Chemometric properties of wines treated with natural extracts as an alternative of sulphur dioxide

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Abstract. Sulphur dioxide (SO₂) is commonly used as an antioxidant and antimicrobial compound during wine production. Phenols naturally existence in plant extracts have been reported to possess antioxidative, antimicrobial activities, and as anti-inflammatory activities. Since few consumers preferred foods treated with natural preservatives, the aim of 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 for red wines (Cabernet sauvignon). The experimental design was achieved by using different plant extracts (grape pomace, rosemary, black blueberry) at different concentrations. As controls were used wine samples produced without natural extracts and second group samples produced without addition of sulphur dioxide (SO₂). At the end of production basic analyzes (total acidity, volatile acidity, pH, dry matter, ash, free and total SO₂), and colorimetric wine analyses (TWP, WC, CD, CI, %R, %Y, %B, Abs280, Abs420, Abs520, Abs620, %dA and tint values) were performed. Analyses on n-dimensional scale demonstrated that each used plant exact have different effects on required SO₂ and wine quality parameters. The lowest concentrations of grape pomace extract caused reduction of SO₂ and maintaining the required wine properties. The highest value of the end of first month of storage for TWP, WC, Abs280, Abs520, %R values were determined for grape pomace treatment wines. The highest value of the TPW were determined for samples treated with grape pomace (1 ml/L extract), rosemary (1 ml/L extract), and blue berry (1 ml/L extract with 25 mg/L SO₂) 2.710, 2.550 and 2.520 respectively. Results emphasized the importance of used plant extracts and their concentrations. The study demonstrated the possibilities of SO₂ optimization on the base of used natural plant extracts.

1. Introduction

During wine production, SO₂ is used as an antioxidant and antimicrobial additive for preventing oxidation and the spread of unwanted organisms (wild yeast, acetic acid bacteria, lactic acid bacteria) [1]. During wine aging, there is a gradual loss of phenolic compounds due to their participation in a number of chemical reactions such as oxidation (with polysaccharides and tannins) and formation of other stable anthocyanin-derived pigments. Used SO₂ could compensate partially the phenolic compounds effects on wines. All these reactions resulted in marked required changes in the colour, mouth feel and flavour properties of red wines [2]. Even these advantages, some negative effects of SO₂ on human health have been subject to researches for many years [3]. The daily intake of sulphite was assumed to be 43 mg/g on average for an individual weighing 60 kg [4]. The quantity SO₂ was gradually reduced due to some health precautions [5]. Recently, uses of natural preservatives, as an alternative of SO₂ have been tested. Some of them included non-thermal processes and some of them proposed using of new chemicals. The most promising natural alternatives of sulphides in wine production are using of natural plant extracts [6]. Phenolic compounds and their derivatives, which are found in the structure of these extracts, have been shown to be effective in preventing auto oxidation [7–9]. It was stated that other phytochemicals (terpenes, alkaloids, lactones, etc.) found in the extract may also contribute to the antioxidative properties of these extracts. Then a rises the question, what are the effects of these compounds on wine chemometric properties. The aims of this study were to determine the effects of different natural plant extracts (grape pomace, blueberry and rosemary) used as an alternative of sulphur dioxide on wine chemometric properties of wines.

2. Material and methods

2.1. 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).
2.2. 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. The density of the must was determined as 1110 g/cm³, if the average pH as 3.8 and the total acid amount as 5.48 g/L (tartaric acid). 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.

2.3. Experimental design and treatments

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

The experimental design was achieved by using different plant extracts (grape pomace, rosemary, black 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 (SO₂). Extracts were added to the wine samples after fermentation. The experimental groups are demonstrated in Table 1.

2.4. Analyses

Wine samples were collected after 1, 2 and 3 month of storage during aging in bottles at 15 ± 2 °C. Basic must and wine analyzes were carried out according to the OIV Compendium of International Methods of wine and must [10]. All analyzes were carried out in duplicate.

2.4.1. Basic wine analyzes

Basic oenological wine analyzes were determined according to recommended methods by International Organization Vine and Wine (OIV) [11]. Alcohol content (% v/v), pH, (direct measurement by using pH meter), total acidity (tartaric acid g/L), volatile acidity amonth (g/L acetic acid), total and free SO₂ (mg/L), dry matter (g/L) and ash (g/L) analyzes were performed.

2.4.2. Chromatic measurements

The absorbance measurements of wine samples were determined by spectrophotometric method (Pharmacia LKB, Novaspec II). Measurements were determined by using 1 mm 1-mm cell path. Absorbance was determined at 280, 420, 520 and 620 nm wavelengths. Required dilutive were done prior analyses [15]. Special wine chromatic parameters were determined by following equations (Equations from 1 to 7):

\[ CD = Abs_{420} + Abs_{520} \]  
\[ CI = Abs_{420} + Abs_{520} + Abs_{620} \]  
\[ T = Abs_{420} / Abs_{520} \]  
\[ \%dA = (Abs_{520} - (Abs_{420} - Abs_{620}))/Abs_{520} \times 100 \]  
\[ \%R = Abs_{520} / CI^{100} \]  
\[ \%Y = Abs_{420} / CI^{100} \]  
\[ \%B = Abs_{620} / CI^{100} \]

For total wine pigment (TWP) analysis, 9 ml of 0.1 N HCl was added to 1 ml of wine samples diluted 10 times with distilled water. After pH <1.0 was obtained, sample

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| Table 1. Experimental Groups. |
|-------------------------------|
| **Group** | **Treatments with wine samples** |
| **SO₂ addition** | **Extract addition** | **Sample codes** |
| mg/L | ml/L |
| 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 |
was allowed to stand for 4.5 hours. Then absorbance was measured at 520 nm. Distilled water was used as blank [13]. Wine colour (WC) analysis carried out with these steps; 2 ml of the 5-fold diluted wine samples were taken with distilled water and 20 µl acetaldehyde (Merck) was added. After waiting 45 minutes, absorbance was measured at 520 nm [13]. HunterLab (coloflex, USA) device was used to determine the colorimetric values of wine samples. CIELab parameters L* (lightness), a* (redness), b* (yellowness) measurements were registered and distilled water as reference [16]. The croma values (C*) and hue angle (H°) of the samples were calculated by using the following formulas; C* = [a² + b²]¹/², H* = (arctan[b/a]).

2.5. 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.

3. Results and discussion

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). The results of the main basic wine analysis results are given in Table 2.

Positive correlations of volatile acidity were found with Abs420 (r = 0.3432, p = 0.026), %Y (r = 0.4054, p = 0.008), and tint (r = 0.4390, p = 0.004). Similar correlations were found between total acidity and colour parameters such as; wine colour (r = 0.40, p = 0.009), Abs520 (r = 0.3918, p = 0.011), CD (r = 0.3253, p = 0.036), R% (r = 0.4190, p = 0.006) and dA% (r = 0.3204, p = 0.039).

3.1. Colorimetric wine analyses

Colour is one of the most important quality parameters of wine and one of the most important factors affecting the total acceptability of consumers [17, 18]. It is known that 99.5% of the wine components are colourless in the visible light spectrum. The colours of red or white wines are known to be due to phenolic compounds found in small percentages [19]. The colour formation in wine takes place in a long period starting from fermentation to the end of storage [20–22]. It was reported that wines treated with phenolic compounds (such as tannin) prevent oxidation and cause sensory perception better than SO₂ in wine to replace SO₂ [23]. However, Bautista-ortan et al., 2005 found that two different tannins have no effect on the colour and other sensory properties of red wine [24]. Chromatic parameters were analyzed at bottling and after 3 months of storage in bottle of wines. The highest value in the first month of storage for TWP, WC, Abs280, Abs520, %R values were determined for grape pomace treatment.

Table 2. Basic wine analyzes results.

| Analyzes & Storage | Experimental Groups*
|-------------------|-------------------
| TK00 | TK25 | GP01 | GP257 | GP253 | GP251 | R01 | R257 | R253 | R251 | Bb01 | Bb257 | Bb253 | Bb251 |
|-------------------|-------------------|
| pH | 3.79 | 3.75 | 3.75 | 3.75 | 3.78 | 3.78 | 3.79 | 3.90 | 3.90 | 3.78 | 3.78 | 3.78 | 3.78 | 3.78 |
| Total acidity (g/L) | 1.54 | 5.90 | 5.10 | 5.50 | 5.00 | 5.50 | 5.00 | 4.70 | 4.50 | 5.70 | 5.60 | 5.10 | 5.50 | 4.90 |
| Free SO₂ (mg/L) | 1.22 | 7.50 | 2.50 | 5.40 | 6.00 | 5.20 | 2.30 | 4.90 | 5.30 | 4.80 | 4.20 | 4.70 | 4.80 | 4.90 |
| Total SO₂ (mg/L) | 5.50 | 32.0 | 6.50 | 33.00 | 30.00 | 31.00 | 11.00 | 27.00 | 11.00 | 30.00 | 6.50 | 31.00 | 33.00 | 32.00 |
| Volatile acidity (g/L) | 0.187 | 0.24 | 0.50 | 0.41 | 0.45 | 0.34 | 0.51 | 0.36 | 0.50 | 0.69 | 0.48 | 0.50 | 0.40 | 0.40 |
| Dry matter (g/L) | 28.1 | 27.20 | 27.90 | 26.60 | 27.20 | 27.40 | 26.10 | 26.70 | 26.30 | 27.30 | 26.80 | 26.30 |
| Ash (g/L) | 4.00 | 4.00 | 4.50 | 4.50 | 4.50 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |

*TK00: without SO₂ sample. TK25: 25 mg/L SO₂ added sample. GP01: 1 ml/L grape pomace extract. GP257: 25 ml/L SO₂ with 0.7 ml/L extract. GP253: 25 mg/L SO₂ with 0.3 ml/L extract. GP251: 25 mg/L SO₂ with 1 ml/L extract. R01: 1 ml/L rosemary extract. R257: 25 mg/L SO₂ with 0.7 ml/L rosemary extract. R253: 25 mg/L SO₂ with 0.3 ml/L rosemary extract. R251: 25 mg/L SO₂ with 1 ml/L rosemary extract. Bb01: 1 ml/L blueberry extract. Bb257: 25 mg/L SO₂ with 0.7 ml/L blueberry extract. Bb253: 25 mg/L SO₂ with 0.3 ml/L blueberry extract. Bb251: 25 mg/L SO₂ with 1 ml/L blueberry extract.
wines. In the first month of storage GP01, R01 and Bb251 wines showed the highest TPW value (2.710, 2.550 and 2.520 respectively) (Table 3). The most remarkable differences during this evolution between the control wines were found for the grape pomace wines. The grape pomace wines showed a higher proportion of Abs280 whereas the rosemary treated wines showed a lower proportion of Abs280. As for the Abs280, no differences were found between the grape pomace and rosemary treated wines. In a study by Zamora (2003), it was shown that the absorbance values at Abs280 for young red wines ranged from 20–80 [25]. The mean values of Abs280 of the wine samples were found to be consistent with young red wines as they ranged from 54.70 to 75.20.

Regarding to the results the experimental group wines showed a lower TWP, CI and Abs520 nm, while showing a higher hue, %Y and T value than the TK00 control wines after 3 month of storage. These colour parameters results demonstrated that the rosemary treated wines showed a lower proportion of %B, %Y and CI and Abs520 nm, while showing a higher hue, %Y and T value than the TK00 control wines. It has been studied by several authors that the reddish colour of the wine becomes orange-red during the aging and maturation of the wine [26–30]. The extract treatment during storage was determined to be significant for all parameters except dry matter, and alcohol value. After 3 months of storage in bottle TWP, WC, Abs280, Abs520, Abs620, CI, CD, %R, %B, and %dA were decreased, while Abs420, %Y, %B, and tint(T) were increased, in agreement with aged wine results at other condition [31–33].

## 4. Concluding remarks
Considering this study, some of these treatments could be used as possible alternatives to SO2 during wine production. After being treatments with plant extracts occurred positive changes in chemometric properties of wines and increases were observed in terms of total wine pigments, red colour, Abs280 and Abs520 values. The result of the experimental group of different parameters with different wines treated with extracts demonstrated

### Table 3. Evaluation of colorimetric values of wines.

| Analyzes & Storage | Experimental Groups* |
|--------------------|----------------------|
|                    | TK00 | TK25 | GP01 | GP257 | GP253 | GP251 | R01 | R257 | R253 | R251 | Bb01 | Bb257 | Bb253 | Bb251 |
| TWP                | 1    | 0.82 | 1.06 | 2.71  | 1.63  | 1.55  | 1.79 | 2.55 | 0.87 | 0.86 | 0.92  | 2.10  | 1.63  | 1.28  | 2.52  |
|                    | 2    | 0.77 | 1.03 | 1.71  | 1.62  | 1.53  | 1.74 | 0.79 | 0.82 | 0.84 | 0.81  | 1.21  | 1.52  | 1.24  | 1.53  |
|                    | 3    | 0.72 | 1.03 | 1.50  | 1.59  | 1.51  | 1.72 | 0.72 | 0.77 | 0.75 | 0.71  | 1.01  | 1.31  | 1.31  | 1.51  |
| WC                 | 1    | 4.40 | 4.60 | 5.50  | 4.85  | 4.75  | 5.25 | 5.45 | 4.60 | 4.55 | 4.65  | 5.10  | 4.95  | 4.75  | 5.40  |
|                    | 2    | 3.60 | 4.00 | 5.00  | 4.40  | 4.30  | 4.50 | 3.75 | 3.95 | 3.85 | 3.80  | 4.10  | 4.25  | 4.15  | 4.35  |
|                    | 3    | 3.20 | 3.75 | 4.00  | 4.15  | 4.10  | 4.25 | 3.15 | 3.65 | 3.60 | 3.55  | 3.70  | 3.85  | 3.80  | 3.95  |
| Abs280             | 1    | 55.40| 57.70| 75.20 | 68.30 | 59.60 | 73.50| 76.40| 72.10| 70.60| 79.70 | 71.10 | 64.40 | 58.70 | 70.60 |
|                    | 2    | 55.10| 57.50| 70.30 | 68.10 | 58.30 | 72.60| 57.40| 57.20| 57.00| 57.50 | 58.00 | 64.00 | 58.20 | 70.40 |
|                    | 3    | 54.94| 57.41| 67.87 | 68.00 | 58.00 | 72.45| 54.77| 56.67 | 56.67 | 54.81 | 57.38 | 57.65 | 57.62 | 70.38 |
| Abs420             | 1    | 3.80 | 3.38 | 3.07  | 3.25  | 3.28  | 3.12 | 3.02 | 3.21 | 3.30 | 2.99  | 3.17  | 3.25  | 3.31  | 3.15  |
|                    | 2    | 4.00 | 3.45 | 3.30  | 3.33  | 3.37  | 3.30 | 3.47 | 3.93 | 3.96 | 3.90  | 3.46  | 3.41  | 3.41  | 3.33  |
|                    | 3    | 4.03 | 3.48 | 3.45  | 3.38  | 3.39  | 3.33 | 3.48 | 4.04 | 4.12 | 3.96  | 3.52  | 3.46  | 3.49  | 3.46  |
| Abs520             | 1    | 6.88 | 7.45 | 7.98  | 7.64  | 7.57  | 7.82 | 7.85 | 7.42 | 7.40 | 7.43  | 7.72  | 7.70  | 7.53  | 7.80  |
|                    | 2    | 6.14 | 6.76 | 7.02  | 6.95  | 6.87  | 7.00 | 6.21 | 6.44 | 6.31 | 6.22  | 6.86  | 6.93  | 6.87  | 6.96  |
|                    | 3    | 6.11 | 6.30 | 6.51  | 6.86  | 6.51  | 6.87 | 6.07 | 6.13 | 6.14 | 6.10  | 6.27  | 6.32  | 6.30  | 6.75  |
| Abs620             | 1    | 1.53 | 1.60 | 2.53  | 1.89  | 1.88  | 2.37 | 1.73 | 1.55 | 1.57 | 1.61  | 2.70  | 2.41  | 2.32  | 2.64  |
|                    | 2    | 1.30 | 1.44 | 1.68  | 1.74  | 1.70  | 2.19 | 1.30 | 1.36 | 1.39 | 1.35  | 2.19  | 1.78  | 1.73  | 2.15  |
|                    | 3    | 1.22 | 1.44 | 1.43  | 1.72  | 1.66  | 2.11 | 1.20 | 1.33 | 1.37 | 1.31  | 1.65  | 1.72  | 1.62  | 2.12  |

*TK00: without SO2 sample, TK25: 25 mg/L SO2 added sample, GP01: 1 mL/L grape pomace extract, GP257: 25 mg/L SO2 with 0.7 mL/L extract, GP253: 25 mg/L SO2 with 0.3 mL/L extract, GP251: 25 mg/L SO2 with 1 mL/L extract, R01: 1 mL/L rosemary extract, R251: 25 mg/L SO2 with 0.7 mL/L rosemary extract, R253: 25 mg/L SO2 with 0.3 mL/L rosemary extract, R257: 25 mg/L SO2 with 0.3 mL/L rosemary extract, Bb01: 1 mL/L blueberry extract, Bb257: 25 mg/L SO2 with 0.7 mL/L blueberry extract, Bb253: 25 mg/L SO2 with 0.3 mL/L blueberry extract, Bb251: 25 mg/L SO2 with 1 mL/L blueberry extract.
the importance of GP, Bb and R treated wines as samples with high colour properties. Rosemary extract with high yellow colour (Y%) and tint value could be accepted as fast auto-oxidation additive after 3 months of storage. The study results demonstrated the possibility of using healthier, nonchemical additives during wine production.

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