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ORIGINAL ARTICLE

Changes in Phenolic Compounds Profile and Anthocyanin Content of cv. Cabernet Sauvignon (*V. vinifera* L.) Induced by Different Doses of Foliar Oak Applications with Urea

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Abstract

Phenolic compounds and anthocyanins had pivotal roles on wine grape quality. In order to enhance contents of these substances, several techniques have been applied to grapevines. In recent years, there has been an increasing concern about using of foliar oak applications for improving wine grape quality. In this study, it was used a low concentration (0.6%) of urea for increasing efficiency of different doses of oak applications. For this aim, it was benefited from different doses of foliar oak applications with or without urea, including: 0 ppm oak (Oa), 1250 ppm oak (Oa), 2500 ppm (Oa), 5000 ppm (Oa), 0 ppm oak (Oa) + 0.6% Urea (Ur), 1250 ppm oak (Oa) + 0.6% Urea (Ur), 2500 ppm oak (Oa) + 0.6% Urea (Ur), 5000 ppm oak (Oa) + 0.6% Urea (Ur). Although yield and wine grape quality characteristics were favorably affected by increasing doses of oak applications; urea added oak applications were especially found to be more effective for both characteristics. As a result, application of 5000 ppm oak (Oa) + 0.6% Urea (Ur) led to best results in terms of yield and wine grape characteristics of cv. Cabernet Sauvignon.

Keywords *Vitis vinifera* L. · Wine grape · Foliar oak application · Foliar urea application · Phenolic compounds · Anthocyanins

Veränderungen bei phenolischen Inhaltsstoffen und des Anthocyananteils bei der Rebsorte 'Cabernet Sauvignon' (*V. vinifera* L.) durch die Blattapplizierung von Eichenextrakt mit Harnstoff in unterschiedlicher Dosierung

Schlüsselwörter *Vitis vinifera* L. · Weinrebe · Blattapplizierung mit Eichenextrakt · Blattapplizierung mit Harnstoff · Phenolverbindungen · Anthocyane

Introduction

Phenolic compounds mainly contain anthocyanins, flavanols, flavonols, stilbenes and phenolic acids (Gougoulias 2010; Kok and Bal 2017a).

Phenolic compounds profile is linked to grape composition, influencing by various factors such as ecological factors (Downey et al. 2006), different viticultural practices (Kok 2011; 2016; Kok et al. 2013; Kok and Bal 2016, 2017b) and grape cultivar (Kok 2017; Kok and Bal 2017a).

Phenolic compounds have important roles as components of wine colour, conducing wine sensory quality. Inside of this family of compounds, anthocyanins are the most considerable molecules, finding in the skins of coloured grapes (Bouzas-Cid et al. 2016).

There have been numerous efforts to rise phenolic compounds composition and anthocyanin content of wine grapes (Pardo-Garcia et al. 2014). Foliar oak applications have recently been used for improving contents of phenolic compounds and anthocyanin.

Newly published scientific papers about utilization of aqueous oak extracts point out that how foliar applications of oak extract modulate the aroma and phenolic compounds

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in wine grapes (Martinez-Gil et al. 2011, 2012; Pardo-Garcia et al. 2014).

Study results about oak applications indicate that foliar oak applications increase phenolic compounds composition and anthocyanin content in wine grapes (Martinez-Gil et al. 2011, 2012, 2013; Pardo-Garcia et al. 2014).

One of the other viticultural practices, affecting wine grape and wine composition is foliar or soil fertilization (Bell and Henschke 2005). There are different favourable (Delgado et al. 2004) or unfavourable (Soubeyrand et al. 2014) research findings about effects of high nitrogen supply on phenolic compounds content of wine grapes. The point that should not be forgotten here is that these conflicting findings seem to be related to nitrogen dose, application time and application numbers, prevailing ecological factors in vineyards (Portu et al. 2015).

Reasons mentioned above, the aim of current study was to determine effects of foliar oak application doses combined with low urea concentration on phenolic compounds composition and anthocyanin content of cv. Cabernet Sauvignon.

Material and Methods

Plant material and characteristics of research area

The research was performed in 2016 growing season in a 7-year old commercial vineyard (40°56′40,81″N; 27°20′15,74″E; 243 m a. s. l) in Tekirdag, Turkey.

The vineyard was planted with cv. Cabernet Sauvignon grafted onto SO4 (*Vitis berlandieri* × *Vitis riparia* No. 4, Selection Oppenheimer No. 4). Grapevine spacing was 1.5 m and row spacing was 3.2 m and grapevines were trained to a guyot training system. In current vineyard, disease and pest controls and other all vineyard operations were conducted according to local standard practices.

The climate of the region where the research was performed is temperate. In 2016 year, annual mean temperature, relative humidity and total precipitation of Tekirdag were successively recorded as 15.54 °C, 80.95% and 791.20 mm.

Yield and wine grape quality characteristics considered in research

In available study, grape length (cm), grape width (cm), grape weight (g) and cluster length (cm), cluster width (cm), cluster weight (g) were measured as yield parameters. Furthermore, total soluble solids content (°Brix), titratable acidity (g/L), pH, total soluble solids content × pH^2, total phenolic compounds content (mg GAE/kg fw) and total anthocyanin content (mg GAE/kg fw) were found out as wine grape quality parameters.

Foliar applications of oak and urea and application times

In present study, uniform grapevines were selected for the foliar applications and it was utilized from different doses of foliar oak applications with urea, consisting: 1–0 ppm oak (Oa), 2–1250 ppm oak (Oa), 3–2500 ppm (Oa), 4–5000 ppm (Oa), 5–0 ppm oak (Oa) + 0.6% Urea (Ur), 6–1250 ppm oak (Oa) + 0.6% Urea (Ur), 7–2500 ppm oak (Oa) + 0.6% Urea (Ur), 8–5000 ppm oak (Oa) + 0.6% Urea (Ur).

In order to prepare aqueous extracts of both oak and urea, powdered Oak (Artu Chemical®, 65% tannin, 24% non-tannin, 5% insoluble solids and 6% humidity) and urea (Sigma-Aldrich, 98%) were used. Foliar spraying applications of oak and urea on leaves of grapevines were carried out two times at verasion period and 15 days after verasion by using a back pump.

Grape sampling and storing of grapes until analyses

When the clusters on control grapevines of cv. Cabernet Sauvignon achieved approximately 23%, the grapes were harvested at technological maturity. After the grapes were harvested, standard measurements and chemical analyses for grapes were immediately managed. However, it was taken advantage of 300-grape samples to appraise total phenolic compounds content and total anthocyanin content. All grape samples were stored at –25 °C up to analyses of total phenolic compounds and total anthocyanin. Prior to these analyses, grape samples were thrown out from –25 °C, allow to thaw overnight at 4 °C and then homogenized in a commercial laboratory blender for 20 s.

Analyses of total phenolic compounds and total anthocyanin compounds by spectrophotometer

In current study, spectrophotometric methods explained by Singleton et al. (1978) and Di Stefano and Cravero (1991) were successively made use of defining total phenolic compounds content and total anthocyanin content in grapes of cv. Cabernet Sauvignon. Results of both analyses were expressed as milligrams of gallic acid equivalent per kilogram of fresh weight (mg GAE/kg fw).

Statistical analysis

All statistical analyses were performed by using TARIST statistical software program. Differences among the means were compared by using Fisher's Least Significant Difference (LSD) multiple comparison test at 5% level.

Results and Discussion

Yield characteristics

As shown in Table 1, grape length and grape weight among the yield parameters were significantly affected by different doses of oak application combined with urea application ($p<0.05$).

Grape cultivars differ in terms of grape sizes (Amone and Simonetti-Bryan 2013). In this study, the different doses of oak and urea applications had significant effects on grape length ($p>0.05$) and the highest means were obtained from applications of 2500 ppm Oa + 0.6% Ur (13.51 mm) and 5000 ppm Oa + 0.6% Ur (13.55 mm) than 0 ppm Oa (12.24 mm) and 0 ppm Oa + 0.6% Ur (12.44 mm) (Table 1).

As far as grape width is concerned, there were no significant differences among the different doses of oak and urea applications ($p<0.05$) and means ranged from 12.05 (0 ppm Oa) to 12.92 mm (5000 ppm Oa + 0.6% Ur) (Table 1).

Concerning grape weight, significant differences were observed among the different doses of oak and urea applications ($p<0.05$) and applications of 2500 ppm Oa + 0.6% Ur and 5000 ppm Oa + 0.6% Ur brought about the highest increases in grape weight (1.55 and 1.57 g) than 0 ppm Oa (1.33 g) (Table 1).

Cluster sizes in grape cultivars can be vary depending on grape cultivar and ecological factors and viticultural practices (Gougoulias and Masheva 2010). In available study, cluster characteristics were not significantly affected by the different doses of oak and urea applications ($p<0.05$). In point of cluster length, means changed from 15.22 (0 ppm Oa) to 17.12 mm (5000 ppm Oa + 0.6% Ur) (Table 1).

As displayed in Table 1, different doses of oak and urea applications had not significant effects on cluster width ($p<0.05$) and the highest mean was recorded for 5000 ppm

Table 1 Yield parameters of cv. Cabernet Sauvignon grapes from different doses of foliar oak and urea applications

Foliar application	Grape length (mm)	Grape width (mm)	Grape weight (g)	Cluster length (cm)	Cluster width (cm)	Cluster weight (g)
0 ppm Oa	12.24^{c}	12.05	1.33^{d}	15.22	9.37	179.19
1250 ppm Oa	12.48^{bc}	12.16	1.41^{bcd}	15.75	9.88	187.73
2500 ppm Oa	12.88^{abc}	12.20	$1.45^{a–d}$	16.60	10.10	191.89
5000 ppm Oa	12.97^{abc}	12.26	1.47^{abc}	16.97	10.52	196.70
0 ppm Oa + 0.6% Ur	12.44^{c}	12.13	1.38^{cd}	16.15	9.48	184.54
1250 ppm Oa + 0.6% Ur	13.38^{ab}	12.44	1.52^{ab}	16.42	10.27	192.57
2500 ppm Oa + 0.6% Ur	13.51^{a}	12.60	1.55^{a}	16.77	10.58	195.99
5000 ppm Oa + 0.6% Ur	13.55^{a}	12.92	1.57^{a}	17.12	11.17	200.27
$LSD_{5\%}$	0.92	N.S.	0.13	N.S.	N.S.	N.S.

Different letters in column point out significant differences in the mean at 5% level by LSD multiple comparison test
Oa Oak, *Ur* Urea, *N.S.* Not significant

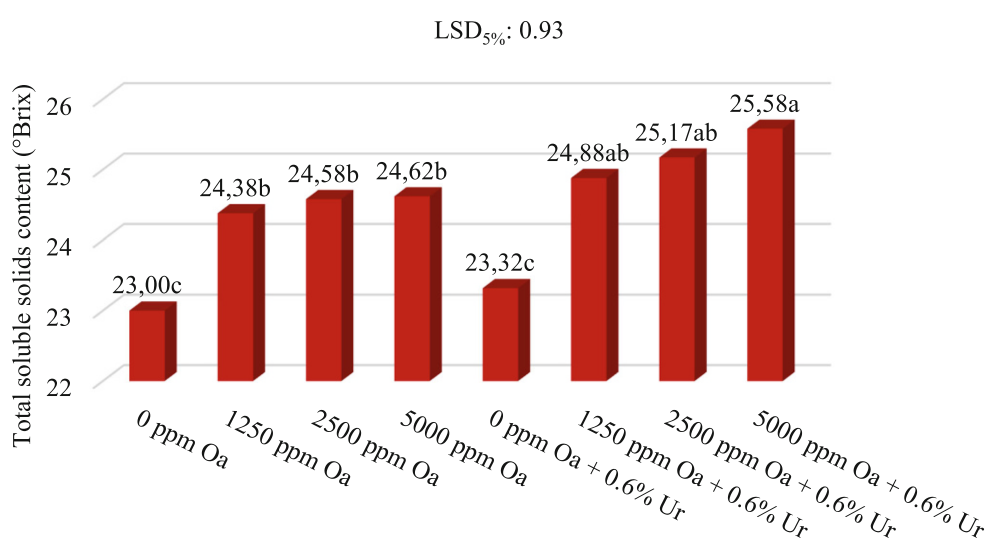

Fig. 1 Effects of different doses of oak and urea applications on total soluble solids content of cv. Cabernet Sauvignon

Oa + 0.6% Ur (11.17 mm) compared to 0 ppm Oa (9.37 mm) (Table 1).

As regard to cluster weight represented in Table 1, significant differences were not observed among the different doses of oak and urea applications ($p < 0.05$). While 0 ppm Oa application was causing the lowest mean (179.19 g); the highest mean was obtained from 5000 ppm Oa + 0.6% Ur application (200.27 g).

Wine grape quality characteristics

Taking into account the wine grape parameters, it was seen that total soluble solids content and total soluble solids content × pH^2 were significantly affected by different doses of oak and urea applications (Figs. 1 and 4; $p < 0.05$).

Grapes are harvested when they achieve a crucial stage for sale or processing. It is utilized from percentage soluble solids by weight as main measure of the grape's suitability for harvest (Janick and Paull 2008). In this study, different doses of oak and urea applications significantly affected total soluble solids content ($p < 0.05$) and 5000 ppm Oa + 0.6% Ur application led to the highest mean (25.58 °Brix), whereas applications of 0 ppm Oa and 0 ppm Oa + 0.6% Ur were the lowest means as 23.00 and 23.32 °Brix (Fig. 1).

Titratable acidity measurement is one of the key determinants on when to harvest grapes and how to treat the harvested grapes and later the wine (Linskens and Jackson 1988). The range for titratable acidity in wine grapes is quite broad from 6 to 10 g/L (Butzke 2010). In actual study, different doses of oak and urea applications had not

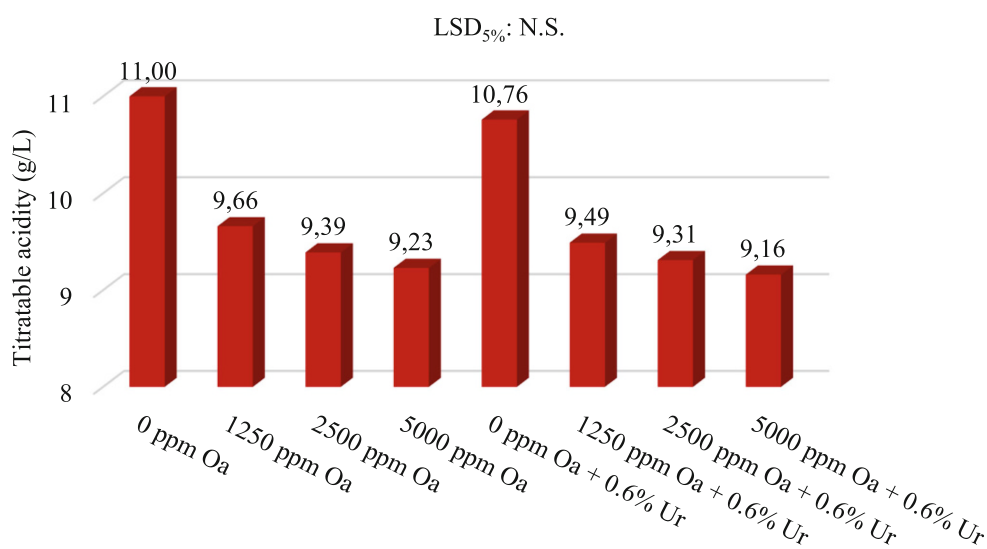

Fig. 2 Effects of different doses of foliar oak and urea applications on titratable acidity of cv. Cabernet Sauvignon

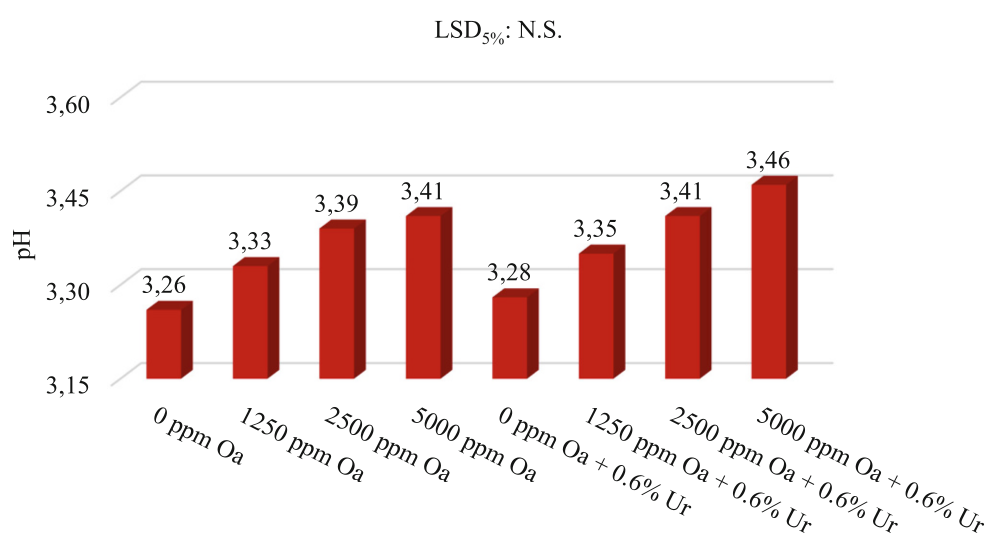

Fig. 3 Effects of different doses of foliar oak and urea applications on pH of cv. Cabernet Sauvignon

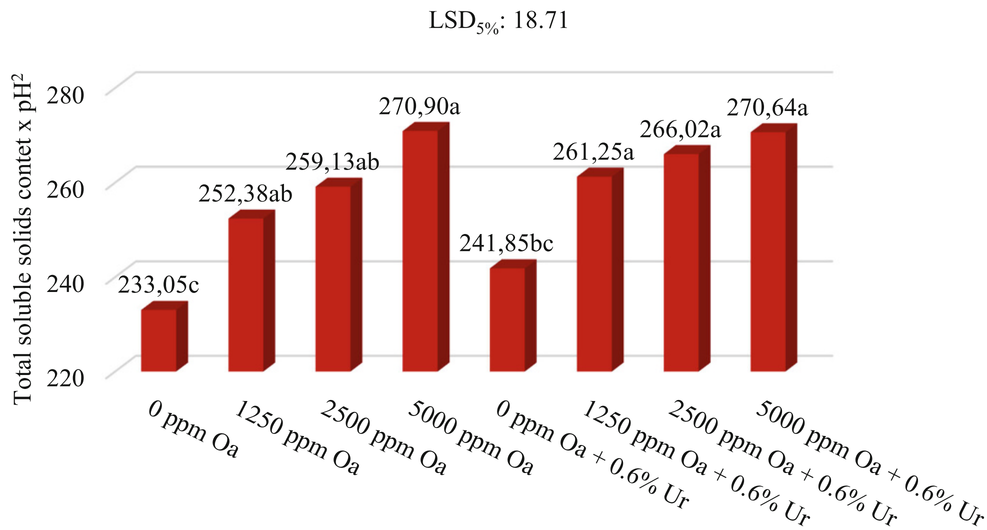

Fig. 4 Effects of different doses of foliar oak and urea applications on total soluble solids content × pH^2 of cv. Cabernet Sauvignon

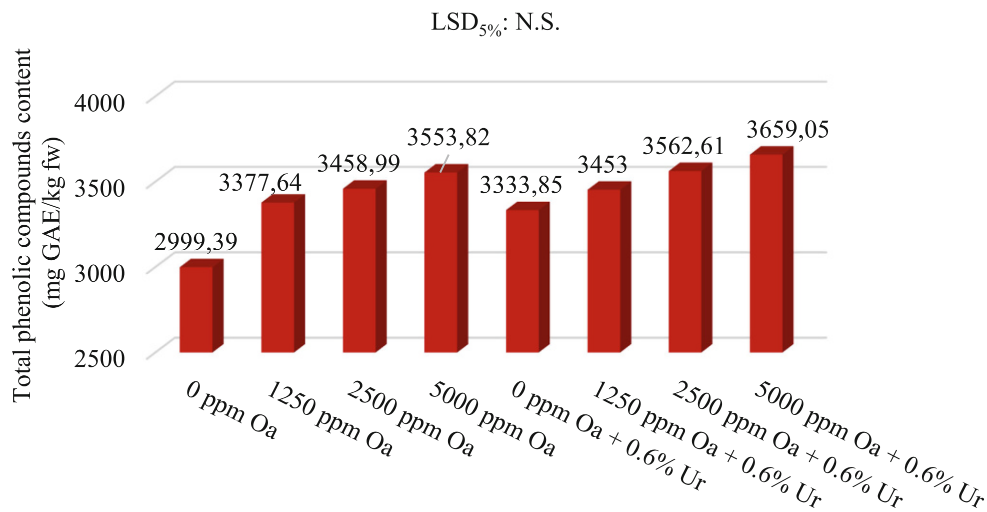

Fig. 5 Effects of different doses of foliar oak and urea applications on total phenolic compounds content of cv. Cabernet Sauvignon

significant effects on titratable acidity of grapes ($p < 0.05$). Fig. 2 show the data for titratable acidity of grapes applied with different doses of foliar oak and urea. Titratable acidity in grapes of grapevines applied with oak combined with urea had lower than other applications. The lowest mean of titratable acidity was 9.16 g/L for 5000 ppm Oa + 0.6% Ur and the highest mean was obtained from 0 ppm Oa application (11.00 g/L).

Another way used to express acidity in grape must is pH. Cooke (2004) inform that suitable ranges for pH for quality winemaking vary between 3.30 and 3.50 in red wine grapes. In present study, although pH means were not affected by the different doses of oak and urea applications ($p < 0.05$); means ranged from 3.26 (0 ppm Oa) to 3.46 (5000 ppm Oa + 0.6% Ur) (Fig. 3).

Coombe et al. (1980) declare that equation of total soluble solids × pH^2 is proper indicator of optimum ripeness in wine grapes. The best wines are made at index values ranging from 200 to 270 in red wine grapes. In this study, different doses of oak and urea applications significantly affected total soluble solids content × pH^2 ($p < 0.05$) and while the lowest mean of total soluble solids content × pH^2 was 233.05 for 0 ppm Oa application, the highest means were successively recorded for applications of 1250 ppm Oa + 0.6% Ur (261.25), 2500 ppm Oa + 0.6% Ur (266.02), 5000 ppm Oa + 0.6% Ur (270.64) and 5000 ppm Oa (270.90) (Fig. 4).

Phenolic compounds are an integral part of grapes and wines, contributing to colour, taste and stability of wines (Botes 2009). There can be important varietal differences within *V. vinifera* L. in terms of phenolic compounds content (Packer 2002; Kok 2017; Kok and Bal 2017a) and phenolic compounds level in grapes is also affected by environmental factors (Downey et al. 2006) and viticultural

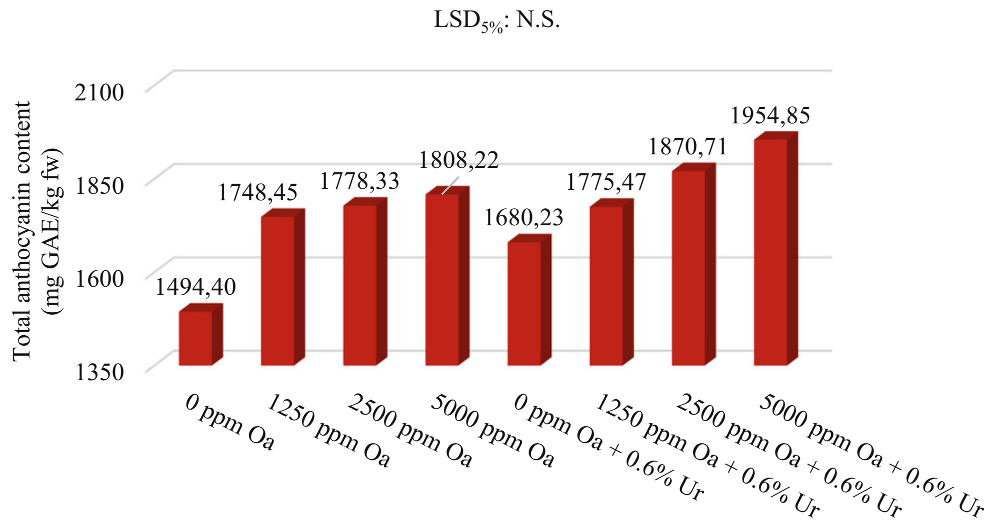

Fig. 6 Effects of different doses of foliar oak and urea applications on total anthocyanin content of cv. Cabernet Sauvignon

practices (Kok 2011; 2016; Kok et al. 2013; Kok and Bal 2016, 2017b). In current study, it was observed that the different doses of oak and urea applications had no significant effects on total phenolic compounds content ($p < 0.05$). Among the applications, 5000 ppm Oa + 0.6% Ur application gave rise to the highest total phenolic compounds content (3659.05 mg GAE/kg fw) than 0 ppm Oa application (2999.39 mg GAE/kg fw) (Fig. 5).

Anthocyanins are red compounds, responsible for the colour of red grapes and wines. These compounds are chiefly located in the vacuoles of grape skins (Tokuşoğlu and Hall 2010). Results of total anthocyanin content are displayed in Fig. 6 and there were no significant differences among the different doses of foliar oak and urea applications ($p < 0.05$). However, 5000 ppm Oa + 0.6% Ur application led to the highest total anthocyanin content (1954.85 mg GAE/kg fw) compared to 0 ppm Oa (1494.40 mg GAE/kg fw).

Conclusion

Phenolic compounds and anthocyanins are very crucial substances for wine grape quality. There have been various applications to improve grape quality and one of these applications used to increase wine grape quality is foliar oak applications.

In present study, it was tried to increase efficiency of foliar oak application's doses by using low concentration of urea application. The research results shown that increasing doses of foliar oak applications combined with low urea concentration enhanced quality parameters of wine grape. From the study findings, it could be concluded that foliar application of 5000 ppm Oa + 0.6% Ur seems to be more effective in wine grape quality of cv. Cabernet Sauvignon.

Conflict of interest D. Kok declares that he has no competing interests.

References

Amone K, Simonetti-Bryan J (2013) Pairing with the masters: a definitive guide to food and wine. Delmar, New York. ISBN 978-1-111-54384-6

Bell SJ, Henschke PA (2005) Implications of nitrogen nutrition for grapes, fermentation and wine. Aust J Grape Wine Res 11(3):242–295

Botes MP (2009) Evaluation of parameters to determine optimum ripeness in Cabernet Sauvignon grapes in relation to wine quality. Master thesis, Stellenbosch University, South Africa, p 111

Bouzas-Cid Y, Portu J, Perez-Alvarez EP, Gonzalo-Diago A, Garde-Cerdan T (2016) Effect of vegetal ground cover crops on wine anthocyanin content. Sci Hortic 211:384390

Butzke CE (2010) Winemaking problems solved. CRC, Boca Raton

Cooke GM (2004) Making table wine at home. Agriculture and natural resources. Publication 21434. University of California, Oakland

Coombe BG, Dundon RJ, Short AW (1980) Indices of sugar-acidity as ripeness criteria for wine grapes. J Sci Food Agric 31:495–502

Delgado R, Martin P, Del Alamo M, Gonzalez MR (2004) Changes in the phenolic composition of grape berries during ripening in relationship to vineyard nitrogen and potassium fertilisation rates. J Sci Food Agric 84:623–630

Downey MO, Dokoozlian NK, Krstic MP (2006) Cultural practice and environmental impacts on the flavonoid composition of grapes and wine. A review of recent research. Am J Enol Vitic 57:257–268

Gougoulias N (2010) Study on phenolics and antioxidant activity of selected wines from some Greek Aegean Sea islands. Oxid Commun 33(1):167–174

Gougoulias N, Masheva L (2010) Effect of gibberellic acid (GA_3) on polyphenols content and antioxidative activity of some table grape varieties. Oxid Commun 33:652–660

Janick J, Paull RE (2008) The encyclopaedia of fruit and nuts. Cambridge University Press, Reading. ISBN 978-0-85199-638-7

Kok D (2011) Influences of pre and post-veraison cluster thinning treatments on grape composition variables and monoterpene levels of Vitis vinifera L. Cv. Sauvignon Blanc. J Food Agric Environ 9(1):22–26

Kok D (2016) Variation in total phenolic compounds, anthocyanin and monoterpene content of 'Muscat Hamburg' table grape variety (V.

vinifera L.) as affected by cluster thinning and early and late period basal leaf removal treatments. Erwerbs-Obstbau 58:241–246

Kok D (2017) Assessment of electrochemical attribute and monoterpene content of twelve aromatic grape cultivars (V. vinifera L.) grown under the ecological conditions of north-western Turkey. Oxid Commun 40(1-II):557–564

Kok D, Bal E (2016) Seedless berry growth and bioactive compounds of cv. 'Recel Uzümü' (V. vinifera L.) as affected by application doses and times of pre-harvest thidiazuron. Erwerbs-Obstbau 58:253–258

Kok D, Bal E (2017a) Compositional differences in phenolic compounds and anthocyanin contents of some table and wine grape (V. vinifera L.) varieties from Turkey. Oxid Commun 40(2): 648–656

Kok D, Bal E (2017b) Leaf removal treatments combined with kaolin film technique from different directions of grapevine's canopy affect the composition of phytochemicals of cv. Muscat Hamburg (V. vinifera L.). Erwerbs-Obstbau. https://doi.org/10.1007/s10341-017-0337-7

Kok D, Bal E, Celik S (2013) Influences of various canopy management techniques on wine grape quality of V. vinifera L. cv. Kalecik Karası. Bulg J Agric Sci 19(6):1247–1252

Linskens HF, Jackson JF (1988) Wine analysis. Springer, Berlin. ISBN 978-3-642-83342-7

Martinez-Gil AM, Garde-Cerdan T, Martinez L, Alonso GL, Salinas MR (2011) Effect of oak extract application to Verdejo grapevines on gape and wine aroma. J Agric Food Chem 59(7):3253–3263

Martinez-Gil AM, Garde-Cerdan T, Zalacain A, Pardo-Garcia AI, Salinas MR (2012) Applications of an oak extract on Petit Verdot grapevines. Influence on grape and wine volatile compounds. Food Chem 132(4):1836–1845

Martinez-Gil AM, Angenieux M, Pardo-Garcia AI, Alonso GL, Ojeda H, Salinas MR (2013) Glycoside aroma precursors of Syrah and Chardonnay grapes after an oak extract application to the grapevines. Food Chem 138(2):956–965

Packer L (2002) Handbook of antioxidants. Marcel Dekker Inc., New York. ISBN 978-0-8247-0547-5

Pardo-Garcia AI, Martinez-Gil AM, Cadahia E, Pardo F, Alonso GL, Salinas M (2014) Oak extract application to grapevines as a plant biostimulant to increase wine polyphenols. Food Res Int 55:150–160

Portu J, Gonzalez-Arenzana L, Hermosin-Gutierrez I, Santamaria P, Garde-Cerdan T (2015) Phenylalanine and urea foliar applications to grapevine: effect on wine phenolic content. Food Chem 180:55–63

Singleton VL, Timberlake CF, Kea L (1978) The phenolic cinnamates of white grapes and wine. J Sci Food Agric 29:403–410

Soubeyrand E, Basteau C, Hilbert G, Van Leeuwen C, Delrot S, Gomes E (2014) Nitrogen supply affects anthocyanin biosynthetic and regulatory genes in grapevine cv. Cabernet Sauvignon berries. Photochemistry 103:38–49

Stefano R di, Cravero MC (1991) Metodi per lo studio dei polifenoli dell'uva. Riv Vitic Enol 2:37–45

Tokuşoğlu Ö, Hall CA (2010) Fruit and cereal bioactives: Sources, chemistry and application. CRC Press, Boca Raton. ISBN 978-1-4398-0667-8