Phenolic compounds extractability from Melnik 55 grape solid parts during fruit maturity

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Abstract. Samples from Melnik 55 grape variety (*Vitis vinifera)* were collected from different stage of grape maturity. Grape seeds and skins from each sample were separated and extracted with model wine solutions with increasing alcohol content simulated alcohol accumulation during wine fermentation. Total phenolic compounds, anthocyanins, skin pigments and tannins in each sample were determined. During grape ripening not only total phenols content of grape skins and seeds are changed but also their extractability. Ethanol released during wine fermentation is strong phenol extragents but its role is getting smaller as riper is the grape. This tendency is more pronounced in seeds phenolics extractability than in skins.

1 Introduction

The phenolic compounds extracted from grape skins and seeds impact in wine quality in various aspects: wine taste and color, antioxidant capacity, wine finning [1, 2, 3, 4, 5, 6, 7, 8]. They also have an important role for the physiological status of the vine especially when the plant is subjected to biotic or abiotic stress factors [9, 10, 11].

The catechins content in solid parts of the grape is mainly genetic feature but also is affected from vine cultivation and climatic factors [12, 13, 14]. Significant dynamic in the content of this group of compounds is also observed during grape ripening. Up to three to seven times more catechins are identified in grapes during the veraison than in ripe grape [15]. This dynamic is even more significant in flavan-3-ols content in grape seeds and stems [16, 17, 18, 19, 20]. Decrease with 50 - 60 % during the grape ripening is also observed in the procyanidins content. The grape ripening also affects some characteristics of polymeric phenolic compounds. Degree of galloylation (DG) and mean degree of polymerization (mDP) of grape seeds and skins phenolic compounds are also changed [12, 16, 17, 21] These changes in mDP and the number of hydroxyl and galloylated groups affect the properties of procyanidins to reacts with proteins and therefore their role in wine astringency [22, 23, 24, 25, 26, 27] and apparently affects phenolic extractability from grape skins and especially from grape seeds [28, 29, 30, 31, 32].

The alcohol released during fermentation also influences on anthocyanin and monomeric and polymeric phenols extraction [33, 34, 35, 36, 37].

The aim of this work is to assess the grape seeds and skins phenolic compounds extractability in relation to the alcohol accumulation during wine fermentation and different stage of grape maturity of Melnik 55 variety.

2 Materials and methods

The acetic acid, Folin Chiocalteu (FC) reagent, sodium dodecyl sulphate, sodium azide, bovine serum albumin were purchased from Merck; the (+) catechin and gallic acid were from Fluka Chemie. All reagents were chemical grade.

The total phenols were detected by the FC colorimetric procedure using gallic acid as a standard. The color characteristics by absorption measurement at 420, 520 and 620 nm in 1 mm cells (undiluted) [38]. The total monomeric anthocyanins were detected by pH change and absorption measurements at 520 nm. Tannins, monomeric, olygomeric and polymeric pigments were detected by Adams procedure [39, 40]. The total rain falls and temperature date were from local meteo station. The physical clay of soil is content of the fraction with particles size under 0.01 mm (separation, drying and weighting).

2.1 Sample preparation

Eight samples each one from 50 grape berries vr. Melnik 55, vintage 2016) were selected in four different stage of the grape maturity (respectively 18th August, 7th and 19th September and 4th October). The berries were collected from five vines typical for the variety and with normal crop (cordon pruning with 4 spur per vine and 8 bunches for one vine). Each sample contains berries uniformly selected from lower middle and upper side of the same bunches. The skins and seeds from the berries of each

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sample were separated manually and weighed (the skin – directly after separation and the seeds – after washing with deionized water and drying). The skins and seeds from one of the samples were used to measure their volume. The rest of the skins and seeds samples were extracted with model solutions with repeating 3 times one of the sample numbers.

2.2 Extraction with model wine solution

Five model wine solutions were prepared. Each contains 5 g/dm^3 tartaric acid, 50 mg/dm^3 SO_2, 50 mg/dm^3 sodium azide and ethanol respectively 0, 3, 6, 9 and 12 %v/v.

The pH in every solution was set up to the pH 3.5 with sodium hydroxide solution. The weighed skins and seeds were flooded with model wine solutions for 24 h in naturally occurring solid parts/juice ratio. The sample with 3% v/v was repeated 3 times. The extracts from each sample were separated after 24 h.

2.3 Extraction with ethanol solution

After the extraction with model wine solutions the solid parts were subjected to three-fold extraction with ethanol/ HCl (0.1%) [41]. Ethanol/ HCl extracts were than mixed and analyzed.

3 Results and discussion

All samples were from the non-irrigated vineyards near to the Kapatovo village (Petrich region, south-west Bulgaria). The content of physical clay (particle size up to 0.01 mm) of the soil (depth from 25 to 50 cm) is 48%. This region is almost the hottest wine region in Bulgaria and average daily temperature more than 10°C is observed even in February (Fig. 1).

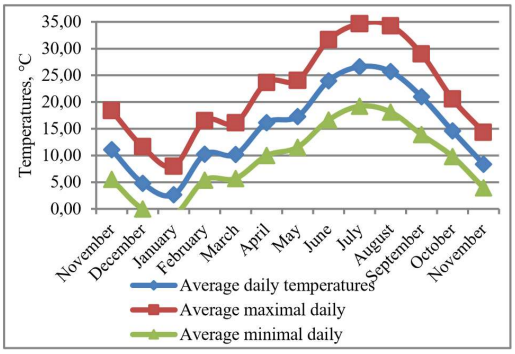

Fig. 1. Average temperatures for the period November 2015 – November 2016

For the active from the vintage 2016 term all quantity of rainfalls is 475.7 dm^3 (Fig. 2).

In these conditions Melnik 55 grape harvest from this region is usually started in the end of September. For the dates of sampling the sugar and acid content of the grape juice is presented in Table 1.

Table 1. Sugar (S), total acidity (TA) and pH of the samples

Date of sample collection	S, g/dm^3	TA, g/dm^3	pH
18.08.2016	210.2	7.81	3.28
07.09.2016	258.4	5.62	3.33
19.09.2016	260.2	5.42	3.54
04.10.2016	268.8	4.98	3.78

During the ripening the phenolic compounds extracted from grape skins decrease (Table 2) from average 278.0 mg/100g berries on August 18th samples to the 204.0 mg/100g berries on October 4th.

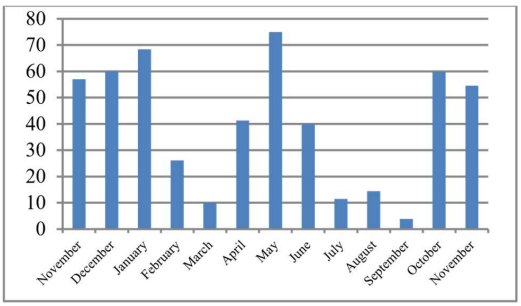

Fig. 2. Average rainfalls for the period November 2015 – November 2016

Table 2. Total phenols extracted from the grape skins

Date of sampling	1	2	3	4 = 2 + 3	5 = (2×100)/4
	Ethanol content in model wine solution, vol %	**Total phenols extracted with model wine solution, mg/100g berries**	**Total phenols extracted with ethanol solution, mg/100g berries**	**Total phenols extracted from the grape skins, mg/100g berries**	**Skin total phenol extractability, %**
18.08. 2016	0	91.4	186.6	278.0	32.88
	3	110.4	165.8	276.2	39.97
	6	106.6	154.6	261.2	40.80
	9	139.8	141.6	281.4	49.68
	12	164.8	128.6	293.4	56.18
			average	**278.0**	
07.09. 2016	0	61.4	151.6	213.0	28.83
	3	75.2	144.8	220.0	34.18
	6	105.4	145.8	251.2	41.96
	9	102.6	131.6	234.2	43.81
	12	104.2	128.2	232.4	44.84
			average	**230.2**	
19.09. 2016	0	67.2	171.6	238.8	28.14
	3	69.2	167.8	237.0	29.20
	6	75.2	140.8	216.0	34.81
	9	102.4	117.4	219.8	46.59
	12	98.8	134.2	233.0	42.40
			average	**228.9**	
04.10. 2016	0	40.4	151.2	191.6	21.09
	3	62.2	156.6	218.8	28.43
	6	64.8	140.8	205.6	31.52
	9	67.2	130.8	198.0	33.94
	12	72.0	134.0	206.0	34.95
			average	**204.0**	

For the both September samples the skins phenolics content are almost the same – 230.2 mg/100g and 228.9 mg/100g respectively for the 7th and 19th September. In this sampling period apparently the seeds phenolic compounds are subjected to more changes and their total phenols are 364.6; 290.2; 287.8 and 239.9 mg/100 g berries respectively for the four dates of sampling (Table. 3). Regarding the results from columns 5 from Table 2 and Table 3 it is interesting to note that extractability of the total phenols with model wine solution decrease during the ripening. This trend is more substantial in seeds extracts where in the different date with the same model wine solutions are extracted up to the 3 times fewer total phenols than in the early date of sampling.

Table 3. Total phenols extracted from the grape seeds

Date of sampling	1	2	3	4 = 2 + 3	5 = (2 × 100)/4
	Ethanol content in model wine solution, vol %	Total phenols extracted with model wine solution, mg/ 100g berries	Total phenols extracted with ethanol solution, mg/ 100g berries	Total phenols extracted from the grape seeds, mg/100g berries	Seed total phenol extrac-tability, %
18.08. 2016	0	32.1	333.4	365.5	8.78
	3	31.0	341.8	372.8	8.32
	6	28.9	334.5	363.4	7.95
	9	49.7	310.0	359.7	13.82
	12	47.7	313.7	361.4	13.20
			average	**364.6**	
07.09. 2016	0	14.7	278.6	293.3	5.01
	3	12.9	264.0	276.9	4.66
	6	20.8	276.8	297.6	6.99
	9	19.6	275.1	294.7	6.65
	12	30.1	258.6	288.7	10.43
			average	**290.2**	
19.09. 2016	0	14.1	269.8	283.9	4.97
	3	13.1	267.4	280.5	4.67
	6	20.2	280.7	300.9	6.71
	9	18.2	269.7	287.9	6.32
	12	21.2	264.8	286.0	7.41
			average	**287.8**	
04.10. 2016	0	5.9	236.6	242.5	2.43
	3	4.7	230.5	235.2	2.00
	6	4.8	234.1	238.9	2.01
	9	7.8	232.3	240.1	3.25
	12	10.1	232.7	242.8	4.16
			average	**239.9**	

May be extraction up to the 6 - 7% from total phenols of the grape seeds phenolics with model wine solution with 9% v/v alcohol content is a good indicator for Melnik 55 ripening. The wines from this native Bulgarian variety are often not very strong colored but often with harsh tannins and the exact timing of the grape harvest is crucial for future wines.

For the same period of sampling monomeric anthocyanin (MA) content of grape skins (tab.4) were changed from 118.5 to 115.1; 148.9 and 136.7 mg for 100 g fresh grape berries respectively for the 18th August, 7th September, 19th September and 4th October dates. In the same time extractability of MA with 0% v/v alcohol content solution increases with 41.63% for the 7th September sample and with 76.40 % for the 19th September sample compared with the August sample. This is essential to know especially when the cold maceration practice is applied. In this practical aspect extractability of MA more than 15% with zero alcohol content solution is good point for the ripening assessment.

From data of absorption at 420, 520 and 620 nm presented in fig. 3 can be seen that up to 19th September sample absorptions at 520 nm are increased. The riper is the grape the greater is the influence of the alcohol content of the model wine solutions. Decrease in A520 is observed at the last sample (4th October) apart from the alcohol content in solutions. In these samples also the A420 absorptions are increased.

Table 4. Monomeric anthocyanins extracted from the grape skins

Date of sampling	1	2	3	4 = 2 + 3	5 = (2 × 100)/4
	Ethanol content in model wine solution, vol %	Total phenols extracted with model wine solution, mg/100g berries	Total phenols extracted with ethanol solution, mg/100g berries	Total phenols extracted from the grape seeds, mg/100g berries	Seed total phenol extrac-tability, %
18.08. 2016	0	13.6	110.8	124.4	10.93
	3	12.9	106.5	119.4	10.80
	6	13.9	102	115.9	11.99
	9	15.6	100.6	116.2	13.43
	12	15.9	100.6	116.5	13.65
			average	**118.5**	
07.09. 2016	0	18.9	103.2	122.1	15.48
	3	18.25	95.8	114.1	16.00
	6	21.3	88.6	109.9	19.38
	9	23.9	89.4	113.3	21.09
	12	23.8	92.3	116.1	20.50
			average	**115.1**	
19.09. 2016	0	30.9	129.4	160.3	19.28
	3	31.9	113.5	145.4	21.94
	6	40.5	103.2	143.7	28.18
	9	48.9	98.4	147.3	33.20
	12	53.6	94.2	147.8	36.27
			average	**148.9**	
04.10. 2016	0	27.6	120.3	147.9	18.66
	3	25.5	115.3	140.8	18.11
	6	23.4	116.4	139.8	16.74
	9	23.4	109.6	133.0	17.59
	12	24.4	97.5	121.9	20.02
			average	**136.7**	

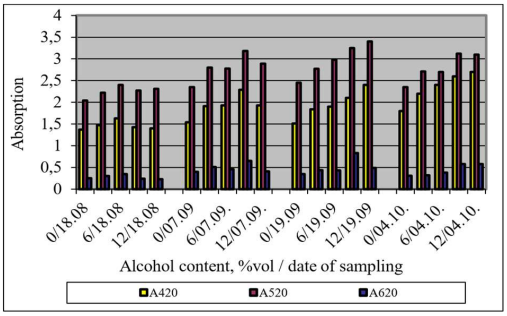

Fig. 3. Color characteristics of the grape skin extracts

The more mature is the grape the higher is percentage of oligomeric and polymeric pigments (Fig.4) although no significant differences can be seen in different pigments as a part of all color between the samples during the grape ripening.

The more significant is the difference between the samples in their tannins content (Fig. 5). In the first date of sampling the content of tannin (the phenolics that are able to precipitate with proteins) is highest.

Although the total phenolic compounds extracted from the seeds with 9 and 12% v/v ethanol solutions is almost 3 time less than total phenols extracted from the skins in the same conditions, the tannins extracted from the seeds are more than from the skins. In the second date of sampling – 7th September the tannins extracted from seeds and skins are almost 2 times smaller than in previous date but still from seeds and skins almost the same quantity of tannins is extracted in the all-alcohol contents of model wine solutions. In the third date – 19th September from grape seeds is already extracted almost 2 time less tannins than from the skins. This difference can be used for more reliable determine of grape ripening than the content of total phenols and anthocyanins.

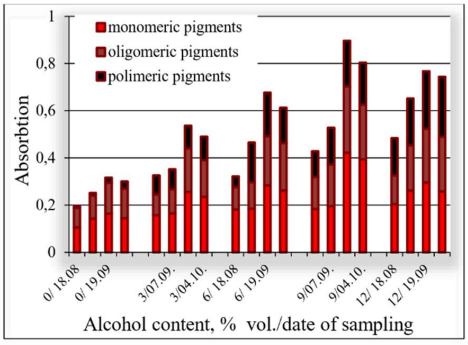

Fig. 4. Monomeric, oligomeric and polymeric pigments in the grape skin extracts

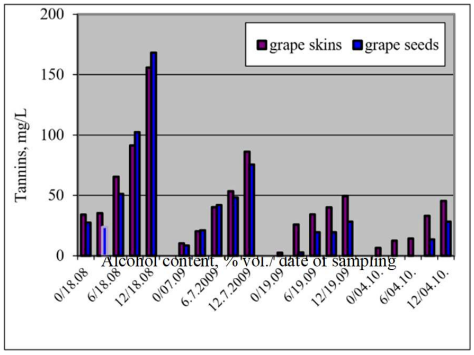

Fig. 5. Tannins content in the grape skin and seed extracts

4 Conclusion

The data shows that from the grape seeds of Melnik 55 variety appropriate in the sough-west part of Bulgaria can be extracted from 3 to 5 times fewer total phenols than from the skins during the grape ripening using model wine solutions as extragents. But in the same time in less ripe grape from the seeds can be extracted almost the same quantity of tannins as from the skins. In more mature grape the tannins extracted from the seeds is almost twice less than these extracted from the skins at 12 % v/v alcohol solution. This more significant change in grape skins and seeds tannins extractability can be used when the exact period of ripening wants to be determined. More data for different vintage, soil and climate conditions can be accumulated so that more precise conclusions for Melnik 55 ripening assessment can be made.

Prunus domestica "Stanley" from village Rumyantsevo, Lovech region could be a source of decent raw material for spirits beverages produces in Bulgaria and should be further explored in future more extensive studies. Both batches obtained from these fruits have excellent parameters and organoleptic qualities.

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