Abstracts

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Studies on Malo-lactic Fermentation

III. Isolation of Malo-lactic Organisms and Inoculation of the Isolates into Musts

Hideo NONOMURA, Yuwao OHARA, Hisashi KAGAMI and Kei-ichi KAZAMA :
Nippon Jozo Kyokai Zasshi (*J. Soc. Brew.* Japan) **59**, 513 (1964)

Three hundred and twenty four strains of malo-lactic organisms were isolated from 26 samples of musts and lees (representing 7 grape varieties) with ROGOSAS tomato juice-liver extract medium modified by the addition of 20 *ppm* actidione, 20 *ppm* eurocidine, 500 *ppm* sorbic acid and 2 % agar. The ratio of the organisms to total isolates increased in the progress of must fermentation. And it was usually greater in red wine lees than in white examined. The representatives, 45 strains, of the isolates were classified into 4 groups (included 9 isolates) of *Lactobacillus*, and 6 (36 isolates) of *Leuconostoc*. Nine strains representing 8 groups out of above 10 were inoculated into the red musts (Black Queen and Merlot) and the white musts (Koshu) respectively, each must was 20 *l* in volume, and contained 50 *ppm* SO_2, 23 or 25 % sugars and 2 % starter (ca. 10^6 cells/*ml*). A strain of heterofermentative *Lactobacillus* and a strain of *Leuconostoc* (sp. related to *citrovorum*) were effectively induce the fermentation in all the three kinds of musts, and the latter strain seemed to cause the fermentation with the greatest regularity. The wines from the musts, in which the fermentation took place by these strains, were lower in acidity, higher in pH value, and better in flavor and taste than the control wines.

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Studies on Japanese Wine Yeasts

VIII. Classification of Flor Yeasts Isolated in Yamanashi Prefecture, Japan.

Isami YOKOTSUKA and Shuki MOROZUMI : *Nippon Jozo Kyokai Zasshi* (*J. Soc. Brew.* Japan) **57**, 836 (1962)

Thirty three typical strains of flor yeasts were selected from 847 cultures isolated from 470 samples collected at 66 wineries and several vineyards in grape and wine producing areas.

They were classified in accordance with the procedures and systems of LODDER and K.-van RIJ as follows : 5 strains of *Saccharomyces cerevisiae* ; 6 of *S. rosei* ; 2 of *S. steineri* ; 2 of *S. exiguus* ; 3 of *S.*

heterogenicus and 15 of *S. oviformis*.

Following two noticeable characteris ics were observed for all strains tested : (1) Pseudomyceliums were not formed and (2) Protuberances resembling to conjugation tubes were always present on the cultures on GORODKOWA agar slant or gypsum block.

IX. Physiological Properties in Fermentation Stage of Flor Yeasts Isolated in Kōfu Valley, Japan.

Isami YOKOTSUKA : *Ibid.*, **57**, 943 (1962)

The comparison of physiol. properties in fermentation stage between flor yeasts (**I**) and non-film forming yeasts (**II**) was conducted by using 33 strains of **I** and 51 of ordinary **II** isolated from many wines made by spontanous fermentation.

The data obtained confirmed the results of many workers, and indicated that **I** resembled ordinary **II** in their temp. for growth or fermenation, their tolerance to SO_2 or pH values, their fermentation velocity, and their abilities of producing alcohol, organic acids, esters and aldehydes. But the results of testing of their wines showed that bad yeasts seemed to be more abundant in **I** than in **II** and that good ones were more in **II** than in **I**.

X. Utilization of Flor Yeasts to Flor Sherry Making and Improvement of the Quality of Dry Table Wine. (1) Flor Film and Its Cells of Flor Yeasts.

Isami YOKOTSUKA : *Ibid.*, **57**, 1197 (1962)

Twenty nine strains isolated in Kofu valley, three (Jerez-5, Xeres-1 and Xeres-2) of typical Spanish and two (D-Douroveu and E-Evora) of Portuguese flor yeasts were cultured on dry wine, and film formation, type of film and its cells were observed as follows ; (1) Each strain had a special type of film which was classified into five groups. (2) Excessive film formation occured frequently on wine contg. below 14% EtOH under favorable environmental condition, which deteriorated the quality of wine. (3) Cells of film did not essentially differ from that in the fermentation stage. They gathered into a flock, but did not form a pseudomycelium.

XI. (2) Changes Brought about in the Composition and the Flavor of Wine by the Flor.

Isami YOKOTSUKA : *Ibid.*, **58**, 82 (1963)

Twenty nine strains of Japanese and five of foreign yeasts mentioned above were cultured on dry dessert wine and dry table wine, and the changes brought about in the compn. and the quality of wines by the flor were estd. as follows : There was no much noticeable differece in the changes brought about in the compn. of wines by the flor of

between the Spanish yeasts and the others. Flor character developed noticeably and the aldehyde (**I**) increased to between 150 and 250 *mg/l* by the fourth to fifth week. The strength of flor character increased as the amount of total **I** increased. But it is likely not the amount of total **I** but the amonnt of fixed **I** that has strong effect. The amount of EtOH used by the flor was only below 1%.

In most of the cultures volatile acids decreased to below 0.2 *g/l* one year after inoculation. A fall in fixed acidity ranged between 0.5 and 2 *g/l*. The pH values of the cultures did not change so much. The amount of sugar used by the flor seemed to be very little. EtOH, glycerol, acetic acid or lactic acid were likely a good carbon source for film growth. Total esters increased a little, but the amount of neutral esters did not almost change. Total **I** increased remarkably and in some case it amounted to about 800 *mg/l* after one year. Fixed **I** increased in wine as total **I** increased, but not in a constant ratio.

XII. (3) The Influence of Environmental Factors and the Composition of the Wine on the Growth of Flor Film : Culture Temperature, and Alcohol Content and pH Value of the Wine.
Isami YOKOTSUKA and Shoji GOTŌ : *Ibid.*, **58**, 169 (1963)

The influence of incubation temp., the alc. content and the pH value of the wine on film formation (**I**) of flor yeasts were examd. under lab. conditions using 27 strains of Japanese and five of foreign yeasts mentioned above. The results obtained were as follows: (1) Temp.:On table wines contg. less than 13% EtOH, the rapid **I** occurred at temp. up to 28°C with most strains used. It has been proved by many investigators that the optimum temp. for **I** on wines contg. between 14 and 15% EtOH is about 20°C, and that at temp. higher than about 23° to 25°C, **I** does not often occur. At low temp. below about 15°C, **I** was usually very solw. At about 10°C on wines contg. less than 12% EtOH, one third of the strains used failed to develop as a film and at temp. down to between 5° and 7°C all strains did not form complete films, and **I**, if it occurred, was restricted to scanty, small patches. (2) The alc. strength of the wine : An optimum alc. content for **I** by most strains used was between 13 and 14%, while the max. for complete films with some strains was above 15%. In this respect four strains resemble quite closely MARCILLA's typical Spanish sherry yeasts. The rate of **I** was increased by lowering the alc. content, but **I** at such low strengths were too much, very unstable and apt to sink. (3) The pH value of the wine: The optimum pH values for **I** was between 3.0 and 4.0. As the pH values of most Japanese ordinary wines are between 3.0 and 4.0, so it is unnecessary to adjust the pH value for **I**.

XIII. (4) The Quantities of Sulfur Dioxide and Sugar in Wine.
Isami YOKOTSUKA, Shoji GOTŌ and Yoshihide YAMAKAWA:
Ibid., **58**, 1221 (1963)

The effects of the quantity of SO_2 added and sugar in wine on the film formation (**I**) by flor yeasts in Delaware white table wine contg. below 12% EtOH were examd. and the results obtained were as follows : (1) The toxity of SO_2 on **I** was very strong. It could not be checked by culture temp. or alc. concn. of wine. (2) The tolerance of **I** could not be raised by training. (3) The quantity of sugar used in **I** on table wine was so small, and usually below 0.2%. (4) Good **I** was observed on the wine, which contd. only below 0.1% of reducing sugar estd. by BERTRAND's method and therefore was lacking in fermentable sugar. No active **I** was caused by adding sugar in the wine on which flor yeasts had completely developed, but it occurred when small quantity of new wine contg. almost no sugar. Accordingly there seems to be better growth factors than sugar.

XIV. (5) The Concentration of Ethanol in Wine.
Isami YOKOTSUKA, Shoji GOTŌ and Yoshihide YAMAKAWA : *Ibid.*, **59**, 84 (1964)

The effect of the strength of EtOH on the development of flor character and the changes in the component of wine brought about by flor was studied. The flavor was tasted and components were analysed on four kinds of dry white wines contg. 15.0, 14.3, 13.5 and 12.5% EtOH, after being stored under the films of Jerez-5, WF-107, W-210 and W-500 for one year respectively. Results obtained were as follows: (1) The amount of EtOH consumed by the flor was so small that it was not a serious problem from economical point of view. (2) Both total acids and non-volatile acids were consumed the most by the flor in the wines contg. 15.0% EtOH and the least in the wines contg. 12.5% EtOH. The amounts of volatile acids consumed by the flor for all wines tested were so much that they amounted from 60 to 70% of their initial amounts. The strength of EtOH under vigorous flor seemed to be check the flor to consume acids in wine. (3) Both the accumulation velocity and the accumulated amount of aldehyde (**I**) under vigorous flor were generally not affected by the strength of EtOH. (4) The flor character (flavor) of the wines under flor increased to some extent as the amount of **I** increased. It was found that the amount of **I** accumulated did not dominate the flavor, and that the flavor depended on the strains. The amounts of **I**, in which superior flavor had always developed under the film of Jerez-5, were always less than those in the wines under the films of the others.

XV. (6) Culture Temperature.
Isami YOKOTSUKA, Shoji GOTŌ and Yoshihide YAMAKAWA : *Ibid.*, **59**, 163 (1964)

The effect of culture temp. on the quality of wines under flor was investigated by observing the film formation, tasting flavor and analysing the components of white dry table wines contg. 12.8% EtOH, on which the films of Jerez-5 and WF-107

had covered for eight months at 10°, 15°, 20° and 25°C. Results obtained were as follows : (1) No film formation at 10°C. Slow and a little formation at 15°C, but complete film cover was never made through the whole period studied. Very rapid at both 20° and 25°C; complete film cover after 20 days and thick films after 30 days, and then the films grew too much. (2) There was no effect of temp. on the consumption of EtOH by the flor. The consumption ot volatile acids increased as temp. rose, but no remarkable difference was recognized in the amount of total acids consumed. The accumulation velosity and the accumulated amount of aldehyde increased as the temp. rose. (3) After one month flor character was developed in all wines except the wine incubated at 10°C. The flavors of the wines incubated at 20° and 25°C were equally strong, but that of the wine at 15°C was comparatively weak. After 40 days they were all satisfactorilly strong, but after 50 days the flavor of the wines incubated at 25°C, smelled of yeast and was inferior to the other wines. It is worth to notice that sufficiently strong flor character was developed in the wines on which the film were only 10 to 15%. The complete thin film cover seemed to be best.

XVI. (7) Strains of Flor Yeasts.
Isami YOKOTSUKA, Shoji GOTŌ and Yoshihide YAMAKAWA : *Ibid.*, **59**, 267 (1964)

The effects of 27 strains of Japanese and three of Spanish yeasts mentioned before, on the quality of both dessert wine and table wine under their films for 90 days were investigated by tasting the wines and analysing their components, and the conclusions were obtained as follows : (1) The strains of the yeasts, whose oxidative activities and decompg. abilities on acids at their film stage were too strong or too weak, could not develop strong fragrant flor character, and most strains of Japanese yeasts tested fell into this category in their abilities. (2) Only the strains, which oxidized alc. to aldehyde, and aldehyde to acids to adequate degrees, and then decompd. acids moderately, much improved the qualiyt of the wines by reducing the acidity and developing strong fragrant flavor.

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Studies on Japanese Plum Liqueur

I. The Infuence of Manufacturing Processes upon the Quality and the Composion of Organic Acids of Liqueur.
Isami YOKOTSUKA and Shoji GOTŌ : *Nippn Jozo Kyokai Zasshi* (*J. Soc. Brew.* Japan) **59**, 633 (1964)

Six kinds of the liqueurs(**I**)were made by the following different processes : (a) Moderately ripe plums and sucrose were put in approx. 35% EtOH for about two

months, then the extract was separated, bottled and matured at room temp. (b) After plums were put in 35% EtOH for two months, the ext. was drawn off, then sucrose put on the same plums for the same period, and the ext. sepd. Both ext. were blended, and the mixture was bottled and matured. (c) Completely crushed plums were used. (d) Extn. was done with approx. 50% EtOH. (e) Over-ripe plums were used. (f) Extn. was done for one year. The compn. of **I** produced was examined by a general chem. analysis, organic acids (**II**) in **I** were detd. by column chromatography, and **I** tasted. Thus the influence of the proces-es applied upon the compn. esp. of **II**, and the quality of **I** we e studied. The results obtained were as follows : (1) There was only little difference in the yields of **I**. (2) Considerable changes were observed in the amounts of **II**, except malic acid, present in **I**. Their amounts depended to some extent on the processes, but the same kinds of **II** were always detected in all **I**. The content of malic acid was considerably affected by the processes. (3) The kinds and the amounts of **II** detected in **I** by column chromatography were approx. as follows (expressed in % to total acids) : citric acid 55, malic acid 33 as a mean value, unidentified acids 10, oxalic acid 2, and acetic acid 1.5. (4) The quality of **I** examd. by tasting was as follows : In 10 months after bottling : **I** (a) was ordinary. **I** (b) was the best. **I** (c) was somewhat too sour and rough in taste and smelled of seeds. In 20 months after bottling : **I** (c) had improved considerably in quality. **I** developed strong fragrant flavour, and was rich in body and rounded in taste. **I** (b) was almost as good as **I** (c), but it was somewhat flat and watery in taste. **I** (e) developed strong fragrant flavour, but was fairly cloudy in appearance. Both **I** (d) and **I** (f) had always fairly cloudy appearance and bad off-odors.

II. Identification of Two Unknown Organic Acids found in Japanese Plum Liqueur.

Isami YOKOTSUKA and Shoji GOTŌ : *Ibid.*, **59**, 636 (1964)

Two unknown organic acids (**I**) found in the liqueur were studied by paper chromatography, column chromatography, gas chromatography, and infrared spectrum analysis. The identification of the neuteal compounds from **I**, and the comparison between the amount of alkali used to neutralize definite amounts of **I** and that used to saponify them were also made from results obtained in these expts., these two **I** were presumed to be monoethylcitrate(**II**) and its isomer, respectively.

Next, **II** was synthesized and its pure crystals were obtained and demonstrated. Then the same expts. as mentioned above were repeated using pure crystals of synthetic **II**, and the data obtained in the former expts. and in the latter ones were compared. Thus it was concluded that **I** were **II** and its isomer, respectively. **II** was found for the first time in the liqueur in this study.