BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 45, 561—563 (1972)

Studies on Mannan and Related Compounds. I. The Purification of Konjac Mannan

Noboru SUGIYAMA, Hideo SHIMAHARA,* and Tetsuo ANDOH*

Department of Chemistry, Tokyo Kyoiku University, Otsuka, Tokyo

**Kabushikikaisha Shimizu Manzo Shoten, Laboratory, Onomichi, Hiroshima*

(Received July 22, 1971)

The preparation of pure konjac mannan is described. Its properties and purity are also studied.

A major component of the konjac flour, which is obtained from the tubers of *Amorphophallus Konjac* C. Koch (*Araceae*), is well known to be a glucomannan called konjac mannan. Traditionally, this flour has been used as the stuff of edible *konnyaku*.

We have succeeded in obtaining pure konjac mannan, which retains the most important intrinsic properties of konjac flour; it can make a transparent colloidal solution and can be used in making a *konnyaku* of good quality. The konjac mannan which are obtained by the methods of Ohtsuki[1-4] and Nishida *et al.*[5] do not exhibit these properties. Ohtsuki's method is to prepare the mannan from a colloidal solution of konjac flour by repeated precipitations with alcohol, while Nishida's method is to take the mannan out through the copper complex by treating the super-heated water extract of konjac flour with the Fehling solution. The latter method has been used up to the present as the general procedure for preparing material for chemical studies of konjac mannan.[6-8] The fact, however, that the mannan obtained by these methods lacks important intrinsic properties suggests that the molecular structure of this mannan suffers a kind of denaturing during the processing, as has also been observed in the case of amylose in an alkaline solution.[9] On the other hand, the retention of its intrinsic properties suggests that the mannan has received little or no denaturing effect.

The present method of purification is composed of extraction, dialysis, and lyophylization.

Results and Discussion

Properties. The purified konjac mannan is a light, voluminous, and white cotton-like product.[10] It readily dissolves in water to give a transparent viscous solution and has a *konnyaku*-forming ability. The viscosity of its colloidal solution is almost the same as that of the mannan before purification. The IR (KBr) of the purified mannan showed bands near

1) M. Mayeda, *Tokyo Igakukai Zasshi*, **18**, 1 (1914).
2) K. Goto, *J. Biochem. Tokyo*, **1**, 201 (1912).
3) T. Ohtsuki, *Acta Phytochim.*, **4**, 1 (1928).
4) S. Miyake, *J. Coll. Agr. Imp. Univ., Hokkaido*, **12**, 164 (1927).
5) K. Nishida and H. Hashima, *Nippon Nogeikagaku Kaishi*, **6**, 991 (1930).
6) F. Smith and H. C. Srivastava, *J. Amer. Chem. Soc.*, **81**, 1715 (1959).
7) K. Kato and K. Matsuda, *Agr. Biol. Chem.*, **33**, 1446 (1969).
8) T. Satoh, A. Moriya, J. Mizuguchi, and S. Suzuki, *Nippon Kagaku Zasshi*, **91**, 1070 (1970).
9) R. E. Reeves, *J. Amer. Chem. Soc.*, **76**, 4595 (1954).
10) S. Kiriyama, H. Morisaki, and A. Yoshida, *Agr. Biol. Chem.*, **34**, 641 (1970).

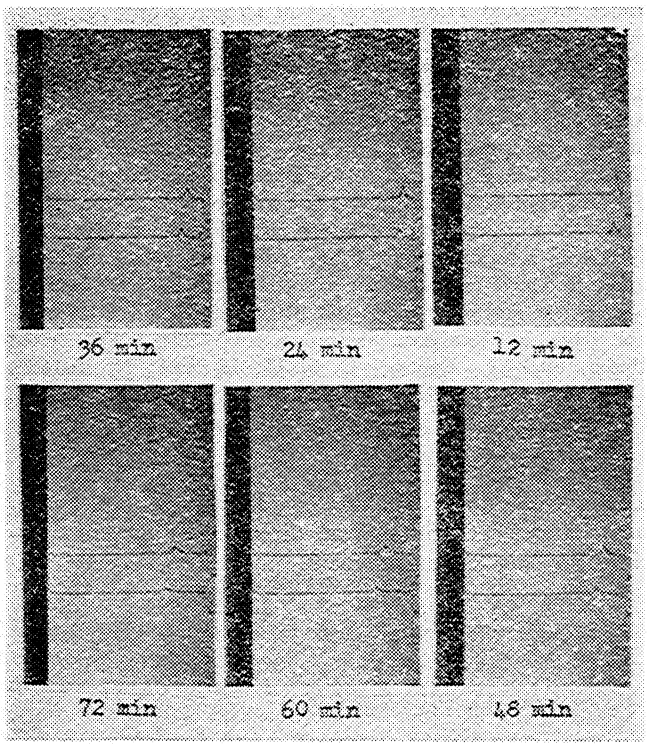

Fig. 1. Sedimentation patterns of purified konjac mannan. Sedimentation analysis was carried out under the following condition: 1.0 mg/m*l* konjac mannan, water, 20°C, 60000 rpm and phase plate angle 70°. Times after reaching the top speed is shown below each diagram. The upper and lower patterns in each diagram are traditional (Zairai-shu) and Chinese species (Shina-shu), respectively. Sedimentation is from light to left.

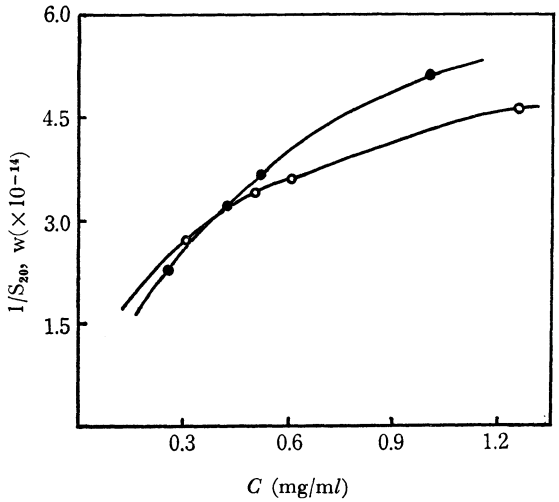

Fig. 2. The sedimentation coefficient as a function of concentration for purified konjac mannan: (○) Traditional species; (●) Chinese species.
Sedimentation analysis was carried out under the follwing condition: water, 20°C and 60000 rpm.

890 (β-glucosidic and β-mannosidic linkages), 1640, and 1720 cm^{-1}. Since the absorptions at 1640 and 1720 cm^{-1} are very weak in the IR chart, they cannot be attributed to uronic acid or the like; rather, it seems that they may be attributed to the trace of absorbed water[11] or to that of protein. However, no entirely reasonable explanation of these absorptions can be given at present. The sedimentation analysis of pure konjac mannan obtained from the traditional species (Zairai-shu) and from the Chinese species (Shina-shu) have been carried out. Each mannan showed only one peak at various intervals of time (Fig. 1). This fact indicates that the konjac mannan purified by the present method consists of ultracentrifugally homogeneous components. Generally, it is known that the linear polymer shows a high concentration dependence of the sedimentation coefficient. Since the purified konjac mannan also shows this phenomenon (Fig. 2), the shape of konjac mannan may be considered to be linear. The difference in the concentration dependence of the mannans suggests that there is some difference in the molecular weight[12] or in the shape of the molecule between the two kinds of mannans.

On complete acid hydrolysis, this purified konjac mannan gave only two spots, corresponding to D-mannose and D-glucose, on paper chromatography. The molar ratio of D-mannose to D-glucose was found to be 1.6 : 1.0 by measuring the spots with a densito-

TABLE 1. PROPERTIES AND RESULTS OF ANALYSES OF THE PURIFIED KONJAC MANNAN

	Konjac flour (intact)	Present method	Nishida and Hashima's method[5,7]	Ohtsuki method[3]
Solubility in water	soluble	soluble	insoluble	insoluble
Solubility in 20% NaOH	insoluble	insoluble	soluble	soluble
Konnyaku forming ability	retained	retained	lost	lost
Reducing power	present	none	none	none
Starch-iodine reaction	positive	negative	negative	negative
C(%)[a]		43.87, 44.01		43.76, 43.47
H(%)[a]		6.16, 6.22		6.47, 6.31
P(%)	0.35	none	trace	
N(%)		trace	0.01—0.02	
Ash(%)	5.23	<0.10	0.10—0.49	0.66—0.81

a) Calculated for $(C_6H_{10}O_5+\frac{1}{8}H_2O)_n$ as indicated by Ohtsuki[3]. C, 43.83%; H, 6.28%.

11) M. Tsuboi, *J. Polymer Sci.*, **25**, 159 (1957).
12) The details of the molecular weight will be described in the following paper.

meter. When the reducing sugar in this acid-hydrolysis mixture was determined by the Bertrand method as modified by Tomoeda, the total content of the reducing sugar was shown to be 96% as D-glucose.

The konjac mannan did not produce any precipitate during the process of the purifying dialysis. This fact and the negligible contents of nitrogen and ashes (Table 1 and Fig. 3) suggest that the inorganic substances and/or the nitrogen-containing substances are not bound with the mannan molecule, as has been mentioned by Kondo and Okimasu.[13]

Purity. This purity was confirmed by elemental analysis, by the ultracentrifuge pattenrs, the ash contentents, and the absence of any reaction of reducing sugars or of starch-iodine. These results are summarized in the above table.

Experimental

Purification Procedure. Ten grams of commercial konjac flour were extracted with three 50 m*l* portions of 50 v/v% ethanol at room temperature for three days. After extraction and filtration, a 50 m*l* portion of 80 v/v% ethanol was added to the residual flour. The flour was then collected and dried at 80—90°C to yield 7.80 g of flour apparently similar to the original flour.

Into 1000 m*l* of water were stirred 4.10 g of the above flour in small portions at room temperature; the stirring was continued for 2—3 hr until the mannan had been completely dissolved. The colloidal solution thus obtained was centrifuged at 15000 rpm for 30 min to remove the insoluble substances. The colorless and transparent supernatant liquid was dialyzed for 72 hr at 9—10°C through a cellulose membrane (VISKING) against running distilled water, and then lyophilyzed. We thus obtained 3.54 g of the purified product.

Preliminary Experiments for the Purification. The above purification procedure was established on the basis of the following experiments.

1) When the commercial konjac flour had been purified without extraction with an ethanol solution, as has been described above, the aqueous solution (0.176%) of the purified product became a slightly yellowish pink after a few days at 9—10°C. Therefore, it seems to have been contaminated with some substance. This substance was removed by extracting the starting flour with 50 v/v% ethanol.

2) The optimum concentration of the dialyzing solution and the necessary time of dialysis have been determined by measuring the ash contents of the lyophilyzed konjac mannan. Figure 3 shows the relationship between the ash content and the dialysis time at various concentrations. These results show that the more concentrated solution is dialyzed more effectively. Thus, the optimum conditions of dialysis were determined to be 0.411 w/w% and 72—96 hr.

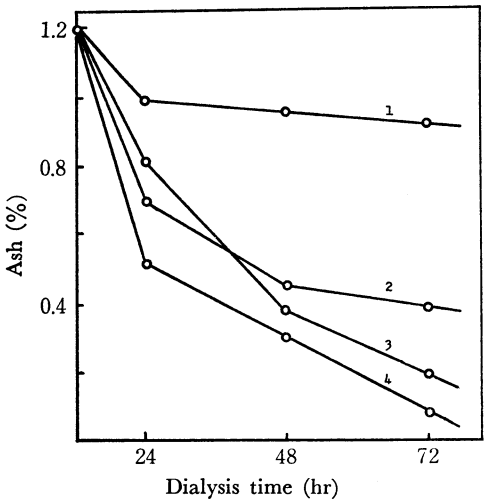

Fig. 3. The Effect of concentration on dialysis.
1: 0.085 2: 0.176 3: 0.252 4: 0.411 w/w%

Hydrolysis and Paper Chromatography. A mixture of 500 mg of the purified konjac mannan and 25 m*l* of N sulfuric acid was heated in a boiling water bath for 24 hr under reflux. According to the usual procedure, the hydrolyzate was paper-chromatographed (Toyo No. 50; *n*-butanol: ethanol : water 40 : 11 : 19; aniline-phthalic acid). The ratio of D-mannose to D-glucose was determined by measuring the area of each intensity integral by means of a densitometer.

The sedimentation patterns were obtained by the use of a Hitachi ultracentrifuge Model UCA-IA.

The authors are grateful to Mr. Koichi Kanna, Tokai Regional Fisheries Research Laboratory, for his measurement of the ultracentrifuge patterns and to Mr. Kyuzaburo Furuhashi, Department of Chemistry, Tokyo Kyoiku University, for the elemental analyses. Thanks are also due to Professor Yutaka Shimizu, Department of Fisheries, Kochi University, his helpful advice and continuous encouragement, and to Mr. Manzo Shimizu, the president of Kabushikikaisha Shimizu Manzo Shoten, for permission to publish this work.

13) Y. Kondo and S. Okimasu, *Journal of Food Science and Technology*, Vol. 14, No. 8, 19 (1967).