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The Effects of Different Plant Extracts on Wine Phenolic Contents and Antioxidant Activities Used as an Alternatives of Sulphur Dioxide During Wine Production

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ABSTRACT

Sulphur dioxide (SO_2) is commonly used as an antioxidant and antimicrobial additive during wine production. Nowadays, consumers preferred foods treated with natural preservatives. The aims this study was to determine the effects of different natural plant extracts as an alternative of sulphur dioxide used in wines. 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 and blueberry) at different concentrations. As control groups were used wine samples produced without addition of SO_2. At the end of production basic oenological analyses (total acidity, volatile acidity, pH, dry matter, ash, free and total SO_2) and specific wine analyses (total phenols, total flavanols, tartaric ester content and antioxidant activity) were performed. Results demonstrated that each used plant exact have different effects on wine quality parameters. The lowest concentrations of grape pomace extract caused reduction of SO_2 and keeping the required wine properties. The highest value of antioxidant activities and total phenols were determined in the wine treated with 25 mg/L SO_2 and 1 ml/L rosemary extract (in the 1st mount of storage) and 25 mg/L SO_2 and 1 ml/L grape pomace extract (in the 2nd and 3rd mount of storage) as 89.92%, 5550.48 mg/l GAE; 88.51%, 5028.65 mg/l GAE; 88.42%, 4974.25 mg/l GAE, respectively. Results emphasized the importance of used plant extracts and their concentrations. The study demonstrated the possibilities of optimization of SO_2 and wines phenols on the base of used natural plant extracts.

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Introduction

SO_2 have been identified as a common chemical preservative that uses in wine production for many years to prevent the oxidation of wine and inhibit the unwanted microorganisms. Besides its antioxidant and antimicrobial effects on wine, today the adverse effects of SO_2 on human health have been subjected to many researches.

SO_2 associated with the many health risk such as asthma, allergic reactions, headache, fatigue, itching, diarrhoea, abdominal pain, and anaphylaxis (Vally and Thompson, 2001,2003; Qin and Meng, 2009; Guerro and Cantos-Villar, 2015). However, it was observed that most of the sulphide-sensitive individuals showed different adverse effect level (ranging from 20 to 50 mg) against SO_2. As a result of various studies, the daily intake of sulphites was assumed to be 43 mg / g on average for an individual weighing 60 kg (Taylor et al., 1986). The Some international authorities have set limits on daily intake of sulphite as 0.7 mg/kg body weight (WHO, 2009). It should be kept in mind that a consumer weighing 60-80 kg who drinks only half a litter of wine can easily overcome this value. The legal regulations and standards have been introduced in national/ international legislation related to SO_2 with the understanding of the adverse effects of SO_2 (IFOAM, 2013; EU Regulation No 203/2012). According to International Organization of Vine and Wine (OIV), these limits are 150 mg/L for wines with sugar content <5 g / L; 200 mg/L for wines with a sugar content ≥ 5 g/L (OIV, 2017). According to U.S. Department of Agriculture (USDA), this limit is 100 ppm for wines labelled as "produced organic grapes" (USDA, 2019).

Especially in the last decade, the importance of alternative methods to chemical additives has increased in winemaking; these technologies have been tried by many researchers. Among these methods are chemical materials such as lysozyme, ascorbic acid and dimethyl decarbonate (Costa et al., 2008; Azzolini et al., 2010; Sonni et al., 2011). However, non-thermal processes such as high hydrostatic pressure (HHP), pulsed electric field (PEF), ultraviolet irradiation (UV), high power ultrasound (HPU) and low electric current (LEC) have been studied as a an

alternatives to SO_2 in wine production (Fredericks and Krügel, 2011; Morata et al., 2015; Delsart et al., 2015a,b; Costantini et al., 2015; Gracin et al., 2016; Briones-labarca et al., 2017). In addition to all these methods, natural alternatives such as eucalyptus and almond skin extracts (Garcia-Ruiz et al., 2013), stilbenes extracts (Raposo et al., 2016a, 2018), thyme essential oil (Freidman et al., 2017), grape and wood tannins (Sonni et al., 2009; Alamo-Sanza et al., 2019; Sánchez-Palomo et al., 2017), hydroxtyasol and oleuropein (Raposo et al., 2016b,c) and glutathione (Hosry et al., 2009) were evaluated. These extracts have been observed that have positive effects on wine quality.

The aim of this study was assessment of changes of red wine phenols and antioxidant activities with addition of different phenolic-rich plant extracts and the possibility of reducing the quantity of SO_2 during wine aging process.

Material and Methods

Plant Material

As materials were used grapes of *Vitis vinifera* L. cv. origin var: *Cabernet sauvignon* from the Menderes/ Gölcükler region of Izmir. 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 from the wine production process of Cabernet sauvignon grapes. The blueberry (Bb) and rosemary (R) extract used in the experimental plan were with *Rosmarinus officinalis* L. and *Vaccinium myrtillus* L. spices origin, respectively. These plants were obtained from the same region of Turkey (Izmir).

Wine Processing

The grapes were transferred to the mill for separation of stems, wastes and foreign materials after weighing process. Crushed fruits were collected in stainless steel tank. As culture was used *Saccharomyces cerevisiae* (20 g/L dose SIHA Active Dry yeast 10). The must was stirred twice daily. The alcoholic fermentation was carried out in controlled conditions. The fermentation process was completed in 12 days at 20-22°C. The pressing operation was done by a mechanical press machine. During alcoholic fermentation, the density and temperature measurements were carried out. Using these data the alcohol and sugar content were determined. At the end of the fermentation, the final sugar content was determined as < 1 g/L. At the end of alcoholic fermentation sterilization procedure were carried out. Obtained wines were stored at 15°C. With the addition of the extracts, the samples were bottled and stored for 3 months.

Experimental Design and Treatments

Natural extracts were prepared as following the path given in Figure 1.

The experimental design was achieved by using different plant extracts (grape pomace, rosemary and blueberry) at different concentrations. As controls were used wine samples produced without added natural extracts and second group samples produced without addition of sulphur dioxide (SO_2). Extracts were added to the wine samples after fermentation. The experimental groups are demonstrated in Table 1.

Wine samples were collected after 1, 2 and 3 months of storage during aging in bottles at 15 ±2°C. Basic must and wine analyse were carried out according to the OIV Compendium of International Methods of wine and must (OIV, 2016). All analyses were carried out in duplicate.

Basic Oenological Wine Analyses

Basic oenological wine analyses were determined according to recommended methods by OIV (International Organization Vine and Wine (OIV, 2017). Alcohol content (% v/v), pH, (direct measurement by using pH meter), total acidity (tartaric acid g/L), volatile acidity (g/L acetic acid), total and free SO_2 (mg/L), dry matter (g/L) and ash (g/L) analyses were performed.

Determination of Total Phenol Content

Total phenol concentration was determined with the Folin–Ciocalteu assay that previously reported by Singleton and Rossi (1965). Total phenol contents of 1:10 diluted wine samples with deionized water were calculated as gallic acid equivalents (GAE). The total amounts of phenolic compounds (mg/ L) of the samples were calculated using the gallic acid standard curve. All results were multiplied by the dilution factor.

Determination of Total Flavanols Content

Total flavanols concentration was measured with the Glorie's method (Gil-Munoz et al., 1998). Total flavanol content of 1:10 diluted wine samples with deionized water were calculated as quercetin equivalents. The total amounts of flavanols (mg/ L) of the samples were calculated using the quercetin standard curve. All results were multiplied by the dilution factor.

Determination of Tartaric Esters Content

Tartaric esters concentration was measured with the Glorie's method (Gil-Munoz et al., 1998). Tartaric ester content of 1:10 diluted wine samples with deionized water were calculated as caffeic acid equivalents. The total amounts of tartaric esters (mg/ L) of the samples were calculated using the caffeic acid standard curve. All results were multiplied by the dilution factor.

Determination of Antioxidant Activity

The antioxidant activity analysis was carried out according to the method described by Kumaran and Karunakaran (2006). Inhibition power of wines was estimated using the dipyridyl method. According to this method a 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was used with spectrophotometric measurements at 517 nm.

All samples were tested after 1:10 dilution with 12% EtOH. The results were expressed as % inhibition of wine samples. % 96 ethanol solutions with 2 ml of 0.1 mM DPPH was used as blank. The absorbance value of the blank was higher than the wine samples. % inhibition was calculated according to the following formula;

$$\% \text{ Inhibition} = (Abs_{(blank)} - Abs_{(sample)}) / Abs_{(sample)} \times 100$$

In all cases, analyses were performed in duplicate, the values were averaged, and the standard deviation was calculated.

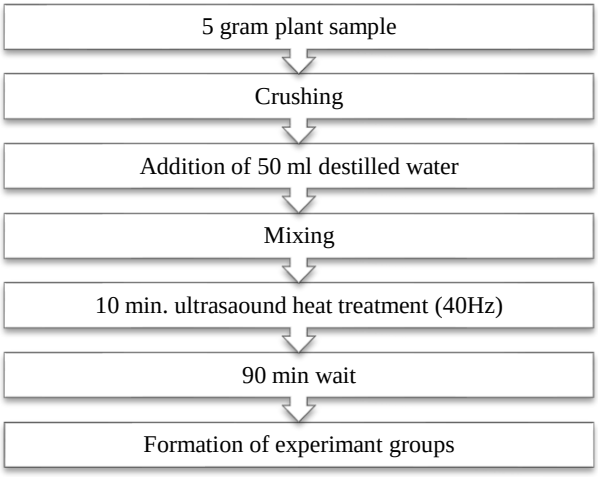

Figure 1 Natural extracts preparation

Table 1 Experimental group formation

Group	Treatments with wine samples		
	SO_2 addition mg/L	Extract addition ml/L	Sample codes
Grape Pomace (Gp)	0	1	GP01
	25	0.7	GP257
	25	0.3	GP253
	25	1	GP251
Rosemary (R)	0	1	R01
	25	0.7	R257
	25	0.3	R253
	25	1	R251
Blueberry (Bb)	0	1	Bb01
	25	0.7	Bb257
	25	0.3	Bb253
	25	1	Bb251
Control 1	0	0	TK00
Control 2	25	0	TK25

Statistical Evaluation

One-way ANOVA was initially used to determine significant differences amongst the samples due to their antioxidant activity, total phenol, total flavanol and tartaric acid content to explore the effect of plant extracts addition. Significant differences between averages were obtained at a 95% significance level. Pearson correlations analyses were used for determined to relations between analyses results. The values were averaged and standard deviation, minimum, maximum and mean values of samples were determined.

Results and Discussion

The study has been concluded by comparing data of individual antioxidant activity, tartaric esters, total flavanols and phenolic compounds of wines treated with natural plant extracts during third month of bottle storage and also by applying statistical analyses. Producing wines with lower sulphur dioxide using compounds naturally obtained from wines or plants such as grape pomace, rosemary or blueberry extracts could provide a healthier wine with added-value since: (i) the amount of SO_2 would be reduced (ii) the concentration of phenols, which has recently accepted as protective compound against oxidative damage in humans, would be increased.

Must Properties

The density of the must was determined as 1110 g/cm^3. The average pH was determined as 3.8 and the total acids were determined as 5.48 g/L (tartaric acid). The average density of the wine was 980 g/ cm^3 and the alcohol value was measured as 13.0 % (v/w) at the end of the fermentation. The reducing sugar content of the wine was calculated as < 1 g/L.

Evaluations of Basic Wine Analyses

The results of the basic wine analysis results are given in Table 2. Statistically, the effect of different concentrations in the same experimental group was not significant but the effect of these groups was found to be significant ($P<0.05$).

In the first month of storage, the highest pH value was determined as 3.90 in R257 wines while the lowest pH value was 3.75 in TK25, GP01 and GP257 wines. In the third month of storage, Bb01 wines showed the highest pH value and the lowest was determined in Bb253 wines as 4.90 and 3.90, respectively. According to total acidity results TK25 wines were higher while R253 wines were lower in the first month of storage (5.90 g/L and 4.50 g/L, respectively). In the third month of storage, GP253 wines showed the highest total acidity value while the lowest were Bb257 wines as 5.40 and 3.90 g / L, respectively.

Considering to volatile acidity results Bb01 wines were higher while TK25 wines were lower in the first month of storage (0.69 g/L and 0.24 g/L, respectively). In the third month of storage, TK00 wines showed the highest total acidity value while the lowest were R251 wines as 0.84 and 0.50 g/ L, respectively. However, the highest and lowest values of the volatile acidity of the samples were determined in the TK00 (0.84 acetic acid g/L) and TK25 (0.24 acetic acid g/L) wines, respectively. At the end of the third month of storage, results indicate that the pH value and the volatile acidity of the samples were increased, while the total acidity value was decreased.

Correlation analysis was used to determine the relation between parameters and within groups. While there were no significant correlation detected between pH and total acidity in the groups, there were a significant correlation between the pH and the volatile acid values ($r= 0.3511$, $p=0.023$). There were also determined correlations between volatile acidity and total phenols content ($r = -0.4032$, $p = 0.08$), tartaric esters ($r = -0.4369$, $p = 0.004$) content and antioxidant activity ($r = -0.3618$, $p = 0.019$).

Evaluations of the Total Phenolic Content of Wines

Phenolic compounds play an important role on the quality characteristics of red wine (Aktan and Yıldırım, 2012). The lowest concentration of total phenolic content in the first month of storage was determined as 2684.8 mg GAE/L in the TK00 wines, while the highest value was determined in the R251 (5550.48 mg GAE/L) and R01 (5380 mg GAE/L) samples (Figure 2). However, in the third month of storage, total phenol content of the same sample groups was determined to be lower value according to the control groups of TK00 (2498.41 mg GAE/L) and TK25 (3345.25 mg GAE/L) wines. In the third month of storage, GP251 wines showed the highest total phenol content, while the lowest value was determined in R01 samples (4974.25 and 2405.2 mg GAE/L, respectively). The results of the total phenol content analyses are given in Figure 2.

Table 2 Basic wine analyses results of wines

Samples* and storage	Analyses														
	pH			Total acidity (g/L)			Volatile acidity (g/L)			Total SO_2 (mg/L)			Free SO_2 (mg/L)		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
TK00	3.79	3.70	3.92	5.40	4.90	4.90	0.68	0.72	0.84	5.50	5.00	4.50	2.20	1.60	1.40
TK25	3.75	3.73	3.94	5.90	5.50	5.30	0.24	0.45	0.69	32.0	16.0	10.0	7.50	5.30	5.00
GP01	3.75	3.96	3.92	5.10	5.60	5.20	0.50	0.69	0.72	6.20	5.00	4.40	2.50	2.00	1.50
GP257	3.75	3.95	3.93	5.30	5.30	5.20	0.41	0.44	0.56	33.00	19.00	9.00	5.40	3.00	2.60
GP253	3.78	3.98	3.92	5.00	6.70	5.40	0.45	0.49	0.59	30.00	18.00	10.0	6.00	4.40	3.00
GP251	3.78	3.95	3.92	5.50	5.00	4.90	0.34	0.40	0.52	31.00	19.00	10.50	5.20	4.00	3.10
R01	3.79	3.73	4.06	5.00	4.70	4.60	0.51	0.55	0.67	6.10	5.00	4.20	2.30	1.50	1.40
R257	3.90	3.72	4.03	4.70	4.70	4.60	0.36	0.40	0.54	34.00	17.00	8.00	4.90	3.50	2.20
R253	3.90	3.82	4.03	4.50	4.80	4.70	0.39	0.48	0.60	33.00	17.00	9.00	5.30	4.50	2.80
R251	3.78	3.75	4.02	5.70	4.80	4.60	0.30	0.36	0.50	30.00	16.00	11.00	4.80	4.10	2.30
Bb01	3.78	3.80	4.90	5.60	4.80	4.70	0.69	0.71	0.74	6.10	5.00	4.50	2.40	2.20	1.50
Bb257	3.79	3.86	3.91	5.10	4.70	3.90	0.48	0.45	0.59	31.00	19.00	9.00	6.20	4.70	2.80
Bb253	3.78	3.83	3.90	5.30	4.80	4.70	0.50	0.60	0.71	33.00	17.00	8.00	6.10	4.70	2.60
Bb251	3.79	3.92	3.91	4.90	4.90	4.70	0.40	0.42	0.57	32.00	18.00	10.00	6.00	4.90	3.10

*TK00: SO_2 addition, TK25: 25 mg/L SO_2 addition, GP01: 1 ml/L grape pomace extract addition, GP257: 25 mg/L SO_2 and 0.7 ml/L grape pomace extract addition, GP253: 25 mg/L SO_2 and 0.3 ml/L grape pomace extract addition, GP251: 25 mg/L SO_2 and 1 ml/L grape pomace extract addition, R01: 1 ml/L rosemary extract addition, R257: 25 mg/L SO_2 and 0,7 ml/L rosemary extract addition, R253: 25 mg/L SO_2 and 0.3 ml/L rosemary extract addition, R251: 25 mg/L SO_2 and 1 ml/L rosemary extract addition, Bb01: 1 ml/L blueberry extract addition, Bb257: 25 mg/L SO_2 and 0.7 ml/L blueberry extract addition, Bb253: 25 mg/L SO_2 and 0.3 ml/L blueberry extract addition, Bb251: 25 mg/L SO_2 and 1 ml/L blueberry extract addition.

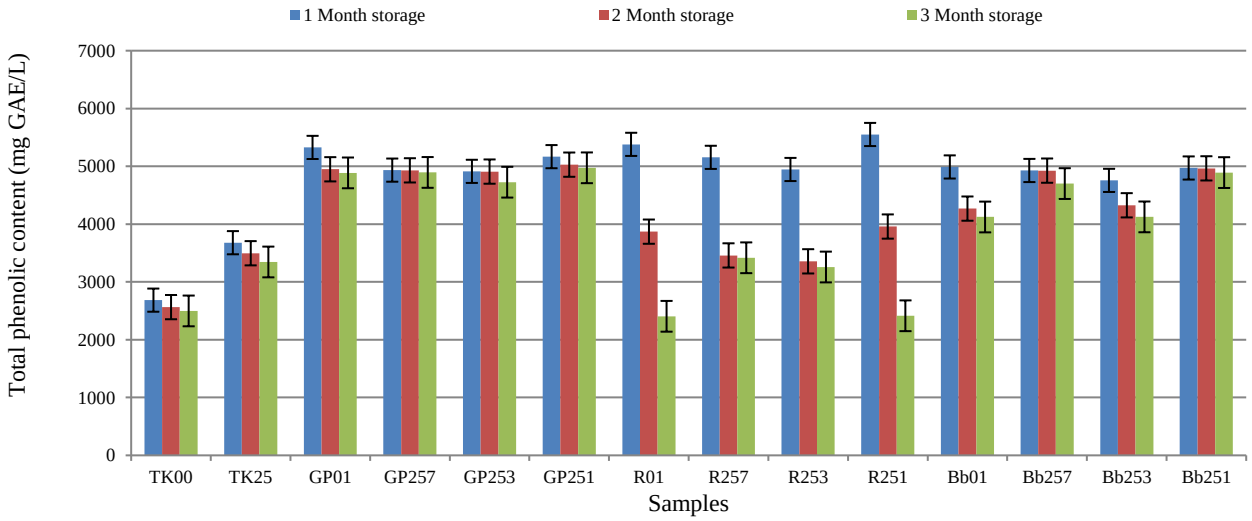

Figure 2 Changes of total phenolic content of wines during storage

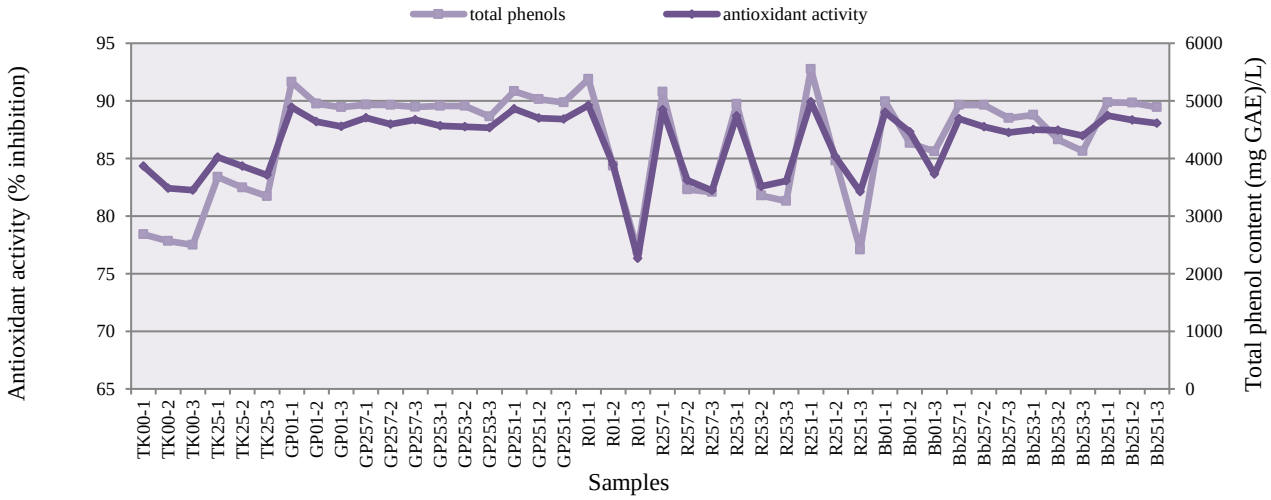

Figure 3 The relationship between total phenol content and antioxidant activity of wines

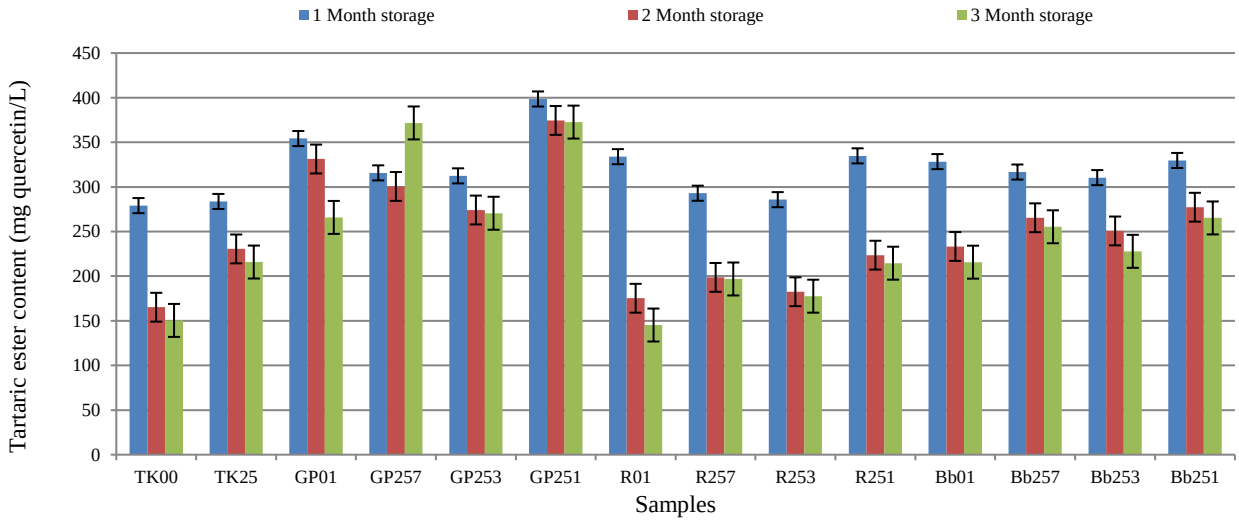

Figure 4 Changes of tartaric esters content of wines during storage

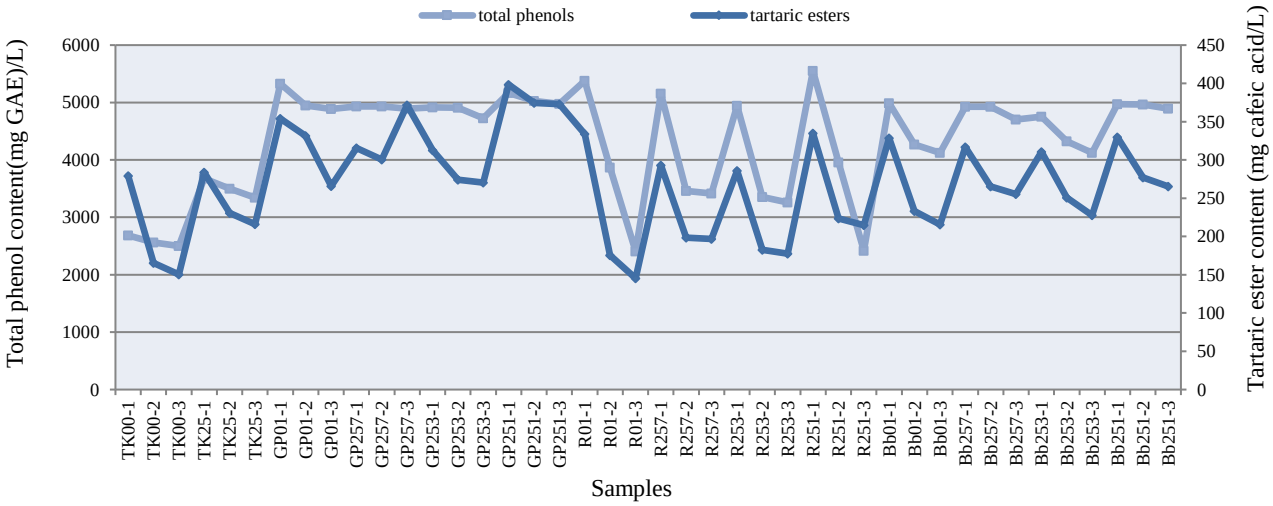

Figure 5 The relationship between total phenol and tartaric esters content of wines

The total phenolic contents of the samples from the highest to the lowest values according to storage were as follows:

- In the 1st month of storage: R251> R01> GP01> GP251> R257> Bb01> Bb251>R253> GP257> Bb257> GP253> Bb253> TK25> TK00.
- In the 2nd month of storage: GP251> Bb251> GP01> GP257> Bb257> GP253> Bb253>Bb01> R251> R01> TK25> R257> R253> TK00.
- In the 3rd month of storage: GP251> GP257> Bb251> GP01> GP253> Bb257> Bb253> Bb01> TK25> R253> R257> TK00> R251> R01.

With this study, it was statistically proven that the wines treated with the grape pomace extracts showed the highest value and with the rosemary extracts showed the lowest value of phenolic compounds in the third month of storage. Based on these results, it was concluded that the wines treated with rosemary extract were rapidly oxidized after three months storage compared to other groups. These findings are consistent with the results of all specific analysis when the compared with wines treated with rosemary extract and the control group wines in the 3rd month of storage. It was determined a high positive correlation between total phenolic compound and antioxidant activity ($r = 0.9306$, $P<0.001$) with Pearson correlations analyses. Similar positive correlation between total phenolic content and antioxidant activity has been reported previously by researchers (Landrault et al, 2001). Considering both the total phenol content and antioxidant activity values of the samples are considered together, it can be seen that these results are consistent with each other (Figure 3). In this case, it has been shown that there is a high positive correlation between the total phenolic compound and antioxidant activity of the samples.

A positive relationship was found between total phenols and antioxidant activity which investigated total phenol levels and antioxidant activity of *Cabernet Sauvignon* grapes obtained from Izmir/Turkey Region ($r = 0.528$, $P<0.05$). In a study it was indicated that the highest antioxidant activity and total phenol levels were determined as grape pomace (82.30% and 82.60%), grape (68.91%) and must (2750 mg/L GAE) (Yıldırım et al., 2006). In a similar study, a positive correlation was found between total phenol levels and antioxidant activity of *Cabernet Sauvignon* grapes in Izmir region ($r = 0.845$, $P=0.034$). In addition, total phenols content in this study was determined as 2.850 mg/L GAE and antioxidant activity was 83.50% (Yıldırım et al., 2007).

Rockenbach et al. (2011) emphasized that the grape pomace (*Cabernet Sauvignon*) extract could be used as an antioxidant agent due to their high amounts of phenolic content (2128 to 16.518 mg GAE / 100 g). It was indicated that especially seed and skins of grapes and blueberries showed high antioxidant capacities; because of their high content of phenolic compounds and tannins (Hayder et al., 2004, 2008; Montoro et al., 2006; Cakir et al., 2004; Romani et al., 1999; Galuska et al., 2013; Rockenbach et al., 2011; González-Paramás, 2004). The high antioxidant activity of blueberries and grape pomace were explained by the fact that they are rich in tannin, phenol, essential oil and fatty acids (Hayder et al., 2004, 2008; Montoro et al., 2006; Cakir et al., 2004; Romani et al., 1999; González-Paramás, 2004). However, Rababah et al. (2004) compared the relationship between the total phenol content and the antioxidant activities of different extracts (such as rosemary, ginger, green and tea and grape seed). According to the findings; while the total phenol content was determined at the highest level in rosemary extract, the highest antioxidant activity was determined in grape seed and green tea extracts.

Evaluations of The Tartaric Esters Content of Wines

In the first month of storage, the highest tartaric esters value was determined in GP251 wines while the lowest tartaric esters value determined in R01 wines at the end of third month of storage (398,54 and 145,27 mg caffeic acid/ L, respectively). Tartaric ester concentrations of wines are given in

Non-flavonoid phenolic compounds found in grapes and wine include hydroxycinamic acid, hydroxybenzoic acids and stilbenes. Hydroxycinamic and hydroxybenzoic acids are also called phenolic acids (Monagas, et al., 2005). These acids are found in the form of tartaric esters in the grape skins and pulp (Ribereau-Gayon, 1965). Our study supports this information due to the tartaric ester content of the samples treated with the grape pomace extract was higher than the other groups at the end of the three month of storage (Figure 4). On the other hand, rosemary (R01) (145.27 mg caffeic acid / L) sample group was indicated the greatest decrease in the amount of tartaric ester compared to the control groups wines TK00 (150.43 mg caffeic acid / L) and TK25 (215.84 mg caffeic acid/L).

The tartaric esters contents of the samples from the highest to the lowest values according to storage were as follows:

- In the 1st month of storage: GP251> GP01> R251> R01> B251> B01> B257> GP257> GP253> B253 > R257> R253> TK25> TK00.
- In the 2nd month of storage: GP251> GP01> GP257> B251> GP253> B257> B253> B01> TK25> R251> R257> R253> R01> TK00.
- In the 3rd month of storage: GP251> GP257 > GP253> GP01> B251> B257> B253> TK25> B01> R251> R257> R253> TK00> R01.

There was a positive correlation between the total phenol content of the samples and the tartaric ester content ($r = 0.8115$, $P<0.001$). Figure 5 show that the amounts of these two different groups of phenolic compounds of the samples were observed to be consistent with each other.

However, a similar positive correlation was observed between the content of the total flavanols and the tartaric ester content ($r = 0.8463$, $P<0.001$). Figure 6 show that the amounts of the tartaric ester and total flavanols of the samples were observed to be consistent with each other.

There was a positive correlation between antioxidant activity and tartaric ester content ($r = 0.8414$, $P<0.001$). Figure 7 show that the amounts of the tartaric ester and antioxidant activity of the samples were observed to be consistent with each other.

Evaluations of The Total Flavanols Content of Wines

In the first month of storage, the highest total flavanols value was determined in GP251 wines while the lowest total flavanols value determined in R01 wines at the end of the third month of storage (135.72 and 58.95 mg quercetin/ L, respectively). Total flavanols concentrations of wines are given in Figure 8.

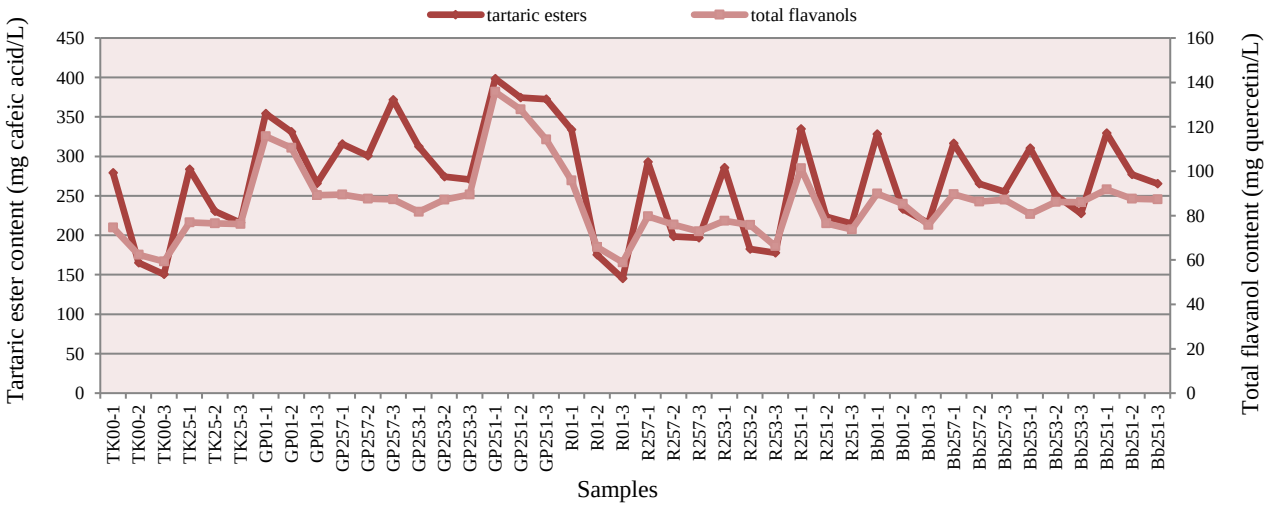

Figure 6 The relationship between total flavanols and tartaric esters content of wines

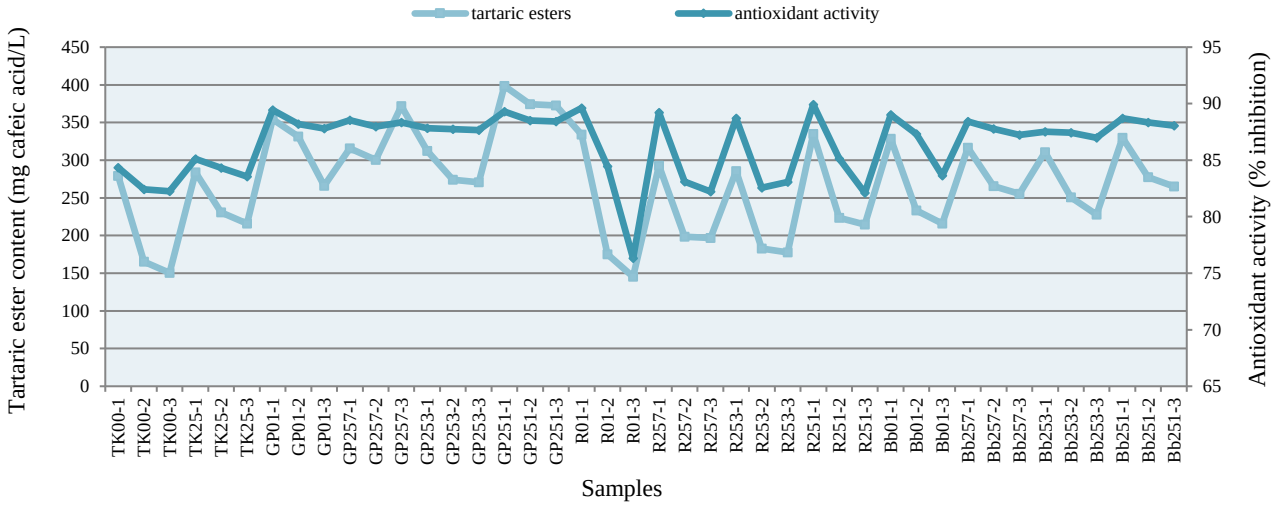

Figure 7 The relationship between antioxidant activity and tartaric esters content of wines

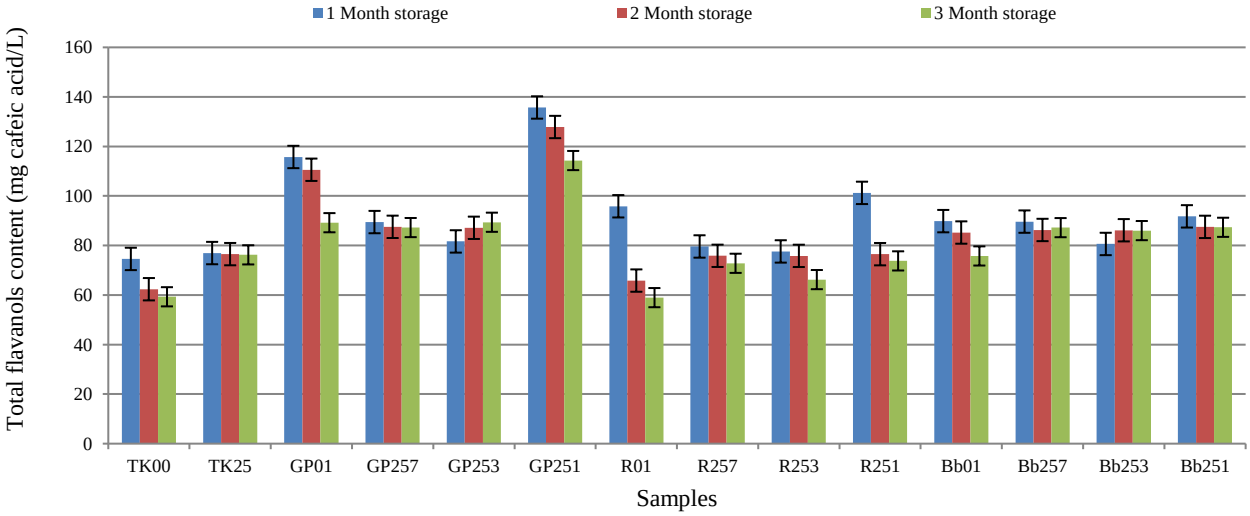

Figure 8 Changes of total flavanols content of wines during storage

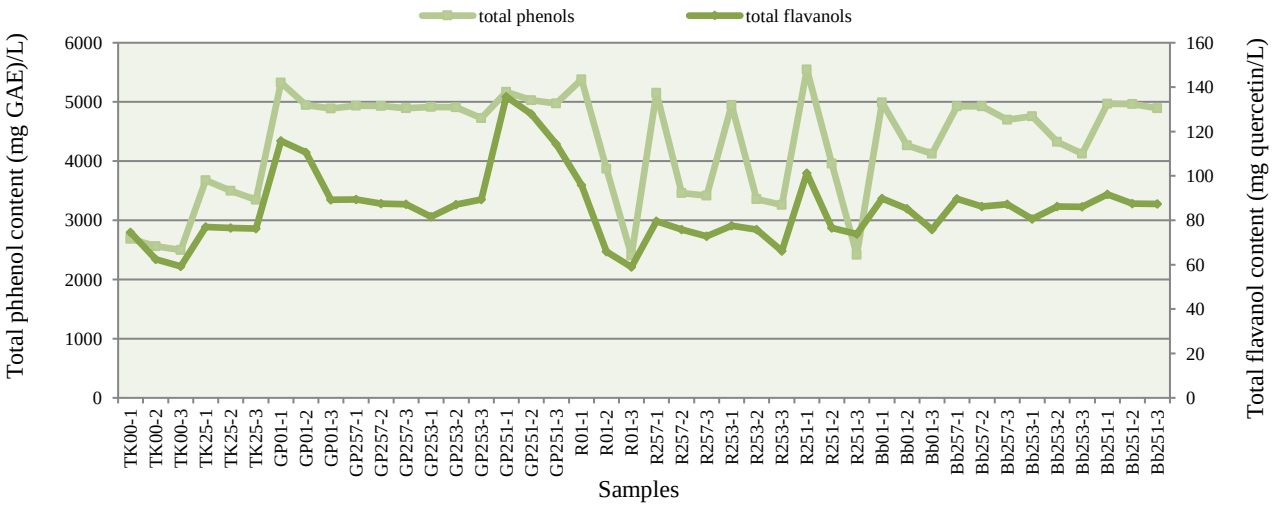

Figure 9 The relationship between total phenols and tartaric esters content of wines

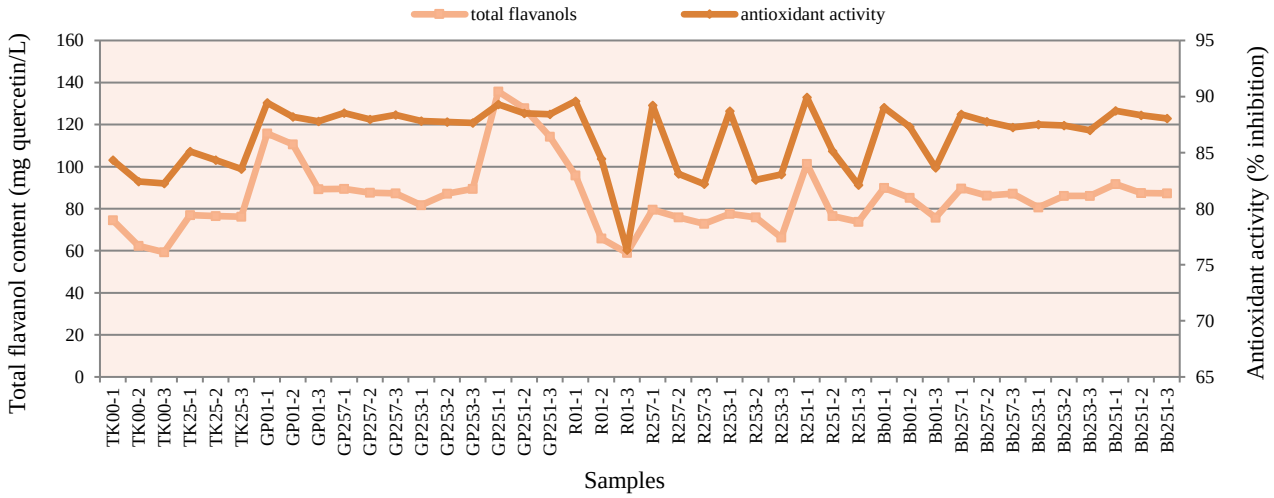

Figure 10 The relationship between antioxidant activity and total flavanol content of wines

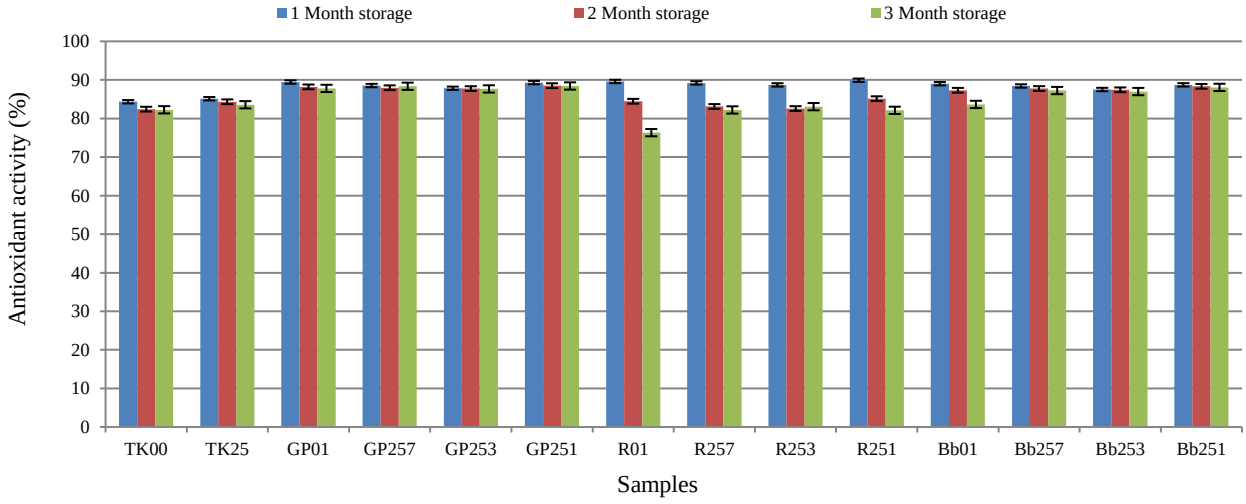

Figure 11 Changes of antioxidant activity of wines during storage

Flavanols (flavan 3-ol) were firstly identified by Freudenberg (1924). These compounds are contribution to astringency flavor of wine. Some flavanol compounds found in grapes and wine are (+) catechins, (-) epicatesins and (-) epigallocatechins (Su and Singleton, 1969). Tannins are the most abundant group of phenolic compounds in grapes (Kennedy et al., 2006). Our study supports this information due to the total flavanol content of the samples treated with the grape pomace extract was higher than the other groups at the end of the three month of storage (Figure 8).

The total flavanols contents of the samples from the highest to the lowest values according to storage were as follows:

- In the 1st month of storage: GP251> GP01> R251> R01> Bb251> Bb01> Bb257> GP257> GP253> Bb253> R257> R253> TK25> TK00.
- In the 2nd month of storage: GP251> GP01> GP257> Bb251> GP253> Bb257> Bb253> Bb01> TK25> R251> R257> R253> R01> TK00.
- In the 3rd month of storage: GP251> GP257> GP253> GP01> Bb251> Bb257> Bb253> TK25> Bb01> R251> R257> R253> TK00> R01.

At the end of the three months storage, the control wines (59.3 mg quercetin / L) contained nearly half amount of total flavanol content when compared to the wines treated with grape pomace extract (114.3 mg quercetin / L). However, while total flavanol concentration in wines treated with rosemary extract decreased significantly, it observed less reduction in wines treated with blueberry extracts. Overall, total flavanol levels decreased at the end of three months of storage for all sample groups. Positive correlations were observed between total flavanol and phenol content ($r = 0.7183$, $P<0.001$) and antioxidant activity ($r = 0.7143$, $P<0.001$). When these results were evaluated together, it was determined that these two parameters showed a consistent distribution as shown in the Figure 9 and 10.

Evaluations of the Antioxidant Activity of Wines

In the third month of storage, GP01 wines showed the highest of antioxidant activity, while the lowest value was determined in R01 samples (88.42 and 76.32 %, respectively). The results of the of antioxidant activity of wines are given in Figure 11.

The lowest concentration of antioxidant activity in the first month of storage was determined as 84.34 % in the

TK00 wines, while the highest value were determined in the R251 (%89,92) samples. However, in the first month of storage, the rosemary experimental group were determined as the highest value, in the third month of storage same group wines were determined as the lowest value of antioxidant activity (Figure 11).

The antioxidant activities of the samples from the highest to the lowest values according to storage were as follows:

In the 1st month of storage: R251> R01> GP01> GP251> R257> B01> Bb251>R253> GP257> Bb257> GP253> Bb253> TK25> TK00.

In the 2nd month of storage: GP251> Bb251> GP01> GP257> B257> GP253> Bb253>Bb01> R251> R01> TK25> R257> R253> TK00.

In the 3rd month of storage: GP251> GP257> Bb251> GP01> GP253> Bb257> Bb253> Bb01> TK25> R253>TK00> R257> R251> R01.

It was indicated that the antioxidant potential of red wines depend to a great extent on their total flavanol content (De Beer et al., 2002). Total flavanol concentration (Figure 8), is highest for treatment GP251 (in the third month of storage) supporting this hypothesis. In addition, the antioxidant activity of a wine is largely dependent on its total phenolic content (De Beer et al., 2002). This hypothesis is also supported by the observed maximum values (Figure 2) of total phenol concentrations of the wines treated with grape pomace extract and blueberry extract in the third month of storage. In agreement with Alonso et al. (2002), the antioxidant activity was found to be strongly correlated with total phenols ($r^2 = 0.5587$) and flavanols ($r^2 = 0.7245$), while the reducing power also exhibited correlation with total phenols ($r^2 = 0.6650$) and flavanols ($r^2 = 0.6521$).

Concluding Remarks

Considering this study used treatments could be used as possible alternatives to SO_2 during wine production. The results of different parameters of different wines treated with extracts demonstrated the importance of grape pomace and blueberry extracts. The study results demonstrated the possibility of using healthier, nonchemical additives during wine production.

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Conflict of Interest

The authors have no conflict of interest to declare.

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