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Changes in the Wild Yeast Flora of Suflited Grape Musts

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Progressive changes in the wild yeast flora at the initial stage of incubation of grape musts added with various concentrations of SO_2 were studied qualitatively and quantitatively. Initial total counts of yeasts in the original grape must were 6.5×10^5 cells/ml and increased rapidly within a short period of time, and after 32 hours fermentation was observed. As early as 4 hours after incubation, total counts of yeasts and the proportion of the five yeast groups (Apiculate, *Saccharomyces*, *Torulopsis*, Film, and Others) were affected by the initial levels of free-SO_2 added. Total counts of yeasts in the must containing 46.7 ppm of free-SO_2 decreased gradually and reached a minimum after 48 hours. After this period, however, total counts of yeasts increased gradually, and this was due to the increase in *Saccharomyces* group. Total counts in the musts containing 97.5 and 228 ppm of SO_2 decreased rapidly to less than 10 cells/ml after 24 and 48 hours. Apliculate yeast group and part of Film and Other yeast groups decreased rapidly in the initial stage of incubation of sulfited grape musts, whereas *Saccharomyces* and *Torulopsis* yeast groups decreased gradually. Yeasts remainded in the sulfited musts were resistant to SO_2.

Yeast flora in the process of wine-making has been a subject of interest for ecologists and enologists. For examples, yeast flora, before fermentation, during the active fermentation and at the end of fermentation, were reported by Ohara and coworkers[1], Peynaud and Domercq[2], Minarik[3], and van Zyl and du Pleesis[4]. Effects of SO_2 on wine yeasts have been reported by Ohara and coworkers[5]. These studies have been carried out with samples taken at long intervals.

In the present work, wild yeast flora at the initial stage of incubation of grape musts added with various concentrations of SO_2 was studied both qualitatively and quantitatively with samples taken at hourly interval.

Materials and Methods

Grape must Pressed grape must (reducing sugar 15.8g/100ml, total acid 0.52g/100ml, and pH 3.3) of a white variety of the Koshu was used. This must (1700ml) was poured into each 2 l glass bottle and four concentrations of SO_2 (0, 46.7, 97.5, and 228.4 ppm) were added immediately. Each bottle was sealed with a glass fermentation lock and incubated at 23℃.

Total counts of yeasts The samples of grape must were diluted 0 to 10^6 fold with sterile water. Medium and methods were the same as described in a previous report[6].

Grouping of yeast isolates All colonies appearing on a plate, approximately 10 to 20 colonies, were picked up on YM agar slants. Methods of purification and grouping of isolates were the same as described in a previous report[6].

Identification of yeast strains Typical isolates, 2 to 7 strains, were selected from the

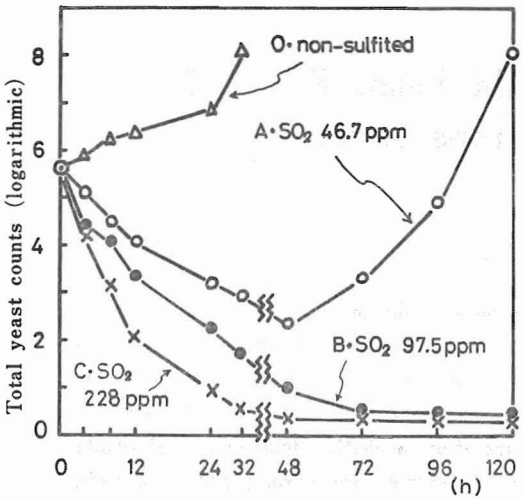

Fig. 1. Progressive changes in the total counts of yeasts in sulfited and non-sulfited grape musts.
Grape musts were prepared by crushing and pressing a white variety of Koshu. Components of grape must were 15.8 g/100 ml of reducing sugar, 0.52 g/100 ml of total acid, and pH 3.17. Pottasium meta-bisulfite was used as a source of SO_2.

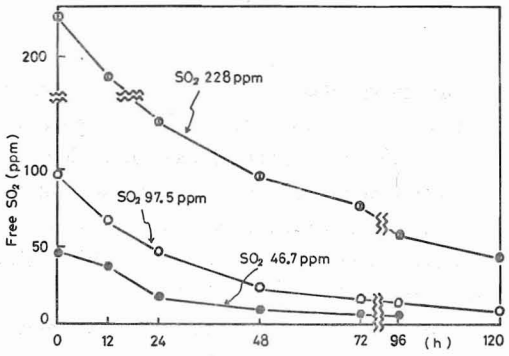

Fig. 2. Reduction in free-SO_2 in grape musts of a white variety of Koshu.
$K_2S_2O_5$ was used as the source of SO_2.

subcultures obtained from each test lot, and a total of 105 strains was identified according to the system of "The Yeasts"[7] and to the methods described by Iizuka and Goto[8].

Analysis of SO_2 The pottasium meta-bisulfite was used as a source of SO_2. Total- and free-SO_2 were analyzed by the Ripper procedure[9].

Results and Discussion

Changes in the total counts of yeasts in the sulfited grape musts Progressive changes in the total counts of yeast in each lot are shown in Fig. 1. Total counts in the original must (lot 0) at 0 time were 6.5×10^5 cells/ml, and after 4 hours slightly increased. After 32 hours, total counts increased to 1.4×10^8 cells/ml and weak fermentation was observed. In the sulfited grape musts, a decrease in the total counts of yeasts was observed, after 4 hours. In the lot A containing 46.7 ppm of free-SO_2, total counts decreased gradually and reached a minimum, 4.7×10^2 cells/ml, after 48 hours. After this period, however, total counts increased gradually and reached 3×10^8 cells/ml after 120 hours. This increase was due to the proliferation of S (*Saccharomyces*) group. In the lots B and C containing 97.5 and 228.4 ppm of initial SO_2, respectively, total counts decreased rapidly and reached levels less than 10 cells/ml after 24 and 48 hours. Wild yeasts were not sterilized completely after 120 hours, but microbial contaminations were not observed. During the incubation time, reduction in free-SO_2 in grape musts was observed, as shown in Fig. 2.

Yeast species of six yeast groups One hundred and five isolates which were randomly selected from those samples of the grape musts added with four concentrations of SO_2 taken at the stated time were classified into 20 species belonging to 10 genera. Details of identification and distribution of isolates will be reported in a separate paqer. The yeast species classified into the six groups, KA, S, T, F, R, and O, are shown in Table 1.

Group KA (apiculate yeast) consisted of *Kloeckera apiculate* and *K. corticis*. The former was the predominant yeast in the original must (lot 0). The latter was isolated at high fre-

Table 1. Yeast species in the six yeast groups.

Group	Yeast	Group	Yeast
KA	*Kloeckera apiculata*	F	*Pichia membranaefaciens*
	K. corticis		*P. terricola*
S	*Saccharomyces cerevisiae*		*Candida valida*
	S. bailii		*Ca. krusei*
	S. bayanus	R	*Rhodotorula rubra*
	S. inconspicuus	O	*Cryptococcus laurentii*
	S. rosei		*Ca. inositophila*
T	*Torulopsis stellata*		*Ca. acutus*
	T. magnoliae		*Saccharomycodes ludwigii*
			Metshnikowia pulcherrima

quency from sulfited must. Group S was composed of *S. cerevisiae, S. bayanus, S. bailii, S. rosei*, and *S. inconspicuus*. *S. bailii* was isolated at high frequency from sulfited grape musts. Group T(*Torulopsis*) consisted of *T. stellata* and *T. magnoliae*. The former was dominant yeasts in this group. Both yeasts were isolated from sulfited musts. Group F (film yeasts) was composed of *Pichia membranaefaciens, P. terricola, Candida valida*, and *Ca. krusei*. *Ca. krusei* and *P. terricola* were predominant yeasts in sulfited grape musts. Group R (*Rhodotorula*) was composed of only one species of *Rh. rubra*. This yeast was isolated from the original must. Group O (other yeasts) was composed of *Saccharomycodes ludwigii, Metschnikowia pulcherrima, Ca. acutus, Ca. inositophila*, and *Cryptococcus laurentii*. *Saccharomycodes ludwigii* was isolated at high frequency from sulfited musts. *Ca. acutus*, described as a new species [10], was isolated from sulfited must added with 228.4 ppm of SO_2.

Progressive changes in the proportion of five yeast groups Progressive changes in the five yeast groups, KA, S, T, F, and O, in grape musts added with three concentrations of initial free-SO_2 (0, 46.7, and 97.5 ppm) are shown in Fig. 3. Group R was included in group O. The proportions of five yeast groups in the original grape must (lot 0 at 0 time) were 50% of KA, 10% of S, T, and O, and 20% of F groups. In the original grape must (lot 0), a rapid increase in the total counts was observed. This increase was mainly due to the proliferation of group S yeasts and the proportion of groups KA, F, and O decreased simultaneously. The proportion of group T increased gradually with the reduction of group KA till 24 hours, but it decreased with the increase in group S. In lot A containing 46.7 ppm of SO_2, after 12 hours, the proportion of group KA decreased rapidly to 15%, and after 24 hours to less than 10%, but the proportion of group S increased gradually. After 48

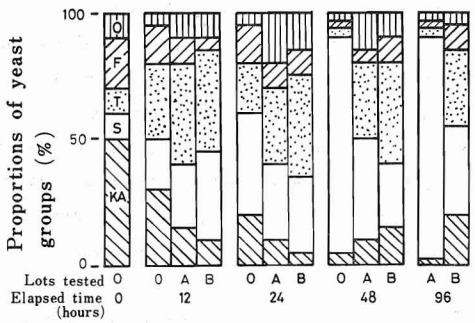

Fig. 3. Progressive changes in the counts of wild yeasts in sulfited and non-sulfited grape musts.
O : non-sulfited must, A : 46.7 ppm of initial free-SO_2, B : 97.5 ppm of free-SO_2, KA : Apiculata yeast group, S : *Saccharomyces* yeast group, T : *Torulopsis* yeast group, F : Film yeast group, O : Other yeastgroup.

hours, total counts of yeasts reached a minimum, but increased gradually after this time. This increase in the total counts was due to the proliferation of group S and the proportion of group S reached 90% after 120 hours. After 12 hours, the proportion of group T increased to 40%. This group, however, decreased rapidly to several percent with the increase of group S after 72 hours. Changes in the proportions of groups F and O also tended to change similarly to that of group T. In lot B containing 97.5 ppm of free-SO_2, the proportion of group KA decreased rapidly to 5% after 24 hours and increased to 15 and 20% after 48 and 96 hours again. After 12 hours, groups S and T increased to 35 and 40 %, respectively, and the proportions of both groups almost did not change thereafter. Proportion of groups F and O almost did not change during the experimental period. In lot C containing 228.4 ppm of SO_2, progressive changes in the proportions of five yeast groups were similar to those of lot B.

In the grape must containing about 50 ppm of free-SO_2, after 8 or 12 hours, a rapid decrease in total counts of yeast and reduction in major wild yeast species were observed. From the about results, and since generaly in wine-making a yeast starter was added at about 8 hours, when an excellent active starter is used, SO_2 less than about 50 ppm will be enough to guarantee safe fermentation.

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