Substances accumulated in “rice-koji” (Report 3)

Changes of the accumulated substance, when the “rice-koji” were incubated at different temperatures and periods.

by

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We investigated the changes of the contents of soluble matters, which were accumulated on the “rice-kojis” in response to the variances of the incubation conditions, mainly by examining the nitrogen-containing substances.

1) At first, the extracting conditions were examined, and it was found that the hot extraction method by J. De Clerck\(^1\) was adequate means. The extractions by the lactate buffers\(^2\) were also used.

2) The ratios of the contents of accumulated matters were changed according to the differences of the incubation temperatures. The highest acidity and formol nitrogen content was observed in the “rice-kojis” incubated at lower temperature. The lowest total nitrogen content was observed in those incubated at higher temperature. The highest content of the 1st fraction of the soluble-nitrogen (protein fraction) was met with in the high temperature “rice-koji”, the highest contents of the 2nd fraction (peptide fraction) in the low temperature ones, and the highest contents of the 3rd fraction (amino acid fraction) in the middle temperature ones.

3) The comparison of the hot extraction and the lactate buffer extraction showed that more compounds were extracted by the latter method. It was assumed that the components of the extracts were different by the two methods; for example, the order of extractability of different fractions of soluble total nitrogen was reversed by the two methods of extraction.

In conclusion, we presumed that the ratios of the components in each N-fractions as well as the ratios of their amounts were changed by the different incubation temperatures.


On Studies of pot still spirits

(Part 5. On the acidity and organic acids in pot still spirits

by

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1) Investigation was made on the acidity of 2278 samples of pot still spirits in the brewing years 1949—1952. The acidity of Moromi-tori spirits (pot still spirits manufactured by mash formula) showed the frequency distribution in 1.5 or lower and that of Kasu-tori spirits (spirits prepared from Sake-cake by pot stills) the frequency distribution from 0.5 to 2.4, when calculated as 25 volume per cent alcohol.

2) By testing the chi-squares on the differences between the quality and the acidity of spirits, they were significant in Moromi-tori spirits, but not in Kasu-tori spirits.

3) There were found different distributions of acidity in pot still spirits prepared from different raw materials, such as sweet potato, rice, rice-bran barley, other cereals, raw sugar, and Sake-cake. Generally the acidity of superior spirits is lower, irrespective of the raw materials.

4) Experiments were made on the fractionation of acidic components of several kinds of pot still spirits, the paper chromatography and Duclaux’s method. Acetic, formic and lactic acids were detected, but no other fatty acids of C₄ or higher were detected.

5) According to the results of t-test, there were no significant differences between Moromi-tori spirits Kasu-tori spirits in the acidities (total, volatile and nonvolatile), and the amount and mutual ratios of main organic acids (acetic, formic and lactic)

6) Total and volatile acidities and the amount of acetic acid were higher in acidic spirits than in normal spirits.

7) It seemed that the ratio of volatile and nonvolatile acidity was 5:5~9:1, and the ratios of the amount of formic, acetic and lactic acids were 0:4:6~1:8:1 in pot still spirits.

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The distribution and growth of the yeast in sake-moromi

by

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(Brewing Experiment Station, Tax Administration Agency)

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As the sake yeast has a top fermenting property, the foamy head of sake-moromi contains a large number of yeasts. The yeast density of head is 12~25×10⁸/g, 10 to 20 times as large as that of wort. At the head-forming stage, therefore, ca. 50% of the total number of yeasts is accumulating in the head, and the other 50% is suspending in the wort.

The growth of yeasts during the sake-moromi fermentation was investigated, with special reference to the sampling error, which is due to the unequal distribution of yeasts. The growth of yeasts ceased at 10th to 12th day after mashing, when alcohol content of wort was 10~12%. At that time, yeast density reached to its maximum, 2.5×10⁸/g, and hereafter decreased slowly by autolysis.

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Studies on the putrefaction of Moromi mash of Sake (Part 6)

On the appearance of bacteria in yeast culture or moromi mash.

by

Masakazu Yamada

(Brewing Experiment Station, Tax Administration Agency)

(Received Aug. 10, 1959)
One loopful of mash was inoculated in koji extract of Be 8° and the amount of acid formed was observed after incubation at 25° for 10 days.

To neutralize 10 ml of the filtrate of yeast culture of old type (Kimoto or Yamahai) before the beginning of alcoholic fermentation 3.1~6.6 ml of 0.1 N NaOH solution was necessary and thus contamination by some bacteria was shown, while to neutralize 10 ml of the filtrate of yeast culture of old type in finished stage or yeast culture of recent type in all stages or moromi mash 1.3~2.1 ml of the same alkaline solution was necessary and thus it was shown that they were all purely fermented by yeasts.

Studies on the chromatic standards of Sake and Synthetic Sake (I)
Proposal of Co-Cr system

by

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For the chromatic standards of Sake and synthetic Sake, we devised a system of Co-solution and Cr-solution, which are prepared as follows:

Co-solution : 50 g of CoCl₂·6H₂O in 1000 cc of N/100 HCl aq
Cr-solution : 5 g of K₂Cr₂O₇ in 1000 cc of N/100 HCl aq.

The whole range of color of Sake can be covered by the mixtures of these two solutions as shown in Fig. 3 and Fig. 4.

These solutions are stable against heat and light at least during six months.

The formation of amino acids in Ki-Moto (Part 4)
On the role of lactic acid and lactic acid bacteria for the protein digestion in Moto.

by

Hiroichi Akiyama and Masuo Chino

(Brewing Experiment Station, Tax Administration Agency)

(Received July 27, 1959)

1) When mass cultures of lactic acid bacteria were added to Moto and the Moto was acidified for a short period, amino acids formed were less than in Ki-moto.

2) The Moto similar to Ki-Moto could be made by adding commercial lactic acid in portions instead of acidification by lactic acid bacteria.

3) From the results of these studies amino acid formation of Ki-Moto might be made by Koji-proteases in connection with pH of Moto mash rather than by peptidase produced by lactic acid
bacteria. Under the strong acidic condition rice protein was decomposed to intermediates like peptides and to small amounts of amino acids. Under the neutral and weakly acidic conditions, rice protein might be decomposed to "amino acid precursors" which were precipitable by trichloracetic acid. By gradual acidification with lactic acid bacteria or lactic acid, 2 or 3 times more amino acids might be formed by decomposition of the precursor than in Sekujo-Moto.

Studies on the production of enzymes by Rice-Koji

(III) The effects of polish rate of rice and of steep water quantities on the production of amylases and proteases.

by

Kenji KATAKURA and Chitoshi HATANAKA (Okayama Univ.)

(Received July 18, 1959)

1) Various kinds of Koji of 26 and 30 hours' cultivation were prepared in flasks by steeping rice of several polish rates (10~50%) in 10~30% water for about 12 hours, sterilizing for 30 min. with Koch's kettle and inoculating at 36°C.

2) Enzyme activities of Koji prepared by a given polish rate were much dependent on the quantity of steep water. The optimum quantity of steep water to get high activity was found to be about 18%, where the total moisture content was about 32%.

3) Enzyme activities of Koji prepared with any given quantity of steep water (10~30%) showed considerable decreases along with the increase in polish rate, especially in the range of 10~30% of polish rate. The rates of decrease in enzyme activities are shown in the following order: acid protease ≈ weak acid protease > α-amylase > alkaline protease > s-amylase.

4) In this case, however, the relative activities of the enzymes with each other were maintained nearly constant, except when the steep water quantity was as small as 10%.

5) At high polish rates of more than 30%, increased moisture content of Koji resulted, when compared with the activity of weak acid protease, in the depression of relative activities of α-amylase, s-amylase, and acid protease, while the relative activity of alkaline protease showed some increase.

6) At very low moisture content (steeped with 10% water), it should be mentioned, the relative activities of the two kind of amylases showed remarkable increases along with the increase in polish rate.

Investigation of protein turbidity of sake (Part 2)

by

Hiroichi AKINOMA

(Brewing Experiment Station, Tex Administration Agency)

(Received Aug. 17, 1959)

Protein turbidity of sake was investigated with respect to its distribution and correlation with components of sake. Similar results as in the previous paper were obtained.
On the so-called Asp. oryzae var. saké
—Oxygen demands of the genus Aspergillus—

Studies on koji (XIX)

by (No. 11, 819)

Hideya Murakami and Kiyomi Takagi
(Received Oct. 15, 1959)

The so-called Asp. oryzae var. sake was compared with various other kinds of the well-known Aspergilli, and it was identified with the Asp. oryzae var. globosus Sakaguchi et Yamada from viewpoints of its great oxygen demands, similarity of morphological characteristics and the strong productive power of chestnut-like aroma. It was clarified that the author's determination method of oxygen demands of molds was correct only for the molds of Asp. oryzae group.

Studies on the cause of protein turbidity in sake

(Report 4) Differences of turbidities in sake brewed under different condition and changes of turbidities during the storage.

by (No. 11, 828)

(Brewing Experiment Station, Tax Administration Agency)
(Received Oct. 1, 1959)

With a view of investigating the correlation between turbidity of sake and its brewing conditions, we brewed 6 kinds of moromi-mash with use of different koji preparations which differed in incubation temperatures (30~33°C and 40~44°C) and periods (41, 46, and 51 hrs).

1.7l of sample was taken from each moromi-mash at various periods, and was added with 0.31 of alcohol, whose concentration was adjusted to be 20% in the mixture.

Samples were all filtered and pasteurized, and their turbidities were observed.

1) The turbidity of sake was greater with koji preparations of incubation at the longer period and the higher temperature. These facts coincide with the results in the 1st report of this series.

2) As in the 2nd report, the turbidity increased remarkably within 20, 40 days, but hereafter the increase of turbidity was quite slow.

3) The aging of crude sake before the pasteurization had a small effect for the diminishing of turbidity, but the aging over a month seemed to have no effect.

4) The degree of turbidity inclined to decrease in proportion to the delay of the time of alcohol addition. This fact was in contradiction to the results of the 1st report, but since there were the exceptional cases, it was difficult to conclude summarily.

5) The substances, which were extractable with 15% alcohol from koji at various stages of its incubation and became turbid by heat pasteurization, increased remarkably after the period of 41 hrs of incubation. As such substance were richer with koji preparations of incubation at the higher temperature, there seemed to be a correlation with the turbid matter in sake.

6) It was also confirmed, as in the 3rd report that the higher the alcohol concentration was, the lesser the precursors of turbid-matter were extracted from koji.
Chemical analysis of the components of rice, which is suitable for saké brewing.

(I) Relationship between the mineral contents and the polishing rates of rice.

by Kenji KATAKURA and Chitoshi HATANAKA (Okayama Univ.)
(Received Oct. 14, 1959)

1) Quantitative analysis of the minerals, P, K, Mg, Ca, Fe, Mn, and Cu were carried out on five kinds of rice and of polished rice (10% polished rice, 30% polished rice and 50% polished rice) of each.

2) There were remarkable decreases in the mineral contents along with the increase in polish rate of rice. The decreasing rates of the minerals, however, were not equal with each other. The order of the decreasing rates was as follows:

Mg > Ca > P > K > Fe > Mn > Cu

3) The ratio of Ca content to total ash was found in every case to be maintained nearly constant independently of the polish rate. But the ratios of the other elements, except that of Mg, to total ash showed slight increases along with the increase in polish rate of rice.

(II) Relationship between polish rates and contents of soluble nitrogen fractions.

1) The soluble nitrogen fractions (I) water-soluble, (II) 3% NaCl-soluble, (III) 70% ethanol-soluble and (IV) N/20 NaOH-soluble nitrogen fraction of the same rice samples used in the preceding study were comparatively studied with reference to the polish rates.

2) The amount of each fraction showed considerable decrease along with the increase in the polish rate. The decreases in the amount of fractions I and III were more remarkable than those of fractions II and IV.

3) The order of the amount of the four fractions was,

IV >> I >> II >> III, in case of brown rice and
IV >> II >> I >> III, in case of polished rice.

From these facts and those reported on soluble proteins, it seems likely that the greater part of water-soluble nitrogen fraction (I) of rice consisted of non-protein nitrogen.

4) The relative content of insoluble nitrogen showed a slight increase along with the increase in polish rate or rice. This seems to imply that there is a close relationship between the insoluble nitrogen and the construction of starch grains in the endosperm of rice.

5) Brief discussion was made on the changes of physiological and chemical properties of rice during storage.

(III) The effects of fertilizers on the quality of “Omachimai” for saké brewing (preliminary report)

1) The rice plant, “Omachimai”, of the best quality for saké brewing in Okayama Prefecture was cultured with several kinds of fertilizers; chemical fertilizers, soybean-cake, farmyard manure, and barnyard manures. The rice seeds were put to quantitative analysis of their chemical components such as mineral elements, soluble nitrogen, and total nitrogen. Rice-Koji prepared
with 30% polished rice of each kind of fertilizer treatment was also put to the examination of the activities of hydrolytic enzymes such as α-amylase, β-amylase, acid protease, weak acid protease and alkaline protease.

2) No appreciable difference could be observed of the contents of chemical constituents. Enzymes, however, of the rice-Koji prepared with the rice treated with soybean cake generally showed higher activities than others.