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STUDIES ON THE UTILIZATION OF GRAPE PART I. COMPOSITION OF GRAPE JUICES*

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Last summer (1953) this work to establish the production method of grape juices for drink was commenced by the request of Miyagi Prefecture. As the first step, the chemical composition of various market and fresh juices obtained in Sendai was analysed, and the paper partition chromatography of organic acids, sugars and amino acids in these juices was performed.

In this country, Sato *et al.* (1), Kawakami *et al.* (2, 3) Brewing Experimental Station, Taxation Bureau (Ministry of Finance) (4), and Kazama *et al.* (5) reported the analytical values of pH, acidity, specific gravity, Brix, reducing sugar and tannin in the grape juices, and Ito *et al.* (6) performed the paper chromatography of sugars and organic acids. The European and American reports on grape juices are too numerous to be mentioned here.

Experimental

[I] *Chemical Composition of Market Grape Juices*

(1) *General Analysis*

The samples for experiments are ten varieties as described below. Six varieties of market juices; "Welch" (U. S. A.), "Bireleys" (Tokyo), "Kikyoya" (Miyagi), "Nisshin" (Yamanashi), "Hatamine" (Yamagata) and "Lemona" concentrated juice (Tokyo), which are indicated by A, B, C, D, E and F in Table 1, respectively. Two varieties of Yamagata *Concord* grape juices which were prepared with the addition of sucrose by Miyagi Agricultural College students; they are shown as I and II. Two varieties of Miyagi *Concord* grape juices, of which one was prepared by pressing without heat and the other with heat, in our laboratory.

Total acidity, pH, refractometer index, reducing sugar (as glucose), invert

* The Japanese report of this part was published in J. Agr. Chem. Soc. Jap., 28, 503 (1954).

Table 1. Chemical Composition of Market and Fresh Grape Juices Obtained in Sendai

		pH	Total acid g/100 ml	Refractometer index	Tannin mg/100 ml	Reducing sugar g/100 ml	Invert sugar g/100 ml	Sucrose g/100 ml	Glucose g/100 ml	Fructose g/100 ml	Total acid Total sugar	Color
Market juice	A	2.5	0.912	15.9	230.95	15.98	—	—	8.32	7.57	5.71	Medicl Crimson (cc180)
	B	2.4	0.377	11.4	12.05	8.94	12.71	3.35	4.93	3.96	2.97	Amaranth Pink (cc170)
	C	2.5	0.890	21.8	156.72	22.21	—	—	11.85	10.18	4.01	Rose Red (cc178)
	D	2.2	0.437	10.8	23.66	11.73	—	—	6.11	5.59	3.73	Delft Rose (cc14)
	E	2.4	0.288	16.5	10.52	10.48	12.92	2.16	6.10	4.32	2.23	Neyron Rose (cc176)
	F	2.3	1.180	—	—	26.30	54.93	26.68	12.60	13.37	—	—
Sugar- added <i>Concord</i> grape juice prepared in Miyagi Agr. College	I	2.5	0.876	18.9	127.09	19.69	—	—	9.98	9.57	4.45	Amaranth Red (cc179)
	II	2.4	1.023	20.2	127.09	20.91	—	—	10.79	9.95	4.89	Medicl Crimson (cc180)
Fresh <i>Concord</i> grape juice	Cold- press	2.7	1.358	—	—	8.49	—	—	4.48	3.93	16.00	—
	Hot- press	2.4	1.332	—	—	8.65	—	—	5.02	3.51	15.39	—

Color is represented according to Dictionary of Colors for Interior Decoration (British Color Council, McCorouodable & Co., Newton-le-Willows and London).

Tannin is determined by Tea Tannin Method of Jikken Nogeikagaku Vol. 2 p. 643 (in Japanese).

sugar, sucrose, glucose, fructose, tannin, color and ratio of acid to sugars were determined.

By paper chromatographic analysis, organic acids in the six varieties (A, B, D, E, I and II) were determined. In this experiment, Toyo filter paper No. 50 and butanol-acetic acid-water (4:1:1) for developing solvent and B. P. B. for spraying reagent were used.

The R_f values of tartaric, malic, and citric acid detected in this procedure were 0.16, 0.41 and 0.36, respectively. Besides, a spot of R_f 0.10 was found in the variety D, but it could not be determined what acid it was.

Simple quantitative determination of these organic acids was also made by the application of paper chromatography. In preliminary experiment, the minimum quantity of pure organic acid required for the color development by B. P. B. was determined. Those of tartaric, malic and citric acid were 5, 15

Table 2. Paper Chromatography of Organic Acids

		Tartaric acid	Malic acid	Citric acid
Market juice	A	+	+	-
	B	-	-	+
	D	-	-	+
	E	+	-	-
Juice prepared in Miyagi Agr. College	I	+	+	-
	II	+	+	-

and 18 γ , respectively. Next, juice was diluted with water successively until each organic acid to be determined did not give the color reaction by B. P. B. Thus, contents of those organic acids in juices were derived by calculation from the dilution degree of juice and the minimum quantity of the corresponding pure acid for color reaction. The micrometer syringe (7) made in England was available for sampling very small amount such as 0.002 ml.

Table 3. Quantity of Organic Acids in Juice 1 ml.

	Tartaric acid, γ	Malic acid, γ	Citric acid, γ
A	4550-5000	3000-3750	-
B	-	-	3000-3600
D	-	-	1800-2250
E	2080-2730	-	-
I	3570-4170	4400-4700	-
II	4170-5000	5000-6000	-

Discussion. From the experimental results, it was supposed that some of market juices contained scarcely any natural grape juice or were prepared by mixing with a great deal of sucrose and organic acids. Therefore, the evaluation of market juices should be made in consideration of the content of tannin, the content and kind of organic acid and sugar, and the proportion of organic acid to sugar.

The manufacturers of grape juices need to pay their attention to the content, kind and combination of organic acid and sugar which should give the most important influence to taste.

[II] Chemical Composition of Fresh Grape Juices

(1) General Analysis

The grapes, harvested in Miyagi and Yamagata Prefecture in September 1953, were used for analysis. The results are summarized in Table 4. However, these juices having low yields owing to squeezing with hands are somewhat different in the analytical values from the juices pressed by laboratory press, which will be shown later (Part II).

Table 4. Chemical Composition of Fresh Grape Juices

	pH	Total acid g/100 ml	Refracto- meter index	Ballg. (15°C) deg.	Tannin mg/100 ml	Amino-N mg/100 ml	Reducing sugar g/100 ml	Invert sugar g/100 ml	Sucrose g/100 ml	Glucose g/100 ml	Fructose g/100 ml
<i>Concord</i> (Yamagata)	2.9	0.916	9.6	9.72	13.00	17.29	8.94	8.95	—	4.58	4.19
<i>Concord</i> (Watari)	3.0	0.445	11.3	11.27	16.45	17.02	10.66	10.60	—	5.26	5.15
<i>Niagara</i> (Watari)	3.0	0.740	—	—	—	22.00	10.81	—	—	5.54	4.41
<i>Delaware</i> (Yamagata)	2.8	0.953	15.2	14.70	72.93	20.49	14.23	14.36	0.124	6.86	7.12
<i>Kōshū</i> (Yamagata)	2.6	1.415	12.8	13.08	6.48	13.62	12.00	12.46	0.256	7.11	4.75

In 1953, the quality of grapes was poor as a result of unreasonable weather. And then, these hand-cold-pressed juices contained much less tannin and organic acids than the hot-pressed juices, which were pressed by the laboratory press after heating to develop the full *Concord* grape color. (cf. Table 1)

(2) Paper Partition Chromatography of Fresh Juices

In this country, Ito *et al.* detected glucose and fructose as sugars, and tartaric and malic as organic acids in eleven varieties of grapes by paper chromatographic analysis, but any report on amino acids could not be found until that time. In foreign country, Lüthi and Vetsch (8) of Switzerland described in detail the paper chromatography of amino acids in "Papierchromatographische Trennung und Bestimmung von Aminosäuren in Traubenmost und Wein". Their preparation method of sample included that amino acids were absorbed to cation exchanger resin, eluted out by hydrochloric acid, and next the salts were removed from the eluted solution. Their paper chromatograms gave a total of ten spots of amino acids as follows: aspartic acid, glutamic acid, serine, glycine, threonine, alanine, arginine, γ -amino-butyric acid, valine and proline. Especially, spots of alanine and glutamic acid were colored intensely.

In our experiments, four fresh juices (two varieties of *Concord*, and *Delaware* and *Kōshū*) were used for paper chromatography.

(a) Paper Chromatography of Organic Acids

The main procedures of sample preparation were as follows. A certain volume of fresh juice was mixed with its equal volume of 95% ethanol and centrifuged. The resulted clear liquor was concentrated under vacuum. Then, it was extracted with ether after pH was adjusted to 2.0 by H_2SO_4 . After the removal of ether, the extract was made up to the original volume with water prior to the application to filter paper.

Through such a procedure, all chromatograms of four fresh juices gave no more than tartaric and malic acid. In the case of *Concord* grape, the spot of malic acid was weaker than the one of tartaric acid.

(b) Paper Chromatography of Sugars

Glucose and fructose were detected in all juices, but sucrose could not by both

aniline-hydrogen-phthalate and resorcinol as spraying reagent.

(c) *Paper Chromatography of Amino Acids*

The sample preparation was as follows. First, 20 to 80ml of fresh juice was mixed with the same volume of 95% ethanol and clarified by centrifugation. The clarified solution was alkalinized by the addition of sodium carbonate. Then, mercuric acetate was added into the solution, the white precipitates were formed. These precipitates were collected, washed with alcohol and then suspended in water. Afterwards, hydrogen sulphide was used for the removal of mercury, and mercury sulphide was filtered off. The filtrate was concentrated to 0.5 to 1.0 ml and neutralized by sodium hydroxide before it was used for paper chromatography.

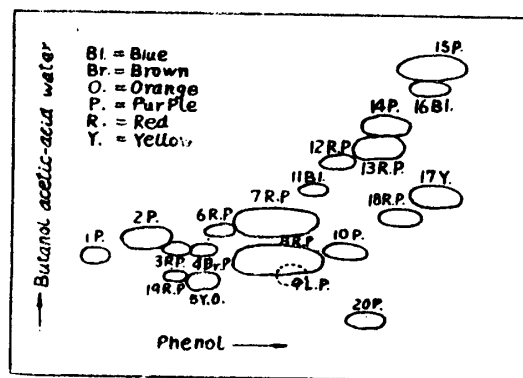
Fig. 1. Paper Chromatogram of Amino Acids in Grape Juice.

Toyo Filter Paper No. 2

Solvents for Two-Dimensional Method: Phenol-Water (4 : 1) & Butanol-Acetic acid-Water (4 : 1 : 5)

Spraying Reagent: Ninhydrine

1. Aspartic acid, 2. Glutamic acid,
3. Serine, 4. Glycine, 5. Asparagine,
6. Threonine, 7. Alanine, 8. Glutamine, 9. Lysine, 10. Arginine, 11. β -Alanine,
12. α -Amino-butyric acid, 13. γ -Amino-butyric acid, 14. Valine, 15. Leucine, 16. Phenylalanine, 17. Proline, 18. ?, 19. ?, 20. ?.



Each amino acid was identified from R_f value, color of spot and agreement with spot of pure amino acid; especially, plutonium chloride reagent was used for the sulphur-containing amino acid, Pauli's reagent for histidine and histamine, and Hopkins-Cole reaction for tryptophane.

However, the pure γ -amino-butyric acid, because it could not be purchased, was prepared in our laboratory from the decarboxylation of 1-glutamic acid by the enzyme which was extracted from the Japanese squash.

The results of chromatographic analyses are summarized in Table 5. Lüthi and Vetsch's data are also quoted for comparison.

Discussion As shown in Table 5, *Concord* (Miyagi) and *Delaware* contain fewer kinds of amino acids than the others. This might be supposed that the sample amount of these grapes was so small that some of amino acids could not give the color reaction.

Alanine is very rich in all the juices. Glutamine is also rich in all the juices except *Kōshū*. As the other main amino acids, it must be noted aspartic acid, glutamic acid, arginine, γ -amino-butyric acid, serine and glycine.

Table 5. Paper Chromatography of Amino Acids in Grape Juices

	Concord (Miyagi)	Concord (Yamagata)	Delaware (Yamagata)	Kōshū (Yamagata)	Lüthi & Vetsch	
					R×S	Räuschling
Aspartic acid	++	++	++	+	××	××
Glutamic acid	++	++	++	+	×××	×××
Serine	++	+	+	++	××	××
Glycine		++		++	×	×
Threonine	+	+	+	++	××	×
Asparagine		+				
Alanine	++++	++++	++++	++++	×××	××××
Glutamine	++++	++++	++++	+		
Lysine				+		
Arginine	++	++	++	++	×	
β-Alanine		+		+		
α-Amino-butyric acid		+		+		
γ-Amino-butyric acid	+	++	+	+++	××	××
Valine		+		+	×	×
Phenylalanine		+				
Leucine or Isoleucine		+				
Proline				++	×	××
Histidine	±	±	±	±		
Tryptophane	±	+	±	+		

± = not sure, + = × = small, ++ = × × = middle, +++ = × × × = large,
++++ = × × × × = very large.

Moreover, γ -amino-butyric acid is rich in *Kōshū* but poor in the others. A considerable amount of proline is in *Kōshū* but none at all in the others.

From those facts, it is thought that the composition of amino acids in *Kōshū* is somewhat different from the other three varieties, and rather similar to the European grapes analysed by Lüthi and Vetsch.

As before described, they reported ten kinds of amino acids in grape juices. On the contrary, a total of seventeen kinds was detected in our experiments. Especially, it was the most important difference from our results that glutamine had not been detected by them. In their preparation method of sample, hydrochloric acid used for the eluent of ion-exchanger might hydrolyse glutamine to glutamic acid during subsequent procedure.

Summary

The composition of market and fresh grape juices obtained at Sendai was analysed, and the paper partition chromatography of organic acids, sugars and amino acids in these juices was carried out.

From the paper chromatograms of amino acids, it was ascertained that seventeen known and three unknown amino acids and large quantity of glutamine

were contained in these juices, although Lüthi and Vetsch reported ten amino acids and none of glutamine, which is supposed to be hydrolysed to glutamic acid during the preparation of sample.

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