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By

Kiyoshi Aso, Toshiyuki Watanabe and Kyoko Yamao

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

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In Japan, the Bee Culture Laboratory of the National Institute of Agricultural Science (1937) (1), and Nakayama *et al.* (1952) (2) reported that glucose, fructose, sucrose and dextrin in honey were determined. But the separation, identification and determination of each sugars in honey by paper chromatography and carbon-Celite column chromatography have not yet been performed.

In foreign countries, Malyoth (3), Täufel *et al.* (4), Vavruch (5), White *et al.* (6) and Goldschmidt *et al.* (7) described that glucose, fructose, sucrose, maltose and melezitose were detected in honey by paper chromatography or carbon-Celite column chromatography. In them, White *et al.* (6) reported in detail that carbohydrate contents of 21 honey samples from 19 floral sources have been analysed by selective adsorption procedure.

We now report on the analyses of 15 honey samples (eleven floral types of honey and four sorts of marketed honey), paper chromatography of the sugars in honey and the fractionation and determination of the sugars of one kind of honey (*Lespedeza bicolor*) by a carbon-Celite column chromatography.

Experimental

I. Analyses of Honey

The 15 honey samples produced mainly in the Tohoku region of Japan were analysed. Tables 1 and 2 represent the properties and sugar compositions.

Analytical methods were as follows. Moisture: Toluol distillation method; invert sugar: after heating with 0.1 per cent HCl on the boiling water bath for 30 minutes and neutralization with NaOH, determined by the Bertrand-Henmi method (as invert sugar); reducing sugar: Bertrand-Henmi method (as glucose); glucose: Willstätter-Schudel method; fructose: reducing

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sugar minus glucose; sucrose: (invert sugar minus reducing sugar) × 0.95. The main constituents were glucose and fructose, and their contents occupied

Table 1. Floral source, Crop year, Productive place, Appearance of Honey.

Sample No.	Floral source	Crop year	Productive place	Color	Appearance
1.	*Lespedeza bicolor*	1956	Fukushima	Pale yellow	Viscous Liquid Small Amount of glucose crystals
2.	Strawberry and Hakuunboku	1956	Yamagata	Yellow	Viscous Liquid No crystal
3.	Clover and Persimmon	1955	Shonai	Yellow	Viscous Liquid Small Amount of glucose crystals
4.	Pumpkin	1955	Hagino	Pale brown	Viscous Liquid Small Amount of glucose crystals
5.	Chestnut	1955	Semi	Brown	Viscous Liquid No crystal
6.	Horse-chestnut	1955	Goshoyama	Yellow	Viscous Liquid Large Amount of glucose crystals
7.	Rape-seed	1955	Yamagata	Pale yellow	Viscous Liquid Large Amount of glucose crystals
8.	Buckwheat	1954	Oharazawa	Dark brown	Viscous Liquid No crystal
9.	*Astragalus sinicus*	1955	Koriyama	Pale brown	Viscous Liquid Small Amount of glucose crystals
10.	Hyotanboku	1955	Urabandai	Pale yellow	Viscous Liquid Small Amount of glucose crystals
11.	Rape-seed	1955	Chiba	Pale brown	Viscous Liquid Large Amount of glucose crystals
12.	Marketed honey A	Unknown	Unknown	Pale yellow	Viscous Liquid Small Amount of glucose crystals
13.	Marketed honey B	Unknown	Unknown	Pale yellow	Viscous Liquid Small Amount of glucose crystals
14.	Marketed honey C	Unknown	Unknown	Pale yellow	Viscous Liquid Large Amount of glucose crystals
15.	Marketed honey D	Unknown	Unknown	Pale yellow	Viscous Liquid Large Amount of glucose crystals

Table 2. Sugar Composition of Honey.

Honey (Floral source)	Moisture	Invert sugar	Reducing sugar	Glucose (G)	Fructose (F)	Sucrose	Quantity ratio of G & F
	(%)	(%)	(%)	(%)	(%)	(%)	
1. *Lespedeza bicolor*	20.53 *	73.41 92.37	67.78 85.29	31.79 40.00	34.68 43.64	3.30 4.15	G<F
2. Strawberry and Hakuunboku	20.29 *	69.75 87.50	66.50 83.43	30.90 38.77	34.38 43.13	0.99 1.24	G<F
3. Clover and Persimmon	20.57 *	77.45 97.51	68.99 88.86	35.11 44.20	33.88 42.65	5.85 7.36	G>F
4. Pumpkin	20.28 *	69.68 87.73	66.01 82.80	32.13 40.30	33.88 42.50	1.13 1.42	G<F
5. Chestnut	23.66 *	64.36 84.31	60.58 79.36	22.06 28.90	35.94 '47.08	1.52 1.99	G<F
6. Horse-chestnut	19.55 *	75.93 94.38	69.63 86.57	33.03 41.06	33.60 41.77	3.53 4.39	G≦F
7. Rape-seed	19.84 *	79.60 99.30	75.11 93.70	38.68 48.25	36.43 45.45	1.83 2.28	G>F
8. Buckwheat	20.50 *	71.81 90.33	66.75 83.96	33.40 42.01	33.35 41.95	2.46 3.07	G≒F
9. *Astragalus sinicus*	26.20 *	72.83 98.69	66.33 89.88	30.77 41.69	35.56 48.18	3.85 5.22	G<F
10. Hyotanboku	19.85 *	74.68 93.18	68.03 84.88	36.82 45.94	31.20 38.93	4.06 5.07	G>F
11. Rape-seed	21.68 *	76.56 97.75	69.75 89.06	39.76 50.77	29.99 38.29	4.14 5.29	G>F
12. Marketed honey A	16.97 *	77.07 92.82	70.73 85.19	22.26 26.81	48.47 58.38	3.61 4.35	G<F
13. Marketed honey B	15.76 *	81.07 96.24	76.10 90.34	34.74 41.24	41.36 49.10	1.79 2.12	G<F
14. Marketed honey C	21.70 *	77.33 98.76	72.36 92.41	31.73 40.52	40.62 51.88	2.20 2.81	G<F
15. Marketed honey D		77.95	72.93	36.07	36.86	2.21	G<F
Average	20.53 *	74.63 93.91	69.17 87.04	32.62 41.05	36.01 45.31	2.83 3.56	

* In dry matter

more than 90 per cent of the total sugar, and the average moisture content was about 20 per cent.

From the results of Table 2, the honeys of high sugar content were rape-seed, *Astragalus sinicus*, clover + persimmon, and two kinds of marketed honey; the honeys of middle sugar content were *Lespedeza bicolor*, horse-chestnut, buckwheat, hyotanboku and two kinds of marketed honey; the

honeys of low sugar content were strawberry + hakuunboku, pumpkin and chestnut. The glucose content is higher than that of fructose in the honeys of clover + persimmon, rape-seed, and hyotanboku; glucose content is less than that of fructose in the honeys of *Lespedeza bicolor*, strawberry + hakuunboku, pumpkin, chestnut and *Astragalus sinicus*; glucose content is almost equal to that of fructose in the honeys of horse-chestnut and buckwheat.

It is remarkable that the moisture contents of honeys produced in Japan are 3–4 per cent more than that in foreign countries. In comparison of the honey used in this experiment with that produced in 1937 (1), the ratio of glucose and fructose in honey obtained from the same kind of flower showed almost the same tendency.

II. Paper chromatography of sugars in honey.

Each honey was prepared by dilution with water 10–15 fold and spotted on filter paper. After irrigating the chromatogram with pyridine: butanol: water (4 : 6 : 3), sugars were located by spraying with aniline hydrogen phthalate and resorcine reagents. Glucose and fructose were detected.

The diluted honey solution was fermented with yeast at 30°C for 48 hours. The fermented solution was filtered and concentrated and examined by paper chromatography, ascending three times and Toyo filter paper No. 2 was used. The results are shown in Fig. 1.

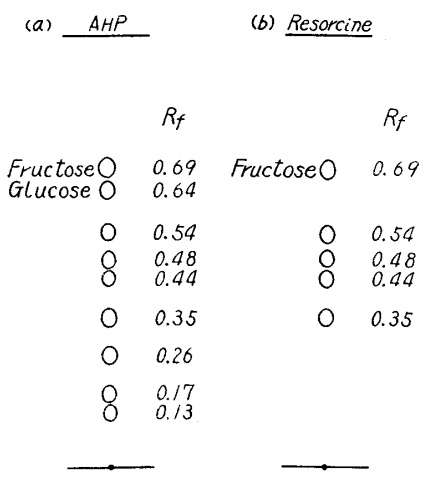

Fig. 1. Multiple paper chromatogram of sugars in honey.

From the results of Fig. 1, fructose, glucose, four di- or tri-saccharides containing ketose and three higher oligosaccharides were detected from the honey of *Lespedeza bicolor*, strawberry + hakuunboku, clover + persimmon, pumpkin, horsechestnut, rape-seed, buckwheat, and *Astragalus sinicus*. It was recognized that the amounts of sugars in the honey were not the same, but the kinds of sugars were almost same.

III. Fractionation and determination of sugars in honey (*Lespedeza bicolor*) by carbon column chromatography.

Three hundred and one and a fifth g of honey (*Lespedeza bicolor*) was dissolved in 2 *l* of distilled water and neutralization with NaOH, the pH adjusted to 6.2. It was poured on a column (550 × 125 mm) composed of the same amount of carbon (Takeda, 800 g) and Celite (No. 545, 800 g) and eluted with water (24 *l*), 2.5% (14 *l*), 5% (18 *l*), 10% (22 *l*), 15% (16 *l*), 20% (16 *l*),

Table 3. Fractionation and determination of the sugars in honey (*Lespedeza bicolor*), by carbon column chromatography.

Fraction No.	Volume of effluent (*l*)	Solvent used for elution	Composition of sugar	Yield (g)
1-2	4	Water	No sugar	...
3-11	20	〃	Fructose, Glucose	185.20 (Fr. 92.53, Glu. 89.97)
12-16	10	2.5% EtOH	Fructose, Glucose	0.035
17-18	4	〃	Sucrose, Oligo. (0.44), Oligo. (0.35)	1.57
19-21	6	5% EtOH	Sucrose, Oligo. (0.48), Oligo. (0.44), Oligo. (0.35)	3.63
22	2	〃	Oligo. (0.48), Oligo. (0.44), Oligo. (0.35),	0.85
23	2	〃	Oligo. (0.54), Oligo. (0.48), Oligo. (0.44), Oligo. (0.35),	0.95
24-25	4	〃	Oligo. (0.59), Oligo. (0.54), Oligo. (0.48), Oligo. (0.35),	2.62
26	2	〃	Oligo. (0.59), Oligo. (0.54), Maltose	0.64
27	2	〃	Oligo. (0.54), Maltose	0.65
28-31	8	10% EtOH	Oligo. (0.54), Maltose, Oligo. (0.36)	2.48
32	2	〃	Oligo. (0.54), Oligo. (0.36), Oligo. (0.22),	0.19
33-34	4	〃	Oligo. (0.54), Oligo. (0.22), Oligo. (0.16)	0.36
35	2	〃	Oligo. (0.54), Oligo. (0.16)	0.09
36	2	〃	Melezitose	0.11
37	2	〃	Melezitose	0.22
38	2	〃	Melezitose, Oligo. (0.34), Oligo. (0.26)	0.30
39-41	6	15% EtOH	Oligo. (0.34), Oligo. (0.26),	0.87
42	2	〃	Oligo. (0.34), Oligo. (0.26), Oligo. (0.17), Oligo. (0.06)	0.79
43	2	〃	Oligo. (0.40), Oligo. (0.17), Oligo. (0.06)	0.91
44-46	6	〃	Oligo. (0.40), Oligo. (0.17)	1.23
47	2	20% EtOH	Oligo. (0.40), Oligo. (0.17),	0.18
48	2	〃	Oligo. (0.40), Oligo. (0.22), Oligo. (0.17)	0.16
49	2	〃	Oligo. (0.40), Oligo. (0.22), Oligo. (0.17), Oligo. (0.05)	0.94
50	2	〃	Oligo. (0.22), Oligo. (0.05)	0.32
51-54	8	〃	Oligo. (0.22), Oligo. (0.09), Oligo. (0.03)	0.78
55-56	4	25% EtOH	Oligo. (0.37), Oligo. (0.22), Oligo. (0.09), Oligo. (0.03)	0.074
57	2	〃	Oligo. (0.37), Oligo. (0.22), Oligo. (0.09), Oligo. (0.05), Oligo. (0.03)	0.087
58-60	6	〃	Oilgo. (0.37), Oligo. (0.09), Oligo. (0.05), Oligo. (0.03)	0.13
61-63	6	〃	Oligo. (0.37), Oligo. (0.01)	0.046
64-67	8	30% EtOH	Oligo. (0.37), Oligo. (0.01)	0.045
68-72	10	〃	Oligo. (0.01)	0.019

The numbers in parentheses represents Rf values.

25% (18 *l*) and 30% (18 *l*) ethanol successively. The eluates were concentrated and examined by paper chromatography as shown in Table 3.

Analyses of sugars in each fraction were estimated. The portion of monosaccharide was determined by the Bertrand-Henmi method and separative determination of glucose and fructose was performed by the Willstätter-Schudel method. The portion of oligosaccharide was determined by the Bertrand-Henmi method after heating with 0.1 per cent HCl on the boiling water bath for 30 minutes and neutralization with NaOH. The obtained values are shown in Table 3.

From the results of Table 3, fructose, glucose, sucrose, maltose, melezitose, eight di- or tri-saccharides and nine higher oligosaccharides were detected in honey. The sucrose content was about 3 per cent. But the true sucrose content must be smaller, because di- or tri-saccharides containing glucose and fructose were determined as sucrose.

Voorst (8) reported that the average of the maltose content was 2–7 per cent and White *et al.* (6) described that the reducing disaccharide content determined as maltose was about 7.11 per cent. But in our experiment, maltose was eluted in Fr. 26–31, and the sugar content in this fraction was 1.25 per cent of whole honey. Maltose content must be less than 1 per cent, because the other three sugars than maltose were eluted in this fraction.

Carbon column chromatographic behavior of sugars in honey (*Lespedeza*

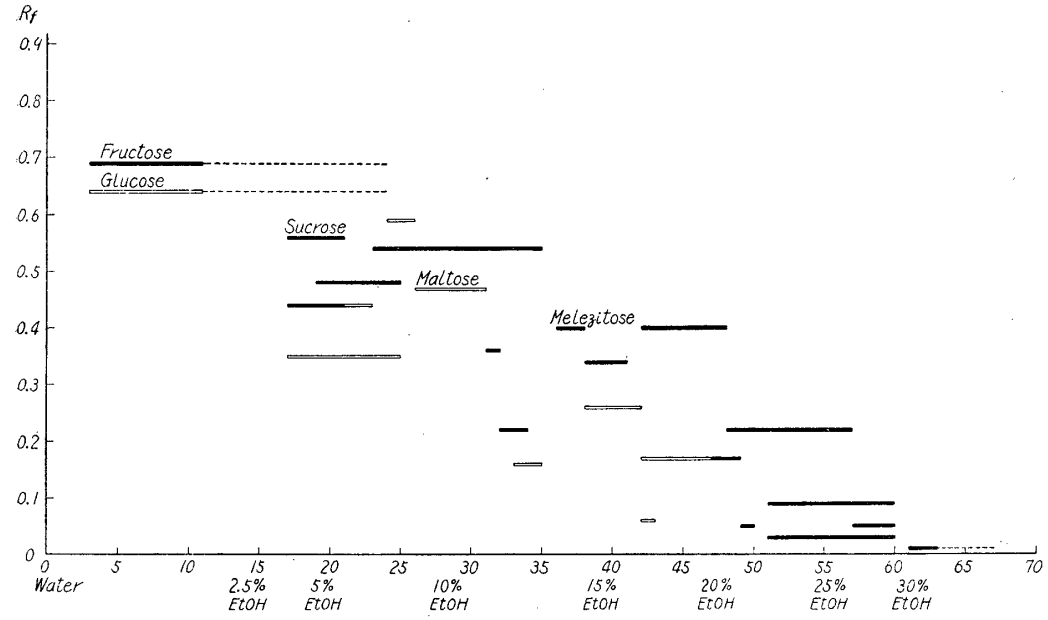

Fig. 2. Carbon column chromatographic behavior of sugars in honey.

bicolor) are shown in Fig. 2.

Glucose and fructose were eluted in water fraction, sucrose in 2.5–5 per cent ethanol fractions, maltose in 5–10 per cent ethanol fractions and melezitose in 10 per cent ethanol fraction. It was recognized by paper chromatography that the sugars of Rf values 0.54, 0.48 and 0.44 were oligosaccharides consisting of glucose and fructose.

From the results in Fig. 2, fructose, glucose, sucrose, maltose, melezitose, eight di- or tri-saccharides and nine higher oligosaccharides were detected in honey fractionated by carbon column chromatography. Fifteen spots of them contained ketose.

Summary

The honeys produced mainly in the Tohoku region of Japan, viz., *Lespedeza bicolor*, strawberry + hakuunboku (*Styrax obassia*), clover + persimmon, pumpkin, chestnut, horse-chestnut, rape-seed, buckwheat, *Astragalus sinicus*, hyotanboku (*Lonicera morrowii*) and four kinds of marketed honey were analysed by the ordinary method and paper partition chromatography.

The main constituents of the honeys were glucose and fructose, and their contents occupied more than 90 per cent of the total sugar, and the average moisture content of the honeys was about 20 per cent. And sucrose content was about 3 per cent, but the true sucrose content must be less; because di- or tri-saccharides containing glucose and fructose were determined as sucrose.

It is remarkable that the moisture contents of honeys produced in Japan are larger than that in foreign countries. In comparision of the honey used in this experiment with that produced in 1937, the ratio of glucose and fructose in the honey obtained from the same kind of flower showed almost the same tendency. The quantities of maltose and melezitose contained in the honeys are less than those described previously by White *et al* and Voorst.

Sugar components of one kind of honey (*Lespedeza bicolor*) were fractionated and determined by carbon column chromatography and paper partition chromatography. By those methods, 22 spots corresponding to glucose, fructose, sucrose, maltose, melezitose, eight di- or trisaccharides and nine higher oligosaccharides were detected, in which 15 spots of them contained ketose.

Acknowledgement

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