Agave Inulin

Generally Recognized as Safe (GRAS) Notice for IMAG Organic®
Agave Inulin Extracted from *Agave tequilana* Weber var. a*zul*

Prepared for

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C O N T E N T S

TABLES:

FIGURES:

1.0 SIGNED STATEMENTS AND CERTIFICATION

1.1 § 170.225(a): Signature

Signed,

Name: Bryan C. Tungland, President, Tungland and Associates, LLC
Date: May 10, 2020

1.2 § 170.225(c)(1): Formal Notification

IMAG (Inulina Y Miel de Agave S.A. de C.V.), through its agent Tungland and Associates, LLC, hereby notifies the U.S. Food and Drug Administration that the identified agave inulin product described below is Generally Recognized As Safe (GRAS) in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act, under the intended conditions of use. This notification is submitted in accordance with 21 CFR § 170 Subpart E.

1.3 § 170.225(c)(2): Name and Address of Notifying Organization and Agent

Notifying Organization:
Inulina Y Miel de Agave S.A. de C.V. (**IMAG**)
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1.4 § 170.225(c)(3): Name of Notified Substance

The trade name of the substance that is the subject of this Generally Recognized as Safe (GRAS) determination is **IMAG Organic®**. This substance represents an inulin-type fructan with a graminan/agavin-type structure, to be used in the U.S. market. This product line is produced by Inulina Y Miel de Agave S.A. de C.V. (**IMAG**), in Capilla de Guadalupe, Jalisco, MX from agave piña or stems (also known as cores, heads or pines) or the agave plant, ***Agave tequilana* Weber var. *azul***, commonly known as blue agave and Weber's blue agave, which is grown and processed in the occidental region of

Mexico. The plant source, manufacturing, specifications and composition of this notified substance is the equivalent to that which is the subject of GRN 854, agave inulin. Other common names for this substance include blue agave inulin, fructans from agave, agave fructans, and inulin tequilana Weber blue agave.

1.5 § 170.225(c)(4): Intended Conditions of Use

IMAG Organic® agave-derived fructans are intended for general addition to foods except non-exempt and exempt infant formula and meat and poultry products, **Table 1.1**. The substance is intended to be used as an alternative for Inufib™ agave inulin. The intended uses are for the same foods and same per serving levels as identified in GRAS Notification to the FDA for Inufib™ agave inulin (GRN 854, FDA 2020).

Table 1.1 Proposed Food Use Categories and Use Levels for IMAG Organic® in the U.S.

Food Category[a]	Reference Amount (mL or g)	Maximum Use Level of IMAG Organic®	
		(%)	(mL or g /serving)[b]
Acidophilus milk (fermented dairy beverages)	240 mL	2	4.8 mL
Bars: all types, including breakfast, granola, energy and meal replacement types	40 to 70 g	10	4 to 7 g
Baby foods: all types of baby foods and beverages, including ready-to-serve and dry baby foods, excludes infant formulas	7 to 60 g	1 g/serving	7 to 60 g
Breakfast cereals: All RTE types	15 to 60 g	5 g/serving[a]	0.75 to 3 g
Beverages, juices and juice drinks: fruit juices and drinks, including ades, cocktails, cider, nectar, and smoothies, vegetable juices, flavored waters, soy drinks, gelatin drinks, and lightly carbonated beverages, including ready-to-drink beverages and dry mixes[c] (excludes citrus juices & highly carbonated beverages)	360 mL	1.5	5.4 mL
Beverages, functional: meal replacement & supplemental, RTD and dry mixes[c]	240 mL	5	12 mL
Beverages, milk-based: RTD & dry mixes[c]	240 mL	1	2.4 mL
Biscuits, reduced energy	55 g	6	3.3 g
Breads, conventional: yeast leavened breads, rolls, & buns	50 g	0.5	0.25 g
Breads, specialty: reduced energy, fiber-enriched or with added calcium, includes muffins and quick breads	55 g	6	3.3 g
Baked goods, lite cakes: fat free/reduced fat/sugar/calorie brownies, pastries cakes	40 to 125 g	5	2 to 6.25 g
Candy (hard dietetic)	15 g	15	8.25 g
Candy (soft dietetic)	30 g	5	1.5 g
Cheese (cream type)	30 g	5	1.5 g
Cheese (processed and cheese products)	30 g	5	1.5 g
Cheese used in pasta fillings	55 g	5	2.75 g
Condiments: major types, including catsup and mustard	15 mL	5	0.75 mL
Cookies, reduce energy, reduced sugar	30 g	8	2.4 g
Crackers: snack-type, including savory, sandwich, whole grain (excluding plain crackers such as saltines, matzo crackers or oyster crackers)	30 g	6	1.8 g
Dessert toppings, lite: fat free/reduced fat marshmallow cream, non-dairy whipped toppings	30 mL	6	1.8 mL
Dessert toppings: excluding whipped toppings	30 mL	2	0.6 mL
French fry coatings: coating on French fries	30 g	1.7[d]	0.51 g
Frozen dairy desserts, lite: fat free/reduced fat/calorie ice creams & dairy-based frozen desserts, novelties and frozen yogurt	161 mL	8	12.88 mL
Icing/glazes, lite: fat free/reduced fat/sugar icings and glazes	30 g	5	1.5 g
Jams and jellies, lite: reduce sugar/energy	20 g	2	0.4 g
Mousse, reduced fat/energy	120 mL	3	3.6 mL
Pasta, fresh: such as spaghetti, fettuccini, tortellini, ravioli, lasagna (excluding noodles)	140 g	4	5.6 g
Pasta, precooked macaroni	140 g	4	5.6 g
Pizza crust	55 g	5	2.75 g
Potatoes, mashed: prepared or frozen (excludes dry mix types)	140 g	3	4.2 g
Pretzels, soft	30 g	5	1.5 g
Salad dressings, lite: fat free/reduced fat/reduced energy, including mayonnaise, salad dressings and mayonnaise-type dressings	15 to 30 g	5	0.75 to 1.5 g

Sauces and gravies: entrée, dipping and condiment sauces such as Alfredo, BBQ, cheese, clam, Hollandaise, pasta, pizza, soy, sweet & sour and white sauces, salsa, gravies, excluding tomato sauce & paste	30 mL	2	0.6 mL
Snack chips, reduced fat: fat free/reduced fat snacks, including chips and extruded snacks	30 g	3	0.9 g
Soups, dry	245 g	3	7.35
Spreads, reduced fat: fat free/reduced fat margarines and margarine-like table spreads	15 mL	10	1.5 mL
Surimi: surimi, imitation crab, and reconstructed seafood	55 g	3	1.65 g
Syrups, lite: reduced energy, including flavored pancake syrups	30 mL	2	0.6 mL
Tortillas, reduced fat	55 g	3	1.65 g
Vegetarian patties/crumbles	85 g	2	1.7 g
Yogurt, reduced fat: fat free/reduced fat refrigerator-type yogurts	170 g	3	5.1 g

RTD = Ready-to-drink; RTE = Ready-to-eat

[a] The food use categories and proposed use levels (%) are adapted from GRN 118 with additional correspondence amendments (U.S. FDA, 2007). Serving sizes correspond to Reference Amounts Customarily Consumed per Eating Occasion, 21 CFR 101.12.

[b] Calculated based on standard serving size and proposed % use level.

[c] Maximum use levels correspond to g IMAG Organic® per 100 g prepared beverage or sauce.

[d] Maximum use level per 100 g coated French fry (as consumed).

1.6 § 170.225(c)(5): Statutory Basis for GRAS Status

IMAG has determined that the intended use of IMAG Organic® is Generally Recognized as Safe (GRAS) for all its intended purposes through scientific procedures in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C.§301 et. seq.) ("The Act"), as described in 21 CFR 170.30(b), thus satisfying the technical element of the GRAS determination. The exposure under the proposed conditions of use is based on knowledge and information that is both publicly available and widely accepted by experts qualified by scientific training and experience as described in 21 CFR 170.30(a). This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience to evaluate the safety of substances added to food .

1.7 §170.225(c)(6): Statement of Exemption from Premarket Approval Requirements

IMAG hereby states that the use of the notified agave inulin product described above and which meets the specification described in Section 2.4, is exempt from pre-market approval requirements of the Federal Food and Drug and Cosmetic Act because IMAG has determined that such use is Generally Recognized As Safe (GRAS) for all intended purposes in accordance with subpart E of 21 CFR 170.

1.8 § 170.225(c)(7):Availability of Information

The data and information within this document that serves as the basis for the GRAS determination is generally available, and will be sent to the FDA upon request, or are available for review and copying at reasonable times at the office of Tungland and Associates, LLC located at 13600 Joseph Avenue, Becker, MN, 55308.

1.9 § 170.225(c)(8): Statement of FOIA Status

IMAG hereby certifies that, to the best of our knowledge, none of the data and information in Parts 2 through 7 of this GRAS notification are exempt from disclosure under the Freedom of Information Act (FOIA).

1.10 § 170.225(c)(9): Statement of Completeness

IMAG hereby certifies that, to the best of our knowledge, this GRAS notification is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the notified substance.

1.11 § 170.225(c)(10): Contact Information for Responsible Official of Agent

Contact: Bryan C. Tungland
President, CEO
Tungland and Associates, LLC
Agent for IMAG
Telephone: 763-350-1590
Email: tungland@sherbtel.net

1.12 § 170.225(c)(11): Statement on Trade Secrets

If the intended conditions of use of the notified substance include use in a product or products subject to regulation by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture, IMAG authorizes FDA to send any trade secrets to FSIS.

2.0 IDENTITY OF THE NOTIFIED SUBSTANCE

2.1 § 170.230(a)(1): Scientific Information Identifying the Notified Substance

IMAG Organic® agave inulin is a liquid or powder formulation consisting of naturally occurring fructose polysaccharides from *Agave tequilana* Weber var. *azul*, that is produced from plants that are between 4 and 7 years of maturity, with a molecular range 2 to about 70 fructose units and a mean degree of polymerization of 11.9, The preparation also contains minor amounts of mono- and disaccharides (fructose, glucose and sucrose). The Chemical Abstracts Service (CAS) has a registry number for inulin as 9005-80-5, although agave inulin does not have a defined CAS registry number.

2.1.1 Chemical Formula and Molecular Structure

Agave inulin is a mixture of naturally occurring carbohydrates under the general name “fructans”, a name for carbohydrates in which one or more fructosyl-fructose links constitute the majority. This refers to polymeric material as well as to oligomers as small as the disaccharide inulobiose. Fructans may be classified into five major types according to the way the β-fructofuranosyl units are linked and position of glucose in the structure (Vijn et al., 1997; Vijn and Smeekens, 1999), although all major groupings, with exception to levans, have primary β(2-1)-fructofuranosyl linkages with lesser β(2-6) side chains, as inulin. These major fructan types include:

1. more linear inulin-type fructans with β(2-1)-fructofuranosyl linkages with a terminal glucose unit that are widely described in the *Asteraceae* or *Compositae* family, which includes the dicotyledon plants, chicory, Jerusalem artichokes, elecampane, dahlia and dandelion;

2. inulin neoseries, which contains a glucose moiety between two fructofuranosyl units extended by β(2-1) linkages, as characterized in onion, garlic, leeks and asparagus, also in the *Asparagales* order;

3. levan (or phlein) with linear β(2-6) linkages with a terminal glucose unit as found in grasses like *Phleum pratense* or are of bacterial origin;

4. levan neoseries, formed by β(2-1)-and β(2-6)-linked fructofuranosyl units on either end of a central sucrose molecule, as reported in oat (*Avena sativa*); alternatively they are composed of two linear β(2-6)-linked fructosyl chains, having an internal glucose moiety, and;

5. mixed fructans (graminans or agavins) containing mainly β (2-1)- fructofuranosyl linkages, as in number 1, having more significant β(2-6) side chains than number 1 (generally, they are branched fructans like those found in wheat (*Triticum aestivum*), and a few members of *Asparagales* order, such as **agave**). The glucose moiety may be terminal, as in graminans or internal, as in agavins (Mancilla-Margalli and Lopez, 2006; Waleckx et al., 2008).

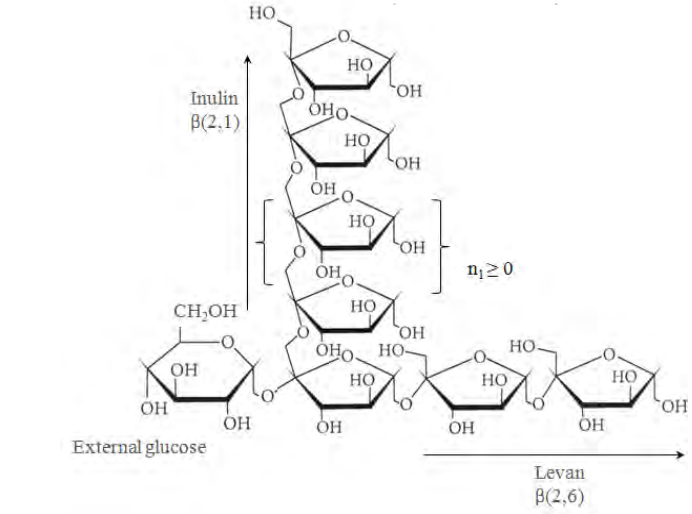
The major groupings that contain a majority of β (2-1)-fructofuranosyl linkages, as in numbers 1, 2, 5, are generally referred to as inulin and inulin-type fructans, while those containing a majority of β (2-6)-linkages are generally referred as being levans.

According to the above system for classification of fructans, the notified substance, IMAG Organic® agave inulin, like that in GRN 854 (FDA, 2020), belongs to the "mixed fructan" group (number 5), based on the two linkage types and chain branching. As mentioned, agave fructans are further categorized as graminans, that are mixed fructans containing branched β(2-1) and β(2-6) linkages and terminal glucose moieties, and agavins, that are branched neo-fructans, characterized by internal α-D-glucopyranose (Mancilla-Margalli and Lopez, 2006).

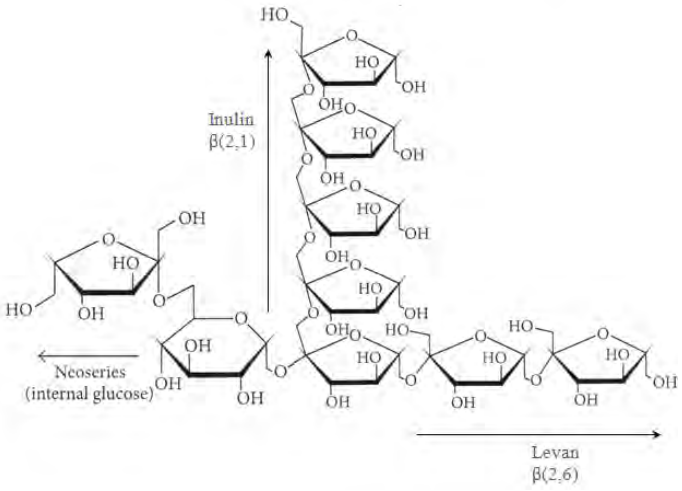
The molecular formula for agave inulin is the same as that of all fructans: $C_6H_{11}O_5(C_6H_{10}O_5)_nOH$. As noted above, IMAG Organic® agave inulin consists of fructan molecules with a DP generally ranging from 2 to about 70.

As **Figure 2.1** shows, the fructans found in monocotyledons, such as agave, are complex and possess a predominate β(2-1) linked inulin-type structure together with a moderate degree of β(2-6) linked branched structure in mature plants. The chemical structures of fructans obtained from mature (5 to 8 year-old) *Agave tequilana* Weber var. *azul* plants, as used to manufacture IMAG Organic®, the same plants used to manufacture Inufib™ in GRN 854 , have been characterized (López et al., 2003; Mancilla-Margalli and López, 2006; Toriz et al., 2007; Mellado-Mojica and Lopez, 2012). Agave fructans are structurally diverse mixtures of fructooligosaccharides (FOS) and fructans that contain both β(2-1), the majority, and β(2-6)-linkages, with internal and external glucose units, which are termed agavin- and graminan-type fructans, respectively (Mancilla-Margalli and López, 2006; Mellado-Mojica and López, 2012). Other researchers have also proposed structures (Franco-Robles and López, 2015; Livingston et al., 1993; Pavis et al., 2001; Sims et al., 1992). In mature agave plants, the agavins are the more abundant of the two fructan types (Mellado-Mojica and López, 2012).

Figure 2.1. Molecular structures of agave mixed type inulin-type fructans of β(2,1)- and β(2,6)-linked graminans and agavins with high degree of branching, modified from Franco-Robles and López (2015) and Mancilla-Margalli and López (2006).



Graminans



Neofructans (Agavins)

Mellado-Mojica and López (2012) determined that, like other inulin-type fructans, the composition of glycosidic linkages of *A. tequilana* fructans differ according to plant age. The authors determined that the average DP of fructans stored in plants 2 to 7 years old range from DP 6 to DP 23, with the latter coinciding with a long degree of polymerization (LDP), about DP = 18-28 for 6-7 year-old plants of this species (González-Cruz et al., 2012; López et al., 2003; López et al., 2014; Mancilla-Margalli and López, 2006). β(2-6) linked branches were absent in 2-year-old plants, emerging at 4-year-old plants, and reached highest degree in fructans from 7-year-old plants. Graminans and agavins were present at all plant ages, but their proportions diverged as plants aged. Toriz and others (2007) determined the native fructans from *Agave tequilana* Weber var. *azul* had a mean DP of 16, with a DP range from 2 - 60. Löppert and others (2009) provided further evidence of the molecular weight and physiochemical characteristics of fructans from *Agave tequilana* Weber var. *azul*, determining that the DP fraction from DP3 - DP12 was 20% of the total distribution, while the polymer fraction from DP 20 - DP 70 had 57% of the distribution, and 20% of the polymers were between DP 12 and DP 20, with a mean DP of about 15.

Mellado-Mojica and López (2012) also found that plants begin with equal proportions of agavins/graminans, moving toward more complex branched structures with more isomeric forms having a higher abundance of agavins than graminans at 7 years (Ratio of agavins/graminans: 0.9 ± 0.3 at 2 yrs. vs 3.6 ± 1.3 at 7 yrs.), and large DP as plants age.

2.1.2 Agave Inulin Composition

IMAG Organic® typically contains 98-100% carbohydrate (dry basis): and more than 92% agave inulin, with up to about 8% mono- and disaccharides, primarily fructose, glucose, and sucrose. This product is equivalent to the agave inulin in GRN 854 (FDA, 2020), Details about the carbohydrate composition and DP can be located in section 2.4 and 8.1.1.

Agave inulin contains no fatty acids. Mineral analyses show sodium levels of approximately 75.0 mg/100g (refer to Table 2.6, section 2.4).

Concentrations of saponins and terpenes are not detected under the same analytical conditions as phthalates (a method with a detection limit of 7 ppb), equal to that shown on page 7 and 10 in GRN 854 (refer to section 2.4, Saponins and terpenes, analysis UNAM).

As the agave used to manufacture IMAG Organic® is organically-grown for U.S. organic food labeling, no pesticides or herbicides were used on the production of the agave crop, and no pesticides were identified in analytical screens of samples of the notified substance at method detection limits (refer to section 2.4, pesticides). The notified substance also does not contain any heavy metals at method detection limits (also refer to section 2.4, heavy metals).

IMAG Organic® is available in both dry spray-dried powder and a stable liquid syrup that is between 68 and 72° Brix. Liquid IMAG Organic® is the purified and filtered agave inulin juice that has been concentrated via evaporation to produce a clear, stable tan syrup, while the dry powdered IMAG Organics® represents the liquid product that has been spray-dried to produce a white to yellowish white powder with a neutral odor. The only difference between the two products is their water content.

2.1.3 Comparison of IMAG Organic® Composition with Other Commercial Agave Fructans

IMAG Organics® can be compared with other purified fructan products extracted from *Agave tequilana* Weber var. Azul (Blue agave), such as Metlin® and Metlos®, agave inulin products of Nekutli S.A. de C.V., MX, or Inufib™ (GRN 854) and Predilife™ (Agave), native agave inulin products from Industrializadora Integral del Agave S.A. de C.V., Jalisco, MX (IIDEA), Agro Corona, S.A. de C.V., Usmajac, Jalisco, MX, respectively. IMAG Organic®, like Inufib® (GRN 854, FDA, 2020) is prepared from *A. tequilana* Weber var. Azul piñas and are manufactured in the same manner. Predilife™ (Agave) is also prepared in a similar manner, although it also undergoes activated carbon and ion exchange treatment steps to eliminate calcium and chelates, as described by Gomez and others (2010). BioAgave®, a product of CP Ingredients S.A. de C.V., (Corn Products Int'l., Inc.), now part of INGREDION, once was supplied as a longer chain agave product, presumably via size fractionation processing. However, it is no longer marketed. A comparison of its fructan distribution with IMAG Organic® is included in **Table 2.1**. The aforementioned agave inulin products are similar to IMAG Organic®, in that they are all derived from piñas of *A. tequilana* Weber Var. Azul (with exception to BioAgave®, which is unknown) and consist of

linear and branched fructan fractions with β(2-1) and β(2-6)-linked fructofuranosyl units. The main difference between Metlin® and Metlos® from Nekutli is their degree of polymerization (DP) distributions. Presumably, the products are produced from native agave inulin molecules that are subjected to additional processing steps, such as membrane ultrafiltration (UF), to produce two fructan fractions based on molecular weight and reduced mono- and disaccharide contents, such as described in Márquez-Aguirre and others (2013, 2016). These researchers defined membrane UF procedures that produced a fructan fraction higher than DP >10 from the retentate of a 3 kDa membrane, whereas fructans with a lower DP < 10 were recovered from the retentate of a 1 kDa membrane. Metlin® contains predominately fructans greater than DP 10 with very-low mono- and disaccharide levels, while Metlos® is a fructooligosaccharide, containing predominately fructans less than DP 10, with slightly higher monosaccharide content. Both products consist of ramified (branched) molecules, so they are highly soluble in cold water. By contrast, IMAG Organic® is a natural mixture of fructans ranging from 2 to about 70 fructose units; predominately shorter chain inulin (DP < 20) and also about 30% fructooligosaccharides (DP ≤ 10), about the same as Inufib™ defined in Table 2, page 7 of GRN 854. About 30% of IMAG Organic® also consists of fractions higher than DP 20. Like the agave inulin defined on page 7 of GRN 854, the fructan mixtures of Metlin® and Metlos® that contain both fructooligosaccharides and longer chain inulin fractions are compositionally comparable to IMAG Organic®.

As mentioned, other refined agave inulin products include Inufib®, BioAgave® and Predilife™ (Agave), **Table 2.1**. Like IMAG Organic®, these agave inulin products represent native, unaltered mixed chain (both β2-1 and β2-6-linked) ramified polyfructan substances with variation due to harvest time and growing location and are produced using similar methods, albeit, as mentioned, Predilife™, uses additional carbon and ion exchange treatments. The DP of IMAG Organic® ranges from 2 to about 60-70, whereas Inufib™ and Predilife™ (Agave) range from 3 to 29, while that from BioAgave® is described as ranging from 25-34. The mean DP for IMAG Organic® is 11.9, while the mean DPs for Inufib® (GRN 854, page 7) is reported to be 19.7, and the mean DP for BioAgave™ and Predilife™ (Agave) are not reported. Although, the mean DP of IMAG Organic® and Inufib™ are different the distribution of the molecular chain is very similar, with IMAG Organic® being somewhat more polydispered (possessing a broader range of fructose moieties than Inufib™. The mean DPs for Metlin® and Metlos® are reported to be 27 and 15, respectively. The relatively narrow molecular weight (DP) range of Inufib™, as compared with the broad range in IMAG Organic®, significantly influences its polydispersity index (IP), a measure of how broad the molecular weight distribution is of a polymeric chain, and is calculated as a ratio of a polymer's weight average molecular weight, Mw, to its average molecular weight, Mn: Mw/Mn. By example, the IP for IMAG Organics® is about 1.7, a moderately polydispersed polymer, and is similar to chicory root inulin (a substance defined in GRN 118), while the IP for Inufib™, another agave inulin, has a significantly narrower distribution, about 1.2, **Table 2.1**.

Table 2.1 Comparison of the Fructan Distribution of IMAG Organic® with Other Agave Fructan Products

Product	Mean (Range) of DP	Distribution of DP	Mean Polydispersion Index
IMAG Organic®	11.9 (2 - 70)	70% > DP10 > 30%	1.7
Inufib™ (GRN 854)	19.7 (3 - 29)	73% > DP10 > 27%	1.2
BioAgave®	NR (25 - 34)	NR	NR
Predilife™ (Agave)	NR (3 - 29)	NR	NR
Metlin®	27 (NR)	84% > DP10 > 16%	2.3
Metlos®	15 (NR)	55% > DP 10 > 45%	3.3
NR = Not reported			

2.2 § 170.230(a)(1): Information on the Biological Source of the Notified Substance

2.2.1 Identification of the Source

Agave inulin (IMAG Organic®) is a natural fructan concentrate that is derived from the piñas (stem or pines) of the agave plant (***Agave tequilana Weber* var. *azul,*** known commonly as blue agave and Weber's blue agave) produced from plants between 4 and 7 years of maturity. ,.

2.2.2 Comparison of Agave Inulin with Inulin from Other Commercial Sources

The physical properties of various commercial fructan products are related to the raw material quality and plant source being used to produce the final fructan product. The agronomic practices and growing conditions, the timing of raw material harvest, its storage conditions, and processing conditions all influence the physicochemical properties shown in **Table 2.2**. Properties shown in the table relate to the typical characteristics observed and specified for each product. Alterations to the previously mentioned conditions influence the properties listed in **Table 2.2**, and as listed, different subcategories exist for the native chicory inulin products, and native agave fructans, such as fractionated short and long chain chicory inulin products, and short chain agave fructans. Data for the chicory, Jerusalem artichoke inulin products and native agave inulin (such as IMAG Organic® or its equivalent defined in GRN 854) listed in **Table 2.2** are associated with native inulin or agave type fructans and are typical characteristics based on currently recorded public domain product specifications.

The majority of commercially available inulin and oligofructose are extracted from chicory roots (*Cichorium intybus* L.). Like agave inulin, the DP of chicory-derived inulin varies with source of the plant and time of harvest. Hot water diffusion is used to extract inulin from the chicory root, and the dried refined inulin product has an average DP of 10-12, ranging from 2 to 60 fructose units, and about 6-10% mono- and disaccharides (glucose, fructose and sucrose).

Agave inulin, sourced from the piña, or stem and produced in a similar manner as inulin from chicory root, has an average DP of about 14-18, and, by some accounts and processes, a distribution from 3 to 29, although others have noted a range from 2 to 70 fructose units for native polydispersed agave inulin. Thus, native fructans from agave and chicory both contain similar degree of polymerization, up to about 10% of mono- and disaccharides, and about 90% inulin (Niness, 1999; Murphy, 2001). Longer-chain chicory inulin fractions, like those from Beneo-Orafti (Orafti® HP) or Sensus Operations (Frutafit® TEX), which are commonly used in the food industry for their ability to form submicron particle gels and

mimic fat, is manufactured by removing the shorter-chain oligomers and residual sugars from native chicory inulin. Thus, they have an average DP of about 25 with a range of about 11 to 60. Oligofructose is derived through water extraction of native chicory root inulin but is treated with endoinulinase to partially hydrolyze the inulin after extract, resulting in chain lengths that range from 2 to 8 and an average DP of 4. By contrast, commercially available inulin from Sigma, as derived from *Dahlia* tubers, has a standardized average DP of 27-29, and is often used to diagnose renal clearance rate and function.

As mentioned previously, the structure of these plant-derived inulins consist mainly of β(2-1)fructosyl-fructose linkages with chicory inulin containing 1-2% β(2-6)fructosyl-fructose branches; dahlia inulin has about 4-5% β(2-6)fructosyl-fructose branches (Hariono et al., 2009), and agave inulin has about 24% β(2-6)fructosyl-fructose branches (Lopez et al., 2003). Neither the β(2-1)- nor the β(2-6)-linkages are susceptible to hydrolytic action by mammalian pancreatic or brush-border digestive enzymes. As a consequence, these fructans reach the colon largely undigested and intact, to serve as fermentation substrate by resident microbiota, particularly the *Bifidobacterium* spp. and other lactic-acid producing bacteria, such as the *Lactobacillus* spp. (Lopez et al., 2003; Munjal et al., 2009; Roberfroid et al., 2010).

High-performance anion exchange chromatography with a pulsed electrochemical detector working in pulsed amperometric detection mode (HPAEC-PED analysis) was used to characterize the chain length distribution of various commercial fructans, including native chicory root inulin (Frutafit® HD, GRN 118), fractionated short-chain chicory inulin (Frutafit® CLR) and fractionated long-chain chicory inulin (Frutafit® TEX), products of Sensus Operations, The Netherlands, native Jerusalem artichoke inulin (Fructanex®), a product from Nexxus Foods, Montreal, Canada, short-chain fructooligosaccharides (scFOS, Actilight®), a product of Eridania Béghin-Say & Meiji (FR JV), chicory oligofructose (Beneo-Orafti® Oligofructose), a product of Beneo-Orafti, BE, div. of Südzucker, DE, and native agave inulin, as in IMAG Organic®, the notified substance, **Table 2.2**. Frutafit® inulin has a range of chain lengths in a range that is characteristic for inulin from DP 2 to greater than 60, with a modal chain length that was ≥ 9 fructose units. Fructanex® has a range of chain lengths in a range that is characteristic for inulin from DP 2 to 28, with a modal chain length that was ≥ 6 fructose units. Native agave inulin from mature plants has a chain distribution that is similar to native chicory inulin from Frutafit®, a GRAS inulin having no questions or objections of its status under GRN 118. The chain length distribution analysis of Frutafit® (native chicory inulin), Fructanex® (native J. artichoke inulin) and IMAG Organic® (native agave inulin) indicate that they are consistent with that of inulin consumed historically by humans in sustenance foods, without change. To this end, the International Organization for Standardization (ISO/TS) 19657:2017, defines inulin from these sources as "natural" for labeling, as they are plant-based source materials and produced by physical and/or enzymatic and/or microbiological processing without alteration of the ingredient from its original source. In addition, these ingredients are also defined as dietary fibers based on recognized physiological effects and have been assessed and approved by the Food Directorate, Health Canada (Health Canada, 2013) and the U.S. Office of Food Labeling FDA (2018b). To an extent, the chain length distribution or the degree of polymerization (DP) influences human tolerance, with short chain fractions, being more easily fermented, resulting in somewhat lower, albeit safe tolerance than longer chain fructan fractions (refer to later section on human tolerance to fructans).

Table 2.2. Physicochemical Properties of Commercially Available Powdered Fructan Products

Physical Properties	scFOS	Oligofructose	Native Chicory Inulin	Fractionated Short Chain Chicory Inulin	Fractionated Long Chain Chicory Inulin	Native Jerusalem artichoke inulin	Native Agave Inulin
Degree of polymerization (DP) range	2 – 4	2 - 8	2 – 60	2 – 20	10 – 60	2 - 28	2 – 70
Average DP	4	5	9	7	25	7	18
Average Mol. Wt. (g/mol)	730	810	1500	1274	4550	1275	2995
< DP3 (%)			5.8	8.8	0.5	7.5	9.9
Kestose (DP 3) (%)	28	27	2.3	5.4	0.2	18	4.4
Nystose (DP 4) (%)	60	31	2.7	6	0.2	17.9	4.9
Fructosylnystose (DP 5) (%)	12	22	3.3	6.4	0.2	8.5	3.7
DP 6 (%)		12	3.3	5.7	0.2	7.6	3.3
DP 7 (%)		3.2	4.6	8.2	0.4	11	4.8
DP 8 (%)			4.4	7.8	0.4	10.4	4.5
DP 9 (%)			4.7	8.1	0.6	10.9	4.7
DP 10 – 14 (%)			20.9	28.5	12.7	8.3	23.9
DP 15 - 19 (%)			15.1	12.6	20.8		17.1
DP 20 - 24 (%)			11	6.3	18.5		11.6
DP 25 – 29 (%)			7.6	3	14.1		6.8
DP 30 – 34 (%)			5.5	1.3	11.4		3.9
DP 35 – 39 (%)			3.8	0.5	8.4		2.5
DP 40 – 44 (%)			2.5	0.2	5.3		1.5
>DP 45 (%)			2.4	0.1	6.2		2.3
DP 3-4 (%)	88	61	6	11.4	0.3	36	9
DP 5-9 (%)	12	39	22	36.2	2	48	21
DP ≤ 10 (%) (excludes < DP 3 fraction)	100	100	28	47.6	2	84	30
DP < 20 (%) (excludes < DP 3 fraction)	100	100	65	88.8	36	100	71
DP > 20 (%)	0	0	29	11.2	64	0	29
Water solubility g/100g @ 20° C w/clarity	>75	>75	9-12	20	2.5	20	> 70
Water viscosity (5% at 10° C)	< 1 mPa	< 1 mPa	1.6 mPa	< 1 mPa	2.4 mPa	< 1 mPa	< 1 mPa
pH (10% soln.)	5 – 7	5 - 7	5 – 7	5 – 7	5 - 7	5 – 7	4 – 6
Mean particle size (μm)	90	230	45	70	50	35	30
Bulk Density (g/L) (tapped)	300	120	600	550	700	770	675
Appearance and Color	White	White	White	White	White	White	White
Relative sweetness (sucrose = 100)	35	35	10	20	0	20	10
Gel formation (particle gels) g/100g	No gel	No gel	30	No gel	10	No gel	No gel

Source: Tungland, 2018.

Agave inulin, as notified on page 9 of GRN 854 and this GRAS notice shares chemical, physicochemical, and nutritional properties with other plant-derived fructans and with fructooligosaccharides produced by enzymatic synthesis from sucrose. Thus, toxicological studies performed with synthetic short-chain fructooligosaccharides (scFOS; avg. DP = 4) are considered to be predictive of the effects of naturally-occurring inulin and oligofructose since the substances are chemically similar with like nutritional properties (Carabin and Flamm, 1999; FDA-GRN 118, 2002a). FDA in GRN 854 found no scientific or toxicological reasons to determine that the chemical structure, physicochemical properties or nutritional attributes of the agave inulin notified as GRAS had any significant bearing on the product's safety to humans under the proposed conditions of use.

2.3 § 170.230(b): Method of Manufacture

Manufacturing processes and analytical methods used by IMAG for the production of IMAG Organic® agave inulin are equivalent to those used for the manufacture of the agave inulin defined starting on page 9 of GRN 854 (Inufib™) from IIDEA and those used for the manufacture of chicory root-derived inulin (GRN 118) and other commercial inulin products. IMAG Organic® is manufactured from agave grown under organic production methods and are consistent with Good Agricultural Practices (GAP) defined in an official field manual for growing and producing foods to be organically labeled. The practices and means of handling of the agave raw material used to satisfy the US National Organic Program (NOP) 7 CFR Part 205 is certified as meeting standards for organic labeled foods by Kiwa BCS

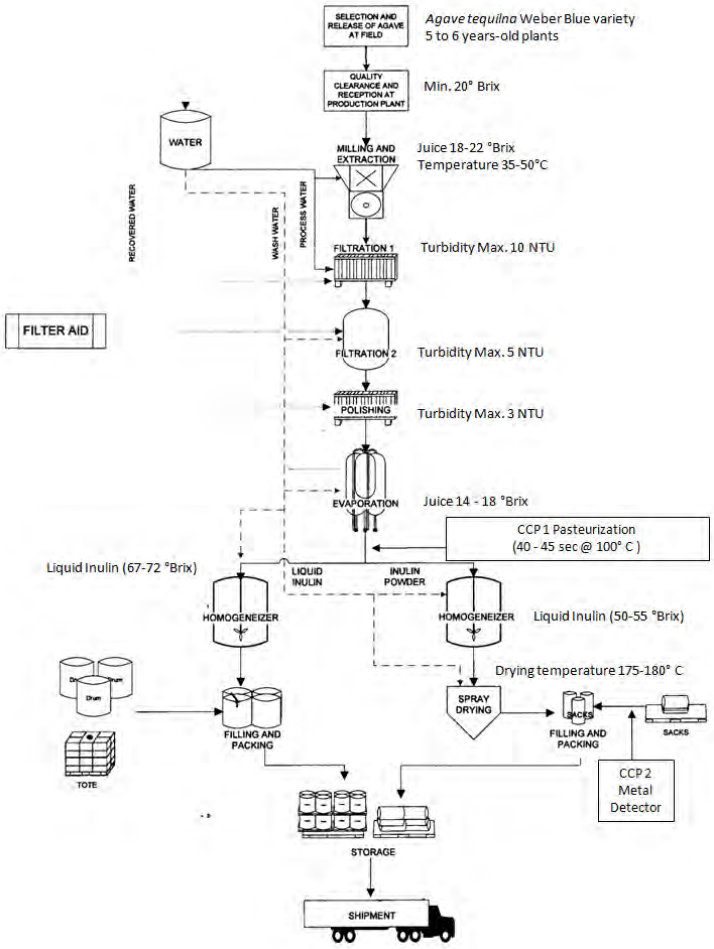
ÖKo-Garantie GmbH under numbers Nr.: A-2007-00676 / 2019-01156. In addition, farmland field soil used to grow the crop and the agave leaves are analyzed in a comprehensive screen for residual pesticides by AGQ Labs Mexico to assure no pesticides have been used and no carryover of pesticides has contaminated the agave crop. Analyzes show no residual pesticides are present in the farmland soil or agave leaves, at the limits of method detection. The finished inulin ingredient is manufactured from the agave raw material in a manner consistent with current Good Manufacturing Practice requirements (cGMP) for human food (21 CFR Part 110).

IMAG Organic® agave inulin is available in both dry spray-dried powder (< 5% moisture) and shelf-stable liquid syrup that is between 68 and 72° Brix. Liquid IMAG Organic® is the purified and filtered agave inulin juice that has been concentrated via evaporation to produce a clear, stable tan syrup, while the dry powdered IMAG Organic® represents the liquid product that has been spray-dried to produce a white to yellowish white powder with a neutral odor. The only difference between the two products is their water content.

IMAG's inulin-type fructan is manufactured in a manner similar to other commercial inulin ingredients from plant sources that have GRAS status, including the preparations described in GRN 118, 392, 477, 576, 582, 687, and 854. The manufacturing process used to make the notified substance is also equal to or similar described starting on page 9 of recent GRN 854 agave inulin produced by IIDEA (Inufib™). Like the other commercial inulins, mmanufacturing to produce IMAG Organic® involves slicing/milling of the raw material (agave piña), followed by water extraction using a counter-current diffusion method, clarification and filtration, concentration, and, finally, drying to a powder (**Figure 2.2)**.

Processing temperatures and contact times do not exceed thermal decomposition limits of the inulin molecule to produce reducing sugars, a primary reactant in the Maillard reaction. Process temperatures, while being relatively high for evaporation and spray drying, are very short contact in duration. Further, pH is above 4.0 and at a level stable to chemical hydrolysis of the inulin molecule (Glibowski and Bukowska, 2011; Tungland, 2018). Moreover, the acidic pH, is nowhere near favorable for Maillard reactions, which primarily take place under alkaline conditions, so the formation of Maillard compounds is negligible, and of no concern. Also, refer to p. 56 of GRN 854, which states that no such products are formed.

Figure 2.2 Process Diagram for Powdered and Liquid IMAG Organic® Products



CCP: critical control point

2.3.1 Pre-processing

During harvesting of agave piña in the fields, the majority of the green upper plant tissue, stalks and long leaves (tops), are removed by cutting near the stalk/root close to leaf/piña interface in agave. The long leaves of the agave plant are manually cut at the leaf-to-piña interface, using a straight, knife-sharp hoe, called a coa, as well as the roots and are left in the fields for soil enrichment. It is important to emphasize that IMAG Organic® agave inulin is derived from the piñas and is not from the leaves, as sap or extracts from some agave leaves are known to contain saponins and raphides of calcium oxalate, known inedible bioactive agents.

The harvested agave piña or stems are typically removed from the field manually by farmers and loaded into trucks. Agave piña (containing IMAG Organic® agave inulin) from trucks are loaded manually onto conveyor belts entering the process facility, allowing extraneous debris, trash and other non-piña material to be manually removed during these transfers, and consequently provides the means to eliminate the extraneous material before it enters the processing facility.

2.3.2 Washing

As the majority of the agave piña grow aerially (above ground), they do not have residual clinging soil, soil bacteria and other soil-related material, however, surfaces of piña are rinsed/washed with reclaimed and pasteurized evaporator condensate water during their transport via conveyor from the truck to slicing operations to remove clinging surface material, such as microbes, insects and fibrous plant matter.

2.3.3 Size Reduction/Slicing/Milling and Scalding

Following the washing step, the agave piña is sliced into appropriate size for the subsequent extraction step.

2.3.4 Extraction

Agave slices are fed into a stainless-steel extraction (diffusion) system where inulin is extracted in a continuous countercurrent mode. Agave fructans are extracted at temperatures between 30 °C and 50 °C. A continuous counter-current diffusion process is used in which the agave slices are conveyed by a feed scroll or tray conveyor, while fresh water is added at the top and fructan-containing juice of increasing concentration is moving in the opposite direction. As the extracted slices reach the end of the diffuser, they are removed from the diffuser and pressed in mechanical presses, and subsequently air dried to produce animal feed, or are burned to produce energy for the manufacture of steam. Agave fructans are soluble so no scalder is used in its processing, unlike that used in manufacturing facilities that produce a less soluble inulin type, such as chicory or Jerusalem artichoke inulin.

2.3.5 Clarification and Purification

The raw agave juice exiting the diffuser, typically about pH 4.5, is transferred to a settling tank (~30 °C) where proteins and pectic substances precipitate. The resulting sludge and particulate matter is separated from the juice *via* successively tighter press filtration beginning with a FDA approved filter medium (Table 2.3) pore size of 10 microns using a polypropylene woven canvas (21 CFR 177.1520 1(i) from Texfyl that is coated with progressively tighter pore size Perlite, a silicate-based porous filter aid material that is allowed for food use by the US FDA under Select Committee on GRAS Substances (SCOGS) ID code 93763-70-3. The final filtration uses cellulose plates from Columbia with a pore size of 1 micron (21 CFR 176.170 and 176.180). These filters remove any microorganisms and fine particulates and are expected to also remove raphides of calcium oxalate, if present, which are 30-500 microns in length (Salinas et al., 2001), if present.

2.3.6 Sterilization, Concentration, Spray Drying

As mentioned, agave juice is polish filtered with a Columbia fiber incorporating food-grade compliant cellulose plates (21 CFR 176.170 & 176.180) at a pore size of 1 micron to remove turbidity to less than 5 NTU. As described in GRN 118, following clarification, the juice is sterilized at 104° C, followed then by concentration to a dry matter of 40-45 percent by multi-effect water evaporation at low temperature (below 90 °C) and reduced air pressure (less than 0.8 Bar). These specific conditions are selected to prevent discoloration of the juice during concentration. The concentrated juice is then pumped to

spray-driers and dried to a final concentration of greater than 95 percent dry matter. The resulting high fructan-containing powder is then packaged for distribution in U.S. Food Grade 25 kilogram multi-walled, poly-lined bags. These bags act as moisture barriers, preventing powder caking during storage. As a liquid agave inulin product, the filtered sterilized juice from above is concentrated to a dry matter content of 68° - 72° Brix and bottled or placed in U.S. Food Grade liquid storage drums or tote container for distribution. Powdered and liquid IMAG Organic® agave inulin products are stored in a conditioned dry, covered warehouse at 25 °C.

2.3.7 Processing Materials and Aids

The IMAG manufacturing process used to produce IMAG Organic® is intended to produce products that meet the condition for U.S. organically labeled foods. Therefore, the process by IMAG to produce IMAG Organic ® does not use any fungicides, slimicides, or other biocides. In addition, the agave crop used for the production of IMAG Organic® is organically grown and uses no pesticides, herbicides or insecticides as condition to meet conditions of the United States Department of Agriculture (USDA)/National Organic Program (NOP) Final Rule (7 Part 205). The raw materials and processing aids used in the manufacture of the IMAG Organic® agave inulin is listed in **Table 2.3**. All raw materials and processing aids used in the manufacture of IMAG's agave inulin product meet food-grade quality specifications, as set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard (e.g., JECFA), and are permitted for use in food by U.S. federal regulation or are GRAS for their respective uses. In addition, other than water, no solvents, and other chemicals or aids, such as pH modifiers, activated carbon, are included in the manufacturing process. Analytical analyses conducted by external laboratory, as shown in section 2.4 (specifications, saponin and terpenes), show concentrations of saponins and terpenes are below 0.1 ppm. None of these bioactive agents are detected, and under the method and conditions of analysis used by the same laboratory used to determine these components in the agave inulin sited on page 10 of GRN 854, the test laboratory concluded that, if the compounds ecogenin and ecogin were present in the analyzed samples, their concentrations would be less than 7 ppb.

Water used for process is pasteurized evaporator condensate as part of the manufacturing water recirculation (water saving) process. Water samples are sent to outside laboratories once per year in accordance with the Mexican Regulations: NOM-127-SSA1-1994 "Environmental Health, Water for Use and Human Consumption". In addition, water quality control analyses are performed internally within IMAG from the process recirculation tank twice per week for: microbiology (coliforms and *E. coli*), pH, conductivity, chlorine and calcium.

As mentioned above, filter medium and filter aids are used to remove particulate material from the extracted agave juice and purify it prior to subsequent evaporation. These processing aids meet current U.S. regulations for human food.

Table 2.3 Raw Materials and Processing Aids Used in the Manufacture of IMAG Organic® Inulin

Material	Function	Regulatory Status
Fresh raw agave piña	Source of raw inulin	Certified under 7 Part 205 organic
Water	Solute for the inulin	Meets NOM-127-SSA1-1994*
Polypropylene canvas filter medium	Process filtration/particulate removal	21 CFR 177.1520 1(i)
Cellulose filter plates	Process filtration/particulate removal	21 CFR 176.170 & 176.180
Perlite silicate-based filter aid	Precoat for filters to improve flux	SCOGS ID code 93763-70-3

*Mexican potable water standard limits : 2 CFU/100 mL total coliforms (no fecal coliforms); 20 units of true color on platinum-cobalt scale; pleasant smell, 5 NTUs of turbidity; Chemical constituents in mg/L: Al (.020), Ar (0.05), Ba (0.70), Cd (0.005), CN (0.07), Chlorine (0.2-1.50), CL (250.00), Cu (2.00), Cr (0.05), Hardness (500.00), Phenolics (0.001), Fe (0.30), F (1.50), Mn (0.15), Hg (0.001), Nitrates (10.00), Nitrites (0.05), Ammonia N (0.50), pH 6.5-8.5), Aldrin and dieldrin (0.03 mcg/L), Chlordane (0.30), DDT (1.00), gamma-HCH (lindane) (2.00), Hexachlorobenzene (0.01), heptachlor and heptachlor epoxide (0.03), hethoxychlor (20.00), 2,4-D (50.00), Pb (0.025), Na (200.00), TDS (1000.00), Sulfates (400.00), SAAM (0.50), Total trihalomethanes (0.20), and Zn (5.00). These are in accordance with the EPA drinking water standards under the Safe Drinking Water Act (SDWA).

2.3.8 Manufacturing Registrations and Certifications

IMAG is registered with the FDA pursuant to section 305 of the U.S. Public Health Security and Bioterrorism Preparedness and Response Act of 2002, and the FDA Registration No. is 12848756546. The agricultural field practices and crop handling comply and are certified as meeting standards under 7 CFR Part 205 for foods to be organically labeled by the US National Organic Program (NOP). The manufacturing process complies with the international GMP standard ISO 22000:2005, ISO/TS 22002-1:2009 and FSSC 22000 v. 4.1 requirements. The production process has been assessed to identify any reasonable potential hazards associated with the process and critical control points established to prevent, eliminate, or reduce potential hazards to acceptable levels. Potential biological, chemical and physical hazards have been addressed by the current Hazard Analysis Critical Control Point (HACCP) Plans in place at IMAG for both and liquid products.

2.4 § 170.230(c): Product Specifications

The specifications for powdered and liquid IMAG Organic® agave inulin, along with analytical data performed by independent third-party testing laboratories from 4 randomly selected non-consecutive lots of each inulin type from 2014-2019 are shown in **Tables 2.4 and Table 2.5.** Data in these tables show both compositional and microbiological analytical results from the sample of lots tested. In addition, heavy metals, another specification, were analyzed by independent laboratories from 2014-2019 using validated analytical methods on 21 lots of representative agave inulin product, which represents about 18-20% of total production. These data are reported in **Table 2.6**. The concentrations of mycotoxins are specified to be not detected at method detection limits. To determine potential levels of mycotoxins levels in the agave inulin, 5 randomly selected production lots of powdered and liquid product from 2015-2019 were sent to Eurofins, an independent testing laboratory located in Madison, WI. Mycotoxins were determined in samples using an ISO-accredited, Eurofins validated stable isotope dilution assay (SIDA) by ultra-high-performance liquid chromatography and mass spectral analysis (UHPLC-MS/MS) [Varga et al., 2012]. Data reported in **Table 2.7** show no detected levels of mycotoxins at method detection limits.

We note that while data for every specification is not provided for each production lot presented in this dossier, testing for ALL specifications will be completed for every commercial lot released to ensure compliance with the specifications required for GRAS status.

Table 2.4 Specifications and Batch Data for Dry IMAG Organic® Powder						
Parameter	**Method**	**Specification**	**Batch Number**			
			CB015913	**CB033913**	**40110512**	**42301412**
Date Manufactured →			**01/15/2019**	**02/02/2019**	**10/01/2015**	**01/23/2014**
Physical Parameters						
Moisture	AOAC 925.45, 945.43, 934.01	≤ 4 g/100 g	3.97	3.47	3.60	3.59
pH	AOAC 981.12	> 5.5	6.88	5.90	7.09	7.34
Color	Observation	White/slight beige	White/slight beige	White/slight beige	White/slight beige	White/slight beige
Taste	Oral test	Slightly sweet	Slightly sweet	Slightly sweet	Slightly sweet	Slightly sweet
Chemical Parameters						
Ash	AOAC 923.03	≤ 0.3 g/100g (d.s. basis)	0.26	0.24	0.24	0.25
Dry Matter (100-moisture)	AOAC 925.45, 945.43, 934.01	≥ 96 g/100g	96.03	96.53	96.4	96.41
Carbohydrate Composition		≥ 99 g/100 g (d.s. basis)	100	100	100	99.94
Inulin[1]	AOAC 997.08	≥ 92 g/100 g (d.s. basis)	95.00	95.10	94.59	94.30
Fructose	AOAC 977.20	≤ 6 g/100 g (d.s. basis)	2.65	2.28	1.89	2.19
Sucrose	AOAC 977.20	≤ 2 g/100 g (d.s. basis)	1.16	1.59	2.00	1.82
Glucose	AOAC 977.20	≤ 2 g/100 g (d.s. basis)	1.18	1.02	1.52	1.63
Average DP Inulin	AOAC 997.08	> 11	18	15	35	35
Heavy Metals	See table 2.6	ND	See table 2.6			
Mycotoxins	See table 2.7	ND	See table 2.7			
Microbiological Parameters						
Total Aerobic Plate Count-CFU/g	FDA-BAM Chap. 3 AOAC 2002.07 (a)	< 1000	50	120	70	100
Yeasts and Molds-CFU/g	FDA-BAM Chap. 18 AOAC 2002.11 (a)	< 10	< 10	< 10	< 10	< 10
Total Coliforms- CFU/g	FDA-BAM Chap. 4 AOAC 2005.03 (a)	Absent	Absent	Absent	Absent	Absent
Escherichia coli-CFU/g	FDA-BAM Chap. 4 AOAC 2005.03 (a)	Absent	Absent	Absent	Absent	Absent
Salmonella (CFU in 25g)	AOAC 989.13 (a)	Absent	Absent	Absent	Absent	Absent

[1]Nondigestible soluble carbohydrates as inulin with 3 or more monomeric units are declared as dietary fiber in compliance with regulatory definition of "dietary fiber" under 2018 FDA guidance 21 CFR 10.30, d.s. - dry substance; (a) Based on NMX-FF-110-SFCI-2008; CFU/g - Colony Forming Units/gram

Table 2.5. Specifications and Batch Data for Liquid IMAG Organic®						
Parameter	**Method**	**Specification**	**Batch Number**			
			CB100824	**CB129922**	**CB032928**	**CB155952**
Date Manufactured →			**04/10/2018**	**05/09/2019**	**02/02/2019**	**06/04/2019**
Physical Parameters						
Moisture	AOAC 925.45, 945.43, 934.01	28.0 - 32.0 g/100 g	31.06	30.81	30.89	30.68
Brix Degrees		68.0 - 72.0° Brix	68.94	69.20	69.11	69.32
pH	AOAC 981.12	≥ 5.5	6.10	6.10	6.15	5.83
Color	Observation	White/slightly amber	White/slightly amber	White/slightly amber	White/slightly amber	White/slightly amber
Taste	Oral test	Slightly sweet	Slightly sweet	Slightly sweet	Slightly sweet	Slightly sweet
Chemical Parameters						
Ash	AOAC 923.03	≤ 0.3 g/100g (d.s. basis)	< 0.14	< 0.14	< 0.14	< 0.14
Dry Matter (100-moisture)	AOAC 925.45, 945.43, 934.01	≥ 96 g/100g	68.94	69.19	69.11	69.32
Carbohydrate Composition		≥ 99 g/100 g (d.s. basis)	100	100	100	100
Inulin[1]	AOAC 997.08	≥ 92 g/100 g (d.s. basis)	93.85	93.82	92.23	93.67
Fructose	AOAC 977.20	≤ 6 g/100 g (d.s. basis)	2.21	2.72	4.18	3.32
Sucrose	AOAC 977.20	≤ 2 g/100 g (d.s. basis)	2.00	1.78	1.97	1.32
Glucose	AOAC 977.20	≤ 2 g/100 g (d.s. basis)	1.95	1.68	1.62	1.69
Average Inulin DP	AOAC 997.08	> 11	18	18	24	18
Heavy Metals	See table 2.6	ND	See table 2.6			
Mycotoxins	See table 2.7	ND	See table 2.7			
Microbiological Parameters						
Total Aerobic Plate Count-CFU/g	FDA-BAM Chap. 3 AOAC 2002.07 (a)	< 1000 CFU/g	< 10	< 10	< 10	< 10
Yeasts and Molds-CFU/g	FDA-BAM Chap. 18 AOAC 2002.11 (a)	< 10 CFU/g	< 10	< 10	< 10	< 10
Total Coliforms-CFU/g	FDA-BAM Chap. 4 AOAC 2005.03 (a)	Absent	Absent	Absent	Absent	Absent
Escherichia coli-CFU/g	FDA-BAM Chap. 4 AOAC 2005.03 (a)	Absent	Absent	Absent	Absent	Absent
Salmonella-CFU/25g	AOAC 989.13 (a)	Absent	Absent	Absent	Absent	Absent

[1]Nondigestible soluble carbohydrates as inulin with 3 or more monomeric units are declared as dietary fiber in compliance with regulatory definition of "dietary fiber" under 2018 FDA guidance 21 CFR 10.30 d.s. - dry substance; (a) Based on NMX-FF-110-SFCI-2008; CFU/g - Colony Forming Units/gram

2.4.1 Heavy Metals

Heavy metal analyses were performed on 21 lots of representative dry inulin product, the most concentrated IMAG Organic® form. These lots represent about 18-20 percent of total production. Analyses were performed by independent laboratories from 2014-2019 using validated analytical methods. Independent laboratories used for these analyses were Medallion Laboratories, Minneapolis, MN using an Inductively Coupled Plasma/Mass Spectrometry method (AOAC 993.14) and Mercury by EPA 7473, CENCON Group Control Center, a central quality control laboratory in Tepatitlán de Morelos, Jalisco, MX using an Official Mexican Standard Atomic Absorption Spectrophotometric method (NOM-117-SSA1-1994), Eurofins Laboratories, Madison, WI using 2011.19 and AOAC 993.14 and Mercury by EPA 7470. Data shown in **Table 2.6,** representing the levels of the heavy metals: arsenic, lead, cadmium and mercury, are all below detection limits of the analytical methods.

Table 2.6 Heavy Metal Levels for Dry IMAG Organic® Powder

Batch Number	Date of Manufacture	Method	Arsenic (Ar)		Cadmium (Cd)		Lead (Pb)		Mercury (Hg)	
			LOD	Result	LOD	Result	LOD	Result	LOD	Result
42301412	01.23.2014	AOAC 993.14/EPA 7473	< 10 ppb	ND	< 10 ppb	ND	< 10 ppb	ND	< 0.2 ppb	ND
40506412	06.05.2014	NOM-117-SSA1-1994	< 19 ppb	ND	< 5 ppb	ND	< 200 ppb	ND	< 30 ppb	ND
41001512	01.10.2015	NOM-117-SSA1-1994	< 19 ppb	ND	< 5 ppb	ND	< 200 ppb	ND	< 30 ppb	ND
40907512	07.09.2015	NOM-117-SSA1-1994	< 19 ppb	ND	< 5 ppb	ND	< 200 ppb	ND	< 30 ppb	ND
40110512	10.01.2015	AOAC 993.14/EPA 7473	< 10 ppb	ND	< 10 ppb	ND	< 10 ppb	ND	< 0.2 ppb	ND
41501612	01.15.2016	NOM-117-SSA1-1994	< 19 ppb	ND	< 5 ppb	ND	< 200 ppb	ND	< 30 ppb	ND
40207612	07.02.2016	NOM-117-SSA1-1994	< 19 ppb	ND	< 5 ppb	ND	< 200 ppb	ND	< 30 ppb	ND
41110612	10.11.2016	AOAC 993.14/EPA 7473	< 10 ppb	ND	< 10 ppb	ND	< 10 ppb	ND	< 0.2 ppb	ND
42710626	10.27.2016	AOAC 993.14/EPA 7473	< 10 ppb	ND	< 10 ppb	ND	< 10 ppb	ND	< 0.2 ppb	ND
40207712	07.02.2017	NOM-117-SSA1-1994	< 19 ppb	ND	< 5 ppb	ND	< 200 ppb	ND	< 30 ppb	ND
CB084813	03.25.2018	AOAC 993.14/EPA 7473	< 10 ppb	ND	< 10 ppb	ND	< 10 ppb	ND	< 0.2 ppb	ND
CB100822	04.10.2018	AOAC 2011.19/993.14	< 10 ppb	ND	< 5 ppb	ND	< 5 ppb	ND	< 5 ppb	ND
CB100824	04.10.2018	AOAC 993.14/EPA 7473	< 10 ppb	ND	< 10 ppb	ND	< 10 ppb	ND	< 0.2 ppb	ND
CB142813	05.22.2018	NOM-117-SSA1-1994	< 19 ppb	ND	< 5 ppb	ND	< 200 ppb	ND	< 30 ppb	ND
CB015913	01.15.2019	AOAC 993.14/EPA 7470	< 10 ppb	ND	< 10 ppb	ND	< 10 ppb	ND	< 0.2 ppb	ND
CB017913	01.17.2019	NOM-117-SSA1-1994	< 19 ppb	ND	< 5 ppb	ND	< 200 ppb	ND	< 30 ppb	ND
CB032928	02.01.2019	AOAC 2011.19/993.14	< 10 ppb	ND	< 5 ppb	ND	< 5 ppb	ND	< 5 ppb	ND
CB033913	02.02.2019	AOAC 2011.19/993.14	< 10 ppb	ND	< 5 ppb	ND	< 5 ppb	ND	< 5 ppb	ND
CB149913	05.29.2019	AOAC 2011.19/993.14	< 10 ppb	ND	< 5 ppb	ND	< 5 ppb	ND	< 5 ppb	ND
CB176913	06.25.2019	NOM-117-SSA1-1994	< 19 ppb	ND	< 5 ppb	ND	< 200 ppb	ND	< 30 ppb	ND
CB279913	10.06.2019	NOM-117-SSA1-1994	< 19 ppb	ND	< 5 ppb	ND	< 200 ppb	ND	< 30 ppb	ND
LOD - Limit of Detection; ppb - Parts per billion; ND - Not detected										

2.4.2 Mycotoxins

To determine potential levels of mycotoxins levels in the agave inulin, 5 randomly selected production lots of powdered and liquid product from 2015-2019 were sent to Eurofins, an independent testing laboratory located in Madison, WI. Mycotoxins were determined in samples using an ISO-accredited, Eurofins validated stable isotope dilution assay (SIDA) by ultra-high-performance liquid chromatography and mass spectral analysis (UHPLC-MS/MS) [Varga et al., 2012]. Data in **Table 2.7** show that mycotoxins levels are below method detection limits for all representative agave inulin samples analyzed by Eurofins.

Table 2.7 Mycotoxin Levels in Powdered IMAG Organic® Agave Inulin

Mycotoxin	Method	LOD (ppb)	Batch Number				
			CB032928	CB033913	43103612	40403512	CB073813
Manufacturing Date →			02.01.2019	02.02.2019	03.21.2016	03.05.2015	04.14.2018
Aflatoxin B1	UHPLC-MS/MS	0.500	ND	ND	ND	ND	ND
Aflatoxin B2	UHPLC-MS/MS	0.500	ND	ND	ND	ND	ND
Aflatoxin G1	UHPLC-MS/MS	0.500	ND	ND	ND	ND	ND
Aflatoxin G2	UHPLC-MS/MS	0.500	ND	ND	ND	ND	ND
Aflatoxin M1	UHPLC-MS/MS	0.500	ND	ND	ND	ND	ND
Aflatoxin M2	UHPLC-MS/MS	0.500	ND	ND	ND	ND	ND
Deoxynivalenol	UHPLC-MS/MS	100	ND	ND	ND	ND	ND
T-2 Toxin	UHPLC-MS/MS	10	ND	ND	ND	ND	ND
HT-2 Toxin	UHPLC-MS/MS	100	ND	ND	ND	ND	ND
Fumonisin B1	UHPLC-MS/MS	25	ND	ND	ND	ND	ND
Fumonisin B2	UHPLC-MS/MS	25	ND	ND	ND	ND	ND
Ochratoxin A	UHPLC-MS/MS	1	ND	ND	ND	ND	ND
Zearalenone	UHPLC-MS/MS	30	ND	ND	ND	ND	ND
ppb - parts per billion; ND - not detected; LOD - Limit of Detection							

Data shown in **Tables 2.5-2.12** confirm that the finished agave inulin products meet or exceed the analytical specifications defined by IMAG, as stipulated in the Mexican Legislation in force for agave inulin (NMX-F-591-SCFI-2010), and demonstrate that the IMAG Organic® manufacturing process results in a consistently reproducible product. Data in Tables 2.8-2.11 further confirm the lack of impurities/contaminants (pesticides, PCBs, dioxins and furans).

2.5 Other Product Attributes

Other quality attributes (nutritionals) are shown in **Table 2.8** from 4 randomly selected non-consecutive lots of dry powder inulin, the most concentrated form, from 2014-2019. In addition, other quality parameters, including residual saponin and terpene, pesticides, PCBs, dioxins and furans, and molecular weight consistency of the IMAG Organic® agave inulin, are also shown in **Tables 2.9** through **Table 2.12** from randomly-selected, non-consecutive lots from 2015-2019, respectively.

2.5.1 Nutritionals

Analyses were also performed on various non-consecutive agave inulin lots to show the relatively consistency of nutritional attributes of the product. Analyses were performed on lots of powdered agave inulin, the most concentrated form, from 2014-2019. Nutritional analytical data provided in **Table 2.8** show no detectable levels of fat (< 0.007 g/100g), including saturated fat, monounsaturated or polyunsaturated fats, trans fatty acids, or cholesterol, and less than 1% total protein expressed on dry basis. The mineral analyses data also show relatively low levels, with sodium content averaging < 0.1% (0.0754 g/100g), similar to that shown on page 11 in GRN 854 for agave inulin.

Table 2.8 Batch Nutritional Data for Dry IMAG Organic® Powder

Parameter	Method	Limit of Detection	Batch Number			
			CB015913	CB033913	41110612	42301412
Date Manufactured →			**01/15/2019**	**02/02/2019**	**10/11/2016**	**01/23/2014**
Fats						
Total fat (d.s. basis)	AOAC 996.06	70 mg/kg	< 70	< 70	< 70	< 70
Saturated fatty acids (d.s. basis)	AOAC 996.06	70 mg/kg	< 70	< 70	< 70	< 70
Monounsaturated fatty acids (d.s. basis)	AOAC 996.06	70 mg/kg	< 70	< 70	< 70	< 70
Polyunsaturated fatty acids (d.s. basis)	AOAC 996.06	70 mg/kg	< 70	< 70	< 70	< 70
Trans fatty acids (d.s. basis)	AOAC 996.06	70 mg/kg	< 70	< 70	< 70	< 70
Cholesterol (d.s. basis)	AOAC 976.26	10 mg/kg	< 10	< 10	< 10	< 10
Elemental Parameters						
Sodium (d.s. basis) (mg/kg)	AOAC 985.01	0.791 mg/kg	1110	776	580	549
Iron (d.s. basis) (mg/kg)	AOAC 985.01	0.065 mg/kg	< 2.60	< 2.58	< 10	< 10
Calcium (d.s. basis) (mg/kg)	AOAC 985.01	0.830 mg/kg	236	259	244	231
Magnesium (d.s. basis) (mg/kg)	AOAC 985.01	2.40 mg/kg	108	138	133	227
Potassium (d.s. basis) (mg/kg)	AOAC 985.01	4.33 mg/kg	1890	2400	1740	1790
Other Nutritional Parameters						
Protein, by Dumas (d.s. basis) (g/100g)	AOAC 992.15	0.648 g/100g	0.80	0.80	< 0.78	< 0.78

d.s. - dry substance; mg/kg - milligrams/kilogram

2.5.2 Saponins and Terpenes

Additional information from scientific literature on these non-carbohydrate bioactive components of agave is contained in Section 8.1.3 and 8.14 of this GRAS Notice. The residual concentration remaining in the finished IMAG Organic® agave inulin samples is the same as that measured in the notified substance in GRN 854, Inufib™ agave inulin, below the limit of the detection in the analytical method used at Department of Analytical Chemistry of the National Autonomous University of Mexico (Universidad Nacional Autónoma de México - UNAM), the same university and method used for the determination of these components for Inufib™ described in GRN 854.

Steroidal saponins and terpenes are two notable non-fructan bioactive components in the raw agave material. Like other edible plants such as oats, peppers, chickpea, tomato seed, alliums, asparagus, and yams, agave plants contain steroidal saponins, with concentrations in agave plant being similar to the saponins in chickpea. The two most predominate steroidal saponins in agave are hecogenin and tigogenin, which are primarily located in the long leaves of the plant, rather than the piña used as the raw material for agave inulin production. Regarding terpenes in the agave plant, several terpenes have been identified, with linalool, a terpene alcohol, being the most predominate.

The presence of steroidal saponins, particularly hecogenin and tigogenin, and terpenes, especially linalool, were determined using a method of hexane extraction developed internally by the Department of Analytical Chemistry of the National Autonomous University of Mexico (Universidad Nacional Autónoma de México - UNAM), an independent laboratory located in University City, Coyoacán, MX, combined with a validated method for the separation and analysis of organic compounds by gas chromatography and detection by mass spectrometry (GC/MS Clave: PT-USA1-FQ-EM-001). This is the same method and laboratory used to identify saponins and terpenes in agave inulin described on page 10 and 16 of GRN 854. The method used to identify potential residual levels of saponin and terpenes in samples of agave inulin described in GRN 854 and IMAG Organic® agave inulin both used liquid-liquid hexane extractions of 4 powdered inulin samples that had been dissolved in water. A report of the analysis of the four (4) extracted powdered inulin samples for free and conjugated saponins and terpenes submitted by the Department showed no detectable amounts of any free or conjugated saponins or terpenes at method detection levels, **Table 2.9.** The same detection limits for these components in GRN 854. Although the analysis was not a quantitative determination (lacking standards), the UNAM laboratory routinely performs analyses using the GC/MS system that require parts per billion (ppb) level determinations. As described in Attachment 2 "Saponins and Terpenes", the aforementioned agave inulin samples were analyzed under the same conditions as phthalates (a method with a detection limit of 7 ppb). As the same method and outside laboratory was used to determine the levels of saponins and terpenes in the agave inulin described in GRN 854 and the notified substance, the method has the same detection limit. Described on page 10 of GRN 854 and in its Attachment 3 "Letter saponins Ext Lab" the test concluded that, if the compounds ecogenin and ecogin were present in the samples, their concentrations would be under 7 ppb.

Table 2.9 Saponins and Terpene Levels in Powdered IMAG Organic® Agave Inulin

Bioactive	Limit of Detection	Batch Number Date of Manufacture			
	(ppb)	40308512 08.03.2015	40505612 05.05.2016	43011412 11.30.2014	CB035813 02.04.2018
Free saponins	≈ 7	ND	ND	ND	ND
Conjugated saponins	≈ 7	ND	ND	ND	ND
Free terpenes	≈ 7	ND	ND	ND	ND
Conjugated terpenes	≈ 7	ND	ND	ND	ND
Ppb - Parts per billion (estimate of detection limit)					

2.5.3 Pesticides

No pesticides or herbicides are used on the *Agave tequilana* crop in Mexico where IMAG Organic® is produced, in accordance to meet current USDA/NOP Final Rule. However, to confirm no pesticide residues are present in the product, comprehensive analytical screens for pesticides were performed by outside laboratory (Cencon), Col. Rancho Pinto, Celaya, Gto, MX on powdered samples, the most concentrated form. These screens did not detect any organohalogens, organophosphates, organonitrogens, carbamates, tebulenozides, glyphosate, herbicides, metabolites, pyrethroids, synergists, neonicotinoid, or any other types at the respective detection limits for each, **Table 2.10**.

Table 2.10 Pesticide Levels in Powdered IMAG Organic® Agave Inulin

Pesticide	Analytical Limit of Detection	Result	Pesticide	Analytical Limit of Detection	Result
3-Hydroxycarbofuran (CB)	0.005 mg/kg	ND	3,4 Dichloroaniline (OC)	0.005 mg/kg	ND
A-BHC a1 b1 (OC)	0.005 mg/kg	ND	A-Endosulfan a1b1 (OC)	0.005 mg/kg	ND
Abamectina (OTROS)	0.005 mg/kg	ND	Acephate a1b1 (OP)	0.005 mg/kg	ND
Acetamiprid (NCN)	0.005 mg/kg	ND	Acrinathrin a1b1 (OC)	0.005 mg/kg	ND
Alachlor a1b1 (OC)	0.005 mg/kg	ND	Aldicarb a1b1 (MET)	0.005 mg/kg	ND
Aldicarb sulfone (MET)	0.005 mg/kg	ND	Aldicarb sulfonoxide (MET)	0.005 mg/kg	ND
Aldrin a1b1 (OC)	0.005 mg/kg	ND	Alfa cypermetrina a1b1 (OC)	0.005 mg/kg	ND
Ametrine a1b1 (ON)	0.005 mg/kg	ND	Amitraz a1b1 (ON)	0.005 mg/kg	ND
Antraquinona (MET)	0.005 mg/kg	ND	Atrazine a1b1 (ON)	0.005 mg/kg	ND
Azinphos ethyl a1 (OP)	0.005 mg/kg	ND	Azoxistrobin a1b1 (ON)	0.005 mg/kg	ND
B-BHC a1b1 (OC)	0.005 mg/kg	ND	B-endosulfan a1b1 (OC)	0.005 mg/kg	ND
Baygon a1b1 (OC)	0.005 mg/kg	ND	Benalaxyl a1b1 (ON)	0.005 mg/kg	ND
Bendiocarb a1b1 (CB)	0.005 mg/kg	ND	Benfluralin a1 (CB)	0.005 mg/kg	ND
Benfuracarb a1 (CB)	0.005 mg/kg	ND	Bensulide a1b1 (OP)	0.005 mg/kg	ND
Bentazone a1 (H)	0.005 mg/kg	ND	Benzoato de emamectina (OTROS)	0.005 mg/kg	ND
Bifenazate a1 (ON)	0.005 mg/kg	ND	Bifenox a1 (H)	0.005 mg/kg	ND
Bifenthrin (a1b1) (PYR)	0.005 mg/kg	ND	Biphenyl (OTROS)	0.005 mg/kg	ND
Bitertanol a1b1 (ON)	0.005 mg/kg	ND	Boscalid a1b1 (ON)	0.005 mg/kg	ND
Bromacil a1 (ON)	0.005 mg/kg	ND	Bromophos methyl (OP)	0.005 mg/kg	ND
Bromuconazole a1b1 (OC)	0.005 mg/kg	ND	Bupirimate a1b1 (ON)	0.005 mg/kg	ND
Buprofezin a1b1 (ON)	0.005 mg/kg	ND	Butachlor a1 (OC)	0.005 mg/kg	ND
Cadusafos a1b1 (OP)	0.005 mg/kg	ND	Captafol a1 b1 (OP)	0.005 mg/kg	ND
Captan a1b1 (OC)	0.005 mg/kg	ND	Carbaryl a1b1 (CB)	0.005 mg/kg	ND
Carbendazim a1b1 (MET)	0.005 mg/kg	ND	Carbetamide (H)	0.005 mg/kg	ND
Carbofuran a1b1 (CB)	0.005 mg/kg	ND	Carbophenothion a1b1 (OP)	0.005 mg/kg	ND
Carbophenothion methyl a1b1 (OP)	0.005 mg/kg	ND	Carbosulfan a1b1 (ON)	0.005 mg/kg	ND
Carboxim a1b1 (ON)	0.005 mg/kg	ND	Chlorantraliniprole a1 (ON)	0.005 mg/kg	ND
Chordane a1b1 (OC)	0.005 mg/kg	ND	Chlordimeform (OC)	0.005 mg/kg	ND
Clorfenapyr a1 (OC)	0.005 mg/kg	ND	Chlorobenzilate (OC)	0.005 mg/kg	ND
Chloroneb (OC)	0.005 mg/kg	ND	Chloropropylate (OC)	0.005 mg/kg	ND
Chlorothalonl a1b1 (OC)	0.005 mg/kg	ND	Chloroxuron (H)	0.005 mg/kg	ND
Chlorpropham a1 (H)	0.005 mg/kg	ND	Chlorprifos a1b1 (OP)	0.005 mg/kg	ND
Chlortal dimethyl a1 (OP)	0.005 mg/kg	ND	Chorfenson (OC)	0.005 mg/kg	ND
Chorfenvinphos a1b1 (OP)	0.005 mg/kg	ND	Cis-chlordane a1 (OC)	0.005 mg/kg	ND
Coumaphos a1b1 (OP)	0.005 mg/kg	ND	Crotoxyphos (OP)	0.005 mg/kg	ND
Cyanazine (ON)	0.005 mg/kg	ND	Cyanophos a1 (OP)	0.005 mg/kg	ND

Pesticide	Analytical Limit of Detection	Result	Pesticide	Analytical Limit of Detection	Result
Cyflutrin a1b1 (PYR)	0.005 mg/kg	ND	Cypermethrin a1b1 (PYR)	0.005 mg/kg	ND
Cyproconazole a1b1 (ON)	0.005 mg/kg	ND	Cyprodinl a1b1 (ON)	0.005 mg/kg	ND
Cyromazine (ON)	0.005 mg/kg	ND	D-BHC a1b1 (OC)	0.005 mg/kg	ND
Deltamethrin a1b1 (PYR)	0.005 mg/kg	ND	Demeton-O a1b1 (OP)	0.005 mg/kg	ND
Demeton-s a1b1 (OP)	0.005 mg/kg	ND	Devrinol a1b1 (ON)	0.005 mg/kg	ND
Deazinon a1b1 (MET)	0.005 mg/kg	ND	Diazinon O analog (MET)	0.005 mg/kg	ND
Dibrom (Nalded) a1b1 (OP)	0.005 mg/kg	ND	Dichlobenyl (H)	0.005 mg/kg	ND
Dechlorfluanid (OC)	0.005 mg/kg	ND	Dichlone (OTROS)	0.005 mg/kg	ND
Dichloran a1b1 (OC)	0.005 mg/kg	ND	Dichlorvos a1b1 (OP)	0.005 mg/kg	ND
Diclobutrazol a1b1 (OC)	0.005 mg/kg	ND	Diclofop methyl a1 (OP)	0.005 mg/kg	ND
Dicrotofos (OP)	0.005 mg/kg	ND	Dieldrin a1b1 (OC)	0.005 mg/kg	ND
Diethofencarb a1 (CB)	0.005 mg/kg	ND	Difenamid a1b1 (ON)	0.005 mg/kg	ND
Difenoconazole a1b1 (CB)	0.005 mg/kg	ND	Difonate a1b1 (OP)	0.005 mg/kg	ND
Dimetametrin (OTROS)	0.005 mg/kg	ND	Dimethachlor a1 (H)	0.005 mg/kg	ND
Dimethoate a1 (ON)	0.005 mg/kg	ND	Dimethomorph (OTROS)	0.005 mg/kg	ND
Dimethomorph a1 (OTROS)	0.005 mg/kg	ND	Dioxacarb a1b1 (CB)	0.005 mg/kg	ND
Dioxathion a1b1 (OP)	0.005 mg/kg	ND	Diphenylamine a1b1 (ON)	0.005 mg/kg	ND
Disulfoton a1b1 (MET)	0.005 mg/kg	ND	Disulfoton sulfone (MET)	0.005 mg/kg	ND
Diuron (H)	0.005 mg/kg	ND	Dmnb a1b1 (ON)	0.005 mg/kg	ND
Edifenphos a1 (OP)	0.005 mg/kg	ND	Endosulfan sulfate a1b1 (OC)	0.005 mg/kg	ND
Endrin a1b1 (OC)	0.005 mg/kg	ND	Endrin aldehido a1b1 (OC)	0.005 mg/kg	ND
EPN a1b1 (ON)	0.005 mg/kg	ND	Epoxiconazole a1b1 (OC)	0.005 mg/kg	ND
Esfenvarelate a1b1 (PYR)	0.005 mg/kg	ND	Etaconazol (OC)	0.005 mg/kg	ND
Ethalfluralin a1b1 (H)	0.005 mg/kg	ND	Ethion a1b1 (OP)	0.005 mg/kg	ND
Ethofumesate a1 (H)	0.005 mg/kg	ND	Ethoprophos a1b1 (OP)	0.005 mg/kg	ND
Ethyl Parathion (OP)	0.005 mg/kg	ND	Etofenprox a1b1 (OP)	0.005 mg/kg	ND
Etoxasole a1 (ON)	0.005 mg/kg	ND	Etrimphos a1b1 (OP)	0.005 mg/kg	ND
Famoxadone a1 (ON)	0.005 mg/kg	ND	Fenarimol a1b1 (OC)	0.005 mg/kg	ND
Fenbuconazole a1 (ON)	0.005 mg/kg	ND	Fenchlorfos a1b1 (OP)	0.005 mg/kg	ND
Fenhexamide a1 (ON)	0.005 mg/kg	ND	Fentrothion a1b1 (OP)	0.005 mg/kg	ND
Fenobucarb (CB)	0.005 mg/kg	ND	Fenotrin (PYR)	0.005 mg/kg	ND
Fenpropathrin a1b1 (ON)	0.005 mg/kg	ND	Fenpropidin a1b1 (ON)	0.005 mg/kg	ND
Fenpropimorph (morpholine) a1 (ON)	0.005 mg/kg	ND	Fenson a1b1 (OC)	0.005 mg/kg	ND
Fenthion a1b1 (OP)	0.005 mg/kg	ND	Fenthion sulfoxide a1 (OP)	0.005 mg/kg	ND
Fenvalerate a1b1 (PYR)	0.005 mg/kg	ND	Fipronil (OC)	0.005 mg/kg	ND
Flamprop isopropil a1 (H)	0.005 mg/kg	ND	Flamprop-methyl (OP)	0.005 mg/kg	ND
Flonicamid a1 (NCN)	0.005 mg/kg	ND	Fluazifop-butyl (H)	0.005 mg/kg	ND
Fluazaifob a1 (H)	0.005 mg/kg	ND	Flubendiamide (OTROS)	0.005 mg/kg	ND
Fluchloralin (H)	0.005 mg/kg	ND	Fludioxonyl a1b1 (OC)	0.005 mg/kg	ND
Flusilazole a1b1 (ON)	0.005 mg/kg	ND	Folicur (Tebuconzole) a1b1 (ON)	0.005 mg/kg	ND
Folpet a1b1 (OC)	0.005 mg/kg	ND	Formothion (OP)	0.005 mg/kg	ND
Fosmet (Imidan) (OP)	0.005 mg/kg	ND	Gamma-cyhalothrin a1 (PYR)	0.005 mg/kg	ND
Guthion (Azinphos methyl) a1b1 (OP)	0.005 mg/kg	ND	H. Epoxido a1b1 (OC)	0.005 mg/kg	ND
HCB a1b1 (OC)	0.005 mg/kg	ND	Heptachlor a1b1 (OC)	0.005 mg/kg	ND
Heptenophos (OP)	0.005 mg/kg	ND	Hexaconazole a1b1 (OC)	0.005 mg/kg	ND
Hexazinone a1 (OC)	0.005 mg/kg	ND	Hexythiazox a1 (OC)	0.005 mg/kg	ND
Imazul a1b1 (OC)	0.005 mg/kg	ND	Imidacloprid a1 (ON)	0.005 mg/kg	ND
Indoxacarb a1b1 (OC)	0.005 mg/kg	ND	Iproodione a1b1 (OC)	0.005 mg/kg	ND
Isazophos a1b1 (OP)	0.005 mg/kg	ND	Isophenphos a1b1 (OP)	0.005 mg/kg	ND
Isoproturon (H)	0.005 mg/kg	ND	Keltane a1b1 (OC)	0.005 mg/kg	ND
Lamdba cyhalothrin a1b1 (PYR)	0.005 mg/kg	ND	Lenacil a1 (ON)	0.005 mg/kg	ND
Leptophos (OP)	0.005 mg/kg	ND	Lindane a1b1 (OC)	0.005 mg/kg	ND
Linuron (H)	0.005 mg/kg	ND	Malathion a1b1 (OP)	0.005 mg/kg	ND
Malaxom a1b1 (OP)	0.005 mg/kg	ND	Mandipropamid (ON)	0.005 mg/kg	ND
Mecarbam a1b1 (OP)	0.005 mg/kg	ND	Mepronil a1b1 (ON)	0.005 mg/kg	ND
Merphos a1b1 (OP)	0.005 mg/kg	ND	Metalaxil a1b1 (ON)	0.005 mg/kg	ND
Metamidophos a1b1 (OP)	0.005 mg/kg	ND	Metasixtox a1 (OP)	0.005 mg/kg	ND
Metazachlor a1b1 (OC)	0.005 mg/kg	ND	Methidathion a1b1 (OP)	0.005 mg/kg	ND
Methiocarb a1b1 (MET)	0.005 mg/kg	ND	Methiocarb sulfone (MET)	0.005 mg/kg	ND
Methomyl a1b1 (CB)	0.005 mg/kg	ND	Methoprotryne (H)	0.005 mg/kg	ND
Methyl chlorpyrifos a1b1 (OP)	0.005 mg/kg	ND	Methyl parathion a1b1 (OP)	0.005 mg/kg	ND
Methyl pirmiphos a1b1 (OP)	0.005 mg/kg	ND	Metolachlor a1b1 (OC)	0.005 mg/kg	ND
Metoxichlor a1b1 (OP)	0.005 mg/kg	ND	Metoxifenozide (OTROS)	0.005 mg/kg	ND
Metribuzin a1 (ON)	0.005 mg/kg	ND	Mexacarbate (CB)	0.005 mg/kg	ND
Mirex a1b1 (OC)	0.005 mg/kg	ND	Monocrothphos a1b1 (OP)	0.005 mg/kg	ND
Myclobutanil a1b1 (ON)	0.005 mg/kg	ND	Napropamide a1 (H)	0.005 mg/kg	ND
Nitrapyrin (OC)	0.005 mg/kg	ND	Nuarimol a1b1 (OC)	0.005 mg/kg	ND
O-Nitrotolueno a1b1 (ON)	0.005 mg/kg	ND	O-PP a1b1 (OTROS)	0.005 mg/kg	ND
O,P DDD a1 (OC)	0.005 mg/kg	ND	O,P DDE a1b1 (OC)	0.005 mg/kg	ND
O,P DOT a1 (OC)	0.005 mg/kg	ND	Omethodate a1b1 (OP)	0.005 mg/kg	ND
Oxadixyl a1b1 (ON)	0.005 mg/kg	ND	Oxamyl a1b1 (CB)	0.005 mg/kg	ND
Oxidiazon a1b1 (OC)	0.005 mg/kg	ND	Oxyfluorfene a1b1 (OC)	0.005 mg/kg	ND
P,P-DDD a1b1 (OC)	0.005 mg/kg	ND	P,P-DDE a1b1 (OC)	0.005 mg/kg	ND
P,P-DDT a1b1 (OC)	0.005 mg/kg	ND	Paclobutrazol a1b1 (OC)	0.005 mg/kg	ND
Parathion a1b1 (OP)	0.005 mg/kg	ND	PCNB a1b1 (OC)	0.005 mg/kg	ND
Penconazole a1b1 (OC)	0.005 mg/kg	ND	Pendimetaline a1 (H)	0.005 mg/kg	ND
Pentachloranili a1 (OC)	0.005 mg/kg	ND	Pentachlorophenol (OC)	0.005 mg/kg	ND

Pesticide	Analytical Limit of Detection	Result	Pesticide	Analytical Limit of Detection	Result
Permethrin a1b1 (PYR)	0.005 mg/kg	ND	Perthane (P,P'-ethyl DDD) (OC)	0.005 mg/kg	ND
Phentoate (OP)	0.005 mg/kg	ND	Phorate a1b1 (OP)	0.005 mg/kg	ND
Phorate oxon a1 (OP)	0.005 mg/kg	ND	Phorate sulfone (OP)	0.005 mg/kg	ND
Phorate sulfone a1 (OP)	0.005 mg/kg	ND	Phorate sulfoxide (OP)	0.005 mg/kg	ND
Phosalone a1b1 (OP)	0.005 mg/kg	ND	Phosdrin a1b1 (OP)	0.005 mg/kg	ND
Phosmet a1b1 (OP)	0.005 mg/kg	ND	Phospamidon a1b1 (OP)	0.005 mg/kg	ND
Piperalin (OTROS)	0.005 mg/kg	ND	Piperonyl butoxide a1b1 (SYN)	0.005 mg/kg	ND
Piperophos a1 (H)	0.005 mg/kg	ND	Pirimicarb a1b1 (ON)	0.005 mg/kg	ND
Pirimiphos ethyl a1 (OP)	0.005 mg/kg	ND	Prochloraz a1 (OC)	0.005 mg/kg	ND
Procymidone a1b1 (OC)	0.005 mg/kg	ND	Profenophos a1b1 (OP)	0.005 mg/kg	ND
Profluralin (H)	0.005 mg/kg	ND	Promecarb a1 (ON)	0.005 mg/kg	ND
Prometon a1 (ON)	0.005 mg/kg	ND	Prometrin (H)	0.005 mg/kg	ND
Pronamide (propizamide) a1b1 (OC)	0.005 mg/kg	ND	Propamocarb (CB)	0.005 mg/kg	ND
Propanil (H)	0.005 mg/kg	ND	Propargite (OTROS)	0.005 mg/kg	ND
Propazine a1 (ON)	0.005 mg/kg	ND	Propetamphos (OP)	0.005 mg/kg	ND
Propham (H)	0.005 mg/kg	ND	Propiconazole a1 (OP)	0.005 mg/kg	ND
Protiophos a1b1 (OP)	0.005 mg/kg	ND	Pyrmetrozin (OTROS)	0.005 mg/kg	ND
Pyraclostrobin (OTROS)	0.005 mg/kg	ND	Pyrazophos a1b1 (OP)	0.005 mg/kg	ND
Pyrimethanil (ON)	0.005 mg/kg	ND	Pryiproxifen a1 (ON)	0.005 mg/kg	ND
Quinalphos a1b1 (ON)	0.005 mg/kg	ND	Quinoxyfen a1 (OC)	0.005 mg/kg	ND
Qizalofob ethyl a1b1 (ON)	0.005 mg/kg	ND	Sethoxydim a1 (H)	0.005 mg/kg	ND
Simazine a1 (OTROS)	0.005 mg/kg	ND	Simetryin a1 (H)	0.005 mg/kg	ND
Spinetoram a1 (OTROS)	0.005 mg/kg	ND	Spinosad a1 (OTROS)	0.005 mg/kg	ND
Spirotetramat a1 (OTROS)	0.005 mg/kg	ND	Sprioxamine (OTROS)	0.005 mg/kg	ND
Sulfotep a1b1 (OTROS)	0.005 mg/kg	ND	Sulprofos (OP)	0.005 mg/kg	ND
Tebuconazole (Folicur) b1 (ON)	0.005 mg/kg	ND	Tebufenozide (OTROS)	0.005 mg/kg	ND
Tefluthrin a1 (OC)	0.005 mg/kg	ND	Temephos (OP)	0.005 mg/kg	ND
Terbacil (H)	0.005 mg/kg	ND	Terbufos a1b1 (OP)	0.005 mg/kg	ND
Terbutrin (H)	0.005 mg/kg	ND	Terrazole a1 (OC)	0.005 mg/kg	ND
Tetraclorvinphos a1b1 (OP)	0.005 mg/kg	ND	Tetraconazole (OC)	0.005 mg/kg	ND
Tetrametryna (PYR)	0.005 mg/kg	ND	Thiabendazole a1 (ON)	0.005 mg/kg	ND
Thiametoxam a1 (NCN)	0.005 mg/kg	ND	Thiametoxam a1 (NCN)	0.005 mg/kg	ND
Thiodicarb a1b1 (CB)	0.005 mg/kg	ND	Thionazin (OP)	0.005 mg/kg	ND
Thiophanate methyl a1 (ON)	0.005 mg/kg	ND	THPI a1 (MET)	0.005 mg/kg	ND
Tiobencarb a1 (H)	0.005 mg/kg	ND	Tolclofos methyl a1b1 (OC)	0.005 mg/kg	ND
Tolyfluanid a1 (OC)	0.005 mg/kg	ND	Triadimefon a1b1 (OC)	0.005 mg/kg	ND
Triallate a1 (OC)	0.005 mg/kg	ND	Triazophos a1b1 (OP)	0.005 mg/kg	ND
Tributyl phosphate a1b1 (OP)	0.005 mg/kg	ND	Trichlorfon a1 (OP)	0.005 mg/kg	ND
Trifloxistrobin a1b1 (OP)	0.005 mg/kg	ND	Triflumizole a1 (OC)	0.005 mg/kg	ND
Trifluralin a1b1 (OC)	0.005 mg/kg	ND	Triforine (OTROS)	0.005 mg/kg	ND
Vegadex CDEC a1 (H)	0.005 mg/kg	ND	Vinclozoline a1b1 (OC)	0.005 mg/kg	ND

Abbreviations: OC (organohalogens), OP (organophosphates), ON (organonitrogens), CB (carbamates), TB (tebulenozide), GLY (glyphosate), H (herbicides), <L.O.Q. (less than limit of quantification, MRL (Maximum residue limit), MET (metabolites), PYR (pyrethroids), SYN (synergist), N.A. (not applicable), OTROS (others), NCN (neonicotinoid), I.A. (Active ingredients), ND (Not Detected, less than limit of detection). Data from lot analysis of 40207612, 40506412, 40907512, 41001512 by CENCON Laboratorio de Analysis de Pesticidas del Bajio, S.A. de C.V., Col. Pinto Celaya, Gto. 2.4.2016.

2.5.4 PCBs, Dioxins and Furans

The term "dioxins" refers to the general name for 210 different polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners. These compounds were determined in the agave inulin samples using a validated EPA 1613B and EPA 1668 modified method, "Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS" and "Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by high resolution gas chromatography/high resolution mass spectral analysis (HRGC/HRMS)", respectively. In addition to the dioxins, some polychlorinated biphenyls (PCBs) possess a planar-type molecular structure and toxicity similar to that of dioxins (dioxin-like compounds) and are referred to as "co-planar PCBs". Data for these toxic, ubiquitous industrial chemical contaminants are shown in **Table 2.11.** The Estimated Detection Limit (EDL), as defined by the EPA methods and the analytical result for each dioxin is provided for each sample. The EDL used in the EPA methods is an estimate that is made by the testing laboratory of the concentration of a given analyte that would have to be present to produce a signal with a peak height of at least 2.5 times the background signal level. The estimate is specific to a particular analysis of the sample and will be

affected by sample size, dilution, etc. Because of the toxicological significance of PCDDs and PCDFs, the EDL value is reported for non-detected analytes rather than reporting the Contract Required Quantitation Limit (CRQL). The method used to express the degree of toxicity of the individual congeners is based on utilization of toxic equivalency factors (TEF), with the toxicity of 2,3,7,8-TCDD set equal to 1. TEF is established by WHO and others by comparing toxicity data for different congeners. Since dioxins and related compounds are usually present in background levels in the environment in the form of a mixture of congeners, the degree of toxicity of the dioxins and their related compounds ingested is expressed as the toxic equivalents (TEQ) by multiplying the amount of each congener by its TEF, and adding up the products. Dioxin toxicity is evaluated internationally on the basis of the TEQ expressed as numerical values. The table also shows data for trace background screens for 12 dioxin-like PCBs, 6 non-like-dioxin PCBs and dioxin and furans. These data show that background PCB levels are well below FDA established food tolerance levels of 0.2 to 3 ppm for all foods (FDA, 2018c).

Table 2.11 PCB, Dioxin and Furan levels in Powdered IMAG Organic® Agave Inulin

"Dioxins"	Method (EPA)	Batch Number Manufacturing Date									
		CB032928		CB033913		43103612		40403512		CB073813	
		02.01.2019		02.02.2019		03.21.2016		03.05.2015		04.14.2018	
		Result	EDL	Result	EDL	Result	EDL	Result	EDL	Result	EDL
Dioxins/Furans		ng/kg	ng/kg	ng/kg	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
2378-TCDD	1613B	ND	0.159	0.0767	0.0712	ND	0.190	ND	0.0790	ND	0.0724
2378-TCDF	1613B	ND	0.133	ND	0.0586	ND	0.150	ND	0.0673	ND	0.0647
12378-PeCDD	1613B	ND	0.153	ND	0.0621	ND	0.133	ND	0.0754	ND	0.0607
12378-PeCDF	1613B	ND	0.108	ND	0.0440	ND	0.0919	0.0835	0.0451	ND	0.0414
23478-PeCDF	1613B	ND	0.105	ND	0.0385	ND	0.0822	ND	0.0414	ND	0.0367
123478-HxCDD	1613B	ND	0.0768	ND	0.0373	ND	0.0541	ND	0.0364	ND	0.0265
123678-HxCDD	1613B	0.143	0.0776	ND	0.0383	ND	0.0550	ND	0.0364	ND	0.0282
123789-HxCDD	1613B	ND	0.0945	ND	0.0456	ND	0.0640	ND	0.0425	ND	0.0307
123478-HxCDF	1613B	ND	0.0448	ND	0.0162	ND	0.0329	ND	0.0196	0.0607	0.0203
123678-HxCDF	1613B	0.0844	0.0500	ND	0.0169	ND	0.0378	0.0472	0.0216	ND	0.0223
123789-HxCDF	1613B	ND	0.0948	ND	0.0261	ND	0.0626	Nd	0.0333	ND	0.0343
234678-HxCDF	1613B	ND	0.0588	ND	0.0206	0.114	0.0434	ND	0.0265	ND	0.0255
1234678-HpCDD	1613B	ND	0.0652	ND	0.0213	0.140	0.0442	0.0944	0.0363	ND	0.0240
1234678-HpCDF	1613B	ND	0.0402	ND	0.0141	ND	0.0335	ND	0.0185	ND	0.0144
1234789-HpCDF	1613B	ND	0.0676	ND	0.0201	ND	0.0471	ND	0.0277	ND	0.0208
OCDD	1613B	ND	0.125	ND	0.0371	0.499	0.0782	ND	0.0581	ND	0.0419
OCDF	1613B	ND	0.180	ND	0.0477	0.118	0.0774	ND	0.0618	ND	0.0527
D/F Toxic Eq.	**1613B**	**0.421**	-	**0.178**	-	**0.410**	-	**0.202**	-	**0.175**	-
WHO 12 dioxin-like PCBs		**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**
PCB77	1668 mod.	ND	0.0654	ND	0.0592	ND	0.0536	0.725	0.0620	ND	0.0530
PCB81	1668 mod.	ND	0.0484	ND	0.0436	ND	0.0394	ND	0.0474	ND	0.0349
PCB105	1668 mod.	0.863	0.0538	0.609	0.0578	ND	0.0555	1.19	0.0650	ND	0.0455
PCB114	1668 mod.	ND	0.0435	ND	0.0452	ND	0.0403	ND	0.0484	ND	0.0337
PCB118	1668 mod.	ND	0.0388	0.924	0.0426	ND	0.0376	1.38	0.0439	0.455	0.0314
PCB123	1668 mod.	ND	0.0380	ND	0.0423	ND	0.0386	ND	0.0431	ND	0.0330
PCB126	1668 mod.	ND	0.0553	ND	0.0652	ND	0.0572	ND	0.0645	ND	0.0457
PCB156	1668 mod.	ND	0.0321	0.359	0.0384	0.367	0.0380	0.593	0.0365	0.113	0.0266
PCB157	1668 mod.	ND	0.0323	ND	0.0429	ND	0.0406	0.210	0.0400	0.0535	0.0317
PCB167	1668 mod.	ND	0.0261	ND	0.0433	ND	0.0450	ND	0.0463	0.112	0.0254
PCB169	1668 mod.	ND	0.0272	ND	0.0344	0.0625	0.0328	ND	0.0344	ND	0.0253
PCB189	1668 mod.	ND	0.0180	ND	0.0271	ND	0.0281	0.179	0.0347	ND	0.0207
PCB Toxic Eq.	**1668 mod.**	**0.00640**	-	**0.00764**	-	**0.00764**	-	**0.00768**	-	**0.00537**	-
6-non-like-dioxins PCBs (ICES-6)		**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**	**pg/g**
PCB28	1668B mod.	2.86	0.0526	3.99	0.0648	4.24	0.0543	4.74	0.0512	3.22	0.0458
PCB52	1668B mod.	ND	0.0364	1.02	0.0422	1.07	0.0466	1.26	0.0568	ND	0.0351
PCB101	1668B mod.	1.00	0.0348	ND	0.0530	ND	0.0462	ND	0.0570	0.994	0.0359
PCB138	1668B mod.	1.57	0.0331	2.28	0.0579	ND	0.0608	ND	0.0588	ND	0.0344
PCB153	1668B mod.	1.78	0.0326	2.87	0.0481	4.15	0.0502	5.36	0.0505	ND	0.0292
PCB180	1668B mod.	2.61	0.0209	3.64	0.0329	6.24	0.0342	8.41	0.0401	2.53	0.0249
ICES-6 SUM	**1668B mod.**	**9.87**	-	**13.9**	-	**15.8**	-	**19.9**	-	**6.84**	-

ng/kg - nanograms/kilogram; pg/g - picograms/gram; ND - not detected; EDL-Estimated Detection Limit; D/F - dioxins/furans

2.5.5 Molecular Weight Analysis of IMAG Organic® and Molecular Chain Consistency

The degree of polymerization (DP) contributes to the product functionality in food, such as its hygroscopicity, solubility, and utilization as a fermentation substrate in the colonic environment. To determine the relatively consistency of the molecular weight distribution of IMAG Organic®, 28 non-consecutive lots of dry powder were analyzed via high performance size exclusion chromatography (HPLC-SEC) over a one-year period, from May 2017 to May 2018, by the Biotechnology Institute from the UNAM (Appendix 9). Data in **Table 2.12** show that, while some variability exists due to differences in plant maturity, seasonality and geography, the mean molecular weight, DP and degree of polymer heterogeneity, its polydispersity (IP), are relatively stable over the year. The polydispersity index (IP), a measure of the heterogeneity of the molecular weight distribution of a polymer, shows that IMAG Organic® is moderately polydisperse (a relatively broad range of molecular weight fractions), which is similar to chicory inulin.

Table 2.12 Molecular Weight (HPLC-SEC) Analysis of 28 Non-consecutive Lots of IMAG Organic®

Lot No.	Concentration (g/L)	Mn (Da)	Mw (Da)	DPn	DPw	IP	DPmax	DPmin
CB059813	10.7	3249	6044	20	37	1.86	72	3
CB061813	11.1	3000	5564	18	34	1.85	73	3
CB083813	12.2	3541	6865	22	42	1.94	72	3
CB094813	11.7	3027	5674	19	35	1.87	73	3
CB109813	11.5	2305	4017	14	25	1.74	73	3
41501712	12.5	3810	6822	23	42	1.79	72	3
72101712	11.3	3945	6993	24	43	1.77	73	3
40902712	12.0	3383	5916	21	36	1.75	70	3
41702712	12.0	2993	5107	18	31	1.71	73	3
41103712	12.3	3206	5321	20	33	1.66	73	3
42203712	12.3	2677	4503	16	28	1.68	72	3
40304712	11.1	2113	3299	13	20	1.56	72	3
42604712	11.8	2991	5255	18	32	1.76	73	3
40205712	11.7	2515	4421	15	27	1.76	72	3
42405712	12.5	2278	3397	14	21	1.49	73	3
40906712	10.9	2887	4319	18	27	1.50	73	3
42606712	12.3	2834	4826	17	30	1.70	72	3
41007712	12.5	2600	4021	16	25	1.55	72	3
42707712	12.6	3670	6382	23	39	1.74	72	3
41708712	11.3	2742	4695	17	29	1.71	72	3
42909712	11.5	3925	6562	24	40	1.67	72	3
40310712	12.2	3206	5298	20	33	1.65	73	3
42910712	12.3	3181	5337	20	33	1.68	73	3
41311712	12.2	2237	4308	14	26	1.93	70	3
43011712	12.9	1900	2974	12	18	1.57	70	3
CB020813	12.0	2618	4529	16	28	1.73	73	3
CB031813	12.1	2727	4650	17	29	1.70	73	3
CB038813	11.0	3812	6543	23	40	1.72	72	3
Mean	**11.9**	**2977.6**	**5130.1**	**18.3**	**31.5**	**1.72**	**72.3**	**3.0**
STD	**0.6**	**562.0**	**1115.5**	**3.4**	**6.8**	**0.12**	**0.9**	**0.0**

Abbrev: Da (Daltons), Mn (avg. number molar mass), Mw (avg. mass of the mass), IP (polydispersion index), DPmax (highest degree of polymerization fraction), DPmin (lowest degree of polymerization fraction), DPn (avg. degree of polymerization number), DPw (avg. degree of polymerization mass).

2.5.6 Analytical Methods Used to Determine IMAG Organic® Specifications

The content of agave inulin and other carbohydrates in IMAG Organic®, as presented in the aforementioned specifications, in addition to microbiological counts and nutrient analyses use official methods as shown in Tables 2.4-Tables 2.7. IMAG Organic® agave inulin has also received certifications from:

- Organic certification from BCS ÖKO GARANTIE GMBH (meets conditions of USDA);
- Food Safety Systems Certification (FSSC 22000);
- Kosher certification (Maguen David);
- Halal certification (Islamic Food and Nutritional Council of America);
- Security certification (C-TPAT);
- Social and Fair-Trade certification (Fair for Life);
- TESTS: Allergen free (CIATEJ/Conacyt), Glycemic index (GI Labs), Gluten free (Acelmex);
- Registers: FDA and the Vegan Society.

As also described on page 13 of GRN 854, the content of agave inulin and other carbohydrates in IMAG Organic®, like those of Inufib™ in GRN 854 are assayed according to the industrial standard "Official Norm NMX-FF-110-SCFI-2008" promulgated by the Government of Mexico (NMX-FF-110-SCFI-2008 Productos Alimenticios - Jarabe de Agave Exlicaciones y Métodos de Prueba). The official Association of Official Analytical Chemists International (AOACI) method for determination of the nondigestible fructan portion of the dietary fiber in foods, food and food products is published as AOAC method 997.08, a high-performance anion exchange chromatographic with pulsed amperometric detection method (HPAEC-PAD). This same method for determination of nondigestible fructan content is published by the European Ministry of Agriculture, Food and Fisheries (MAFF) and by the American Association of Cereal Chemists (AACC) as method 32-31. The specific method of analyses for each analyte is listed in each table.

2.5.7 Product Stability

The effective or useful life of a food product is an estimate of the time a product has to appropriately fulfill the function that it was created without change to its safety, sensory, physicochemical and microbiological properties, and complies with any statement on the nutritional panel when stored under recommended storage conditions (IFST, 1993). This storage life is related to a balance between the microbiological expiration, and the physicochemical and sensory properties of the product. In addition to the food product's expiration, is the time limit a food can be stored before it loses its properties. The useful storage life is the period from the food's manufacture to the date it expires, meaning the time period that the food maintains all of its qualities. With respect to this GRAS Notice, the safe storage life depends on maintaining minimum levels of contamination, and preserving its physicochemical properties (its homogeneity, stability, and structure). Data on the product’s safety over shelf life, are provided, which consider its moisture or humidity content, as these influence microbiological contamination, microbiological changes, as these specifically can influence product safety, and changes in sugar profile.

IMAG Organic® agave inulin is manufactured using similar or equal processes as Inufib™ agave inulin, (GRN 854). IMAG Organic® also has equal to or higher inulin content and equal to or better microbiological and simple sugar specifications, and like, Inufib™ in GRN 854, is shown to meet or exceed these specifications from analysis of randomly selected, non-consecutive product lots. In GRN 854, IIDEA, the manufacturer of Inufib™, stated on page 14, section 2.4.2. "stability", that "on the basis of the data and review by the HACCP program, which compared the Inufib™ products to the shelf life of similar products, shelf lives of 3 years for the dry Inufib™ and 3 months for the liquid Inufib™ were assigned. Given the mentioned similarity between the agave inulin products, it is expected that IMAG Organic® will have equal to or better product stability as Inufib™ described in GRN 854.

In order to determine the shelf life for powdered agave inulin, both an accelerated and a concurrent real-time stability test was performed using 2 kg per sack of lot number 40502712 (manufactured 02.05.2017) by the Center for Research and Assistance in Technology and Design of the State of Jalisco, AC (CIATEJ) located in Guadalajara, Jalisco, MX, and internally by IMAG in the Quality and Innovation Laboratory, respectively. Packaging used in the studies was of paper and double-lined poly pouch, as is currently used in commercial production at IMAG. The packaging was obtained from the process currently used by the company Inulina y Miel de Agave S.A. de C.V. (IMAG). The accelerated storage study was performed to determine, among other attributes such as color and flavor, effects on the product's physicochemical properties (% humidity and microbiological levels of mesophilic aerobic bacteria, yeast and molds and coliform bacteria) of the powdered agave inulin product at 20°, 30 °C and 40 °C, using analyses performed from 10 samplings over a 6 month period. These data are shown in **Table 2.13**. The methods used for the analysis of samples during the testing were: microbiology [total aerobic count (NOM 092 SSA1 1994), total coliforms (NOM 113 SSA1 1994), yeast and molds (NOM 111 SSA1 1994)]; percent humidity (AOAC 925.45 1925); and sugars (NOM 002 SAGARPA 2016).

The IMAG real-time shelf life study was performed to determine inulin stability under ambient temperature (25-30°C), a cool dry environment protected from sunlight for a much longer 18-month period; conditions more typifying actual commercial practice. **Table 2.13** also shows these data. To assure self-stability, additional real-time self-life studies of other lots are also being assessed.

All physical determinations were carried out in triplicate in the different measurements, while the sensory determinations and microbiological parameters were determined in duplicate.

2.5.7.1 Results of the Shelf Life Studies

C.F.U./g = Colony Forming Units/gram, Analysis time frequency is months (T1 = 1 mon, T2 = 2 mon, etc.). *Salmonella* and *E. coli* not measured in accelerated study.

Table 2.13 Data from Accelerated and Real-time Shelf-life Studies

Part 1: 20° C - Accelerated Shelf-life Study Only:

Determination	Storage Temperature (20°C)						
	Sampling						
	T0	T1	T2	T3	T4	T5	T6
Aerobic mesophilic bacteria	320	280	250	180	190	170	180
Molds	<10	<10	<10	<10	<10	<10	<10
Yeasts	<10	<10	<10	<10	<10	<10	<10
Coliform bacteria	AB	AB	AB	AB	*	AB	*
% moisture/humidity	4.00	4.50	4.45	4.50	4.50	4.50	4.97

* - Not tested at this sampling point. AB = Absent

Part 2: 40° C - Accelerated Shelf-life Study Only:

Determination	Storage Temperature (40°C)						
	Sampling						
	T0	T1	T2	T3	T4	T5	T6
Aerobic mesophilic bacteria	320	230	180	130	180	140	210
Molds	<10	<10	<10	<10	<10	<10	<10
Yeasts	<10	<10	<10	<10	<10	<10	<10
Coliform bacteria	AB	AB	AB	AB	*	AB	*
% moisture/humidity	4.00	4.00	3.80	3.95	3.95	4.00	3.22

* - Not tested at this sampling. AB = Absent

Part 3: 30° C – Both Accelerated **(A)** and Real-time **(R)** Shelf-life Studies:

Analysis	Storage Temperature (30°C)																		
	Sampling (A/R)																		
Months →	T0 A/R	T1 A/R	T2 A/R	T3 A/R	T4 A/R	T5 A/R	T6 A/R	T7 A/R	T8 A/R	T9 A/R	T10 A/R	T11 A/R	T12 A/R	T13 A/R	T14 A/R	T15 A/R	T16 A/R	T17 A/R	T18 A/R
Aerobic mesophilic bacteria	320/ 230	230/ 210	180/ 210	150/ 230	180/ 200	120/ 220	180/ 200	-/ 190	-/ 190	-/ 200	-/ 170	-/ 160	-/ 180	-/ 160	-/ 120	-/ 150	-/ 120	-/ 110	-/ 110
Molds	<10/ <10	<10/ <10	<10/ <10	<10/ <10	<10/ <10	<10/ <10	<10/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/< 10	-/ <10	-/ <10
Yeasts	<10/ <10	<10/ <10	<10/ <10	<10/ <10	<10/ <10	<10/ <10	<10/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10	-/ <10
Coliform bacteria (per g)	AB/ AB	AB/ AB	AB/ AB	AB/ AB	*/ AB	-/ AB	*/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB
***E. coli*[1] (per 1g)**	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB
Salmonella (per 25g)	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB	-/ AB
% moisture	4.00/ 3.62	4.10/ 3.55	4.20/ 3.60	4.30/ 3.55	4.30/ 3.65	4.30/ 3.72	4.40/ 3.70	-/ 3.80	-/ 3.75	-/ 3.77	-/ 3.80	-/ 3.84	-/ 3.87	-/ 3.93	-/ 4.00	-/ 3.93	-/ 4.00	-/ 4.01	-/ 4.07
Inulin	-/ 92.59	-/ 92.52	-/ 92.58	-/ 92.50	-/ 92.42	-/ 92.41	-/ 92.43	-/ 92.39	-/ 92.45	-/ 92.39	-/ 92.39	-/ 92.30	-/ 92.25	-/ 92.28	-/ 92.20	-/ 92.25	-/ 92.23	-/ 92.16	-/ 92.15
Sucrose	-/ 1.22	-/ 1.20	-/ 1.25	-/ 1.23	-/ 1.28	-/ 1.29	-/ 1.27	-/ 1.30	-/ 1.31	-/ 1.30	-/ 1.30	-/ 1.32	-/ 1.31	-/ 1.30	-/ 1.33	-/ 1.34	-/ 1.33	-/ 1.40	-/ 1.43
Fructose	-/ 4.60	-/ 4.60	-/ 4.60	-/ 4.64	-/ 4.60	-/ 4.62	-/ 4.60	-/ 4.60	-/ 4.60	-/ 4.60	-/ 4.62	-/ 4.61	-/ 4.60	-/ 4.59	-/ 4.60	-/ 4.60	-/ 4.60	-/ 4.60	-/ 4.60
Glucose	-/ 1.40	-/ 1.47	-/ 1.52	-/ 1.57	-/ 1.59	-/ 1.58	-/ 1.57	-/ 1.60	-/ 1.60	-/ 1.60	-/ 1.60	-/ 1.61	-/ 1.62	-/ 1.63	-/ 1.65	-/ 1.68	-/ 1.71	-/ 1.72	-/ 1.72

-Not tested in accelerated study; * - Not tested at this sampling point. AB = Absent

2.5.7.2 Conclusions of Key Product Stability Indicators from Shelf-life Studies

Microbiology:

A fundamental parameter that determines the stability of a product is its safety against contamination by pathogenic bacteria, molds, and/or yeasts, as this influences the quality of the food, decreases its nutritional contribution, and poses a risk to the health of the consumer. Data from both the CIATEJ accelerated storage study, and the real-time IMAG study show NO increase in any microbiological parameter, regardless of storage temperature, that would indicate product decomposition or contamination during the storage period. Aerobic mesophilic bacteria decreased across all storage temperatures, yeast and molds remained constant throughout the storage periods with colony forming units (CFUs) found to be less than 10 CFU/g, while coliforms were absent in all samples analyzed , Parts 1-3 of **Table 2.13.** Although not determined in the 6-month accelerated shelf study, E. *coli* (a coliform) and *Salmonella* were determined to be absent in samples stored over 18-months in the real-time study.

% Water/humidity:

Humidity is another useful parameter that determines the shelf life of a product, which can be used as an indirect measure of the water activity (Aw) of the ingredient and therefore the possibility of contamination of the ingredient by microbes. As expected, percent moisture in the product increases as a function of temperature, as inulin is hygroscopic. It is due to this fact that within the specification for the ingredient the statement, "Avoid contact with moisture as it is a highly hygroscopic product", is defined. Data from both shelf-life studies show that the agave inulin product stored at 20° and 30 °C had a slight increase in moisture, while product stored at 40 °C had moisture decrease from 4.00 to 3.22%. All these values are below 5% and consistent with moisture levels for commercial inulin currently sold worldwide, and below that recorded by Mexican Legislation in force for agave inulin.

Carbohydrate Content and Profile:

The carbohydrate content and profile of lot 40502712 made in Feb. 2017 was not followed as a condition of the CIATEJ study. However, IMAG determined this quality parameter in the same production lot as part of their real-time 18-month shelf life study performed at room temperature between 25-30° C.

Samples collected over the 18-month shelf life were analyzed using a peer-reviewed method in common use by experts in the field, which employs High Performance Anionic Exchange Chromatography coupled to a Pulsed Amperometric Detector (HPAEC-PAD) in a Dionex-ICS3000/ICS5000 analytical system. Separations were carried out using a CarboPac PA100 column and guard column, and carbohydrates were eluted using a gradient of sodium acetate/sodium hydroxide, as prescribed by Mellado-Mojica and others (2012, 2015). Carbohydrate quantifications were performed according to retention times of each compound from calibration curves of standards with commercial purity of 99.5%.

Data presented in Parts 1-3 of **Table 2.13** show a slight increase in glucose and sucrose content from T0 over the 18-month storage period, ranging from 1.40 to 1.72 g/100g (DS) and 1.22 to 1.43 g/100g (DS), respectively. The fructose content was relatively stable over the 18-month storage period and did not

show any significant trend that might relate to hydrolysis of the polyfructan (inulin) molecule. The overall analysis of total simple sugars (the contribution of glucose, fructose and sucrose) reflects a similar behavior, as there is only a minor tendency to increase over storage time from 7.30 g/100g DS to 7.82 g/100g DS. However, the maximum carbohydrate values are maintained well within the limits established by IMAG specifications and as stipulated in the Mexican Legislation in force for agave inulin (NMX-F-591-SCFI-2010).

In addition to the simple sugar content of the lot during the IMAG study, the inulin/dietary fiber content was also monitored using the same HPAEC-PAD system as for the simple sugar determinations. Data in **Table 2.13** show a slight, but gradual inverse correlation between inulin content and time. The inulin/dietary fiber content ranged from 92.59 g/100 g (DS) at the start of the real-time study (T0) to 92.14 g/100g DS for samples analyzed after 18 months of storage, within acceptable levels based on IMAG specifications and Mexican legislation in force for commercial agave inulin (NMX-F-591-SCFI-2010).

Further, in addition to the T0 - T18 month real-time analyses, analysis of carbohydrate profiles was also performed at 25 months and 36 months of storage. These 25-month and 36-month follow-up carbohydrate tests of the stored product that had been protected from sunlight, under cool, dry conditions, showed a stable carbohydrate profile for the powdered agave inulin product, having inulin contents of 92.12 and 92.10 g/100 g DS, respectively. The balance of the inulin content between the initial time and the last sampling point varies in a range of 92.59% to 92.10%, thus reflecting a slight decrease of approximately 0.49% of the component over 36 months (3 years) of storage.

Data presented in **Table 2.14** show changes in the carbohydrate profiles over 18, 25 and 36 months of real-time product storage for IMAG Organic® production lot 40502712.

Table 2.14 Carbohydrate Levels of IMAG Organic® Powder Stored for Various Times

Storage Period (Months)	Sucrose	Glucose	Fructose	Inulin (Dietary fiber)
0	1.22	1.40	4.60	92.59
18	1.43	1.72	4.59	92.14
25	1.50	1.67	4.70	92.12
36	1.52	1.63	4.74	92.10
Change over 36 mon.	+ 0.30	+ 0.23	+ 0.14	- 0.49

2.5.7.3 Conclusions of Shelf-Life Studies and Product Stability

Equal to the product stability data presented on page 14 and in Attachment 17 of GRN 854, the information provided in **Table 2.14** regarding the content and profile of carbohydrates in the 100% of IMAG Organic® agave inulin powder show no significant changes to carbohydrate parameters established to define a period of at least 36 months (3 years) of shelf life. Agave inulin has not lost significant amounts of any prebiotic dietary fiber listed on the label. All these parameters are within those established by IMAG specifications and the Mexican Legislation in force for agave inulin.

Data from the accelerated shelf study of product performed in an outside independent laboratory, that was stored at 20, 30 and 40° C for 6-months, and a real-time shelf life study performed at IMAG, also showed no significant stability issues (microbiology, % humidity, or carbohydrate profiles) for the powdered agave inulin product, IMAG Organic®.

Date suggest that the notified powdered agave inulin product is stable for at least 3 years, the same stability as described on page 14 and Attachment 17 of GRN 854 for similar agave inulin from *A. tequilana* Weber *azul.*

2.6 § 170.230(d): Intended Physical or Technical Effect

Inulin and inulin-type fructans, having typically molecular structures as shown in earlier figures for inulin, graminan/agavin, oligofructose and neoseries structures, possesses several unique physicochemical properties that allow them to be used effectively to add texture, body and mouthfeel to processed foods. In addition, they also possess beneficial characteristics to health when consumed as an ingredient in food products. Because these fructans have excellent moisture management properties and are reduced-calorie carbohydrates (i.e. dietary fiber) with a slightly sweet taste, they may be used to replace fat and sugar as a "bulking agent" in a wide variety of food products, with a resultant reduction in the energy content of the food. In addition, as a result of the β 2-1 and β 2-6 linkages in inulin and inulin-type fructans, they are not hydrolyzed or absorbed from the human intestinal tract, they result in reduced energy content of foods they are added, and can serve as a source of fermentable carbohydrate in the diet for saccharolytic microorganisms, such as the genera *Bacteroides, Bifidobacterium, Ruminococcus, Eubacterium, Lactobacillus* and *Clostridium*. In the 1980s, Japanese researchers (Yazawa et al., 1978; Mituoska et al., 1987) had already demonstrated that specific non-digestible oligosaccharides (especially fructo-oligosaccharides) were selectively fermented by bifidobacteria and had the capacity, upon feeding, stimulating their growth in human feces. These observations were confirmed and further expanded by Gibson and Roberfroid who introduced the concept of prebiotics in 1995 (Gibson and Roberfroid, 1995) and have published reviews of the research which includes more recent development (Gibson et al., 2004; Roberfroid et al., 2010).

The legal caloric value ascribed to inulin for labeling on food products is defined by the country of use, but in most countries, including the U.S. and Canada, this is 2 kcal/g. The reduction in calories is predicted based on the lack of digestion in the small intestine, and as such these fructans are bulking agents or dietary fiber.

There are numerous recent reviews on the positive physiological benefits of inulin and related fructans (Roberfroid et al., 2010; Grizard and Barthomeuf 1999; Carabin and Flamm 1999; Flamm et al. 2001; Boeckner et al. 2001; Kaur and Gupta 2002; Roberfroid 2007; Kelly 2008, 2009; Tungland, 2018). Inulin from all sources is a well-recognized and established prebiotic agent, as defined by Gibson and others in 2017 as a selective dietary fiber that adds low caloric (dietary fiber) bulk to foods to which it is added. Agave inulin's prebiotic fiber properties are the intended physical or technical effect described on page 14 or GRN 854 for the similar agave inulin produced from IIDEA.

3.0 § 170.235 DIETARY EXPOSURE

3.1 § 170.235(a): Dietary Exposure from the Intended Use and Sources in the Diet

The proposed uses of IMAG Organic® will not result in an increase in the overall consumption of inulin because they are intended to provide an alternative source of agave inulin for use in selected foods. This GRAS Notice provides the intended food uses and conditions of IMAG Organic® agave inulin, which are identical as those food uses specified on pages 2 and 3 of GRN 854 by IIDEA (Industrializadora Integral del Agave, SA de CV) for Inufib™, another agave piña-derived inulin (FDA, 2020) which cited the exposure assessment presented in GRN 118 by Imperial Sensus, LLC for Frutafit®, a chicory root-derived inulin product (FDA, 2002a and 2007 amendment). On page 15 of GRN 854 they estimated the combined average intake of inulin by the general U.S. population (consumers two years of age and older) from all uses of Inufib™ would be 10.1 g inulin/person-day. The 90th percentile was estimated to be 19.2 g inulin/person-day. For U.S. consumers (non-breastfeeding children) from one year up to two-years of age, the combined average intake of inulin from all uses of fortified product was estimated to be 7.6 g inulin/person-day, and the 90th percentile intake was estimated to be 13.7 g inulin/person-day. For non-breastfeeding infants under 1 year of age the combined average intake of inulin from all proposed use categories was estimated to be 2.3 g inulin/person-day, and the 90th percentile intake was estimated to be 5.7 g inulin/person-day.

Because the level of inulin in both Frutafit® chicory inulin from GRN 118 and Inufib™ agave inulin from GRN 854 has essentially the same level of inulin as IMAG Organic® from IMAG, the same levels of each when added to the same proposed foods will result in about the same levels of inulin per serving. As a consequence, the estimated intake of inulin from the same proposed uses of IMAG's agave inulin products will be comparable to or less than that of the current GRAS inulin products that were subject of GRN 118 for chicory-derived inulin and the recent GRN 854 for agave-derived inulin, particularly as use in meat and poultry, which a proposed use for GRN 118 is not being considered as part of this GRAS Notice for IMAG Organic®. Amounts of inulin from agave to be added to the proposed foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods, as required by FDA regulations.

Humans have seen significant historical exposure to native inulin and inulin-type fructans or shorter chain inulin products, such as oligofructose or FOS, through a variety of foods, including agave piña and leaves, several kinds of tubers that served as staple crops, and grains such as wheat, oats, rye and barley. Inulin-type fructans are present in a number of foods that are currently eaten on a daily basis throughout the world, **Table 3.1.**

In the United States, the most commonly consumed non-digestible fructan-containing foods include bananas, garlic, onions, tomatoes and several of the cereal grains, predominately wheat (**Table 3.2**), with exception to tomatoes all are monocotyledon (monocots) plants that store inulin-type fructans possessing a branched graminan/agavin structure, (similar to those in IMAG Organic®), rather than a linear inulin structure, such as from the dicotyledon plant, chicory root, as in Frutafit® GRN 118.

Table 3.1. Fructan Content of Edible Mono- and Dicot Plants

Plant	Scientific Name and Type of Plant (mono- or dicotyledon)	Edible Part	Fructan Content %
Agave	*Agave americana* & *Agave tequilana* (monocots)	stems/piña	7 – 10 16 - 25
Asparagus (Safed musli) (Shatwaar)	*Asparagus racemosus* (monocot) *Asparagus officinalis* (monocot)	tubers	2 - 3
Asphodel	*Asphodelus sp.* (monocot)	leaf/stem/tuber	Detected
Banana	*Musa acuminate* (monocot)	fruit	0.3 – 0.7
Barley	*Hordeum vulgare* (monocot)	cereal	0.5 – 1.5
Burdock	*Arctium* sp. (dicot)	roots	3.5 – 4.0
Camas	*Camassia* sp. (monocot)	bulb	12 – 22
Chicory	*Cichorium intybus* (dicot)	roots	15 – 20
Chicory coffee powder	*Cichorium intybus* (dicot)	extract	20 - 60
Comfrey	*Symphytum sp.* (monocot)	Leaf	Detected
Dahlia	*Dahlia* sp. (dicot)	tubers	15 – 20
Dandelion	*Taraxacum officinale* (dicot)	leaves	12 - 15
Elecampane	*Inula helenium* (dicot)	tubers	19 – 44
Garlic	*Allium sativum* (monocot)	bulb	9 – 16
Globe artichoke	*Cynara cardunculus* (dicot)	leaves/heart	3 – 10
Jerusalem artichoke	*Helianthus tuberosus* (dicot)	tubers	14 – 19
Jicama	*Pachyrhizus erosus* (dicot)	root	5
Kuth	*Saussurea lappa* (dicot)	root	18 – 20
Leek	*Allium ampeloprasum* (monocot)	bulb	3 - 10
Meadow cabbage	*Symplocarpus foetidus* (monocot)	root	Detected
Murnong	*Microseris lanceolate* (dicot)	root	8 – 13
Oats	*Avena sativa* (monocot)	cereal	Detected
Onion	*Allium cepa* (monocot)	bulb	2 - 6
Palm Lily	*Cordyline stricta* (monocot)	tuber	Detected
Raisin	*Vitis vinifera* (dicot)	fruit	4
Rampion	*Campanula rapunculus* (monocot)	root	Detected
Rye	*Secale cereal* (monocot)	cereal	0.5 – 1.0
Salsify	*Tragopogon* sp. (dicot)	root	15 – 20
Spanish salsify	*Scorzonera hispanica* (dicot)	root	8.15 – 10.75
Tomato	*Solanum lycopersicum* (dicot)	fruit	0.15
Wheat	*Triticum sp.*(monocot)	cereal	1 - 4
Yacon	*Smallanthus sonchifolius* (dicot)	root	3 – 19

Sources: Van Loo et al., 1995; Incoll & Bonnett, 1993, Roberfroid et al., 1993; Partida et al., 1998; Petkova, N., 2018; Kuniyal et al., 2005.

As reviewed by Van Loo and others (1995), inulin-type fructans are present in a variety of edible fruits and vegetables in appreciable amounts. The most common sources of inulin-type fructans are wheat, onions, bananas, garlic and leek. Inulin-type fructans content of edible plants ranges from < 1% to > 20% of the wet weight. In 1999, Coussement estimated that the daily intake of fructans (defined as inulin and oligofructose) in a Western-type diet was up to about 10g/day, and range between 1 and 4 g/d for the 97th percentile in the U.S. In 1999, the consumption level in Europe was estimated to range from 3-11 g/d (Van Loo et al., 1999; Coussement 1999). Moshfegh and others (1999) estimated the average inulin and oligofructose intake in the U.S. was 2.6 g, of which, about 95% was from wheat and onions. In both cases, the estimates were based on foods that primarily store fructans having a graminan/agavin structure as their reserve carbohydrate, like the agave inulin in GRN 854 and IMAG Organic®, rather than a more linear type structure as in inulin, like those from the dicotyledon chicory, as in Frutafit® GRN 118.

3.2 § 170.235(b): Dietary Exposure to Substances Formed in or Around Food

All inulin-type fructans are stable to heat at pH > 4. At pH values of < 4, hydrolysis occurs depending on temperature and heating time (Glibowski and Bukowska, 2011; Sensus America, LLC internal correspondence). The notified substance, IMAG Organic®, is a high carbohydrate-containing product

composed of primarily of fructose moieties that undergo hydrolysis to monomers (i.e. fructose and glucose) upon prolonged heating at pH values of 4 or less. As stated in the previous Section 3.1, and as stated on page 16 of GRN 854 for Inufib™, the use of IMAG Organic® its intended foods will not result in an increase in the overall consumption of inulin or its hydrolysis products, as it provides an alternative source of inulin for use in process food manufacturing.

3.3 § 170.235(c): Dietary Exposure to Other Substances

IMAG Organic® is manufactured to a highly purified inulin of agave piñas, as shown from its chemical and microbiological specifications in Tables 2.4 and 2.5, and as shown by analysis of potential residual pesticides, heavy metals, aflatoxins, and microbes. Agave is also known to contain steroidal sapinogens and terpenes. However, concentrations of these saponins and terpenes in IMAG Organic® have been evaluated using the same method and laboratory as was used for the agave inulin, Inufib™ described in GRN 854, and, like the agave inulin in GRN 854, were shown to be negligible, below 0.1 ppm for terpenes and below the 7 ppb method detection limit for saponins (refer to page 16 of GRN 854). Consequently, concern for these potential substances is not expected to increase due to the intended uses of IMAG Organic®. Maillard reaction products, such as furfural, 5-(hydroxymethyl) furfural and methyl-2-furoate, that are known to be produced from inulin decomposition, take place at high temperatures (above 100 °C), for long durations (up to 32 hours) of cooking agave for tequila production (Mancilla-Margalli and Lopez, 2002), and under alkaline conditions. Processing conditions that favor the production of these compounds, do not occur in the production of refined agave inulin, which include short duration vacuum evaporation and spray drying at acid pH levels (4.5). These conditions have been documented in the literature as ones that provide resistance of the inulin molecule to acid hydrolysis (Glibowski and Bukowska, 2011; Tungland, 2018). As agave processing conditions do not favor inulin decomposition and the pH is maintained near 4.5, a stable range for the inulin molecule, and not in the alkaline range (a favorable condition necessary for the Maillard reaction), the formation of Maillard reaction compounds is negligible.

3.4 § 170.235(d): Source of Food Consumption Data

Dietary exposure to IMAG Organic®, as an alternative to the agave inulin determined GRAS in GRN 854,is the same as that for the notified chicory inulin in GRN 118 by Imperial Sensus (FDA, 2002a and 2007 amendment) and the agave inulin in the recent GRN 854 by IIDEA (FDA, 2020). .

3.5 § 170.235(e): Assumptions Made in Estimating Dietary Exposure

Because the expected dietary exposure to IMAG Organic® is the same as that from GRN 854 (FDA, 2020), and will not result in any additional cumulative exposure, no other additional assumptions were made when estimating dietary exposure.

4.0 § 170.240: SELF-LIMITING LEVELS OF USE

Suggested serving and use levels in food of the notified substance are identical to those listed in the GRAS Notification submission to the U.S. FDA for Inufib™ agave inulin (GRN 854), Frutafit® chicory inulin (GRN 118 and 2007 amendment) and fructooligosaccharide (GRN 44), which were determined as GRAS without questions or objections by FDA (FDA 2020; FDA 2000b; FDA 2003). As no known self-limiting levels of use are associated with the notified substance, self-limiting use levels are not applicable to this GRAS Notice.

5.0 § 170.245: COMMON USE OF THE NOTIFIED SUBSTANCE IN FOODS

Fructans, like starch found in corn, rice, or potato, serve as storage polymers in over 15% of the global angiosperm flora numbering over 36,000 fruits and plants, including 1,200 native grasses belonging to 10 families (Hendry, 1987; Hendry and Wallace, 1993). After starch, the fructans are the most plentiful carbohydrates occurring in the plant kingdom (Carpita et al., 1989; Van den Ende et al., 2013; van Loo et al., 1995).

Fructans are found in many edible plants including the *Liliaceae*, *Campanulaceae, Goodeniaceeae, Lobelianceae, Stylidiaceae, Agavaceae, Vilaceae, Graminae* (grass) and *Compositae* (sunflower/daisy) families, **Table 3.1**. The occurrence of the storage carbohydrate fructan in a significant portion of the world's flora (Hendry, 1987; Hendry and Wallace, 1993) has all but guaranteed that nondigestible fructans were consumed by Pliocene and Pleistocene ancestors millions of years ago. Fructan-rich plants have been sources of sustenance for indigenous populations, including Dacopa, a beverage from roasted Dahlia tubers (*Dahlia* sp.), Yacon tuber (*Smallanthus sonchifolius,* also called Peruvian ground apple); Jerusalem artichoke tubers (*Helianthus tuberosus*); Chicory root (*Cichorium intybus*); Murnong (*Microseris lanceolate*, a.k.a yam daisy) and Camas root (*Camassia* sp.). Decades of large-scale archaeological evidence from dry cave deposits in the northern Chihuahuan Desert shows extensive consumption of inulin-type fructans having graminan and agavin-type structures from fructan-rich plants, such as agave (*Agave lechuguilla*), sotol (*Dasylirion* spp.), camas (e.g. *Camasia quamash, C. leichtlinii*), and wild onion (*Allium* spp.) occurred over a span of over 10,000 years (Leach and Sobolik, 2010). Ancient cooking features, stable carbon isotope analysis of human skeletons, and well-preserved coprolites and macrobotanical remains reveal a plant-based diet that included a dietary intake of ~135 g/d of inulin-type fructans, principally from agave, sotol and onions, by the average adult male, and about 108 d/day for adult females, based on about 20% less energy (Leach and Sobolik, 2010). While a great number of fructan-bearing plants were known as food sources among the prehistoric and historic groups of North America (Wandsnider 1997), these particular plants by far provide the oldest evidence of inulin-type fructan consumption in North America, dating back over thousands of years. Throughout Western Europe, similar remains of massive cooking facilities are known to occur in Wales, England, Scotland, Ireland, and Scandinavia and were constructed with in the last 6,000 years. Jerusalem artichokes were consumed by Western European populations in the 16th century as a substitute for white potatoes, and the consumption of inulin in these populations was estimated to have reached 25-32 g/d (FDA, 2002a, GRN 118). In Australia, more than 800 plant foods have been known to be eaten for

tens of thousands of years by Aborigines, many containing inulin-type fructans, such as murnong (Brand-Miller, 1998; van Loo et al., 1995).

There is common knowledge that Agave fructans have a long history of human consumption. The agave plant has been used as a source of food and fiber production for at least 10,000 years, and it was exported and used as a food source to Europe since 1520 A.D, and was mentioned as a food of Aztecs and natives in the Florentine Codex of 1580 (IOAA, 2009). Agave concentrates produced from the agave plant, such as various syrups, aguamiel (its sap), and inulin have been safely used for decades and even centuries. Many examples of various food products containing agave are currently marketed in the U.S. and throughout the world. Due its organic status and high direct solubility, demand for agave inulin has increased for a variety of food applications as a source of labeled dietary fiber, including the U.S., Canada and Europe.

Agave spp. (agave) and *Dasylirion spp.* (sotol) are succulent monocotyledon plants and members of the *Agavaceae* family, with 8 genera. The agave genus alone includes about 275 species that belong to the *Asparagales* order. Edible parts of the agave, including the flowers, leaves, stem (piña) or basal rosettes, and the sap, or aguamiel (Prescott, 1843 adapted), have supported the pre-Columbian Mesoamerican civilization since the first inhabitants (more than 9000 years ago), culminating in substantial intensification around 1,250 years ago until present time (Colunga-García Marín et al., 1993; Leach, 2005). Analyses of several species of agave plant have shown that nonstructural, water soluble carbohydrates, known as "fructosans" are the major fraction and a concentrated in the stem (Srinivasan and Bathia, 1953; Srinivasan and Bathia, 1954). Agave use predates the arrival of the Spaniards. To prehispanics, agave was referred to as *Metl* in the náhuatl language and manuey in old Spanish sources (de Sahagún et al., 1970). The botanical diversity for this plant is the result of a prehistoric human selective breeding (Parsons and Darling, 2000). Certain prehistoric tribes learned to cook agave plants and use them to resist dehydration in the desert climate. These tribes understood that the hydrated cooked agave could ferment, producing a desirable beverage. This method was used for centuries to produce a variety of agave-derived beverages (Cedena, 1995). In modern times, Kolbye and others (1992) reported that inulin and oligofructose had a long history of use pre-1958.

Indigenous populations cooked the softer parts of the agave by direct fire or with hot water for > 40 hours prior to consumption. Castetter and others (1938), and Wandsnider (1997) described a communal practice of indirect pit baking in northern Mexico and the American southwest to cook agave for human consumption. The cooked material is eaten immediately, or pounded into sheets, dried and stored or later consumption (Dering, 1999). The moist cooking environment of the earth oven reduces their steroidal sapinogen-based (see section on agave bioactive compounds for more information) potential as an antinutritent and improves the nutritional profile (Wandsnider, 1997). It is known that the juice from several agave species, mainly its leaves, can cause contact dermatitis from calcium oxalate raphides, which is also reduced by cooking. The intensification of inulin-containing foods in southern North America around 1,250 years ago (specifically the American Southwest) coincides with increased reliance on crops such as corn (*Zea mays*), squash (*Cucurbita* sp.) and beans (*Phaseolus* sp.) and large-scale growth in human population (Lu, 2006). Analysis of 359 human coprolites dated by carbon 14 techniques by Callen (1965) as reported by Sobolik (1996) showed that between 7000 B.C.

and 1500 A.D. agave formed 25-60% of the studied material. Stable carbon isotope analysis on skeletal material recovered from various deposits in Chihuahuan Desert area, near present Del Rio, Texas, suggested that 45 to 68% of the diet may have been derived from C^4 and CAM (Crassulacean acid metabolism) plants, with CAM plants (e.g. agave) making the greater contribution (Huebner, 1991; Lopez et al., 2003; Mancilla-Margalli and Lopez, 2006). Webb and Starr, 2015 citing work by Achmann (1959) noted from archeological reports that edible agaves were very important resources for Indians of California, comprising 28 percent of their annual food consumption, which rose to 45 percent in springtime when other forms of vegetables were in short supply. Evidence suggests that agave and sotol may have collectively contributed up to 80 to 90 percent of the inulin-type fructans in the diet (Leach and Sobolik 2010). Archaeological research in the northern Tucson Basin has confirmed that species of agave were cultivated in extensive agricultural fields marked by the presence of rock piles, terraces and check dams. Researchers estimate that ~10,000 agaves were harvested annually from a standing population of >100,000 cultivated plants in larger fields (Leach, 2007). Stems (a.k.a piñas) of the agave were also hydrolyzed by heat and fermented around 1300 B.C., when the Aztec civilization fermented sap (aguamiel, or honey water) from the agave stems to produce a viscous beer, Pulque (Noble, 1988), both are popular beverages in the south of the Sonoran Desert (Debnath et al., 2010). In the late 15th and early 16th century, Spaniards colonizing north central Mexico introduced distillation process, giving rise to current distillated beverages such as tequila, mescal, and bacanora having higher alcohol content than Pulque. The sweetish sap from agave is also consumed as a nutritious beverage, especially for diabetic patients, as a low glycemic, high fructose-containing product.

Agave and sotol are indigenous to southern and western U.S., Mexico, and in central and tropical South America. When mature, typically after 7 years, agave has over 80 weight percent of its carbohydrate content as fructans, and agave piña can contain up to about 60%, but more typically about 24-32% inulin-type fructans on an as is basis. As mentioned, the main source of food in the agave and sotol is the soft starchy white meristerm, in the short stem and the bases of the leaves, excluding the green portion, although, as mentioned, the fructans from the head or piña of the agave are typically used for commercial inulin and agave syrup, and fermented to produce fermented alcoholic beverages like mescal and tequila. Originally, blue agave (*Agave tequilana* var. *azul*) was selectively bred and utilized in a short maturation cycle to promote caramelized aroma and flavor qualities and ease of processing in baking.

In addition to agave inulin, these fructans serve as precursors for other common agave-derived food and beverage products, made by additional processing. According to the Natural Standard Review Research Collaboration for agave indicates that agave syrup is also useful as a sugar alternative as fructans are 90% fructose and have a low glycemic index (Hackman et al., 2006). By cooking the piñas or treating the resulting fructan polysaccharides with enzymes or heat to hydrolyze them to fructose monomers, provides a means to produce high fructose-containing syrups. Such syrup was developed and became regulated by the Mexican government in the 1990s. Agave syrups are manufactured by several different agave species, including the most popular, *Agave tequilana*, as well as *Agave salmiana, Agave americana* and *Agave mapisaga* (Debnath et al., 2010). In addition, chunks of roasted agave piña are current sold in markets in Mexico as a sweet tasting treat.

As mentioned, the juice from roasted agave piña, of several different agave species is also the starting material for distilled spirits, such as mescal and tequila, although tequila is only manufactured from *Agave tequilana* Weber *azul*, due to Mexican NOM regulations. Several local varieties of mescal are made in Mexican villages within the agave habitat; the most well know variety being, Bacanora, named after the Sonoran village (Debnath et al., 2010). Tequila is recognized as the most well-known distilled spirit derived from agave in the world.

Food and beverages products that are made from agave vary by species used as the raw material for the product's manufacturing, as well as the amount and type of processing used in their manufacture. By example, *Agave salmiana, A. potatorum*, and *A. angustifolia* are used in mescal production (Michel-Cuello et al., 2008; Pena-Alvarez et al., 2004), while *Agave atrovirens, Agave americana*, and *Agave salmiana* are all sources of aguamiel and Pulque production, and, as mentioned, only *A. tequilana* Weber var. *azul* (the species used to manufacture the Inufib™ in GRN 854 and IMAG Organic®), that is grown near the town of Tequila in Jalisco, MX, can be legally used for tequila production.

Agave as a raw material has been applied in several patents, including the use of its native fructans as a prebiotic agent with natural fiber levels; as a sweetener with improved nutritional properties; and in foodstuffs and cosmetic applications. The hydrolyzed fructans have also been incorporated in organic sports beverages and as a bulking agent in sugar replacement food systems to reduce calories and lower the system's glycemic response. The hydrolysis step, involving either thermal, acid or enzymatic treatments, or a combination thereof, is not utilized to manufacture agave inulin.

6.0 BASIS FOR CONCLUSION OF GRAS STATUS

6.1 § 170.250: Safety Narrative and Description of Relevant Data

The same information used as the basis for the conclusion of GRAS for Inufib™, page 19 - 39 of GRN 854, is relevant to document the basis for GRAS status of IMAG Organic®; IMAG Organic® uses agave inulin manufactured using a similar production process, is made from the same plant source, piñas of *Agave tequilana* Weber var. *azul*, grown in same geographical location, and is intended for use in the same foods and at the same inclusion levels. Comparison of specifications and compositional analyses of the agave inulin in GRN 854 with the notified substance show that the two agave inulin products are essentially chemically equivalent. Both products are intended for U.S. organic food labeling.

Additionally, several inulin and inulin-type fructans have GRAS status for use as food ingredients, including (sc-FOS) short-chain fructooligosaccharides (GRNs 44, 537, 605, 623 and 717) (FDA 2000b, 2015a, 2016b,c, 2018a), oligofructose (GRNs 392 and 576) (FDA 2012, 2015b), long chain inulin (GRN 477)(FDA 2014b), native chicory inulin (GRN 118)(FDA 2002a and 2007 amendment), and Jerusalem artichoke inulin (GRN 849)(FDA, 2019a).

Studies pertaining to infants and/or the use of infant formula are included in this summary to support human safety and the safety of IMAG Organic®, as also described on page 21 of GRN 854 for Inufib™, an agave inulin made from the same raw material, possessing similar or equal molecular structure, specifications, and manufacturing processes as IMAG Organic®, for the general populations including

infants. Additionally, as described on page 21 of GRN 854, the safety and tolerance studies conducted with Metlin®, Metlos® and their mixtures, BioAgave® or Predilife®, are relevant to addressing the safety and tolerance of IMAG Organic®, because: 1) all of these products, including the Inufib™ (the subject of GRN 854, which is equal to or similar to IMAG Organic®) consist of mixtures of fructooligosaccharides and fructans obtained from agave, having the same basic molecular structures, with variable degrees of polymerization (DP), but overlapping with IMAG Organic® in the proportion of polymerization. Irrespective of DP and demineralization, all the agave fructans pass through the digestive tract largely intact, where they reach the colon, undergo equivalent metabolic processing, and are well tolerated orally.

Thus, in order to provide a comprehensive review of the pertinent literature, pivotal data for agave inulin, as well as corroborative data for the closely related inulin-type fructan substances, are reviewed.

Some of the studies report functional effects, such as bifidogenic properties, which are not intended to assess safety. However, as described on page 20 of GRN 854, these studies are included because they indicate the substantial history of long-term fortification of diets with inulin-type fructans without evidence of any adverse safety concerns.

Published data, as well as reviews conducted by various regulatory agencies, support the conclusion that inulin and inulin-type fructans, including those from agave, as described in this GRAS Notice and in GRN 854, are safe for use as a human food ingredient. Human tolerance to inulin-type fructans has been thoroughly evaluated in historical and contemporary diets and in clinical study employing bolus, short-term, and long-term exposure. Therefore, IMAG has determined that their agave inulin product, IMAG Organic®, is safe and GRAS for its intended uses.

6.1.1 Metabolic fate

Studies on the metabolic fate of inulin and inulin-type fructans from all plant sources, including those from chicory, Jerusalem artichoke and agave, and for short chain fructooligosaccharides (scFOS) show that these fructans act as substrate for fermentation and promote the growth of colonic microbiota, which in turn produce short chain fatty acids, gases (hydrogen, carbon dioxide and methane) and additional bacterial mass.

The absorption, distribution, metabolism and excretion of inulin type fructans, are well characterized and have been previously described in detail in previous GRAS Notices(GRN 44, pg. 57-104 and GRN 118, pg. 45-55), and in recent published literature (Tungland, 2018). Fructans that are predominately β(2-1)-linked and with β(2-6)-linked side chains are not absorbed and are resistant to digestion by salivary amylase, human pancreatic or intestinal enzymes. Available animal and human metabolic and safety information indicate that inulin from all sources will be similarly metabolized and processed by the body following consumption. As inulin is largely not digested or absorbed in the upper gastrointestinal tract, the majority of the molecules reach the large intestine primarily intact where resident microbiota ferment them as substrate. As example, studies in ileostomy patients show that inulin-type fructans (DP>2) isolated from Jerusalem artichoke are practically indigestible in the small intestine of humans (Bach Knudsen and Hessov, 1995). The recovery of inulin in ileal effluent was 87 percent at both a low

(10 grams inulin product) and a high (30 grams of inulin product) intake level which confirmed earlier human (Rumessen et al., 1990) and rat studies (Nilsson et al., 1988) that showed inulin is virtually indigestible in the small intestine.

In 1997 and 1998, Rossi and others evaluated inulin digestion in ileal and fecal samples in 5-8-week old, cannulated piglets weaned at 28 days of age and fed a diet containing 10% inulin. Following fermentation and hindgut parameters, the authors found that inulin digestion was low in the small intestine (7.5% ± 11.4%) but inulin had been completely fermented in the large intestine.

During fermentation by the colonic microflora, inulin and inulin-type fructans are metabolized to SCFA (Tokunaga et al., 1986, 1989; Wang and Gibson 1993; Oku 1986; Roberfroid et al., 1993; Tungland, 2018). The stoichiometry of this metabolic conversion, as measured both *in vitro* using a fecal microbiota and *in vivo* in the cecum of inulin (defined by the authors as a FOS fraction with a DP of 8) fed rats has been defined as follows (Roberfroid et al., 1993): 1 mol fructosyl unit in FOS produces about 1 mol SCFA (0.9 mol acetate, 0.12 mol propionate and 0.06 mol butyrate) and 0.3 mol L (+)-lactate. In terms of C-atoms, the overall balance is 40 percent SCFA, 15 percent L(+)-lactate, 5 percent CO_2 , and about 40 percent bacterial mass, predominately bifidobacteria. The significance of the production of the SCFA is in their resorption through the colonic epithelium into the portal blood, thus becoming a source of energy and systemic effects for the host. As mentioned, butyrate is metabolized by the colonocytes. Most of the propionate and L (+)-lactate are completely metabolized in the liver; propionate being transformed into propyl-CoA and then to methylmalonyl-*S*CoA and then succinyl-CoA and L (+)-lactate being a precursor in gluconeogenesis. Acetate is only partly metabolized in the liver to acetyl CoA, a precursor to cholesterol; the remaining fraction is metabolized in peripheral tissues, mainly muscle (Roberfroid et al., 1993). There are several physiological consequences of microbial fermentation. Most notably are a decreased colonic and fecal pH that occurs from production of SCFA by the colonic microflora (Roberfroid et al., 1993; Oku et al., 1984; Oku 1986; Nilsson and Björck 1988). This decrease in pH is thought to be a result of fermentation by colonic bacteria, particularly bifidobacteria, which produce both the strong acids, acetic and lactic acid.

The fermentation processes not only provide energy for the bacterial proliferation, but they also produce gases (H_2, CO_2, CH_4), which are not of metabolic value to the host, and small organic acids such as acetate, propionate, butyrate (SCFA), and L (+)-lactate). The SCFA (except for butyrate) are largely absorbed through the intestinal wall, reach the portal circulation and are transported to the liver where they are utilized. Approximately 95% of the butyrate and about 25% of the propionate is utilized by the cells lining the colon as energy. Part of the acetate (25 to 50 percent) is transported via the systemic circulation to the peripheral tissues, predominantly muscle. Thus, the bacterial fermentation of SCFA provides the host with energy (Roberfroid et al. 1993; Tungland 1998, 2003, 2018).

Malabsorption of fermentable substrates, like the inulin-type fructans, results in increased hydrogen (H_2) production by the colonic flora. Stone Dorshow and Levitt (1987) measured breath H_2 excretion during human ingestion of inulin (Neosugar® at a level of 15 gram per day (5 grams, 3 times/day) for 12 days. Breath H_2 after 10 grams of inulin was similar to that of 10 grams of lactulose, suggesting near total malabsorption of the inulin. Breath H_2 was also increased (not statistically significant) by 50% after

a 12-day period on the inulin. This H_2 is absorbed and excreted in expired air and breath. Studies in humans measuring breath H_2 release indirectly demonstrate that inulin reaches the colon and is subsequently fermented by the microflora. By example, Rumessen and others (1990) showed that inulin from Jerusalem artichoke is virtually indigestible in the small intestine and measured breath H_2 excretion in eight healthy subjects after consumption of 5, 10, or 20 grams of inulin from Jerusalem artichoke to determine its fermentability in the colon. The increase in breath H_2 suggested that inulin was substantially fermented.

Since all β(2-1)-linked fructans with varying degrees of β(2-6)-linked branches are substantially chemical equivalent from a fermentation standpoint, they ultimately are expected to be physiologically equivalent.

6.1.2 Safety Studies in Humans with Agave Inulin

Clinical studies documenting the safety and tolerance of agave inulin are incorporated by reference from page 21-23of GRN 854 (FDA, 2020) and summarized below. Three human studies are cited in GRN 854 comprising infants and healthy adults in short term (6 hours) and longer-term durations (3 weeks and 6 months) with a dose range of 0 - 24 gram/day. These studies and three additional human studies, along with those reviewed in 1999 by Carabin and Flamm show that inulin-type fructans are safe for human consumption under their intended conditions of use as a reduced calorie bulking agent (dietary fiber), and that up to 20 g/d is well tolerated. In a large randomized double-blind controlled (RDBC) study involving term-born healthy infants, López-Velázquez and others (2013) studied the effect of fructans obtained from *Agave tequilana* Weber var. *azul* on the frequency of adverse effects on the gastrointestinal tract, including changes in bowel habit (bowel consistency and incidence of colic, abdominal distention, flatus effects, and regurgitations). It is important to note that this study and others that utilized inulin and inulin-type fructans and pertain to infants are included to support human safety, and the safety of IMAG Organic® for the general population, including infants, although this notification does not include infant formula as an intended food application. In their RDBC study, López-Velázquez and others (2013) assigned healthy infants (20 ± 7 days) to one of six groups (100/group). The infants in 3 of the groups received a formula containing a Lactobacillus probiotic (10^7 CFU) and 0.5 g/100 mL agave fructans, while one group received formula with only probiotic, and one group received formula without probiotics or fructans, and one group received human breast milk. Of the three groups receiving agave fructans, one group was fed formula containing a short chain agave fructooligosaccharides, as trade name Metlos®, an agave product having a DP < 10, while another of the groups infants received formula containing agave inulin, as trade name Metlin®, an agave inulin having a DP > 10, and the third group was fed formula containing a mixture of Metlos® and Metlin® (50:50)[2]. During the treatment phase of the study, the mean daily formula intake over the last 2 months of the six-month study ranged from 1423 - 1510 mL/day, representing an average daily agave inulin dose of 7.1 - 7.5 g/day, during the period of maximum formula consumption. Monthly case report forms were used to measure gastrointestinal effects for six months. Infants receiving formula containing the mixture of agave fructans and agave inulin with a DP > 10 (Metlin®), had no significant changes in stool consistency or increases in incidence of colic, abdominal distention, daily stool frequency, or daily regurgitation episodes as compared with infants fed only breast milk. However, infants fed formula containing the

agave fructooligosaccharide (Metlos®; DP < 10), experienced a significant increase in the number of daily flatulence episodes, but no significant changes in stool consistency, and no significant increases in the incidence of colic, abdominal distention or daily regurgitations were observed. The researchers reported that agave fructans, when provided under the conditions of the study, are safe for use as a nutritional supplement in healthy infants. A later study by this research group investigated the effects of fructans from Mexican agave inulin in 600 newborns fed with enriched formula (López-Velázquez et al., 2015). This study demonstrated the efficacy as prebiotics of agave inulin DP >10 and agave FOS DP < 10 from samples taken from healthy infants at 20 ± 7 days, and three (3) months of age. The study showed that both treatments supported the efficacy of the agave inulin products as prebiotics in humans based on statistically significant outcomes of immune response, serum ferritin, C-reactive protein, bone metabolism, and gut bacteria changes.

In a later randomized, double-blind, placebo-controlled, crossover study in adults, Holscher and others (2014) investigated the tolerance and utilization of native agave inulin (BioAgave®), an agave inulin with similar DP range as IMAG Organic®. The study involved 29 healthy men and women aged 20-36 that consumed 0, 0.5, or 7.5 g/d of the native agave inulin in a single daily serving of chocolate chews for three 21-day treatment periods that were separated by 7-day washout periods. Daily assessments for severity of gastrointestinal symptoms, such as abdominal pain, bloating, burping, flatulence, nausea, reflux, rumblings were recorded on a scale of 0 (absent) to 3 severe, while weekly assessments for the frequency of symptoms and diarrhea was reported on a scale of 0 (occurs no more than usual) to 2 (occurs much more than usual). The daily assessments of gastrointestinal symptom severity showed statistically significant increases in the mean scores for abdominal pain, bloating, flatulence, and rumblings for adults in the treatment group as compared with the placebo group, but scores indicated only mild severity, ranging from a mean score of 0.2 for abdominal pain to a mean score of 1.2 for flatulence in the high dose group. Weekly assessment reports similarly showed statistically significant increases in the mean scores measuring the frequency of bloating, flatulence and rumblings compared with the placebo group, although reported scores showed only slight increases in frequency, ranging from 0.4 for rumblings to 1.0 for flatulence in the high dose (7.5 g/d) treatment group. Further, while bowel habit, including bowel frequency, ease of defecation, stool consistency, and the percent of bowel dry matter were influenced by agave inulin consumption, the magnitudes of these effects were minimal. By example, the mean number of daily bowel movements increased from 1.2 in the placebo group to 1.4 in the high 7.5 g/d dose group, and the mean stool consistency score increased from 3.4 to 3.6 in the placebo and high dose groups, respectively, with higher scores representing softer stools. The researchers reported that daily consumption of between 5 - 7.5 g native agave inulin in a single bolus serving was generally well tolerated in adults with only mild flatulence being reported as the most common untoward effect.

Bonnema and others (2010) using a randomized, double-blind, controlled, crossover human study to investigate the gastrointestinal tolerance of chicory inulin products in twenty-six men and women. Subjects consumed 5 and 10g doses of shorter-chain chicory fructans (oligofructose) and native chicory inulin in bagels, cream cheese and orange juice. Questionnaires administered at t=0, 2, 4, 24 and 48 hrs. following the fiber challenge indicated that the two chicory inulins tended to increase GI symptoms mildly. Most frequently reported symptoms were flatulence followed by bloating. The 10-g dose of

oligofructose substantially increased GI symptoms compared to a control. The authors concluded that doses up to 10 g/d of native inulin and up to 5g/d of oligofructose were well-tolerated in healthy, young adults.

Also, in 2010 Tarini and Wolever studied the effects of inulin on postprandial glucose, insulin, short-chain fatty acids, free fatty acids, and gut hormone responses in healthy subjects. Healthy subjects (n=12) after an overnight fast were studied for 6 hours following the consumption of 400 mL of drinks containing either 80 g high-fructose corn syrup (80 HFCS), 56 g HFCS (56 HFCS), or 56 g HFCS plus 24 g inulin (HFCS+I), using a randomized, single-blind, crossover experimental design. A standard lunch was served 4 hours after the test drink. The researchers found that glucose and insulin response after the HFCS+I treatment did not differ significantly from those after the 80HFCS or 56HFCS treatments. Serum short-chain fatty acid levels of acetate, propionate and butyrate were significantly higher after the HFCS+I treatment, as compared with the HFCS-only containing drinks from 4-6 h. Free fatty acids fell at a similar rate after all 3 test drinks, although there were lower after the HFCS + I treatment than after the 56HFCS at 4 h (0.40 ± 0.06 vs. 0.51 ± 0.06 mmol/L; $p < 0.05$). When compared with the 56HFCS treatment, the HFCS + I treatment significantly increased plasma glucagon-like peptide-1 concentrations at 30 min. while reducing ghrelin at 4.5 h and 6 h. The researchers concluded that inulin reduces postprandial free fatty acid rebound and reduces the serum ghrelin response after a subsequent meal, related to events associated with enhanced colonic short-chain fatty acid production.

In recent study twenty-eight (28) obese volunteers were used to investigate the effects of agave inulin from *Agave tequilana* Weber var. *azul* on body fat mass, body weight control, lipid profile, and physical tolerability in obesity (Padilla-Camberos et al., 2018). Researchers reported the 96 mg/body weight of agave inulin administered for 12 weeks significantly reduced the body mass index (BMI) of agave inulin treated group, as compared with those in the untreated placebo group. In addition, the agave inulin treated group had a decrease of 10% in total body fat, resulting in a statistically significant difference in the final versus baseline measurements between the agave inulin treated group and the placebo control group. The authors also noted that serum triglycerides were significantly reduced in the treatment group, and the agave inulin intake was safe and well tolerated throughout the duration of the study.

Although, not reflecting on intake and tolerance of agave inulin in humans, Carabin and Flamm (1999) reviewing and summarizing clinical information in these attributes, noted that the effects that potentially develop from the use of non-digestible fructans in the diet, i.e., flatulence, bloating, abdominal distention, and rumbling, are the same as those symptoms associated with the intake of fruits and vegetables and are related to the influence of fructans on osmotic pressure in the colon. They noted that the chain length of the fructan influences osmotic pressure to differing degrees, shorter chain molecules resulting in higher osmotic pressure, while long chain molecules are typically more slowly fermented and more easily tolerated than faster fermenting compounds. Thus, the potential for osmotic pressure-related diarrhea is greater with shorter chain fructooligosaccharides having an average DP of 3 than with inulin having an average DP of 10. Carabin and Flamm (1999) concluded that inulin-type fructans are safe for human consumption under their intended conditions of use as a reduced calorie bulking agent (dietary fiber), and that up to 20 g/d of inulin and/or oligofructose is well tolerated.

6.1.3 Toxicity/Tolerance Studies in Rodents with Agave Inulin

6.1.3.1 Repeated Dose Studies (cited in GRN 854 pages 23-25)

Recently, Rivera-Huerta and others (2017) fed mouse diets containing agave inulin from Mexican-grown *A. tequilana* Weber var. *azul* to groups of adult BALB/CAnNhsd mice (a model for colon cancer) for periods of time ranging from one (1) to nine (9) months. To induce tumors the mice were injected with a single dose of 10 mg/kg of the tumor-promoting azoxymethane at the beginning of the study and were then treated with 2% dextran sulfate sodium over a four-day period. The agave inulin content in the rodent diet was not specified in the publication. After the treatment period with inulin, the mice were euthanized and colon and jejunum specimens were extracted from both the control and treated mice and subsequently analyzed by ELISA for concentrations for the cytokines, tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10). Intestinal specimens were also reviewed histopathologically. The revealed that concentrations of TNF-α were significantly decreased in the inulin-treated groups compared to controls, and as compared to controls, treatment with inulin was associated with diminished intestinal polyps.

In 2013, Márquez-Aguirre and others investigated *in vitro* and *in vivo* the safety of agave fructans from *Agave tequilana* Weber var. *azul* having differing degree of polymerization (DP) and demineralization and their metabolic effects on body weight gain and intestinal microbiota profiles of seventy (70) obese male C57BL/6 mice. At the onset of the *in vivo* study to investigate metabolic effects, mice were fed high-fat diets to induce obesity, and then given 5 g/kg body weight daily gavage doses of agave inulin derived from *Agave tequilana* Weber var. *azul* for 12 weeks. During the treatment phase of the study, one group received agave inulin, a short chain fructooligosaccharide (scFOS) having a DP < 10 (Metlos®), while another received agave inulin with a DP > 10 (Metlin®). Additional groups received native agave inulin, without any fractionation with or without ion exchange demineralization processing. In addition, a commercial chicory inulin blend (Beneo Orafti Synergy 1™; a 50:50 mixture of chicory oligofructose and long-chain chicory inulin, avg. DP 23) was also utilized as a prebiotic reference. Controls not containing any fructans, employing either high fat or standard mouse diets, were also included. Results showed that, regardless of DP or mineralizations, *Agave tequilana* fructans were not mutagenic or toxic, and were safe even at a dose of 5 g/kg b.w. However, DP and demineralization appeared to influence body weight and blood lipid control, and the count of fecal Bifidobacteria. Obese mice receiving agave scFOS had a significant decrease in body weight gain, fat mass and total cholesterol without increasing fecal Bifidobacteria counts. Whereas obese mice receiving longer chain agave fructans (DP > 10), and no demineralization showed decreased triglycerides and an increased Bifidobacteria count. Although obese mice receiving demineralized long chain agave fructans did not show changes in body weight gain, fat tissue, total cholesterol or triglycerides, they did show an increase in Bifidobacteria counts.

A follow up study by this research group in 2016 investigated the effect of unfractionated and fractionated agave fructans (DP > 10 and DP < 10) from *Agave tequilana* in high-fat diet-induced obese mice (Márquez-Aguirre et al., 2016). The study determined fructans with a DP < 10 decreased weight gain by 30%, body fat mass by 51%, hyperglycemia by 25% and liver steatosis by 40%. Interestingly, unfractionated branched agave fructans decreased glucose and triglycerides, whereas fractionated

fructans with a DP > 10 decreased triglycerides, but not glucose; in contrast, agave fructans with a DP < 10 decreased glucose, but not triglycerides. Linear fructans from chicory (Beneo Orafti Synergy 1™, a 50:50 mixture of oligofructose and long chain chicory inulin, as in the first study) exhibited similar effects on glucose to unfractionated branched agave fructans, decreasing hyperglycemia by 19% versus 20%, respectively. This research indicates that both higher and lower DP agave fructans have complementary effects in metabolic disorders related to obesity. Therefore, it is desirable to have branched agave fructans that contain both higher and lower DP, that typify those in IMAG Organic®, to achieve positive effects in all metabolic disorders related to obesity. Like the earlier study, all fructans chain lengths were well tolerated and posed no overtly adverse health effects in mice.

Urías-Silvas and others (2008) compared the physiological effects of inulin-type fructans (graminan/agavin structures) from *Agave tequilana* Gto. and *Dasylirion spp.* (plant family Asparagaceae), a plant similar to agave morphologically, its geographical distribution and pollen characteristics. with chicory oligofructose (Orafti® P95) in male C57B1/6J mice over a 5-week period. Groups of 8 mice per group were given diets supplement with 10% fructans or a control standard mouse diet. Of significance is that the dietary concentration of fructans was higher than the upper limit of 5% of the total diet recommended to avoid nutritional imbalances in long term studies. Measures taken two-times per week included body weights and food intake for the 5-week period, and 24-hour fecal collections were performed on the mice three (3) times during the course of the study. Blood samples were taken once (1) per week for serum glucose, triacylglycerol cholesterol and nonesterified fatty acid determinations. Portal vein blood samples were used to determine the glucagon-like peptide-1 (GLP-1) levels. Cecum and proximal, medial and distal colon segments were collected for mRNA and GLP-1 analysis. Cecum samples that were full and empty, along with liver and epididymal fat tissue were weighed, and livers from euthanized mice were kept for histological analysis. In addition to the blood measurements for triacylglycerol cholesterol and nonesterified fatty acids, liver samples were also used to determine these components.

The authors reported that all three (3) fructan treatments were well tolerated by the rodents. Effects of the agave fructan supplemented diet were reported to be qualitatively similar to those obtained with diets supplemented with the two other fructans from different plant sources (chicory and *Dasylirion* spp.), and previously reported effects of fructan consumption, namely, a decrease in energy intake and body weight gain, and a decrease in glycemia. The researchers reported that mice fed fructan-containing diets for 5-weeks had significantly (≈30%) lower body weight gain and a 10% decrease in energy intake than the group of mice fed standard diets. The serum glucose level was reduced 15%, and serum cholesterol about 20%, similar in magnitude to the reductions in the other fructan-supplemented diet groups. Daubioul and others (2002) noted that mechanistically, the effects of non-digestible fructans are generally related to energy intake and related sequelae (e.g. body weight, adipose tissue mass, and lipid metabolism) and are indirect effects, being attributed to short-chain fatty acids, which are dependent on production from fermentation in the caeco-colon. The authors found that the total cecum weight and cecum wall weight were increased 100% and 77%, respectively, most significantly in the agave fructan-supplemented diet group. Although, the weight increases were similar in magnitude to the chicory and Dasylirion diet groups, which the authors attributed to increased bacterial fermentation and a corresponding increase in short-chain fatty acid production. Significant changes

compared to controls found in the agave, but not in the other two fructan-supplemented diet groups included, increased fecal excretion (17% dry basis), decreased epididymal fat weight as a surrogate for adipose tissue weight (27%), and decreased liver weights (13%). These changes were likely secondary to the reduction in body weight gain, which was reported to be more pronounced in the agave group than from the other two fructan sources. Authors reported no significant histological differences of hepatic tissue between the fructan treated groups or compared with controls, and no adverse effects were reported. GLP-1 and its precursor, proglucagon mRNA content in the different colon segments were found to be higher in all three (3) fructan supplemented groups than controls. In addition, GLP-1 concentrations in portal vein blood was also increased 1.5 to 2-fold in the treatment groups as compared with controls. This result suggests that fermentable fructans are able to promote the production of satietogenic/incretin peptides in the colon. Of noteworthy significance, is that even though the study compared the physiological effects of fructans from three different sources, the study did not reveal any adverse effects in mice consuming diets containing 10% fructans, a level that is significantly higher than is recommended for long term studies (Urias-Silvas et al., 2008).

6.1.3.2 Acute toxicity Studies

Studies show that agave inulin does not elicit acute toxicity in mice or rats. An OECD Guideline 425 compliant acute toxicity test, involving twenty-five (25) male Balb/c mice given a single gavage doses of branched agave fructans from *Agave tequilana* Weber var. *azul* at concentrations of 175, 550, 1750 and 5000 mg/kg b.w. was used to assess the acute toxicity of agave inulin (Márquez-Aguirre et al., 2013). In addition, a chicory inulin blend, possessing a mixture of fructan chains covering the chain distribution embodied within IMAG Organic®, was also evaluated. Effects on blood cells and cell components (red blood cells, hemoglobin, hematocrit, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils) blood analytes, including aspartate aminotransferase, alanine aminotransferase, glucose and creatine, and body weight gain were determined.

At the highest single gavage fructan dose of 5000 mg/kg (regardless of average DP or plant source), no mortality and no statistically significant changes were reported in any of the measured hematological or blood chemistry parameters when compared to untreated control mice. In addition, no change in body weight gain was observed. The researchers reported that the fructan treatments did not affect the general state of health, although no further details were provided regarding other health effects that had been assessed in the single-dose assay.

Also in 2013, Garcia and others performed an acute toxicity study using groups of 5 male and 5 female Hsd:ICR mice (4-5 weeks of age) and 5 male and 5 female Hsd:WI rats (8-9 weeks of age) given a single gavage dose of either a low DP (< 10) agave fructan (trade name Metlos®) or a long chain agave fructan (DP > 10), trade name Metlin® derived (molecule chain fractions embodied in IMAG Organic®) from *Agave tequilana* Weber var. *azul* at 17.5, 55, 175, 550, 1750, or 5000 mg/kg. Animals were observed for effects for a 14-day post-treatment period. Results show no mortality, adverse clinical observations, changes in body weight, or histopathological findings from examined stomach, large intestine, small intestine, and liver. The researchers reported that agave fructan products given orally to rodents at up to 5000 mg/kg b.w. are non-toxic.

In summary, no adverse effects were identified following acute oral gavage dosing of branched agave inulin up to 5000 mg/kg b.w. in rats and mice, nor was there any difference in toxicity compared with a chicory inulin having mixed short and long chain inulin fractions.

6.1.3.3 Genotoxicity Studies

Studies show that agave inulin is non-mutagenic based on *in vitro* study. A bacterial reverse mutation assay was conducted by Márquez-Aguirre and others (2013) on *Salmonella typhimurium* strains TA98, TA100, and TA102, using branched agave fructans from *Agave tequilana* Weber var. *azul* with similar molecular chain fraction of IMAG Organic® at a concentration of 800 µg/plate. The fructans did not significantly increase the frequency of mutations relative to negative controls, regardless of metabolic activation with Arochlor-1254 induced S9 mixture. Although the study did not conform to OECD Guideline 471 and FDA Redbook, it was compliant to methods described in Maron and Ames (1983). The protocol deviated from current standardized guidelines by: 1. not including at least five strains of bacteria, including *S. typhimurium* TA1535 and TA1537 or TA97a or TA97 in addition to *S. typhimurium* strains used in the study, TA98, TA100, and TA102, and 2, failing to use a recommended maximum concentration of 5 mg/plate for noncytoxic substances. However, even though the study did not conform to standardized guidelines, based on structure-activity considerations, agave inulin is not expected to interact with DNA, and the mutagenic potential is expected to be negligible.

Also, in 2013, Garcia and others performed *in vivo* chromosomal aberration and micronucleus assays with Hsd:ICR mice to evaluate the genotoxicity of branched agave inulin derived from *Agave tequilana* Weber. In these assays, both a low DP agave fructan (DP < 10; trade name Metlos®) and a long chain agave fructan (DP > 10; trade name Metlin®), products having inulin chain fractions embodied in IMAG Organic®, were used. Male mice (4-5 weeks old) were grouped with 5 mice per treatment group and given intraperitoneal injections of 143, 357.5, or 715 mg/kg of either the low DP agave fructan or the long chain agave fructan, while two other groups were either injected with Mitomycine-C, to represent a positive control or a phosphate buffer solution (PBS) to represent a negative control. After 24 hours post-treatment, peripheral blood from the tail vein was collected for analysis. Following this collection, mice were euthanized, and femur bone marrow was extracted from each. A chromosome aberration study was performed using 100 bone marrow cells in metaphase from each animal and scores for alterations in the chromosomes and chromatids were recorded, which the researchers reported is a protocol compliant to OECD Guideline 475 and EPA OPPTS 870.5385. A micronucleus assay was also performed using erythrocytes from the tail vein blood. Stained cells were evaluated for frequencies of micronucleated polychromatic erythrocytes using a fluorescence microscope. Based on methodology, the micronucleus assay is compliant to OECD Guideline 474. Based on comparisons with negative controls, the chromosome aberration study reveals that there was no significant increase in the number of cells with deletions, fragments, translocations, or gaps among the two agave fructan treated groups. Analysis of stained erythrocytes in the micronucleus assay showed that the mean frequency of micronucleated cells was not significantly increased by either the low DP or long chain DP agave fructan treatments at any dose, when compared with the negative control group. Agave fructans from *Agave tequilana* Weber were deemed non-genotoxic in mice.

6.1.4 Corroborative Tolerance Studies in Fructans Derived from Non-Agave Sources

6.1.4.1 Human Studies

The gastrointestinal (GI) tolerance of native chicory inulin and shorter chain length oligofructose, as well as fructooligosaccharides derived from enzymatic action on sucrose has been evaluated in human study. In general, there are no safety concerns with the ingestion of inulin-type fructans and they are well-tolerated, also by very young children and infants (Kim et al., 2007; Yap et al., 2008; Bonnema et al., 2010; Holscher et al., 2012). Marteau and others (2010) reported that inulin can initially cause some untoward side effects, most notable are flatulence, bloating, rumbling, cramps, and loose stools, caused by gas formation and osmotic effects of short chain fatty acids (SCFAs) and lactate formed in the cecum and colon. Untoward side effects may be minimized by distribution of the fructan intake over the course of a day and ingestion with solid foods (meals), as well as adaptation of a desirable intestinal microbiota population with the use of probiotic bacteria, such as the bifidobacteria and lactobacilli that produce low gas levels on inulin (Wang and Gibson, 1993; Marteau and Florié. 2001). However, inter-individual variability in response of using probiotic bacteria exists (Marteau and Florié, 2001), as these bacteria do not produce significant gas from fermentation and produce significant levels of short chain fatty acids that lower the colonic pH to levels that suppress gas-producing microbiota. The pH for the colon in healthy humans is acidic, approximately 5.7, while that in the large intestine is only slightly acidic, approximately 6.5-6.7 (Fallingborg, 1999; Nugent et al., 2001). In an imbalanced gut microbiome, that has low levels of lactic-acid bacteria (LAB), the pH is typically more alkaline, around 7.5 or higher, an optimal range for gas-producing opportunistic pathogenic microorganisms (Edwards, 1985; Gibson and Wang, 1994; Gibson and Roberfroid, 1995). Molecular chain length influences the rate of fermentation and potential side effects, with longer chain fructan molecules (~DP 23-25) reducing the overall potential for any untoward side effect, as these molecules are fermented about 50% lower than that of short chain inulin molecules (Roberfroid et al., 1998; Coussement, 1999). Roberfroid and others (1998) reported that the activity of microbial inulinases, which are the necessary enzymes for inulin fermentation and SCFA production and gas production, is influenced *in vitro* by the degree of polymerization. Van Hoeij and others (1997) and Botham and others (1998) showed *in vitro* that inulin produced much more favorable mean SCFA/gas volume ratios than shorter chain FOS or oligofructose or soy polysaccharides, resistant starch from peas or potatoes, oat β-glucans, or arabic gums. Favorable SCFA/gas ratio indicates that inulin only results in modest gas production while producing relatively high quantities of the SCFA, an important factor in patient tolerance for supplemented enteral nutrition formula (van Hoeij et al., 1997). The rate of fermentation also defines intestinal tolerance and SCFA-mediated systemic responses such as mineral absorption, carbohydrate and lipid effects, and osmotic laxation (Roberfroid et al., 1998). Other researchers have indicated that if not taken in excess over 80 g/d, inulin-type fructans are completely fermented in the colon, resulting in the production of SCFA (Clausen et al., 1998).

Bonnema and others (2010) confirmed that chicory inulin and oligofructose are well tolerated in moderate doses (5 to 10 g/d), as compared to a placebo control. Twenty-six (26) healthy men and women aged 18-60 years with no history of GI issues consumed diets with typical fiber amounts. The chicory and agave inulin fibers only produced mild GI symptoms, with most frequent symptoms report

as flatulence, followed by bloating. The 10 g dose of the shorter chain oligofructose substantially increased GI symptoms when compared to the control group. Doses up to 10g/day of native chicory inulin and up to 5 g/ay of oligofructose were well-tolerated in the healthy-young adults.

The safety and tolerance of oligofructose ingestion by infants is documented in a Japanese nationwide survey of 20,742 infants ingesting formula containing 0.32 g scFOS/100 ml (Japanese Infant Formula Survey 1993). This results in an estimated mean and 90th percentile consumption of 3.0 g and 4.2 g scFOS/day, respectively. The estimated daily intake of inulin from all of the proposed uses of prebiotic fructans for infants below 1 yr. of age, as calculated by ENVIRON (2002) as part of GRN 000118, was 2.3 g and 5.7 g, as the mean and 90th percentile, respectively. López-Velázquez and others (2015) reported another large infant tolerance study involving 600 healthy term infants (20 ± 7 days) consuming standard infant formula, human breast milk or enriched with either native agave inulin-type fructans, a short chain agave inulin-type fructan, or a dual inulin-type fructan system containing both native agave fructans and short chain agave fructans. In 66,120 days of total follow-up, there were no differences on the frequency of stool transit and stool consistency was similar between human breast milk and prebiotic supplemented formula infant groups. Also, the frequency of gastrointestinal symptoms (frequency of abdominal distension, flatulence, regurgitations, or vomiting) was significantly low between these groups. Inulin-type fructans derived from agave and added to infant formula are safe and well tolerated by Mexican healthy term infants.

However, there is less data on the tolerance of inulin-type fructans for children, but daily doses of inulin of about 1.5 g appear to be well tolerated in infants (Kim et al., 2007; Yap et al., 2008; Holscher et al., 2012) and 5 g/d in children aged 7-8 years (Lien et al., 2009). Moreover, Absalonne and others (1995) observed that oligofructose in dosages of 6-12 g/d apparently does not lead to too many untoward side effects in children aged 6-12 years, and digestive tolerance is influenced by the type of food (differing mainly between solid and liquid food) and the way of consumption (isolated consumption outside meal-times favors symptoms). No adverse effects were observed in children aged 6 m -10 years that consume 1 -5 g/d of native inulin (Szajewska et al., 2012). Recently, Liber and others (2013) also observed that even higher dosages of oligofructose, 8 g/d in children aged 7-11 years or 15 g/d in children aged 12-18 years, did not produce any untoward side effects.

The results of these studies have conclusion that the consumption of the naturally-occurring, inulins, such as Frutafit® chicory inulin (GRN 118, FDA 2002a and 2007 amendment) and Inufib™ (GRN 854, FDA 2020), and that IMAG Organic®, the notified substance having similar manufacturing processes, molecular structure and specifications, from foods is tolerated at levels higher than that of shorter chain higher fructooligosaccharide-containing inulin molecules found in Raftilose® oligofructose or Neosugar®. In addition, as mentioned previously, human tolerance to inulin has been demonstrated to be greater when inulin is consumed as part of the regular diet as opposed to consumption as a bolus dose. Even in the case of Neosugar®, a very short chain, fructooligosaccharide-containing inulin product, which caused adverse effects such as diarrhea when initially consumed of large amounts, greater tolerance was achieved with continued consumption (Oku, 1986).

A review of the earliest clinical studies from 1874 through 1955, as reviewed in GRN 118 (FDA 2002a and 2007 amendment), show utilizing inulin from various sources was well tolerated up to about 200 grams

per day. In addition, Gibson and others (1995) evaluated gastrointestinal tolerance of 15 g/day of Raftilose®, a shorter chain chicory fructooligosaccharide, in 8 healthy volunteers with a mean age of 33.8. Flatulence and mild abdominal pain were the only symptoms reported with the conclusion that 15 g/day was well tolerated by the subjects. Bruhwlyer and others (2009) compared the digestive tolerance of inulin-type fructans, administered for 2 weeks, at different doses. Eighty-four healthy volunteers (aged 18-45), having a mean body mass index 25.1 kg/m^2 and mean total fiber consumption of 12 g were included in a double-blind, placebo controlled, randomized, cross-over study comparing shorter chain inulin (Fibrulose®), and oligofructose, at 5g/d and 20 g/d), native chicory inulin (Fibruline® instant (5, 10 and 20 g/d)), and long chain chicory inulin (Fibruline® XL, 10 g/d), equal to degrees of polymerization of 2-20, 2-60 (avg. 10), and 12-60 (avg >20) to placebo. The three products tended to increase digestive symptoms of: flatulence; rumbling; bloating; abdominal pain; abdominal cramps; nausea; stool frequency and/or stool consistency, whatever the dose but the change was mild (maximum, +19 mm on the 800-mm scale) and significant ($P<0.001$) for inulin (2-60) at 20 g/d only. At 20 g/d, a statistically significant difference between inulin (2-60) and shorter chain inulin (2-20) was demonstrated ($P=0.011$). There was a dose—effect relationship for both shorter chain inulin ($P>0.05$) and inulin ($P=0.042$).

Ripoll and others (2009) assessed the effect of 2 doses of an inulin-rich roasted soluble chicory extract (IRSCE) on overall gastrointestinal discomfort after short-term ingestion and the effect on gastrointestinal symptoms of long-term consumption of IRSCE administrated at a dose compatible with its future commercial use. The researchers used a double-blind, crossover study involving 18 healthy subjects receiving in a randomized order a morning coffee drink including 10 g sucrose alone (control period) or with IRSCE at 2 doses (8.9 and 14 g containing 5.0 and 7.8 g of inulin, respectively) during three consecutive 6-d periods to assess the overall gastrointestinal discomfort of IRSCE. Thirty-five subjects were followed during a randomized, double-blind, protocol that provided an instant coffee drink twice per day containing IRSCE (8.1 g/d containing 5.0 g/d of inulin) or sucrose 8.1 g/d for 4 weeks. In the first study, a significant slight increase ($P = 0.05$) in overall abdominal discomfort was observed with the morning coffee drink containing 7.8 g of inulin after 1 week of consumption. In the second study, no significant differences between the IRSCE and placebo groups were evidenced with respect to gastrointestinal symptoms during the consumption period.

The maximum dose of Neosugar®, a very short chain fructooligosaccharide-containing fructan, as reported in GRNs 44 (FDA, 2000b) and GRN 118 (FDA 2002a and 2007 amendment), demonstrated not to cause diarrhea, termed the maximum tolerated dose, was demonstrated to be approximately 21 to 24 grams per day (Takahashi et al., 1986; Hata and Nakajima, 1985). Lower daily doses of 15 grams of Neosugar® per day did result in flatulence and other mild gastrointestinal effects (Stone-Dorshow and Levitt, 1987). However, as discussed previously, the results of the Neosugar® studies for determining human tolerance to inulin (i.e., Frutafit®) is limited by the molecular weight of this fraction (DP 3 to 5).

6.1.5 Toxicological Studies with Short-Chain Fructooligosaccharides

A limited number of *in vitro* and *in vivo* animal tests on the inulin-type fructans containing nondigestible β(2-1) and β(2-6) fructose linkages have been published to determine both acute and genotoxicity, **Table 6.1**. These studies were performed on non-digestible fructan products that contain short chain fructans

similar to those found in IMAG Organic®. Studies shown in Table 6.1 include a genotoxicity battery, teratology studies and rat subchronic and carcinogenicity assays. No specific safety issues have been raised in studies using scFOS (Clevenger et al., 1988; Sleet and Brightwell, 1990; Hasman et al., 1990; Tokunaga et al., 1986), chicory oligofructose (Boyle et al., 2008), or both short or native inulin-type fructans from *Agave tequilana* Weber var. *azul* (Huazano-García and López, 2013).

In a critical review by Carabin and Flamm in 1999 of animal experimental toxicity data and clinical studies of inulin and oligofructose (fructans), concluded that these fructans have not shown evidence of mortality, morbidity, target organ toxicity, reproductive or developmental toxicity, mutagenicity or carcinogenicity. They cited that the LD_{50} for FOS is more than 9 g/kg for acute dosing. In addition, no treatment-related chronic toxicity has been reported for oral doses of 4.5 g/kg for six weeks. In animal experiments, the authors reported that FOS showed no toxicity compared with existing sugars commonly used in the food supply and no observable negative effects on pregnant rats or the development of fetuses and newborns. Moreover, no untoward effects were seen in the animal studies when dose levels have ranged from 10 to 25 percent inulin in the diet for 3 to 4 weeks or 5 to 20 percent of FOS (DP$\leq$ 8) in the diet for up to 5 weeks. Results from subchronic and chronic toxicity and carcinogenicity studies in rats (Tokunaga et al., 1986; Clevenger et al. 1988; Haseman et al., 1990) demonstrate that there are no significant adverse effects up to 2,664 mg/kg/day. The No Observed Effect Level (NOEL) for chronic administration of Neosugar® FOS is 2,664 mg/kg/day. The only effect noted was the occurrence of soft stools or diarrhea after ingestion of large quantities of Neosugar® (more than 5 percent in the diet of rats). In more recent study, Jain and others (2019) showed that short-chain FOS (FOSSENCE™), a FOS product from Tata Chemicals Limited, Tamilnadu, India, at intakes of up to 9000 mg/kg b. wt. in acute and 14-day studies, did not cause any mortality or clinical signs or changes in body weights, feed consumption, or gross pathology. In 90-day study, no treatment-related clinical signs or mortalities were observed, even at the upper dose of 9000 mg/kg b. wt. Also, no treatment-related toxicological or biological significant changes were observed in body weights, feed consumption, ophthalmological findings, neurological effects, hematology, clinical chemistry, urinalysis, and gross pathological findings. However, there was a significant increase in cecum weight was noted, which is considered a trophic effect and not toxic, and is often attributed to positive health effects from the fermentation of non-digestible fructan consumption (Tungland, 2018).

The FDA also has stated that FOS, produced from fermentation of sucrose (Neosugar®), and inulin, produced from chicory roots (Frutafit®) and agave piña (Inufib™), are GRAS for their intended purposes (GRN 44, FDA 2000b; GRN 118, FDA, 2002a and 2007 amendment; GRN 854, FDA 2020), respectively.

Table 6.1. Summary of Toxicology Studies of Non-Agave Fructans

Study	Subject(s)	Route, Dose & Duration	Results & Effects
		In vitro studies	
Takeda and Niizato, 1982, as reported in Carabin and Flamm, 1999	Rat oral toxicity study. 6-week study.	scFOS used at rates up to 9 g/kg gavage and at 10% FOS in diet.	No treatment-related toxicity up to a dose of 4.5 g/kg (gavage) and 10% FOS in the diet relative to control diets containing existing sugars in use in the food supply. Oral LD_{50} for FOS was greater than 9 g/kg.
Clevenger et al., 1988 (reported findings from Meiji Seika Kaisha).	Ames assay using *S. typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, & TA	Neosugar® scFOS used at a rate of 50 to 5,000 µg/plate	No increase in frequency of mutation per plate in any bacterial strain with or without metabolic activation vs. control. FOS did not possess mutagenic activity.

Table 6.1. Summary of Toxicology Studies of Non-Agave Fructans			
Study	**Subject(s)**	**Route, Dose & Duration**	**Results & Effects**
	100, & an E. *coli* WP2 uvrA assay		
Clevenger et al., 1988. (reported findings from Meiji Seika Kaisha).	Mouse lymphoma L5178Y cells	Cells exposed to 2,000 to 5,000 µg/ml of Neosugar® scFOS	No increase observed in the mutation frequency either with or without metabolic activation.
Clevenger et al., 1988.(reported findings from Meiji Seika Kaisha).	HeLa S3 epitheloid cells assayed for DNA damage by Unscheduled DNA synthesis (UDS)	Neosugar® scFOS exposed to HeLA cells at 25 to 51,200 µg/ml. % of cells in DNA repair quantified.	UDS was significantly increased at one Neosugar® concentration, 1600 µg/ml, without metabolic activation. No dose response observed, and no significant increase was observed at any concentration either with or without metabolic activation in a repeat test. In all three *in vitro* assays, Neosugar® did not possess genotoxic potential under the conditions of the tests.
		In vivo studies in animals	
Meiji Seika Kaisha 1982. (follow up to data reported by Clevenger et al. 1988).	Exp 1 and 2.: Rats fed FOS diets to elucidate previous FOS work.	Exp 1: Rats fed scFOS up to 15% in diet to assess if FOS increases cecum and colon weight & is fermented by colonic bacteria, as colon bacteria known to play role in colon carcinogenesis (micro adenoma test) Exp 2: Rats fed scFOS 7.5 or 15% (7.2 and 16.7 g/kg/d) for 13 wks to determine subchronic effects on liver granulation and parameters.	Exp 1: Micro adenoma assay indicated that at levels up to 15% in rat diets, scFOS did not significantly modify the number of aberrant crypts and did not act as a promoter of chemically-induced carcinogenesis. Exp 2: Intake did not modify the total content of retinol, retinol palmitate and tocophenol or glutathione in rat liver, indicating no effect on these hepatic free-radical scavengers. The hepatic cytochrome P450 and cytochrome P450-dependent testosterone metabolism was not modified by scFOS.
Clevenger et al., 1988. reported findings from Meiji Seika Kaisha).	F-344 rats (50/sex/group) known to have high incidence of neoplastic lesions.	scFOS tested for genotoxicity and carcinogenicity using 0, 8000 (341 to 419 mg/kg/d), 20,000 (854 to 1045 mg/kg/d), or 50,000 ppm (2170 to 2664 mg/kg/d) for 104 weeks.	No genotoxic potential observed and no difference observed in the onset of cancer in F-344 rats between control or FOS, with exception of pituitary adenomas in male rats, although this tumor was not considered related to scFOS, as incidence of all groups was within historical control range (mean of 31%, range 17-49%), and only equivocal evidence of a dose-response trend using the Cochran-Armitage trend test was observed.
Henquin, 1988.	Wistar female rats.	Diets containing 20% scFOS during gestation	No developmental toxicity
Sleet and Brightwell, 1990.	Crl CD (SD) BR rats	Diets containing 0, 5, 10, or 20% scFOS during gestation.	No adverse postnatal developmental effects observed or negatively affect the pregnancy outcome or in utero. Moderate reduction in maternal body weight observed in the 20% FOS group.
Haseman et al., 1990	F-344 rats, rats known to have high incidence of neoplastic lesions	scFOS tested for genotoxicity using 0, 8000 (341 to 419 mg/kg/d), 20,000 (854 to 1045 mg/kg/d), or 50,000 ppm (2170 to 2664 mg/kg/d).	Incidence of pituitary adenomas was 20, 26, 38, and 44%, respectively (incidence was significant for intakes at 20K and 50K ppm).
Carabin and Flamm 1999.	Pregnant rats and fetuses	Rats fed diets containing 4.5 g/kg to 9.0 g/kg chicory inulin or 3-4 wks or oligofructose for up to 6 wks.	Concluded that the LD_{50} for oligofructose and inulin are more than 9 g/kg for acute dosing. No treatment-related chronic toxicity was reported for oral doses of 4.5 g/kg for 6 wks. Inulin and oligofructose showed no toxicity compared with existing sugars used in the food supply. No observable negative effects on pregnant rats or development of fetuses or in newborns.
Boyle et al., 2008.	Rats	Rats fed chicory oligofructose in 4 doses ranging from 0.55% to 9.91% of diet for 13 wks. Safety evaluated using *in vitro* mutagenicity tests.	Cecal weights and bifidobacteria increased in dose-related manner. No consistent differences in gross pathology or histopathology related to oligofructose intake and did not induce a positive response in the Ames test or chromosomal aberration test with CHO cells. The No Adverse Effect Observed Level (NOAEL) of oligofructose was 9.91% of diet.
Roldan-Marin et al., 2009	F344 rats	Rats fed diets containing 7% fructan extract from onions or control for 4-weeks.	Significant decrease ($P<0.05$) in the hemoglobin concentration; significant increase ($P< 0.05$) in glutathione reductase and glutathione peroxidase activities in erythrocytes of rats fed the fructan diet, and rats fed the fructan diet; significantly lower ($P<0.01$) hepatic glutathione peroxidase activity, although glutathione reductase activity was unchanged; no DNA damage as measured in liver and white blood cells. No significant difference was reported in gastrointestinal transit time for the

Table 6.1. Summary of Toxicology Studies of Non-Agave Fructans			
Study	**Subject(s)**	**Route, Dose & Duration**	**Results & Effects**
			treatment group when compared with the control group. The treatment group also did not show any alteration of hepatic gene expression of Gr, Gpx1, catalase, 5-aminolevaulinate synthase and AD(P)H:quinone oxidoreductase. The fructan treatment significantly increased fermentation as compared with the group rats by decreased fecal pH, increased short chain fatty acid production (butyrate and propionate), and an increase in the cecal microbiota enzyme activities of β-glucosidase and β-glucuronidase
Rendón-Huerta et al., 2012	Diabetic and obese Wistar rats	Agave, J. artichoke and chicory fructans fed in feeds at 15%, corresponding to 7-9 g/kg b.w/d for 6-weeks.	Modest reductions in body wt. for all fructans. Significant increase ($P<0.05$) in lactobacilli and bifidobacteria for all fructans. All fructans well tolerated with no adverse effects.
Hijová et al., 2013	Sprague-Dawley rats	Rats fed oligofructose enriched inulin (Synergy 1) at 8% in diet for 28-weeks.	Fructan treatment was well tolerated and no adverse effects were attributed to the treatment diet.
Jain et al., 2019	Wistar rats	Rats fed FOSSENCE™ (a scFOS from India) at 0, 2000, 5000, and 9000 mg/kg b.wt. in acute, 14-day and subchronic (90-day) toxicity studies.	Intake in acute and 14-day studies did not cause any mortality or clinical signs or changes in body weights, feed consumption, or gross pathology at any of the doses. In 90-day study, no treatment-related clinical signs or mortalities were observed. Also, no treatment-related toxicological or biological significant changes were observed in body weights, feed consumption, ophthalmological findings, neurological effects, hematology, clinical chemistry, urinalysis, and gross pathological findings. However, there was a significant increase in cecum wt. was noted, which is often attributed to positive health effects from non-digestible fructan consumption.

6.1.6 Allergenicity

A review of published literature shows no cases of allergenicity or hypersensitivity in association with consumption of agave inulin. Natural sources of agave juice concentrates, such as agave inulin and syrup, have been safely consumed for more than two decades. There is also no evidence in the literature that the inulin content is implicated as allergenic in any foods of the 8 major food allergens (milk, eggs, fish, crustacean shellfish, tree nuts peanuts, wheat and soya) identified in the U.S, which were identified in section 202 (findings, 21 USC 343 note) of the 2004 Food Allergen Labeling and Consumer Protection Act (FALCPA, 21 USC 301 note) that found that the eight (8) major foods or food groups accounted for 90% of the food allergies in the U.S (FDA, FALCPA, 2004). To this end, third party laboratory analyses of randomized sampling of the notified substance, lot number 180908 at CIATEJ, Jalisco, Mexico in 2008 showed no detectable levels of the 8 major known food allergens (milk, egg, peanut, soy, tree nut, fish/shellfish and wheat), **Table 6.2**.

As further described on page 30 of GRN 854 (Inufib™ Agave Inulin), emphasis is placed on the plant part where IMAG Organic® and Inufib™ are derived, the piñas, as sap or extracts from the leaves from some agave species have been associated with saponins and raphides, in contrast to juice extracted from the piñas. Contrary to liquid from the piñas, liquid sap from the leaves of some agave species, including *Agave tequilana*, can produce skin irritation and contact dermatitis when it contacts human skin, which is associated with the presence of sharp, needle-like calcium oxalate crystals, called raphides (Salinas et al., 2001). Tequila distillery workers and workers on agave plantations have experienced irritant

contact dermatitis due to these raphides. After isolating and purifying the raphides from leaves of *A. tequilana,* Salinas and others (2001) determined that the calcium oxalate crystals were sharpened on both ends and had a length of 30-150 μm. The authors found that a single drop of leaf juice of *A. tequilana* contained 100-150 of the raphides.

Table 6.2. Allergen Determination of IMAG Organic® Agave inulin.

Allergen determination	Units	Result (detection limit)
Milk	ppm	Negative (< 1 ppm)
Egg	ppm	Negative (< 2.5 ppm)
Peanut	ppm	Negative (< 5 ppm)
Soy	ppm	Negative (< 3.5 ppm)
Almond (Tree nut)	ppm	Negative (< 5 ppm)
Fish/Shellfish	ppm	Negative (< 5 ppm)
Wheat	ppm	Negative (< 10 ppm)
CIATEJ Report 2008		

With exception to the reported agave-induced irritant dermatitis associated with agave plantation and tequila distillery workers, Ricks and others (1999) reported that such dermal irritations are relatively rare. The researchers reported a case of Agave-induced purpura on the anterior legs in an otherwise healthy patient that resulted from cutting down an ornamental *Agave americana* plant during a landscaping project. A punch biopsy of the purpura and histopathologic examination was consistent with hypersensitivity vasculitis. Published literature cites twelve (12) cases of irritant contact dermatitis provoked by the ornamental plant, *A. americana*, also known as the "Century Plant" (Hackman et al., 2006).

Like the manufacturing process used to produce the similar Inufib™ agave inulin, the subject of GRN 854, IMAG Organic® agave inulin is water extracted from the pines ("piñas") of the agave plant, defined as stems with their leaves removed. Once harvested, the leaves and plant roots are removed and left in fields for soil enrichment. As the majority of saponins and raphides of calcium oxalate are located in the leaves and not in the pines, the resulting inulin product does not contain these non-fructan bioactive compounds. Further, due to the rigorous production methods, its good manufacturing practices, and the quality standards used in the manufacture of IMAG Organic®, hypersensitivity is not a safety concern for agave inulin.

In addition to these effects, only a few of allergic episodes of anaphylactic reactions to non-agave inulin are reported in literature, indicating that allergy to inulin is highly rate given its widespread presence and use in human food (Bacchetta et al., 2008; Franck et al., 2005; Gay-Croisier, 2000; Streeks et al., 2017). Bacchetta and others (2008) described hypersensitivity to inulin as a rare and mostly benign event, but cited anaphylaxis due to renal hypersensitivity to inulin and IgA nephropathy, and following administration of inulin (long chain) for determining the glomerular filtration rate (Chandra and Barron, 2002; Tsinalis and Thiel, 2009). An anaphylactic response was also reported by Gay-Croisier (2000) in a 39 year old butcher who had four (4) episodes of anaphylaxis a few minutes after ingesting salsify, globe

artichoke leaves, a margarine (Brunch, Migros, Geneva) containing a long inulin chain chicory inulin (Beneo Orafti [Raftiline®] HP), and a candy (Actilife Toffee orange-carrot, Migros) containing chicory inulin (either long chain Beneo Orafti HP or Beneo Orafti oligofructose P95). He also had local wheal-and-flare reactions after touching globe artichokes. All four episodes occurred within a two-year period. Although, subsequent skin-prick testing in the patient produced very strong reaction to long chicory inulin (Beneo Orafti HP; avg. DP 23) and strong reactions to salisfy, globe artichoke, a margarine containing chicory inulin (Brunch), candy (Actilife Toffee), and short chain oligofructose (Beneo Orafti P95), results from skin-prick tests with the long chain chicory inulin in 10 control subjects and intradermal tests with Inutest 25% and the chicory oligofructose in 3 controls were negative. In addition, an open oral challenge to 40 g of oligofructose was negative in the patient.

In a case of allergic reaction to chicory root inulin, Franck and others (2005) reported that a 50-year-old woman with a past history of allergy to globe artichoke had two episodes of immediate allergic reactions, one of which was a severe anaphylactic shock after eating two types of health foods containing chicory-derived inulin for its bifidogenic properties: 0.38 g in one biscuit and 2.5 g in a yogurt product. Both food products containing added long chain chicory inulin Beneo Orafti HP; avg. DP 23, a chicory root inulin from *Cichorium intybus*, plant family *Compositae* (also called *Asteraceae*) Dot blot and dot blot inhibition assay techniques identified specific IgEs to globe artichoke, to yogurt F, and to a heated BSA + inulin product, suggesting possible inulin binding to food proteins during heating. Due to the potential for cross-reactivity, the authors concluded that consumers of health foods containing chicory inulin with any history of allergy to other members of the Asteraceae *(Compositae)* plant family, *viz*., globe artichoke (genus *Cynara*) or endive (genus *Cichorium*), should be warned of possible allergic reaction.

Lastly, Streeks and others (2017) recently reported a case of a young child with anaphylaxis to inulin on multiple exposures. The inulin allergy was confirmed by percutaneous skin testing using a powdered form of chicory inulin and to globe artichoke, which also contains inulin, with appropriate positive and negative controls.

In conclusion, inulin allergy is exceedingly rare, the only reported cases are associated with inulin derived from chicory root (*Cichorium intybus* L.) from the plant family Asteraceae, and globe artichoke, whereas no instances of allergy from agave inulin have been reported even though inulin from multiple plant sources has been utilized in a wide range of foods worldwide as a labeled dietary fiber for more than two decades. It is therefore reasonable to expect that IMAG Organic will not induce allergic reactions in consumers.

6.1.7 Regulatory Status of the Notified Substance and Similar Substances in Other Jurisdictions

Inufib™ agave inulin, GRN 854, received a Letter of No-Objection for GRAS (FDA, 2020). Chicory-derived inulin and oligofructose (e.g. Frutafit® inulin and Frutalose® oligofructose), Jerusalem artichoke inulin, and scFOS produced via enzymatic synthesis of sucrose are marketed in the U.S. and have GRAS status (GRN 44:FDA 2002b, GRN 118:2003, GRN 392: 2012, GRN 477: 2014, GRN 537: 2015, GRN 576: 2015, GRN 605 and GRN 623: 2016, GRN 717: 2018, GRN 849: 2019,).

Inulin is approved for use as an acceptable food or food ingredient in most countries worldwide, including all EU countries, Australia, Canada, and Japan (Franck, 2002). In the EU inulin is an allowable food ingredient under the European Directive 95/002 on Food Additives (EC, 1995), and all EU countries list inulin as having food ingredient status. The safe use of scFOS derived from enzymatic action on sucrose, an alternative to FOS derived from inulin, both being short-chain fractions/subsets of inulin, was also evaluated by the Foods Standards Australia New Zealand (FSANZ) in 2008 (FSANZ, 2008), concluding that it is as safe as inulin-derived substances (IDS) that are already permitted as additives to foods and infant formula, infant foods and formulated supplementary foods for young children either alone or in combination with IDS and GOS (galacto-oligosaccharides) up to the currently permitted maximum levels. Food manufactures have been adding inulin-derived substances to the general food supply in Australia and New Zealand since the mid-1990s. In Japan, scFOS (as Neosugar®), also defined as inulin, has a long history of safe use as a general food use low-calorie sweetener since 1983, and is listed in the Japan Ministry of Health, Labor and Welfare (MHLW) as FOSHU (Foods For Specified Health Issues). FOS is listed in the Approved FOSHU products list as oligosaccharides and classified as "foods to modify gastrointestinal conditions". Foods included in this list are reviewed for their effectiveness in attaining given health functions by the Council on Pharmaceutical Affairs and Food Sanitation (Japan MHLW, 2020).

As a food or food ingredient, inulin can be used without limitation in food and beverages. The Association of Official Analytical Chemists (AOAC) mentions two methods of analysis for fructans (AOAC 997.08) and (AOAC 999.03) to accurately measure the content of inulin and oligofructose in food and food products.

Inulin from *Agave tequilana* Weber var. *azul,* blue agave head, chicory root, and Jerusalem artichoke tuber are among food ingredients considered "natural" based on technical specification as defined under International Organization for Standardization (ISO/TS) 19657:2017, "food ingredients obtained from plant-based source materials by physical and/or enzymatic and/or microbiological processing without alteration of the ingredient from its original source".

The technological purpose for adding inulin to food is to emulsify or thicken food, or for its nutritional properties, such as for their prebiotic effects (Tungland, 2018) or as a dietary fiber. To this end, inulin from chicory root, Jerusalem artichoke and agave are defined as dietary fibers based on recognized physiological effects and have been assessed and approved by the Food Directorate, Health Canada (Health Canada, 2013) and the U.S. Office of Food Labeling FDA (2018b). Since 2001, inulin has appeared in a wide range of foods and is predominantly labeled as dietary fiber (FSANZ, 2008).

6.1.8 Safety Data Summary

Inulin (non-digestible fructans), including those from agave, have been consumed for over 10,000 years from various sustenance foods. As extracted and refined fructans, these food ingredients have been utilized as reduced energy (dietary fibers) and prebiotics in various foods and have been the subject of evaluations by many legal authorities worldwide for decades, including the United States Food and Drug Administration (FDA), resulting in separate "No Questions Letters" for their use in many conventional foods, infant formulas and medical foods, as described in the Conditions of Use in each GRAS Notice

(GRN 44, 2000b; GRN 118, 2003; GRN 392, 2012; GRN 477, 2014b; GRN 537 and GRN 576, 2015a,b; GRN 605, 2016b; GRN 623, 2016c; GRN 717, 2018b; GRN 849, 2019; GRN 854, 2020). All of these admissions of safe use in various foods have been for inulin and inulin-type fructan molecules that have the majority of their molecule comprising linear β(2-1)-linked fructofuranosyl units with varying degrees of β(2-6)-linked molecular branching. These GRAS approved inulin-type fructans, span the range of the degree of polymerization of IMAG Organic® agave inulin. IMAG Organic®, the notified substance of this GRAS determination, has equal molecular bonding as those substances receiving no questions from FDA, and similar molecular structure and degree of β(2-6) molecular branching as the agave inulin substance described in GRN 854 (FDA, 2020).

Studies conducted and published that support the evaluation of the safety of agave inulin, regardless of the degree of polymerization (DP) or its plant derivation, include those from *in vitro* and *in vivo* animal studies, as well as clinical studies in humans. These safety studies show no acute oral toxicity in mice and rats (at maximum dosage of >5 g/kg b.w.) and an absence of mutagenicity and clastogenicity with test substances having DP spanning the DP range of IMAG Organic®. Fructan-enriched diets used in rodent studies resulted in only modest reduction in body weight gain and mild diarrhea, when dosage was at high levels of 5 to 10% of the total enriched diet.

In humans, no adverse effects have been reported, other than mild, transient untoward gastrointestinal (GI) effects such as flatulence and abdominal discomfort at high bolus doses. It is noted here that these untoward GI effects are also documented using other plant-based fructans at similar dose levels (e.g. chicory inulin) and are the same effects as those symptoms associated with the consumption of fruits and vegetables that contain non-digestible/fermentable carbohydrates, including dietary fructans.

In summary, healthy individuals in general population, including healthy infants and children, and adult men and women have shown minimal to no gastrointestinal symptoms from the daily consumption of up to 7.5 g of agave inulin over study periods of three (3) or six (6) months. In addition, available public domain data on non-agave fructans have demonstrated no evidence of toxicity based on both animal and clinical studies. Any signs of untoward effects from inulin and inulin-type fructan consumption are primarily flatus in nature or abdominal discomfort, which would result in self-regulation. These untoward effects of gastrointestinal intolerance are observed with intakes above 20-30 gram, particularly stemming from bolus intake, but nondigestible fructans are better tolerated when consumed with solid food and when given in divided doses through the day.

6.2 § 170.250(a)(1): Basis for Conclusion of GRAS Status for the Notified Substance

IMAG has determined that IMAG Organic®, their agave inulin product, is Generally Recognized as Safe (GRAS) for all its intended food purposes through scientific procedures in accordance with the Federal Food, Drug, and Cosmetic Act (21 U.S.C.§301 et. seq.) ("The Act"), as described in 21 CFR 170.30(b), thus satisfying the technical element of the GRAS determination. This GRAS claim, like that described on page 34 of the recent GRN 854 for a similar agave inulin, Inufib™, is based on a combination of: 1) a general recognition of its safety through scientific procedures based on generally available and accepted scientific data, and 2) experience based on extensive common use of the substance in food prior to January 1, 1958. The claim represented in item 1 is based on studies conducted and published in

support of a safety evaluation of agave inulin that includes *in vitro*, *in vivo* animal studies and clinical studies in humans, as corroborated by an Expert GRAS panel review described in Section 9 of this notification. To this end, the data used to define the GRAS Status of IMAG Organic® includes the same elements as that used for GRN 854 (FDA, 2020):

- The estimated daily intake (EDI) of the notified substance in the general population is not expected to increase as a result of the intended use. The EDI will be equal to or less than the EDI of inulin products identified for identical general food use (GRN 118, chicory inulin and GRN 854, agave inulin), and the notified substance will serve as an alternative source, rather than an additional source of agave inulin for use in food (see additional discussion below).

- The studies conducted and published in public domain, in support of the evaluation of the safety of agave inulin, including *in vitro* and *in vivo* animal studies, as well as human clinical studies, report no safety concerns at doses up to 1000 mg/kg-day in rats and at typical consumption levels in humans, respectively.

- Agave inulin and related inulin-type fructans have a long history of human consumption and safe use based on the narrative presented in section 5 of this notification.

This GRAS determination is for an identical general food use of agave inulin to the current agave inulin product as described in GRN 854 (FDA, 2020) and for the chicory inulin described (excluding meat and poultry) in GRN 118 (FDA, 2002a). Therefore, the basis used to define the EDI for the notified substance will be the same as that described by IIDEA on page 34 of GRN 854, as their Inufib™ agave inulin product has virtually equal the level of inulin (≈90%) as the level of inulin in IMAG's IMAG Organic® agave inulin, and that from Imperial Sensus, LLC's Frutafit® chicory inulin, the inulin product IIDEA used for their EDI assessment. In their EDI assessment, IIDEA rationalized on page 34 of GRN 854 that given equal inulin levels as Frutafit® in GRN 118, the same levels added to food will result in the same levels of inulin per serving. Thus, the EDI of inulin from the proposed uses of IIDEA's products will be equal to or less than that of the chicory inulin in GRN 118, since the use of meat and poultry was not part of the GRN 854 GRAS Notification. IMAG also uses this same logic when determining the EDI for its agave inulin product, IMAG Organic®. IMAG Organic® from IMAG is also an agave inulin that has virtually equal inulin content (≈90%) as both the chicory inulin in GRN 118 and the agave inulin in GRN 854, has similar molecular structure and degree of molecular branching as the agave inulin in GRN 854, and the same intended food list as GRN 854. Therefore, the level of inulin from IMAG Organic® agave inulin to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods, as required by FDA regulation, and will be the same or less than that of the current GRAS agave-derived inulin product that was subject of GRN 854 or the chicory-derived inulin product that was subject of GRN 118.

For reference, Imperial Sensus, LLC, in GRN 118, estimated the combined average intake of inulin by the general U.S. population (non-breastfeeding infants up to two years of age through consumers two years of age and older) from all uses of Frutafit® chicory inulin, those foods in general use (including meat and poultry) would be 2.3 to 10.1 g inulin/person/day, and the 90th percentile intake was estimated to be 5.7 to 19.2 g inulin/person/day.

The proposed use of IMAG Organic® will not result in an increase in the overall consumption of inulin to the populations but will rather provide an alternative source of inulin for use in food.

The studies described in Section 6.1 (Safety Narrative and Relevant Data) of this GRAS determination, and as described beginning on page 19 of GRN 854, show low acute oral toxicity in both rats and mice, and absence of genotoxicity, and no adverse effects when evaluated in human clinical studies and rodent feeding studies. Data also show no adverse effects following OECD guideline compliant acute oral dosing tests using *Agave tequilana*-derived inulin up to 5000 mg/kg in rats and mice (Marquez-Aguirre et al., 2013; Gracia et al., 2013), nor was there any difference in acute oral toxicity compared with chicory inulin(GRN 118, FDA 2002a and 2007 amendment). There was also an absence of mutagenicity *in vitro* (Marquez-Aguirre et al., 2013 and clastogenicity *in vivo* (Gracia et al., 2013). In human clinical studies, no adverse were noted for the consumption of agave inulin up to 7.5 g/d in infants (López-Velázquez et al., 2013) and adults (Holscher et al., 2014), other than mild untoward effects, such as flatulence and abdominal discomfort, which have been documented with other plant fructans (Bonnema et al., 2010; GRN 118, FDA 2002a and 2007 amendment) and are the same as the untoward effects experienced from consumption of fruits and vegetables.

There is scientific basis for any concern for purified inulin from any source regardless of differences in chemical structure, in other words, the length of its polymeric chain and the degree of molecular β(2,6) fructosyl branching. Literature in the public domain to the present has not revealed any studies that report safety concerns. The U.S. FDA and Health Canada have evaluated possible effects of plant-derived inulin concentrates on calcium and magnesium absorption and allergenicity and their reports demonstrate the absence of significant safety concerns when inulin is consumed as part of the normal diet.

In conclusion, a majority of commonly consumed foods throughout human history contain non-digestible fructans, including agave, that are of the structure typical of those in IMAG Organic®. Agave inulin is extractable by mechanical means and has been demonstrated to be safe for oral intake through standard animal toxicity studies, genetic toxicity studies and clinical studies, and through their long history of safe human consumption, dating more than 10,000 years. The effects inulin, including agave inulin, on human physiology and metabolism, such as its prebiotic and related gastrointestinal effects, and effects are well described and understood. Signs of untoward gastrointestinal effects, such as flatulence, borborygmus, or bloating, have been seen with inulin and oligofructose intakes above 20-30 g, particularly in a bolus dose, but these effects are not considered harmful to human health.

6.3 § 170.250(a)(2): Statement Regarding the Status of All Data and Information Used to Establish Safety

As mentioned herein in this GRAS determination, all data and information are available in the public domain. None of the information used in this assessment is confidential or unavailable to the general public.

6.4 § 170.250(b): Basis for GRAS Conclusion of Notified Substance Among Qualified Experts to be Safe Under the Conditions of its Intended Use

In accordance with section 201(s) (21 U.S.C. § 321 (s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et. Seq.) ("the Act"), the determination if a substance may be considered GRAS is set forth in 21 CFR 170.30. This regulation states that:

> *The general recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either through scientific procedures or in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food.*
>
> *The general recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food. The general recognition of safety based upon scientific procedures shall require obtaining approval of a food additive regulation for the ingredient. The general recognition of safety through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data and information.*

IMAG has applied these criteria to determine if the use of its agave-derived inulin, IMAG Organic®, for use in food for human consumption is GRAS based upon scientific procedures. All data used in this GRAS determination is based on data generally available in the public domain and generally known as pertaining to the safety agave-derived inulin, a substance already receiving no objections from FDA for its GRAS status in GRN 854 (FDA, 2020). As a consequence, the criteria for meeting the "general recognition" standard under the FD&C Act, is satisfied. The safety of related chicory derived inulin and oligofructose, Jerusalem artichoke inulin and short-chain fructooligosaccharides (scFOS) also have received ten (10) additional FDA GRAS recognitions in GRNs 44, 118, 392, 477, 537, 579, 605, 623, 717, and 849.

Further, these previously mentioned criteria have been expressed in an opinion of an Expert Panel shown in Section 9 in the evaluation of the safety of inulin, oligofructose and fructooligosaccharides. The general recognition standard of the "Act" has been satisfied. All relevant data and information used to establish safety is established and documented herein and is publicly available from published peer-reviewed literature, international regulatory agencies, such as FDA GRNs, history of its use, and a consensus among qualified scientific experts. The determination of the safety of agave inulin has already been established by an Expert Panel of scientists as described beginning on page 66 of GRN 854 (Opinion of an Expert Panel on the Generally Recognized as Safe Status of Agave Inulin for Use in Human Food), who reviewed a dossier prepared by IIDEA's agent, NSF International, as well as other information available to them. This panel of experts (the Expert Panel) was qualified by scientific training and experience to evaluate the safety of food ingredients. Based on their review of all the available data and information found that the intended use of agave inulin in food, produced in a manner consistent with cGMP and meeting the specifications described in GRN 854, was GRAS based on scientific procedures. In 2020, IIDEA agreed with the Expert Panel conclusion, that agave inulin is GRAS under all the intended conditions of use on the basis of scientific procedures: and, therefore, it was

excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without any announcement as a food additive under Title 21 of the CFR.

Based on this earlier Expert Panel review described in GRN 854, and FDA acceptance of the GRAS position stated therein, an Expert Panel consisting of Drs. Dietrich Conze, Fred Lozy and Claire Kruger was convened by Spherix Consulting Group, Inc. on November 12, 2020 to independently and critically evaluate all data and information presented herein in this GRAS Notice. This independent panel of experts has also concluded that IMAG Organic® agave inulin as manufactured by IMAG is GRAS for use in intended foods, as described in Section 1.4 based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of IMAG Organic® agave inulin is presented in Section 9.

6.5 § 170.250(c): Statement Regarding Data Inconsistent with the Conclusion of GRAS Status

IMAG is aware of only a few studies pertaining to the GRAS position that may be considered as inconsistent with a finding that the proposed uses of IMAG Organic® agave inulin in food for human consumption is GRAS. However, these studies were also described on pages 36 and 37 of GRN 854, an agave inulin that has received a Letter of Non-Objection of its GRAS status by the FDA (FDA, 2020).

These studies, among others, include those involving C57BL/6J $Apc^{Min/+}$ mice that we exposed to inulin enriched diets. In these studies, performed using diets enriched with 10% polydispersed inulin for three (3), six (6), or nine (9) weeks resulted in a 20% higher adenoma incidence and a 44% increase in adenoma size in the small intestine (Pajari et al., 2003). Misikangas and others (2005) also found increased cellular expression of nuclear β-catenin, and in later study increased cyclin D1 during the formation of these adenomas, when compared to similar mice on a control diet (Misikangas et al., 2008). The researchers generally concurred that the consumption of dietary inulin can activate the normal-appearing mucosa β-catenin signaling, which can induce adenoma growth in APC deficient mice that have enhanced susceptibility to tumorigenicity.

However, as described on page 37 of GRN 854, Pool-Zobel (2005) reported that adenocarcinoma are seldom observed in Apc Min deficit mice, and the progression from pre-neoplastic lesions to carcinoma has never been established in this rodent model of enhanced tumorigenicity. Moreover, Shoemaker and others (1997) reported that the K-ras mutations observed in many human tumors are not detected in Min mice polyps, and Fazeli and others (1997), further noted that the inactivation of p53 gene, a commonly reported gene in human cancers, did not increase the tumor expression in Min mice. Using these types of mutant models also carries the disadvantage of only having tumors that are expressed in the small intestine, not the colon (Pool-Zobel, 2005). Since the conditions of fermentation by intestinal microbiota are significantly different between the two GI areas, and as the small intestine is not a target tissue of human carcinoma (Pool-Zobel, 2005), it is questionable if inulin-induced adenomas in the small intestine of $Apc^{Min/+}$ mice has any relevance.

Recent study by Moen and others (2016) has indicated that more relevant data can be achieved by using a better model Min mouse model ($A/J^{Min/+}$) for colon cancer, as these mice spontaneously develop a considerable number of colonic adenomas that transition into carcinomas in older individuals, and are

also more susceptible to colon carcinogenesis induction with azoxymethane (AOM) than using C57BL/6J Min/+ mice. Their research using AOM-treated A/J$^{Min/+}$ mice that were fed a diet enriched with 15% of a commercial long-chain inulin (DP ≥ 23) showed that treated mice had significantly lower colon tumor incidence as compared with concurrent groups of A/J Min/+ mice fed either cellulose or brewer's grain. The authors concluded that dietary inulin does not result in increased tumor incidence relative to similar fibers in mice predisposed to the more human-relevant genetic model for colon cancer (A/J$^{Min/+}$), in contrast to C57BL/6J Apc$^{Min/+}$mice that are predisposed to non-human relevant small intestinal adenoma.

IMAG concludes, as also described on page 37 of GRN 854, that given the aforementioned peer-reviewed literature above, that indicate increased incidence of adenoma in inulin-fed C57BL/6J Apc$^{Min/+}$ mice, do not contradict the GRAS-status of inulin-type fructans. Recent reviews of the scientific literature on the subject have revealed no other potential adverse health concerns.

6.6 § 170.250(d): Statement Regarding Exemption of Data from Disclosure under the FOIA

No data provided herein in this GRAS Notice are exempt from disclosure under the FOIA.

6.7 § 170.250(e): Statement Regarding Non-Public Safety Data and Information

This section does not apply, as all data and information herein in this GRAS Notice were made available to the Expert Panel for their review.

7.0 LIST OF SUPPORTING DATA AND REFERENCES

7.1 § 170.250: List of Safety Studies Cited in Part 6

Studies supporting the safety of IMAG Organic® agave inulin, as described in section 6, are summarized in **Tables 7.1** and **7.2** below. Data on relevant studies of non-agave derived inulin are also included, as these food ingredients are chemically similar to the notified substance and have been shown to have like physiological and metabolic consequences (Tungland, 2018).

Table 7.1 Summary of Safety Studies on Inulins Derived from *Agave tequilana* Weber var. *azul* as the Notified Substance

Study	Subject(s)/study type	Route, Dose & Duration	Results & Effects
Urias-Silvas et al., 2008	Male C57BL/6J mice, 8 mice/group; 5-week repeated dose feeding study.	Groups of 8 mice per group fed diets with 10% *A. tequilana* Gto. fructans or a standard control mouse diet for 5 weeks.	Reduced body weight gain and liver weights; no histological finding in liver.
Tariani and Wolever, 2010	Healthy human adults (n=12), clinical trial	Single dose of 24 g agave inulin with 56 g HFCS in 400 mL drink after overnight fast. 6-hr observation post-treatment.	No adverse effects.
Gracia et al., 2013	Hsd:ICR male mice (4-5 weeks old), 5/ group; Chromosome aberration	Single intraperitoneal injections of 143, 357.5, or 715 mg/kg of DP < 10 agave fructan, DP > 10 agave fructan (treatments) or Mitocycine-C (positive control) or phosphate buffer solution (negative control). 24 hr post-treatment femur bone marrow collections.	Non-clastrogenic.
Garcia et al., 2013	Hsd:ICR male mice (4-5 weeks old), 5/ group; micronucleus assay	Single intraperitoneal injections of 143, 357.5, or 715 mg/kg of DP < 10 agave fructan, DP > 10 agave fructan (treatments) or Mitocycine-C (positive control) or phosphate buffer solution (negative control). 24 hr post-treatment tail vein collections.	Non-mutagenic

Table 7.1 Summary of Safety Studies on Inulins Derived from *Agave tequilana* Weber var. *azul* as the Notified Substance

Study	Subject(s)/study type	Route, Dose & Duration	Results & Effects
Garcia et al., 2013	10 Hsd:ICR mice, 5 male/5 female and 5 male/5 female Hsd:ICR mice (8-9 weeks old); acute toxicity	Single gavage dose of DP < 10 agave fructans or DP >10 agave fructans at 17.5, 55, 175, 1750 or 5000 mg/kg body weight. Observed for 14-day post-treatment.	No mortality, adverse clinical observations, body weight changes or histophathological findings at levels up to 5000 mg/kg b.w. (non-toxic).
López-Velázquez et al., 2013	Human infants (20 ± 7 days), clinical trial	Six groups of 100 infants. 3 groups received formula with Lactobacillus probiotic + 0.5 g/100 mL agave fructans (7.1-7.5 g/day), 1 group only probiotic, 1 group with only formula and 1 group human breast milk. 6-month study.	No adverse effects reported.
Marquez-Aguirre et al., 2013	Ames assay using *S. typhimurium* strains, TA 98, TA 100 & TA 102. Bacterial reverse mutation assay.	In vitro study. Concentrations of agave fructans up to 800 µg/plate. Used negative controls.	Non-mutagenic. Fructans did not increase frequency of mutations relative to negative controls, regardless of metabolic activation with Arocholor-1254 induced S9 mixture.
Marquez-Aguirre et al., 2013	25 BALB/C Mice, acute toxicity study	Single dose of 175, 550, 1750 and 5000 mg/kg b.w. with 14-day observations post-treatment.	No mortality or adverse effects reported.
Marquez-Aguirre et al., 2013	Obese male C57BL/6J mice, repeated dose gavage study.	Gavage 5 g/kg/d dose. 12-week study.	No adverse effects reported.
Holscher et al., 2014	Human adult clinical trial. 29 healthy men and women aged 20-36.	Subjects consumed 0, 0.5 or 7.5 g/d native agave fructans in single daily serving of chocolate chews for3-21-day periods separated by 7-d washout periods.	High dose associated with mild GI effects; characterized as non-adverse.
López-Velázquez et al., 2015	Human healthy term infant clinical study (20 ± 7 days). 600 infants.	Subjects consumed std. infant formula, human breast milk or enriched with either agave inulin (DP >10), a short-chain agave inulin (DP < 10) or dual inulin system with both agave chain lengthed products. 66,120 day follow up.	No adverse effects reported. Infant formulas enriched with agave inulin are safe and well tolerated by Mexican healthy term infants.
Rivera-Huerta et al., 2017	BALB/CAnNhsd mice (a model for colon cancer). Repeated dose feeding study.	No dose specified. Doses feed from 1 to 9 months.	No adverse effects reported.
Padilla-Camberos et al., 2018	Obese adult clinical study (28 volunteers).	Ninety-six (96) mg/kg b.w. for 12 weeks vs placebo.	Treatment significantly reduced body mass index, decrease of 10% body fat and serum triglycerides, compared with placebo. No adverse effects, well tolerated throughout study duration.

Table 7.2 Summary of Safety Studies on Inulins Derived from Other Sources

Study	Subject(s)/study type	Route, Dose & Duration	Results & Effects
Rao et al., 1965	Albino rats, unknown source of inulin, feeding study	Rats fed diet containing 5% inulin for 6 weeks.	Treatment resulted in 13% reduced body weight gain, no other adverse effects reported.
Meiji Seika Kaisha 1982.	Exp 1 and 2.: Rats fed FOS diets to elucidate previous FOS work.	Exp 1: Rats fed scFOS up to 15% in diet to assess if FOS increases cecum and colon weight & is fermented by colonic bacteria, as colon bacteria known to play role in colon carcinogenesis (micro adenoma test) Exp 2: Rats fed scFOS 7.5 or 15% (7.2 and 16.7 g/kg/d) for 13 wks to determine subchronic effects on liver granulation and parameters.	Exp 1: scFOS did not significantly modify the number of aberrant crypts and did not act as a promoter of chemically-induced carcinogenesis. Exp 2: The hepatic cytochrome P450 and cytochrome P450-dependent testosterone metabolism was not modified by scFOS. No adverse effects reported.
Yamashita et al., 1984	Human clinical trial with 18 non-insulin dependent diabetics.	Neosugar® (scFOS) of GRN 44 fed daily as single dose for 14 days.	No GI or other intolerance or adverse effects reported.
Hata and Nakajima, 1985	Human clinical trial with 85 healthy (51 men/34 women) volunteers.	For men, increasing doses from 12 to 50 g Neosugar® (scFOS) (GRN 44)and for women, 10 to 41 g FOS administered as a single dose.	Neosugar® (scFOS) well tolerated at levels up to 17 and 14 g in men and women, respectively. Early signs of diarrhea (9% incidence) as noted in mean at 25 g and in women at 26g.
Takahaski et al., 1986	Human clinical trial with 9 adults with chronic liver failure	6 grams of Neosugar® (GRN 44) per day in diet for 1 year.	No adverse effects reported.
Hidaka et al., 1986	Clinical trial:	1. 25 g Neosugar® as single dose 2. 8 g Neosugar®/day for 2 weeks	No GI or other intolerance or adverse effect reported.

Table 7.2 Summary of Safety Studies on Inulins Derived from Other Sources			
Study	**Subject(s)/study type**	**Route, Dose & Duration**	**Results & Effects**
	1. Healthy adult subjects; 2. 23 senile patients ages 50-90 yrs.; 3. 21 senile patients ages 54-88 yrs.; 4. healthy adults	3. 1, 2 and 4 g Neosugar®/day 4. 8 g Neosugar®/day for 2 months	
Stone-Dorshaw and Levitt, 1987	Human clinical trial with 15 healthy volunteers aged 21-65 yrs.	Day 1: all subjects consumed 10 g Neosugar®; Days 2-13: 10 subjects consumed 5 g Neosugar® per meal vs. 5g sucrose control; Day 14: all subjects consumed 10 g Neosguar®	Treatment increased prevalence for GI issues, abdominal discomfort, flatulence and bloating vs. sucrose control. No difference in severity for diarrhea, nausea or headaches for treated vs. control.
Clevenger et al., 1988. reported findings from Meiji Seika Kaisha).	F-344 rats (50/sex/group) known to have high incidence of neoplastic lesions. Feeding study.	scFOS tested for genotoxicity and carcinogenicity using 0, 8000 (341 to 419 mg/kg/d), 20,000 (854 to 1045 mg/kg/d), or 50,000 ppm (2170 to 2664 mg/kg/d) for 104 weeks.	No genotoxic potential observed and no difference observed in the onset of cancer in F-344 rats between control or FOS, with exception of pituitary adenomas in male rats, although this tumor was not considered related to scFOS, as incidence of all groups was within historical control range (mean of 31%, range 17-49%), and only equivocal evidence of a dose-response trend using the Cochran-Armitage trend test was observed.
Henquin, 1988.	Female rats. Feeding study	Diets containing 20% scFOS during gestation	No adverse maternal effects or *in utero* or early postnatal developmental effects.
Sleet and Brightwell, 1990.	Pregnant rat feeding study.	Diets containing 0, 5, 10, or 20% scFOS during gestation.	No adverse maternal effects or *in utero* or early postnatal developmental effects.
Gibson et al., 1995	Human clinical study with 8 volunteers	Consumed 15 g/d chicory oligofructose.	Well tolerated with transient complaints of flatus an abdominal distension.
Garleb et al., 1996	Clinical study with 27 male university students	Double-blind study of scFOS at 5 g/liter and 10g/liter with control group. Formula containing FOS as sole source of nutrition for 14 days with total consumption of 15 or 31g/d FOS.	No intolerance and no adverse effects on serum chemistry reported. Less than 5% of patient days had reports of any complaints and no severe complaints reported. Flatus reported at higher freq. in high dose but adapted after about 4 days.
Molis et al., 1996.	Human clinical study with 6 healthy volunteers (3 men/3 women.	Consumed 20g scFOS per day in three divided doses following meals for 11 days.	No adverse GI effects noted.
Kleesen et al., 1997.	Human clinical study with 10 elderly patients with constipation	Consumed 20 g/d chicory-derived inulin for 7 days followed by 40 g/d for another 12 days.	Only mild-moderate flatulence reported, no discomfort. Increased stool frequency in 8/10 patients. No other adverse effects reported.
Pederson et al., 1997.	Human clinical study with 64 healthy women (age 20-36 yrs)	Consumed 14 g/d chicory inulin for 2-4-week periods without washout.	Symptoms of rumbling in stomach and gut, flatulence and cramping, although mean values ranged from 0.3-1.2, with 1 being a weak effect. No other adverse effects reported.
Davidson et al., 1998.	Human clinical study with 21 men and women.	Consumed 18g chicory inulin/day RDBC design with two 6-week treatment periods and a 6-week washout.	GI discomfort attributed to increased flatulence, cramping, bloating, and changes in freq. and consistency of bowel movements.
Carabin and Flamm 1999.	Pregnant rats and fetus feeding study.	Rats fed diets containing 4.5 g/kg to 9.0 g/kg chicory inulin or 3-4 wks or oligofructose for up to 6 wks.	No treatment-related chronic toxicity was reported for oral doses of 4.5 g/kg for 6 wks. Inulin and oligofructose showed no toxicity compared with existing sugars used in the food supply. No observable negative effects on pregnant rats or development of fetuses or in newborns.
Buddington et al., 2002	B6C3F1 mouse feeding study	Mice fed diets enriched with 10% chicory inulin (≈100 g/k/g) for 6 weeks.	No adverse effects reported.
Boyle et al., 2008.	Rat feeding study.	Rats fed chicory oligofructose in 4 doses ranging from 0.55% to 9.91% of diet for 13 wks. Safety evaluated using *in vitro* mutagenicity tests.	Cecal weights and bifidobacteria increased in dose-related manner. No consistent differences in gross pathology or histopathology related to oligofructose intake and did not induce a positive response in the Ames test or chromosomal aberration test with CHO cells. The No Adverse Effect Observed Level (NOAEL) of oligofructose was 9.91% of diet.
Bruhwyler et al., 2009.	Human clinical study with 84 healthy subjects (aged 18-45 yrs, BMI 25.1 kg/m^2 and total fiber intake of 12 g).	Consumed either chicory-derived oligofructose at 5 and 10 g/d, native chicory-derived inulin at 5, 10, 20g/d, and long chain chicory-derived inulin at 10g/d for 2-five-week periods.	Increased GI symptoms (flatulence, rumbling, bloating, cramps stool freq.). Higher dose yielded more symptoms when comparing oligofructose vs native or long chain inulin. No other adverse effects reported.

Table 7.2 Summary of Safety Studies on Inulins Derived from Other Sources			
Study	**Subject(s)/study type**	**Route, Dose & Duration**	**Results & Effects**
Ripoll et al., 2009	Human clinical study with 18 subjects.	RDC-placebo controlled design. Morning coffee in drink with 10 g sucrose or 5 g or 7.8 g chicory-derived inulin for 3-6-day periods.	Slight, but statistically significant increase in overall abdominal discomfort reported at 7.8 g inulin dose after 1 week. No other adverse effects reported.
Roldan-Marin et al., 2009	F344 rat feeding study.	Rats fed diets containing 7% fructan extract from onions (*Allium cepa* L.) or control for 4-weeks.	No adverse effects reported.
Bonnema et al., 2010.	Human adult clinical study with 26 healthy men and women (aged 18-60 yrs) w/ no history of fiber-related GI issues.	5 g/d or 10 g/day of native chicory inulin or oligofructose consumed for 5 days.	Mild increase in bloating and flatulence; 10 g dose of oligofructose had substantially greater GI symptoms than placebo group. Doses up to 10 g/d of native chicory inulin and up to 5 g/d of oligofructose well tolerated in healthy-young adults. No other adverse effects reported.
Rendón-Huerta et al., 2012	Diabetic and obese Wistar rats, feeding study.	Agave (*A. angustifolia*), J. artichoke (*Helianthus tuberosus*) and chicory fructans (*Cichorium intybus*) fed in feeds at 15% enrichment, corresponding to 7-9 g/kg b.w/d for 6-weeks.	Modest reductions in body wt. for all fructans. All fructans well tolerated with no adverse effects.
Hijová et al., 2013	Sprague-Dawley rat feeding study.	Rats fed oligofructose enriched inulin from chicory root (Synergy 1) at 8% in diet for 28-weeks.	Fructan treatment was well tolerated and no adverse effects were attributed to the treatment diet.
Dávila-Céspedes et al., 2014	Wistar rat feeding study	Rats fed 10% dietary fructans from *A. salmiana* for 13 weeks.	No adverse effects reported.

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8.0 APPENDIX

8.1 Scientific Literature on Chemical Identity of Agave Inulin and Raw Material

8.1.1 Carbohydrate Composition and Degree of Polymerization

Agave fructans are structurally diverse mixtures of fructooligosaccharides (FOS) and fructans that contain both β(2-1), the majority, and β(2-6)-linkages, with internal and external glucose units, which are termed agavin- and graminan-type fructans, respectively (Mancilla-Margalli and López, 2006; Mellado-Mojica and López, 2012). Other researchers have also proposed structures (Franco-Robles and López, 2015; Livingston et al., 1993; Pavis et al., 2001; Sims et al., 1992). In mature agave plants, the agavins are the more abundant of the two fructan types (Mellado-Mojica and López, 2012).

Mellado-Mojica and López (2012) determined that, like other inulin-type fructans, the composition of glycosidic linkages of *A. tequilana* fructans differ according to plant age. The authors determined that the average DP of fructans stored in plants 2 to 7 years old range from DP 6 to 23, with the latter coinciding with the DP = 18-28 previously reported for 6-7 year-old plants of this species (López et al., 2003; Mancilla-Margalli and López, 2006; Arrizón et al., 2010). Authors noted that β(2-6) linked branches are absent in 2-year-old plants, emerging at 4-year-old plants, and reached highest degree in fructans from 7-year-old plants. Arrizón and others (2010) reported that 2-year old plants contained the highest levels of free monosaccharides (fructose and glucose) and fructan molecules (DP 3-6) with a total fructan content that comprised 69% of the total carbohydrate content. The authors reported that older plants (4 and 6.5 years old) had a fructan content of 97%, while the simple sugars, fructose, glucose and sucrose each accounted for < 1% of the total carbohydrate content. Graminans and agavins are present at all plant ages, but their proportions diverged as plants aged.

Toriz and others (2007) determined the native fructans from *Agave tequilana* Weber var. *azul* had a mean DP of 16, with a DP range from 2 - 60. Löppert and others (2009) provided further evidence of the molecular weight and physiochemical characteristics of fructans from *Agave tequilana* Weber var. *azul*, determining that the DP fraction from DP3 - DP12 was 20% of the total distribution, while the polymer fraction from DP 20 - DP 70 had 57% of the distribution, and 20% of the polymers were between DP 12 and DP 20, with a mean DP of about 15. Mellado-Mojica and López (2012) also found that plants begin with equal proportions of agavins/graminans, moving toward more complex branched structures with more isomeric forms having a higher abundance of agavins than graminans at 7 years (Ratio of agavins/graminans: 0.9 ± 0.3 at 2 yrs. vs 3.6 ± 1.3 at 7 yrs.), and large DP as plants age.

Waleckx and others (2008) further evaluated the water-soluble carbohydrates from the heads of mature (aged not specified) *Agave tequilana* Weber var. *azul*. Water soluble carbohydrates were extracted in hot water from six mature heads of the plants. The content of the carbohydrates in the agave heads was 28.3 g/100g (fresh weight) ± 0.1% and 86.7 g/100g (dry weight) ± 1.3%. The authors noted that HPLC analyses showed that 93.4% of the carbohydrates consisted of fructans with a DP ≥ 3, with free mono- and disaccharides consisting of 2% sucrose, 0.8% glucose, and 3.8% fructose. The average DP of the fructans in the water extract was 13.6 ± 1.3.

Thus, as noted from Mellado-Mojica and López (2012), the average DP of agave inulin from Agave tequilana Weber var. azul plants from 2-7 years ranges from approximately 6 to 23, while the average DP is centered around 14-18, with some variation based on the plant's age, climatic or environmental conditions during the growing and harvesting period, and the region of cultivation. This degree of polymerization is consistent with that of other inulins consumed by humans, including native chicory root inulin, which has an average DP of 10-20 and a range 2-60 (Roberfroid and Delzenne, 1998).

IMAG Organic® represents a substance from agave piña that is produced from plants that are between 4 and 7 years of maturity.

8.1.2 Other Non-carbohydrate Constituents from the Tissue of the *Agave tequilana* Stems

Several compounds from the tissue of *Agave tequilana* piñas, including terpenes and fatty acids, contribute to the characteristic flavors of alcoholic beverages made from agave. Pena-Alvarez and others (2004) analyzed three (3) species of agave (*A. tequilana* Weber var. *azul*), *A. salmiana* and *A. angustifolia*), species used to make the beverages tequila and mescal. Terpene and fatty acid contents were determined using steam distillation extraction-solid-phase microextraction coupled to GC-MS and as fatty acid esters by Bligh-Dyer extraction-derivatization coupled with GC-MS, respectively. The predominant fatty acids found in *A. tequilana* were linoleic acid (448 μg/g) and palmitic acid (about 257 μg/g), followed by oleic acid and linolenic acid (about 100 μg/g each). The authors also determined other fatty acids in all three species ranged from about 5 to 30 μg/g, including lauric acid, myristic acid, pentadecylic acid, palmitoleic acid, margaric acid and stearic acid. The total fatty acid content in Agave tequilana was found to be about 0.1% (985 μg/g). The authors also noted that terpenes were difficult to identify and quantify because of their relatively low concentration in agave and poor resolution by GC, although they identified thirty-two (32) terpenes in *A. tequilana*, with the main terpene in all three species, linalool, albeit they were not quantified.

8.1.3 Chemical Constituents in Other Food Products Derived from Agave Piña (Stems)

In tequila production, agave piñas (stems) are cooked and crushed to extract the juice, and then the high carbohydrate-containing juice is fermented to produce alcohol. Distilled tequila contains about 200 different compounds, from the raw material undergoing many chemical and biochemical reactions, which depends on plant maturity, how and for how long it is cooked, what microorganism is used to fermented its sugars and how the alcohol is distilled. Aroma and flavor compounds that impact the beverage's characteristic organoleptic attributes include alcohols, fatty acids, esters, aldehydes, terpenes, phenols, lactones and thiols. An analysis of non-carbohydrate components of tequila that was produced using traditional processes that utilized long cooking in ovens to hydrolyze fructans prior to fermentation, was performed by Avila-Fernádez and others (2009) using GC-MS. Most notably terpenoids, including linalool were determined. The authors found the combined concentration of linalool and its oxides was 0.4 mg/L of tequila, and, in addition, free fatty acids and fatty acid esters (100-150 mg/L); alcohols and esters (200-250 mg/L); cyclic oxygenated compounds (20-50 mg/L); and terpenoids (1-3 mg/L).

In 2004, Peña-Alvarez and others characterized the terpenes of three agave species via steam distillation solid-phase microextraction coupled to gas chromatography-mass spectrometry. They determined that different terpenes were identified in the three Agave plants: nine (9) in *A. salmiana*, eight (8) in *A. angustifolia* and thirty-two (32) in *A. tequilana* Weber var. *azul*. Of those characterized from *A. tequilana*, the most abundant is linalool.

Linalool is a terpene alcohol that is found in more than two hundred different species of plants. It is an important constituent in many essential oils, such as lavender, myrrh, lemongrass, frankincense and other oils. It has two stereoisomers - (S)-(+)-linalool is known as coriandrol, while (R)-linalool is known as licareol. The compound has a floral fragrance and is commonly used in cleaning products, lipsticks, perfumes, shampoos and bubble bath applications.

Linalool is linked to the production of vitamin E and is therefore an important terpene to human function. Linalool is an anxiolytic agent, with potent anti-stress effects, and has direct sedative effects, its primary function in essential oils. In addition to these effects, linalool is used as a decongestant and anti-inflammatory when added to aromatherapy inhalers to relieve respiratory issues. The compound has a broad range of biological properties, including cancer chemopreventative effects, antibacterial, antifungal, and antiviral effects, antihyperglycemic, anti-inflammatory, and antiparasitic activities (Paduch et al., 2007). The authors also reported that terpenes act as skin penetration permeability enhancers and agents involved in the prevention and therapy of several inflammatory diseases (Paduch et al., 2007). In a 2003 study, linalool was determined to have additional analgesic properties (Peana et al., 2003). In later studies, the researchers determined anti-inflammatory mechanisms of linalool in pain reduction, reporting that linalool influences adenosine A1 and A2A receptors in (-)-linalool-induced anti-nociception to block pain sensation (Peana et al., 2006b) and that the terpene inhibits NO formation *in vitro*, citing probable involvement in the antinociceptive activity of the compound (Peana et al., 2006a).

Linalool is regarded as GRAS as a flavoring agent by the FDA at levels < 0.25%, its cytotoxic level reported in literature, and it is considered safe as an ingredient in cosmetics as well, including aromatherapy oils as a fragrance, although oil oxidation can render it a potential contact irritant, so proper oil storage is important. It is acceptable for use as a flavoring agent and a fragrance in Europe, but it is included on the list of allergenic substances, meaning its presence must be highlighted on labels of topical products at a concentration of more than 0.001% or if present in rinse-off products at concentrations of > 0.01%.

Regarding human intake of linalool from tequila, assuming that ripe agave heads average 50-60 kg and yield about 7.1 to 8.5 liters of tequila, and all of the linalool in tequila originates in the agave plant, one agave head can contain about 3.6 - 4.3 mg linalool/head (0.5 mg linalool/L x 7.1 - 8.5 L). Assuming about 800 tons of raw agave yields about 150 tons of pure, dried inulin (Nutraingredients, 2005), substances, such as linalool can be concentrated about 5.33-fold (800/150) during refined inulin production. Using the average agave head weight and linalool content, the dried refined inulin might be expected to contain about 21 mg/head or about 0.4 mg/kg. No terpenes were detected in the powdered IMAG Organic® agave inulin product.

As mentioned, many chemical and biochemical reactions take place during the production of tequila. By example, Amadori rearrangement compounds created by Maillard reaction are generated by reactions of amino acids with a reducing sugar from thermal processing of *A. tequilana* Weber var. *azul* during its production (Mancilla-Margalli and Lopez, 2002). Because of the long cooking times of the piñas (up to 32 hrs.) and high cooking temperatures (100 °C), non-enzymatic browning reactions produce many Amadori rearrangement compounds, such as methyl-2-furoate and 5-(hydroxymethyl) furfural, and 2,3-dihydroxy-3,5-dihydro-6-methyl-4(H)-pyran-4-one. In 2000, Frank and Hofmann also noted that furfural was also formed from thermal processing of other fructan-containing crops, such as from the small grains wheat, rye and barley, and from roasting chicory root for coffee extension. The Maillard reaction products impart sweet notes and contribute significantly to the characteristic flavor of tequila. However, refined agave inulin production does not involve temperatures that would thermally hydrolyze the inulin molecule, and as the pH of the process juices is above 4.0, the inulin molecule is stable to chemical hydrolysis and the production of reducing sugars (reactants in the Amadori reactions). Further, at the processing pH of 4.5, Maillard reaction products are negligible. Consequently , refined agave inulin does not contain any notable Amadori rearrangement compounds.

In addition to tequila, agave syrup (a.k.a. blue agave syrup and agave nectar) is also produced *via* water extraction of fructans from agave piñas and subsequent hydrolysis of the extracted agave juice fructans by thermal and/or enzymatically-treatment to fructose monomers, followed by evaporative concentration to a syrup (Macilla-Margalli and Lopez, 2002). In addition to its high fructose content, this agave syrup product has been found to contain other sugars, amino acids and Amadori compounds from the Maillard reaction. Pätzold and Brückner (2005) determined the simple sugars, sucrose, glucose and fructose using enzymatic assay and amino acids by enantioselective GC-MS in agave syrup, juice and other plant syrups (maple, pomegranate, grape and palm). The principal Amadori compound, 5-(hydroxymethyl)furfural, which serves as an indicator of high temperature thermal processing and Maillard reaction was assayed colorimetrically after it had been derivatized using a barbituric acid/*p*-toluidine mixture. The amino acid D-alanine was determined in all plant products and at 13.5% (relative to L-alanine + D-alanine) in agave syrup. The D-alanine content in agave syrup was found to be similar to that in pomegranate, palm and grape syrups, while in Canadian maple syrups the content of the amino acid was about 2.5-fold (33-34%). No other D-amino acids were determined in agave or grape concentrate (Arrope), although several other D-amino acids were measured in the other syrups and juices. As for simple sugars, the concentrations of glucose and fructose in agave syrup were 19.9 and 55.6%, respectively, while sucrose was not detected. The concentration of the Amadori rearrangement compound, 5-(hydroxymethyl)furfural, ranged from 7 mg/100g in the agave syrup to more than 14.5% by weight in Arrope (grape concentrate).

Five commercial agave syrup (agave nectar) products have also been analyzed for total antioxidant content, in comparison with major U.S. brands of other natural sweeteners, such as refined white sugar and corn syrup (Phillips et al., 2009). Analysis using a ferric-reducing ability of plasma (FRAP) assay revealed that the five agave syrup products contained only minimal antioxidant capacity, which were comparable to the other refined white sugar and corn syrup products. Data show that two of the blue agave nectar brands (Molina Real and Live Superfoods) contained 0.034 mmol FRAP/100g and 0.143

mmol FRAP/100g, respectively. The other commercial agave syrups analyzed, representing "light", "raw" and "amber" syrups (Madhava), had an antioxidant capacity of less than 0.03 mmol FRAP/100g

In 2009, enzymatically-hydrolyzed syrups were analyzed from manufacturing methods used to optimize the manufacture of fructose-rich syrup products (García-Aguirre et al., 2009). During this research, the authors used fresh "pines" (plans without leaves) of *Agave tequilana* Weber var. *azul,* and an enzyme having inulinase activity from the yeast the *K. marxianus*, a yeast obtained from Aguamiel from traditional rural producers of pulque in the Mexican state, Guanajuato. Analysis of the obtained fructose-rich syrups showed an average fructose concentration of 95%, with a glucose concentration of 5%, and contained no sucrose. As these products were not subjected to either thermal or acid hydrolytic processes, analysis of the products for Amadori compounds revealed that they were free of contaminates, such as hydroxymethylfurfural, which may be present in products obtain by the more harsh hydrolytic processes. As neither thermal or acid processes are not used in the production of refined agave inulin, hydroxymethyl furfural and related Amadori products are not present. The Aguamiel, the sweet sap obtained from *Agave mapisaga* plants, which are called “maguey pulquero”, has as its main sugars, glucose, sucrose, fructose and several pentoses (Sanchez-Marroquin and Hope, 1953, as cited by Tovar et al., 2008).

The sugars in Aguamiel were determined by Ortiz-Basurto and others (2008), along with the amino acid content. The sap contained a dry matter content of 11.5%, which was made up of about 75% sugars. Of these sugars, 32% by weight was fructose and 26% by weight was glucose, followed by fructooligosaccharides that accounted for 10% by weight, with the remaining 9% made up from sucrose. Analysis of the sap further showed the protein content was 3% by weight, and the free amino acids were 0.3% by weight, which included gamma-amino butyric acid, glycine, asparagine/asparate and glutamine/glutamate.

In addition the sugars contained in Aguamiel, Tovar and others (2008) also determined the phytase activity and nutrient levels of ascorbic acid and the minerals, iron, zinc, calcium, magnesium and selenium in several pulque and aguamiel samples from the Mexican states of Tlaxcala, Puelba and Hidalgo. Official methods were used to determine each component. The ascorbic acid (vitamin C) levels in two of the liquid samples of pulque was 2.66 ± 0.12 mg/100 mL, and was negligible in another sample, while the levels of this nutrient in two (2) different aguamiel samples was 2.01 ± 0.10 mg/100 mL, which indicates that ascorbic acid is part of the nutrient make up of agave. The content of the minerals, calcium, magnesium, selenium and iron in fresh pulque was 20.4, 16.4, 1.3, and 0.03 μg/100g, respectively, while those in aguamiel were similar at 25.8, 13.8, 1.3, and 0.03 μg/100g, respectively. The antioxidant mineral, zinc, was not detected in either pulque or aguamiel. Kuhnlein (2004) noted that a typical 500 mL serving of pulque contains 30 mg of ascorbic acid (vitamin C), 0.1 mg of thiamin (vitamin B1), 0.1 mg of riboflavin (vitamin B2), and 3.5 mg of iron, while containing about 4-6% of ethanol. Tovar and others (2008) determined that phytase activity was found in both pulque and aguamiel and suggested that the enzyme, originating from live microbiota in the pulque, dephosphorylates phytate, improving the bioavailability of iron and zinc. Phytic acid is a known component in plants that has a strong affinity to bind minerals. This results in precipitation, making the minerals unavailable for absorption in the intestines (Ekholm et al., 2003; Cheryan, 1980). The amount of phytic acid is

commonly reduced in animal feeds by adding histidine acid phosphate type of phytases to them (Kumar et al., 2012). By dephosphorylating phytase, the microbiota-derived phytase, improves dietary mineral bioavailability (Tovar et al., 2008; Schlemmer et al., 2009).

8.1.4 Non-Carbohydrate Bioactive Constituents of Whole Agave Plants, Roots, Leaves and Fruits

In addition to the carbohydrates, terpenes, such as linalool, trace nutrients, such as vitamins and minerals, and antioxidants, agave plants, mainly the long spiked-like leaves, contain liquid that contain sharp, needle-like calcium oxalate crystals, called raphides (Ricks, et al., 1999; Salinas et al., 2001). These raphides are known to produce dermal irritations when it the agave liquid is in contact with human skin. Contact dermatitis stemming from this event, is well known to workers in tequila distilleries and on agave plantations (Ricks et al., 1999; Cherpelis and Fenske, 2000; High, 2003; de la Cueva et al., 2005). Various cases of contact dermatitis provoked by leaves of *Agave spp.* were also described by Brenner and others (1998), with either systemic signs and symptoms or with abnormal laboratory results. A case of occupational allergic bronchial asthma cause by *Agave americana* has also been reported with the detection of specific IgE antibodies (Hagemeyer, 1993).

The calcium oxalate crystals isolated from the long leaves of *A. tequilana* by Salinas and others (2001) were characterized as 30-500 μm in length and sharpened on both ends. The authors found that a single drop of pressed juice from the leaves contained 100-150 of the needle-like crystals.

Most of the bioactivity from raw agave products, including those from *Agave tequilana*, has been attributed to saponins (Blunden et al., 1978, 1980, 1986; Dewidar and el-Munajjed, 1970; Kintja et al., 1975; Anwar and Hussain, 2017; Santos-Zea et al., 2012). In agave, these compounds are glycosylated derivatives of plant steroids, that include smilagenin, gitogenin, chlorogenin, hecogenin, tigogenin, sarsapogenin and hongguanggenin, with hecogenin, tigogenin and diosgenin being the most predominate. These compounds are used for the production of contraceptives, corticosteroids, and steroidal diuretics, among other therapeutic applications (Crabbe, 1979; Bedour et al., 1979; Garcia, 2000; Narvaez-Zapata and Sanchez-Teyer, 2009; Ruvalcaba-Ruiz and Rodriquez-Garay, 2002). Saponins are potentially toxic, but have been shown to have anti-cancer activities in several human cell lines (Chen et al., 2011; Ohtsuki et al., 2004; Sati et al., 1985; Yang et al., 2006; Yokosuka and Mimaki, 2009), have anti-inflammatory activities (de Silva et al., 2002; Monterrosas-Brisson et al., 2013; Peana et al., 1997; Salazar-Pineda et al., 2017), and anti-microbial properties (Anwar and Hussain, 2017; Salazar-Pineda et al., 2017; Verástegui et al., 2008; Yang et al., 2006). Crude extracts of agave plants have also been shown to contain two utero-active compounds, one of these exerting pharmacological actions similar to acetylcholine, however having a structure of an acyl derivative of choline different from acetylcholine (Basilio, et al., 1989).

Da Silva and others (2002), working in vivo in BALB/c mice, showed that sarasapogenin glycoside from *Agave attenuata* significant potential to reduce acetic acid-induced vascular permeability almost at the same levels as an indomethacin, but at concentrations ten-fold higher.

Although saponins have potential to be toxic and have been linked to poor growth, bloating, feed intake and reproduction in foraging animals (Birk and Puri, 1980; Francis et al., 2002), it takes massive doses to

create such problems. Certain saponins, due to their interaction with cholesterol have also be reported to be able to lyze erythrocytes, but the action is weak (Birk and Puri, 1980; Scott et al., 1985; Hronek et al., 1989). Saponins are also known to inhibit certain enzymes, such as succinate dehydrogenasae, a key enzyme in the Kreb cycle (Birk and Puri, 1980), and reduce the activity of trypsin and chymotrypsin, key digestive enzymes (Liener, 1994). Research has also indicated that saponins may be goitrogenic and increase risk for enlargement of the thyroid (Kimura et al., 1976), although many other bioactive agents found in plants, such as isoflavones, coumestans, lignans, gossypol glycosides and other goitrogens may also be significant contributors to thyroid effects.

Steroidal saponins, in addition to being found in agave, are also found in many other edible plants, such as oats, capsicum peppers, chick pea, aubergine, tomato seed, alliums (onions, leeks), lettuce, spinach, asparagus, yams, most legumes and beans, paprika, alfalfa, fenugreek, and ginseng. The concentration of steroidal saponins in agave is similar to that for triterpenoid saponins in chickpea, a vegetative fruit having high human consumption. However, these effects are related to consuming bioactive saponins, which are quickly destroyed under heat, while cooking, as takes place to extract and purify agave inulin-type fructans for commercial use in human food production.

Saponins are typically isolated from leaves of *Agave lecheguilla, A. sisalana, A. lophantha, A. parasana, A. utahensis* and *A. americana* (Bedour et al., 1979); from flowers of Maguey (*Agave salmiana*)(Sotelo et al., 2007); from fruits of *A. cantala* (Uniyal et al., 1991); roots and seeds of *A. lechuguilla* and from whole plants of *A. utahensis*. , but not typically from piña, where they are in lesser amounts. Isolation from substrates is typically by methanolic or organic solvent extraction. Many saponins have been identified in the various species of Agave and have already been presented extensively on page 62, Table A-1 of GRN 854. Cripps and Blunder (1978) determined the two most abundant sapogenins in mature leaves of *A. sisalana* as hecogenin and tigogenin from acetylated leaf and leaf juice derivatized extracts using a gas-liquid chromatographic method. They found that the hecogenin content of leaf extract was about 1%, while that of leaf juice was about 0.14%. The tigogenin content was found to be about one-tenth that of the hecogenin content.

No saponins have been detected in stem (piña) extracts of the *Agave tequilana* plant, nor have they been determined in the inulin fraction of the same plant species. There is no evidence of the presence of any toxic saponins in agave inulin, either from compositional analysis of agave piña extracts or the long history of use of Agave stems for food and spirits dating back about 10,000 years.

In addition to saponins and raphides, a few species of the genus *Agave* have been studied for their phenol composition and related biological activities (Almaraz-Abarca et al., 2013). The most ubiquitous phenolic compounds occurring in almost all parts of the plants are the flavonoids. Many of those have several biological properties with medical implications (Mouren et al., 1994; Zhang and Cui, 2005). The primary species of Agave studied for their phenol composition, contents of phenols and flavonoids and their biological activities include: *Agave amerciana* L., *Agave barbadensis* Trel., *Agave sisalana*, *Agave attenuata* Salm-Dick, and *Agave tequilana* Weber, the raw material associated with the notified substance.

Chen and others (2009) isolated three known flavones: 5,7dihydroxyflavanone, kaempferol 3-rutinoside-4-glucoside, and kaempferol 3-(2G rhamnosylrutinoside); and seven homoisoflavonoids: 7-*O*-methyleucomol, 3-deoxysappanone, (±)-3,9-dihydroeucomin, dihydro-bonducellin, 7-hydroxy-3-(4-hydroxy-benzyl) chromane, 5,7-dihydroxy-3-(4-hydroxy-benzyl)-4-chromanone, and 5,7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)-4-chromanone), from methanolic extracts of the leaves of *Agave sisalana*. Morales-Serna and others (2010) also studied the leaves and piñas of *Agave tequilana* for their phenol composition. The authors identified three homoisoflavanones (5,7-dihydroxy-3-(4'-methoxybenzyl)-chroman-4-one, 7-hydroxy-3-(4'-hydroxybenzyl)-chroman-4-one, and 4'-dimethyl-3,9-dihydropunctatin) via NMR in plants growing in Mexico. The 5,7-dihydroxy-3-(4'-methoxybenzyl)-chroman-4-one was found also in *A. barbadensis* (Tinto et al., 2005). The three homoisoflavanones identified by Morales-Serna and others in *A. tequilana* were found to be different than those identified by Chen and others (2009) for *A. sisalana*.

The phenol composition of and biological activities of *Agave* species have seen significant study in recent history (Almaraz-Abarca et al., 2013; López-Romero et al., 2017; Santos-Zea et al., 2012). The agave offer important and diverse biological activities related to these phenolic compounds, particularly as sources of flavonoids, homoisoflavonoids, and phenolic acids with importance as antioxidants, antibacterial and antifungal compounds, immunomodulator substances and antinematod components, which could be regarded as nutraceutical products with applications in food and beverages, and as further substances with potential to develop medicinal compounds for humans and animals. The biological activities of these compounds are health-promoting rather than toxic, and as a consequence should not elicit any concern for human health.

8.1.5 Classification of Agave Inulin amongst Edible Plant Fructans

Fructans may be classified into five major types according to the way the β-fructofuranosyl units are linked and position of glucose in the structure (Roberfroid, 2005; Vijn et al., 1997; Vijn and Smeekens, 1999), although all major groupings, with exception to levans, have primary β(2-1)-fructofuranosyl linkages with lesser β(2-6) side chains, as inulin. These major fructan types include:

1. more linear inulin-type fructans with β(2-1)-fructofuranosyl linkages with a terminal glucose unit that are widely described in the *Asteraceae* or *Compositae* family, which includes the dicotyledon plants, chicory, Jerusalem artichokes, elecampane, dahlia and dandelion;

2. inulin neoseries, which contains a glucose moiety between two fructofuranosyl units extended by β(2-1) linkages, as characterized in onion, garlic, leeks and asparagus, also in the *Asparagales* order;

3. levan (or phlein) with linear β(2-6) linkages with a terminal glucose unit as found in grasses like *Phleum pratense* or are of bacterial origin;

4. levan neoseries, formed by β(2-1)-and β(2-6)-linked fructofuranosyl units on either end of a central sucrose molecule, as reported in oat (*Avena sativa*); alternatively they are composed of two linear β(2-6)-linked fructosyl chains, having an internal glucose moiety, and;

5. mixed fructans (graminans or agavins) containing mainly β (2-1)- fructofuranosyl linkages, as in number 1, having more significant β(2-6) side chains than number 1 (generally, they are branched fructans like those found in wheat (*Triticum aestivum*), and a few members of *Asparagales* order, such as **agave**). The glucose moiety may be terminal, as in graminans or internal, as in agavins (Mancilla-Margalli and Lopez, 2006; Waleckx et al., 2008).

According to the above system for classification of fructans, agave inulin belongs to the "mixed fructan" group (number 5), based on the two linkage types and chain branching. As mentioned, agave fructans are further categorized as graminans, that are mixed fructans containing branched β(2-1) and β(2-6) linkages and terminal glucose moieties, and agavins, that are branched neo-fructans, characterized by internal α-D-glucopyranose (Mancilla-Margalli and Lopez, 2006).

Most fructans originating from plants, either exist as oligomers or polymers, are highly specific and influenced by environmental conditions during maturity and the plant's developmental stage (Sims, 2003; Sims et al., 2001). Plants that store fructans make up over 15% of the global angiosperm flora numbering over 36,000 monocotyledonous and dicotyledonous plant species (Hendry 1987; Hendry and Wallace, 1993).

Regardless of plant source, the length of the plant-derived fructan polymeric chain, or its degree of polymerization (DP) as well as the types of linkage, which predominate in the fructan molecule, depends on the type of fructan biosynthetic enzymes present in the plant source. Phylogenetic analysis based on the presence of two such enzymes, vacuolar invertases and fructosyltransferases, places *Agave tequilana* within the *Asparagales* order, closely related to *Allium cepa*, the common onion and *Asparagus officinalis*, asparagus (Van den Ende et al., 2011). In addition to a plant's genetics, the DP also is variable and depends on the time of harvest and the duration and conditions of post-harvest storage. Depending on the source, fructans can contain from 2 to more than 100,000 fructose units linked by β-2,1-fructosyl-fructose linkages (inulin-type) or β-2,6-fructosyl-fructose linkages (levan-type) glycosidic bonds (Banguela and Hernandez (2006), albeit the highest DP typically found in plants comes from the family of *Asteraceae* found in globe artichoke (*Cynara scolymus*), with roots reaching up to 200 fructose residues (Okey and Williams, 1920; Praznik and Beck, 1985; Vijn and Smeekens, 1999; Hellwege et al., 2000). By comparison, bacterial levan has a much higher DP (up to 100,000), as compared to fructans from plant origin. The common DP range of the fructans from chicory root and agave piña in commercial use both have a DP range from 3 to 60 or 70 fructose residues, while fructans from Jerusalem artichoke tubers, have a DP range of 3 to 20 fructose residues. The molecular weight distribution is 527-4739 Da, which corresponds to a range of DP from 3 to 29 (Lopez et al., 2003), with a small fraction that has a DP from 30 to 60 fructose units (Toriz et al., 2007). The average fructan content from the blue agave plant (*A. tequilana* Weber var. *azul*) has been shown to range from approximately 6 to 23 (Mellado-Mojica and Lopez, 2012) and is typically centered around 14-18 (Waleckx et al., 2008; Toriz et al., 2007; Mancilla-Margalli and Lopez, 2006) with some variation based on the age of the plant and the region of cultivation.

By general definition, fructan polysaccharides are called inulins (from the plant that it was first isolated from, *Inula helenium*) when the DP is > 10 fructose units. As fructans from mature blue agave plants

have mean DP > 10 fructose units, the product is referred to as agave inulin. Toriz and others (2007) reported that the low molecular weight fructans of mature agave plants (DP 3 to 5 fructose units) account for only about 9% of the total, while mono- and disaccharides typically account for < 10% of the total carbohydrate content. The DP for agave inulin is consistent with that of other inulins consumed by humans, including native chicory root inulin, which has a DP of 10-20 and a range of 2 to 60 (Roberfroid and Delzenne, 1998).

8.2 Effects of Non-Carbohydrate Bio-active Constituents of the Agave Plant

As mentioned in the previous section, a few notable non-carbohydrate bioactive constituents are known in agave. These include terpenes, such as linalool, fatty acids, such as linoleic, palmitic, oleic and linoleic acids, raphides (calcium oxalate crystals), flavonoids, homoisoflavonoids and phenolic acids. Some of the effects of these bioactive agents have already been discussed.

A single dose (po) up to 6g of a leaf extract of *A. intermixta* Trel. did not cause any adverse effects (Garcia et al., 2000).

Kuhnlein (2004) noted that moderate pulque consumption in the maternal diet in the central highlands of Mexico is associated with better infant birth size and growth than mothers not consuming pulque. Pulque consists of about 5% ethanol, and a 500 mL serving provides significant nutrients and minerals that include the vitamins, C (ascorbic acid), B1 (thiamin), B2 (riboflavin) and the mineral, iron.

Raphides (sharp needle-like crystals of calcium oxalate) have been reported in the liquid sap from the leaves of Agave plants, which, along with acrid oils and saponins, are known to cause contact dermatitis among workers in tequila distilleries and on agave plantations. During investigative work to isolate and identify effects of raphides from leaves of *A. tequilana*, Salinas and others (2001) found that a single drop of sap contained 100-150 needle-like crystals, which were found to produced dermatitis similar to that of the workers within an hour of contact with an aqueous suspension of the crystals. The authors also noted that only exposed skin areas where workers skin had direct contact with the plants developed irritation. When the raphide suspension was passed through single or double layered cotton cloth, 75 and 92% of the crystals, respectively, were removed. Clothing, to minimize skin exposure to the sap, is suggested as an effective barrier to prevent irritation and dermatitis.

Incidence of agave-induced irritant dermatitis has also been reported in landscaping workers, although occurrence is relatively rare (Ricks et al., 1999). Hackman and others (2006) reported twelve (12) cases of such dermatitis by the *Agave americana* (the century plant), a popular plant used in landscaping as an ornamental. In addition, Ricks and others (1999) report a single case of Agave-induced purpura on the anterior legs in an otherwise healthy patient following cutting down an *A. americana* plant with a chain saw.

Reports in Mexico of skin irritation from the use of scouring pads (estropajo) during bathing are known (Salinas et al., 2001). The scouring pads, made from fibers of *A. lechugia* leaves, which contain raphides in their leaves are typically used for washing dishes. When Salinas and others (2001) examined estropajos from local markets in Guadalajara, Jalisco, MX, they found raphides in all products tested.

A case of occupational allergic bronchial asthma cause by *Agave americana* has also been reported with the detection of specific IgE antibodies (Hagemeyer, 1993).

Agave leaves and roots, not the stems (piña) contain steroidal saponins, and have various biological activity. Certain saponins, due to their interaction with cholesterol are also able to lyze erythrocytes, but the action is weak (Birk and Puri, 1980; Scott et al., 1985; Hronek et al., 1989). Saponins are also known to inhibit certain enzymes, such as succinate dehydrogenasae, a key enzyme in the Kreb cycle (Birk and Puri, 1980), and reduce the activity of trypsin and chymotrypsin, key digestive enzymes (Liener, 1994; Sotelo et al., 2007). Sotelo and others (2007) determined that trypsin inhibitors in *Agave salmiana* flowers were 1.11 ± 0.10 Trypsin unit inhibited/mg sample, which is very low when compared with the content in most legume seeds. The authors also reported very low concentrations of hemaglutinnins and agglutinations.

Research has also indicated that saponins may be goitrogenic and increase risk for enlargement of the thyroid (Kimura et al., 1976), although many other bioactive agents found in plants, such as isoflavones, coumestans, lignans, gossypol glycosides and other goitrogens may also be significant contributors to thyroid effects. However, these effects are related to consuming bioactive saponins, which are quickly destroyed under heat, while cooking, as takes place to extract and purify agave inulin-type fructans for commercial use in human food production. In addition, bioactive materials that have been extracted and isolated from Agave plants have been studied extensively, but the piña, the part of the Agave plant used to make refined inulin products, was not the source of the material investigated in any of these studies, nor was the *Agave tequilana* Weber var. *azul* the species of Agave used.

As in many other bioactive molecules, the sugar moiety of saponins plays an important role in many different human beneficial effects, such as influence in membrane-permeabilizing, immunostimulation, cholesterol reduction, anti-inflammatory, anti-fungal and anti-carcinogenic properties (Santos-Zea et al., 2012).

The steroidal saponins from agave have proven anti-cancer properties against several different cell lines (Chen et al., 2011; Ohtsuki et al., 2004; Sati et al., 1985; Yang et al., 2006; Yokosuka et al., 2009; Yokosuka and Mimaki, 2009), is an potential anti-inflammatory (da Silva et al., 2002; Peana et al., 1997) and anti-fungal properties (Anwar and Hussain, 2017; Yang et al., 2006).

Working *in vitro*, several researchers have proven that several steroidal saponins from *Agave* spp. have anti-cancer effects on various human cell lines. Chen and others (2011) working with hecogenin from *Agave sisalana* found that the saponin was cytotoxic to NCI-H460, MCF-7, and SF268 human cell lines when doses of 4.0 - 6.5 µM, 5.3 - 9.5 µM and 8.2 - 11.9 µM were used, respectively. In 2009, Yokosuka and Mimaki determined that gitogenin from whole *Agave utahensis* induced cytotoxic effects on the HL-60 cell line at doses between 5.5 to 12.3 µg/mL. The saponin, smilagenin from whole *Agave utahensis* was also found to be cytotoxic to the HL-60 cell line at doses between 4.9 - 7 µg/mL (Yokosuka et al., 2009). Isolates from *Agave americana* containing the saponins, hongguanggenin or tigogenin, were also used to induce cytotoxicity in BT-549 and HepG2 cell lines at doses of 10 µg/mL and 4.8 µg/mL, respectively (Yang et al., 2006). Ohtsuki and others (2004) found that chlorogenin hexasaccharide isolated from *Agave fourcroydes* produced cytotoxic and cell cycle inhibitory activities on the HeLa cell

line at a dose of 13.1 µg/mL. These researchers also determined that similar inhibitory activities were provided in HeLa cell lines using isolates of tigogenin and hecogenin at doses of 4.8 µg/mL and 5.2 µg/mL, respectively. In early research, Sati and others (1985) showed that the glycoside cantalasaponin 1 (hongguanggenin), a novel spirostanol bidesmoside from *Agave cantala*, was cytotoxic to the JTC-26 cell line at an unspecified dose.

Several saponins from *Agave* spp. also have anti-inflammatory properties. Peana and others (1997) showed that aqueous extracts of *A. americana,* and genins (steroidial sapogenins) isolated from them, showed good anti-inflammatory properties when administered by the intraperitoneal route at doses equivalent to 200 and 300 mg/kg of fresh plant starting material. The researchers also demonstrated that doses of genins (total steroidal sapogenins, hecogenin and tigogenin) equivalent to the amount in the lyophilized extracts produced an anti-edematous effect, which was much stronger and more efficacious than that obtained with an i.p. administration of 5 mg/kg of indomethacin or dexamethansone 21-phosphate at a dose equivalent to the molar content of hecogenin administration. At the doses used to evaluate the anti-inflammatory activity, the genins did not have any harmful effects on the gastric mucous membranes. However, lesions occurred when significantly higher doses of hecogenin were given, but gastric damage was still less than that caused by the drugs used for comparative purposes (Peana et al., 1997). Oral administration (300 and 500 mg/kg) of a leaf extract of *A. intermixta* Trel. into carrageenan-induced endemic rats were also shown to produce a marked anti-inflammatory effects (81.4 ± 4.1% inhibition; $P<0.001$), which was comparable or greater than that of the reference compound, dexamethasone, used in the study (García et al., 2000). García and others (2000) also found that topical application of the extract (2 and 5 mg/mouse ear) also produced a 50% reduction in tetradecanoylphorbol acetate-induced edema in mice. Salazar-Pineda and others (2017) also recently found that dichloromethane and acetone extracts of *Agave cupreata* provided an inhibitory effect on the formation of edemas of 64.29% (ED_{50} = 107.55 mg/kg b.wt.) and 48.82%, respectively when inflammation was induced with λ-carrageenan and being induced by TPA it was 62.47% (ED_{50} = 1.21 mg/ear) and 40.82%. Hexane and dichloromethane extracts of the agave plants also showed a significant antibacterial effect against the pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 16 g/mL dose.

In later study, Da Silva and others (2002), working *in vivo* in BALB/c mice, showed that sarasapogenin glycoside from *Agave attenuata* significant potential to reduce acetic acid-induced vascular permeability almost at the same levels as an indomethacin, but at concentrations ten-fold higher.

Steroidal saponins of agave also are known to have anti-microbial properties. Dried ethanol extracts of the roots of *Agave lecheguilla* Torr. (Agavaceae) were found to have activity against several pathogenic bacteria and fungi with minimal inhibitory concentrations ranging from 3.3 - 12 mg/mL (Verástegui et a., 1996). Two of the tetratriacontanol derivatives exhibit significant anti-bacterial activity (Anwar and Hussain, 2017). The two major steroidal saponins in agave, tigogenin and hecogenin, have also been found to provide anti-fungal properties to several microbial organisms. Yang and others (2006) determined *in vitro* that tigogenin, extracted from *Agave americana*, inhibited the yeasts, *Candida albicans* and *Candida glabrata* at a dose of 10 µg/mL, *Candida krusei* at 20 µg/mL and *Cryptoccocus neoformans* at 1.25 µg/mL. Ingestion of *Agave brittoniana* leaf extracts and semi-purified fractions

have also been evaluated for growth inhibitory activity against the human parasite *Trichomonas vaginalis*, which is the causative agent in Trichomoniasis or (trich). Guerra and others (2008), as reported by Mehriardestani and others (2017) demonstrated that a crude extract of the agave leaves inhibited the parasite *in vitro* growth by 99% at 24 and 48 hours at 10 µg/mL.

Researchers Misra and Varma (2017) showed in mice that an extract of *Agave americana* markedly improved wound healing, the rate of epithelialization for 10% hydroalcoholic extract (10% HEAA) was almost comparable to a standard Soframycin ointment treatment. The plant contains flavonoids, tetratriacontanol and homoisoflavanoids that help in wound healing. The authors also mention that the plant also contains genins that help in reducing the inflammatory process. Like other studies involving agave and anti-inflammatory properties, a recent follow-up study by the Misra research group showed that an hydroalcoholic extract of *A. americana* improved the percentage inhibition of edema in experimental animal paws in graded doses. Doses of 200 and 400 mg/kg of the extract significantly ($P<0.001$) reduced weights of granuloma as compared to a control in cotton pellet-induced granuloma model (Misra et al., 2018). The 400 mg/kg dose was almost comparable to aspirin, and is comparable to the standard, indomethacin in carrageenan-induced paw edema model.

8.3 Pertinent Scientific Literature Search Strategy

A minimum of 17 literature databases were searched using electronic search tools for this GRAS assessment on IMAG Organic® using searched words and terms follow that follow, as no Chemical Abstract Registry Number (CASRN) is available for agave inulin by itself:

> *Agave inulin, agave fructans, agave polyfructosans, agave fructosans, agave carbohydrates, agavins, graminan, GRAS fructans, prebiotic fructans, fructans, fructans and agave, fructans functional foods, inulin, oligofructose or fructooligosaccharides or prebiotic fructans, FOS or scFOS or sc-FOS, chicory inulin or levans or Jerusalem artichoke or murnong or yacon or dahlia or camas or elecampane or onion or garlic or agave, or Synergy 1 or Synergy One, or oligofructose-enriched inulin or Nutraflora or Neosugar or Actilight or Meioligo.*

Electronic Databases Used to Retrieve Literature and Provider(s)

- AGRICOLA (National Agricultural Library)
- AGRIS (FAO, United Nations)
- BIOSIS® Toxicology
- CAB Abstracts (CABI)
- ChemID Plus
- Chemical Abstracts Service (American Chemical Society)
- Chemical Carcinogenesis Research Information System (CCRIS)
- Cochrane Library (Wiley Interscience)
- EMBASE (Elsevier)
- GENE-TOX
- Google Scholar (Google)
- GoPubMed (Transinsight)
- Hazardous Substances Data Bank (HSDB)
- Integrated Risk Information System (IRIS)
- Medline (*via* PubMed)
- PubMed (NIH, NLM)

- Registry of Toxic Effects of Chemical Substances (RTECS)
- Science Direct (Elsevier)
- Scopus (Elsevier)
- SpringerLink (Springer)
- Toxline - Core and Special
- TRI (Toxics Release Inventory)

The literature search for this chemical was initially conducted in 2002 during the development of GRN 118 for chicory inulin, albeit agave terms were not used. The literature search was again performed, with agave terms, between March 2016 and March 2017 as a comprehensive review for "Microbiota in Health and Disease: Pathogenesis to Therapy" (Tungland, 2018), and was performed again, and updated in April, May, and June 2020. This document includes all relevant information retrieved as a result of these searches.

In addition to these searches, a search on the FDA website for possible relevant information pertaining to the term, Agave yielded 26 hits. All 26 FDA entries were reviewed, and the following items were considered relevant to this GRAS Notice. These items have been addressed where appropriate within this document.

1. FDA poisonous plants database lists five species of Agave, as having poisonous constituents that are primarily located in the leaves and roots, as described in the Safety section of this document. This list does not include *Agave tequilana* Weber var. *azul*, the genus and species of the notified substance, and the stems or piñas are used from *A. tequilana* to manufacture the notified substance, not the leaves or roots. The five agave species listed in the FDA poisonous plants database include: *A. americana, A. atrovirens* (maguey), *A. fourcroydes* (henequen), *A. sisalana* (sisal), and *A. victroiae-reginae*. (http://accessdata.fda.gov/scripts/Plantox/detail.CFM?ID=5850). Retrieved 5.19.2020.

2. Agave nectar, not agave inulin, has been included in a couple products that were the subject of FDA-initiated recalls due possible health risk. On August 23, 2011 FDA initiated recall of Xymogen Bars that may contain undeclared peanut protein. On July 19, 2016 FDA initiated recall of "Agave Dream" brand Cappuccino Ice Cream due to potential *Listeria monocytogenes* contamination. The subject of this GRAS Notice is agave inulin, not hydrolyzed agave inulin syrup, agave nectar.

3. In addition, two Department of Health and Human Services Warning Letters were issued in 2015, one (5.12.15) pertaining to the inaccurate description of the term "organic agave nectar" in labeling for "Laughing Giraffe Organics" Pineapple Snakaroons product. The other, for Nikki's Ginger Tea, LLC (3.18.15), for among other stated issues, failing to declare the common or usual name of agave syrup in accordance with 21 CFR § 101.4. Again, neither warning letters pertain to agave inulin, the subject of this GRAS Notice.

4. Two alerts were reported by FDA on October 1, 2010, pertaining to agave inulin products, described as "Fiber Agave Inulina" from Agaviotica S.A. de C.V.; Distrito B 4 No. 433,

Monterrey, MX. The products were defined as unapproved new drug or misbranded drug and subject to Detention without Physical Examination. The alerts were related to label claims, and the firm and its agave inulin product are not the manufacture or substance of this GRAS Notice.

5. In addition, a single agave inulin appeared on an FDA import refusal on November 10, 2010, regarding agave inulin produced by Agaviotica S.A. de C.V.; Distrito B 4 No. 433, Monterrey, MX for a new drug without an approved new drug application. The product is not manufactured by IMAG, the manufacturer of IMAG Organic®, the notified substance of this GRAS Notice.

6. Further, there were import refusal reports for 3 agave syrups and 2 agave honeys. These violations were due to the presence of pesticides between August 2007 and January 2009 and the manufacturer listed was "Extrusiones Home S de RL De CV, Juan Valdivia 36, Col 5 De Mayo, Guadalajara, MX." Products manufactured by IMAG were not subject of any of these violations.

7. Agave syrup was included in the final Risk Assessment for the final evaluation of risk for International Adulteration (May 2016), and final rule 21 CFR 117.5(2)(g)(2)(xx) syrups.

8. Agave syrup from Mexico has been monitored for pesticide residues. No residues were found out of approximately 44 syrup samples monitored.

9. Agave inulin was included in the "Review of the Scientific Evidence on the Physiological Effects of Certain Non-Digestible Carbohydrates, June 2018, citing "the strength of the evidence supports that inulin-type fructans extracted from chicory root or extracted inulin-type fructans from all sources, as well as synthetic inulin-type fructans, have a beneficial physiological effect on bone mineral density and absorption of calcium. The evidence from which scientific conclusions could be drawn supports our decision to propose to include inulin and inulin-type fructans in the definition of dietary fiber and, until of such a rulemaking, to consider enforcement discretion for declaring the amount of inulin and inulin-type fructans as dietary fiber."

9.0 Expert Panel Consensus Statement on GRAS Status of Agave Inulin for Use in Human Foods

9.1 GRAS Criteria

As it applies to food ingredients, safe or safety is defined by FDA in Title 21, volume 3 of the Code of Federal Regulations, revised April 1, 2019, Title 21 CFR 170.3(i) as:

> "...a reasonable certainty in the minds of competent scientists that the substance is not harmful under the conditions of its intended use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance." (FDA, 2019b)

Further, FDA, in 21 CFR 170.3(i)(1) and (2), acknowledges that safety may be determined by scientific procedures or by general recognition of safety, and that determining safety should include the probable consumption of the substance and of any substance that is formed in or on food because of its use, and the cumulative effect of the substance in the diet, while taking into account any chemically or pharmacologically related substance or substances in such diet (FDA, 2019b). FDA further notes in 21 CFR 170.3(i)(3) that in regard to the general recognition of safety of a substance, this safety requires common knowledge of the substance throughout experts in the scientific community that are qualified by scientific training and experience to evaluate the safety of food and food ingredients. According to 21 CFR 170.3 about "Substances Generally Recognized as Safe", FDA defines the requirements of common knowledge in the scientific community as:

- a general availability of data and information used to establish safety, most commonly established utilizing peer-reviewed scientific public domain literature, and;
- a basis to conclude a consensus among qualified scientists that a substance is safe for its intended use, through review of secondary scientific literature, such as published review articles, textbooks, or compendia, or by obtaining opinions of Expert Panels or opinions from authoritative bodies, such as the National Academy of Sciences.

By using terms such as "appreciable", "at the time" and "reasonable certainty", imprecise terms, FDA concedes that providing inequitable safety is not possible in this area (Lu, 1988; Renwick, 1990; Rulis and Levitt, 2009).

9.2 Introduction

Foods and beverages manufactured from the *Agave tequilana* Weber var. *azul* plant have a substantial history of human consumption. Data in literature show from human remains dating back at least 10,000 years that agave was used as food and fiber. In 1520, agave was exported to Europe and was mentioned in the Florentine Codex of 1580 as a food of Aztecans and natives. Fructan-containing products derived from agave and other plants have been commercially available and sold on-line and in health food stores in the U.S for more than three (3) decades, beginning in the 1990s. Inulin is legally classified as food or a food ingredient in most world countries, including all EU countries, Australia, Canada, and Japan (Franck, 2002). The EU Standing Committee meeting of June 1995 also confirmed oligofructose, a partially-hydrolyzed inulin, as a food ingredient (EC, 1995). Eleven GRAS notifications for plant-derived (inulin and oligofructose) and non-plant derived (short chain fructooligosaccharides, scFOS) have been issued for use as food ingredients for addition to several specific conventional food and beverage applications along several different food categories, including non-exempt and exempt infant formulas (GRN 44 [2000], 118 [2002a], 392 [2012], 477 [2014b], 537 [2015a], 576 [2015b], 605 [2016b], 623 [2016c], 717 [2018a], 849 [2019a], and 854 [2020]). Seven of these GRAS Notices that are specific to inulin have been submitted to FDA (GRN 44, FDA 2000; GRN 392, FDA 2012; GRN 477, FDA 2014b; GRN 537, FDA 2015a; GRN 849, FDA 2019a; GRN 854, FDA 2020). GRAS Notice 854 is for agave inulin, similar to the notified substance in this GRAS Notice. Without exception all GRAS notifications were accepted with no questions. The safe use of scFOS derived from enzymatic action on sucrose, an alternative to FOS derived from inulin, both being short-chain fractions/subsets of inulin, was also evaluated by the

Foods Standards Australia New Zealand (FSANZ) in 2008 (FSANZ, 2008), concluding that "there is a history of safe use of inulin-derived substances in food in Australia and New Zealand, and that it is as safe as inulin-derived substances (IDS) that are already permitted as additives to foods and infant formula, infant foods and formulated supplementary foods for young children either alone or in combination with IDS and GOS (galacto-oligosaccharides) up to the currently permitted maximum levels. "So, food manufacturers do not need express permission to add these substances to the general food supply" (FSANZ, 2008). Since 2001, inulin has appeared in a wide range of foods and is predominantly labeled as dietary fiber (FSANZ, 2008).

In Japan, scFOS (as Neosugar®), also defined as inulin, has a long history of safe use as a general food use low-calorie sweetener since 1983, and is listed in the Japan Ministry of Health, Labor and Welfare (MHLW) as FOSHU (Foods For Specified Health Issues). FOS is listed in the Approved FOSHU products list as oligosaccharides and classified as "foods to modify gastrointestinal conditions". Foods included in this list are reviewed for their effectiveness in attaining given health functions by the Council on Pharmaceutical Affairs and Food Sanitation (Japan MHLW, 2020).

As a food or food ingredient, inulin can be used without limitation in food and beverages. The Association of Official Analytical Chemists (AOAC) mentions two methods of analysis for fructans (AOAC 997.08) and (AOAC 999.03) to accurately measure the content of inulin and oligofructose in food and food products.

Inulin from *Agave tequilana* Weber var. *azul,* blue agave head, chicory root, and Jerusalem artichoke tuber are among food ingredients considered "natural" based on technical specification as defined under International Organization for Standardization (ISO/TS) 19657:2017, "food ingredients obtained from plant-based source materials by physical and/or enzymatic and/or microbiological processing without alteration of the ingredient from its original source".

The food ingredient evaluated by the Expert Panel is IMAG Organic® agave inulin, extracted and manufactured by IMAG without modification from this native, polydispersed chain distribution originally existing within the piñas of *Agave tequilana* Weber var. *azul*, which is compositionally equivalent to the agave inulin described in GRN 854 (FDA, 2020). The evaluation of the safety of IMAG Organic®, was accomplished through review of the extensive database on the safety of inulin and related β(2-1)-linked fructans, oligofructose and fructooligosaccharides. This review included the product's manufacturing process, gastrointestinal and metabolic fate, animal studies and human exposure.

The pivotal information supporting the safety of IMAG Organic® is summarized in GRN 854 (FDA, 2020), and corroborated by information available for other related inulin-type fructans. Therefore GRAS status is based on the chemical and compositional similarities of all β(2-1)-linked fructans, published studies supporting the safety of other fructan preparations, the expected levels in the diet of fructans from agave inulin, and the safety and tolerability of agave inulin as demonstrated from animal toxicity studies and human clinical trials,.

IMAG requested that an independent panel of scientists (the "Expert Panel"), qualified by their scientific training and relevant experience to evaluate the safety of food ingredients, be convened to conduct a

critical and comprehensive evaluation of the available pertinent data related to the use of IMAG Organic® agave inulin manufactured by IMAG as a food ingredient. For the purposes of their evaluation of the scientific data, the terms "safe" and "safety" imply that no reasonable doubt exists that agave inulin from IMAG would cause any harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR § 170.3(i).

The Expert Panel, provided an independent and collective comprehensive evaluation of IMAG Organic® incorporated into foods, through review of extensive information and data on the safety of inulin and related β(2-1)-linked fructans, including plant-derived native inulin, oligofructose, non-plant-derived short chain fructooligosaccharides prepared from a comprehensive search of the scientific literature. This review also included both favorable and unfavorable data and information, as well as details pertaining to the method of manufacture and product specifications, supporting analytical data, intended conditions of use of IMAG Organic® in food, estimated exposure under the proposed food uses (estimated daily intake or EDI). The Panel also evaluated other information deemed pertinent or appropriate.

The safety of InufibTM is predicated on multiple factors which include:

- The similarity of the composition of agave inulin to that in GRN 854 as well as to other GRAS inulin-type fructans;
- The high degree of purity of IMAG Organic where an estimated 99% of the product is inulin and other carbohydrates and impurities from the agave plant---such as saponins and terpenes--- are at a very low level;
- The expected levels in the diet of fructans from agave inulin; and
- The safety and tolerability of agave inulin as demonstrated by animal studies and human experience.

Following its comprehensive evaluation of the available data and information, the Panel convened on November 12, 2020, and unanimously concluded that IMAG Organic® agave inulin, as manufactured by IMAG, meeting food- grade specifications, and manufactured according to current Good Manufacturing Practice (GMP), is GRAS under all conditions of intended use, This GRAS determination was based on scientific procedures, and further basis for the Expert Panel's conclusion is provided in the follow summary.

9.2.1 Composition of IMAG Organic® agave inulin and similarity to other plant-derived fructans

After reviewing the manufacturing process used to produce the IMAG Organic® agave inulin product, its food grade specifications and batch analyses, the Panel agrees that IMAG's manufacturing and analytical procedures provide significant documentation that the IMAG Organic® agave inulin product is food grade.

Inulin substances described in other GRAS Notices that have received no questions from FDA (44, 537, 605, 623, 717, 849 and 854) are chemically similar Short-chain (scFOS) substances described in GRN 44,

537, 605, 623 and 717 are all manufactured in a similar manner, utilizing a food grade enzyme to biotransform sucrose to fructose oligomers having a β(2-1)-fructofuranosyl linkage, which sometimes end in a glucosyl unit, like those in the GRAS Notices for inulins from chicory root, Jerusalem artichoke tubers and agave piña, as described in GRNs 118, 849 and 854, respectively. These plant derived inulins (native, polydispersed inulins [GRN 118, GRN 849 and GRN 854], partially hydrolyzed chicory inulin as oligofructose [GRN 392, 576], and long chain chicory inulin from cooling crystallization precipitation [GRN 576]), all begin from water extraction of native inulin-containing plant material, followed by filtration. The oligofructose embodied within GRN 392 and 576, and the long chain inulin embodied within GRN 477 and 576 undergo further hydrolysis or fractionation to yield shorter chain oligomers or longer chain inulin molecules, respectively. However, all plant-derived fructans, including agave inulin described in GRN 854 and the substance in this GRAS Notice, as well as synthetically-produced fructooligosaccharides have the same basic β(2-1)-fructofuranosyl linkages, with varying degrees of β(2-6)-branching. Due their basic structural similarities, their common metabolic pathways, i.e. fermentation by the resident microbiota of the colon, are all the same. Carabin and Flamm (1999) proposed a working definition for inulin/oligofructose and fructooligosaccharides after a thorough review of published literature, as determined by the degree of polymerization (DP), which comprises the number of individual monosaccharides that constitute the molecule. In review of the available public-domain studies on a variety of oligosaccharides with differing DP, degree of molecular branching and plant source, concluded that none of the fructans demonstrated any toxicological differences.

The Panel further reviewed the chemical compositions of two similar inulins (from chicory root and agave piña) that have attained GRAS status (GRN 118:FDA, 2002 and GRN 854: FDA, 2020) and notes that fructans extracted from both plant sources contain nearly identical inulin concentrations (about 90%) and mono-disaccharide levels, consisting of fructose, glucose and sucrose (10%). Furthermore, historical human consumption information reviewed from the public domain (Tungland, 2018; Van Loo et al., 1995) shows that fructan-containing plant species have been commonly consumed as part of the normal diet of humans for centuries. The same molecular linkages in these commonly consumed vegetables and grains (e.g. agave, asparagus, garlic, leek, onion, Globe artichoke, tomatoes, Jerusalem artichoke, scorzonera, wheat, rye, barley, and chicory roots) vary only quantitatively, not qualitatively.

IMAG Organic® agave inulin is produced in a similar manner as the other GRAS approved plant-derived fructans. The manufacturing process is conducted in accordance with current Good Manufacturing Practices (GMP), Food Safety System standards: ISO 22000:2005, ISO/TS 22002-1:2009 and FSSC 22000 v. 4 standards), and the principles of Hazard Analysis and Critical Control Point (HACCP) and (see Appendices 2, 3, 4, and 5, respectively). In summary, IMAG's agave inulin manufacturing process is similar or equal to that utilized to produce the agave inulin described in GRN 854, an agave inulin receiving no questions from FDA. Like that of Inufib™ in GRN 854, IMAG Organic® agave inulin utilizes all physical purification techniques involving mechanically slicing/milling agave piña, followed by water extraction via counter-current diffusion, multi-step physical solid-liquid separation via filtration using progressively tighter porosity and then juice concentration in a multi-effect evaporator to produce liquid agave inulin syrup, or can be subsequently spray dried to high purity powdered agave inulin. IMAG agave inulin manufacturing does not utilize any solvents, other than water or other chemicals in its production. All materials used in the production of this agave inulin meet food-grade and organic

labeling quality specifications and are permitted for use in food and organic-food labeling by U.S. federal regulation or are GRAS for their respective uses. The production process has many certifications, and analyses of random, non-consecutive production lots show that no pesticides or biocides are present, and that IMAG Organic® agave inulin meets or exceeds all microbiological, heavy metal, and dioxins and PCB standards.

Based on its raw materials, production methods, and available compositional analyses, the Expert Panel agrees that IMAG's agave inulin manufacturing process utilizes manufacturing processes that are typical for extracting and purifying carbohydrate-based food ingredients and produces a fructan product that is consistent with the composition of other food-grade inulin-type fructan preparations as discussed above. Further IMAG's agave inulin preparation is chemically representative of other GRAS inulin and inulin-type fructans sources. The Expert Panel also concludes that no novel processes are employed during the production of IMAG Organic® agave inulin from IMAG that would introduce new reaction products or impurities to the notified ingredient.

IMAG Organic® agave inulin typically contains 98-100% carbohydrate on a dry basis, with approximately 90% as refined inulin and up to 10% mono- and disaccharides, of which, are mainly fructose, glucose and sucrose. Secondary metabolites are only present in trace amounts, being below limits of method detection. Saponins and terpenes are not detected at levels as low as 7 ppb) and fatty acids are not detected at levels as low as 70 ppm. By comparison with data from 2004 by Pena-Alvarez and others of three agave plant species, that include the species of this GRAS Notice and that in GRN 854, *A. tequilana* Weber var. *azul,* the concentrations of fatty acids in the stems (piña) of *A. tequilana* was 985 μg/g (about 0.1%). Regarding terpene levels in agave, Pena-Alvarez and others (2004) identified thirty-two (32) types but did not quantify the terpenes due to their very low concentrates. The principal terpene in agave piña, which is also found in most edible fruits, herbs and spices, is GRAS approved by FDA and utilized accepted as a flavoring agent up to 25 mg/kg b.w. by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and utilized as such in foods with estimated daily consumption from these sources of 40 to 140 μg/kg-d (OECD, 2002).

In later study, Ávila-Fernández and others (2009) showed that tequila, a product made from *A. tequilana* Weber var. *azul,* had a combined terpene/terpenoid level of 1-3 mg/L and a linalool content of 0.5 mg/L. Data calculated based on the linalool level in tequila and extrapolated to dried agave inulin, as shown in section 8.1.3, show that the potential levels in the dried product is about 0.4 mg/kg. If consumed at 20 g/d, the intake of linalool from the dried agave inulin would total 7.6 μg/d or < 0.15 μg/kg-d for a 70 kg person. The terpene is classified as a moderate skin irritant, but it's potential to cause sensitivity is weak. Linalool (CAS No. 78-70-6) is not considered mutagenic or carcinogenic. There is no evidence of bioaccumulation, as it is relatively rapidly excreted. The Organization for Economic Cooperation and Development (OECD) lists the acute oral mammalian toxicity of linalool low with a rat oral LD_{50} of close to 3,000 mg/kg b.w. (2,790 mg/kg b.w.), and the acute dermal toxicity that is ≥ 2,000 mg/kg b.w. Based on data from a 28-day oral rat study using 72.9% linalool and its effects of linalool on liver and kidney, the OCED lists a NOAEL of 160 mg/kg b.w/d (equivalent to 117 mg/kg b.w/d linalool) was derived (OECD, 2002). The Panel concludes that the estimated concentration of linalool in the piña tissue is well below concentrations posing any safety concern.

Saponins normally present in and isolated from leaves, roots and fruit of agave, have not been detected in the stems (piñas), the source of the agave plant utilized to produce agave inulin, the subject of this GRAS Notice or that described previously in GRN 854 and receiving no questions by FDA (FDA, 2020).

9.3 Evidence of Safety from Animal and Human Studies

The Expert Panel has reviewed extensive data published in literature on agave inulin and its similar fructans, such as chicory and Jerusalem artichoke inulin, oligofructose and fructooligosaccharides. These data include information from animal and clinical studies, comprehensive critical reviews on inulin and inulin-related fructans, international regulatory summaries, and previous GRAS notification submissions on inulin and fructooligosaccharides. In addition, the during the preparation of this GRAS Notice, Tungland and Associates, LLC, a consulting group with extensive scientific experience in food and food ingredient research, food chemistry, regulatory processes, and a world inulin expert, conducted comprehensive literature and databank search in 2002 during the development of GRN 118 for chicory inulin, albeit agave terms were not used. The literature search was again performed, with agave terms, between March 2016 and March 2017 as a comprehensive review for "Microbiota in Health and Disease: Pathogenesis to Therapy" (Tungland, 2018), and was performed again, and updated in April, May, and June 2020 for this GRAS Notice involving IMAG Organic® agave inulin. This document includes all relevant information retrieved as a result of these searches. The Expert Panel has reviewed all relevant information presented in this Notice regarding these studies and those presented in GRN 854, an agave inulin with equivalent manufacturing processes, specifications and analytical analyses as the agave inulin in this GRAS Notice, and also performed a critical review of the IMAG Organic® production process, and compared it with that of Inufib®, the agave inulin described in GRN 854. These efforts resulted in the Panel concluding that IMAG Organic® agave inulin, manufactured by IMAG at usage levels described herein is Generally Recognized as Safe (GRAS) for its intended uses in human foods.

In the following the Panel has summarized the critical elements taken into consideration in the evaluating the safety of inulin, oligofructose and fructooligosaccharides from evidence in animal and human studies.

1. The **structure of agave inulin** consists of linear and branched inulin and levan type fructans that are composed of fructose units join by β(2→1) and β(2→6)-glycosidic linkages, with one glucose moiety per molecule, with either a terminal (external), as in graminans or and internal position, as in neofructans (agavins). The types of linkages in these fructans vary only quantitatively, not qualitatively, as they are similar. The Panel finds no reason to believe that the branched carbohydrate would present any systemic toxicity concern. As described in the authoritative review of fructooligosaccharides and inulin, the only issue of safety in humans are primarily untoward flatus effects due to slight digestive intolerance, which is self-limiting. As defined in GRN 854 and reviewed in Section 1.5 of this GRAS Notice, scientific evidence supports the conclusion that agave inulin will be well-tolerated at the proposed use levels.

2. The **metabolic fate** of agave inulin from metabolism and gastrointestinal tract (GI) studies show that the molecule is largely resistant to hydrolysis by human digestive enzymes and

that it is transported mostly intact to the colon where resident microbiota utilize the substrate as an energy source *via* fermentation. The absorption, distribution, metabolism and excretion of inulin and inulin-type fructans along with the physiological effects on the gastrointestinal tract and systemic effects on metabolism related to inulin and inulin-type fructan consumption is well characterized and has been previously described in detail in GRAS notifications (GRN 44 [2000b], 118 [2002a], 392 [2012], 477 [2014b], 537 [2015a], 576 [2015b], 605 [2016b], 623 [2016c], 717 [2018], 849 [2019a], and 854 [2020]) and by qualified scientific experts in published comprehensive reviews (Roberfroid et al., 2010; Roberfroid and Delzenne 1998; Grizard and Barthomeuf 1999; Carabin and Flamm 1999; Flamm et al. 2001; Boeckner et al. 2001; Kaur and Gupta 2002; Roberfroid 2007; Kelly 2008, 2009; Tungland, 2018). Generally, scFOS and related β(2→1)-linked fructans, including those from agave, are not absorbed and are resistant to digestion by salivary amylase, human pancreatic or intestinal enzymes. Agave inulin, like the other β(2→1)-linked fructans, reaches the large intestine largely intact where resident microbiota utilizes them as substrate for fermentation. Colonic fermentation results in gases (methane, carbon dioxide and hydrogen), short chain fatty acids (acetate, butyrate and propionate), heat, and additional bacterial mass. Any unfermented non-digestible fructan is excreted in the feces. Since all β(2→1)-linked fructans are qualitatively equivalent, they are handled in an equal physiological manner.

3. **Animal toxicity studies** on agave inulin show an oral LD_{50} value of > 5 g/kg b.w. in rats and mice, a low order of toxicity. Diets enriched with 10% agave inulin that were consumed by mice in a 5-week study showed no toxicity in a comparison with a 50:50 blend of chicory oligofructose-enriched with long chain chicory inulin (Beneo-Orafti Synergy 1), a product currently in wide use in the world food supply. Another mouse comparison study with the same chicory inulin product also showed no toxicity at gavage doses of 5 g/kg-b.w./d over 12-weeks of feeding. Fructooligosaccharides (Neosugar®, scFOS) have shown low order of toxicity in all animal studies (no significant adverse effects up to 2,664 mg/kg/day (the No Observed Effect Level (NOEL) for chronic administration of Neosugar® FOS), which included no significant effects on reproduction, developing fetuses or on newborns. In addition, scFOS is also shown to yield negative skin sensitivity in guinea pig studies.

4. ***In vitro* genotoxicity and mutagenicity studies** on Neosugar® (short-chain fructooligosaccharides), carboxymethyl inulin, and agave inulin have all shown no *in vitro* mutagenesis or clastogenesis.

5. **Animal carcinogenicity study** in rats showed no evidence of carcinogenicity or incidence of neoplasms at dietary concentrations of scFOS up to 50,000 ppm after 2 years. The study also did not show any significant dose-related effects on body weight, food intake, mortality, growth, blood chemistry or hematology, or organ weights.

6. **Human clinical studies** show that inulin-type fructans, including agave inulin, are well tolerated, in historical and contemporary diets, and in clinical studies that used bolus,

short-term and long-term exposures. Consensus in the scientific community is that inulin-type fructans are non-digestible oligosaccharides that positively influence the composition and metabolic activity of human intestinal microbiota. Studies that have been reviewed by FDA for inulin's inclusion in the list of approved dietary fibers in the U.S. have shown that these fructans increase calcium and magnesium absorption, and have shown significant decreases in total serum cholesterol, blood glycemia, serum triglyceride and low density lipoprotein cholesterol levels.

9.3.1 Human Digestive Tolerance to Dietary Agave Inulin

The totality of the publicly available literature investigating the consumption and tolerance of inulin-type fructans in human subjects has been the subject of several comprehensive evaluations by several notifiers, independent expert panels and the FDA during previous reviews on the GRAS status of scFOS, as described in GRN 44 [2000b]; 537 [2014]; 605 [2016]; 623 [2016]; and 717 [2018a], oligofructose in GRN 392, long chain inulin GRN 477 [2014], oligofructose and inulin in GRN 576 [2015], and native chicory inulin in GRN 118 [2002a], Jerusalem artichoke inulin in GRN 849 [2019a], and native agave inulin in GRN 854 [2020]. By example, the first GRAS notification for scFOS [Neosugar] submitted to the FDA 18 years ago, GTC Nutrition concluded that the AIL (Acceptable Intake Level) for the intake of FOS in the general population, excluding infants that were less than one year of age, was 20 g/d, while the AIL for infants less than one year old is 4.2 g/d (GTC Nutrition, 2000). Repeated daily ingestion of agave inulin has also been shown to be well tolerated in adults over three 21-day periods when evaluated at doses of 5.0 or 7.5 g/d (Holscher et al., 2014). The first GRAS notice for inulin (native, polydispersed chicory inulin) submitted to the FDA 16 years ago, revealed that Imperial-Sensus, LLC (now Sensus America, LLC), concluded that the AIL for the intake of inulin in the general population, excluding infants less than one year of age, was 40 g/d, and was a conservative estimate of inulin tolerance as studies suggested that up to 70 g inulin/d, consumed as a regular part of the diet, may be well tolerated (FDA, 2002).

In the GRAS notice in 2002, a 1993 Japanese Infant Formula Survey noted that the safety and tolerance of ingestion of FOS by survey of 20,742 infants ingesting formula containing 0.32 g FOS/100 mL resulted in a mean and 90th percentile intake of 3.0 and 4.2 g FOS/d. A higher level of agave inulin was also well tolerated in infants when administered daily via infant formula for > 5 months at a concentration of 0.5 g/100 mL or approximately 7.5 g/d (López-Velázquez et al., 2013). The estimated daily intake (EDI) of inulin from all of the proposed used of IMAG Organic® for infants below 1 yr. of age were calculated to be 2.3 g/user/d and 5.7 g/user/d as the mean and 90th percentile, respectively, according to methodology of ENVIRON for Frutafit® (GRN 118, FDA, 2002a). They further calculated that the estimated 2-day mean and 90th percentile intake of chicory inulin by the U.S. population ages 2 years and older from all GRAS proposed use categories was 10.1 g/user/d and 19.2 g/user/d, respectively. Based on these estimated exposures the Panel concludes that IMAG Organic® agave inulin, for the proposed food uses and at the levels specified herein, is GRAS. Given that this GRAS determination excludes infant formula applications for agave inulin, the cited studies did not identify any safety concerns, and they further support human safety and the safety of IMAG Organic® for the general population.

9.4 Common Knowledge Requirements of the GRAS Conclusion

One of key provisions of a conclusion of GRAS for a substance is that the data and information used to establish safety be generally available, which is most typically established by utilizing peer-reviewed scientific literature published in public-domain journals. The majority of data and information used to establish safety in this GRAS Notice for IMAG Organic® are from such scientific literature and is widely available. The general common use of agave inulin, and all related inulin-type fructans and their use in food on a global basis and their absence of any significant harmful effects are commonly-known to scientists in the field from published information, including human clinical studies that support the their safety.

Numerous scientific reviews from well-known experts in the field of food toxicology and dietary fiber (e.g. Boeckner et al., 2001; Carabin and Flamm 1999; Flamm et al., 2001; Grizard and Barthomeuf 1999; Kaur and Gupta 2002; Kelly 2008; 2009; Kolbye et al., 1992; Roberfroid, 2007; Roberfroid et al., 2010, Slavin, 2013; Schaafsma and Slavin, 2015; Tungland, 2018) that have published critical and comprehensive reviews of available data and information, have unanimously concluded that, under the conditions of intended use in foods, inulin-type fructans that include agave inulin, is GRAS based upon the scientific studies reviewed. As these reviews have noted, there is no evidence in the public-domain that inulin-type fructans induce any acute chronic, reproductive or developmental toxicity, carcinogenicity or genotoxicity in tests at dose levels that are considerably higher than the anticipated human exposure.

Human intake and tolerance clinical studies of inulin-type fructans have shown some modest intolerance when dietary intake are above 20-30 grams. Recently, in critical review of available data and information in peer-reviewed literature on human inulin-type fructan tolerance, Schaafsma and Slavin (2015) noted that:

> *"the saccharolytic fermentation of inulin-type fructans increases bacterial mass and defecation frequency and helps to prevent constipation. All these effects are physiological and beneficial and are typical dietary fiber effects. Undesirable side effects (flatulence, bloating, borborygmus), frequently called intestinal discomfort, may occur at higher doses, but generally do not occur at doses below 20 g/d, when the dose is spread over the day and taken with meals."*

In addition to requiring that the data and information be generally available to make a GRAS assessment, a GRAS determination requires that there is consensus among qualified scientists that inulin-type fructans are safe for all its intended food uses. Reviews from qualified scientists, as noted previously, all conclude that without exception, inulin-type fructans are GRAS.

Furthermore, along with evidence provided in safety reviews by Roberfroid and Delzenne (1998) and Carabin and Flamm (1999), Kolbye and others (1992), an Expert Panel convened by Beneo-Orafti (Belgium) in their GRAS self-affirmation of inulin and oligofructose, noted that these inulin-type fructans are GRAS for their intended purposes. Although, not published peer-review literature, the GRAS self-affirmation for the inulin-type fructans are referred to in peer-reviewed literature and GRAS Notices (Coussement, 1999; GRN 44, 2000b; GRN 118, FDA 2002a). In citing their conclusions, the Expert Panel

composed of Kolbye and others (1992) found that the metabolic fate of inulin and oligofructose was that of non-hydrolysis in the stomach or small intestine, but complete fermentation by resident microbiota in the colon, preferrentially as substrate for growth of *Bifidobacteria* into harmless metabolites (notably, short-chain fatty acids, fermentation gases CO_2, H_2, and CH_4), heat and bacterial mass). The authors concluded that available animal toxicity studies did not show any expected adverse effects from use in foods at intended use levels. Furthermore, the authors concluded that inulin and oligofructose are dietary fibers by definition and their nutritional properties, although from a regulatory perspective for food labeling in the U.S., this was not officially approved until 2018. They further concluded that intake of inulin and oligofructose to be self-limiting as any untoward flatus response in the colon will prevent over-usage. Kolbye and others (1992) further concluded that the safety of inulin and oligofructose was based on a long human history of exposure from consuming inulin-containing foods, as well as an evaluation of their use in scientific studies. Related to their long history of human intake from commonly consumed foods, the Panel concluded that they had no reason to suspect that inulin and oligofructose would provide a significant risk to public health when they were used in foods as intended. The Expert Panel at the time summarized their self-affirmation of GRAS by concluding that the inulin-type fructans are Generally Recognized as Safe (GRAS), both by a long-established history of use in human foods and in the opinion of experts qualified by scientific training and experience in food safety after a thorough review of the available scientific evidence.

Furthermore, the global regulatory bodies such as the EU Commission (EU), the Food and Drug Administration (US-FDA), Food Standards Australia and New Zealand (FSANZ-Australia and New Zealand), the Ministry of Health, Labor and Welfare (MHLW-Japan), Healthy Canada (HC-Canada) have provided further consensus elements for inulin-type fructans.

In the U.S., chicory inulin (native inulin, oligofructose and short- and long-chain inulins), Jerusalem artichoke inulin, native agave inulin and short-chain fructooligosaccharides produced by enzymatic synthesis from sucrose), essentially spanning all commercial plant sources and inulin degrees of polymerization have all been determined to be GRAS without questions by FDA in eleven separate GRAS Notices: (sc-FOS) short-chain fructooligosaccharides (GRNs 44, 537, 605, 623 and 717) (FDA 2000b, 2015a, 2016b,c, 2018a), oligofructose (GRNs 392 and 576) (FDA 2012, 2015b), long chain inulin (GRN 477)(FDA 2014b), native chicory inulin (GRN 118)(FDA 2002a and 2007 amendment), Jerusalem artichoke inulin (GRN 849)(FDA, 2019a) and agave inulin (GRN 854) (FDA, 2020).

Inulin has food ingredient legal status in many world countries, including all EU countries, Australia and New Zealand, Canada, and Japan (Franck, 2002). As a food ingredient, inulin is used without any specific limitations in foods and beverages. The EU Standing Committee meeting in 1995 recognized chicory oligofructose as a food ingredient (EC, 1995), and inulin is classified as such, and not as a food additive according to the European Directive 95/002 on food additives (EC, 1995). Inulin from chicory roots, Jerusalem artichoke tubers and agave piñas is listed as an acceptable source of dietary fiber for human food by the Canadian Food Inspection Agency (CFIA, 2011), the U.S. Food and Drug Administration (FDA, 2018b), and for animal feed and pet food by the American Association of Feed Control Officials (AAFCO, 2018).

Moreover, because of its wide global acceptance as a safe food ingredient, food manufacturers have been adding inulin-type fructans to the general food supply, predominately as a dietary fiber since the mid-1990s, including the in the U.S. In fact, the 2008 FSANZ report declared that inulin-derived substances have a history of safe use in food in Australia and New Zealand, so food manufacturers do not need to express permission to add these substances to the general food supply (FSANZ, 2008).

Several other Expert Panel reviews of chicory, Jerusalem artichoke and agave inulin, chicory oligofructose and short-chain fructooligosaccharides made by enzymatic synthesis from sucrose have all agreed that there exists a consensus that these fructans are safe for their intended use in human food. The Panel reviews are included in previous GRAS Notices submitted to FDA for inulin and fructooligosaccharides, of different degrees of polymerization and degrees of molecular β(2,6)-branching, various plant sources and different methods of manufacture, including those by:

- GTC Nutrition company on sc-FOS made via enzymatic synthesis from sucrose (Neosugar®)(GRN 44, FDA 2000b);
- Imperial Sensus, LLC on native chicory inulin (Frutafit®) (GRN 118, FDA 2002);
- Pfizer Nutrition and BENEO-Orafti on chicory oligofructose (GRN 392, FDA 2012);
- Danone Trading B.V. on long chain chicory inulin (GRN 477, FDA 2014b);
- Nutrica North America, Inc. on chicory oligofructose and long chain inulin (GRN 576, FDA 2015b);
- Intrinsic Organics, LLC on Jerusalem artichoke inulin (GRN 849, FDA 2019a);
- Industrializadora Integral del Agave SA de CV (IIDEA) on agave inulin (GRN 854, FDA 2020).

All these food ingredients have received no questions by FDA. These and other in-depth reviews by experts qualified by scientific training and experience to evaluate safety of substances to be added to foods from data and information in published and unpublished sources, along with many global regulatory agency approvals and favorable positions for inulin's use in food and beverages, as presented throughout this GRAS Notice, all support the conclusion that inulin is safe for use in food.

9.5 Expert Panel Conclusions

We, the members of the Expert Panel, have carefully, independently and collectively, critically evaluated the information summarized in this document and the specific product data available from IMAG in concert with the potential human exposure to this substance and concludes that the proposed uses of IMAG Organic® in foods described elsewhere herein in this GRAS document, when produced in compliance with Good Manufacturing Practices requirements, and meeting appropriate food-grade specifications established by IMAG as presented in this document, is safe and suitable and GRAS based on scientific procedures, under the conditions of intended use in foods as described in GRN 854 for a similar or equal agave inulin. This declaration is made in accordance with FDA's standard for agave inulin safety, meaning that there is reasonable certainty of no harm under the intended conditions of use.

It is our opinion that other qualified experts would concur with these conclusions.

December 22, 2020

Dietrich B. Conze, PhD
Managing Partner
Spherix Consulting Group, Inc.

Date

December 22, 2020

Claire L. Kruger, PhD, DABT
Managing Partner
Spherix Consulting Group, Inc.

Date

December 22, 2020

Fred Lozy, PhD
Project Manager
Spherix Consulting Group, Inc.

Date

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER | DATE OF RECEIPT
ESTIMATED DAILY INTAKE | INTENDED USE FOR INTERNET
NAME FOR INTERNET
KEYWORDS

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*
☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person Francisco Young		Position or Title Sales and Marketing Director	
Organization *(if applicable)* Inulina Y Miel de Agave S.A. de C.V. (IMAG)			
Mailing Address *(number and street)* Calle Extramuros No. 125			
City Capilla de Guadalupe	State or Province Jalisco	Zip Code/Postal Code CP 47700	Country Mexico
Telephone Number +52 (378) 7010 112, 7010 377	Fax Number	E-Mail Address fyong@imag.mx	

1b. Agent or Attorney *(if applicable)*

Name of Contact Person Bryan Tungland		Position or Title President/CEO	
Organization *(if applicable)* Tungland and Associates, LLC			
Mailing Address *(number and street)* 10306 74th St., NE			
City Otsego	State or Province MN	Zip Code/Postal Code 55301	Country USA
Telephone Number 763-350-1590	Fax Number	E-Mail Address tungland@charter.net	

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term: **IMAG Organic®**

2. Submission Format: *(Check appropriate box(es))*
- ☐ Electronic Submission Gateway
- ☒ Electronic files on physical media
- ☐ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

- ☒ a) GRAS Notice No. GRN 44, 118, 392, 477, 537, 576, 605, 623, 717, 849, 854
- ☐ b) GRAS Affirmation Petition No. GRP _____
- ☐ c) Food Additive Petition No. FAP _____
- ☐ d) Food Master File No. FMF _____
- ☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

- ☐ Yes *(Proceed to Item 8*
- ☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

- ☐ Yes, information is designated at the place where it occurs in the submission
- ☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

- ☐ Yes, a redacted copy of the complete submission
- ☐ Yes, a redacted copy of part(s) of the submission
- ☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance. Intended for general addition to foods except non-exempt and exempt infant formula and meat and poultry products, as per Table 1.1 of the GRAS Notice. The same as GRN 854. Intended foods and max. use in food: Acidophilus milk (2%); Bars, all types (10%); Baby foods, all types 1g/serv.; Breakfast cereals, RTE (5 g/serv.); Beverages, juices & juice drinks (1.5%); Beverages, functional (5%); Beverages, milk-based (1%); biscuits, reduce energy (6%); Breads, conventional (0.5%); Breads, specialty (6%); baked goods, lite cakes (5%); candy, hard dietetic (15%); candy, soft dietetic (5%); cheese, cream-type (5%); cheese, processed and cheese products (5%); cheese, pasta fillings (5%); condiments (5%); cookies, reduced energy (8%); crackers (6%); dessert toppings, lite (6%); dessert toppings, excluding whipped toppings (2%); French fry coatings (1.7%); frozen dairy desserts (8%); icings/glazes, lite (5%); jams and jellies, lite (2%); mousse, reduced fat/energy (3%); pasta, fresh (4%); pasta, precooked macaroni (4%); pizza crust (5%); potatoes, mashed (3%); pretzels, soft (5%); salad dressings, lite (5%); sauces and gravies (2%); snack chips, reduced fat (3%); soups, dry (3%); spreads, reduced fat (10%); surimi, reduced fat (3%); syrups, lite (2%); tortillas, reduced fat (3%); vegetarian patties/crumbles (2%); yogurt, reduced fat (3%).

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture? *(Check one)*

Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture? (Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

☐ ☐

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

- [x] PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).
- [x] PART 3 of a GRAS notice: Dietary exposure (170.235).
- [x] PART 4 of a GRAS notice: Self-limiting levels of use (170.240).
- [x] PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).
- [x] PART 6 of a GRAS notice: Narrative (170.250).
- [x] PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

[x] Yes [ ] No

Did you include this other information in the list of attachments?

[x] Yes [ ] No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Inulina Y Miel de Agave S.A. de C.V. *(name of notifier)*

has concluded that the intended use(s) of IMAG Organic® *(name of notified substance)*

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Inulina Y Miel de Agave S.A. de C.V. *(name of notifier)* agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

Calle Extramuros No. 125, Capilla de Guadalupe, Jalisco, MX CP 47700 *(address of notifier or other location)*

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
[redacted]	Bryan C. Tungland, President/CEO, Tungland and Associates, LLC	12.28.2020

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
1	Insert / Clear GRAS Notification for IMAG Organic® Agave Inulin Extracted from Agave tequilana Weber var. azul	Submission
2	Insert / Clear References	Submission
	Insert / Clear	
	Insert / Clear	
	Insert / Clear	
	Insert / Clear	
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Add Continuation Page

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, PRAStaff@fda.hhs.gov. (Please do NOT return the form to this address). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

Sixty-seven pages have been removed in accordance with copyright laws. The removed reference citations are:

Bach Knudsen, K.E. and Hessov, I. 1995. Recovery of inulin from Jerusalem artichoke (Helianthus tuberosus L.) in the small intestine of man. Br J Nutr 74:101-103. Banguela, A., and Hernandez, L. 2006.

Bedour, M.S., Elgamal, M.H., and El-Tawil, B.A. 1979. Steroid sapongenins, part XV, The constituents of Agave utahensis var. nevadensis, A. lophantha and A. parasana. Planta Med 36(2):180-181.

IOAA (International Organic Agave Alliance). 2009. Retrieved from https://opuntiads.com/oblog/wp-content/uploads/2015/10/Agave-Ancient-History.pdf. Accessed July 30, 2018.

Lien, D.T.K., Nhung, B.T., Khan, N.C., Hop, L.T., Nga, N.T.Q., Hung, N.T., Kiers, J., Shigeru, Y., te Biesebeke,

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The Agency Response letter GRAS notice GRN 000623 was also removed. It can be found at https://www.fda.gov/food/generally-recognized-safe-gras/gras-notice-inventory

From: Bryan Tungland
To: Hall, Karen
Subject: [EXTERNAL] RE: Questions Regarding GRN 1019
Date: Monday, April 18, 2022 12:26:05 PM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png

Hello Karen,

Thank you for your comments. I have addressed each comment below in yellow.

Kind regards,

Bryan

From: Hall, Karen <Karen.Hall@fda.hhs.gov>
Sent: Wednesday, April 06, 2022 4:34 PM
To: tungland@charter.net
Subject: Questions Regarding GRN 1019

Good Afternoon,

During our review of GRN 1019, which you submitted for agave inulin extracted from *Agave tequilana* Weber var. *azul*, we noted concerns that need to be addressed. Please provide a response to the below questions within 10 business days. If you are unable to complete the response within that time frame, please contact me to discuss further options. If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

1. On p. 12, the Notifier states that the mean/range degree of polymerization is higher than that of the subject material of GRN 854. Additionally, OFAS notes that the subject agave inulin is composed of mixed fructans, relative to linear inulin molecules isolated from chicory root with increased mean/range of polymerization.

- Please clarify that these differences are unlikely to result in distinct physiological effects or raise safety concerns relative to other dietary fructooligosaccharides.

Response: As noted on p. 12, Table 2.1 of GRN 1019, the mean degree of polymerization (DP) of the notified substance is lower than that of the mixed agave fructan in GRN 854, while having a higher DP range. The mean DP and range of the notified substance is similar to that found in chicory root, as notified in GRN 118, and is consistent with an agave fructan from mature plants. In the human body, all DP fractions of inulin are readily fermentable by resident colonic microbiota that have

fructosidase enzymes to hydrolyze the beta 2,1 and beta 2,6 molecular linkages to ferment the resulting simple sugars to produce products of that fermentation, i.e. short chain fatty acids, gases, more microbiota and some heat. Literature shows that longer chain fractions take somewhat longer to fully ferment, while short chain fractions less time. From a tolerance standpoint, as expressed in that section of GRN 854 and GRN 1019, any tolerance differences between the various fractions is dependent on these rates, with slightly higher tolerance (reduced flatus effects) being favored for longer chain fractions, while somewhat less tolerance being associated with shorter chain fractions. The differences in the DP between the agave mixed fructans in GRN 854 and GRN 1019 are unlikely to result in any distinct physiological effects or raise any safety concerns. One would expect more noticeable flatus for short chain products, such as fructooligosaccharides (as in GRN 44 and others), as the fermentation rate can be more rapid, producing more gas per unit time than a fructan product having longer chain fractions.

2. On p. 18 the notifier describes clarification and purification procedures which are expected to remove potential calcium oxalate contaminants. However, no information is provided related to the assessment of calcium oxalate levels in the subject agave inulin. Increased consumption of calcium oxalate can lead to adverse events such as renal toxicity.
   - Please provide information that supports that levels of calcium oxalate in your subject material will not present a safety concern.

Response:

The agave fructan notified in GRN 1019 is produced from the same raw material (agave pina), has the same composition, has the same intended uses and concentrations, has the same specifications, and has the same method of manufacturing as that defined in GRN 854, a mixed agave fructan receiving a LONO by FDA.

As expressed in GRN 854 and GRN 1019, although raphides (calcium oxalate crystals) are known to be present in the leaves of Agave species and cause contact dermatitis, their concentration is much lower in pina or stem (the plant part used to produce refined agave fructans) than in the leaves, which are left in the fields during harvest. Hence, the distribution of oxalate within plants is also uneven, with oxalate being highest in leaves, then seeds, and is lowest in stems (Osweiler et al., 1985). Further, as calcium oxalate and many other calcium salts are significantly less soluble in water at elevated temperatures, these salts precipitate in the aqueous extract and a majority are filtered out prior to and/or after evaporation, when temperatures are near boiling. Because the agave fructan defined in GRN 854 and the notified agave fructan in GRN 1019 represent highly refined food ingredients, oxalate concentrations have not been a safety concern as part of normal diet that typically has many sources of naturally-occurring background oxalate (Noonan and Savage, 1999). According to the University of Chicago, the typical American diet contributes to upwards of 200-300 mg of oxalate per day (Harris, 2022), while a mean English diet is calculated to contribute between 70-150 mg/d (Noonan and Savage, 1999).

Osweiler, GD, Carson, TL, Buck, WB, et al. 1985. Clinical and diagnostic veterinary toxicology, 3rd Ed. Iowa: Kendall/Hunt Dubuque, IA.

Noonan, SC and Savage, GP. 1999. Oxalate content in foods and its effect on humans. Asia Pacific J Clin Nutr 8(1):64-74.

Harris, J. 2022. How to eat a low oxalate diet. Univ. Chicago. https://kidneystones.uchicago.edu/author/jharris/

To put this into perspective, Lippmann (2009) showed the calculation of an oxalate ADI based on animal toxicity data and utilizing an uncertainly factor for developmental toxicity studies of 0.2 mg/kg/d, reproduction studies of 2 mg/kg/d and for chronic studies of 3 mg/kg/d (Table 7.11, p. 221). For an average adult of 70 kg, that range (0.2 mg/kd/d to 3 mg/kg/d) represents an ADI of 14 mg to 210 mg oxalate per day.

Lippmann, M. Environmental Toxicants: Human Exposure and Their Health Effects, 3rd Ed., Morton Lippmann, ed., Wiley & Sons, 2009. Chapter 7, section 7.6 Constituents and Contaminants of Natural Origins (7.6.1.1 Intrinsic components of foods: oxalate.

Lippmann states that the average intake from naturally-occurring dietary sources is 4 to 17 times the ADI for developmental effects and 16 times the ADI for reproductive effects. The background oxalate levels from naturally-occurring foods far exceed the ADI. To illustrate the potential calcium oxalate toxicity from the agave fructan substance notified in GRN 1019, the calcium content of the powdered fructan, as shown in Table 2.8, p. 25, was utilized. As shown in the table, the average calcium content of the 4 batches shown is 242 mg/kg dry product. Based on the molar ratio of calcium and oxalate in calcium oxalate, and assuming all of the calcium in the notified substance is as oxalate the oxalate level that relates to the calcium would be about 530 mg/kg dry product. Using an estimated daily intake of the dry notified substance of between 20-30 grams/d, the daily oxalate intake would be approximately 10 mg to 16 mg. These values are far below the ADI of up to 210 mg/d defined in the reference above, and significantly lower than the background oxalate levels occurring in the diet from natural sources. Further, the calculated EDI for oxalate from the agave fructan intake, is likely highly overestimated, as it is more likely that a majority of the calcium in the notified substance is in the carbonate or chloride forms, which are more common anions, rather than oxalate.

3. In the notice, you provide the specification parameters, and the analytical methods used to assess these parameters, in Tables 2.4 and 2.5 for the powder and liquid forms of agave mixed fructans from Agave tequilana Weber var. azul. You also provide data from 4 non-consecutive batches of both the powder and liquid forms to demonstrate that the products meet the specifications. In Table 2.6 you provide data for heavy metal levels for 21 batches of the powder form of your ingredient that were tested in the years ranging from 2014 to 2019, using various analytical methods. We note that heavy metals were not listed in your specification

parameters and that you did not provide heavy metal analyses data for the liquid form of the ingredient. Please revise your specifications to include heavy metals and provide the current analytical method used to assess these specifications for both the liquid and powder form. In addition, please provide batch data for the liquid form to show that the liquid form of the ingredient meets the product specifications.

Response: The comment that the specifications for both powdered and liquid products did not include heavy metal levels is clear. The following table shows the specified values for heavy metals for each product type of the notified substance. Note that these specifications are the same as those defined for the mixed agave fructans in GRN 854. The heavy metal levels shown in Table 2.6 of GRN 1019 for all of the 21 batches of powdered product were below the detection limit using each official method utilized to determine the respective heavy metal. Heavy metal content in liquid product is not currently available. However, note from GRN 1019 manufacturing section that the liquid and powdered products are made from the same process stream (clarified thin agave fructan containing juice). The stream is either evaporated to about 45 Brix for subsequent spray drying or to about 72 Brix for storage as liquid agave fructan. The two products differ by their respective water content (5% vs. 28% water). As none of the 21 batches of powder fructan resulted in any detectable levels of heavy metals, the liquid, a more dilute version of the powder, would certainly be lower, to also yield undetectable levels. All production batches going forward of each type will be analyzed to show that they meet specified levels using the atomic absorption method in NOM-117-SSA1-1997, as was used to determine heavy metal content for the mixed agave fructans in GRN 854.

Heavy metal (mg/kg)	Powder	Liquid
Lead (Pb)	<0.015	<0.02
Mercury (Hg)	<0.003	<0.01
Cadmium (Cd)	<0.001	<0.01
Arsenic (As)	<0.020	<0.03

Kind Regards,
Karen

Karen M. Hall (she/her/hers)
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Karen.Hall@fda.hhs.gov

From: Bryan Tungland
To: Hall, Karen
Subject: [EXTERNAL] RE: Regarding GRN 1019
Date: Thursday, November 10, 2022 12:27:59 PM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png

Good morning, Karen,

Thank you for your continued review of GRN 1019. I apologize for the confusion.

Please review my responses below each of your questions.

Kind regards,

Bryan

From: Hall, Karen <Karen.Hall@fda.hhs.gov>
Sent: Monday, November 07, 2022 3:13 PM
To: tungland@charter.net
Subject: Regarding GRN 1019

Good Afternoon,

Thank you for your patience as we continue to review GRN 1019. The review team is asking for clarification on a few chemistry items.

1. You provide the heavy metal specifications for agave mixed fructans in the amendment dated April 26, 2022 and note that the heavy metal specifications are the same as the specifications in GRN 000854. We note in GRN 000854 that the heavy metal specifications for the powder form (i.e., the more concentrated form of the ingredient) are lower than the liquid form. However, in an amendment to GRN 000854 dated September 4, 2019, the notifier indicated that the powder form would have the same specifications for heavy metals as the liquid form. In addition, the specification for arsenic in your notice in lower than that in GRN 000854, which is consistent with the Agency policy to ensure that dietary exposure to heavy metals is as low as possible. Please clarify the heavy metal specifications for the liquid and powder form of agave mixed fructans in your GRAS notice.

Response: To clarify: The powdered form of the agave mixed fructans is the most concentrated form of the product. As I mentioned in a previous email, the liquid and powdered product forms

are both made from the same product stream. They only differ by water content. The liquid form is concentrated to a syrup in an evaporator, while the powdered form is spray dried. Because the liquid form is a diluted version of the powdered form, it will always contain less heavy metals than the powdered version.

2. On page 6 of the notice, you state that the intended uses for agave mixed fructans are for the same foods and same per serving levels as GRN 000854. In Table 1.1, for the food category "candy (hard dietetic)," you list the reference amount of 15 g, and the maximum use levels of 15% or 8.25 g/serving. We note that based on the provided reference amount and percent maximum use level, the use level is calculated to be 2.25 g/serving. Please clarify the maximum use level in hard candies in relation to the reference amounts listed in 21 CFR 101.12.

Response: I apologize for miscalculating this item's use level. The calculated use level is indeed 2.25 g/serving, not the 8.25 g/serving as listed in Table 1.1. The reference amount 15g x 0.15 = 2.25 g, not 8.25.

Please respond within 10 business days. If you need additional time, feel free to contact me.

Kind Regards,
Karen

Karen M. Hall (she/her/hers)
Regulatory Review Scientist

Division of Food Ingredients
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Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Karen.Hall@fda.hhs.gov

From:	Bryan Tungland
To:	Hall, Karen
Subject:	[EXTERNAL] New GRN 1019 heavy metal specification table
Date:	Wednesday, November 16, 2022 10:20:29 AM
Attachments:	image001.png image002.png image003.png image004.png image005.png image006.png GRAS Notification 1019 new heavy metal specification table.docx

Hi Karen,

Thank you for your groups time today to discuss GRN 1019 heavy metal specifications. Attached is a revised table showing that the powdered and liquid forms of the agave mixed fructans in the notification will have equal heavy metal specifications.

Regards,

Bryan

From: Hall, Karen <Karen.Hall@fda.hhs.gov>
Sent: Wednesday, November 16, 2022 9:09 AM
To: Bryan Tungland <tungland@charter.net>
Subject: table

Karen M. Hall (she/her/hers)
Regulatory Review Scientist

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Heavy Metal	GRN 1019 Powder	GRN 1019 Liquid
Arsenic	<0.020 mg/kg	<0.020 mg/kg
Cadmium	<0.001 mg/kg	<0.001 mg/kg
Mercury	<0.003 mg/kg	<0.003 mg/kg
Lead	<0.015 mg/kg	<0.015 mg/kg