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SUPPLEMENTARY MATERIAL TO

Chemical and sensory characterization of plum spirits obtained from cultivar Čačanska Rodna and its parent cultivars

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TABLE S-I. Contents of volatile compounds (mean ± standard deviation, g hL^{-1} a. a.) in six plum spirits (processing with stones) produced from the fruits with stones of Čačanska Rodna and its parent cultivars; A–C: differ significantly ($p \leq 0.05$) for processing with stones; ČR – Čačanska Rodna, ST – Stanley, PO – Požegača; 3M1B – 3-methyl-1-butanol; 2M1B – 2--methyl-1-butanol; 2M1P – 2-methyl-1-propanol; 1-P – 1-propanol; 2-PE – 2-phenylethanol; 1-H – 1-hexanol

Compound	Year	Cultivar		
		ČR	ST	PO
Methanol	2011	697.07±19.24^{C}	816.93±5.72^{B}	931.30±41.56^{A}
	2012	673.37±13.05^{C}	934.90±10.21^{A}	746.87±9.64^{B}
1-Propanol	2011	25.70±0.20^{B}	22.27±0.68^{C}	73.17±1.87^{A}
	2012	75.23±1.16^{B}	35.17±0.55^{C}	77.33±0.91^{A}
1-Butanol	2011	3.17±0.25^{B}	1.40±0.61^{C}	10.90±0.52^{A}
	2012	2.20±0.78^{B}	1.90±0.96^{B}	6.17±0.12^{A}
2-Butanol	2011	1.93±0.12	2.07±0.06	2.50±0.46
	2012	1.50±0.00^{B}	1.78±0.23^{B}	2.57±0.29^{A}
2-Methyl-1-propanol	2011	25.43±0.57^{B}	21.53±0.99^{C}	36.30±1.06^{A}
	2012	40.23±0.55^{A}	26.27±0.50^{C}	35.87±0.49^{B}
2-Methyl-1-butanol	2011	19.60±0.44^{B}	15.40±0.36^{C}	23.60±0.27^{A}
	2012	23.97±0.42^{A}	14.20±0.30^{C}	21.33±0.06^{B}
3-Methyl-1-butanol	2011	83.83±1.50^{A}	71.40±0.80^{B}	70.93±1.21^{B}
	2012	97.20±1.23^{A}	45.53±0.72^{B}	44.70±0.26^{B}
1-Hexanol	2011	1.18±0.13^{B}	1.32±0.25^{B}	2.69±0.32^{A}
	2012	1.01±0.19^{B}	2.65±0.10^{A}	2.86±0.43^{A}
2-Phenylethanol	2011	2.06±0.08^{A}	1.00±0.10^{C}	1.17±0.01^{B}
	2012	2.42±0.15^{A}	0.77±0.09^{C}	1.38±0.30^{B}

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TABLE S-I. Continued

Compound	Year	Cultivar		
		ČR	ST	PO
Higher alcohols total	2011	162.91±2.55^{B}	136.28±2.02^{C}	221.26±5.30^{A}
	2012	244.46±2.10^{A}	128.25±2.32^{C}	192.21±1.20^{B}
3M1B/2M1B ratio	2011	4.28±0.02^{B}	4.64±0.11^{A}	3.01±0.02^{C}
	2012	4.09±0.03^{A}	3.21±0.03^{B}	2.10±0.02^{C}
(3M1B+2M1B)/2M1P	2011	4.07±0.03^{A}	4.04±0.15^{A}	2.60±0.04^{B}
ratio	2012	3.03±0.07^{A}	2.27±0.06^{B}	1.84±0.02^{C}
(3M1B+2M1B)/1-P	2011	4.02±0.08^{A}	3.90±0.15^{A}	1.29±0.01^{B}
ratio	2012	1.62±0.04^{B}	1.70±0.01^{A}	0.85±0.01^{C}
2M1P/1-P ratio	2011	0.99±0.03^{A}	0.97±0.06^{A}	0.50±0.00^{B}
	2012	0.53±0.00^{B}	0.75±0.02^{A}	0.46±0.01^{C}
2-PE/1-H ratio	2011	1.76±0.26^{A}	0.77±0.08^{B}	0.44±0.05^{C}
	2012	2.45±0.32^{A}	0.29±0.03^{B}	0.48±0.06^{B}
Ethyl acetate	2011	59.47±1.33^{C}	89.83±0.55^{B}	145.77±2.72^{A}
	2012	28.60±0.44^{C}	146.30±4.15^{B}	162.00±1.32^{A}
Ethyl butanoate	2011	0.07±0.03^{B}	0.07±0.03^{B}	0.20±0.00^{A}
	2012	0.10±0.00	0.09±0.02	0.10±0.00
Ethyl hexanoate	2011	0.10±0.00^{C}	0.20±0.01^{B}	0.30±0.00^{A}
	2012	0.20±0.00	0.10±0.01	0.13±0.06
Ethyl octanoate	2011	0.33±0.06^{B}	0.47±0.06^{A}	0.47±0.06^{A}
	2012	1.07±0.12^{A}	0.33±0.06^{B}	0.27±0.05^{B}
Ethyl decanoate	2011	0.32±0.09^{B}	0.30±0.07^{B}	0.52±0.06^{A}
	2012	0.94±0.03^{A}	0.16±0.11^{C}	0.34±0.04^{B}
Ethyl dodecanoate	2011	0.00±0.00^{B}	0.05±0.01^{A}	0.00±0.00^{B}
	2012	0.13±0.04^{A}	0.05±0.02^{B}	0.05±0.02^{B}
Ethyl tetradecanoate	2011	0.00±0.00^{B}	0.08±0.03^{B}	0.20±0.07^{A}
	2012	0.04±0.01^{AB}	0.08±0.04^{A}	0.00±0.00^{B}
Isoamyl acetate	2011	0.20±0.00^{B}	0.30±0.00^{A}	0.27±0.06^{AB}
	2012	0.30±0.00^{A}	0.20±0.00^{B}	0.20±0.00^{B}
Diethyl succinate	2011	0.07±0.02^{C}	0.20±0.05^{B}	0.37±0.06^{A}
	2012	0.11±0.04	0.48±0.35	0.82±0.41
Ethyl lactate	2011	12.63±0.34^{B}	12.10±0.23^{B}	66.96±2.62^{A}
	2012	15.20±0.53^{C}	71.96±1.52^{A}	62.11±1.67^{B}
Esters total	2011	73.19±1.68^{C}	103.58±0.76^{B}	215.04±4.20^{A}
	2012	46.68±0.96^{C}	219.75±5.11^{B}	226.02±0.47^{A}
Esters total –	2011	13.72±0.36^{B}	13.75±0.22^{B}	69.28±2.62^{A}
ethyl acetate	2012	18.08±0.56^{C}	73.45±1.48^{A}	64.02±1.67^{B}
Esters total – ethyl	2011	1.10±0.03^{C}	1.65±0.06^{B}	2.31±0.03^{A}
acetate – ethyl lactate	2012	2.88±0.06^{A}	1.49±0.39^{B}	1.91±0.35^{B}
Hexanoic acid	2011	0.82±0.11^{B}	0.80±0.09^{B}	1.40±0.18^{A}
	2012	0.78±0.04^{B}	0.58±0.03^{C}	0.89±0.09^{A}
Octanoic acid	2011	2.31±0.03^{B}	2.76±0.21^{A}	2.42±0.07^{B}
	2012	1.84±0.06^{A}	1.48±0.12^{B}	1.39±0.24^{B}
Decanoic acid	2011	2.25±0.18	2.66±0.33	2.34±0.04
	2012	1.96±0.00	1.99±0.30	2.07±0.12

TABLE S-I. Continued

Compound	Year	Cultivar		
		ČR	ST	PO
Acids total	2011	5.38±0.32^{B}	6.21±0.45^{A}	6.16±0.14^{A}
	2012	4.58±0.10	4.05±0.38	4.35±0.28
Acetaldehyde	2011	8.50±1.23^{B}	12.13±0.71^{A}	11.93±0.45^{A}
	2012	7.47±0.42^{B}	7.93±1.11^{B}	17.07±0.67^{A}
Benzaldehyde	2011	1.07±0.06^{C}	2.93±0.06^{A}	1.80±0.10^{B}
	2012	2.93±0.21^{A}	3.17±0.20^{A}	2.10±0.11^{B}
Aldehydes total	2011	9.57±1.17^{B}	15.06±0.70^{A}	13.73±0.51^{A}
	2012	10.40±0.62^{B}	11.10±1.31^{B}	19.17±0.57^{A}

TABLE S-II. Contents of volatile compounds (mean ± standard deviation, g hL^{-1} a. a.) in six plum spirits (processing without stones) produced from the fruits without stones of Čačanska Rodna and its parent cultivars; a–c: differ significantly ($p \leq 0.05$) for processing without stones; ČR – Čačanska Rodna; ST – Stanley, PO – Požegača; 3M1B – 3-methyl-1-butanol; 2M1B – 2-methyl-1-butanol; 2M1P – 2-methyl-1-propanol; 1-P – 1-propanol; 2-PE – 2-phenylethanol; 1-H – 1-hexanol

Compound	Year	Cultivar		
		ČR	ST	PO
Methanol	2011	668.67±15.41^{b}	831.97±15.97^{a}	804.66±9.71^{a}
	2012	715.03±13.37^{b}	939.37±11.21^{a}	709.30±7.76^{b}
1-Propanol	2011	33.37±0.71^{b}	25.34±0.78^{c}	72.27±1.42^{a}
	2012	52.33±1.02^{b}	27.23±0.29^{c}	68.17±1.80^{a}
1-Butanol	2011	3.57±0.12^{b}	1.40±0.17^{c}	9.87±0.67^{a}
	2012	2.30±0.10^{b}	1.77±0.06^{c}	3.33±0.12^{a}
2-Butanol	2011	1.90±0.10^{b}	2.00±0.10^{b}	2.67±0.06^{a}
	2012	2.07±0.06	2.57±0.58	2.07±0.12
2-Methyl-1-propanol	2011	24.93±0.51^{b}	22.60±0.35^{c}	32.40±0.46^{a}
	2012	37.67±0.78^{b}	26.53±0.15^{c}	42.43±0.72^{a}
2-Methyl-1-butanol	2011	19.23±0.78^{b}	16.60±0.10^{c}	21.47±0.50^{a}
	2012	23.13±0.21^{b}	17.93±0.06^{c}	26.56±0.06^{a}
3-Methyl-1-butanol	2011	81.64±3.87^{a}	76.03±0.76^{b}	62.63±1.27^{c}
	2012	89.63±1.01^{a}	58.60±0.17^{c}	63.47±0.21^{b}
1-Hexanol	2011	1.47±0.01^{b}	1.77±0.72^{ab}	2.69±0.37^{a}
	2012	1.09±0.27^{b}	3.89±1.04^{a}	1.49±0.31^{b}
2-Phenylethanol	2011	1.82±0.12^{a}	0.98±0.10^{c}	1.47±0.19^{b}
	2012	2.86±0.13^{a}	1.00±0.04^{c}	1.84±0.14^{b}
Higher alcohols total	2011	167.92±4.51^{b}	146.71±0.56^{c}	205.47±2.96^{a}
	2012	211.08±2.52^{a}	139.52±0.52^{b}	209.36±2.17^{a}
3M1B/2M1B ratio	2011	4.24±0.05^{b}	4.58±0.07^{a}	2.92±0.03^{c}
	2012	3.87±0.02^{a}	3.27±0.00^{b}	2.39±0.01^{c}
(3M1B+2M1B)/2M1P	2011	4.05±0.25^{a}	4.10±0.06^{a}	2.60±0.05^{b}
ratio	2012	2.99±0.03^{a}	2.88±0.02^{b}	2.12±0.04^{c}
(3M1B+2M1B)/1-P	2011	3.02±0.14^{b}	3.66±0.12^{a}	1.16±0.02^{c}
ratio	2012	2.16±0.03^{b}	2.81±0.04^{a}	1.32±0.04^{c}

TABLE S-II. Continued

Compound	Year	Cultivar		
		ČR	ST	PO
2M1P/1-P ratio	2011	0.75±0.01[b]	0.89±0.02[a]	0.45±0.00[c]
	2012	0.72±0.01[b]	0.97±0.01[a]	0.62±0.01[c]
2-PE/1-H ratio	2011	1.24±0.08[a]	0.60±0.18[b]	0.55±0.07[b]
	2012	2.71±0.57[a]	0.27±0.06[c]	1.28±0.29[b]
Ethyl acetate	2011	55.10±3.10[c]	100.83±4.99[a]	86.63±1.70[b]
	2012	45.73±1.00[c]	150.77±1.25[b]	166.33±1.82[a]
Ethyl butanoate	2011	0.00±0.00[c]	0.07±0.03[b]	0.20±0.00[a]
	2012	0.09±0.02	0.09±0.02	0.10±0.00
Ethyl hexanoate	2011	0.13±0.06	0.13±0.06	0.23±0.06
	2012	0.20±0.00	0.13±0.06	0.20±0.00
Ethyl octanoate	2011	0.40±0.10	0.43±0.06	0.53±0.05
	2012	0.93±0.06[a]	0.37±0.05[b]	0.43±0.06[b]
Ethyl decanoate	2011	0.36±0.06	0.34±0.08	0.68±0.38
	2012	0.87±0.04[a]	0.23±0.10[c]	0.50±0.16[b]
Ethyl dodecanoate	2011	0.00±0.00	0.06±0.01	0.10±0.07
	2012	0.17±0.06[a]	0.04±0.02[b]	0.06±0.01[b]
Ethyl tetradecanoate	2011	0.00±0.00[b]	0.07±0.01[b]	0.23±0.06[a]
	2012	0.04±0.01	0.10±0.03	0.13±0.06
Isoamyl acetate	2011	0.20±0.00[b]	0.30±0.01[a]	0.17±0.05[b]
	2012	0.30±0.01	0.30±0.00	0.30±0.01
Diethyl succinate	2011	0.06±0.02[b]	0.27±0.04[a]	0.37±0.11[a]
	2012	0.08±0.03	0.43±0.04	0.96±0.74
Ethyl lactate	2011	15.25±0.27[c]	19.63±0.75[b]	57.01±1.37[a]
	2012	20.76±0.28[b]	59.61±1.05[a]	10.22±0.38[c]
Esters total	2011	71.50±3.33[c]	122.12±4.66[b]	146.15±3.27[a]
	2012	69.18±1.17[c]	212.07±0.05[a]	179.24±1.79[b]
Esters total – ethyl acetate	2011	16.40±0.46[c]	21.29±0.72[b]	59.52±1.72[a]
	2012	23.45±0.37[b]	61.30±1.21[a]	12.90±0.68[c]
Esters total – ethyl acetate – ethyl lactate	2011	1.15±0.21[b]	1.66±0.07[b]	2.51±0.44[a]
	2012	2.68±0.10	1.69±0.18	2.68±0.74
Hexanoic acid	2011	0.91±0.19	1.02±0.22	1.35±0.19
	2012	0.96±0.12[b]	0.73±0.02[c]	1.26±0.06[a]
Octanoic acid	2011	2.67±0.12	3.20±0.85	2.94±0.30
	2012	1.99±0.08	2.04±0.13	2.16±0.22
Decanoic acid	2011	2.29±0.17	3.32±1.32	2.29±0.17
	2012	2.28±0.11[b]	2.39±0.27[b]	3.08±0.02[a]
Acids total	2011	5.87±0.25	7.54±2.40	6.58±0.43
	2012	5.22±0.31[b]	5.16±0.36[b]	6.50±0.23[a]
Acetaldehyde	2011	6.43±0.95[c]	11.30±0.92[b]	15.33±0.49[a]
	2012	12.33±0.61[a]	8.23±0.35[c]	10.53±0.61[b]
Benzaldehyde	2011	0.33±0.29	0.50±0.10	0.37±0.32
	2012	0.13±0.23	0.43±0.38	0.57±0.11
Aldehydes total	2011	6.76±1.21[c]	11.80±0.85[b]	15.70±0.20[a]
	2012	12.46±0.61[a]	8.66±0.50[c]	11.10±0.72[b]

CHARACTERISTICS OF PLUM CULTIVARS FOR SPIRIT PRODUCTION

In the production of plum spirits with distinctive aromatic characteristics, old widespread plum cultivars (such as plum cultivars Požegača) have been traditionally used.[1,2] Depending on the country in which it is grown, cultivar Požegača has various synonyms: Hauszwetschge, Bistrica, Bistriţa, Bystricka, Kyustendilska Sinya (Кюстендилска синя), Wegierka zwyykla, Besztercei, Quetsche and German Prune. In recent years, in some countries and areas, some previously rarely used autochthonous, introduced or newly developed plum cultivars have been used more intensively as a raw material in the production of spirits. The main reasons are: *i*) replacement of old cultivars with cultivars that are more resistant or tolerant to plant diseases; *ii*) utilization of market surpluses of fruit cultivars, primarily intended for fresh consumption or processing into other products; *iii*) satisfying modern consumers' needs for the spirits with specific varietal and regional features. The suitability of less widespread and rarely used plum cultivars for spirit production is usually determined experimentally, based on the content of the volatile compounds and sensory characteristics of distillates obtained.[3–8] Besides the cultivar, differences in microflora during spontaneous fermentation are one of the important reasons for the occurrence of differences in the content of certain ingredients and aroma of monovarietal fermented mash[9] and the final distillates.[10] The quality of the plum spirits depends on the concentration and the relationship of the individual volatile components, which have different origins.

Methanol is toxic ingredient of plum spirit generated by the action of pectin methylesterase on the fruit pectin. Differences in the content of methanol in monovarietal plum spirits may be caused by different share of pectin fractions in total fruit pectin, degrees of its esterification by methanol and different activity of pectin methylesterase,[11–13] as well as the ratio of fermentable sugars and pectin in fruits.[14]

Yeasts form most of the analysed higher alcohols during fermentation from sugars and/or corresponding amino acids (leucine, valine, isoleucine, phenylalanine).[15] From this group of amino acids, leucine had the largest share in total amino acid in the fruits of Čačanska Rodna, Stanley and Požegača cultivars, followed by valine, and isoleucine.[16] During fermentation, 2-butanol derives from 2,3-butanediol (a by-product of sugar metabolism in yeast cells), by the activity of the yeast *Saccharomyces spp*. or, more commonly by the activity of bacteria *Lactobacillus spp*.[17] 1-Hexanol is the product of the enzymatic degradation of linoleic acid (lipoxygenase pathway) from the fruits during processing and fermentation of grapes and fruits.[18]

Higher alcohols are characterized by different odours: oily-floral odour (1-propanol and 2-methyl-1-propanol), unpleasant fusel-ton odour (1-butanol, 2/3--methyl-1-butanol and 2-butanol) and pleasant odour of rose (2-phenylethanol).

Scholten and Kacprowski[19] found non-characteristic unpleasant aroma of plum spirits at concentrations of 1-propanol higher than 362 g hL^{-1} a. a., of 2-butanol higher than 228 g hL^{-1} a. a. and of 2/3-methyl-1-butanol higher than 290 g hL^{-1} a. a. 1-Hexanol in lower concentrations contributes to odour freshness while at higher concentrations (> 10 g hL^{-1} a. a.) gives distillates an unpleasant odour of green part of plants (grass-like odour).[20]

Ethyl acetate is the most common ester in distilled beverages. Its share in total esters is usually greater than 50 %, often ranging even up to 95 %.[15] Lower concentrations of ethyl acetate contribute to fruity aroma, while at higher concentrations (above 218 g hL^{-1} a. a.) it negatively affects the odour of spirit due to its characteristic, "solvent-like" odour.[19] Ethyl acetate in spirits is primarily produced by acetic acid bacteria. Also, apiculate yeasts found in microflora at the beginning of spontaneous fermentations, produce to 10 times more ethyl acetate than the yeasts of genera *Saccharomyces*.[21]

The lactic acid bacteria, present in the indigenous microflora, form ethyl lactate during malolactic fermentation. This microbiological process may run simultaneously with alcoholic fermentation in wine spirit production.[22] Batagglia *et al.*[23] found similar during spontaneous alcoholic fermentation of plum mash. According to Scholten and Kacprowski,[19] the concentration of ethyl lactate above 208 g hL^{-1} a. a. exerts negative effects on the sensory characteristics of plum spirits.

Ethyl esters of fatty acids (ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate and ethyl tetradecanoate) as well as diethyl succinate and isoamyl acetate have pleasant fruity odours, and also contribute to the characteristic aroma of plum spirit.[24] These esters are mostly formed by yeasts during alcoholic fermentation.[15]

Besides higher alcohols and esters, volatile fatty acids are a significant part of the plum spirits aromatic complex.[24] Fatty acids are mostly formed by yeasts during alcoholic fermentation.[15] These compounds are characterized by unpleasant odours: the stable-like (hexanoic acid), the goat-like (octanoic acid) and the sweat-like (decanoic acid).

Acetaldehyde is the major aldehyde in spirit drinks, most commonly formed by yeast as a by-product of sugar metabolism during alcoholic fermentation. Acetic acid bacteria can also oxidize ethanol to acetaldehyde.[15] At lower concentrations, acetaldehyde contributes to the pleasant fruit aroma of spirits, while in larger concentrations it has a sharp grass-like and apple-like odour, so it may be responsible for the appearance of an unpleasant (head fraction-like) odour of plum spirit.[19]

Benzaldehyde is characterized by the specific odour of bitter almonds (a stone-like tone). This compound is considered as a characteristic component of plum spirits, especially those obtained by processing fruits with stones.[15,24]

There are no limits of the benzaldehyde content in plum spirits prescribed by the EU regulations.[25] Hence, it is up to producers and consumers to produce and consume spirits with more or less pronounced stone odour, depending on the personal preferences. On the other hand, Serbian regulations[26] have until recently limited the content of benzaldehyde in plum spirits to a maximum of 10 g hL^{-1} a. a. It was considered that greater benzaldehyde content adversely affected the sensory characteristics of spirits. In other words, the incidence of over-expressed stone-like tone in plum spirit is considered a defect in Serbia.

Traditional plum spirit production in Serbia includes the processing of plums with stones. Plum stones contain cyanogenic glycoside amygdalin, which is a precursor of toxic HCN and benzaldehyde. Stone removal (destoning) during processing of plums is the simplest way to decrease the contents of these ingredients in plum spirit.[27,28] Since benzaldehyde has a specific bitter almond odour, processing of plums with or without stone may affect the occurrence of differences in sensory characteristics of the spirits produced. Still, whether the spirit obtained from mash with or without stones will be consumer acceptable, it depends on the cultivar. Spirits produced from Požegača fruits with stones always have significantly higher sensory grades than the plum spirits obtained from fruits destoned prior to fermentation.[29] On the other hand, Schehl *et al.*[28] found that the presence or absence of stones during processing of the plum cultivar Ersinger had no significant influence on the assessors' preference and plum spirit attractiveness, but it was the matter of personal taste of each assessor. Effect of the presence or absence of stones during processing of the Čačanska Rodna and Stanley cultivars on sensory characteristics of plum spirits has not been investigated so far.

EXPERIMENTAL DETAILS

Plum fruits collection and its characteristics

The fruits of the plum cultivars Čačanska Rodna (ČR), Stanley (ST) and Požegača (PO) were harvested at full maturity from the same age trees in an experimental orchard of the Fruit Research Institute Čačak, at site Preljinsko Brdo (43°92′41″N, 20°44′75″E) in two consecutive years – 2011 and 2012. All the trees in the orchard were checked every year during June, to control the presence of plum pox virus. The fruits for the experiment were taken exclusively from the healthy trees. About 140 kg of the fruits of each cultivar were picked from six randomly selected trees in a row. Fruit processing was done immediately after harvesting. Only healthy and undamaged fruits were used.

On a randomly selected sample of 30 fruits of each cultivar, basic characteristics of plums (fruit weight, stone ratio, soluble solid content and pH value) were determined (Table S-III) according to the standard methods.[30]

Plum spirits production

For the processing with stones (W), 60 kg of fruit of each cultivar was used. Twenty kilograms of whole plum fruits with stones were placed in three 30 L polyethylene (PE) vessels for alcoholic fermentation (three replications). For the processing without stones

(WO), the stone was removed manually from every single fruit. In the majority of the fruits, fruit halves remained together after destoning thus enabling the mashes without stones characteristics similar to the mash with stones. Upon manual removing of stones, 20 kg of fruits were distributed in three 30 L polyethylene (PE) vessels (three replications) for alcoholic fermentation.

TABLE S-III. Basic characteristics of plums for spirit production

Characteristics	Year	Cultivar		
		Čačanska Rodna	Stanley	Požegača
Fruit weight, g	2011	35.71	39.40	20.17
	2012	32.97	46.28	19.33
Stone ratio, %	2011	3.80	5.72	4.30
	2012	4.78	5.15	4.53
Soluble solids content, %	2011	20.0	18.5	22.0
	2012	25.1	17.8	21.0
pH	2011	3.60	3.56	3.86
	2012	3.44	3.66	3.72

Spontaneous alcoholic fermentation of plum mashes was conducted by the indigenous microflora of plum fruits. During alcoholic fermentation in the open vessels, surface layers of mashes were in constant contact with air. Mash temperature during fermentation was 20±2 °C. Each day a reduction of soluble solids content (SSC) was measured in the mash, using 3828 Carl Zeiss manual refractometer. Alcoholic fermentation was considered completed if there was no decrease of soluble solids content in the mash during the two consecutive days. Table S-IV shows soluble solids contents in the unfermented and fermented mashes and duration of fermentation.

TABLE S-IV. Soluble solid content (mean±standard deviation, %) in unfermented and fermented mashes and the duration of alcohol fermentation; SSC – soluble solid content, W – processing with stones, WO – processing without stones; ČR – Čačanska Rodna, ST – Stanley, PO – Požegača; 1 – year 2011, 2 – year 2012

Characteristics	Year	Processing					
		W			WO		
		ČR	ST	PO	ČR	ST	PO
SSC in unfermented	1	20.0±0.0	18.5±0.0	22.0±0.0	20.0±0.0	18.5±0.0	22.0±0.0
mash, %	2	25.1±0.0	17.8±0.0	21.0±0.0	25.1±0.0	17.8±0.0	21.0±0.0
SSC in fermented	1	10.7±0.8	9.5±0.0	10.6±0.1	9.8±0.3	9.6±0.4	10.6±0.1
mash, %	2	12.8±0.2	9.1±0.4	11.4±0.7	13.0±0.1	10.6±0.6	11.3±0.7
Duration of alcoholic	1	11	11	11	9	8	9
fermentation, days	2	10	9	11	10	9	11

A double distillation, traditionally used in the production of plum spirit in Serbia, was performed. Fermented mashes were distilled immediately after completion of alcoholic fermentation. A 25 L copper pilot pot still of traditional construction (alembic) was used for distillation. A gas burner was used for direct heating of the boiler. During the first distillation (distillation of the fermented mashes), no fractions were being separated; the content of ethanol in the obtained monovarietal distillates was 28.0±0.3 vol. %. The second distillation

(re-distillation) was performed in the same alembic. During second distillation (re-distillation of the first distillate), the cutting of the first fraction (head) from the middle fraction (heart) was performed in the same way; the volume of the head fraction was 1 % of the volume of the first distillate, with 28.0±0.3 vol. % of ethanol, placed in the distilling pot to redistill. The middle fractions (hearts) were cut from the tail fractions in the same way; the volume of the heart was about 40 % (39.55–41.33 %) of the volume of the first distillate, with 28±0.3 vol. % ethanol, placed in the pot to redistill, so that the contents of ethanol in all obtained monovarietal heart fractions were 60.0±0.3 vol. %. In traditional plum spirit production in Serbia, using double distillation method in alambic, the middle fraction (heart) is often collected with ethanol content of 60.0±0.3 vol. %. For the analysis of volatile compounds and sensory analysis only middle fractions (hearts) were used. Since only the middle fraction (heart), obtained during second distillation (re-distillation), is used for the production of plum brandy in Serbia, the tail fraction, that was collected after the separation of the heart fraction, was not analyzed.

Chemicals and reagents

Analytical grade chemicals, manufactured by Merck (Darmstadt, Germany), Sigma–Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland) and Carl Roth (Karlsruhe, Germany) were used.

GC analysis of volatile compounds

The quantification of the major volatile compounds (methanol, 1-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, acetaldehyde and benzaldehyde) has been performed using the headspace method.[28,31] Briefly, a headspace gas chromatograph (model HS 40, GC 8420 Perkin Elmer, Überlingen, Germany) equipped with a packed crossbond phenyl methyl polysiloxane column (Rtx volatiles; 60 m×0.32 mm i.d. film thickness 1.5 μm, Resteck GmbH, Bad Homburg, Germany), a flame ionisation detector (FID), and a CLASS VP 4.2 integrator (Shimadzu, Duisburg, Germany) was used. Setting the headspace injector: the quantity of sample 3 ml; transfer line temperature 90 °C; time of pressure rise 3 min; sample temperature 70 °C; GC cycle time 45 min; retention time 0.5 min; needle temperature 90 °C; thermostat time 30 min; injection time 0.08 min. Temperature program of gas chromatograph oven: 2 min at 60 °C; 2 °C/min to 70 °C; 8 °C/min to 160 °C; 2 min at 160 °C; 4 °C/min to 200 °C; 15 °C/min to 250 °C; 10 min at 250 °C. Injector temperature was 260 °C and detector temperature was 270 °C. Carrier gas was helium (115 kPa). Gases for combustion were hydrogen (100 kPa) and synthetic air (160 kPa). As an internal standard, 2-pentanol was used.

The quantitative analysis of 2-phenylethanol, 1-hexanol, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, ethyl lactate, diethyl succinate, hexanoic acid, octanoic acid and decanoic acid was performed using polar column (HP-INNOWax column (30 m×0.32 mm *i.d.*, film thickness 0.25 μm, Agilent Technologie) with direct injection gas chromatography.[31] Briefly, a gas chromatograph Shimazu (model AOC-20, GC 17) equipped with a flame ionisation detector (FID), and a CLASS VP 4.2 integrator (Shimadzu, Duisburg, Germany) was used. Temperature program: 2 min at 60 °C; 5 °C/min to 100 °C; 10 °C/min to 250 °C; 10 min at 250 °C. Injector temperature was 260 °C and detector temperature was 280 °C. Carrier gas was helium (50 kPa). Gases for combustion were hydrogen (60 kPa) and the synthetic air (80 kPa). As an internal standard, 2-ethylbutyric acid was used. All samples of the plum spirit were analysed in triplicate.

Sensory analysis

For sensory analysis, ethanol content in middle fractions (hearts) was diluted with deionized water from 60.0±0.3 to 45.0±0.3 vol. %. Sensory analysis of the produced plum spirits was carried out by 5 members of the expert panel. Panel members are highly experienced (between 10 and 30 years) in the sensory evaluation of fruit spirits. Buxbaum method of positive ranking used for sensory analysis is based on four sensorial characteristics of plum spirit (clearness 0–1 points, colour 0–2 points, odour 0–7 points, taste 0–10 points) rated by maximum 20 points.

Statistical analysis

A statistical package program Statistica 7 (StatSoft Inc., Tulsa, OK, USA) was used for statistical analysis. Results of the gas chromatographic analysis and sensory analysis of the spirits were subjected to one-way analysis of variance (ANOVA). Spirits produced in the same manner (with or without stones) from the plums of Čačanska Rodna and its parent cultivars, during the same year, were compared. For plum spirit ingredients or sensory characteristics by which ANOVA showed statistically significant differences, a comparison was performed using Duncan's test ($p \leq 0.05$).

For determining similarities and differences among plum spirits, a cluster analysis was performed as well, using the same statistical package. Plots were scaled to a standardized scale $D_{link}D_{max}^{-1}\times10^2$ (D – distance, link – linkage, max – maximum of linkage Euclidean distance), and that ratio on the ordinate axis is a quantitative measurement of dissimilarities among plum spirits (expressed in %).

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