Pure Appl. Chem., Vol. 74, No. 7, pp. 1245–1251, 2002.

Present status and future of functional oligosaccharide development in Japan*

Teruo Nakakuki

Research Institute, Nihon Shokuhin Kako Co., Ltd., 30 Tajima, Fuji, Shizuoka, 417-8530, Japan

Abstracts: Several oligosaccharides such as glycosylsucrose, fructo-oligosaccharides, malto-oligosaccharides, isomalto-oligosaccharides (branched-oligosaccharides), galacto-oligosaccharides, xylo-oligosaccharides, isomaltulose (palatinose), and lactosucrose have been produced on an industrial scale. Recent developments in industrial enzymology have made possible a series of new starch oligosaccharides such as β-1,6 linked gentio-oligosaccharides, α,α-1,1 linked trehalose, α-1,3 linked nigero-oligosaccharides, and branched-cyclodextrins. Some new sweeteners, including trehalose and nigero-oligosaccharides, are being developed as food ingredients with physiologically unique functions such as superoxide dismutase-like activity and immunological activity. Also, soybean oligosaccharides containing raffinose, stachyose, and other oligosaccharides mentioned above are now used in beverages, confectionery, bakery products, yogurts, daily products, and infant milk. In 1991, the Japanese government legislated for Foods for Specified Health Use (FOSHU). FOSHU increased the total to 223 items of which more than 50 % incorporate oligosaccharides as the functional components. Furthermore, the Ministry of Health, Labor, and Welfare published the proposal for a new system named Foods with Health Claims (FHC), which was carried out in April 2002.

INTRODUCTION

Oligosaccharides are an important group of polymeric carbohydrates that are found either free or in combined forms in all living organisms. The generic term “oligosaccharides” is customarily used for saccharides having the degree of polymerization of 2–10 [1]. Structurally, oligosaccharides are composed of 2–10 monosaccharide residues linked by glycosidic bonds that are readily hydrolyzed to their constituent monosaccharides either by acids or by specific enzymes.

Starch oligosaccharides, which represent the fragments of original polysaccharides, are composed of α-D-glucopyranosyl units linked by α-1,4 and/or α-1,6 bonds. Oligosaccharides containing only α-1,4 glucosidic linkages are called malto-oligosaccharides, while those containing both α-1,4 and α-1,6 glucosidic linkages are called branched-oligosaccharides or isomalto-oligosaccharides in Japan. Also, cyclodextrins are cyclic α-1,4 linked malto-oligosaccharides containing 6-12 glucose units. The discovery of novel enzymes that produce specific oligosaccharides such as maltotriose, maltotetraose, maltopentaose, and maltohexaose has made possible the production of syrup with a high content of each malto-oligosaccharide [2]. Furthermore, recent developments in industrial enzymology have made possible a series of new starch oligosaccharides such as β-1,6 linked gentio-oligosaccharides [3], α, α-1,1 linked trehalose [4], α-1,3 linked nigero-oligosaccharides [5] and branched-cyclodextrins having glucosyl, maltosyl, or hydoxypropyl residue [6].

**Pure Appl. Chem.* Vol. 74, No. 7, 2002. A special topic issue on the science of sweeteners.

Various types of oligosaccharides have been found as natural components in many common foods including fruits, vegetables, milk, honey, and Japanese traditional foods such as sake and sweet sake used as seasonings. Also, oligosaccharides are functional food ingredients that have a great potential to improve the quality of many foods. In addition to providing useful modifications to physicochemical properties of foods, it has been reported that these oligosaccharides have various physiological functions such as the improvement of intestinal microflora based on the selective proliferation of bifidobacteria, stimulation of mineral absorption, non- or anticariogenicity, and the improvement of both plasma cholesterol and blood glucose level.

PRODUCTION, PROPERTIES, AND APPLICATIONS OF OLIGOSACCHARIDES

Research on the production of oligosaccharides for foods was started around 1970–1975 in Japan, and several oligosaccharides such as starch-related, sucrose-related, and lactose-related oligosaccharides have been developed, as shown in Table 1.

Also, xylo-oligosaccharides, agaro-oligosaccharides, manno-oligosaccharides, and chitin/chitosan oligosaccharides have been produced from various polysaccharides such as xylan, agar, mannan, chitin, and chitosan as the raw materials. Functional properties evaluated until now are summarized in Table 2. At present, these oligosaccharides have been widely utilized in foods, beverages, and confectionery processing applying these various properties of oligosaccharides.

Table 1 Various kinds of oligosaccharides.

Raw material	Product
Starch	Malto-oligosaccharides: maltose ~ maltoheptaose Isomalto-oligosaccharides: isomaltose, panose, isomaltotriose Cyclodextrins (CDs): α-CD, β-CD, γ-CD, HP- β-CD, branched CDs Others: maltitol, gentio-oligosaccharides, trehalose, nigerose
Sucrose	Glycosylsucrose, fructo-oligosaccharides, palatinose (isomaltulose), lactosucrose, xylosucrose, raffinose, stachyose, trehalulose
Lactose	Galacto-oligosaccharides, lactosucrose, lactulose, lactitol
Xylan, agar, mannan, chitin, chitosan, etc.	Xylo-oligosaccharides, agaro-oligosaccharides, manno-oligosaccharides, chitin/chitosan oligosaccharides, etc.

Table 2 Properties of oligosaccharides.

Physicochemical property	Sweetness, bitterness, hygroscopicity, water activity, reinforcement agent for drinks, stabilization of active substances (protein, flavor, color, etc.), inclusion capability, etc.
Biological property	Digestibility, nondigestibility, noncariogenicity, anticariogenicity, bacteriostatic action, selective proliferation of bifidobacteria, improvement of serum lipids, and blood glucose, etc.
Other properties	Specific substrate for enzymes, enzyme inhibitors, elicitors, etc.

Starch-related oligosaccharides

Since 1970, several novel microbial enzymes producing specific gluco-oligosaccharides have been discovered as listed in Table 3. Using these new enzymes, it is now possible to produce various new sweeteners from starch on an industrial scale. Among these microbial enzymes, maltotriohydrolase from *Microbacterium imperiale*, maltotetraohydrolase from *Pseudomonas stutzeri*, and maltopentaose-forming amylase from *Bacillus licheniformis* are used for the production of maltotriose, maltotetraose, and maltopentaose syrups, respectively. Recently, the production method of trehalose has been developed by the Hayashibara Biochemical Laboratories using two kinds of glucosidases, such as malto-oligosyltrehalose synthase (MTSase) and malto-oligosyltrehalose trehalohydrolase (MTHase). MTSase is a kind of glycosyltransferase, and MTHase should be classified as an α-amylase. Also, nigero-oligosaccharides-containing syrup has been developed using the transfer reaction of α-glucosidase from *Acremonium* sp.

Cyclodextrins are produced from starch due to the action of cyclodextrin glucanotransferase (CGTase) from various origins as shown in Table 4. In this table, *Bacillus macerans*, *B. stearothermophilus*, *B. coagulans,* and alkalophilic *bacillus* are utilized for the industrial production of cyclodextrins in Japan.

Table 3 Novel microbial enzymes.

Enzyme	Product	Microbial origin
MTSase(glycosyltransferase)	Trehalose	*Arthrobacter sp.*
MTHase(α-amylase)		*Sulfolobus sp.*
α-Glucosidase	Nigero-oligosaccharide	*Acremonium sp.*
Maltotriohydrolase	α-Maltotriose	*Streptomyces. griseus*
		B. subtilis
		M. imperiale
Maltotetraohydrolase	α-Maltotetraose	*Ps. stutzeri*
		Ps. saccharophila
		B. circulans
Maltopentaose-forming amylase	α-Maltopentaose	*B. licheniformis*
		B. subtilis
		B. cereus
		Pseudomonas sp.
Maltohexaohydrolase	α-Maltohexaose	*Klebsiella pneumoniae*
		B. subtilis
		B. circulans F-2, G-6

Table 4 Properties of CGTases from some microorganisms.

	Type	Optimum pH	Optimum temperature (°C)
B. macerans	α	5.0–5.7	55
B. amyloliquefaciens	α	4.0–7.0	70
B. stearothermophilus	α, β	5.0–5.5	75
B. coagulans	α, β	6.0–6.5	65
B. circulans	β	5.2–6.1	55
B. megaterium	β	5.0–5.7	55
B. ohbensis	β	5.0–6.0	60
Alkalophilic *bacillus*	β	4.5–9.5	65
K. pneumoniae	α	5.0–6.0	–

Currently produced starch oligosaccharides are shown in Fig. 1. Both isomalto-oligosaccharides (branched-oligosaccharide)-containing syrup, and nigero-oligosaccharide-containing syrup are produced from maltose as the raw material using the transfer reaction of α-glucosidase. Furthermore, gentio-oligosaccharide-containing syrup is produced from glucose using the condensation and transfer reaction of β-glucosidase.

Standard saccharide compositions of various kinds of malto-oligosaccharide-containing syrups are shown in Table 5. These syrups are products having low sweetness. They impart resistance to retrogradation of starch gel and prevent the crystallization of sucrose. Their reduced browning tendency results in the improvement of heat stability. They have begun to be used as property enhancers for various foods, powdering materials, saccharides for dry milk, liquid diets for patients, and viscosity-increasing agents for refreshing drinks.

Cyclodextrins are capable of forming inclusion complex with various organic compounds by incorporating them into the cavity of their cyclical structure. This can lead to desirable changes in the physical and chemical properties of the incorporated compounds.

Branched-oligosaccharide syrups are mildly sweet and have relatively low-viscosity, high-moisture-retaining properties, and lower water activity, which reduces microbial growth. Isomaltose, panose, and isomaltotriose cannot be digested by yeast. Therefore, these syrups are effectively used for traditional fermented foods in Japan. The intake of these syrups not only improves the consistency of feces

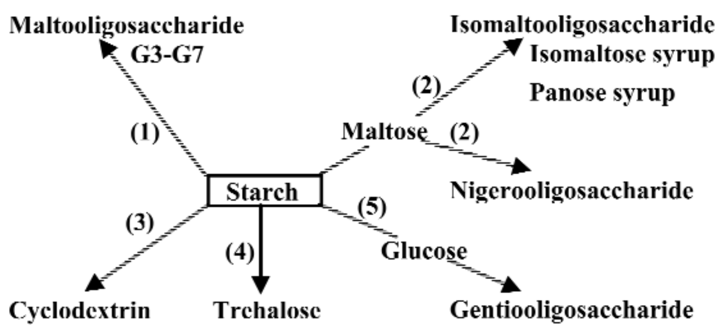

Fig. 1 Currently produced starch oligosaccharides. (**1**) Malto-oligosaccharide-forming amylase; (**2**) α-Glucosidase; (**3**) CGTase; (**4**) MTSase, MTHase; (**5**) β-Glucosidase.

Table 5 Standard saccharide compositions of various kinds of malto-oligosaccharides-containing syrups.

Saccharide	Fuji-oligo G3	Fuji-oligo G4		Fuji-oligo
	#360	#450	#470	G67
Glucose	4.0	1.0	2.0	4.4
Maltose	21.7	7.8	8.5	13.4
Maltotriose	60.0	10.2	11.0	7.9
Maltotetraose	7.6	50.5	72.0	7.6
Maltopentaose	1.4	2.5	1.0	7.6
Maltohexaose	3.3	1.3	0.2	21.1
Maltopentaose	1.2	1.2	0.8	18.9
Others (≧DP 8)	0.8	25.5	4.5	19.1

but also increases *Bifidobacterium* count in human intestine, thus effecting an improvement in colonic conditions. Also, branched-oligosaccharides such as isomaltose and panose inhibit the synthesis of water-insoluble glucan from sucrose, and these syrups are effectively used as anticariogenic saccharides [7]. With these specific characteristics as background, it can be understood why these syrups are used in various fields of foods and beverages processing such as baking, confectionery, soft drinks, and sake making and seasonings.

Trehalose has a wide application to food products utilizing its physicochemical properties. Gentio-oligosaccharide-containing syrup having the unique property of bitter taste is used for beverages as a taste-improving saccharide. Also, it has been revealed that gentio-oligosacchrides have the improving effects on human intestinal microflora and also on the absorption of calcium. Furthermore, a new nigero-oligosaccharide-containing syrup has begun to be used for various kinds of foods and beverages as a taste-improving and color-stabilizing saccharide.

Sucrose-related oligosaccharides

Various sucrose-related oligosaccharides such as glycosylsucrose (malto-oligosylsucrose), fructo-oligosaccharides, palatinose (isomaltulose), lactosucrose, and so on have been developed.

Glycosylsucrose is produced by subjecting the mixture of starch hydrolysate and sucrose to the action of cyclodextrin glucanotransferase (CGTase). Because the saccharides have a small amount of reducing sugars, it induces less Maillard reaction with protein. Also, it is an anticariogenic sweetener that not only hardly induces dental caries, but also inhibits the cariogenicity of sucrose.

Fructo-oligosaccharides are produced from sucrose by the transfructosylation of fungal β-fructofuranosidase. And, the saccharides have been efficiently produced by the continuous reaction system using an immobilized enzyme. It has been verified that the saccharides are nondigestible sweeteners and have low cariogenicity. Also, the saccharides are selectively utilized by human intestinal *Bifidobacterium* to improve the microflora. Furthermore, it has been revealed that the saccharides have physiological properties such as cholesterol reduction and the improvement of constipation.

Palatinose (isomaltulose) is an isomer of isomaltose, and the production of the saccharide from sucrose has been carried out using immobilized α-glucosyltransferase from *Protaminobacter rubrum* origin. The process produces two kinds of products, crystalline palatinose and palatinose syrup. It is generally accepted that both products are effective as sucrose substitutes for caries prevention, and that these saccharides are suitable for children because palatinose is digestible in the small intestine without diarrhea and flatulence. Also, there are soybean oligosaccharides including raffinose and stachyose having the same physiological properties as fructo-oligosaccharides.

Lactose-related oligosaccharides and others

Several kinds of oligosaccharides, such as galacto-oligosaccharides, lactosucrose, lactulose, and lactitol, have been developed from lactose as the raw material. Almost all of these saccharides have the same characteristics as those produced from sucrose.

Other oligosaccharides such as xylo-oligosaccharides, agaro-oligosaccharides, manno-oligosaccharides, and chitin/chitosan oligosaccharides are produced from various polysaccharides using microbial enzymes. Xylo-oligosaccharides are low-digestible sugars and utilized by most *Bifidobacterium* species. Also, the new markets for foods applied to the novel oligosaccharides such as agaro-oligosaccharides, manno-oligosaccharides, and chitin/chitosan oligosaccharides have been developed.

The demand for oligosaccharides in Japan is shown in Fig. 2. Among these oligosaccharides, the demand for starch oligosaccharides is the largest, as well as for their production amount. Also, at present, the market for oligosaccharides is expected to be about 20 billion yen/year.

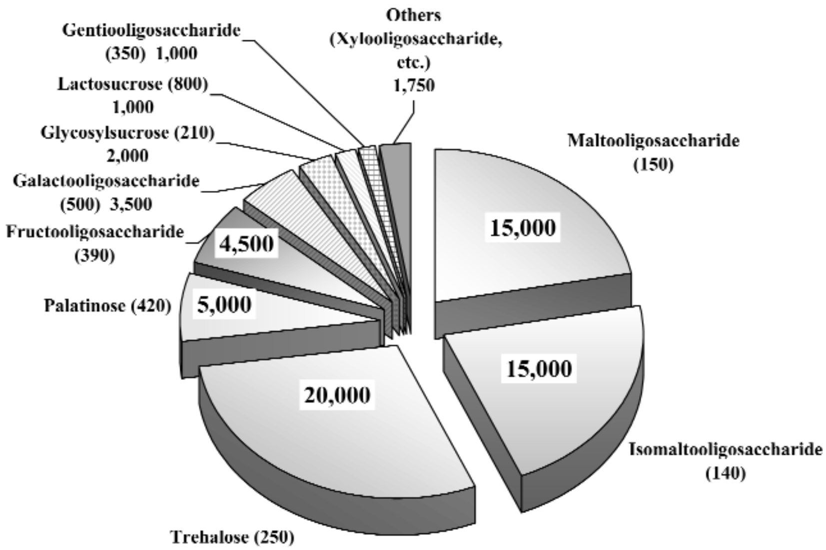

Fig. 2 Demand of oligosaccharides in Japan (ton/year). ( ) Average price per 1 kg of each product.

Future of functional oligosaccharides

As mentioned above, research on the production of oligosaccharides for foods was started around 1970–1975 in Japan, and several oligosaccharides were produced on an industrial scale from the early 1980s to the late 1990s. Research and development of novel oligosaccharides with physiologically functional properties is now continuing. The market for oligosaccharides is already substantial and continues to expand gradually. Among the markets, the production amount of starch oligosaccharide reaches the highest level along with sales amount. At present, Japanese companies dominate worldwide oligosaccharide production, as well as the research and development of oligosaccharides. However, some problems remain to be solved in the development of oligosaccharides, as follows.

The first problem to be solved is that there is a need to achieve continuous and efficient production of oligosaccharide syrup and to maintain a low cost in high-purity oligosaccharides. Second, oligosaccharides with highly functional properties must be developed. Third, physiologically functional saccharides need to be designed and the evaluation system of the functional properties needs to be established. By breaking through these problems, the market for oligosaccharides will progress more rapidly.

On the other hand, the Japanese government legislated for Foods for Specified Health Use (FOSHU), taking the initiative in the world in 1991. FOSHU increased the total to 223 items of which more than 50 % incorporate oligosaccharides as the functional components. Furthermore, the Ministry of Health, Labor, and Welfare published the proposal of a new system named Foods with Health Claims (FHC), which was carried out in April 2001. FHC consists of both FOSHU and FNFC (Foods with Nutrient Function Claims).

With increasing consumer health consciousness and also increasing awareness of physiologically functional foods, the future for products containing oligosaccharides seems to be greatly promising. These functional oligosaccharides may play an important role especially for the reduction of lifestyle-related diseases in the near future, as well as the improvement of human health.

REFERENCES

l. T. Nakakuki (Ed.). *Oligosaccharides: Production, Properties and Applications*, Japanese Technology Reviews, Vol. 3, No. 2, Gordon and Breach, Switzerland (1993).
2. M. Okada and T. Nakakuki. *Oligosaccharides in Starch Hydrolysis Products*, pp. 335–366, VCH, New York (1992).
3. T. Unno, T. Nakakuki, S. Kainuma, K. Kataura, G. Okada. *Oyo Toshitsu Kagaku* **41**, 317–324 (1994).
4. M. Shiosaka. *FFI J.* **172**, 97–102 (1997).
5. M. Konishi and K. Shindo. *Biosci. Biotech. Biochem.* **61**, 439–442 (1997).
6. N. Nakamura and K. Hara. “Cyclodextrins”, in *Oligosaccharides*, pp. 25–49, Gordon and Breach, Switzerland (1993).
7. W. Aono, Y. Tamura, T. Minami, T. Takei, M. Matsumoto, T. Ooshima, S. Sobue. *Shohni Shikagaku* **31**, 903–910 (1993).