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Antimicrobial Treatment of Grapes Using Sodium Hypochlorite in Winemaking and Its Effects on the Chemical and Sensory Characteristics of Wines

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This study was performed to examine the use of NaOCl as an alternative antimicrobial compound in winemaking because of the potential health problems that may arise as a result of the use of SO_2. For this, the blank (non-treated), control (SO_2-added), and sample (NaOCl-treated) wines were made, and microbial and chemical changes including sensory characteristics were analyzed during the fermentation periods. Treatment of grapes with NaOCl decreased the initial contaminating microbial population in grape must, resulting in higher growth of yeast and lactic acid bacteria. After 200 days of fermentation, the chemical analysis of sample wine revealed that it had higher ethanol content, redness (*a), and concentrations of fruity ester compounds and lower total acidity than the control. In the sensory analyses, the sample wine obtained a higher overall acceptability score (5.70) than the control (4.26). This result reveals that NaOCl can be used as an alternative to SO_2 in winemaking for inhibiting the growth of contaminating microorganisms.**

Keywords: Wine, sulfur dioxide, sodium hypochlorite, antimicrobial, sensory characteristic

Sulfur dioxide (SO_2) is the main preservative used in vinification to protect wine from alterations utilizing antimicrobial and antioxidant activities that inhibit non-enzymatic as well as enzymatic browning [25, 26]. SO_2 has long been used in winemaking to prevent the growth of undesirable microorganisms (wild yeast, acetic and lactic acid bacteria) and to inhibit oxidation [28]. However, because of the potential health problems that may arise as a result of the use of SO_2, its use in wine has recently come under review. SO_2 has been associated with allergic reactions and food intolerance symptoms [5, 8, 10]. The FDA establishes levels of 10 mg/l sulfite as that which must be indicated on the label and also does not permit the use of this compound in meat in the USA [11]. The upper limit of SO_2 allowed in wine in the USA is 350 mg/l, whereas in Europe, it is 160 mg/l in red wine and 210 mg/l in white or rose wines [10]. Winemakers are, therefore, seeking an effective substitute for the antimicrobial action of SO_2 in winemaking.

Various substances and methods were tested to inhibit the growth of spoilage microorganisms and antioxidants in wine, such as natural substances, chemical substances, and engineering technologies. The natural substances, such as nisin (bacteriocin), are used against food spoilage bacteria. A previous study showed that nisin and metabisulfite have a synergistic inhibitory effect on lactic acid bacteria (LAB) growth [32]. Lysozyme was used to prevent heterolactic fermentation during the biological aging of red wine; its use in winemaking may inhibit *Lactobacillus* species during alcoholic fermentation, reducing the risk of increased volatile acidity and delaying or blocking the onset of malolactic fermentation (MLF) [18]. Phenolic compounds are natural constituents of grapes and wines. It inhibited the growth of spoilage microorganisms and antioxidants in wine [14, 35]. In addition, organic acids are naturally found in a variety of fruits and fermented foods. The antimicrobial activity of organic acids was shown against Gram positive and negative bacteria, but the inhibition of microorganisms by organic acids depends upon several factors such as pH, acid, chain length, cell physiology, and metabolism [2]. The chemical substance dimethyl dicarbonate may be an effective chemical for inhibition of microorganisms in wine as a replacement for SO_2. Sorbic acid was also used as an antimicrobial and antioxidant in the wine industry [27]. As a physical technology, thermal processing was used in grape juice [15]. Pulsed electric field is one of the most novel technologies used for pasteurization because it can inactivate pathogenic and spoilage microorganisms [22, 29]. Ultrahigh pressure

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can impact the life processes of living bacterial cells, thus causing the bacteria to die [4]. Ultraviolet irradiation affected the initial microbial counts in wine [13]. High-power ultrasound uses frequencies in the range of 20 to 100 kHz and has the ability to cause the formation and collapse of high-energy micro-bubbles and can be used in food processing to inactivate microbes [4]. Moreover, physical removal of microorganisms through filtration of juice or wine can also be used. However, further research is necessary to know in more detail the effect of those treatments on the sensorial properties of the wine, and to validate the applicability of those technologies in wineries.

Recently, consumption of fresh produce has increased mainly because of heightened awareness of the benefits of a healthy diet. However, fresh-cut surfaces discharge nutrients, which can be used for growth of microorganisms, and pathogens have been shown to attach to fruit skin, leaves, and internal tissue [17]. Previously, a study revealed that unwashed cilantro (plant leaf) shows typically high initial microbial loads (aerobic mesophilic bacteria, 7.00 ± 0.12 log CFU/g; yeast, 4.57 ± 0.37 log CFU/g) [3]. For the surface-sterilization of food substances, sodium hypochlorite (NaOCl) is widely used as a disinfecting agent. NaOCl is widely used in the food industry to kill bacteria and disinfect. The KFDA establishes the level of 200 mg/l NaOCl for disinfecting fruits and vegetables [18]. When used in concentrations of 5.25%, NaOCl presents high antimicrobial activity against aerobic microorganisms such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans* [34].

Accordingly, this study was carried out to investigate the effectiveness of NaOCl as an alternative antimicrobial agent to SO_2 in red winemaking. For this, grape berries were soaked in NaOCl solution, and after washing, wine was made and microbial and chemical changes, including sensory characteristics of sample wine (NaOCl-treated), were compared with the blank (non-treated) and control (SO_2-added) wines during fermentation periods.

Materials and Methods

Materials

The *Campbell Early* grapes used in this study were harvested in 2008 in the Cheongwon-gun, Chungbuk Province, Republic of Korea. Sucrose was added in grape must and it was purchased from CJ (Seoul, Korea). Commercial yeast [*Saccharomyces cerevisiae* (*S. cerevisiae*), Lalvin K1-V1116, Lallemand, Montreal, Canada] was used as a starter culture. For microbial growth, trypticase soy broth, yeast extract, YM, and MRS media were purchased from Difco (Sparks, MD, USA) and sheep blood was from Hanil Komed (Seongnam, Korea).

Antimicrobial Activity of NaOCl on Grapes

To examine the antimicrobial activity of NaOCl on grape skin, 300 g of grape berries were soaked in 100–200 mg/l NaOCl solution for different time periods. The number of microbial cells that survived was counted on TSA medium (4% trypticase soy broth + 2% agar + 5% sheep blood).

Winemaking

For the red wine production, the destemmed and crushed grape berries (10 kg each) were divided into different batches (blank and control) and placed in a 10 l fermenter. For blank (non-treated) wine, sucrose was added to make 24 °Brix of grape must, and fermentation was initiated by adding a rehydrated inoculum of commercial *S. cerevisiae* in a proportion of 10^7 CFU/ml. Primary (alcoholic) fermentation was performed for 7 days at 25°C with 3 daily pumpings. After removing skins and seeds by cotton filtering, MLF was performed for another 28 days at 20°C. After cold stabilization (7 days at 0°C), the wines were transferred to 0.75 l bottles, which were stoppered and stored at 15°C for 4 months until analysis [21, 31]. For preparation of control wine, 100 mg/l SO_2 was added to the must and the other steps of winemaking were the same as for those of the blank wine. For sample wine production, 10 kg of grapes was soaked in 200 mg/l NaOCl solution for 30 min, washed with enough water, and the next steps for winemaking were the same as those for the blank wine.

Microbiological Analysis

Samples were serially diluted (10^{-0}, 10^{-1}, 10^{-3}, and 10^{-5}) with sterile physiological saline (NaCl 0.85%), and 0.05 ml of each of the diluents was spread onto 2 different media: YM and MRS media. YM was used to cultivate yeast on yeast extract agar at 30°C for 48 h. MRS agar was used to cultivate LAB at 30°C for 48–72 h [16].

Chemical Analysis

Alcohol concentration was measured by densitometry at 15°C after recovering the alcohol fraction using a distiller. Wine pH was measured with a pH meter (IQ 240; IQ Scientific Instruments, San Diego, CA, USA). Soluble solid concentration (°Brix) was measured using a hand refractometer (ATAGO, Tokyo, Japan). Organic acid contents were measured by high performance liquid chromatography (HPLC) (TSP US/Spectra system, San Jose, CA, USA) with an Aminex HPX87-H column (I.D., 300 × 7.8 mm; Bio-Rad, Richmond, USA); the flow rate of 0.008 N H_2SO_4 was 0.6 ml/min. Color components of the *a** (redness) value of red wines were measured using a Chroma Meter CR-400 (Minolta, Osaka, Japan). Every measurement was performed in triplicate, and the average values are reported.

Volatile Compounds Analysis

Volatile compounds were isolated from the wine samples, using a Head-Space Auto Sampler (Agilent 7694E, Agilent Technologies, Santa Clara, CA, USA) [19, 23]. Wine (10 ml) and the internal standard (4-methyl-2-pentanol, 50 µl) were added to a 20 ml vial, and volatile compounds were isolated at 80°C for 30 min. The injection loop temperature was 90°C and the transfer line temperature was 100°C. Gas chromatography–mass spectrometry (GC–MS) analysis was carried out using a Hewlett-Packard 6890N Network gas chromatograph coupled to a Hewlett-Packard 5973 quadrupole mass spectrometer equipped with a DB-FFAP fused silica capillary column (I.D., 30 m × 0.25 mm; film thickness, 0.25 µm; J&W Scientific Inc., Folsom, CA, USA). The carrier gas was ultrapure helium with a flow rate of 1 ml/min, and the pressure was set at 7.5 kPa. The

oven temperature was programmed from 50°C (5 min) to 150°C at 2°C/min, 230°C at 3°C/min. The injector and transfer lines were heated to both 250°C and 280°C. The ionization voltage applied was 70 eV, and mass spectra were obtained in a scan range from 40 to 350 m/z. All mass spectra were compared with the library database (Wiley 275) [6].

Sensory Evaluation

The sensory characteristics of wines were evaluated by 23 panels. The panels were faculty members and students of the Department of Food Science and Technology, Chungbuk National University, comprising 11 women and 12 men, aged 20 to 40 years. The preferences for aroma, color, sweetness, tartness, astringency, and overall acceptability were determined using a 9-point hedonic scale. Wine samples were stored at 10°C and presented at 15°C for the detection of odor and aroma. Each wine was coded with a 3-digit random number and 20 ml of wine in glasses were presented in random order to the panel.

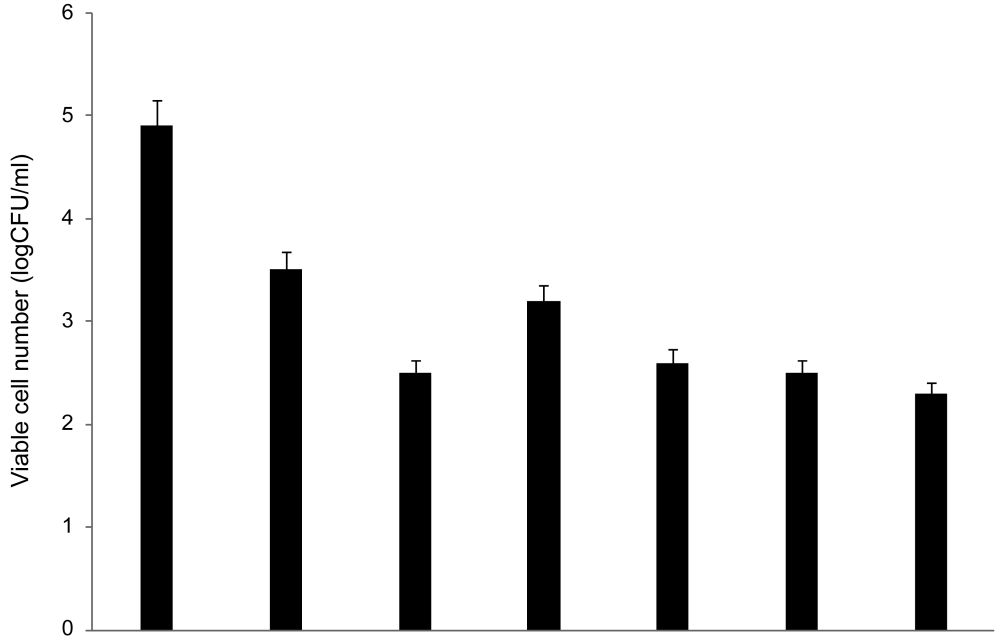
Statistical Analysis

All GC and sensory data were statistically analyzed using the SPSS Version 12.0K statistical package for Windows (SPSS, Chicago, IL, USA). Descriptive data were analyzed by mixed model one-way analysis of variance (ANOVA) to determine the effects of wine and judge [21].

RESULTS AND DISCUSSION

Antimicrobial Activity of NaOCl on Grapes

To examine the antimicrobial activity of NaOCl on grape skin and to determine the optimal treatment condition, grapes were soaked in 100–200 mg/l NaOCl solution for different time periods, and the viable cell numbers were compared with the control case in which grapes were soaked in 100 mg/l SO_2 solution (Fig. 1). When total microbes in 1 ml of raw grape must were counted, 4.9×10^4 viable cells were detected. After washing grape berries with tap water, the viable cell number reduced to 3.5×10^3, and after soaking in 100 mg/l SO_2, it reduced to 2.5×10^2. Meanwhile, when grape berries were soaked in 100 mg/l NaOCl solution for 5 and 30 min, viable cell numbers reduced to 3.2×10^3 and 2.6×10^2, respectively. When 200 mg/l NaOCl was used for 5 and 30 min, the cell counts reduced to 2.5×10^2 and 2.3×10^2, respectively. These results show that soaking grape berries in 200 mg/l NaOCl solution for 30 min provides the same sterilization effect as that achieved with adding 100 mg/l SO_2 in grape must. Remaining chlorine concentrations were reduced to 0.05–0.21 mg/l after washing with enough tap water. Accordingly, in the next experiment,



Chemical concentrarion (mg/l)	Blank[a]	H_2O[b]	SO_2	NaOCl			
	.	.	100	100	100	200	200
Time (min)	.	.	60	5	30	5	30
Viable cell numbers (log CFU/ml)	4.9	3.5	2.5	3.2	2.6	2.5	2.3
Remaining chlorine (mg/l)	.	.	.	0.05±0.003	0.15±0.003	0.16±0.01	0.21±0.015

Fig. 1. Changes of microbial cell numbers after various treatments on grapes or must and their remaining chlorine concentration after washing.
[a]Blank, non-treated. [b]H_2O, washed with tap water.

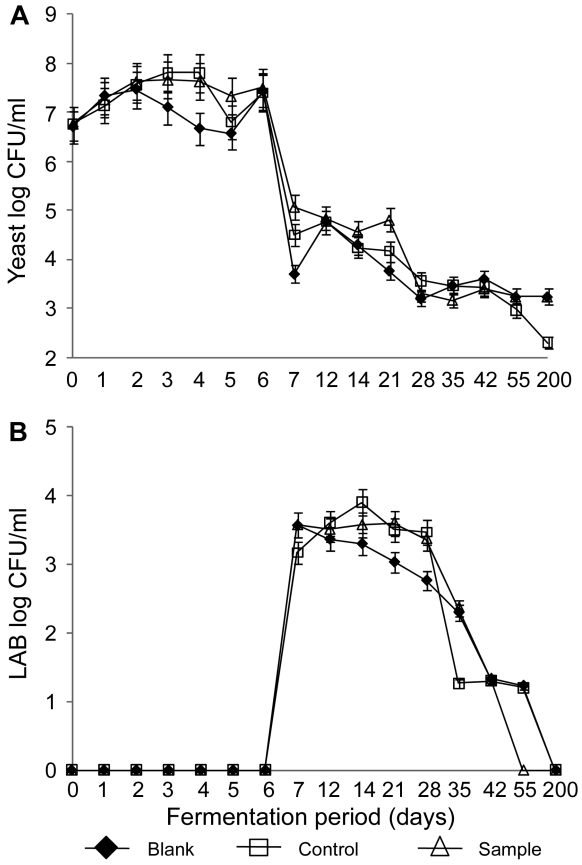


Fig. 2. Population changes in yeast (**A**) and lactic acid bacteria (**B**) during wine fermentation.

grapes were treated with 200 mg/l NaOCl solution for 30 min to sterilize contaminating microorganisms.

Microbial Changes During Wine Fermentation

The blank, control, and sample wines were prepared as described in Materials and Methods, and microbial changes were monitored during the fermentation and incubation periods. As shown in Fig. 2, dynamic changes in the microbial population were observed in the wine batches, but with slightly different patterns. In detail, yeast cell numbers increased for 7 days, decreased suddenly after the press-filtering operation, and decreased slowly during the following incubation period (Fig. 2A). Although yeast made a vivid growth for 7 days in the control and sample wines, its growth was retarded in the blank wine. Considering the antimicrobial activities of SO_2 and NaOCl, this result can be explained in that sterilization of contaminating bacteria using those chemicals provided a better condition for yeast growth in the control (SO_2-added) and sample (NaOCl-treated) wines. In the case of LAB, they started to grow from the 7th day of fermentation, and different rates of decline were observed after the 28th day, depending on wine samples (Fig. 2B).

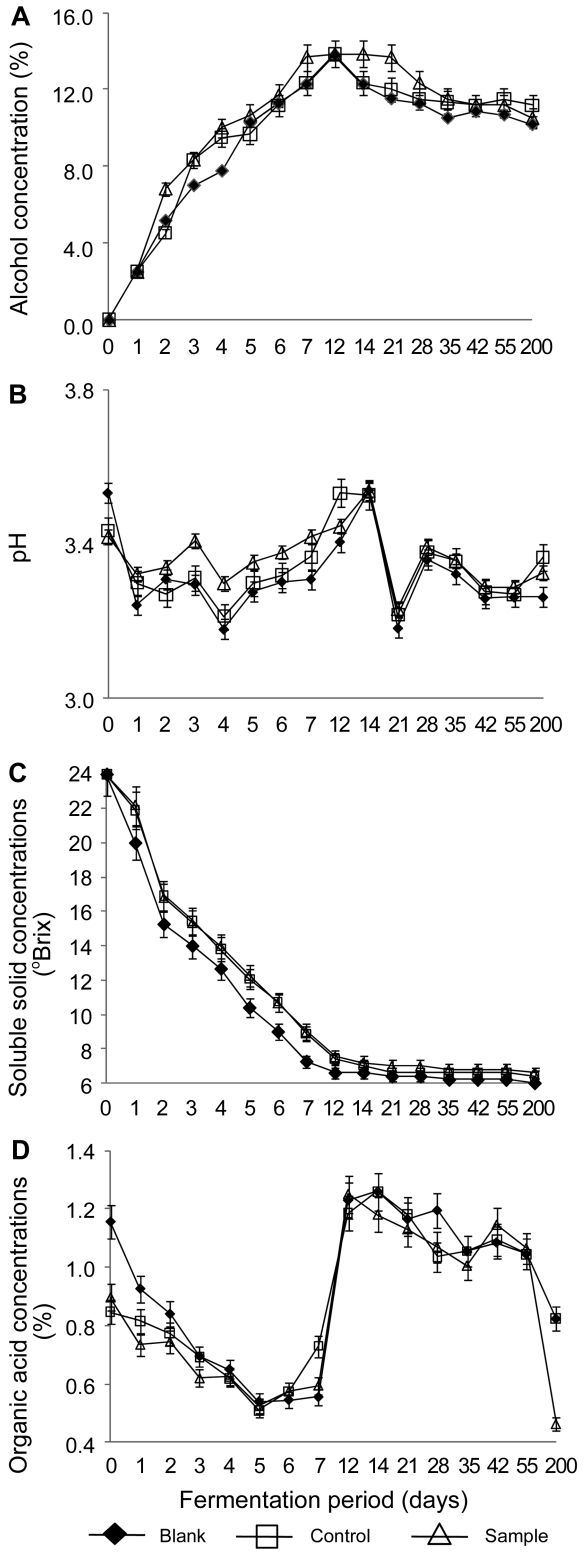


Fig. 3. Time courses of changes in alcohol (**A**), pH (**B**), soluble solids (**C**), and organic acid contents (**D**) during the fermentation of studied wines.

Blank, non-treated wine; Control, 100 mg/l SO_2-added wine; Sample, wine made by soaking grapes in 200 mg/l NaOCl solution for 30 min before crushing.

Changes in General Wine Characteristics During Wine Fermentation

The chemical changes in the 3 wines during fermentation are shown in Fig. 3, which presents alcohol concentrations (%), pH, soluble solid concentrations (°Brix), and organic acid concentrations (%). As shown in Fig. 3A, the alcohol concentration gradually increased during alcoholic fermentation (7 days), and the sample wine showed a higher alcoholic level (13.5%) than the control wine (12.5%). In Fig. 3B, the pH of the 3 wines changed between 3.2 and 3.6 with similar patterns, but the blank wine showed a lower pH (3.26) than the control (3.38) and sample (3.33) wines after 200 days. The initial sugar contents of the blank, control, and sample wines were 24 °Brix as shown in Fig. 3C, and they rapidly decreased for 7 days during alcoholic fermentation until they reached constant levels (6.0, 6.4, and 6.6 °Brix, respectively). In Fig. 3D, the organic acid compositions show generally the same trend of changes for 42 days; however, the sample wine shows lower acid content (0.46%) than the control wine (0.82%) after 200 days.

The red color intensities (a*) of the 3 wines are shown in Fig. 4. The color changed rapidly before the filter-pressing on the 7th day, and thereafter, the a* values of the control and sample wines stabilized at higher levels than that of the blank wine. The reason for the greater redness intensity of the sample wine is not clear, but regarding the role of SO_2 to stabilize the wine color [10, 33], it can be said that NaOCl played the same role as SO_2.

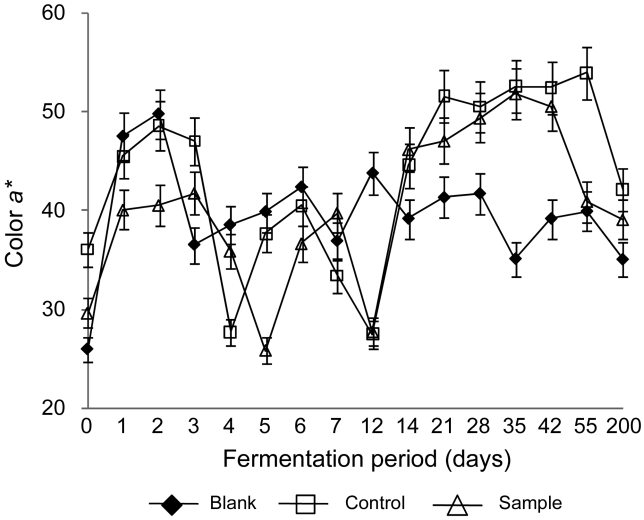


Fig. 4. Time courses of changes in red color (a*) during fermentation.
Blank, non-treated wine; Control, 100 mg/l SO_2-added wine; Sample, wine made by soaking grapes in 200 mg/l NaOCl solution for 30 min before crushing.

Volatile Compounds Analysis

Wine aroma is an important factor that contributes to wine quality. Esters and higher alcohols are produced during alcoholic fermentation. Table 1 shows the presence of volatile compounds in the 3 wine samples after 55 days of fermentation. In all, 8 volatile compounds were identified by GC–MS analysis, including 5 esters (ethyl acetate, ethyl propanoate, ethyl butanate, ethyl pentanoate, and ethyl hexanoate) and 3 higher alcohols (isoamyl alcohol, isobutyl alcohol, and phenylethyl alcohol). When their total concentrations were compared, the control and sample wines revealed significantly higher levels of esters and higher alcohols than the blank; for blank, control, and sample wines, 86.27, 145.89, and 174.30 mg/l in esters, respectively, and 162.62, 183.45, and 194.65 mg/l in higher alcohols, respectively.

Table 1. General composition of volatile compounds of the 3 studied wines[a].

Volatile compounds (mg/l)		Molecular formula	Blank[b]		Control[c]		Sample[d]		Aromas Reference [9]
			Mean	SD	Mean	SD	Mean	SD	
Esters	Ethyl acetate	$C_4H_8O_2$	61.93	1.12	115.99	1.95	141.08	4.91	Fruity, vinegar
	Ethyl propanoate	$C_5H_{10}O_2$	.	.	.	.	1.31	0.12	Apple, banana
	Ethyl butanoate	$C_6H_{12}O_2$	10.34	0.02	11.93	0.59	13.92	0.60	Fruity
	Ethyl pentanoate	$C_7H_{14}O_2$	0.97	0.13	1.10	0.02	1.11	0.10	Apple
	Ethyl hexanoate	$C_8H_{16}O_2$	13.03	0.54	16.87	0.64	16.88	0.75	Fruity
	Total esters		86.27		145.89		174.30		
Higher alcohols	Isoamyl alcohol	$C_4H_{10}O$	154.44	2.44	175.20	3.09	187.21	6.71	Grass
	Isobutyl alcohol	$C_5H_{12}O$	6.79	0.06	8.25	0.05	6.69	0.25	Ethereal
	Phenylethyl alcohol	$C_8H_{10}O$	1.39	0.40	.	.	0.75	0.25	Rose, sweet
	Total higher alcohols		162.62		183.45		194.65		

[a]Compounds are listed in order of elution from the DB-FFAP fused silica capillary column.
[b]Blank, non-treated wine.
[c]Control, 100 mg/l SO_2-added wine.
[d]Sample, wine made by soaking grapes in 200 mg/l NaOCl solution for 30 min before crushing.

Various ester compounds can be synthesized *via* the esterification of alcohols and acetyl-CoA in yeast, and generally, they are known to give a fruity aroma: ethyl acetate (fruity and vinegar), ethyl propanoate (banana and apple), ethyl butanoate (fruity), ethyl pentanoate (fruity), and ethyl hexanoate (fruity) [1]. Ethyl acetate is known to be produced by yeast and LAB as a secondary product of sugar metabolism during alcoholic fermentation as well as MLF [23]. In the sample wine, ethyl propanoate was detected as an additional ester compound.

Among the volatile aromas, higher alcohols such as isoamyl alcohol, isobutyl alcohol, and phenylethyl alcohol were detected in the 3 wines. Higher alcohols are formed *via* the amino acid metabolism of yeast during fermentation and they do not change much during incubation [30]. The concentrations of isoamyl alcohol were relatively higher (154.44, 175.20, and 187.21 mg/l) in the 3 samples. Generally, *Saccharomyces* are good producers of isoamyl alcohol [15]. Isobutyl alcohol (ethereal odor) was detected in the narrow range of 6.69−8.25 mg/l in the 3 wines. Small amounts of phenylethyl alcohol were also detected in the blank and sample wines (1.39 and 0.70 mg/l, respectively).

Indeed in sensory tests, the control and sample wines achieved significantly higher aroma preference scores than the blank wine, and this result was consistent with a previous result [37], suggesting that consumers generally prefer fruity aroma wines containing higher amounts of ester compounds. The above analysis results implicate that the antimicrobial activities of SO_2 (in control wine) and NaOCl (in sample wine) provided a proper condition in wines for yeast and LAB to synthesize esters and higher alcohols during the fermentation and incubation periods.

Sensory Test

The sensory characteristics of red wines were evaluated by 23 panels. The preferences of aroma, color, sweetness, sourness, astringency, and overall acceptability were determined using a 9-point hedonic scale. The sample wine made by grapes after soaking in NaOCl solution obtained significantly ($p<0.05$) higher color, sweetness, sourness, and overall acceptability scores (6.43, 5.09, 5.09, and 5.70, respectively) than the control wine (6.17, 3.87, 4.00, and 4.26, respectively) (Table 2).

These results are also consistent with previous findings that consumers generally prefer wines that are sweet, flat, mildly acidic, and fruity [36]. Previous sensory tests have revealed that acidity is an important factor for wine taste, and generally a less acidic wine is preferable to a highly acidic wine [21]. Analysis performed by Campos *et al.* [7] presented that tartaric and malic acids in grape must were slowly consumed by yeast during the alcoholic fermentation stage, and when MLF began, citric, lactic, and acetic acids were newly synthesized by LAB.

Table 2. Sensory test result of wine samples.

Attributes of wine	Wine samples		
	Blank	Control	Sample
Aroma	4.00^{b}	5.39^{a}	6.09^{a}
Color	5.39^{b}	6.17^{ab}	6.43^{a}
Sweetness	3.52^{b}	3.87^{b}	5.09^{a}
Sourness	4.09^{bc}	4.00^{c}	5.09^{ab}
Astringency	4.52^{a}	4.61^{a}	5.35^{a}
Overall acceptability	4.09^{b}	4.26^{b}	5.70^{a}

$^{a-c}$Mean score given by the panel. Values within a row not sharing a superscript letter are significantly different ($p < 0.05$, Duncan's multiple range test).
A commercial product of red wine was used as the positive control.

Conclusively, simply soaking grape berries in 200 mg/l NaOCl for 30 min before winemaking reduced the initial bacterial population, which was the same level as that in SO_2-added wine, and this pretreatment provided a better condition for the growth of starter yeast during alcoholic fermentation. The dominant growth of yeast resulted in the production of wine with a higher concentration of ethanol and volatile aroma compounds. Furthermore, when LAB were grown in the NaOCl-treated wine, alcohol-tolerant bacteria grew well during MLF. This might have resulted in wine with lower acidity and more volatiles. Additionally, the NaOCl treatment affected wine color by increasing the red color intensity. The overall changes described above resulted in production of a better tasting wine. NaOCl is approved as a food additive by the FDA and KFDA, and it is commonly used in the food industry [12, 18]. This study is the first report on the use of NaOCl treatment to sterilize contaminating microorganisms for winemaking, and it was found that 200 mg/l NaOCl can be used as an alternative to SO_2 to inhibit spoilage microorganisms and give wine a superior taste and improved overall acceptance.

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