Turkish Journal of Agriculture and Forestry

Volume 40 | Number 1 Article 9

1-1-2016

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TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2016) 40: 62-67
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doi:10.3906/tar-1412-54

Correlation between browning degree and composition of important Turkish white wine grape varieties

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Received: 15.12.2014 • **Accepted/Published Online:** 08.04.2015 • **Final Version:** 01.01.2016

Abstract: This study was undertaken to investigate the correlation between browning degree and composition of important Turkish white wine grape varieties during the 2006 and 2007 seasons. Large seasonal and cultivar variations were found in all measured variables. The highest browning degree was found in the Narince cultivar in both years, followed by Emir and Sultaniye, with Narince being the most susceptible to browning and Sultaniye being the least. No correlation ($P < 0.05$) was found between browning degrees and cysteine, glutathione, polyphenol oxidase activity, or total phenolic contents. Effects of harvest year and variety on all measured parameters, except total phenolics, were statistically significant ($P < 0.01$).

Key words: Browning, composition, grape, polyphenol oxidase, white wine

1. Introduction

Oxidative browning encountered during the vinification of white wine adversely affects quality attributes of wine, such as color, flavor, and aroma. Therefore, it is considered undesirable in young table and sparkling wines. The initiating mechanism of browning of wine can be enzymatic or nonenzymatic. Enzymatic browning is initiated by the action of polyphenol oxidase (PPO, EC 1.14.18.1) when grapes are crushed or pressed during vinification of white wine. Nonenzymatic browning can occur both in grape must and wine (Macheix et al., 1991; Cheynier et al., 1994; Li et al., 2008; Kallithraka et al., 2009). PPOs are cupric oxidoreductases, catalyzing the hydroxylation of monophenols to *o*-diphenols and the dehydrogenation of *o*-diphenols to *o*-quinones. Subsequent polymerization of o-quinones leads to the formation of brown-black pigments (López-Miranda et al., 2011). Browning intensities of individual phenolics vary.

The predominant phenolic compounds in freely expressed grape juice are caffeoyl tartaric (caftaric) acid and coumaroyl tartaric (coutaric) acid, which are also good substrates for PPOs. Enzymatic oxidation of caftaric and coutaric acid results in the formation of caftaric acid *o*-quinone, which then reacts with glutathione to form 2-S-glutathionyl caffeoyl tartaric acid (also called grape reaction product, GRP). The formation of GRP, which is a colorless compound and not oxidizable by PPO, prevents *o*-quinones from participating in polymerization, implying that the formation of brown pigments by polymerization of *o*-quinones will be reduced. Glutathione is a naturally occurring tripeptide in grapes with an –SH group. (Cheynier et al., 1988, 1994; Cheynier and Hulst, 1988). It has been postulated that cysteine, which has long been known to be a PPO inhibitor, can have similar effects (Singleton et al., 1985; Ünal et al., 2010). Susceptibility of grape varieties to browning varies, which might be attributed to the differences in the content of reductive species that can react with quinines, such as glutathione and ascorbic acid (Moreno-Arribas and Polo, 2009), as well as cysteine.

The Emir, Narince, and Sultaniye grapes used in this study are important grape varieties grown in Turkey. Emir, which is cultivated in the Nevşehir-Ürgüp (Cappadocia) region, is an important white grape variety for the wine industry (Ünal and Şener, 2006). It comprises around 25% of the total vineyards of the region. Narince is another important white variety commonly grown in the Tokat region (Ünal and Şener, 2014). Sultaniye, which is mainly cultivated in the Aegean region, is sold as fresh fruit as well as used in wine production (Ünal et al., 2007). This study is aimed at determining browning potentials, phenolic compounds, cysteine and glutathione contents, and PPO activities of Narince, Sultaniye, and Emir, and examining the relationship between browning potential

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and composition. Until now, no such research has been carried out on these varieties.

2. Materials and methods

2.1. Plant materials

The investigation was conducted during the years 2006 and 2007, using Sultaniye, Emir, and Narince grape varieties. Sultaniye grapes were obtained from Denizli Province, Emir from Nevşehir Province, and Narince from Tokat Province of Turkey. Fruit samples of around 20 kg were collected in a random fashion at the time of optimum harvest maturity as determined by Turkish wine producers. The grapes were transferred to the laboratory in a cool Styrofoam box, frozen at -25 °C, and stored in a freezer until further analysis.

2.2. Reagents

Catechol, Triton X-100, Folin–Ciocalteu reagent, sodium metabisulfite, polyvinylpolypyrrolidone, sodium acetate, and acetic acid were purchased from Merck (Germany). Polyethylene glycol, phenylmethylsulfonyl fluoride, acetone, acetonitrile, ammonium sulfate, cellulose membrane (76 × 49 mm), cysteine, cysteic acid, ascorbic acid, 2-nitrobenzoic acid, NADPH, and yeast glutathione reductase were purchased from Sigma-Aldrich (USA). All chemicals were of analytical grade.

2.3. General composition

Grape berries (300 g) were homogenized in a Waring blender (Model HGB2WTS3, Waring, USA), followed by centrifugation at 6000 × *g* for 8 min at 10 °C. The supernatant was analyzed for pH, total acidity, and total soluble solids (degrees Brix) (Ough and Amerine, 1988).

2.4. Total phenolic content

The total phenolic concentration in the samples was determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method using gallic acid as a standard (Ough and Amerine, 1988). The total phenolic content was expressed as gallic acid equivalents in mg/L.

2.5. Browning degree

Grape berries (200 g) collected from several clusters were homogenized in a Waring blender for 1 min at low speed. The slurry was vacuum-filtered through filter paper. The homogenate was then filtered through cheesecloth. The juice was incubated at room temperature for 24 h and centrifuged at 10,000 × *g* for 10 min at room temperature. Optical density was then measured at 420 nm. Juice prepared with added ascorbic acid (1%) served as the control. Browning degree (OD_{420}) was obtained by subtracting optical density at 420 nm of the control from that of the sample. The measurements were carried out in triplicate and the results are expressed as means (Lee and Jaworski, 1988).

2.6. Assay of enzyme activity

Enzymes were extracted from 200 g of frozen berries as described elsewhere (Ünal and Şener, 2006; Ünal et al., 2007). PPO activity was determined in 1.0 mL of assay mixture in a spectrophotometer by measuring the increase in absorbance at 410 nm at 30 °C. The initial rate was calculated from the slope of the absorbance–time curve. Unless otherwise stated, the standard reaction mixture consisted of 0.1 mL of enzyme solution and 0.9 mL of catechol. Enzyme activity for PPO was performed in triplicate and the results are expressed as means. In all experiments, control reactions without the enzyme were conducted and no significant oxidation of the substrate was observed during the short period employed to measure PPO activity. One unit of enzyme activity was defined as the amount of enzyme that caused an increase of 0.001 in the absorbance value per min under the assay conditions (Ünal and Şener, 2006).

2.7. HPLC analysis of cysteine

Berries of the Emir and Narince cultivars were manually deseeded. Deseeding was not required for Sultaniye, as it is a seedless variety. Cysteine extraction and sample preparation was carried out according to Hermosín et al. (2003) and Gómez-Alonso et al. (2007). Cysteine content of the cultivars was determined using Agilent 1100 HPLC chromatography (Agilent, USA) with photodiode array detector. Separation was carried out with an ACE C18 column (Agilent, UK) (5 µm, 250 mm × 4.6 mm) at 16 °C. Detection was at 280 nm with a diode array detector. Elution solvents were acetonitrile (A) and acetate buffer (25 mm) at pH 5.8 with 0.02% sodium azide (B). Elution was performed with a gradient program of 6% A; 16% A (13 min); 18% A (13.5 min); 18% A (17 min); 22% A (20 min); and 32% A (32 min). Flow rate was 1.0 mL/min. The internal standard used was γ-aminobutyric acid. Cysteine was identified by comparison of the retention times of the pure compound. The amounts of cysteic acids in the samples were quantified by using calibration curves obtained from known concentrations of cysteic acid (Bozdogan and Canbas, 2011). The cysteic acid concentrations were then converted to cysteine by using the conversion rate of cysteine to cysteic acid that was experimentally determined from 5 different cysteine concentrations. The conversion rate was found to be 59.1% (Varga-Visi et al., 2000).

2.8. Glutathione extraction and analysis

Undamaged grape berries (150 g) were snipped from clusters. Manually deseeded berries were lyophilized using a freeze dryer (LP3 model, Jouan, France). The lyophilized samples were homogenized for 2 min using a prechilled Waring blender. Four grams of the berry powder was extracted with 16 mL of 5% perchloric acid. The slurry was allowed to stand until it reached room temperature

and was then further homogenized using a glass bar. The extract was then centrifuged at 7000 × *g* for 20 min at 4 °C. An aliquot of supernatant (800 µL) was neutralized to pH 3.5 with 190 µL of 2 M potassium hydroxide. After allowing the potassium perchlorate to precipitate for 15 min, 60 mg of PVPP was added and the slurry was centrifuged at 13,600 × *g* for 5 min. The supernatant obtained was used directly in the assay (Adams and Liyanage, 1991).

The reaction mixture consisted of 680 µL of 0.1 M phosphate buffer (pH 7.5) containing 5 mM EDTA. Yeast glutathione reductase (100 µL) was diluted from the stock to give approximately 15 IU/mL, 100 µL of DTNB (2.4 mg/mL), and 100 µL of NADPH (1.9 mg/mL). The reaction was started by adding 20 µL of the PVPP-treated extract and following the rate of absorption change at 412 nm in a spectrophotometer. The amount of glutathione in the extract was determined by comparing the rate of absorption change to a standard curve, which was generated using the complete reaction mixture and starting the reaction with an appropriately diluted glutathione solution prepared just prior to use. A standard curve over the range of 20 to 100 ng gave suitable rates (Adams and Liyanage, 1991).

2.9. Statistical analysis

The statistical methods used for the data analysis were two-way analysis of variance (ANOVA). Mean differences was compared using Duncan's multiple range test ($P < 0.05$). Pearson's correlation test also was used. Data processing was conducted with statistical software (SPSS 10.0 for Windows; SPSS Inc., USA).

3. Results and discussion

3.1. Chemical composition

Chemical compositions of the grape varieties used in this study in terms of degrees Brix, pH, and total acidity are given in Table 1. Sultaniye had the highest Brix value in both years. Brix values in all varieties were found to be higher in 2007 compared to the previous year. pH values of the grape varieties in the year 2006 were close to each other. Narince must had the highest total acidity in both years.

3.2. Total phenolics

Table 2 shows total phenolic contents of the cultivars examined. There were differences among the cultivars with respect to the total phenolics that also showed seasonal variations. The highest phenolic content was found in Emir at 227.2 mg/L in 2006, whereas in 2007 it was observed in Narince at 218.4 mg/L. In grape must, enzymatic browning is largely correlated with the content of hydroxycinnamates, such as caffeoyl tartaric acid (caftaric acid) and *p*-coumaroyl tartaric acid (coutaric acid), and is promoted by flavanols. Caffeic acid is one of the most common hydroxycinnamate acids in wine. Caftaric acid, the most abundant hydroxycinnamate found in grapes, consists of caffeic acid bound to tartaric acid (Li et al., 2008; Fracassetti et al., 2011).

Table 1. General composition of the grape cultivars.

	Emir		Narince		Sultaniye	
	2006	2007	2006	2007	2006	2007
Brix	18.0	24.8	20.2	22.8	24.6	35.0
pH	3.98	3.74	3.73	3.75	3.90	4.12
Total acidity*	3.16 ± 0.089	3.50 ± 0.027	4.33 ± 0.147	3.89 ± 0.019	3.49 ± 0.136	3.31 ± 0.026

*As g tartaric acid/L.

Table 2. Total phenolics, browning degrees, and cysteine contents of grape cultivars.

Cultivar	Total phenolics* (mg/L)		Browning degree (OD_{420})		Cysteine (mg/L)	
	2006	2007	2006	2007	2006	2007
Emir	227.2 ± 4.5	202.7 ± 10.5	0.73 ± 0.12	1.28 ± 0.02	13.1 ± 0.50	17.8 ± 0.60
Narince	205.5 ± 1.0	218.4 ± 13.9	1.10 ± 0.06	1.35 ± 0.02	20.9 ± 2.34	12.8 ± 0.51
Sultaniye	217.5 ± 6.2	190.8 ± 7.40	0.35 ± 0.02	0.23 ± 0.02	7.7 ± 0.76	14.0 ± 0.50

*As gallic acid.

3.3. Browning degree

Wide variations in browning degrees were apparent among the cultivars studied and harvest years (Table 2). The highest browning degree was observed in Narince in both years, followed by Emir and Sultaniye, with Narince being the most susceptible to browning and Sultaniye being the least.

3.4. Cysteine content

Cysteine content also varied depending on the cultivar and season. The highest cysteine content in 2006 was in Narince at 20.9 mg/L, while that in 2007 was found in Emir at 17.8 mg/L. Cysteine contents of Emir and Sultaniye cultivars increased in 2007, while that of Narince decreased (Table 2). Cysteine is a thiol compound, which is a strong nucleophile and suppresses enzymatic browning mainly via the formation of colorless addition products with *o*-quinones (Robinson, 1991). In a study carried out by our research group on PPO extracted from Narince grapes over two consecutive years, cysteine at 0.1 mM (12.116 mg/L) inhibited PPO activity between 47.2% and 68.0% (Ünal and Şener, 2014). We also investigated the inhibition of Emir and Sultaniye PPOs by cysteine; according to our results, cysteine at 0.1 mM inhibited PPO at varying degrees (unpublished data). Therefore, the amounts of cysteine in the cultivars studied are expected to inhibit enzymatic browning reactions in must to certain degree.

3.5. Glutathione content

Glutathione, which is a tripeptide composed of L-glutamate, L-cysteine, and glycine, plays an important role in the oxidation of musts by trapping *o*-quinones, formed during oxidation, to limit the amount of browning pigments. It also protects various aromatic compounds in wine. Its biological significance is mostly related to its free sulfhydryl moiety of the cysteine residue, which confers unique redox and nucleophilic properties (Kritzinger et al., 2013). The glutathione content of grapes generally increases markedly during ripening, coinciding with veraison (Adams and Liyanage, 1993). Cultivars with high hydroxycinnamic acid contents but low glutathione contents have a heightened browning potential (Jackson, 2008).

The glutathione contents of the cultivars are given in Table 3. Emir was the richest in terms of glutathione in both years, ranging between 38.7% and 27.8 µg/kg fresh weight. Maggu et al. (2007) reported that glutathione concentrations in Sauvignon Blanc juice ranged between 36 and 40 mg/L, which is much higher than those found in our study.

3.6. PPO activity

PPO activities of the grape extracts are given Table 3. PPO activities varied across the cultivars and seasons. PPO activity was highest in the Narince cultivar in both years, followed by Emir in 2006 and Sultaniye in 2007. Wissemann and Lee (1980) investigated PPO activity during grape maturation and wine production in 11 white grape varieties grown in the northeastern United States. They reported that PPO activity for any one cultivar fluctuated throughout the ripening period and varied among the cultivars. Hooper et al. (1985), who investigated PPO activity in 14 Australian white grape varieties, reported varietal and regional variations in terms of PPO activity.

3.7. Statistical analysis

Statistical analyses are given in Table 4. Browning degree, specific activity, glutathione, and cysteine varied significantly due to year and variety ($P < 0.01$). However, there were no significant differences between total phenol values of samples due to year and variety according to Duncan's multiple range test.

No correlation ($P < 0.05$) was found between browning degrees and cysteine, glutathione, PPO activity, or total phenolic contents. In a study carried out by Cheng and Crisosto (1995) on browning potential, phenolic composition, and PPO activity of buffer extracts of peach and nectarine skin tissue, a significant correlation was found between chlorogenic acid and (-) - epicatechin content and browning potential in the first hour of incubation. However, they did not find any significant correlation between PPO activity and browning potential

Table 3. Glutathione contents and PPO activities of grape cultivars.

Cultivar	Glutathione (µg/kg fresh weight)		Specific PPO activity (U/mg protein)	
	2006	2007	2006	2007
Emir	38.7 ± 1.0	27.8 ± 0.3	81,864	689,384
Narince	21.9 ± 0.5	25.2 ± 2.0	508,193	879,583
Sultaniye	27.3 ± 0.7	14.9 ± 1.1	322,865	716,015

Table 4. Effect of grape cultivar and harvest year on browning degree and other parameters.

	Browning degree (OD_{420})	Specific activity (U/mg protein)	Glutathione (µg/kg)	Cysteine (mg/L)	Total phenol (mg/L)
Year	**	**	**	**	NS
2006	0.73[b]	304,307.1[b]	29.3[a]	13.88[b]	216.73
2007	0.95[a]	761,660.7[a]	22.62[b]	14.85[a]	203.97
Cultivar	**	**	**	**	NS
Emir	1.00[b]	385,623.7[c]	33.24[a]	15.43[a]	214.95
Narince	1.23[a]	693,888.1[a]	23.55[b]	16.83[a]	211.95
Sultaniye	0.29[c]	519,439.8[b]	21.09[c]	10.84[b]	204.15

*Means in the same column with different letters are significantly different according to Duncan test ($P < 0.05$).
*Significant at the 0.05 significance level, **Significant at the 0.01 significance level, NS: No significant differences.

of the peaches and nectarines they examined. Lee et al. (1990), on the other hand, reported a correlation between degree of browning and PPO activity of individual peach cultivars. They also reported that browning degree was correlated with total phenolics. With regard to browning rates of individual phenolics in white grapes, Lee and Jaworski (1988) reported that browning rates varied among the phenolics; for example, catechin and epicatechin had the fastest browning rates, while those of acidic phenolics were the lowest.

3.8. Conclusion

Large seasonal and cultivar variations were observed for all measured variables. The highest browning degree was found in the Narince cultivar in both years, followed by Emir and Sultaniye, with Narince being the most susceptible to browning and Sultaniye being the least. No correlation ($P < 0.05$) was found between browning degrees and cysteine, glutathione, PPO activity, or total phenolics contents. Effects of harvest year and variety on all measured parameters except total phenolics were found to be statistically important ($P < 0.01$).

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