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Improvement of anthocyanin content in the cv. Öküzgözü wines by using pectolytic enzymes

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Abstract

The effect of the addition of two commercial pectolytic enzymes on the anthocyanin and chemical composition of Öküzgözü wines was studied. A rapid HPLC-diode array detection (DAD) method was developed for the analysis of anthocyanins in wines. Direct injection of filtered wine samples followed by selective detection at 520 nm allowed quantitation of these compounds in red wines. Thirteen anthocyanin compounds were detected in wines and, addition of the two enzyme preparations improved the extraction of anthocyanins. Moreover, the wines treated with enzymes had higher values in total phenolics, tannins, and colour intensity than the control wines.

Keywords: Maceration enzymes; Öküzgözü; Red wine; Phenolic compounds; Anthocyanins

1. Introduction

Colour is one of the most important quality characteristics of red wines. Anthocyanins are the main pigments responsible for the colour of red grapes and wines (Mazza, 1995; Revilla, Pérez-Magariño, González-Sanjosé, & Beltrán, 1999). They are located in the skin of grapes. Anthocyanin composition of wines depends on several factors, such as grape variety, ripening, maceration condition (time and temperature), and winemaking conditions (Delfini, 1994; Ribéreau-Gayon & Glories, 1987; Sun, Spranger, Rogue-do-Vale, Leandro, & Belchior, 2001; Spayd, Tarara, Mee, & Ferguson, 2002).

The addition of commercial pectolytic enzymes is a common practice in winemaking, to increase the phenolic content of wines, especially anthocyanins. These enzymes may also improve the stability, taste, and structure of red wines, because not only anthocyanins are released from the skins, but also tannins bound to cell walls may be extracted because of enzymatic action. In the literature, this technique generally provides good results for red wine colour (Amrani-Joutei & Glories, 1995; Bautista-Ortin, Martinez-Cutillas, Ros-Garcia, López-Roca, & Gómez-Plaza, 2005; Canall-Llaubères & Pouns, 2002; Capounova & Drdak, 2002), but some authors have reported the opposite (Revilla & González-Sanjose, 2001; Wightman, Price, Watson, & Wrolstad, 1997; Zent & Inama, 1992).

Öküzgözü is a native red grape variety of *Vitis vinifera* L. grown in eastern Turkey, especially Elazig, Malatya, and Diyarbakir provinces. It is an important red grape variety for Turkey, which produces well-balanced and characteristics wines, with fruity notes such as strawberry, cherry, and blackberry-like odours (Cabaroglu, Canbas, Lepoutre, & Gunata, 2002). Anthocyanin composition of Öküzgözü wines and influence of different maceration times have already been studied by Kelebek et al. (2006). The data obtained in this study indicate that cv. Öküzgözü has a low anthocyanin content, compared to other common red wines, so enhancement of anthocyanin extraction would be useful trial for this cultivar.

The aim of the present study was therefore to investigate the effect of enzyme addition on the anthocyanin content of Öküzgözü wines.

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2. Materials and methods

2.1. Grapes and winemaking

Healthy grapes of cv. Öküzgözü (1600 kg) were manually harvested at optimum maturity in the 2003 vintage in Elazig province and transported to the Experimental Winery at the Department of Food Engineering, University of Cukurova, Adana, Turkey. Öküzgözü must had 6.7 g/l of titratable acidity (as tartaric acid), a pH of 3.2, and 195.8 g/l of reducing sugar. After harvest, grapes were destemmed and crushed in a commercial grape destemmer-crusher (Sarksan Ltd., Sti, Ankara, Turkey). The crushed grapes (replicates) were then homogeneously transferred into six stainless-steel tanks. Each of the two tanks were assigned for three treatments: control (no enzyme addition), Rapidase Ex Color, and Vinozym G. Sulfur dioxide (20 mg/kg) was added to all treatments. Enzyme codes and application doses described according to the producer procedure were: Rapidase Ex Color (E1, DSM/Gist Brocades, Seclin-France; activity of $\geqslant$15.000 AVJP/g) 3 g/hl, and Vinozym G (E2, Novozymes, Bordeaux-France; activity of 4000 FDU/g) 3 g/hl. Enzyme preparations were dissolved in must and then added into E1- and E2-treatments before the inoculation of yeast. The maceration of all treatments took place at 20–22 °C for 8 days. After destemming, approximately 500 kg of grape mash was used for each treatment. After maceration, the mash was pressed gently in a horizontal press. Alcoholic fermentations were conducted using Zymaflore F10 yeast culture (Lafford Oenologie, Bordeaux, France) at room temperature. During alcoholic fermentation, temperature and must density were monitored twice a day for 10 days. After fermentation, the wines were racked and kept at 20 °C to stimulate malolactic fermentation. Malolactic fermentation was judged by the degradation of malic acid, using paper chromatography, according to the procedure of Cartesio and Campos (1988). When the malolactic fermentation was completed the wines were racked and 80 mg/l sulfur dioxide was added. Finally, wines were bottled and stored at 15 °C for 6 months prior to analysis.

2.2. Chemical analysis

Total acidity, pH and reducing sugar analyses were performed in the musts and wines (OIV, 1990). Additionally, the wines were analysed for density, ethanol, volatile acidity, total phenolic compounds (280 index), and tannins (OIV, 1990; Ough & Amerine, 1988).

A direct measurement of absorbance (Abs) of the wines at 420, 520, and 620 nm was carried out using a Uvikon 922 (Kontron Instruments) spectrophotometer. Colour intensity (CI) was calculated as the sum of 420 nm, 520 nm, and 620 nm absorbances; tint was calculated by dividing the absorbance at 420 by the absorbance at 520 nm; proportion of yellow (Ye%), red (Rd%) and blue colour (Bl%) were calculated by dividing the absorbances at 420, 520 and 620 nm, by the colour intensity (CI), respectively. The proportion of red colour produced by the flavylium cations of free and bound anthocyanins (dA%) was calculated using the formula described by Glories (1984): $(dA\%) = (1 - (Abs_{420} + Abs_{620})/2 \times Abs_{520}) \times 100$.

2.3. HPLC analysis of anthocyanins

The chromatograph was a Beckman System Gold HPLC 126, with Diode Array Detector 168, fitted with New Gold software. The column used was an RP-18 XTerra™ (Waters Corporation, Milford, MA, USA: 4.6 mm × 100 mm, 3.5 μm equipped with a pre-column 4.6 mm × 4.5 cm). Solvents used were water acidified with formic acid (5%) (A) and methanol acidified in the same manner (B). Anthocyanin compounds were eluted under the following conditions: 1 ml/min flow rate, elution with linear gradients from 0% to 5% B in 1 min, from 5% to 40% B in 29 min, from 40% to 100% B in 10 min, followed by washing and reconditioning of the column. The ultra-violet–visible spectra (scanning from 200 nm to 600 nm) were recorded for all peaks. Identification of anthocyanins was obtained using authentic standards and by comparing the retention times and spectra with those found in the literature (Bakker & Timberlake, 1985; Hebrero, Santos-Buelga, & Rivas-Gonzalo, 1988; Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999; Revilla, Ryan, & Martin-Ortega, 1998; Wulf & Nagel, 1978). Quantification of anthocyanins was based on peak areas at 520 nm. Delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside, obtained from Extrasynthèse (Lyon, Genay-France), were used as standards. The linear calibration curves were obtained by injecting different concentration of standards. Unknown concentrations were determined from linear regression equations.

2.4. Statistical analysis

The data were submitted to one-way analysis of variance, to test the effect of different pectolytic enzymes on the chemical and anthocyanin composition of wines. In addition, Duncan multiple range test was used to compare the means. The statistical analyses were performed using SPSS statistics software version 11.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. The effect of enzyme addition on general wine composition

The general composition of wines obtained with or without enzymatic treatments is given in Table 1. Enzyme addition had no significant effect on density, ethanol, pH, total acidity nor reducing sugars; however, slight differences were detected in volatile acidity. As expected, wines produced by enzyme treatment were higher in total phenolics, tannins,

Table 1
General composition of Öküzgözü wines

Analysis	Wines			
	Control	E1	E2	Sig.
Density (20 °C/20 °C)	0.9950 ± 0.00	0.9954 ± 0.00	0.9958 ± 0.000	ns
Ethanol (%, v/v)	11.5 ± 0.01	11.4 ± 0.01	11.6 ± 0.00	ns
Total acidity[A] (g/l)	6.3 ± 0.02	6.2 ± 0.01	6.0 ± 0.01	ns
pH	3.40 ± 0.02	3.35 ± 0.01	3.38 ± 0.04	ns
Volatile acidity[B] (g/l)	0.35 ± 0.03	0.43 ± 0.06	0.41 ± 0.04	ns
Residual sugar (g/l)	2.0 ± 0.08	2.1 ± 0.07	2.2 ± 0.02	ns
Free SO_2 (mg/l)	5.0 ± 0.15	5.8 ± 0.42	4.4 ± 0.18	ns
Bound SO_2 (mg/l)	63.8 ± 1.21	67.1 ± 1.52	66.3 ± 1.02	ns
Total phenolics (280 index)	65.2 ± 0.02[a]	73.1 ± 0.05[b]	75.4 ± 0.06[b]	**
Tannin (g/l)	4.3 ± 0.01[a]	4.5 ± 0.00[a]	5.0 ± 0.02[b]	**
Colour intensity	0.986 ± 0.04[a]	1.062 ± 0.06[b]	1.105 ± 0.05[c]	**
Tint	0.64 ± 0.01[b]	0.58 ± 0.02[a]	0.60 ± 0.01[a]	**
%Ye	33.8 ± 0.10	35.4 ± 0.13	33.1 ± 0.16	ns
%Rd	54.6 ± 0.09	54.9 ± 0.11	54.4 ± 0.13	ns
%Bl	11.6 ± 0.07	9.7 ± 0.12	12.5 ± 0.11	ns
%dA	58.5 ± 0.22	58.1 ± 0.18	59.2 ± 0.14	ns

Results are the means of three analyses. Sig., significance at which means differ, as shown by analysis of variance.
[A] As tartaric acid.
[B] As acetic acid.
[a,b,c] Different superscripts in the same row indicate statistial differences at the 0.01 ($^{**}p < 0.01$) level. ns, Not significant.

and colour intensity, but lower in tint value than the control wines. Levels of total phenolics, tannins, and colour intensity were highest in wines obtained using E2. Similar results have been reported in other studies (González-Sanjosé, Izcara, Pérez-Magarino, & Revilla, 1998; Muñoz, Sepulveda, & Schwartz, 2004; Pardo, Salinas, Alonso, Navarro, & Huerta, 1999; Revilla, Luisa, & González-Sanjozé, 2003a, Revilla, Luisa, & González-Sanjozé, 2003b; Zimman, Joslin, Lyon, Meier, & Waterhouse, 2002). The increase in colour intensity by enzymatic treatments may be due to an increase in polymeric anthocyanin content and/or due to co-pigmentation effects caused by the enhanced extraction of other phenolic fractions (Watson, Goldberg, Chen, Mcdaniel, & Price, 1999). Enzymatic treatment did not have a significant influence on Ye%, Rd%, and Bl% in the wines. As previously reported, the optimal ratio between the components of red wine colour was considered to be Ye:35%, Rd:55%, and Bl:10% (Glories, 1984). Our findings are very similar to those reported by Glories. As can be seen in Table 1, the dA% values in the control, E1- and E2-treatments were, 58.5, 58.1, and 59.2, respectively, and enzyme treatments had no significant effect on this parameter.

3.2. Effect of enzyme treatments on anthocyanin composition of the Öküzgözü wines

Fig. 1 shows the chromatograms recorded at 520 nm of Öküzgözü wines, obtained with or without enzymatic treatments. Thirteen different peaks were quantitated and were assigned to the 3-glucosides of delphinidin (1), cyanidin (2), petunidin (3), peonidin (4), malvidin (5), the 3-acetyl-glucosides of delphinidin (6), petunidin (7), peonidin (8), malvidin (9), and the 3-*p*-coumaroyl-glucosides of delphinidin (10), petunidin (11), peonidin (12) and malvidin (13), on the basis of their retention times and ultra-violet–visible spectra, compared with authentic standards or data found in the literature (Bakker & Timberlake, 1985; Hebrero et al., 1988; Revilla et al., 1998; Wulf & Nagel, 1978).

Table 2 shows the effect of the two pectolytic enzyme additions on the anthocyanin content of Öküzgözü wines, expressed as the means (mg/l) of three analytical replicates. It was observed that the anthocyanin profiles of all wines were similar and enzymatic treatment did not produce a selective effect on any anthocyanin. However, this process increased the total concentration of anthocyanins in wines, compared to the control wine. Our data indicated that all anthocyanin compounds were readily released from grape skin cell-wall matrices to the wines, using both enzyme preparations. Similar findings have been reported in other studies (Amrani-Joutei & Glories, 1995; Bakker, Bellworty, Reader, & Watkins, 1999; Capounova & Drdak, 2002; Muñoz et al., 2004; Pardo et al., 1999). The total amount of anthocyanins in control, E1- and E2-treated wines were 161.3, 176.9, and 202.1 mg/l, respectively. E2-treated wines contained the highest amount of all individual anthocyanins, except for peonidin-3-glucoside acetate and total anthocyanins, followed by the E1-treated wine.

As can be seen in Table 2, monoglucoside derivatives of anthocyanins predominate. Among these, malvidin-3-glucoside was the most dominant in the wines. In addition to this compound, Öküzgözü wines contained significant levels of petunidin-3-glucoside and delphinidin-3-glucoside, and lesser amounts of cyanidin-3-glucoside and peonidin-3-glucoside. Their levels significantly increased with enzymatic treatment, except for peonidin-3-glucoside. Similar results were also found in other *Vitis vinifera* varieties (Bakker & Timberlake, 1985; Núñez, Monagas, Gomez-Cordovés, &

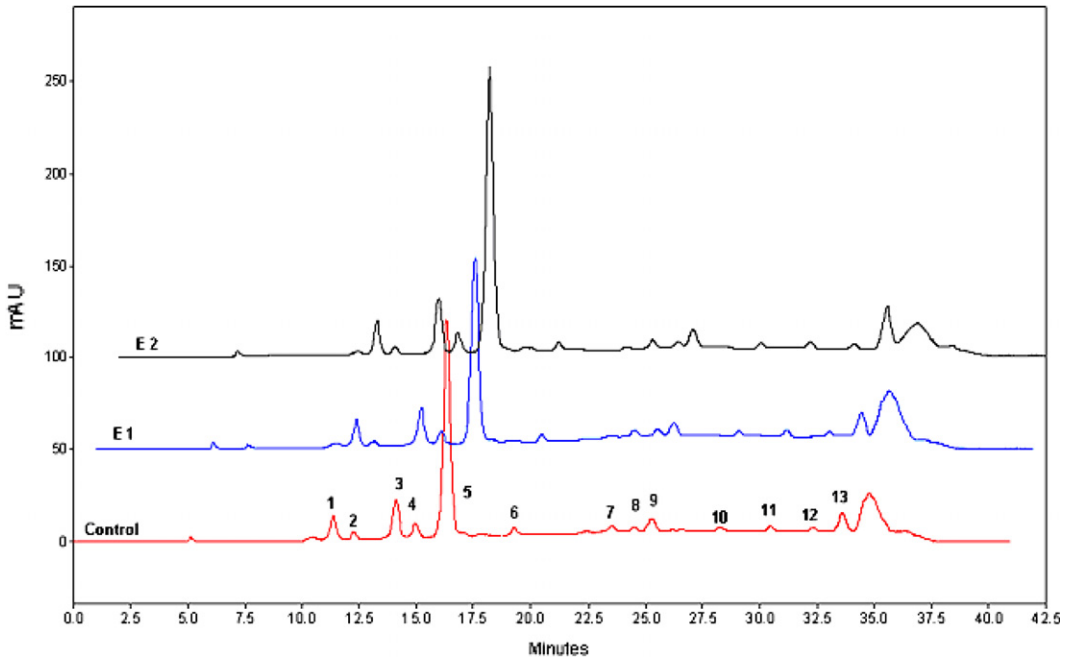

Fig. 1. HPLC chromatogram of the Öküzgözü wine anthocyanins (at 520 nm).

Table 2
Effect of enzymes treatments on anthocyanin compounds levels of Öküzgözü wines (mg/l)

Peak no.	Compounds	Wines			
		Control	E1	E2	Sig.
1	Delphinidin-3-glucoside	9.1 ± 0.83^{a}	10.1 ± 1.63^{b}	10.9 ± 1.76^{b}	*
2	Cyanidin-3-glucoside	2.6 ± 0.03^{a}	3.2 ± 0.02^{b}	3.5 ± 0.05^{b}	**
3	Petunidin-3-glucoside	15.0 ± 1.18^{a}	17.0 ± 1.54^{ab}	18.6 ± 1.4^{b}	*
4	Peonidin-3-glucoside	5.8 ± 0.21	5.3 ± 0.34	6.3 ± 0.28	ns
5	Malvidin-3-glucoside	97.6 ± 5.06^{a}	101.2 ± 5.12^{b}	115.9 ± 5.32^{c}	**
6	Delphinidin-3-glucoside acetate	3.1 ± 0.10^{a}	3.4 ± 0.11^{b}	3.9 ± 0.13^{c}	*
7	Petunidin-3-glucoside acetate	2.9 ± 0.16^{a}	3.5 ± 0.12^{b}	4.8 ± 0.14^{c}	**
8	Peonidin-3-glucoside acetate	1.7 ± 0.01^{a}	3.4 ± 0.01^{b}	2.9 ± 0.01^{a}	**
9	Malvidin-3-glucoside acetate	5.9 ± 0.35^{a}	7.0 ± 0.43^{b}	8.4 ± 0.22^{c}	**
10	Delphinidin-3-glucoside *p*-coumarate	3.0 ± 0.02^{a}	3.3 ± 0.01^{a}	3.8 ± 0.03^{b}	*
11	Petunidin-3-glucoside *p*-coumarate	2.8 ± 0.01^{a}	3.7 ± 0.03^{b}	4.4 ± 0.05^{c}	**
12	Peonidin-3-glucoside *p*-coumarate	1.6 ± 0.01^{a}	1.8 ± 0.01^{a}	2.3 ± 0.02^{b}	*
13	Malvidin-3-glucoside *p*-coumarate	10.2 ± 0.73^{a}	14.0 ± 0.70^{b}	16.4 ± 0.77^{c}	**
	Total anthocyanins	161.3	176.9	202.1	

Results are the means of three analyses. Sig., significance at which means differ, as shown by analysis of variance.
[a,b,c] Different superscripts in the same row indicate statistical differences at the 0.05 ($^{*}p < 0.05$) and 0.01 ($^{**}p < 0.01$) levels. ns, Not significant.

Bartolome, 2004; Revilla et al., 1999; Revilla & González-Sanjose, 2001; Ribéreau-Gayon, 1982). The total monoglucoside levels were higher in E2-treated wine, compared to both the control and E1-treated wines. The control wine contained 97.6 mg/l of malvidin-3-glucoside, and the concentration was increased by 3.7% and 18.6%, using E1- and E2-treatments, respectively. The results are in agreement with other studies, namely by Bakker et al. (1999) and Canall-Llaubères and Pouns (2002). Within the monoglucoside compounds, cyanidin-3-glucoside was a minor component, compared to the other monoglucosides. In addition, the acylated and *p*-coumaroylated conjugates of cyanidin-3-glucoside were not detected in all wines. The control, E1- and E2-treated wines contained 2.6, 3.2, and 3.5 mg/l of cyanidin-3-glucoside, respectively. Enzymatic treatment resulted in a significant increase in concentration of this compound. Similarly, some authors indicated that the amount of cyanidin-3-glucoside was found in the lowest concentration in *Vitis vinifera* varieties, with some exceptions (Darné & Glories, 1988; Mazza et al., 1999; Núñez et al., 2004; Revilla et al., 1998). González-Neves, Gómez-Cordéves, and Barreiro (2001) and the references therein also pointed out that cyanidin is the precursor of other anthocyanidins in the metabolism of vine.

Among the acylated and *p*-coumaroylated anthocyanins, malvidin-3-glucoside acetate and malvidin-3-glucoside *p*-coumarate were the most abundant anthocyanins in Öküzgözü wines. E1- and E2-enzyme preparations

significantly increased the level of these compounds between 18.6% and 60.8%. Moreover, similar data for other acylated and *p*-coumaroylated anthocyanins were obtained (Table 2). Mazza (1995) reported that malvidin-3-glucoside acetate, along with malvidin-3-glucoside *p*-coumarate are the most important derivatives for the characterization of varieties. Like monoglucosides, E2-treated wine contained the highest amount of these compounds, with the exception of peonidin-3-glucoside acetate.

3.3. Sensory evaluation

Wines were evaluated using a preference test (Amerine & Roessler, 1976; Roessler, Pangborn, Sidel, & Stone, 1978). The wine made without enzyme treatment was used as a control. The wines were easily distinguished ($p < 0.05$) by the judges (ten judges from our Food Engineering Laboratory). In the preference test, the most preferred wine was the one produced with E2, because of better colour, visual aspect and general flavour properties, followed by E1, then the control wine.

4. Conclusions

The use of pectolytic enzyme preparations led to wines that are richer in anthocyanin compounds and with a better visual aspect. These results are in agreement with previous studies of Bakker et al. (1999), Bautista-Ortin et al. (2005), Canall-Llaubères and Pouns (2002), but not with studies of Wightman et al. (1997). These disparities could be due to the fact that each grape variety has different pectic composition (Nunan, Sims, Bacic, Robinson, & Fincher, 1997). Hence, it is necessary to study the effect of each commercial pectolytic preparation on each grape variety and winemaking process. According to data obtained in the present study, it should be noted that Öküzgözü grape variety is rich in readily extractable anthocyanin compounds during winemaking using enzymatic preparations.

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