Studies on the Water Extracted Mucilage from *Ficus Awkeotsang* Makino

Part 1. Water Extracted Substances from *Ficus Awkeotsang* Makino

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The authors investigated the viscosity and the chemical components of the water extracts from the dried fruits of *Ficus Awkeotsang* Makino. The dried fruits were divided into three groups, red filaments, ovules-like particles and its ground pieces. The water extracted solution from the red filaments was the most viscous. However, there was no great differences in their chemical components between the red filaments and the ovules-like particles. Of ash contents, water insoluble fractions were abundant in calcium. Of free amino acids, leucine, valine, tyrosine, proline, alanine, glutamic acid, threonine, aspartic acid, cystine and other ninhydrine positive substances were detected by paper chromatography. Of free sugars, fructose, glucose, galactose, sucrose and unknown sugars were identified by paper chromatography. The results of acid hydrolysis and fractionation by DEAE-cellulose column chromatography showed that the mucilage polysaccharide of water soluble fraction was one kind of polyuronic acid.

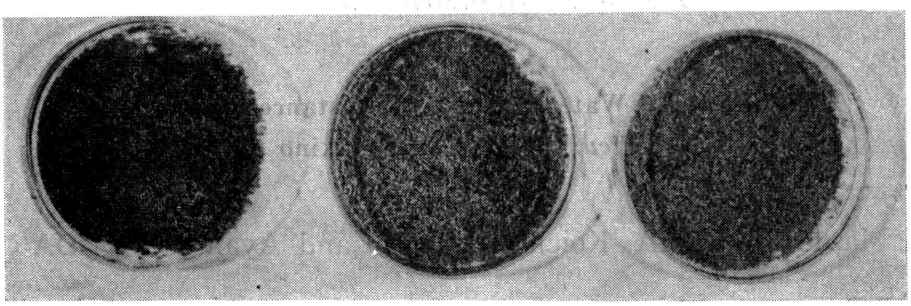
Introduction

Ficus Awkeotsang Makino, not seen in Japan, grows in Taiwan. When the fruits of *Ficns Awkeotsang* Makino were ripe to yellow green from August to November, collected, opened and dried in the sun. Both red filaments and ovules-like particles were taken out of the dried fruits and extracted with warm water. Adding sugars to water extracted solution and cooling, the solution forms an agar gel. People in Taiwan eat this jelly-like food in summer. Water extracted mucilage from Ficus Awkeotsang Makino had been studied by S. Miyake[1] and S. Ohno. They got pectic substances by alcohol precipitation. However, they didn't clarify the relationship between the viscosity and the chemical components. We schemed our experiments to clarify this problem.

Materials and Methods

Ficus Awkeotsang Makino: The dried fruits of *Ficus Awkeotsang* Makino were

divided into three groups, the red filaments (R), the ovules-like particles (O) and its ground pieces (G) (see Fig. 1.). Subsequent experiments were carried out to investigate each of these three groups and the abbreviations, (R.), (O.), (G.) are used in this paper.



R	O	G
Red filaments.	Ovules-like particles.	Its ground pieces.

Fig. 1. Three Groups of the Dried Fruits of *Ficus Awkeotsang* Makino.

Viscosity measurement: Viscosity measurement was performed with Lion VT-01 Viscometer at 15°C.

Quantitative analysis of ash:

Ashing.. Ashing was performed in usual way at 550–600°C.

Phosphorus.. Modification of Molybdovanadophosphate Method.[2]

Iron.. Modification of O-Phenanthroline Method.[3]

Calcium and Magnesium.. Modification of EDTA Titration Method.[4]

Manganese.. Modification of Potasium Periodate Method.[5]

Paper chromatography: Paper chromatography was done on Toyo Filter Paper No. 50 with ascending method using the solvents, n-butanol-pyridine-water (6:4:3) for sugars, n-butanol-acetic acid-water (4:1:1) for amino acids. Localization of sugars was detected with naphthoresorcinol reagent and amino acids were detected with ninhydrine reagent.

Gas chromatography analysis: Trimethylsilyl derivatives of sugars were analyzed with gas chromatography. The analysis was carried out using Shimazu Gas Chromatograph G.C.-4A. The column consisted of 2 meters of glass tubing packed with 3% SE52 on Chromosorb W. The carrier gas was helium, flowing at 1.1 Kg/cm^2. The oven temperature was set at 190°C and raised after injection to 250°C. The detector was F.I.D.

Acid hydrolysis: After 60 mg of dried matters were hydrolyzed in 6 ml of 1N-H_2SO_4 at 100°C for 120 mins., the solution was neutralized with $BaCO_3$ and then, filtrated. The filtrate was dried under reduced pressure and extracted with 85% ethanol.

The extracts were subjected to identify the sugar components by paper chromatogrophy.

Fractionation of the mucilage polysaccharides: In order to investigate the mucilage polysaccharides, water soluble fraction was applied to column chromatography on DEAE-cellulose equilibrated with 0.01M $Na_2B_4O_7$ (column size 1.5×20 cm, flow rate: 100 m*l*/hr.). Step wise elution was performed with 0.01M, 0.1M, saturated $Na_2B_4O_7$, 0.01N and 0.5N-NaOH respectively. Taking 18 m*l* fractions, each fraction was colorimetrically analyzed by phenol-sulfuric acid method.

Experiments and Results

1. Viscosity and yields. In order to investigate the yields and the viscosity, the samples were prepared as shown in Fig. 2.

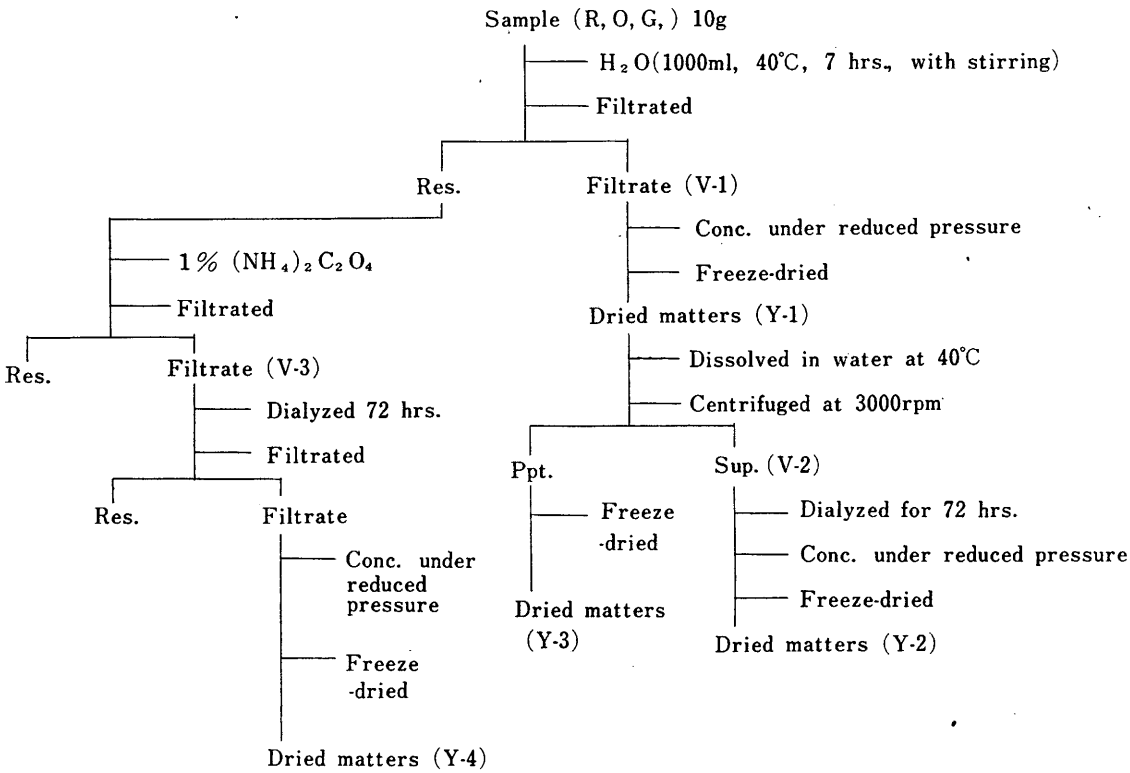


Fig. 2. Sample Preparation 1.

Table I and Table II show the yields and the viscosity on each sample preparation procedure. The mucilage of *Ficus Awkeotsang* Makino could be extracted with water. Water extracted solution from red filaments (R-V-1) was the most viscous. When we tried to dissolve the R and O-Y-1 in water, they were hard to be dissolved and highly insoluble precipitates appeared. The G-V-2 was in suspension and didn't precipitated by centrifugation at 3000 rpm for 10 mins.

Table I.

The Yields on each Sample Preparation Procedure.

R-Y-1	2038 mg	O-Y-1	1183 mg	G-Y-1	1672 mg
R-Y-2	210	O-Y-2	190	G-Y-2	590
R-Y-3	720	O-Y-3	940	G-Y-3	74
R-Y-4	120	O-Y-4	110	G-Y-4	191

Table II.

The Viscosity on each Sample Preparation Procedure.

R-V-1	27.0 c.p.	O-V-1	15.3 c.p.	G-V-1	7.5 c.p.
R-V-2	1.8	O-V-2	1.5	G-V-2	6.0
R-V-3	2.0	O-V-3	1.5	G-V-3	1.5

c.p.: centi-poise

2. Identification of the chemical components. In order to investigate the chemical components of the water extracted substances, the samples were prepared as shown in Fig. 3.

Table III.

Ash Contents on each Sample Preparation Procedure.

	Ash mg	P_2O_5 mg	FeO mg	CaO mg	MgO mg	HCl insoluble mg
R-Y-1	143	6.92	0.672	17.4	3.79	6.60
R-So.	54.0	trace	trace	31.9	8.47	
R-In.	131	trace	trace	97.5	6.68	
O-Y-1	164	14.0	0.539	11.9	1.65	7.90
O-So.	54.5	2.55	trace	27.4	9.28	
O-In.	180	trace	trace	137	12.2	
G-Y-1	123	22.6	0.178	18.5	9.60	6.62
G-So.	70.7	trace	trace	14.7	13.9	
G-In.	265	7.70	trace	138	5.13	

The results were converted into per 1 g of freeze-dried matter.
Y-1: See sample preparation 1, Fig. 2.
So.: Soluble fraction (see sample preparation 2, Fig. 3).
In.: Insoluble fraction (see sample preparation 2, Fig. 3).
The content of MnO was O in all samples.

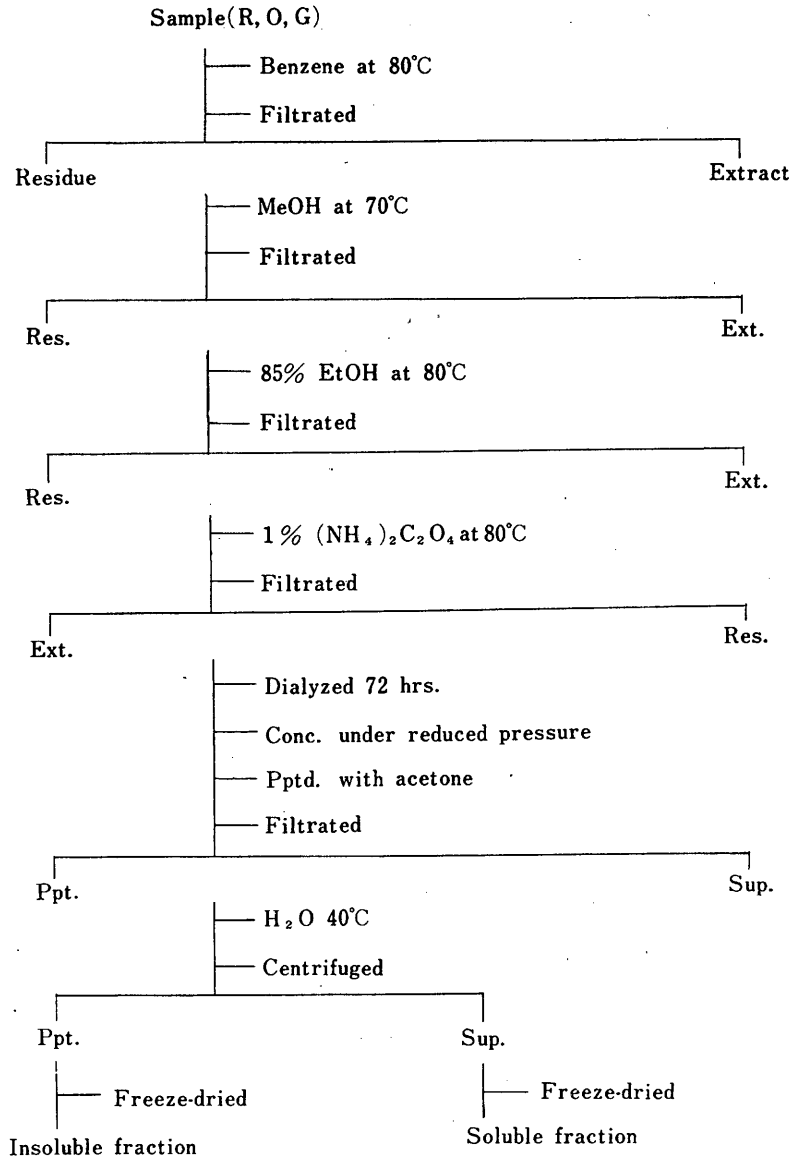


Fig. 3. Sample Preparation 2

Ash contents: The ash contents of the Y-1 (see Fig. 2), the soluble fractions and the insolube fractions (see Fig. 3) were analyzed. Experimental results are shown in Table III. Though there was not a great difference among three groups (R, O, G), insoluble fractions were rich in calcium more than soluble fractions. These results were very interesting but it cannot be concluded that high calcium content affects the insoluble character.

Free amino acids and sugars: Samples were prepared as shown in Fig. 4.

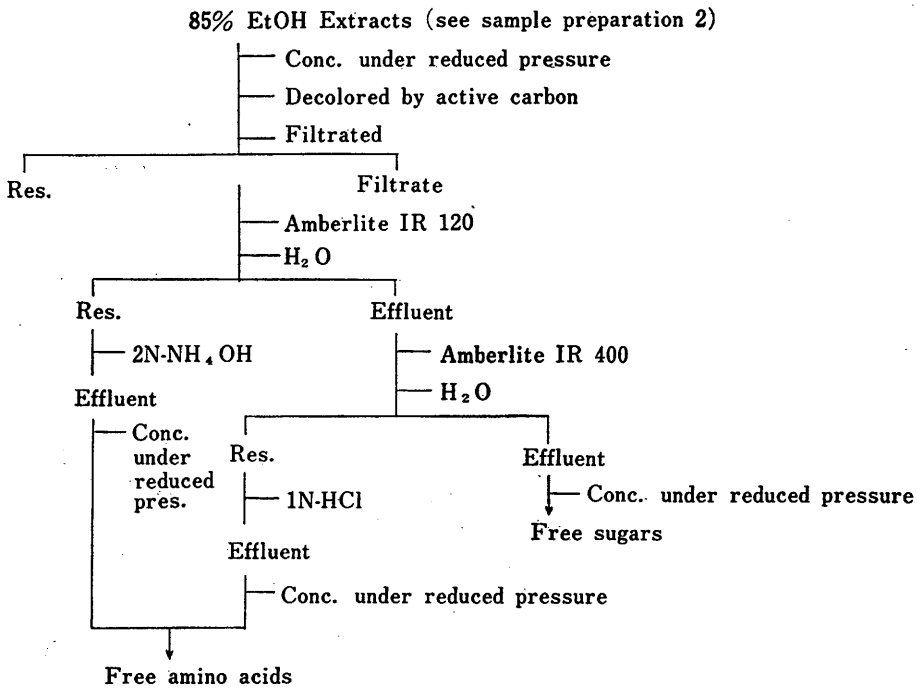


Fig. 4. Sample Preparation of Free Amino Acids and Sugars

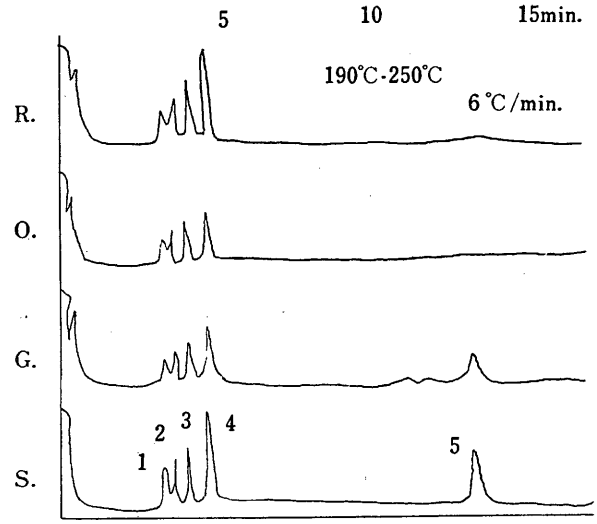
Free amino acids.. Free amino acids were identified by paper chromatography. Experimental results are shown in Table IV. As there was not a great difference among three groups, only the G. was expressed. Many amino acids, leucine, valine, tyrosine, proline, alanine, glutamic acid, threonine, aspartic acid and cystine, were detected on paper chromatogram.

Free sugars.. Free sugars were identified by paper chromatography and gas chromatography. The results are shown in Fig. 5 and Fig. 6. Fructose, glucose and galactose were identified in three groups and only in the sample G. sucrose and unknown sugars were detected on paper chromatogram as red spots.

Mucilage polysaccharides: The mucilage polysaccharides of the water soluble fractions (see Fig. 3) were investigated

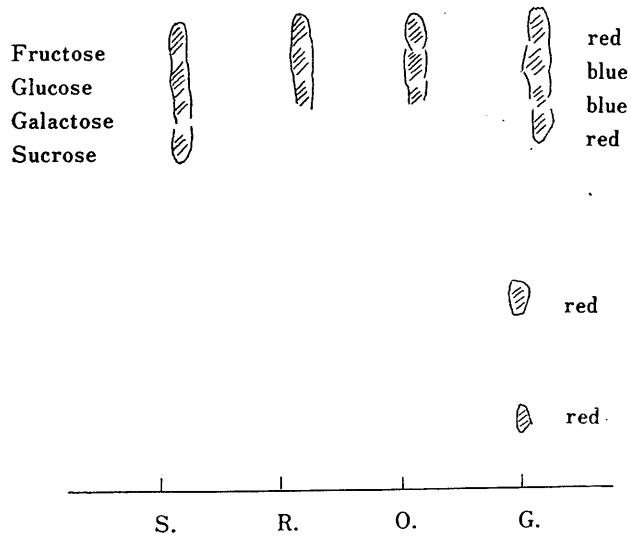
Table IV.
The Rf. Values on Paper Chromatogram of Free Amino Acids.

Amino acids	Rf.	Sample Rf.
Leucine	0.82	0.82
Valine	0.69	0.69
Tyrosine	0.59	0.59
Proline	0.54	0.54
Alanine	0.50	0.50
Glutamic acid	0.46	0.46
		0.42
Threonine	0.40	0.39
		0.37
Aspartic acid	0.30	0.31
		0.28
Cystine	0.19	0.19



1 : Fructose. 2 : Galactose. 3 : Glucose (α).
4 : Glucose(β) 5 : Sucrose.

Fig. 5. Gas Chromatogram of Free Sugars.



S : Standards. R : Red filaments. O : Ovules-like particles. G : Its ground pieces.

Fig. 6. Paper Chromatogram of Free Sugars.

by acid hydrolysis and column chromatography.

Acid hydrolysis.. Paper chromatogram of hydrolyzed soluble fractions are shown in Fig. 7. All samples gave one spot detectable with trichloroacetic acid-aniline reagent. It coincides in position with galacturonic acid.

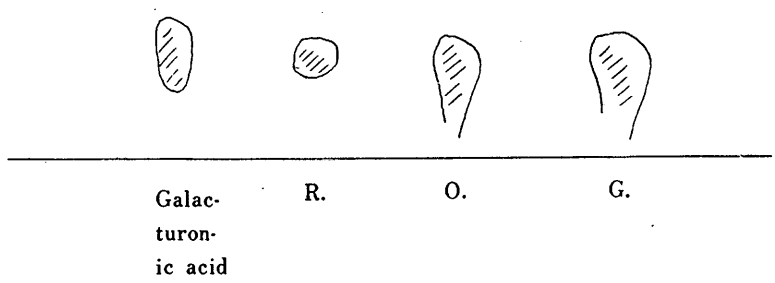


Fig. 7. Paper Chromatogram of Hydrolyzed Soluble fraction.

Fractionation of water soluble fractions.. As the results of hydrolysis show the water soluble fractions of three groups to contain similar polysaccharides, the sample R. and G. were fractionated on DEAE-cellulose column chromatography. The results are shown in Fig. 8. Both of sample R. and G. gave the same single peak at 0.1M-$Na_2B_4O_7$ effluent. In the sample R., the red solution effluented at 0.1M-NaOH but didn't react with phenol-sulfuric acid. So, it was considered not to be a sugar. This red substance could not be separated from polysaccharide on our previous reports.[6]

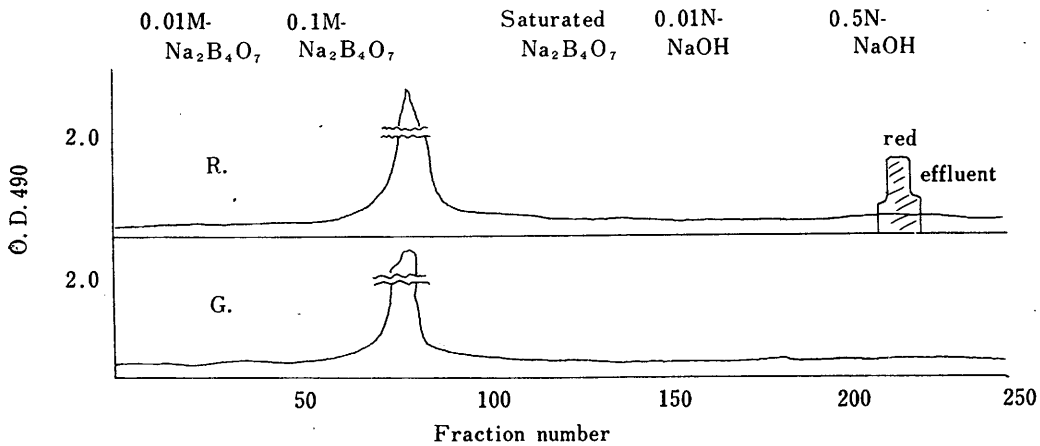


Fig. 8. Column Chromatogram of Soluble Fraction.

Discussion

On this paper, we schemed to investigate the factor of its viscosity by measuring the viscosity and identifying the chemical components of the water extracts and moreover, separated the mucilage polysaccharides. The water extracted solution from the red filaments was the most viscous and we got the highest yield of the three groups. The results show that the larger quantities of the water extracts increase its gelation. The remarkable result is that highly insoluble precipitates appeared when freeze-dried products or acetone precipitates were redissolved in water. This phenomenon is based

on the character of the mucilage polysaccharides in itself or the relationship between the mucilage and some other chemical components. As this insoluble fraction is abundant in calcium, calcium content may affect the insolubility. The results of acid hydrolysis and fractionation by DEAE-cellulose column chromatography expect that the soluble fraction contains one kind of polysaccharide, composed of galacturonic acids. Now, we have studied on this polysaccharide, but nothing is known at present concerning the purification and the isolation, and this problem must be await for future studies.

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