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Food Standards Australia New Zealand
Boeing House
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03 February 2017

Attention: Standards Management Officer

RE: Submission – A1123 - Isomalto-oligosaccharide as a Novel Food, 13 December 2016 [31-16]

This submission is made by Ingredion ANZ Pty Ltd (Ingredion).

Ingredion is a global ingredient solutions company manufacturing sweeteners, starches and unique ingredients from plant sources such as corn, tapioca, rice, sago and potato. The business is focused on providing critical ingredients to the food, beverage, industrial, pharmaceutical and personal care industries.

Ingredion **supports Option 1** - Prepare a draft variation to the Code to permit the use of IMO as a novel food in all foods except infant formula products, infant foods, and formulated supplementary food for young children.

Ingredion **does not support** the proposed specification for IMO.

Ingredion notes the requirement for FSANZ to have regard to the promotion of consistency between domestic and international food standards. However, while internationally, IMO is recognised in a number of jurisdictions the proposed approach will not promote consistency with such approvals – the specification proposed by FSANZ is not aligned with some countries such as Korea which specify IMO content should be more than 10% (Refer to Attachment 1).

In addition, the US Pharmacopoeia is currently working on drafting IMO specifications to be published in the Food Chemicals Codex[1]. The proposed Isomaltooligosaccharides monograph first-round review published in Dec 2016 is provided as Attachment 2. The draft IMO specifications are IG2+P+IG3 more than 17%.

Ingredion request FSANZ consider alignment of the proposed IMO specification with the proposed FCC monograph.

Please do not hesitate to contact me if we can provide any further information in support of this submission.

Best regards,

[redacted]

Technical Service Manager

[redacted]

[1] http://www.usp.org/food/notices/early-input-sought-proposed-isomaltooligosaccharides-monograph, accessed 02.02.2017

10. Oligosaccharides

1) Definition:

Oligosaccharides refer to products such as fructo-, isomato-, galacto-, malto-, xylo-, or gentio-oligosaccharides which are produced by the processing of sugar solutions.

2) Requirements of Raw Material

3) Manufacturing and Processing Standards

4) Food Type

(1) Fructo-Oligosaccharide

Fructo-Oligosaccharide refers to a liquid or powdery form of product that uses sugar solution that is enzymatically processed to have at least one fructose molecule binding at its structure by using sugary base materials, which is then processed by following processing steps of filtration, purification, and concentration.

(2) Isomalto-Oligosaccharide

Isomalto-Oligosaccharide refers to a liquid or powdery form of product that is processed by filtration, purification, and concentration steps by using sugar solution produced by enzymatic digestion on sugary base materials, which rearranges the molecular structures to be glucose based form.

(3) Galacto-Oligosaccharide

Galacto-Oligosaccharide refers to a liquid or powdery product that is produced by using trans-galacto-oligosaccharide sugar solution produced by enzymatic digestion of sugary base materials, or the product which is processed by filtration, purification and concentration of raffinose and stachyose sugar solution extracted from sugar beet or soybeans.

(4) Malto-Oligosaccharide

Malto-Oligosaccharide refers to a liquid and powdery form of product that is processed by using sugar solution produced by enzymatic digestion of 100% sugary base materials to produce 3~10 linearly bound glucose molecules in its structure.

(5) Xylo-Oligosaccharide

Xylo-Oligosaccharide refers to a liquid and powdery form of product that is processed by using sugar solution produced by enzymatic digestion of xylan used as a base material.

(6) Gentio-Oligosaccharide

Gentio-Oligosaccharide refers to a oligosaccharide manufactured through filtration, purification, and concentration of sugar solution obtained from enzymatic treatment to induce beta-binding of glucose molecules to carbohydrate components.

(7) Other Oligosaccharides

Other Oligosaccharides refer to oligosaccharide products manufactured by adding foods or food additives to one of the above oligosaccharides (1)~(6).

5) Specifications

(1) Oligosaccharide Content (%)

① Fructo-, Isomalto-, Galacto-, Xylo-, and Gentio- oligosacchrides should have more than 10.0

② Malto-oligosaccharide : More than 40

③ Mixed-oligosacchride : It requires more than the content of individual oligosaccharide.

(2) Lead : Not more than 1.0.

6) Test Method

(1) Oligosaccharides

① Fructo-oligosaccharide

A. Equipment

High Performance Liquid Chromatography(HPLC)

B. Regents

Ⓐ Acetonitrile : HPLC grade

Ⓑ Glycerol : Special grade

Ⓒ Ethanol : Special grade (99.5%)

Ⓓ Diatomic Earth(filter-preparative purpose)

Ⓔ 80% Ethanol : It is prepared by mixing 99.5% ethanol and distilled water at the ratio of 80:20 (v/v).

Ⓕ 5% Glycerol : It is prepared by mixing 5 g of exactly weighed glycerol with 50 mL ethanol in a 100 mL mess flask to fully dissolve the glycerol. Then distilled water is added to make total volume of the solution to be 100 mL, and the preparation is used as internal standard

(Arabinose could be used as an internal standard also).

C. Preparation of standard solution

0.4 g of 1-Ketose (GF_2), Nystose (GF_3), and 1-F Fructofuranosyl Nystose (GF_4) of all regents are weighed and mixed with 10 mL of distilled water in 20 mL mess flask to dissolve these regents completely, and additional distilled water is added to meet the 20 mL marking. 10 mL mess flasks are used to transfer 1, 2, 3, 4 and 5 mL of standard solution to each flask, and 1 mL of 5% glycerol is added to all 5 flasks and distilled water is added to fill up the flask up to the 10 mL marking, which is used as standard solutions.

D. Preparation of Sample solution

Ⓐ For samples, holding negligible amounts of fats.

A sufficient amount of sample is exactly weighed to hold approximately 0.2-2 g of Fructo-oligosaccharide and 20 mL of distilled water is used to dissolve and extract it in the 100 mL mess flask(If necessary, heating or ultrasonication can be used to mix the sample completely). After the complete dissolution of the sample, 20 mL of 5% glycerol solution is added and filled up with distilled water up to 100 mL marking. If necessary, the solution can be used after filtration (No.5B). The exact volume of prepared 25 mL sample solution is transferred into a 50 mL mess flask, and diluted by ethanol by filling the flask with 80% ethanol solution up to the 50 mL marking of the flask. If the solution develops precipitation or increases the turbidity, it is filtered with (No.5B) filter paper to be used as a sample solution.

NOTE1: If sample holds large amount of insoluble matters, It is filtered with (No.5B) filter paper. The

filtered solution is transferred into a 100 mL mess flask, and the same analysis method is followed.

Ⓑ For samples, containing large amount of fats.

For the analysis of liquid form of samples, an approximate amount of samples that will contain 0.2-2 g of Fructo-oligosaccharide is exactly weighed, and neutralized if necessary (For this manipulation, keep in mind that directly heating the acidic sample can cause hydrolysis of the fructo-oligosaccharides). So, diatomic earth is added and mixed well (The proper amount of diatomic earth is when the added earth turns into crumbly form). The preparation is dried under decompressed atmospheric condition for 2-4 hours, and whole dried amount is transferred into a paper filter tube to undergo 8-16 hours of fat extraction using a soxhlet fat extractor using petroleum ether. After the fat extraction, whole amount in the paper filter tube is followed by the sugar extraction method used above for samples holding negligible amount fats. If the sample for fat extraction is in powder form, diatomic earth is not added, but the whole extraction procedure is same.

E. Test Procedure

Ⓐ High Performance Liquid Chromatography operation condition

- Column : μ- Bondapak CH, p-nitrophenol hydroxylation 2-10 (PNH_2-10) equivalents (Biosil Amino 5S, Shodex Rspak DC-613, Nueleosil NH_2, Lichrosorb NH_2, etc.)
- Mobile phase : Acetonitrile:water (65:35)

  The ratio of acetonitrile and water can be adjusted from 65:35 to 70:30 and small volume of ethyl-alcohol can be added for the adjustment.
- Flow rate : 1.0 mL/min
- Detector : Refractive Index detector (RI)

Ⓑ Quantitative test

It is tested by injecting 10 μL standard solutions of Fructo-oligosaccharides and 10 μL of internal standard solutions to have their own chromatograms, each peak area and height of the peak is recorded to have a standard curve. 10 μL of test solution and internal solution are injected to have the histograms of fructo-oligosacchride and internal standard's to have their peak area and height to calculate the total amount of fructo-oligosaccharides (GF_2+GF_3+GF_4) by following equation.

<Calculation>

$$\text{Total fructo-oligosaccharides content (\%)} = (A + B + C) \times D \times \frac{100}{E} \times \frac{100}{1000}$$

A : Concentration (mg/mL) of GF_2 in test solution obtained from standard curve

B : Concentration (mg/mL) of GF_3 in test solution obtained from standard curve

C : Concentration (mg/mL) of GF_4 in test solution obtained from standard curve

D : Dilution factor

E : Sampling weight (g)

② Isomalto-oligosaccharide

A. Equipment

High Performance Liquid Chromatography

B. Regents

Ⓐ 50% Ethyl alcohol

Ⓑ Activated Carbon: the grade used for rice wine fermentation or sugar solution bleaching

Ⓒ Ion-exchange resin: Enforced acid type(H-type) or Enforced alkaline type(OH-type)

Ⓓ Perlite

Ⓔ Acetonitrile : HPLC grade

Ⓕ Standard sugars

Monosaccharides - Glucose, Fructose

Disaccharides - Maltose, *Isomaltose, Sucrose, *Kojibiose, *Nigerose

Cellotriose - Maltotrisose, *Pannose, *Isomaltotriose

Cellotetrose - Maltotetrose, *Isomaltotetraose

Pentose - Maltopentaose, *Isomaltopentaose

Hexose － Maltohexaose, *Isomaltohexaose

Heptaose － Maltoheptaose, *Isomaltoheptaose

(* indicates sugar substance diverged from Isomato-oligosaccharides)

C. Preparation of Standard solution

Ⓐ Exclusion ion chromatography system: Each degree of polymerized linear sugars is exactly weighed to 50 mg, and mixed with 50 mL distilled water in a 100 mL mess flask. If there is a problem in dissolving the sample, additional heating can be used and later filled up with distilled water to 100 mL marking after cooling.

Ⓑ Reverse phase ion chromatography : Each 100 mg of isomalto-oligosaccharide diverged sugar is dissolved with 1 mL of distilled water to be used as a standard solution for the sugar.

D. Preparation of test solution

Ⓐ For samples, containing negligible amount of fats

Approximate amount of sample (0.5-5 g) that will contain the proper amount of isomalto-oligosaccharides approximately 500 mg is exactly weighed, and 30 mL of distilled water or 50% ethanol solution (For sample that contains protein) is mixed and heated to completely dissolve the solution to extract the sugar. The extracted solution is purified by 0.5 g of activated carbon and 1.5 g of ion-exchange resin which was formulated by mixing cation and anion in the ratio of 1: 2. The purified solution is filled up with distilled water to have total volume of 500 mL.

Ⓑ For samples, containing large amount of fats

Approximate amount of sample (0.5-5 g) powdery form of isomalto-oligosaccharide containing sample is exactly weighed and directly transferred into fat extractor for fat extraction, if the sample is in powder form. If the sample is liquid type, it can be neutralized when necessary (outer range of pH 4-10) and then Perlite is added until it turns to crumble form, which will allow the sample to be well dispersed during the following fat extraction for 8-16 hours. After the fat extraction, whole

amount of sample is followed by the next operation described in above.

NOTE 1. For the sugar samples, which are mixed with sugars, having 2-5 degree of polymerization such as sugar, lactose, fructo-oligosaccharide, coupling sugar, and galacto-oligosaccharide were mixed in the samples, Each sugar content has to be determined individually by following the individual sugar measurement methods for the sugar samples. However, the presence of starchy sugars such as isomerized sugars, glucose, and molasses do not interfere the analysis of Isomalto-oligosaccharides.

E. Test Procedure

Ⓐ High Performance Liquid Chromatography operation condition

- Exclusion ion chromatography system
- Column : ∮8 mm× 200 mm MCI GEL CKO 4S equivalents
- Guard column : ∮8 mm× 50 mm MCI GEL CKO 8S equivalents
- Column temperature : 65℃
- Mobile phase : Water
- Flow rate : 0.4 mL/min
- Detector : Refractive Index detector

Ⓑ Reversed phase

- Column : ∮4.6 mm× 250 mm TTSK GEL NH_2 -60 equivalents
- Column temperature : 35℃
- Mobile phase: Acetonitrile : Water(63:37)
- Flow rate : 0.8 mL/min
- Detector : Refractive Index detector

B. Quantitative test

Ⓐ Standard curve preparation: Standard solutions of 5, 10, 15, 20, and 25 mL are transferred into each 50 mL mess flask, and filled up with distilled water to the marking to make standard solutions, that will contain 25, 50, 75, 100, and 125 mg of sugar in each 50 mL preparation. 10㎕L of each preparation is injected into the HPLC system, and Peak area and height is recorded to formulate a standard curve.

Ⓑ Calculation

ⓐ The peak area and height gathered from the exclusion ion chromatography will be used to earn the sugar concentration by the degree of polymerization from the standard curve, and the result is calculated as below.

Degree of Polymerization DP1 (Monosaccharide) : A
Degree of Polymerization DP2 (Disaccharide) : B
Degree of Polymerization DP3 (Cellotriose) : C
Degree of Polymerization DP4 (Cellotetrose) : D
Degree of Polymerization DP5 (Pentose) : E
Degree of Polymerization DP6 (Pentose) : F
Degree of Polymerization DP7 (Pentose) : G

Ⓒ The sugar content (%) yielded by analyzing the data produced by reverse phase ion chromatography

DP1 Fructose	A1
DP1 Glucose	A2
DP2 Maltose	B1
DP2 Sucrose	B2
DP2 Isomaltose	B3
DP2 Nigerose	B4
DP2 Gojibiose	B5
DP3 Maltotriose	C1
DP3 Pannose	C2
DP3 Isomaltotriose	C3
DP3 Unidentified (Diverged sugar)	C4
DP4 Maltotetraose	D1
DP4 Isomaltotetraose	D2
DP5 Maltopentaose	E1
DP5 Isomaltopentaose	E2
DP6 Higher polymerization than 6	F
DP6 Isomaltohexaose	F2
DP7 Maltoheptaose	G1
DP7 Isomaltoheptaose	G2
DP8 Higher polymerization	H

(NOTE : Isomalto-oligsaccharides do not include linear degree of polymerization more than DP4 and any compounds, having more than degree of polymerization than DP8).

<Calculation>

DP1 Fructose $A\ mg \times \frac{A_1}{A_1+A_2} = a_1\ mg$

DP1 Glucose $A\ mg \times \frac{A_2}{A_1+A_2} = a_2\ mg$

DP2 Maltose $B\ mg \times \frac{B_1}{B_1+B_2+B_3+B_4+B_5} = b_1\ mg$

DP2 Sucrose $B\ mg \times \frac{B_2}{B_1+B_2+B_3+B_4+B_5} = b_2\ mg$

DP2 Isomaltose $B\ mg \times \frac{B_3}{B_1+B_2+B_3+B_4+B_5} = b_3\ mg^{※}$

DP2 Negerose $B\ mg \times \frac{B_4}{B_1+B_2+B_3+B_4+B_5} = b_4\ mg^{※}$

DP2 Kojibiose $B\ mg \times \frac{B_5}{B_1+B_2+B_3+B_4+B_5} = b_5\ mg^{※}$

DP3 Maltotriose $C\ mg \times \frac{C_1}{C_1+C_2+C_3+C_4} = c_1\ mg$

DP3 Pannose C mg × $\frac{C_2}{C_1+C_2+C_3+C_4}$ = c_2 mg※

DP3 Isomaltotriose C mg × $\frac{C_3}{C_1+C_2+C_3+C_4}$ = c_3 mg※

DP3 Unidentified (Diverged sugar) C mg × $\frac{C_4}{C_1+C_2+C_3+C_4}$ = c_4 mg※

DP4 Maltotetraose D mg × $\frac{D_1}{D_1+D_2}$ = d_1 mg

DP4 Isomaltotetraose D mg × $\frac{D_2}{D_1+D_2}$ = d_2 mg※

DP5 Maltopentaose E mg × $\frac{E_1}{E_1+E_2}$ = e_1 mg

DP5 Isomaltopentaose E mg × $\frac{E_2}{E_1+E_2}$ = e_2 mg※

DP6 Higher polymerization than 6 F mg = f mg

DP6 Maltohexaose F mg × $\frac{F_1}{F_1+F_2}$ = f_1 mg

DP6 Isomaltohexaose F mg × $\frac{F_2}{F_1+F_2}$ = f_2 mg※

DP7 Maltoheptaose G mg × $\frac{G_1}{G_1+G_2}$ = g_1 mg

DP7 Isomaltoheptaose G mg × $\frac{G_2}{G_1+G_2}$ = g_2 mg※

DP8 Higher polymerization H mg = h mg

Substantially, defining the sampling weight and dilution factor as I mg and L, respectively, and considering the *marked sugar represent diverged sugar, the diverged sugar content (%) could be calculated by = (b3 + b4 + b5 + c2 + c3 + c4 + d2 + e2+f_2 + g_2)× L÷ I× 100.

③ Galacto-oligosaccharide (trans-galacto-oligosaccharide)

A. Equipment

High Performance Liquid Chromatography

B. Regents

Ⓐ 20% Sulfosalicylic acid : Special grade regent

20 g of sulfosalicylic acid is dissolved with distilled water to make total volume of 100 mL solution.

Ⓑ Ribose : Special grade

Ⓒ Arabinose : Special grade

C. Preparation of standard solution

2 g of galacto-oligosaccharide is exactly weighed and dissolved with distilled water to make final

volume of 50 mL.

D. Preparation of test solution.

Proper amount of sample is exactly weighed to hold approximately 0.1-1 g of galacto-oligosaccharide, and it is dissolved with distilled water to make final solution of 50 mL. The prepared solution is filtered with 0.45 um filter and used as test solution.

NOTE 1. For the samples, requiring protein removal procedure due to the use of several drops of sulfosalicylic acid which activates the formation of insoluble proteins, it is removed by centrifugation or filtered by passing through Sep pak C_{18} cartridge. When these procedures are required, Ribose and Arabinose is used as internal standard.

NOTE 2. For the samples, containing large amount of fats, The pre-treatment procedure used for Fructo-oligosaccharide is used for these samples' pre-treatment.

NOTE 3: If oligosaccharides of DP3 to DP6 are included in the sample, use appropriate enzymes to digest such oligosaccharides into monosaccharides or disaccharides and then, perform the analytical procedures. However, the presence of isomerized sugar, sugar, or polysaccharides does not interfere with the analysis of galacto-oligosaccharide.

However, the presence of sugars like isomerized sugar and polysaccharides do not interfere the analysis of galacto-oligosaccharide.

E. Test Procedure

Ⓐ High Performance Liquid Chromatography operation condition

- Column: Shodex Ionpak KS-802 equivalents or Reverse phase amino columns
- Column temperature : 80℃
- Mobile phase : Water
- Flow rate : 0.4 mL/min
- Detector: Refractive Index (RI) detector

Ⓑ Quantitative test

2, 4, 6, 8, and 10 mL of standard solutions of galacto-oligosaccharide are transferred into 20 mL mess flasks, respectively, and filled up with distilled water to have final volume of 20 mL. From the prepared standard solution, 10 μL is withdrawn from each preparation to inject into High Performance Liquid Chromatography to acquire peak area and height to produce standard curve. The content of galacto-oligosacchride (%) in the sample is calculated by following equation.

$$\text{Galacto-oligosaccharide (\%)} = B\ \text{mg/mL} \times \frac{50\ \text{mL}}{Ag} \times \frac{100}{1000}$$

A: Sampling weight (g)

FS1B: Concentration of galacto-oligosaccharide obtained from the standard curve (mg/mL)

④ Galacto-oligosacchrides (Raffinose, Stachyose)

A. Regents

Ⓐ Sulfosalicylic acid

Ⓑ Galacto-oligosaccharides(Raffinose, Stachyose) standard solution

Each oligosacchrides of 0.6 g was exactly weighed and dissolved with distilled water to make final volume of 20 mL.

B. Preparation of test solution

Ⓐ For samples, holding negligible amount of fats.

Approximate amount of sample that will contain proper amount (0.07-0.7 g of each galacto-oligosaccharide) is exactly weighed, and dissolved with distilled water to make final volume of 50 mL or extracted. The whole preparation is filtered with 0.45 um filter.

Ⓑ For samples, holding large amount of fats.

If the sample is in liquid form, the same procedure of Ⓐ is used after exactly weighing the sample weight. If necessary, the sample could be neutralized, and diatomic earth is mixed well. Then, the preparation is dried under decompressed atmospheric condition at 60± 2℃ for 2-4hours.

After the drying, the whole amount is transferred into a paper filter tube for the fat extraction by petroleum ether using a soxhlet fat extractor for 8-16 hours. After the removal of fat, the whole amount is taken from the thimble filter and the process of the above Ⓐ is repeated. If the sample is in the powder form, it is directly put into the thimble filter without addition of diatom and then, processed according to the same procedures.

NOTE 1. For the protein containing samples, several drops contained 0.2 g/ mL sulfosalicylic acid is added to remove the protein. If the sample is mixed with large quantity of dextrin, it is removed by the addition of glucoamylase.

C. Test Procedure

Ⓐ High Performance Liquid Chromatography operation condition

- Column: Shodex Ionpak KS-802 equivalents
- Column temperature : 70℃
- Mobile phase : Water
- Flow rate : 1.0 mL/min
- Detector : Refractive Index (RI) detector

Ⓑ Quantitative test

1, 2, 3, 4, and 5 mL of standard solutions of galacto-oligosaccharide are transferred into 5 sets of 10 mL mess flasks, and filled with distilled water to have a final volume of 10 mL. From the prepared standard solution, 10 μL is withdrawn from each preparation to inject into High Performance Liquid Chromatography to acquire peak area and height to produce standard curve. The content of galacto-oligosacchride (%) in the sample is calculated by following equation.

$$\text{Galacto-oligosaccharide (\%)} = (A + B) \times \frac{50}{C} \times \frac{100}{1000}$$

A : Concentration of galacto-oligosacchride obtained from the standard curve (mg/mL)

B : Concentration of galactor-oligosacchride in test solution, obtained from the standard curve (mg/mL)

C : Sampling weight (g)

⑤ Malto-oligosaccharide

A. Equipment

Ⓐ High Performance Liquid Chromatography

B. Regents

Ⓐ 50% Ethyl-alcohol

Ⓑ Activated carbon: grade for the manufacture of the refined rice wine or the bleaching of the sugar solution.

Ⓒ Ion-exchange resin: Enforced acid type (H-type) or Enforced alkaline type (OH-type)

Ⓓ Perlite

Ⓔ water : HPLC grade

Ⓕ Standard sugars

Cellotriose (DP3) - Maltotriose

Cellotetrose (DP4) - Maltotetraose

Pentose (DP5) - Maltopentaose

Hexose (DP6) - Maltohexaose

Heptose (DP7) - Maltoheptaose

C. Preparation of standard solution

50 mg of linear sugars of each degree of polymerization is accurately weighed to dissolve with 50 mL of deaerated distilled water. If necessary, the standard sample may be heated to facilitate dissolution. After the heating, it is cooled and distilled water is added to achieve final volume of 100 mL, that is used as a standard solution.

D. Preparation of test solution

Ⓐ For samples, containing negligible amounts of fats.

4 g of sample is exactly weighed and 30 mL distilled water is added or the same amount of 50% ethanol is added for those samples, which contain proteins. And then heating is applied for dissolution or extraction of sugars. The extracted solution is purified by mixing with 0.5 g of activated carbon and ion-exchange resin (1.5 g that is mixed in the ratio of 1:2 of cation to anion), and filled up with distilled water to make the final volume of 100 mL to be used as a test solution.

Ⓑ For samples, containing large amounts of fats.

4 g of sample in powder form is exactly weighed, and fat extracted with a soxhlet fat extractor. If the sample is in liquid form, recording outer pH range between pH 4-10, it is neutralized and Perlite is added until it turns into crumbly form before the fat extraction for 8-16 hours, After the fat extraction, the whole sample in thimble filter is recovered to follow the same procedures as described in ⓐ after the extraction.

NOTE1. For the sugar molecules as like sugar, lactose, fructo-oligosaccharides, coupling sugar, and galacto-oligosaccharides have a similar the degree of polymerization between 2-5, If such sugar molecules are present in a mixed form in the sample, These individual sugar content is independently analyzed, but the starchy sugars as like isomerized sugars, glucose, and molasses are present in a mixed form. Those sugar types do not interfere the analysis of malto-oligosaccharide.

E. Test Procedure

Ⓐ High Performance Liquid Chromatography operation condition

- Column: Aminex HPx42A(Bio Rad) or equivalent exclusion ion-exchange type.
- Column temperature: 85℃
- Mobile phase: Water
- Flow rate: 0.6 mL/min
- Detector: Refractive Index (RI) detector

Ⓑ Quantitative test (Standard curve)

Each concentration of standard solution 10 μL is injected and analyzed to have a curve. The standard curve is plotted in a way that the horizontal axis represents the concentrations of malto-oligosaccharide (mg) and the vertical axis represents the peak area.

Ⓒ Calculation

Read the area of malto-oligosaccharide and calculate the content of malto-oligosaccharide in the sample solution.

$$\text{Malto-oligosaccharide content (\%)} = \frac{(A + B + C + D + E)\ \text{mg}}{\text{Sampling weight (mg)}} \times \text{Dilution factor} \times 100$$

DP3	Maltotriose	A mg
DP4	Maltotetraose	B mg
DP5	Maltopentaose	C mg
DP6	Maltohexaose	D mg
DP7	Maltoheptaose	E mg

⑥ Xylo-oligosaccharide

A. Equipment

Ⓐ High Performance Liquid Chromatography

B. Regents

Ⓐ Water : HPLC grade

Ⓑ Acetonitrile : HPLC grade

Ⓒ Perlite

Ⓓ Standard sugars

Disaccharide - xylobiose

Cellotriose - xylotriose

Cellotetrose - xylotetraose

Pentose - xylopentaose

Hexose - xylohexaose

C. Preparation of Standard solution

Proper amount of samples containing 50 to 100 mg of xylo-oligosaccharides with different degree of polymerization are exactly weighed and dissolved in deaerated 50 mL distilled water. The

preparations are filtered with 0.45 um filter to be used as standard solutions.

D. Preparation of Test solution

Ⓐ For samples, holding negligible amounts of fats

3 g of samples are weighed and dissolved in water to make the final volume of 30 mL. The solution is filtered with 0.45um filter to be used as a test solution.

Ⓑ For samples, holding large amounts of fats.

3 g of powdery form of samples are exactly weighed, and fat extracted with a soxhlet fat extractor. If samples are in liquid form, pH neutralization is conducted, Perlite is added into the solution until it turns into crumbly form, and then, fat extraction is conducted for 8-16 hours. After the fat extraction, the whole amount of sample in a thimble filter is completely recovered to prepare the test solution according to the same procedure as used in ⓐ.

E. Test Method

Ⓐ High Performance Liquid Chromatography operation condition

- Column: Carbohydrate column 4.6 × 250 mm or equivalent columns
- Column temperature : Room temperature
- Mobile phase : Acetonitrile: Water (75:25)

(The ratio is adjustable according to the type and condition of column)

- Flow rate : 1.2 mL/min
- Detector: Refractive Index (RI) detector

Ⓑ Quantitative test

Each concentration of standard solution 10 μL is injected and analyzed to have a curve. The standard curve is plotted in a way that the horizontal axis represents the concentrations of xylo-oligosaccharide (mg) and the vertical axis represents the peak area. The xylo-oligosaccharide content (%) in the test solution is calculated by following equation

$$\text{Xylo-oligosaccharide content}(\%) = \frac{(A + B + C + D + E)(mg)}{\text{Sampling weight}(mg)} \times \text{Dilution factor} \times 100$$

xylobiose	A mg
xylotriose	B mg
xylotetraose	C mg
xylopentaose	D mg
xylohexaose	E mg

⑦ Gentiooligosaccharides

A. Equipment

Ⓐ HPLC

B. Reagents

Ⓐ Water: HPLC grade

Ⓑ Sodium Hydroxide

Ⓒ Sodium acetate

Ⓓ Perlite

Ⓔ Standard

Gentiobiose, Cellobiose

C. Preparation of standard solution

Appropriate amount of sample is taken to assure that it contains about 50-100 mg of gentiobiose and cellobiose and it is dissolved in 1 L of de-aerated water. The solution is filtered through 0.45 ㎛ filter, which is used as the standard solution.

D. Preparation of Test solution

Ⓐ For sample containing negligible amount of fat

100 mg of sample is accurately taken and dissolved in water to make the final volume of 1 L. This solution is filtered through 0.45 ㎛, which is used as the test solution.

Ⓑ For sample containing large amount of fat

100 mg of sample is accurately taken. For powdered sample, it is put into the extractor. For liquid sample, it is neutralized, if necessary, and added with Perlite until it becomes crumbly. Then, fat extraction is performed for 8-16 hours. After fat extraction, the whole amount in the thimble filter is recovered and then, the procedures of Ⓐ are repeated.

E. Test procedure

Ⓐ HPLC operation condition (1)

- Column: PA-1 Column 4.6 × 250 mm or equivalent one
- Column temperature : Room temperature
- Mobile phase :

A: 150 mM Sodium Hydroxide

B: 150 mM Sodium Hydroxise + 600 mM Sodium Acetate

- Flow rate : 1.0 mL/min
- Detector : Pulsed Amperometric Detecter

Ⓑ HPLC operation condition (2)

- Column : Aminex HPX-42A Column 7.8 × 300 mm or equivalent exclusion ion-exchange type
- Column temperature : 85℃
- Mobile phase : Water
- Flow rate : 0.6 mL/min
- Detector : Refractive Index detector (RI)

Ⓒ Quantitative test

ⓐ Standard curve

Each concentration of standard solution 10 μL is injected and analyzed to have a curve. The horizontal axis represents concentration of gentio-oligosaccharide in mg and the vertical axis of the curve represents the peak area.

ⓑ Calculation

The area values of gentio-oligosaccharide in different concentration of standard solutions are used

to obtain a standard curve and the content of malto-oligosaccharide from sample is calculated from the curve.

Degree of Polymerization DP1 (Monosaccharide): A

Degree of Polymerization DP2 (Disaccharide): B

Degree of Polymerization DP3 or higher (Trisaccharide or higher levels): C

Sugar contents (%) of samples analyzed with use of PA-1 column are as follows;

DP1 Fructose	A_1
DP1 Glucose	A_2
DP2 Maltose	B_1
DP2 Gentiobiose	B_2
DP2 Cellobiose	B_3
≥ DP3	C

<Calculation>

DP1 Fructose A mg × $\frac{A_1}{A_1+A_2}$ = a_1 mg

DP1 Glucose A mg × $\frac{A_2}{A_1+A_2}$ = a_2 mg

DP2 Maltose B mg × $\frac{B_1}{B_1+B_2+B_3}$ = b_1 mg

DP2 Gentiobiose B mg × $\frac{B_2}{B_1+B_2+B_3}$ = b_2 mg

DP2 Cellobiose B mg × $\frac{B_3}{B_1+B_2+B_3}$ = b_3 mg

Content of gentio-oligosaccharide(%)=(b_2 + b_3)mg÷ sample amount mg× dilution factor× 100

(3) Lead

It is tested according to 7.1. Harmful Metal described in 10. General Testing Methods.

BRIEFING

Isomaltooligosaccharides. Because there is no existing *FCC* monograph for this food ingredient, a new monograph is proposed, based in part on the GB/T 20881-2007 Isomaltooligosaccharide monograph from the China National Food Standard, as well as on data and information received. The following tests or specifications are the main differences between this proposed monograph and the GB/T 20881-2007 Isomaltooligosaccharide standard:

1. An IC-PAD method is proposed for Identification, Assay, and the Glucose and Maltose impurity test, as well as Glucose Content after Hydrolysis.
2. The proposed specification for Isomaltose, Isomaltotriose, and Panose Content is based on commercial sample testing results which had a mean and standard deviation of 42 and 12, respectively, calculated on the anhydrous basis. This specification is different from the GB standard of NLT 35 for IMO-50 and NLT 45 for IMO-90. Comments are welcome concerning this specification.
3. Specifications for Glucose and Maltose limits are proposed using data and information received, in which 3 of 12 samples had glucose content higher than the limit, and 4 of 12 samples had maltose content higher than the limit. Comments are welcome concerning these specifications.
4. Specifications for Glucose Content after Hydrolysis and Water are based on testing data of commercial sample analysis. Comments are welcome concerning these specifications.
5. Specifications for Arsenic and Lead limits, and Residue on Ignition (Sulfated Ash) are proposed, based on published available data on commercial products. Comments are welcome concerning these specifications.

The proposal is targeted for publication in the *Third Supplement* to *FCC 10*.

(FI: L. Chen) C169451

Add the following:

▪Isomaltooligosaccharides

Table 1 provides a list of typical saccharides in Isomaltooligosaccharides (IMO).

Table 1. Typical Saccharides in Isomaltooligosaccharides

Individual Saccharide Name	DP[a]	Chemical Name	Chemical Formula	CAS	Formula Wt.
Glucose	1	D-Glucose	$C_6H_{12}O_6$	[50-99-7]	180.16
Maltose	2	4-*O*-α-D-Glucopyranosyl-D-glucose	$C_{12}H_{22}O_{11}$	[69-79-4]	342.30
Isomaltose	2	6-*O*-α-D-Glucopyranosyl-D-glucose	$C_{12}H_{22}O_{11}$	[499-40-1]	342.30
Panose	3	4-*O*-(6-*O*-α-D-Glucopyranosyl-α-D-glucopyranosyl)-D-glucose	$C_{18}H_{32}O_{16}$	[33401-87-5]	504.44
Isomaltotriose	3	6-*O*-(6-*O*-α-D-Glucopyranosyl-α-D-glucopyranosyl)-D-glucose	$C_{18}H_{32}O_{16}$	[3371-50-4]	504.44
Maltotriose	3	4-*O*-(4-*O*-α-D-Glucopyranosyl-α-D-glucopyranosyl)-D-glucose	$C_{18}H_{32}O_{16}$	[1109-28-0]	504.44
Isomaltotetraose	4	6-*O*-[6-*O*-(6-*O*-α-D-Glucopyranosyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-D-glucose	$C_{24}H_{42}O_{21}$	[35997-20-7]	666.58
Maltotetraose	4	4-*O*-[4-*O*-(4-*O*-α-D-Glucopyranosyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-D-glucose	$C_{24}H_{42}O_{21}$	[34612-38-9]	666.58

[a]DP stands for degree of polymerization.

DESCRIPTION

Isomaltooligosaccharides occur as a white amorphous powder, or as a colorless pale yellow transparent viscous syrup. They are a mixture of glucose oligomers, along with disaccharides. The characteristic saccharides of this ingredient are di- and oligosaccharides with α-1,6-glucose linkages, mainly isomaltose, panose, and isomaltotriose, with some products containing smaller amounts of isomaltotetraose and other higher branched oligosaccharides. Other typical saccharides in isomaltooligosaccharides include glucose, maltose, maltotriose, and maltotetraose. Isomaltooligosaccharides are often produced by enzyme-catalyzed reactions on starch-based ingredients through multiple manufacturing processes.

Function: Source of isomaltooligosaccharides; low-calorie sweetener

Packaging and Storage: Store in a well-closed container.

IDENTIFICATION

- **PROCEDURE**

Analysis: Following the *Assay* procedure, identify major peaks of isomaltose, isomaltotriose, and panose in the *Sample solution* chromatogram using the *System suitability solution* chromatogram.

Acceptance criteria: The chromatogram of the *Sample solution* in the *Assay* exhibits major peaks for isomaltose,

isomaltotriose, and panose at retention times corresponding to those in the chromatogram of the *System suitability solution*.

ASSAY

• **ISOMALTOSE, ISOMALTOTRIOSE, AND PANOSE CONTENT**

Solution A: High-purity deionized water (resistivity NLT 18.2 MΩ · cm)

Solution B: 250 mM sodium acetate solution

Solution C: 250 mM sodium hydroxide solution

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	86	2	12
2	86	2	12
23	72	16	12
42	23	55	22
42.1	0	90	10
46	0	90	10
46.1	18	2	80
49	18	2	80
49.1	86	2	12
60	86	2	12

System suitability solution: 20 µg/mL of USP Isomaltooligosaccharides System Suitability RS

Standard solution: 5 µg/mL of USP Maltose RS

Sample solution: 20 µg/mL

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography, ion chromatography

Detector: Pulsed amperometric

Working electrode: Gold

Reference electrode: Silver–silver chloride

Columns

Analytical: 4-mm × 25-cm; consisting of 10-µm diameter nonporous beads covered with a fine latex of functionalized resin[1]

Guard: 4-mm × 5-cm with the same resin phase with the analytical column

Column temperature: 22°

Flow rate: 1.0 mL/min

Injection volume: 25 µL

System suitability

Sample: *System suitability solution*

Suitability requirement 1: The relative standard deviations of the isomaltose, isomaltotriose, panose, and maltose peak areas are NMT 2.0% for six replicate injections.

[NOTE—The approximate retention times of isomaltose, isomaltotriose, maltose, and panose are 14.3 min, 17.4 min, 20.2 min, and 23.6 min, respectively.]

Suitability requirement 2: The tailing factor for isomaltose, isomaltotriose, panose, and maltose is NMT 2.0.

Analysis: Separately inject equal volumes of the *System suitability solution*, *Standard solution*, and *Sample solution* into the chromatograph. Use the chromatograms of the *System suitability solution* to identify the peaks of isomaltose, isomaltotriose, panose, and maltose listed in *Table 3*.

Table 3. Chromatographic Profile

Compound	Relative Retention Time	Relative Response Factor
Isomaltose	0.70	1.12
Isolaltotriose	0.86	1.03
Maltose	1	1.00
Panose	1.16	0.94

Calculate, separately, the percentages of isomaltose, isomaltotriose, and panose in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area of individual oligosaccharide in the *Sample solution*

r_S = peak area of maltose in the chromatogram of the *Standard solution*

C_S = concentration of maltose in the chromatogram of the *Standard solution* (µg/mL)

C_U = concentration of the sample in the *Sample solution* (µg/mL)

F = relative response factor (see *Table 3*)

Acceptance criteria: The sum of the percentages for isomaltose, isomaltotriose, and panose is NLT 17%, calculated on the anhydrous basis.

IMPURITIES

Inorganic Impurities

• **ARSENIC**, *Arsenic Limit Test,* Appendix IIIB

Sample solution: Prepared as directed for organic compounds.

Acceptance criteria: NMT 1 mg/kg, calculated on the anhydrous basis

• **LEAD,** *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIIB

Sample solution: Prepare as directed for organic compounds, using a 5-g sample.

Acceptance criteria: NMT 0.5 mg/kg, calculated on the anhydrous basis

Organic Impurities

• **GLUCOSE AND MALTOSE**

Solution A, Solution B, Solution C, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 1 µg/mL of USP Dextrose RS and 1.6 µg/mL of USP Maltose RS

Sample solution: 20 µg/mL

System suitability

Sample: *Standard solution*

[1] CarboPac PA10 column (Dionex, http://www.thermoscientific.com), or equivalent.

Suitability requirement 1: The relative standard deviations of the dextrose and maltose peak areas are NMT 2.0% for six replicate injections.
[NOTE—The approximate retention times of dextrose and maltose are 8.5 min and 20.3 min, respectively.]
Suitability requirement 2: The tailing factor for dextrose and maltose is NMT 2.0.
Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak areas.
Acceptance criteria The peak areas of glucose and maltose for the *Sample solution* are not greater than those for the *Standard solution*. (NMT 5% for glucose, NMT 8% for maltose, calculated on the anhydrous basis)

SPECIFIC TESTS

- **GLUCOSE CONTENT AFTER HYDROLYSIS**
  Solution A, Solution B, Solution C, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.
  Standard solution: 2 µg/mL of USP Dextrose RS
  Acetate buffer: 0.1 mol/L of sodium acetate. Adjust the pH to 4.5 with acetic acid.
  Amyloglucosidase solution: Amyloglucosidase from *Aspergillus niger*, 1200 IU/mL in *Acetate buffer.*[2] Centrifuge at 3000 rpm/min for 5 min, and use the supernatant to prepare the *Sample solution*.
  Carrez I solution: 36 mg/mL of potassium hexacyanoferrate (II) trihydrate
  Carrez II solution: 72 mg/mL of zinc sulfate heptahydrate
  Sodium hydroxide solution: 0.1 M
  Sample stock solution: 0.1 mg/mL
  Sample solution: Pipet 5 mL of *Stock sample solution* and 5 mL of *Amyloglucosidase solution* into a 50-mL centrifuge tube, vortex for 30 s to mix well, then put in a shaker and incubate at 58° for 24 h, with constant shaking at 60 rpm/min. After incubation, cool to room temperature, then add 5 mL of *Carrez I solution*, 5 mL of *Carrez II solution*, and 5 mL of 0.1 mol/L *Sodium hydroxide solution*. Mix vigorously after each addition, and finally vortex to mix well. [NOTE—Add solutions to the centrifuge tube in the order specified.] Centrifuge at 10,000 rpm/min for 10 min, then pipet 1 mL of the supernatant into a 10-mL volumetric flask, dissolve, and dilute with water to volume. Filter with a 0.2-µm membrane. Final concentration is 2 µg/mL.
  Blank: Pipet 5 mL of water and 5 mL of *Amyloglucosidase solution* into a 50-mL centrifuge tube, vortex for 30 s to mix well, then put in a shaker and incubate at 58° for 24 h. Cool to room temperature, then add 5 mL of *Carrez I solution*, 5 mL of *Carrez II solution*, and 5 mL of 0.1 mol/L *Sodium hydroxide solution*. Mix vigorously after each addition, and finally vortex to mix well. Centrifuge at 10,000 rpm/min for 10 min, then pipet 1 mL of the supernatant into a 10-mL volumetric flask, dissolve, and dilute with water to volume. Filter with a 0.2-µm membrane.
  System suitability
  Sample: *Standard solution*
  Suitability requirement 1: The relative standard deviations of the dextrose peak areas are NMT 2.0% for six replicate injections.
  Suitability requirement 2: The tailing factors for dextrose are NMT 2.0.
  Analysis: Separately inject equal volumes of the *Standard solution*, *Sample solution*, and *Blank* into the chromatograph, record the chromatograms, and measure the peak areas.
  Calculate the percentage of glucose after hydrolysis:

$$\text{Result} = [(r_U - r_B)/r_S] \times (C_S/C_U) \times 100$$

  r_U = peak area of glucose in the *Sample solution*
  r_B = peak area of glucose in the *Blank*
  r_S = peak area of dextrose in the *Standard solution*
  C_S = concentration of dextrose in the *Standard solution* (µg/mL)
  C_U = concentration of the *Sample solution* (µg/mL)
  Acceptance criteria NLT 99%, calculated on the anhydrous basis
- **RESIDUE ON IGNITION (SULFATED ASH)**, Appendix IIC
  Sample: 2 g
  Acceptance criteria: NMT 0.3%, calculated on the anhydrous basis
- **pH**, *pH Determination,* Appendix IIB
  Sample solution: 30% (solids, calculated as 100% minus percentage in *Water* determination) in fresh, neutral, distilled water at room temperature
  Acceptance criteria: 4.0–6.0
- **SOLUBILITY (FOR POWDER PRODUCTS ONLY)**
  Sample: 5000.0 mg
  Analysis: Pre-dry a quantitative filter paper in a tared dish to a constant weight at 105° ± 2° and record the weight. Put the *Sample* in a 50-mL beaker, add the appropriate amount of 35°–40° water to dissolve the *Sample*. Filter the solution with the pre-dried quantitative filter paper, and wash the beaker and the quantitative filter paper 3–4 times thoroughly with a total amount of 50 mL of water. Place the filter paper with the residue in a pre-dried tared dish, dry to a constant weight at 105° ± 2° (approximately 3 h of drying time), and record the weight. Determine the weight of the residue (R).
  Calculate the solubility, in mass percentage (%):

$$\text{Result} = 100 - (R \times 100)/[(1 - W) \times S]$$

  R = weight of the residue on the quantitative filter paper (g)
  W = percent water content of the *Sample*, determined in *Water* test, and expressed as a decimal
  S = weight of the *Sample* taken (g)
  Acceptance criteria: NLT 99%

[2] 100 IU is the amount of amyloglucosidase that catalyzes the conversion of water soluble starch into 1 mg of glucose per min, at 40° and pH 4.6. EC Number is 3.2.1.3.

- **TRANSMITTANCE (FOR SYRUP PRODUCTS ONLY)**
  Sample solution: 30% (solids, calculated as 100% minus percentage in *Water* determination) in fresh, neutral, distilled water at room temperature
  Analysis: Measure transmittance at 440 nm using a suitable spectrophotometer. Conduct a blank test that follows the *FCC General Provisions and Requirements*.
  Acceptance criteria: NLT 95%
- **WATER**, *Water Determination, Method I,* Appendix IIB
  Acceptance criteria
  Powder products: NMT 5%
  Syrup products: NMT 45%■3S (*FCC10*)