ON THE SUGAR COMPOSITION OF AMASAKE (A SWEET SUGARY LIQUOR MADE FROM RICE).

ON THE SUGAR COMPOSITION OF AMASAKÉ (A SWEET SUGARY LIQUOR MADE FROM RICE).*

By

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Amasaké, which is made from ancient times as described in the Nihon Shoki, the old book of Japan, was a sweet sugary liquor made from rice. The producing method of Amasaké is follows: the mixture of rice Koji and boiled rice (Katazukuri) or rice Koji and rice gruel (Yawarakazukuri) are saccharified for several hours at 45-55°C.

Takahashi (1) and Yamada (2) have reported on the analyses of Amasaké as shown in Table 1.

Table 1. Analyses of Amasaké

		Moisture (%)	Dry matter (%)	Sugar (%)	Crude starch (%)	Dextrin (%)	Total acid (%)	Ash (%)
Takahashi	Katazukuri	45-40	60-55	32-28	5-3	12-10	0.3-0.2	0.3-0.2
	Yawarakazukuri	80-60	40-20	26-15	1.5-0.3	9-2.5	0.1-0.07	0.1-0.06
Yamada	Kataneri	64.52	25.48	25.29	0.43	7.82	0.09	0.08
	Yawarakaneri	73.02	26.98	18.64	1.67	2.86	0.05	0.08

We now report on the analyses of total sugar, reducing sugar, total nitrogen, amino nitrogen, total acid, total phosphoric acid and inorganic phosphoric acid, paper chromatography (PPC) of sugars, separative determination of the sugar solution of Amasaké by the paper chromatographic method, and then compared with the sugar composition in Saké (3, 4), rice Koji juice (5) and Mirin (6, 7) produced from rice.

* The original Japanese report was published in Hakkô Kôgaku Zasshi (J. Fermentation Technology) **38**, 464-469 (1960).

Experimental

I. Analyses of rice and rice Koji.

The crushed common and waxy rice was sorted out with a 40 mesh screen followed by analysed. Analyses was carried out by "Jikken Nōgei Kagaku, Tōkyō university". The results are shown in Table 2 and Table 3.

Table 2. Analyses of rice.

	Moisture (%)	Crude starch (%)	Crude protein (%)	Total sugar (%)	Reducing sugar (%)	Water soluble total nitrogen (%)	Amino nitrogen (%)
Common rice	15.60	74.19	7.19	1.51	0.70	0.078	0.003
	—	87.90	8.52	1.79	0.83	0.092	0.004
Waxy rice	15.38	73.26	6.94	1.94	0.94	0.072	0.003
	—	86.80	8.20	2.29	1.11	0.085	0.004

Table 3. Analyses of rice Koji.

	Moisture (%)	Total sugar (%)	Reducing sugar (%)	Crude protein (%)	Crude fat (%)	Crude ash (%)
Common rice Koji	28.87	64.51	11.30	6.63	0.34	0.29
	—	90.69	15.89	9.32	0.48	0.41

II. Producing of Amasaké and preparation of sugar solution.

The 2160 *ml* of distilled water was added to 1200 g of common rice (Chōkai) and waxy rice (Sasashigure) Koji in the enameled tub respectively, and these were saccharified at 45-55°C. The 200 *ml* of saccharified solution was taken every one hour, heated at 70°C and centrifuged. The supernatant was used for analyses.

III. General analyses.

(1) *Total sugar*

After heating with 2.27 per cent HCl on the boiling water bath for 2.5 hours it was neutralized with NaOH followed by the Bertrand-Henmi method (calculated as glucose). The results are shown in Table 4.

Table 4. Analyses of total sugar.

Hours of saccharification	1	2	3	4	5	6	7	8
Amasaké made from common rice Koji (g/100 *ml*)	22.48	28.54	30.14	31.54	32.76	33.84	34.72	35.12
Amasaké made from waxy rice Koji (g/100 *ml*)	27.24	31.12	32.64	33.18	33.84	34.00	34.50	35.26

After one hour of saccharification, the total sugar in the sugar solution of Amasaké made from waxy rice Koji was about 5 g/100 *ml* more than that of common rice Koji, but after eight hours, there was no difference between common rice and waxy rice Koji.

(2) *Reducing sugar*

Reducing sugar was determined by the Bertrand-Henmi method (calculated as glucose). The results are shown in Table 5.

Table 5. Analyses of reducing sugar.

Hours of saccharification	1	2	3	4	5	6	7	8
Amasaké made from common rice Koji (g/100 *ml*)	19.76	25.34	28.10	29.50	30.14	31.66	32.20	33.08
Amasaké made from waxy rice Koji (g/100 *ml*)	18.65	24.27	26.84	28.36	29.15	30.12	31.36	32.25

The reducing sugar of common rice Koji was about 0.5—1 g/100 *ml* more than that of waxy rice Koji.

(3) *Total nitrogen*

Total nitrogen was determined by the Kjeldahl method. The results are shown in Table 6.

Table 6. Analyses of total nitrogen.

Hours of saccharification	1	2	3	4	5	6	7	8
Amasaké made from common rice Koji (g/100 *ml*)	0.143	0.155	0.161	0.172	0.184	0.192	0.203	0.211
Amasaké made from waxy rice Koji (g/100 *ml*)	0.143	0.157	0.170	0.176	0.186	0.191	0.197	0.206

There is no difference between common rice Koji and waxy rice Koji.

(4) *Amino nitrogen*

Amino nitrogen was determined by the Soerensen-Formol titration method. The results are shown in Table 7.

Table 7. Analyses of amino nitrogen.

Hours of saccharification	1	2	3	4	5	6	7	8
Amasaké made from common rice Koji (g/100 *ml*)	0.043	0.056	0.064	0.071	0.082	0.091	0.095	0.105
Amasaké made from waxy rice Koji (g/100 *ml*)	0.069	0.076	0.081	0.087	0.091	0.093	0.097	0.100

The ratio of amino nitrogen to total nitrogen are shown in Table 8.

Table 8. Ratio of amino nitrogen to total nitrogen.

Hours of saccharification	1	2	3	4	5	6	7	8
Amasaké made from common rice Koji(%)	30.34	36.12	40.00	41.04	44.78	47.29	46.89	49.95
Amasaké made from waxy rice Koji (%)	48.83	48.75	47.71	49.62	48.79	48.70	48.92	48.58

From the results of Table 8, the ratio of amino nitrogen to total nitrogen in common rice Koji was 30-50 per cent but the ratio in waxy rice Koji was 48-49 per cent.

(5) *Total acid*

Titration acidity was determined by $N/10$ NaOH, calculated as succinic acid with an indicator of phenolphthalein. The results are shown in Table 9.

Table 9. Analyses of total acid.

Hours of saccharification	1	2	3	4	5	6	7	8
Amasaké made from common rice Koji (g/100 *ml*)	0.091	0.099	0.109	0.113	0.117	0.119	0.121	0.124
Amasaké made from waxy rice Koji (g/100 *ml*)	0.096	0.100	0.105	0.107	0.108	0.113	0.115	0.120

There is no difference between common rice and waxy rice Koji.

(6) *Determination of total phosphoric acid and inorganic phosphoric acid.*

Total and inorganic phosphoric acid were determined by the Allene method (8, 9). The standard curve of KH_2PO_4 was made by the Allene method. The obtained curve is shown in Fig. 1.

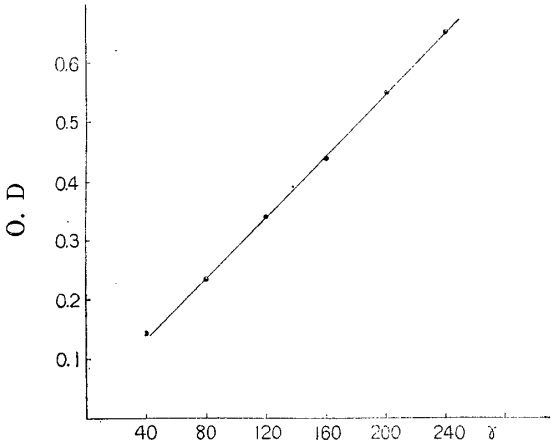

Fig. 1. Standard curve of KH_2PO_4.

Total and inorganic phosphoric acid contents were calculated from the standard curve of KH_2PO_4. The results are shown in Table 10.

Table 10. Analyses of total and inorganic phosphoric acid.

Hours of saccharification		1	2	3	4	5	6	7	8
Amasaké made from common rice Koji	Total P_2O_5 (g/100 *ml*)	0.016	0.019	0.021	0.022	0.024	0.028	0.030	0.033
	Inorganic P_2O_5 (g/100 *ml*)	0.013	0.016	0.018	0.019	0.019	0.020	0.021	0.022
Amasaké made from waxy rice Koji	Total P_2O_5 (g/100 *ml*)	0.015	0.016	0.017	0.020	0.021	0.021	0.025	0.027
	Inorganic P_2O_5 (g/100 *ml*)	0.010	0.012	0.014	0.016	0.016	0.017	0.017	0.019

The ratio of inorganic phosphoric acid to total phosphoric acid are shown in Table 11.

Table 11. Ratio of inorganic phosphoric acid to total phosphoric acid.

Hours of saccharification	1	2	3	4	5	6	7	8
Amasaké made from common rice Koji (%)	80.6	86.4	88.0	83.8	78.1	70.9	67.8	66.1
Amasaké made from waxy rice Koji (%)	70.3	73.2	83.5	80.5	77.2	77.8	70.2	69.8

From the results of Table 11, the ratio of inorganic phosphoric acid to total phosphoric acid in Amasaké made from common rice Koji was 66-88 per cent (average 78.4 per cent) and the ratio in waxy rice Koji was 70-83 per cent (average 75.3 per cent).

The ratio of total and inorganic phosphoric acid to total acid are shown in Table 12.

Table 12. Ratio of total and inorganic phosphoric acid to total acid.

Hours of saccharification		1	2	3	4	5	6	7	8
Total phosphoric acid/Total acid	Amasaké made from common rice Koji (%)	17.0	18.6	19.2	19.7	20.7	23.1	24.9	26.2
	Amasaké made from waxy rice Koji (%)	15.1	15.7	16.2	18.2	19.1	18.7	21.3	22.1
Inorganic phosphoric acid/Total acid	Amasaké made from common rice Koji (%)	13.7	16.2	17.0	16.5	16.2	16.4	16.9	17.3
	Amasaké made from waxy rice Koji (%)	10.6	11.5	13.5	14.7	14.8	14.6	14.9	15.4

From the results of Table 12, the ratio of total phosphoric acid to total acid in Amasaké made from common rice Koji was 17.0-26.2 per cent (average 21.2 per cent) and the ratio in waxy rice Koji was 15.1-22.1 per cent (average 18.3 per cent). And the ratio of inorganic phosphoric acid to total acid in common rice Koji was 13.7-17.3 per cent (average 16.3 per cent) and the ratio of waxy rice Koji was 10.6-15.4 per cent (average 13.8 per cent).

An equal amount of phosphoric acid (as KH_2PO_4 and H_3PO_4) in Amasaké was added to a soluble starch solution and this solution was saccharified under the same condition of producing of Amasaké. After saccharification, the reducing power was determined by the Bertrand-Henmi method. The quantity of sugars was less by one per cent to soluble starch.

IV. PPC of sugars in Amasaké.

Amasaké was prepared by dilution with 10 fold water and spotted on the Tôyo filter paper No. 2. After irrigating the chromatogram with pyridine-butanol-water (4:6:3), ascending three times, the sugars were located by spraying with aniline hydrogen phthalate reagent. The results are shown in Fig. 2.

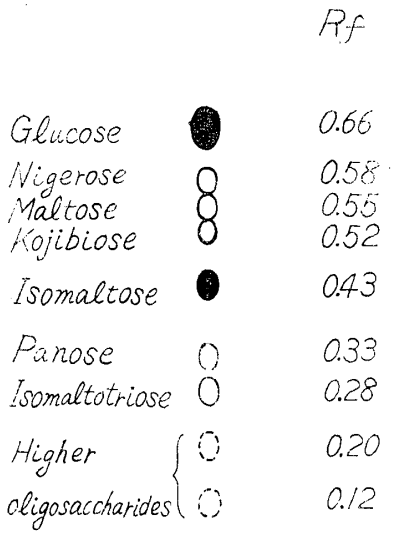

Fig. 2. Multiple paper chromatogram of sugars in Amasaké.

From the results of Fig. 2, glucose, kojibiose, nigerose, maltose, isomaltose, panose, isomaltotriose and two higher oligosaccharides were detected in Amasaké.

It was recognized that Amasaké contained maltose just as in Mirin, while Saké did not contain maltose.

V. Separative determination of sugars in Amasaké.

Since glucose, kojibiose, nigerose, maltose, isomaltose, panose and isomaltotriose were detected in Amasaké as mentioned above, these sugars were determined separately by PPC followed by the Somogyi method. The diluted Amasaké solution was spotted on the Tôyo filter paper No. 51. After irrigating the chromatogram with pyridine-butanol-water (4:6:3), ascending three times, guid strips were cut off from both sides of the chromatogram and the position of the sugars were located by aniline hydrogen phthalate. The zones corresponding to monosaccharides and oligosaccharides were cut off and eluted with water and oligosaccharides

fractions were hydrolysed with acid, followed by neutralization with NaOH, and determined by the Somogyi method. The results are shown in Table 13.

Table 13. Separative determination of sugars in Amasaké

			Glucose	Nigerose Maltose Kojibiose	Isomaltose	Higher oligo-saccharides
Amasaké made from common rice Koji	After 4 hours of saccharification	g/100*ml*	26.19	2.92	3.30	3.03
		%	73.90	8.25	9.31	8.54
	After 8 hours of saccharification	g/100*ml*	27.35	3.08	3.01	3.04
		%	75.35	8.32	8.12	8.21
Amasaké made from waxy rice Koji	After 4 hours of saccharification	g/100*ml*	25.85	2.52	3.76	4.54
		%	70.48	6.87	10.26	12.39
	After 8 hours of saccharification	g/100*ml*	26.34	2.84	3.92	4.09
		%	70.83	7.62	10.54	11.01

From the results of Table 13, the glucose content was about 74-75 per cent of the total sugar and kojibiose, nigerose, maltose fraction, isomaltose fraction and higher oligosaccharides fraction in Amasaké made from common rice Koji were 8-9 per cent, respectively. And the glucose content in Amasaké made from waxy rice Koji was about 70 per cent of the total sugar and higher oligosaccharides was 11-12 per cent.

Summary

Total sugar, reducing sugar, total nitrogen, amino nitrogen, total acid, total phosphoric acid and inorganic phosphoric acid of two samples of Amasaké produced from common and waxy rice Koji were analysed.

On the paper chromatogram of sugars in Amasaké, nine spots corresponding to glucose, kojibiose, nigerose, maltose, isomaltose, panose, isomaltotriose and two higher oligosaccharides were detected and determined. The main sugar was glucose and its content was 70-75 per cent of the total sugar.

From the above results, it was recognized that Amasaké contained maltose just as in Mirin, while Saké did not contain maltose.

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