek dessert wine were identified and quantified using targeted metabolomics based on the importance of the metabolite for wine quality, covering the major classes of low molecular weight phenolic compounds. Phenolic compounds were grouped into five classes on the basis of their chemical structure: benzoic acid derivatives (hydroxybenzoic acids (HBA) and phenolic aldehydes), cinnamic acid derivatives (hydroxycinnamic acids (HCA) and their esters), flavan-3-ols, stilbenes and flavonols (Table 3). There was wide variability in the phenolic composition and critical Fisher coefficients of 18 wine samples (Table 4). Significant differences in phenolic compounds were found by wine type, variety and vintage ($p < 0.05$). The expected large variability among varieties was confirmed for almost all phenolic compounds and vintages. The ANOVA results for the vintage showed that the reduction of significant phenolic compounds reduced from 45 to 34. The vintage showed no significant differences for following eleven compounds: PHBA, CA, FA, cfA, tPic, cPic, Ast, Iso, Phl, Ar and iPP.

The effect of variety was clearly evident for 27 phenolic compounds. However, only 11 of these were significantly different between dessert and dry wines: ellagic acid, *trans*-caftaric acid, *trans*-coutaric acid, epigalocatechin, gallocatechin, procyanins B2 + B4, kaempferol, quercetin, isorhamnetin, syringetin-3-glucoside + syringetin-3-galactoside and quercetin-3-glucuronide. The complete lack of kaempferol, myricetin, larticitrin, myricitrin and isorhamnetin in Pošip is probably due to berry color and consistent with a classification of red and white grape varieties based on six flavonol aglycones not found in white varieties (Mattivi et al., 2006).

The following discussion focuses on those phenolic compounds that significantly distinguish wine type (Table 4). Eight non-flavonoids (4-aminobenzoic acid, *p*-hydroxybenzoic acid, syringaldehyde, ellagic acid, *p*-coumaric acid, ferulic acid, *trans*-caftaric acid, and *trans*-coutaric acid) and 15 flavonoid compounds (catechin, epicatechin, epigallocatechin, gallocatechin, procyanidin B2 + B4, procyanidin B3, kampferol, quercetin isorhamnetin, syringetin-3-glucoside + syringetin-3-galactoside, quercetin-3-glucuronide, kaempferol-3-glucuronide, caffeic acid + catechin condensation, quercetin-3-gluconoride + quercetin-3-galactoside and quercetin-3,4-diglucoside) were extracted.

The low molecular weight flavan-3-ols are important compounds in red wines because their polymerization reactions lead to tannins, which

Table 3
Phenolic classes of 45 compounds observed in wine samples with their abbreviations.

Phenolic group	Sample	Abbreviation
Phenolic acid-HBA	anthranilic acid	ANA
	4-aminobenzoic acid	PABA
	p-hydroxybenzoic acid	PHBA
	vanillic acid	VA
	gallic acid	GA
	ellagic acid	EA
Phenolic acid-HCA	*p*-coumaric acid	pCA
	caffeic acid	CA
	ferulic acid	FA
	trans-caftaric acid	cfA
	trans-fertaric acid	ferA
	trans-coutaric acid	t couA
Phenolic aldehyde	vanillin	V
	syringaldehyde	SYAL
Stilbenes	*trans*-piceide	tPic
	cis-piceide	cPic
	astringin	Ast
	isorhapontin	Iso
	pallidol	Pal
	isohopeaphenol	iPP
Flavan-3-ols	catechin	Cat
	epicatechin	EC
	epigallocatechin	EGC
	gallocatechin	GC
	procyanidin B1	B1
	procyanidin B2 + B4	B2B4
	procyanidin B3	B3
	caffeic acid + catechin condensation	CA CC
Hydroquinone	arbutin	Ar
Flavonols	kaempferol	Ka
	quercetin	Q
	taxifolin	T
	isorhamnetin	IS
	myricetin	My
	laricitrin	Lar
	syringetin	Syr
	quercetin-3-rhamnoside	Q 3 Rha
	syringetin-3-glucoside + syringetin-3-galactoside	syr 3 Glc
	quercetin-3-gluconoride	Q3G
	kaempferol-3-glucuronide	Ka_3G
	myricitrin	Myr
	quercetin-3-glucoside + quercetin-3-galactoside	que 3 glc
	isorhamnetin-3-glucoside	iso 3 Glc
	quercetin-3,4-diglucoside	Q 3 4 diG
Dehydroflavonol	phlorizn	Phl

Table 4
ANOVA results and mean values of phenolic compounds observed in different wine samples (two varieties, two wine types, and four vintages).

	Variety			Wine type			Vintage				
Compounds	PM	P	F_{cal}	dessert	dry	F_{cal}	2007	2008	2010	2011	F_{cal}
ANA	0.002	0.001	0.033	0.001	0.002	2.961	n.d.	0.001	0.002	0.004	7.157*
PABA	0.002	0.001	2.916	0.001	0.002	5.227*	0.001	0.001	0.001	0.004	10.150*
PHBA	0.191	0.146	0.621	0.235	0.117	6.374*	0.176	0.212	0.037	0.243	2.636
V	0.025	0.017	1.133	0.018	0.027	1.782	0.016	0.023	0.009	0.050	16.899*
VA	0.390	0.067	45.211*	0.345	0.219	2.316	0.344	0.281	0.029	0.415	4.214*
GA	3.705	0.036	24.377*	1.787	3.178	1.731	3.532	0.783	0.073	6.192	58.919*
SYAL	0.054	0.020	2.228	0.020	0.066	5.417*	0.031	0.022	0.011	0.141	58.982*
EA	0.319	n.d.	3.743*	0.047	0.379	4.802*	0.173	n.d.	n.d.	0.930	49.082*
pCA	0.229	0.267	0.153	0.404	0.079	54.645*	0.241	0.396	0.058	0.117	4.217*
CA	0.465	0.367	0.602	0.423	0.441	0.022	0.363	0.427	0.638	0.375	0.872
FA	0.049	0.089	1.398	0.097	0.027	6.154*	0.038	0.112	0.034	0.039	1.824
cfA	9.670	2.356	27.924*	5.192	9.272	4.579*	7.903	5.234	4.398	12.720	3.369
ferA	1.928	1.732	1.795	1.809	1.917	0.577	1.741	1.886	1.569	2.353	11.362*
t_couA	6.198	1.227	11.017*	2.672	6.410	5.601*	5.303	3.398	0.511	9.332	5.296*
tPic	0.864	0.020	10.711*	0.602	0.564	0.015	0.367	0.847	0.023	1.048	2.214
cPic	2.832	0.046	10.968*	1.825	1.982	0.023	1.122	2.686	0.069	3.734	2.625
Ast	0.262	0.009	9.647*	0.220	0.135	0.797	0.111	0.277	0.018	0.270	1.778
Iso	0.235	n.d.	9.933*	0.208	0.105	1.433	0.141	0.259	0.000	0.141	1.474
Phl	0.215	0.030	13.808*	0.103	0.204	2.946	0.172	0.137	0.025	0.276	2.298
Cat	2.389	0.442	4.218	0.211	3.268	22.004*	1.954	0.297	0.885	5.053	9.041*
EC	0.647	0.072	4.239	0.029	0.883	17.227*	0.565	0.043	0.144	1.373	7.540*
EGC	0.170	0.009	5.290*	0.012	0.221	14.409*	0.175	0.017	0.018	0.296	4.588*
GC	1.163	0.029	6.425*	0.110	1.461	13.481*	1.225	0.160	0.058	1.885	4.404*
B1	4.717	0.013	5.221*	0.016	6.282	15.453	4.686	0.024	0.027	9.448	7.443*
B2B4	1.266	0.005	4.531*	0.007	1.685	12.552*	1.028	0.010	0.010	2.988	11.029*
B3	0.382	0.001	4.068	0.006	0.504	10.174*	0.271	0.009	0.001	0.969	12.414*
Ka	0.010	n.d.	6.023*	0.002	0.011	7.731*	0.013	n.d.	n.d.	0.012	6.122*
Q	0.176	0.002	12.402*	0.053	0.183	5.991*	0.228	0.007	0.000	0.238	24.672*
T	1.736	0.096	30.495*	0.850	1.528	2.305	1.482	0.618	0.089	2.845	32.223*
IS	0.011	n.d.	6.610*	0.002	0.013	9.980*	0.013	n.d.	n.d.	0.017	8.059*
My	0.070	n.d.	15.433*	0.030	0.064	2.425	0.089	0.022	n.d.	0.061	5.277*
Lar	0.044	n.d.	9.949*	0.021	0.037	1.015	0.069	0.004	n.d.	0.030	18.556*
Syr	0.014	0.001	8.470*	0.012	0.007	1.047	0.021	0.003	n.d.	0.011	7.451*
Q_3_Rha	0.362	0.002	21.837*	0.155	0.329	2.874	0.269	0.156	0.004	0.597	9.239*
syr_3_Glc	0.951	n.d.	13.659*	0.320	0.948	4.675*	0.643	0.335	n.d.	1.847	17.016*
Q3G	2.285	0.001	4.990*	0.004	3.044	14.404*	1.900	0.002	0.001	5.336	13.563*
Ka_3G	0.068	n.d.	4.165	n.d.	0.090	11.260*	0.048	n.d.	n.d.	0.175	16.421*
Ar	0.024	0.002	5.757*	0.023	0.011	1.577	0.014	0.024	0.002	0.023	0.940
CA_CC	1.134	n.d.	3.955	n.d.	1.513	10.506*	0.730	n.d.	n.d.	3.079	21.441*
Pal	0.245	0.039	2.834	0.098	0.255	1.727	0.054	0.147	n.d.	0.657	16.327*
iPP	1.030	0.066	5.015*	1.128	0.289	4.074	0.384	1.432	n.d.	0.622	2.400
Myr	0.686	n.d.	17.189*	0.302	0.612	2.160	0.461	0.341	n.d.	1.140	6.436*
que_3_glc	1.198	n.d.	2.382	0.049	1.548	4.729*	0.245	0.025	n.d.	4.251	170.172*
iso_3_Glc	0.221	n.d.	1.908	n.d.	0.295	4.331	0.020	n.d.	n.d.	0.846	98.847*
Q_3_4_diG	0.183	n.d.	4.456	0.024	0.221	6.287*	0.074	0.036	n.d.	0.514	50.816*

PM – Plavac mali; P – Pošip; *significance level $p < 0.05$, n.d. – not detected.

are responsible for wine astringency (McRae and Kennedy, 2011). The dessert wines had much lower concentrations of low molecular weight flavan-3-ols than the dry wines (0.21 mg/L (+)-catechin in dessert wines compared to 3.27 mg/L in dry wines). Dimers of procyanidin B3 were less abundant in sweet wines at only 0.01 mg/L while dry wines had 0.51 mg/L. Similarly, the concentration of the procyanidins B2 + B4 averaged 0.01 mg/L in sweet wines but 1.69 mg/L in dry wines. Lower concentrations of flavan-3-ols in dessert wines suggest that these compounds become substrates for polymerization or degradation reactions during grape drying or subsequent vinification and wine ageing. Marquez et al. (2013) found very low concentrations of flavan-3-ols in sweet wines produced from red grapes subjected to traditional sun drying: only 1.6 mg/L (+)-catechin and 4.6 mg/L (−)-epicatechin. A significant decreasing in flavan-3-ols was observed in sweet red wine during one year of ageing (Marquez et al., 2014). Likewise, some high molecular weight derivatives of flavan-3-ols can originate from low molecular weight flavan-3-ols in the dehydrated grapes (Serratosa et al., 2008). High molecular weight tannins were not included in this study, but further investigation might lead to determination of polymers in Prošek wines. Moreover, flavan-3-ols participate in polymerization and copigmentation reactions, so decreased concentrations could be due to their conversion into high molecular weight proanthocyanidins during bottle storage of sweet wines (Fulcrand et al., 2006; Marquez et al., 2014).

Among flavonols, the dessert wine Prošek and dry wines from Plavac mali and Pošip contained the aglycones kaempferol, quercetin, myricetin, myricitrin, laricitrin, isorhamnetin and syringetin. Dehydroflavonol taxifolin was the most abundant compound in this phenolic class at 0.85 mg/L. Similar results were found by Marquez et al. (2013), where in production of red dessert wines the major flavanol quercetin had concentrations from 0.69 to 1.97 mg/L depending on the grape drying method applied before vinification. The concentrations of berry flavonols in the resulting wines are known to depend on the grape variety, cultural practices and environmental factors (Mattivi et al., 2006; Downey et al., 2006; Petropoulos et al., 2011). Partial hydrolysis reactions of grape flavonols substantially decreased concentrations of berry-derived 3-O-glycosides in favor of the corresponding aglycones during storage of dessert wine (Castillo-Muñoz et al., 2007; Marquez et al., 2014). There were statistically lower concentrations of the flavonol aglycones kaempferol, quercetin, and

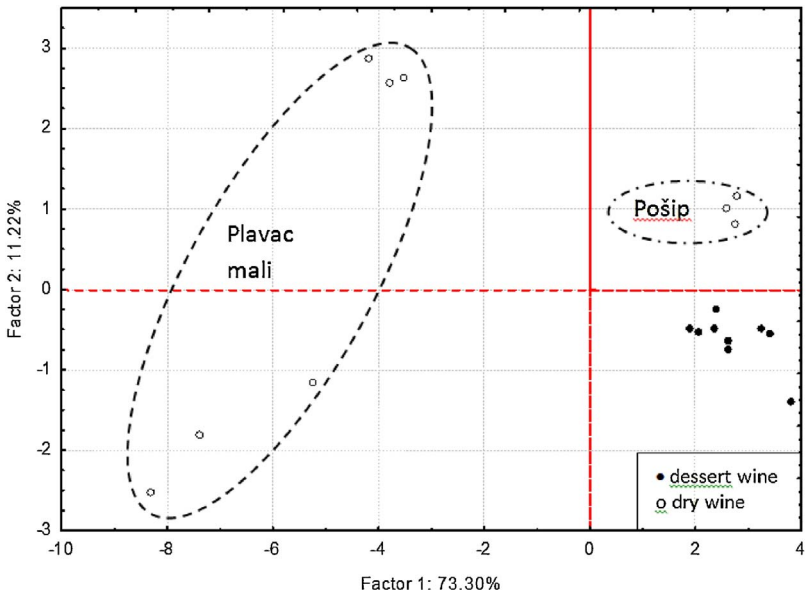

Fig. 1. PCA grouping of wines based on the type (dry, dessert) based on the 23 significant components from Table 4. PC factor 1 and PC factor 2 together comprise 84.5% of total variability.

Table 5
Average values and standard deviations of five phenolic groups in different wine types.

Wine type	phenolic acid-HBA	phenolic acid-HCA	phenolic aldehyde	flavan-3-ols	flavonols
	(mg/L)	(mg/L)	(μg/L)	(mg/L)	(mg/L)
dessert	0.3 ± 0.1[a]	8.4 ± 5.6[a]	19.8 ± 9.8 [a]	0.4 ± 0.6[a]	0.5 ± 0.4[a]
dry	0.5 ± 0.5[a]	15.8 ± 8.2[b]	66.1 ± 58.8[b]	9.5 ± 6.8[b]	6.1 ± 5.4[b]

Different letters in the same column show significant differences regarding the wine type.

isorhamnetin in Prošek than in the dry wines.

3.3. Differentiation of dessert and dry wine using multivariate analysis

Factor analysis (FA) was applied to identify the most important phenolic compounds and reduce the input data set of 45 variables to group and validate clustering based on the phenolic components. The FA identified 23 phenolic compounds as important for separating the wine types. Those percentages show the dominance of the phenolic components 4-aminobenzoic acid, *p*-hydroxybenzoic acid, syringaldehyde, ellagic acid, *p*-coumaric acid, ferulic acid, caftaric acid, *trans*-coutaric acid, catechin, epicatechin, epigallocatechin, gallocatechin, procyanidin B2 + B4, procyanidin B3, kampferol, quarcetin, isorhamnetin, syringetin-3-glucoside, quercetin-3-glucuronide, kaempferol-3-glucuronide, caffeic acid + catechin condensation, quercetin-3-glucoside + quercetin-3-galactoside and quercetin-3,4-diglucoside.

To identify patterns in experimental data, group or separate the phenolic compounds and screen for possible outliers, we used principal component analysis (PCA). This multivariate tool will derive a small number of independent linear combinations (principal components, PCs) for our observed set of variables, retaining as much information as possible. PCA was applied to detect patterns between specific phenolic compounds that can be used to distinguish among wines whether produced using standard winemaking processes for white or red wine or using drying of grapes before alcoholic fermentation to make dessert wine. Grouping of dessert and dry wines was efficient, accounting for 84.5% of all variance in the data set. PCA showed that the first three PCs (based on the eigenvalues 1 criterion) account for over 90.6% of the variation in the whine phenolic compounds data set PC1 (73.3% of the total variance) correlates with phenolic compounds as catechin, quercetin-3-glucuronide as well as epicatechin, kaempferol-3-glucuronide and caffeic acid + catechin condensation. PC2 (11.2%) is described with three phenolic acid-HCA: *p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, and PC3 (6.2%) by two flavonols: kaempferol and quercetin.

The dessert wines grouped in the fourth quadrant based on significant differences in seven phenolic acids (4-aminobenzoic acid, *p*-hydroxybenzoic acid, ellagic acid, *p*-coumaric acid, ferulic acid, *trans*-caftaric acid and *trans*-coutaric acid), a phenolic aldehyde (syringaldehyde), no stilbenes, seven flavan-3-ols (catechin, epicatechin, epigallocatechin, gallocatechin, procyanidin B2 + B4, procyanidin B3 and caffeic acid + catechin condensation), and eight flavonols (kaempferol, quarcetin, isorhamnetin, syringetin-3-glucoside + syringetin-3-galactoside, quercetin-3-glucuronide, kaempferol-3-glucuronide, quercetin-3-glucuronide, and quercetin-3,4-diglucoside). Dry wines were distributed in the remaining three quadrants grouped based on the variety. Thus, dry Pošip wine grouped in the first quadrant, while the dry Plavac mali wines were in the second or third quadrant, depending on the vintage (Fig. 1).

Using the dominant phenolic components that separated dry and dessert wines, validation was conducted on all 18 wine samples with 98% efficacy. Linear discriminant analysis was applied to derive a classification function for effective recognition of dry and dessert wines.

To evaluate which group of phenolic compounds was dominant in the wine separations, a PCA was conducted on the summarized grouped components (HBA, HCA, phenolic aldehyde, flavan-3-ols, and flavonols). The Kaiser-Meyer-Olkin measure of sampling adequacy for grouped phenol components was middling (KMO = 0.7327). Those variables are used to present average values for significant phenolic components in different phenolic groups (Table 5).

Dry wine samples have significantly higher concentrations of significant components from four phenolic groups: the phenolic acid HCA (2.3 to 16.9 mg/L in dessert wines and 4.8 to 23.3 mg/L in dry, respectively); the phenolic aldehyde syringaldehyde; flavan-3-ols (catechin, epicatechin, epigallocatechin, gallocatechin, procyanidin B2 + B4, procyanidin B3, and caffeic acid + catechin condensation)

and flavonols (kaempferol, quercetin, isorhamnetin, syringetin-3-glucoside + syringetin-3-galactoside, quercetin-3-glucuronide, kaempferol-3-glucuronide, quercetin-3-glucoside +quercetin-3-galactoside and quercetin-3,4-diglucoside).

The classification performance by wine type was evaluated by LDA using 23 variables (isolated as the significant phenolic compounds). The first two roots explained 100% of the properties of different wine types, which means that classification of dry and dessert wines was completely successful. Those findings upheld the utility of multivariate tools in classification, even of complex samples such as wines that differ in their phenolic composition by type, vintage and variety. De Villiers et al. (2005) successfully classified wines according the grape variety, but our study showed that the phenolic profile of wines can be used to differentiate dessert from dry wines.

4. Conclusions

The concentrations of 45 phenolic compounds were determined in three dessert and three dry wines produced from micro-scale vinification of autochthonous grape cultivars used in Croatia for production of Prošek dessert wine. ANOVA indicated that the concentrations of these compounds in the wines were affected by variety, vintage and wine type. The Prošek dessert wines had significantly lower concentrations of phenolic acids, syringaldehyde, flavanols and flavonols than dry wines produced from the same varieties. The differences in phenolic compounds between dessert and dry wines were sufficient to successfully differentiate the samples by wine type, regardless of vintage or variety.

Acknowledgments

This study was supported by the Ministry of Science, Education and Sports of the Republic of Croatia, Project “Biotechnological Parameters of Premium-Quality Dalmatian Dessert Wine–Prošek”—(091-0910468-0452).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfca.2017.05.015.

References

Aguilera, Y., Martin-Cabrejas, M., De Mejia, E.G., 2016. Phenolic compounds in fruits and beverages consumed as part of the Mediterranean diet: their role in prevention of chronic diseases. Phytochem. Rev. 15, 405–423.
Arapitsas, P., Perenzoni, D., Nicolini, G., Mattivi, F., 2012. Study of Sangiovese wines pigment profile by UHPLC-MS/MS. J. Agric. Food Chem. 60, 10461–10471.
Bindon, K., Varela, C., Kennedy, J., Holt, H., Herderich, M., 2013. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. Food Chem. 138, 1696–1705.
Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., Hermosín-Gutiérrez, I., 2007. Flavonol profiles of Vitis vinifera red grapes and their single-cultivar wines. J. Agric. Food. Chem. 55, 992–1002.
De Torres, C., Diaz-Maroto, M.C., Hermosin-Gutierrez, I., Perez-Coello, M.S., 2010. Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin. Anal. Chim. Acta 660, 177–182.
De Villiers, A., Majek, P., Lynen, F., Crouch, A., Lauer, H., Sandra, P., 2005. Classification of South African red and white wines according to grape variety based on the non-coloured phenolic content. Eur. Food Res. Technol. 221, 520–528.
Downey, M.O., Dokoozlian, N.K., Krstic, M.P., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. Am. J. Enol. Viticult. 57, 257–268.
Fulcrand, H., Duenas, M., Salas, E., Cheynier, V., 2006. Phenolic reactions during winemaking and aging. Ame. J. Enol. Viticult. 57, 289–297.
Herjavec, S., Jeromel, A., Da Silva, A., Orlic, S., Redžepović, S., 2007. The quality of white wines fermented in Croatian oak barrels. Food Chem. 100, 124–128.
López de Lerma, N., Moreno, J., Peinado, R.A., 2014. Determination of the optimum sun-drying time for Vitis vinifera L: cv. Tempranillo grapes by E-nose analysis and characterization of their volatile composition. Food Bioprocess Technol. 7, 732–740.
Letaief, H., Rolle, L., Zeppa, G., Orriols, I., Gerbi, V., 2007. Phenolic characterization of grapevine cultivars from Galicia (Spain): Brancellao Merenzao and Mencia (Vitis vinifera L.). Ital. J. Food Sci. 1 (19), 111–119.
Loizzo, M.R., Bonesi, M., Di Lecce, G., Boselli, E., Tundis, R., Pugliese, A., Menichini, F., Frega, N.G., 2013. Phenolics, aroma profile, and in vitro antioxidant activity of Italian dessert Passito wine from Saracena (Italy). J. Food Sci. 78, 703–708.
Lorrain, B., Chira, K., Teissedre, P.L., 2011. Phenolic composition of merlot and cabernet-sauvignon grapes from Bordeaux vineyard for the 2009-vintage: comparison to 2006, 2007 and 2008 vintages. Food Chem. 126, 1991–1999.
Marquez, A., Serratosa, M.P., Merida, J., 2013. Quality improvement in sweet red wines through an alternative grape-drying system. S. Afr. J. Enol. Vitic. 34, 252–261.
Marquez, A., Serratosa, M.P., Merida, J., 2014. Influence of bottle storage time on colour: phenolic composition and sensory properties of sweet red wines. Food Chem. 146, 507–514.
Mattivi, F., Guzzon, R., Vrhovšek, U., Stefanini, M., Velasco, R., 2006. Metabolite profiling of grape: flavanols and anthocyanins. J. Agric. Food Chem. 54, 7692–7702.
McRae, J.M., Kennedy, J.A., 2011. Wine and grape tannin interactions with salivary proteins and their impact on astringency: a review of current research. Molecules 16, 2348–2364.
Moreno, J.J., Cerpa-Calderón, F., Cohen, S.D., Fang, Y., Qian, M., Kennedy, J.A., 2008. Effect of postharvest dehydration on the composition of Pinot noir grapes (Vitis vinifera L.) and wine. Food Chem. 109, 755–762.
OIV, 2005. Compendium of International Methods of Wine and Must Analysis, Paris, International. Organisation of Vine and Wine, 2005 ed. .
Panceri, C.P., Gomes, T.M., De Gois, J.S., Borges, D.L.G., Bordignon-Luiz, M.T., 2013. Effect of dehydration process on mineral content: phenolic compounds and antioxidant activity of Caberent Sauvignon and Merlot grapes. Food Res. Int. 54, 1343–1350.
Petropoulos, S., Kallithraka, S., Paraskevopoulos, I., 2011. Influence of some viticultural practices on the polyphenolic content of wines produced from cv. Agiorgitiko (Vitis vinifera L.). J. Int. des Sciences de la Vigne et du Vin 45, 235–243.
Piljac, J., Martinez, S., Valek, L., Stipčević, T., Kovačević Ganić, K., 2005. A comparison of methods used to define the phenolic content and antioxidant activity of Croatian wines. Food Technol. Biotechnol. 43, 271–276.
Rangel-Huerta, O.D., Pastor-Villaescusa, B., Aguilera, C.M., Gil, A., 2015. A systematic review of the efficacy of bioactive compounds in cardiovascular disease: phenolic compounds. Nutrients 7, 5177–5216.
Rastija, V., Srečnik, G., Medić-Šarić, M., 2009. Polyphenolic composition of Croatian wines with different geographical origins. Food Chem. 54–60.
Rodriguez-Mateos, A., Vauzour, D., Krueger, C.G., Shanmuganayagam, D., Reed, J., Calani, L., Mena, P., Del Rio, D., Crozier, A., 2014. Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. Arch. Toxicol. 88, 1803–1853.
Sen, I., Tokatli, F., 2016. Differentiation of wines with the use of combined data of UV–visible spectra and color characteristics. J. Food Compos. Anal. 45, 101–107.
Serratosa, M.P., Lopez-Toledano, A., Merida, J., Medina, M., 2008. Changes in color and phenolic compounds during the raisining of grape Cv: pedro Ximenez. J. Agric. Food. Chem. 56, 2810–2816.
Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A., Qian, M.C., 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of Vitis vinifera L. cv. Pinot noir wine. Food Chem. 15 (173), 424–431.
Stefani, M., Rigacci, S., 2014. Beneficial properties of natural phenols: highlight on protection against pathological conditions associated with amyloid aggregation. Biofactors 40, 482–493.
Tamborra, P., Esti, M., Minafra, M., Sinesio, F., 2003. Phenolic compounds in red-berry skins of uva di Troia and Bombino nero grapes. Ital. J. Food Sci. 15, 347–357.
Vinković Vrček, I., Bojić, M., Žuntar, I., Mendaš, G., Medić-Šarić, M., 2011. Phenolic content: antioxidant activity and metal composition of Croatian wines deriving from organically and conventionally grown grapes. Food Chem. 124, 354–361.
Vrhovšek, U., Masuero, D., Gasperotti, M., Franceschi, P., Caputi, L., Viola, R., Mattivi, F., 2012. A versatile targeted metabolomics method for rapid quantification of multiple classes of phenolics in fruit and beverages. J. Agric. Food Chem. 60, 8831–8840.