

EXTRACTABILITY OF GRAPE SEED AND SKIN PHENOLIC COMPOUNDS DURING GRAPE MATURITY

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Introduction

Grape phenolic compounds play a very important role in wine quality in various aspects: wine color (Gonzales-San-Jose 1990, Mirabel 1999, Saucier 1997, Timberlake 1980), astringency and bitterness (Arnold 1978, Arnold 1980, Brossaud 2001, Freitas 2001¹, Sarni-Manchado 1999), haze formation and interaction with proteins during wine finning (Maury 2001, Ricardo-da-Silva 1991, Siebert 1996, Yokotsuka 1987), oxidation and browning, and color stability (Cheynier 1991, Yokotsuka 2001, Singleton 1992).

The extraction of grape skin and seed phenolic compounds and their transfer into must is an essential part of red wine production. Recently, the evolution of catechins and proanthocyanidins in bunch stems during berry development has been the object of many studies. Monomeric and polymeric phenolic compounds have been found to decrease during grape maturation both in the skins and in the seeds (Kennedy 2000, Kennedy 2001, Freitas 2000, Jordao 2001). Seed tannins that are partly galloylated procyanidins based on (+)-catechin, (-)-epicatechin and (-)-epicatechin 3-gallate show larger proportions of galloylated units (from 13,2 % to 30,3 %) than skin tannins (from 2,7 % to 6,0 %) which also contain prodelfenidins. This procyanidin characteristic varied during grape maturation: (-)-epicatechin 3-gallate units decreased from 38 to 18 % of total terminal units in seed procyanidins, and from 13,8 % in green berries to 3,7 % in red berries in skin proanthocyanidins (Kennedy 2000, Kennedy 2001). The molecular weight is higher in skin tannins (from 3,4 to 83,3) than in seed tannins (from 2,3 to 18,6) (Prieur 1994, Souquet 1996). The mean degree of polymerization (mDP) of proanthocyanidins is also changed during grape maturation.

Changes that phenolic compounds undergo during fruit ripening are crucial for their extractability and transfer from the solid parts of bunches into wine. On the other hand, the chemical structure of phenolic compounds, degree of polymerization and galloylation of proanthocyanidins influence their affinity to interact with proteins and their pigmentation and copigmentation properties (Artz 1987, Hagerman 1998, Porter 1984, Freitas 2001², Mazza 1989, Mirabel 1999).

The aim of our work is to assess the phenolic compounds extracted from grape skin and seed depending on the alcohol accumulation at must fermentation in different stages of grape maturation.

Materials and Methods

The FC reagent, acetic acid, sodium dodecyl sulfate (SDS), sodium azide, bovine serum albumin (BSA), and hydrochloric acid were purchased from Merck Darmstadt, Germany; and the (+)-catechin and gallic acid – from Fluka Chemie, Germany. All the other reagents were chemical grade.

The total phenols were detected by the FC colorimetric method using gallic acid as a standard. The color characteristics were detected according to Glories as follows: the visible color was the sum of 420, 520 and 620 nm absorbance and the hue was the ratio of 420 nm absorbance divided by 520 nm absorbance (1 mm cells, wine pH, undiluted). The total monomeric anthocyanins were established by pH change. The total acidity, pH, and sugar content in must were determined according to the standard for wine and must chemical analysis procedures (Ivanov 1979). Tannins in the samples were detected by Adams procedure (Harbertson 2002).

Sample preparation: In order to assess the extractability of grape seed and skin phenolic compounds during alcohol accumulation, two kinds of seed and skin extracts were prepared:

Extracts with model wine solution: Five model wine solutions were prepared. Each of them contained 5g/dm³ tartaric acid, 50mg/dm³ SO₂, 50 mg/dm³ sodium azide and ethanol respectively 0, 3, 6, 9 and 12 % v/v; the pH in every solution was corrected to pH 3,3 using a sodium hydroxide solution. Six samples, each one consisting of 60 grape berries (vr. Cabernet Sauvignon, vintage 2002), were prepared in three different stages of grape maturity (the 1st one on September 18, 2002, the 2nd one on September 25, 2002 and the 3rd one on October 03, 2002). The samples were collected from five vines typical of the *Cabernet Sauvignon* variety and with normal crop loading. Six berries were taken from the same place on grape bunch – one for each sample. The berries were collected from the vines uniformly from the lower, middle and upper place of the vine. The skins and seeds from all 60 berries constituting one sample were separated manually. The skins were weighed and the seeds were washed with deionized water three times, then dried on filter paper and weighed. The weighed skins and seeds were flooded with model wine solutions from 1 to 5 at the naturally occurring solid parts/ grape juice correlation (the 6th sample was used for the measuring the volume of berries, skins and seeds by water displacement). The extragent from each sample was separated after 24 hours. *Spirit extracts preparation:* After the model wine solutions were separated, the solid parts, i.e. skins and seeds, were subjected to three-fold extraction with ethanol (Singleton 1983).

Results and Discussion

Data for the sugar content, total acidity and pH of the samples is shown on table 1:

Sugar, total acidity and pH of must table 1

Date of sample harvest	Sugar content of must %	Total acidity g/dm ³ as tartaric acid	pH
Sept. 18,2002	20.5	9.43	3.12
Sept. 25,2002	20.5	7.80	3.21
Oct. 03,2002	21.0	7.35	3.23

The analysis of total phenols in the model wine solutions and spirits extracts for the three different stages of grape maturation are shown in table 2 for skin extracts and in table 3 for seed extracts.

Total phenols in skin extracts prepared from grape harvested on Sept. 18, 2002: 1.*,
Sept. 25, 2002: 2.* and Oct. 03, 2002: 3.*

table 2

1	Model wine solution extracts					Spirit extracts			10 = 6+9	11 =6/10x100
	2	3	4	5	6	7	8	9		
sample	Ethanol content in the model wine extracts % v/v	Extragent volume ml	Total phenols, as gallic acid equivalent # mg/dm ³	Rate of extraction in comparison with sample *.5 %	Total phenols in the extragent mg	Extragent volume ml	Total phenols, as gallic acid equivalent # mg/dm ³	Total phenols in the extragent mg	Total phenols extracted from the skins mg	% of total phenols transferred in the model wine solutions %
1.1	0	44	925	59,18	41	120	690	83	124	33,06
1.2	3	44	1078	68,97	47	120	600	72	119	39,50
1.3	6	43	1401	89,64	60	120	550	66	126	47,62
1.4	9	44	1536	98,27	68	120	517	62	130	52,31
1.5	12	44	1563	100,00	69	120	517	62	131	52,67
2.1	0	41	988	67,49	41	120	742	89	130	31,54
2.2	3	43	1033	70,56	44	120	652	78	122	36,07
2.3	6	41	1203	82,17	49	120	560	67	116	42,24
2.4	9	42	1293	88,32	54	120	517	62	116	46,55
2.5	12	41	1464	100,00	60	120	496	60	120	50,00
3.1	0	46	674	57,31	31	120	688	83	114	27,19
3.2	3	46	799	67,94	37	120	665	80	117	31,62
3.3	6	45	934	79,42	42	120	688	83	125	33,60
3.4	9	44	1105	93,96	49	120	625	75	124	39,52
3.5	12	44	1176	100,00	52	120	539	65	117	44,44

- n = 4, p < 0,05, r = ± 26.9

The total phenols extracted from the skins (column 4) increased with the increase in the alcohol content of the model wine solution extracts for all stages of grape maturation and decreased with the increase in grape maturity. The extraction rate of total phenols in comparison with the sample with alcohol content 12 %v/v - *.5 (column 5) averaged 98 % for the sample 1.4. For samples 2.4 and 3.4 this mean was respectively 88.32 % and 93.96%. The 90 % extraction rate was reached even in sample 1.3 for the first harvest. There was also a decrease in total phenols transferred in the model wine solution extracts with the increase in grape maturity (column 11). The data confirm that there is a decrease in the total phenol pool of the skins with the increase in grape maturation and indicate that these are not the only changes in the skin total phenols. The extraction ability of phenolic compounds decrease with the increase in grape maturity. The mean degree of polymerization (mDP) of skin proanthocyanidins increase during grape maturation (Kennedy 2001) and this, as well as the possible association with polysaccharides, make grape skin proanthocyanidins more difficult to be extracted at must fermentation.

Total phenols in seed extracts prepared from grape, harvested on Sept. 18, 2002: 1.*,
Sept. 25, 2002: 2.* and Oct. 03, 2002: 3.*

table 3

1	Model wine solution extracts					Spirit extracts			10 = 6+9	11 =6/10x100
	2	3	4	5	6	7	8	9		
sample	Ethanol content in the model wine extracts % v/v	Extragent volume ml	Total phenols, as gallic acid equivalent # mg/dm ³	Rate of extraction in comparison with sample *.5 %	Total phenols in the extragent mg	Extragent volume ml	Total phenols, as gallic acid equivalent # mg/dm ³	Total phenols in the extragent mg	Total phenols extracted from the seeds mg	% of total phenols transferred in the model wine solutions %
1.1	0	44	289	29,31	13	40	3430	137	150	8,67
1.2	3	44	546	55,38	24	40	4131	165	189	12,70
1.3	6	43	447	45,33	19	40	3484	139	158	12,03
1.4	9	44	806	81,74	35	40	3664	147	182	19,23
1.5	12	44	986	100,00	43	40	3646	146	189	22,75
2.1	0	41	526	76,45	22	40	4677	163	185	11,89
2.2	3	43	465	67,59	20	40	4518	181	201	9,95
2.3	6	41	460	66,86	19	40	4434	177	196	9,69
2.4	9	42	661	96,08	28	40	4539	182	210	13,33
2.5	12	41	688	100,00	28	40	4224	169	197	14,21
3.1	0	46	280	52,14	13	40	4257	170	183	7,10
3.2	3	46	216	40,22	10	40	3736	149	159	6,29
3.3	6	45	266	49,53	12	40	3664	147	159	7,55
3.4	9	44	318	59,22	14	40	3448	138	152	10,14
3.5	12	44	537	100,00	24	40	3574	143	167	14,37

- n=4, p < 0,05, r = ± 26.9

No clear relationship was observed between the alcohol content in the model wine solutions and the phenolic compounds extracted from the seeds up to 6 %v/v (column 2 and column 4 – table 3). The total phenols extracted were higher at 9 and 12 % v/v ethanol content. For the grape harvested on 03.10.2002, the rate of extraction at the 9 % v/v alcohol content did not exceed 60 % in comparison with sample 3.5. For the samples harvested on September 25, this mean exceeded 60 % for all alcohol content in the model wine solution extracts but the percentage of phenols transferred in the model wine solution was comparable with samples 3.*. The total phenols in the model wine solutions and their transfer in wine was the highest for 1.* samples. The phenols transferred into the model wine solution decreased with grape maturation. The extraction rate decreased in the same manner (column 11) with the change in the influence of alcohol content during grape maturation.

The quantity of monomeric anthocyanins extracted from the skins and their extraction rate also decreased during grape maturation (the data is not shown). The percentage of monomeric anthocyanins transferred into the model wine solutions varied from 39.39 to 50.00,

from 40.38 to 54.90 and from 31.11 to 50.98 % respectively for the samples harvested on September 18, September 25 and October 03, 2002.

Visible color characteristics of the samples (n = 4; p < 0.05; r = ± 0.05)

table 4

Sample №	Samples from Sept. 18, 2002					Samples from Sept. 25, 2002					Samples from Oct. 03, 2002				
	A ₄₂₀	A ₅₂₀	A ₆₂₀	I	T	A ₄₂₀	A ₅₂₀	A ₆₂₀	I	T	A ₄₂₀	A ₅₂₀	A ₆₂₀	I	T
*.1	2.60	5.19	0.88	8.67	0.501	2.57	4.86	0.87	8.30	0.529	1.64	2.45	0.49	4.58	0.669
*.2	2.86	5.75	0.94	9.55	0.497	2.51	4.29	0.76	7.56	0.585	2.10	3.59	0.70	6.36	0.585
*.3	3.19	6.52	1.13	10.84	0.489	2.95	5.50	0.97	9.42	0.536	2.45	4.07	0.75	7.27	0.602
*.4	3.46	7.20	1.18	11.84	0.481	2.88	5.73	0.95	9.56	0.503	2.82	4.88	0.88	8.58	0.578
*.5	3.36	6.67	1.08	11.11	0.504	3.15	6.04	1.02	10.21	0.522	2.85	5.21	0.90	8.96	0.547

The color characteristics of the samples are shown on table 4. The color intensity increased with the increase in the alcohol content (except for samples 1.4 and 1.5). In the period studied, the color intensity decreased during grape maturation. The variation in anthocyanin content during maturation has been discussed by other authors as well (Gonzales-San Jose 1990). An assessment of the climatic conditions and a longer study period would be needed to evaluate anthocyanin evolution during maturation and their influence in the wine color. There is no alcohol fermentation in the sample preparation for the purposes of our study, therefore the role of the acetaldehyde formed during fermentation is not discussed here. The acetaldehyde effect on wine polymeric pigment formation, co-pigmentation and the nature of grape pigment during maturation will be treated in our next work.

In this study, the tannin content is assessed by means of precipitation with bovine serum albumin (Herbertson 2002). The results are shown on table 5.

Tannins in grape skin and seed model wine solution extracts table 5

Sample №	Tannins, mg/l catechin equivalent					
	Sept. 18, 2002		Sept. 25, 2002		Oct. 03, 2002	
	Skin extracts	Seed extracts	Skin extracts	Seed extracts	Skin extracts	Seed extracts
*.1	48	30	n.d.	25	2	n.d.
*.2	12	5	6	5	n.d.	n.d.
*.3	21	1	15	3	3	n.d.
*.4	108	166	36	124	24	4
*.5	189	240	87	125	24	98

n.d. – not detected

The tannins extracted from the solid parts – skins and seeds - during alcoholic fermentation cause precipitation of salivary proteins that provide lubrication in the mouth. This is associated with the astringent taste in red wine. The ability of tannins to precipitate proteins depends on many tannin characteristics: mDP, degree of galloylation,

structural pattern, which involves too much analysis. The study of all these characteristics for wine phenolic compounds is too expensive and not always possible. Although the tannin-protein precipitation is also influenced by protein characteristics, the tannins detected by means of these methods may be related to wine astringency.

In spite of the relatively steady increase in total phenols in the skin and seed model wine solution extracts, the tannins were much more in the extracts with alcohol content 9 and 12 % v/v. Tannins decreased both in the skin and seed extracts during grape maturation. Although the total phenols were more in the skin extracts, the tannins were more in the seed extracts, which corresponded to the seed tannin contribution to wine astringency formation. The alcohol content influence on the seed and skin tannin extraction decreased with grape maturation. The riper the grapes, the more prolonged the pomace contact may be in red wine producing.

The percentage of total phenol (TPh) decrease for the samples with alcohol content 12 % v/v with grape maturation ($[(TPh_{1.5}^{18.09} - TPh_{2(3).5}^{25.09(03.10)}) / TPh_{1.5}^{18.09}] \times 100$) was 6,33 and 24,76 % for skin extracts, and 30,22 and 45,54 % for seed extracts. When the percentage of tannin decrease was calculated for the same extracts, the values were respectively 53,97 and 87,30 % for skin extracts and 47,92 and 59,17 % for seed extracts. This data indicate a more rapid decrease in total phenols in the seed extracts during maturation in comparison with the skin extracts and a more rapid decrease in the tannins in the skin extracts at the same time. The decrease in the tannins, however, was more rapid than the decrease in total phenols both in the skin and the seed extracts. This may be associated with oxidation and the interaction of

phenolic compounds with polysaharides, which decrease the ability of phenols to precipitate with proteins. These processes probably occur more easily in the grape skins than in the seeds.

Conclusion

The total phenols and tannins of the grape seeds and skins decreased during fruit ripening. Their extractability also changed in this period. Although the total phenols were higher in the skins, the tannins were higher in the seeds model wine extracts.

This variation in the phenolic compounds of grape seeds and skins, the rate of their decrease during fruit ripening, the different extractability in relation to alcohol accumulation during wine fermentation, and the wine color characteristic have to be considered when grapes in different maturity stages are used in winemaking. The pomace contact with different duration may be used in relation to grape maturity and the desired wine character.

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