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Effect of Sulphur Dioxide, Ethyl Alcohol, Maceration Temperature and Time on Extraction of Phenolic Compounds of *Vitis vinifera* L. cvs Öküzgözü and Bogazkere Grapes in Model Wine Solutions

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The effect of sulphur dioxide (SO_2), ethyl alcohol, maceration temperature and time on the extraction of phenolic compounds of Öküzgözü and Bogazkere grapes in model wine solution was investigated. Data was evaluated using variance analysis technique. Significant differences were observed among all of the variables according to SO_2, ethyl alcohol, maceration temperature and time. Quantity of non-coloured phenolic compounds with increasing the temperature was higher than that of anthocyanin. Furthermore, extraction of anthocyanin from grape pomace was more than that of non-coloured phenolic compounds in model wine solutions which contained SO_2 and alcohol. The extraction of non-coloured phenolic compounds continued during maceration. In contrast, anthocyanin content reached maximum between day 3 and 5 for Bogazkere and between day 2 and 5 for Öküzgözü and then decreased.

Key Words: Phenolic compounds, SO_2, Ethyl alcohol, Maceration temperature and Time.

INTRODUCTION

Phenolic compounds, one of the most important components of grapes and wines, are responsible for all of the colour differences between grapes and the resultant wines[1,2]. They also contribute to the sensory properties of wines, such as colour, flavour, astrigency and bitterness. Recently, there is much interest on the phenolic compounds because of their health benefits, such as antioxidant activity[3,4].

Anthocyanins and tannins are the most important phenolic compounds. Anthocyanins give characteristic colour to wine. Tannins especially are responsible for the bitterness and astrigency of wine[5,6]. During maceration of red wine, phenolic compounds are transferred from solid parts of the grape cluster into wine. The rate of transfer depends on various factors, such as phenolic concentration of grapes, maceration time and temperature, alcohol and SO_2 levels[1,7-9]. The quantity and composition of phenolic compounds in grapes vary according to variety, maturity and climate conditions in vegetation period[10,11].

Maceration time has an important impact on the extraction of phenolic compounds. Total phenolic compounds and tannin generally increase with extending maceration time[12,13]. However, the amount of anthocyanins increases for evident time and then a decrease is observed[5,8]. There is a relationship between the maceration temperature and the extraction of phenolic compounds. Coloured and non-coloured phenolic compounds increase with high temperature[14,15].

Alcohol produced during the maceration contributes to extraction of phenolic compounds. It has been reported that red wines which contain high levels of alcohol, have high tannin and deep colour[1,2,9]. SO_2 is widely used during wine making as an antioxidant and an inhibitor of undesirable microbial growth. Furthermore, SO_2 contributes to the extraction of phenolic compounds of grapes during the vinification[16,17].

As described above, the extraction of phenolic compounds during vinification occurs in a complex medium and under the effects of various conditions. In this complex medium, it is difficult to determine the factors which affect the extraction of phenolic compounds. Therefore, in previous studies in order to determine the effects of these factors, model solutions were used[7,18,19]. Öküzgözü and Bogazkere are important black grape varieties in Turkey which are used for production of quality red wines[20,21].

The aim of this study was to investigate the effects of maceration conditions (temperature and time), SO_2 and ethyl alcohol levels on various phenolic compounds of Bogazkere and Öküzgözü grapes in wine-like model solution.

EXPERIMENTAL

Öküzgözü and Bogazkere cultivars (each 24 kg) were obtained from Elazig Region of Eastern Turkey. Grape stems were manually seperated. They were mixed for homogenization and then divided into 24 seperate 1 kg lots. Each lot was carefully crushed by hand and pressed with a basket type manual press. About 700 mL juice was separated. The pomace was mixed with 700 mL synthetic solution in a glass jar. Maceration process was conducted at 20 and 30 °C for 14 d. The content of the jar was mixed daily before taking the sample. After each sampling, model wine solution (10 mL) was added immediately into every jar. Samples were centrifuged at 5500 rpm for 10 min and analyzed. Assays were performed in duplicate.

Preparation of model wine solution: In maceration process, a wine-like synthetic medium containing 0, 5 and 10 % ethyl alcohol with either 0 or 100 mg/L SO_2 addition was used. Model solution was prepared by mixing 5 g/L of tartaric acid with necessary amount of ethyl alcohol. pH of the solution was adjusted to 3.2 by addition of 1 N sodium hydroxide. This value was chosen as an average pH of grape juices. Potassium sorbate was added into model wine solution in order to prevent fermentation.

Spectrophotometric determination: The analytical variables measured were: total phenolic compounds, total anthocyanin and colour density. Absorbance measurements

were done on a Shimadzu UV-1201 (Japan) spectrophotometer. Total phenolic compounds were calculated using Folin-Ciocalteu reagent (Merck) and the results were expressed as grams per litre of gallic acid[22]. The content of anthocyanin was analyzed according to method of Ough and Amerine[22]. Colour of the juice and model wine solution was determined by measuring the absorbance at 520 and 420 nm. Colour density was calculated[5] as the sum of absorbance at 420 and 520 nm. Each analysis was performed in duplicate.

Statistical analysis: Analysis of variance was carried out on chemical analysis data using SAS General Linear Model Procedure and means were compared by Duncan test (Version 1998, SAS Institute Inc, Cary, NC, USA).

RESULTS AND DISCUSSION

The initial characteristics of Öküzgözü and Bogazkere grape juice are given in Table-1.

TABLE-1
GENERAL COMPOSITION OF ÖKÜZGÖZÜ AND BOGAZKERE GRAPE JUICE

	Öküzgözü grape juice	Bogazkere grape juice
Reducing sugar (g/L)	198	186
Density (20 ºC)	1089	1083
pH	3.1	3.0
Total acidity (meq/L)	72	85
Total phenolic compounds (g/L)*	0.98	0.87
Total anthocyanin (mg/L)	207	167
Colour density ($OD_{420} + OD_{520}$)	0.103	0.125
Colour tint (OD_{420} / OD_{520})	1.28	1.19

*As gallic acid.

The changes in phenolic compounds during pomace maceration in model wine solution were followed by total phenolic compounds, anthocyanin and colour density analysis. Table-2 shows the p-values for each factor (SO_2, alcohol, maceration time and temperature). Significant differences were found for all simple effects of the spectrophotometric data. Generally, second-order effect on extraction of phenolic compounds and colour density were found to be significant at $p < 0.01$.

Influence of different parameters on extraction of phenolic compounds of model wine solutions

Maceration temperature: Figs. 1-4 show the influence of temperature on extraction of phenolic compounds in Öküzgözü and Bogazkere grape juice. Increasing the mace-ration temperature from 20 to 30 ºC significantly increased total phenolic compounds. Quantity of phenolic compounds increased as 53% for Bogazkere grape juice and 38 % for Öküzgözü grape juice (Table-3).

Tannin is the most abundant phenolic compound in wines. Therefore, the amount of total phenolic compounds may also reflect the amount of tannin. For this reason, there is a significant relationship between maceration temperature and wine style

TABLE-2
PROBABILITY VALUES OF STATISTICAL ANALYSIS FOR THE SPECTROPHOTOMETRIC DATA (ANOVA test $p < 0.01$)

Effect		Bogazkere grape juice			Öküzgözü grape juice		
		Total phenolic compd.	Anthocyanin	Colour density	Total phenolic compd.	Anthocyanin	Colour density
Simple effect	T	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
	A	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
	S	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
	Ti	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
Second-order effect	T×A	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
	T×S	$p < 0.01$	0.0287(ns)*	0.0349(ns)	$p < 0.01$	$p < 0.01$	0.8402(ns)
	A×S	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
	T×Ti	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
	A×Ti	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
	S×Ti	0.0877(ns)	$p < 0.01$	$p < 0.01$	0.065(ns)	$p < 0.01$	$p < 0.01$
Third-order effect	T×A×S	0.0222(ns)	$p < 0.01$	0.0196(ns)	$p < 0.01$	$p < 0.01$	$p < 0.01$
	T×A×Ti	$p < 0.01$	0.0246(ns)	0.0332(ns)	$p < 0.01$	0.0122(ns)	$p < 0.01$
	T×S×Ti	0.0222(ns)	$p < 0.01$	0.2329(ns)	$p < 0.01$	$p < 0.01$	$p < 0.01$
	A×S×Ti	$p < 0.01$	0.93(ns)	0.0338(ns)	0.0137(ns)	0.1509(ns)	$p < 0.01$
	T×A×S×Ti	$p < 0.01$	0.11(ns)	0.9982(ns)	$p < 0.01$	0.7907(ns)	0.1467(ns)
Fourth-order effect	T×A×S×Ti	$p < 0.01$	0.11(ns)	0.9982(ns)	$p < 0.01$	0.7907(ns)	0.1467(ns)

T = Temperature; A = Alcohol; S = SO_2, Ti = Time; *ns = Not significant.

TABLE-3
MEAN VALUES OF THE SPECTROPHOTOMETRIC DATA (ANOVA ANALYSIS)

Effect		Bogazkere grape juice			Öküzgözü grape juice		
		Total phenolic compd. (g/L)	Anthocyanin (mg/L)	Colour density ($D_{420} + D_{520}$)	Total phenolic compd. (g/L)	Anthocyanin (mg/L)	Colour density ($D_{420} + D_{520}$)
Temp.	20 °C	(1.28^{b})*	436.86^{b}	0.51^{b}	1.52^{b}	439.15^{b}	0.53^{b}
	30 °C	1.76^{a}	593.80^{a}	0.83^{a}	2.34^{a}	594.66^{a}	0.84^{a}
Alcohol	0°	1.24^{c}	393.92^{c}	0.53^{c}	1.64^{c}	412.18^{c}	0.65^{c}
	5°	1.55^{b}	530.99^{b}	0.68^{b}	1.91^{b}	507.13^{b}	0.68^{b}
	10°	1.76^{a}	621.09^{a}	0.79^{a}	2.25^{a}	631.41^{a}	0.74^{a}
SO_2	0 mg/L	1.40^{b}	439.41^{b}	0.64^{b}	1.80^{b}	445.66^{b}	0.66^{b}
	100 mg/L	1.64^{a}	591.26^{a}	0.69^{a}	2.06^{a}	588.15^{a}	0.72^{a}
Time (d)	1	0.99^{h}	428.38^{e}	0.36^{e}	1.20^{h}	434.42^{f}	0.32^{f}
	2	1.34^{g}	561.71^{b}	0.66^{cd}	1.65^{g}	601.04^{a}	0.66^{e}
	3	1.48^{f}	591.42^{a}	0.71^{b}	1.73^{f}	561.83^{b}	0.72^{bc}
	4	1.51^{ef}	580.38^{ab}	0.73^{ab}	1.77^{f}	562.79^{b}	0.68^{de}
	5	1.53^{d}	578.54^{ab}	0.75^{a}	1.85^{e}	564.00^{b}	0.75^{a}
	6	1.59^{e}	530.67^{c}	0.71^{b}	1.94^{d}	535.83^{c}	0.74^{ab}
	7	1.62^{cd}	494.75^{d}	0.65^{d}	2.09^{c}	533.42^{c}	0.74^{ab}
	8	1.65^{bc}	524.04^{c}	0.73^{ab}	2.19^{b}	504.88^{d}	0.77^{a}
	10	1.67^{ab}	486.42^{d}	0.71^{b}	2.25^{a}	499.13^{d}	0.76^{a}
	12	1.67^{ab}	445.42^{e}	0.69^{bc}	2.27^{a}	460.88^{e}	0.74^{ab}
	14	1.69^{a}	446.96^{e}	0.65^{cd}	2.28^{a}	427.75^{f}	0.70^{cd}

*Means separated by Duncan's multiple range test at the ($p < 0.01$) levels; not significantly different belonging to variable effect when followed by the same letter.

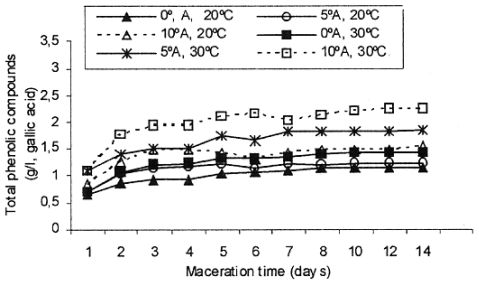


Fig.1. Effect of temperature and ethyl alcohol on the extraction of phenolic compounds in pomace of Bogazkere grape (without SO_2)

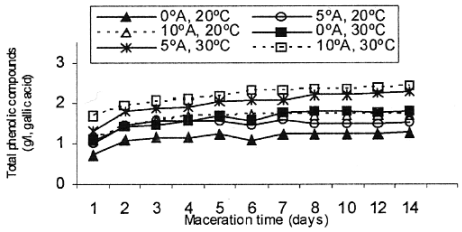


Fig.2. Effect of temperature and ethyl alcohol on the extraction of phenolic compounds in pomace of Bogazkere grape (with 100 mg/L SO_2)

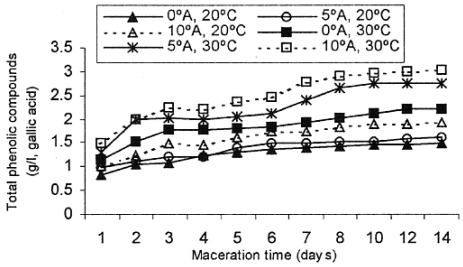


Fig.3. Effect of temperature and ethyl alcohol on the extraction of phenolic compounds in pomace of Öküzgözü grape (without SO_2)

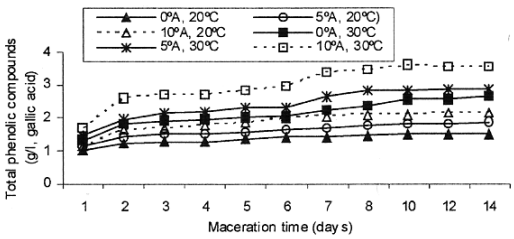


Fig. 4. Effect of temperature and ethyl alcohol on the extraction of phenolic compounds in pomace of Öküzgözü grape (with 100 mg/L SO_2)

and quality. Similar results were obtained by Merida *et al.*[15] and Gil-Munoz *et al.*[23]. Ramey *et al.*[14] reported that catechin increased 160 %, tannin 31% and total phenolic compounds 42 % with increasing temperature from 5 to 15 °C for 72 h.

The influence of temperature on extraction of anthocyanin in Bogazkere and Öküzgözü grape juice is shown in Figs. 5-8. Maceration temperature greatly affected the extraction of anthocyanin from grape pomace to model wine solution. The influence of temperature on samples treated with 100 mg/L SO_2 is higher than that of without SO_2.

Total anthocyanin levels in all Bogazkere and Öküzgözü grape juice samples increased during maceration, usually reaching a maximum in 2-3 d for both grape varieties. Changes of colour density during maceration was similar to changes in anthocyanin. Gao *et al.*[24] reported that colour density was increased with increasing temperature from 20 to 30 °C.

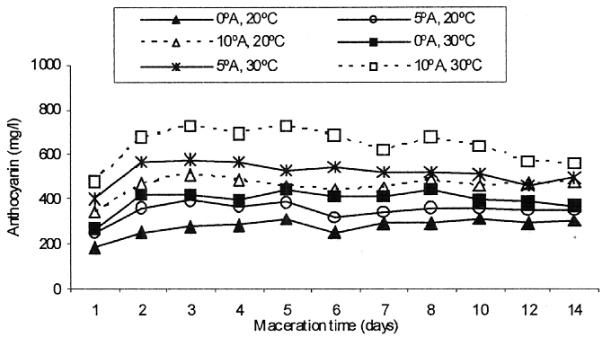


Fig. 5. Effect of temperature and ethyl alcohol on the extraction of anthocyanin in pomace of Bogazkere grape (without SO_2)

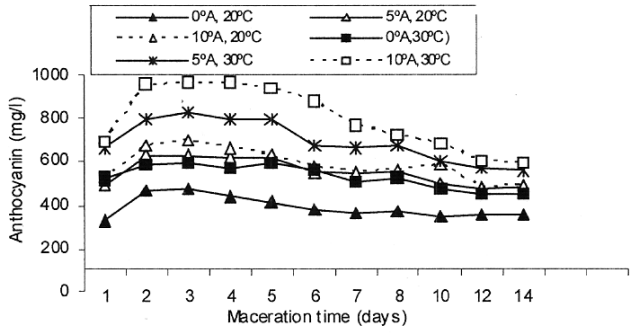


Fig. 6. Effect of temperature and ethyl alcohol on the extraction of anthocyanin in pomace of Bogazkere grape (with 100 mg/L SO_2)

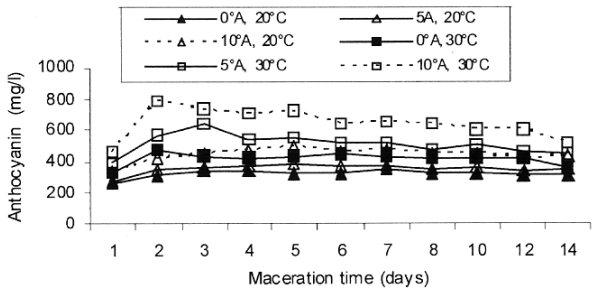


Fig. 7. Effect of temperature and ethyl alcohol on the extraction of anthocyanin in pomace of Öküzgözü grape (without SO_2)

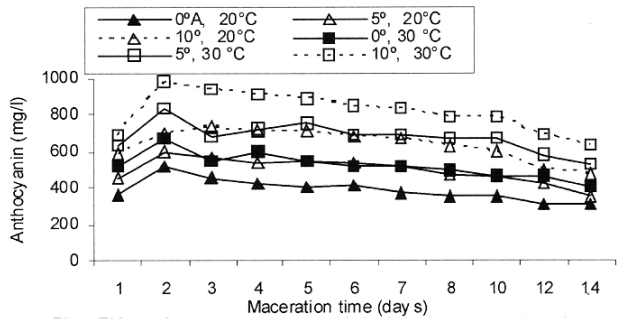


Fig. 8. Effect of temperature and ethyl alcohol on the extraction of anthocyanin in pomace of Öküzgözü grape (with 100 mg/L SO_2)

Ethanol concentration: Increasing alcohol content greatly influenced the rate of total phenolic compounds extraction during maceration (Figs. 1-4). Total phenolic compounds increased as 21 % with 5° alcohol and 36 % with 10° alcohol for Bogazkere. These values for Öküzgözü grape were 17 and 37 % with 5° and 10° alcohol, respectively (Table-2). These findings are in agreement with the results of previous studies[7,9,18].

Anthocyanin levels exhibited different in model wine solutions containing 0°, 5° and 10° ethanol. In Bogazkere samples which contained 5 and 10 % ethanol at 20 °C, anthocyanin level reached the maximum within 3 d compared to sample without alcohol. Release of anthocyanin in Öküzgözü pomace was different between trials. The level of anthocyanin increased 23 % in a trial containing 5 % alcohol and 53 % in trial containing 10 % alcohol (Table-3). Results are in good agreement with previous findings[2,7,25]. Changes of colour density in maceration was also found similar to anthocyanin.

Sulphur dioxide (SO_2): Total phenolic compounds significantly increased with SO_2 addition in all samples. The rates of extraction of total phenolic compounds were similar in experiments with 20 °C + 5 % alcohol and 20 °C + 0 % alcohol +

100 mg/L SO_2. Total phenolic compounds in model wine solution with 100 mg/L SO_2 increased 17 % for Bogazkere and 16 % for Öküzgözü samples (Table-3). In a study carried out by Canbas[26], it was found that anthocyanin levels increased 10 % with the addition of 100 mg/L SO_2 compared to control which SO_2 was not added in Dimrit grape variety.

Amounts of anthocyanin significantly increased in sample with the addition of 100 mg/L SO_2 compared to the one without SO_2. In Bogazkere pomace without SO_2 at 20 ºC, anthocyanin extractions after 1, 3 and 10 d of maceration was 59, 89 and 100 %, respectively. In the samples with 100 mg/L SO_2 addition, anthocyanin extractions after 1 and 3 d were 70 and 100 %, respectively. Similar results were also obtained with Öküzgözü sample. Dallas and Laureano[16] reported that the addition of 0, 75 and 150 mg/L SO_2 to Tinta baracco, Tinta roriz and Periquita grapes increased the amount of anthocyanin during maceration.

With regard to colour density, significant differences were found between the model wine solution with 100 mg/L SO_2 and without SO_2. Colour density in sample with 100 mg/L SO_2 greatly increased compared with sample without SO_2. This is attributed to the effect of SO_2 on the extraction of anthocyanin in accordance with previous data[1,2].

Maceration time: Amount of total phenolic compounds increased during maceration. However, with extended maceration, an increase in total phenolic compounds slowed down (Figs. 1-4). The lowest value of total phenolic compounds was observed with maceration at 20 ºC with no alcohol and SO_2. In contrast, the highest value of total phenolic compounds was obtained at 30 ºC with 10 % alcohol + 100 mg/L SO_2. The level of total phenolic compounds at both temperatures (20 and 30 ºC) significantly increased between days 1 and 4. This increase continued at 30 ºC, whereas it slowed down at 20 ºC.

On the other hand, the level of anthocyanin increased until 5 day and then decreased in both varieties. This is due to the fact that the amount of tannin in grape is 10 times more than anthocyanin[2]. Results in this experiment was in good agreement with previous findings[1,27].

In present experimental conditions, the anthocyanin level begun to increase on the first days for Öküzgözü and Bogazkere samples. On the other hand, means of anthocyanin reached a maximum between days 2 and 5 of maceration for Öküzgözü sample. After this time, the reason for the decrease in anthocyanin level after reaching a maximum is attributed to the absorption of anthocyanin to pomace and complex formation with tannin[5,28]. The highest value of colour density was generally observed on the 4th-5th days of maceration for Öküzgözü and Bogazkere samples.

Conclusion

During the maceration, all the factors (SO_2, alcohol, maceration time and temperature) increased quantity of phenolic compounds and colour density, but effects of these factors on coloured and non-coloured phenolic compounds varied for

Bogazkere and Öküzgözü samples. Quantity of non-coloured phenolic compounds with increasing the temperature rised higher than that of anthocyanin. On the other hand, in model wine solution which contained SO_2 and alcohol, extraction of anthocyanin from grape pomace was more than that of non-coloured phenolic compounds. The extraction of non-coloured phenolic compounds throughout the maceration continued for Bogazkere and Öküzgözü samples. In contrast, an increase in anthocyanin content reached a maximum between days 3 and 5 for Bogazkere sample and between days 2 and 5 for Öküzgözü sample and then decreased.

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