A Survey of Commercially Available Isomaltooligosaccharide-Based Food Ingredients

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Abstract: Isomaltooligosaccharides (IMOs) are included in many commercially available food products including protein/fiber bars, shakes, and other dietary supplements. Marketed as "high fiber," "prebiotic soluble fiber," and/or as a "low-calorie, low glycemic sweetener," IMO may be present in significant amounts, for example, more than 15 g/item or serving. Herein, high-pressure anion exchange chromatography with pulsed amperometric detection and high-pressure liquid chromatography with differential refractive index detection are used to compare 7 commercially available IMO-containing bulk food ingredients. The ingredients are typical of those produced either (a) via bacterial fermentation ("fermented" IMO or MIMO) of sucrose in the presence of a maltose acceptor mediated by a glucosyltransferase enzyme (dextransucrase), or (b) via transglycosylation of hydrolyzed starch with α-glucosidase ("industrial" IMO). Analysis of the results with respect to digestibility suggests that the potential glycemic impact of the ingredients and products containing "industrial" IMO may be inconsistent with the product labeling and/or certificates of analysis with respect to overall fiber content, prebiotic fiber content, and glycemic response and are thus inappropriate for diabetic patients and those on low-carbohydrate (for example, ketogenic) diets.

Keywords: carbohydrates diabetes, dietary fiber, oligosaccharides, prebiotics

Practical Application: This analysis comparing 7 commercially available isomaltooligosaccharide-based food ingredients demonstrates that most of these products are, by way of definition, and particularly with respect to content of "oligosaccharides" and "dietary fiber," mislabeled. This is significant because claims, such as "low glycemic," "zero calorie," and the like, are certainly false, and may pose a health hazard to certain populations (diabetic patients and epileptic patients on ketogenic diets, in particular) while misleading others (those on low carbohydrate diets). We conclude that labeling requirements should be reconsidered for products of this type.

Introduction

Oligosaccharides are small polysaccharides ranging in degree of polymerization (DP) from 3 to 9 or "oligosaccharides DP > 2" (Jones 2014), or as "carbohydrates consisting of between 2 disaccharides and 10 monosaccharides connected by either an alpha- or beta-glycosidic link" (National Library of Medicine 2011). Isomaltooligosaccharides (IMOs) are glucooligosaccharide (GlcOS) homooligomers, and can be subdivided into 3 types. Type 1, known as "6-O-α-isomaltooligosyl-D-maltose," "IMOMs," "maltosylisomaltooligosaccharides," or "MIMOs," are linear α-1,6 oligoglucan chains terminated to glucose via an α-1,4 glycosidic linkage. Panose is the representative trisaccharide, and homologs are referred to as PAN-type oligosaccharides, here. Type 2, also known as oligodextrans, are linear α-1,6 oligoglucans that include isomaltotriose (IMT) as the representative trisaccharide, and are, henceforward, referred to as IMT-type oligosaccharides. Type 3 are linear chains of either the type 1 or type 2, but are also branched to glucose via α-1,2, α-1,3, or α-1,4 linkages. These side chains may extend beyond a single residue, usually via α-1,6 linkages, or the novel centose-type (Shi and others 2016), originally proposed by Goffin and others (2010).

The production of IMOs studied herein is accomplished via 1 of 2 methods. Method A involves the transglycosylation (TG) of saccharified vegetable starch (typically corn or cassava/tapioca) with an α-glucosidase enzyme, typically from *Aspergillus niger spp* (Kwon and others 2011). This method yields a mixture of type 1 and type 2 IMOs. This is the method by which "industrial" IMOs (6 exemplars) are produced. Resistant maltodextrin (RMDx) should also be evident as a side product resulting from the hydrolysis of starch via α-amylase, for example, limit dextrins. Because this approach can yield a significant amount of glucose, this is typically removed via secondary fermentation with yeast (Zhong and others 2005). Method B involves the fermentation of sucrose with a maltose acceptor in the presence of a bacterial organism capable of expressing the dextransucrase (1,6-α-D-glucan 6-α-D-glucosyltransferase [GT]) enzyme (Day and Chung 2004). This method yields IMOs of type 1 (very small amounts), but types 2 and/or 3 predominate, and broths may contain, depending on the organism employed, a significant quantity of fructose and/or mannitol (depends on the metabolic profile). The branching pattern, or lack thereof, of the oligomeric product is likewise dependent on the organism employed, and can be sufficiently specific as to facilitate speciation. Commercial products manufactured this way are usually purified to remove color bodies and acidic (lactic, acetic acids, and so on) metabolites that can contribute to off-flavors.

IMOs produced using the methods A and B (linear) are currently available on the commercial market in the United States.

Materials and Methods

Several bulk IMO food ingredients were acquired and used as-is. The materials included 1 "fermented" IMO and 6 "industrial" IMOs. ISOThrive™ Prebiotic Nectar (ISOThrive, LLC., Healdsburg, Calif. U.S.A. and Manassas, Va., U.S.A.), the only

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"fermented" IMO, is made up of a mixture of mainly linear type 2/PAN-type oligosaccharides (DP 3 to 8, 85% PAN-type IMO) and a very small fraction of type 1/IMT-type (DP 3 to 4, <1% IMT-type IMO) prepared via fermentation of a sucrose donor in the presence of a maltose acceptor via dextransucrase (*Leuconostoc citreum* NRRL B-742) as described by Madsen and others (2015). The 6 "industrial" IMOs manufactured via TG of starch hydrolysate and typically followed by a yeast fermentation to remove residual glucose include (product name and description provided by manufacturer):

- IMO-900 powder (Baolingbao Biotechnology, Co. LTD., Yucheng Shandong, China, >90% IMO).
- AdvantaFiber 90 powder, non-GMO soluble fiber sweetener (Top Health Ingredients, Inc. (Edmonton Canada) 2014; country of manufacture: China, 90% IMO).
- Wako IMOs (Wako Pure Chemical Industries, LTD., Osaka, Japan).
- FiberYum™ Prebiotic sweetener, sugar free (Raw Indulgence LTD., Hawthorne, N.Y., U.S.A.; packed in the United States, 100% fiber/dry solids based on 5 g total carbohydrate = 5 g fiber, from tapioca starch).
- VitaFiber™ prebiotic fiber sweetener, powder from corn (China), and syrup from tapioca (Indonesia, Bioneutra Global Corp., Edmonton, 96% carbohydrate, 91% fiber).

Sample preparation: Each IMO exemplar was diluted by a factor of 4 with deionized water (DI water, 18 MΩ, house system, Hydro Service & Supplies, Inc., Gaithersburg, MD). The refractive dry solids (RDSs) were measured using an Atago PAL1 critical-angle refractometer, as g RDS/100 g material (% w/w) relative to sucrose (Sigma S7903). Serial dilutions were prepared therefrom, in order, for analysis via high-pressure liquid chromatography with differential refractive index detection (HPLC-RID; Agilent 1100, details below) and high-pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD; ThermoDionex ICS-5000+, details below). First, the amount of the primary diluted material (typically 15% to 25% RDS) needed was calculated as 500/brix. The calculated amount was diluted by mass to 1 g in a 1.5 mL Agilent-type autosampler vial (target 0.5% w/w RDS, HPLC-RID). Second, 25 μL of the 0.5% solution was transferred by pipette to a tared 1.5 mL Agilent-type autosampler vial. The mass was recorded. To this was added 25 μL of internal standard (IS, L-arabinose, Sigma, 99+%, 500 μg/g), and the total mass was recorded. To the latter was added DI water to a total of 1 g (target 15 to 25 μg/g per analyte, HPAEC-PAD).

Instrumental analysis: Bulk DP 1 to 3, mannitol, glycerol, and organic acids were quantified via HPLC-RID (Agilent 1100, 20 μL on-column, BioRad Aminex HPX-87H 7 × 300 mm @ 65 °C, isocratic, 0.008N H_2SO_4, 0.6 mL/min, runtime 25 min, RID @ 45 °C) using external standards (0.2% w/w per analyte, see Figure 3). The sugar alcohols, mono-, di-, and oligosaccharides, were quantified/confirmed via HPAEC-PAD (ThermoDionex ICS-5000+, all PEEK, 10 μL on column, Carbopac PA-100 (4 × 250 mm) and guard @ 35 °C, NaOH > pH 12.50, 100 mMol, 5 min isocratic then acetate gradient to 250 mM, runtime 25 min, including 2 min equilibration prior to injection, PAD at 25 °C with standard gold electrode compared with Ag/AgCl running the standard Au quad waveform). All compounds listed, except for PAN-type IMOs ≥ DP 3 were quantified using L-arabinose as an IS relative to bona-fide compounds (15 ppmw each). It is important to first analyze any new matrix without IS to make sure that the sample does not contain L-arabinose. PAN-type IMOs were quantified by approximation using the relative response factors (RRFs) for the corresponding DP in a known maltodextrin ladder (DP 3 to 7: Supelco Oligosaccharides kit, DP 8 to 10: Elicityl Oligotech).

Electrode surface effects dictate that RRF compared with external standards can drift over time, so an IS was used. However, because RRFs are not necessarily the same relative to L-arabinose for a homologous series of oligosaccharides with different linkage

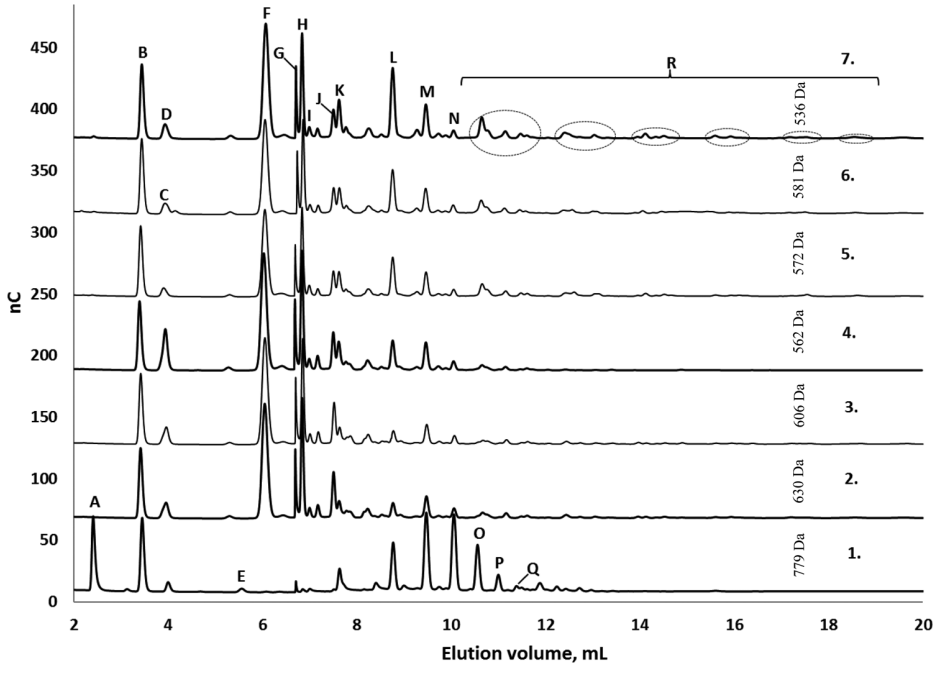

Figure 1–Overlaid (offset by 60 nC) HPAEC-PAD chromatograms of 1. ISOThrive®, 2. IMO-900, 3. AdvantaFiber 90P, 4. Wako IMO, 5. FiberYum, 6. VitaFiber syrup, and 7, VitaFiber powder. A: D-mannitol, B: L-arabinose (IS), C: unknown, possibly D-mannose, D: D-glucose, E: D-leucrose, F: isomaltose, G: sucrose, H: isomaltotriose, I: unknown disaccharides, J: isomaltotetraose, K: maltose, L: D-panose, M-Q: PAN-type IMO DP 4 to 8, and R: resistant maltodextrins in clusters of increasing molecular weight.

Table 1–HPAEC-PAD results for 7 isomaltooligosaccharide bulk food ingredients. Note that the sucrose observed in all but ISOThrive appears to be a coeluting unknown. MWD is M_w or mass-average molecular weight of DP 2 to 10 (to avoid skew) and IMO content the sum of components from DP 3 to 10.

Compound, %/brix	ISOThrive nectar	IMO-900 powder	AdvantaFiber 90P	Wako IMO	FiberYum prebiotic	VitaFiber IMO syrup	VitaFiber IMO powder
Exemplar #	1	2	3	4	5	6	7
brix:	63.06	95.18	96.24	96.98	75.58	75.93	97.49
Erythritol	0.15	0.00	0.00	0.00	0.00	0.00	0.00
Mannitol	6.80	0.00	0.00	0.00	0.05	0.06	0.09
Glycerol	0.40	0.00	0.32	0.00	1.79	1.71	1.86
Glucose	0.90	1.58	1.81	5.32	1.16	0.77	1.36
Fructose	0.12	0.00	0.00	0.09	0.00	0.00	0.01
Leucrose	2.07	0.00	0.00	0.00	0.00	0.00	0.00
Isomaltose	0.13	32.14	29.83	33.62	24.13	23.85	21.84
Sucrose	1.15	10.06	9.73	12.11	6.71	8.20	7.02
Maltose	3.80	3.36	3.27	6.08	5.27	5.15	5.28
Isomaltotriose	0.40	13.98	12.12	14.69	10.41	10.08	8.32
Isomaltotetraose	0.51	9.47	8.53	7.91	5.26	5.21	4.13
Maltotriose	3.17	1.18	2.07	1.79	1.41	2.92	0.00
Maltotetraose	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Panose (MIMO-DP3)	11.18	3.76	3.41	7.30	9.49	10.12	11.75
MIMO-DP4	22.48	6.55	5.95	8.24	7.01	7.24	6.76
MIMO-DP5	23.77	2.99	2.65	2.76	2.19	2.93	1.79
MIMO-DP6	14.48	0.77	0.71	0.40	0.38	0.54	0.00
MIMO-DP7	4.58	0.00	0.00	0.00	0.00	0.00	0.00
MIMO-DP8	1.73	0.00	0.00	0.00	0.00	0.00	0.00
MIMO-DP9	0.61	0.00	0.00	0.00	0.00	0.00	0.00
IMT IMO	0.90	23.45	20.66	22.60	15.66	15.29	12.46
PAN IMO	78.83	13.30	12.01	18.30	18.70	20.29	20.30
Total IMO	79.74	36.75	32.67	40.91	34.36	35.58	32.76
Total	98.42	85.07	79.70	99.91	75.27	78.23	70.23
Balance:	1.58	14.93	20.30	0.09	24.73	21.77	29.77
MWD, Da:	779.06	630.18	606.27	562.07	572.46	580.81	536.04

types (despite identical molecular weight and number of reducing end groups (Goffin and others 2009), an RRF correction factor was based on the peak areas normalized over mass between the corresponding DP 3 types (panose in this case, and the calibrating compound, maltotriose). Typically, this factor is in the range of 0.71 to 0.75 for the PAN/IMT series DP 3 to 10. We found that standards DP 8 to 10 were inadequately fractionated and did not reflect the indicated purities (likely done via area % rather than mass), so care must be taken to qualify the individual standards prior to use.

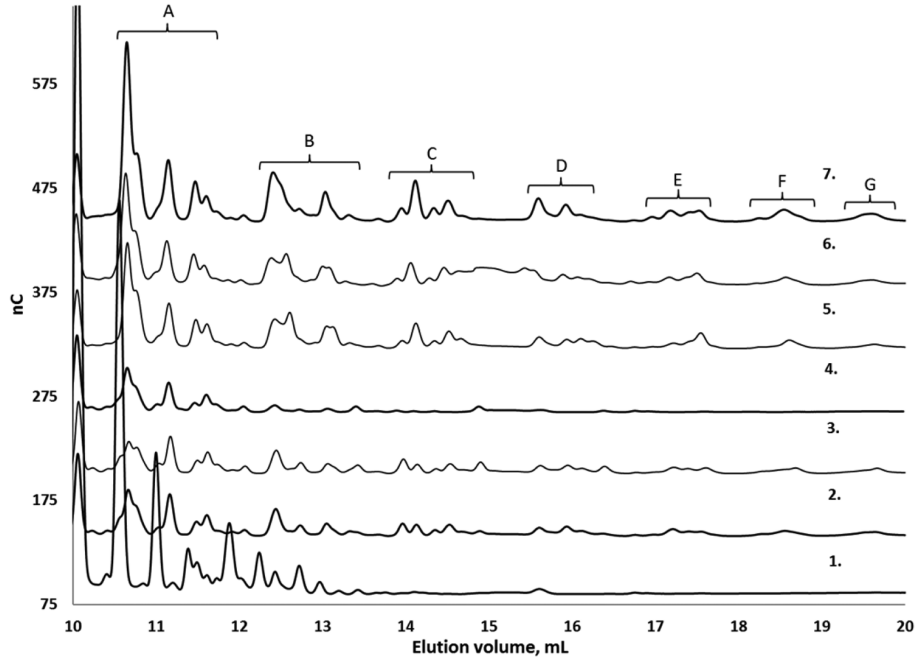

Figure 2–Overlaid HPAEC-PAD chromatograms of 1. ISOThrive, 2. IMO-900, 3. AdvantaFiber 90, 4. Wako IMO, 5. FiberYum, 6. VitaFiber Syrup, and 7. VitaFiber powder; A-G indicate resistant maltodextrins in clusters of increasing molecular weight.

Refractive components, given in g refractive material/100 g sample (brix), were measured using an Atago PAL1 refractometer. When compared with true dry solids (oven), calculation over brix (for homologues equivalent to the calibrated standards DP 3 to 10 only) yields excellent closure on balance of mass, for example, 97% to 103%, and total % w/w is usually within ± 2.5%.

Upon examination of the results, the authors decided to perform a brief ($N = 2$) set of experiments comparing the effects of glucose and a representative "industrial IMO" (FiberYum) on their blood sugar. Blood sugar was measured using a contour next EZ (Bayer, Ascensia Diabetes Care, Parsippany, NJ) blood glucose meter and contour next test strips (#7312). After a 12 h fast, baseline blood glucose was measured for 1 h in 15 min interval. At the 1 h mark, a 70 g aqueous solution containing either 20.00 ± 0.001 g glucose (Fisher D14) or 26.50 ± 0.05 g FiberYum (Raw Indulgence LTD.) IMO syrup (equivalent to 20.02 ± 0.05 g glucan) was rapidly ingested and chased with 50 mL tap water. Blood sugar was measured in triplicate every 15 min. Testing was concluded once blood sugar either returned to baseline or it became evident that it would not. The results ($N = 2$) were averaged, integrated, and expressed as glycemic index (GI) where glucose = 100.

Results and Discussion

It becomes clear (Figure 1, Table 1) that commercially available IMO can be sorted into 1 of 4 discrete types. TG type 2a consists predominantly of DP 4 and includes exemplars 2 (IMO 900) and 3 (AdvantaFiber™). TG type 2b features an even bias on PAN-type DPs 3 and 4, and includes exemplar 4 (Wako). TG type 2c features a heavy bias on DP 3 (panose), and includes exemplars 5 to 7 (FiberYum, VitaFiber syrup and powder). All TG types contained a significant amount of isomaltose, for example, 28 ± 5%/solids and a relatively even distribution of IMT- and PAN-type oligosaccharides, 18.4 ± 4.5%/solids and 17.2 ± 3.6%/solids, respectively (35.5 ± 3.1% total IMO). GT type 1a, or exemplar 1, appears distinct from exemplars 2 to 7 (which are quite similar upon inspection of gross composition), featuring a predominance of DP 5, and the presence of oligosaccharides up to DP 9. Exemplar 1 did not contain a significant amount of either isomaltose or IMT-type oligosaccharides, 0.13%/solids and 0.90%/solids, respectively. The IMOs were almost exclusively PAN-type comprising 79% of the solids (80% total IMO). Exemplar 1 was further differentiated by the presence of mannitol (6.8%/solids), a byproduct of the fermentation process, and leucrose (5-O-α-D-glucopyranosyl-D-fructose), a byproduct specific to IMOs made via donor–acceptor chemistry mediated by dextransucrase(s).

In Figure 2, exemplars 2 to 3 (IMO-900, AdvantaFiber) and 5 to 7 (FiberYum, VitaFiber syrup and powder) contain a significant quantity of unidentified material evident as clusters of increasing molecular weight. This is RMDx. Determined as the difference of the whole from 100%, exemplars 2 and 3 and 5 to 7 contain 22.3 ± 5.5% leading to mass closure of 77.7 ± 5.5%. Interestingly, exemplar 4 contained virtually no RMDx and the mass balance closed to 99.9%, indicating that the missing mass for the other exemplars (2 and 3, 5 to 7) was likely the result of inadequate quantitation of the resistant components. We suppose that the saccharification was more complete, perhaps via use of isoamylase/pullalanase debranching enzymes prior to treatment with amylase.

Within the scope of this work (types 2a and 2b are made from corn and type 1a uses high-purity maltose), TG type 2c can be further differentiated into products made from corn/maize (TG2cm) and those made from tapioca (TG2ct). The exemplars made from tapioca typically demonstrated a higher molecular weight (from DP 6), relative to those made from corn, of about 10 Da. While the difference is too small, in terms of mass-average molecular weight, to decisively differentiate them, the RMDx profiles are both discrete and reproducible (Figure 2).

It can be seen from the fingerprint of the RMDx fractions that exemplar 1 (ISOThrive) is clearly discrete (it has no RMDx because is not made from starch), exemplars 2 and 3 (IMO-900, AdvantaFiber) are similar (TG2a), exemplar 4 (Wako) is unique (TG2b) by virtue of containing little, if any RMDx while belonging to the "industrial IMO" group, exemplars 5 and 6 (FiberYum, VitaFiber syrup) are essentially identical (TG2ct) and discrete from 7 (VitaFiber powder, TG2cm). In Figure 2, groups B, D, and E are

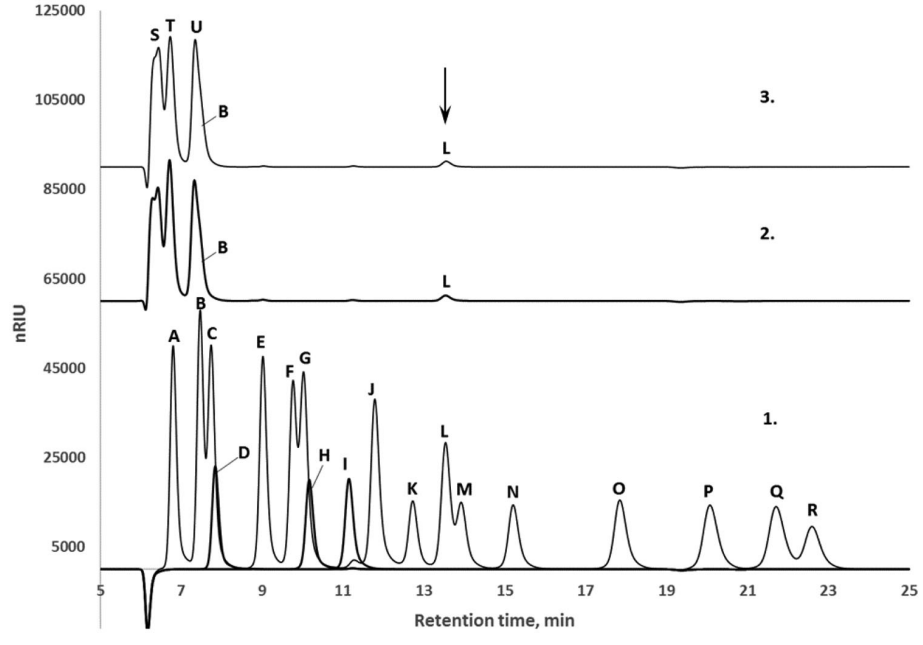

Figure 3–HPLC-RID chromatograms of 1. Standard mixture 0.2% w/w per analyte, and 0.5% w/w each of 2. VitaFiber syrup, and 3. FiberYum syrup; where A: maltotriose, B: maltose, C: leucrose, D: maltitol, E: glucose, F: fructose, G: mannitol, H: sorbitol, I: xylitol, J: erythritol, K: lactic acid, L: glycerol, M: formic acid, N: acetic acid, O: propionic acid, P: iso-butyric acid, Q: n-butyric acid, R: ethanol, S: DP >3, T: isomaltotriose, and U: isomaltose.

Table 2–Comparison of available analytical data with this work.

Constituent group	ISOThrive nectar	IMO-900 powder	AdvantaFiber 90P	Wako IMO	FiberYum Prebiotic	VitaFiber IMO powder	VitaFIber IMO syrup
Solids, %	63-67[i], 63.1	**>95[b], 95.2**	95.3[h], 96.2	**97.0**	**75.6**	96[f], 97.5	75[g], 75.9
Glucose, %	<1.5[i], 0.9	<5[a],4[b]; 3.8[c], 1.6	1.6[h], 1.8	**5.3**	**1.2**	<5[f], 1.4	**<5[g], 0.8**
Fructose, %	**<0.2[i], 0.1**	**n/a**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**
Isomaltose %:	**0.1**	22.8[c], 32.1	**29.8**	**33.6**	**24.1**	**21.8**	10-15[a], 24.0
Isomaltose + panose + isomaltose	**11.7**	>45[b,d], 49.9	**45.0**	**55.6**	**44.0**	**42.0**	**44**
DP 3 IMT, %	**0.4**	**14.0**	**12.1**	**14.7**	**10.4**	**8.3**	**10.1**
DP 3 PAN, %	**11.2**	**3.8**	**3.4**	**7.3**	**9.5**	**11.8**	**10.1**
DP 3 total, %	**11.6**	16.7[c], 17.8	**15.5**	**22.0**	**19.9**	**20.1**	20-25[a], 20.2
DP 4 IMT, %	**0.5**	**9.5**	**8.5**	**7.9**	**5.3**	**4.1**	**5.2**
DP 4 PAN, %	**22.5**	**6.6**	**6.0**	**8.2**	**9.5**	**6.8**	**7.2**
DP 4 total, %	**23.0**	17.7[c], 16.1	**14.5**	**16.2**	**14.8**	**10.9**	15[a], 12.4
DP 5, %	**23.8**	7.2[c], 3.0	**2.6**	**2.8**	**2.2**	**1.8**	7.9[a], 2.9
DP 6, %	**14.5**	1.7[c], 0.8	**0.71**	**0.4**	**0.4**	**0.0**	4-5[a], 0.5
DP 7, %	**4.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
DP 8, %	**1.7**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
DP 9, %	**0.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
RMDx†	**1.6**	**14.9**	**20.3**	**0.1**	**24.7**	**29.8**	**21.8**
Total IMO DP 3 to 9	>75[i], 79.7	>90[a,b], 37.5	90.8[h], 33.4	**n/a*,41.3**	100[e‡], 34.7	91[f], 32.8	93.3[g], 36.1
Total IMO DP 2 to 9	**79.8**	**69.6**	**63.2**	**74.9**	**58.8**	**54.6**	**60.1**
Total IMO DP 2 to 9 + RMDx	**81.4**	**84.5**	**83.5**	**75.0**	**83.5**	**84.4**	**81.9**
TOTAL, all compounds	**98.4**	**85.1**	**79.7**	**99.9**	**75.3**	**70.2**	**78.2**
Mw, Da	**779**	**630**	**606**	**562**	**572**	**536**	**581**
DP	**4.72**	**3.80**	**3.65**	**3.37**	**3.44**	**3.21**	**3.49**

*no available data, †RMDx approximated by difference based on total of all compounds by mass, ‡derived from given information, for example, carbohydrate 5g and fiber 5g from product label.
[a]Bioneutra Table 3 GRAS document (Zhu, March 12, 2005)[22]; [b]Baolingbao Biology Co., LTD[23]; [c] Kaneko and others[18] (1992); [d]Dancheng Caixin Sugar Industry Co. LTD, specification of isomaltooligosaccharide (IMO)[24]; [e]Packed in the United States for Raw Indulgence LTD. FiberYum, Nutritional Facts from product container; [f]Bioneutra Nutritional Data (May 22, 2013) VitaFiber-IMO powder, declared as "VitaSugar Isomalto-oligosaccharide food ingredient," on bill of lading (non-GMO corn[25], China); [g]syrup (tapioca[26], Indonesia); [J]Bioneutra Table 3 GRAS document (Zhu, March 12, 2005)[22]; [h]Top Health Ingredients, Inc. AdvantaFiber 90P, Certificate of Analysis #1090-14071631, July 17, 2014, non-GMO corn[27], manufactured by Baolingbao Biology Co. Ltd, China; [i]ISOThrive, LLC, ISOThrive Prebiotic Nectar, GRAS internal document available upon request with NDA.

Table 3–Comparison of oligosaccharide descriptions given in the product profiles with this work.

	IMO powder	IMO syrup	Syrup solids
From product profiles			
Nondigestible oligos*	55	42	55
Digestion resistant oligos**	16	13	17
Digestible oligos†	20	15	20
Dietary fiber (TDF)	91	70	92
This work			
Nondigestible oligos*	12.7	12.1	15.9
Nondigestible + RMDx	42.5	28.6	37.7
Digestion resistant oligos**	20.1	15.3	20.2
Digestible oligos†	21.8	18.1	23.8
Dietary fiber (TDF)	84.4	62.1	81.7

diagnostic for differentiating TG2ct from TG2cm, and may be sufficiently discrete as to be fingerprints of the specific process/place of manufacture, and/or of the lot. That is, the signature may vary due to slight inconsistencies in the saccharification process. It appears that exemplars 5 and 6 may have been manufactured in the same Indonesian factory (6 is known to be of Indonesian origin). Accordingly, the fingerprints suggest that exemplars 2 and 3 were likely manufactured either in the same plant, or using the same IP (Baolingbao Biology Co. Ltd 2015). Additionally, in Figure 3, it was noted that samples 5 to 7 also contained similar amounts of glycerol, 1.79%/solids, 1.86%/solids, and 1.71%/solids, respectively. Unlike sample 1, where some glycerol remains as a metabolic byproduct, its presence in samples 5 to 7 likely originated with the enzyme cocktails (glycerol, sorbitol, and so on) (Elliot and McKay 2002) used to perform the TG. Because most enzyme cocktails are proprietary formulations (and can vary widely in composition), the consistency in glycerol quantity suggests that these products were manufactured using a similar enzyme preparation.

Discussion of claims and nutritional information

Because MIMO (as in exemplar 1, ISOThrive) only became available for purchase in 2016, most of the prior literature regarding digestibility/fermentability of IMO dealt with products similar to exemplars 2 to 7. Of these, IMO-900 (2) and Wako IMO (4) claim the greatest coverage. With respect to popularity and online presence, however, VitaFiber is perhaps the best known IMO-based food ingredient and FiberYum is gaining popularity because it is less expensive in bulk. Bioneutra claims that "oligosaccharides are short-chained carbohydrates composed of DP 2 to 7, or more, glucosyl units" (BioNeutra). Furthermore, they claim that said oligosaccharides "constitute nondigestible soluble dietary fiber" by citing AACC (March 2001) that, interestingly, defines oligosaccharides as "chains with a DP between 3 and 10," (AACC 2001). Thus, by way of proper definition, exemplars 2 to 7 do not contain >90% IMO. If one takes the definition of oligosaccharide to include only DP > 2, then these products contained approximately 36% (of both IMT and PAN types via HPAEC-PAD). It is further claimed that the composition is prebiotic (Bioneutra citing Rycroft and others 2001) by virtue of demonstrated bifidogenesis *in-vitro* (Bioneutra citing Kohmoto and others 1988) and *in-vivo* using 20 g/d doses (containing 13.5 g IMO at purity described in the paper, but only 7.35 g where 4.69 g is IMT type, 2.66 g is PAN type, and the $M_W = 619.54$ Da or DP = 3.73, according to this work) of Isomalto-900(R) composition.

Interestingly, it was later determined by Oku and Nakamara (2003) via testing of breath hydrogen and methane that a 20 g bolus dose of an IMO composition (Isomalto-900(R), Showa Sangyo

Co., Ltd, Tokyo, Japan, composition verbatim from Kaneko and others (1995) and similar to IMO type 2a; IMO-900) "hardly reaches the large intestine and does not produce gas." This corroborated the work of Kohmoto and others (1988) who estimated, via studying digestibility of ^{13}C-labeled IMOs, that the tested material (prepared in-house and congruent with Isomalto-900 [Showa Sangyo Co., Ltd]) was largely digestible and delivered 70% to 80% of the calorific value of an equivalent dose of maltose. Kaneko and others (1995) discovered via "Rat Jejunum Loop Method," that IMOs with increasing molecular weight became increasingly indigestible, and that "the digestibility of disaccharides in IMO (for example, isomaltose) is similar to those of sucrose or maltotriose." It can thus be implied that IMOs with higher molecular weight would be less glycemic.

These authors also noted that in large doses, the brush-border enzymes (sucrase-isomaltase) would saturate with the preferred substrate (isomaltose) allowing some IMO to pass. So, considering digestibility, the 20 g dose administered by Kohmoto and others (1988) would have delivered, at best, a potential prebiotic dose of, perhaps, 1.5 to 2.2 g. Inasmuch as prebiotic specificity is known, the dose administered would have been of relatively low molecular weight, according to Hu and others (2013), who tested VitaSugar (Bioneutra Inc., Edmonton) as a sole carbon source for *L*actobacillus *reuteri* and *Bifodobacteria spp*., and found that DP $\leq$ 4 would favor *Lactobacillus spp*. only, and thus it is less likely representative of a bifidogenic prebiotic.

It is also claimed, based on a publication in the *Chinese Journal of Clinical Nutrition* (Bioneutra citing Sheng and others 2006), that the composition exhibits low GI and is thus suitable for use by those on restrictive diets (diabetics (Evert et al. 2014), low-carb ketogenic (Lee and Kossof 2011), and so on). However, as noted above, these compositions, particularly in the large doses needed to reach the colon (effectively saturating the brush border enzymes), can be expected to have a GI of at least 70% of an equivalent dose of maltose (which is completely digestible), for example, 14 g maltose/20 g dose IMO or about 56 kcal/serving.

These are claims typical of those purveyed by IMO manufacturers/importers including:

VitaFiber: "VitaFiber^{TM} is a sweet natural fiber providing low calorie and soluble prebiotic fiber for human digestive health . . . VitaFiber is greater than 90% soluble fiber . . . a prebiotic . . . Maintain healthy blood sugar levels . . . low GI" (Bioneutra.ca/products 08-09-2016).

AdvantaFiber: "Adds prebiotic, soluble fiber . . . 90% IMO (Isomalto oligosaccharide) Fiber" (http://www.tophealthingredients.com/products/advantafiber/ 08-09-2016).

FiberYum: "super low-glycemic alternative sweetener with awesome amounts of soluble prebiotic fiber . . . Make special foods for glucose intolerance . . . low glycemic . . . sugar free . . . 5 g soluble fiber per serving" (a serving is 5 g; http://shop.rawrev.com/product-p/clearancefiberyum2.5a.htm 08-09-2016).

ISOThrive: "Naturally Fermented Prebiotic Soluble Fiber . . . a type of complex carbohydrate you cannot digest . . . has no calories and does not cause blood sugar spikes" (https://www.isothrive.com 08-09-2016).

It appears that all of the commercially available products are making similar claims that are in line with the beneficial effects of prebiotics on human health (indeed, the first use of the term "prebiotic") described by Gibson and Roberfroid (1995).

Therefore, when comparing the analytical results from this work with the declared fiber content within the context of the given claims, the data suggest that not all ingredients labeled as "IMOs" are quantitatively equal. It appears that most manufacturers have adopted a loose definition that is neither based on an accurate quantitative analysis nor consequence of metabolic impact.

In Table 2 below, values available from product certificates of analysis (COA)/specifications or abstracted from Table 1 are compared with this work, given in bold numbers.

In general, for all components up to DP 4, the analytical values from this work were similar to the values reported from the various cited sources. For DP 5 and higher, our results were always significantly lower (factor of 2.43 $\pm$ 0.31) than indicated. Ketabi and others (2011) noted similar findings where the starting material "IMO obtained from BioNeutra Inc." indicated that a composition of where DPs 2 to 8 were present in (DP2) 18% to 25%, (DP3) 15% to 23%, (DP4) 14% to 22%, (DP5) 8% to 10%, (DP6) 6% to

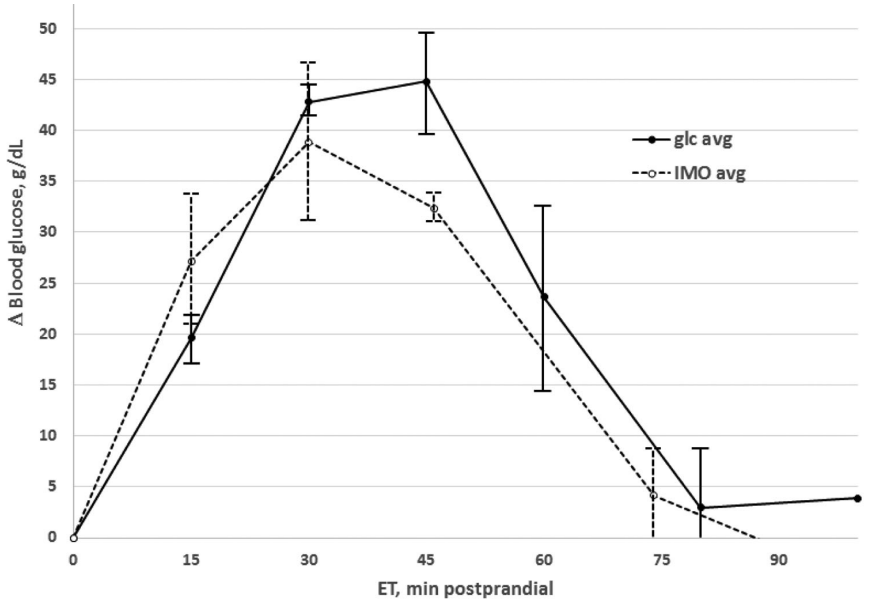

Figure 4–Effect of glucose and IMO on blood sugar over time in 2 human subjects. Triplicate measurements (each subject and time) demonstrated that the test meter/strip returned standard deviations ranging from ± 0.71 to 6.24 g/dL (average ± 3.05).

Table 4–Average ($N = 2$) results and integrated values normalized to glucose (GI = 100).

GLUCOSE averages	GLC eq., g: glc g/dL	20.00 stdev	Δg/dL	stdev	FiberYum averages	GLC eq., g: glc g/dL	20.02 stdev	Δ g/dL	stdev
0	89.18	1.32	0.0	0.0	0	89.84	0.58	0.0	0.0
15	108.83	1.18	19.6	2.5	15	117.00	7.07	27.2	6.5
30	132.00	2.83	42.8	1.5	30	128.67	7.07	38.8	7.7
45	134.00	6.60	44.8	5.3	46	122.17	0.24	32.3	0.8
60	112.83	10.61	23.6	9.3	74	94.00	3.77	4.2	4.4
78	92.17	4.48	3.0	5.8	89	89.17	5.42	−0.7	4.8
95	93.08	3.65	3.9	5.0	98	82.67	0.47	−7.2	1.1
integral	2470	744			integral	2074	361		
GI, % glc	**100**				**GI, % glc**	**83.97**			

8%, (DP7) 2% to 4%, and (DP8) 2% to 3%, respectively, was used to formulate rat-chow and found only DPs 3 to 5 and a trace of 6, as indicated in this work. Thus, we conclude that the analytical method that is being use to qualify the IMO product (and likely the others, given similarity in the COAs) does not fractionate the individual IMO types by DP, and includes the RMDx in with the IMT and PAN-type oligosaccharides of similar molecular weight. Further, this becomes evident when we add everything up, for example, total IMO DP 3 to 9 = 32.9%. Adding the isomaltose gives us 60.1%. Adding RMDx (determined by difference from 100 of the sum of all accountable components given in Table 1) brings the balance to reasonable closure at 84.4%. Clearly, this is an approximation, as direct quantitation of a similar product (exemplar 4) containing little if any RMDx closes to 99.9%. This helps us to better understand the information given in BioNeutra's product profiles, for example, Table 3.

Here, it appears evidently that the convention seems to follow Ketabi and others (2011) (citing Kohmoto and others 1992 and Kaneko and others 1995) assertion that "Isomaltose is hydrolyzed by the brush border enzymes in the intestinal epithelium, the digestibility of IMT and panose is unclear, and longer chain oligosaccharides are considered nondigestible." When tested, "digestible oligos" are similar to the given values when our results for isomaltose are used. It is important to differentiate disaccharides such as isomaltose from the oligosaccharide class. Biologically, there are discrete enzymes for dealing with disaccharides (disaccharidases such as sucrase-isomaltase, glucoamylase, cellobiase, and so on) and for dealing with oligosaccharides, for example, oligo glucan-α-1,6-glucosidases, and that the former is found in abundance in the small intestine. "Digestion-resistant oligos" are likewise similar because sucrase-isomaltase is somewhat promiscuous in that it can hydrolyze a glucose unit from either IMT or panose to yield isomaltose or maltose, respectively, and that both products are completely digestible, and therefore glycemic. This explains why digestibility decreases with increasing molecular weight (that is, kinetically limited).

Interestingly, though, adding up all DPs ≥ 4 gives a low result compared to the given data for all exemplars 2 to 7. Adding the approximated value for RMDx to this amount gives us a far more reasonable result with respect to the given data. When summed, we get reasonable closures of mass, for example, 84.4%/solids and 81.7%/solids.

A review of the literature differentiated the digestibility of RMDx from resistant starch, a high molecular weight polyglucan that is either retrograded, granular (and hence resistant to amylolytic activity), or chemically modified so as to be indigestible (Sajilata and others 2006; Fuentes-Zaragoza and others 2010). RMDx can be subdivided as (1) an indigestible, highly branched glucan made from starch via heat, acid, and/or α and β-amylases, (2) other enzymes that transglycosylate starch to yield a variety of linkage types (Lee et al. 2013), or (3) known as "limit dextrins," are relatively small, glycogen-like α-(1,4) glucans that are α-(1,6) branched so as to resist the further action of α-amylase (α-limit dextrin can also be made). α-D-1,6-glucan-6-hydrolases (dextranase), pullulan α-1,6-glucanhydrolases (pullulanase), and so on, should be able to quickly reduce it into a digestible form, for example, oligo amylose (maltodextrin). Given the distribution of products that results, it appears that the RMDx found in these products is of the latter type, for example, limit dextrin. Thus, the RMDx described here is digestible, and is not likely to be a constituent of the fiber content, soluble, or not.

Therefore, we conclude that the definition of "IMOs" must not include digestible constituents including disaccharides (isomaltose) and α-limit dextrins. That is, the definition of "oligosaccharide" must be made to include DP ≥3, only. This is the difference between a COA that indicates >90% soluble fiber for a product that actually contains approximately 36% DP 3–10 (actually DP 3 to 5 with an average of 3.8). Inclusion of DP 3 constituents into this definition requires further study, but the literature suggests that these are digestible as well. In order to test this, the authors studied the effect, relative to equivalent glucose, of low molecular weight (labeled as 5 g dietary fiber per 5.5 g syrup dose, FiberYum) IMO on fasting blood glucose levels.

Postprandial blood glucose levels

The authors' results were averaged ($N = 2$) and the increase in measured blood glucose over time is shown in Figure 4 and the tabulated results, including integrals relative to glucose (GI = 100) are shown in Table 4.

Although an $N = 2$ experiment is not statistically significant, the standard deviations (between subjects) were relatively small and permitted some comparison. Our observations suggest that the GI of the "industrial" IMO exemplar (5), which is typical of the compositions commonly encountered on the commercial market, is at least 80% (84% observed here, see Table 4) as digestible, and hence, glycemic, as anhydrous glucose on an equivalent weight/weight basis. Based on transit time, absorption likely began in the stomach (15 min postprandial) and the observed amount was unlikely to have reached the colon (absorption complete within 90 min). Another interesting feature that was observed in both subjects with IMO is that the "sugar crash" was significantly larger and more prolonged than that elicited via equivalent dosage of glucose.

Using scientifically sound definitions facilitates fair comparison between products allowing downstream manufacturers to make improved formulations while providing a more accurate accounting of ingredients and nutritional information. The latter is critical

for consumers who require a low-glycemic product. If the nutritional information given for bulk ingredient is incorrect, it can lead to downstream products that are mislabeled. The modern consumer gauges what they can eat (this is especially true of those on restrictive diets) by reading the ingredients and nutritional information given on product labels. It is therefore imperative that they are correct.

Conclusion

This analysis comparing 7 commercially available IMO-based food ingredients demonstrates that most of these products are, by way of definition, and particularly with respect to content of "oligosaccharides" and "dietary fiber," mislabeled. When comparing the analytical results from this work with the declared fiber content within the context of the given claims, the data suggest that not all ingredients labeled as "IMOs" are quantitatively equal. It appears that most manufacturers have adopted a loose definition that is neither based on an accurate quantitative analysis nor consequence of metabolic impact. This is significant because claims, such as "low glycemic," "zero calorie," and the like, are certainly false, and may pose a health hazard to certain populations (diabetic patients and epileptic patients on ketogenic diets, in particular) while misleading others (those on low carbohydrate diets). Therefore, we conclude that the definition of "IMOs," and certainly, "fiber," must not include digestible constituents such as disaccharides (isomaltose) and α-limit dextrins. That is, the definition of "oligosaccharide" must be made to include DP $\geq$ 3, only. This is the difference between a COA that indicates >90% soluble fiber for a product that actually contains approximately 36% DP 3 to 10 (actually DP 3 to 5 with an average of 3.8). Inclusion of DP 3 constituents into this definition requires further study, but the literature suggests that these are digestible, as well. We conclude that labeling requirements should be reconsidered for products of this type. If the nutritional information given for bulk ingredient is incorrect, it can lead to downstream products that are mislabeled. The modern consumer gauges what they can eat (this is especially true of those on restrictive diets) by reading the ingredients and nutritional information given on product labels. It is therefore imperative that they are correct.

Acknowledgments

The authors report a business relationship as partners in ISOThrive LLC, a manufacturer of one of the ingredients analyzed in the manuscript. The report is intended to provide an accurate accounting of the composition of each product, while bringing to light and averting potential health hazards to certain patient populations due to the discovered mislabeling of ingredients. Every effort has been made to maintain unbiased reporting of facts with respect to all ingredients analyzed. The authors have listed methods so that results can be replicated by any interested party. We would like to acknowledge Savana Gilman for administrative assistance in the preparation of this manuscript.

Author Contributions

L. Madsen designed the study, collected test data, interpreted the results, and drafted the manuscript; S. Stanley collected test data; P. Swann interpreted the results and edited the manuscript; and J. Oswald assisted in the design of the study, interpreted the results, and edited the manuscript.

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