

Volatile Compounds in Prošek Dessert Wines Produced from White and Red Grapes

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Abstract

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Prošek dessert wines produced from dried grapes of two native Croatian varieties, one white and one red, had complex volatile compositions. Various categories of volatile compounds were identified using headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS). Sixty-one individual volatile compounds of Prošek were identified. Of these, nine compounds comprised over 95% of the total peak area of volatile aromas detected in Prošek: five esters and four alcohols. The remaining volatile compounds consisted of 11 alcohols, 11 esters, 10 terpenes, 8 aldehydes, 10 ketones, 4 acids, 1 lactone, and 1 norisoprenoide. Only 13 of the 61 aroma compounds showed significant differences between the varieties investigated. These results suggest that the characteristic aroma of Prošek is very complex and determined predominantly by the unique process of the grape drying.

Keywords: aroma; HS-SPME-GC-MS; cv. Pošip; cv. Plavac mali

Prošek is a highly appreciated Croatian dessert wine traditionally produced in the coastal region and islands of Dalmatia and is considered to be one of the area most important traditional food products. Prošek is produced through alcoholic fermentation of the juice obtained from dried grapes. The drying process increases the concentration of sugar, yielding sweet wines characterised by their high sugar concentration (PANCERI *et al.* 2013). Although the quality of Prošek wines is determined by their aroma and their production has a long history, there is little information concerning the volatile composition of this unique Croatian dessert wine. This is due in part to the official regulations defining Prošek production as based on traditional procedures, which have recently been established as part of new wine regulations in the EU.

The volatile composition, defined generally as aroma, is an important factor in characterising wine. Wine aroma, together with colour, is of fundamental importance for consumer attraction and enjoyment. During the key grape drying step in the dessert wine production, metabolic changes occur and volatile

compounds are formed from the major grape constituents through various biochemical pathways (CONSTANTINI *et al.* 2006). The yeasts involved in alcoholic fermentation are responsible for the wine fermentation aromas. Alcohols, esters, volatile fatty acids, carbonyls and sulphur-containing compounds are produced as secondary products of the yeast metabolism (REGODÓN MATEOS *et al.* 2006). In addition, yeast contributes to the overall aroma by liberating grape-derived volatile compounds from odourless glycosidic precursors, producing terpenes, C-13 norisoprenoids, benzenoids, aliphatic compounds, and volatile phenols (FERREIRA *et al.* 2008).

Dessert wines obtained from grapes dried under various conditions are produced along the Mediterranean (GUARRERA *et al.* 2005; FIGUEIREDO-GONZÁLEZ *et al.* 2013). Recent studies have examined sweet wines to determine their volatile composition (MÁRQUEZ *et al.* 2008; DUGO *et al.* 2014; LOPEZ DE LERMA *et al.* 2014). Various categories of volatile compounds were identified using headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass

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spectrometry (GC-MS). Prošek is still not mentioned by name in the international rules and standards, so reliable analytical reference values are missing. There is one work describing the aroma compounds in Prošek (BUDIĆ-LETO *et al.* 2010), but the discussion is limited to just a few compounds and only one red grape cultivar, Plavac mali. In contrast, this work compares the volatile profiles of two very distinct Prošek wines: a white and a red, produced from the widely used native cultivars Pošip and Plavac mali, respectively.

MATERIALS AND METHODS

Experimental wines. The original technology for Prošek wine making involves drying the grapes, however, commercial wines produced by different methods are currently on the Croatian market. To equalise the production conditions of the samples and facilitate direct comparison, we produced experimental wines under controlled conditions. The experiments were conducted in 2008 using two grapevine cultivars: the white Pošip and red Plavac mali (both *Vitis vinifera* L.), grown in commercial vineyards situated on the island of Korčula and the Pelješac peninsula of Dalmatia. Both vineyards are ~20 years old.

Production of white Prošek from Pošip grapes. Amount of 490 kg of grapes cv. Pošip (26.6 °Brix, total acidity 5.06 g/l, pH 3.93) were harvested and dried in a greenhouse. The grapes were dried for five days (September 10–15, 2008). The greenhouse was equipped with a system for temperature control and ventilation; the maximum temperature during drying was 40°C. The sugar concentration in the must after drying was ~32 °Brix, the total acidity was 6.01 g/l, and the pH was 4.07. The fruit weight loss was 33%. The weight loss was calculated using the equation: %WL = [(Wi – Wf)/Wi] (100), where: %WL – percentage weight loss; Wi – initial fruit weight in g; Wf – final fruit weight in g. The dried grapes were crushed, destemmed and sulphited with potassium metabisulphite (15 g/hl). After 4 h skin contact at 15°C, the must was racked into a 25-l glass container. The must was inoculated with active dry yeast (Lalvin EC 1118, *Saccharomyces cerevisiae* var. *bayanus*) to start the fermentation. Alcoholic fermentations were done in triplicate. The Prošek was racked twice, at one and six months from the beginning of fermentation, and then bottled.

Production of red Prošek from Plavac mali grapes. 400 kg of grapes cv. Plavac mali (22 °Brix, total acidity

4.3 g/l, pH 3.66) was harvested in Pelješac, Croatia. The grapes were dried in a greenhouse for 18 days (October 10–29, 2008). The fruit weight loss was 55%, calculated as described above. The dried grapes were crushed, destemmed, and the pectolytic enzyme Endozym cultivar (AEB S.p.A., Brescia, Italy, 30 g/100 kg) and potassium metabisulphite (15 g/hl) were added. The sugar concentration in the must after drying was ~32 °Brix, the total acidity was 6.6 g/l, and the pH was 3.60. The must was divided into three equal parts and put into inox containers. Maceration took five days. Alcoholic fermentation was done using dry yeast (Lalvin EC 1118, *Saccharomyces cerevisiae* var. *bayanus*). After maceration, the pomace was pressed on a hydraulic press at < 2 bar. The must was put in 25-l glass vessels, where alcoholic fermentation continued. The first racking was done on day 29 and the second on day 184 (six months) from the beginning of fermentation. After the second racking, the Prošek was bottled.

HS SPME- GC/MS analysis. Ten ml wine, 10 µl acetophenone-d8 (cat. No. 296732-1G; Sigma Aldrich, St. Louis, USA) (internal standard, 0.1 mg/l standard solution in methanol) and 3 g NaCl were introduced into a 20-ml vial. The extraction of volatiles was carried out using a solid-phase microextraction (SPME, Stable Flex) fiber coated with Carboxen/polydimethylsiloxane sorbent (1 cm long, 85 µm thick; both Supelco, Bellefonte, USA). The analysis was conducted on a GC 7890A gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a MPS2 Multipurpose autosampler (Gerstel, Baltimore, USA) and 5975C mass spectrometer (Agilent Technologies). The sample vials were conditioned in a temperature-controlled heating module at 45°C for 51 min and agitated at 600 rpm. The volatile compounds were desorbed into a GC injector port at 250°C in splitless mode for 2 minutes. The gas chromatograph was fitted with a ZB-WAX capillary column, 60 m × 0.32 mm *i.d.* with 1 µm film thickness. Helium was used as the carrier gas at a flow rate of 1.2 ml/min at 40°C. The oven temperature was programmed as follows: initial temperature 40°C held for 5 min, then 4°C/min to 230°C. The volatile compounds were identified with a mass selective detector (5975C; Agilent Technologies). The detector operated in the *m/z* range between 30 and 250, the ion source and quadrupole temperature were maintained at 250 and 150°C, respectively. The identification of the compounds was performed by comparison of their mass spectra with those of the NIST mass spectral database (National Institute of Standards

Table 1. Experimental Kovats index values and peak area of volatile compounds identified in Prošek

No	Compound	Kovats index	Pošip	Plavac mali
Aldehydes				
1	Acetaldehyde	700	143.06	217.42
2	Hexanal	1 101	1.85	2.24
3	Heptanal	1 184	9.45*	6.03*
4	2-Hexenal	1 148	151.15	88.53
5	Octanal	1 307	0.76	0.86
6	Nonanal	1 402	44.18*	233.86*
7	Benzaldehyde	1 525	15.04	31.64
8	Phenyl acetaldehyde	1 650	15.14	15.98
Alcohols				
9	2-Methyl-1-butanol	1 225	1 408.18	1 323.85
10	3- Methyl-1-butanol	1 229	5 205.15	4 814.94
11	1-Pentanol	1 255	28.48	36.99
12	Hexanol	1 373	939.85	1 202.61
13	3-Hexene-1-ol (<i>Z</i>)		1.40	2.76
14	3-Ethoxy-1-propanol		15.08	12.78
15	2-Hexene-1-ol (<i>E</i>)	1 405	61.11	38.64
16	1-Heptanol	1 460	5.41*	40.35*
17	Ethylhexanol	1 483	7.41	10.53
18	2,3-butandiol		348.33	539.73
19	1-Octanol	1 562	22.75	27.41
20	1-Nonanol	1 670	17.28	18.07
21	2-Nonen-1-ol	1 742	4.54	3.35
22	2-phenylethanol	1 935	12 558.67	10 494.31
23	(<i>Z</i>)-6-Nonen-1-ol		2.28	5.80
Esters				
24	Ethylacetate	905	11 185.13	10 738.89
25	Isoamylacetate	1 140	19 136.93	13 132.20
26	Ethyl crotonate	1 037	6.03	4.29
27	Ethyl hexanoate	1 235	2 014.76	1 132.40
28	Hexylacetate	1 275	143.23	130.13
29	Ethyl heptanoate	1 331	25.40*	56.10*
30	Methyl octanoate	1 413	28.13	23.19
31	Ethyl octanoate	1 457	10 345.37*	3 885.07*
32	3-Methylbutyl hexanoate	1 450	59.91*	32.97*
33	Ethyl nonanoate	1 559	32.28	37.29
34	Ethyl decanoate	1 636	3 270.52*	1 238.50*
35	3-Methylbutyl octanoate	1 682	141.83	68.28
36	Ethyl-2-phenylacetate	1 867	6.47*	31.70*
37	Methyl 2-hydroxybenzoate		1.89	3.15
38	Ethyl dodecanoate	1 864	145.78	96.79
39	Ethyl tetradecanoate		21.49	52.61

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Table 1 to be continued

No	Compound	Kovats index	Pošip	Plavac mali
Terpenes				
40	α -Terpinene	1 200	0.26	2.10
41	Limonene	1 224	4.91	4.50
42	β -Phellandrene	1 246	0.25	0.91
43	<i>cis</i> -Ocimene	1 255	14.27	30.21
44	Cinnamene	1 273	9.60	17.00
45	<i>p</i> -Cymene	1 282	25.78	26.92
46	Terpinolen	1 297	1.16	1.71
47	Linalool	1 544	8.45*	2.13*
48	4-Terpineol	1 606	1.10*	15.78*
49	Nerol	1 792	4.20	8.61
Ketones				
50	Heptan-2-one	1 160	2.17*	2.98*
51	3-Octanone	1 279	5.42*	19.24*
52	3-Hydroxy-2-butanone (or acetoin)	1 322	5.77	17.44
53	6-Methyl-5-heptene-2-one	1 366	0.98*	3.73*
54	2-Nonanone	1 415	29.90	23.06
55	2-Decanone	1 523	0.66	1.28
C-13 norisoprenoids				
56	β -Demascenone	1 835	72.23	44.23
Lactone				
57	γ -Butyrolactone	1 640	20.99	21.17
Acids				
58	Acetic acid	1 481	337.45	555.78
59	Pentanoic acid	1 698	203.63	155.56
60	Heptanoic acid	1 900	2.65	4.27
61	Octanoic acid	2 090	300.40	138.65
Σ Total volatiles			68 623.93	50 929.50

Results are expressed as relative concentrations of the individual compounds in comparison to the peak area of the internal standard ($\times 10^5$) calculated for three replications (RSD \leq 5%); *indicate significant differences at a 95% confidence level

and Technology, USA). The results were expressed as the relative concentration of compounds, as calculated from the peak area of the individual compounds in comparison with the internal standard. The repeatability of the experimental method was determined by performing three replicate analyses of each Prošek sample. Calculated relative standard deviations (RSD %) of the peak areas were below than 5%.

Statistical analysis. Statistical analysis was performed to compare Prošek wines made from different grape varieties. An analysis of variance (ANOVA) was made for each aroma compound studied ($P < 0.05$). The software used was Statistica 8.0 (StatSoft, Inc., Tulsa, USA).

RESULTS AND DISCUSSION

We analysed the volatile aroma compounds of Prošek wines produced by using traditional procedures from partially dried grapes of the two Croatian native grape cultivars Plavac mali and Pošip.

The two Prošek wines yielded 61 peaks corresponding to volatile compounds (Table 1). Quantification of the compounds was performed based on FID% area related to SPME fiber uptake. Absolute concentrations would have to be obtained using standard compounds, a highly demanding procedure considering the complexity of Prošek. The 61 identified compounds were divided into eight groups: 16 esters, 15 alcohols, 10 terpenes, eight

aldehydes, 6 ketones, 4 acids, 1 C-13 norisoprenoid, and 1 lactone, each being present in each Prošek. Esters were the most abundant volatile compounds making 67.7% in Pošip and 60.2% in Plavac mali wines. This was followed by alcohols (30.1% in Pošip and 36.5% in Plavac mali). Acids content was 1.2% in Pošip and 1.7% in Plavac mali. Aldehydes content was less than 1% and that of terpenes was 0.1% in Pošip and 0.2% in Plavac mali. Ketones and C-13 norisoprenoid were found in the amount of ~ 0.1% and lactone in the amount of < 0.1% in both wines. These values are the percentages of the sum of the peak areas of the identified volatile compounds.

Acetate esters are the major esters in Prošek. Isoamyl acetate, with a characteristic banana odour, was the most abundant single compound in Prošek with 25.8% in Plavac mali and 27.9% in Pošip. Ethyl acetate was the second most abundant ester making 16.3% in Pošip and 21.1% in Plavac mali. The wines produced from sun-dried grapes had high concentrations of isoamyl acetate and ethyl acetate (MÁRQUEZ *et al.* 2008). Ethyl acetate and acetic acid are usually very abundant in Passito and Vin Santo wines, but the flavour note is buffered by the high sugar concentration (BELLINCONTRO *et al.* 2004). Ethyl acetate and isoamyl acetate were major ester compounds in fino sherry wine (CASTRO *et al.* 2004). Ethyl octanoate, ethyl decanoate, and 3-methylbutyl hexanoate were statistically more abundant ($P < 0.05$) in Prošek from cv. Pošip than in that from cv. Plavac mali, while ethyl-2-phenylacetate and ethyl heptanoate were less abundant.

The most abundant alcohol (apart from ethanol) in Prošek wines was 2-phenylethanol, 18.3% in Pošip and 20.6% in Plavac mali. 2-Phenylethanol was a primary volatile compound in Andalusian sweet wines, Malvasia delle Lipari, Fino, and Pedro Ximenez (MURATORE *et al.* 2007; CAMPO *et al.* 2008; MÁRQUEZ *et al.* 2008). 3-Methyl-1-butanol (7.6% in Pošip and 9.5% in Plavac mali) and 2-methyl-1-butanol (2.1% in Pošip and 2.6% in Plavac mali) were other abundant alcohols in Prošek. Due to its strong rose and honey notes, 2-phenylethanol has a positive influence on the sweet wine aroma (GENOVESE *et al.* 2007; LÓPEZ DE LERMA & PEINADO 2011).

There were significant differences ($P < 0.05$) between Plavac mali and Pošip wines in 13 of the 61 identified compounds. Although the wine aroma is predominantly determined by the variety, different terroir may also influence the aroma. The effects on the wine aroma originating from agronomic practices have been also well documented (GONZÁLEZ-RODRÍGUEZ *et al.*

2011; GONZÁLEZ-ÁLVAREZ *et al.* 2012; NOGUEROL-PATO *et al.* 2012b). Wine techniques that affect temperature, oxygen and other conditions during drying, maceration, and alcoholic fermentation also significantly influence the wine aroma (GONZÁLEZ-ÁLVAREZ *et al.* 2013; NOGUEROL-PATO *et al.* 2013; REBOREDO-RODRÍGUEZ *et al.* 2015).

Despite the very clear differences in aroma and other sensory attributes of white and red Prošeks (data not shown), the differences in chemical composition were very small. Despite substantial differences in the fermentation of white and red Prošeks, there were no differences between the contents of C₆ alcohols, C₆ aldehydes, and β-damascenone: the volatiles originating from the berry skin (NOGUEROL-PATO *et al.* 2012a). Instead, the primary differences between white and red Prošeks were in the major volatile compounds, particularly certain esters. White Prošek wines had more esters than the red ones. Ethyl esters of fatty acids, mainly ethyl hexanoate and ethyl octanoate, are produced by yeast during alcoholic fermentation of most red and white wines, but in Prošek, which is produced from dried grapes, acetate esters, isoamyl acetate and ethyl acetate dominate.

Sweet wines generally have lower concentrations of volatile terpenes, β-damascenone, alcohols, and ethyl esters of fatty acids, especially those containing 6 to 10 carbon atoms, due to the lower metabolic activity of yeasts. Dry wines produced from the same red grape cultivar, Garnache Tintorera, formed ketones such as acetoin, and aldehydes like 2-furfural and 5-methylfurfural, during fermentation through the microbial activity of lactic acid bacteria and yeasts (NOGUEROL-PATO *et al.* 2012a). The two latter aldehydes were not detected in our samples of Prošek.

Hexanol, a C₆ alcohol compound originating from grape-derived precursors, comprised > 1% of the identified volatile compounds in Prošek. These compounds are of varietal or pre-fermentative origins being scarcely affected by the fermentation, and are important contributors to the green, grassy, and fresh aroma (DE TORRES *et al.* 2010). Small amounts of C₆ alcohols were also determined in two Sicilian passito wines, Malvasia delle Lipari and Moscato di Noto (GUARRERA *et al.* 2005).

CONCLUSIONS

The volatile profiles of Prošek wines made from grapes of two native cultivars grown in Dalmatia,

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red Plavac mali and white Pošip, were characterised. The screening analysis was used to isolate 61 volatile compounds. Higher alcohols made up ~ 33% of the total volatiles; this group included 2-phenylethanol, 3-methyl-1-butanol, 2-methyl-1-butanol and hexanol. Acetate and ethyl esters formed 64% of total volatiles, of which the amount of acetate was 45%. The higher ester components included isoamyl acetate, ethyl acetate, ethyl octanoate, ethyl decanoate, and ethyl hexanoate. Despite the different colours of the grapes used, one white and one red, and the different duration of maceration, the aromas of the wines were very similar. Differences were determined in only 13 of 61 compounds identified, most of which were present in very low relative proportions of the total volatiles.

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