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Quality Evaluation of Anthocyanin Extract Obtained from Wine Grape Pomace

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Summary

Extraction of anthocyanins from wine grape pomace (grape variety »Vranec«) with water containing 0.05–0.5% SO_2 was investigated. A multistage extraction was performed using minimal pomace to solvent mass ratio 1:3 that enabled soaking of pomace, as well as a mass ratio 1:5. Content of anthocyanins and ballast-tannins and nitrogen compounds, were determined in the extracts. The degree of extract purity was estimated by so called quality indicator (QI) value. It indicates the difference between the anthocyanin yield and the average ballast yield. The extracts purity increases as SO_2 concentration increases. The extracts obtained with mass ratio 1:5 were purer.

Keywords: anthocyanins, grape pomace, multistep extraction, purity of the extract

Introduction

A group of synthetic colours for food products have potential mutagenic effects *(1)*. The restriction of the use and prohibition of some red colouring additives have spurred the interest in natural pigments. More recent publications report about plant pigment application as food colorants *(2–6)*. As a source of red pigments miscellanous raw materials and extraction methods have been studied. The most frequently investigated raw material as relatively inexpensive source of anthocyanins has been grape pomace remaining after wine or juice production. Philip *(7)* proposed a tartaric acid acidified methanolic solution for continuous process of anthocyanins extraction from grape waste. Metivier *et al. (8)* proved that acidified methanol is 20% more effective than the corresponding acidified ethanol. Peterson and Jaffe *(9)* patented a process of anthocyanin extraction from berry and fruit with water or ethanol containing 200–2000 ppm SO_2. Pompei *et. al. (10,11)* found deterioration of extract quality in the course of grape pomace extraction with aqueous SO_2(400–2000 ppm) if pH is corrected to 2.5 with tartaric acid. A proportion of the anthocyanins and tannins of the new wine/extract are associated with macromolecules of proteins and polisaccharides as Ribereau-Gayon *(12)* noted. Thus formed colloidal fractions of the colouring matter are precipitated during wine/extract cooling. The condensation reaction leads to purification of the anthocyanin extracts, and a drop in anthocyanin content. Bocevska and Stevčevska *(13)* found that candy and jelly mass coloured with partially purified anthocyanin extract of grape wine pomace are more colour-stable than if unpurified extract is used.

In the current study anthocyanins were extracted from the wine grape pomace. The influence of SO_2 concentration in water, step of extraction and pomace to solvent mass ratio on the anthocyanin extract purity was investigated.

Materials and Methods

Raw Material

Grape pomace was taken after the intensive fermentation of grape variety »Vranec« in wine industry »Skovin-Skopje«. The stems and the greatest part of the seeds were separated by hand. Some seeds remained captured within the hulls. These hulls were about 52% of the initial pomace. They were frozen and stored at –18 °C. The day before experiments they were thawed by placing in refrigerator (+4 °C) overnight. The hulls were ground and the particles with dimensions in the range of 1 mm to 1.6 mm were used for anthocyanin extraction. Their approximate composition was: 43.6% dry matter, 6.4% water soluble substances, 3.323 g/kg anthocyanins, 26.3 g/kg tannins, 1.534 g/kg nitrogen and 2.14% tartaric acid.

Extraction Procedure

In the initial phase 10 g of pomace was extracted for 24 h at 30 °C with periodical stirring. A 1:5 mass ratio of pomace to water containing 0.05% to 0.5% SO_2 was used. Content of anthocyanins in the extracts was measured every 15 min during the first hour, later on every two or four hours. In the second phase a six-step extraction was performed. The steps continued for 24 h. The extracts were filtered on a fritted glass filter and allowed to be stabilized overnight in the refrigerator. Then they were centrifuged at 2500 rpm. In the supernatants, content of anthocyanins, tannins, nitrogen, reducing sugars, pH, and DI values were determined. All experiments were duplicated.

Analytical Methods

Total anthocyanins content and Degradation Index (DI) values were determined by the spectrophotometric pH differential method of Fuleki and Francis *(14,15)* at the wavelength 520 nm. Calculation was performed by expressing anthocyanins as a mixture of dominant malvidin-3-glucoside and pelargonidin-3-glucoside, identified by ascending chromatography *(16)*. Tannins were measured by the permanganometric procedure of Leventa, cited by Harlamova and Kafka *(17)*. Nitrogen was determined by the micro-Kjeldahl method *(18)*. The content of dry matter and aqueous soluble substances in the pomace were measured by the gravimetric methods, drying the samples at 105 °C to the constant mass. Titratable acidity was determined by titrating the sample to an end point of pH = 7 with 0.1 M NaOH by the use of pH meter, with the result expressed as percent tartaric acid. Reducing sugars were determined according to the procedure of Miller *(19)*.

Results and Discussion

Single Extraction of Wine Grape Pomace

Wine grape pomace (pomace to solvent mass ratio = 1:5) was macerated for 24 h with periodical stirring. The influence of SO_2 concentration in water on anthocyanins yield *vs* time of extraction is illustrated in Fig. 1. It must be stressed that in the course of the first hour anthocyanins were rapidly extracted in all cases. Later the rate of extraction was very slow if 0.05% SO_2 aqueous solution was used, slightly faster with 0.1% SO_2 obtaining only about 10% to 12% of total anthocyanins after 24 h. For the same time anthocyanins yields of about 19.5, 21 and 24% were attained with 0.2, 0.3 and 0.5% SO_2 solutions, respectively. It means that only aqueous solutions containing SO_2 over 0.1% caused destruction of the hull cells that favour extraction and increased anthocyanins yield.

Multiple Extraction of Wine Grape Pomace

Multiple extraction of wine grape pomace was conducted to determine the effect of pomace to solvent mass ratio, SO_2 concentration and extraction step on the extractibility of some hull compounds. This was necessary for evaluating the degree of extract purity. Namely, it is

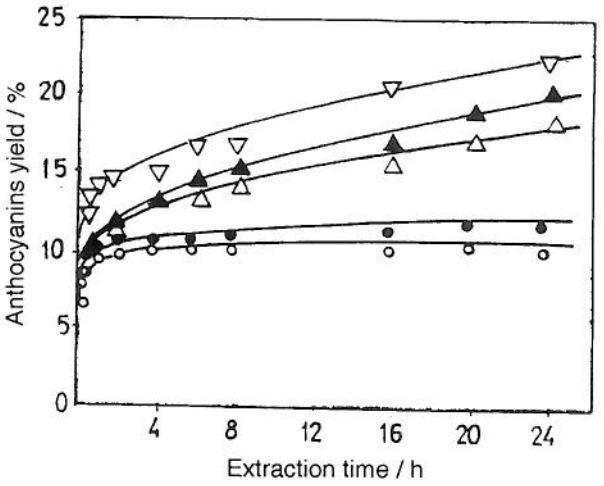

Fig. 1. Extraction yield of anthocyanins depending on SO_2 concentration in water and extraction time (pomace/solvent mass ratio 1:3) (○ – 0.05% SO_2; ● – 0.1% SO_2; △ – 0.2% SO_2; ▲ – 0.3% SO_2; ▽ – 0.5% SO_2)

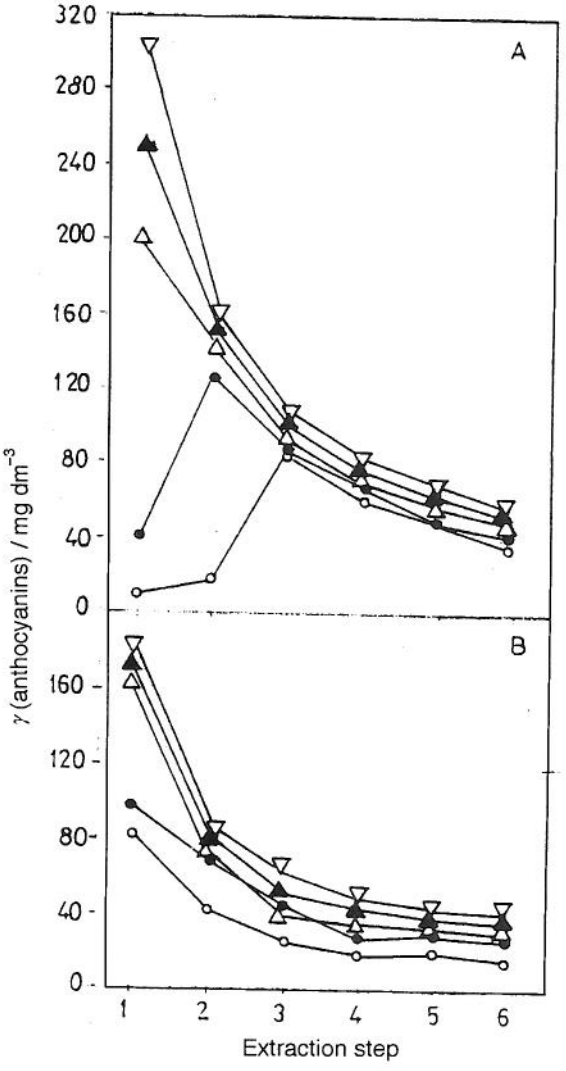

Fig. 2. Effect of multiple extraction of wine grape pomace with water containing SO_2 on anthocyanins content in the extracts. Pomace/solvent mass ratio: A) 1:3; B) 1:5 (The curves are marked like in Fig. 1)

desirable to obtain the extract with maximum anthocyanins and minimum undesirable ballast compounds that affect anthocyanins precipitation and loss. Extraction was performed with minimal mass ratio 1:3 that enabled soaking of pomace, as well as a mass ratio 1:5. Anthocyanin concentration in the extracts obtained after each extraction step is shown in Fig. 2. When mass ratio of 1:3 and SO_2 concentrations below 0.1% were used just a small part of anthocyanin transferred into the medium of the first and the second extraction step. When SO_2 concentrations over 0.1% were used the greatest amount of anthocyanins occurred in the extracts of the first extraction step. The rate of anthocyanin extraction from the third to the sixth extraction step was almost equal for all SO_2 concentrations that is seen from the slopes of the curves.

When mass ratio 1:5 was used the most of the anthocyanins were extracted at the first four extraction steps. The rate of anthocyanin extraction was different for every step and SO_2 concentration.

In Tables 1 and 2 pH values of the extracts are presented. It is clear that pH was falling from step to step. In pH range from 2 to 3.5 any alteration in the composition and the decrease in the content of anthocyanins are avoided.

Tables 1 and 2 also give information about DI values of the extracts. When 1:5 mass ratio, or 1:3 mass ratio with SO_2 concentrations over 0.1% was used, DI values increased from step to step. The first and second extraction step with mass ratio 1:3 and SO_2 concentrations below 0.1% gave the highest DI values. These changes are related to tannins content in the extracts. Tannins content increased with every extraction step while the content of more easily extractable anthocyanins were decreasing (Fig. 3).

The highest extraction of nitrogen compounds occurred in the first extraction step corresponding to SO_2 concentrations. In subsequent extraction steps the content of nitrogen compounds was falling without strong dependence on SO_2 concentration (Fig. 4).

Content of reducing sugars in the extracts obtained by the all SO_2 concentrations was in traces (results are not presented).

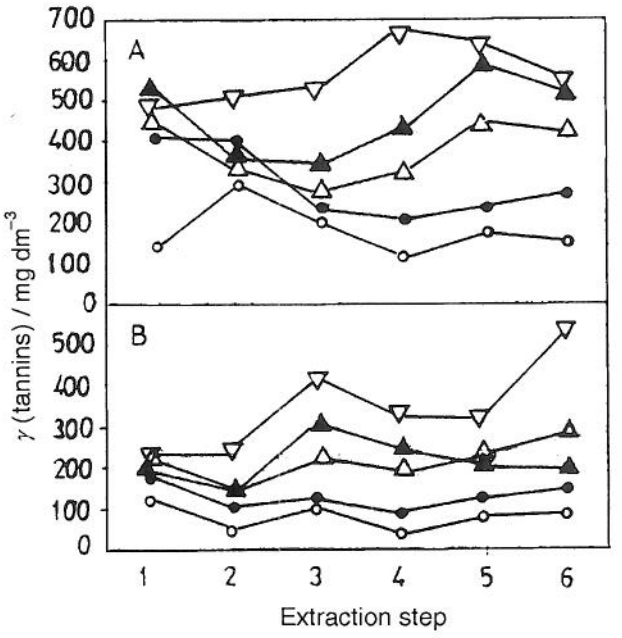

Fig. 3. Effect of multiple extraction of wine grape pomace with water containing SO_2 on tannins content in the extracts. Pomace/solvent mass ratio: A) 1:3; B) 1:5 (The curves are marked like in Fig. 1).

Table 1. pH and DI values of the extracts obtained with water containing different SO_2 concentrations (pomace to solvent mass ratio = 1:3)

Extraction step	w (SO_2)/%									
	0.05		0.10		0.20		0.30		0.50	
	pH	DI	pH	DI	pH	DI	pH	DI	pH	DI
1	3.40	1.60	3.30	1.21	3.20	1.08	3.13	1.07	3.03	1.05
2	3.40	1.50	3.28	1.12	3.07	1.12	2.91	1.10	2.61	1.10
3	3.30	1.17	3.11	1.17	2.74	1.17	2.49	1.17	2.15	1.22
4	3.31	1.26	2.88	1.28	2.31	1.30	2.13	1.31	2.03	1.31
5	3.07	1.28	2.66	1.34	2.30	1.33	2.16	1.31	2.03	1.32
6	2.28	1.30	2.39	1.30	2.18	1.33	2.08	1.33	1.90	1.36

Table 2. pH and DI values of the extracts obtained with water containing different SO_2 concentrations (pomace to solvent mass ratio = 1:5)

Extraction step	w (SO_2)/%									
	0.05		0.10		0.20		0.30		0.50	
	pH	DI	pH	DI	pH	DI	pH	DI	pH	DI
1	3.44	1.08	3.35	1.08	3.23	1.05	3.13	1.07	3.03	1.04
2	3.30	1.16	3.30	1.10	3.18	1.09	2.99	1.11	2.35	1.10
3	3.45	1.22	3.26	1.20	2.78	1.19	2.78	1.23	2.32	1.20
4	3.19	1.25	2.88	1.21	2.39	1.20	2.29	1.23	2.10	1.23
5	3.06	1.33	2.59	1.25	2.27	1.23	2.29	1.25	2.00	1.27
6	2.99	1.42	2.59	1.35	2.26	1.29	2.01	1.33	2.01	1.36

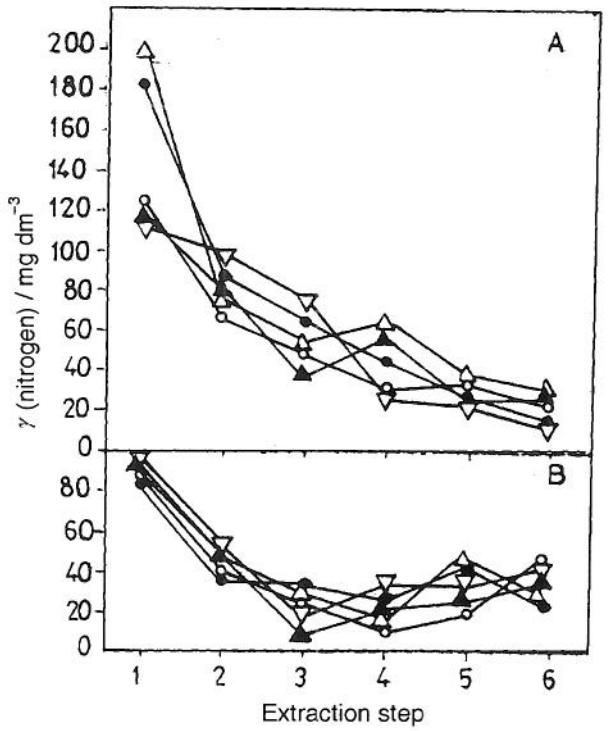

Fig. 4. Effect of multiple extraction of wine grape pomace with water containing SO_2 on nitrogen content in the extracts. Pomace/solvent mass ratio A) 1:3; B) 1:5 (The curves are marked like in Fig. 1).

Evaluation of Extract Quality

On the basis of the results of these extraction studies an attempt was made to choose number of extraction steps, mass ratio and SO_2 concentration that enabled obtaining the purest anthocyanin extract. The difference between the anthocyanins yield and the average yield of the other (tannins and nitrogen), so called ballast compounds, gives a quality indicator (QI) value. It is clear that the extract is purer if QI value is higher; that means a greater percentage of anthocyanins are extracted than undesirable compounds.

The yields of anthocyanins and ballast compounds, tannins and nitrogen are given in Fig. 5.

The data about QI values of the extracts obtained with different SO_2 concentrations and mass ratios are shown in Table 3.

The anthocyanin preparations obtained by mass ratio 1:3, SO_2 concentrations of 0.05, 0.1 and 0.2% expressed negative QI values. Negative QI value also occurred at 1:5 mass ratio and 0.05% SO_2 concentration. It is evident that QI values increased as SO_2 concentration increased. This agrees with the results obtained by Palamideas and Markakis *(20)*. According to their evaluation the extract of grape wine pomace with 500 ppm SO_2 solution gave purer anthocyanins than the water extract.

The extracts obtained by mass ratio 1:3 have shown lower QI values compared with that obtained with the same SO_2 concentration by mass ratio 1:5. The gratest QI value (13.3) was obtained after the third extraction step with 0.5% SO_2, 1:5 mass ratio, yielding 41% of anthocyanins. The same anthocyanin recovery, with slightly less purity, was obtained as early as after the fourth extraction step with 0.3% SO_2, mass ratio 1:5.

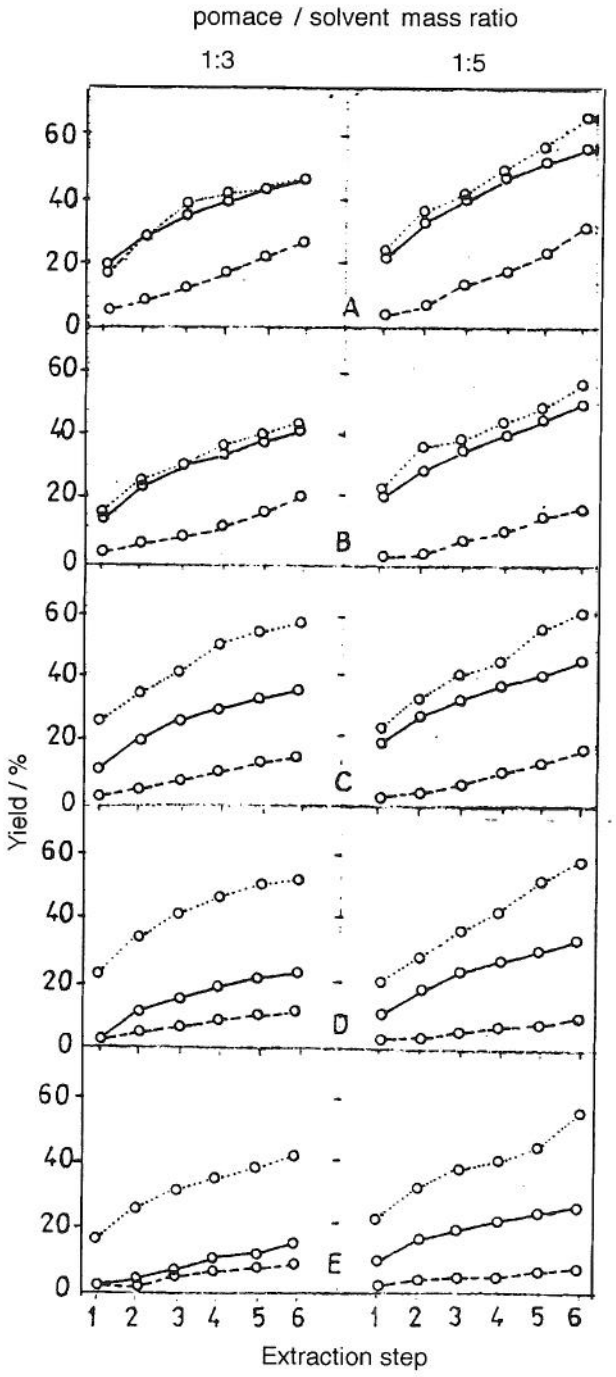

Fig. 5. Total yields of anthocyanins, tannins and nitrogen after each extraction step in dependence on the pomace/solvent mass ratio and SO_2 concentration in water. (Extract obtained with water containing: A 0.5% SO_2; B) 0.3% SO_2; C) 0.2% SO_2; D) 0.1% SO_2 and E) 0.05% SO_2 (_____ anthocyanins; ..... tannins; _ _ _ _ nitrogen)

Conclusions

Anthocyanin extraction from wine grape pomace is intensified with increasing SO_2 concentration in water,

Table 3. Quality indicator (*QI) values of the extracts

Extraction step	1	2	3	4	5	6
Pomace/solvent mass ratio			1:3			
w (SO_2)/%			QI values			
0.05	–9.6	–12.9	–11.6	–10.2	–10.8	–9.3
0.10	–10.4	–9.4	–8.8	–8.1	–7.9	–7.4
0.20	–2.2	0.3	1.4	–0.2	–0.2	–0.7
0.30	5.7	8.3	10.4	10.3	10.3	8.7
0.50	8.6	9.9	9.2	10.0	10.0	9.8
Pomace/solvent mass ratio			1:5			
w (SO_2)/%			QI values			
0.05	–1.9	–1.1	–1.9	–1.0	–1.6	–5.3
0.10	–0.3	2.4	3.0	2.9	1.3	0.2
0.20	7.1	9.1	8.9	10.2	7.4	5.7
0.30	7.4	9.7	12.2	12.9	13.1	12.6
0.50	8.4	10.9	13.3	13.0	11.2	6.6

*QI = yield (anthocyanins)/%-1/2 yield (tannins+nitrogen)/%

and pomace to solvent mass ratio. Extraction process proceeds with simultaneous transfer of tannins and nitrogen compounds into the extraction medium that affects extract purity. The extract purity increases as SO_2 concentration increases. Mass ratio of 1:5 gives purer anthocyanin extracts. When 0.5% SO_2 in aqueous solution is used a three-step extraction is recommended, although the anthocyanin yield is only about 41%. Further extraction gives less pure extracts. The same yield of anthocyanins with slightly lower QI values is achieved with 0.3% SO_2 already after four extraction steps. Because of a lower solvent cost an aqueous 0.3% SO_2 is preferred as an extraction medium for anthocyanin extraction at 30 °C.

References

1. M. Vargova, *Cesk. Hyg. 31* (1986) 36.
2. J. H. Main, F. M. Clydesdale, F. J. Francis, *J. Food Sci. 43* (1978) 908.
3. R. E. Buckmire, F. J. Francis, *ibid. 43* (1978) 908.
4. J. Debicki-Pospišil, T. Lovrić, K. Szabo, I. Topolnjak, *Prehrambeno-tehnol. rev. 17* (1979) 3.
5. L. Freedman, F. J. Francis, *J. Food Sci. 49* (1984) 1212.
6. F. J. Francis, *ibid. 50* (1980) 1640.
7. T. Filip, *ibid. 39* (1977) 589.
8. R. P. Metivier, F. J. Francis, F. M. Clydesdale, *ibid. 45* (1980) 1099.
9. R. G. Peterson, E. B. Jaffe, Berry and fruit treating process. *US Patent 3 484 254* (1969).
10. C. Pompei, M. Lucisano, E. M. Casiraghi, *Riv. Viticol. Enol. 36* (1983) 381.
11. C. Pompei, M. Lucisano, E. M. Casiraghi, *ibid. 36* (1983) 400.
12. P. Ribereau-Gayon, The Anthocyanins of Grapes and Wines, In: *Anthocyanins as Food Colors*, P. Markakis (Ed.), Academic Press, N.J. (1982) p. 237.
13. M. Bocevska, V. Stevčevska, *Hrana i ishrana, 30* (1989) 115.
14. T. Fuleki, F. J. Francis, *J. Food Sci. 33* (1968) 72.
15. T. Fuleki, F. J. Francis, *ibid. 33* (1968) 83.
16. M. Bocevska, *Optimizacija na nekoi parametri pri ekstrakcija na antocijani (crveni pigmenti) od ostatokot posle dobivanjeto na ovošni sokovi*, M Sc Thesis, Faculty of Technology and Metallurgy, Skopje, Macedonia (1988) p. 50.
17. O. A. Harlamova, V. V. Kafka: *Naturalni piščevi krasiteli, Piševaja promišlenost*, Moskow (1979) p. 102.
18. J. Trajković, M. Mirić, J. Baras, S. Šiler, *Analiza životnih namirnica, Beograd* (1983) p. 75.
19. G. L. Miller, *Anal. Chem. 31* (1959) 426.
20. N. Palamidis, P. Markakis, *J. Food Sci. 40* (1975) 1047.

Ocjenjivanje kakvoće ekstrakta antocijanina iz grožđane komine

Sažetak

Ispitana je ekstrakcija antocijanina iz grožđane komine (grožđe vrste Vranec) s vodom koja je sadržavala 0,05–0,5% SO_2. Višestupanjska ekstrakcija provedena je dvojako: minimalnim omjerom komine prema otapalu 1:3 (što je omogućilo namakanje) te s masenim omjerom 1:5. U ekstraktima je utvrđen udjel antocijanina i balastnih tvari (tanin i dušikovi spojevi). Stupanj čistoće ekstrakta određen je prema vrijednosti pokazatelja kakvoće. Pokazatelj kakvoće upućuje na razliku iskorištenja antocijanina i prosječnog iskorištenja balastnih tvari. S povećanjem koncentracije SO_2 postiže se veća čistoća ekstrakta pa su oni dobiveni masenim omjerom 1:5 čišći.