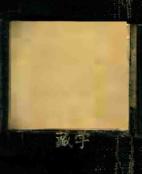
PRODUCTION PROPERTIES AND APPLICATIONS



Ofigosaccharides

Production, Properties, and Applications

Edited by

Teruo Nakakuki, 1993

Nihon Shokuhin Kako. Ltd

Shizuoka, Japan

Gordon and Breach Science Publishers

Japanese Technology Reviews

Editor in Chief

Toshiaki Ikoma, University of Tokyo

Section Editors

Section A: Electronics

Toshiaki Ikoma, University of Tokyo

Section B: Computers

and Communications

Tadao Saito, University of Tokyo

Kazumoto Iinuma, NEC Corporation, Kawasaki

Section C: New Materials

Hiroaki Yanagida, University of Tokyo

Noboru Ichinosé, Waseda University, Tokyo

Section D: Manufacturing

Engineering

Fumio Harashima, University of Tokyo

Section E: Biotechnology

Isao Karube, University of Tokyo Reiko Kuroda, University of Tokyo

Section E: Biotechnology

Volume 1, Number 1

Production of Nucleotides and Nucleosides by Fermentation

Sadao Teshiba and Akira Furuya

Volume 1, Number 2

Recent Progress in Microbial Production of Amino Acids Hitoshi Enei, Kenzo Yokozeki and Kunihiko Akashi

Volume 2. Number 1

Recent Advances in Japanese Brewing Technology

Takashi Inoue, Jun-ichi Tanaka and Shunsuke Mitsui

Volume 2, Number 2

Antibiotics I: β-Lactams and Other Antimicrobial Agents Isao Kawamoto and Masao Miyauchi

Volume 3, Number 1

Antibiotics II: Antibiotics by Fermentation

Sadao Teshiba, Mamoru Hasegawa, Takashi Suzuki, Yoshiharu Tsubota, Hidehi Takebe, Hideo Tanaka, Mitsuyasu Okabe and Rokuro Okamoto

Volume 3, Number 2

Oligosaccharides: Production, Properties, and Applications Edited by Teruo Nakakuki

Preface to the Series

Modern technology has a great impact on both industry and society. New technology is first created by pioneering work in science. Eventually, a major industry is born, and it grows to have an impact on society in general. International cooperation in science and technology is necessary and desirable as a matter of public policy. As development progresses, international cooperation changes to international competition, and competition further accelerates

technological progress.

Japan is in a very competitive position relative to other developed countries in many high technology fields. In some fields, Japan is in a leading position; for example, manufacturing technology and microelectronics, especially semiconductor LSIs and optoelectronic devices. Japanese industries lead in the application of new materials such as composites and fine ceramics, although many of these new materials were first developed in the United States and Europe. The United States, Europe and Japan are working intensively, both competitively and cooperatively, on the research and development of high-critical-temperature superconductors. Computers and communications are now a combined field that plays a key role in the present and future of human society. In the next century, biotechnology will grow, and it may become a major segment of industry. While Japan does not play a major role in all areas of biotechnology, in some areas such as fermentation (the traditional technology for making sake), Japanese research is of primary importance.

Today, tracking Japanese progress in high technology areas is both a necessary and rewarding process. Japanese academic institutions are very active; consequently, their results are published in scientific and technical journals and are presented at numerous meetings where more than 20,000 technical papers are presented orally every year.

However, due principally to the language barrier, the results of academic research in Japan are not well-known overseas. Many in the United States and in Europe are thus surprised by the sudden appearance of Japanese high technology products. The products are admired and enjoyed, but some are astonished at how suddenly these products appear.

With the series Japanese Technology Reviews, we present stateof-the-art Japanese technology in five fields:

Electronics

Computers and Communications

New Materials

Manufacturing Engineering

Biotechnology

Each tract deals with one topic within each of these five fields and reviews both the present status and future prospects of the technology, mainly as seen from the Japanese perspective. Each author is an outstanding scientist or engineer actively engaged in relevant research and development.

The editors are confident that this series will not only give a deep insight into Japanese technology but will also be useful for developing new technology of interest to our readers.

As editor in chief, I would like to sincerely thank the members of the editorial board and the authors for their contributions to this series.

Тоѕніакі Ікома

Preface

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 a degree of polymerization of two to ten. Structurally, oligosaccharides are composed of between two and ten monosaccharide residues joined by glycosidic bonds that are readily hydrolyzed to their constituent monosaccharides in acid solution or by the reaction of specific enzymes.

Research into the production of oligosaccharides for food was started around 1970–1975 in Japan and several oligosaccharides discussed in this book were produced on an industrial scale during the 1980s. Recently, various new biologically active oligosaccharides have been developed and some are now produced on a large scale using bioreactor systems. Improving health when used as food additives, these oligosaccharides are creating a sensation in Japan. With the increasing health consciousness of consumers and their increasing awareness of the bioavailability of food, the future for products containing oligosaccharides looks very bright.

Reviewing the recent progress of oligosaccharides in Japan is a rewarding task for the contributors, all outstanding biochemists actively engaged in relevant research and development. The details of production, properties, and applications of the respective oligosaccharides are described.

I wish to thank the coauthors for their contributions to this book. I also would like to express my gratitude to Prof. Isao Karube for giving me the opportunity to review the status of development of oligosaccharides in Japan.

TERUO NAKAKUKI

Contributors

Shigeaki Fujikawa

Suntory Ltd Tokyo, Japan

Koki Fujita

Ensuiko Sugar Refining Co. Ltd Yokohama, Japan

Kozo Hara

Ensuiko Sugar Refining Co. Ltd Yokohama, Japan

Tatsuhiko Kan

Yakult Honsha Co. Ltd Tokyo, Japan

Sumio Kitahata

Osaka Municipal Technical Research Institute Japan

Youichi Kobayashi

Yakult Honsha Co. Ltd Tokyo, Japan

Kunimasa Koga

Suntory Ltd Tokyo, Japan

Yasushi Koga

The Calpis Food Industry Co. Tokyo, Japan

Toshiaki Kono

Meiji Seika Kaisha Ltd Tokyo, Japan

Akio Kuroda

Yakult Honsha Co. Ltd Tokyo, Japan

Keisuke Matsumoto

Yakult Honsha Co. Ltd Tokyo, Japan

Yoshikazu Nakajima

Mitsui Sugar Co. Ltd Kawasaki, Japan

Teruo Nakakuki

Nihon Shokuhin Kako. Co. Ltd Shizuoka, Japan

Nobuyuki Nakamura

Nihon Shokuhin Kako. Co. Ltd Shizuoka, Japan

Koji Nishio

Mitsui Sugar Co. Ltd Kawasaki, Japan

Robert O'Brien

The Calpis Food Industry Co. Tokyo, Japan

Shigetaka Okada

Ezaki Glico Co. Ltd Osaka, Japan

Takanobu Shibata

The Calpis Food Industry Co. Tokyo, Japan

Yasuo Sumihara

Yakult Honsha Co. Ltd

Tokyo, Japan

Ryuichiro Tanaka

Yakult Honsha Co. Ltd Tokyo, Japan Sadao Ueyama

Yakult Honsha Co. Ltd

Tokyo, Japan

Taichi Usui

Shizuoka University

Japan

Tsunekazu Watanabe

Yakult Honsha Co. Ltd

Tokyo, Japan

Tsuneya Yatake

Showa Sangyo Co. Ltd

Ibaraki, Japan

Contents

Pre	face to	the Series	xiii
Pre	face		xv
List	t of Co	ontributors	xvii
1.		tooligosaccharides o Nakakuki	1
	1.1.	Immobilization of Maltotetraose-Forming	
		Amylase and Pullulanase	3
		1.1.1. Enzymes	3
		1.1.2. Immobilization of enzymes	3
	1.2.	Properties of Immobilized Enzymes and Effects of Operating Conditions on the Continuous	
		Production of Maltotetraose Syrup	6
	1.3.	Continuous Production of Maltotetraose Syrup	7
		1.3.1. Bench-scale bioreactor system	9
		1.3.2. Bioreactor system on a commercial scale	14
	1.4.	Properties of Maltotetraose Syrup	14
		1.4.1. Sweetness	15
		1.4.2. Viscosity	15
		1.4.3. Colorability	16
		1.4.4. Moisture retainment and moisture	
		absorptivity	18
		1.4.5. Freezing point	18
		1.4.6. Effect of maltotetraose syrup on human	
		intestinal microflora	19
		1.4.7. Antimicrobial activity of maltotetraose	21
	1.5.	Applications of Maltotetraose Syrup	22
	1.6.	Conclusion	. 22
	1.7	Acknowledgements	24

2.		elodextrins uyuki Nakamura and Kozo Hara	25
	2.1.	α -, β -, and γ -Cyclodextrin	25
		2.1.1. Cyclomaltodextrin glucanotransferase	27
		2.1.2. Production of cyclodextrins	25
		2.1.3. Applications of cyclodextrins	35
	2.2.	Branched Cyclodextrins	37
		2.2.1. Introduction	37
		2.2.2. Types of branched cyclodextrins	39
		2.2.3. Properties of branched cyclodextrins	44
		2.2.4. Preparation of branched cyclodextrins	44
		2.2.5. Application of branched cyclodextrins	47
3.		ctooligosaccharides	50
		niaki Kono	
	<i>3.1.</i>	Chemical Structures and Distribution	50
	3.2.	Production	52
		3.2.1. Enzyme for fructooligosaccharide	
		production	53
		3.2.2. Production using β -fructofuranosidase	
		from Aspergillus niger ATCC 20611	56
		3.2.3. Highly purified fructooligosaccharide	
		production by chromatographic	
		separation	62
	3.3.	Properties of Fructooligosaccharides	62
		3.3.1. Physical and chemical properties	62
		3.3.2. Sensory evaluation	65
		3.3.3. Biological properties	66
4.	Ano	malously Linked Oligosaccharides	79
	Tsun	eya Yatake	
	4.1.	Manufacturing Procedure	79
	4.2.	Physical Properties and Application to the Food	tool seem
		Industry	80
	`4.3.	Physiological Properties	82
		4.3.1. Improvement of intestinal flora	82
		4.3.2. Caries inhibitory effect	85

5.	Gala	actoolig	osaccharides	90
	Keisı	ike Mats	rumoto, Youichi Kobayashi, Sadao	
	Ueya	ma, Tsu	nekazu Watanabe, Ryuichiro Tanaka,	
	Tatsi	uhiko Ka	n, Akio Kuroda and Yasuo Sumihara	
	5.1.	Produc	tion Process	91
		5.1.1.	Transgalactosylation reaction	91
		5.1.2.	Industrial production process	93
	5.2.	Physiol	logical Properties	94
		5.2.1.	Safety	94
		5.2.2.		95
		5.2.3.	Indigestibility	96
			Utilization by intestinal bacteria	96
		5.2.5.	Effects of ingestion on intestinal	
			microflora	99
		5.2.6.	Clinical effects associated with changes	
			in the intestinal flora	99
	5.3.	Propert	ties and Use of Galactooligosaccharides	
		in Food	d Processing	104
		5.3.1.	Properties relating to food processing	104
		5.3.2.	Application in foods	105
	5.4.	Conclu	sion	106
6.	Ison	naltulos	S.P.	107
٠.			akajima and Koji Nishio	107
	6.1.		e and Immobilization	108
	0.1.	6.1.1.		108
		6.1.2.	Immobilization of α -glucosyltransferase	109
		6.1.3.	Properties of immobilized α-glucosyl-	107
		0.7.5.	transferase	110
	6.2.	Produc	tion and Properties of Isomaltulose	
	0.2.	Produc		111
		6.2.1.	Production process	112
		6.2.2.	Product descriptions	112
	6.3.	W. A. ST. T. ST. T.	tion of Caries-inducing Properties	113
	0.5.		In vitro experiments	113
		6.3.2.	In vivo experiments	115
		0.0.2.	in the experiments	110

-				ė.
	on	te	n	t

	6.4.	Nutritional Properties	116
	6.5.	Isomaltulose and Bifidobacteria	117
	6.6.	Safety	117
7.		Itooligosylsucrose etaka Okada and Sumio Kitahata	118
	7.1.	Production, Purification, and Properties of	
		CGTases	119
	7.2.	Action of Cyclomaltodextrin Glucanotransferase	121
	7.3.	Effects of Various Reaction Conditions on	
		Coupling Sugar Production	122
	7.4.	Properties of Coupling Sugar	124
		7.4.1. Physical properties	124
		7.4.2. Physiological properties	126
	7.5.	Applications	129
8.	Xylo	poligosaccharides	130
	Kuni	masa Koga and Shigeaki Fujikawa	
	8.1.	Production of Xylooligosaccharides	130
		8.1.1. Raw material	130
		8.1.2. Enzymatic Hydrolysis	132
		8.1.3. Enzymatic hydrolysis by the batch	
		method	132
		8.1.4. Enzymatic hydrolysis by the bioreactor	133
		8.1.5. Purification of Xylooligosaccharides	136
	8.2.	Application of Xylooligosaccharides	136
		8.2.1. Sweetness	136
		8.2.2. Stability	136
		8.2.3. Water Activity	137
		8.2.4. Antifreezing Activity	137
		8.2.5. Growth Factor of Intestinal	1.10
		Bifidobacterium	142
		8.2.6. Digestibility	143

9.		ooligos i <i>Usui</i>	accharides	144
	9.1.	Microbi	ial Production of GlcNAc2	145
	9.2	GlcNA	c6 and GlcNAc7 Production	146
	/121		Chitinase	147
			Lysozyme	147
			Proposed mechanism of the	
			transglycosylation mode of chitinolytic	
			enzymes	150
	9.3.	Lysozy	me-mediated p-Nitrophenyl Penta-N-	
	,,,,,	acetyl-f	3-chitopentaoside Production	152
			Enzymatic synthesis	152
		9.3.2.	Transglycosylation mode of lysozyme	153
		9.3.3.	Novel substrate for lysozyme assay	155
10	Xvle	sucrose	, Isomaltosucrose, and	
10.		tosucro		158
			ata and Koki Fujita	
		Levan		159
	10.1.	10.1.1.	Production, purification, and properties	
		10.11.	of levan sucrase	159
		10.1.2.	Action of levan sucrase	160
			Effects of various reaction conditions	
	,		on xylsucrose production	161
		10.1.4.	Anti-cariogenic properties of xylsucrose	
			and isomaltosucrose	163
	10.2	. β-Fruc	tofuranosidase	165
		10.2.1.	Production, purification, and properties	
			of β-fructofuranosidase from	
			Arthrobacter sp. $K-1$	166
		10.2.2.	. Action of β-fructofuranosidase from	
			Arthrobacter sp. K-1	167
		10.2.3	. Effects of various reaction conditions	1/0
			on lactosucrose production	168
-		10.2.4	. Properties of lactosucrose	171

x Contents

11.	Soybean Oligosaccharides Yasushi Koga, Takanobu Shibata, and Robert O'Brien	175
	11.1. Saccharide Composition	175
	11.2. Soybean Oligosaccharide Manufacturing Process	178
	11.3. Soybean Oligosaccharide Physiological	
	Properties in Humans	178
	11.3.1. Nondigestible characteristics	178
	11.3.2. Promotion of bifidobacteria growth	180
	11.3.3. Suppression of putrefactive metabolites	183
	11.3.4. Improved bowel evacuation	188
	11.4. Safety	190
	11.4.1. Acute toxicity test	190
	11.4.2. Subacute toxicity test	190
	11.4.3. Mutagenicity test	190
	11.4.4. Maximum noneffective dose for	
	diarrhea	190
	11.5. Physical and Chemical Properties	191
	11.5.1. Relative sweetness and taste	191
	11.5.2. Viscosity	191
	11.5.3. Osmotic pressure	191
	11.5.4. Depression of freezing point	193
	11.5.5. Temperature and acid stability during	
	heating	193
	11.5.6. Color formation	197
	11.5.7. Relative humidity	198
	11.5.8. Water activity	198
	11.5.9. Storage	199
	11.6. Applications	199
	11.6.1. Yogurt	199
	11.6.2. Beverages	200
	11.6.3. Bread products	201
	11.6.4. Other applications	201
	11.7. Conclusion	202

12.	Neoagarooligosaccharides Toshiaki Kono			
	12.1. Agar and Its Oligosaccharides	205		
	12.2. Production Method of			
	Neoagarooligosaccharides	205		
	12.2.1. Agar-degrading enzymes (agarases)	206		
	12.2.2. Neoagarooligosaccharide production			
	from agar	206		
	12.3. Properties of Neoagarooligosaccharides	211		
	12.3.1. General properties	211		
	12.3.2. Strong prevention of starch			
	retrogradation	211		
	12.3.3. Bacteriostatic action	213		
	12.3.4. Non-digestibility and non-utilizability by			
	intestinal microorganisms	217		
Rej	rences	218		
Au	hor Index	231		
Sul	ect Index	233		

CHAPTER 1

Maltooligosaccharides

Teruo Nakakuki

The production of starch sweeteners by enzymatically decomposing starch started many years ago, but, industrially, starch syrup, glucose, etc., were first produced by acid hydrolysis. However, because of the discovery of various kinds of amylases produced by microorganisms, the acid hydrolysis process has given way to the enzymatic process of starch hydrolysis in most cases. Glucose production by the enzymatic process started with the discovery by Fukumoto et al.1 in 1959 of Rhizopus-origin glucoamylase, which produced glucose by 100% decomposition. This triggered the growth of the glucose industry. Then, with the discovery²⁻³ of debranching enzymes such as pullulanase and isoamylase, it became possible to produce pure-grade maltose through their combined use with β -amylase. Then, the situation in which the use of synthetic sweeteners such as sodium cyclohexylsulfamate was prohibited resulted in stepping up the production of high-fructose corn syrup, which was expected to have a stronger sweetness than glucose. Although studies on the production of high-fructose corn syrup were focused on chemical isomerization at the beginning, various microorganism-origin glucose isomerases have been discovered, 4-6 following the discovery of glucose isomerase produced by microorganisms by Marshall and Kooi⁷ in 1957. Thus, the technology for high-fructose corn-syrup production by the enzymatic method was established. The technique was long a glimmer in the eyes of hydrolysis researchers. A continuous isomerization process by immobilized glucose isomerase is now in wide use. Also, a process for separating glucose and fructose by using affinity liquid chromatography with cation-exchange resins has been established.8 As a result, starch hydrolyzates account for about 60% of starch consumption.

As is seen from the foregoing, the starch sweetener industry has developed together with the discovery of new enzymes, though there has been a dynamic flow of domestic and international economic factors and various changes in needs in its background. It can be said that technological applications of enzymes have progressed in parallel with the advance of the starch-hydrolyzing industry.

Starch syrup, glucose, and maltose produced from starch, and their hydrogenated saccharides – hydrogenated syrup, sorbitol, and maltitol – high-fructose corn syrup, and cyclodextrins are almost established for commercial manufacture and application. However, maltooligosaccharides of more than DP3 are expected as newcomer saccharides in the starch-sweetener production industries. Under these circumstances, new enzymes have been recently discovered one after another, including various oligosaccharide-forming amylases that specifically produce maltotriose (G3), maltotetraose (G4), maltopentaose (G5), and maltohexaose (G6) by acting on starch or amylase ⁹⁻²⁵ (Table 1). Therefore, it is possible to produce a high content of every maltooligosaccharide using these new amylases.

Table 1. Various maltooligosaccharide-forming amylases.

Product	Origin	References	
^a α-maltotriose	Streptomyces griseus	14	
	Bacillus subtilis	21	
α-maltotetraose	Pseudomonas stutzeri	9	
	P. saccharophila	23	
	B. circulans	24	
α-maltopentaose	B. licheniformis	11	
		12	
		16	
		17	
	B. subtilis	13	
	Pseudemonas sp.	20	
	B. cereus	22	
α-maltohexaose	Klebsielia pneumoiae	10	
	B. subtilis	15	
	B. circulans F-2	18	
	B. circulans G-6	19	
	Bacillus sp.	25	

^aα: anomeric configuration.