STUDIES ON BEER I. ON THE SUGAR COMPOSITION OF BEERS

STUDIES ON BEER
I. ON THE SUGAR COMPOSITION OF BEERS

By

Kiyoshi Aso, Toshiyuki Watanabe, Ko Sasaki and Yoshie Motomura

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received, July 26, 1961*)

No one has hitherto reported on the sugar composition of beers in Japan. But in foreign countries, Gjertson (1) has reported on the fractionation of sugars in bottom fermented beers by carbon-Celite column, and ribose, xylose, arabinose, galactose, maltose, isomaltose, two unidentified sugars, maltotriose, isomaltotriose, maltotetraose and higher oligosaccharides were detected by paper chromatography (PPC). Stöckli (2, 3) has detected maltose, isomaltose, maltotriose and three kinds of unidentified sugars by PPC. Randoin (4) has described that the average contents of each carbohydrate in beers are as follows. Reducing sugar (as maltose): 20—30 g, dextrin: 60—75 g and pentosan: 6—8 g per 100g of the dry matter in the review of beer. Silbereisen *et al.* (5) have reported that the sugars of beers were fractionated by carbon-Celite column and xylose, arabinose, ribose, trisaccharides and higher glucose polymers were detected by PPC. Montreuil *et al.* (6) have fractionated the sugar composition of six kinds of beers by dialysis. Pentose (ribose, xylose and arabinose), hexose (glucose and galactose), unidentified sugars X_1 and X_2, maltose, isomaltose, maltotriose, isomaltotriose, maltotetraose, isomaltotetraose maltopentaose, maltohexaose, and ten kinds of higher glucosans were detected and determined by PPC. The undialysable fraction gave on hydrolysis glucose, xylose and arabinose. MacWilliam *et al.* (7) have detected nigerose and malturose by PPC in the beer produced by a strain of *Saccharomyces cerevisiae* (National Collection of Yeast Culture No. 447), isolated them by cellulose column chromatography and identified them as turanosazone and maltosazone.

We now report on the analyses of four Japanese and three imported beers and two imitation beers in Japan, PPC of the sugars in beers and separative determination of the sugars by paper chromatographic method (Somogyi method).

The original Japanese report was published in Nippon Nogei-kagaku Kaishi. **35**, 1073 (1961).

Experimental

1) Analyses of Beers.

The general analyses of bottled beers (the content of each bottle is 633*ml*) of trade mark "Asahi Gold", "Sapporo", "Kirin", "Takara" (produced in Japan), "Dortmunder Actien Beer (DAB)", "Löwenbräu München Beer (LMB)" (these two are produced in Germany), "San Miguel Beer (SMB)" (in the Philippines), canned beers of the first three, and "Lavie" and "Liner" (these two are imitation beers in Japan) were carried out. These results are shown in Table 1.

Table 1. General analyses of sugars.

Trade mark		Date of purchase	Gas pressure kg/cm²	Specific gravity	Alcohol	Total sugar g/100*ml*	Reducing sugar g/100*ml*	Total acid g/100*ml*	Total N g/100*ml*	Amino N g/100*ml*	pH
Asahi Gold		1960 Jan.	1.7	1.009(13°C)	—	3.35	0.85	0.118	0.065	0.006	4.20
		1959 Sep.	1.4	1.008(17°C)	3.93	3.45	1.13	0.114	0.062	0.008	—
		1959 Jun.	—	1.008(21°C)	—	2.91	0.63	0.114	0.077	0.011	—
		average	1.55	1.008		3.24	0.87	0.115	0.068	0.008	
	(a)	1959 Nov.	—	1.008(16°C)	3.88	3.13	0.98	0.119	0.062	0.007	
Sapporo		1960 Jan.	1.7	1.010(13°C)	—	3.52	1.16	0.105	0.046	0.004	4.30
		1959 Oct.	1.8	1.008(21°C)	3.81	3.68	1.43	0.089	0.046	0.006	—
		1959 Jun.	—	1.008(21°C)	—	3.50	1.38	0.109	0.050	0.009	—
		average	1.75	1.009		3.57	1.32	0.101	0.047	0.006	
	(a)	1959 Dec.	—	1.010(9°C)	3.81	3.65	1.18	0.094	0.046	0.005	—
Kirin		1960 Jan.	1.5	1.009(20°C)	—	3.46	1.40	0.096	0.050	0.007	4.38
		1959 Sep.	—	1.010(18°C)	3.58	4.04	1.84	0.109	0.077	0.010	—
		1959 Jun.	—	1.009(23.5°C)	—	3.65	1.60	0.102	0.058	0.010	—
		average		1.009		3.72	1.61	0.102	0.062	0.009	
	(a)	1960 Jun.	—	1.009(23.5°C)	3.78	3.06	1.11	0.128	0.049	0.007	4.24
Takara		1960 Jan.	2.0	1.006(20°C)	—	2.90	0.68	0.088	0.065	0.010	4.50
		1959 Oct.	1.6	1.006(20°C)	3.75	3.28	1.05	0.099	0.046	0.010	—
		1959 Jun.	—	1.010(21°C)	—	3.83	1.39	0.109	0.054	0.008	—
		average	1.8	1.007		3.34	1.04	0.099	0.055	0.009	
D A B		1960 May.	1.4	1.008(19°C)	—	3.23	0.97	0.193	0.031	0.002	4.32
L M B		1960 May.	1.8	1.015(20°C)	3.84	4.39	1.51	0.159	0.062	0.012	4.61
S M B		1960 Jan.	1.8	1.012(13°C)	3.87	4.22	1.47	0.092	0.055	0.007	4.20
Lavie		1960 Jun.	1.6	1.010(23°C)	3.58	4.03	1.50	0.142	0.018	0.002	3.80
Liner		1959 Sep.	1.1	1.002(13°C)	5.57	2.95	1.70	0.079	0 015	0.006	—
Pare beer	(b)	average	1.75	1.0075	3.53	1.93 (Dex-trin%)	0.85 (%)	0.120 (%)	0.0520 (%)	0.0145 (%)	

(a) : Represents canned beers, these contents are 350*ml*, respectively.
(b) : J. Nakagawa's Industrial Examination Method of Beer (1955), in Japan.

Analytical methods were as follows.

Gas pressure : Gas pressure meter.

Specific gravity : Standard specific gravity meter.

Alcohol: 100g of beer was distilled, the distillate was filled up to 100g, the specific gravity was measured with a picnometer, and alcohol (wt.%) was calculated by Windisch's alcohol table.

Total reducing sugar: After heating with 2.27 per cent HCl on the boiling water bath for 2.5 hr and neutralization with NaOH, it was determined by the Bertrand-Henmi method (as glucose).

Reducing sugar: Bertrand-Henmi method (as maltose).

Total acid: Titration acidity (as lactic acid) with mixed indicator of Brom-thymol blue and Neutral red.

Total N: Kjeldahl method.

Amino N: Sörensen-Formol titration method.

pH: Hitachi pH meter.

From the above results, total sugar contents of LMB and SMB were a little larger than those of the Japanese beers, but no remarkable difference was observed as shown in Table 1.

2) PPC of sugars in beers.

Each beer was spotted on Toyo filter paper No. 2.

After developing with pyridine: butanol: water (4:6:3), the sugars were

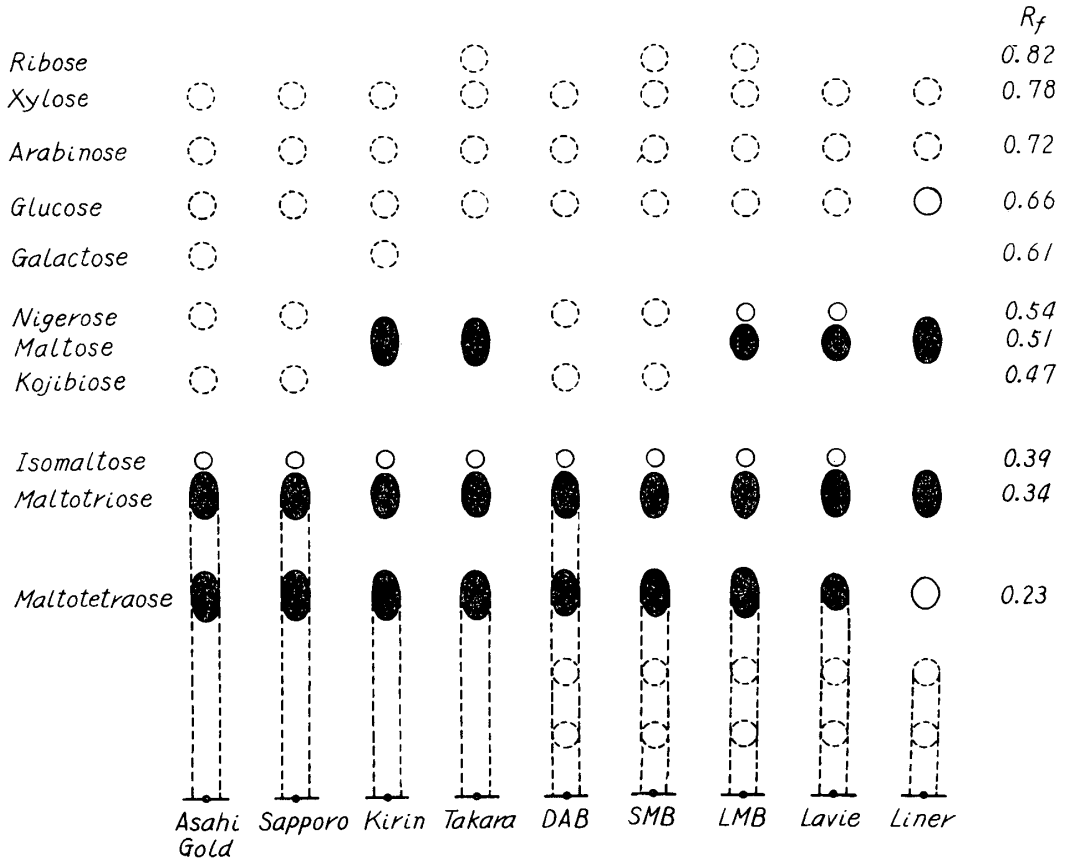

Fig. 1. PPC of sugars in beers.

located by spraying with aniline hydrogen phthalate and resorcinol reagents. These results are shown in Fig. 1.

As shown in Fig. 1, ribose, xylose, arabinose, glucose, maltose (or nigerose and kojibiose), isomaltose, maltotriose, maltotetraose and other oligosaccharides were detected in all of the samples.

According to the sugar composition of the disaccharides fraction, the beers may be divided into two types of a) KN-type, which contains kojibiose and nigerose but almost no maltose as in Asahi Gold, Sapporo, DAB and SMB, and b) M-type, which contains maltose and a small amount of nigerose but no kojibiose as in Kirin, Takara and LMB.

In Takara, LMB and SMB, a small amount of ribose was detected, and in Asahi Gold and Kirin, galactose was slightly detected.

In Lavie, xylose, arabinose, glucose, nigerose, maltose, isomaltose, maltotriose, maltotetraose and some other oligosaccharides were detected. Locating with the resorcinol reagent, a small amount of fructose was detected in DAB and SMB but not clearly in Japanese beers. In each maker's beer one unknown sugar, which seems to contain ketose, *Rf* 0.43 developed three times with pyridine : butanol:water (4 : 6 : 3) was detected.

The amounts of maltotriose, maltotetraose and higher oligosaccharides were comparatively larger in Asahi Gold, Sapporo, DAB and SMB. In Kirin, Takara, LMB and Lavie, a considerable amount of maltose was contained besides the above sugars.

3) Separative determination of sugars.

Since xylose, arabinose, glucose, nigerose, maltose, kojibiose, isomaltose, maltotriose, maltotetraose and other oligosaccharides were detected in beers as mentioned above, these sugars were determined separatively by PPC followed by the Somogyi method.

0.5*ml* of each beer was spotted on filter paper and developed three times with pyridine : butanol : water (4 : 6 : 3) as the developing solvent. Guid strips were cut off from both sides of the chromatogram and the position of the sugars were located by aniline hydrogen phthalate. The corresponding zones 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 value of each sugars calculated as glucose, are shown in Table 2. But, xylose and arabinose were calculated from the standard curve of the authentic samples.

In Table 2, higher oligosaccharides fractions in Asahi Gold, Sapporo, Kirin, Takara and DAB contain maltotetraose, maltopentaose, maltohexaose and other higher oligosaccharides. But, higher oligosaccharides fractions in SMB, LMB and Lavie, contain maltopentaose and other higher oligosaccharides.

Table 2. Separative determination of sugars.

	Xylose	Arabinose	Glucose	Nigerose	Maltose	Kojibiose	Isomaltose	Maltotriose	Maltotetraose	Higher oligo-saccharides
	%	%	%	%	%	%	%	%	%	%
Asahi Gold	0.12	0.40	0.18	1.24	—	1.41	2.48	5.66	88.51	
Sapporo	0.43	0.63	0.63	0.70	—	0.70	1.82	19.08	76.01	
Kirin	0.31	0.35	0.30	—	11.19	—	2.68	11.48	73.69	
Takara	0.35	0.54	0.21	—	8.76	—	2.60	7.53	80.01	
DAB	0.29	0.45	0.97	1.34	—	2.00	3.40	7.21	84.35	
SMB	0.17	0.12	0.53	1.15	—	1.85	1.69	17.27	12.13	65.09
LMB	0.13	0.17	0.32	0.18	6.59	—	1.25	9.73	16.20	65.43
Lavie	0.26	0.24	0.38	1.02	7.30	—	0.89	14.77	6.71	68.43

The position of isomaltose was very close to that of maltotriose, and in this case clear separation of isomaltose from the maltotriose was not successful, because of the presence of a large amount of maltotriose. Therefore, the contents of the above two sugars shown in Table 2 somewhat contaminated each other.

In Takara, SMB and LMB, ribose was contained in the xylose fraction and in Asahi Gold and Kirin, galactose was contained in the glucose fraction.

In the beers of M-type, the maltose content was 6.59—11.19 per cent of the total sugar and in the beers of KN-type, the kojibiose and nigerose contents were 1.40—3.34 per cent of the total sugar. The gluco-biose contents in the beers of M-type were larger than those of the beers of KN-type.

The contents of mono-, di-, tri-, tetra- and higher oligosaccharides in beers are shown in Table 3.

Table 3.

	Mono-saccharides	Disaccharides	Trisaccharides	Tetra-saccharides	Higher oligo-saccharides
	%	%	%	%	%
Asahi Gold	0.70	5.13	5.66	88.51	
Sapporo	1.69	3.22	19.08	76.01	
Kirin	0.96	13.87	11.48	73.69	
Takara	1.10	11.36	7.53	80.01	
DAB	1.71	6.73	7.21	84.35	
SMB	0.82	4.69	17.27	12.13	65.09
LMB	0.62	8.02	9.73	16.20	65.43
Lavie	0.88	9.21	14.77	6.71	68.43

From the results of Table 3, tetra and higher oligosaccharides content was 73.69—88.51 per cent of the total sugar. In SMB and LMB, maltotetraose content

was 12.13—16.20 per cent and higher oligosaccharides (over pentasaccharide) content was 65 per cent. Maltotriose content was 5.66—19.08 per cent. In the M-type (Kirin and Takara), the content of the disaccharides was larger than that of the trisaccharides. But, in the KN-type just on the contrary, the latter was larger than the former. Monosaccharide content was 0.62—1.71 per cent.

The sugar composition of Lavie (imitation beer) closely resembled beers. In Lavie, higher oligosaccharides content was 68.43 per cent and the quantities of other sugars were in the sequence of tri->di->tetra->monosaccharides.

The contents of each sugars in beer are shown in Table 4.

In Table 5, the results of separative determination by Montreuil *et al.* are shown for reference.

According to the results of Montreuil *et al.*, although it is not certain to what type their sample belong, the contents of maltose, 0.5—3 g/*l*, X_1 (corresponding to *Rf* value of nigerose), 0.43—2.80 *g*/*l* and X_2 (corresponding to *Rf* value of kojibiose) were 0.35—1.80 g/*l*. But according to our results, the contents of maltose 2.9—4.2 g/*l* and nigerose were 0.08 g/*l* in the beers of M-type, but in the beers of KN-type, the contents of nigerose 0.25—0.49 g/*l* and kojibiose were 0.25—0.78 g/*l*.

The maltose content in our samples was larger than that of Montreuil *et al.* whereas the contents of other disaccharides, maltotriose and maltotetraose were smaller than those reported by Montreuil *et al.* But, higher oligosaccharides contents of our samples were larger than that of Montreuil *et al.*

However, it is not certain whether these higher oligosaccharides contents are really larger. Because, Montreuil *et al.* have dialysed their sample followed by the treatment with ion exchanger, whereas we have not treated our samples in this way.

4) Sugar composition in oligosaccharides fraction.

Oligosaccharides fraction of each beers was cut off from paper chromatogram and eluted with water and hydrolysed with acid (2.27 per cent HCl, 2.5hr), followed by neutralization with NaOH, and passed through a column of Amberlite IR-120 and IRA-410. The deionized solution was concentrated and examined by PPC.

A large amount of glucose and small amounts of xylose and arabinose were detected in both Japanese and foreign beers. In Takara, two sugars were detected besides the above three sugars.

Summary

The general analyses of bottled beers of the trade mark "Asahi Gold", "Sapporo", "Kirin", "Takara", "Dortmunder Actien (DA)", "Löwenbräu München (LM)", "San Miguel (SM)", "Lavie", "Liner" and canned beers of the first three

Table 4. Content of each sugars in beers. (g/100*ml*)

	Total sugar	Reducing sugar	Xylose	Arabinose	Glucose	Nigerose	Maltose	Kojibiose	Isomaltose	Malto-triose	Malto-tetraose	Higher oligo-saccharides
Asahi Gold	29.13	7.11	0.04	0.12	0.05	0.36	—	0.41	0.72	1.65	25.78	
Sapporo	35.81	11.35	0.15	0.23	0.23	0.25	—	0.25	0.65	6.83	27.22	
Kirin	37.08	13.99	0.11	0.13	0.11	—	4.15	—	0.99	4.26	27.33	
Takara	35.85	13.21	0.13	0.19	0.08	—	3.14	—	0.93	2.70	28.68	
DAB	32.28	9.66	0.09	0.14	0.31	0.43	—	0.64	1.10	2.34	27.23	
SMB	42.17	14.72	0.07	0.05	0.22	0.49	—	0.78	0.71	7.28	5.12	27.45
LMB	43.94	15.18	0.06	0.07	0.14	0.08	2.90	—	0.55	4.27	7.12	28.75
Lavie	40.29	14.99	0.11	0.10	0.15	0.41	2.94	—	0.36	5.95	2.70	27.57

Table 5. Contents of each sugars in beer. (dialysable fraction) (g/100*ml*)

	Total sugar	Pentose (Xylose Arabinose)	Fructose	Glucose	Galactose	X_1	Maltose	X_2	Isomaltose	Maltotriose	Isomaltotriose	Maltotetraose	Maltopentaose	Maltohexaose	Glucosan
Table	14.16	0.10	0	traces	0.18	1.08	0.50	1.08	1.35	1.92	0.76	2.30	0.60	0.25	4.04
Bock	22.95	0.12	0	0.12	0.10	1.62	0.80	1.80	1.12	2.39	0.62	4.78	1.30	0.50	7.68
Lux	33.97	0.24	0	0.12	traces	0.43	1.22	0.35	1.40	6.48	1.38	8.45	2.10	0.85	10.95
Pils	38.71	—	0	traces	traces	0.82	2.20	0.56	2.70	8	2.31	8.32	3.20	0.85	9.75
Scotch	46.83	—	0	0.25	traces	2.80	2.88	0.56	2.20	8.80	2.90	11.12	2.72	0.90	11.70
Speciale	30.70	—	1.50	1.52	traces	1.62	0.80	1.04	1.40	4	1.08	5.70	1.44	1.14	9.46

were carried out and, especially, their sugar components were analysed by the paper chromatographic method.

As the results, xylose, arabinose, glucose, nigerose, maltose, isomaltose, maltotriose, maltotetraose, higher oligosaccharides and a maltulose-like sugar were detected in each of the samples. There were two types of beers, a) KN-type which contains kojibiose and nigerose but almost no maltose as in Asahi Gold, Sapporo, DA and SM and b) M-type which contains maltose but a small amount of nigerose and no kojibiose as in Kirin, Takara and LM.

Ribose was detected in Takara, LM and SM; galactose in Asahi Gold and Kirin; fructose in DA and SM.

The contents of monosaccharides, disaccharides, trisaccharides and higher oligosaccharides were found to be 0.62—1.71 per cent, 3.22—13.87 per cent, 5.66—19.08 per cent and 73.69—88.51 per cent respectively. In the M-type, the content of the disaccharides was larger than that of the trisaccharides but in the KN-type just on the contrary, the latter was larger than the former. The oligosaccharides of the beers consisted of a large amount of glucose and small amounts of xylose and arabinose.

Acknowledgement

The authors gratefully acknowledge for the gift of "San Miguel Beer" from Mr. Peaty and that of "Lavie" from Dr. S. Iida, and wish to acknowledge their indebtedness to Messrs. T. Nakayama, K. Yamamoto and N. Miyadai for their kind advices.

References

1) Gjertson, P.G. (1955). Proc. Eur. Brew. Conv., Baden-Baden 37.
2) Stöckli, A. (1956). Schweiz. Brau-Runds., **67**, 1.
3) Stöckli. A. (1956). ibid. **67**, 62.
4) Randoin, L., S. Billaud (1956). Bull. Soc. Scient. Hyg. Alim., **44**, 3.
5) Silbereisen, K., J. Bielig, F. Just. A. Kallman (1958). Brauerei, Wissensch, Beil, **11**, 66.
6) Montreuil, J., R. Scriban, S. Mullet (1959). Brauerei, **14**, 64
7) MacWilliam, I. C., A. W. Phillips (1959). Chem. & Ind., 364.