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Effects of leaf removals on must and wine chemical composition and phenolic compounds of Narince (*Vitis vinifera*) grape cultivar

Tuba Bekar[a,*], Mustafa Bayram[b], Rüstem Cangi[a], Nusret Genc[c], Mahfuz Elmastas[c]

[a] Gaziosmanpasa University, Faculty of Agriculture, Horticulture Department, 60240 Tokat, Turkey
[b] Gaziosmanpasa University, Faculty of Engineering and Natural Sciences, Food Eng. Dept., 60240 Tokat, Turkey
[c] Gaziosmanpasa University, Faculty of Science and Literature, Chemistry Department, 60240 Tokat, Turkey

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ABSTRACT

Leaves are the vital components of grapevines. Leaves are removed with summer pruning to improve grape and wine quality. Leaves of some grape cultivars are harvested at different periods for stuffed grape leaves or brined vine leaves. Since the leaves of Narince grape cultivar supplies an income as much as the grapes, the cultivars are commonly exposed to excessive leaf harvests. The present study was conducted in 2014–2015 to investigate the effects of 4 different leaf harvest treatments (LHT) (control, 2, 4 and 6 harvests) on must and wine quality of Narince grape cultivar grown in Tokat province of Turkey. The grapes harvested at technological ripening level were processed into wine with microvinification method. The pH, total soluble solid contents (TSSC), titratable acidity, specific gravity, ethyl alcohol, volatile acid, reducing sugar, total sulphur dioxide, total phenolics, total flavonoids and some phenolic compounds of the must and wines were determined. LHTs increased TSSC and total phenolics of the must; pH, ethyl alcohol, total phenolics and total flavonoids of the wines increased in six LHT as compared to control treatment. While LHTs increased phenolic compounds of catechin, epicatechin and caffeic acid as compared to control treatment, coumaric acid was not observed in wines. Wine sensory scores were the highest in control treatment and the lowest in six leaf harvest treatments.

1. Introduction

Researchers have long been carried out about the cultural practices influencing berry quality in grapevines (Teixeira et al., 2013). As a cultural practice, leaf removal is routinely practiced as a part of summer pruning in both table and wine grapes. Sufficient and on time leaf removal may not have any negative impacts on grapevines and may even have various positive impacts like better berry coloration of colored grapes, better air circulation around clusters especially in precipitated regions and somehow prevention of cluster diseases (Nicolosi et al., 2012; Verzera et al., 2016). Of these cultural practices, leaf removal and cluster thinning carried out to change leaf area/yield ratio and microclimate around clusters potentially improve fruit quality (Delgado et al., 2004; Guidoni et al., 2008). Excessive leaf removal may negatively influence product quantity and quality. Higher leaf area/yield ratios improve grape quality and there should be sufficient leaf area per unit weight of the product in table, dry and wine grape production (Kliewer and Antcliff, 1970).

There are several researches about the effects of leaf removal on must and wine quality of wine grape cultivars. In those researches, leaf removal was practiced as removal of 5 leaves from the tip of shoots before and after blooming (Sivilotti et al., 2016); leaf removal 20–25 days after blooming from the basal 36 cm section (Main and Morris, 2004); removal of entire leaves 14 days after blooming (Friedel et al., 2015); removal of 5 basal leaves 25 days after blooming (Mosetti et al., 2016); removal of the initial 6 basal leaves and all leaves at fruit set period (Kotseridis et al., 2012; Gatti et al., 2012); removal of the initial 6 leaves between fruit set and veraison (Verzera et al., 2014); removal of the initial 6 leaves when the berries reached to a size peas (Pisciotta et al., 2012); removal of leaves in the cluster area after veraison (Palliotti et al., 2013); removal of 50% leaves on shoots (Peña-Olmos et al., 2013); removal of 50 and 100% of lateral shoots when the berries reached to a size peas (Feng et al., 2015); removal of 4 and 8 leaves at veraison (Kozina et al., 2008).

Since brined vine leaves are quite rich in sugars, organic acids, phenolic compounds and some vitamins, they have a significant place in human nutrition. Stuffed grape leaf has been a famous dish of Turkish cuisine for centuries (Yerasimos, 2002; Dogan et al., 2015). The leaves collected from different grape cultivars in Turkey bring as much income as the grape of those vineyards. Excessive leaf removals from

* Corresponding author.
E-mail addresses: tubabekar@gmail.com (T. Bekar), mustafa.mbayram@gop.edu.tr (M. Bayram), rcangi@hotmail.com (R. Cangi), nusretgenc@gmail.com (N. Genc), mahfuz.elmastas@gop.edu.tr (M. Elmastas).

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wine grapes may result in some criticisms in wine industry.

The present study was conducted to investigate the effects of leaf removal for brined vine leaf on grape, must and wine quality of Narince grape cultivar, which is a significant white wine cultivar of Turkey.

2. Material and method

2.1. Experimental site, plant material and experimental design

Experiments were conducted in a producer vineyard established with Narince/1103P in Tokat central town (40°19′59″ N, 36°15′48″ W) in Middle Black Sea Region of Turkey during the growing seasons of 2014–2015. Narince is a white table grape cultivar grown in Kazova/Tokat region (VIVC, 2017). Experimental vineyard is located at 677 m altitude, established in 1989 with 3.0 m x 1.75 m planting density. Vines are cultivated in bilateral cordon system and spur pruned. The cordon height from the soil surface was 25–40 cm. The leaves of Narince cultivar are commonly collected for stuffed grape leaves and brined vine leaves. Therefore, leaf collection was expressed as harvest in this study. There were 4 different harvest treatments in this study (control, 2, 4 and 6 harvests). Each harvest had 3 replications and each replication included 10 grapevines. The grapevines were spur pruned through leaving 1–2 buds over annual canes (20 ± 2 bud/vine) in winter pruning of both years.

Leaf harvest periods were determined in accordance with phenological stages of grapevines specified by Eichorn and Lorenz (1977) (Table 1). Generally the young leaves over apical sections of summer canes (the leaves reached to 2/3 size of a mature leaf) were harvested in 7–10 day intervals. With harvested leaves, number of leaves per hectare, leaf yield and leaf area (m^2) were determined. Control grapevines were not subjected to any leaf removals and summer pruning. Together with control, 2, 4 and 6 leaf harvests (LHT: Leaf Harvest Treatments) were performed and must and wines of the grapes harvested from the same grapevines were subjected to quality analyses.

2.2. Grape harvest and microvinification

The grapes subjected to different treatments were harvested on September 12–17, 2014 and on September 15–19, 2015 by taking their total soluble solid contents (TSSC), titratable acidity, maturation index and environmental factors into consideration and they were processed into wine in the same day. In each vegetation year, wines were produced from 24 different samples in 3 L Erlenmeyer flasks.

Wines were produced by adding commercial wine yeast to the must through microvinification method. The must, obtained after stalk separation, crushing and pressing, was filled into 3 L Erlenmeyer flasks at 80% and each flask was then supplemented with 30 ppm SO_2 and 20 g/100 L wine yeast *(Saccharomyces cerevisiae*, Oenobrands, Montpellier, France) and left for fermentation at 18 °C. Temperature and density measurements were performed throughout the fermentation process. When the density decreased to 1.045 g/cm^3, 20 g/100 L yeast feeding (Nutristart, Laffort, France) was performed. Fermentation was terminated when the density dropped below 1 g/cm^3 and clarification was initiated through adding 50 ppm SO_2. During the clarification process, wines were supplemented with 0.3 g/L bentonite and they were transferred 10 days later.

Table 1
Leaf harvest periods and phenological stages.

Harvest Periods	2014		2015	
	Date	Phenological Stage	Date	Phenological Stage
1st Harvest	24 May	19th Stage	30 May	19th Stage
2nd Harvest	31 May	23rd Stage	6 June	21–23rd Stage
3rd Harvest	7 June	27th Stage	13 June	27th Stage
4th Harvest	14 June	29th Stage	17 June	29th Stage
5th Harvest	21 June	31st Stage	27 June	31st Stage
6th Harvest	27 June	33rd Stage	30 June	33rd Stage

19th Stage: Immediate pre-bloom, 21st Stage: First bloom, 23rd Stage: Full bloom, 27th Stage: Fruit set (about 3–4 mm), 29th Stage: Post fruit set (about 4–5 mm), 31st Stage: Buckshot berries. 33rd Stage: Berry touch/bunch closure.

2.3. Must and wine chemical composition

The must and wine produced from the harvested grapes were subjected to pH, TSSC (%), titratable acidity (g/L), specific gravity, ethyl alcohol (%), volatile acid (g/L), reducing sugar (g/L) and total sulphur dioxide (mg/L) analyses (OIV, 2016a; OIV, 2016b). Analyses on wines were performed 2 months after bottling.

Total phenolics of the must and wines were determined with Folin-Ciocalteu reactive. Following 4.5 mL distilled water supplementation to 100 µL sample, 100 µL Folin-Ciocalteu reactive was added, left for 3 min and 2% 300 µL sodium carbonate (Na_2CO_3) was added. The mixture was vortexed and incubated under normal room conditions for 2 h. Then, sample absorbances were read in a spectrophotometer at 760 nm. Results were expressed in gallic acid equivalent (mg/L) by using a calibration curve prepared with different concentrations of gallic acid standard (Slinkard and Singleton, 1977).

For flavonoids of the must and wines, 100 µL samples were supplemented with distilled water as to have a volume of 4.3 mL, then 0.1 mL 10% $Al(NO_3)_3$ and 0.1 mL 1 M NH_4CH_3COO were added. Following vortexing, samples were incubated under room conditions for 40 min. Sample absorbances were read in a spectrophotometer at 415 nm. Results were expressed in quercetin equivalent (mg/L) by using a calibration curve prepared with different concentrations of quercetin standard (Chang et al., 2002; Kosalec et al., 2005).

For phenolics composition of the must and wines, cinnamic acids (*p*-coumaric acid, caffeic acid, ferulic acid), benzoic acids (vanillic acid and gallic acid), flavonols (catechin, epicatechin and quercetin) were quantitatively determined with a Shimadzu Prominence brand high pressure liquid chromatography device (HPLC) in accordance with the method developed by Lee and Scagel (2009) with some modifications. Before the analyses, extracts were filtered through 0.45 µ filters with the aid of a syringe and 20 µL of filtrate was directly analyzed. Quantitative analyses of phenolic acids were performed with UV–vis/DAD detector at 280 nm by using internal standards. Calibration curve was drawn for these standard compounds and sample quantities (mg/L) were determined based on this calibration curve (Table 2).

Column Characteristics:

Prontosil C18-EPS 3 µm Reversed-Phase HPLC Columns (Reverse phase HPLC column)

Dimensions: ID * Length = 4.6* 150 mm

2.4. Sensory analyses

A degustation panel was formed with 5 panelists for sensory analyses of wines. The method of scoring out of 20 full score developed by International Organization of Vine and Wine (OIV) was employed (OIV,

Table 2
Gradient system mobile phase flow concentration.

Minutes	% water (with 0.1% formic acid)	% ACN
0 min	100	0
3 min	100	0
8 min	85	15
13 min	75	25
26 min	74	26
35 min	0	100
40 min	100	0

Flow rate: 1 mL/dk; Column temperature: 40 °C.