STUDIES ON THE DIOSPYROS KAKI (JAPANESE PERSIMMON) II. ON THE TOTAL SUGAR DETERMINATION OF THE SUBSTANCES CONTAINING FRUCTOSE

STUDIES ON THE *DIOSPYROS KAKI* (JAPANESE PERSIMMON)
II. ON THE TOTAL SUGAR DETERMINATION OF THE SUBSTANCES CONTAINING FRUCTOSE*

By

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Previously, we (1) have reported on the analysis of fresh and dried *Diospyros Kaki* (flesh and white powder). Paper chromatography (PPC) of sugars, sugar alcohols and organic acids was also carried out.

The major constituents in the white powder of dried *Diospyros Kaki* are glucose and fructose, and the content ratio was about 90 : 10. Mannitol was not detected. The major sugars in the flesh of dried *Diospyros Kaki* were also glucose and fructose, and the content ratio was about 60 : 40. The fructose content in the flesh increased during the drying period, while the glucose content decreased.

On the paper chromatogram of the sugars in the fresh fruit, four spots corresponding to glucose, fructose, sucrose and an unidentified oligosaccharide were detected. Malic acid was detected as the sole organic acid.

In the dried fruit, sucrose and the above oligosaccharide could not be detected.

During the course of analysis of the sugar contents of Kaki fruit, it was found that the so-called total sugar determination (2), after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours, gave a lower value than the total invert sugar or reducing sugar as shown in Table 1.

Table 1. Composition of the fresh *Diospyros Kaki* juice in Miyagi Prefecture in 1953.

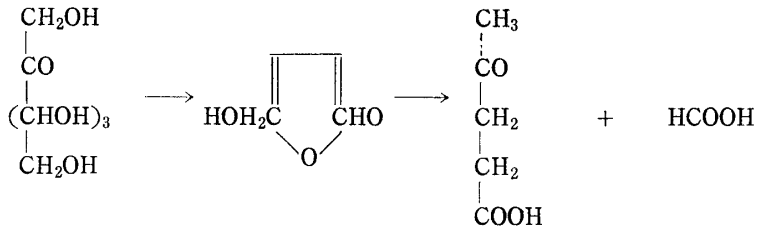
Total sugar (g/100 *ml*)	Total invert sugar (g/100 *ml*)	Reducing sugar (g/100 *ml*)	Glucose (g/100 *ml*)	Fructose (g/100 *ml*)	Sucrose (g/100 *ml*)
12.82	15.71	14.58	9.77	4.42	1.04

* The original Japanese report was published in Nippon Nôgei Kagaku Kaishi, **30**, 191-195 (1956).

The object of this study was to find the cause of this discrepancy.

Paper chromatography of sugars and the determination of each sugar in the fresh *Diospyros Kaki* juice were carried out after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours.

From the results of the above experiment, it was found that most part of the fructose was converted into hydroxymethylfurfural (HMF) and a small amount of HMF was further converted into levulinic acid during the hydrolysis under the above condition.



Fructose — Hydroxymethyl-furfural — Levulinic acid — Formic acid

Hydroxymethylfurfural showed less reducing power than fructose in Bertrand-Henmi method and behaved as an aldose in Willstätter-Schudel method. Therefore, when substances containing fructose are used as samples for the quantitative determination of sugars, it may be possibly observed that the total sugar value is less than the total invert sugar and the reducing sugar value and glucose value by Willstätter-Schudel method is estimated too large while fructose value is too small in estimation.

Shichiji *et al.* (3), (4) have reported that 40~50 per cent of fructose was decomposed during the process of total sugar determination. Therefore, when substances containing fructose are used as samples for the determination of sugars, the total sugar value is often less than the total invert sugar and reducing sugar value. But they have not investigated on the decomposed product.

Nomura (5) has reported that fructose was more easily decomposed into HMF than glucose, but the determination of HMF was not performed.

In Europe and America, Mathews *et al.* (6) have determined the decomposition ratio of fructose by specific rotation and observed that fructose solution was very stable at pH 3.3. Pictet *et al.* (7) have reported that fructose was converted into hetero levulosan and its dimer.

We now report on the estimation of decomposition ratio of fructose solution by Bertrand-Henmi method and Willstätter-Schudel method under the condition of hydrolysis applied for total sugar determination. The decomposition ratio of glucose, xylose and arabinose solution after lacid hydrolysis was

also determined as well as the decomposition ratio of fructose solution with H_2SO_4 instead of HCl.

Experimental

I. Analyses of mixture of glucose and fructose solution.

The mixture of glucose and fructose solution was made and the total sugar was determined by Bertrand-Henmi method after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours, reducing sugar was determined by Bertrand-Henmi method. The separative determination of glucose and fructose was carried out by the combination of Bertrand-Henmi and Willstätter-Schudel methods. The results are shown in Table 2.

Table 2. Analyses of mixture of glucose and fructose solution before and after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours.

Before (g/100 *ml*) (Reducing sugar)		After (g/100 *ml*) (Total sugar)	
5.70		4.97	
aldose (g/100 *ml*)	ketose (g/100 *ml*)	aldose (g/100 *ml*)	ketose (g/100 *ml*)
3.72	1.95	4.51	0.43

As shown in Table 2, the total sugar value was less than the reducing sugar value and fructose content decreased while glucose content increased. Because, fructose was decomposed to HMF under the condition of total sugar determination. HMF behaved as an aldose in Willstätter-Schudel method.

II. Heating of glucose solution.

About seven per cent of glucose solution was prepared and heated under the same condition as above. And the total and reducing sugar, before and after heating, were determined by Bertrand-Henmi method. Before heating 7.08 g/100 ml, after heating 7.07 g/100 ml. There was no change between before and after heating.

III. Heating of fructose solution.

Fructose solution was prepared and the same analyses were determined by Bertrand-Henmi method. The separative determination of aldose and ketose was carried out by the combination of Bertrand-Henmi and Willstätter-Schudel methods. The results are shown in Table 3.

Table 3. Analyses of fructose solution before and after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours.

Before (g/100 *ml*)		After (g/100 *ml*)	
2.23		1.40	
aldose (g/100 *ml*)	ketose (g/100 *ml*)	aldose (g/100 *ml*)	ketose (g/100 *ml*)
0.07	2.16	1.04	0.36

As shown in Table 3, the total sugar value was about 63 per cent of the reducing sugar, and fructose content decreased while glucose content increased.

Paper chromatography of fructose solution after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours was carried out.

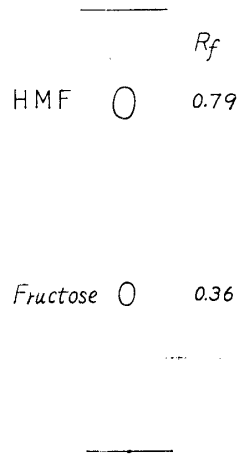


Fig. 1. Paper chromatogram of fructose solution after heating on the boiling water bath with 2.5 per cent HCl for 2.5 hours.
solvent; pyridine: butanol: water
4 : 6 : 3
spray reagent; aniline hydrogen phthalate, resorcin

The fructose solution after acid hydrolysis was spotted on the Tôyo filter paper No. 2. After irrigating the chromatogram with pyridine-butanol-water (4 : 6 : 3), the sugars were located by spraying with aniline hydrogen phthalate and resorcinol reagents. The results are shown in Fig. 1.

The spot of R_f 0.79 was identified as HMF synthesized from sucrose (9). And levulinic acid was detected by PPC in fructose solution after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours.

The above results indicate that fructose was decomposed and the most part was converted into HMF under the condition of total sugar determination. HMF showed considerable reducing power and behaved as an aldose in Willstätter-Schudel method. Therefore, the aldose value increased as shown in Table 3.

The determination value of HMF synthesized from sucrose was 89.41 per cent by Bertrand-Henmi method calculated as fructose and 38.84 per cent as aldose by Willstätter-Schudel method.

The determination of HMF was carried out by the dilution method of PPC. The colored limiting quantity of HMF by micrometer syringe (8) was about 0.7 γ. The results are shown in Table 4.

Total sugar was determined by Bertrand-Henmi method after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours.

Table 4. Determination of HMF and fructose in fructose solution after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours.

	Total sugar 1.40 g/100 *ml*		
	Willstätter-Schudel method (g/100 *ml*)		Dilution method (g/100 *ml*)
Aldose	1.04	HMF	0.78
Fructose	0.36		

Analyses of glucose and fructose solution before and after heating with 0.1 per cent HCl on the boiling water bath for 30 minutes were carried out. The results are shown in Table 5.

Table 5. Analyses of glucose and fructose solution before and after heating with 0.1 per cent HCl on the boiling water bath for 30 minutes.

Glucose solution

Before (g/100 *ml*) as glucose		After (g/100 *ml*) as invert sugar	
5.00		5.24	
aldose (as glucose) (g/100 *ml*)	ketose (as fructose) (g/100 *ml*)	aldose (g/100 *ml*)	ketose (g/100 *ml*)
4.97	0.03	5.01	0.02

Fructose solution

Before (g/100 *ml*) as fructose		After (g/100 *ml*) as invert sugar	
4.49		4.68	
aldose (g/100 *ml*)	ketose (as fructose) (g/100 *ml*)	aldose (g/100 *ml*)	ketose (as fructose) (g/100 *ml*)
0.34	4.13	0.45	4.04

Fructose did not undergo any change under the condition of hydrolysis applied for total invert sugar determination, namely heating with 0.1 per cent HCl on the boiling water bath for 30 minutes.

On the paper chromatogram, HMF was not detected in the fructose solution after heating with 0.1 per cent HCl on the boiling water bath for 30 minutes.

IV. Isolation and identification of HMF from heated fructose solution.

Since fructose was changed into HMF under the condition of total sugar determination, isolation and identification were attempted.

50 *ml* of 2.5 per cent HCl was added to 4 g of fructose and heated on the boiling water bath for 2.5 hours followed by neutralization with calcium carbonate and filtration. The filtrate was extracted with ether, and after removal of the ether, about 0.5 g of HMF (b.p. 145~150°C/15 mm Hg) was obtained and identified with HMF synthesized from sucrose (9). Levulinic acid was detected by PPC, but isolation and identification was not performed.

V. Comparison of the decomposition power of HCl and H_2SO_4 to fructose solution.

Since fructose was decomposed into HMF by heating with 2.5 per cent HCl for 2.5 hours, the decomposition ratio of fructose by HCl and H_2SO_4 was examined. The results are shown in Table 6.

Table 6. The decomposition of fructose by HCl and H_2SO_4 (heating time 2.5 hours)

	Bertrand-Henmi method		Willstätter-Schudel method			
original solution	Reducing sugar (as fructose) (g/100 *ml*) 4.59		aldose (g/100 *ml*) 0.41		fructose (g/100 *ml*) 4.18 (100.00)	
	HCl (g/100 *ml*)	H_2SO_4 (g/100 *ml*)	HCl (g/100 *ml*)	H_2SO_4 (g/100 *ml*)	HCl (g/100 *ml*)	H_2SO_4 (g/100 *ml*)
1.0%	4.02	4.42	1.44	0.72	2.58 (61.72)	3.70 (88.52)
1.5%	3.69	4.37	1.93	0.98	1.76 (42.11)	3.39 (81.10)
2.0%	3.25	4.30	2.18	1.17	1.07 (25.60)	3.13 (74.88)
2.5%	2.86	4.24	2.19	1.34	0.67 (16.03)	2.90 (69.38)

The numbers in parentheses represent the decomposition ratio of fructose solution when the original solution is 100.00.

As shown in Table 6, about 83.9 per cent of fructose was decomposed after 2.5 per cent HCl hydrolysis for 2.5 hours, and about 30.6 per cent of fructose was decomposed after 2.5 per cent H_2SO_4 hydrolysis for 2.5 hours. H_2SO_4 was also capable to decompose fructose, but its power was less than half of that of HCl. And about 38 per cent of fructose was decomposed when the fructose solution was heated with one per cent HCl for 2.5 hours.

The decomposition ratio of fructose solution after heating with 0.1, 0.2, 0.5 per cent HCl for 2.5 hours were estimated. The results are shown in Table 7.

As shown in Table 7, about 15 per cent of fructose was decomposed after heating with 0.5 per cent HCl on the boiling water bath for 2.5 hours. Therefore, when the HCl concentration is over 0.5 per cent, we can not expect the accurate results.

Table 7. The decomposition of fructose by heating with 0.1, 0.2 and 0.5 per cent HCl on the boiling water bath for 2.5 hours.

	Bertrand-Henmi method	Willstätter-Schudel method	
	Reducing sugar (as fructose) (g/100 *ml*)	aldose (g/100 *ml*)	fructose (g/100 *ml*)
original solution	4.49	0.21	4.28 (100.00)
0.1% HCl	4.50	0.26	4.24 (99.07)
0.2% HCl	4.48	0.45	4.03 (94.16)
0.5% HCl	4.39	0.75	3.64 (85.05)

The decomposition ratio of fructose solution after heating with 2.5 per cent HCl for 30 minutes, one hour, 1.5 hours, two hours and 2.5 hours was determined. The results are shown in Table 8.

Table 8. The decomposition of fructose solution after heating with 2.5 per cent HCl on the boiling water bath for 0.5, 1.0, 1.5, 2.0 and 2.5 hours.

	Bertrand-Henmi method	Willstätter-Schudel method	
	Reducing sugar (as fructose) (g/100 *ml*)	aldose (g/100 *ml*)	fructose (g/100 *ml*)
original solution	4.59	0.41	4.18(100.00)
30 min	4.35	0.92	3.43 (82.06)
1.0 hr	4.09	1.59	2.50 (59.81)
1.5 hr	3.71	1.83	1.88 (44.98)
2.0 hr	3.26	2.08	1.18 (28.23)
2.5 hr	2.86	2.19	0.67 (16.03)

Table 9. Analyses of fructose solution before and after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours.

Before (g/100 *ml*)		After (g/100 *ml*)	
2.23		1.40	
aldose (g/100 *ml*)	ketose (g/100 *ml*)	aldose (g/100 *ml*)	ketose (g/100 *ml*)
0.07	2.16	1.04	0.36
4.30		2.78	
aldose (g/100 *ml*)	ketose (g/100 *ml*)	aldose (g/100 *ml*)	ketose (g/100 *ml*)
0.16	4.14	2.04	0.74
8.94		5.68	
aldose (g/100 *ml*)	ketose (g/100 *ml*)	aldose (g/100 *ml*)	ketose (g/100 *ml*)
0.33	8.61	4.14	1.54

As shown in Table 8, about 18 per cent of fructose was decomposed after heating with 2.5 per cent HCl for 0.5 hours.

The analyses of about two, four and nine per cent of fructose solution before and after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours were carried out. The results are shown in Table 9.

As shown in Table 9, the decomposition of fructose was about 82~83 per cent. There was no remarkable difference due to the sugar concentration.

VI. Heating of pentose solution.

Since xylose and arabinose were more easily decomposed than glucose, the analyses of xylose and arabinose solution before and after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours were carried out.

About five per cent of xylose and arabinose solution were made, and determined by Bertrand-Henmi method. The separative determination of aldose and ketose were performed by Willstätter-Schudel method. The results are shown in Table 10.

Table 10. Analyses of xylose and arabinose solution before and after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours.

	Before (g/100 *ml*)		After (g/100 *ml*)	
Xylose solution	4.86		4.89	
	aldose (g/100 *ml*) 4.98	ketose (g/100 *ml*)	aldose (g/100 *ml*) 4.78	ketose (g/100 *ml*) 0.11
Arabinose solution	4.67		4.66	
	aldose (g/100 *ml*) 4.98	ketose (g/100 *ml*)	aldose (g/100 *ml*) 4.87	ketose (g/100 *ml*)

As shown in Table 10, xylose and arabinose did not undergo any change in the condition of hydrolysis applied for total sugar determination.

Discussion

About 83 per cent of fructose was decomposed and the most part was converted into HMF under the condition of hydrolysis applied for determining total sugar, namely heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours.

The results of Table 4 indicate that 55.60 per cent of fructose was converted into HMF, after heating with 2.5 per cent HCl for 2.5 hours. Determination

of HMF was performed by the dilution method with PPC. This method is as follows. On the paper chromatogram, the disappearance point of the standard HMF was estimated and the same point of HMF produced from fructose solution after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours was determined. The HMF content in the original solution is calculated from the above both values. This method is capable of error more easily than the spectrophotometric method. But the value of HMF by the dilution method was almost agreed with the value calculated from molecular weight.

When the fructose solution was heated with 2.5 per cent HCl on the boiling water bath for 2.5 hours, HMF value by the above dilution method was about 56 per cent of the total sugar. However, when the same solution was determined by Willstätter-Schudel method, the obtained value was about 47 per cent calculated as aldose, which is about 10 per cent less by the dilution method. The aldose value of standard HMF synthesized from sucrose was determined by Willstätter-Schudel method. The obtained value was about 39 per cent.

When the fructose solution was heated with 0.5, 1.0, 1.5, 2.0 and 2.5 per cent HCl on the boiling water bath for 2.5 hours, the change of values of aldose and ketose were examined. The ratio of increased aldose and decreased ketose was at the maximum between 1.0 per cent and 1.5 per cent.

In the case of H_2SO_4, when the acid concentration was over one per cent, the ratio of increase of aldose and decrease of ketose was gradually lenient.

For the examination of the difference according to the sugar concentration, the analyses of about two, four and nine per cent of fructose solution before and after heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours were carried out. The decomposition of fructose was about 82~83 per cent. There are no remarkable difference due to the sugar concentration.

Summary

Fructose did not undergo any change in the condition of hydrolysis applied for total invert sugar determination, namely heating with 0.1 per cent HCl on the boiling water bath for 30 minutes. But, about 83 per cent of fructose was decomposed and the most part was converted into HMF under the condition of hydrolysis applied for total sugar determination, namely heating with 2.5 per cent HCl on the boiling water bath for 2.5 hours. HMF showed less reducing power than fructose in Bertrand-Henmi method and behaved as an aldose in Willstätter-Schudel method. Therefore, when substances containing fructose are used as samples for the quantitative determination of sugars, it may be possibly observed that the total sugar value is less than the total invert sugar and reducing sugar value, and the fructose value is to little in estimation. On the contrary, glucose, xylose and arabinose were not affected by the same condition of hydrolysis. And H_2SO_4 was also capable to decompose fructose, but its power was less than half of that of HCl.

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