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Comparison of Composition of Wines Made Using Liquid Starters of *Saccharomyces cerevisiae* W3 and OC2 and Active Dry Yeast Forms of the Same Strains

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Active dry wine yeasts were prepared from two yeasts, *Saccharomyces cerevisiae* W3 and OC2, commonly used in Japan for winemaking. White table wines were made from Koshu grapes using liquid starters and dry wine yeasts from the same two strains. The fermentation activities of the dry yeasts were slightly lower than those of the liquid starters, and those of *S. cerevisiae* W3 in the two forms (dry and liquid) were higher than those of *S. cerevisiae* OC2.

Chemical and sensory analyses showed that there was an appreciable difference in higher alcohol composition between the wines made using the liquid and dry forms of the same strains, although the overall quality of the wines was not different. On the other hand, there were some differences in chemical composition, especially in higher alcohols, amino acids and acetaldehyde between the wines made using *S. cerevisiae* W3 and OC2.

Active dry wine yeast (ADY) has found increasing use in the major wine-producing regions of the world in place of pure-culture liquid yeast and spontaneous fermentation because ADY is probably more certain, convenient, and economical, and is now commercially available, although the use of ADY is not very common yet in Japan. Pure cultures of many different strains of wine yeast have been studied as to their suitability for winemaking and as sources for the production of ADY. Among those commercially available are the well-known Burgundy and Champagne wine yeasts as well as those of Montracht, Tokay, and Steinberg, and these have been used as the most desirable strains in many countries of the world. On the other hand, there has been a report showing that the use of local yeast strains gave better wine quality than either spontaneous fermentation or the use of standard dry yeast cultures(1). Thus, it is still necessary to investigate local wine yeasts in order to produce regional wines with an individual character through a combination of local grapes, yeasts, and vinification techniques.

S. cerevisiae W3(2) and OC2(3) have been used as local wine yeasts in Japan and are still among the most important wine yeasts in the Japanese wine industry. However, few detailed studies have been made on the composition of wines made with these strains, and there has been no report on the production of ADYs from the same strains or of winemaking with them.

MATERIALS AND METHODS

Stock cultures of *S. cerevisiae* W3 and OC2 were sent to Novo Nordisk Ferment Ltd. (Dittingen, Switzerland), and two ADYs were produced from those according to the method described in "Biotechnology Applications in Beverage Production"(4). The compositions of the ADYs produced were analyzed in our laboratory. The contents of moisture, total nitrogen, crude fat, and ash in the ADYs were analyzed according to the methods described in "Methods for Analysis of Musts and Wines"(5) and "Jikken Nogeikagaku"(6).

Koshu grapes (240kg), harvested at our Institute vineyard in 1990, were destemmed and crushed with a horizontal-type destemmer-crusher. The crushed grapes were pressed with a small Vaslin-type press and 161*l* of must were obtained. After adding potassium pyrosulfite (16g) to the must, it was allowed to stand overnight at room temperature. The precipitate formed was removed by decantation and the supernatant (128 *l*) was obtained. Sugar (10.6kg) was added to the supernatant to give a Brix of 23 degrees. The must was divided into 8 portions (16*l* each) in 20-*l* glass carboys numbered from 1 to 8. No.1 and No.2 carboys were used for fermentation using a liquid starter of *S. cerevisiae* W3, No.3 and No.4 for the ADY from the same strain, No.5 and No.6 for a liquid starter of *S. cerevisiae* OC2, and No.7 and No.8 for the ADY from the same strain. Thus, all fermentations were conducted in duplicate; the values given are the averages of two carboys. A liquid starter (2ml) of each strain or the ADY with the same number of viable cells as the corresponding liquid starter was added to the carboy. The numbers of viable cells in the liquid starters or the ADYs were about 4.0 X 10^8 for *S. cerevisiae* OC2 and 4.5 X 10^8 for *S. cerevisiae* W3 in both the liquid starter and the ADY of each strain. The numbers of viable cells were estimated from spread plates of serial dilutions on malt extract agar. The musts were fermented at 15℃ in the carboys with one-piece glass airlocks. When the fermentation completed, the wines were racked and potassium pyrosulfite (50mg/*l*) was added to the finished wines. Several months after racking, the wines were filtered through a membrane filter (0.8 μm), followed by physical and chemical analyses.

The general composition of the wines was analyzed by the methods described in "Methods for Analysis of Musts and Wines"(5). Total phenols, and flavonoid and non-flavonoid phenols were determined by the methods of Slinkard and Singleton(9), and Kramling and Singleton(10), respectively. Amino acids were analyzed according to our previous paper(11). Volatile components were determined by modified methods of Nelson and Acree(12), and Killian and Ough(13).

RESULTS AND DISCUSSION

The moisture content of the ADY of *S. cerevisiae* W3 (ADY-W3) and that of *S. cerevisiae* OC2 (ADY-OC2) was 7.2 and 5.4 % respectively, while the nitrogen content was 48.0 and 61.0mg/g. Multiplication by a factor of 6.25 gave "crude protein" contents of about 30 and 38%, respectively. The moisture and protein levels of the two ADYs were similar to the reported values (about 8% for moisture and 31-50% for protein) in a common, stable and active dry wine yeast(4,5). The crude fat content was about 1% (determined before acid hydrolysis) in both ADYs. The ash content of ADY-W3 and ADY-OC2 was 3.3 and 4.9%, respectively. These values were also similar to the reported values (2-5%)(7,8). The number of viable cells of ADY-W3 and ADY-OC2 was 4.10 X 10^{10}/g (rate of viable cells, 68.6%) and 1.55 X 10^{10}/g (41.1%), respectively, and was similar to the reported values (10-40 X 10^9 cells/g)(4).

As shown in Fig. 1, during fermentation, small differences were found in the rates of alcohol production in the musts between the fermentations with the liquid starters and those with the ADY of the same strain, but there were large differences between *S. cerevisiae* W3 and OC2 in both liquid and dry forms. The largest number of viable cells in the fermenting musts two days after the

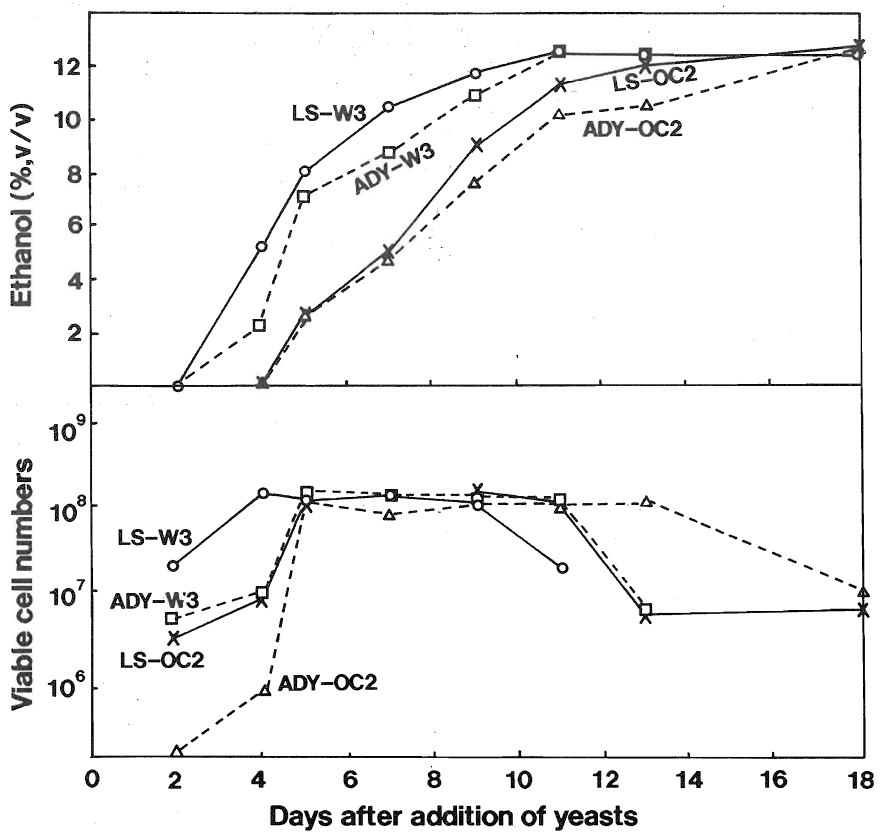

FIG.1. Changes in ethanol and viable cell numbers during fermentation.

addition of the yeasts was found in the must with the liquid starter of *S. cerevisiae* W3 (LS-W3), followed by that with ADY-W3, the liquid starter of *S. cerevisiae* OC2 (LS-OC2), and ADY-OC2, respectively, although the initial numbers of viable cells (the number of viable cells added) were similar (Fig. 1). The order of the number of viable cells in the musts was consistent with that of the rates of alcohol production. The fermentation activity of *S. cerevisiae* W3 was much higher than that of *S. cerevisiae* OC2. The fermenting musts with LS-OC2 and ADY-OC2 foamed much more than those with LS-W3 and ADY-W3, while there was little difference in the degree of foaming between LS-OC2 and ADY-OC2.

Both sensory evaluations (data not shown) and chemical analyses (Table 1) of the finished wines showed that there were no significant differences in quality between the wines made with the liquid starters and those produced with the ADY from the same strain, but there was an appreciable difference in higher alcohols such as isobutyl alcohol, isoamyl alcohol and 2-phenyl alcohol. On the other hand, there were considerable differences in proline, acetaldehyde, and isoamyl alcohol between the wines made with *S. cerevisiae* W3 and OC2 in both liquid and dry forms. The fermentation activity and number of viable cells of the two ADYs hardly changed before and after the ADYs were stored at −20 ℃ for 3 years.

The results show that the two strains in both liquid and dry forms differed with respect to fermentation activity and wine composition,

TABLE 1. Composition of Koshu wines made by liquid starters of *S. cerevisiae* W3 and OC2 and active dry yeasts from the same strains.

	LS[a]–W3	LS[a]–OC2	ADY[a]–W3	ADY[a]–OC2
pH	3.31	3.31	3.26	3.31
Total acids (g/100ml, as tartaric acid)	0.68	0.70	0.65	0.68
Volatile acid (g/ml as tartaric acid)	0.03	0.03	0.02	0.04
Ethanol (%,v/v)	12.8	13.1	12.6	12.6
Extract (%)	1.30	1.35	1.30	1.35
Reducing sugar (g/100ml, as glucose)	0.09	0.10	0.09	0.15
Ash(g/*l*)	1.77	1.81	1.79	1.76
Total phenol (mg/*l*,GAE)[b]	269	279	270	267
Flavonoids	57	58	56	46
Non–flavonoid phenolics	212	221	214	211
Total SO_2 (mg/*l*)	112	117	108	102
Free SO_2	51	41	45	34
Pro(mg/*l*)	362	260	376	263
Ala	20	17	22	18
Glu	11	16	11	16
Other amino acids	59	33	68	37
Acetaldehyde(mg/*l*)	37	43	33	42
Methanol	46	42	44	47
Isobutyl alcohol (mg/*l*)	49	49	50	45
Isoamyl alcohol[c]	156	133	131	118
2–Phenyl ethanol	11	13	9	11
Other higher alcohols	3	3	3	3
Ethyl acetate (mg/*l*)	20	21	21	20
Ethyl lactate	10	12	10	12
Diethyl succinate	4	5	4	7
Other esters	4	4	4	4

[a] LS:Liquid starter,ADY:Active dry yeast.
[b] Gallic and equivalent.
[c] The values also include a small amount of active amyl alcohol.

but the liquid and dry forms of the same strain gave similar fermentation curves and wine composition except for higher alcohols.

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