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Changes in Color Parameters during Fermentation and Storage of Red Wine Using Thai Roselle under Different pHs and SO_2 Concentrations

Koki YOKOTSUKA,[1] Mizuo YAJIMA,[2] Tatsuji SEKI[3] and Mark MATTHEWS[4]

[1] *The Institute of Enology and Viticulture, Yamanashi University, Kofu, Yamanashi 400, Japan*
[2] *Asama Chemical Co., Ltd., 20-3, Nihonbashi-Kodenma-cho, Chuo-ku, Tokyo 103, Japan*
[3] *International Center of Cooperative Research in Biotechnology, Osaka University, Suita, Osaka 565, Japan*
[4] *Department of Viticulture and Enology, University of California, Davis, CA 95616, USA*

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A red wine was made from must consisting of dried roselle calyces, water, yeast extract, sugar, and $NH_4H_2PO_4$. The pHs of wine samples were adjusted to various values within the range 2.5 to 4.0, or SO_2 was added prior to yeast addition to give concentrations ranging from 0 to 250 mg/*l*, and the wines were stored at 25°C. After storage for 35 months, the pH of each wine was regularized, and the wines were then analyzed to determine their general composition and various red color parameters. There was little difference in general composition among the wines stored under different pHs and containing various concentrations of SO_2. There were, however, appreciable differences in color density, color hue, and polymeric pigment color, but little difference in the wine color measured at wine pH and at pH 0.25, among the wines stored under different pHs. On the other hand, there were noticeable differences in all the above color parameters, except for color hue, among the wines stored under various SO_2 concentrations. Wine color and anthocyanin color were more stable in wines stored at lower pHs or at lower concentrations of SO_2, whereas the degree of contribution of polymeric pigment to wine color (at pH<0.5) increased with increasing storage pH and was lowest in the wines to which 100 or 150 mg SO_2/*l* was added. Sensory analysis indicated that the addition of SO_2 at 100 mg/*l* when bottling imparted the best overall quality to the roselle wines stored under the various conditions tested.

Keywords: roselle red wine, color parameters, anthocyanins, pigments in wine

Roselle (*Hibiscus sabdariffa* L.) is very important in several tropical countries both as a medicinal plant and as a beverage coloring and flavoring additive (Leclerc, 1938; Sharaf, 1962; El-Merzabani *et al*., 1979; Perry, 1980; El-Shaveb & Mabrouk, 1984). In Thailand, dried roselle calyces are commercially available and are used as a raw material for red wine because of the contribution of their attractive red color and flavor to the wine. Shibata and Furukawa (1969) reported the presence of cyanidin-3-glucoside (Cn-3G), delphinidin-3-glucoside (Dp-3G), and delphinidin-3-sambubioside (Dp-3GX) as red pigments in the dried calyx and bract of roselle. Du and Francis (1973) identified cyanidin-3-sambubioside (Cn-3GX) as well as Cn-3G, Dp-3G, and Dp3GX in fresh roselle calyces. The color of red pigments such as anthocyanins in red wine is strongly influenced by the pH and the concentration of SO_2 (Somers & Evans, 1974, 1977; Jackson *et al*., 1978; Yokotsuka, 1995). The color of red wine made using roselle is labile and subject to rapid decolorization, but there has hitherto been no report on the stability of red pigments in the wine. The present study was conducted to determine the general composition of red wine made using roselle must consisting of water, roselle calyces, and sugar; the changes in total phenol and red pigment color during fermentation; and the effects of adjusting the pH and the amount of SO_2 added just prior to bottling on the composition and color of the wine.

Materials and Methods

Winemaking Dried roselle (calyces) was purchased at a market in Bangkok, Thailand, brought to Japan, and stored in vinyl bags at 4°C until use.

Sugar (20%, w/v), $NH_4H_2PO_4$ (0.05%, w/v), and yeast extract (0.10%, w/v) were dissolved in 24 *l* water. The solution was divided into 12, 2-*l* portions in 3-*l* glass carboys fitted with glass air locks, and dried roselle (3%, w/v) was added to each. One of three treatments was then instituted: potassium metabisulfite was added to give either 50 or 100 mg/*l* as free SO_2, or no potassium metabisulfite was added (SO_2 of 0 mg/*l*).

All the musts were allowed to stand overnight at room temperature. After the addition of SO_2, the total acidity of the 12 musts was 0.68% as H_2SO_4 equivalent, and their pHs ranged from 2.73 to 2.76. Active dry wine yeast of *Saccharomyces cerevisiae* W3 (2%, w/v) (Yokotsuka & Matsudo, 1992) was placed in warm water (30-35°C), and the suspension was allowed to stand for 2 h at room temperature. This suspension (5 ml) prepared just before use was added to each carboy with gentle stirring.

Fermentation was conducted at 25°C. Fermenting must samples (20 ml) were taken at appropriate time intervals and centrifuged at 31,000×g at 4°C for 20 min. The supernatant obtained was filtered through a 0.45-μm membrane filter. The absorbance of the filtrate at 520 and 420 nm was measured and analyzed for total phenols (Figs. 1 and 2). The fermenting

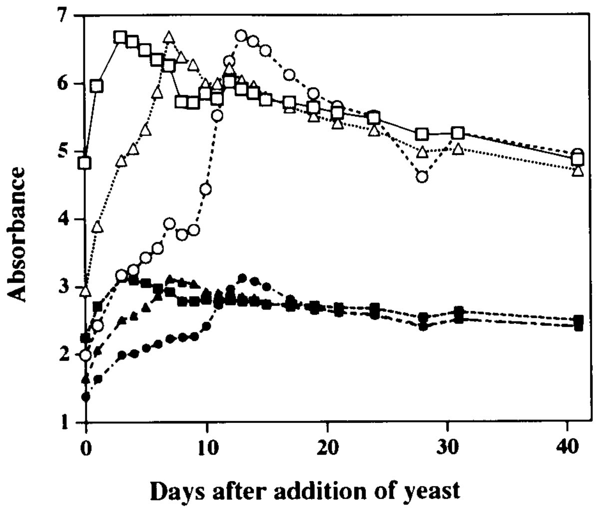

Fig. 1. Changes in absorbances at 520 nm and 420 nm during vinification of roselle red wine with various concentrations of added SO_2. □, A_{520} at 0 mg SO_2/l; △, A_{520} at 50 mg SO_2/l; ○, A_{520} at 100 mg SO_2/l; ■, A_{420} at 0 mg SO_2/l; ▲, A_{420} at 50 mg SO_2/l; ●, A_{420} at 100 mg SO_2/l.

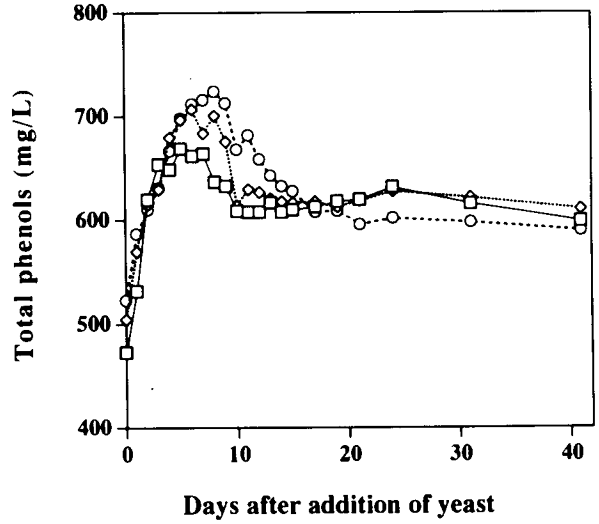

Fig. 2. Changes in total phenols during vinification of roselle red wine with SO_2 added in various concentrations of added SO_2. □, 0 mg SO_2/l; ◇, 50 mg SO_2/l; ○, 100 mg SO_2/l.

must was pressed with a small, hand-operated basket press made of oak wood a week after the beginning of fermentation (when the formation of carbon dioxide was observed through the air locks). The fermentation was continued until the specific gravity reached about 1.00 when measured with a hydrometer. It was then stopped by removing yeast cells by centrifugation at 12,000×g followed by filtration through a 0.8-μm membrane filter. The general composition of the wines was analyzed in terms of specific gravity, alcohol, total and volatile acids, reducing sugars, total and free SO_2, the absorbances at 520 and 420 nm, and total phenols immediately after filtration, according to the methods described by Amerine and Ough (1980).

In order to investigate the effects of SO_2 and pH on the stability of the red color in roselle wine during storing, 30 l of the same must previously described was used. An active dry yeast suspension of *Saccharomyces cerevisiae* W3 (15 g/50 ml), but no SO_2, was added to the must. Fermentation was conducted in a 50-l stainless-steel tank at 25°C, and the fermenting must was pressed as already described a week after the addition of the yeast (specific gravity, 1.027). The fermentation was stopped by removing yeast cells by centrifugation at 9,700×g with a type-H-600S centrifuge (Kokusan Enshinki Co., Ltd., Tokyo) equipped with a continuous-flow head (50 l/h) followed by filtration through a 0.8-μm membrane filter when the specific gravity of the fermenting must became 1.00. The yield of the finished wine was 29.1 l.

Storage of roselle red wine at different pHs and in the presence of various concentrations of SO_2 The finished wine was split into 20 aliquots of 1.44 l each. The pH of the finished wine, pH 2.81, was adjusted to various values ranging from 2.50 to 4.00 with aqueous KOH or HCl solution in the absence of SO_2. Because the volume of KOH or HCl solution added to the 1.44-l aliquots was only between 3 and 9 ml, the changes in the volumes of the wine samples were very small. Potassium metabisulfite was added to other aliquots to give concentrations of 144 mg (total SO_2 44 mg/l, measured one day after addition), 288 mg (84 mg), 432 mg (146 mg), or 720 mg (245 mg), or no potassium metabisulfite was added (SO_2, 0 mg/l). The wines of different pHs and with various concentrations of SO_2 were then bottled in 720-ml screw-cap glass bottles, stored in an air temperature-controlled room at 25°C, and analyzed for their general composition, pigment parameters, and sensory characteristics 35 months after the beginning of storage.

Phenolic and spectral analyses, and sensory evaluation Duplicate analyses of wine components were carried out, and the analytical values reported are the averages of the two samples. Total flavonoid and non-flavonoid phenols were determined according to the methods of Slinkard and Singleton (1977) and Kramling and Singleton (1969), respectively. Spectral analyses were carried out according to the methods of Somers and Evans (1974, 1977) and Yokotsuka (1995). Sensory evaluation of the wines was performed as described by Jackson *et al.* (1978).

Results and Discussion

Changes in wine color and total phenols during vinification of roselle red wine In grape must, fermentation generally begins within a few days after the addition of yeast if a normal dose of SO_2 is added, the must is allowed to stand overnight, and the yeast is then added. In the case of the roselle must, fermentation began one day after the addition of yeast without SO_2, but not until after 7 days with 50 mg added SO_2/l and after 11–12 days with 100 mg added. This may be because the roselle must contained only small amounts of compounds, such as acetaldehyde, pyruvic acid, and α-ketoglutaric acid, which normally react with SO_2 to form bisulfite complexes (Burroughs & Whiting, 1960), and hence, the added SO_2 left in free form probably suppressed the growth of yeast cells.

The absorbance of the fermenting musts at 520 nm increased with time after the addition of roselle calyces to the medium consisting of water, yeast extract, sugar, and

$NH_4H_2PO_4$ (Fig. 1). The maximum values of the absorbance at 520 nm were obtained a few days after the beginning of fermentation, after which the absorbance gradually decreased. The trend in the changes in the absorbance at 420 nm was similar to that at 520 nm, although the day on which the maximum values occurred slightly differed depending on the concentration of SO_2 added to the must before the addition of yeast. The maximum absorbance at 520 nm was delayed by SO_2 addition; the higher the SO_2 concentration, the longer the delay, accompanied by a slight drop in the maximum values (Fig. 1). A larger amount of SO_2 also delayed the maximum total phenols (Fig. 2), but the days when the maximum total phenol values occurred did not coincide with those for the maximum absorbances at 520 nm. The maximum values of total phenols were higher in the musts with a higher dose of SO_2. These results indicated that SO_2 bleached the red pigments extracted from roselle calyces, although it also helped release the pigments from the tissues.

Changes during storage in the general composition of roselle red wine with different pHs and different concentrations of SO_2 The above results showed that the addition of SO_2 to the must greatly delayed the start of fermentation. Because no spoilage of roselle wines made from the musts without SO_2 occurred during the vinification and storage processes (data not shown), roselle red wine was made without adding SO_2 to the must in order to investigate the effects of pH and SO_2 on the stability of the red pigments during storage. The pH and the absorbances at 520 and 420 nm of the red wine produced were 2.72, and 14.92 and 6.79, respectively.

Wines of different pHs (2.50 to 4.00) in the absence of SO_2 and with different concentrations of added SO_2 (0 to 250 mg/l) at the same pH (2.72) were stored at 25°C, and their general composition was analyzed 35 months after the beginning of storage. The results are shown in Table 1. A high storage temperature (25°C) was used to promote changes in the red pigments during a relatively short period. Little differences in specific gravity, alcohol, volatile acid, and reducing sugar were observed between the wines when bottled and after storage for 35 months. However, among the wines in which the pH was adjusted, the pH values of the two wines with the highest pHs prior to storage changed from 3.50 to 3.38 and 4.00 to 3.82, respectively. In the case of SO_2 treatment, when a higher amount of SO_2 was added, a slightly higher volatile acid content was found. When less than 150 mg/l of SO_2 was added at bottling, wines stored for 35 months had very little free SO_2. The addition of 150 mg/l of SO_2 gave 16 mg/l of free SO_2 in the stored wine, which is a common concentration of free SO_2 in grape wine.

Changes during storage in color parameters of roselle red wines of different pHs and with different concentrations of SO_2 Red wine color at wine pH is due to anthocyanins and oligomeric and polymeric pigments (Somers & Evans, 1977; Jackson *et al.*, 1978; Yokotsuka, 1995). As red wine ages, the oligomeric and tannin-pigment polymers or polymerized compounds increase. The addition of SO_2 to finished wine results in temporary color reduction by a reversible reaction between SO_2 and the carbon 4 of a colored flavylium ion of anthocyanin to form colorless anthocyanin-4-bisulfite (Jurd, 1964). On the other hand, it prevents the formation of insoluble polymeric pigments due to oxidative browning reactions or polymerization during aging. The effect of SO_2 on the red pigments or color parameters of the roselle wines was thus investigated (Table 2).

Red wine color (WC, A_{520}) at each wine pH and at pH 3.70 (the pH of all the wines was adjusted to the same pH for comparison) and color density (CD, $A_{520}+A_{420}$) at wine pH of the stored wines decreased with increasing pH among the wines of different pH and also among the wines with different SO_2 concentrations (Table 2). As expected, the wine color at pH 0.25 (WCA, A_{520}) and total phenols increased with increasing SO_2 concentration. From these results, it was concluded that SO_2 undoubtedly helps protect red pigments and other flavonoid phenolics in wine by preventing the formation of insoluble complexes due to oxidative polymerization. Thus, wine color (WC) and polymeric pigment color (PPC, A_{520} at wine pH in the presence of excess SO_2) significantly decreased with an increase in SO_2 among the wines with different SO_2 concentrations. The bisulfite anion reacts with the colored anthocyanin cation to form a colorless

Table 1. General composition of roselle red wines.

	pH	Specific gravity	Alcohol (%, v/v)	Total acid (as H_2SO_4, g/100 ml)	Volatile acid (as acetic acid, g/100 ml)	Reducing sugar (as glucose, g/100 ml)	Total SO_2 (mg/l)	Free SO_2 (mg/l)
pH of wine at the beginning of storage[a]								
2.5	2.51	1.002	10.9	1.07	0.057	1.48	—	—
2.8	2.79	1.002	10.6	0.93	0.057	1.49	—	—
3.0	2.95	1.002	10.5	0.86	0.058	1.49	—	—
3.5	3.38	1.002	10.6	0.64	0.055	1.48	—	—
4.0	3.82	1.002	10.7	0.47	0.057	1.44	—	—
SO_2 (mg/l) added at the beginning of storage[b]								
0	2.79	1.002	10.6	0.93	0.057	1.48	0	0
50	2.81	1.002	10.5	0.93	0.058	1.43	3	1
100	2.79	1.002	10.6	0.95	0.064	1.38	43	3
150	2.79	1.002	10.6	0.95	0.066	1.41	88	16
250	2.79	1.002	10.4	0.96	0.073	1.41	193	72

The wines were analyzed after storage at 25°C in the dark for 35 months.

[a] The pH was adjusted to a value between 2.5 and 4.0 just before bottling and storage after fermentation at 25°C using roselle must with a pH of 2.81 and without SO_2.

[b] Various amounts of SO_2 (as solid metabisulfite) were added to wine with a pH of 2.79 just before bottling and storage.

Table 2. Spectral and phenolic analyses of roselle red wines.

	pH of wine[a]					Concentration of SO_2 (mg/*l*)[b] added to wine of pH 2.8				
	2.5	2.8	3.0	3.5	4.0	0	50	100	150	250
Measured 35 days after the addition of yeast										
WC[c] (A_{520} at wine pH)	6.34	5.74	5.23	3.76	2.55	5.63	2.35	1.76	1.51	1.31
CD[d] ($A_{520}+A_{420}$ at wine pH)	9.09	8.34	7.72	5.94	4.50	8.23	4.06	3.32	2.99	2.73
CH[e] (A_{420}/A_{520} at wine pH)	0.43	0.45	0.48	0.58	0.76	0.46	0.73	0.89	0.99	1.08
Measured 35 months after the addition of yeast										
WC (A_{520}) at wine pH	1.90	1.73	1.62	1.49	1.49	1.73	1.83	1.75	1.53	1.09
WC (A_{520}) at pH 3.7	1.68	1.50	1.40	1.42	1.42	1.50	1.60	1.42	1.28	1.08
CD ($A_{520}+A_{420}$ at wine pH)	4.05	3.81	3.63	3.47	3.47	3.81	3.92	3.68	3.21	2.38
CH (A_{420}/A_{520} at wine pH)	1.13	1.20	1.24	1.33	1.33	1.20	1.14	1.10	1.10	1.18
PPC[f] (A_{520} at wine pH)	1.24	1.14	1.12	1.18	1.24	1.14	1.18	0.90	0.82	0.66
WCA[g] (A_{520} at pH<0.5)	2.00	1.86	1.80	1.70	1.78	1.86	1.95	2.04	2.22	2.44
α[h] (A_{520} at pH 3.7/WCA)	84	81	78	84	80	81	82	70	58	44
AC[i] (WC at wine pH−PPC)	0.66	0.59	0.50	0.31	0.25	0.59	0.65	0.80	0.71	0.43
CAW[j] (PPC/WC at wine pH)	0.65	0.66	0.69	0.79	0.83	0.66	0.64	0.51	0.54	0.61
Total phenols (mg GAE[k]/*l*)	605	611	657	624	619	611	667	664	701	731
Flavonoid (mg GAE/*l*)	4	35	75	48	43	35	44	50	56	121
Non-flavonoid (mg GAE/*l*)	601	576	582	576	576	576	623	614	645	610

[a,b] See footnote of Table 1. [c] Wine color. [d] Color density. [e] Color hue. [f] Polymeric pigment color. [g] Wine color in acid (at pH 0.25). [h] Degree of pigment coloration. [i] Anthocyanin color. [j] Chemical age at wine pH. [k] Gallic acid equivalent.

species, anthocyanin-4-bisulfite (Jurd, 1964). Because the carbon 4 of anthocyanin-4-sulfite is involved in bond formation between anthocyanin and other flavonoids, such sulfites are less reactive, and the formation of polymeric pigments might thus be reduced.

Color hue (CH, A_{420}/A_{520} at wine pH), a measure of wine browning, increased with increasing pH among the wines of different pH, but did not appreciably vary among the wines with different SO_2 concentrations. The color hues of the wines in the SO_2-treated group ranged from 1.10 to 1.20. These values are similar to those of aged or over-aged grape wines (Somers & Evans, 1977; Yokotsuka, 1995). The higher color hue observed in the SO_2-treated wines soon after vinification (35 days after the addition of yeast) may be due to a reduction of the A_{520} by the temporary decolorization of red pigments.

The degree of coloration of total wine pigments at pH 3.7 (α) (Somers & Evans, 1974, 1977; Timberlake, 1982; Bakker & Timberlake, 1986) did not vary in the wines stored under different pHs, but decreased with increasing SO_2 in the SO_2-treated wines. The degree of coloration was strongly correlated with the concentration of free SO_2.

Two color parameters, the chemical age at wine pH (CAW, $A_{520}^{SO_2}/A_{520}^{pH<0.5}$) (Somers & Evans, 1974, 1977) and the anthocyanin color (AC), are related to the contribution of polymeric pigments or anthocyanins to wine color. With increasing pH in the wines, AC decreased, but CAW increased. This means that the contribution of polymeric pigments to wine color increased with increasing storage pH. On the other hand, after storage for 35 months in the presence of various concentrations of SO_2, the lowest CAW and highest AC values were obtained at 100 mg SO_2/*l*.

The main stability problem with phenols, including red pigments in red wine, is directly attributable to oxidation problems. In general, a lower pH and higher SO_2 make wine less liable to oxidation because the oxidation reaction of the phenols proceeds more rapidly at a higher pH, and oxygen in wine is reduced by SO_2. In the present work, as expected, the total, flavonoid and non-flavonoid phenols increased with increasing SO_2 in the SO_2-treated wine. In contrast, in the wines with pHs of 3.0, 3.5, and 4.0 at the beginning of storage, the total and flavonoid phenols decreased with increasing pH. The wines with pHs of 2.5 and 2.8 had less total and flavonoid phenols than those with higher pHs (3.0, 3.5, and 4.0). Although no definite explanation for this can be advanced, it may be related with the finding that grape seed polymeric tannins remained less soluble in a wine-like model solution (10% ethanol) with a pH of 2.5 than in solutions with higher pHs (3.0 to 4.5) (Yokotsuka & Singleton, 1987, 1995). The degree of polymerization of phenols in the five wines with different pHs is thought to have been rather high; the proportion of polymeric pigments in the wine color (WC) at wine pH reached 65 to 83%, with an average of 72% (Table 2).

Table 3. Taster's scores for roselle red wines.

	Color (0–5)	Aroma (0–5)	Flavor (0–10)	Overall quality (0–20)
pH of wine at the beginning of storage[a]				
2.5	3.3	2.6	3.5	9.4
2.8	3.4	2.6	3.5	9.5
3.0	3.0	2.6	3.6	9.2
3.5	2.7	2.6	3.7	9.0
4.0	2.6	2.6	2.9	8.1
SO_2 (mg/*l*) added at the beginning of storage[b]				
0	3.4	2.6	3.5	9.5
50	3.0	2.8	3.5	9.3
100	3.4	2.9	3.7	10.0
150	3.2	2.6	3.3	9.1
250	2.6	2.2	3.3	8.1

Color (5 good, 3 fair, 0 poor); Aroma (5 good, 3 fair, 0 poor); Flavor (10 complex/good, 5 not deficient/fair, 0 flat/poor); Overall quality (20 excellent, 15 good, 10 fair, 5 poor).
[a,b] See footnote of Table 1.

Quality rating Somers and Evans (1974, 1977) reported strong correlations between color density and quality ranking in young Australian wines. Also, in comparative studies of young and aging red wines made from Cabernet Sauvignon and Muscat Bailey A grapes in Japan, Yokotsuka (1995) found that visual assessment of color significantly correlated with taster assessment of overall quality and with instrumental measurements of pigment parameters at wine pH, such as wine color, color density, and polymeric pigment color. In the roselle wines adjusted to different pHs from 2.5 to 4.0 or to which different amounts of SO_2 from 0 to 250 mg/*l* were added when bottled, there was always a good relationship [correlation coefficient (r)=0.863, $p<0.10$, for the 5 wines stored under different pHs; $r=0.869$, $p<0.10$, for the 5 wines stored with different concentrations of SO_2] between taster assessment of overall quality (color+flavor+aroma) (Table 3) and anthocyanin color [AC (anthocyanin color)=WC (wine color at wine pH)−PPC (polymeric pigment color at wine pH)] (Tables 2 and 3). There was also a good correlation ($r=0.859$, $p<0.10$, for the 5 wines stored under different pHs; $r=0.918$, $p<0.05$, for the 5 wines stored with different concentrations of SO_2) between visual assessment of color and overall quality. Red wine color is influenced by pH and SO_2: the higher the pH and the higher the SO_2 concentration under which the red wines are stored, the lower the intensity of the red color. Instrumental measurement of color parameters is, therefore, very important in investigating the effects of pH and SO_2 on wines during storage. In the present work, spectral analysis of pigment parameters and visual assessment of red wine color significantly correlated with taster assessment of overall quality.

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