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journal or publication title	Tohoku journal of agricultural research
volume	3
number	1
page range	185-218
year	1952-10-25
URL	http://hdl.handle.net/10097/29078

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By

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(*Received June 30, 1952*)

Introduction

Sake-wine is a widely used alcoholic beverage in Japan. It is brewed in so thick suspension of steamed rice, with Kôji as an amylolytic reagent, that there are many difficulties in controling the Sake fermentation, where saccharification of the rice starch proceeds in parallelism with the yeast fermentation.

The fermentation process of Sake brewing consists of two, i.e. "Moto-process" (Starter-making) and "Moromi-process" (Main fermentation). In both processes, steamed rice, rice Kôji, and water are used as the basal matters. Table 1 shows an additional rate of the materials in Moto- or Moromi-preparation. In the Sake-breweries, two different kinds of the rice Kôji are prepared by means of controling humidity in the manufacturing room. When higher humidity is

Table 1. Additional amounts of the materials in a batch of Sake-Moto, and of Sake-Moromi.[1)]

A. A batch of Sake-Moto.

Kinds of Moto	Steamed rice (Kg)	Rice for Kôji (Kg)	Water (L)	Lactic acid (75%) (ml/18 L)
Ki-moto	70.0	34.0	108.0	—
Sokujô-moto	70.0	34.0	108.0	120-130

B. A batch of Sake-Moromi.

Materials / Preparation for	Steamed rice (Kg)	Rice for Kôji (Kg)	Water (L)
Moto	70.0	35.0	108.0
The first addition	154.0	61.6	216.0
The second addition	308.0	92.4	514.8
The third addition	546.0	133.0	1051.2
Total	1078.0	322.0	1890.0

supplied, Aspergillus oryzae grows abunduntly both on the surface and inside of rice granules (Sôhaze-Kôji), while its growth on the surface of the granules is very poor, but rich within, when in low humidity (Tsukihaze-Kôji). The former type of Kôji is ordinarily used in most of Sake-breweries, and the latter one is prepared in special batch of Sake-brewing, especially for the best qualities of Sake. The former Kôji has an intensive amylolytic power, which acts momently on the rice starch, and on the contrary, the latter's amylolytic power is rather weaker but of continuity. According to whether the former Kôji or the latter one is used, it affects the fermentation state both in Moto- or Moromi-processes.

In the early stage of Moto-process, Kôji-amylase saccharifies the rice starch and the sugar is accumulated in 25% or more, so that the growth of bacteria, invading from air and water, is blocked, and only Lactobacilli are able to grow and acidify the Moto-solution. Then the Sake-yeast is inoculated and allowed to grow. This kind of the Moto preparation process is called "Ki-moto" or "Yamahai-moto" (naturally acidified Moto), while the Moto, acidified with addition of the lactic acid instead of adoption of naturally occurring action of Lactobacilli, is called "Sokujô-moto" (artificially acidified Moto).

When the fermentation of the Moto attains to its maximum, which is always judged from the bublling state in the breweries (so-called "Wakitsuki"), the Moto is left to stand still without any such treatment, as heating or agitating, till its supply to the Moromi-process (so-called "Karashi" or "Yasumi", which means resting).

In the Moromi-process, the materials, together with the Moto prepared, are mixed in three steps in order to facilitate the yeast growth. It takes three days for completion. After the first mixing, the preparation is incubated for a day and then the following mixings are made at intervals. The fermentation then occurs during the three steps-mixing process, and proceeds from the first day after completion of mixing the matters. It becomes gradually more vigorous, so that the aspect and the hight of bubble in the Moromi vary according to the following day. The brewing worker detects the fermentation stage only by observation of the bubbling state. Alcohol is accumulated up to about 18% and more, as the fermentation preceeds.

Since a mild fermentation is required throughout the process for Sake-wine of best qualities, the fermentation temperature is kept as low as 10–12°C, and highly polished rice (40–60% polished) is used, which is poorer in the nitrogenous matters derived from the nitrogen-rich outer layer of the rice granule. The so-called "Ginjô-shu" (Sake-wine of super-refined qualities), attained by this means, is almost colorless and has mild taste with a good flavor like a fruit essence. In this case difficulties in the brewing process are increased. Above all, it often

occurrs that the fermentation, which has passed away normally in the earlier stage of the Moromi-process, ceases suddenly to proceed on its course and falls into spoilage without any attack of bacteria, though this case is also due to the bacterial infection. The cause of this phenomenon is obscure, but it is clear that in this case there happens an abnormal change in the fermentative ability of the yeast. Scientific data on the Sake-yeast fermentation in the breweries may be able to supply a clue to the researches on such an abnormal fermentation in the labratories. But the breweries are apt to overlook it, because the government admits to add the alcohol to the Sake-Moromi to save vain expense of the rice, which is a notable foodstuff for the Japanese, and, as above mentioned, they always still engage themselves only in observation of the bubbling state in the fermentation process. On the other hand, laboratorians should take the responsibility of this case, when they stand just on the chemical point and take no notice of the physiological aspects of the yeast.

It was done by us to investigate ecological aspects of the yeast in the Sake-brewing process, and, as a result, a simple method to estimate the fermentative ability of the Sake-Moto or -Moromi was deviced. This might supply a useful help in scientific control of the yeast fermentation in the Sake-breweries. And some physiological aspects of the Sake-yeast were also investigated. The results might be available in elucidation of the ecological results above obtained.

PART I. ECOLOGICAIL ASPECTS OF THE SAKE YEAST IN THE BREWING PROCESS

Fermentative ability of the sake-yeast separated directly from the Moromi

By

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Introduction

On the physiological properties of the Sake-yeast we have so many research works in literature, where it is a customary method to analyse the yeast isolated by cultivation on an artificial medium. As it is well known that the experimental circumstances give influences upon the physiological properties of the yeast, the cultivated yeast would be to distinguish on its properties from the yeast of state in nature. Especially, the state of the Sake-Moromi (Sake wine

mash), which is extremely viscous and dense in its components, is supposed to affect not only on the yeast growth, but also on its fermentative ability.

Unless actual activity of the brewing microbes in the natural circumstances is clarified, the brewages are always left mysterious. But there are few studies on this respect, and indeed, the eclogical research might give a way to elucidate it.

Thus, we separated the yeast directly from the Sake-Moromi by differential centrifugation and adopted it to observe its fermentative ability. Several results are cited in this report.

Experimental Part

1. *Samples of the Sake-Moromi.*

Five kinds of samples of the Sake-Moromi were collected from different breweries in Sendai districts in 1949. The collection was made twice. i.e. the Moromi in earlier stage and in its later stage. All batches of the Moromi, from which the samples were collected, showed a normal fermentation in earlier stage, while in later stage two of them (Sample A and B) had fallen somewhat into putrefaction caused by bacterial infection. Sample C – batch is prepared for Sake of first class quality and, on the contrary, others are for Sake of second class quality.

2. *Experimental Method.*

a) Analysis of Moromi and its filtrate. pH was measured by quinhydron electrode. Relative viscosity of the Moromi and its filtrate was shown by the time (second) to pass through between two menusci marked on the 10 ml glass pipetts provided with wide or narrow opening respectively. Acidity was a titer of N/10 NaOH as lactic acid. Volatile and non-volatile acid were shown as acetic and lactic acid respectively. Soluble sugars and also dextrin (HCl-hydrolyzable

Table 2. Components

Sample	Time elapsed after the materials mixing (Day)	pH	Relative viscosity of		Ethanol (%)	Total Nitrogen (mg/ml)
			Moromi (Second)	Filtrate (Second)		
A	8	3.9	2.8	27.4	6.8	1.81
B	6	3.9	2.5	27.0	5.5	1.29
C	4	3.8	2.8	27.2	7.0	1.25
D	6	4.3	6.3	27.3	5.3	0.96
E	4	4.6	3.4	27.2	0.0	—
A'*	23				9·8	—
B'*	21				10.0	1.59
C'*	17				8.3	1.28
D'*	19				13.8	0.98
E'*	17				12.0	0.86

* Samples A'~E' were collected at the later stage of the samples A~E.

part of the Moromi-filtrate) were estimated by ordinary Bertrand's method. For estimation of NH_2-N and total-N Van Slyke's and Kjeldahl's methods were used respectively. Ethanol was estimated by oxidation method with $K_2Cr_2O_7$. Number of the yeast was measrued by hemacytometer.

b) Separation of Moromi-yeast. The samples of the Moromi were at first centrifugated at 3000 r.p.m. for 30 minutes and supernatant liquid was supplied to analysis. The precipitate was then suspended in sterile water and recentrifugated at 2000 r.p.m. for 10 minutes. The supernatant was discarded. The precipitate was resuspended in water and recentrifugated at 1000 r.p.m. for 5 minutes. This supernatant liquid and also the washings of the precipitate contained mainly yeast. Then both liquids were mixed together, centrifugated at 3000 r.p.m. for 30 minutes and washed thrice with sterile water and prepared for the yeast suspension, which contained still a little solid matter and bacteria.

c) Estimation of the fermentative ability of the Moromi-yeast. For the measurement of the fermentative ability was used a thick-glass injection tube (20–50 ml. in volume). When gas was evolved, the inner rod of the tube was lifted off and the gas space was measured. The tube was shaken at 80 r.p.m. during the fermentation. Q_{CO_2}, shown as the fermentative ability, is the gas volume (μl) evolved for an hour per unit number of the yeast cell, instead of its dry weight, because the yeast suspension so prepared was somewhat contaminated.

Results and Discussion

1. *Chemical and Other Properties of the Moromi-samples.*

In earlier stage. Among the Moromi-samples, C was in the most diluted and

of the Moromi.

NH_2-N (mg/ml)	Acid			Sugar (%)	Dextrin (%)	Number of the yeast cell (10^8/ml)
	Total acid (mg/ml)	Non-volatile acid (mg/ml)	Volatile acid (mg/ml)			
1.14	3.4	2.0	1.5	6.77	1.93	3.2
0.72	2.1	1.7	0.4	3.88	1.80	3.9
0.75	1.7	1.6	0.1	3.06	2.40	1.5
0.54	1.1	0.7	0.4	5.21	2.48	6.7
0.72	0.9	0.5	0.4	10.02	2.17	2.7
				5.34		8.1
				4.96		9.7
				3.74		6.1
				1.82		11.0
				2.80		12.5

macerated state, in which, as shown in Table 2, the alcohol was accumulated in the highest amounts and the soluble sugar in the smallest amounts, in spite of the fewest number of the yeast cell. D and E were the densest and contained abunduntly the solid matters and also higher amounts of soluble sugars and dextrin, where, accordingly, values of pH, and of the relative viscosity were higher. A and B were in medium macerated state but their higher value in acidity seemed to be rather abnormal, judging from their fermentation stage. Since each Moromi-filtrate showed almost the same value in the relative viscosity, which, on the contrary, was considerably distinguished in Moromi itself according to the samples, the viscosity would be mainly dependent on the viscous solid matters contained in the Moromi.

In the later stage. Moromi A and B were somewhat contaminated with the acidifying bacteria, but rescued from the putrefaction. In the year 1949 at many Sake-breweries occurred a type of putrefaction, so-called "Kan-sampai" caused by contamination of the acidifying bacteria. In this putrefaction, the Sake-Moromi fell into an abnormal acidification and reduced its fermentation till it stopped suddenly, so that the acid and sugars were simultaneously accumulated at extremely high levels. Above both samples showed a little such a putrefied tendency in their component, as compared with other samples, and were microscopically detected still the bacterial contamination, while in other samples was observed the yeast cell alone.

2. *Nitrogen Content of Moromi-yeast.*

Moromi-yeast (the yeast fraction separated directly from the Moromi by differential centrifugation) in each sample was prepared in suspension. This suspension was treated in like maner with the true yeast one, though contaminated a little. Table 3 shows the nitrogen content of the yeast cell in each sample. The data per unit dry weight showed a different tendency from the one per unit

Table 3. Nitrogen contents of the yeast separated from the Moromi.

Sample	Number of the yeast cell (X) (10^8/ml)	Dry weight of the yeast (Y) (mg/10ml)	Y/X (mg.10^{-7})	Nitrogen contents of the yeast (mg/10ml)	Nitrogen contents per	
					dry weight (%)	Number of the yeast cell (mg.10^{-9})
A	1.6	179	11.2	9.6	5.4	6.0
B	1.2	108	9.0	6.0	5.1	5.0
C	0.5	90	18.0	3.2	3.5	6.0
D	1.2	113	9.4	4.7	4.1	3.9
E	0.6	113	13.8	3.2	3.9	5.3
A′	8.1	237	33.7	13.5	5.9	1.7
B′	9.7	319	32.9	14.7	4.6	1.5
C′	6.1	323	53.0	24.6	7.6	4.0
D′	11.0	335	30.5	24.6	7.9	2.4
E′	12.5	211	16.8	24.1	11.4	1.9

number of the cell. On referrence with the former, the value in each sample in earlier stage is smaller than in later stage. But in the latter, contrary was the case. If one attaches great importance to the latter result, it seems to be concluded that the nitrogen content of the Moromi-yeast in earlier stage is higher than in later stage. But this conclusion is still doubtful, since the suspension in earlier stage was more contaminated than the one in later stage, where only few matters, other than the yeast cell were recognized microscopically. At any rate, it is correctly concluded that the nitrogen content of the yeast varies according to the fermentation stage in the Moromi-process.

3. *Fermentative Ability (Q_{co_2}) of the Moromi itself, and of the Moromi-yeast.*

As shown in Table 4, Q_{co_2} of the Moromi (fermentative ability of the yeast present in the Moromi) in the later stage was considerably reduced, comparing

Table 4. Fermentative ability of Moromi itself and of its yeast.

Sample	Moromi		Moromi-yeast			
	Q_{co_2} ($\times 10^{-8}$)	Sugar (%)	Q_{co_2} ($\times 10^{-8}$)	Q_{co_2} in the air ($\times 10^{-8}$)	Q_{co_2} against Moromi-filtrate ($\times 10^{-8}$)	
A	206	6.97	1060	1380	A*—CF	1440
B	333	3.88	700	830	B*—BF	1010
C	1240	3.06	600	1200	C*—CF	1120
D	254	5.21	580	750	D*—DF	805
E	444	10.02	630	1500	E*—EF	1850
A′	72.5	5.34	272			
B′	79.1	4.96	256			
C′	138.0	3.74	302			
D′	49.7	1.82	265			
E′	61.8	2.80	246			
Contents of the injection tube	5 ml of the Moromi		YS 10 ml, GP 2 ml. (FSC1.67%)	YS 2 ml, GP 5 ml, air 3 ml. (FSC 7.15%)	YS 10 ml, MF 2 ml, added with GP up to 4 ml. (FSC 1.67%)	

Abbreviation YS: Yeast suspension, GP: Glucose-phosphate buffer solution.
FSC: Final sugar concentration. MF: Filtrate of Moromi.
*A—C^F, *B—B^F, etc. indicates Q_{co_2} of the Moromi A -yeast (*) in the presence of the Moromi filtrate C (C^F), and so on.

with the earlier stage. This reduction would be mainly responsible to the quantities of the alcohol accumulated. The yeast growth phase in the Moromi-process should be also accounted for it, since Q_{co_2} of the Moromi-yeast suspension in the earlier stage showed a larger value in each sample than Q_{co_2} in the later stage.

The other factor, which gives a considerable influence upon the fermentative ability of the Moromi is its viscosity. The Moromi C, which was the least viscous, showed the largest value of Q_{co_2} in both stages, though the smallest was

its content of the yeast cell, Q_{co_2} of which was almost the same or rather lower, as compared with other ones. Especially, in the earlier stage, where the viscosity of the Moromi was considerably distinguished, this dominance in Q_{co_2} was remarkable.

The inhibitory effect of viscosity of the Moromi was also seen in comparison of Moromi-Q_{co_2} with its yeast-Q_{co_2} in both stages. In Table 5, the comparison rate of them in the earlier stage ($Q^{M\text{-}I}_{co_2}/Q^{S\text{-}I}_{co_2}$) was larger than the one in the later stage ($Q^{M\text{-}II}_{co_2}/Q^{S\text{-}II}_{co_2}$) with exception of the Moromi A. That is to say, in the

Table 5. The ratio between the values of Q_{co_2} obtained under various conditions.

Sample	$Q^{M\text{-}I*}_{co_2}/Q^{S\text{-}I'}_{co_2}$ (%)	$Q^{air**}_{co_2}/Q^{S\text{-}I'}_{co_2}$ (%)	$Q^{F''}_{co_2}/Q^{S\text{-}I}_{co_2}$ (%)	$Q^{F''}_{co_2}/Q^{air**}_{co_2}$ (%)	$Q^{M\text{-}II*}_{co_2}/Q^{S\text{-}II'}_{co_2}$ (%)	$Q^{M\text{-}II*}_{co_2}/Q^{M\text{-}I*}_{co_2}$ (%)	$Q^{S\text{-}II'}_{co_2}/Q^{S\text{-}I'}_{co_2}$ (%)
A	19	130	A-CF 135	104	27	34	26
B	48	118	B-BF 144	122	31	24	36
C	207	200	C-CF 177 C-AF 224	95 121	46	11	50
D	44	129	D-DF 139	107	19	20	45
E	71	238	E-EF 294	123	25	14	39

* $Q^{M\text{-}I}_{co_2}$ is Q_{co_2} of the Moromi at the earlier stage (sample A, B, etc.) and $Q^{M\text{-}II}_{co_2}$ is the Q_{co_2} of the one at the later stage (Samples A′, B′, etc.).

′ $Q^{S\text{-}I}_{co_2}$ is Q_{co_2} of the Moromi-yeast at the earlier stage in the presence of glucose added and $Q^{S\text{-}II}_{co_2}$ is the Q_{co_2} of the one at the later stage in the presence of glucose added.

** $Q^{air}_{co_2}$ is Q_{co_2} within air.

″ $Q^{F}_{co_2}$ is Q_{co_2} against the Moromi-filtrate.

earlier stage the recovery rate in the fermentative ability of the yeast was larger when it was made free from the inhibitory factors contained in the Moromi, while in the later stage the yeast would be damaged irreversibly by the components, such as alcohol. Exceptional activity of the Moromi A is not elucidated, because in this case various treatments to rescue it from the putrefaction had been done in the breweries.

It is very interesting to note that, as already mentioned, the Moromi-Q_{co_2} in C was startlingly larger than its yeast-Q_{co_2} in the earlier stage. From this fact, it was assumed that there would be a fermentation promoting factor contained in the Moromi. Then, the effect of the Moromi-filtrate was researched. When added with the Moromi-filtrate, the fermentative ability of the Moromi-yeast in each sample was considerably promoted unexceptionally. Each data on this promoting effect was in accordance with the result in the aerobic fermentation, though both experimental conditions differed, as shown in the Table 3.

Because the accord was much better than to say a chance, the promoting effect would be concerned with the aerobic fermentation. If it were true, it is not reasonable, however, that the Moromi C contained other specific promoting factors, since the Moromi-filtrate A showed also the promoting effect on the fermentation of the Moromi-yeast C as same as the Moromi-filtrate C. All these values of Q_{CO_2} in this effect referred to the Moromi C were almost the identical. Therefore, it may be correctly mentioned that the viscosity of the Moromi C was too low to affect inhibitorily on its fermentation, and from the results above cited, it is also found that a fermentation inhibiting factor was not contained in each Moromi-filtrate, but the fermentation was inhibited by the viscosity of the Moromi itself.

Summary

Five samples of the Sake-Moromi, collected from different breweries in Sendai districts in 1949, were analyzed, and fermentative ability of the yeast which was separated directly from each Moromi-sample, was investigated. It was concluded that the yeast fermentation was inhibited by the viscosity of the Moromi, the nltrate of which would contain a fermentation promoting factor.

PART II. CHANGES IN THE FERMENTATIVE ABILITY OF THE YEAST AND ITS RESISTANT ABILITY AGAINST THE ALCOHOL DURING THE SAKE-BREWING PROCESS

By

Tsuneji SUDÔ, Kenjirô TAKAGI, Yoshitsugu FUJII, Chôseki FURUSAKA, & Teijirô UEMURA

Introduction

As preliminarily reported, we have attained to provide a method to investigate ecological aspects of the yeast in Sake-brewing process. The yeast was separated directly from the Sake-Moromi by differential centrifugation. At the present report, changes in the fermentative ability of the yeast and its tolerance against 15% alcohol during the Sake-fermentation process were periodically observed by this means. The latter ability was investigated, either when incubated in the alcohol, or fermented in the presence of it. Samples were collected from three Sake-batches in Hôzan brewing manufactory, attached

to Sendai Sake-Making Company, Ltd. in 1950.

Experimental Part

1. *Samples*

Following are series of the samples in the Sake-fermentation process, which were collected from the brewery at two or three days interval.

Series No. 8: A batch for brewing the Sake of the first class quality, used with Sokujô-Moto.

Series No. 13.: A batch for brewing the Sake of the first class quality, used with Yamahai-Moto.

Series No. 12: A batch for brewing the Sake of the second class quality, used with Yamahai-Moto incubated in resting for a long time (3 weeks resting).

In each batch was used Tsukihaze-Kôji (a Kôji with an abundunt growth of Asp. oryzae only in the inside of the rice granule).

The additional rates of the materiales for the preparation of each batch were as follows (Table 6).

Table 6. Additional amounts of the materials for the preparation of Moromi.

Materials / Preparation for	Steamed rice (Kg)	Rice for Kôji (Kg)	Water (L)
Moto	77.0	37.8	126.0
the first addition	168.0	67.2	216.0
the second addition	336.0	98.0	558.0
the thrid addition	637.0	119.0	1080.0
Total	1218.0	322.0	1980.0

2. *Experimental Method.*

a) Analysis of the Sake-Moto, or of its Moromi.

The supernatant of centrifugate of the samples was applied to an analysis. Measurement of pH, and of acidity, and also determination of soluble nitrogen, soluble sugar, dextrin as well as of alcohol were done by the methods reported preliminarily. On estimation of acidity, which was shown as lactic acid in the Moto-samples and as succinic acid in the Moromi-samples, was used phenolphthalein as an indicator, so that dissolved CO_2 had in part the response to it.

b) Yeast-counting and measurement of its fermentative ability.

The number of yeast was hemacytometrically measured.

The fermentative abilities of the Moto, the Moromi, or their yeast were measured by injection tube method at 30°. 10ml of the samples were contained in injection tube (50 ml in volume). From the gas volume (ml) when evolved linearly, was calculated Q_{co_2} per a cell.

c) Preparation of the Moto- and the Moromi-yeast.

The preliminary method to separate the yeast was partly modified in attempting to prepare the yeast suspension with less contaminant.

The sample was diluted with water and filtered through gauze to get rid of the solid matters, such as unmacelated rice granules. The filtrate was centrifugated at 1000 r.p.m. for 3–5 minutes. The precipitate was distinctive in two layers. The grayish white colored layer of the yeast was covered by a thin white layer. The yeast layer was stripped off from the upper one which was scraped off with a spatula. Then, it was suspended in the sterile water and centrifugated again at 1000 r.p.m. for 3 minutes. This stripping treatment was repeated several times. At last the yeast layer was washed twice with water and prepared in suspension, in which almost only the yeast cell was present. Difficultics in the stripping came in the stage of Moromi just after completion of mixing the materials. As in this case the yeast layer was as white as the starchy granule layer, it was difficult to distinguish both layers.

d) Estimation of the tolerance of the yeast fermentative ability against 15% alcohol.

The yeast suspension prepared as above was supplied to estimate the tolerance against 15% alcohol. An injection tube containing 1 ml of 30% alcoholic solution of M/10 glucose and M/10 pottasium phosphate was added with 1 ml of the yeast suspension. Then the fermentative ability in the presence of 15% alcohol was estimated at 30°.

The inhibitory effect produced by soaking in 15% alcohol was also observed. The injection tube containing the above cited components, excluding the glucose, was incubated at 30°C for about 20 hours, during which it was detected whether or not the endogenous fermentation occurred. Then the content of the tube was centrifugated and the yeast was separated, which was then adopted to estimate its fermentative ability. From the loss in its activity, as compared with the original one, was shown the inhibitory effect by soaking in 15% alcohol.

e) Emergency of the microbes in the samples.

The sample was diluted 10^5 times, inoculated into Kôji-juice agar medium and allowed to grow on it at 30°C. Number of the colonies emerged on it and their kind were detected respectively.

f) Growth test on the Moto- or the Moromi-yeast as inoculum.

0.1 ml of the Moto- or the Moromi-yeast suspension was added to 2 ml of Kôji-juice and contained in the injection tube (50 ml in volume). To the content of the tube was added air up to 5 ml in all, and then incubated at 30°C for about 24 hours on shaking. Time of the lag (hour), volume of the gas evolved (ml), and number of the yeast multiplied were measured respectively.

Results

a) Changes in components in the fermentation process.

In the brewery was inoculated the yeast into the Moto, on the 13th day (Sample No. 8) or on the 12th day (Sample No. 12 and No. 13), from which the samples were collected. In each Moto-sample, the sugar as well as the soluble nitrogen were accumulated in higher level of the quantities before the inoculation of the yeast, as shown in Tables 7, 8, and 9. Especially in the Sokujô-Moto, pH of which was lower than others at the initial and kept almost in equilibrium throughout the process, the amounts of the sugar accumulated, attained already to 20% and more over in the initial stage of the process. In this

Table 7. Changes in components of No. 8-Moto (Sokujô-Moto).

Time elapsed after the materials-mixing (Day)	pH	Acid (%)	Sugar (%)	Dextrin (%)	Alcohol (vol. %)	Soluble nitrogen (mg. %)
3	3.8	0.47	20.09	10.11		69.0
6	3.8	0.49	23.10	8.11		146.4
10	3.7	0.52	25.42	6.17		154.8
12	3.7	0.53	25.50	6.45		171.6
13 (0)*	3.8	0.54	25.93	4.18		
15 (2)	3.9	0 57	25.04	4.40	0.27	175.4
18 (5)	3.3	0.73	23.03	4.37	1.41	162.6
21 (8)	3.3	0.86	13.09	7.31	3.98	142.6
24 (11)	3.7	0.86	9.45	3.83	7.10	145.2
26 (13)	3.7	0.85	8.24	4.02	9.23	143.2

* Figures in ( ) show the time (day) elapsed after the inoculation of the yeast into Moto.

Table 8. Changes in componenfts of No. 12-Moto (Yamahai-Moto).

Time elapsed after the materials-mixing (Day)	pH	Acid (%)	Sugar (%)	Dextrin (%)	Alcohol (vol. %)	Soluble nitrogen (mg. %)
3	5.8	0.09	16.87	7.97		27.2
6	5.7	0.10	18.89	6.65		47.2
9	4.4	0.21	20.85	5.45		85.0
12	4.3	0.29	22.90	6.33		
15 (0)*	4.4	0.37	25.16	2.58		162.0
18 (3)	3.8	0.51	25.81	1.58	0.27	139.0
21 (6)	3.7	0.79	21.96	2.01	2.37	174.5
24 (9)	3.8	0.86	10.98	3.60	5.55	156.0
30 (15)	3.7	0.87	8.66	3.63	11.14	167.6
33 (18)	3.7	0.86	8.04	3.65	9.06	152.0
39 (24)	3.6	0.86	9.05	3.06	8.23	158.4
45 (30)	3.8	0.84	8.88	3.47	9.15	

* Figures in ( ) show the time (day) clasped after the inoculation of the yeast into Moto.

Table 9. Changes in components of No. 13-Moto (Yamahai-Moto).

Time elapsed after the materials-mixing (Day)	pH	Acid (%)	Sugar (%)	Dextrin (%)	Alcohol (vol. %)	Soluble nitrogen (mg. %)
3	5.8	0.09	16.74	8.93		14.8
6	5.8	0.11	18.84	6.59		48.6
9	4.3	0.20	21.82	3.18		82.6
12	4.3	0.28	23.62	2.41		87.2
15 (0)*	4.2	0.36	25.73	4.02		162.6
18 (3)	4.1	0.48	26.19	3.31	0.27	191.6
21 (6)	3.7	0.86	21.52	2.87	3.21	178.0
24 (9)	3.7	0.96	11.69	3.48	6.32	169.8
28 (13)		0.93	9.46	3.97	9.83	172.4

* Figures in ( ) show the time (day) elapsed after the inoculation of the yeast into Moto.

case, accumulation of other components progressed also more rapidly than in Yamahai-Moto, pH of which became lower day by day before the inoculation of the yeast, where Lactobacilli were growing on. In this stage of both Moto-processes, there only occurred a faint alcoholic fermentation, or none.

The yeast was then inoculated, and compelled to grow on such a dense medium. Alcoholic fermentation became graudally vigorous and attained to its maximum. This was a fermentation stage in the Moto-process, in which the sugar and the soluble nitrogen were decreased in their quantities respectively, while the alcohol as well as acidity were increased. The sugar was depressed almost to half, but the alcohol accumulation did not perfectly correspond to this depression. On the contrary, there was a little decrease in the soluble nitrogen.

Table 10. Changes in components of No. 8-Moromi.

Time elapsed after the materials-mixing (Day)	pH	Acid (%)	Sugar (%)	Dextrin (%)	Alcohol (vol. %)	Soluble nirogen (mg. %)
28*			10.85	8.38	1.68	
31 (3)**	4.3	0.12	6.79	6.33	2.16	35.4
34 (6)	3.8	0.16	5.17	6.94	4.23	29.4
37 (9)	3.9	0.20	4.70	7.07	5.70	52.8
40 (12)	4.0	0.24	4.36	6.26	6.79	69.0
43 (15)	3.9	0.28	2.76	4.33	9.83	75.6
46 (18)	3.8	0.31	3.72	5.15	11.23	87.0
51 (23)	3.9	0.38	2.99	4.46	13.56	100.6
56 (28)	3.9	0.39	3.08	3.45	14.06	120.4
61 (33)	4.0	0.43	2.74	2.86	16.72	113.4
66 (38)	4.2	0.38	2.28	2.24	18.62	110.2

* Just before the second addition of the materials for preparation of the Moromi.

** Figures in ( ) show the time (day) elapsed after the mixing process of the materials for preparation of the Moromi.

Table 11. Changes in components of No. 12-Moromi.

Time elapsed after the materials-mixing (Day)	pH	Acid (%)	Sugar (%)	Dextrin (%)	Alcohol (vol. %)	Soluble nitrogen (mg. %)
47*	4.0	0.40	11.87	5.62	3.56	116.0
48**	4.3	0.40	10.84	6.65	1.82	69.0
50 (3)***	4.6	0.11	8.60	5.53	2.51	39.0
53 (6)	4.0	0.17	6.56	5.59	4.80	
56 (9)			5.45	5.30	7.58	
59 (12)	4.0	0.30	4.31	4.91	10.79	81.8
62 (15)	4.0	0.42	3.76	3.84	13.44	112.7
65 (18)	4.1	0.42	3.31	3.64	15.36	120.3
70 (23)	4.1	0.46	3.01	2.91	16.07	129.6

* Just before the second addition of the materials for preparation of the Moromi.

** Just before the third addition of them.

*** Figures in ( ) show the time (day) elapsed after the mixing process of the materials for preparation of the Moromi.

Table 12. Changes in components of No. 13-Moromi.

Time elapsed after the materials-mixing (Day)	pH	Acid (%)	Sugar (%)	Dextrin (%)	Alcohol (vol. %)	Soluble nitrogen (mg. %)
31*	4.0	0.21	11.70	7.30	2.02	130.5
32**	4.2	0.20	9.50	7.63	1.34	73.5
34 (3)***	4.5	0.13	9.63	5.90	1.48	46.4
37 (6)	3.9	0.12	9.88	5.04	1.54	29.6
40 (9)	3.8	0.20	6.17	6.10	4.65	58.6
43 (12)	3.8	0.24	5.09	5.18	6.17	77.6
46 (15)	3.9	0.15	4.84	5.16	9.72	91.8
49 (18)	3.9	0.38	4.20	4.07	12.41	103.4
54 (23)	3.9	0.42	3.54	3.70	14.27	117.8
59 (28)	4.0	0.44	3.25	3.39	15.70	122.6
64 (33)	4.2	0.39	2.62	2.04	19.34	96.0

* Just before the second addition of the materials for preparation of the Moromi.

** Just before the third addition of them.

*** Figures in ( ) show the time (day) elapsed after the mixing process of the materials for preparation of the Moromi.

The resting stage ("Karashi" or "Yasumi") was followed to the fermentation one. During this stage, the fermentation became weaker and weaker, till it seemed to stop, and all the components scarcely showed a visible change, as seen from the figures in the later period of the sample No. 12 in the Table 8.

In the Moromi-process, the fermentation proceeded accompanied with the saccarification from its initial stage, so that there was not encountered as high amount of sugar accumulated as in the Moto-process (Tables 10, 11, 12). During mixing of the materials and in 2 or 3 days after it, the sugar was accumulated in comparatively high level of quantities (8-11%), and then

gradually decreased, as the fermentation proceeded. The soluble nitrogen was also decreased parallel with the sugar in the earlier stage, while, on the contrary, in the later stage it was then increased parallel with the alcohol. This fact showed the luxuriant growth of the yeast during the vigorously occurring fermentation. The acidity varies in like manner with the soluble nitrogen. The alcohol continued to be accumulated till the last. pH varied slightly in the initial stage, and then was kept almost in equilibrium.

b) Changes in the fermentative abilities of the Moto, and of the Moromi.

In the Moto-process, the fermentative ability of the Moto as well as the number of the yeast emerged in it were able to be measured for the first time on the 3rd day after the inoculation of the yeast. This fact marks decisively a characteristic aspect of the Moto, distinguished from the Moromi-process. Despite the use of Kôji, on which the yeast had somewhat adhered, it was not able to achieve a remarkable growth, because the initial temperature of the Moto was too low to admit its growth, while even at this low temperature the saccahrification could proceed on. At the period, when the temperature of the Moto became higher by means of heating and thus more favourable conditions to the yeast grwoth was prepared, the inoculation was done, so that it might be indiscernable whether the yeast crowding was derived from the inoculum, or from the wild. But it is ordinarily accepted to facilitate the yeast growth by the inoculation and to be able to protect the Moto from the spoilage.

Table 13 showed that the maximum fermentative ability of the Moto (X_{co_2}) reached about on the 5th or 9th day after the inoculation, dependently on the yeast population, whilst the maximum value of Q_{co_2} per a cell appeared in earlier period, already on the 3rd day after the inoculation, corresponding to the growth period of the yeast. In long-incubated sample No. 12, X_{co_2} was decreased gradually but more rapidly than the Q_{co_2}-depression in the later stage of the Moto-process.

During the three steps-mixing of the materials for preparation of the Moromi, considerable number of the yeast was crowded, showing also the comparatively high value of X_{co_2} (see Tables 14 & 15). The first peak of X_{co_2} was followed on the 3rd or 6th day after the completion of the materials-mixing. During the mixing process and the following initial stage of the Moromi-fermentation, the level of order in the number of the yeast in each sample at the intervals tested, was indeed almost unchanged (10^8/ml.), though the yeast population present in the Moto used in preparation was diluted by mixing a large amount of the materials, such as steamed-rice, Kôji, or water. This fact seems to be rather curious, but it might be well mentioned, that in the preparation process for the Moromi, the yeast was treated to burst into its multiplication as soon as the level in its population was disrupted by the mixing, and to recover the

Table 13. Changes in the fermentative ability of the Moto

Time elapsed after the materials-mixing (Day)	No. 8-Moto			
	X_{CO_2} (μ1/hr/ml)	number of yeast cells (.10^8/ml)	Q_{CO_2} (μ1/ml .10^8)	X_{CO_2} (μ1/hr/ml)
9				10
12	140			20
13*	140			
15**	380			0
18	1720			490
21	1260	5.2	242	1300
24	1030	6.6	162	1320
26	1040	4.8	217	
28				
30				800
33				750
39				410
45				660

* Inoculation of the yeast into No. 8-Moto.

** Inoculation of the yeast into No. 12- and No. 13-Moto.

level soon after. Even in the sample No. 12, in which the long-resting Moto was used and accordingly, the multiplicating ability of the yeast would be weakened anyhow, this recovery in the initial stage of the Moromi-proccess was also observed.

However, although this was the case, the yeast in the early stage of the Moromi-process continued to grow, being supplied with the sugar and soluble nitrogen by the Kôji-enzymes. Thus, the second peak of X_{CO_2}, which emerged on

Table 14. Changes in the fermentative ability of No. 8- and No. 13-Moromi against number of the yeast cells present in them.

Time elapsed after the materials-mixing (Day)	No. 8-Moromi			No. 13-Moromi		
	X_{CO_2} (μ1/hr/ml)	Number of yeast cells ($\times 10^8$/ml)	Q_{CO_2} (μ1/ml .10^8)	X_{CO_2} (μ1/hr/ml)	Number of yeast cells ($\times 10^8$/ml)	Q_{CO_2} (μ1/ml. 10^8)
28	800	5.3	150			
30				800	2.6	308
31	1650			1380	1.8	767
34	1910	7.0	273	1710	2.2	737
37	1600	4.4	364	2660	4.4	605
40	1750	8.8	199	1380	4.4	416
43	2450	13.2	184	2350	16.4	143
46	1320	7.9	167	1540	7.4	208
49				1080	6.6	164
51	1330	4.4	302			
54				700	6.4	109
56	590	6.8	87			
59				400	5.7	70
61	310	5.5	56			
64				200	4.6	43
66	130	4.3	30			

against number of the yeast cells present in it.

No. 12-Moto		No. 13-Moto		
number of yeast cells ($.10^8$/ml)	Q_{CO_2} (μl/ml $.10\mu$)	Q_{CO_2} (μl/hr/ml)	number of yeast cells ($\times 10^8$/ml)	Q_{CO_2} (μl/ml $.10^8$)
		10		
		20		
		0		
0.3	1630	480	0.44	1090
4.4	295	1380	4.4	406
4.8	275	1440	5.7	253
		790		
3.1	242			
4.4	91			
4.1	162			

the 9th or 15th day after the completion of mixing in each sample, would be rather due to the ripe yeast crowding at the maximum rate.

On the other hand, the peak of Q_{CO_2} (per a cell) of the Moromi did not always periodically accord with the one of its X_{CO_2}, because Q_{CO_2}-value was not affected by the yeast-population but mainly by the grwoth period of the yeast and the components of the Moromi, whilst X_{CO_2}-value was dependent on these three factors, the effective rate of which varied according to the fermentation periods in the Moromi-process.

In the later stage of the Moromi-process, X_{CO_2} of the Moromi as well as its Q_{CO_2} were decreased considerably, though they did not become null until the last, while the number of the yeast in it was depressed rather slightly, and kept almost in equilibrium. It is obvious that the decrease in the fermentative ability

Table 15. Changes in the fermentative ability of No. 12-Moromi against number of the yeast cells present in it.

Time elapsed after the materials-mixing (Day)	X_{CO_2} (μl/hr/ml)	Number of yeast cells ($\times 10^8$/ml)	Q_{CO_2} (μl/ml $.10^8$)
47	1530	3.5	437
48	1860	3.1	600
50	2630	6.6	398
53	2200	4.2	524
56		7.6	
59	1670	5.7	298
62	2100	5.2	404
65	960	5.1	186
70	420	4.6	91

of the Moromi was caused on account of high amounts of alcohol accumulated.

c) Fermentative ability of the yeast separated directly from the Moto or the Moromi, its nitrogen contents, and its tolerance against 15% alcohol.

The yeast separated directly from the Moto or the Moromi by differential centrifugation, are designated Moro- or Moromi-yeast. A few profits were derived methodologically by detection on the properties of such a yeast. Above

Table 16. Changes in the fermentative ability, nitrogen
the Moto or the Moromi

Time elapsed after the materials-mixing (Day)	Yeast suspension (/ml)			
	X_{CO_2} (μ1/hr/ml)	Number of yeast cells (.10^8/ml)	Q_{CO_2} (μ1/ml .10^8)	Nitrogen contents (mg/10^8)
18	1700			
21	5680	4.2	1350	0.195
24	3160	5.0	630	0.264
26	2200	3.6	610	0.253
28	2560	2.8	910	0.375
31 (3)*	1480	0.4	3700	
34 (6)	3200	4.4	730	0 304
37 (9)	5700	7.2	790	0.218
40 (12)	4460	8.0	560	0.229
43 (15)	7640	15.2	500	0.211
46 (18)	4460	9.2	480	0.309
51 (23)	2580	3.6	720	0.372
56 (28)	2480	2.4	1030	0.500
61 (33)	2180	3.2	680	0.378
66 (38)	2520	7.3	350	0.210

* Figures in ( ) show the (day) elapsed after the mixing process of

Table 17. Changes in the fermentative ability,
separated from the Moto or the

Time elapsed after the materials-mixing (Day)	Yeast suspension (/ml)			
	X_{CO_2} (μ1/hr/ml)	Number of yeast cells ($\times 10^8$/ml)	Q_{CO_2} (μ1/ml .10^8)	Nitrogen contents (mg/10^8)
21	1700	2.8	610	0.447
24	2580	4.3	600	0.174
30	2660	4.6	580	0.302
33	2560	4.4	580	0.259
39	1860	3.2	580	0.431
45	2060	3.6	570	0.683
47	2240	1.2	1720	0.550
48	1620	0.8	2030	1.288
50 (3)*	2180	2.4	910	0.450
53 (6)	2820	3.6	790	
59 (12)	5820	6.0	970	0.360
62 (15)	4980	9.0	550	0.240
65 (18)	4660			
70 (23)	4040	10.2	400	0.226

* Figures in ( ) show the (day) elapsed after the mixing

all, measurement of its fermentative ability, or of its nitrogen contents served to detect correctly the growth phase of the yeast present in the fermmentation mash, since it was not available for acknowledgement on the growth period of the organism, which might afford us to elucidate the complicated phenomena in the brewing process, to count the number of the cells in such a dense and viscous mash as the Moto or Moromi.

contents and alcohol tolerance of the yeast separated from in the Sample No. 8.

In the presence of 15% alcohol		When soaked in the 15% alcohol	
X_{CO_2} (μl/hr/ml)	Rate of inhibition (%)	X_{CO_2} (μl/hr/ml)	Rate of inhibition (%)
140	91.8	0	100
0	100	0	100
1180	62.7	0	100
0	100	240	89.1
580	77.3	440	82.8
0	100	0	100
840	29	320	90.0
1540	73.0	0	100
1120	74.9	1120	74.9
2420	68.3	1080	85.9
980	88.0	3720	16.6
600	76.7	1580	38.8
620	75.0	1480	40.3
480	78.0	1320	40.9
540	78.6	740	70.6

the materials for preparation of the Moromi.

notrogen contents and alcohol tolerance of the yeast Moromi in the Sample No. 12.

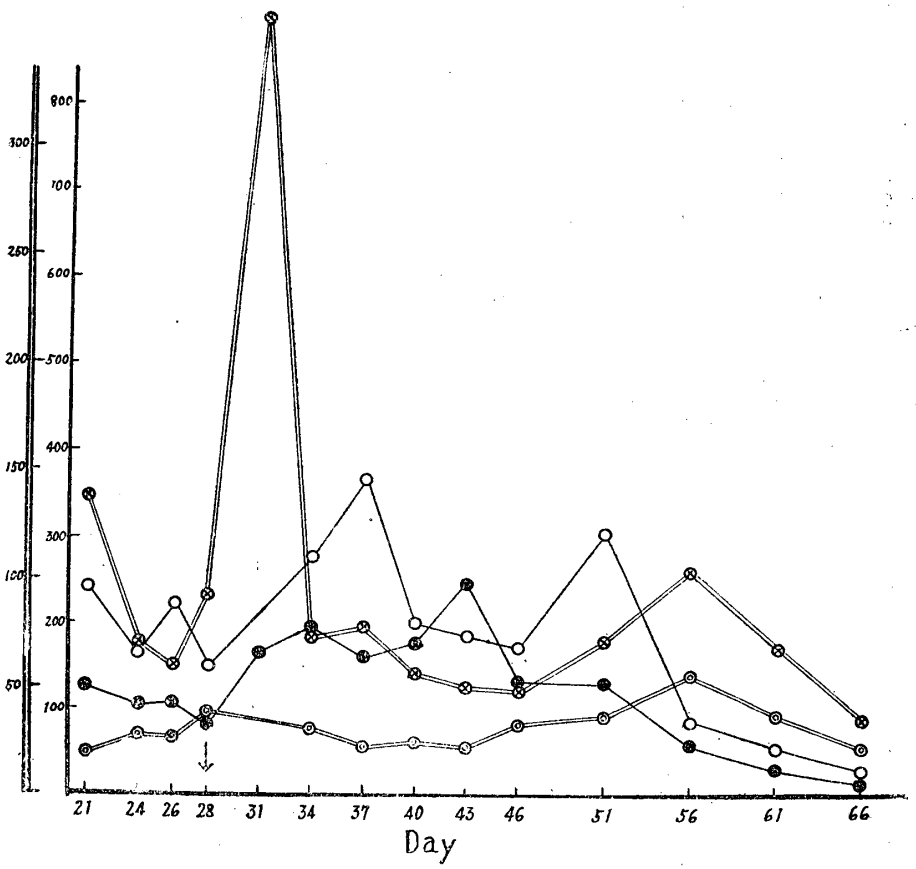
In the presence of 15% alcohol		When soaked in the 15% alcohol	
X_{CO_2} (μl/hr/ml)	Rate of inhibition (%)	X_{CO_2} (μl/hr/ml)	Rate of inhibitiou (%)
0	100	120	92.9
140	94.6	20	99.2
980	63.2	1620	39.1
1500	41.4	620	75.8
500	73.1	1400	24.7
700	66.0	1340	35.0
240	89.3	0	100
60	96.3	0	100
100	95.4	0	100
380	86.5	600	78.7
1380	76.3	4900	15.8
1300	73.9	3140	16.9
1140	75.5	2940	36.9
800	80.4	1440	64.9

process of the materials for preparation of the Moromi.

Table 18. Changes in the fermentative ability, yeast separated from the Moto or the

Time elapsed after the materials-mixing (Day)	Yeast suspension (/ml)			
	X_{CO_2} (μl/hr/ml)	Number of yeast cells ($\times 10^8$/ml)	Q_{CO_2} (μl/ml. 10^8)	Nitrogen contents (mg/10^8)
21	1740	3.6	430	0.328
24	2580	4.3	600	0.328
28	1620	2.4	680	0.425
31	2000	2.4	830	0.550
32	1500	1.2	1250	0.550
34 (3)*	400	0.4	1000	1,250
37 (6)	1980	3.9	510	0.200
40 (9)	2620	13.2	200	0.089
43 (12)	3940	10.0	394	0.168
46 (15)	3340	5.6	600	0.273
49 (18)	5300	5.2	1020	0.408
54 (23)	3600	4.4	820	0.532
59 (28)	3580	3.6	990	0·603
64 (33)	2200	6.9	320	0.183

* Figures in ( ) show the (day) elapsed after the mixing process of



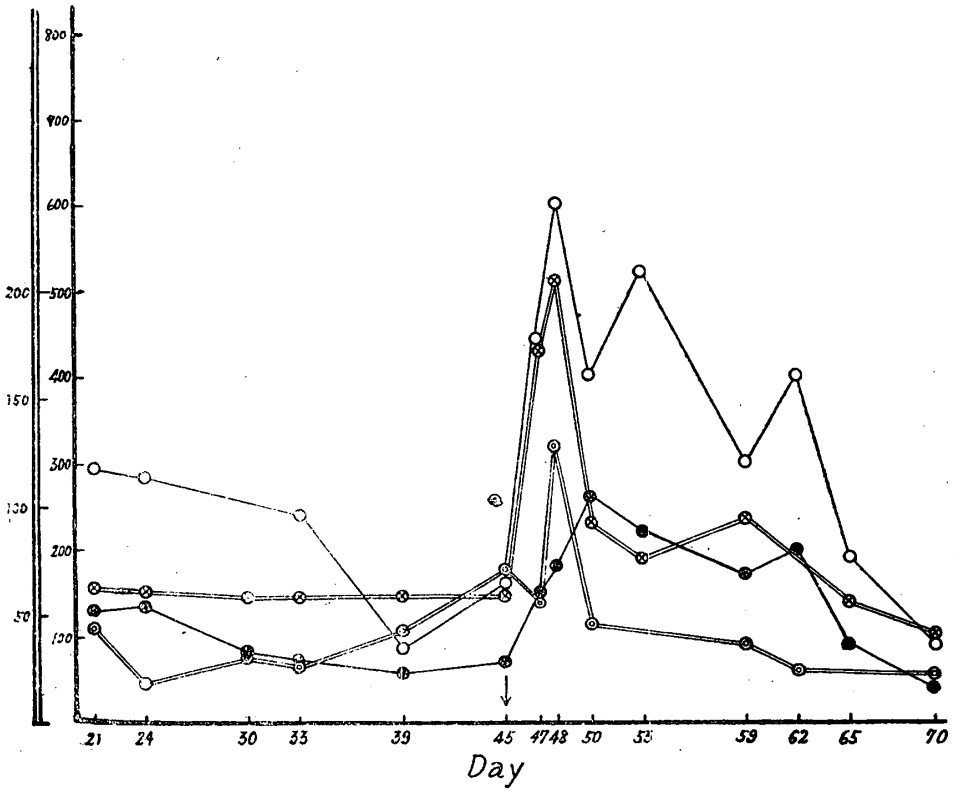
○——○ Q_{CO_2} of Moto and Moromi ; ●——● X_{CO_2} of Moto and Moromi ; ◎═══◎ Q_{CO_2} of their yeast suspension ; ⊗═══⊗ nitrogen contents (mg/ml) of their yeast suspension. The days in the abscissa before ↓ shows the Moto-process and the days after ↓ the Moromi-process.

Fig. 1. Changes in the fermentative ability of Moto, Moromi and their yeasts. (No. 8–Moto and –Moromi)

nitrogen contents and alcohol tolerance of the Moromi in the Sample No. 13.

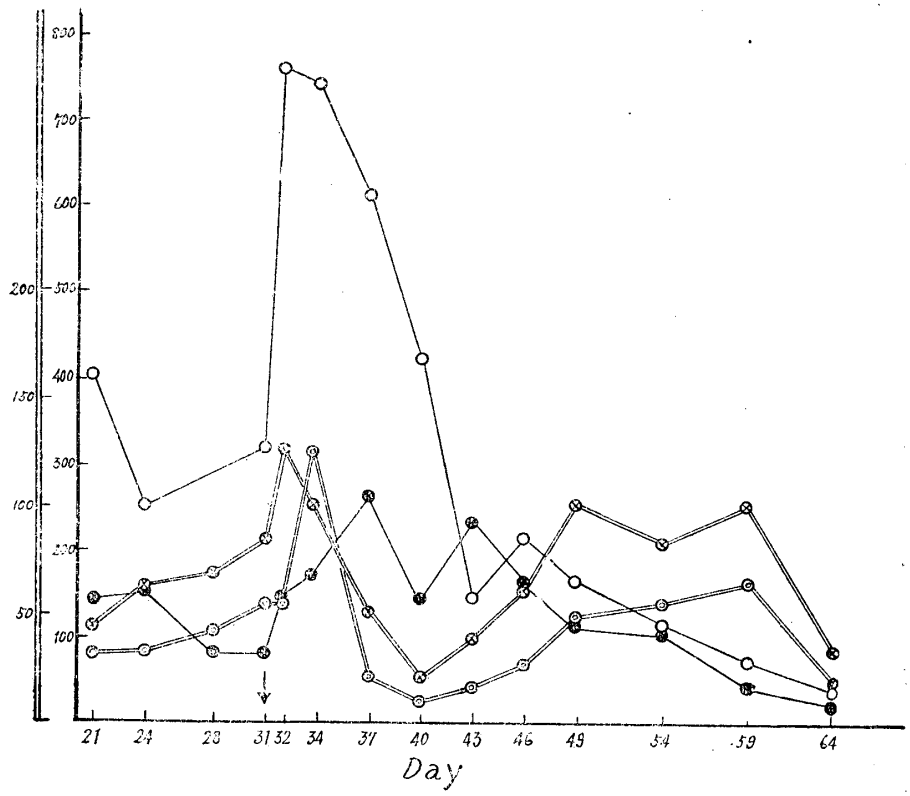
In the presence of 15% alcohol		When soaked in the 15% alcohol	
X_{CO_2} (μl/hr/ml)	Rate of inhibition (%)	X_{CO_2} (μl/hr/ml)	Rate of inhibition (%)
140	92.0	500	71.3
140	94.6	0	100
480	70.3	0	100
100	95.0	40	98.0
40	97.3	180	88.0
0	100	220	45.0
200	89.9	620	68.7
640	75.6	200	92.4
1110	70.6	340	91.4
1020	69.5	120	96.4
1180	77.7	300	94.3
860	76.1	1200	66.7
900	74.8	1580	55.9
420	80.9	460	79.1

the materials for preparation of the Moromi.



○—○ O_{CO_2} of Moto and Moromi; ●—● X_{CO_2} of Moto and Moromi; ◎═◎ Q_{CO_2} of yeast suspension; ⊗═⊗ nitrogen contents (mg/ml) of yeast suspension. The days in the abscissa before ↓ shows the Moto-process and the days after ↓ the Moromi-process.

Fig. 2. Changes in the fermentative ability of Moto, Moromi and their yeasts. (No. 12–Moto and –Moromi).



○—○ Q_{co_2} of Moto and Moromi; ●—● X_{co_2} of Moto and Moromi; ◎═◎ Q_{co_2} of their yeast suspension; ⊗═⊗ nitrogen contents (mg/ml) of their yeast suspension. The days in the abscissa before ↓ shows the Moto-process and the days after ↓ the Moromi-process.

F:g. 3. Changes in the fermentaive ability of Moto, Moromi and their yeasts. (No. 13-Moto and -Moromi).

In the early stage of the Moto-process, though considerable fermentation occurred, it was hardly successful in separation of the yeast by the centrifugation on account of the abundunt contaminants, which were colorded as white as the younger yeast layer. The Moto-yeast was then cropped at the vigorously fermenting period or the following resting period, because the ripe yeast layer was colored dark gray, and accordingly, easily distinguishable from the less quantities of the white-colored contaminants than in earlier stage. Thus, so far as changes in Q_{co_2}-values of the Moto-yeast as well as in its nitrogen contents are concerned, it was yet difficult to presume their tendencies. However, it was well mentioned that Q_{co_2}-values of the Moto-yeast were higher than those of the Moto itself, as shown in Tables 16, 17 and 18 (sea also Figures 1, 2 and 3).

In long-resting Moto (Sample No. 12), Q_{co_2}-values were almost unchanged

and constant even in incubation for 20 days. As already mentioned, all the components of the Moto were also kept in equlibrium during such a resting period. But the nitrogen contents of the Moto-yeast were increased and its fermentative ability became more tolerable against 15% alcohol, especially when soaked in it (see Table 16).

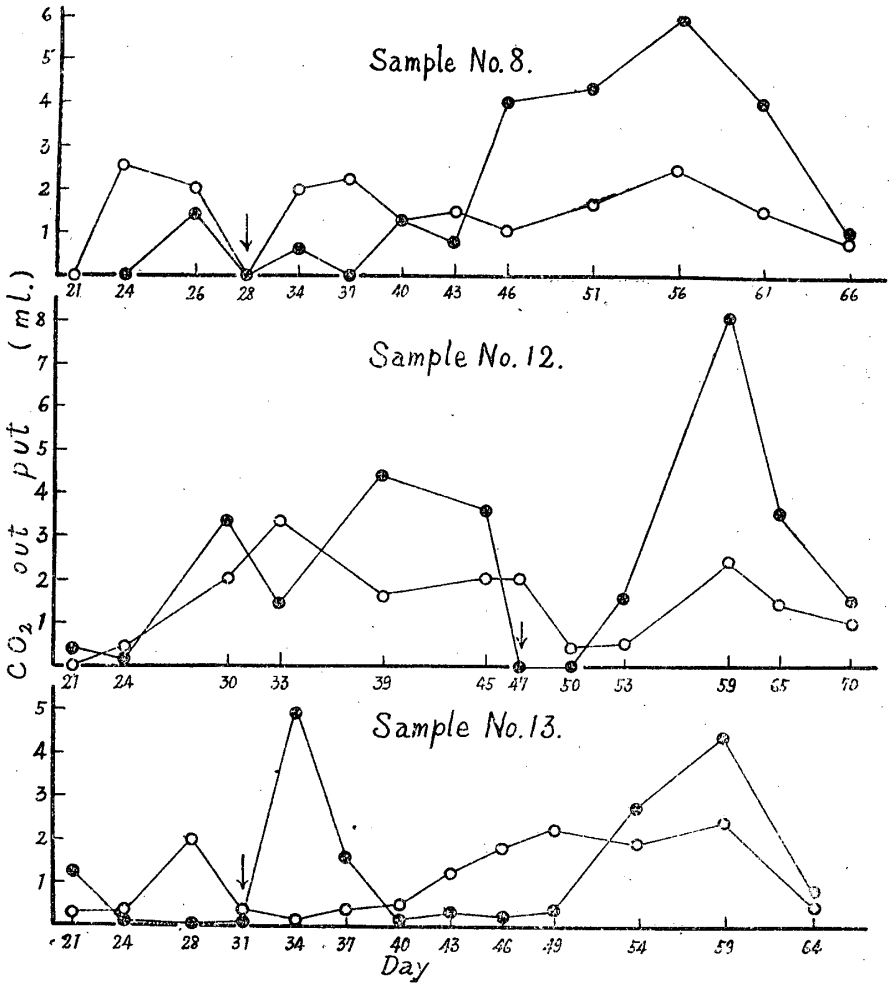
The resting process of the Moto is also one of the characteristic aspects of Sake-brewing. Its purpose is to kill the infecting bacteria including Lactobacilli by the acid and alcohol accumulated in the Moto-process. It is customary to say, too, that the yeast would be ripened during the resting of the Moto. This might be interpreted in the fact, in which the Moto-yeast acquired abundantly the nitrogenous substances in its body with faintly but continuously occurring fermentation during the resting period. The more purified and ripened Moto-yeast thus obtained, is often favorable when used in the Moromi-porocess, because such a yeast is able to grow sluggishly and accordingly, to cause safely a mild fermentation in the Moromi-process.

Some technical difficulties were also encountered in preparation of the Moromi-yeast in its earlier periods such as on the 3rd day, or just after completion of the materials-mixing. The preparations in these periods were much contaminated by the strach granules. But, as a large amount of the yeast was present in such a Moromi, as already mentioned, the separation was attained successfully. The Moromi-yeast thus obtained, showed the highest values in Q_{co_2} and in their nitrogen contents throughout the process, as seen in Figs. 1, 2 and 3. On the other hand, their tolerance against 15% alcohol were very weak, or almost null.

Then, Q_{co_2} and the nitrogen contents of the Moromi-yeast were remarkably decreased, whereas it became more resistant against the alcohol. Particularly, the tolerance tested when fermented in the presence of 15% alcohol was gradually obtained in it. This stage was in a bottom of Q_{co_2}-curve. Nitrogen contents of the yeast were the lowest throughout the process, too.

Against the following period were ascending these curves (see Fig. 4). As already indicated, early in this stage of the Moromi-process emerged the second peak in the X_{co_2} (Moromi itself)-curve. When this X_{co_2}-curve was inclined to descend, Q_{co_2} (Moromi-yeast)-curve attained to the second top, where the alcohol-tolerance of the yeast increased to its maximum. The nitrogen contents of the Moromi-yeast varied almost corresponding to its Q_{co_2}, though their maximum rate appeared a little later.

On the 33rd day after the mixing process, Q_{co_2} of the yeast as well as its nitrogen contents were considerably decreased and its alcohol-tolerance was also depressed. The Moromi-yeast then showed aspects of the last stage in its number and also in its activities.



○—○ Q_{CO_2} of yeast within 15% alcohol soln.; ●—● Q_{CO_2} of the yeast preliminarily steeped in 15% alcohol soln. The day in the abscissa before ↓ shows the Moto-proces and the day after ↓ the Moromi-process.

Fig. 4. Changes in the alcohol-tolerance of the Moto- and Moromi-yeasts.

These changes in Q_{CO_2} of the Moromi-yeast and in its nitrogen contents showed to be in a good agreement with variations in properties of the cultivated yeast due to its growth phases. Because in the early stage of the Moromi-process, the saccharification caused by the Kôji-enzyme proceeded on as actively as the multiplication of the yeast, the growing phase would be fairly prolonged and indeed elapsed for 7 or 10 days in each sample. In the Moromi of the sample No. 12, in which the long-resting Moto was used, the yeast passed through each growth period in haste. This fact indicated that the suitable resting period would be necessary for the preparation of a good starter.

d) Detection on the organisms present in the Moto, or the Moromi.

The samples, diluted in the fixed volume, were inoculated into the Kôji-juice agar medium and incubated at 30°C for 3 days long, in order to detect the microbes present in the samples.

Asp. oryzae, which was derived from the rice Kôji, was alive in the Moto-samples till about on the 13th day after the materials-mixing, and a few colonies of Penicillia were also observed in this stage. On the other hand, many strains of Lactobacillus were isolated for the first time on the 6th or 9th day after the mixing in the Yamahai-Moto, and yet until its later resting period, whilst they were scarcely found in the Sokujô-Moto, in which, however, a few strains of Hansenula, or of red yeast were isolated.

After the yeast inoculation in each Moto-sample, there appeared yeast colonies abunduntly on the agar media, on which then, neither bacterial colonies other than Lactobacillus, nor mold crowdings were observed any longer. As shown in Table 19, number of the yeast colony emerged, attained to the maximum on

Table 19. Number of the yeast colony grown on the Koji-juice agar medium in the Moto- and Moromi-process.

No. 8		No. 12		No. 13	
Time elapsed after the materials-mixing (Day)	Number of yeast colony ($\times 10^5$)	Time elasped after the materials-mixing (Day)	Number of yeast colony ($\times 10^5$)	Time elapsed after the materials-mixing (Day)	Number of yeast colony ($\times 10^5$)
13	0.02	15	1.35	18	12
24	214	18	18	24	177
26	172	24	196	28	204
28	205	30	206	30	102
31	82	33	175	31	47
34	286	39	153	34	9
40	568	45	293	46	290
43	612	47	103	49	534
56	641	48	93	54	771
61	75	50	420	59	381
66	53	53	790	64	55
		56	4870		
		59	203		
		62	288		

the 10th or 14th day after the yeast inoculation, and thereafter kept at about this level of magnitude. For an instance, in the sample No. 12, which was long-resting Moto, this level in number of the yeast colony was observed till its last stage.

In each Moromi-sample, the number of the yeast colony after the completion of the materials-mixing was rather smaller than during the mixing process. And then it became larger according to the elapsing time. In the Moromi, in which shorter-resting Moto was used, its maximum magnitude was attainable

on the 15th to the 20th day after the mixing process, while in the Moromi No. 12, in which long-resting Moto was used, it was attained on about the 10th day after the mixing process. In the latter case, the earlier emergency of the maximum level in the number of the yeast colony would be dependent not only on the time of resting as the Moto was prepared, but also on the polishing rate of the rice used in the preparation of the Moromi which might be more effective. After the number of the yeast colony attained to its maximum level in each Moromi-sample, only a few bacterial colonies were observed.

The microbes isolated from the Moto in early stage were supplied to test on its growing ability on the dense Moto-filtrate. The Moto just before the yeast inoculation was centrifugated and its supernatant liquid was filtered off through Berkefeld's filter. The microbes tested were inoculated into this sterile Moto-filtrate and incubated at 30°C for 5 days. All of the aerobic bacteria (10 strains) were unable to grow on it at all, whereas a faint growth of the Lactobacillus (4 strains) was obtained. Hansenula-yeast (2 strains) and *Asp. oryzae* could show an abundunt growth on it.

Thus, the growth of the bacteria invading from the air or the materials was inhibited, when high amounts of the sugar as well as of the acid were accumulated and resulted in the dense and acidified state in the early stage of the Moto. And *Asp. oryzae* also could not grow, because the surface of the Moto was ruptured continuously by agitating vigorously every day, though the time of agitating was not longer than an hour, while the yeast alone could grow on the Moto, when the yeast-starter was inoculated into it[2].

e) Growing test on the Moto- or Moromi-yeast on the Kôji-juice.

The Moto- or Moromi-yeast separated from the samples in various stages

Table 20. Growing test on the

No. 8				No.	
Time elapsed after the materials-mixing (Day)	Lag pericd* (hr)	Number of yeast inoculated ($\times 10^7$/ml)	Mean** volume of the gas evolved (ml/hr)	Time elapsed after the materials-mixing (Day)	Lag period* (hr)
21	2.3	4.2	1.9	21	3.1
24	2.4	5.0	2.1	24	3.2
26	3.2	3.6	1.2	33	2.2
28	2.3	2.8	1.6	39	2.9
31	4.2	0.4	1.4	45	2.5
34	2.1	4.4	2.2	47	3.5
37	1.6	7.2	1.7	48	2.6
46	1.8	9.2	1.6	50	2.7
51	2.0	3.6	1.5	53	2.4
61	2.7	3.2	1.7	59	1.1

* The time elapsed before linear gas evolution.

** The mean value of the gas evolved linearly.

were inoculated in 3 ml of Kôji-juice in sterile injection-tube, in which 2 ml of the air was supplied, and incubated at 30°C for 24 hours with continuous shaking. Table 16 showed the results obtained, in which the time of lag period (the time necessary for the gas evolution, when calculated in the figure of the gas volume-time curve) and mean volume of the gas evolved (ml per hour subtracted by the time of lag period, i.e. the mean value of gas volume against the time in the linear part of the gas volume-time curve) were indicated.

As seen in the Table 20, the periodical changes in the growing abilities of the Moto-yeast on the Kôji-juice did not differ significantly from each other, and even the yeast obtained from the long-resting Moto (No. 12) did not vary its growing ability till the last. However, the Moromi-yeasts showed considerably various aspects in the periodical changes in their growing ability according to the samples, especially in their earlier stage. For instance, the values indicated in the table referring to the yeast from the Moromi-sample No. 12 just after the completion of materials-mixing (0 day) corresponded to the ones referring to the yeasts from the sample No. 8 and No. 13 on the 3rd day after the mixing process. It is also mentioned, that the values of the No. 12-yeast just after the mixing process were almost the same with the one on the 3rd day after the mixing process, though there were considerable differences in the number of the yeast cells. These distinct characters of the Moromi-sample No. 12 might be also attributable to the use of the long-resting Moto and the rice of lower polishing rate in preararation of it.

Discussion

It is well known that the Moto process as a starter-making one is an exce-

Moto- or Moromi-yeast.

12		No. 13			
Number of yeast inoculated ($\times 10^7$/ml)	Mean** volume of the gas evolved (ml/hr)	Time elapsed after the materials-mixing (Day)	Lag period* (hr)	Number of yeast inoculated ($\times 10^7$/ml)	Mean** volume of the gas evolved (ml/hr)
2.8	1.5	21	2.5	3.6	1.8
4.3	1.9	24	3.0	4.3	1.6
4.4	1.8	28	3.1	2.4	1.4
3.2	1.9	30	2.9	2.4	1.9
3.6	1.8	31	3.3	1.2	1.4
1.2	1.4	34	4.6	0.4	1.3
0.8	1.6	37	3.3	3.9	2.1
2.4	1.7	40	2.3	13.2	2.2
3.6	1.7	49	1.5	5.2	2.0
6.0	2.0	59	2.0	3.6	1.5

llent selection technique by maintenance of sugar and acid in so high amounts, that the invading bacteria cannot grow any more. Accordingly, the Sake-yeast strain, which is more tolerable against above both constituents, is used in the brewing process. The present data showed this fact distinctly. It is also well mentioned, that in the Moto-process, the saccharification and the acidification occurred in its primary stage and then were followed by the abundunt growth of the yeast after its inoculation. Therefore, in this process, the saccharification stage (the primary stage of the Moto process) and the yeast growth one (the later stage of the Moto process) are periodically distinguished, indifferently to the initial presence of a small number of the yeast cells adhering to the Kôji, which was used as a material for preparation of the Moto. The yeast, thus, which was obliged to grow on extremely high amounts of sugar accumulated in the process, seemed to be less tolerable against the alcohol. This might cause to accumulate the alcohol in the volume less than the yeast could be tolerable against it in the last stage of the process, and to reserve the sugar in the considerable volume, a small amount of which was consumed by the yeast, but easily recovered by the volume derived from saccharified sugar in this stage. The inhibitory effect of the alcohol would be enforecd by the lowered value of pH in it. The last stage of the Moto process is characteristic as a resting period, whereupon the fermentative ability of the yeast was almost unvaried but became more tolerable against the alcohol and the nitrogen contents of the yeast, which was able to assimilate the nitrogenous matters in the milieu by the fermentation occuring a little, were increased more and more. During this resting period, the purity of the yeast culture was increased, so far as the invading bacterial strains, including Lactobacilli were killed by the lowered pH value and the alcohol accumulated in ca. 10% in the Moto. This effect might be maintained during the period, when the nitrogen content of the yeast is increased. In the Moto process of the samples, this effective time of resting was as long as 20 days.

In the Moromi-process, mixing the materials in three steps for preparation of the Moromi, and parallelism between the saccharification and the yeast growth then occurring from the initial stage of the fermentation process, where the number of the yeast was continuously increased, so that the dilution effects was not able to be observed considerably, might be also mentioned as characteristic. As shown from the present data, the Moromi-process was obviously distinguished into the following various growth phases of the Moromi-yeast.

I. The first phase. Its primary stage (during the period from the day just after the mixing process to the 2nd or the 3rd day after it) would correspond to the lag phase of the yeast growth period, where the nitrogen contents of the the yeast and its fermentative ability were the highest throughout the Moromi-

process, though the fewest number of the yeast.

II. The second phase, The growing phase of the Moromi-yeast was affected by abundunt supplement of the saccharified sugar occurred continuously and thus caused to prolong its conversion period. Correspondingly, the fermentative ability of the Moromi itself in this phase was maintained in high level of its magnitude though its varying aspects differed with each other according to the samples. However, the fermentative ability and the nitrogen contents of the Moromi-yeast were reduced and then converted to increase gradually, so that their aspects showed distinctly the reflecting statue of the growing phase. It took about 10 days or more in this phase, where the yeast had not yet acquired full tolerance against the alcohol, as corresponding to its growth phase. Therefore, the low level in it succeeding the preliminary phase, was still maintained throughout this phase. At any rates, it is well stated that the young growing yeast in this phase could ferment the sugar vigorously and cause the most actively bubbling state of the Moromi (so-called "Taka-awa"). However, the time elapsing in the Taka-awa-stage, which takes usually about a week, would be rather partly included in the growing phase of the Moromi-yeast.

III. The third phase. Then, the third phase of the Moromi-process, i.e. the equilibrium phase of the yeast growth followed stepwise as the ripening stage of the Moromi-process. The Moromi-yeast possessed again the nitrogenous matters more abunduntly and acquired the tolerance against the alcohol considerably. Its fermentative ability was increased again more and more, whilst, on the contrary, the fermentative ability of the Moromi itself proceeded on its reduction course, which was not direct, but zigzag and stepwise so far as the alcohol-tolerance of the yeast was increased, which caused to overcome the inhibitory effect of the higher amounts of alcohol accumulated in this phase. And indeed, the fermentative ability of the Moromi was rather increased at the primary stage of this phase, attaining to the second peak. The Moromi-yeast in this phase could obtain characteristically the alcohol-tolerance, which would be otherwise unattainable. Its fermentative ability was maintained still in high level of its magnitude. Even in the presence of 15% of alcohol the yeast could ferment the sugar with the 25% of its activity and lost its activity only at 20 to 55% when soaked in the 15% alcohol solution at 30°C for 24 hours long. This remarkable alcohol tolerance of the Moromi-yeast resulted in the specific cultivation method occurred in the Sake-fermentation process, that is, the yeast grew in the earlier stage on not so high amounts of the sugar supplied, but its adequately smaller amounts derived continuously by the saccharification, and at the later stage, continuing always to ferment the sugar though at reduced rate, it was ripened in the milieu, where the abundant volumes of the nitrogenous maters have been accumulated together with smaller amounts of the sugar.

About 10 days or more elapsed during this phase.

IV. The forth phase. The forth and the last stage of the Moromi-process is the decaying phase of its yeast, which showed a declining aspect in its various activities as well as its nitrogen contents in this phase. Since the Moromi-yeast possessed the ability enogh to ferment the sugar yet being accumulated a little and the alcohol-tolerance sufficiently even in this phase, a faint fermentation occurred and the alcohol was accumulated still furthermore up to ca. 18%. In the breweries, the final amount of the alcohol accumulated in the Moromi is usually preliminarily projected, and when the volume of the alcohol accumulated has attained nearly to the projected amounts, the fermentation is stopped by further addition of the 30% alcoholic solution, because the qualities of the ripened Moromi are damaged, when the fermentation proceeds on to the end. It is very interesting that the fermentation ability (Q_{CO_2}) of the Moromi-yeast just before the filtration process was considerably high though its value had been lowered up to about one-tenth of its maximum even when it was soaked for 5 days long in as high amounts of the alcohol thus attained, as 18 %. The fermentation caused by the Moromi-yeast in this stage, as shown in the table, would be served to adjust the taste and the flavor of the ripened Moromi adequately.

Referring to the studies on the Sake-brewing process, some acknowledgments on the changes of the chemical constituents, such as sugars,[3] dextrin,[4] nitrogenous matters,[5] etc. as well as on the emergencies of several kinds of bacteria[6] or of the yeasts, have been much accumulated, whilst on the fermentative ability of the Sake-yeast, which displays a main role in the brewing process, it is yet obscure. The present work might give a supplement to this blank spot in the references to the Sake-brewing.

Summary

The yeast was separated by the differential centrifugation from the Sake-Moto, or -Moromi at various fermentation stages in three batches of Sake-brewing, and supplied to estimate its fermentative ability, its nitrogen content, and its alcohol-tolerance. Thereupon, several kinds of their chemical constituents, which varied according to the time, were also analyzed. As a result, the Moto- or the Moromi-process, i.e. these fermentation processes in the Sake-brewing were obviously distinguished into several steps by the growth phases of the yeast, which varied as being affected by various factors contained in both processes. This led to the perception that the measurement of the fermentative ability of the Moto or the Moromi had a great significance in the breweries.

PART III. SIMPLE MEASUREMENT OF FERMENTATIVE ABILITY IN SAKE-MOROMI

By

Kenjirô TAKAGI, Tokuo MIYAGI, and Teijirô UEMURA

Introduction

It was well mentioned, as preliminarily reported, that the fermentative ability of the yeast in the Sake-fermentation process varied according to its growth periods, and its varying aspect especially in the Moromi-process was also affected by the treatments done, or the materials used in the breweries. This means that the direct measurement of the relative ability of the fermenting mash has a great significance to judge its fermentation course. The injection tube method, which was at first deviced by Dr. H. Tamiya in 1927 to estimate the gas metaboilic activities of the microbes, has been thereupon also recognized to be quite suitable. In order to adopt the method more easily in treatment in the brewing manufactories, the measuring injection tube was somewhat designed and the method was simplified. The results obtainced in some Sake-breweries, showed that the designed method is available.

Methods and Results

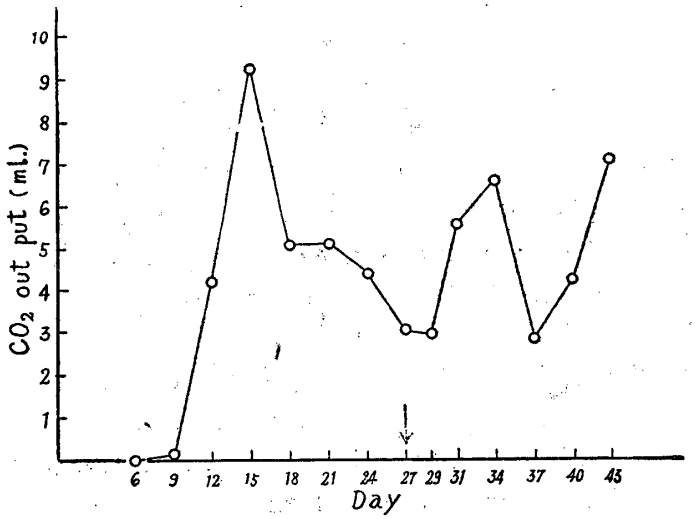
The spit of the ordinary thick-glass injection tube was enlarged in order that the granulous sample could pass through more easily and a small gum-stopper was fitted up to the fixed depth of this spit. 2 ml of the sample were taken in the tube (in the range of a fixed distance between two red menusci of the tube). Its volume was changed in 3 ml at present. (See Fig. 5). The tube adopted with the sample was incubated at 37°C for 2 hours. (At present, the incubation is done at 30°C for 3 hours). And then the volume of the gas evolved was measured.

Fig. 5. The modified injection tube, designed for measurement of the fermentative ability of the Sake-Moto or -Moromi.

Samples were collected in 1951 from two brewing manufactories, attatched to the Sendai Sake-Making Comp. Ltd. One series of them were collected from a batch for brewing the Sake of the ordinary qualitiy and the other series were from one of the best quality (Ginzyô-Shikomi), as followed.

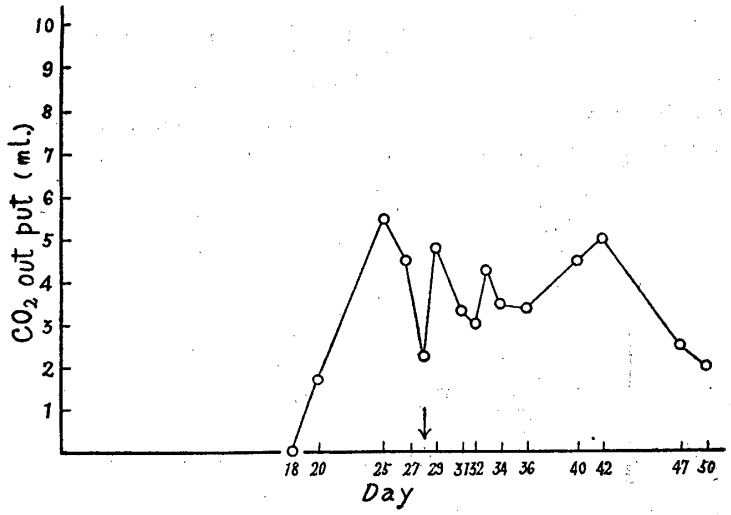
H-manufactory

No. 14: Ordinary batch (Fig. 6), No. 29: Special batch (Fig. 7).



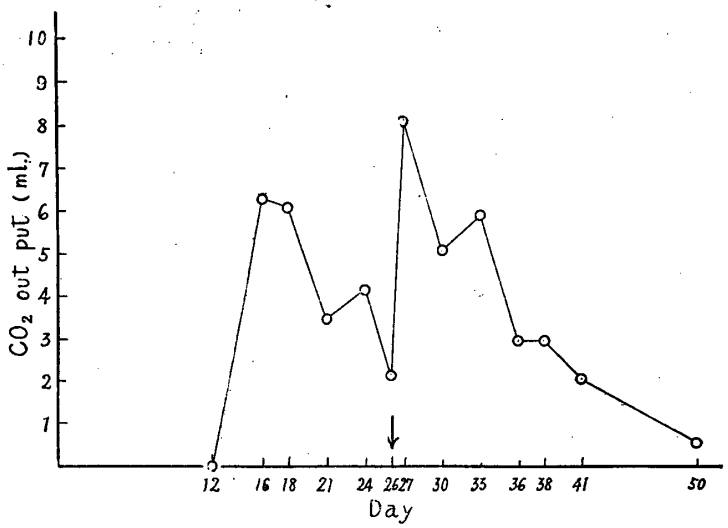
The day in the abscissa before ↓ shows the Moto-process and the day after ↓ the Moromi-process.

Fig. 6. Changes in the fermentative ability of the Moto and Moromi. (H. No. 14).



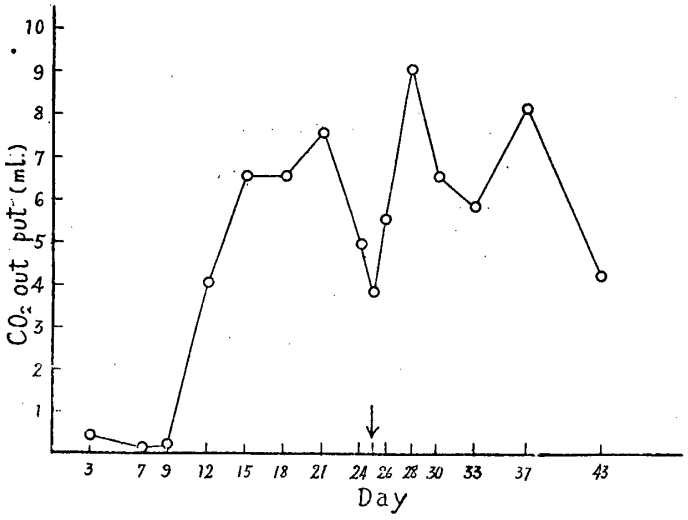
The day in the abscissa before ↓ shows the Moto-process and the day after ↓ the Moromi-process.

Fig. 7. Changes in the fermentative ability of the Moto and Moromi. (H. No. 29).



The day in the abscissa before ↓ shows the Moto-process and the day after ↓ the Moromi-process.

Fig. 8. Changes in the fermentative ability of the Moto and Moromi. (K. No. 1).



The day in the abscissa before ↓ shows the Moto-process ans the day after ↓ the Moromi-process.

Fig. 9. Changes in the fermentative ability of the Moto and Moromi. (K. No. 7).

K-manufactory

No. 1: Ordinary batch (Fig. 8), No. 7: Special batch (Fig. 9).

In all these samples, the Sokuzyô-Moto was used.

As shown in Figs. 6, 7, 8 and 9, the varying aspects of the fermentative

ability were different according to the samples. However, some shapes of the curves were distinguishable approximately in the figures. In the Moto-process of H-samples, the peak of the curve was sharper, especially in the Ginzyô-series, while in K-samples a mild and wide descending range was observed. In the Moromi-process, one shape of the curve was that the second peak appeared on the 12th or the 15th day after the mixing process of the materials. This character was seen presumably in the Ginzyô-series indifferently to the origin of the sample. The other shape was that the first peak declined down mildly and even by steps, so that the second peak was not distinguished, as seen in Fig. 4. The causes might be attributable to the sort of the Kôji used and heating rate of the Moromi.

Furthermore, in other cases, the fermentative ability-curve in the Sake-Moto and -Moromi was affected by the sort of the water used in their preparations, where other materials added and the treatments done were quite the equal, and the caused alcohol production-curves were indistinguishable. It has been also recognized that the fermentative ability-curves were obtained in the same shape, when all the materials used were the identical and the processes were treated in the same ways. The measuring method was designed in so simplified manner, that it gave to the brewing workers no unbearable tasks in its treatment.

Summary

A simplified method for determination of the fermentative ability of the Sake-Moto, or of the Sake-Moromi has been designed by using a thick-glass injection-tube somewhat deviced. Several sorts of samples in two breweries in Sendai, were attempted to the test, and it was recognized that this new method is useful in management of Sake-brewing.

The authors wish to thank Hôzan brewing manufactory attached to Sendai Sake-Making Company, LTD., for delivery of the Moromi- or Moto-samples. The work was aided in part by a grant from the Scientific Research Expenditure of the Ministry of Education.

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