

## **Selected Scientific Literature**

### a) IMO as a Dietary Fiber:

The carbohydrate compositional analysis by HPLC confirmed that IMO is a mixture of short-chains carbohydrates consists of glucose units in 2-7 or more. Therefore, it is defined as a short-chained fiber molecule (resistant oligosaccharide) (AACC Definition, 2000). The routine analysis of fiber content in IMO is being performed using HPLC-RI system and is based upon the published scientific papers;

#### **1) “The Definition of Dietary Fiber”**

[AACC Report (March 2001) "The Definition of Dietary Fiber", Vol. 46, No. 3, Page 112]

Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, **oligosaccharides**, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.

#### **2) “Quantitative Analysis of commercial Isomalto-oligosaccharide”**

[Taisuke N., et al, (2006) An improved method for the quantitative analysis of commercial IMO products using the calibration curve of standard reagents. J. Appl. Glycosci. 53;215-222]

An improved method for the quantitative analysis of IMO products by HPLC with polymer-based amino column was developed. A commercial IMO was analyzed and results showed the mixture of short chain oligosaccharide with variable quantity of individual components.

#### **3) “Nondigestible Oligo- and Polysaccharides (Dietary Fiber)”**

[B.C. Tunland and D. Meyer; Comprehensive Reviews in Food Science and Food Safety; "Nondigestible oligo- and polysaccharides (dietary Fiber): Their physiology and role in human health and food", 2002, vol. 1, page 73-92]

Non-digestible oligosaccharides occur naturally in many foods. The physicochemical and biological properties of these compounds, as they relate to dietary fiber, are associated with physiological actions in the small and large intestine, having important implications in human health. These properties include water dispensability and solubility, viscosity effects, bulk, absorption and fermentability, and binding of other compounds. These features may lead to various physiological actions such as reducing cholesterol and attenuating blood glucose, maintaining gastrointestinal health and positively affecting calcium bioavailability and immune function. Mover, based on their physicochemical properties, any of the new oligosaccharide can help improve the organoleptic properties and nutritional value of foods.

#### 4) “Determination of IMO in sake by HPLC”

[Hayakawa K., et al., (2000) "Determination of saccharides in sake by HPLC with polarized photometric detection", Biomed. Chromatogr 14:72-76]

A high-performance liquid chromatographic method has been developed for the determination of saccharides in sake, an alcoholic beverage brewed from rice. Saccharides in sake were separated on a normal phase (carbamoyl bonded silica) column using a linear gradient elution of water in acetonitrile. Seven saccharides, glucose, maltose, isomaltose, maltotriose, panose, isomaltotriose and ethyl  $\alpha$ -D-glucoside, were determined by a polarized photometric detector. Unidentified peaks suggesting saccharides with polymerization degrees over 4 were also observed. The proposed method did not require any sample clean-up treatment. As an application, saccharide compositions in various kinds of sake were compared.

#### b) IMO as a Prebiotic:

##### 1) **IMO was effective at increasing numbers of *Bifidobacterium* and *Lactobacillus* whilst generating the least gas ..... A Fermentation Study”**

[Rycroft, C.E.; Jones, M.R.; Gibson, G.R.; Rastall, R.A. 2001. A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. J Appl Microbiol 91(5):878-887]

Comparison of *in-vitro* fermentation properties of commercial prebiotic oligosaccharides, including IMO was studied. Populations of predominant gut bacterial groups were monitored over 24 hrs of batch culture through fluorescent *in-situ* hybridization. Gas production and short-chain fatty acid were also measured. Gas production was lowest on IMO and highest on inulin. IMO was effective at increasing numbers of *Bifidobacteria* and *Lactobacilli* whilst generating the least gas.

##### 2) **“*Bifidobacterium* and the *Bacteroides* group from human intestine could utilize IMO but *E. coli* and other bacteria could not ..... A Human Study”**

[Kohmoto, T.; Fukui, F.; Takaku, H.; Machida, Y.; Arai, M.; Mitsuoka, T. 1988. Effect of isomalto-oligosaccharides on human fecal flora. Bifidobacteria Microflora 7(2):61-69]

The effect of IMO on human fecal flora was studied. *Bifidobacteria* and the *Bacteroides* group from human intestine could utilize IMO but *E. coli* and other bacteria could not. After the administration of IMO (13.5 g daily for 2 weeks) to 6 healthy adult men & 18 elderly individuals, the numbers of *Bifidobacteria* in the feces observed to be increased several fold. On average, an approximate 12 –fold increase was observed in the number of *Bifidobacteria* ( $10^{8.3}$  to  $10^{9.4}$  *Bifidobacteria*/g feces) in the 12 subjects.

##### 3) **“IMO-induced stimulation of *Bifidobacterium* growth and a suppression of *Clostridium* growth ..... A Laboratory Animal Study”**

[Kaneko, T.; Kohmoto, T.; Fukui, F.; Akiba, T.; Suzuki, S.; Hirao, A.; Nakatsuru, S.; Kanisawa, M. 1990. [Acute and chronic toxicity and mutagenicity studies on isomaltooligosaccharides, and the effect on peripheral blood lymphocytes and intestinal microflora]. *Shokuhin Eiseigaku Zasshi* 31(5):394-403]

In male Wistar rats provided 3% of an IMO mixture in the drinking water (2.7 to 5.0 g IMO/kg body weight/day) for a period of 12 months, a significant increase was observed in the levels of intestinal *Lactobacilli* following 3 months of treatment. The authors also noted an IMO-induced stimulation of *Bifidobacterium* growth and a suppression of *Clostridium* growth.

**4) “Studies showed increased levels of *Bifidobacterai* and *Lactobacilli*, and inhibition of *Clostridim perfriengenes* growth ..... A Human & Laboratory Animal Study”**

[Qing, G.; Yi, Y.; Guohong, J.; Gai, C. 2003. [Study on the regulative effect of isomaltooligosaccharides on human intestinal flora]. *Wei Sheng Yan Jiu* 32(1):54-55]

IMO was administered to groups of 10BABL/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 *Bifidobacterai* and *Lactobacilli*, and inhibition of *Clostridim perfriengenes* growth. Analysis of human fecal samples (15/sex) revealed similar results following 7 days of IMO consumption at a dose of 15 g per day.

**5) “IMO increased fecal bacterial mass in diarrhea patients ..... A Human Study”**

[Chen, H.-L.; Lu, Y.-H.; Lin, J.-J.; 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]

In a group of 7 elderly males experiencing diarrhea, ingestion of up to 24g 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.

**6) “Significant increase in levels of *Bifidobacteria*, *Lactobacilli* & *Eubacteria* ..... A Human Study”**

[Kaneko, T.; Komoto, T.; Kikuchi, H.; Shiota, M.; Yatake, T.; Iino, H.; Tsuji, K. 1993. [Effects of isomaltooligosaccharides intake on defecation and intestinal environment in healthy volunteers]. *Nihon Kasei Gakkaishi* 44(4):245-254]

A group of 7 subjects was provided 10 g of an IMP preparation per day for 3 consecutive weeks followed by 1-week IMO-free interval. The IMO product was reinstated in the final week of the study (week 5). At the end of weeks 3 & week 5, significant increases in levels of *Bifidobacteria*, *Lactobacilli* and *Eubacteria* were observed in comparison to values recorded in the first week.

**7) “Minimum effective dosage of IMO to increase number of fecal *Bifidobacterium* ..... A Human Study”**

[Kohmoto, T.; Fukui, F.; Takaku, H.; Mitsuoka, T. 1991. Dose-response test of isomaltooligosaccharides for increasing fecal bifidobacteria. *Agric Biol Chem* 55(8):2157-2159]

In a dose-response studies of IMO for increasing fecal *Bifidobacterium*, the minimum effective dosage of IMO was found 10 gm/day for a period of 14 days consumed by 9 healthy individuals, and the result was a significant increase in the number of fecal *Bifidobacterium*.

**8) “An intake of IMO resulted significant increase of Bifidobacterial number in feces..... A Human Study”**

[Kaneko, T., Kohmoto, T., Kikuchi, H., Shito, M., Iino, H. and Mitsuoka, T. (1994) [Effect of isomaltooligosaccharides with different degrees of polymerization on human fecal bifidobacteria] *Biosci. Biotech. Biochem.* 58(12), 2288-2290]

The administration of IMO in amount from 5 to 20 g/day increased human intestinal *Bifidobacteria* in dose-dependent manner. An IMO intake of 10g/day and 5g/day produced a significant increase of *Bifidobacterial* number in feces and the ratio in fecal microflora within 12 days.

**9) “IMO was found to be well utilized by the five strains of Bifidobacteria..... In-vitro culturing Study”**

[Claire L. Vernazza, et al., (2006) "Carbohydrate preference, acid tolerance and bile tolerance in five strains of Bifidobacteria" *J. Appl. Microbiol.* 100;846-853]

Five strains of Bifidobacteria were analyzed for their carbohydrate preference from 12 substrates. GOS and IMO were well utilized by all the test species. This study allows the comparison of the properties of bifidobacteria, allowing a cost effective screen for the best species for use in symbiotic products to allow better survival and efficacy.

c) IMO Exhibit Low Glycemic Index (GI)

**1) “IMO has low glycemic index in adult healthy human.....A Human Study**

Sheng, G. E., Dong-lian, C. A. I. & Wan, Li-li. (2006) [Determination of glycemic index of xylitol and isooligosaccharide] *Chin. J. Clin. Nutr.*, 14(4) 235-237

Twelve healthy adults were randomly divided into xylitol group and isomaltooligosaccharide group. Each group was orally administered 50 g of xylitol or 50 gm IMO & 50 gm of glucose (as control). Blood glucose was analyzed at different intervals after oral intake of xylitol, IMO or glucose. This study repeated continuously for 3 days, and glycemic index were calculated. The glycemic index for IMO was  $34.66 \pm 7.65$  which represent a low GI.

d) IMO Relives Constipation & Lowered Blood Cholesterol

**1) “Supplementation of IMO into diets may be practical in relieving constipation in the elderly population..... A Human Study”**

Hsiao-Ling Chen, et al., (2001) [Effects of Isomalto-oligosaccharides on bowel functions and indicators of nutritional status in constipated elderly men] J Amer. College. Nutri., 20 (1), 44-49

Seven older male subjects participated in this study that consisted of a 30-day control low fiber period followed by a 30-day IMO-supplemented (10 gm) experimental period. Bowel functions such as defecation, enema use and bloating were monitored daily. Incorporation of IMO significantly increased the defecation frequency, wet stool output and dry stool weight by twofold, 70% & 55%, respectively. Consumption of IMO effectively improved bowel movement, stool output and microbial fermentation in the colon without any adverse effect observed in this study.

**2) “IMO is effective in increasing bowel frequency, improving constipation, lowering total cholesterol and triglycerides and in raising HDL-C in hemodialysis patients .....A Human Study”**

Hsueh-Fang Wang, et al., (2001) [Use of isomaltooligosaccharide in the treatment of lipid profiles and constipation in hemodialysis patients] J. Renal Nutri., 11 (2) 73-79

This clinical trial study evaluates the therapeutic efficacy of IMO in the treatment of chronic severe constipation and its effect on lipid profiles in 20 hemodialysis (HD) patients. After a 2-week basal period, these patients were received 30 g of IMO for a 4-week period. After the study period, those patients receiving IMO had reductions in levels of total cholesterol -17.6% , triglycerides -18.4%, and elevation of levels of HDL-C by +39.1%. Also, IMO induced a significant increase in number of bowel movements and hence improvement of constipation 76.3% + 30.9% of patients during the 4-week treatment. In conclusion; IMO once a day is effective in increasing bowel frequency and improving constipation in HD patients. In addition, IMO treatment was effective in lowering total cholesterol and triglycerides and in raising HDL-C in HD patients.