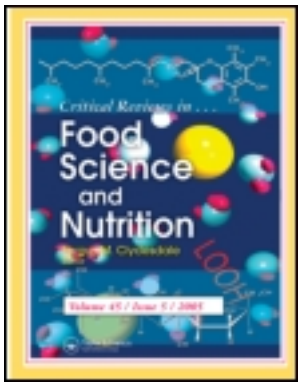

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Will Isomalto-Oligosaccharides, a Well-Established Functional Food in Asia, Break through the European and American Market? The Status of Knowledge on these Prebiotics

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Will Isomalto-Oligosaccharides, a Well-Established Functional Food in Asia, Break through the European and American Market? The Status of Knowledge on these Prebiotics

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This critical review article presents the current state of knowledge on isomalto-oligosaccharides, some well known functional oligosaccharides in Asia, to evaluate their potential as emergent prebiotics in the American and European functional food market. It includes first a unique inventory of the different families of compounds which have been considered as IMOs and their specific structure. A description has been given of the different production methods including the involved enzymes and their specific activities, the substrates, and the types of IMOs produced. Considering the structural complexity of IMO products, specific characterization methods are described, as well as purification methods which enable the body to get rid of digestible oligosaccharides. Finally, an extensive review of their techno-functional and nutritional properties enables placing IMOs inside the growing prebiotic market. This review is of particular interest considering that IMO commercialization in America and Europe is a topical subject due to the recent submission by Bioneutra Inc. (Canada) of a novel food file to the UK Food Standards Agency, as well as several patents for IMO production.

Keywords isomalto-oligosaccharides, gluco-oligosaccharides, functional food, prebiotics, transglucosylation

INTRODUCTION

In the last three decades the functional food market has been deeply ingrained in Asia and is a fast growing segment of the food industry in the United States and Europe (Tomomatsu, 1994; Hasler, 1996, 2000; Milner, 2000; Arai, 2002; Roberfroid, 2002). Regulation of the microbial ecology of the colon through the use of probiotics and prebiotics, has for decades gained special interest in the scientific consortium as well as among consumers (Fooks et al., 1999; Kolida et al., 2000; Rastall and Maitin, 2002; Lucas, 2002; Saarela et al. 2002; Manning and Gibson, 2004; Fedorak and Madsen, 2004; Rastall et al., 2005; Douglas and Sanders, 2008; Vasiljevic and Shah, 2008). However, in recent years, prebiotics tend to supersede probiotics due to various advantages such as resistance to digestive barrier, being cheaper, carrying less risks, providing new techno-functionalities, and being easier to incorporate into the diet (Roberfroid, 2002; Tuohy et al., 2005; Ouwehand et al., 2005; Manning and Gibson, 2004; Macfarlane et al., 2006). Prebiotics

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are nondigestible dietary components that pass through the digestive tract to the colon and selectively stimulate proliferation and/or activity of desired populations of bacteria indigenous to the human or animal colon in situ (Gibson and Roberfroid, 1995; Loo et al., 1999; Roberfroid, 2008; Wang, 2009). Among these, nondigestible oligosaccharides have received the most attention (Gibson and Roberfroid, 1995; Grizard and Barthomeuf, 1999; Roberfroid and Slavin, 2000; Delzenne, 2003; Rastall and Hotchkiss, 2003; Swennen et al., 2006; Mussatto and Mancilha, 2007). Research on the production of oligosaccharides for foods was started between 1970–1975 in Japan and several oligosaccharides were produced on an industrial scale from the early 1980s to the late 1990s (Nakakuki, 2003).

Among these functional oligosaccharides preparations, isomalto-oligosaccharides (IMOs) have over the years been the most developed in Asian countries, thanks to their many favorable properties for application in the food industry. IMOs are low-digestibility glucosyl "ALOs" (Anomalously Linked Oligosaccharides) (Takaku, 1958; Yatake, 1993) considered as prebiotics and anticariogenic agents (Hamada and Torii, 1980; Ooshima et al., 1988; Koga et al., 1988; Kohmoto et al., 1988; 1991; Kanno, 1990; Yatake, 1993; Kaneko et al., 1994 and 1995b; Rycroft et al., 2001; Gu et al., 2003; Zhang et al., 2003; Thitaram et al., 2005). Recent data obtained on human subjects support the involvement of dietary oligosaccharides in physiological processes in the different intestinal cell types and also outside the gastrointestinal tract (e.g., hormone production, lipid and carbohydrate metabolism immune response, etc.). (Delzenne and Williams, 2002). The composition (linkage types and DP) of prebiotic mixtures is the key factor for their efficiency throughout the structure/function relationship as well as the possible partial digestion by indigenous enzymes that can occur before reaching the colon (Sanz et al., 2005). IMOs are also known for their potential to activate the immune system, thereby enhancing the resistance to diseases and improving lipid metabolism as well as the functions of the liver and the kidneys (Wang et al., 2001; Mizubuchi et al., 2005; Li et al., 2009).

IMOs are found naturally in various fermented foods such as miso, sake, or soy sauce but also in honey (Playne and Crittenden, 2004). Commercial IMOs are produced enzymatically and are the market leader in the dietary carbohydrate sector of functional foods in Japan (Mountzouris et al., 2002). They are generally obtained industrially from starch hydrolysates (maltose and maltodextrins) through the action of the α-transglucosidase (EC 2.4.1.24) from *Aspergillus* sp. (Roper and Koch, 1988). An alternative method for producing isomalto-oligosaccharides is using an α-amylase (EC 3.2.1.3) and an α-glucosidase (EC 3.2.1.20), combined with a pullulanase (EC 3.2.1.41) (Yasuda et al., 1986). Branched IMOs or gluco-oligosaccharides (GOSs) can also be produced from sucrose by an acceptor-reaction catalyzed with dextransucrase (EC 2.4.1.5) (Paul et al., 1992; Remaud-Simeon et al., 1994). Due to the substrate nature and the transglucosylation reaction, IMO syrups often present a significant amount of low DP, i.e., deleterious digestible oligosaccharides and monosaccharides. Various methods of purification have been applied such as yeast fermentation (Pan and Lee, 2005), membrane separation process, and chromatography (Demuth et al., 2002).

As reported in a previous article (Goffin et al., 2009), High Performance Anion Exchange Chromatography (HPAEC) appears to be the only chromatographic method able to resolve complex IMO mixtures composed of structurally close isomers. However, graphitized carbon stationary phases also display interesting chromatographic stereo-selective properties compatible with the separation of geometric isomers and other closely related compounds (Goffin et al., unpublished data).

This article aims at providing evidence about the potential place of IMOs, well known functional oligosaccharides in Asia, as emergent prebiotics in the American and European functional food market. For that purpose, a detailed review of the current state of knowledge on isomalto-oligosaccharides was carried out including the description of different types of IMOs, their enzymatic routes, their methods of characterization and purification, as well as their specific properties. This review is of particular interest considering that IMO commercialization in America and Europe is a topical subject due to the recent submission by Bioneutra INC. (Canada) of a novel food file to the UK Food Standards Agency as well as several patents for IMO production (Mu et al., 2005a; 2005b). The Frost and Sullivan Institute evaluated a European market for prebiotics used in foods and beverages costing €295.5 million in 2008, and representing nearly 92,000 tons and predicted that by 2015, this figure will increase to €767m, or 205,000 tons—a compound annual growth rate of 14%.

DEFINITION AND STRUCTURE OF ISOMALTO-OLIGOSACCHARIDES

Although in a strict sense, IMO means glucosyl saccharides with only α-(1→6) linkages, commercial IMO syrup is generally accepted as a mixture of glucosyl saccharides with both α-(1→6) linkages and α-(1→4) linkages (Yun et al., 1994a). Moreover, this definition has been extended in these past years to glucooligosaccharides linked by α-(1→6) linkage and/or in a lower proportion α-(1→3) (nigerooligosaccharides) or α-(1→2) (kojioligosaccharides) glucosidic linkages found in commercial IMO syrup but also produced through other enzymatic routes (Konishi and Shindo, 1997; Chaen et al., 2001; Kobayashi et al., 2003; Yamamoto et al., 2004). Moreover, branched IMOs produced with dextransucrase, known as glucooligosaccharides (GOSs) (Paul et al., 1992; Remaud-Simeon et al., 1994), oligodextran produced by controlled-hydrolysis of dextran (Mountzouris et al., 2002), and non-reducing IMO-alditols produced via dextransucrase-catalyzed glucosylation of alditols such as glucitol, mannitol, maltitol, or Isomalt^{R} (Demuth et al., 2002) are also considered as IMOs. Indeed, all these compounds are considered as "ALOs" (Anomalously Linked Oligosaccharides) and therefore

present low digestibility by endogenous enzymes as well as providing beneficial effects to the host (Tsunehiro et al., 1999; Murosaki et al., 1999 and 2002; Yamashita et al., 2001; Chung, 2002; Yoshitaka et al., 2004; Sanz et al., 2005). Isomaltulose or palatinose (α-D-Glc$p$-(1→6)-α-D-Fru$f$) has also been described as an IMO (Moynihan, 1998). Finally, cyclic IMOs have been enzymatically produced (Oguma et al., 1994; Nishimoto et al., 2002; Mukai et al., 2005). The proportion of different linkage types depends on the substrate and the origin of the transglucosidase which specifies its tendency to form specific linkages (Pazur and French, 1951; McCleary and Gibson, 1989; Yun et al., 1994b; Wang and Rakshit, 2000; Kato et al., 2002; Kuriki et al., 1992; Nakakuki, 2005). Thus, IMO structure is characterized at the same time by their DP value (from 2 to ~10), linkages types (α-1-2, 3, 4, or 6), and the proportion and position of each type of linkage (only α-(1-6) or combined types). The most abundant IMOs are isomaltose/α-D-Glc$p$-(1→6)-α-D-Glc$p$, panose/α-D-Glc$p$-(1→6)-α-D-Glc$p$-(1→4)-D-Glc$p$, isomaltotriose/α-D-Glc$p$-(1→6)-α-D-Glc$p$-(1→6)-D-Glc$p$, kojibiose/α-D-Glc$p$-(1→2)-D-Glc$p$, nigerose/α-D-Glc$p$-(1→3)-D-Glc$p$, Glc-maltotriose/α-D-Glc$p$-(1→6)-(α-D-Glc$p$-(1→4))$_2$-D-Glc$p$, Glc-panose/α-D-Glc$p$-(1→6)-α-D-Glc$p$-(1→6)-α-D-Glc$p$-(1→4)-D-Glc$p$, and isomaltotetraose/α-D-Glc$p$-(1→6)-(α-D-Glc$p$-(1→6))$_2$-D-Glc$p$. Recently, Goffin and co-workers (2009) developed structural determination methods for unknown IMOs using 1D and 2D NMR special spectral feature and MS^2 specific fragments after GCC-LC-MS (Goffin et al., under revision).

ENZYMATIC ROUTES FOR ISOMALTO-OLIGOSACCHARIDES PRODUCTION

Commercial IMOs are enzymatically produced, as extraction from natural sources is not economic (Chung, 2002). Regio- and stereo-specificity of enzymatic synthesis is the key advantage for the production of oligosaccharides as a complementary tool to the chemical approach (Crout and Vic, 1998; Monsan and Paul, 1995). Approaches available to-date are based on two major classes of enzymes: glycosyl-transferases and glycosidases (Plou et al., 2007). Glycosyl-transferases or transglucosidases (EC 2.4.1.) were first described by Doudoroff and co-workers in 1947. This specific general class comprises of several subclasses of interest for "ALO" mixture production such as: 1,4-α-glucan 6-α-glucosyltransferase (EC 2.4.1.24), dextransucrase (EC 2.4.1.5), dextrin dextranase (EC 2.4.1.2), mutansucrase (EC 2.4.1.5.), or alternansucrase (EC 2.4.1.140). These enzymes can use simple carbohydrates of agricultural origin as glycosyl donors (Monsan and Paul, 1995). Glycosidases are hydrolytic enzymes which can catalyze either reverse hydrolysis (thermodynamic control) or transglycosylation (kinetically-controlled process) synthesis reactions to a certain extent (Perugino et al., 2004).

Various endo- or exo-enzymes, wild or genetically modified and produced by different bacteria or mold are able to produce the IMOs from various substrates at a different rate and different yield. Their activity and origin determine their specificity and therefore the degree of polymerization, linkage types, and linkage sequence of the IMOs produced (See Table 1).

Transferases

Transferase enzymes catalyze group-transfer reactions even in dilute reaction conditions. Transferase producing IMOs are non-Leloir enzymes that require neither co-factors nor activated substrates, as they directly employ the free energy of starch, dextran, or sucrose osidic bond cleavage which is stored in the covalent glycosyl-enzyme intermediate (Plou et al., 2002). Besides such intermolecular transfer, the donor molecule can also play the role of acceptor to yield an intramolecular transfer reaction producing cyclic IMOs (Oguma et al., 1994; Nishimoto et al., 2002; Plou et al., 2002; Mukai et al., 2005).

In the classical production method, which is the cheapest and the most used industrially, starch is first liquefied with a thermostable bacterial α-amylase derived from *Bacillus licheniformis* or *Bacillus stearothermophilus* (EC 3.2.1.1.), then maltogenic enzymes such as fungal α-amylase, β-amylase (EC 3.2.1.2.), and optionally-debranching enzymes such as pullulanase (EC 3.2.1.41) are used to obtain high maltose solution with residual low DP maltodextrins. IMOs are then produced through the action of a fungal α-transglucosidase (EC 2.4.1.24) from *Aspergillus sp.* (Roper and Koch, 1988; Lee and Chen, 1997). This enzyme catalyzes both hydrolytic and transfer reactions on incubation with α-D-gluco-oligosaccharides. Transglucosylation can occur even at quite low substrate concentrations (McCleary and Gibson, 1989). The non-reducing D-glucosyl residue of maltose may be transferred to water (hydrolysis), to D-glucosyl residues released by hydrolysis, or to the nonreducing residue of maltose or every α-glucooligosaccharide present in the solution. The transfer occurs most frequently to the 6-OH group of the non-reducing glucose unit, producing isomaltose from D-glucose, or panose from maltose. The enzyme can also transfer to the 2-OH or 3-OH group to form kojibiose or nigerose from glucose, or scarcely back to 4-OH to reform maltose. Maltose and maltodextrins can actually act as both glucosyl donor and acceptor in the reaction. The transglucosidase from *Aspergillus niger* acts only on oligosaccharides with a low degree of polymerization (DP) (Barker and Carrington, 1953; Pazur et al., 1978; Benson et al., 1982; McCleary and Gibson, 1989). The Hayashibara Corporation in Japan developed this transglucosylation reaction to an industrial process (Yoneyama et al., 1992).

Various transferases from different origins have been identified as branched IMOs producing enzymes using saccharose as substrate, as glucopyranosyl-fructofuranosyl linkage is a relatively high-energy bond (Côté and Tao, 1990):dextransucrase (EC 2.4.1.5), mutansucrase (EC 2.4.1.5) and alternansucrase (EC 2.4.1.140). These enzymes exhibit specific activities and are presented in Table 1.

Glucosyltransferase (EC 2.4.1.5) polymerizes the glucosyl moiety of sucrose to form dextran, an α-(1→6) linked glucan with optionally α-(1→2), α-(1→3), or α-(1→4) branch

Table 1 Examples of prebiotic "ALO" mixture producing enzymes

Glycosides formed	Enzyme(s) trivial name and EC No.	Source	Glycosyl donor(s)	Linkages formed	Branching	References
			Transferases			
IMOs	Glucosyltransferase, 2.4.1.24	*Aspergillus ssp.*	α-D-gluco-oligosaccharides	(1→6), (1→2),(1→3)	/	Duan et al., 1995
GOS	dextransucrase, 2.4.1.5	*Leuconostoc, Streptococcus spp.*	saccharose (and) co-substrates	(1→6), (1→2),(1→3)	(1→2)	*
Alternan-oligosaccharides	alternansucrase, 2.4.1.140	*Leuconostoc mesenteroides*	saccharose	α-(1→3) alterning with α-(1→6)	(1→3)	Côté and Robyt, 1982; Côté et al., 2003 Argüello-Morales et al., 2000
Mutan-oligosaccharides	mutansucrase, 2.4.1.5	*Streptococcus spp.*	saccharose	α-(1→3)	(1→6)	Mooser, 1992; Binder et al., 1983
			Cyclo-transferases			
Cyclic IMOs, linear IMOs	CycloIMO glucanotransferase	Bacillus spp.,	dextran, isomaltodextrins	(1→6)	/	Oguma et al., 1994
Cyclic maltosyl-(1→6)-maltose	CycloIMO glucanotransferase	*Arthrobacter globiformis*	starch	(1→6), (1→4)	/	Mukai et al., 2005
Cyclic tetrasaccharide	glucosyl- and glucano-transferase	*Bacillus globisporus*	α -(1→4) glucan	(1→6), (1→3)	/	Nishimoto et al., 2002
			Glycosidases			
IMOs	α-glucosidase, 3.2.1.20	fungi, bacteria, plants	α-glucosides, free D-Glc	various	/	**
Oligodextrans	glucodextranase, 3.2.1.70	*Arthrobacter globiformis*	dextran, isomaltodextrins	(1→6)	(1→2)	Kitahata et al., 1981
Oligodextrans	Isomaltodextranase, 3.2.1.94	*Arthrobacter globiformis*	dextran, isomaltodextrins	(1→6)	(1→2)	Sawai and Niwa,1975
Oligodextrans	endodextranase, 3.2.1.11	*Penicillium lilacinum*	dextran	(1→6)	(1→2)	Mountzouris et al., 2002
Oligodextrans	Neopullulanase, 3.2.1.135	*modified Bacillus stearother-mophillus*	pullulan	(1→6)	/	Takata et al., 1992

*Monsan, 2001; Kitaoka and Robyt, 1999; Lee and Chen, 1997; Seo et al., 2007; Robyt and Eckland, 1983; Demuth et al., 2002; Smiley et al., 1982; Dols–Lafargue, 1998.

**Faijes and Planas, 2007; Kato, 2002; Yamamoto et al., 2004; Duan et al., 1994;1995; Pazur et al.,1977; Vetere et al., 2000; Yamasaki et al., 1973; Kobayashi, 2003; Kita et al., 1991; Benson et al., 1982; Pazur and French, 1951; Pazur et al., 1978; Chiba, 1973;1988; Fernadez-Arrojo et al., 2007; Yamasaki, 1976.

linkages depending on the producing enzyme (Jeanes et al., 1954). When, in addition to sucrose, other carbohydrates such as glucose (Smiley et al., 1982; Tanriseven and Dogan, 2002), maltose (Killey et al., 1955; Dols et al., 1998; Kitaoka and Robyt, 1999), and isomaltose (Walker, 1973; 1977), etc., are present, some of the glucosyl groups are transferred to the carbohydrates through an "acceptor reaction" diverting the dextran formation to yield acceptor products (Koepsell et al., 1953; Robyt and Walseth,1978; Robyt and Eckland, 1983; Paul et al., 1986; Demuth et al., 2002; Plou et al., 2002). The saccharose concentration is also an important factor for the yield of the reaction (Seo et al., 2007). Details on the mechanism of glucansucrase action are presented in a review by Monchois and co-workers (1999) and by Remaud-Simeon (2000).

The dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F synthesizes a dextran containing 95% α-(1→6) and 5% α-(1→3) osidic bonds, while the *L. mesenteroides* B-1299 dextran contains 65% α-(1→6), 30% α-(1→2) and 5% α-(1→3)-branching linkages (Paul et al., 1992; Remaud et al., 1994; Dols et al. 1998; 2001). The obtained GOS usually presents a DP from 2 to 7. GOS rich in α-(1→2) linkages are of particular interest for their very low digestibility and are now produced on an industrial scale (40 metric tons/year) for their dermocosmetic and nutritional (animal and human) applications (Plou et al., 2002).

Moreover, Goulas and co-workers (2004a; 2004b) proposed a step-forward method for IMO and oligodextrans production by combining the synthesizing activity of dextransucrase and the hydrolytic activity of dextranase. This method was further improved and simplified through the use of a fusion enzyme constructed with both the enzymes and producing only linear IMOs (Kim et al., 2009).

Glycosidases

α-Glucosidases (EC 3.2.1.20, α-D-glucoside glucohydrolase) catalyze liberation of glucose from non-reducing ends of α-glucosides, α-linked oligosaccharides, and α-glucans. They show diverse substrate specificities (Chiba, 1988; Frandsen and Svensson, 1998). Theoretically α-glucosidases are capable of catalyzing transglycosylation, since they are retaining glycosyl

hydrolase (GH) (Chiba, 1997), and some α-glucosidases indeed exhibit clear transglycosylation activity (Kato et al., 2002; Faijes and Planas, 2007). This activity can be expressed by either the direct coupling of glycosyl moieties by simple reversion of the hydrolysis reaction, or the transfer of a glycosyl residue from an activated donor onto an acceptor. In the first case, shifting such reaction equilibrium from hydrolysis towards synthesis can be obtained using specific conditions such as increased reactant concentration, decreased water activity, or the removal of the reaction product by precipitation, extraction, or transformation into another product (Monsan and Paul, 1995; Watt et al., 1997). In the second case, a covalent enzymes-substrate intermediate is very often observed leading to the transfer of a glycosyl moiety from a glycoside derivative onto an acceptor molecule containing a hydroxyl group.

Various types of α-glucosidases from mammals, insects, plants, fungi, and bacteria have been purified and investigated (Yamamoto et al., 2004) and present wide substrate specificities. For example, *Aspergillus niger* (Yamasaki et al., 1973; Pazur et al., 1977; Pazur et al., 1978; Benson et al., 1982; Kita et al., 1991; Duan et al., 1995; Vetere et al., 2000; Kobayashi et al., 2003), *Aspergillus oryzae* (Pazur et al., 1951), *Aspergillus nidulans* (Kato et al., 2002), and *Saccharomyces logos* (Chiba et al., 1973), and α-glucosidases preferentially catalyze the formation of α-(1→6) glucosidic linkages in addition to hydrolysis, resulting in the production of isomaltose, panose, and tetrasaccharides with α-1,6 linkages from maltose. *Aspergillus carbonarious* α-glucosidase catalyzes α-(1→6) linkage formation but also exhibits an α-(1→4) specificity (Duan et al., 1994). Buckwheat α-glucosidase produces kojibiose, nigerose, maltose, and isomaltose from soluble starch (Chiba, 1988), while α-glucosidases from *Bacillus stearothermophilus* and brewer's yeast produce oligosaccharides consisting of α-(1→3), α-(1→4), and α-(1→6) linkages (Mala et al., 1999). *Paecilomyces lilacinus* α-glucosidase form nigerosyl- and kojibiosyl-oligosaccharides from maltose (Kobayashi et al., 2003). *Xantophyllomyces dendrorhous* synthesizes oligosaccharides with α-(1→2), α-(1→4), and α-(1→6) bonds and presented no hydrolytic activity towards α-(1→6) bonds (Fernadez-Arrojo et al., 2007). *Mucor javanicus* α-glucosidase also transfers α-glucosyl residue from maltose to the hydroxyl groups of riboflavin, pyridoxine, esculin, and rutin to synthesize the respective α-glucosides (Yamasaki et al., 1973). Moreover, *Acremonium implicatum* specifically secretes an α-glucosidase having the high ability to synthesize α-(1,3)-glucosidic linkage producing nigerosyl-oligosaccharides from maltooligosaccharides and soluble starch (Konishi and Shindo, 1997; Yamamoto et al., 2004). The transglycosylation activity of the α-glucosidases has been applied in industries together with α-amylase (EC 3.2.1.3) and pullulanase (EC 3.2.1.41) (Yasuda et al., 1986) to produce IMOs but also to conjugate sugars to biologically useful materials, aiming to improve their chemical properties and physiological functions (Yamamoto et al., 1990; Murase et al., 1997). A process with immobilized *Aureobasidium pullulans* cells has also been described (Yun et al., 1994a; 1994b).

IMOs can also be obtained from transglucosylation reactions using a neopullulanase (EC 3.2.1.135) from *Bacillus stearothermophillus*, which has been modified by site-directed mutagenesis (Kuriki et al., 1993a; 1993b; 2005). This enzyme has a hydrolyzing activity toward pullulan and proved to have a transglucosylation activity on hydrolyzed starch when it acts on high concentration of the substrate (Takata et al., 1992). The yield of IMOs was increased to more than 60.0%, compared to 45.0% obtained with the conventional system. The transglycosylation reaction of neopullulanase also produces branched IMOs (Kuriki et al., 1993b).

Finally, oligodextrans can be generated via controlled enzymatic depolymerization of dextran (Khalikova et al., 2005) using an endodextranase (EC 3.2.1.11) from *Penicillium lilacinum* (Mountzouris et al. 1999; 2002), a glucodextranase (EC 3.2.1.70) from *Arthrobacter globiformis* (Kitahata et al., 1981), or an isomalto-dextranase (EC 3.2.1.94) from *Arthrobacter globiformis* (Sawai and Niwa, 1975). Oligodextrans have considerably larger DP (Mountzouris et al., 1999) and, hence, would be less digestible than the commercially available IMOs.

Besides the enzymatic route, chemical synthesis of isomaltose and other α-glucopyranosides has been described by Berry and Dutton (1974). More recently, a stereoselective one-pot synthesis of IMOs was described by Pastuch and co-workers (2003).

PURIFICATION METHODS

There are some disadvantages in enzymatic production methods. Some of these processes are multi-step requiring gelatination, liquefaction, saccharification, and purification or hydrolysis of polysaccharides leading to the production of undesirable mono-, and/or oligosaccharides in the final product (Yoo, 1997). These digestible by-products can significantly reduce the health benefits and commercial value of the product. Membrane separation process is a purification method which removes mono- and disaccharides from the product and keeps the other oligosaccharides with higher DP (Goulas et al., 2003; Grandison et al., 2002; Charcosset, 2006). However, this process needs expensive equipment, has low efficiency, and leads to a loss of disaccharides active compounds. Another method is the adsorption-separation process which involves the removal of mono- and di-saccharides by ion exchange chromatography or monosaccharides by activated carbon column (Nakanishi et al., 1983). In particular, isomaltose can be separated from the IMO mixture using zeolite or desaluminated β-zeolite (Berensmeier and Buccholz, 2004; Holtkamp et al., 2008). However, the column capacity is low and therefore multiple recycle adsorption processes are needed. Moreover, the less expensive and the most used purification method consist of a selective fermentation of the undesired sugars. Crittenden and Playne (2002) used immobilized cells of *Zymomonas mobilis* to remove glucose, fructose, and sucrose from the unpurified IMO mixture

while Pan and Lee (2005) used *Saccharomyces cerevisae* to remove glucose and *Saccharomyces carlbergensis* to remove glucose, maltose, and maltotriose from IMO mixtures produced either from rice crumbs or tapioca flour. Previously, a branched oligosaccharides mixture was prepared from liquefied corn starch using *Bacillus licheniformis* maltogenic amylase and was purified using *Saccharomyces cerevisae* to remove glucose and maltose (Yoo et al., 1995). Furthermore, a Canadian patent (Mu et al., 2005a) from Bioneutra Inc., describes a method involving mixing the IMO syrup with yeast and carbamide as nitrogen source and culturing for 20–30 hours in order to eliminate monosaccharides. In 2003, Yoon and co-workers studied the specificity of *Saccharomyces cerevisiae* yeast on the removal of carbohydrates by fermentation. They found that D-glucose and D-fructose were completely removed, disaccharides maltose and sucrose were completely removed while isomaltose was partially removed, trisaccharides maltotriose was partially removed, and isomaltotriose was completely resistant, and so also the tetrasaccharides maltotetraose and isomaltotetraose.

Finally, a patent application was recently submitted by Goffin et al. (2008), presenting an original method which consisted of the enzymatic conversion of the deleterious glucose to gluconic acid which has been recognized as a prebiotic (Tsukahara et al., 2002). New prebiotic products can thus be produced with the synergistic action of IMO and gluconic acid. Furthermore, this uncharged glucose conversion to gluconic acid considerably facilitates its removal using anion-exchange resins. These new methods permitted the detection of new unknown IMOs which have not been described previously (Goffin et al., 2010).

ANALYSIS METHODS FOR ISOMALTO-OLIGOSACCHARIDES

Until recently, there was no original paper in the literature presenting a method able to fully separate the numerous homologues present in enzymatically produced IMO syrups. However, some references exist on the analysis of individual IMOs such as panose and isomaltose in different matrix such as serum, plasma, sake, honey, etc. (Wolfrom and Schwab, 1969; Vitek and Vitek, 1973; Mirmira et al., 1993; Perlné et al., 2000; Hayakawa et al., 2000; Cai et al., 2005; Guddat et al., 2005;), structurally close molecules or separate IMO families (one type of linkage at a time, (Schmidt and Enevoldsen, 1978; Robyt and Mukerjea, 1994)) and present a different degree of relevancy. Moreover, numerous articles relate production methods and include analytical methods to estimate the efficiency of transglucosylation (Kuriki et al., 1993b; Oguma et al., 1994; Tanriseven and Dogan, 2002; Kubik et al., 2004; Goulas et al., 2004a; 2004b) but the methods presented such as isocratic liquid chromatography with refractive index detection are unable to separate homologues.

The literature dedicated to IMO characterization is very limited. Robyt and Mukerjea (1994) presented an effective method using Thin Layer Chromatography (TLC) separation method for isomalto- and malto- oligosaccharides which could give quantitative results when using a densitometer in the reflective mode but eventually proved to be very plodding.

A Genencor patent (Li et al., 2004) described a chromatographic method using two different columns (Rezex RHM monosaccharide and Shodex Rspak oligosaccharides DC-613). The first enables the separation of oligosaccharides by their DP value while the second the separation of isomers. However, this method does not allow the full separation of IMO mixtures. Chromatography on the aminopropyl-silicagel column (LiChroSpher 100 NH2) or the strong cation exchanger column in silver form (Aminex HPX 65 A) (Paskach et al., 1991; Demuth et al., 2002) as well as C18 columns (Dols-Lafargue et al., 2001) suffered from the same loophole. Schmidt and Enevoldsen (1978) used gel filtration chromatography on isomalto-, malto-oligosaccharides and their alditols. However, this technique proved to be effective on the separate families but not on the mixture of those found in IMO preparations. Similarly, Koizumi et al. (1989) presented different HPAEC-PAD methods for the separation of each separate glucooligosaccharide family possessing α-(1-2,3,4,6) linkages respectively. Furthermore, Vinogradov and Bock (1998) studied the structure and the branching pattern of IMOs naturally present in beer. They used various techniques such as reversed phase HPLC, HPAEC-PAD, gel chromatography, and amino phase HPLC of aminocoumarin derivatives with success while Ammeraal et al. (1991) studied pullulan fragments by HPAEC-PAD. However, IMOs naturally present in beer and pullulan oligosaccharides present simplified profiles compared to enzymatically-produced IMO preparations as they are produced after hydrolysis of starch and pullulan respectively rather than through transglucosylation reaction. More recently, a study proposed an improved method using HPLC with a polymer-based amino column using the calibration curve of RI-detector response against a concentration of standard IMO and maltooligosaccharides (Nakanishi et al., 2006). However, it demands two analyses and only simple IMOs with a limited degree of complexity are efficiently separated.

Finally, a step-forward method using HPAEC-PAD, a system presenting the advantages of high sensitivity, simplicity, organic solvent freedom, and one without any need for derivatization (Paskach et al., 1991; Jahnel et al., 1998; Cataldi et al., 2000) has thus been described and validated by Goffin et al. (2009). This method is the only known one able to separate the large majority of the structurally-close oligosaccharides found in IMO preparations besides another method using graphitized carbon micro-liquid chromatography described by the same authors (Goffin et al., under revision). Indeed, graphitized carbon columns have already proved their unique ability to resolve isomeric and closely related compounds and in particular, complex oligosaccharide mixtures (Koizumi et al.,1991; Koizumi, 1996; Fan et al., 1994; Karlsson et al., 2004; Ruhaak et al., 2009).

ISOMALTO-OLIGOSACCHARIDES PROPERTIES

IMOs have been found to be of interest in the fields of foods, pharmaceuticals, and cosmetics due to their unique properties.

The most significant property and the key to their success in particular in Asian countries, is their prebiotic character, leading to better global intestinal health, mineral absorption, cholesterol regulation, immunity, as well as prevention and resistance to various diseases.

Indigestibility of IMO and Dose-Dependency Activity

Indigestibility is a key parameter in the prebiotic mechanism of oligosaccharides (Wong and Jenkins, 2007). Indeed, prebiotics pass through the small to the large intestine where they can be fermented by intestinal beneficial bacteria, act on various metabolic parameters, and be absorbed (Buchholz and Seibel, 2003). The indigestibility of prebiotics comes from the specific configuration of their linkage and the substrate specificity of the digestive gastrointestinal enzymes (Roberfroid et al., 1997; Conway, 2001). IMOs produced from hydrolyzed starch do not conform strictly to the non-digestibility criterion of potential prebiotics as they are partially digested by isomaltase in the human jejunum while the residual oligosaccharides are fermented by bacteria in the colon (Gibson et al., 2004). Kaneko et al. (1992) first used digestive system models containing artificial gastric acid, rat intestinal mucosa, or human salivary or hog pancreatic α-amylase. IMOs were not hydrolyzed by the in vitro digestive system except in the model containing rat intestinal mucosa. The same authors later confirmed these previous findings using the in situ rat jejunum loop method, demonstrating the slow and partial digestion of IMOs in the jejunum (Kaneko et al., 1995a), components with a higher DP being less digestible and hydrogenated IMO derivatives nearly indigestible. This last assertion was confirmed by Tsunehiro et al. (1999). The jejunum was chosen because it exhibits the highest digestive enzyme activities (Grizard and Barthomeuf, 1999). As such, IMOs enters the colon in variable amounts. However, the expiration rates of excess $^{13}CO_2$ and breath hydrogen in healthy humans while sedentary and while taking physical exercise indicated that a proportion of ^{13}C-labeled IMOs remains available for fermentation by intestinal bacteria (Kohmoto et al., 1992) as these gases cannot be produced by human cells. Moreover, physical exercise enhanced the usage efficiency of IMOs. Later, Oku and Nakamura (2003) compared the digestibility of fructooligosaccharides, galactosyl-sucrose, and IMOs using, again, breath hydrogen tests. IMOs appeared to be relatively highly hydrolyzed by small intestinal enzymes and high dosage was required to observe fermentation. Because of this partial digestibility, composition and dosage in the diet are primary parameters for the prebiotic potential of IMO preparations. Indeed, Kaneko and co-workers (1994) studied the effects of a commercial IMO preparation as well as two of its fractions, IMO2 and IMO3, obtained by preparative HPLC and containing mainly disaccharides (86.4%) and trisaccharides (89.9%), respectively. A dose-dependent increase of human intestinal bifidobacteria was observed within 12 days of administration and for at least an intake of 10 and 5 g/day of IMO2 and IMO3, respectively. Previously, Kohmoto et al. (1988) observed an effect on human fecal flora after a 2 weeks administration of 13.5 g/day and concluded a minimum intake of 8 to 10 g/day (1991). In an 8-day double-blind, randomized, placebo-controlled, parallel-group study, Bouhnik and co-workers (2004) found no bifidogenic effect of IMO at a 10 g/day dose. However, the treatment period is probably too short for this minimum dosage. Palframan et al. (2002), in an anaerobic batch culture fermentation inoculated with fresh fecal slurry also demonstrated a dose-dependent and pH-dependent bifidogenic effect of IMO while Ushida and Sakata (1998) found a pH-dependent bifidogenic effect on pig caecum digesta in vitro. Moreover, oral LD_{50} was estimated to be >44 g/kg and no adverse biochemical effects were noted when rats were given 3% IMO (IMO-900P; Showa Sangyo Co., Ltd.) in drinking water corresponding to an intake of 5 g per kg body weight per day. Mutagenicity tests on bacteria proved negative (Kaneko et al. 1990).

On the other hand, branched IMOs (GOS) produced from saccharose and maltose by *Leuconostoc mesenteroide* enzymes were tested in vitro through substrate utilization tests with several human gut bacteria (Djouzi et al., 1995; Wichienchot et al., 2003) and in vivo in gnotobiotic rats inoculated with human fecal flora (Djouzi and Andrieux, 1997). Branched oligomers were resistant to both gastrointestinal enzymes and utilization by pathogenic microorganisms. α-(1$\rightarrow$2) bonds were more resistant than α-(1$\rightarrow$6) in pH-controlled fermentation kinetic studies. Previously, Valette et al. (1993) showed that GOSs were only 20% digested by gnotobiotic rats, and Djouzi et al. (1995) maintained that the main component of the GOS mix, a pentasaccharide, is fully resistant to digestion. Flickinger et al. (2000) confirmed this low digestibility in the small intestine and the occurrence of bacterial fermentations in the large intestine of ileal-cannulated dogs and furthermore the rapid production of short-chain fatty acids in human fecal material. This evidence is in agreement with previous findings demonstrating the importance of IMO structure on prebiotic potential. In a more theoretical study, Sanz et al. (2005) brought into light the influence for disaccharide of the linkage type on prebiotic selectivity in vitro and found a decreasing prebiotic index for α-gluco-disaccharides with α-(1$\rightarrow$2), α-(1$\rightarrow$6), α-(1$\rightarrow$3), and α-(1$\rightarrow$4) bonds respectively.

Bifidogenic Effects

A number of in vitro and in vivo (animal and human) studies have suggested the bifidogenic properties of IMO. Kohmoto et al. (1988) were the first to conduct an in vitro pure culture study in which they tested isomaltose, isomaltotriose, panose, and the commercial product Isomalto-900 (Hyashibara Co. Ltd, Okayama, Japan). They found that *Bifidobacterium adolescentis*, *B. longum*, *B. breve*, and *B. infantis* (not *B. bifidum*) metabolized the test sugars at least as well as raffinose. IMOs were also metabolized by Bacteroides, *Enterococcus faeca*lis and *Clostridium ramnosum* but not by a range of other enteric

bacteria. Confronted by the multiplication of emerging prebiotic oligosaccharides, Palframan and co-workers (2003) developed a quantitative equation (prebiotic index, PI) to evaluate their prebiotic fermentation using in vitro methods. Their PI equation is based on the changes as related to their starting levels in key bacterial groups during fermentation (bifidobacteria, lactobacilli, clostridia, and bacteroides). The equation assumes that an increase in the populations of bifidobacteria and/or lactobacilli is a positive effect while an increase in bacteroides and clostridia (histolyticum subgroup) are negative. They included previously published prebiotic studies carried out by their research group (Rycroft et al., 2001; Palframan et al., 2003). IMOs obtained high PI values compared to other presumed prebiotics. Soon, Vulevic and co-workers (2004) ameliorated the PI by including more bacterial groups (positive bacteria eubacteria and negative bacteria *Escherichia coli*, and sulphate-reducing bacteria) fermentation end products, such as short-chain fatty acids (SCFA), and substrate assimilation aiming to compare their fermentation profiles on a quantifiable basis. IMOs again obtained a positive PI. More recently, the fastest growth rate for *B. longum* was observed with IMO supplementation (Vernazza et al., 2006).

In an animal in vivo experiment, IMO was administered to groups of 10 BABL/c mice at dose levels up to 7.5 g/kg body weight for a period of 7 days. Analysis of fecal samples revealed increases in the levels of *Bifidobacteria* and *Lactobacilli*, and inhibition of *Clostridium perfriengenes* growth (Gu et al., 2003). Moreover, in order to characterize the influence of prebiotics, including IMOs, on gut microbial ecology in mice and the persistence of the observed effects, Santos and co-workers (2006) realized the first long-term study (6 months) in mice. This study suggested that the benefits of a prebiotic-enriched diet disappeared quickly, so that a continuous prebiotic diet must be followed to maintain the reported benefits. It is worth noting that IMOs have been successfully used on broiler chickens (Zhang et al., 2003; Chung and Day, 2004; Thitaram et al., 2005; Yang et al., 2009; Rehman et al., 2009).

Studies were also carried out in vivo in humans. According to Kohmoto et al. (1988), after IMO administration (13.5 g daily for 2 weeks) to 6 healthy adult men and 18 elderly individuals, *Bifidobacteria* numbers in feces increased, on average, approximately 12-fold ($10^{8.3}$ to $10^{9.4}$ *Bifidobacteria*/g feces). IMOs could also reduce numbers of *Clostridium perfringens* and Enterobacterriaceae in vivo (Nakakuki, 1993). In another study, a group of 7 subjects was provided 10 g/day of an IMO preparation for 3 consecutive weeks followed by 1-week IMO-free interval. The IMO product was reinstated in the final week of the study. At the end of weeks 3 and 5, significant increases in levels of *Bifidobacteria*, *Lactobacilli,* and *Eubacteria* were observed in comparison to values recorded in the first week (Kaneko et al., 1993). Furthermore, in a group of 7 elderly males experiencing diarrhoea, ingestion of up to 24 g of an IMO mixture for a period of 30 days was associated with a significant increase in fecal bacterial mass in comparison to pre-treatment values (Chen et al., 2001). Additionally, Gu and co-workers (2003), after their study on BABL/c mice, collected human fecal samples (15/sex) and revealed similar results following 7 days of IMO consumption (15 g/day).

All these studies brought into light the fact that the bifidogenic effect appears to be very selective giving some opportunities for target treatments and pointing out the importance of considering each prebiotic compound for its specificity.

Beneficial Effects on Bowel Functions and Metabolism

A study was conducted on the effects of IMOs and other oligosaccharides on blood glucose and serum lipid composition in streptozotocin-induced diabetic rats by Chai and Rhee (2001). Results suggested improving the effects of dietary oligosaccharides on carbohydrate and lipid metabolism in diabetic rats. A similar study has been conducted recently on growing pigs (Li et al., 2009). IMOs did not influence the growing performance of pigs; however, it tended to increase dry matter and calcium digestibility and decreased glucose concentration in serum. IMOs also lowered the diarrhoea index and content of fecal ammonia in weaned piglets (XueLan et al., 2003). Furthermore, serum levels of triglycerides and non-esterified fatty acids were significantly lower in rats fed with 20% IMO (Kaneko et al., 1992).

Human trials have also been conducted for evaluating the overall effects of IMOs on bowel functions and metabolism. First, seven elderly males suffering from constipation went on a 30-days low fiber control diet followed by a 30-day period where diet was supplemented with IMOs (10 g/day). During the IMO ingestion, the defecation frequency increased significantly and no complaints of bloating or diarrhoea were noted. The mean wet fecal weight increased by 70% and the mean dry fecal weight by 55% (Chen et al., 2001). Then, the therapeutic efficacy of IMOs in the treatment of chronic severe constipation and its effect on lipid profiles in 20 haemodialysis patients was estimated (Wang et al., 2001). After a 2-week basal period, these patients received 30 g of IMO for a 4-week period. After the study period, reductions in total cholesterol (–17.6%) and triglycerides (–18.4%) levels were observed as well as elevation of HDL-C levels (+39.1%). The IMOs also induced a significant increase in a number of bowel movements, hence the improvement of constipation. The same group investigated the effects of IMOs on biochemical parameters, constipation, and fecal putrefactive metabolites in hyperlipidaemic subjects (ShengDun et al., 2005). After a 6 weeks treatment period, improved lipid profiles and positive effects on constipation as well as fecal putrefactive metabolites were observed. Results from Lee et al. (2003) further suggested that IMOs are more effective than fructo-oligosaccharides for preventing constipation. Moreover, IMO glycemic index was evaluated by Sheng and co-workers (2006). Twelve healthy adults were randomly divided into a xylitol group and a IMO group. Each group was orally administered 50 g of xylitol or 50 g IMO or 50 g of glucose (as control). Blood glucose was analyzed at different intervals, repeated continuously for 3 days. The glycemic index for IMOs was 34.66 ± 7.65, which represents a low GI.

IMOs were also discovered to be effective non-competitive inhibitors of α-glucosidase and therefore used to delay digestion of starch and saccharose in a therapeutic application for several diseases, including obesity, diabetes mellitus, prediabetes, gastritis, gastric ulcer, duodenal ulcer, caries, cancer, and viral diseases such as hepatitis B and C, HIV, and AIDS. A diet with 5–20% IMOs was also shown to reduce the abdominal fat tissue in mammals. (Chung, 2002). Finally, IMOs also stimulate the growth of many livestocks such as piglets and calves, and improves the feed efficiency (Kanno, 1990)

Anti-Cariogenic Properties

Another important property of IMOs is their anti-cariogenic character. Koch and Miller proposed in 1882 that dental caries are caused by insoluble glucan gums forming on the surface of teeth (plaque), and the formation of acids under this plaque which attacks the tooth enamel (Hamada, 2002). Studies with animal models showed that IMOs in place of sucrose reduce the amount of plaque formed and also reduces the amount of enamel attacking acids formed (Tsunehiro et al., 1997; Minami et al., 1989). Indeed, IMOs are metabolized to acid to a much lesser extent than glucose and sucrose when incubated with *Streptococcus mutans* in vitro (Moynihan, 1998), and plaque pH studies have shown that IMO mixes are less acidogenic compared with glucose or sucrose, but may result in a fall in pH to below 5.0 (Kaneko et al., 1995b). It has been reported that IMO mixes inhibit glucan synthesis from saccharose (Koga et al., 1988) and also inhibit the sucrose-dependent adherence of *Streptococcus mutans* on the tooth surface in vitro (Hamada and Torii, 1980). Ooshima et al. (1988) reported on the dental effects of panose- and maltose-rich sugar in vitro and in experiments on rats. *Streptococcus mutans* and *S. sobrinus* did ferment this panose rich IMO mix and produced acid in a similar way to glucose and maltose; however, the mixture was not a substrate for *S. mutans* or *S. sobrinus* glucosyltransferase and therefore did not participate in the synthesis of glucan and was shown to significantly inhibit its synthesis from sucrose, as well as inhibiting growing cells adherence to a glass surface. Animal studies also demonstrated reduced cariogenic potential of this panose-rich oligosaccharide and showed that it induced significant but minimal caries in rats which have been super-infected with either *S. mutans* or *S. sobrinus*. The same observations have been made for isomaltose-rich sugar mixture by the same group (Minami et al., 1989).

Effects on Immunity

There is increasing evidence that prebiotics like IMOs have immunomodulatory effects on systemic immune response including gut-associated lymphoid tissues system (Sung et al., 2004; Buchholz and Seibel, 2003). Studies in mice gave strong evidence that IMO might stimulate the immune system (Watanabe et al., 2002). The mechanisms by which IMOs affect immune functions are still a subject of debate. Mizubuchi and co-workers (2005) observed greater levels of IgA in feces in mice fed for 4 weeks with a diet supplemented with 20% IMO, while Sung et al. (2004) found no difference with control for a 5% supplementation. Interferon-γ production by intestinal intraepithelial lymphocytes in response to T-cell receptor triggering was greater in IMO group, indicating T helper-1 polarization of intestinal immunity by IMOs. The same interferon-γ production was observed for liver mononuclear cells together with an increased production of natural killer T-cells also found in the spleen. These results suggest that IMOs, in synergy with lactobacilli, up-regulate the Th1 response and beneficially modulate host defence. Moreover, rat and human studies demonstrated that SCFA increase the activity of natural killer cells (Pratt et al., 1996; Chiang et al., 2000) as well as IgA excretion. Butyrate has also been reported as a suppressor of the cytokine induced expression of the transcription factor NFκB in colonic cell line (Inan et al., 2000). In a recent article, Li et al. (2009) emphasized the synergistic beneficial effect of IMOs and the probiotic Bacillus OJ on intestinal microbial population, immune responses, and resistance to white spot syndrome virus in shrimp. Finally, as for the bifidogenic effect, short-chain fatty acid profiles in plasma and urine depend on the type of prebiotic ingested, pointing out the importance of considering each prebiotic compound for its specificity (Verbeke et al., 2008).

Techno-Functional Properties

Besides their beneficial effects on the intestinal tract and metabolism, IMOs have gained interest as food additives because they can replace partially or totally, liquid sugar syrups, giving new functionalities to the product. Indeed, IMOs are about half as sweet as saccharose and therefore can be used to produce different sweetness profiles. They can also be added to beer as nonfermentable sugar syrups to regulate sweetness and mouthfeel. They have been identified as good humectants with low viscosity and water activity but high moisture retaining capacity (Takaku et al., 1988). They are, thus, able to maintain texture, prevent microbial spoilage, and retard degradation in food (Yoo et al., 1995). A recent study on the quality characteristics of sponge cake formulated using, in various proportions, IMOs as a sweetener to replace saccharose, which gave positive physicochemical, microbiological, and sensory evaluations (Ching-Ching et al., 2008).

Other Imo Types

On the other hand, GOSs can be used as effective prebiotics for both birds and mammals (Chung, 2002; Chung and Day, 2004) and as an alternative to antibiotics in poultry. They have been shown to be utilized readily by *bifidobacteria* and *lactobacilli* in a pure culture study but not by *Salmonella spp*.

or *E. coli.* The fermentation properties of these oligosaccharides have been studied by Djouzi et al. (1995), who carried out a pure culture study and found that GOSs were utilized by *Bifidobacterium breve*, *B. pseudocatenulatum*, and *B. longum,* but not by *B. bifidum*. They were utilized also by *Bacteroides spp.* and *Clostridium spp.* but not by *lactobacilli*. They then carried out artificial mixed culture studies in anaerobic culture vessels using *B. thetaiotomicron*, *B. breve,* and *C. butyricum*. Gluco-oligosaccharides were then fed to germ-free rats inoculated with the three cultures used in vitro (Djouzi et al. 1995). In this model, the GOS had no effect on bacterial populations.

A similar approach, taking advantage of the bifidogenic effect of GOS, has been applied to the skin microbial flora ecology (Monsan and Paul 1995). In fact, the presence of lactic bacteria on the skin is essential for preventing the growth of detrimental microorganisms which induce skin problems (acnea, bad smell, irritation, etc.). It is possible to efficiently promote the growth of such positive bacteria by incorporating GOS in dermocosmetic formulations (Lamothe et al., 1991).

Furthermore, the results from Boucher et al. (2003) suggest the role of GOS in carbohydrate metabolism regulation. Indeed, mice, which received GOS supplemented diet showed increased glucose utilization after a 1 g/kg load of glucose, compared with control.

On the other hand, Olano-Martin et al. (2000) studied the prebiotic effect of different molecular size oligodextran produced by controlled-enzymatic depolymerization of industrial-grade dextran (Mountzouris et al., 1999). A low-molecular-mass oligodextran appeared to promote the growth of bifidobacteria and lactobacilli while producing butyrate. Using fecal batch cultures, and FISH using group specific 16S rRNA targeted probes, Rycroft et al. (2001) showed that oligodextran acting as the sole carbon source resulted in a significant increase in numbers of bifidobacteria after 24 h of batch culture. A significant increase in lactic acid and acetate was also observed.

Concerning nigerosyl-maltooligosaccharides (NOS) having α-1,3-glucosidic linkages various studies relate their implication in immunomodulatory mechanisms and systemic immune response. NOS showed immunopotentiating activities for T-helper 1-like cells immune response and for natural killer activity of hepatic mononuclear cells in mice (Murosaki et al., 1999; 2002). Yoshitaka et al. (2004) also reported the ability of NOS to increase mitogen-induced proliferation and to suppress activation-induced apoptosis of human peripheral blood mononuclear cells. Moreover, cyclic nigerosylnigerose also proved to have immunopotentiating properties (Hino et al., 2006). Anticariogenic activity was also described (Imai et al., 1984) on *Streptococcus mutans* as well as an enhancement activity of dietary calcium plasma concentration in neonatal chicks (Yamashita et al., 2001).

Recently, a new strategy has been proposed to benefit from IMO potential properties in preventing the progression of type 2 diabetes mellitus. It consists of a transglucosidase (α-glucosidase, EC 3.2.1.20) oral administration in order to produce active oligosaccharides from ingested starch in situ inside the digestive tract (Sasaki et al., 2007). The method proved to be effective for the decrease or delay of glucose absorption due to the transformation of intestinal substrate to indigestible IMOs. Furthermore, these IMOs will then be able to develop their functional properties further in the intestinal tract.

CONCLUSIONS AND PERSPECTIVES

Besides the classical IMO widely commercialized in Asia and produced from hydrolyzed starch, a wide variety of compounds have been classified as IMOs, such as koji-oligosaccharides, nigero-oligosaccharides, gluco-oligosaccharides (branched IMO), cyclic IMOs, as well as isomaltulose. Indeed, all these compounds are taken under the common denomination of "ALO" mixtures. It thus brings a great disparity in the IMO structures and composition of final products and therefore the need for step-forward analytical methods. IMOs present the advantages of being produced from highly available and relatively low-cost plant material (hydrolyzed starch, saccharose, etc.) through a simple enzymatic process compared to other functional oligosaccharides extracted from plants. However, the use of hydrolyzed starch implies the presence of residual digestible low DP malto-oligosaccharides and the co-production of glucose which not only considerably lowers the global added value of the product but also its beneficial effect on the host. Specific purification methods such as fermentation, filtration, chromatography, and more recently enzymatic conversion are therefore needed. Concerning their properties as functional food ingredients, IMOs have proved their beneficial effects on bifidogenic flora, bowel functions and metabolism, as well as on the immune system. The critical point about IMOs is their partial digestibility by endogenous enzymes leading to a lessening activity as a prebiotic. However, the choice and the mastery of the substrate and the production method can allow obtaining specific structural profiles leading to less digestible IMOs. On the other hand, a complemented dosage can be easily adjusted as IMOs can replace other oligosaccharides without changing significantly the physico-chemical properties of the final product. Moreover, the prebiotic effect specificity has been proved to be driven by the nature and the structure of the oligosaccharides. Indeed, different types of prebiotic oligosaccharides stimulate or inhibit the development of different bacteria, produce different SCFA profiles, and regulate the immune system and metabolism of the host in different ways. In this context, IMOs have their place in the American and European market and new products containing several different prebiotic oligosaccharides should be tested in order to bring into light their potential synergistic effects. Moreover, IMOs are anti-caricnogenic and present some unique techno-functional properties such as non-fermentability, low sweetness, low viscosity, and high moisture retaining properties which enable them to maintain texture, prevent microbial spoilage, and retard degradation in food.

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REFERENCES

Ammeraal, R.N., Delgado, G.A., Tenbarge, F.L., and Friedman, R.B. (1991). High-performance anion-exchange chromatography with pulsed amperometric detection of linear and branched glucose oligosaccharides. *Carbohyd Res.* **215**: 179–192.

Arai, S. (2002). Global view on functional foods: Asian perspectives. *Br J Nutr.* **88**: 139–143.

Argüello-Morales, M.A., Remaud-Simeon, M., Pizzut, S., Sarcabal, P., Willemot, R.M., and Monsan, P. (2000). Sequence analysis of the gene encoding alternansucrase, a sucrose glucosyltransferase from Leuconostoc mesenteroides NRRL B-1355. *FEMS Microbiol Lett.* **182**: 81–85.

Barker, S.A. and Carrington, T.R. (1953). Studies of *Aspergillus niger.* Part II. Transglycosidation by *Aspergillus niger. J Chem Soc. (Lond.)* **1953**: 3588–3593.

Benson, C.P., Kelly, C.T., and Fogarty, W.M. (1982). Production and quantification of transglucosidase from Aspergillus niger. *J Chem Tech Biotechnol*. **32**: 790–798.

Berensmeier, S. and Buchholz, K. (2004). Separation of isomaltose from high sugar concentrated enzyme reaction mixture by dealuminated β-zeolite. *Sep Purif Technol.* **38**: 129–138.

Berry, J.M. and Dutton, G.G.S. (1974). Synthesis of isomaltose and other α-D-glucopyranosides. *Carbohydr Res.* **38**: 339–345.

Binder, T.P., Côté, G.L., and Robyt, J.F. (1983). Disproportionation reactions catalyzed by Leuconostoc and Streptococcus glucansucrases. *Carbohydr Res*. **124**(2): 275–286.

Boucher, J., Daviaud, D., Siméon-Remaud, M., Carpéné, C., Saulnier-Blache, J.S., Monsan, P., and Valet P. (2003). Effect of non-digestible gluco-oligosaccharides on glucose sensitivity in high fat diet fed mice. *J Physiol Biochem.* **59**(3): 169–174.

Bouhnik, Y., Raskine, L., Simoneau, G., Vicaut, E., Neut, C., Flourie, B., Brouns, F., and Bornet, F.R. (2004). The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double blind, randomized, placebo-controlled, parallel-group, dose-response relation study. *Am. J Clin Nutr.* **80**(6): 1658–1664.

Cai, Y., Liu, J., Shi, Y., Liang, L., and Mou, S. (2005). Determination of several sugars in serum by high-performance anion-exchange chromatography with pulsed amperometric detection *J Chrom A*, **1085**: 98–103.

Cataldi, T.R.I., Campa, C., and De Benedetto, G.E. (2000). Carbohydrate analysis by high-performance anion-exchange chromatography with pulsed amperometric detection: The potential is still growing. *Fresenius J Anal Chem.* **368**: 739–758.

Chaen, H., Nishimoto, T., Nakada, T., Fukuda, S., Kurimoto, M. and Tsujisaka, Y. (2001). Enzymatic synthesis of kojioligosaccharides using kojibiose phosphorylase. *J Biosci Bioing.* **92**(2): 177–182.

Charcosset, C. (2006). Membrane processes in biotechnology: An overview. *Biotechnol Adv.* **24** (5): 482–492.

Chen, H.-L., Lu, Y.-H., Lin, J.-J., and Ko, L.-Y. (2001). Effects of isomalto-oligosaccharides on bowel functions and indicators of nutritional status in constipated elderly men. *J Am. Coll. Nutr.* **20**(1): 44–49.

Chiang, B.L., Sheih, Y.H., Wang, L.H., Liao, C.K., and Gill, H.S. (2000). Enhancing immunity by dietary consumption of a probiotic lactic acid bacterium (Bifidobacterium lactis HN019): optimization and defnition of cellular immune responses. *European Journal of Clinical Nutrition* **54**: 849–855.

Chiba, S., Sam, T., and Shimomura, T. (1973). Substrate specificity of saccharomyces logos α-Glucosidase. *Agr Biol. Chem.* **37**(8): 1831–1836.

Chiba, S. (1988). α-Glucosidases. **In:** Handbook of Amylases and Related Enzymes. pp. 104–105. The Amylase Research Society of Japan, Ed., Pergamon Press, Oxford, UK.

Chiba, S. (1997). Molecular mechanism in α-glucosidase and glucoamylase. *Biosci Biotechnol Biochem.* **61**: 1233–1239.

Ching-Ching, L., Hsueh-Fang, W., and Sheng-Dun, L. (2008). Effect of Isomalto-oligosaccharide syrup on quality characteristics of sponge cake. *Cereal Chem*. **85**(4): 515–521.

Chung, C.H. (2002). A potential nutraceutical from leuconostoc mesenteroïde B-742 (ATCC 13146); production and properties, PhD thesis, Sejong University.

Chung, C.-H. and Day, D.F. (2004). Efficacy of *Leuconostoc mesenteroides* (ATCC 13146)

Isomalto-oligosaccharides as a Poultry Prebiotic. *Poultry Science* **83**: 1302–1306.

Conway, P.L. (2001). Prebiotics and human health: The state-of-the-art and future perspectives. *Scandinavian J Nutr*. **45**: 13–21.

Côté, G.L. and Robyt, J.F. (1982). Isolation and partial characterization of an extracellular glucansucrase from Leuconostoc mesenteroides NRRL B-1355 that synthesizes an alternating (1→6), (1→3)-α-**D**-glucan. *Carbohydr Res.* **101**(1): 57–74.

Côté, G.L. and Tao, B.Y. (1990). Oligosaccharide synthesis by enzymatic transglycosylation. *Glycoconjugate J.* **7**: 145–162.

Côté, G.L., Holt, S.M., and Miller-Fosmore, C. (2003). Prebiotic oligosaccharides via Alternansucrase acceptor reactions. **In:** Oligosaccharides in Food and Agriculture, vol. 849, pp. 77–89. Eggleston, G. and Côté, G., Eds., ACS Symposium Series, American Chemical Society, Washington DC.

Côté, G.L. and Sheng, S. (2006). Penta-, hexa-, and heptasaccharide acceptor products of alternansucrase. *Carbohydr Res.* **41**(12): 2066–2072.

Crittenden, R.G. and Playne, M.J. (2002). Purification of food-grade oligosaccharides using immobilised cells of Zymomonas mobilis. *Appl Microbiol Biotechnol*. **58**: 297–302.

Crout, D.H. and Vic, G. (1998). Glycosidases and glycosyl transferases in glycoside and oligosaccharide synthesis. *Curr Opin. Chem Biol.* **2**: 98–111.

Delzenne, N. and Williams, C. (2002). Prebiotics and lipid metabolism. *Curr Opin. Lipidol.* **13**: 61–67.

Delzenne, N.M. (2003). Oligosaccharides: State of the art. *Proc. Nutr. Soc.* **62**: 177–182.

Demuth, K., Jordening, H. J., and Buchholz, K. (2002). Oligosaccharide synthesis by dextransucrase: new unconventional acceptors. *Carbohyd. Res.* **337**: 1811–1820.

Djouzi, Z., Andrieux, C., Pelenc, V., Somarriba, S., Popot, F., Paul, F., Monsan, P., and Szylit, O. (1995). Degradation and fermentation of α-gluco-oligosaccharides by bacterial strains from human colon – in vitro and in vivo studies in gnotobiotic rats. *J Appl Bact*. **79**(2): 117–127.

Djouzi, Z. and Andrieux, C. (1997). Compared effects of three oligosaccharides on metabolism of intestinal microflora in rats inoculated with a human fecal flora. *Brit. J Nutr.* **78**: 313–324.

Dols-Lafargue, M., Remaud-Simeon, M., Willemot, R.M., Vignon, M., and Monsan, P. (1998). Structural characterization of the maltose acceptor-products synthesized by Leuconostoc mesenteroides NRRL B-1299 dextransucrase. *Carbohydr Res.* **305**: 549–559.

Dols-Lafargue, M., Willemot, R.M., Monsan, P.F., and Remaud-Simeon, M. (2001). Factors affecting alpha-(1–2) glucooligosaccharide synthesis by Leuconostoc mesenteroides NRRL B-1299 dextransucrase. *Biotechnol Bioeng.* **74** (6): 498–504.

Doudoroff, M., Hassid, W.Z., and Barker, H.A. (1947). The mechanism of action of sucrose phosphorylase as a glucose-transferring enzyme (transglucosidase). *J Biol. Chem.* **168**: 725–32.

Douglas, L.C. and Sanders, M.E. (2008). Probiotics and Prebiotics in Dietetics Practice *J Am. Diet. Assoc*. **108**: 510–521.

Duan, K.J., Sheu, D.C., Lin, M.T., and Hsueh, H.C. (1994). Reaction mechanism of isomalto-oligosaccharides synthesis by α-glucosidase from *Aspergillus carbonarious*. *Biotechnol Lett.* **16** (11): 1151–1156.

Duan, K. J., Sheu, D.C., and Lin, C.T. (1995). Transglucosylation of a fungal α-glucosidase. The enzyme properties and correlation of

isomaltooligosaccharide production. *Ann N Y Acad. Sci.* **750**: 325–328.

Faijes, M. and Planas, A. (2007). In vitro synthesis of artificial polysaccharides by glycosidases and glycosynthases. *Carbohydr Res.* **342**: 1581–1594.

Fan, J.Q, Kondo, A., Kato, I. and Lee, Y.C. (1994). High-performance liquid chromatography of glycopeptides and oligosaccharides on graphitized carbon columns. *Anal Biochem.* **219**: 224–229.

Fedorak, R.N. and Madsen, K.L. (2004). Probiotics and prebiotics in gastrointestinal disorders. *Curr Opin. Gastroenterol.* **20**: 146–155.

Fernandez-Arrojo, L., Marrn, D., Gomez De Segura, A., Linde, D., Alcalde, M., Gutierrez-Alonso, P., Ghazi, I., Plou, F.J., Fernandez-Lobato, M., and Ballesteros, A. (2007). Transformation of maltose into prebiotic isomaltooligosaccharides by a novel a-glucosidase from *Xantophyllomyces dendrorhous*. *Process Biochem.* **42**: 1530–1536.

Flickinger, E.A., Wolf, B.W., Garleb, K.A., Chow, J., Leyer, G.J., Johns, P.W., and Fahey, G.C. (2000). Glucose-based oligosaccharides exhibit different in vitro fermentation patterns and affect in vivo apparent nutrient digestibility and microbial populations in dogs. *J Nutr.* **130**: 1267–1273.

Fooks L.J., Fuller, R., and Gibson, G.R. (1999). Prebiotics, probiotics and human gut microbiology. *Int. Dairy J.* **9**: 53–61.

Frandsen, T.P. and Svensson, B. (1998). Plant α-glucosidases of the glycoside hydrolase family 31. Molecular properties, substrate specificity, reaction mechanism, and comparison with family members of different origin. *Plant Mol Biol*. **37**: 1–13.

Gibson, G.R. and Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr.* **125**: 1401–1412.

Gibson, G.R., Probert, H.M., Van Loo, J., Rastall, R.A. and Roberfroid, M.B. (2004). Dietary modulation of the human colonic microbiota : Updating the concept of prebiotics. *Nutr. Res. Rev.* **17**: 259–275.

Goffin, D., Robert, C., Wathelet, B., Blecker, C., Malmendier, Y., and Paquot, M. (2009). A Step-forward method of quantitative analysis of enzymatically produced isomaltooligosaccharide preparations by AEC-PAD. *Chromatogr.* **69**: 287–293.

Goffin, D., Paquot, M., Blecker, C., and Robert, C. Process for the production of a composition, the composition and the use there-of as food additive. European Patent No. 09150869.7, submitted in january 2009

Goffin, D.,Bystricky, P., Shashkov, A.S., Lynch, M., Hanon, E., Paquot, M., and Savage, A.V. (2009). A systematic NMR determination of α-D-glucooligosaccharides, effect of linkage type, anomeric configuration and combination of different linkages type on ^{13}C chemical shifts for the determination of unknown isomalto-oligosaccharides. *Bull. Korean Chem Soc.* **30** (11): 2535–2541.

Goffin, D.,Karlsson, N.G., Paquot, M., and Savage, A.V. (2009). Structural analysis of α-gluco-oligosaccharides by negative ion, graphitized carbon microliquid chromatography/electrospray ionization mass spectrometry, as a tool to identify unknown Isomalto-oligosaccharides (Under revision).

Goffin, D., Wathelet, B., Blecker, C., Malmendier, Y., Paquot, M. (2010). Comparison of the glucooligosaccharide profiles produced from maltose by two different transglucosidases from *Aspergillus niger*. BASE.

Goulas, A.K., Grandison, A.S., and Rastall, R.A. (2003). Fractionation of oligosaccharides by Nanofiltration. *J Sci. Food Agric.*, **83**: 675–680.

Goulas, A.K., Fisher, D.A., Grimble, G.K., Grandison, A.S., and Rastall, R.A. (2004a). Synthesis of isomalto-oligosaccharides and oligodextrans by the combined use of dextransucrase and dextranase. *Enzyme and Microbial Technology* **35**: 327–338.

Goulas, A.K., Cooper, J.M., Grandison, A.S., and Rastall, R.A. (2004b). Synthesis of isomalto-oligosaccharides and oligodextrans in a recycle membrane bioreactor by the combined use of dextransucrase and dextranase. *Biotechnol Bioeng.* **88** (6): 778–787.

Grandison, A.S., Goulas, A.K. and Rastall, R.A. (2002). The use of dead-end and cross-flow nanofiltration to purify prebiotic oligosaccharides from reaction mixtures. *Songklanakarin J Sci. Technol.* **24**: 915–928.

Grizard, D. and Barthomeuf, C. (1999). Non-digestible oligosaccharides used as prebiotic agents: mode of production and beneficial effects on animal and human health. *Reprod. Nutr. Dev.* **39**: 563–588.

Gu, Q., Yang, Y., Jiang, G., and Chang, G. (2003). Study on the regulative effect of isomalto-oligosaccharides on human intestinal flora. *J Hyg. Res.* **32**: 54–55.

Guddat, S., Thevis, M., and Schänzer, W. (2005). Identification and quantification of the plasma volume expander dextran in human urine by liquid chromatography–tandem mass spectrometry of enzymatically derived isomaltose. *Biomed. Chrom.* **19**: 743–750.

Hamada, S. and Torii, M. (1980). Interaction of glucosyltransferase from *S. mutans* with various glucans. *J General Microbiol.* **116**: 51–59.

Hamada, S. (2002). Role of sweeteners in the etiology and prevention of dental caries. *Pure Appl Chem.* **74**(7): 1293–1300.

Hasler, C.M. (1996). Functional foods: The western perspective. *Nutr. Rev.* **54**: 6–10.

Hasler, C.M. (2000). The changing face of functional foods. *J Am. Coll. Nutr.* **19**: 499–506.

Hayakawa, K., Ando, K., Yoshida, N., Yamamoto, A., Matsunaga, A., Nishimura, M., Kitaoka, M., and Matsui, K. (2000). Determination of saccharides in sake by high-performance liquid chromatography with polarized photometric detection. *Biomed. Chrom.* **14**(2): 72–76.

Hino, K., Kurose, M., Sakurai, T., Inoue, S., Oku, K., Chaen, H., Kohno, K., and Fukuda, S. (2006). Effect of dietary cyclic Nigerosylnigerose on intestinal immune functions in mice. *Biosci Biotech. Biochem.* **70**(10): 2481–2487.

Holtkamp, M., Erhardt, F.A., Jördening, H.-J., and Scholl, S. (2008). Reaction-integrated separation of isomaltose by ad- and desorption on zeolite. *Chem Eng. Process*. **48**(4): 852–858.

Imai, S., Takeuchi, K., Shibata, K., Yoshikawa, S., Kitahata, S., Okada, S., Araya, S., and Nishizawa, T. (1984). Screening of sugars inhibitory against sucrose-dependent synthesis and adherence of insoluble glucan and acid production by Streptococcus mutans. *J Dent. Res.* **63**: 1293–1297.

Inan, M.S., Rasoulpour, R.J., Yin, L., Hubbard, A.K., Rosenberg, D.W., and Giardina, C. (2000). The luminal short-chain fatty acid butyrate modulates NF-κB activity in a human colonic epithelial cell line. *Gastroenterology* **118**(4): 724–734.

Jahnel, J., Ilieva, P. and Frimmel, F. (1998). HPAE-PAD – a sensitive method for the determination of carbohydrates. *Fresenius J Anal Chem.* **360**: 827–829.

Jeanes, A.R., Haynes, W.C., Wilham, C.A., Rankin, J.C., Melvin, E.H., Austin, M.J., Cluskey, J.E., Fisher, B.E., Tsuchiya, H.M., and Rist C.E. (1954). Characterization and classification of dextrans from ninety six strains of bacteria. *J Am. Chem Soc*. **76**: 5041–5052.

Kaneko, T., Kohmoto, T., Kikuchi, H., Fukui, F., Shiota, M., Yatake, T., Takaku, H., and Iino, H. (1992). Digestibility of isomalto-oligosaccharides by rats and effects on serum lipids. *Journal of the Japan Society for Bioscience Biotechnology and Agrochemistry* **66**(8): 1211–1220.

Kaneko, T., Kohmoto, T, Kikuchi, H., Shiota, M., Iino, H., and Mitsuoka, T. (1994). Effects of isomalto-oligosaccharides with different degrees of polymerization on human fecal bifidobacteria. *Biosci Biotechnol Biochem.* **58**(12): 2288–2290.

Kaneko, T., Yokoyama, A., and Suzuki, M. (1995a). Digestibility characteristics of isomalto-oligosaccharides in comparison with several saccharides using rat jejunum loop method. *Biosci Biotechnol Biochem.* **59**: 1190–1194.

Kaneko, T., Matsukubo, T., Yatake, T., Muramatsu, Y., and Takaesu, Y. (1995b). Evaluation of acidogenicity of commercial isomalto-oligosaccharides mixture and its hydrogenated derivative by measurement of pH responses under human dental plaque. *Biosci Biotechnol Biochem.* **59**: 372–377.

Kanno, T. (1990). Some functional properties of so-called isomalto-oligosaccharides and their applications to food industry. *Denpun Kagaku* **37**: 87–97.

Kato, N., Suyama, S., Shirokane, M., Kato, M., Kobayashi, T., and Tsukagoshi, N. (2002). Novel α-Glucosidase from *Aspergillus nidulans* with strong transglycosylation activity. *Appl Environ Microbiol.* **68**: 1250–1256.

Karlsson, N., Wilson, N., Wirth, H-J., Dawes, P., Joshi, H., and Packer, N.H. (2004). Negative ion graphitised carbon nano-liquid chromatography/mass spectrometry increases sensitivity for glycoprotein oligosaccharide analysis. *Rapid Commun. Mass Spectrom.* **18**: 2282–2292.

Khalikova, E., Susi, P., and Korpela, T. (2005). Microbial dextran-hydrolyzing enzymes: Fundamentals and applications. *Microbiol Mol Biol. Rev.* **69**(2): 306–325

Killey, M., Dimler, R.J., and Cluskey, J.E. (1955). Preparation of panose by the action of NRRL B-512 dextransucrase on a sucrose-maltose mixture. *J Am. Chem Soc.* **77**(12): 3315–3318.

Kim, A.S. and Floch, M.H. (2008). Probiotics in clinical trials from 2004 to 2007: A review of current literature. *Gast.* **134**: 694–694.

Kim, Y.-M., Seo, M.-Y., Kang, H.-K., Atsuo, K., and Kim, D. (2009). Construction of a fusion enzyme of dextransucrase and dextranase: Application for one-step synthesis of isomalto-oligosaccharides. *Enz Microb. Technol.* **44**: 159–164.

Kita, A., Matsui, H., Somoto, A., Kimura, A., Takata, M., and Chiba, S. (1991). Substrate specificity and subsite affinities of crystalline α-glucosidase from *Aspergillus niger*. *Agric Biol Chem.* **55**: 2327–2335.

Kitahata, S., Brewer, C.F., Genghof , D.S., Sawai, T., and Hehre, E.J. (1981). Scope and mechanism of carbohydrase action stereocomplementary hydrolytic and glucosyl-transfering actions of glucoamylase and glucodextranase with α- and β- D-glucosylfluorides. *J Biol. Chem.* **256**: 6017–6026.

Kitaoka, M. and Robyt, J.F. (1999). Mechanism of the action of *Leuconostoc mesenteroides* B-512FMC dextransucrase: kinetics of the transfer of D-glucose to maltose and the effects of enzyme and substrate concentrations. *Carbohydr Res.* **320**: 183–191.

Kobayashi, I., Tokuda, M., Hashimoto, H., Konda, T., Nakano, H., and Kitahata, S. (2003). Purification and characterization of a new type of a-glucosidase from Paecilomyces lilacinus that has transglucosylation activity to produce α-(1,3)- and α-(1,2)-linked oligosaccharides, *Biosci Biotechnol Biochem.* **67**: 29–35.

Koepsell, H.J. Tsuchiya, H.M. Hellman, N.N. Kazenko, A., Hoffman, C.A., Sharpe, E.S., and Jackson, R.W. (1953). Enzymatic synthesis of dextran. *J Biol. Chem.* **200**: 793–801.

Koga, T., Horikoshi, T., Fujiwara, T., and Hamada, S. (1988). Effects of panose on glucan synthesis and cellular adherence by *Streptococcus mutans. Microbiol Immunol*. **32**(1): 25–31.

Kohmoto, T., Fukui, F., Takaku, H., Machida, Y., Arai, M., and Mitsuoka, T. (1988). Effect of isomalto-oligosaccharides on human fecal flora. *Bifidobacteria Microflora* **7**: 61–69.

Kohmoto, T., Fukui, F., Takaku, H., and Mitsuoka, T. (1991). Dose-response test of isomalto-oligosaccharides for increasing fecal bifidobacteria. *Agric Biol Chem.* **55**(8): 2157–2159.

Kohmoto, T, Tsuji,K., Kaneko, T., Shiota, M., Fukui, F, Takaku, H, Nakagawa, Y., Ichikawa, T., and Kobayashi, S. (1992). Metabolism of ^{13}C- isomalto-oligosaccharides in healthy human. *Biosci Biotechnol Biochem.* **56**(6): 937–940.

Koizumi, K., Kubota, Y., Tanimoto, T., and Fukuda, M. (1989). High performance anion-exchange chromatography of homogeneous D-gluco-oligosaccharides and poly-saccharides (polymerization degree >50) with pulsed amperometric detection. *J Chrom.* **464**: 365–373.

Koizumi, K., Okada, Y., and Fukuda, M. (1991) High-performance liquid chromatography of mono- and oligo-saccharides on a graphitized carbon column. *Carbohydr Res.* **215**: 67–80.

Koizumi, K. (1996). High-performance liquid chromatographic separation of carbohydrates on graphitized carbon columns. *J Chrom A* **720**: 119–126.

Kolida, S., Tuohy, K., and Gibson, G.R. (2000). The human gut flora in nutrition and approaches for its dietary modulation. *British Nutrition Foundation Nutr. Bull.* **25**: 223–231.

Konishi, Y. and Shindo, K. (1997). Production of nigerose, nigerosylglucose, and nigerosylmaltose by Acremonium sp. S4G13. *Biosci Biotechnol Biochem.* **61**: 439–442.

Kubik, C., Sikora, B., and Bielecki, S. (2004). Immobilization of dextransucrase and its use with soluble dextranase for glucooligosaccharides synthesis. *Enzyme and Microbial Technology* **34**: 555–560.

Kuriki, T., Tsuda, M., and Imanaka, T. (1992). Continuous production of panose by immobilized neopullulanase. *J Ferm Bioeng.* **73**(3): 198–202.

Kuriki, T., Yanase, M., Takata, H, Imanaka, T., and Okadawa, S. (1993a). Highly branched oligosaccharides produced by the transglycosylation reaction of neopullulanase. *J Ferm Bioeng.* **76**(3): 184–190.

Kuriki, T., Yanase, M., Takata, H., Takesada, Y., Imanaka, T., and Okada, S. (1993b). A new way of producing isomalto-oligosaccharide syrup by using the transglycosylation reaction of neopullulanase. *Appl Environ. Microbiol.* **59**: 953–959.

Kuriki, T., Hondoh, H., and Matsuura Y.(2005). The conclusive proof that supports the concept of the α-amylase family: structural similarity and common catalytic mechanism. *Biologia, Bratislava*, **60** (16): 13–16.

Lamothe, J.P., Marchenay, Y., Monsan, P., Paul, F., and Pelenc, V. (1991). Compositions cosmétiques contenant des glucooligosaccharides. French patent FR 2678166.

Lee, S.-L. and Chen, W.-C. (1997). Optimization of medium composition for the production of glucosyltransferase by *Aspergillus niger* with response surface methodology. *Enz Microb.Tech.* **21**: 436–440.

Lee, M.R., Lee, K.A., and Ly, S.Y. (2003). Improving effects of fructooligosaccharides and isomalto-oligosaccharides contained in sponge cakes on the constipation of female college students. *J Korean Soc. Food Sci. Nutr.*, **32** (4): 621–626.

Li, F., Vadakoot, J., Duan, G., and Shetty, J.K. (2004). Grain compositions containing pre-biotic isomalto-oligosaccharides and methods of making and using same. Genencor International, Inc. World Patent WO 2004/081022.

Li, J., Tan, B., and Mai, K. (2009). Dietary probiotic Bacillus OJ and isomalto-oligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (*Litopenaeus vannamei*). *Aquacult.* **291**: 35–40.

Loo, J.V., Cummings, J., Delzenne, N., Englyst, H., Franck, A., Hopkins, M., Kok, N., Macfarlane, G., Newton, D., Quigley, M., Roberfroid, M., Vliet, T.V., and Heuvel, E. (1999). Functional food properties of non-digestible oligosaccharides: A consensus report from the ENDO project (DGXII AIRII-CT94–1095). *Brit. J Nutr.* **81**: 121–132.

Lucas, J. (2002). European Union-funded research on probiotics, prebiotics and new foods. *Digest. Liver Dis.* **34**(2): 98–104.

Macfarlane, S., Macfarlane, G.T., and Cummings, J.H. (2006). Review article: Prebiotics in the gastrointestinal tract. *Aliment. Pharmacol. Ther.* **24**: 701–714.

Mala, S., Dvorakova, H., Hrabal, R., and Kralova, B. (1999). Towards regioselective synthesis of oligosaccharides by use of α-glucosidases with different substrate specificity. *Carbohydr Res.* **322**: 209–218.

Manning, T.S. and Gibson, G.R. (2004). Prebiotics. *Best Pract. Res. Clin Gastroenterol.* **18**(2): 287–298.

McCleary, B. and Gibson, T. (1989). Purification, properties, and industrial significance of transglucosidase from *Aspergillus niger*. *Carbohydr Res.* **185**: 147–162.

Milner, J.A. (2000). Functional foods: The US perspective. Am. *J Clin Nutr.* **71**: 1654–1659.

Minami, T., Fujiwara, T., Kabawata, S., Izumitani, A., Ooshima, T., Sobue, S., and Hamada, S. (1989). Caries-inducing activity of isomaltooligosugars (IMOs) in vitro and in rat experiment. *Shoni Shikagaku Zasshi* **27**(4): 1010–1017.

Mirmira, S.R., Schreiber, M.A., and Guessford, S.A. (1993). High performance ion chromatographic analysis of glucose, isomaltose, and maltose in hydroxyethyl starch. *J. Liq. Chrom Rel.Technologies.* **16**(12): 2631–2638.

Mizubuchi, H., Yajima, T., Aoi, N., Tomita, T., and Yoshikai, Y. (2005). Isomalto-oligosaccharides polarize Th1-like responses in intestinal and systemic immunity in mice. *J Nutr.* **135**: 2857–2861.

Monchois, V., Willemot, R.-M., and Monsan, P. (1999). Glucansucrases: Mechanism of action and structure-function relationships. *FEMS Microbiol Rev.* **23**: 131–151.

Monsan, P. and Paul, F. (1995). Enzymatic synthesis of oligosaccharides. *FEMS Microbiol Rev.* **16**: 187–192.

Monsan, P., Bozonnet, S., Albenne, C., Joucla, G., Willemot, R.M., and Remaud-Simeon, M. (2001). Homopolysaccharides from lactic acid bacteria. *Int. Dairy J* **11**: 675–685.

Mooser, G. (1992). Glycosidase and glycosyltransferases. **In:** The Enzymes. Vol. 20, p. 187. Boyer, P.D., Ed., Academic Press, London.

Mountzouris, K.C., Gilmour, S.G., Grandison, A.S., and Rastall, R.A. (1999). Modeling of oligodextran production in an ultrafiltration stirred-cell membrane reactor. *Enz Microb Tech* **24**: 75–85.

Mountzouris, K.C., Gilmour, S.G., and Rastall, R.A. (2002). Continuous production of oligodextrans via controlled hydrolysis of dextran in an enzyme membrane reactor. *J Food Sci*., **67**(5): 1767–1771.

Moynihan, P.J. (1998). Update on the nomenclature of carbohydrates and their dental effects. *J Dent*. **26**(3): 209–218.

Mu, X., Xu, X., Zhong, Z., Zhu, J. and Li, X., and Thompson D. (2005a). A method for the removal of monosaccharide in oligosaccharides production. Canadian Patent CA 2474999.

Mu, X., Zhu, J., Li, X., Xu, X., Zhong, Z., and Thompson, D. (2005b). A method to control the distribution of the starch sugars molecular weight in oligosaccharides production. Canadian Patent CA 2475817.

Mukai, K., Watanabe, H., Kubota, M., Chaen, H., Fukuda, S., and Kurimoto, M. (2005). Purification, characterization, and gene cloning of a novel maltosyltransferase from an arthrobacter globiformis strain that produces an alternating α-(1,4)- and α-(1,6)-Cyclic tetrasaccharide from starch. *Appl Environ Microbiol.* **72**(2): 1065–1071.

Murase, H., Yamauchi, R., Kato, K., Kunieda, T., and Terao, J. (1997). Synthesis of a novel vitamin E derivative, 2-(α-D-glucopyranosyl) methyl-2,5,7,8-tetramethylchroman-6-ol, by α-glucosidase-catalyzed transglycosylation. *Lipids* **32**: 73–78.

Murosaki, S., Muroyama, K., Yamamoto, Y., Kusaka, H., Liu, T., and Yoshikai, Y. (1999). Immunopotentiating activity of nigerooligosaccharides for the T helper 1-like immune response in mice. *Biosci Biotechnol Biochem*. **63**: 373–378.

Murosaki, S., Muroyama, K., Yamamoto, H., Liu, T., and Yoshikai, Y. (2002). Nigerooligosaccharides augments natural killer activity of hepatic mononuclear cells in mice, *Int. Immunopharmacol*. **2**: 151–159.

Mussatto, S.I. and Mancilha, I.M. (2007). Non-digestible oligosaccharides: A review. *Carbohyd. Polym.* **68**: 587–597.

Nakakuki, T. (1993). Oligosaccharides. Production, properties and applications. **In:** Japanese Technology Reviews Vol. 3, No. 2, p. 204–229. Gordon & Breach Science Publishers, Aarborg, Norway.

Nakakuki, T. (2003). Development of functional oligosaccharides in Japan. *Trends Glycosci Glycotech.* **15**: 62–63.

Nakakuki, T. (2005). Present status and future prospects of functional oligosaccharides in Japan. *J Appl Glycosci.* **52**: 267–271.

Nakanishi, K., Matsuno, R., Torii, K., Yamamoto, K., and Kamikubo, T. (1983). Properties of immobilized β-**D**-galactosidase from *Bacillus circulan*s. *Enz Microb Technol.* **5**: 115–120.

Nakanishi, T., Nomura, S., and Takeda, Y. (2006). An improved method for the quantitative analysis of commercial isomalto-oligosaccharides products using the calibration curve of standard reagents. *J Appl Glycosci.* **53**: 215–222.

Nishimoto, T., Aga, H., Mukai, K., Hashimoto, T., Watanabe, H., Kubota, M., Fukuda, S., Kurimoto, M., and Tsujisaka, Y. (2002). Purification and characterization of glucosyltransferase and Glucanotransferase involved in the production of cyclic tetrasaccharides in Bacillus globisporus C11. *Biosci Biotechnol Biochem*. **66**(9): 1806–1818.

Oguma, T., Tobe, K., and Kobayashi, M. (1994). Purification and properties of a novel enzyme from Bacillus spp. T-3040, which catalyzes the conversion of dextran to cyclic isomalto-oligosaccharides. *FEBS Lett.* **345**: 135–138

Olano-Martin, E., Mountzouris, K.C., Gibson, G.R., and Rastall, R.A. (2000). *In vitro* fermentability of dextran, oligodextran and maltodextrin by human gut bacteria. *Brit J Nutr.* **83**: 247–255.

Ooshima, T., Fujiwara, T., Takei, T., Izumitani, A., Sobue, S., and Hamada, S. (1988). The caries inhibitory effects of GOS-sugar in vitro and in rat experiments. *Microbiol Immunol*. **32**: 1093–1105.

Ouwehand A.C., Derrien M., de Vos W., Tiihonen K., and Rautonen, N. (2005). Prebiotics and other microbial substrates for gut functionality. *Curr Opin Microbiol.***16**: 212–217.

Palframan, R.J., Gibson, G.R., and Rastall, R.A. (2002). Effect of pH and dose on the growth of gut bacteria on prebiotic carbohydrates in vitro. *Anaerobe* **8**: 287–292.

Palframan, R., Gibson, G.R., and Rastall, R.A. (2003). Development of a quantitative tool for the comparison of the prebiotic effect of dietary oligosaccharides. *Lett Appl Microbiol.* **37**: 281–284.

Pan, S.C., Andreasen, A.A., and Kolachov, P. (1950). Enzymatic conversion of maltose into unfermentable carbohydrate. *Science* **112**: 115–117.

Pan, Y.-C. and Lee, W.-C. (2005). Production of high-purity isomalto-oligosaccharides syrup by the enzymatic conversion of transglucosidase and fermentation of yeast cells. *Biotechnol Bioeng.* **89**(7): 797–804.

Pastuch, G., Wandzik, I., and Szeja, W. (2003). Stereoselective one-pot synthesis of isomalto-oligosaccharides. *Pol J Chem.* **77** (8): 995–999.

Paul, F., Oriol, E., Auriol, D., and Monsan, P. (1986). Acceptor reaction of a highly purified dextran sucrase with maltose and oligosaccharides. Application to the synthesis of controlled-molecular-weight dextrans. *Carbohydr Res.* **149**: 433–441.

Paul, F., Lopez-Munguia, A., Remand, M., Pelenc, V., and Monsan, P. (1992). Procédé de préparation enzymatique d'oligodextranes utiles dans la fabrication de substituts de sucre, et nouveaux oligodextranes. European Patent EP 0325872.

Paul, F., Lopez-Munguia, A., Remaud, M., Pelenc, V., and Monsan, P. (1992). Method for the production of α-(1,2) oligodextrans using *Leuconostoc mesenteroides* B-1299. US Patent 5141858.

Paskach, T., Licker, H.P., Reilly, P.J., and Thielecke, K. (1991). High-performance anion-exchange chromatography of sugars and sugar alcohols on quaternary ammonium resins under alkaline conditions. *Carbohydr Res.* **215**: 1–14.

Pazur, J.H. and French, D. (1951). The Transglucosidase of *Aspergillus oryzae*. *J Am Chem Soc.*, **73**(7): 3536.

Pazur, J. and French, D. (1952). The action of transglucosidase of Aspergillus oryzae on maltose. *J Biol Chem.* **196** (1): 265–272.

Pazur, J.H., Cepure, A., Okada, S., and Forsberg, L.S. (1977). Comparison of the action of glucoamylase and glucosyltransferase on D-glucose, maltose, and maltooligosaccharides. *Carbohydr Res.* **58**: 193–202.

Pazur, J.H., Tominaga, Y., DeBrosse, C.W., and Jackman, L. M. (1978). The synthesis of 1,6-anhydro-β-**D**-glucopyranose and **D**-glucosyl oligosaccharides from maltose by a fungal glucosyltransferase. *Carbohydr Res.* **61**: 279–290.

Perlné, M.I., Horváth, K., and Katona, Z. (2000). The possibilities of GC/MS and HPLC in the analysis of sugars and acids in natural matrices. *Acta Pharma Hung.* **70**(3–6): 231–238.

Perugino, G., Trincone, A., Rossi, M., and Moracci, M. (2004). Oligosaccharide synthesis by glycosynthases. *Trends Biotechnol.* **22**(1): 31–37.

Playne, M.J. and Crittenden, R.G. (2004). Part II Biotechnology strategies for producing specific food ingridents. **In:** Bioprocesses and Biotechnology for Functional Foods and Nutraceuticals, pp. 120–121, Neeser, J.R. and German, J.B., Eds., CRC Press, Boca Raton, FL.

Plou, F.J., Martín, M.T., Gomez de Segura, A., Alcalde, M., and Ballesteros, A. (2002). Glucosyltransferases acting on starch or sucrose for the synthesis of oligosaccharides. *Can J Chem.* **80**: 743–752.

Plou, F.J., Gomez de Segura, A., and Ballesteros, A. (2007) Application of glycosidases and transglycosidases in the synthesis of oligosaccharides. **In:** Industrial Enzymes, Chap. 9, pp. 141–157. Polaina, J. and MacCabe, A.P. Eds., Springer, Netherlands.

Pratt, V.C., Tappenden, K.A., McBurney, M.I., and Field, C.J. (1996). Short-chain fatty acid-supplemented total parenteral nutrition improves nonspecific immunity after intestinal resection in rats. *J Parenteral Enteral Nutr.* **20**(4): 264–271.

Rastall, R.A. and Maitin, V. (2002). Prebiotics and synbiotics: Towards the next generation. *Curr Opin Biotechnol.* **13**: 490–496.

Rastall, R.A. and Hotchkiss, A.T. (2003). Potential for the development of prebiotic oligosaccharides from biomass. **In:** Oligosaccharides in Food and Agriculture. pp. 44–53. Eggleston, G. and Côté, G.L., Eds., ACS Press, Washington, DC.

Rastall, R.A., Gibson, G.R., Harsharnjit, S.G., Guarner, F., Klaenhammer, T.R., Pot, B., Reid, G., Rowland, I.R., and Sanders, M.E. (2005).

Modulation of the microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: An overview of enabling science and potential applications. *FEMS Microbiol Ecol.* **52**: 145–152.

Rehman, H., Vahjen, W., Kohl-Parisini, A., Ijaz, A., and Zentek, J. (2009) Influence of fermentable carbohydrates on the intestinal bacteria and enteropathogens in broilers. *World's Poultry Sci J.* **65**: 75–90.

Remaud-Simeon, M., Lopez-Munguia, A., Pelec, V., Paul, F. and Monsan, P. (1994). Production and use of glucosyltransferases from Leuconostoc mesenteroides NRRL B-1299 for the synthesis of oligosaccharides containing α-(1,2) linkages. *Appl Biochem Biotechnol*. **44**: 101–117.

Remaud-Simeon M., Willemot, R.-M., Sarcabal, P., Potocki de Montalk, G., and Monsan, P. (2000). Glucansucrases: Molecular engineering and oligosaccharide synthesis. *J Mol Catal B: Enzym.* **10**: 117–128.

Roberfroid, M.B. (1997). Health benefits of non-digestible oligosaccharides. **In:** Dietary Fiber in Health and Disease, pp. 211–219. Kritchevsky and Bonfield, Eds., Plenum Press, New York.

Roberfroid, M. and Slavin, J. (2000). Nondigestible oligosaccharides. *Crit. Rev. Food Sci Nutr.* **40**(6): 461–480.

Roberfroid, M.B. (2002). Global view on functional foods: European perspectives. *Br J Nutr.* **88**: 133–138.

Roberfroid, M. (2002). Functional food concept and its application to prebiotics. *Digest Liver Dis.* **34** (2): 105–110.

Roberfroid, M.B. (2008). Prebiotics: Concept, definition, criteria, methodologies, and products. **In:** Handbook of Prebiotics, pp. 40–60. Gibson, G.R. and Roberfroid, M.B., Eds., CRC Press, Boca Raton, FL.

Robyt, J.F. and Walseth, T.F. (1978). The mechanism of acceptor reactions of leuconostoc mesenteroides B-512F dextransucrase. *Carbohydr Res.* **61**: 433–445.

Robyt, J.F. and Ecklund, S.H. (1983). Relative, quantitative effect of acceptors in the reaction of *Leuconostoc mesenteroides* dextran sucrase. *Carbohydr Res.* **121**: 279–286.

Robyt, J.F. and Mukerjea, R. (1994). Separation and quantitative determination of nanogram quantities of maltodextrins and isomaltodextrins by thin-layer chromatography. *Carbohydr Res.* **251**: 187–202.

Roper, H. and Koch, H. (1988). New carbohydrate-derivatives for biotechnical and chemical processes. *Starch* **40**: 453–459.

Ruhaak, L.R., Deelder, A.M., and Wuhrer, M. (2009). Oligosaccharide analysis by graphitized carbon liquid chromatography−mass spectrometry. *Anal Bioanal Chem.* **394**: 163–174.

Rycroft, C., Jones, M., Gibson, G., and Rastall, R. (2001). The role of prebiotics in human gut microbiology. Prebiotic oligosaccharides. *J Appl Microbiol*. **91**: 878–887.

Saarela, M., Lahteenmaki, L., Crittenden R., Salminen, S., and Mattila-Sandholm, T. (2002). Gut bacteria and health foods—the European perspective. *Int J Food Microbiol.* **78**: 99–117.

Santos, A., San Mauro, M., and Diaz, D.M. (2006). Prebiotics and their long-term influence on the microbial populations of the mouse bowel. *Food Microbiol.* **23**: 498–503

Sanz, M.L., Gibson, G.R., and Rastall, R.A. (2005). Influence of disaccharide structure on prebiotic selectivity in vitro. *J Agric Food Chem.* **53** (13): 5192–5199.

Sasaki, M., Joh T., Koikeda, S., Kataoka, H., Tanida, S., Oshima, T., Ogasawara, N., Ohara, H., Nakao, H., and Kamiya, T. (2007). A novel strategy in production of oligosaccharides in digestive tract: Prevention of postprandial hyperglycemia and hyperinsulinemia. *J Clin Biochem Nutr.* **41**: 191–196.

Sawai, T. and Niwa, Y. (1975). Transisomaltosylation activity of a bacterial isomalto-dextranase *Agric Biol Chem*. **39**(5): 1077–1083.

Seo, E.-S., Nam, S.-H., Kang, H.-K., Cho, J.-Y.,. Lee, H.-S, Ryu, H.-W., and Kim, D. (2007). Synthesis of thermo- and acid-stable novel oligosaccharides by using dextransucrase with high concentration of sucrose. *Enzyme Microb Technol.* **40**: 1117–1123.

Smiley, K.L., Slodki, M.E., Boundy, J.A., and Plattner, R.D. (1982). A simplified method for preparing linear isomalto-oligosaccharides. *Carbohydr Res.* **108**: 279–283.

Schmidt, F. and Enevoldsen, B.S. (1978). Comparative studies of malto- and Isomalto-oligosaccharides and their corresponding alditols. *Carbohydr Res.* **61**: 197–209.

Swennen, K., Courtin, C.M., and Delcour, J.A. (2006) Non-digestible Oligosaccharides with Prebiotic Properties. *Crit Rev Food Sci Nutr.* **46**: 459–471.

Takaku, H. (1988). Anomalously linked oligosaccharides mixture. **In:** Handbook of Amylases and Related Enzymes: Their Sources, Isolation Methods, Properties and Applications. pp. 215–217. The Amylase Research Society of Japan, Osaka, Japan, Ed., Pergamon Press, New York.

Takata, H., Kuriki, T., Okada, S., Takesada, Y., Lizuka, M., Minamiura, N., and Imanaka ,T. (1992). Action of neopullulanase: Neopullulanase catalyzes both hydrolysis and transglycosylation at α-(1–4)- and α-(1–6)-glucosidic linkages. *J Biol Chem.* **267**: 18447–18452.

Tanriseven, A. and Dogan, S. (2002). Production of isomalto-oligosaccharides using dextransucrase immobilized in alginate fibres. *Process Biochem.* **37**: 1111–1115.

Thitaram, S.N., Chung, C.H., Day, D.F., Hinton, A., Bailey, J.S., and Siragusa, G.R. (2005). Isomaltooligosaccharide increases cecal bifidobacterium population in young broiler chickens. *Poultry Sci.,* **84**: 998–1003.

Tomomatsu, H. (1994). Health effects of oligosaccharides. *Food Technol.* **48**: 61–65.

Tsukahara, T., Koyama, H., Okada, M., and Ushida, K. (2002). Stimulation of butyrate production by gluconic acid in batch culture of pig cecal digesta and identification of butyrate-producing bacteria. *J Nutr.* **132**: 2229–2234.

Tsunehiro, J., Matsukubo, T., Shiota, M., and Takaesu, Y. (1997). Caries-inducing activity of the hydrogenated derivative of an isomaltooligosaccharide mixture in rats. *Biosci Biotechnol Biochem.* **61**(8): 1317–1322.

Tsunehiro, J., Okamoto, K., Furuyama, Y., Yakate, T., and Kaneko, T. (1999). Digestibility of the hydrogenated derivative of an Isomaltooligosaccharide mixture by rats. *Biosci Biotechnol Biochem.* **63**(9): 1515–1521.

Tuohy, K.M., Rouzaud, G.C.M., Brück, W.M., and Gibson, G.R. (2005). Modulation of the human gut microflora towards improved health using prebiotics: Assessment of efficacy. *Curr Pharm Des.* **11**: 75–90.

Valette, P., Pelenc, V., Djouzi, Z., Andrieux, C., Paul, F., Monsan, P., and Szylit, O. (1993). Bioavailability of new synthesized glucooligosaccharides in the intestinal tract of Gnotobiotic rats. *J Sci Food Agric.* **62**: 121–127.

Vasiljevic, T. and Shah, N.P. (2008). Probiotics—from Metchnikoff to bioactives. *Int Dairy J* **18**: 714–728.

Verbeke, K., Ferchaud-Roucher, V., Preston, T., Small, A.C., Henckaerts, L., Priebe, M., and Vonk, R.J. (2008). Short chain fatty acid profiles in plasma and urine of healthy volunteers depend on the type of indigestible carbohydrate. *Gast* **134**: 692–692.

Vernazza, C.L., Gibson, G.R., and Rastall, R.A. (2006). Carbohydrate preference, acid tolerance and bile tolerance in five strains of Bifidobacterium. *J Appl Microbiol*. **100**: 846–853.

Vetere, A., Gamini, A., Campa, C., and Paoletti, S. (2000). Regiospecific transglycolytic synthesis and structural characterization of 6-O-a-glucopyranosyl-glucopyranose (isomaltose). *Biochem Biophy Res Commun.* **274**: 99–104.

Vinogradov, E. and Bock, K. (1998). Structural determination of some new oligosaccharides and analysis of the branching pattern of isomaltooligosaccharides from beer. *Carbohydr Res.* **309**: 57–64.

Vitek, V. and Vitek, K. (1973). Chromatography of sugars in body fluids IV. Separation of isomaltose and lactose in urine by paper chromatography. *Biochem Med.* **7**: 119–127.

Vulevic, J., Rastall, R.A. and Gibson, G.R. (2004). Developing a quantitative approach for determining the in vitro prebiotic potential of dietary oligosaccharides. *FEMS Microbiol Lett.* **236**: 153–159.

Walker, G.J. (1973). Preparation of isomaltose oligosaccharides labelled with ^{14}C in the non-reducing terminal unit, and their use in studies of dextranase activity. *Carbohydr Res.* **30**: 1–10

Walker, G.J. (1977). Synthesis of oligosaccharides of the isomaltose series labelled with ^{14}C at the reducing end. *Carbohydr Res.*, **53**: 263–267.

Wang, X. and Rakshit, S. (2000). Iso-oligosaccharide production by multiple forms of transferase enzymes from Aspergillus foetidus. *Process Biochem.* **35**: 771–775.

Wang, H.-F. , Lim, P.-S. , Kao, M.-D., Chan, E.-C , Lin, L.-C., and Wang, N.-P. (2001). Use of isomalto-oligosaccharide in the treatment of lipid profiles and constipation in hemodialysis patients. *J Ren Nutr.* **11**(2): 73–79.

Wang, Y. (2009). Prebiotics: Present and future in food science and technology. *Food Res Int.* **42**: 8–12.

Watanabe, T., Watanabe, M., and Kageyama, S. (2002) Prophylactic or ameliorating agent for immunological dysfunction, Prophylactic or ameliorating agent for microbism, tumor immunological enhancer and Prophylactic or ameliorating agent for in vivo various dysfunctions and functional food comprising $\alpha(1\rightarrow6)$-bonded chain glucose oligomer as active ingredient. Japanese Patent, JP 2002161039.

Watt, G.M., Lowden, P.A.S., and Flitsch, S.L. (1997). Enzyme-catalyzed formation of glycosidic linkages. *Curr Opin Struct Biol.* **7**: 652–660.

Wichienchot, S., Prasertsan, P., Hongpattarakere, T., Gibson, G.R., and Rastall, R.A. (2003). In vitro fermentation of mixed linkage glucooligosaccharides produced by gluconobacter oxydans NCIMB 4943 by the human colonic microflora. *Curr Issues Intestinal Microbiol.* **7**: 7–12.

Wolfrom, M.L. and Schwab, G. (1969). Quantitative analysis of gentibiose and isomaltose in admixture and its application to the characterization of dextrins. *Carbohydr Res.* **9**: 407–413.

Wong, J.M.W. and Jenkins, D.J.A. (2007). Carbohydrate digestibility and metabolic effects. *J Nutr.* **137**: 2539S-2546S.

Yamamoto, I., Muto, N., Nagata, E., Nakamura, T., and Suzuki, Y. (1990). Formation of a stable l-ascorbic acid α-glucoside by mammalian α-glucosidase- catalyzed transglucosylation. *Biochim Biophys Acta*, **1035**: 44–50.

Yamamoto, T., Unno, T., Watanabe, Y., Yamamoto, M., Okuyama, M., Mori, H., Chiba, S., and Kimura, A. (2004). Purification and characterization of Acremonium implicatum a-glucosidase having regioselectivity for α-(1,3)-glucosidic linkage. *Biochim Biophys Acta* **1700**: 189–198.

Yamasaki, Y., Miyake, T., and Suzuki, Y. (1973). Properties of crystalline α-glucosidase from *Mucor javanicus*. *Agric Biol Chem.* **37**: 251–259.

Yamasaki, Y., Suzuki, Y., and Ozawa, J. (1976). Purification and properties of α-glucosidase from *Penicillium purpurogenum*. *Agric Biol Chem.* **40**: 669–676.

Yang,Y., Iji, P.A., and Choct, M. (2009). Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poultry Sci J.* **65**: 97–114.

Yasuda, E.H, Takaku, H., and Matsumoto, H. (1986). Japanese Patent, JP 61–212296.

Yatake, T. (1993). Anomalously linked oligosaccharides in oligosaccharides. **In:** Production, Properties and Application. pp. 79–89. Nakakuki, T., Ed., Gordon and Breech Science Publishers, Tokyo, Japan.

Yoneyama, M., Shibuya, T., and Miyake T. (1992). Saccharides sous forme de poudre, préparation et utilisations. French Patent, FR 2677359.

Yoo, S.H., Kweon, M.R., Kim, M.J., Auh, J.H., Jung, D.S., Kim, J.R., Yook, C., Kim, J.W., and Park, K.H. (1995). Branched oligosaccharides concentrated by yeast fermentation and effectiveness as a low sweetness humectant. *J Food Sci.* **60**(3): 516–519.

Yoo, S.K. (1997). The production of glucooligosaccharides by *Leuconostoc mesenteroides*

ATCC 13146 and *Lipomyces starkeyi* ATCC 74054. Ph.D. Dissertation, Louisiana State University.

Yoon, S.-H., Mukerjea, R., and Robyt, J.F. (2003). Specificity of yeast (*Saccharomyces cerevisiae*) in removing carbohydrates by fermentation. *Carbohydr Res.* **338**: 1127–1132.

Yun, J., Lee, M., and Song, S. (1994a). Continuous production of isomalto-oligosaccharides from maltose syrup by immobilized cells of permeabilized *Aureobasidium Pullulans*. *Biotechnol Lett.* **16**: 1145–1150.

Yun, J., Suh, J.H., and Song, S. (1994b). Kinetic study and mathematical model for the production of isomalto-oligosaccharides from maltose by transglucosylation of *Aureobasidium pullulans. J Korean Inst Chem Eng.* **32**(6): 875–880.

Zhang, F., Li, D.F., Lu, W.Q., and Yi, G.F. (2003). Effects of isomalto-oligosaccharides on broiler performance and intestinal microflora. *Poultry Sci.* **82**: 657–663.