KOREAN J. FOOD SCI. TECHNOL.
Vol. 10, *No.* 4 (1978)

◆ *Review* ◆

Present Status and Prospect of Starch Utilization in Japan*

Shigeo Suzuki
National Food Research Institute, Ministry of Agriculture and Forestry, Tokyo, Japan

日本에 있어서 澱粉利用의 現況과 展望

鈴 木 繁 男
日本 農林省 食品總合研究所

Abstract

Since 1950 there has been a dramatic progress in rationalization of the production of sweet potato and potato starch in Japan. This enabled dextrose industry by enzymatic process to develop rapidly due to the success of enzymatic liquefaction and saccharification. Isomerization of glucose to fructose has been studied, and the immobilization of isomerases prompted its products on industrial scale in 1970. Another advance is the development of effective methods of producing high purity maltose. A malto-hexaose forming amylase was discovered in 1971 and attempts are being made for its pharmaceutical utilization. Saccharification of cellulose by cellulase has been studied. Conversion of starch to other polysaccharides is another example for the numerous Japanese activities.

1. Progress of Starch Technology in Japan since 1950[1,2]

Soon after World War II was over in 1945, there was a boom of small mills manufacturing starch from sweet potatoes and potatoes. Most of those small mills based on batch operation were inefficient in process where starch was sedimented and washed in a tank. Since early 1950's, however, there has been a dramatic progress in modernizing the industrial production of sweet potato starches in Japan. The most significant progress was the introduction of centrifugal separation system for separation and washing of starch. One example is Shihoro Potato Starch Plant in Hokkaido, where a Westfalia separator was introduced from Germany for the very first time, and they demonstrated a successful continuous process of potato starch production. This led other plants to establish such modernized plants one after another.

Table 1 shows the changes in the number of starch mills in Japan. As a result of the technical innovations, Japan's starch production recorded its surplus by the middle of 1950's. That is to say, in 1957, the Japanese Government supported and purchased sweet potato and potato starches according to the Agricult-

Table 1. Number of starch factories in Japan

Year	Sweet Potato	Potato	Corn	Wheat
1955	1,495	1,908	1	45
1965	1,203	583	8	45
1975	119	58*	22	13

* 30 factories of 58 are modernized factories
Maximum capacity: potato starch 180~365 t/day
corn starch 500~530 t/day
wheat starch 50~70 t/day

* A special lecture at the 21st Scientific Meeting of this Society held in Daegu on October 7, 1978

ural Product Stabilization Act, amounting to 200,000 tons.

Fig. 1 shows the production of different starches in Japan. Evidently there was a drastic decrease of sweet potato starch that took place after its peak production of 740,000 t in 1963, whereas corn starch rapidly increased. Potato starch leveled off at the range of 200,000~250,000 t with marginal changes, while wheat starch shows constant figures. Increasing sweet potato and potato starch surplus was primarily responsible for establishing enzymic saccharification industries in Japan.

Fig. 2 shows the consumption of starch in Japan. Technological development in starch sweetener industry in Japan is shown in Table 2. Starch liquefaction and saccharification by means of enzymes on an ind-

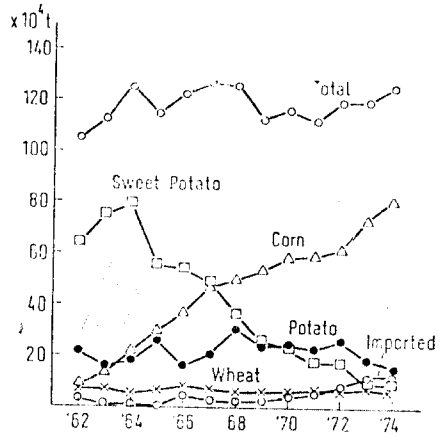


Fig. 1. Production of various starches in Japan

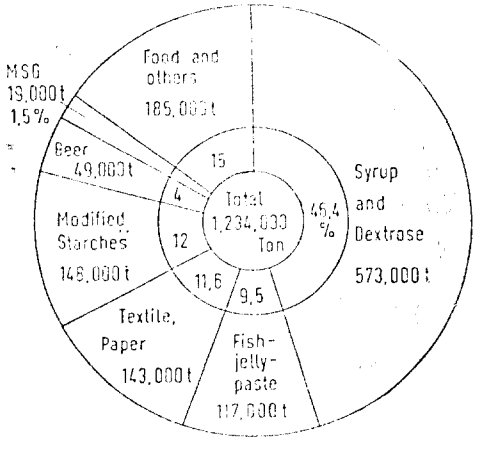


Fig. 2. Consumption of starch in Japan (Oct. 1973~Sep. 1974)

Table 2. Technological development in starch sweetener industry in Japan

Year	Technology
1950	Acid hydrolysis
	Dextrose
	Starch syrup
1960	Enzyme saccharification
	Bacterial α-amylase
	Fungal glucoamylase
	Dextrose
	Fine powder dextrose (1959)
	Glucose isomerization
	Alkaline process (1960)
	Enzymic process (1960)
1970	Industrial production
	Immobilized enzyme process (1976)

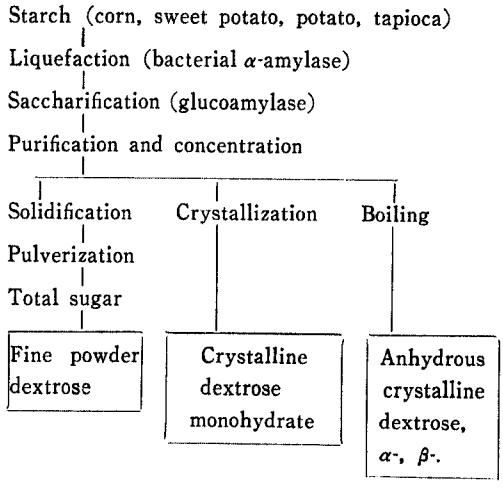


Fig. 3. Enzymatic process of dextrose production in Japan

ustrial scale took over acid hydrolysis in 1959, and the basis for starch sweetener industry was founded. In 1963, the total production capacity of dextrose reached already over 400,000 t per year. Fig. 3 shows the present industrial enzymatic process of dextrose production in Japan. We undertook research on the isomerization of dextrose to fructose to find ways to expand the utilization of surplus dextrose. Using alkaline process in the pilot plant in our Institute, we succeeded for the first time in continuous isomerization process[3].

The results obtained in the pilot plant studies are shown in Table 3. Our alkaline process proved to be

successful in continuous isomerization of high concentration dextrose solution in short time. It was discovered that 33~35% of fructose content was the maximum for the minimum fructose degradation. However, we found in kinetic studies that it is theoretically possible to isomerize up to 50% fructose content.

Worldwide sugar shortage at that time required to increase fructose content of isomerized dextrose. Over ten years since 1960, Dr. Tsumura and his colleagues have been engaged in the studies on microbial strains of glucose isomerase with high enzymic activity and stability[4]. This method was developed by Dr. Takasaki[5] and the Japanese strains were transferred to USA, where the first industrial plant was built and the modified processes have been widely exploited both in Europe and Japan.

Fig. 4 shows enzymic isomerization process presently used in Japan. Since 1976, continuous isomerization by immobilized enzyme systems has rapidly advanced and taken over the batch processes in the past. Continuous processes have such advantages over batch operations as reduced reaction time, lower coloration and high productivity.

In passing the past, present and future prospect of isomerized syrup production in Japan should be discussed. In 1970, one Japanese mill started batch operation using self-supplied isomerase. Production on dry basis amounted to 30,000 t in 1972, 40,000 t in 1973 and 50,000 t in 1974. In 1975, 75,000 t were marked

Table 3. Reaction conditions for continuous glucose isomerization and their products

Conditions	
Conc. of glucose	40-65% (w/v)
NaOH added	0.8-1.0%
Temperature	70-90°C
Time	5-75 min
Results	
1) Percent of fructose formation	33-35%
2) Degree of glucose degradation	1-3%
3) Specific conductivity of the ion-exchange resin purified solution	3×10^6 Ω cm

Products: colorless and clear, sweetness like invert sugar

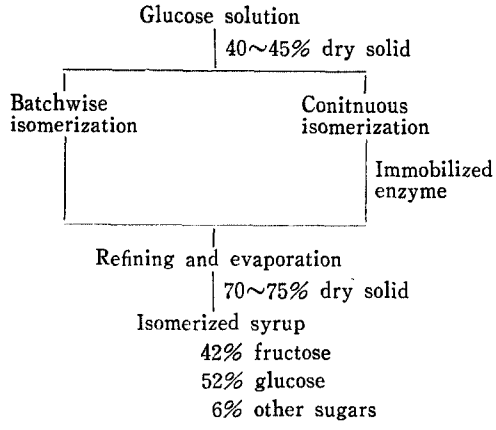


Fig. 4. Enzymatic isomerization of glucose

due to the world-wide price hike of sugar in 1974. The annual consumption of cane and beet sugar in Japan is about 3,000,000 t at present, and I assume that at least 10% or 300,000 t of the consumption would be replaced by isomerized syrup.

There is no statistics available on the consumption of isomerized syrup, but it is estimated that out of 100,000~110,000 t of 1976 production, 40,000 t are consumed for lactic acid bacteria-fermented milk, 30,000 t for bread as major areas of consumption. The rest of 20,000 t are used for soft drinks, confectionary, canned foods and cooking sauce. The figures are all based on dry basis. The products commercially traded in the market have 25% moisture content, and are mixed with sucrose or kept at 40°C to prevent crystalization due to low temperature. At present, 16 mills are operating in isomerized syrup production in Japan, out of which 8 mills produce more than 90% of the total production. Production capacity of each of these 8 mills is 50~100 tons per day respectively. All of the mills use immobilized enzyme, and one mill uses selfsupplied enzyme, three of them use Japanese-brand enzymes. Three use the enzyme from NOVO industries, and one employs the adsorption method using aluminium oxide obtained from Corning Glass Co.

Quality of isomerized syrup is to meet Japan Agricultural Standard (JAS) which was enacted in 1977. There are two kinds of products that are in common with 30% moisture, 70% or more of reducing sugar and pH 4.5~5.5. They are classified into two different types, A and B, according to fructose content. A-group

contains over 42% fructose and less than 8% oligosaccharides. B-group contains over 35% fructose and less than 15% oligosaccharides. B-group is only for limited uses and A has a larger demand in most cases.

2. Recent Advances and Future Prospect in the Utilization of New Carbohydrases

The future prospect in utilization of new carbohydrases and relating three research projects that are being undertaken in our Institute at present are to be described and discussed.

2.1. Production of maltooligosaccharides

Recently, maltooligosaccharides-forming amylases have been given considerable attention from the point of view of action pattern of these new amylases and also of the possible utilization of pure maltooligosaccharides[6]. Due to the difficulties of production, maltooligosaccharides except maltose have not been studied on either chemical and physical properties of their useful properties.

Table 4 shows a list of oligosaccharides-forming amylases, both conventional and newly discovered[7]. Recently, new amylases from microorganisms have been discovered. The initial work in this area was undertaken by Drs. Robyt and French, who discovered microbial β-amylase[8] and *Pseudomonas stutzeri* maltotetraose-forming amylase[9]. The latter enzyme discovered in 1970 was the third exo-enzyme after β-amylase and glucoamylase.

In 1971, Dr. Kainuma discovered maltohexaose-forming exo-amylase as the fourth exo-amylase[10]. After these two new amylases, several other new amylases were discovered in Japan, for example, microbial β-amylase, maltotriose-forming exo-amylase, α-amylase which forms mainly maltopentaose. We will be able to produce different maltooligosaccharides, starting from maltose up to maltohexaose in the large scale. Isoamylase is also useful to produce a linear chain from amylopectin and glycogen. Here, some notes will be given to properties of maltohexaose-forming amylase.

This enzyme was discovered in *Aerobacter aerogenes*, and the fourth exo-amylase after glucoamylase, β-amylase and *Pseudomonas* maltotetraose-forming amylase. This enzyme produces maltohexaose from starch,

Table 4. Starch and pullulan as sources of oligosacchardies (Whelan 1971)

Substrate	Enzyme	Product
Starch	Glucoamylase	Glucose
Starch	β-Amylase	Maltose
	Microbial amylases[1)]	Maltose
Starch	Streptomyces amylase[1)]	Maltotriose
Pullulan	Pullulanase	Maltotriose
Pullulan	"Isopullulanase"	Isopanose
Starch	*Ps. stutzeri* amylase	Maltotetraose
Starch	*Bacillus licheniformis* amylase[(1)]	Mainly maltopentaose
Starch	*A. aerogenes*	Maltohexaose
Starch	Isoamylase	Linear chains

1) Supplemented by K. Kainuma (1975)

amylose and amylopectin exclusively. The most unusual character of the enzyme is that it degrades β-limit dextrin of amylopectin and glycogen easily by bypassing the branch point. Dr. Kainuma separated the branched oligosaccharides, which is formed by the action of the enzyme on β-limit dextrin of amylopectin and analyzed the structure. He concluded that this enzyme mimics an α-1, 6 glucosidic linkage as an α-1, 4-glucosidic linkage in β-amylase limit dextrin.

Fig. 5 shows the action of G_6-forming amylase on amylopectin. As it is seen, maltohexaose is the only product at the first stage, then this is hydrolyzed slowly into maltose and maltotetraose. Fig. 6 shows the results of the studies on the action pattern of the enzyme on the reducing end $-C^{14}$ labeled maltosaccharides. The sixth glucosidic bond of the oligosaccharides was exclusively hydrolyzed.

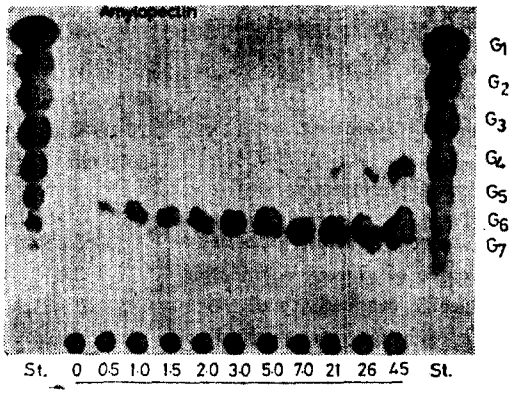


Fig. 5. Action of G_6-forming amylase on amylopection

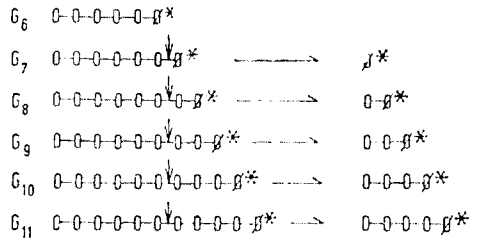


Fig. 6. Action of G_6-producing amylase on maltooligosaccharides

Table 5. Cyclodextrin synthesizing enzymes

Bacillus sp.	Major product	Reference
Bacillus macerans	α-CD	Tilden and Hudson (1939)
Bacillus stearothermophilus	α-CD	Shiosaka and Bunya (1973)
Bacillus megaterium	β-CD	Okada et al. (1972)
Alkalophilic *Bacillus* sp.	β-CD	Horikoshi (1973)

A needle crystal of maltohexaose[12] was obtained as the first crystal of maltooligosaccharides higher than DP 3. We are looking for the special use of oligosaccharides, mainly in the clinical and pharmaceutical fields.

2. 2. Production of cyclodextrin

Cyclodextrin is a cyclic polysaccharide obtained from starch, and is formed by the action of cyclodextrin synthetic enzyme which is produced by *Bacillus macerans*[18]. It is an α-1, 4 linked cyclic oligosaccharides having 6 glucose moieties forming a cavity. The cavities are the hydrophobic regions which take different types of oily substance and form inclusion compounds. This particular feature finds uses in vast varied aspects such as stabilization of unstable materials, retaining of volatile compounds, or solubilization of insoluble compounds.

Bacillus macerans and *B. circulans* are known to produce cyclodextrin synthesizing enzyme. But, as in Table 5, many other strains such as *B. stearothermophilus*[14], *B. megaterium*[15] and alkalophilic *Bacillus sp.*[16] have recently been discovered in Japan. β-cyclodextrin can be easily produced with these enzymes.

Since yield of cyclodextrin was low, complex formation and precipitation was employed in the past by using the characteristics of cyclodextrin that forms a

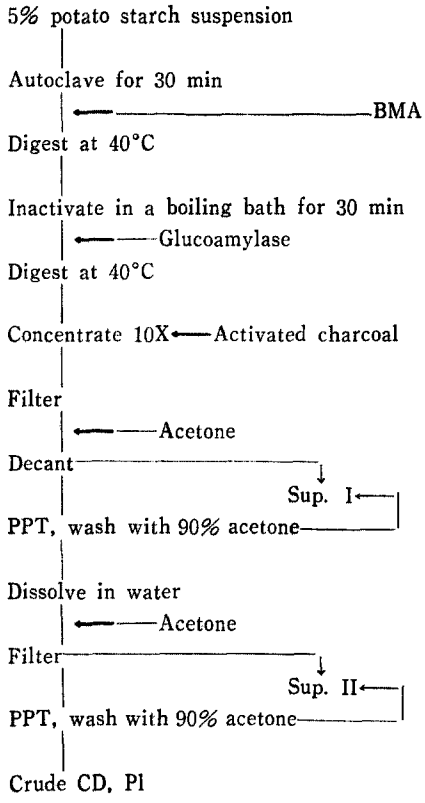


Fig. 7. Preparation of cyclodextrin

complex with alcohol or other organic solvents[17,18]. However, in order to overcome its cumbersome handling, a simpler method of glucoamylase treatment-acetone precipitation method was discovered by our group[19].

As shown in Fig 7, the principle of this method is to hydrolyze polysaccharides except cyclodextrin into glucose by glucoamylase, and to precipitate directly by acetone.

Cyclodextrin is obtained as mixture of α-and β-cyclodextrin by this method. Since β-cyclodextrin is crystallized from concentrated aqueous solution, it is easy to separate it from α-cyclodextrin. The crystal of α-cyclodextrin was obtained by adding n-propanol to the mother liquor after β-cyclodextrin was separated. β-cyclodextrin was obtained from aqueous solution in the form of prism-like crystal.

2. 3. Miscellaneous enzyme utilizations

Recently, in Japan, Hayashibara Biochemical Laboratories have achieved remarkable success in developing new products from starch by means of enzyme

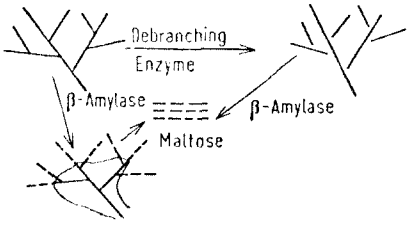


Fig. 8. Debranching of starch

applications[27]. The first is the industrial production of pure maltose. They are successful in producing maltose with high yield by the combination of pullulanase, isoamylase and β-amylase[28]. Fig. 8 shows the widely known principle of the formation of linear molecule by starch debranching and splitting into maltose by β-amylase. Based on this principle, they built a large industrial plant.

Pure crystalline maltose is used for the injection, and green which is obtained after the separation of crystalline maltose produces maltose for confectionary and other food uses, or is used for dietetic food as maltitol, which is manufactured by hydrogenation.

The second achievement is the production of pullulan. *Pullularia pullulans* and *Pullularia fermentans* were incubated for 7 days using hydrolysate of starch digest as cultural media. The product then was precipitated in solvent to obtain white powder. This is quite soluble in water and the solution is highly viscous. It is possible to obtain the product with molecular weight between 50,000~2,000,000 easily by changing cultural conditions.

Pullulan can be formed like plastic, and it is easy to form into film or fibre. Also, since the product is bio-degradable, various ways of application are being developed. Pullulan film is particutarly strong in gas barrier, especially in oxygn barrier. Therefore, it is quite useful for film wrapping of foods, fat and oil or vitamins that are oxidized rather easily. Pullulan film coating on the surface of various foods by spraying or dipping is also effective. Present production amounts to 20 t per month and is expected to become 200 t within 1977.

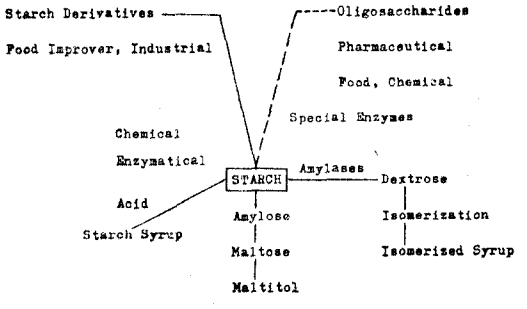
The last and the latest achievement to be stressed is coupling sugar as new sweetener. As shown below, the transfer product can be obtained by the reaction of *Bacillus megaterium*, *B. circulans*, *B. stearothermophilus* on the mixture of soluble starch and sucrose. Dr. Okada and his collaborators named the product "coupling sugar"[29]. The enzyme that is produced by *B. megaterium* has higher transfer activity to sucrose than *B. macerans*, and its higher enzyme productivity allows industrial use. Normally, the ratio between sucrose and soluble starch is 1:1, but there are other ratios including 1:2.

Starch + Sucrose —(Cyclodextrin Glycosyl transferase, PH 5.0~6.0, Temp. 50~60°C)→ G−(G....G)$_n$ -Suc.

B. megaterium
B. circulans
B. stearothermophilus
B. macerans

Coupling sugar has several specific characteristics. The most interesting one is that the product has a good possibility to prevent dental caries. Dental caries starts when sucrose forms insoluble glucan by *Streptococcus mutans* and adheres on the surface of teeth. Coupling sugar has the same structure with sucrose at the one end of molecule and has possibility to inhibit competitively the formation of glucan. The sweetness of this product is about 80% of that of sucrose, and its natural and soft sweetness is quite palatable. Since no coloration takes place by heating, wide uses in the food industry in future is forese eable.

To conclude, a map of starch utilization in which I anticipate great progress in the future is shown below. The straight line indicates the ones that are already in use at present. The dotted line is for those that are being investigated[30]. With dextrose, not only the products from starch but the ones from cellulose resources[31] are also anticipated.



3. Utilization of Cellulose

In Japan, three years ago, the Ministry of Agriculture and Forestry started a considerably large project on reclamation of cellulosic wastes and their utilization. One third of the world population including Japanese are fed on rice but the great bulk of rice husk and straw are left over without any utilization. We are studying the utilization of cellulosic wastes as new food resources. Rice production in Japan is approximately 12,000,000 t per year, and the amount of husk reaches roughly 2,400,000 t per year, containing 1,000,000 t of cellulose and 670,000 t of xylan.

The purpose of our investigation is to hydrolyze cellulose from rice husk into glucose in a manner comparable with conversion of starch into glucose. Glucose solution obtained by cellulose degradation may be used as material for isomerized sugar, single cell protein production and fermentation of amino acids, nucleotides or antibiotics, etc. A variety of methods on conversion of cellulose into glucose by means of cellulase have been reported, but the higher conversion into glucose by the enzyme still poses some problems because a strong crystalline structure of cellulose resists enzymic attack. Judging from many reports on cellulose degradation by enzyme, we considered that the key of the complete enzymic hydrolysis of cellulose lies in the pretreatment of cellulose which destroys the cellulose micel structure and removes the interaction between glucose chains.

As for the treatment procedures, there are mainly two approaches, physical degradation and chemical degradation[20~24]. Recently, the Natick Group of USA developed a new and interesting physical procedure, so-called "milling", which has been highly effective in destroying the cellulose structure and permitting a high degree of saccharification over 90% by *T. viride* mutant cellulase.

On the other hand, chemical degradation of cellulose structure has been studied by Dr. Sasaki in our Institute[25,26]. The purpose of chemical treatment of cellulose is the removal of the crystalline structure of cellulose by solubilizing and converting it into amorphous structure of cellulose may be greatly more susceptiable than any of the crystalline structure. He developed a new procedure of dissolving cellulose with acid as one of cellulose solvents and the precipitation of cellulose with organic solvents to recover cellulose.

This procedure has many advantages including no salt formation, and is very simple and rapid. The organic solvent used for precipitation can be recovered easily. The treated cellulose materials could be rapidly degraded to a soluble fraction within one hour and hydrolyzed to glucose with over 95% of recovery within 24 hours by commercial cellulase which is containing Cx-glucosidase only. This sugar solution supported good growth of yeast and gave good yield for amino acid fermentation.

References

1) Suzuki, S.: *Stärke*, **16**, 285 (1964).
2) Suzuki, S.: *J. Jap. Soc. Starch Sci.*, **17**, 155 (1969).
3) Kainuma, K. and Suzuki, S.: *Stärke*, **19**, 60 (1967); **19**, 66 (1976).
4) Tsumura, N. and Sato, T.: *Agr. Biol. Chem.*, **29**, 1129 (1965).
5) Takasaki, Y. and Tanabe, O.: *Japan. Patent*, 484 472 (1965).
6) Kainuma, K.: *J. Jap. Soc. Starch Sci.*, **21**, 222 (1974).
7) Whelan, W. J.: *Proceedings of the International Symposium on Conversion and Manufacture of Foodstuffs by Microorganisms* (Kyoto), p. 87 (1971).
8) Robyt, J. and French, D.: *Arch. Biochem. Biophys.*, **104**, 338 (1964).
9) Robyt, J. and Ackermann, R. J.: *Arch. Biochem. Biophys.*, **145**, 105 (1971).
10) Kainuma, K., Kobayashi, S., Ito, T. and Suzuki, S.: *FEBS Letter*, **26**, 281 (1972).
11) Kainuma, K., Wako, K., Kobayashi, S., Nogami, A. and Suzuki, S.: *Biochem. Biophys. Acta*, **410**, 333 (1975).
12) Kainuma, K.: *Kagaku to Seibutsu*, **11**, 146 (1973).
13) French, D.: *Advan. Carbohyd. Chem.*, **12**, 189 (1957).
14) Shiosaka, M. and Bunya, H.: *Amylase Symposium* (Japan), **8**, 43 (1973).

15) Kitahata, S. and Okada, S.: *Agr. Biol. Chem.*, **38**, 2413 (1974).
16) Nakamura, N. and Horikoshi, K.: *Agr. Biol. Chem.*, **40**, 753 (1976).
17) Cramer, F. and Henglein, F. M.: *Chem. Ber.*, **91**, 308 (1957).
18) French, D., Pulley, A. O. and Whelan, W. J.: *Stärke*, **8**, 280 (1963).
19) Kobayashi, S., Kainuma, K. and Suzuki, S.: *J. Jap. Soc. Starch Sci.*, **22**, 6 (1975).
20) Reese, E. T., Gilligan, W. and Norkrans, B.: *Phy. Plant*, **5**, 379 (1952).
21) Ghose, T. K.: *Biotech. Bioeng.*, **11**, 239 (1969).
22) Spano, L. A., Medeiros, J. and Mandels, M.: *Enzymatic Hydrolysis of Cellulosic Wastes to Glucose* (Natic Development Center, USA) 8 Sept. (1975).
23) Adams, E.: *Textile Res. J.*, **20**, 71 (1950).
24) Walseth, C. S.: *Tappi*, **35** (5), 233 (1952).
25) Sasaki, T., Mikami, N., Kainuma, K. and Suzuki, S.: *Japanese Starch Science Abstracts* (1975).
26) Tanaka, T., Sasaki, T., Nanbu, T., Kainuma, K. and Suzuki, S.: *Japanese Agricultural Chemistry Abstracts* (1976).
27) Sugimoto, K. and Hirao, M.: *J. Jap. Soc. Starch Sci.*, **21**, 314 (1974).
28) Suzuki, S.: *Proceedings of 3rd International Congress of Food Science and Technology*, p. 484 (1971).
29) Okada, S. and Kitahara, S.: *J. Jap. Soc. Food Sci. Technol.*, **22**, 420 (1975).
30) Kainuma, K.: *Food Industry*, **19** (22), 29 (1976).
31) Sasaki, T.: *Food Industry*, **19** (24), 2 (1976).