STUDIES ON THE SUGARS IN APPLE JUICE

STUDIES ON THE SUGARS IN APPLE JUICE

By

Kiyoshi ASO and Kazuo MATSUDA

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

(*Received June 25, 1951*)

Many investigations have been made on the sugar composition of apple juice, and the presence of fructose, glucose and sucrose has long been established. Among these sugars, fructose has the largest content and glucose the least,[1] but the ratio of these contents is different according to kind, maturity and days of storage. According to Mackinnis[2] no pentose either free or combined is contained in apple juice.

We analysed these three sugars in several Japanese apple juice and then tried to detect the sugars by the method of paper partition chromatography. In addition to the spots corresponding to fructose, glucose and sucrose, another spot corresponding to xylose was discovered to be present. This spot remained on the paper chromatogram after the juice was fermented by brewery yeast, whereas the other spots entirely disappeared. The presence of xylose was confirmed by deriving the unfermented sugar to its phenylosazone.

In investigating the paper chromatography of sugars using Partridge's aniline hydrogen phtalate reagent,[5] we found that the heating temperature and the time to reveal the spots as well as the Rf values and the color of the spots are useful to identify sugars.

Sucrose content in long stored apple juice was extremely decreased, and on the paper chromatogram of over 6-months stored apple juice, sucrose could not be detected by this reagent, but a spot corresponding to galacturonic acid appeared, which is considered to be derived from pectic substance.

It is interesting that pentose in apple juice is not arabinose considered to be derived from galacturonic acid, but xylose.

Experimental

1) *Determination of sugars in apple juice.*

As the largest part of sugars in apple juice consists of fructose, glucose and sucrose, these three sugars were separately determined neglecting the other sugars. The total sugar and reducing sugar were determined by Bertrand's

method. The sucrose content was indicated as 0.95 × nonreducing sugar (difference between the above two values). Then glucose was determined according to the method described by Willstätter and Schudel.[3] Fructose corresponds to the difference between the reducing sugar and glucose. An example of the data are shown in Table I.

Table I.

Veriety	* Total sugar (g/100ml.)	Sucrose (〃)	Glucose (〃)	Fructose (〃)
Jonathan	11.16	2.97(1.17)**	2.39(1)	5.13(2.15)
Ralls Janet	12.06	3.97(1.47)	2.70(1)	4.96(1.65)
Starking	13.72	4.41(1.56)	2.82(1)	5.35(1.90)
Golden Delicious	12.68	4.50(2.81)	1.60(1)	5.64(3.53)
Escollon	14.43	6.31(2.47)	2.56(1)	4.92(1.92)

* Total sugar is calculated as invert sugar.
** The values in ( ) show the ratio of other sugars to glucose.

2) *Detection of sugars by paper partition chromatography*

Two-dimensional chromatography was carried out by Partridge's method[4),5)] using phenol and butanol-acetic acid as the developing solvents. 30×30cm. sheets of Toyo filter paper No. 2 and No. 50 were employed. After removal of the solvent, the developed paper sheets were sprayed with the anilline hydrogen phtalate reagent and heated in an oven at 105~110°C for 5 minutes. For further detection of sucrose the sheets were heated at 120~130°C for 15~20 minutes. When anilline hydrogen phtalate was used as the revealing reagent, not only the color of the spots, but also the time to reveal the spots is fairly different according to the kind of sugars. For example the spots of aldopentose appeared most rapidly (in 1.5 minutes), aldohexose next rapidly (in 2 minutes), but ketose such as fructose took more than 3 minutes.

The yielded chromatogram of apple juice showed three spots corresponding to fructose, glucose and xylose under heating at 105~110°C., but when heated at 120~130°C. for 15~20 minutes, another spot corresponding to sucrose appeared. The total results are shown in Table II.

Reviewing the results, the presence of fructose, glucose and sucrose is hitherto reported, but the presence of xylose is different from the result reported by Mackinnis.[2)]

To confirm the presence of xylose, the apple juice was fermented by brewery yeast (No. 396) and the unfermented sugar was chromatographed. On the chromatogram of fermented juice the spots corresponding to fructose, glucose and sucrose were not observed but the spot corresponding to xylose clearly remained. The analytical data are as follows:

Table II.

Spot	Rf		Color	Corresponding sugar
	Phenol	Butanol-acetic acid		
A	0.44～0.45	0.11～0.15	Yellowish brown	Fructose
B	0.28～0.29	0.09～0.10	Brown	Glucose
C	0.36～0.37	0.15～0.17	Pink	Xylose
D	0.33～0.35	0.04～0.05	Brown	Sucrose

Before fermentation

Total sugar 12.82 g/100ml. (as invert sugar)
Reducing sugar 8.88 〃 (as fructose)
pH 3.6

* Chromatogram

Spot	Rf		Corresponding sugar
	Phenol	Butanol-acetic acid	
A	0.30	0.12	Glucose
B	0.42	0.17	Fructose
C	0.37	0.21	Xylose

* Sucrose is not detected, because the paper was heated at 105°C

After fermentation

Remained sugar 0.15 g/100ml. (as xylose)
pH. 3.6

Chromatogram

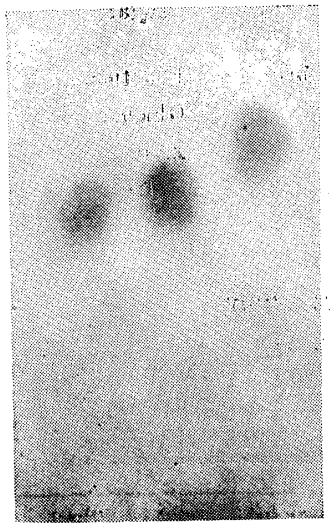
Spot	Rf		Corresponding sugar
	Phenol	Butanol-acetic acid	
	0.37	0.18	Xylose

After being kept in storage over 6 months, the sucrose content in apple juice was extremely decreased (less than 1g/100ml.), and the spot corresponding to sucrose could not be detected by anilline hydrogen reagent, but another spot corresponding to galacturonic acid (Rf 0.09 by phenol, 0.08 by butanol-acetic acid) appeared, which was also unfermentable by brewery yeast.

3) *Identification of xylose*

2L. of apple juice was fermented by brewery yeast (No. 396) for 5 days, filtered, and the largest parts of the ionic materials were removed by passage through ion-exchange resin and then the filtrate was concentrated under reduced

pressure to ca. 500ml. The concentrate was treated with a slight excess of Pb acetate, filtered, and after removal of the remaining Pb by H_2S, it was further concentrated to a syrup. The syrup was then extracted with 5 volume of 90 per cent alcohol and the extract was again concentrated to a syrup. Though after repeating this treatment three times, the syrup could not be crystallized.



A B C

Fig. 1 Paper chromatogram of the unfermented sugar of apple juice.
A Unfermented sugar
B Xylose
C Arabinose

The syrup showed a clear green color by Bial's orcine-$FeCl_3$ reagent and its paper chromatogram showed exactly the same position with the authentic xylose (Fig. 1).

We next tried to identify the sugar as phenylosazone.

1 gram. of syrup, 2 grms. of phenylhydrazine hydrochloride and 3 grms. of Na acetate were dissolved in 20 ml. of water and heated in a boiling water bath for one hour. After cooling, the obtained yellow crystals were filtered and washed with water. After recrystallization twice from hot water, the melting point of plenylosazone was 159~160°C and the mixed melting point with authentic phenylxylosazone, showed no depression.

Summary

The sugars in apple juice were detected by the method of paper partition chromatography and simlutaneously these sugars were analyzed by the usual method.

I) The sugar content of Japanese apple juice was as follows:
Total sugar (calculated as invert sugar) 11~14.5 g/100ml.,
the ratio of glucose : fructose : sucrose 1 : 1.7~3.5 : 1.2~2.8.

II) On the paper chromatogram, 4 spots corresponding to glucose, fructose, sucrose and xylose were observed. Xylose was not hitherto detected in apple juice. On the paper chromatogram of long stored apple juice, sucrose was not detected by the anilline hydrogen phtalate reagent, but another spot corresponding to galacturonic acid appeared.

III) On the paper chromatogram of the juice fermented by yeast, the spots corresponding to glucose, fructose and sucrose disappeared, but the spot corresponding to xylose remained, The xylose was identified as its phenylosazone.

Acknowledgement.

This study was supported by a grant from the technical experiment station of Aomori Prefecture. We express our deep gratitude to the late Mr. Masami Komiyama, the former superintendent of the technical experiment station of Aomori Prefecture, for his assistance and kindness during the cource of the present experiments.

References

1) J. R. Eott : Ind. & Eng. Chem., 9, pp 587 (1917)
2) R. B. Mackinnis : J. Am. Chem. Soc., 50, pp 1911 (1928)
3) R. Willstätter, G Schudel : Ber., 51, pp 780 (1918)
4) S. M. Partridge : Nature, 158, pp 270 (1946)
5) 〃 Ibid., 164, pp 443 (1949)