Improvement in Sensory Characteristics of *Campbell Early* Wine by Adding Dual Starters of *Saccharomyces cerevisiae* and *Oenococcus oeni*

Yoo, Ki-Seon[1], Ji Eun Kim[1], Eunyoung Seo[1], Yu Jin Kim[1], Hwa Young Choi[1], Hyang-Sik Yoon[2], Myoung-Dong Kim[3], and Nam Soo Han[1*]

[1]*Department of Food Science and Technology, Research Center for Bioresource and Health, Chungbuk National University, Cheongju 361-763, Republic of Korea*
[2]*Chungcheongbuk-do Agricultural Research and Extension Services, Ochang 363-883, Republic of Korea*
[3]*School of Bioscience and Biotechnology, Kangwon National University, Chuncheon 200-701, Republic of Korea*

This study was performed to investigate the effects of adding a dual starter on the chemical and sensory characteristics of red wine made of *Campbell Early* grape. The yeast starter, *Saccharomyces cerevisiae,* and lactic acid bacteria (LAB) starter, *Oenococcus oeni*, were used for inoculation in the winemaking process for alcoholic fermentation and malolactic fermentation (MLF), respectively. After 200 days of incubation, the chemical compositions of yeast/LAB-added wine (YL-wine) were compared with those of no-starter-added wine (control) and yeast-added wine (Y-wine). The results show that no significant differences were observed in pH, total sugar, and alcohol content among the wine samples, but the malic acid content in YL-wine was significantly reduced, and various esters and higher alcohols were synthesized. The sensory test revealed that the addition of dual starters resulted in improved overall acceptability in wine. This study emphasizes the importance of *O. oeni* in addition to yeast in making *Campbell Early* wine.

Keywords: *Saccharomyces cerevisiae, Oenococcus oeni,* malolactic fermentation, *Campbell Early* grape, sensory test, red wine

The *Campbell Early* grape belongs to the *Vitis labrusca* variety and is the major table grape in Korea. Previous studies on winemaking with *Vitis labrusca* were carried out using varieties of *Campbell Early*, *Gerbong*, and *Muscat Bailey* A (MBA) at various fermentation conditions and using various processing techniques [10, 15, 24]. In addition, the sensory characteristics of red wines made of various *Vitis labrusca* varieties were evaluated using a descriptive analysis and ranking test [12]. Generally, *Campbell Early* grapes have a higher concentration of water and titratable acidity and a lower concentration of sugar compared with other varieties. This grape also has a simple aroma composition and a higher concentration of ethyl acetate (vinegar odor) and isoamyl alcohol (grass odor) [13]. Therefore, in order to make high-quality wine by using the *Campbell Early* grape, the establishment of a sophisticated and well-designed fermentation process is required.

Winemaking can be summarized as the biotransformation of must into wine, which is performed mainly by *Saccharomyces cerevisiae* strains during the alcoholic fermentation process. The yeast species of *S. cerevisiae*, *Zygosaccharomyces*, and *Brettanomyces* have been used in fermenting alcoholic beverages, where it converts the sugars present in grape must into alcohol [18]. In the previous study, they observed differences in the quantities of the volatile compounds produced by the different species of *S. cerevisiae* [14].

Malolactic fermentation (MLF) is a biodeacidification process that is often encouraged, because it improves microbiological stability and final wine quality. MLF is one of the main stages in the elaboration of red wines, among which the most important is the transformation of malic acid into lactic acid and CO_2. As a result, wines that have undergone MLF exhibit lower titratable acidity, which enables greater microbiological stability and better taste owing to the loss of astringency and bitterness. *O. oeni* (previously known as *Leuconostoc oenos*) is the main species responsible for MLF, as it is very well adapted to fermentation conditions, such as low pH and the presence of ethanol [3, 8].

The aim of this study was to improve the sensory characteristic of *Campbell Early* wine by adding *S. cerevisiae* and *O. oeni* dual starters. To investigate the effects of dual starters on the quality changes along with alcoholic

*Corresponding author
Phone: +82-43-261-2567; Fax: +82-43-271-4412;
E-mail: namsoo@cbnu.ac.kr

fermentation and MLF, analyses of the microorganism, general composition, volatile composition, and sensory test were performed.

MATERIALS AND METHODS

Grape and Winemaking

The 2008 vintage of *Campbell Early* grape was taken from a vineyard in Cheongwon-gun, Chungbuk, Republic of Korea. After removing brawlers and washing with tap water, the total mass of 10 kg of grapes was crushed and the resulting 8 l of grape must was transported to a 10-l plastic jar fermenter. The must was treated with 100 ppm SO_2 for 6 h, and sucrose (CJ, Seoul, Korea) was added to make 24 °Brix must. A rehydrated inoculum of *S. cerevisiae* starter (Lalvin K1-V1116, Lallemand, Montreal, Canada) was inoculated in the grape must in a proportion of 10^7 cells/l, and alcohol fermentation was continued for 7 days at 20^oC with 3 daily pumpings. After removing skins and seeds by filtering, MLF was performed by adding commercial *O. oeni* starter (Lalvin EQ 54, Lallemand, Toulouse, France) in a proportion of 10^8 cells/l for 7 days at 20^oC. After MLF, wines were matured at 12^oC for 28 days and cold stabilized at 0^oC for 7 days. Finally, wines were transferred to 0.75-l bottles, stopped, and stored at 15^oC for 4 months [18]. For comparison of additional effects of starters, 3 different wine samples were made: yeast/LAB-added wine (YL-wine), yeast-added wine (Y-wine), and no-starter-added wine (control).

Microbiological Analysis

Samples were serially diluted (10^{-0}, 10^{-1}, 10^{-3}, and 10^{-5}) with sterile physiological saline (0.85% NaCl) and 0.05 ml of each of the diluents was spread onto 3 different media: YM, MRS, and AT. YM was used to cultivate yeast on yeast extract agar (Becton Dickinson, MD, U.S.A.) at 30^oC for 24−48 h. MRS agar (Becton Dickinson, MD, U.S.A.) was used to cultivate LAB at 30^oC for 24 to 48 h. AT medium contained (per liter) 10 g of bacto peptone, 10 g of glucose, 5 g of yeast extract, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $MnSO_4 \cdot 4H_2O$, 250 ml of tomato juice, and 20 g of agar, and it was used to cultivate *O. oeni* anaerobically in gas pack jars at 37^oC for 48−72 h [9].

Chemical Analysis

The alcohol fraction was recovered using a distiller, and the alcohol concentration was measured by a densitometer at $15°C$. The wine pH was measured with an IQ 240 pH meter (IQ Scientific Instruments, San Diego, U.S.A.). Soluble solid concentrations (°Brix) were measured using a hand refractometer (ATAGO, Tokyo, Japan). Organic acid concentrations (%) were measured by HPLC (TSP, US/Spectra System, MA, U.S.A.) with an Aminex HPX87-H column (300×7.8 mm ID; Bio-Rad, Richmond, U.S.A.) with a 0.6 ml/min flow rate of 0.008 N H_2SO_4. Color components of *L** (lightness), *a** (redness), and *b** (yellowness) values of red wines were measured using a Minolta Chroma Meter CR-400 (tristimulus method) (Minolta, Osaka, Japan). Every measurement was replicated 3 times and the average values are reported.

Volatile Compounds Isolation

The volatile compounds were isolated using a Head-Space Auto Sampler (Agilent 7694E; Agilent Technologies, CA, U.S.A.) and a

gas chromatography−mass spectrometry (GC−MS) (HP 6890N; Hewlett-Packard, PA, U.S.A.) was used for analysis. Wine (10 ml) and the internal standard (4-methyl-2-pentanol, 50 μl) were added to a 20-ml vial. The isolation of the volatile compounds was carried out for each vial at 80^oC for 30 min, with the injection loop at 90^oC and the temperature transfer line at 100^oC [2, 13, 20]. GC−MS analysis was carried out using a Hewlett-Packard 6890N Network gas chromatograph coupled to a Hewlett-Packard 5973 quadrupole mass spectrometer which was equipped with a DB−FFAP fused silica capillary column (30 m×0.25 mm ID and 0.25 μm film thickness; J&W Scientific Inc., Folsom, CA, U.S.A.). The carrier gas was ultrapure helium with a flow rate of 1 ml/min and the pressure was at 7.5 kPa. The oven temperature was programmed from 50^oC (5 min) to 150^oC at 2^oC/min, and 230^oC at 3^oC/min. The injector and the transfer line were heated both at 250^oC and 280^oC. The ionization voltage applied was 70 eV and the mass spectra were obtained in a scan range from 40 to 350 *m/z*. All mass spectra were also compared with the data system library database (Wiley 275) [4, 16, 22].

Sensory Evaluation

The sensory characteristics of wines were evaluated by a 23-membered panels after 55 days of incubation. The preferences for aroma, color,
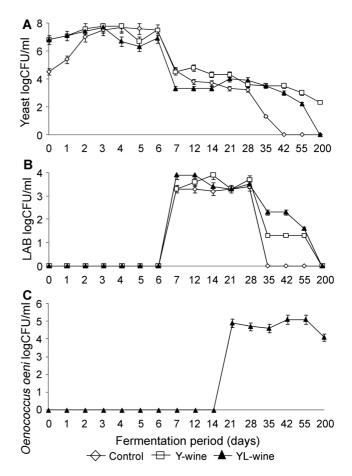

Fig. 1. Changes in yeast (**A**), LAB (**B**), and *O. oeni* (**C**) populations during wine fermentation.

sweetness, tartness, astringency, and overall acceptability were determined by 9-point hedonic scale (1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely). The sensory evaluation panel included professors and students of the Department of Food Science and Technology at Chungbuk National University. The sensory panel consisted of 11 women and 12 men, aged from 20 to 50 years. Each wine was coded with a 3-digit random code and 20 ml of wines in glasses were presented in random order to the panel.

Statistical Analysis
Significant differences (sensory and GC−MS analyses) among wines for each of the parameters analyzed were assessed with a one-way analysis of variance (ANOVA) using the SPSS Version 12.0K statistical package for Windows (SPSS, Chicago, U.S.A.) [13].

RESULTS AND DISCUSSION

Microbial Growth During Wine Fermentation
S. cerevisiae and *O. oeni* are the most usuful strains during the alcoholic fermentation and MLF processes. The control wine (no-starter-added wine), the yeast-added wine (Y-wine), and the yeast/LAB-added wine (YL-wine) were prepared as described in Materials and Methods, and the microbial changes were monitored along with the fermentation process. When wine samples were spread on the selective agar plates (YM, MRS, and AT), different types of colonies grew and the microscopic observation of those cells showed that they were typical yeast, lactic acid bacteria, and *O. oeni*. As shown in Fig. 1, during the wine fermentation and incubation period of 200 days, a dynamic

change of microbial population was observed. First, yeast grew during the alcoholic fermentation period (for 7 days), followed by a sudden reduction of cell counts after the press-filtering process, and it then made a slow decline after the 35th day (Fig. 1A). In the case of the control wine, the viable cell number of yeast reached 10^7 CFU/ml on the 3rd day, but they disappeared on the 42nd day, showing a shorter survival term (about 40 days) compared with yeasts in Y- or YL-wines (over 200 days in Y-wine and 55 days in YL-wine). LAB started to grow from the 7th day of fermentation just after press-filtering of the wines, and different rates of declines were observed after the 35th day depending on wine samples (Fig. 1B); LAB in the control wine disappeared on the 35th day, but LAB in Y- and YL-wines disappeared after the 55th day. In the case of *O. oeni*, the species was only detected in the YL-wine in which the *O. oeni* starter was inoculated on the 14th day, and its population size was maintained at a constant level during the fermentation period (Fig. 1C) [3].

Chemical Analysis
The chemical changes in the 3 different types of wines during fermentation are shown in Table 1, where alcohol concentrations (%), pH, soluble solid concentrations (°Brix), organic acid concentrations (%), and colorimetric (L^*, a^*, and b^*) analysis results are presented. First, the ethanol concentration in the control wine increased up to 15.5% for 12 days and then slowly decreased to 11.5%; other starter-added wines showed similar patterns. Secondly, the pH levels in the 3 wines were slightly changed along the fermentation period: from 3.45 to 3.28 in the control, and from 3.45 to 3.37 or 3.38 in Y- and YL-wines, respectively.

Table 1. Time courses of general compositions during fermentation of the control (A), Y-wine (B), and YL-wine (C).

A

Days	Alcohol (%)	pH	Brix (°Brix)	L^*	a^*	b^*	Tartaric acid (%)	Malic acid (%)	Citric acid (%)	Lactic acid (%)	Acetic acid (%)	Total acids (%)
0	0.0	3.45±0.00	24.0±0.0	13.1±0.2	25.2±0.4	9.8±0.1	0.46±0.06	0.42±0.00	.	.	.	0.88±0.05
1	1.0	3.32±0.00	22.2±0.0	12.5±0.5	26.4±0.2	10.6±0.2	0.38±0.06	0.41±0.04	.	.	.	0.79±0.02
2	1.5	3.39±0.00	21.3±0.1	10.0±0.2	27.6±0.2	5.7±0.1	0.37±0.00	0.38±0.01	.	.	.	0.74±0.03
3	8.0	3.32±0.00	20.4±0.0	19.6±0.5	46.2±0.3	28.8±0.3	0.39±0.04	0.38±0.03	.	.	.	0.77±0.02
4	8.0	3.19±0.00	15.6±0.0	20.0±0.1	41.0±0.4	26.3±0.1	0.37±0.02	0.36±0.01	.	.	.	0.73±0.02
5	8.5	3.29±0.00	12.3±0.1	24.2±0.1	30.7±0.2	14.1±0.1	0.28±0.00	0.26±0.02	.	.	.	0.54±0.03
6	12.5	3.32±0.01	10.2±0.1	15.6±0.2	27.0±0.3	11.4±0.2	0.31±0.02	0.21±0.01	.	.	.	0.52±0.01
7	14.0	3.34±0.00	8.4±0.0	24.4±0.2	42.4±0.4	18.0±0.3	0.34±0.03	0.21±0.02	.	.	.	0.55±0.03
12	15.5	3.58±0.00	7.2±0.1	21.3±0.1	33.8±0.3	0.0	0.51±0.02	0.32±0.01	0.12±0.01	0.29±0.02	.	1.24±0.01
14	12.5	3.66±0.00	7.2±0.0	34.7±0.3	50.3±0.2	13.4±0.2	0.49±0.00	0.35±0.02	0.15±0.01	0.30±0.04	0.02±0.00	1.29±0.01
21	12.0	3.15±0.00	7.0±0.0	42.8±0.2	52.8±0.5	11.1±0.1	0.42±0.01	0.32±0.01	0.14±0.02	0.26±0.02	0.02±0.00	1.14±0.02
28	11.5	3.32±0.00	7.0±0.0	31.2±0.1	46.9±0.3	13.4±0.3	0.43±0.02	0.35±0.02	0.14±0.00	0.28±0.00	0.02±0.00	1.21±0.01
35	11.5	3.32±0.02	7.0±0.0	34.9±0.2	51.9±0.4	14.7±0.1	0.37±0.03	0.29±0.03	0.13±0.02	0.23±0.01	0.02±0.00	1.03±0.00
42	12.0	3.24±0.01	7.0±0.0	35.7±0.2	52.5±0.3	15.4±0.2	0.40±0.02	0.33±0.02	0.15±0.01	0.27±0.02	0.02±0.00	1.16±0.01
55	12,.0	3.26±0.01	7.0±0.0	31.5±0.1	50.1±0.2	12.5±0.1	0.39±0.02	0.34±0.02	0.15±0.01	0.27±0.02	0.02±0.00	1.16±0.00
200	11.5	3.28±0.00	6.8±0.0	31.4±0.1	46.3±0.3	11.1±0.1	0.28±0.01	0.15±0.02	0.10±0.02	0.14±0.01	0.01±0.00	0.66±0.02

Table 1. Continued.

B

Days	Alcohol (%)	pH	Brix (°Brix)	L*	a*	b*	Tartaric acid (%)	Malic acid (%)	Citric acid (%)	Lactic acid (%)	Acetic acid (%)	Total acids (%)
0	0.0	3.44±0.00	24.0±0.0	17.1±1.1	36.1±1.3	11±0.6	0.37±0.02	0.47±0.00	.	.	.	0.84±0.04
1	2.5	3.30±0.01	22.0±0.1	15.9±0.2	45.5±0.9	23.3±1.2	0.35±0.04	0.45±0.03	.	.	.	0.80±0.09
2	4.5	3.27±0.00	17.0±0.1	17.4±0.2	48.6±0.2	28.1±0.1	0.34±0.03	0.42±0.07	.	.	.	0.76±0.10
3	8.5	3.31±0.00	15.4±0.0	16.3±0.4	47.1±0.8	25.1±0.8	0.33±0.01	0.34±0.01	.	.	.	0.66±0.02
4	9.5	3.21±0.01	13.8±0.1	17.1±0.1	27.8±1.0	9.3±0.2	0.30±0.02	0.33±0.00	.	.	.	0.62±0.05
5	9.5	3.3±0.00	12.1±0.0	24.9±0.3	37.7±1.1	10.9±0.1	0.24±0.00	0.28±0.04	.	.	.	0.51±0.03
6	11.0	3.32±0.00	10.7±0.2	13.1±0.0	40.5±0.6	18.6±0.4	0.30±0.02	0.28±0.02	.	.	.	0.58±0.01
7	12.5	3.37±0.00	8.9±0.0	21.8±0.2	33.5±0.3	9.4±0.0	0.43±0.06	0.30±0.01	.	.	.	0.73±0.04
12	14.5	3.53±0.01	7.4±0.0	19.1±0.1	27.5±0.4	0.0	0.46±0.02	0.38±0.02	0.14±0.00	0.20±0.02	0.04±0.01	1.23±0.03
14	12.5	3.53±0.01	7.0±0.0	39.5±0.6	44.6±0.2	4.1±0.3	0.48±0.05	0.38±0.02	0.17±0.04	0.23±0.01	0.02±0.00	1.28±0.01
21	12.0	3.22±0.00	6.6±0.0	47.7±0.3	51.6±0.3	4.1±0.4	0.41±0.02	0.38±0.06	0.15±0.01	0.24±0.00	0.02±0.00	1.20±0.03
28	11.5	3.38±0.00	6.6±0.0	34.5±0.3	50.5±0.1	9.6±0.1	0.38±0.03	0.33±0.01	0.13±0.01	0.20±0.01	0.02±0.00	1.06±0.02
35	11.5	3.36±0.01	6.6±0.0	37.3±0.1	52.5±0.8	9.8±0.2	0.37±0.00	0.35±0.03	0.14±0.03	0.20±0.03	0.02±0.00	1.08±0.04
42	11.0	3.28±0.00	6.6±0.0	36.3±0.2	52.4±0.3	9.8±0.2	0.38±0.02	0.37±0.01	0.14±0.01	0.21±0.02	0.02±0.00	1.12±0.04
55	11.5	3.27±0.00	6.6±0.0	41.9±0.3	53.9±0.5	4.6±0.1	0.34±0.03	0.35±0.02	0.14±0.02	0.22±0.01	0.02±0.00	1.07±0.02
200	11.0	3.37±0.00	6.4±0.0	26.4±0.1	42.1±0.1	4.4±0.1	0.19±0.04	0.35±0.01	.	0.28±0.03	0.01±0.00	0.54±0.01

C

Days	Alcohol (%)	pH	Brix (°Brix)	L*	a*	b*	Tartaric acid (%)	Malic acid (%)	Citric acid (%)	Lactic acid (%)	Acetic acid (%)	Total acids (%)
0	0.0	3.43±0.00	24.0±0.1	23.1±0.7	30.2±0.5	6.7±0.1	0.45±0.03	0.39±0.02	.	.	.	0.84±0.07
1	1.5	3.38±0.00	20.6±0.2	16.8±0.3	48.1±0.3	26.8±0.2	0.38±0.01	0.40±0.04	.	.	.	0.77±0.03
2	6.0	3.31±0.00	15.0±0.1	20.5±0.3	47.1±0.3	27.4±0.2	0.42±0.03	0.41±0.01	.	.	.	0.83±0.04
3	8.0	3.34±0.00	14.4±0.3	13.8±0.0	44.4±0.4	21.3±0.1	0.42±0.02	0.35±0.02	.	.	.	0.77±0.02
4	8.5	3.31±0.00	12.6±0.0	12.9±0.1	38.2±0.5	13.7±0.2	0.42±0.02	0.38±0.03	.	.	.	0.80±0.02
5	9.5	3.32±0.00	10.0±0.2	29.3±0.2	51.5±0.1	15.3±0.1	0.33±0.01	0.24±0.00	.	.	.	0.57±0.01
6	11.5	3.34±0.00	8.8±0.2	19.8±0.3	45.3±0.2	23.0±0.2	0.33±0.02	0.21±0.03	.	.	.	0.54±0.03
7	14.0	3.38±0.01	7.2±0.1	13.1±0.1	42.7±0.1	17.3±0.2	0.35±0.04	0.20±0.02	.	.	.	0.55±0.00
12	14.5	3.48±0.00	6.6±0.1	25.6±0.3	36.9±0.3	2.1±0.3	0.50±0.03	0.33±0.01	.	0.26±0.03	0.04±0.02	1.28±0.11
14	12.0	3.57±0.01	6.6±0.0	38.2±0.2	47.0±0.3	7.2±0.1	0.49±0.04	0.29±0.02	0.13±0.01	0.31±0.01	0.02±0.00	1.24±0.07
21	13.0	3.30±0.00	6.4±0.0	43.3±0.2	50.7±0.2	7.7±0.1	0.44±0.02	0.27±0.00	0.11±0.00	0.28±0.00	0.02±0.00	1.13±0.04
28	12.5	3.47±0.01	6.2±0.2	34.0±0.3	49.4±0.2	10.7±0.0	0.41±0.01	0.24±0.01	0.11±0.02	0.26±0.03	0.02±0.01	1.03±0.04
35	11.5	3.43±0.00	6.2±0.0	38.5±0.2	51.8±0.1	11.1±0.1	0.39±0.02	0.25±0.01	0.12±0.03	0.26±0.02	0.02±0.00	1.04±0.02
42	12.0	3.36±0.00	6.2±0.0	38.5±0.3	51.5±0.3	10.7±0.2	0.42±0.04	0.28±0.02	0.12±0.02	0.29±0.01	0.02±0.00	1.13±0.06
55	11.5	3.34±0.00	6.2±0.0	36.9±0.1	49.2±0.2	5.3±0.1	0.39±0.02	0.27±0.00	0.12±0.01	0.28±0.00	0.02±0.00	1.09±0.03
200	10.5	3.38±0.00	6.2±0.0	35.3±0.3	43.3±0.2	5.7±0.2	0.16±0.01	0.16±0.02	.	0.18±0.01	0.01±0.00	0.51±0.02

Control, no-starter-added wine; Y-wine, yeast-added wine (*S. cerevisiae*); YL-wine, yeast/LAB-added wine (*S. cerevisiae* and *O. oeni*).
Values are mean±SD of triplicate determinations.

Next, the soluble solid concentrations (°Brix) in the control wine decreased from 24 to 7 during the alcoholic fermentation period (7 days) and its level was not changed thereafter. The Y- and YL-wines showed the same total sugar change patterns as the control. In the case of colorimetric analysis of the wines, the lightness value (L*) of the control wine was increased from 13 to 42.8 for 21 days and the level was maintained thereafter. Whereas redness (a*) followed a similar pattern to L*, the yellowness (b*) showed a small variation along with fermentation period.

When organic acids were analyzed in wines, the total acidity content in the control wine decreased from 0.88% during the alcoholic fermentation period, but with the growth of LAB (Fig. 1B) on the 12th day, its level increased and decreased again to 0.66% after 200 days. Only tartaric and malic acids were detected in the grape must and their levels did not change much for 7 days during alcoholic
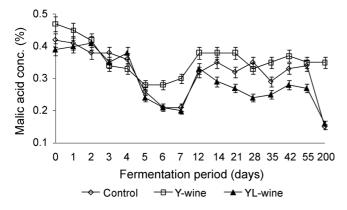
Fig. 2. Changes in malic acid concentration over time during wine fermentation.

fermentation; however, after the 7th day, citric, lactic, and acetic acids were synthesized as LAB started to grow. The change in the profiles of organic acids in the 3 types of wines was generally identical; however, the malic acid content in the YL-wine decreased significantly after the addition of the *O. oeni* starter on the 7th day, as shown in Fig. 2. This result suggests that malic acid digestion occurred in the YL-wine because of the MLF induced by *O. oeni*, and this reaction resulted in a lower malic acid content.

Volatile Compounds Analysis

Wine aroma is an important characteristic that contributes to wine quality and it depends on the grape variety, ripeness, yeast activity, environment, climate, fermentation condition, vinification procedures, and aging in the bottle. Wine aroma is created by alcohols, esters, aldehydes,

ketones, ethers, acids, hydrocarbons, benzene compounds, monoterpenes, and other compounds [19].

Table 2 represents the volatile compounds of the 3 wine samples. Totally, 12 volatile compounds were identified from GC−MS analysis, including 6 esters, 4 alcohols, and 2 other compounds. With regard to the esters, ethyl acetate, ethyl propanoate, and ethyl hexanoate were detected in the 3 wines and 2-methyl-ethyl-propanoate was additionally detected in the starter-added wines. The enzyme-induced reaction between alcohols and acetyl-CoA can result in 2-methyl-ethyl-propanoate, which gives rise to a fruity aroma of banana or apple [1, 8]. Among the volatile compounds, higher alcohols such as isobutyl alcohol, isoamyl alcohol, 1-hexanol, and phenylethyl alcohol were partly detected in the 3 wine samples: 3 in the control, 2 in Y-wine, and 4 in YL-wines. YL-wine also showed high levels of ethyl acetate and a low level of isoamyl alcohol. Alcoholic fermentation by yeast is known to produce esters and higher alcohols, and MLF is involved in the reduction of herbaceous and vegetable aromas and the appearance of other fruity and floral aromas [21]. In our results, synthesis of esters and higher alcohols was increased in YL-wine (the yeast/LAB-added wine) compared with Y-wine (the yeast-added wine), and this result strongly suggests that dual starters of *S. cerevisiae* and *O. oeni* worked together to produce volatile compounds in wine.

Sensory Test

The 3 types of red wines were evaluated by a panel. The preferences for aroma, color, sweetness, tartness, astringency, and overall acceptability were determined by a 9-point hedonic scale (Table 3). Surprisingly, Y-wine showed similar or less sensory scores than the control wine in most

Table 2. General composition of the volatile compounds in the studied wines on the 55th day.

Volatile compounds (mg/l)		Molecular formula	Control		Y-wine		YL-wine	
			Mean	SD	Mean	SD	Mean	SD
Esters	Ethyl acetate	$C_4H_8O_2$	98.48	1.72	115.99	1.95	124.42	2.17
	Ethyl propanoate	$C_5H_{10}O_2$	1.28	0.08	1.25	0.01	1.12	0.13
	Ethyl butanoate	$C_6H_{12}O_2$	11.79	0.32	11.93	0.59	10.58	0.50
	Ethyl pentanoate	$C_7H_{14}O_2$	0.61	0.21	1.10	0.02	0.95	0.06
	Ethyl hexanoate	$C_8H_{16}O_2$	10.52	0.32	16.87	0.64	17.23	0.06
	2-Methyl-ethyl-propanoate	$C_{12}H_{15}ClO_3$	-	-	0.50	0.01	0.62	0.05
			-	-	-	-	-	-
Higher alcohols	Isobutyl alcohol	$C_4H_{10}O$	10.18	0.14	11.93	0.59	8.14	0.05
	Isoamyl alcohol	$C_5H_{12}O$	239.61	4.18	175.20	3.09	199.05	1.72
	1-Hexanol	$C_6H_{14}O$	-	-	-	-	0.98	0.06
	Phenylethyl alcohol	$C_8H_{10}O$	0.72	0.01	-	-	0.75	0.27
			-	-	-	-	-	-
Other compounds	4-Methyl-2-pentanone	$C_6H_{12}O$	0.62	0.04	-	-	0.65	0.01
	Linalool	$C_{10}H_{18}O$	0.95	0.01	-	-	-	-

Control, no-starter-added wine; Y-wine, yeast-added wine (*S. cerevisiae*); YL-wine, yeast/LAB-added wine (*S. cerevisiae* and *O. oeni*).

Table 3. Sensory test results of studied wines determined on a 9-point hedonic scale on the 55th day.

Attributes of wine	Wine samples		
	Control	Y-wine	YL-wine
Aroma	4.43^b	5.39^a	5.78^a
Color	6.35^a	6.17^b	6.60^a
Sweetness	3.91^b	3.87^b	4.35^a
Tartness	4.26^{bc}	4.00^c	5.00^a
Astringency	4.61^b	4.61^b	5.43^a
Overall acceptability	4.65^{bc}	4.26^c	5.26^a

Mean score obtained by panel. Values within a row not sharing a superscript letter are significantly different ($p<0.05$, Duncan's multiple range test). Control, no-starter-added wine; Y-wine, yeast-added wine (*S. cerevisiae*); YL-wine, yeast/LAB-added wine (*S. cerevisiae* and *O. oeni*).

sensory attributes, except for the aroma preference, and this result revealed that the addition of the *S. cerevisiae* starter was not effective in sensory improvement of *Campbell Early* wine. In contrast, YL-wine generally showed superior scores as compared with the control, with significantly ($p<0.05$) higher preferences in aroma, sweetness, tartness, astringency, and overall acceptance. This result strongly suggests that the dual starter addition of *S. cerevisiae* and *O. oeni* resulted in an overall improvement in sensory attributes in *Campbell Early* wine. The tested wines did not differ with regard to soluble solid concentration (°Brix) between wines; thus, aroma, tartness, and astringency are regarded as the major factors affecting the overall acceptability of tested wines. In our previous study [23], it was disclosed that Korean consumers generally prefer wines that are sweet (3.7% of sugar content), flat (0.14% of tannin), mildly acidic (0.56% of total acidity), and have a fruity aroma. In this study, the panel's preference for the aroma of Y- or YL-wines can be explained from the above-mentioned results of volatile compounds analysis, in Table 2; 2-methyl-ethyl-propanoate ester was additionally synthesized in starter-added wines and various higher alcohol compounds were produced in YL-wine. In addition, the panel's tartness preference for the YL-wine can be explained by the MLF that eliminated malic acid, which gives a very sour taste, resulting in a milder tasting wine.

The *Campbell Early* grape has been regarded to be inappropriate for winemaking because of its high water content, high acidity, low sugar content, and simple aroma compositions. In this study, dual starter addition of commercial *S. cerevisiae* and *O. oeni* was employed in the winemaking process of *Campbell Early* grapes to overcome the above defects and thus to improve the wine quality. General composition analysis showed no significant differences in pH, total sugar, and alcohol content among the wine samples. However, the malic acid content was significantly reduced in the dual starter wine possibly because of MLF. This reaction might confer a mild taste,

which was preferred by the panel. The dual starter wine showed broad profiles of esters and higher alcohols, and this result possibly resulted in an acceptable fruity aroma. Accordingly, the addition of dual starters improved wine sensory characteristics by changing the volatile compound and malic acid concentration.

This study emphasizes the importance of *O. oeni* in addition to *S. cerevisiae* in winemaking. However, interestingly, *O. oeni* was not detected in the control or Y-wine, even though it was well known to exist in grapes and play an important role in natural wine fermentation. Moreover, the detection or isolation of *O. oeni* has not yet been reported in any kind of Korean wines including grape or rice wines. Therefore, further investigation on the existence of *O. oeni* species in Korean wines or their raw materials and their influence on wine quality should be carried out. Furthermore, this study suggests that an *O. oeni* starter culture should be used in the winemaking process using *Campbell Early* grapes.

Recently, Nehme *et al.* [15] reported that, when a special combination of *S. cerevisiae* D strain and *O. oeni* X strain was inoculated together at the beginning of alcoholic fermentation, this coculture method resulted in an increased consumption of malic acid. Instead, in this study, we added the dual starters by sequential manner, which is a traditional procedure for inoculating *O. oeni* after completion of alcoholic fermentation, because the coculture strategy has not been widely adopted by winemakers so far, owing to the high occurrences of high acidity in wine resulting from a large consumption of sugar by *O. oeni* [7, 10]. The coculture strategy with a special species pair may be interesting for winemaking using *Campbell Early* grape, and thus further work is required to determine their exact effect on malic acid consumption, microbial growth, ethanol production, and risk of over-acidification.

Acknowledgments

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