Natural alternatives of Sulphur dioxide used in wine and their effects on aromatic compounds

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Keywords:
Wine
Extract
Sulfur dioxide
Red wine
Aromatic

Article history:

Received 10.03.2020
Received in revised form 14.06.2020
Accepted 27.12.2020

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DOI:10.24263/2304-974X-2020-9-4-12

Abstract

Introduction. The aims of this study were to determine the effects of different natural plant extracts used as an alternative of Sulphur dioxide on wine aroma compounds.

Materials and methods. The wine production was done according to the accepted conventional method of red wines (*Cabernet Sauvignon*). The experimental design was achieved by using different plant extracts (grape pomace, rosemary, black blueberry) at different concentrations. As the first control group was used wine samples processed without natural extracts and Sulphur dioxide treatments and as the second group was used wines produced with 25mg/L Sulphur dioxide addition.

Results and discussion. The highest total amount of volatile compounds was achieved by applying blueberry extract and grape pomace extract. The combined application of Sulphur dioxide and blueberry extract increased the wine volatile complexity. The best results related to higher alcohols synthesis and their accumulation in wines was obtained by using Sulphur dioxide (25 mg/L) and plant extracts (0.3 mL). The terpenes were dominated by geraniol. The highest value was obtained in sample treated with grape pomace.

The ester fraction was represented by 9 identified compounds. The highest total ester content (169.13 mg/L) was found in sample obtained with combined treatment of 25 mg/L Sulphur dioxide and rosemary extract 0.3 mL. The other three variants of rosemary treatments demonstrated quantitatively close ester content. In samples containing grape pomace extract was found the lowest total ester content compared to all others. From this group, only sample including25 mg/L Sulphur dioxide and grape pomace extract 0.3 mL (40.62 mg/L) was distinguished. Methyl alcohol was found in all tested wines. The methyl alcohol levels were very low and not pose a risk to the consumer.

Conclusions. The study demonstrated the possibilities of optimization of Sulphur dioxide by using natural plant extracts.

Introduction

During wine production, SO_2 is used as an antioxidant and antimicrobial additive in wine production. It uses for preventing oxidation and the spread of unwanted organisms such as wild yeasts, acetic acid bacteria and lactic acid bacteria [1]. Aging process causes gradual loss of phenolic compounds due to some oxidation reactions with polysaccharides and tannins leading to formation of other stable anthocyanin-derived pigments. These reactions can result in some changes in the color, taste and flavor properties of red wines [2, 3]. Even these advantages, negative effects of SO_2 on human health have been subject to researches for many years [4].

A number of studies have been indicated as an alternative of SO_2. Most of them proposed non-thermal processes, or using of new chemicals. One of the most promising natural alternatives to sulphides in wine production are using of natural plant extracts [5]. The flavonoids, phenolic compounds and their derivatives, which are naturally found in the structure of these extracts, have been shown to be effective in preventing auto-oxidation [6,7]. It is emphasized that some phytochemicals such as terpenes, alkaloids, lactones, etc. found in the extract may contribute to the prevention from auto-oxidation of wine. The aroma is an essential characteristic determining the quality of the wine. It is due to the significant diversity of volatile compounds (over 800) and the variation in their total content (up to 800–1200 mg/L) [8]. Numerous factors influence the formation of the final wine aroma: the genetic ability of the vine variety to synthesize and accumulate volatile aromatic components in the grapes, climatic, soil and geographic characteristic of the vine growing area, agro-technical measures and phytosanitary status of the vine, the technique and technology of wine making, metabolic potential of yeast and malolactic microflora, processes during wine aging [9, 10, 11, 12, 13, 14, 15].The final wine aroma contains various volatile compounds belonging to several major groups such as esters, higher alcohols, aldehydes, terpenes and methoxypyrazines [16, 17]. The ester compounds have a significant contribution to the wine aroma. This is due to the low threshold of aromatic perception of the esters. This group of compounds is also formed in the grapevine, accumulating in very low amounts (10–30 mg/L) in the grapes [18]. The subsequent organic yeast ester synthesis is realized during alcoholic fermentation. It leads to significant ester accumulation (up to 500 mg/L) in young wines [12]. The third phase of their accumulation takes place in the aging process called esterification which is due to the chemical bonding between the available alcohols and the acids of the wine. This stage proposed the significantly increased of the total ester content of the wine (792–800 mg/L) and formed wine bouquet [19]. The higher alcohols are a group of aromatic compounds with a lesser aromatic effect. This is due to their higher thresholds of aromatic perception. They are, however, an important factor of the aroma profile of wine, since they cause formation of various esters with the wine acids [11]. The higher alcohols are the product of yeast amino acid metabolism and accumulate in red wines up to 600 mg/L [12]. The important representatives are 3-methyl-1-butanol, phenyl ethanol, hexanol, isobutyl alcohol and others [20]. The terpenes are mainly represented by terpene alcohols – linalool, α-terpineol, β-citronellol, nerol and geraniol [21]. These compounds have a significant contribution to the wine aroma [22]. Then the common question, what is the effect of natural plant extracts on wine aromatic profile in case of application of different methods during production?

The aims of this study were to determine the effects of different natural plant extracts used as an alternative of SO_2 on wine aroma compounds.

Materials and methods

Plant material

As materials were used grapes of *Vitisvinifera* L. cv. origin var: Cabernet Sauvignon from the Menderes/ Gölcükler region of Izmir (Sevilen Winery vineyards). 100 kg grapes were processed in Ege University Food Engineering Department (Izmir/ Turkey) within 24 h of hand-harvest.

The grape pomace (GP) extract was supplied as waste in the normal wine production process of Cabernet Sauvignon grapes. The blueberry (BB) and rosemary (R) extract used in the experimental groups belongs to *Rosmarinus officinalis* L. and *Vacciniummyrtillus* L. spices, respectively. These plants were obtained from the Aegean region in Izmir/ Turkey.

The selection of *Cabernet Sauvignon* pomace and blueberries was done on the base of our previous studies [23, 24, 25, 26, 27, 28, 29] in which higher total phenols and antioxidant activities were determined. The choice of rosemary was done after evaluation of our project results related to conservation of foods by using rosemary extracts (unpublished). All these plants even have a different origin possess similar properties related to the protection of food as materials with higher phenolic content and higher antioxidant activities.

Used plants in the experiment were evaluated on the wet basses. They were not dried before using in experiments.

Wine processing method

The grapes transferred to the mill for separation from stems, wastes and foreign materials after weighing process. Crushed fruit and juices were collected in stainless steel tank. The density of the juice was determined as 1110 g/L, the average pH was 3.8 and the total acid amount was 5.48 g/L (as tartaric acid). *Saccharomyces cerevisiae* Fermivin strain was added in the tank as commercial yeast (20 g/L dose SIHA Active Dry yeast 10). Fermentation process was completed in 12 days at 20-22 °C. The must was stirred twice daily. All fermentation process was carried out at controlled conditions. The separation of must was done by a mechanical press machine. During fermentation some measurements were carried out regularly such as alcohol content, density and sugar content of the product. At the end of the fermentation, the final sugar content was < 2 g/L. When the fermentation was completed, the wine was transferred and stabilized in the cold. The wines were stored at 15 °C and the preparation of the extraction agents was started. With the addition of the extracts, the samples were bottled and stored during 3 months.

Experimental design and treatments

The *Cabernet Sauvignon* grapes were gowned in Aegean region of Turkey-İzmir. After processioning of wine the pomace was used for the experiment as described. Blueberry (BB) and rosemary (R) plants were supplied from the same region of Turkey- Aegean region-İzmir. The botanical evaluation of plants was done by an expert from Ege University. Natural extracts were prepared as following the path given in Figure 1. Different plant extracts such as grape pomace, rosemary and black blueberry were used at different concentrations for experimental design. As the first control group was used as wine samples processed without natural extracts and SO_2 treatments and as the second group was used wines produced with 25mg/L SO_2 addition.

The main reason of adding these extracts after the fermentation step is inhibition effect of these extract in the negative direction during alcohol fermentation by inhibiting the yeast strain in the must. The wine was divided into five batches, in which the different treatments were carried out. For each batches the treatments were created with addition of the extracts at different concentrations. The experimental groups are presented in Table 1.

Analyses

For each experimental group, wine samples were analyzed under three main topics; classic wine analyzes aromatic compounds and statistical analyzes. Basic must analyzes were carried out according to the OIV Compendium of International Methods of wine and must [30,31]. All analyzes were carried out in triplicate.

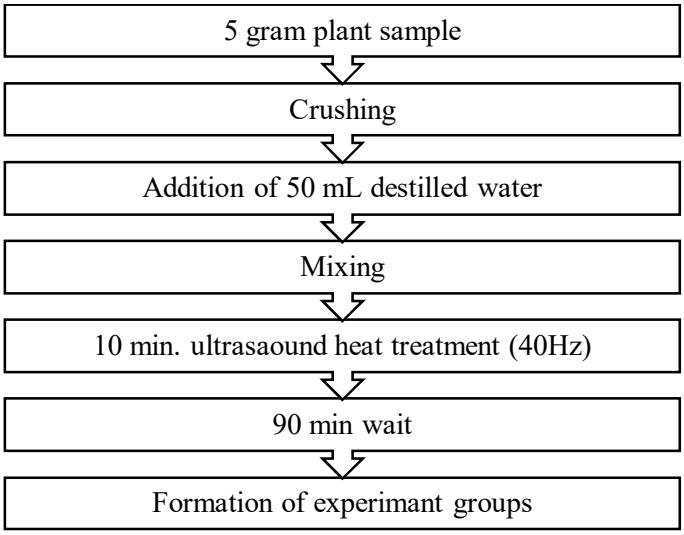

Figure 1. Natural extracts preparation

Classic wine analyzes. Classic oenological wine analyzes were determined according to recommended methods by International Organization of Vine and Wine (OIV). Alcohol content (% v/v), pH (direct measurement by using pH meter), total acidity (tartaric acid g/L), volatile acidity amount (g/L acetic acid), total and free SO_2 (mg/L), dry matter (g/L) and ash (g/L) analyzes were performed.

Table 1

Experimental groups

PLANT EXTRACTS	Group 1 GRAPE POMACE (GP)	Group 2 ROSEMARY (R)	Group 3 BLUEBERRY (BB)	Control Group 1	Control Group 2
Set 1 (0 mg/L SO_2+ 1 mL extract)	0 mg/L SO_2+ 1 mL extract (GP01)	0 mg/L SO_2+ 1 mL extract (R01)	0 mg/L SO_2+ 1 mL extract (BB01)		
Set 2 (25 mg/L SO_2+ 0,7 mL extract)	25 mg/L SO_2+ 0,7 mL extract (GP257)	25 mg/L SO_2+ 0,7 mL extract (R257)	25 mg/L SO_2+ 0,7 mL extract (BB257)	non SO_2 and extract addition (TKOO)	SO_2 treatment (TK25)
Set 3 (25 mg/L SO_2 +0,3 mL extract)	25 mg/L SO_2 +0,3 mL extract (GP253)	25 mg/L SO_2 +0,3 mL extract (R253)	25 mg/L SO_2 +0,3 mL extract (BB253)		
Set 4 (25 mg/L SO_2 +1 mL extract)	25 mg/L SO_2 +1 mL extract (GP251)	25 mg/L SO_2 +1 mL extract (R251)	25 mg/L SO_2 +1 mL extract (BB251)		

Notes:

Grape Pomace (GP)
- 0 mg/L SO_2+ 1 ml (GP01)
- 25 mg/L SO_2+ 0,7 ml (GP257)
- 25 mg/L SO_2+0,3 ml (GP253)
- 25 mg/L SO_2 + +1 ml (GP251)

Rosemary (R)
- 0 mg/L SO_2 + 1 ml (R01)
- 25 mg/L SO_2 + 0,7 ml (R257)
- 25 mg/L SO_2 + 0,3 ml (R253)
- 25 mg/L SO_2 + 1 ml (R251)

Blueberry (Bb)
- 0 mg/L SO_2 + 1 ml (BB01)
- 25 mg/L SO_2 + 0,7 ml (BB257)
- 25 mg/L SO_2 + 0,3 ml (BB253)
- 25 mg/L SO_2 + 1 ml (BB251)

Control groups (TK)
- Control group without SO_2 (TK00)
- Control group containing 25mg/L SO_2 (TK25)

Aromatic content determination by GC-FID. The aromatic profiles of samples were determined by gas chromatographic technique equipped with FID. The content of major volatile aromatic compounds was determined on the basis of stock standard solution. The purity of standard solution used in this study was as > 99.0%. The sample quantity was determined to be 2 µl. For analyses was used gas chromatograph Varian 3900 (Varian Analytical Instruments, Walnut Creek, California, USA) with a capillary column VF max MS (30 m, 0.25 mm ID, DF = 0.25 µm), equipped with a flame ionization detector (FID). The used carrier gas was He. Hydrogen to support combustion was supplied to the chromatograph via a hydrogen bottle. The injection was manually by microsyringe.

The parameters of the gas chromatographic determination were: injector temperature – 220 °C; detector temperature – 250 °C, initial oven temperature – 35 °C/retention 1 min, rise

to 55 ºC with step of 2 ºC/min for 11 min, rise to 230 ºC with step of 15 oC/min for 3 min. Total time of chromatography analysis – 25.67 min. The identified retention times of the compounds in standard solution were: acetaldehyde (3.141), ethyl acetate (3.758), methanol (3.871), 2-propanol (5.170), isopropyl acetate (5.975), 1-propanol (6.568), 2-butanol (7.731), propyl acetate (9.403), 2-methyl-propanol (10.970), 1-butanol (11.509), isobutyl acetate (11.662), ethyl butyrate (12.710), butyl acetate (12.752), 2-methyl-1-butanol (13.054), 4-methyl-2-pentanol (13.629), 3-methyl-1-butanol (13.840), 1-pentanol (15.180), isopenthyl acetate (15.965), pentyl acetate (16.033), 1-hexanol (16.276), ethyl hexanoate (16.376), hexyl acetate (16.510), 1-heptanol (16.596), linalool oxide (16.684), phenyl acetate (18.055), ethyl caprylate (18.625), α-terpineol (19.066), 2-phenyl ethanol (19.369), nerol (19.694), β-citronellol (19.743), geraniol (19.831), ethyl decanoate (19.904). An internal standard octanol was used. After determination of the retention times of aromatic compounds in the standard solution, we proceed to the identification and quantification of the volatile aromatic substances in the wines. The aromatic compositions of samples were determined by using 2 µl of samples.

Statistical evaluation

Significant differences between averages were obtained at a 95% significance level. The values were averaged and standard deviation, minimum, maximum and mean values of samples were determined. The least significant differences (LSD) and correlations were also performed. Statistical analysis was performed using the PC (SPSS 15) software package.

Results and discussion

General evaluation of wines

Wine quality is closely related to aroma content of wine. Many aromatic compounds contribute to the flavour, taste and odour of wines. Some studies have demonstrated the relation of aroma compounds related to flavour characters of wines [32,33].

The statistical evaluation of results indicated the significant differences among same samples ($p<0.05$). Correlation analysis was used to determine the relation between parameters and within groups. The mean values with their standard deviation, minimum and maximum values were analyzed. The highest pH and total acidity value were detected in BB01 (4.90) and GP253 (6.70 tartaric acid g/L) groups, respectively. The lowest value of pH and total acidity were detected in TK00 (3.70) and BB257 (3.90 tartaric acid g/L), respectively. The highest and the lowest value of volatile acidity of samples were detected in TK00 (0.84 acetic acid g/L) and TK25 (0.24 acetic acid g/L) groups, respectively. While there was no significant correlation detected between pH and total acidity in the groups. However, there were a significant correlation between the pH and the volatile acid values ($r= 0.3511$, $p=0.023$).

In our study 18 aromatic compounds were determined in order to evaluate the effects of treatment on characters of Cabernet Sauvignon. These compounds included methanol, 1-butanol, 2-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol, 1-hexanol, 1-heptanol, 2-phenyl ethanol, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, isobutyl acetate, ethyl butyrate, ethyl hexanoate, pentyl acetate and phenyl acetate.

The identified and quantified higher alcohols, esters and terpenes compounds by GC-FID are presented in Figures 2, 3, and Table 2 respectively. All established concentrations

corresponded to the quantitative ranges typical for young red wines. The data in this aspect were correlated with other studies [34].

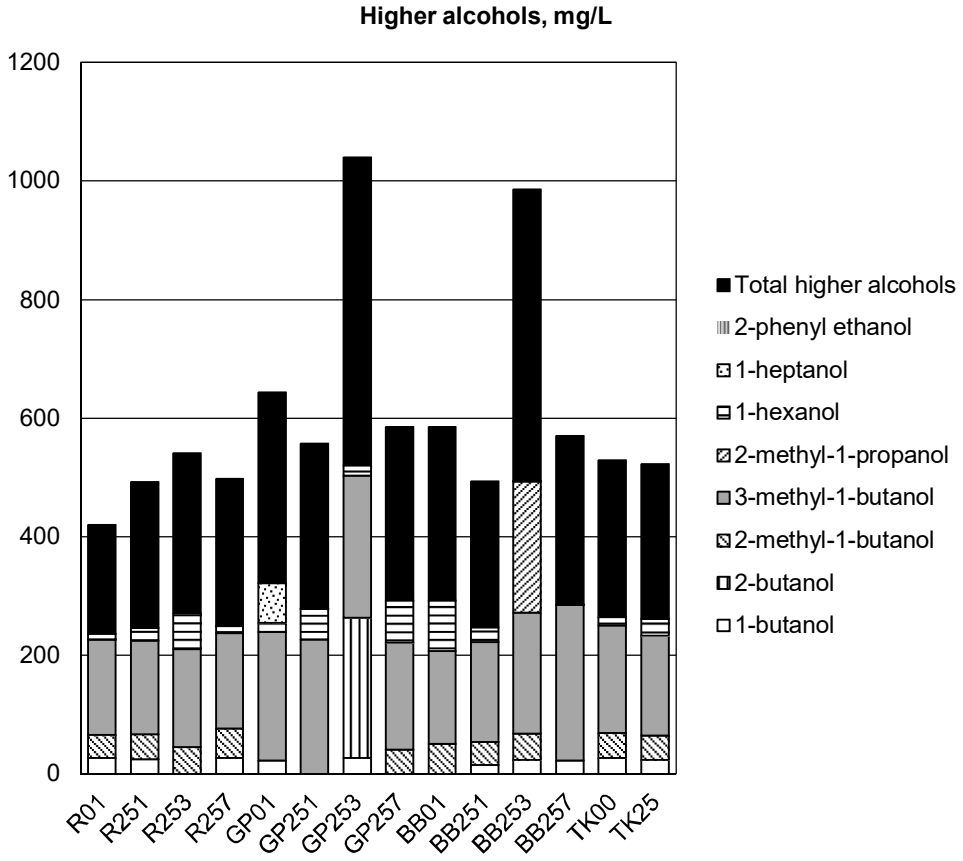

Figure 2. Identified and quantified (by GC-FID) higher alcohols compounds of wines with different added extracts. Average ethanol and methanol value of samples were determined as vol. 13% and 16,7 mg/L respectively

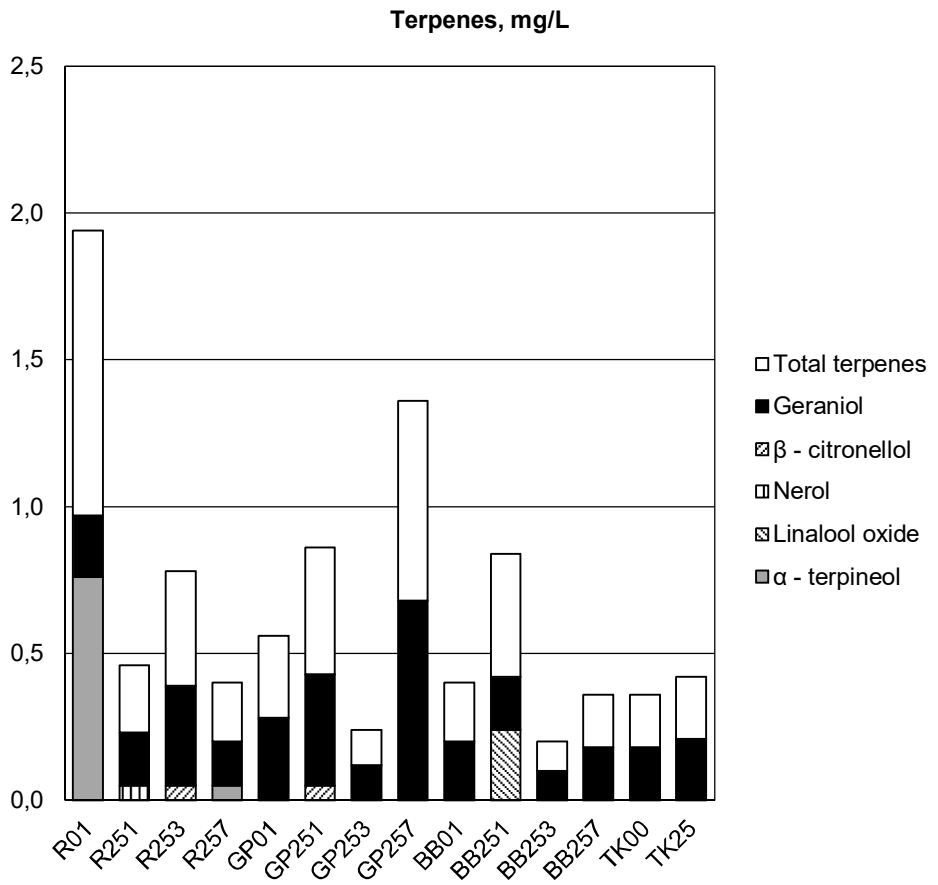

Figure 3. Identified and quantified (by GC-FID) terpenes compounds of wines with different added extracts

Effects of treatments on the total volatile content and total higher alcohols in wine samples

The highest total content of volatile compounds was found in samples BB251 (579.31 mg/L) and GP253 (579.00 mg/L). Compared to the TK00 (296.29 mg/L) and TK25 (311.91 mg/L) controls, they have almost a two-fold higher total amount of synthesized volatile compounds. The incorporated two extracts (blueberry and grape pomace) applied in the indicated amounts have a positive effect on the wine total volatile composition. The lowest amount of total volatile compounds was found in the sample without SO_2, but with the addition of 1 mL of rosemary – R01 (242.63 mg/L).

The complete absence of SO_2 in this sample has affected negatively the fermentation process. This resulted in decreased secretion of metabolic yeast products, which reflected in the low total volatile content of the wine. This can be explained by uncontrolled fermentation,

without added sulfur dioxide, which not inhibits the activity of wild yeasts and other microflora. It is known that SO_2 has the responsible for inhibit the growth of harmful yeasts and bacteria and ensure the normal course of fermentation [18]. High total content of volatile components were also found in the samples with extracts of blueberry BB01 (545.78 mg/L) and rosemary R253 (453.92 mg/L). It is noteworthy that the blueberries extract was a positive effect on the wine volatile composition, even in the absence of sulfur dioxide – BB01 sample. However, the results shown that the combined effect of sulfur dioxide and blueberry extract (BB253) influenced the wine volatile composition with increasing of its complexity.

The lowest established total higher alcohols content (182.67 mg/L) was observed in wine R01, obtained with added rosemary extract (1 mL) and without SO_2. In the other three variants (R251, R253 and R257), where SO_2 and rosemary extract were added at different concentrations, higher total levels of higher alcohols were observed. This demonstrated an improved sulphitation efficiency of the rosemary extract when it was combined with low SO_2 levels. This synergy reflected on increased yeast aromatic metabolites.

Table 2

Identified and quantified (by GC-FID) esters compounds and total volatile content of wines with different added extracts

IDENTIFIED COMPOUNDS mg/L	WINES							
	R01	R251	R253	R257	GP01	GP251	GP253	GP257
Ethyl acetate	15.98	17.28	15.12	11.64	20.15	20.25	11.53	18.95
Propyl acetate	26.87	0.05	0.05	27.75	0.05	0.05	29.09	0.05
Isopropyl acetate	ND	ND	0.05	ND	ND	ND	ND	ND
Butyl acetate	ND	26.44	95.09	ND	ND	ND	ND	ND
Isobutyl acetate	ND	ND	58.82	ND	ND	ND	ND	ND
Ethyl butyrate	ND	ND	ND	ND	ND	ND	ND	0.05
Ethyl hexanoate	0.05	ND	ND	ND	ND	ND	ND	ND
Pentyl acetate	0.05	ND	ND	ND	ND	ND	ND	ND
Phenyl acetate	ND	ND	ND	ND	ND	ND	ND	ND
Total esters	**42.95**	**43.77**	**169.13**	**39.39**	**20.20**	**20.30**	**40.62**	**19.05**
Total Volatile Content	**242.63**	**306.88**	**453.92**	**301.40**	**357.11**	**313.99**	**579.20**	**334.07**

Table 2 *(continue)*

IDENTIFIED COMPOUNDS mg/L	WINES					
	BB01	BB251	BB253	BB257	TK00	TK25
Ethyl acetate	37.72	4.98	19.83	18.43	14.12	9.63
Propyl acetate	0.05	21.54	0.05	0.05	0.05	24.30
Isopropyl acetate	ND	ND	ND	ND	ND	ND
Butyl acetate	ND	ND	ND	ND	ND	ND
Isobutyl acetate	ND	ND	ND	ND	ND	ND
Ethyl butyrate	ND	ND	ND	ND	ND	ND
Ethyl hexanoate	ND	ND	ND	ND	ND	ND
Pentyl acetate	ND	ND	47.44	ND	ND	ND
Phenyl acetate	105.31	ND	ND	ND	ND	ND
Total esters	**143.08**	**26.52**	**67.32**	**18.48**	**14.17**	**33.93**
Total Volatile Content	**545.78**	**286.00**	**579.31**	**325.55**	**296.29**	**311.91**

Total higher alcohols in wines with rosemary extract was not differ significantly from those found in the control samples (TK00 and TK25). The best result for this group of experimental wines was observed in variant R253 (270.32 mg/L). The synergic effect of 25 mg/L SO_2 + 0.3 mL rosemary extract had a positive effect on the accumulation of higher alcohols in the wine.

In the following variants (grape pomace extract) in GP253, the highest total content of higher alcohols (519.86 mg/L) of all the wines analyzed was found. The results obtained with this type of extract showed a trend of increased amounts of higher alcohols compared to the two control samples. It was noticed that the combined effect of SO_2 and grape pomace extract (GP253) caused increasing of the concentration of higher alcohols in the wine. It was interesting to noted that in the variant of treatment with grape pomace without SO_2 (GP01), a good effect on the total content of higher alcohols (321.88 mg/L) was observed. It was significantly better than the same variant but with the addition of rosemary extract (R01).

With the addition of blueberry extracts, the best quantitative accumulation of higher alcohols was found in sample coded as BB253 (492.94 mg/L). This confirmed the hypothesis that the combined effects of SO_2 and extract could improve the synthesis of higher alcohols in the wine. The results for total higher alcohols in the other three samples (BB01, BB251 and BB257) were almost comparable to those found in the control variants (TK00 and TK25).

The results obtained for the total content of higher alcohols in wines with incorporated extracts found that in all variants the best result was obtained with a combination of SO_2 (25 mg/L) and extracts (0.3 mL) in samples coded as R253, GP253 and BB253.

Effects of treatments on individual higher alcohols in wine samples

Eight higher alcohols were identified in the experimental wines. The dominant were 1-butanol, 2-methyl-1-butanol (active amyl alcohol), 3-methyl-1-butanol (isoamyl alcohol) and 1-hexanol.

The 3-methyl-1-butanol was a major component of higher alcohols group. The lowest concentration (157.14 mg/L) was found in BB01 wine – with the addition of blueberry extract. The highest amount (262.22 mg/L) was identified in sample BB257, obtained with the addition of blueberry extract. The lowest amounts of this compound were observed in the samples obtained with the addition of rosemary extract.

The experimental wines obtained with the addition of grape pomace extract had higher amounts of isoamyl alcohol than the samples with rosemary. The highest amount of 3-methyl-1-butanol of this group (239.75 mg/L) was distinguished in experimental wine coded as GP253. In this case the positive effect of the combination of SO_2 + extract on synthesis of 3-methyl-1-butanol was observed. In the samples with addition of blueberry extract a gradual increase in the levels of established 3-methyl-1-butanol from BB01 (157.14 mg/L) to BB257 (262.22 mg/L) was realized. The result confirmed the effect of the extract with certain doses of SO_2.

The 3-methyl-1-butanol is an important aromatic compound in red wines. It was found to be a an important component of Californian and Australian red wines from Merlot and Cabernet Sauvignon varieties subjected to aging in stainless steel tanks [11,35]. This compound formed the malt and whiskey flavor in wines [20].

The 2-methyl-1-butanol (active amyl alcohol) was found in the lowest quantities in sample coded as R01 (38.43 mg/L) obtained with the addition of rosemary extract. Its highest amount (49.81 mg/L) was found in sample BB01 – with addition of blueberry extract. The active amyl alcohol was not identified in three of the samples: GP01, GP251 and GP253.

Another representative with a significant presence in the wines studied was 1-butanol. Its concentrations in all tested wines corresponded to those found in the two control samples. In two of the samples (GP251 and GP257) it was identified in very low amounts. In R253 and BB01 it was not established. In the remaining samples the content of this component ranged within 14.83 mg/L (BB251) to 26.99 mg/L (R257). The content of 1-butanol in wine ranges in the concentration range of 1.00 – 64.00 mg/L [12]. This study corresponded to these quantitative variations.

The 1-hexanol was found in all experimental wines. This compound gives a herbaceous tone of wine aroma. It accumulates when the leaves and bunches are affected by the crushing process [12]. In most samples analyzed in this study, elevated levels of this alcohol were observed. The added extracts affect the final content of 1-hexanol in the wine. Significantly high levels were observed in the samples treated with grape pomace extract. The observed concentration of 1-hexanol in these wines ranged from 15.69 mg/L (GP01) to 71.99 mg/L (GP257). The highest content was observed in BB01 wine (85.81 mg/L). The best in terms of this indicator were two of the wines containing rosemary extract – R01 (10.42 mg/L); R257 (11.57 mg/L) and blueberry extract – BB253 (0.05 mg/L); BB257 (0.05 mg/L).

The 1-heptanol was identified in a substantial amount (67.27 mg/L) only in the GP01 sample. The 2-phenylethanol (aromatic alcohol) was found in small quantities in the experimental wines coded as R01, BB251 and in the TK25 control. This compound closely related to rose aroma in wines [36].

Considering the 2-methyl-1-butanol, 3-methyl-1-butanol and butyl acetate compounds were determined significant differences between wines treated with grape pomace extract and rosemary extract ($p<0.05$). There were also determined significant differences between wines treated with blueberry and rosemary extract according related to butyl acetate content ($p<0.05$).

Effects of treatments on esters in wine samples

The ester fraction was represented by 9 identified compounds. The highest total ester content (169.13 mg/L) was found in sample coded as R253 with combined treatment of 25 mg/L SO_2 + rosemary extract (0.3 mL). The other three variants of rosemary treatments demonstrated quantitatively close ester content. In samples containing grape pomace extract was found the lowest total ester content compared to all others. From this group, variant GP253 (40.62 mg/L) was distinguished.

In variants with blueberry extract were found high concentrations of total ester content. The highest amount of esters was identified in a sample without SO_2 – BB01 (143.08 mg/L) obtained only with the blueberry extract. This result revealed the potential of blueberry extracts as an alternative approach for sulphitation with enough ester accumulation. A satisfactory result was also established with sample BB253 (67.32 mg/L), also. This confirmed the good synergic action between SO_2 and discussed extract.

The ethyl acetate was found in all samples tested. It presents have a positive influence by providing a pleasant fruity aroma (concentrations of 50.00 – 80.00 mg/L) [37]. At high concentrations it has a negative effect [38]. The concentrations of this ester found in our study met the criteria corresponding to its positive effect. This ester was found in the lowest amount (9.63 mg/L) in the control sample TK25. The highest content of ethyl acetate (37.72 mg/L) was found in the wine sample coded as BB01 variant.

Another representative of the ester fraction identified in all the wines examined was propyl acetate. In most samples, it was found in low amounts (0.05 mg/L). The highest amount was observed in the GP253 sample with quantity of 29.09 mg/L.

Butyl acetate was identified only in wine samples numbered as R251 (26.44 mg/L) and R253 (95.09 mg/L). This compound was observed only in wines produced with the addition of rosemary extract.

Isobutyl acetate was identified only in wine samples R253 (58.82 mg/L). The phenyl acetate was found in two of the wines samples: R01 (0.05 mg/L) and BB253 (47.44 mg/L). Phenyl acetate was another identified ester. It was only observed in variant BB01 (105.31 mg/L). This ester gives fruity and floral characters to the wine aroma [39].

Effects of treatments on terpenes in wine samples

From the group of terpenes, were identified five representatives – α-terpineol, linalool oxide, nerol, β-citronellol and geraniol. A remarkable high total terpenic content (0.97 mg/L), significantly greater than the two control samples, was found in wine coded as R01 which was produced without SO_2 and treated with rosemary extract. The higher total terpenic content was probably due to the transition of terpene compounds to wine from the rosemary extract. Wines treated with grape pomace extract, sample coded as GP257 demonstrated the highest total terpene content (0.68 mg/L). When a blueberry extract was applying, higher values of total terpenes were observed in sample BB251 (0.42 mg/L). The dominant terpene was geraniol. It was found in all tested wines. The highest concentration (0.68 mg/L) was found in the sample GP257 obtained by sulphitation and addition of grape pomace extract. The concentrations of this terpene found in the remaining experimental wines were not significantly different from those of the TK00 and TK25 controls. Geraniol has a strong aromatic effect in the muscat grapes and was found to be dominant (24.2%) [40].

The α-terpineol was identified in two of the experimental wines numbered as R01 (0.76 mg/L) and R257 (0.05 mg/L). This terpene alcohol gives the wine melon and lily aromas [41].

Linalool oxide was identified only in the wine with blueberry extract BB251 (0.24 mg/L). The nerol was identified only in R251 sample and β-citronellol was detected in R253 and GP251 samples. The last two terpenes were found in very low concentrations.

Effects of treatments on methanol content in wine samples

Methyl alcohol were found in all tested wines. It results from degradation of pectins in fruits with the action of the pectolytic enzyme complex [42]. In red wines, it is formed in concentrations ranging from 60.00 to 230.00 mg/L [18]. A remarkable quantitative presence of this alcohol (109.74 mg/L) was recorded in experimental wine sample coded as BB01 produced with addition of blueberry extract and without SO_2. This is explained by the added effect of the pectin content of the blueberry fruit. In the remaining samples the amount of methanol was not different from responding control groups. The methyl alcohol levels were very low and not pose a risk to the consumer.

Correlation analyses were used to determine the relation between parameters and within groups. In this study, the regression correlations of compounds were significantly different and those compounds contributed to the wine aroma content. This indicates that each aroma compounds have different flavor characteristics in wines.

While there was no positive correlation detected between pH and total acidity or volatile acidity in the groups, there were a positive correlation between the pH and 1-hexanol, ethyl acetate and phenyl acetate values (respectively, $r=0.598$, $r=0.814$ and $r=0.9771$, $p<0.05$). There were also determined correlations between volatile acidity and total SO_2 content

(r = -0.772, p <0.05), total SO_2 and free SO_2 value (r = 0.763, p <0.05), dry matter and 2-methyl-1-butanol (r = -0.547, p <0.05), ash and 1-butanol value (r = -0.576, p <0.05).

Considering the butyl acetate, there were detected positive correlation between isobutyl acetate and isopropyl acetate (r = 0.679, p <0.05). It was also determined significant correlations between 3-methyl-1-butanol and 2-methyl-1-butanol (r =-0.889), 1-hexanol and 1-butanol (r = -0.9084), ethyl acetate and 1-butanol (r = -0.544), isopropyl acetate and ethanol vol.% (r = -0.578), isobutyl acetate and ethanol vol.% (r = -0.578), 1-hexanol and ethyl acetate (r = -0.566), propyl acetate and ethyl acetate (r = -0.552), propyl acetate and 2-phenyl ethanol (r = 0.624), phenyl acetate and ethyl acetate (r = 0.862) and ethyl hexanoate and phenyl acetate (r = 0.679) (p <0.05).

Conclusions

The results of the study demonstrated the influence of different plant extracts on the aromatic profile of Cabernet Sauvignon wines. The highest total amount of volatile compounds (twice higher than the TK00 and TK25 controls) was achieved by applying blueberry extract and grape pomace extract treatments- variants BB251 (579.31 mg/L) and GP253 (579.20 mg/L). The blueberry extract affected positively the volatile composition, even when it was applied alone – BB01. The combined application of SO_2 and blueberry extract (sample BB253) increased the wine volatile complexity. The best effectivity on higher alcohols synthesis and their accumulation in wines was obtained by using SO_2 (25 mg/L) and plant extracts (0.3 mL) in samples coded as R253, GP253 and BB253 and eight higher alcohols have been identified. 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 1-hexanol were determined as dominant. The highest total ester content was found in sample R253 (169.13 mg/L). A high amount of individual esters was identified in BB01 sample (143.08 mg/L), obtained only with blueberry extract, without SO_2. This revealed the potential of blueberries as an alternative approach for sulphitation, realizing good ester accumulation in wines. The terpenes were dominated by geraniol. The highest value was obtained in sample GP257 with concentration of 0.68 mg/L. Methyl alcohol was found in all wines tested. Its quantity was within normal limits. Results demonstrated the importance of treatment of plant extracts and their concentrations in red wine. The study indicated the possibilities of optimization of SO_2 in wine production and wines quality by treatment with natural plant extracts.

Acknowledgments. This study was a part of the project titles “The Effects of Different Plant Extracts on Wine Quality Which May be an Alternative to Sulfur Dioxide” supported by Ege University Projects Coordination Centre of Scientific Research with project number 2017-MUH-013.

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