ORIGINAL SUBMISSION

#587

NSF International

December 16, 2016

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-255)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Nadine Bewry

RE: GRAS Notification – Premium Agave Inulin – Resubmission

Dear Dr. Bewry:

On behalf of IIDEA of Tlaquepaque Jalisco, México, we are resubmitting for FDA review a GRAS notification (original submission Nov. 11, 2011; first resubmission May 19, 2015) for Premium Agave Inulin, trade name, Inufib™, a soluble dietary fiber which is to serve as a bulking agent or source of reduced energy carbohydrate. Please find enclosed one hard copy and one virus-free electronic copy (on CD). This submission was originally assigned a GRAS notification number of 582 but was withdrawn on November 25, 2015 pending an updated Expert Panel review.

In the current resubmittal, we have reformatted the GRAS notification to comply with the requirements of the 2016 Final Rule (i.e. See Food and Drug Administration (2016), Substances Generally Recognized as Safe, Final Rule. August 17, 2016 Federal Register, 81 FR 54960). Specifically, the content of the GRAS notification has been reorganized into the seven-part format described in the Final Rule and the section headers have been revised to correspond to each of the seven parts.

Additionally, in the current resubmittal, we have included an updated declaration of safety from the Expert Panel.

We appreciate your careful consideration of our revised GRAS notification, and we look forward to your feedback.

Sincerely,

Bradley J. Lampe, MPH
Senior Research Toxicologist
NSF International
Tel: 734-913-5759
E-mail: blampe@nsf.org

Agave Inulin

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTIFICATION

Prepared for

IIDEA (Industrializadora Integral del Agave SA de CV)
Av. Periférico Sur 7750,
Tlaquepaque Jalisco, México

Prepared by

NSF International
Ann Arbor, MI

July 2016

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1.0 SIGNED STATEMENTS AND CERTIFICATION

1.1 § 170.225(a): Signature

Signature:

(b) (6)

Date:

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

IIDEA (Industrializadora Integral del Agave SA de CV), through its agent NSF International, 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.

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

Name and Address of Notifying Organization:
IIDEA (Industrializadora Integral del Agave SA de CV)
Av. Periférico Sur 7750,
Tlaquepaque Jalisco, México
FDA registration number: 13439186334

Name and Address of Agent for Notifying Organization:
NSF International
789 N. Dixboro Rd.
Ann Arbor, MI 48105

1.4 § 170.225(c)(3): Name of the Notified Substance

The name of the substance that is the subject of this Generally Recognized as Safe (GRAS) determination is Inufib™, the trade name used by IIDEA, for the inulin-type fructans prepared from the piñas[1] (stems, also known as cores, heads, or pines) of the agave plant, ***Agave tequilana* Weber var. *azul***, commonly known as blue agave and weber's blue agave. Inulin (synonym: inulina) or agave inulin (synonym: inulina de agave) are the common names of the fructans derived from the piñas of ***Agave tequilana* Weber var. *azul***, commonly known as blue agave and Weber's blue agave, grown and processed in the occidental region of Mexico. Other common names include blue agave inulin, fructans from agave, and inulin tequilana Weber blue agave.

[1] Stems, also known as cores, hearts, or pines.

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

Agave-derived fructans are intended for general addition to foods except infant formula and meat and poultry products (Table 1). It will be added to the same foods at per serving levels as identified in the GRAS Notification submission to the U.S. FDA (FDA) for chicory inulin (GRN 118 and 2007 amendment) and fructooligosaccharide (GRN 44), which were determined to be GRAS without questions or objection by FDA (FDA, 2000b; FDA 2003). Inulin added to foods serves as a general purpose bulking agent, texturizing agent, or source of reduced energy carbohydrate for uses as a sugar replacer, fat-replacer and/or texture modifier. The amount used will not exceed the amount reasonably required to accomplish its intended technical effect.

Table 1. Intended Food Use Categories and Use Levels of Inufib™

Food Category	Maximum Use Level of Inufib™ (g per 100 g food)
Baby foods: all types of baby foods and beverages, including ready-to-serve and dry baby foods (excluding infant formula)	1 g/serving[a]
Baked goods, lite cakes: fat free/reduced fat/sugar/calorie baked goods including cakes, brownies, and pastries	5
Baked goods, lite cookies: fat free/reduced fat/sugar/calorie cookies	8
Bars: all types, including breakfast bars, granola bars, energy bars, and diet/meal replacement bars	10
Beverages, fermented milks: kefir, buttermilk, yogurt drinks	2
Beverages, functional: meal replacement beverages and meal supplement beverages, including ready-to-drink beverages and dry beverage mixes[b]	5
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* beverage mixes[b] (excluding citrus juices and highly carbonated beverages)	1.5
Beverages, milk-based: dairy-based beverages, including ready-to-drink beverages and dry beverage mixes[b]	1
Biscuits. reduced fat: fat free/reduced fat biscuits	6
Breads, conventional: conventional yeast breads, rolls, and buns	0.5
Breads, specialty: specialty types such as breads reduced in calories or fat and/or containing added fiber or added calcium	6
Candy, hard dietetic	15
Candy, soft dietetic	5
Condiments: catsup and mustard	5
Cream cheese, reduced fat: fat free/reduced fat cream cheese	5
French fry coatings: coatings on French fries	1.7[c]
Frozen dairy desserts, lite: fat free/reduced fat/sugar/calorie ice creams and dairy-based frozen desserts, including novelties and frozen yogurt	8
Icings/glazes, lite: fat free/reduced fat/sugar icings and glazes	5
Jams and jellies, lite: reduced sugar calorie jams and jellies	2
Mousse, reduced fat	3
Pancake syrup, lite	2

Food Category	Maximum Use Level of Inufib™ (g per 100 g food)
Pasta fillings: fillings used in pasta, such as tortellini, ravioli and manicotti fillings	5
Pasta, fresh: fresh pasta, such as spaghetti, fettuccini, linguini, tortellini, ravioli, or lasagna (excluding noodles)	4
Pasta, precooked macaroni	4
Pizza crust	5
Potatoes, mashed: prepared or in frozen meals (excluding dry mix types)	3
Pretzels, soft	5
Processed cheese, reduced fat: fat free/reduced fat processed cheese and cheese products	5
Pudding mix: regular and reduced sugar/calorie pudding mix	7
RTE breakfast cereals, all types of ready-to-eat (RTE) breakfast cereals	5 g/serving[a]
Salad dressings, lite: fat free/reduced fat/calorie dressings, including mayonnaise, salad dressings and mayonnaise-type dressings	5
Sauces and gravies: entree, dipping and condiment sauces such as Alfredo, BBQ, cheese, clam, Hollandaise, pasta, pizza, soy, sweet & sour and white sauces, salsa, and gravies, including prepared sauces and dry sauce mixes[a] (excluding tomato sauce and paste)	2
Snack chips, reduced fat: fat free/reduced fat snacks, including chips and extruded snacks	3
Snack crackers: savory snack, sandwich, and whole grain crackers (excluding plain crackers such as saltines, matzo crackers or oyster crackers	4
Soups, dry	3
Spreads. reduced fat: fat free/reduced fat margarines and margarine-like spreads	10
Surimi, imitation crab, and reconstructed seafood	3
Toppings, dessert: toppings used on desserts (excluding whipped toppings)	2
Tortillas, reduced fat	3
Vegetarian patties/crumbles	2
Whipped toppings, lite: fat free/reduced fat/sugar non-dairy whipped cream toppings	6
Yogurt, reduced fat: fat free/reduced fat refrigerator-type yogurts	3
[a] Serving sizes correspond to Reference Amounts Customarily Consumed per Eating Occasion; 21CFR 101.12 [b] Maximum use levels correspond to g Inufib™ per 100 g prepared beverage or sauce [c] Maximum use level per 100 g coated French fry (as consumed) Note: unless otherwise indicated all food categories include both regular and lite versions of all food products	

Agave inulin is a prebiotic ingredient that belongs to a class of fibers known as fructans. Agave inulin is an organic dietary soluble fiber which is extracted from the *A. tequilana* Weber plant. A prebiotic is a non-digestible food ingredient that beneficially affects the body by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon to improve body health. Agave inulin is not digested in the upper gastrointestinal tract, thereby contributing to reduced caloric intake. Consumption will not lead to a rise in serum glucose or simulate insulin secretion. In addition, agave inulin aids to increase calcium and magnesium absorption. Agave inulin has a neutral, sweet, clean flavor and is used to improve the mouth feel, stability and acceptability of low fat foods. It can be used to fortify foods with fiber and to improve the flavor and sweetness of low calorie foods. Agave inulin also improves the texture of fat-reduced foods. Agave inulin is highly soluble in cold water and can easily be incorporated into

beverages, bakery products, and dairy products. Agave inulin has a unique ability to add textural properties to food. Inulin gels are very creamy and fat-like, and as such can be used as a bulking agent and in fat reduction and fat replacement. Agave inulin also serves as a source of reduced energy carbohydrates for use as a sugar replacer.

Roberfroid et al. (1998) describe the commercial use of inulin-type fructans and oligofructose in the U.S., Japan and Europe, where they are added to foods for their nutritional properties and dietary fiber content (typically 3–6 g per portion). As a macronutrient substitute, inulin is used to replace fat (0.25 g inulin replaces 1 g of fat) such that inulin concentrations are 2–6 g per portion. Likewise oligofructose is used as a sugar substitute mainly in dairy products and bakery products, at typically 2–6 g per portion (Coussement 1999)[2]. Fructans are also used as texturing agents, foam stabilizers, or for improved mouth feeling in miscellaneous food products (e.g., fermented dairy products; desserts such as jellies and ice creams; bakery products including biscuits, breads, and pastries; spreads; and infant formulas) (Roberfroid and Delzenne, 1998). In other applications, inulin or oligofructose is added to allow a specific nutritional claim regarding the bifidogenic activity (typically 3 – 8 g per portion).

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

IIDEA has determined that the intended use of Inufib™ is Generally Recognized As Safe (GRAS) through scientific procedures in accordance with in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. IIDEA's GRAS determination for the intended uses of agave-derived fructans is based on scientific procedures as set forth in 21 CFR § 170.30(b), thus satisfying the "technical" element of the GRAS determination. The safety of intake exposure under the proposed conditions of use is based on knowledge and information that is both publically available and widely accepted by experts qualified by scientific training and experience to evaluate the safety of substances in food, thereby meeting the common knowledge element 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.

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

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

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

The data and information that serve as the basis for this 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 offices of NSF International located at 789 N. Dixboro Rd, Ann Arbor, MI, 48105.

[2] See "Typical Use Levels of Fructooligosaccharide, at:
http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154400.htm

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

IIDEA 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

IIDEA 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: Bradley J. Lampe, MPH
Senior Research Toxicologist
NSF International
Agent for IIDEA
Telephone: 734-913-5759
Email: blampe@nsf.org

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, IIDEA authorizes the 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

The notified substance consists of naturally occurring fructose polysaccharides with a range of 3 to 60 fructose units and mean of 19.7, based on the analysis of samples from 6 lots of Inufib™. The preparation also contains minor amounts of monosaccharides (fructose and glucose) and disaccharide (sucrose). The Chemical Abstracts Service Registry Number (CASRN) for inulin is 9005-80-5; agave inulin does not have a distinct CASRN.

2.1.1 Chemical/Structural Formulas

Chemically, agave inulin is a mixture of naturally occurring oligo- and polysaccharides known as fructans having fructose as the repeating unit. Fructose moieties are joined by β(2→1)- and β(2→6)-glycosidic bonds, with typically one terminal 6-linked glucose moiety or an internally linked glucose moiety per molecule. The fructan molecules vary with respect to their length (degree of polymerization or DP), and degree of branching. The molecular weight distribution is 527-4739 Da, corresponding to a range of DP from 3 to 29 (Lopez et al. 2003), with a small fraction

having DP from 30 to 60 (Toriz et al. 2007). The average degree of polymerization (i.e., the mean number of fructose units) in fructans from the blue agave plant (*A. tequiliana* Weber var. *azul*) range from approximately 6 to 23 (Mellado-Mojica and Lopez, 2012) and is 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 convention, fructan polysaccharides are called inulins when the DP is greater than 10 and referred to as fructo-oligosaccharides or oligofructose when the DP is ≤ 10 (Niness, 1999; Corradini et al., 2004; Ortiz-Basurto et al. 2008). Since fructans from the mature blue agave plant have a mean DP of >10, the product is referred to as agave inulin. Low molecular weight fructans (DP range 3-5) account for approximately 9% of the total (Toriz et al. 2007). Monosaccharides and disaccharides typically account for <10% of the mixture. The degree of polymerization (DP) for agave inulin is consistent with that of other inulins consumed by humans, including native chicory root inulin, which has an average DP of 10-20 and range of 2 to 60 (Roberfroid and Delzenne, 1998).

The molecular formula for agave inulin is $C_{6n}H_{10n+2}O_{5n+1}$. The trisaccharide subunits (Figure 1) that comprise the Agave fructans are 1-kestose, the same linear inulin subunit found in chicory root, and neokestose (synonyms: 6_G-kestotriose or Fru2,6Glcα1,2βFru), a fructan with an internal glucose moiety that is common to oats and onion. Fructans having the 6G-kestotriose backbone with repeating fructose units on both ends have also been termed neo-inulin- and neo-levan-type fructans, and are common to oat, *Asparagus*, and *Lolium* sp. as well as *Agave* (Pavis et al., 2001; Van den Ende, 2013). The degree of branching of the agave-derived fructans appears to be high relative to that of chicory root and onion, but the differences are quantitative, and fructans from these plant sources are qualitatively similar.

The chemical structures of fructans obtained from mature (5 to 8 year-old) *Agave tequilana* Weber var. azul plants have been characterized (Lopez et al., 2003; Mancilla-Margalli and Lopez, 2006; Toriz et al. 2007; Mellado-Mojica and Lopez, 2012). Agave fructans are structurally diverse mixtures of fructo-oligosaccharides (FOS) and fructans containing β(2→ 1) and β(2→ 6) linkages, with internal and external glucose units, termed agavin- and graminan-type fructans, respectively (Mancilla-Margalli and Lopez, 2006; Mellado-Mojica and Lopez, 2012).

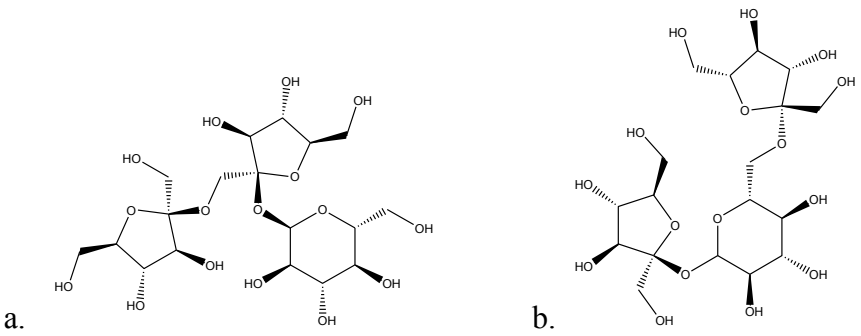

Figure 1. a. 1-kestose; b. neokestose (synonym, 6_G-kestotriose)

2.1.2 Agave Inulin Composition

Inufib™ typically contains 98 - 100% carbohydrate (dry basis); approximately 90% inulin with up to approximately 10% monosaccharides and disaccharides, primarily fructose, glucose, and sucrose. Details regarding the Carbohydrate Composition and Degree of Polymerization can be found in Section 8.1.1.

The fatty acid content of agave inulin accounts for approximately 0.1% of the composition. Mineral analysis showed sodium content to be 0.0353 g/100 g (Attachment 1, Analysis Eurofins).

Concentrations of terpenes are below 0.1 ppm, and saponins have not been detected at levels as low as 7 ppb (Attachment 2 "Saponins and Terpenes"; Attachment 3 "Letter saponins Ext Lab").

Inufib™ is available for use in two forms, dry and liquid. Liquid Inufib™ is the concentrated, filtered agave stem juice. Dry Inufib™ is the filtered agave stem juice concentrate that is spray dried to produce a white or yellowish white powder with a neutral odor.

2.1.3 Comparison of Inufib™ Composition with other Agave Fructan Products

Inufib™ can be compared with other purified fructan products extracted from the Blue Agave species such as Metlin® and Metlos®. The latter are similar to Inufib™ in that they are both derived from *A. tequilana* var Weber *piñas* and consist of linear and branched fructans with β (2 → 1) and β (2 → 6) linked fructofuranosyl units. The Metlin® and Metlos® preparations are distinguished from each other by their degree of polymerization (DP) distributions. Presumably an additional processing step is used to separate the fructans in Metlin® and Metlos® by size, whereas Inufib™ is the natural mixture of fructans ranging in DP from 3 to 29; predominantly long chain inulin (DP > 10) and also some fructooligosaccharide content (DP < 10) (see Table 2). Therefore, the fructan mixtures of Metlin® and Metlos® containing both fructo-oligosaccharides and long chain inulin are compositionally comparable to Inufib™.

Table 2. Comparison of the composition of Inufib™ with other agave fructan products[a]

Product	Mean (Range) Degree of Polymerization	Distribution	Mean Polydispersion index
Inufib™	19.7 (3-29)	73% > DP10 > 27%	1.2
Metlin®	27 (NR)	84% > DP10 > 16%	2.3
Metlos®	15 (NR)	55% > DP10 > 45%	3.3
BioAgave®	NR (25-34)	NR	NR
Predilife (Agave)	NR (3 – 29)	NR	NR

[a] This table and the associated text is included in response to an FDA comment to describe in detail the similarities and differences in composition and biological activities between Inufib™ and other purified agave fructan products that are discussed in Safety (Section 5 of this document).
NR = not reported

Other purified agave fructan products include BioAgave® and "Predilife (Agave)" (Table 2). Like Inufib™, Predilife (Agave) was obtained from *A. tequilana* Weber var. azul piñas and prepared in the same manner as Inufib™ except for the addition of a treatment step with activated carbon and ion exchangers to eliminate calcium and chelates. BioAgave® is also derived from the agave plant, however, no information was reported or located regarding the Latin binomial or identity of the originating plant species. Both BioAgave® and Predilife (Agave) products consist of linear and branched fructans with β (2 → 1) and β (2 → 6) linked fructofuranosyl units, consistent with Inufib™. The DP of Inufib™ and Predilife range from 3 to 29, whereas the DP for BioAgave® is described as 25-34. The mean DP of Inufib™, based on analysis of six nonsequential lots, is 19.7 (Attachment 4 – size exclusion chromatography); the mean DPs of Predilife (Agave) and BioAgave® were not reported or located.

The safety and tolerability studies discussed in Section 5 that were conducted with Metlin®, Metlos®, mixtures of Metlin® and Metlos®, BioAgave® or Predilife, are relevant to addressing the safety and tolerability of Inufib™ because all of these products consist of mixtures of fructo-oligosaccharides and fructans obtained from agave piñas, having the same basic molecular structures, with variable degrees of polymerization (DP), but overlapping with Inufib™ in the proportion of polymerizations. Based on studies in obese mice, the DP of agave fructans or a demineralization processing step can influence the effect of oral intake on body weight gain, serum cholesterol, serum triglycerides, and the relative populations of intestinal bacteria, whereas no influence was found on acute oral toxicity or mutagenicity by such variations in agave fructan composition (Marquez-Aguirre et al. 2013). Irrespective of DP and demineralization, the agave fructans in Table 2 all pass into the colon mostly intact, undergo equivalent metabolic processing, and are well tolerated orally.

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

2.2.1 Identification of the Source

Agave inulin (Inufib™) is a natural fructan concentrate derived from the piñas (plant stem) of the agave plant, ***Agave tequilana* Weber var. *azul***, commonly known as blue agave and Weber's blue agave.

2.2.2 Comparison of Agave Inulin with Inulin from Other Sources

Most commercially available inulin and oligofructose are extracted from chicory roots (*Cichorium intybus*). As with agave inulin, the degree of polymerization (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 product has an average DP of 10-12 with a chain length distribution from 2 to 60 and 6-10% content of free sugars as sucrose, fructose, and glucose.

Agave inulin, sourced from the plant stem and produced in a manner similar to inulin from chicory, has an average degree of polymerization of about 14-18 and distribution primarily from 3 and 29. Thus fructans extracted from chicory roots and agave stems contain (as dried wt%) up to ~10% of combined mono and disaccharides, mainly sucrose and fructose, and approximately 90% inulin (Niness, 1999; Murphy, 2001). The product Raftiline HP, commonly used in the food industry

because of its fat mimetic properties, is manufactured by removing the shorter-chain oligomers and residual sugars from chicory-derived inulin; it has an average DP of 25 with a molecular distribution range from 11 to 60. Oligofructose is derived in the same way as inulin, with the addition of an enzymatic hydrolysis step after extraction, such that chain lengths range from 2 to 10 with an average DP of 4. The commercially available inulin from Sigma is derived from *Dahlia tubers* and is standardized to have an average DP of 27-29 (Zuleta and Sambucetti, 2001).

Structurally, these plant-derived inulins consist mainly of β(2→1)fructosyl–fructose links with chicory inulin containing 1-2% β(2→ 6)fructosyl–fructose branches; Dahlia inulin having 4-5% β(2→6)fructosyl–fructose branches (Hariono et al., 2009), and agave inulin having approximately 24% β(2→6)fructosyl–fructose branches (Franck and de Leenheer, 2004). β(2→1) and β(2→ 6)fructosyl–fructose linkages cannot be hydrolyzed by pancreatic or brush-border digestive enzymes. Therefore, these fructans reach the colon undigested, where they are fermented by *Bifidobacterium spp*. and other lactic acid-producing bacteria (Lopez et al. 2003; Munjal et al. 2009).

Agave inulin shares chemical, physico-chemical, and nutritional properties with other plant-derived fructans and with fructo-oligosaccharides produced by enzymatic synthesis from sucrose; therefore, toxicological studies performed with synthetic fructo-oligosaccharides (average DP = 4) are considered to be predictive of the effects of naturally occurring inulin and oligofructose since the substances are chemically similar entities with like nutritional properties (Carabin and Flamm, 1999).

2.3 § 170.230(b): Method of Manufacture

Manufacturing processes and analytical methods used by IIDEA for the production of Inufib™ are similar to those used for the manufacture of chicory-derived inulin (Franck, 2002) and other inulin products on the market. Inufib™ is manufactured in a manner consistent with current Good Manufacturing Practices requirements (cGMP) for food (21 CFR Part 110).

Production of inulin from premium agave involves the mechanical extraction of the juice from the pine (piñas) of the blue agave without the use of solvents or other chemicals. Inufib™ is mechanically extracted from the pines ("piñas") of the agave plant. When the plants are harvested, the leaves and roots are cut off and left in the fields for soil enrichment. It is important to emphasize that the agave inulin Inufib™ is derived from the piñas and is not derived from the leaves of the agave, because sap or extracts from the leaves from some agave species have been noted to contain saponins and raphides of calcium oxalate, thereby rendering them inedible (see Appendix Table A-1).

The harvested piñas are transported by conveyor into a mill where the piñas are subject to a series of extractions for sieving and squeezing. The inulin juice falls into tubs while the resulting bagasse is separated and removed. The extracted inulin juice undergoes three filtration steps, and the juice is then concentrated by evaporation. The filters retain foreign matter, such as small stones, insects, soil, fiber, plastic and metal particles. Filters of 0.5 microns remove any microorganisms that are possibly present and can be expected to remove raphides of calcium oxalate, which are 30–500 µm in length (Salinas et al. 2001), if present. The filters used are manufactured with materials that

are approved by the FDA. (See Attachment 5 "Ficha Tecnica Bolsas", "FDA Datos acerca de bolsas de FS" and "Betafine – filters cartridges"). After a final filtration step the resulting liquid product is bottled, or alternatively, the concentrated juice is spray dried to a final concentration of greater than 95% dry matter. Please refer to Attachment 6 "HACCP Plan – Inulin" for the process flow charts and process steps for Dry and Liquid product, as well as dried and liquid product specifications.

As a processing aid, IIDEA adds Perlite as a filter aid in the production of Inufib™. No other chemicals or processing aids are used. No processing aids or additives are included in the final Inufib™ products, and no proprietary or coloring ingredients are added. Based on gas chromatography-mass spectrometric analysis (method PT-USAI-FQ-EM-001) of IIDEA's purified agave inulin product, Inufib™, conducted by an external laboratory, concentrations of saponins and terpenes are below 0.1 ppm (see Attachment 2 "Saponins and Terpenes"). No saponins were detected, and under the conditions of analysis, the test laboratory concluded that, if the compounds ecogenin and ecogin were present in the sample, their concentrations would be <7 ppb (see Attachment 3 "Letter saponins Ext Lab").

No fungicides, slimicides or other biocides are used by IIDEA in the production of Inufib™.

IIDEA 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 13439186334 (see Certificate of Registration Attachment 7 "FDA-IIDEA 2010 – 2011"). The manufacturing process complies with the international GMP standard ISO 21 000. 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 Hazard Analysis Critical Control Point (HACCP) Plan (Attachment 6 "HACCP Plan – Inulin"), which has been certified by Global Standards Certification (see Attachment 8 "HACCP Certificate") and Silliker Global Certification Services (see Attachment 9 "Silliker Audit Recognition").

2.4 § 170.230(c): Product Specifications

Properties and food grade specifications for IIDEA's agave inulin (Inufib™) are presented in Table 3. See Attachment 10 "Data sheet – powder inulin premium" and Attachment 6 "HACCP Plan – Inulin." Dry Inufib™ is a fine, white powder. The results of compositional and microbiological analyses of four non-consecutive lots of dry Inufib™ powder are presented in Table 4.

Table 3. Properties and Specifications for Dry and Liquid Inufib™

Properties/Specifications	Dry Product	Liquid Product
Physical Chemical Properties		
Moisture:	0.5 – 4.0%	27 – 31%
Density:	0.6 – 0.8 g/mL	1.34 – 1.36 g/mL
Concentration	NA	69° – 73° Brix
pH:	4.0 – 6.0 (1%)	4.0 – 6.0
Color:	White powder	300 – 1000 ICUMSAS[a]

Storage stability:	Stable, hygroscopic	Stable, hygroscopic
Taste:	Slightly sweet	Slightly sweet
Aroma:	Neutral	Not reported
Product Specifications		
Ash content:	Max. 5.0 %	< 0.7 %
Dry matter	98.0-100 % total carbohydrates	≥ 98.0 % carbohydrates
Composition:	≥ 88.0% inulin	≥ 80.0% Inulin
	≤ 10.0% fructose	≤ 15.0% Fructose
	≤ 3.5% glucose	≤ 5.0% Glucose
	≤ 2.0% disaccharides	≤ 2.0% Disaccharides
Microbiological Contaminants		
Mesofilic	Max. 2,500 UFC	≤2,500 UFC/g
Coliform	Max. 10 UFC	≤10 UFC/g
Yeast and molds	Max. 100 UFC	≤100 UFC/g
Shelf Life		
Shelf life from date of manufacture	3 years [3]	3 months[4]
NA = not applicable [a] The method is based on the NMX-ff-110-SCFI-2008; Accessed February 29, 2016 at http://dof.gob.mx/nota_detalle.php?codigo=5089984&fecha=12/05/2009.		

Additional analyses of organic agave inulin by Eurofins Analytics showed total fat content to be <0.5% of the composition. Fatty acid composition showed that ~2/3 is saturated fatty acids and ~1/3 is monounsaturated fatty acids. The following components were all <0.05%: docosadienoic acid C22:2 (n-6) – ω6; polyunsaturated fatty acids; total trans-fatty acids; omega-3 fatty acids; and omega-6 fatty acids. Non quantifiable fatty acids were also <0.05%. Mineral analysis showed sodium content to be 0.0353 g/100 g. The complete analytical results are provided in the Attachment 1 – "Analysis Eurofins." The results of compositional and microbiological analyses of three lots of liquid Inufib™ are presented in Table 5.

Table 4. Compositional analysis of dry Inufib™ powder

	Specification per HACPP March 2011	4NIPP11023	4NIPP10041	4NIPP10271	4NIPP11020
Appearance		Creamy white fine powder	Creamy white fine powder	Creamy white fine powder	Creamy white fine powder
Total carbohydrates (%)	Min 98.0%	99.02%	99.89	99.02	99.13
Inulin (%)	≥ 88.0	91.98	90.00	90.98	91.47
Fructose (%)	≤ 10.0	4.95	4.73	5.66	4.62
Dextrose (i.e. glucose) (%)	≤ 3.5	0.45	2.32	0.60	0.71
Sucrose (glucose-fructose disaccharide) (%0	≤ 2.0 disaccharides	0.60 saccharose	0.93 saccharose	0.69 saccharose	1.18 saccharose
Other carbohydrates (%)	Max 6.0	1.03	1.91	1.09	1.15
Microbiological					

[3] As stated in Section 6.1.5, HACCP Plan – Inulin. See also "Data Sheet – Powder Inulin Premium"; "Shelf Life Inulin"; and "Shelf Life External Analysis," attached.
[4] As stated in section 6.2.5, HACCP Plan - Inulin.

Mesofilic (total count)	Max. 2,500	10	10	260	13
Coliform (UFC/g)	Max. 10	<10	<10	<10	<10
Yeast (UFC/g)	Max. 100	<10	<10	<10	<10
Mold (UFC/g)	Max. 100	<10	<10	<10	<10
Salmonella (in 25 g)		Absent	Absent	Absent	Absent

Table 5. Compositional analysis of Inufib™ liquid

	Specification per HACPP March 2011	IL150111	IL140111	IL110111
Appearance		Light amber	Light amber	Light amber
Total carbohydrates (%)	Min 98.0%	98.04%	98.27	98.92
Inulin (%)	≥ 80.0	89.63	89.89	90.00
Fructose (%)	≤ 15.0	4.59	3.13	4.54
Dextrose (i.e. glucose) (%)	≤ 5.0	0.50	1.57	1.65
Sucrose (glucose-fructose disaccharide) (%0	≤ 2.0 disaccharides	0.62 saccharose	1.09 saccharose	1.11 saccharose
Other carbohydrates (%)	No specification	2.70	2.59	1.62
Microbiological				
Mesofilic (total count)	Max. 2,500	414	338	359
Coliform (UFC/g)	Max. 10	<10	<10	<10
Yeast (UFC/g)	Max. 100	<10	<10	<10
Mold (UFC/g)	Max. 100	<10	<10	<10

Agave inulin powder from IIDEA is analyzed for heavy metals (arsenic, lead, mercury, and cadmium), aflatoxins, and an extensive list of pesticides, dioxins, and PCBs (see Attachment 11 "Analysis Status"). For Agave inulin powder, finished product specifications for the assay of heavy metals and aflatoxin are presented in Table 6.

Table 6. Finished Dry Inufib™ specifications for heavy metals and aflatoxins

Assay	Specification
Heavy Metals	
Lead	< 0.015 mg/kg
Mercury	< 0.003 mg/kg
Arsenic	< 0.002 mg/kg
Cadmium	< 0.001 mg/kg
Antimony	< 1.0 mg/kg
Aflatoxins	
Aflatoxin B1	< 0.5 µg/kg
Aflatoxin B2	< 0.2 µg/kg
Aflatoxin G1	< 0.5 µg/kg
Aflatoxin G2	< 0.2 µg/kg
Total Aflatoxins	< 1.4 µg/kg
Deoxynivalenol	< 100 µg/kg

Ocratoxina A	< 0.5 µg/kg
Zearalenona	< 20 µg/kg

The analytical (chemical and microbiological) results for agave inulin summarized in Tables 4, 5 and 6, and included in the CoAs and Technical Data Sheets in Attachments 14 and 15 confirm that the finished products meet the analytical specifications, demonstrate that the Inufib™ manufacturing process results in a consistently reproducible product, and confirms the lack of impurities/contaminants (heavy metals, pesticides, microbiological toxins). Analytical reports listing the individual contaminants, their respective analytical methods, and the results of the analyses, are provided in the Attachment 12 "Pesticides Silliker" and Attachment 1"Analysis Eurofins." The results of the analyses indicate that contaminants are not present at levels of concern.

2.4.1 Analytical Methods used to Determine Inufib™ Specifications

The contents of agave inulin and other carbohydrates in Inufib™ 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 Explicaciones y Métodos de Prueba). Other assay methods used are NOM-092-SSA1-1994 for total count of mesophylic aerobic microorganisms; NOM-112-SSA1-1994 for coliform microorganisms; NOM-111-SSA1-1994 for yeasts and molds; NOM-114-SSA1-1994 for Salmonella; NOM-117-SSA1-1997 for heavy metals; and NMX-F-591-SCFI-2010 for foreign matter. The fatty acid composition is determined with method EN ISO 15304; EN ISO 5508; EN ISO 5509. Other assay methods employed in the production of Inufib™ are referenced in Attachment 13 "Laboratory analyses."

Microbiological analyses of the dry product include coliform, yeast, mold and Salmonella (see Attachment 14 "Certificate of Quality" 1, 2, 3 and 4 for the dried product and "Test Report – Microbiological). Microbiological analysis of the liquid product includes coliform, yeast, and mold (see Attachment 15 "Certificate of Quality" 1, 2 and 3 for the liquid product).

Inufib™ production has received the following certifications:

- Organic product certified by the United States Department of Agriculture (USDA)[5], ECOCERT, BCS ÖKO-GARANTIE GMBH, Japanese Agricultural Standards (JAS) and Naturland
- Kosher certified by The Badatz Igud Rabbonim KIR
- Halal certified by the Islamic Food and Nutrition Council of America

2.4.2 Stability

A study of sensory attributes plus microbiological analyses of the powder Inufib™ at room temperature, 35 and 45°C was performed. The data support a room temperature shelf life of 275 days for odor, flavor, and fluidity; however, the product did not change with respect to appearance,

[5] Organic foods production act of 1990, at http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5060370&acct=nopgeninfo

color, rancidity, or microbiological contamination during the study period (see Attachment 16, "Shelf Life External Analysis"). 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, respectively (see Attachment 17).

2.4.3 Specifications for Similar Inulin Products on the Market

The majority (>95%) of food-grade inulin available on the worldwide market is produced from chicory (Orafti, 2007). The Canadian Food Inspection Agency (2011) lists "chicory root inulin" as a traditional fiber source with the following specifications required for labeling:

> *Specifications for standard inulin from chicory root (dwb): Appearance: white powder; Total fiber: 90% up to >98% (AOAC 997.08 or AOAC 999.03 method); Sugars: 5-11%; Max 2% if desugared; Degree of polymerization (DP) range: 2-60 (2-44 for late harvest); Average DP: 7-14; Molecules with DP < 10: 30-36%, up to 59% for late harvest; Molecules with DP < 20: 63-71% (up to 88% for late harvest); Molecules with DP = 20: 29-37% (min 12% for late harvest).*

Under the USDA National Organics Program (NOP), inulin (CAS # 9005-80-5: synonym "inulin oligofructose enriched"), is listed on NOP §205.606 which lists the only nonorganic agricultural ingredients that are allowed to be used in organic products. These nonorganic ingredients may only be used when the organic form is not commercially available. Organically produced inulin may be used to replace the nonorganic ingredients allowed in NOP § 205.606. NOP certified organic inulin from agave is among the products registered under USDA's "606organic" web site of organic sources for agricultural ingredients listed on NOP § 205.606.
(See http://606organic.com/results.php?product=Inulin-oligofructose%20enriched)

2.5 § 170.230(d): Intended Physical or Technical Effect

The following description of inulin from premium agave, or Inufib™, is given on the IIDEA Company's website[6]

> *Inulin is a prebiotic ingredient that belongs to a class of fibers known as fructans. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon to improve host health.*
>
> ***Prebiotic Properties***
>
> • *Resistance to digestion*

[6] http://www.iidea.com.mx/en/

• Hydrolysis and fermentation by colonic microflora
• Selective stimulation of growth of one or a limited number of bacteria in the feces
• May repress the growth of pathogens for overall beneficial health

The agave fructans are an important and emerging group of prebiotics. On an industrial scale, inulin is extracted from chicory, agave, or artichoke with physical treatments.

3.0 DIETARY EXPOSURE

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

This GRAS determination for Inufib™ is intended to apply to food uses of agave inulin that are identical to those uses specified in GRN 118 by Imperial Sensus (FDA, 2002 and 2007 amendment) for the chicory-derived inulin product, except that Inufib™ is not proposed for use in meat and poultry products. Imperial Sensus, in GRN 118, estimated the combined average intake of inulin by the general U.S. population (consumers two years of age and older) from all uses of Frutafit® (i.e., general food use including meat and poultry) would be 10.1 g inulin/person-day. The 90th percentile intake 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 nonbreast-feeding 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.

Given that the level of inulin in the Frutafit® product is virtually the same as the level of inulin in IIDEA's Inufib™, the same levels added to food will result approximately in the same levels of inulin per serving. The estimated intake of inulin from the proposed uses of IIDEA's products will be comparable to or less than that of the current GRAS chicory-derived inulin product that was the subject of GRN 118, since use in meat and poultry is not being considered as part of this GRAS Notification for Inufib™. The amounts of inulin from premium agave 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. In summary, the proposed uses of Inufib™ will not result in an increase in the overall consumption of inulin but will simply provide an alternative source of inulin for use in food.

As reviewed by Roberfroid and Delzenne (1998), inulin-type fructans are present in a variety of edible fruits and vegetables in appreciable quantities. The most common sources are wheat, onions, bananas, garlic, and leek. The inulin-type fructan content of edible plants ranges from <1% to >20% of the wet weight. In populations consuming a Western-style diet, the intake of inulin-type fructans has been estimated at up to 10 grams per day (Coussement, 1999) and range between 1 and 4 g/d for the 97th percentile in the U.S. In Europe, estimated consumption ranges from 3-11 grams per day (Van Loo et al. 1999; Coussement 1999). Moshfegh et al. (1999)

estimated the average inulin and oligofructose ingestion in the U.S. diet was 2.6 g, and approximately 95% of that amount was attributable to wheat and onions.

Roberfroid et al. (1998) describe the commercial use of inulin-type fructans and oligofructose in the U.S., Japan, and Europe, where they are added to foods for their nutritional properties and dietary fiber content (typically 3–6 g per portion). As a macronutrient substitute, inulin is used to replace fat (0.25 g inulin replaces 1 g of fat) such that inulin concentrations are 2–6 g per portion. Likewise oligofructose is used as a sugar substitute mainly in dairy products and bakery products, at typically 2–6 g per portion (Coussement 1999). Inulin-type fructans from various botanical sources have been sold under various brand names and incorporated in a wide variety of food and beverage products to replace fat and sugar. Fructans are also used as texturing agents, foam stabilizers, or for improved mouth feeling in miscellaneous food products. Consumption of inulin-type fructans incorporated into baked goods, dairy products, baby foods, infant formulas, meat products and a large variety of processed foods and beverages is commonplace, at least since 1992, at which time the fat replacing potential of inulin was discovered and patented by Orafti (Roberfroid and Delzenne, 1998). In other applications, inulin or oligofructose are added to allow a specific nutritional claim regarding the bifidogenic activity (typically 3 – 8 g per portion).

In 2011, customers of IIDEA are consuming inulin from premium agave at an average rate of 25,000 kg of per month.[7]

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

The notified substance is composed of fructan carbohydrates that can undergo hydrolysis to monosaccharides (i.e., fructose and glucose) upon prolonged heating (Appendix 8.1.3). The intended uses of Inufib™ in food will not result in an increase in the overall consumption of inulin or inulin hydrolysis products, but will simply provide an alternative source of inulin for use in food.

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

Inufib™ is a highly pure preparation of agave inulin, as shown by analysis for pesticides, heavy metals, aflatoxins, and microbial species. The potential for the presence of saponins and terpenes due to their natural occurrence in parts of the agave plant have been evaluated and shown to be negligible (see section 2.1.2; Attachment 2 “Saponins and Terpenes”; Attachment 3 “Letter saponins Ext Lab”). Concentrations of terpenes are below 0.1 ppm, and saponins have not been detected at levels as low as 7 ppb. As such, dietary exposures to these potential substances of concern are not expected to increase as a result of the intended use of Inufib™.

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

The anticipated dietary exposure to the notified substance resulting from its intended use is the same as or less than that identified in GRN 118 by Imperial Sensus (FDA, 2002 and 2007

[7] Email correspondence from Martin A. Sanchez, MASO Consulting LLC on Sept 6, 2011

amendment) for a chicory-derived inulin product. The consumption rates of agave inulin from dietary sources were derived from data available in the published literature.

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

Since the anticipated dietary exposure to the notified substance resulting from its intended use is the same as or less than that identified in GRN 118 by Imperial Sensus (FDA, 2002 and 2007 amendment) for a chicory-derived inulin product, no additional assumptions were made in estimating dietary exposure.

4.0 SELF-LIMITING LEVELS OF USE

The suggested serving levels and use levels in food of the notified substance will be identical to those identified in the GRAS Notification submission to the U.S. FDA (FDA) for chicory inulin (GRN 118 and 2007 amendment) and fructooligosaccharide (GRN 44), which were determined to be GRAS without questions or objection by FDA (FDA, 2000b; FDA 2003). As there are insufficient data to identify a threshold use level in food that would adversely affect palatability, self-limiting levels of use are not applicable to this GRAS notification.

5.0 COMMON USE OF THE NOTIFIED SUBSTANCE IN FOOD

There is common knowledge of a long history of human consumption of Agave fructans. The agave plant has been used for food and fiber for at least 10,000 years, and it was exported as a food source to Europe since 1520. Natural sources of agave juice concentrates, such as syrup, aguamiel, and inulin, have been safely consumed for decades. Numerous food products containing agave are currently marketed in the U.S. and around the world. Agave fructans have become a desirable ingredient for addition to a variety of food products as a source of soluble fiber.

The agave genus includes about 275 species belonging to the Asparagalus order and Agavaceae family. Four major parts of the agave are edible: the flowers, the leaves, the stem or basal rosettes, and the sap, called *aguamiel* (Davidson, 1999), and all have been used by indigenous peoples for food and beverage since pre-Columbian times (Slauson, 2001). Analyses of several species of agave plant have shown that nonstructural, water soluble carbohydrates known as fructosans are the major fraction and are concentrated in the stem (Srinivasan and Bathia, 1953; Srinivasan and Bathia, 1954). There are several commonly used foods and beverages that originate from the juice or sap of the agave piñas. *Blue tequilana* Webber (or Weber) agave, known as blue agave, is grown in the state of Jalisco and is best known for its juices, or aguamiel, which is the base for distilling tequila. Originally blue agave was selectively bred for its short maturation cycle, flavorful baking qualities and ease of processing.

The freshly extracted juice or sap is drunk as a beverage known as aguamiel (honey-water), and the fermented beverage from this juice is a nutrient-rich brew known as pulque; both are popular beverages in the south of the Sonoran Desert (Debnath et al. 2010). Pulque is used as a regular dietary item in the central highlands of Mexico; it is mildly alcoholic and is consumed especially during festivals and significant cultural events such as religious holidays and weddings. Pulque has been studied extensively for its nutritional potential among traditional and indigenous

populations, and serves as an example of how local food-based strategies can be used to ensure micronutrient nutrition (Kuhnlein, 2004; Hackman et al. 2006).

Human remains dating back at least 10,000 years show early uses of agave for food and fiber. The food uses of the agave plant have long been part of human culture dating back to the pre-colonial era. It was exported to Europe by 1520 and was mentioned as a food of Aztecs and natives in the Florentine Codex of 1580 (IOAA, 2009).

A number of fructan-rich plants have been food sources for indigenous peoples, including Dacopa, a beverage from roasted Dahlia tubers, Yacon tuber (also called Peruvian ground apple); Jerusalem artichoke tuber; Chicory root (*Cichorium intybus*); Murnong, (*Microseris scapigera,* also called the yam daisy) and Camas root. Detailed paleodietary studies demonstrate that prehistoric populations of the semi-arid northern Chihuahuan Desert consumed a wide variety of plants including *Agave lechuguilla* (agave), *Dasylirion sp.* (sotol) and *Allium drummondii* (onion). Conservative estimates of the contribution of inulin-bearing plants in the diet suggest that the average male hunter–forager from this population would have consumed about 135 g per day, and adult females about 108 g/day (based on about 20% less energy) (Leach and Solbok, 2010). Jerusalem artichokes were consumed by some populations as a substitute for white potatoes, and the consumption of inulin by these populations was estimated to have reached 25 to 32 grams per day (FDA, 2002, GRN 118). Fructan-containing products derived from many of these plants are presently commercially available and sold online and in health food stores in the U.S.

Agave plants serve as a food source in some states of Mexico, and their use predates the arrival of the Spaniards. Certain tribes learned to cook agave plants and use them as food to compensate for the lack of water in the desert. These tribes discovered that cooked agave soaked in water could ferment, producing a desirable beverage. This method was used for centuries to produce a variety of beverages from agave (Cedena, 1995). In the modern era, Kolbye et al. (1992) states that inulin and oligofructose have a "history of long-term use before 1958."

The freshly extracted juice of agave is a source of fructans, a form of nondigestable soluble fiber such as that found in oat, wheat, and chicory, as well as agave. The degree of polymerization (DP) of fructans varies from 2 to 60. By convention the fructans are called inulins when the DP is greater than 10, and they are referred to as fructo-oligosaccharides or oligofructose when the DP is $\leq$ 10 (Niness, 1999; Corradini et al., 2004; Ortiz-Basurto et al. 2008). Fructans are virtually unabsorbed from the gastrointestinal tract and are not hydrolyzed by human digestive enzymes. Agave inulin has become a desirable ingredient for addition to a variety of food products as a source of dietary soluble fiber, and products are sold by other companies under the trade names BioAgave® Fructagave, Vivagave, Agavina and Predilife (Gomez et al. 2010) and Olifructine-SP.

In addition to agave inulin, agave fructans serve as the starting material for other common agave-derived food and beverage products, and they are made by further processing of the fructans. The Natural Standard Review for agave indicates that agave is a useful sugar alternative since fructans are 90% fructose and have a low glycemic index (Hackman et al. 2006). Cooking the piña or otherwise treating the fructan polysaccharides to hydrolyze them into their component fructose monomers is a method of commercial fructose production and doing so can also produce fructose-based syrups. Agave syrup was developed and regulated by Mexico in the 1990s. Agave syrups

are made from at least half a dozen plant varieties, the most popular being blue agave, *Agave salmiana, Agave americana* and *Agave mapisaga* (Debnath et al. 2010). The roasted agave piña is sweet and is sold in markets in Mexico in chunks to be eaten.

Juice of agave piñas is the starting material for distilled spirits. Mescal is made by steaming and mashing the piñas, allowing the juice to ferment with added liquid for several days, and distilling the resulting fluid. Several local varieties of mescal are made in Mexican villages within the agave habitat; the most well-known variety is called Bacanora, named after the Sonoran town (Debnath et al. 2010). Tequila is perhaps the most well-known of the distilled spirits derived from agave.

The food and beverage products from agave differ with respect to the species of agave used as raw material and the degree and types of processing steps used to produce the final product. Thus, *Agave vera-cruz* is grown as a commercial source of fructose; *Agave salmiana, A. potatorum*, and *A. angustifolia* are used in the production of mescal (Michel-Cuello et al., 2008; Pena-Alvarez et al., 2004); *Agave atrovirens, Agave americana*, and *Agave salmiana* are the sources of aguamiel and pulque; and only agave of the species *A. tequilana* Weber blue variety, grown near the town Tequila in Jalisco, can be used for tequila production. Foods and beverages derived from the agave piñas that are presently available in the U.S. include fructose and fructose-based syrups, inulin, and tequila.

The *A. tequilana* plant is used for the production of three main products that are ingested; the alcoholic beverage, tequila, the natural sugar substitute, agave syrup, as well as the subject of this notification, the natural fructan, agave inulin. Tequila and agave syrup differ from agave inulin in an important respect---specifically in the production of tequila and agave syrup, both involve the hydrolysis of the fructans into their component fructose monomers. The hydrolysis step, accomplished by thermal, acid or enzymatic treatments, or some combination thereof, is not applied in the case of agave inulin production.

Several patents have been developed for applications of agave as a raw material which include the use of fructans from agave as a natural prebiotic with high natural fiber content; as a sweetener having improved nutritional properties; and as an additive in foodstuffs and cosmetic preparations. Fructose syrup from agave has proposed applications for an organic sports drink and a sugar replacement based on reduced calories and low glycemic index.

6.0 BASIS FOR CONCLUSION OF GRAS STATUS

6.1 § 170.250: Safety Narrative and Description of Relevant Data

In order to provide a comprehensive review of the pertinent literature, data for agave inulin, as well as data for the closely related substances, have been reviewed. A summary of the most relevant studies is presented below. Some of these studies have reported on potential therapeutic or beneficial aspects of fructans, including bifidogenic properties, and were not intended to assess safety. Nevertheless, they are included in this discussion because they show considerable experience with long-term fortification of diets with inulin-type fructans without evidence of associated adverse effects.

Studies with inulin derived from other sources, as well as fructo-oligosaccharides, were considered relevant to the safety of agave inulin, based on evidence that these substances are biologically equivalent. For example, the bifidogenic potential of fructans from *Agave tequilana* Weber var. *azul* have been investigated *in vitro* and compared with prebiotic properties of chicory derived inulin and fructo-oligosaccharides. Specifically, the ability to selectively increase the number of bifidobacteria and alter colonic short-chain fatty acid profiles was determined (Gomez et al., 2010). Agave inulin produced significantly increased growth of bifidobacteria and lactobacilli, similar to the effect observed for established inulin-type prebiotics derived from chicory root. Total short chain fatty acid production was also increased by agave inulin with significant increases in acetate and propionate. The increases in short chain fatty acid production by agave inulin were qualitatively and quantitatively similar to those produced by the chicory-derived inulin products (Gomez et al. 2010), supporting the biological equivalence of agave inulin with these related products. Furthermore, an *in vitro* assessment of the prebiotic effect of fructans showed an efficient stimulation of growth of Bifidobacteria and Lactobacilli by several agave fructans including *A. tequilana* Gto. (Lopez and Urias-Silvas, 2007). Fructans from *A. tequilana* exhibit a similar bifidogenic potential *in vitro* as compared with a short-chain fructan derived from chicory roots inulin (Raftilose®Synergyl). Carabin and Flamm (1999) considered synthetic fructo-oligosaccharides and plant-derived inulin and oligofructose to be toxicologically equivalent, due to their chemical similarity. Recent toxicological studies with agave inulin support this assertion and indicate that the variations in fructose linkage type and degree of polymerization that exist among the edible plant fructans do not influence toxicity.

The published data, as well as reviews conducted by regulatory agencies, support the conclusion that inulin and inulin-type fructans, including agave inulin, 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 studies employing bolus, short-term, and long-term exposures. *In vitro* studies show that agave inulin promotes the growth of colonic microflora, which in turn produce short chain fatty acids, in a manner that is similar to existing chicory-derived inulin products and other non-digestible polysaccharides. These results are corroborated by the *in vivo* study in mice showing increased cecum and cecum wall weights. No adverse effects were identified following repeated oral dosing of agave inulin or other fructans.

The available animal and human metabolism and safety information indicate that inulin from all sources will be similarly metabolized and processed by the body following consumption. Agave inulin is a fructan, defined as naturally occurring mixture of oligo- and polysaccharides with fructose as the repeating unit. It is a form of nondigestable soluble fiber such as that found in oat, wheat, and chicory, as well as agave. The $\beta(2\rightarrow1)$ and $\beta(2\rightarrow6)$ linkages present in agave inulin and other fructans are resistant to hydrolysis by human digestive enzymes and, therefore, fructans are largely unabsorbed and pass intact into the colon where they are subject to fermentation by colonic microflora (Lopez et al. 2003). This fermentation results in the production of gases such as hydrogen, carbon dioxide, and methane, as well as short-chain fatty acids. The short-chain fatty acids are utilized locally as an energy source by the resident flora, taken up systemically *via* the colonocytes and transported to the liver for caloric utilization by the host, or excreted in the feces.

6.1.1 Studies in Humans with Agave Inulin

López-Velázquez et al. (2013) studied the effect of fructans obtained from *Agave tequilana* var Weber on the frequency of gastrointestinally adverse events (including changes in stool consistency and incidence of colic, abdominal distention, flatulence, and regurgitations) in a randomized double blind clinical controlled trial in term-born infants. Healthy infants[8] 20 ±7 days of age at study onset were assigned to one of six groups (100 per group). Three groups were fed formula containing a probiotic (Lactobacillus, CUF = 107) and fructans (0.5 g/100 mL), while one group each was fed formula containing probiotic only, formula containing no probiotic or fructans, or human breast milk. Among the three groups fed formula containing both probiotic and fructans, one group was fed formula containing agave fructo-oligosaccharides (trade name Metlos®; DP predominantly <10); one group was fed formula containing agave inulin (trade name Metlin®; DP predominantly >10), and the third group was fed formula with a fructan mixture containing both Metlos® and Metlin®[9]. For all three treatment groups, the mean daily formula intake over the last two months of the six month study duration ranged from 1423 - 1510 mL/day for an average daily fructans dose of 7.1 – 7.5 g/day during the period of maximum formula consumption. Gastrointestinal effects were evaluated *via* Case Report Forms once per month until the babies reached 6 months of age. Among infants fed formula containing the probiotic and the fructan mixture, as well as infants fed formula containing the probiotic and Metlin® only, there were no significant changes in stool consistency or increases in the incidence of colic, abdominal distention, number of daily flatulence episodes, and number of daily regurgitation episodes compared with infants that were fed only breast milk. Among infants fed formula containing the probiotic and Metlos® only, there was a significant increase in the percentage of infants with > 10 flatulence episodes per day, but there were no significant changes in stool consistency, nor were there any significant increases in the incidence of colic, abdominal distention or number of daily regurgitations. The authors concluded that agave fructans, when given under the conditions of this study, is safe for use as a nutritional supplement in infants.

A randomized, double-blind, placebo-controlled, crossover study was conducted by Holscher et al. (2014) to assess tolerance and utilization of agave inulin in healthy adults. Study participants consisted of 29 healthy men and women aged 20-36, who consumed daily doses of 0, 5.0, or 7.5 g agave inulin (BioAgave® Agave inulin fiber) in a single serving (administered *via* one chocolate chew per day) for three 21-day treatment periods separated by 7-day "washout" periods. The severity of gastrointestinal symptoms (abdominal pain, bloating, burping, flatulence, nausea, reflux, and rumblings) as well as the consistency and ease of bowel movements was recorded daily.

[8] This and other studies that pertain to infants and/or the use of infant formula are included to support human safety, and the safety of Inufib for the general populations including infants. Note that this notification does not include infant formula applications.

[9] The safety and tolerability studies discussed in Section 5 that were conducted with Metlin®, Metlos®, mixtures of Metlin® and Metlos®, BioAgave® or Predilife, are relevant to addressing the safety and tolerability of Inufib™, because all of these products consist of mixtures of fructooligosaccharides and fructans obtained from agave piñas, having the same basic molecular structures, with variable degrees of polymerization (DP), but overlapping with Inufib™ in the proportion of polymerizations. The DP of agave fructans or a demineralization processing step can influence the effect of oral intake on body weight gain, serum cholesterol, serum triglycerides, and the relative populations of intestinal bacterial in obese mice, whereas no influence was found on acute oral toxicity or mutagenicity by such variations in agave fructan composition (M´arquez-Aguirre et al. 2013). Irrespective of DP and demineralization, the agave fructans in Table 2 all pass into the colon mostly intact, undergo equivalent metabolic processing, and are well tolerated orally.

In addition, weekly assessments of the frequency of abdominal pain, bloating, flatulence, nausea, rumblings, and diarrhea were completed by questionnaire. In the daily assessments, the severity of symptoms was reported on a scale of 0 (absent) to 3 (severe), and in the weekly assessments, the frequency of symptoms was reported on a scale of 0 (occurs no more than usual) to 2 (occurs much more than usual). The daily assessments revealed statistically significant increases in the mean scores measuring the severity of abdominal pain, bloating, flatulence, and rumblings among the treated groups compared with the placebo control group, but the reported scores indicated 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. Similarly, in the weekly assessments there were statistically significant increases in the mean scores among treated groups measuring the frequency of bloating, flatulence and rumblings compared with the placebo group, but the reported scores indicated only slight increases in frequency, ranging from 0.4 for rumblings to 1.0 for flatulence in the high dose group. Stool characteristics, including number of bowel movements, ease of stool passage, stool consistency, and percent dry matter were affected by agave inulin consumption. However, the magnitudes of these effects were very small; for example, the mean number of daily bowel movements increased from 1.2 in the placebo group to 1.4 in the high 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 reflecting softer stool consistency. The authors concluded that a daily consumption of 5 – 7.5 g agave inulin in a single serving is generally well tolerated in adults with mild flatulence reported as the most common side effect.

Tarini and Wolever (2010) studied the effects of inulin on postprandial glucose, insulin, short-chain fatty acids, free fatty acids, and gut hormone responses in healthy subjects. Overnight-fasted healthy subjects (n = 12) were studied for 6 hours after consuming 400 mL drinks containing 80 g high-fructose corn syrup (80HFCS), 56 g HFCS (56HFCS), or 56 g HFCS plus 24 g inulin (HFCS+I), using a randomized, single-blind, crossover design. A standard lunch was served 4 hours after the test drink. Glucose and insulin responses after HFCS+I did not differ significantly from those after 80HFCS or 56HFCS. Serum acetate, propionate, and butyrate were significantly higher after HFCS+I than after HFCS-alone containing drinks from 4–6 h. Free fatty acids fell at a similar rate after all 3 test drinks, but they were lower after HFCS + I than after 56HFCS at 4 h (0.40 ± 0.06 vs. 0.51 ± 0.06 mmol/L; $p < 0.05$). Compared with 56HFCS, HFCS+I significantly increased plasma glucagon-like peptide-1 concentrations at 30 min while reducing ghrelin at 4.5 h and 6 h. The authors concluded that inulin reduces postprandial free fatty acid rebound and reduces the serum ghrelin response after a subsequent meal, events associated with increased colonic short-chain fatty acid production.

Clinical information on the intake and tolerance of fructans in humans was reviewed and summarized by Carabin and Flamm (1999). Effects that potentially develop from the use of 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 colonic pressure. The effect of inulin, oligofructose and synthetic fructose oligosaccharides on the gastrointestinal tract differ as a function of their chain lengths. In this regard, smaller molecules have a higher osmotic colonic pressure, and slower fermenting compounds are more easily tolerated than faster fermenting compounds. The potential for osmotic diarrhea is greater with fructo-oligosaccharides 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 intended conditions of use as a dietary fiber, and that up to 20 g/day of inulin and/or oligofructose is well tolerated.

6.1.2 Studies in Rodents with Agave Inulin

6.1.2.1 Repeated Dose Studies

Marquez-Aguirre et al. (2013) studied the effect of the degree of polymerization (DP) and a demineralization processing of agave fructans on body weight gain and gut bacterial profiles of obese mice. Seventy male C57/BL/6 mice (9 weeks old at study onset) were fed a high-fat diet to induce obesity, and they were given daily gavage doses of 5 g/kg body weight agave fructans derived from *Agave tequilana* Weber var. azul for a 12-week period. Among the treated groups, one group received agave fructans with a DP < 10, and another group received agave fructans with a DP > 10. Additional groups received total agave fructans with or without demineralization by ion exchange chromatography. A commercial chicory fructan (OraftiSynergy1™) was employed as a reference to the prebiotic effect of fructans. Controls for high fat diet and normal diets without fructan-supplementation were included. At the end of the treatment period all animals were sacrificed, and gross pathological examinations were performed on all organs. Blood serum was analyzed for total cholesterol and triglycerides, and fat tissue was measured by the excision and weighing of white adipose tissue. In addition, all mice were observed throughout the duration of the treatment period for mortality, body weight effects, and clinical signs, although further details were not provided. Quantification of *Lactobacilli* and *Bifidobacteria* in mice fecal samples recovered from the colon immediately following sacrifice was performed by real-time PCR.

Agave fructan treatments were well tolerated, and no adverse effects were reported. In all fructan treatment groups, there were reduced body weight gains in animals given the high fat diet; this reduction achieved statistical significance only in the group treated with low-DP agave fructans. Relative to the high fat diet controls, the low-DP agave treatments resulted in significantly reduced fat tissue and total serum cholesterol, and long-chain and total agave fructan treatments resulted in significantly reduced serum triglycerides. A bifidogenic effect, defined as an increased relative abundance of intestinal *bifidobacterium* to *lactobacillus* when compared with control animals was observed in obese mice treated with the demineralized total agave preparation and the OraftiSynergy1™ group. The authors concluded that agave fructans with low DP can prevent body weight gain and fat tissue accumulation associated with a high fat diet without bifidogenic activity. Regardless of the source of fructan, DP and demineralization status, consumption of 5 g/kg-day fructans was not associated with any overtly adverse health effects in mice.

The physiological effects of *A. tequilana*-derived fructans in the diet of mice over 5 weeks was studied (Urias-Silvas et al. 2008) to compare them with other prebiotic fructans, including commercially available chicory-derived inulin (i.e. Raftilose®Synergyl) and fructans from the *Dasylirion* spp (plant family Asparagaceae), which is similar to agave with respect to plant morphology, geographical distribution and pollen characteristics. Groups of eight mice were given fructan supplemented diets (10%) or standard diet (controls) for 5 weeks. It is noted that the concentration of the fructans in the diets was higher than the upper limit of 5% of the total diet recommended to avoid nutritional imbalances in long term studies. Body weights and food intake were measured two times per week, and 24-hour feces collections were performed three times

during the course of the experiment. Blood samples were taken once per week for measurement of serum glucose, triacylglycerol cholesterol and nonesterified fatty acids. Glucagon-like peptide-1 (GLS-1) was measured in terminal portal vein blood samples. Segments of the cecum and proximal, medial and distal colon collected for mRNA and GLS-1 analysis. Full and empty cecum, liver and epididymal fat tissue were weighed, and livers were kept for histological analysis. Hepatic triacylglycerol cholesterol and nonesterified fatty acids were determined.

The three fructan treatments were reported to be well tolerated. The effects of the *A. tequilana* fructan supplemented diet were qualitatively similar to those obtained with the diets supplemented with the chicory and *Dasylirion* spp sources of fructans and previously described effects of fructan consumption, namely, a decrease in energy intake and body weight gain, and a decrease in glycemia. Significant changes in mice receiving the *A. tequilana* fructan supplemented diet compared with the standard diet included an 10% decrease in energy intake; body weight gain was ~30% of the standard diet group (body weight was not reported). Serum glucose concentration was reduced 15%, and serum cholesterol ~20%, similar in magnitude to the reductions in the other fructan-supplemented diet groups. Mechanistically, the effects of fructans generally on energy intake and related sequelae (e.g., body weight, adipose tissue mass, and lipid metabolism) are indirect and have been attributed to fermentation-dependent production of short-chain fatty acids in the caeco-colon (Daubioul et al. 2002). Total cecum weight and cecum wall weight were increased 100% and 77%, respectively, in agave diet group, which was similar in magnitude to the chicory and *Dasylirion* diet groups and were attributed by the authors to increased bacterial activity and an increase in short-chain fatty acid production through fermentation by colonic bacteria. The changes that were statistically significant in the agave, but not other fructan-supplemented diet groups, were increased feces excretion (17% dry basis), decreased epidydymal fat weight as a surrogate for adipose tissue weight (27%), and decreased liver weights (13%), relative to controls. The changes were likely secondary to the reduction in body weight gain, which was more pronounced in the agave group than the other fructan groups. However, histological analysis of the liver did not reveal any differences between the groups or compared with controls, and the effects were not considered adverse. Mouse diets supplemented with the all three types of fructans also induced a higher concentration of glucagon-like peptide-1 (GLP-1) and its precursor, proglucagon mRNA, in the different colonic segments. GLP-l concentrations measured in the portal vein were increased 1.5 to 2-fold in the test groups relative to controls. On the basis of these findings, the authors suggested that fermentable fructans are able to promote the production of satietogenic/incretin peptides in the colon. While the focus of this detailed study was a comparison of the physiological effects of fructans from agave, chicory, and *Dasylirion,* the study did not reveal adverse effects in mice of diets supplemented with 10% fructans for 5 weeks (Urias-Silvas et al. 2008).

6.1.2.2 Acute Toxicity Studies

In an acute toxicity test stated as compliant to OECD Guideline 425, 25 male Balb/c mice were given single gavage doses of agave fructans derived from *Agave tequilana* Weber var. azul at concentrations of 175, 550, 1750, and 5000 mg/kg (Marquez-Aguirre et al., 2013). A commercial chicory fructan (OraaftiSynergy1™) was also evaluated. Following a 14-day observation period, the following parameters were assessed:

Red blood cells, hemoglobin, hematocrit, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, aspartate aminotransferase, alanine aminotransferase, glucose, creatinine, and body weight gain.

Among mice treated with a single gavage dose of 5000 mg/kg fructans (regardless of average DP or plant source), there were no mortality and no statistically significant changes in any of the measured hematological or blood chemistry parameters compared to untreated controls. Body weight gain was similarly unaffected. The authors further stated that treatment did not affect "general state of health," although no further details regarding which health effects were assessed in the single-dose assay were provided.

In an acute toxicity study reported by Gracia et al. (2013), 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) were given a single gavage dose of agave fructans derived from *Agave tequilana* Weber at 17.5, 55, 175, 550, 1750, or 5000 mg/kg. The animals were treated with either a low DP (< 10) or a high DP (> 10) agave fructan preparation (trade names Metlos® and Metlin® respectively). Animals were observed for a 14-day post-treatment period. No mortality, adverse clinical observations, changes in body weight, or histopathological findings the tissues examined (stomach, large intestine, small intestine, and liver) were reported. The authors concluded that the agave fructan products given orally to rodents at up to 5000 mg/kg is non-toxic.

In summary, there were no adverse effects identified following acute oral dosing of agave inulin up to 5000 mg/kg in rats and mice, nor was there any difference in toxicity compared with chicory inulin.

6.1.2.3 Genotoxicity Studies

Agave inulin has been shown to be non-mutagenic *in vitro*. In a bacterial reverse mutation assay conducted on *S. typhimurium* strains TA98, TA100, and TA102, agave fructans derived from *Agave tequilana* Weber var. azul at a concentration of 800 µg/plate did not significantly increase the frequency of mutations relative to negative controls, both with and without metabolic activation with Arochlor-1254 induced S9 mixture (Marquez-Aguirre et al., 2013). The study protocol was stated as compliant to methods described in Maron and Ames (1983) but deviated from current standardized guidelines (including OECD Guideline 471 and FDA Redbook) in the following ways: (1) testing did not include at least five strains of bacteria, including *S. typhi* TA1535 and TA1537 or TA97a or TA97 in addition to *S. typhi* strains TA98, TA100, and TA102 and (2) for noncytotoxic substances a maximum concentration of 5 mg/plate is recommended, which is below the 800 µg/plate concentration used in the study. Although this study does not conform to standardized guidelines and no rationale was provided for the noted deviations, based on structure-activity considerations, agave inulin is not expected to interact with DNA, and the mutagenic potential is expected to be negligible.

In vivo chromosomal aberration and micronucleus assays were conducted with Hsd:ICR mice by Gracia et al. (2013) to evaluate the genotoxicity of agave fructans derived from *Agave tequilana* Weber. Both a low DP (< 10) and a high DP (> 10) agave fructan preparation (trade names Metlos® and Metlin®, respectively) were used in the study. Groups of male mice 4-5 weeks of

age (5 per treatment group) were given intraperitoneal injections of 143, 357.5, or 715 mg/kg of Metlin® or Metlos®, while two additional groups were given Mitomycine-C or phosphate buffer solution (PBS) as a positive and negative control, respectively. Twenty-four hours after treatment, 5 µL of peripheral blood from the tail vein were collected from each animal. Subsequently, the animals were euthanized and bone marrow was extracted from the femur of each animal. For the chromosome aberration study, 100 bone marrow cells in metaphase from each animal were scored for alterations in the chromosomes and chromatids. For the micronucleus assay, erythrocytes from tail vein blood were stained and examined for frequencies of micronucleated polychromatic erythrocytes in a fluorescence microscope. The chromosome aberration assay was stated by the authors as compliant to OECD Guideline 475 and EPA OPPTS 870.5385. No specific guideline was cited by the authors for the micronucleus assay, although, based on the methodology described, it is compliant to OECD Guideline 474. It is noted that no rationale for the selected doses was provided. In the chromosome aberration assay, the number of cells with deletions, fragments, translocations, or gaps was not significantly increased among the Metlin® and Metlos® treated groups compared to negative controls. Similarly, in the micronucleus assay, the mean frequency of micronucleated cells was not significantly increased by treatment with Metlin® and Metlos® at any dose, compared with the negative control group. The authors concluded that agave fructans derived from *Agave tequilana* Weber is non-genotoxic in mice.

6.1.3 Studies with Fructans Derived from Non-Agave Sources

6.1.3.1 Human Studies

The gastrointestinal tolerance of native chicory inulin and its shorter chain length oligofructose was evaluated at 5 and 10 g doses compared to a placebo control (Bonnema et al. 2010). Twenty-six healthy men and women ages 18 to 60 years participated in the study. Healthy subjects with no history of gastrointestinal conditions consumed diets with typical amounts of fiber. The two inulin fibers tended to increase gastrointestinal symptoms mildly. Most frequently reported symptoms were flatulence followed by bloating. The 10 g dose of oligofructose substantially increased GI symptoms compared to control. Doses up to 10 g/day of native chicory inulin and up to 5 g/day of oligofructose were well-tolerated in healthy, young adults.

6.1.3.2 Rodent Studies

Dávila-Céspedes et al. (2014) conducted a 13-week dietary study of fructans from *Agave salmiana* in Wistar rats (n=36) to assess the effect on azoxymethane-induced carcinogenesis. Two intraperitoneal injections of 15 mg/kg azoxymethane were administered with a one week interval between injections, and administration of either standard diet or diet containing 10% fructans derived from either *A. salmiana* or *C. intybus* continued for a 13-week period following the second injection. Fructan treatments were well tolerated, and no adverse effects attributable to the test diets were reported. The authors reported that the number of aberrant crypt foci found in the colon of azoxymethane-treated rats fed the fructan fortified diets were significantly lower than the corresponding control group fed a standard diet

In a 28-week dietary study, Hijová et al. (2013) evaluated the effect of oligofructose-enriched inulin derived from chicory root (Orafti Synergy1) at 8% in the diet on bacterial activity, cytokine

levels, and the expression of chemopreventive markers cyclooxygenase-2 (COX-2) and nuclear transcription factor kappa beta (NFkB) in Sprague-Dawley rats exposed to a cancer-causing agent. The fructan treatment was well tolerated, and no adverse effects attributable to the test diet was reported.

In a 6-week feeding study (Rendón-Huerta et al. (2012)), the effect of fructans from three sources (*Agave angustifolia, Helianthus tuberosus,* and *Cichorium intybus*) was studied in diabetic and obese Wistar rats (sex not specified). The fructans were administered at a concentration of 15% in feed, corresponding to 7-9 g/kg bw – day. Body weight reductions in agave fructan fed rats were modest (<6% relative to the corresponding controls). Regardless of obesity or diabetic status, consumption of fructans resulted in significantly increased fecal concentrations of *Lactobacillus* and *Bifidobacterium* compared with controls ($p<0.05$). The agave and other fructan treatments were well tolerated, and no adverse effects were reported.

The effect of fructans extracted from onion (*Allium cepa* L) was studied in male F344 rats in a 4-week feeding study (Roldan-Marin et al. 2009). Groups of 8 rats were given diets containing 7% of the fructan extract or control diets. A semiquantitative size distribution analysis of the fructans in the extract indicated that > 90% had ten fructose residues or less, and > 60% had five residues or less with very small amounts of longer chain fructans present. Fructan treatment was well tolerated. There was a significant decrease ($P<0.05$) in the hemoglobin concentration in treated rats compared with the rats in the control group, consistent with a previously noted anemia caused by onions fed to rodents. Antioxidant enzyme activities were measured in erythrocytes and in liver. There was a significant increase ($P<0.05$) in glutathione reductase and glutathione peroxidase activities in erythrocytes of rats fed the test diet while hepatic glutathione peroxidase activity was significantly decreased ($P<0.01$), and hepatic glutathione reductase activity was unchanged compared with controls. There was no DNA damage as measured in liver and leukocytes by the comet assay. There was no significant difference in gastrointestinal transit time in the test diet group compared to the control group. The test diet had prebiotic effects as evidenced by decreased pH, increased butyrate and propionate production and an increase in the cecal microbiota enzyme activities, β-glucosidase and β-glucuronidase. Hepatic gene expression of Gr, Gpx1, catalase, 5-aminolevulinate synthase and AD(P)H:quinone oxidoreductase were not altered in the test group.

Buddington et al. (2002) reported studies with B6C3F1 mice in which diets containing nondigestible β-fructans provided protection against various health challenges. In the control diet, the sole source of fiber was 100 g cellulose/kg; in the test diets, the cellulose was replaced with oligofructose (Raftilose P95; Orafti, Tienen, Belgium) or inulin (Raftiline HP; Orafti, Tienen, Belgium). These levels of dietary fiber (10%) are comparable to levels of fiber commonly recommended for human intake. Test and control diets were fed for a 6-week period before the challenges to allow for full adaptation of the gastrointestinal tract ecosystem, and the diets continued to be fed throughout the challenge period. Concurrent studies with B6C3F1 mice verified that diets containing 100 g/kg inulin or oligofructose increased the densities of lactic acid-producing bacteria. Enteric and systemic defense functions were assessed. β-Fructan treatments were well tolerated, and no adverse effects attributable to the test diets were reported. Under the conditions of this study, feeding inulin or oligofructose to mice prior to enteric challenges and systemic bacterial infections resulted in an increased host resistance to the challenges. The authors

concluded that gastrointestinal tract bacteria remain responsive to long-term feeding of fructan prebiotics.

Rao et al. (1965) conducted 6-week feeding studies in albino rats to evaluate the effects of polyfructosans from the stems of the *Agave vera-cruz* plant compared with inulin (Merck & Company). Test diets were supplemented with 5% agave fructosans or 5% inulin at the expense of starch, and controls were given basal diets (n=8). Food intake, body weights, total cholesterol in liver and plasma, fecal steroids, and excreted bile acids were determined in rats. Body weight gains were lower in the groups given fructosan (10%) and inulin (13%) diets compared to controls. Agave fructosan (fructan) and inulin treatments were well tolerated. Fructosan and inulin were largely unutilized based on an increase in fecal bulk in rats given the test diets relative to controls. The only adverse effect reported was mild diarrhea in the rats fed agave fructosan. Highly significant reductions in plasma cholesterol levels were observed in fructosan (35%) and inulin (22%) treated groups, and hepatic cholesterol was reduced ~10% in each group. The average fecal sterol excretion was 16.3 mg/day in the fructosan diet group compared with 10.4 and 11.2 mg/day in the basal diet and inulin diet, respectively. Mean fecal excretion of bile acids was unchanged in the fructosan group (16.8 mg/day) compared with controls (17.0 mg/day) and was reduced to 11.9 mg/day in the inulin diet group, however, the difference was not significant.

6.1.4 Toxicological Studies with Fructo-oligosaccharides

In a critical review of the animal toxicology data and clinical studies of inulin and oligofructose (fructans), Carabin and Flamm (1999) concluded that these fructans have not shown evidence of mortality, morbidity, target organ toxicity, reproductive or developmental toxicity, mutagenicity or carcinogenicity. The authors defined the scope of their review as including any inulin-type fructan which contains molecules with an average DP 10 and above (referred to as inulin); any inulin-type fructan containing only molecules with a DP less than 10 and derived from chicory (referred to as oligofructose); and substances synthesized from sucrose with an average DP 3.5 (referred to as fructo-oligosaccharides or FOS). The authors considered toxicological studies performed with FOS to be predictive of the effects of naturally occurring inulin and oligofructose since the substances are chemically and physiologically similar entities with like nutritional properties. The following is a brief summary of the studies discussed by Carabin and Flamm (1999):

The rat oral LD_{50} for FOS was determined to be greater than 9 g/kg. In a rat 6-week oral toxicity studies, conducted by gavage and dietary feeding routes, respectively, there was no treatment-related toxicity in any FOS-treated groups up to a dose of 4.5 g/kg (gavage) and 10% FOS in the diet relative to control diets containing existing sugars commonly used in the food supply (Takeda and Niizato, 1982).

In a 2-year carcinogenicity study with male and female Fischer 344 rats (Clevenger *et al.,* 1988), given FOS with their diet at concentrations up to 50,000 ppm (equivalent to 2170 mg/kg/day and 2664 mg/kg/day, respectively, for male and female rats), there were no significant dose-related effects on body weight, food consumption, survival, growth, hematology, blood chemistry, or organ weights, nor did the treatment affect the incidence of neoplasms.

Maternal and developmental toxicity was evaluated in Wistar rats (Henquin, 1988) and Crl CD (SD) BR rats (Sleet and Brightwell, 1990). Dietary supplementation with FOS at concentrations up to 20% did not cause adverse effects or negatively affect the pregnancy outcome or *in utero* or early postnatal development of the rat. The only treatment-related effect was a moderate reduction in maternal body weight observed in the 20% FOS groups.

Fructo-oligosaccharides exhibited no genotoxic activity in three assays conducted with and without metabolic activation, which included the bacterial reverse mutation assay with *Salmonella typhimurium* (strains TA1535, TA1537, TA1538, TA98, and TA100) and *Escherichia coli* WP2 *uvr* A, the L5178Y mouse lymphoma TK6 mammalian cell mutation assay, and an unscheduled DNA synthesis assay in human epithelioid cells (Clevenger *et al.,* 1988).

As concluded by the Carabin and Flamm (1999):

> *"No evidence of treatment-related toxicity, carcinogenicity, or genotoxicity was observed from standard toxicity tests conducted at doses far above anticipated human exposure. Human and animal studies demonstrated that inulin-type fructans do not adversely affect mineral absorption, glycemic control, lipid metabolism, or intestinal flora."*

Fructo-oligosaccharides induce less diarrhea than the disaccharide maltitol and significantly less than the monosaccharide sorbitol (Takeda and Niizato, 1982). Inulin, as a slower fermenting compound, has better gastrointestinal tolerance than fructo-oligosaccharides or oligofructose, and similarly, agave inulin can be expected to be better tolerated than the shorter chain molecules with respect to gastrointestinal symptoms.

6.1.5 Toxicological Studies with Carboxymethyl Inulin

Johannsen (2003) reviewed the toxicological properties of carboxymethyl inulin, a material used as an anti-scalant in food processing applications that is synthesized by carboxylation of a chicory-derived inulin. Several studies conforming to international test guidelines were reviewed. In brief, a rat 4-week toxicity study by the gavage route showed no treatment-related effects in body weight, food consumption, mortality, hematology, clinical blood chemistry, organ weights or gross or microscopic pathology up to the highest dose of 1000 mg/kg-day. Females in the 1000 mg/kg-day group showed a modest increase in motor activity, however, this finding was not considered toxicologically significant. A guinea pig Magnusson–Kligman maximization test showed no evidence of dermal sensitization with carboxymethyl inulin. It was also not genotoxic in either bacterial reverse mutation assays conducted with Salmonella strains TA1535, TA1537, TA98 and TA100 or in Escherichia coli WP2uvrA, and did not induce chromosomal aberrations Chinese hamster ovary cells *in vitro.*

6.1.6 Allergenicity

No cases of allergenicity or hypersensitivity reported in association with consumption of Agave inulin were located in the published literature. Natural sources of agave juice concentrates, such as agave inulin and syrup, have been safely consumed for decades. There is no evidence that inulin

content is implicated as allergenic in any the foods of the 8 major food allergens (FDA 2010). The lack of available information and absence from FDA's list leads to the conclusion that allergic reactions to agave inulin are not of concern.

It is important to emphasize that the agave inulin Inufib™ is derived from the piñas and is not derived from the leaves of the agave, because sap or extracts from the leaves from some agave species have been noted to contain saponins and raphides of calcium oxalate. In contrast to the juice extracted from the piñas of the agave plant, the leaves can produce a liquid that can be irritating when it comes in contact with human skin. Workers in tequila distilleries and on agave plantations may develop an irritant contact dermatitis, which was determined by Salinas et al. (2001) to be attributable to the presence of sharp, needle-like calcium oxalate, known as raphides, in the plant. Salinas et al. (2001) isolated and purified calcium oxalate crystals from the leaves of *A. tequilana*. The crystals were characterized as 30–500 μm in length, sharpened at both ends. One drop of juice pressed from the leaves contained 100 – 150 of the needle-like crystals.

Outside of agave plantations and tequila distilleries, agave-induced irritant dermatitis is relatively rare (Ricks et al., 1999). Twelve cases of irritant contact dermatitis provoked by the popular ornamental plant, *Agave americana*, have been reported (Hackman et al. 2006). Ricks et al. (1999) presented a case report of Agave-induced purpura on the anterior legs in an otherwise healthy patient. The condition developed as a result of landscaping work during which an *A. americana* plant was cut down with a chain saw. Histopathology examination of punch biopsy was consistent with hypersensitivity vasculitis.

Inufib™ is mechanically extracted from the pines ("piñas") of the agave plant, defined as the stems without the leaves. Indeed, when the plants are harvested, the leaves and roots are cut off and left in the fields for soil enrichment. The resulting inulin does not contain saponins or raphides of calcium oxalate. Due to the rigorous production methods, good manufacturing practices, and quality standards used in the manufacture of Inufib™, hypersensitivity is not a safety concern for Agave inulin.

Only two cases of anaphylaxis to inulin in food have been published (Gay-Croisier, 2000 and Franck et al. 2005), indicating that allergy to inulin is extremely rare given its widespread presence and use in food. The two cases were related to consumption of Raftiline®HP, a chicory root inulin (from *Cichorium intybus,* plant family Compositae (also called Asteraceae*)*. In the chicory inulin case reported by Franck et al. (2005), a 50-year-old woman with a past history of allergy to artichoke presented with two episodes of immediate allergic reactions, one of which was a severe anaphylactic shock after eating two types of health foods containing inulin. Both food products had added inulin (Raftiline®HP) for its bifidogenic effect: 0.38 g in one biscuit and 2.5 g in the yoghurt. Specific IgE to an inulin-protein compound was identified using dot blot and dot blot inhibition techniques, 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 Raftiline with any history of allergy to other members of the Asteraceae (Compositae) plant family, *viz.*, artichoke (genus *Cynara*) or endive (genus *Chicorium*), should be warned (Franck et al. 2005). While inulin allergy is exceedingly rare, the only cases are associated with inulin from chicory (*Cichorium intybus)* from the plant family Asteraceae, whereas no instances of allergy from agave

inulin, plant family Asparagaceae, are known. Hence, the above referenced warning for products containing Raftiline would not apply to agave inulin.

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

Chicory-derived fructan concentrates (e.g., Frutafit® inulin and Frutalose® oligofructose) and FOS are marketed in the U.S. and have GRAS status (FDA 2002b, 2003, 2012, 2014 and 2015). In common with agave inulin, these GRAS products are fructose oligo- and polysaccharides covering a range of DP, from <10 for the fructooligosaccharide products (e.g., GRN 44 and 392) and >10 for inulin products (e.g., GRN 118, 477 and 537). Agave inulin differs from these products in the amount of branching of the fructosyl linkages and internal glucose moieties, however, these differences are quantitative rather than qualitative. Inufib™ (~90% agave inulin content) has been on the market for over 15 years, and no safety concerns have been reported.

The FDA, and Health Canada have reviewed the safety of inulin and fructans from chicory and other plant sources and found their use to be safe based on the available animal, *in vitro*, and human safety data. Inulin from a variety of plant species including *Agave azul tequilana*, Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intybus*) are used in the food industry (Roberfroid and Delzenne, 1998) and have been the subject of numerous published scientific evaluations and regulatory reviews (FDA, 2000a, 2002, 2011, 2013; 2014 Health Canada, 2013).

In the U.S., chicory inulin was determined to be GRAS without questions by FDA (FDA 2003, GRN 118) and fructooligosaccharide (FOS), a shorter chain length fructan produced by enzymatic synthesis from sucrose, was determined to be GRAS without questions by FDA (FDA, 2000b GRN 44). More recently, other chicory inulin products (GRN 382 and 477) and short chain FOS produced by enzymatic synthesis from sucrose (GRN 537) were determined to be GRAS without questions by FDA (FDA, 2012, 2014, 2015).

Inulin has been approved for use as an acceptable food or food ingredient in most countries including all EU countries, Australia, Canada, and Japan (Franck, 2002). As a food or food ingredient, inulin can be used without specific limitations as ingredients in foods and drinks. A specific Association of Official Agricultural Chemists (AOAC) method of analysis was developed for fructans (AOAC 997.08) to accurately measure the content of inulin and oligofructose (Coussement, 1999).

Inulin from Blue agave, chicory root, and Jerusalem artichoke tuber are among the dietary fibers that were assessed and found acceptable by the Food Directorate, Health Canada (Health Canada, 2013).

Inulin is classified as 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.

Food manufacturers have added inulin-derived substances to the general food supply in Australia and New Zealand since the mid-1990s. The technological purpose for addition to food is to emulsify or thicken food, or for nutritional reasons, such as for their prebiotic effect or as dietary

fiber. 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

Studies conducted and published in support of the evaluation of the safety of agave inulin, regardless of DP, include *in vitro* and *in vivo* animal studies as well as clinical studies in humans. These studies show low acute oral toxicity in mice and rats (>5 g/kg bw) and an absence of mutagenicity and clastogenicity with test substances having DP spanning the range of Inufib DP. In rodent studies employing fructan-enriched diets, the most consistent observation was a modest reduction in body weight gain and mild diarrhea, observed when doses were high at 5 to 10% of the total diet.

In humans, no adverse effects were noted other than mild, transient gastrointestinal (GI) effects such as flatulence and abdominal discomfort. It should be noted that these GI effects have also been documented with other plant fructans (e.g., chicory-derived inulin) and are the same as those symptoms associated with the intake of fruits and vegetables which contain dietary fructans. Most notably, in a critical review of the animal toxicology data and clinical studies of inulin-type dietary fructans considering both inulin and oligofructose, Carabin and Flamm (1999) concluded that these fructans have not shown evidence of mortality, morbidity, target organ toxicity, reproductive or developmental toxicity, mutagenicity or carcinogenicity. The authors considered toxicological studies performed with synthetic fructo-oligosaccharides (average DP = 4) to be predictive of the effects of naturally occurring inulin and oligofructose since the substances are chemically similar entities with like nutritional properties. Literature searches up to the present have revealed no studies that report safety concerns confirming Carabin and Flamm's (1999) earlier findings.

All nondigestible carbohydrates including inulin-type fructans may cause intestinal discomfort and possible laxative action that is dose-related as a result of fermentation in the large bowel. Roberfroid and Delzenne (1998) concluded from their review of the published data that:

> *"In a liquid food product, a single daily dose of 10 g will not cause a transient appearance of mild symptoms of intestinal discomfort, whereas a single daily dose of 20 g may, and the single daily dose likely to cause major discomfort in most individuals (except very resistant high-fiber consumers) is 30 g. However, if the dose is split through the day into several individual servings, symptoms of discomfort will be reduced and, in most cases, will disappear, even for total daily doses as high as 20-30 g. Liquid food products containing inulin-type fructans are always more likely to induce intestinal discomfort than solid formulations are, and the risk of an effect is reduced if the food product is consumed as part of a complete meal. Finally, it must be underscored that a small percentage (1-4%) of the population might have a higher-than-average sensitivity to these intestinal discomforts. But these highly sensitive individuals are also likely to be very sensitive to the intestinal discomfort caused by sugar alcohols, any nondigestible carbohydrates, or even fermented dairy products."*

A committee of experts concluded that increased exposure to inulin and oligofructose is likely to be of negligible biological significance even for a consumer at the 90th percentile dietary exposure level (Kolbye et al, 1992), and this conclusion has stood for over two decades.

The potential allergenicity of agave inulin was researched in the available literature, and no cases of allergenicity or hypersensitivity reported in association with consumption of Agave inulin were located despite a substantial history of human consumption. There is no evidence that inulin content is implicated as allergenic in any the foods listed among the 8 major food allergens (FDA 2010). Hypersensitivity is not a safety concern for dietary Agave inulin produced using the rigorous production methods, practices and quality standards such as those used in the manufacture of Inufib™.

In a review of the applications of inulin and oligofructose in health and nutrition, Kauer and Gupta (2002) summarized the effect on various human health parameters of inulin/oligofructose in the diet. In addition to the bifidogenic effect of fructans and effects on serum lipids, oligofructose and inulin relieved constipation, lowered blood glucose levels, and increased the absorption of calcium.

In summary, healthy adult men and women, and healthy infants showed minimal to no gastrointestinal symptoms associated with the daily ingestion of up to 7.5 g agave inulin over periods of three or six months. The available data on inulin and oligofructose have demonstrated no evidence of toxicity based on animal and clinical studies. Signs of gastrointestinal intolerance are observed with intakes above 20–30 g; however, fructans are better tolerated when given with solid food and when given in divided doses throughout the day.

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

IIDEA had determined that their Agave Inulin product Inufib™ is GRAS based on a combination of: (a) general recognition of safety through scientific procedures based on generally available and accepted scientific data, and (b) experience based on common use of the substance in food prior to January 1, 1958. Item (a) is based upon studies conducted and published in support of the evaluation of the safety of agave inulin which include *in vitro* and *in vivo* animal studies as well as clinical studies in humans, and is corroborated by the GRAS panel review described in Section 9 of this notification. More specifically:

- The estimated dietary 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 an inulin product for an identical general food use (GRN 118), and the notified substance will serve as an alternative source, rather than an additional source, of agave inulin for use in food (see further discussion below).
- Studies conducted and published in support of the evaluation of the safety of agave inulin, including *in vitro* and *in vivo* animal studies as well as clinical studies in humans, report no safety concerns up at doses of 1000 mg/kg-day in rats and at typical consumption levels in humans, respectively.
- Agave inulin and inulin-related 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 (excluding meat and poultry) to the current chicory-derived inulin product as described in GRN 118 (FDA, 2002). Imperial Sensus, in GRAS Notification 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® (i.e., general food 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. Given that the level of inulin in the Frutafit® product (~90%) is virtually the same as the level of inulin in IIDEA's Inufib™, the same levels added to food will result in the same levels of inulin per serving. The estimated intake of inulin from the proposed uses of IIDEA's products will be the same as or less than that of the current GRAS chicory-derived inulin product that was the subject of GRN 118, since use in meat and poultry is not being considered as part of this GRAS Notification for Inufib™. Therefore, the proposed uses of Inufib™ will not result in an increase in the overall consumption of inulin but will simply provide an alternative source of inulin for use in food.

The studies described in Section 6.1 of this notification show low acute oral toxicity in both rats and mice, an absence of genotoxic effects, and no adverse effects when evaluated in human clinical studies and rodent feeding studies. In human clinical studies (López-Velázquez et al. (2013); Holscher et al. (2014)), no adverse effects were noted at consumption levels up to 7.5 g fructans/day in infants and adults respectively, other than mild, transient gastrointestinal (GI) effects such as flatulence and abdominal discomfort. These GI effects have also been documented with other plant fructans such as chicory-derived inulin (Bonnema et al., 2010) and are the same as those symptoms associated with the intake of fruits and vegetables. Agave inulins are similarly well tolerated in rodents. Mice fed *A. tequilana*-derived fructans at a dietary exposure of 10% for 5 weeks (Urias-Silvas et al. 2008) or given gavage doses of *A. tequilana*-derived fructans at doses of 5 g/kg-day for 12 weeks Marquez-Aguirre et al. (2013) were healthy throughout their respective exposure periods with no adverse effects reported. OECD guideline compliant toxicity tests revealed a lack of acute toxicity in mice and rats at doses up to 5000 mg/kg (Marquez-Aguirre et al., 2013; Gracia et al., 2013), and a lack of mutagenicity *in vitro* (Marquez-Aguirre et al., 2013) and clastogenicity *in vivo* Gracia et al. (2013).

There is no basis for any concern for purified inulin from any source regardless of differences in chemical structure, i.e. number of fructose units per molecule and degree of branching with β(1→6) linkages. Literature searches up to the present have revealed no studies that report safety concerns. Evaluations of the possible effects of plant-derived inulin concentrates on calcium and magnesium absorption and allergenicity have also been considered by U.S. FDA and Health Canada, and these reports demonstrated the absence of significant safety concerns when consumed as part of the normal diet.

In conclusion, the fermentable carbohydrates that occur naturally in the agave plant, referred to as agave inulin, are extractable by mechanical means and have been demonstrated to be safe for oral intake through standard animal toxicity studies, genetic toxicity studies, clinical studies, and through their history of safe human ingestion. Their prebiotic and related gastrointestinal effects are well described and understood. Signs of gastrointestinal intolerance, such as flatulence, and bloating, have been seen with intakes of inulin and/or oligofructose above 20–30 g, but these effects are not considered harmful to health.

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

All data used in this GRAS determination are publicly available. None of the information used in this assessment is confidential or unavailable to the general public.

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

The regulatory framework for determining whether a substance can be considered generally recognized as safe (GRAS) 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"), is set forth at 21 CFR 170.30, which states:

> *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 (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food.*
>
> *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. General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. 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.*

These criteria are applied in this analysis to determine whether the use of an agave-derived inulin for use in food for human consumption is GRAS based upon scientific procedures. All data used in this GRAS determination are publicly available and generally known, and therefore meet the "general recognition" standard under the FD&C Act.

The aforementioned critical elements were articulated in the opinion of the Expert Panel (Exhibit 1, Section 9) in the safety evaluation of inulin, oligofructose and fructo-oligosaccharides. The common knowledge element of the "general recognition" standard required under the Federal Food, Drug, and Cosmetic Act has been met. The data and information relied upon to establish safety is generally available from the peer-reviewed scientific literature, international review/authorizations, FDA GRNs, history of use, and a consensus among qualified scientists is established and documented herein. Determination of the safety and GRAS status of the agave inulin product described above for direct addition to food under its intended conditions of use was made through deliberation of an Expert Panel consisting of Richard Kraska, Ph.D., DABT, (chair), Robert S. McQuate, Ph.D., and Robert W. Kapp, Jr., Ph.D., Fellow ATS, ERT-UK, who reviewed

a dossier prepared by IIDEA's agent, NSF International, as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They individually and collectively critically evaluated published data and information pertinent to the safety of agave-derived inulin and unanimously concluded that the intended use of agave inulin in food, produced in a manner consistent with cGMP and meeting the specifications delineated herein for Inufib™, is "generally recognized as safe" (GRAS) based on scientific procedures. The Panel's GRAS opinion is included as Exhibit 1 to this document.

IIDEA agrees with the Expert Panel and has concluded that agave inulin is GRAS under the intended conditions of use on the basis of scientific procedures; and, therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

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

IIDEA is not aware of any information that would be inconsistent with a finding that the proposed use of agave inulin in food for human consumption meeting appropriate specifications and used according to Good Manufacturing Practice is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

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

None of the data and information presented in this GRAS notification are exempt from disclosure under the FOIA.

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

Since all of the data and information presented in this GRAS notification were available to the Expert Panel during their determination of GRAS status, this section is not applicable.

7.0 LIST OF SUPPORTING DATA AND REFERENCES

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

A list of studies described in Section 6 that support the safety of the notified substance are listed in Tables 7a and 7b below. Table 7b includes studies on inulins not derived from Agave *tequilana* Weber var. *azul* but are chemically similar to the notified substance and thus deemed relevant.

Table 7a: List of Safety Studies on the Notified Substance (Inulins derived from *Agave tequilana* Weber var. *azul*)

Species	Study Type	Study Duration	Doses	Results	Reference
Human Infants	Clinical Trial	6 months	7.1 - 7.5 g/day	No adverse effects reported.	López-Velázquez et al. (2013)
Human Adults	Clinical Trial	75 days (2 intermittent 7-day washout periods)	0, 5.0, or 7.5 g/day	High dose associated with mild GI effects; characterized as non-adverse.	Holscher et al. (2014)
Human Adults	Clinical Trial	Single dose with 6-hour observation	24 g	No adverse effects reported*	Tarini and Wolever (2010)
Obese Male C57/BL/6 Mice	Repeated Dose Gavage Study	12 weeks	5 g/kg-day	No adverse effects reported.	Marquez-Aguirre et al. (2013)
Mice (unspecified strain)	Repeated Dose Feeding Study	5 weeks	10% dietary concentration	Reduced body weight gain and liver weights; no histological findings in the liver.	Urias-Silvas et al. (2008)
Balb/c Mice	Acute Toxicity	Single dose with 14-day observation	175, 550, 1750, and 5000 mg/kg	No mortality or adverse effects reported.	Marquez-Aguirre et al. (2013)
Hsd:ICR Mice	Acute Toxicity	Single dose with 14-day observation	17.5, 55, 175, 550, 1750, or 5000 mg/kg	No mortality or adverse effects reported.	Gracia et al. (2013)
S. typhimurium strains TA98, TA100, and TA102	Bacterial Reverse Mutation Assay	N/A; in vitro study	Up to 800 µg/plate	Non-mutagenic	Marquez-Aguirre et al. (2013)
Mice (unspecified strain)	Chromosome Aberration	Single dose	143, 357.5, or 715 mg/kg	Non-clastogenic	Gracia et al. (2013)
Mice (unspecified strain)	Micronucleus Assay	Single dose	143, 357.5, or 715 mg/kg	Negative for induction of micronucleated cells	Gracia et al. (2013)

Table 7b: List of Safety Studies on Inulins Derived from Other Sources

Species	Test Substance	Study Type	Study Duration	Doses	Results	Reference
Human Adults	Native chicory inulin	Clinical Trial	5 days	5 g/day or 10 g/day	Mild increase in bloating and flatulence; no other adverse effects reported.	Bonnema et al. 2010
Wistar Rats	Fructans from Agave salmiana	Feeding Study	13 weeks	10% dietary concentration	No adverse effects reported.	Dávila-Céspedes et al. (2014)
Sprague-Dawley Rats	Oligofructose-enriched inulin derived from chicory root	Feeding Study	28 weeks	8% dietary concentration	No adverse effects reported.	Hijová et al. (2013)
Obese Wistar Rats	Fructans from Agave angustifolia, Helianthus tuberosus, and Cichorium intybus	Feeding Study	6 weeks	7 - 9 g/kg-day	No adverse effects reported.	Rendón-Huerta et al. (2012)
Male F-344 Rats	Fructans extracted from onion (Allium cepa L)	Feeding Study	4 weeks	7% dietary concentration	No adverse effects reported.	Roldan-Marin et al. 2009
B6C3F1 Mice	Chicory root inulin	Feeding Study	6 weeks	10% dietary concentration (approximately 100 g/kg)	No adverse effects reported.	Buddington et al. (2002)
Albino rats	Inulin, unknown source	Feeding Study	6 weeks	5% dietary concentration	13% reduced body weight gain, no other adverse effects reported	Rao et al. (1965)
Rat (unspecified strain)	FOS	Feeding and Gavage Studies	6 weeks	4.5 g/kg and 10% dietary concentration	No adverse effects reported.	Takeda and Niizato, 1982
F344 rats	FOS	Feeding Study	2 years	2170 – 2664 mg/kg-day, for males and females (respectively)	No adverse effects or increased incidence of neoplasmas.	Clevenger et al., 1988
Wistar Rats and Crl CD (SD) BR rats	FOS	Feeding Study	During pregnancy	Up to 20% dietary concentration	No adverse maternal effects or *in utero* or early postnatal developmental effects	Henquin, 1988; Sleet and Brightwell, 1990

7.2 References

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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

The molecular weight distribution of fructans from 8 year-old *Agave tequilana* Weber var. azul plants is 527-4739 Da, corresponding to a range of DP from 3 to 29 (Lopez et al. 2003). A similar range of DP predominantly 3 to 29, with a small fraction having DP from 30 to 60 was reported by Toriz et al. (2007) for fructans from *Agave tequilana* Weber var. azul plants (age of plant not specified). Low molecular weight fructans (DP range 3-5) accounted for approximately 9% of the total. Mean molecular weight (Mn) was 2690 g/mol with mean DP=16 (Toriz et al, 2007). Other than fructose and glucose, no other monosaccharides were identified in the analysis of *Agave*

tequilana Weber var. azul. (Mancilla-Margalli and Lopez, 2002; Lopez et al. 2003; Ávila-Fernández et al., 2009).

Mellado-Mojica and Lopez, (2012) analyzed the fructan content of *Agave tequilana* Blue variety throughout plant development and reported the average degree of polymerization (DP) by plant age. The fructan content increased from 2- to 5-year-old plants and remained at relatively constant levels between 5- and 7-year-old plants in the field. From 2- to 7-year-old plants, the fructan mean DP progressively increased from 6 to 23. The fructan content of the young plants consists of shorter DP molecules, which become substrates for biosynthesis of longer DP branched molecules over the maturation cycle of the plant in the field. The increase in fructan content with age of plant was similar to that described by Arrizon et al. (2010), who reported that 2-year old plants contained the highest levels of free monosaccharide (fructose and glucose) and low molecular weight fructans (DP 3 – 6) with total fructan content comprising 69% of the total carbohydrate content. Fructan content of the 4 and 6.5 year old plants was 97% while free fructose, glucose and sucrose each accounted for < 1% of the carbohydrate content.

The water soluble carbohydrate composition and fructan structures of 6 year old *Agave tequilana* plants grown in different regions of Mexico were investigated by Mancilla-Margalli and Lopez (2006). Carbohydrate content varied with climatic conditions. Fructan fractions from *A. tequilana* grown in Jalisco consisted mainly of molecules with a high degree of polymerization, and an estimated average DP of 18.12, with fructooligosaccharides not detected. Monosaccharides were exclusively glucose and fructose. Both internal (4 – 7%) and terminal (1 – 8%) glucose moieties were present in fructans from Agave species, in proportions dependent on the region of cultivation.

Water soluble carbohydrates from the heads of mature (age not otherwise specified) *Agave tequilana* Weber var. azul were evaluated by Waleckx et al. 2008. Pulp produced from the transversal cutting of six mature A. tequilana heads were placed in a mixer with distilled water at 80°C and agitated for 5 minutes to extract a suspension of water soluble carbohydrate, which was filtered. The water soluble carbohydrate content of the agave heads was 28.3 g/100 g (fresh weight) ± 0.1% and 86.7 g/100 g (dry weight) ± 1.3%. Based on high performance liquid chromatography analysis, 93.4% of the carbohydrates consisted of fructans having DP ≥ 3; free disaccharides accounted for 2%, free glucose 0.8%, and free fructose, 3.8%. The average DP of fructans in the extract was 13.6 ± 1.3.

Thus, the average degree of polymerization of agave inulin from *Agave tequilana* plants ranges from approximately 6 to 23, and is concentrated around 14-18, with some variation based on the age of the plant and the region of cultivation. The degree of polymerization for agave inulin is consistent with that of other inulins consumed by humans, including native chicory root inulin, which has an average DP of 10-20 (range 2-60) (Roberfroid and Delzenne, 1998).

8.1.2 Other Constituents from the Tissue of the *Agave tequilana* Piñas

Pena-Alvarez et al., (2004) analyzed *Agave tequilana* Weber var. azul, *A. salmiana*, and *A. angustifolia* for terpenes and fatty acid content which contribute to the characteristic flavors of the alcoholic products of agave. Terpene content was determined on 160 g samples of fresh or frozen agave piñas tissue using steam distillation extraction–solid-phase microextraction coupled to GC–

MS; identification was accomplished by comparison of retention times with those of standards, Kovats Index and the NIST mass spectrometry library. Fatty acids as their ethyl esters were determined on 50 g samples of agave piñas tissue by Bligh–Dyer extraction–derivatization coupled with gas chromatography, identified with external standards and confirmed by mass spectra. In all the Agave species tested, ten fatty acids were identified. With the quantities found in the *Agave tequilana* samples presented in parentheses, the predominant fatty acids were linoleic acid (448 µg/g) and palmitic acid (~257 µg/g) followed by oleic acid and linolenic acid (~ 100 µg/g each). Others included lauric acid, myristic acid, pentadecylic acid, palmitoleic acid, margaric acid and stearic acid, present at concentrations ranging from ~5 to 30 µg/g. Total fatty acid content in *Agave tequilana* was 985 µg/g; or approximately 0.1%. The authors considered it likely that some of the fatty acids found in tequila came from the *Agave* raw material and did not undergo any modification during the cooking, fermentation and distillation process. Terpenes were difficult to identify due to their low concentrations in the plants and poor resolution by gas chromatography. Thirty-two terpenes were detected in *A. tequilana*, but they were not quantified. The main terpene in the three Agave plants was linalool (Pena-Alvarez et al., 2004).

8.1.3 Chemical Constituents of Other Food Products Derived from Agave Piñas

Linalool is also present in tequila, which is produced by the hydrolysis of the fructans obtained from the piñas of *A. tequilana*. Since it was detected in the *A. tequilana* plant (Pena-Alvarez et al, 2004), it is likely that some if not all of the linalool content in tequila originates from the raw material, although some may be introduced through the production process. In tequila production, the agave piñas (agave cores) are cooked, crushed to extract the juice, and then fermented to produce alcohol. The raw material undergoes numerous chemical and biochemical reactions leading to a distilled tequila product containing approximately 200 different compounds. The composition of the product is dependent on plant maturity, cooking, yeast fermentation and distillation processes. Some of the compounds that impart aroma and flavor characteristics are alcohols, fatty acids, esters, aldehydes, terpenes, phenols, lactones, sulfur compounds. Ávila-Fernández et al., (2009) measured the concentrations noncarbohydrate components of tequila by GC-MS analysis; notably of terpenoids including linalool. Tequila was produced by the traditional process involving exclusively thermal hydrolysis of fructans prior to fermentation. The combined concentration of linalool and its oxides was 0.5 mg/L tequila. Other constituents that were quantified included free fatty acids and fatty acid ethyl 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).

Most ripe agave heads average 50-60 kg and can yield 7.1 to 8.5 liters of tequila[10]. If all of the linalool in tequila originates in the plant, one ripe agave head can be estimated to contain (0.5 mg linalool/L x 7.1 – 8.5 L) or 3.6 – 4.3 mg linalool/head. This value can be used as the basis for estimating the quantity of linalool in the pure dried inulin produced from agave piñas. Assuming a ratio of raw agave to pure dried inulin of 5.33:1[11] substances in the raw agave can theoretically be concentrated 5.33-fold during the production of pure dried inulin. If the concentration of linalool in agave is 4 mg/55 kg, the dried inulin might be expected to contain ~21 mg/55 kg or 0.38 mg/kg (0.4 mg/kg, rounded).

[10] "In search of the blue agave" (http://www.ianchadwick.com/tequila/production.htm)

[11] The article "Inulin answers agave surfeit problem" indicates that 800 tons of raw agave yield 150 tons of pure, dried inulin. http://www.nutraingredients-usa.com/Industry/Inulin-answers-agave-surfeit-problem

Agave inulin does not contain Maillard compounds. Maillard compounds are generated from thermal processing of *A. tequilana* Weber var. *azul* during tequila production (Mancilla-Margalli and Lopez, 2002). After cooking the plant stems at 100°C for 4 to 32 hours, the most abundant Maillard compounds generated were the furans, methyl-2-furoate and 5-(hydroxymethyl) furfural, and the pyran, 2,3-dihydroxy-3,5-dihydro-6-methyl-4(H)-pyran-4-one. Also present was furfural, shown to be formed from the thermal processing of other fructan containing crops including wheat, rye, barley, and chicory (Frank, and Hofmann 2000). These Maillard products impart sweet notes contributing to the flavor of tequila. Since the production of agave inulin does not involve the thermal hydrolysis of fructans, agave inulin does not contain Maillard compounds.

Hydrolyzed agave juice from *Agave salmiana* Otto ex Salm-Dick was analyzed for sugar content by high performance liquid chromatography with refractive index detection. The only sugars identified were xylose, fructose, glucose, sucrose, and maltose (Michel-Cuello et al., 2008).
Agave syrup (also known as blue agave syrup and agave nectar) is also produced from the juice of agave piñas that has been heated or treated enzymatically to hydrolyze the fructans to fructose monomers, and subsequently concentrated to syrup (Mancilla and Lopez, 2002). The taste and consistency of agave nectar are similar to corn syrup owing to the high fructose content. Agave syrup was among the plant syrups and juices that were analyzed for sugar content, amino acid content and 5-(hydroxymethyl)furfural concentration, to assess amino acid racemization through formation of fructose-amino acids (Amadori compounds) formed during the Maillard reaction. Sucrose, D-glucose, and D-fructose were determined using an enzymatic assay and amino acids by enantioselective gas chromatography-mass spectrometry. 5-(Hydroxymethyl)furfural served as an indicator for heat treatment and progress of the Maillard reaction and was assayed colorimetrically after derivatization with barbituric acid/p-toluidine. D-Ala was detected in all plant products and amounted to 13.5% D-Ala (relative to L-Ala + D-Ala) in agave syrup; similar D-Ala was found in pomegranate, palm and grape syrups, while mean D-Ala content in Canadian maple syrups ranged from 33-34%. No other D-amino acids were also detected in Agave or grape concentrate (Arrope); whereas several other D-amino acids were found in the other syrups and juices. The quantities of glucose and fructose in agave syrup were 19.9 and 55.6%, respectively. Sucrose was not detected. 5-(Hydroxymethyl)furfural concentration ranged from 7 mg/100 g in Agave syrup to 14.5 g/100 g in Arrope (Pätzold and Brückner, 2005).

García-Aguirre et al. (2009) investigated methods to optimize the production of fructose-rich syrups *via* enzymatic hydrolysis of agave fructo-oligosaccharides. The substrate was fructo-oligosaccharides in agave juice obtained from fresh "heads" or "pines" (plants without leaves) of *Agave tequilana* Weber var. *azul*. The source of the enzyme having inulinase activity was the yeast Kluyveromyces marxianus, endogenous to Aguamiel obtained from traditional rural producers of pulque of Guanajuato state in Mexico. Conditions were optimized for maximum inulinase synthesis and hydrolysis of agave fructo-oligosaccharides. HPLC analysis of the fructose-rich syrups obtained at these optimal conditions showed an average composition of 95% of fructose and 5% of glucose and the absence of sucrose. The analysis also revealed that the syrups are free of contaminants such as hydroxymethylfurfural, which may be present in products obtained by thermal or acid hydrolysis. Since thermal and acid hydrolytic processes are not relevant to the production of agave inulin, hydroxymethylfurfural and related contaminants are not present.

The total antioxidant content of Agave nectar as compared with other natural sweeteners and refined sugar was determined using the ferric-reducing ability of plasma (FRAP) assay. Major brands of sweeteners, refined white sugar and corn syrup were sampled from retail outlets in the United States. Five agave nectar products were analyzed and found to contain minimal antioxidant capacity, comparable to refined white sugar or corn syrup. The two brands of blue Agave nectar analyzed contained 0.034 mmol FRAP/100 g (Molina Real) and 0.143 mmol FRAP/100 g (Live Superfoods). The antioxidant capacity of the other three Agave nectar products brands analyzed: "light", "raw", and "amber" Agave nectars (Madhava), was <0.03 mmol FRAP/100 g (Phillips et al., 2009).The main sugars identified in aguamiel are glucose, sucrose, fructose and several pentoses (Sanchez-Marroquin and Hope, 1953, as cited by Tovar et a. 2008).

Aguamiel from Agave mapisaga plants was analyzed to determine its chemical composition by Ortiz-Basurto et al. (2008). It contained 11.5 wt % of dry matter, which consisted mainly of sugars (75 wt %). Fructose (32 wt %) and glucose (26 wt %) were the principle components followed by fructo-oligosaccharides which accounted for 10 wt % and sucrose at 9 wt %. Protein accounted for 3 wt%. Free amino acids constituted 0.3 wt % and included most essential amino acids and γ-aminobutyric acid, glycine, asparagine/aspartate and glutamine/glutamate.

Agave derived pulque and aguamiel were analyzed for phytase activity and ascorbic acid, iron, zinc, calcium, magnesium and selenium contents. Pulque and aguamiel samples from several producers located in the states of Tlaxcala, Puebla and Hidalgo were pooled and stored at 4 °C. Iron, zinc, calcium and magnesium in the samples were quantified by flame atomic absorption spectroscopy according to the AOAC (method 985.35) and selenium was determined by hydride generation. Ascorbic acid was determined in samples of liquid pulque and aguamiel, according to the AOAC (method 967.21). The ascorbic acid content of two of the liquid samples of pulque from different dates was 2.66±0.12 mg/100 ml, and was negligible in a third sample. Ascorbic acid content in two different samples of aguamiel was 2.01± 0.10 mg/100 ml, indicating that ascorbic acid is an endogenous metabolite of the Agave. The concentrations of calcium, magnesium, selenium and iron in fresh pulque were 20.4, 16.4, 1.3, and 0.03 µg/100 g, respectively; and in aguamiel were 25.8, 13.8, 1.3, and 0.03 µg/100 g, respectively. Zinc was not detected in pulque or aquamiel. Phytase activity was also found the pulque and aquamiel samples and the authors proposed that phytase from live bacteria in pulque dephosphorylates phytate in the gastrointestinal tract of humans, improving the bioavailability of iron and zinc (Tovar et al. 2008). A typical 0.5 L serving of pulque contains 30 mg of ascorbic acid, 0.1 mg of thiamin, 0.1 mg of riboflavin, and 3.5 mg of iron, and contains approximately 4%–6% ethanol (Kuhnlein, 2004).

8.1.4 Chemical Constituents of Agave Whole Plants, Roots, Leaves and Fruits

Agave plants typically have long spine-like leaves with needles along the edges. Leaves can produce a liquid that can be irritating when it comes in contact with human skin. Workers in tequila distilleries and on agave plantations may develop an irritant contact dermatitis, which was determined by Salinas et al. (2001) to be attributable to the presence of sharp, needle-like calcium oxalate crystals, known as raphides, in the plant. Salinas et al. (2001) isolated and purified calcium oxalate crystals from the leaves of *A. tequilana*. The crystals were characterized as 30–500 µm in

length, sharpened at both ends. One drop of juice pressed from the leaves contained 100 – 150 of the needle-like crystals.

The agave genus is an important source of steroidal sapogenins, among them, hecogenin, tigogenin and diosgenin, 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 are present in many other edible plants including lettuce, onions, oats, spinach, most beans and legumes, paprika, and alfalfa. Agave saponins have been investigated for their antimicrobial and antifungal properties, as well as anti-inflammatory and immune-stimulating properties. Saponins are characterized structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. The aglycone is referred to as the sapogenin and steroid saponins are called saraponins. The combination of the nonpolar sapogenin and the water soluble side chain are the basis for the foaming properties of saponins and their use for soaps (Cornell, 2009).

Saponins are typically isolated from Agave leaves or roots by methanolic or organic solvent extraction. Steroidal sapogenins have been isolated from leaves of *Agave lecheguilla, A. sisalana, A. lophantha,* and *A. parasana* and *A. utahensis*, and *A. Americana* (Bedour et al., 1979; Blunden et al. 1974)*;* from the flowers of Agave salmiana (Maguey) (Sotelo et al. 2007); from the fruits of *A. cantala* (Uniyal et al. 1991); from the roots and seeds of *A. lechuguilla* and from the whole plants of *A. utahensis. Agave lechuguilla* contains a saponin in the rootstocks and leaves, which are used locally as soap substitutes and in shampoo mixtures. Saponins that have been identified in the various species of Agave are presented in the Appendix Table A-1. Chen et al. (2009) isolated three known flavones: 5,7-dihydroxyflavanone, 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-hydroxybenzyl) 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 A. sisalana (Debnath et al. 2010).

Hecogenin and tigogenin are the two most abundant sapogenins in the mature leaves of *A. sisalana* (Cripps and Blunder, 1978). Their concentrations in extracts of the leaf and leaf juice were determined using a gas-liquid chromatographic method of the acetylated derivatives. Hecogenin content was up to approximately 1% of the leaf extract and 0.14% in the leaf juice. The corresponding tigogenin contents were approximately one tenth of the hecogenin content.

Saponins and flavones have not been detected in stem extracts of *Agave tequilina* plant, nor have they been reported in the inulin fraction of *Agave tequilana*. There is no evidence of the presence of 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.

8.1.5 Classification of Agave Inulin among Edible Plant Fructans

Five major types of fructans have been identified in nature according to the type of β-fructofuranosyl linkages and position of glucose in the structure (Vijn et al. 1997): (i) linear inulin

with β(2→1) -fructofuranosyl linkages and a terminal glucose, commonly found in chicory and other plants in the Asteraceae family, (ii) neoseries inulin, which contains an internal glucose moiety between two fructofuranosyl units extended by β(2→1) linkages, characterized in onion (*Allium cepa)* and asparagus *(Asparagus officinalis),* (iii) levans with linear β(2→ 6) linkages with a terminal glucose, found in grasses like *Phleum pratense*, (iv) Neoseries levans, formed by β(2→1)- and β(2→ 6)-linked fructofuranosyl units on either end of a central glucose molecule, which has been reported in oat (*Avena sativa)*; alternatively they are composed of two linear β(2→ 6)-linked fructosyl chains, having an internal glucose moiety, and (v) Mixed fructans containing β(2→1) and β(2→ 6) linkages; generally the fructans of this group are branched like those found in wheat (*Triticum aestivum)* and agave. The glucose moiety may be terminal (graminans) or internal (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 based on the two linkage types and chain branching. Agave fructans were further categorized as graminans, (mixed fructans containing branched β(2→1) and β(2→6) linkages and terminal glucose moieties), and agavins (branched neo-fructans, characterized by internal α-D-glucopyranose) (Mancilla-Margalli and Lopez, 2006).

The degree of polymerization as well as the types of linkage which predominate in the fructan molecules depends on the type of fructan biosynthetic enzymes present in the plant. Phylogenetic analysis based on the presence of two such enzymes, vacuolar invertases and fructosyltransferases, places *Agave tequilana* within the Asparagales branch, closely related to Allium cepa (common onion) and Asparagus officinalis (asparagus) (Van den Ende, et al., 2011).

Analysis of the structural diversity of fructan-rich plants indicates quantitative more than qualitative differences among the species. For example, plants from the Asterales order such as chicory and dahlia contain predominantly linear polysaccharides with β(2→1) linkages (i.e., "linear inulins" group), but branched fructans and β(2→6) linkages are also present (Van Loo et al., 1995; Roberfroid and Delzenne, 1998; Mancilla-Margalli and Lopez, 2006). Likewise, a small fraction of the total agave fructan contains fructans from the linear inulin series (Waleckx et al. 2008). The term "inulin" for fructans of chain length > 10 has been applied generically to the various fructans owing to the fact that they were first isolated from *Inula helenium* (Toriz et al. 2007).

8.2 Effects of Non-Carbohydrate Constituents of the Agave Plant

Moderate pulque consumption in the central highlands of Mexico as a part of the maternal diet is associated with better infant birth size and growth than non-use of pulque (Kuhnlein, 2004). It is approximately 5% ethanol, and a 0.5 L serving provides significant nutrients and minerals, including ascorbic acid, thiamin, riboflavin and iron.

Agave plants typically have long spine-like leaves with needles along the edges. Several known irritants are present in sap of agave leaves, including calcium oxalate raphides, acrid oils, and saponins. Irritant contact dermatitis was relatively common among workers in tequila distilleries and on agave plantations. During their investigation of these workers, Salinas et al. (2001) isolated and purified calcium oxalate crystals from the leaves of *A. tequilana*. The crystals were characterized as 30–500 μm in length, sharpened at both ends, and one drop of juice pressed from

the leaves contained 100 – 150 of the needle-like crystals. The investigators developed dermatitis similar to that of the workers within an hour of contact with aqueous suspensions of the isolated crystals. Previously, Sakai et al (1984) determined that raphide crystals longer than 180 mm in length caused irritation. Salinas et al. (2001) further confirmed that irritation occurred only at body locations where workers had direct skin contact with the plants. When the raphide suspension was passed through single and double layered cotton cloth, 75 and 92% of the crystals, respectively, were removed. The authors proposed that clothing could be an effective barrier to the calcium oxalate raphides.

Outside of agave plantations and tequila distilleries, agave-induced irritant dermatitis is relatively rare (Ricks et al., 1999). Twelve cases of irritant contact dermatitis provoked by the popular ornamental plant, *Agave americana*, have been reported (Hackman et al. 2006). Ricks et al. (1999) presented a case report of Agave-induced purpura on the anterior legs in an otherwise healthy patient. The condition developed as a result of landscaping work during which an *A. americana* plant was cut down with a chain saw. Histopathology examination of punch biopsy was consistent with hypersensitivity vasculitis.

In Mexico, leaves of *A. lechugia* are used to make fibers used in ‘‘estropajo,’’ or scouring pads for washing dishes. Salinas et al. (2001) reported that when estropajos were used while bathing there were complaints about skin irritation. They examined estropajos purchased in local markets in Guadalajara, Jal., Mexico, and found raphides in all of the products examined. Raphides were also found in the leaves of *A. lechugia.*

Roots and leaves of various species of agave contain steroidal saponins, which vary in their biological activities. Santos et al. (1997) investigated the hemolytic activity of saponins which had been extracted and isolated from *Agave sisalana* leaf juice. Crude extracts from the leaves of *Agave americana* contain two utero-active compounds with properties similar to the neurotransmitter acetylcholine or other choline derivatives (Basilio et al. 1989). Steroids derived from the sisal plants *Agave sisilana* and *Agave americana* have been used in the preparation of antifertility agents anordin and dinordin (Crabbe, 1979). Bioactive materials that have been extracted and isolated from Agave plants have been studied extensively, however, the piñas were not the source of the material in any of these studies, nor was the blue Agave used.

Yokosuka et al. (2009) evaluated several steroidal saponins for their cytotoxic activity against HL-60 human promyelocytic leukemia cells. The saponins were isolated from hot methanol extracts of the whole plants of Agave utahensis. Relative to the etoposide control, furostanol saponins were non cytotoxic (IC50 >20 μg/mL) and spirostanol saponins were non- to moderately cytotoxic (IC50 values of 5.5 to >20 μg/mL).

Ohtsuki et al. (2004) evaluated a chlorogenin hexasaccharide isolated from the leaves of Agave fourcroydes for its cytotoxic and cell cycle inhibitory activities. The isolated saponin was cytotoxic against HeLa cells, and showed a cell cycle inhibitory effect at the G2/M stage at the concentrations of 7.5 and 10 μg/mL, respectively.

The hecogenin saponins, including agavacides A and B, obtained from leaves of *Agave americana* were evaluated and found to have some antifungal activity against agricultural pathogens such as

Piricularia oryzae and the human pathogenic yeast *Candida* species (Yang et al. 2006). The antifungal activity of the hecogenin saponins was found to be largely dependent on the composition of sugar moiety, and no activity was detected when the sugar moiety is less than four monosaccharide units.

The Agave plant was evaluated for its antimicrobial activity by Verástegui et al. (1996). The roots of Agave *lecheguilla* Torr. (Agavaceae) were extracted with ethanol and dried. The material was found to have the activity against several pathogenic bacteria and fungi with minimal inhibitory concentrations ranging from 3.3 – 12 mg/mL.

The extracts of several species, including *Agave americana* L. and *A. intermixta* Trel have reported anti-inflammatory activities. Lyophylized aqueous extracts of the leaves of *Agave americana* L. containing hecogenin and ticogenin were reported to have anti-inflammatory activity in rats at doses that did not harm the gastric mucosa (Peana et al. 1997).

Extracts of the leaves of *A. intermixta* Trel. were evaluated by Garcia et al. (2000) for anti-inflammatory activity. The estimated LD_{50} in male albino mice (intraperitoneal) for an extract from the leaf of *A. intermixta* Trel. was 543 ± 132 mg of dry residue / kg bw, equivalent to 19.3 ± 4.7 g of plant/kg bw. In the carrageenan-induced edema-rat paw model, oral administration of *A. intermixta* (300 and 500 mg/kg) produced a marked anti-inflammatory effect (81.4± 4.1% inhibition; $P<0.001$) which was comparable or greater than that of the reference compound, dexamethasone. Topical application (2 and 5 mg/mouse ear) also produced a 50% reduction in tetradecanoylphorbol acetate-induced edema in mice (Garcia et al., 2000).

Edible flowers from Agave salmiana (Maguey), were analyzed for nutritional content, Trypsin inhibitors and hemaglutinnins, alkaloids, saponins and cyanogenic glucosides (Sotelo et al. 2007). The studied flowers showed a good macronutrient composition and a high quality of essential amino acids. Cyanogenic glycosides were not detected in any of the flowers. But saponins, as expected, were present. Trypsin inhibitors in Agave salmiana flowers were measured at 1.11±0.10 Trypsin unit inhibited/mg sample; very low when compared with the content in most legume seeds. Also very low was the concentration of hemaglutinnins and agglutination observed (Sotelo et al. 2007).

An extract from the roots of *Agave lecheguilla* (amole) was been evaluated in human volunteers, as a potential treatment for patients with vitreous hemorrhage (Segura et al., 1996). Previously, a single dose (po) up to 6 g did not cause adverse effects (Garcia et al. 2000). Twelve healthy male volunteers, aged 25 – 35 participated in a short term study of oral amole. Prior to the beginning of the study, subjects were examined for clinical history, electrocardiogram, chest X-ray, sperm count and stool guaiac test for occult bleeding. Subjects were hospitalized during the treatment periods for daily medical examinations. Ten subjects were given 500 mg capsules of amole, at 12 hour intervals for 10 days, and two received control capsules. Clinical symptoms and blood chemistries, including glucose, creatinine, urea, cholesterol, uric acid, total protein, albumin, ALP, total bilirubin, direct bilirubin, ALT, AST, LDH, hematocrit, and hemoglobin, as well as white blood count and mean corpuscular hemoglobin concentration were assessed prior to the initiation of the treatments, after 10 days of treatment and at 90 days. There were no adverse gastric, cardiovascular or respiratory symptoms. There were no changes in the muscular and nervous

systems. Blood glucose levels were slightly decreased after 10 days of treatment compared to levels in the same volunteers 10 days following the end of the treatment period; however changes remained within the normal range, and were not significantly different from the placebo controls.

Table A-1. Saponins identified in *Agave* *

Source	Compound	Reference
Agave americana leaves	Three known saponins and bisdesmosidic spirostanol saponin, (25R)-3 β, 6 α-dihydroxy-5 α-spirostan-12-one 3,6-di-O-β-D-glucopyranoside	Yokosuka, A.; Mimaki, Y.; Kuroda, M. et al. A new steroidal saponin from the leaves of Agave americana. Planta Med. 2000;66(4):393-396.
Agave americana methanolic extract of leaves	Two new saponins, agavasaponin E and agavasaponin H Agavasaponin E is 3-O-[[beta]-d-xylopyranosyl-(1-->2glc1)-[alpha]-l-rhamnopyranosyl-(1-->4)-[alpha]-l-rhamnopyranosyl-(1-->3glc 1)-[beta]-d-glucopyranosyl-(1-->4)-[beta]-d-glucopyranosyl-(1-->4)-[alpha]-d-galactopyranosyl]-(25R)-5[alpha]-spirostan-12-on-3[beta]-ol; agavasaponin H is 3-O-[[beta]-d-xylopyranosyl-(1-->2 glc 1)-[alpha]-l-rhamnopyranosyl-(1-->4)-[alpha]-l-rhamnopyranosyl-(1-->3 glc 1)-[beta]-d-glucopyranosyl-(1-->4)-[beta]-d-glucopyranosyl-(1-->4)-[beta]-d-galactopyranosyl]-26-O-[[beta]-d-glucopyranosyl]-(25R)-5[alpha]-furostan-12-on-3[beta],22[alpha],26-triol.	Wilkomirski, B., V.A. Bobeyko, P.K. Kintia 1975. New steroidal saponins of Agave americana, Phytochemistry 14 (12) 2657-2659.
Agave attenuate leaves	Steroidal saponin: (3β,β,25S)-spirostan-3-ylO-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside	Mendes, T. P.; Silva, G. M.; da Silva, B. P. et al. 2004. A new steroidal saponin from Agave attenuata. Nat. Prod. Res. 18(2):183-188.
Agave attenuata Salm-Dyck leaves	Steroidal saponin: (3β, 5 β, 22alpha, 25S)-26-(β-D-glucopyranosyloxy)-22-methoxyfurostan -3-yl O- β-D-glucopyranosyl-(1-->2)- β-D-glucopyranosyl-(1-->2)-O-[β-D-glucopyranosyl-(1-->3)]-β-D-glucopyranosyl-(1-->4)-β-D-galactopyranosi de	da Silva, B. P.; de Sousa, A. C.; Silva, G. M. et al. A new bioactive steroidal saponin from Agave attenuata. *Z. Naturforsch. C.* 2002;57(5-6):423-428.
Agave aurea, A. avellanidens, A. cerulata, A. cerulata ssp. subcerulata, A. cocui, A. goldmaniana, A. shawii leaves	hecogenin and tigogenin were the major sapogenins isolated. Gitogenin was found in the extracts of all the leaf samples, except that of A. shawii, and manogenin and 9-dehydromanogenin in all but that of A. cocui. Chlorogenin was isolated from A. cocui, but was not detected in any of the other species examined. Qualitative and quantitative variations were found in the sapogenin contents of extracts of different regions of the same leaves of A. cocui and F. macrophylla. In particular, hecogenin predominated in the basal regions and tigogenin in the apical.	Blunden, G., A. Carabot, C. K. Jewers 1980. Steroidal sapogenins from leaves of some species of Agave and Furcraea, Phytochemistry 19 (11) 2489-2490.
Agave cantala fruits	steroidal glycoside, agaveside D: 3 β-(α-L-rhamnopyranosyl-(1→2),β-D-glycopyranosyl- (1→3)-β-D-glucopyranosyl[β-D-xylopyransoyl-(1→4)-α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl)-25R-5 α-spirostane	Uniyal, G. C.; Agrawal, P. K.; Sati, O. P. et al. 1991. A spirostane hexaglycoside from Agave cantala fruits. Phytochemistry 30(12):4187-4189
Agave cantala fruits	agaveside A and B 3 beta-O-[beta-D-xylopyranosyl-(1----2),beta-D-xylopyranosyl-(1----3), beta-D-glucopyranosyl-(1----3)-[beta-D-xylopyranosyl-(1----3)-beta-D-galactopyranosyl-(1----2)]-beta-D-glucopyranosyl]-(25R)-5 alpha- spirostane and 3 beta-O-[beta-D-xylopyranosyl-(1----2), beta-D-xylopyranosyl-(1----3)-beta-D-glucopyranosyl-(1----3)- [beta-D-galactopyranosyl-(1----2)]-beta-D-glucopyranosyl]-(25R)-5 alpha- spirostane.	Uniyal GC Agrawal PK Thakur RS Sati OP 1990. Steroidal glycosides from Agave cantala. In: Phytochemistry 29(3):937-40
Agave cantala fruits	agaveside C: 3[beta]-{[alpha]-l-rhamnopyranosyl-(1-->2)-[beta]-d-glucopyranosyl-(1-->3)-[beta]-d-glucopyranosyl-[[beta]-d-xylopyranosyl-(1-->4)-[alpha]-l-rhamnopyranosyl-(1-->2)]-[beta]-d-glucopyranosyl}-2[alpha]-hydroxy-25R-5[alpha]-spirostane	Girish C. Uniyal, Pawan K. Agrawal, Raghunath S. Thakur, Om P. Sati, Agaveside C, a steroidal glycoside from Agave cantala, Phytochemistry, Volume 30, Issue 4, 1991, Pages 1336-1339,
Agave cantala aerial part	gitogenin-3-O-[beta]-D-glucopyranosyl (1 --> 3)-[beta]-D-glucopyranoside.	Jain, D.C. 1987. Gitogenin-3-O-[beta]-D-laminaribioside from the aerial part of Agave cantala, Phytochemistry 26(6) 1789-1790.

Agave cantala ethanolic extract of the roots	3-O-[[beta]-d-glucopyranosyl]-6O-[[beta]-d-glucopyranosyl]-(25R)-5[alpha]-22[alpha]-O- spirostan-3[beta], 6[alpha]-diol.	Sharma, S.C. O.P. Sati, 1982. A spirostanol glycoside from Agave cantala, Phytochemistry 21(7) 1820-1821
Agave decipiens methanolic extract of the leaves	3-O-[alpha]-l-rhamnopyranosyl-(1-->2)-[[alpha]-l-rhamnopyranosyl-(1-->4)]-[beta]-d-glucopyranosyl-26-O-[beta]-d-glucopyranosyl-22[alpha]-methoxy-(25R)-furost-5-ene-3[beta],26-diol (1), neoruscogenin 1-O-[beta]-d-glucopyranosyl-(1-->3)-[[alpha]-l-rhamnopyranosyl-(1-->2)]-[beta]-d-glucopyranosyl-(1-->4)-[beta]-d-galactopyranoside (2), 1-O-[alpha]-l-rhamnopyranosyl-(1-->2)-[[alpha]-l-rhamnopyranosyl-(1-->4)]-[beta]-d-glucopyranosyl-26-O-[beta]-d-glucopyranosyl-22-O-methylfurosta-5,25(27)-diene-1[beta],3[beta],22,26-tetraol (3) and neohecogenin 3-O-[beta]-d-glucopyranosyl-(1-->3)-[[beta]-d-xylopyranosyl-(1-->3)-[beta]-d-xylopyranosyl-(1-->2)]-[beta]-d-glucopyranosyl-(1-->4)-[beta]-d-galactopyranoside (4).	Abdel-Gawad, M.M. El-Sayed, E.S. Abdel-Hameed 1999. Molluscicidal steroidal saponins and lipid content of Agave decipiens, Fitoterapia, 70 (4) 371-381.
Agave fourcroydes leaves	A new chlorogenin hexasaccharide saponin: chlorogenin 3-O-[[alpha]--rhamnopyranosyl-(1 --> 4)-[beta]--glucopyranosyl-(1 --> 3)-{[beta]--glucopyranosyl-(1 --> 3)-[beta]--glucopyranosyl-(1 --> 2)}-[beta]--glucopyranosyl-(1 --> 4)-[beta]--galactopyranoside]	Ohtsuki, T. T. Koyano, T.Kowithayakorn, S. Sakai, N.Kawahara, Y.Goda, N.Yamaguchi, M. Ishibashi 2004. New chlorogenin hexasaccharide isolated from Agave fourcroydes with cytotoxic and cell cycle inhibitory activities, Bioorganic & Medicinal Chemistry 12 (14) 3841-3845
Agave lecheguilla Leaves, roots, and seeds	1.0% smilagenin, dry matter basis. The roots contained about 1.0% total genin, of which about 80% was smilagenin and the rest gitogenin. The seeds contained 1 5 to 2% hecogenin with some manogenin	Wall ME Warnock BH Willaman JJ. 1962. Steroidal sapogenins. LXVIII. Their occurrence in Agave lecheguilla. Econ Bot 16:266-269
Agave lecheguilla Torrey leaves	two steroidal sapogenin diols: (25R)-spirost-5-ene-2 α, 3 β-diol (yuccagenin) and (25R)-5 β-spirostane-3 β , 6 α-diol (Ruizgenin)	Blunden, G.; Carabot, A.; Cripps, A. L. et al. 1980. Ruizgenin, a new steroidal sapogenin diol from Agave lecheguilla. Steroids 35(5):503-510
Agave lechuguilla roots	$C_{27}H_{44}O_{12}$: Hydrolysis yields galactose and an end-sapogenin identical to that obtained from Yucca filamentosa	Johns C.O. et al. 1922. A saponin from Agave lechuguilla Torrey. Journal of Biological Chemistry 52:335-347.
Agave lophantha Schiede leaves	(25R)-5 β-spirostan-3 β-ol-3-O-(β-D-apiofuranosyl(1→4) β-D -glucopyranosyl(1→3)[β-D-glucopyranosyl(1→2)] β-D -galactopyranoside) and 26-O- β-D-glucopyranosyl(25R)-5 β-furost-20(22)-ene-3 β, 26-diol-3-O-(β-D-xylopyranosyl(1→3)-[β-D-glucopyranosyl(1→2)] β-D-galactopyranoside),	Abdel-Khalik, S. M.; Miyase, T.; Melek, F. R. et al. 2002. New steroidal saponins from Agave lophantha Schiede and their pharmacological evaluation. Pharmazie 57(8):562-566.
Agave shrevei Gentry leaves	Steroidal saponin: 26-(β-D-glucopyranosyloxy)-22-methoxy-3-(O-β-D-glucopyranosyl-(1-->2)O-[O-β-D-glucopyranosyl-(1-->4)-O-[O-β-D-glucopyranosyl-(1-->6)]-O- β-D-glucopyranosyl(1-->4)-β-D-galactopyranosyl]oxy)-(3β, 5α, 25 R)-furostane	da Silva, B. P.; Parente, J. P. 2005. A new bioactive steroidal saponin from Agave shrevei. *Z. Naturforsch. C.* 60(1-2):57-62
Agave shrevei leaves	Steroidal saponin: 3-[O- β-D-glucopyranosyl-(12)-O-[O-β-D-glucopyranosyl-(14)-O-[O-β-D-glucopyranosyl-(16)]-O-β-Dglucopyranosyl-(14)-β-D-galactopyranosyl)-oxy]-(3β, 5α,25R)-spirostane.	Pereira de Silva et al. 2006. A new steroidal saponin from Agave shrevei. Natural Products Research 20(4)385-390.
Agave sisalana leaves	Barbourgenin, a steroidal sapogenin	Blunden, G.; Patel, A. V.; Crabb, T. A. 1986. Barbourgenin, a new steroidal sapogenin from Agave sisalana leaves. *J. Nat. Prod.* 49(4):687-689
Agave sisalana forma Dong No. 1 - dried fermented residues of leaf-juices	Five steroidal saponins, named dongnosides C (3), D (2), E (1) B (4) and A (5).	Ding, Y.; Tian R. H.; Yang, C. R. et al. 1993. Two new steroidal saponins from dried fermented residues of leaf-juices of Agave sisalana forma Dong No. 1. *Chem. Pharm. Bull. (Tokyo)* 41(3):557-560
Agave sisalana form Dong No 1 methanol extracts of the fermented residues of leave-juices	Three new steroidal saponins, dongnosides E, D and C: tigogenin-3-O-[beta]-d-xylopyranosyl(1-->2)[[beta]-d-glucopyranosyl(1-->3)][beta]-d-glucopyranosyl(1-->4)[beta]-d-galactopyranoside, tigogenin-3-O-[beta]-d-xylopyranosyl(1-->3)[beta]-d-xylopyranosyl(1-->2)[[beta]-d-glucopyranosyl (1-->3)[beta]-d-glucopyranosyl(1-->4)[beta]-d-galactopyranoside and tigogenin-3-O-[alpha]-l-rhamnopyranosyl (1-->4)[beta]-d-xylopyranosyl(1-->2)[[beta]-d-glucopyranosyl (1-->3)][beta]-d-glucopyranosyl(1-->4)[beta]-d-galactopyranoside, respectively.	Ding Yi, Chen Yan-Yong, Wang De-Zu, Yang Chong-Ren, 1989. Steroidal saponins from a cultivated form of Agave sisalana, Phytochemistry,28 (10) 2787-2791
Agave sislana	Saponin, Hecogenin (IV) was used as the starting material for cortisone manufacture.	Fazli, F. R. 1968. Contraceptives and other steroid drugs: their production from steroidal sapogenins. Pak. J. Sci. 20(1 and 2):64-67.

Agave sisalana Leaf extract and leaf juice	hecogenin 3[beta]-hydroxy-(25R)-5[alpha]-spirostan-12-one and tigogenin (25R)-5[alpha]-spirostan-3[beta]-ol	Cripps, A.L. and G. Blunden. 1978. A quantitative gas-liquid chromatographic method for the estimation of hecogenin and tigogenin in the leaves, juice and sapogenin concentrates of agave sisalana, Steroids 31(5) 661-669
Agave utahensis whole plants	isolation of 15 steroidal saponins including five spirostanol saponins and three furostanol saponins	Yokosuka, A. and Mimaki, Y. 2009. Steroidal saponins from the whole plants of Agave utahensis and their cytotoxic activity. Phytochemistry,70(6)807-815.
*Summarized and updated from Sigma Aldrich Life Science Nutrition Research learning center – plant profiler for *Agave sissalana* http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler/agave.html accessed June 3, 2011 and updated from the current literature search.		

8.3 Literature Search Strategy

The literature search strategy employed for this GRAS assessment on Inufib™ was based on the following search terms, as no Chemical Abstract Service Registry Number (CASRN) was available for agave inulin per se:

> Agave inulin, agave fructans, agavins, GRAS fructans, prebiotic fructans, fructans *Agave tequilana* Weber, agave fructosan, agave polyfructosan, agave carbohydrates, *Agave tequilana* Weber var. *azul*, fructans and agave, fructans functional foods, prebiotic fructans agave.

As a minimum, the following data banks were searched:

- ChemID Plus
- Registry of Toxic Effects of Chemical Substances (RTECS)
- Hazardous Substances Data Bank (HSDB)
- GENE-TOX
- Environmental Mutagen Information Center (EMIC)
- Developmental and Reproductive Toxicology (DART)
- TOXLINE – Core and Special
- TRI (Toxics Release Inventory)
- Chemical Carcinogenesis Research Information System (CCRIS)
- Medline (*via* PubMed)
- Integrated Risk Information System (IRIS)
- Syracuse Research Corporation Online Toxic Substance Control Act Database (TSCATS)

The literature search for this chemical was initially conducted on April 13, 2011, and was updated on October 3, 2011, April 8, 2015, and February 29, 2016. This document includes all relevant information retrieved as a result of those searches.

The FDA website with the search term "Agave" yielded 24 hits. All 24 entries were reviewed and the following items were considered relevant to this GRAS notification. Where appropriate they have been addressed within this document.

1. Five species of Agave are listed in the FDA poisonous plants database; *A. Americana*, *A. atrovirens* (maguey), *A. fourcroydes* (henequen), *A. sisalana* (sisal), and *Agave victoriae-reginae* [http://www.accessdata.fda.gov/scripts/Plantox/Detail.CFM?ID=5850] The

poisonous constituents that have been characterized in Agave are primarily associated with the leaves and roots, as discussed within the document.

2. Agave nectar is an ingredient in The Xymogen Bars that were the subject of an FDA initiated recall because the Xymogen Bars may contain undeclared peanut protein (Enforcement report, August 24, 2011). The subject of this GRAS notice is agave inulin, not agave nectar.
http://www.fda.gov/Safety/Recalls/EnforcementReports/ucm269605.htm

3. Two import alerts were reported on Oct 1, 2010 for agave inulin products described as "Fiber Agave Inulina" from the firm "Agaviotica S.A. De C.V.; Distrito B 4 No 433, Monterrey, Mexico.**"** Products were subject to Detention without Physical Examination (DWPE) under this Import Alert (a.k.a. Red List) (unapproved new drug – misbranded drug). *41 B - - 99* Foods with Supplemental Nutrients Added, with or without Artificial Sweeteners; *62 B - - 99* Anti-Hyperlipidemic N.E.C. These alerts are related to label claims, and the firm charged is not the manufacturer of the product that is the subject of this GRAS notice.

4. There are import refusal reports for six agave products including 3 agave syrups, 2 agave honeys and 1 agave inulin. For all of the syrup and honey products, the violation was 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, Mexico." The single charge made against the agave inulin (November 10, 2010) was "The article appears to be a new drug without an approved new drug application" and the manufacturer listed is Agaviotica S.A. De C.V.; Distrito B 4 No 433, Monterrey, Mexico." Products manufactured by "IIDEA" were not the subject of any of these violations.

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

9.0 Exhibit 1: OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE STATUS OF AGAVE INULIN FOR USE IN HUMAN FOOD

9.1 GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "…reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of 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."[12]

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance, and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that

> **"…**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."[13]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following components:[14]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon 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.

The apparent imprecision of the terms "appreciable", "at the time" and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety in this or any other area (Lu 1988; Renwick 1990, Rulis and Levitt, 2009).

[12] See 21 CFR 170.3(i).
[13] See 21 CFR 170.30(a).
[14] See Food and Drug Administration (2016), Substances Generally Recognized as Safe, Final Rule. August 17, 2016 Federal Register, 81 FR 54960.

9.2 Introduction

Foods and beverages made from the ***Agave tequilana*** **Weber var. azul** plant have a substantial history of human consumption. Human remains dating back at least 10,000 years show early uses of agave for food and fiber. It was exported to Europe by 1520 and was mentioned in the Florentine Codex of 1580 as a food of Aztecs and natives. Today, fructan-containing products derived from agave and other plants are commercially available and sold online and in health food stores in the United States. Inulin is legally classified as food or a food ingredient in most countries including all EU countries, Australia, Canada, and Japan (Franck, 2002). The EU Standing Committee meeting of June 1995 confirmed oligofructose as a food ingredient (EC, 1995). Five GRAS notifications for inulin have been submitted to FDA (FDA, 2000a, GRN 44; FDA, 2002, GRN 118; FDA 2011, GRN 392; FDA 2013; GRN 477; FDA, 2014, GRN 537) with "no questions" responses from the FDA (FDA, 2000b; FDA, 2003; FDA 2012; FDA 2014; FDA 2015). In March, 2006, Canada's Health Products and Food Branch approved the classification of inulin as a dietary fiber in Canada. Food manufacturers have added inulin-derived substances to the general food supply in Australia and New Zealand since the mid-1990s. Since 2001, inulin has appeared in a wide range of foods and is predominantly labeled as dietary fiber. The FSANZ (Food Standards of Australia and New Zealand) Food Standards Assessment Report dated July 16, 2008 declared that "There is a history of safe use of inulin-derived substances in food in Australia and New Zealand, so food manufacturers do not need express permission to add these substances to the general food supply (FSANZ, 2008). Literature searches conducted to the present have revealed the absence of studies that report safety concerns with inulin. The most well-known food industry use of this plant is for the production of tequila which is the distilled product of fermented inulin-containing agave juice.

Evaluation of the safety of Inufib™, incorporated into foods as a bulking or bifidogenic agent, was accomplished through a review of the extensive database on the safety of inulin and related β(2→1) fructans, oligofructose and fructooligosaccharides. This review included the production process, gastrointestinal fate, animal studies and human exposure.

The safety of Inufib™ is predicated on multiple factors which include:

- The similarity of the composition of agave inulin to other plant fructans;
- The high degree of purity of Inufib™ where 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.

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

The Panel has reviewed the manufacturing procedure, food grade specifications and batch analyses for Inufib™ and agrees that IIDEA's manufacturing and analytical procedures provide ample documentation that the product is food grade.

Inulin, oligofructose, and fructooligosaccharide are chemically similar entities demonstrating like nutritional properties. Their chemical and nutritional similarities are due to the basic structural similarities:

- β(2 →1) linkage of fructosyl units which sometimes end with a glucosyl unit, and
- their common metabolic pathway, i.e., fermentation by the microflora of the colon.

In a thorough published review, a working definition was proposed for inulin/oligofructose and fructooligosaccharide as determined by the degree of polymerization (which is the number of individual monosaccharide units constituting the molecule) (Carabin and Flamm, 1999). These authors discussed the studies on a variety of oligosaccharides with different chain lengths, degrees of branching and plant source and concluded that there are no demonstrated toxicological differences.

The Panel reviewed the composition of chicory inulin which has attained GRAS status (FDA, 2002) and notes that fructans extracted from chicory roots and agave stems contain nearly identical quantities of inulin (~90%) and combined mono- and disaccharides consisting of mainly sucrose, glucose and fructose (~10%). Moreover, fructan-containing plant species are commonly eaten as vegetables (e.g., asparagus, garlic, leek, onion, artichoke, Jerusalem artichoke, scorzonera, and chicory roots (Van Loo et al., 1995). The types of linkages in these fructans vary quantitatively but are qualitatively similar. The Panel considers the fructan composition of agave inulin to be sufficiently similar to other edible fructans, including chicory inulin, and agrees that it is reasonable to conclude that the same consumption limitations placed on the related fructans should apply to agave inulin.

Inufib™ is a very pure preparation of well characterized carbohydrates. It typically contains 98 - 100% carbohydrate (dry basis); approximately 90% inulin with up to approximately 10% monosaccharides and disaccharides which are primarily fructose, glucose, and sucrose. There are only small traces of secondary metabolites from the plant; the concentrations of terpenes and saponins are below 0.1 ppm (Attachment 2 "Saponins and Terpenes"), and saponins were not detected at levels as low as 7 ppb (Attachment 3 "Letter saponins Ext Lab). For comparison with data from the literature, when three species of agave plants, including *A. tequilana* Weber var. *azul,* were characterized by Pena-Alvarez et al. (2004), the concentration of fatty acids in the stem tissue of *A. tequilana* was determined to be 985 µg/g; or approximately 0.1%. Additionally, thirty-two terpenes/terpenoids types were detected in the piñas tissue but were not quantified because they were found at extremely low concentrations (Pena-Alvarez et al., 2004). The principal terpene in the piñas was linalool which is ubiquitous in edible fruits, herbs and spices and is used as a food flavoring agent, with estimated consumption from these sources of 40 to 140 µg/kg-day (OECD, 2002). The analysis of tequila by Ávila-Fernández et al. (2009) showed a combined terpene/terpenoid content of 1-3 mg/L and linalool content of 0.5 mg/L. The estimated potential concentration of linalool in dried agave inulin, based on its measurement in tequila, is ~0.4 mg/kg (see Section 8.1.3 for derivation). If the dried inulin was consumed at 20 g/day, the ingested amount of linalool from this source would be 7.6 µg/day or <0.13 µg/kg-day for a 60 kg individual. Linalool has been accepted by JECFA as a flavoring agent with an acceptable daily intake of 0.25 mg/kg bw (WHO, 1969). Linalool is a moderate skin irritant but has a weak sensitizing potential. It is not mutagenic and not suspected to have carcinogenic activity. It is excreted relatively rapidly, and there is no tendency for bioaccumulation. The overall toxicity of linalool is low with a rat oral LD_{50} of 2790 mg/kg and a 4-week rat oral gavage NOAEL of 160 mg/kg/day (OECD, 2002). It

is concluded that the estimated concentration of linalool in the piñas tissue is far below concentrations posing any safety concern.

The bioactive saponins that have been isolated from the leaves, roots, and fruit of agave (Appendix Table A-1) have not been detected in the piñas or in agave inulin.

The production process for Inufib™ from premium agave involves the mechanical extraction of the juice from the pine (piñas) of the blue Agave without the use of solvents or other chemicals. The production process has numerous certifications, and analyses reveal that no pesticides or biocides are present, and the resulting Inufib™ meets all microbiological and heavy metal standards.

9.3 Safety evidence in animal and human studies

The Expert Panel has reviewed the substantial body of data in the published literature on agave inulin and closely-related inulin fructans, such as oligofructose and fructooligosaccharide, from animal and clinical studies, the major comprehensive critical reviews on inulin and inulin-related fructans, the international regulatory summaries, and the previous GRAS submissions on inulin and the fructooligosaccharide, while also considering the FDA "no questions" response letters. In addition, the Panel conducted a comprehensive literature and databank search in August, 2016 and also undertook a critical review of the Inufib™ production process. These efforts resulted in the Panel conclusion that IIDEA's Inufib™ at the usage levels described herein is generally recognized as safe (GRAS) in human foods.

The following is a brief summary of the critical elements that were taken into consideration in the safety evaluation of inulin, oligofructose and fructooligosaccharides.

- **Chemical structure studies** show that agave fructan consists of linear and branched inulin-levan type fructans, composed of fructose units joined by β(2→1) and β(2→6) glycosidic linkages, with one glucose moiety per molecule which is consistent with a terminal or internal position. The types of linkages in these fructans vary quantitatively but are qualitatively similar. The Panel finds that there is no reason to believe that the branched carbohydrate would present a systemic toxicity issue. As stated in an authoritative review of fructooligosaccharides and inulin, the only issue of safety in the diet of humans is possible digestive tolerance (Carabin and Flamm, 1999). As reviewed in Section 1.5, the scientific evidence supports the position that agave inulin will be well-tolerated at the proposed use levels.
- **Metabolism and gastrointestinal tract studies** show that agave inulin is resistant to hydrolysis by human digestive enzymes and that it will pass largely intact to the colon where it is subject to fermentation by colonic microflora.
- **Animal studies** on agave inulin reveal a low order of toxicity in animal testing, yielding an oral LD_{50} value >5 g/kg bw in rats and mice. A 5-week mouse study with diets enriched with 10% agave fructans showed no toxicity compared with chicory-derived inulin (i.e., Raftilose® Synergy l) currently used in the food supply; and a 12-week mouse study exhibited no toxicity at gavage doses of 5 g/kg-bw/day compared with a commercial chicory fructan (Orafti®Synergy 1). Fructooligosaccharides display a low

order of toxicity in all animal testing, and this extends to the absence of significant effects on reproduction or developing fetuses and newborns; furthermore, the subject fructooligosaccharides testing was negative in skin sensitization studies in guinea pigs.

- **Genotoxicity and mutagenicity** studies on fructooligosaccharides, carboxymethyl inulin, and agave inulin have shown no *in vitro* mutagenesis or clastogenesis.
- **Carcinogenicity** was not evident after a 2-year rat carcinogenicity study at dietary concentrations of fructooligosaccharides up to 50,000 ppm. This study revealed no significant dose-related effects on body weight, food consumption, survival, growth, hematology, blood chemistry, or organ weights, nor did the treatment affect the incidence of neoplasms.
- **Clinical studies** show tolerance to inulin-type fructans in historical and contemporary diets and in clinical studies employing bolus, short-term, and long-term exposures. By consensus, inulin-type fructans have been classified as "nondigestible" oligosaccharides, which positively affect the composition and metabolic activity of the intestinal microflora of humans. Studies have shown that fructans cause increases in calcium and magnesium absorption, as well as significant decreases in total cholesterol, blood glucose level, triglycerides and low density lipoproteins.

9.3.1 Human tolerance to dietary agave inulin

Human tolerance to inulin has been thoroughly evaluated in historical and contemporary diets and in clinical studies employing bolus, short-term, and long-term exposures (FDA, 2002). Data reviewed on both oligofructose and fructooligosaccharides indicate that ingestion of up to 40 g inulin/day is safe and well-tolerated (Grühn, 1994). Any adverse effects that occur are expected to be gastrointestinal in nature and are not expected to endanger the health of the individual. Repeated daily ingestion of agave inulin was well-tolerated in adults over three 21-day periods when evaluated at doses of 5.0 or 7.5 g/day (Holscher et al. 2014). Other studies have suggested that up to 70 g of inulin/day, consumed as a regular part of the diet, may be well-tolerated (FDA, 2002).

The safety and tolerance of fructooligosaccharide ingestion by infants is documented in a Japanese nationwide survey of 20,742 infants ingesting formula containing 0.32 g/100 mL (Yamamoto and Yonekubo, 1993). This results in an estimated mean and 90th percentile consumption of 3.0 and 4.2 g fructooligosaccharides/day. 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/day (López-Velázquez et al. 2013). The estimated daily intake (EDI) of inulin from all of the proposed uses of Inufib™ for infants below 1 yr of age were calculated to be less than 2.3 and 5.7 as the mean and 90th percentile, respectively, according to methodology of ENVIRON for Frutafit® (GRN 118, FDA, 2002). Based upon these estimated exposure values and the Japanese infant survey (Yamamoto and Yonekubo, 1993), the Panel believes the food uses at the levels specified herein are GRAS. Recognizing that the current GRAS determination excludes infant formula applications for agave inulin, the cited studies did not identify any safety concerns, and they support human safety and the safety of Inufib™ for the general population, including infants.

9.4 Common knowledge elements of the GRAS conclusion

The first common knowledge element for a GRAS determination is that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing published, peer-reviewed scientific journals for the safety assessment. The majority of the studies reviewed in this safety assessment have been published in the scientific literature. The common use of agave inulin and its associated components in food on a global basis and the associated absence of harm are based upon published information of all types including clinical studies which support the safety assessment and these clinical investigations themselves have also been published in the scientific literature.

Major critical reviews of well-known experts in the field of food toxicology (e.g., Roberfroid and Delzenne, 1998; Carabin and Flamm, 1999) published comprehensive and critical reviews of the available data and information---both published and unpublished---and unanimously concluded that, under the conditions of intended use in foods, inulin-type fructan is GRAS based on scientific studies that the authors reviewed. These reviews clearly note that there is no evidence of acute, chronic, reproductive or developmental toxicity, carcinogenicity or genotoxicity in tests at dose levels considerably higher than the anticipated human exposure.

Clinical studies on the intake and tolerance of inulin-type fructans show signs of gastrointestinal intolerance with dietary intakes above 20–30 g. Roberfroid and Delzenne (1998) indicate that fructans are better tolerated when given with solid food and when given in divided doses throughout the day. Carabin and Flamm (1999) summarized 20 published studies analyzing the gastrointestinal symptoms and tolerance of inulin-type fructans and concluded that they are safe for human consumption under intended conditions of use as a dietary fiber, and that up to 20 g/day of inulin and/or oligofructose are well tolerated. More recently, Holscher et al. (2014) demonstrated that healthy adults who consumed daily doses of 7.5 g agave inulin fiber in a single serving (highest dose given) for three consecutive 21-day periods did not report any serious gastrointestinal symptoms, and López-Velázquez et al. (2013) reported that infants who consumed formula containing 0.5 g/100 mL *Agave tequilana* fructans for a six-month period did not experience a significant increase in GI symptoms such as colic, abdominal distention, flatulence, and regurgitation.

The second common knowledge element for a GRAS determination requires establishing that a consensus exists among qualified scientists about the safety of the substance with its intended use. As previously noted, in 1998 (Roberfroid and Delzenne, 1998) and in 1999 (Carabin and Flamm, 1999) literature reviews and analyses of all available data (published and unpublished) were published in peer-reviewed publications; both of these thorough assessments concluded that agave inulin-type fructans are GRAS.

Further consensus evidence is provided from a committee of experts convened by BENEO-Orafti (Belgium) in 1992 to conduct a GRAS self-affirmation of inulin and oligofructose (Kolbye et al., 1992). While the report was not published in a peer-reviewed journal, the committee members were imminently qualified, and their findings are still referred to in many published articles and regulatory documents on inulin-type fructans (i.e., Coussement, 1999, GRN-44 [FDA, 2000a]; GRN-118 [FDA 2003]). The committee was composed of Albert C. Kolbye, Herbert Blumenthal,

Barbara A. Bowman, John H. Byrne, C. Jelleff Carr, John C. Kirschman, Marcel B. Roberfroid and Morris A. Weinberger. This expert committee found the following:

- Inulin and oligofructose are not hydrolyzed in the stomach or small intestine but are fermented completely into harmless metabolites in the colon, where they are specific substrates for the growth of *Bifidobacteria*.
- Available animal toxicity studies are consistently free of any suggestions of adverse effects to be expected from such proposed levels of use in foods.
- Inulin and oligofructose are dietary fibers by definition and by their nutritional properties; intake is self-limiting because of a gaseous response in the colon that prevents over-usage.
- The safety of inulin and oligofructose is based on the long human experience of consuming inulin-containing foods as well as evaluation of available scientific evidence relating to inulin and its hydrolysis products. Further, since inulin and oligofructose have been natural components of many foods consumed safely by humans over millennia, the committee had no reason to suspect a significant risk to the public health when used in foods as intended by the notifier.
- These food substances are generally recognized as safe, both by a long-established history of use in 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.

Additional consensus elements include the reviews of the global expert bodies such as the EU and the FDA, as well as the authorities in Australia, New Zealand, Canada, and Japan.

In the U.S., chicory inulin was determined to be GRAS without questions by FDA (GRN 118, FDA 2002), and fructooligosaccharide (which is a shorter chain length fructan produced by enzymatic synthesis from sucrose), was determined to be GRAS without questions by FDA (GRN 44, FDA, 2000). The FDA had no questions about either of these GRAS assessments (FDA, 2000b; FDA, 2003).

Inulin is legally classified as a food or food ingredient in most countries including all EU countries, Australia, Canada, and Japan (Franck, 2002). As a food or food ingredient, inulin can be used without specific limitations as ingredients in foods and drinks. The EU Standing Committee meeting of June 1995 confirmed oligofructose as a food ingredient (EC, 1995). Inulin is classified as a food ingredient and not a food additive according to the European Directive 95/002 on Food Additives (EC, 1995), and all the EU countries list inulin as having food ingredient status. Inulin from agave is listed as an acceptable source of dietary fiber by the Canadian Food Inspection Agency (see: http://www.inspection.gc.ca/food/labelling/food-labelling-for-industry/nutrition-labelling/elements-within-the-nutrition-facts-table/eng/1389206763218/1389206811747?chap=5).

Food manufacturers have added inulin-derived substances to the general food supply in Australia and New Zealand since the mid-1990s. Since 2001, inulin has appeared in a wide range of foods and is predominantly labeled as dietary fiber. The FSANZ Food Standards Assessment Report dated July 16, 2008 declared that "There is a history of safe use of inulin-derived substances in food in Australia and New Zealand, so food manufacturers do not need express permission to add these substances to the general food supply (FSANZ, 2008).

The Expert Panel also acknowledges that several other reviews support the contention that a consensus exists regarding the safety of the intended human food uses of inulin-related fructans. These include previous GRAS notices submitted to FDA: 1) by the GTC Nutrition Company on short-chain fructooligosaccharides, 2) by Imperial-Sensus, LLC. on Frutafit® inulin 3) by Pfizer Nutrition and BENEO-Orafti on Oligofructose, and 4) by Danone Trading B.V. on long-chain inulin, all of which received "no questions" agency responses. The various in-depth reviews by multiple highly qualified experts found in published and unpublished sources, coupled with the numerous global regulatory agency approvals for uses in food and beverages, along with the favorable positions taken by regulatory bodies in the U.S., Canada, Mexico, Japan, Australia and New Zealand, all support the conclusion that inulin is safe for use in food (References)

9.5 Panel Conclusions[15]

The Expert Panel has carefully reviewed and evaluated the publicly available information summarized in this document and the specific product data available from IIDEA in concert with the potential human exposure to this substance and concludes that the proposed uses of Inufib™ in foods described elsewhere within this notification---when produced in compliance with Good Manufacturing Practices requirements and which meets the specifications established by IIDEA as presented in this document---are generally recognized as safe at dietary levels expected from the proposed uses.

The Expert Panel concurs with IIDEA's conclusion that Inufib™ is GRAS based upon the findings described within this dossier. This declaration is made in accordance with FDA's standard for agave inulin safety, i.e., reasonable certainty of no harm under the intended conditions of use.

This declaration is made in accordance with FDA's standard for agave inulin safety, i.e., reasonable certainty of no harm under the intended conditions of use.

(b) (6)

Richard C. Kraska, Ph.D., DABT
Chair

(b) (6)

Robert S. McQuate, Ph.D.

(b) (6)

Robert W. Kapp, Ph.D.

[15] The detailed educational and professional credentials for two the individuals serving on the Expert Panel can be found on the GRAS Associates website at www.gras-associates.com. Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Kapp's curriculum vitae can be accessed at http://www.biotox.net. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety. Each individual has previously served on multiple GRAS Expert Panels. Dr. Kraska served as Chair of the Panel.

10.0 ATTACHMENTS

10.1 Attachment 1

eurofins | analytics

Sample code Nr.	**E4-370-02518274**	**Date**	**07/04/2011**	**Page 1/3**
Analytical Report Nr.	**AR-11-AA-029715-01 / E4-370-02518274**			

Industrializadora Integral del Agave S.A. de C.V.
For the attention of **Mrs Carolina De los santos Gonzales**
Periférico Sur 7750
Colonia Sta. María Tequepexpan
45601 Tlaquepaque
MEXIQUE

Email carolina.santos@iidea.com.mx

Technical contact for your orders : Marie Jaillais

Our reference :	E4 370 02518274 / AR 11 AA 029715 01	**Type :**	EX
Client reference :	**4NIPP10338**		
Sample described as :	Organic Agave inulin INDULINA PREMIUM EN POLVO		
Packaging :	120g plastic bag		
Sample reception date :	21/03/2011	**Analysis starting date :**	21/03/2011
Sampling/Transport :	FedEx		
Analyses requested :	PAG35: PCDD/F (17) + PCB (12) ~ food / feed PAL1E: Nutritional Labelling Group EC 1 PAL2A: Nutritional Labelling Group EC2 complement J5001: Fructanes : calc. as Inuline CYP07: dry matter		

Energy values			Results	(uncertainty)
AAC99	**AA**	**Energy values according to EC 2008/100**		
	Energy value (kJ)		1313	kJ/100 g
	Energy value (kcal)		313	kcal/100 g
AAC90	**AA**	**Energy values according to EC 90/496**		
	Energy value (kJ)		1014	kJ/100 g
	Energy value (kcal)		239	kcal/100 g

Compositional analyses			Results	(uncertainty)
AAC00	**AA**	**Carbohydrate content**		
	Available carbohydrates (by difference)		59.7	g/100 g
A7359	**AA**	**Moisture oven dry 70°C, Vacuum Method : Arrêté du 8 septembre 1977 adapté**		
	Moisture		2.7	(± 0.5) g/100 g
	Total solids		97.3	(± 0.8) g/100 g
AA009	**AA**	**Ash Method : Arrêté du 8 septembre 1977 adapté**		
	Ash		0.37	(± 0.10) g/100 g
A6201	**AA**	**Proteins Method : Internal method, Continuous flow**		
	Proteins (Nx6.25)		<0.5	g/100 g
AAC08	**AA**	**Fatty acids in 100 g product calculation**		
	Fatty acids, monounsat. (/product)		<0.5	g/100 g
	Fatty acids, polyunsat. (/product)		<0.5	g/100 g
	Fatty acids, saturated. (/product)		<0.5	g/100 g
	Fatty acids, trans. (/product)		<0.5	g/100 g
A6204	**AA**	**Total fat (acid hydrolysis) Method : AOCS Am 5-04**		
	Fat		<0.5	g/100 g
J5001	**JK**	**Fructanes : calc. as Inuline Method : Internal Method**		
(a)	calculated as Inulin		37.3	g/100 g
A7488	**AA**	**Sugar profile (IC) Method : Internal method, I.C.**		
	Fructose		63.2	(± 7.0) g/kg
	Glucose		5.4	(± 0.9) g/kg
	Lactose		<1.5	g/kg
	Maltose		<1.5	g/kg
	Maltotriose (IC)		<1.5	g/kg
	Saccharose		<1	g/kg

Compositional analyses			Results	(uncertainty)
A7488	**AA**	**Sugar profile (IC) Method : Internal method, I.C.**		
	Sum of sugars (mono and disaccharides)		6.9	(± 1.3) g/100 g
AA210	**AA**	**Total Dietary Fiber Method : AOAC 985.29 2003**		
	Fiber content (according to AOAC 985.29)		<0.5	g/100 g

Minerals - Oligoelements			Results	(uncertainty)
AA622	**AA**	**Sodium Method : Arrêté du 8 septembre 1977 adapté**		
	Sodium (Na)		0.0353	(± 0.0050) g/100 g

Fatty acid profile (exp. % total)			Results	(uncertainty)
AA251	**AA**	**Fatty acid composition (GC) Method : EN ISO 15304; EN ISO 5508; EN ISO 5509**		
	Docosadienoic acid C22:2 (n 6) ω6		<0.05	%
	Saturated fatty acids (%total)		65.8	%
	Monounsaturated fatty acids (%total)		34.2	%
	Polyunsaturated fatty acids (%total)		<0.05	%
	Total trans fatty acids (%total)		<0.05	%
	Not quantifiable fatty acids		<0.05	%
	Omega 3 fatty acids (%total)		<0.05	%
	Omega 6 fatty acids (%total)		<0.05	%

Dioxins			Results	(uncertainty)
CYP07	**GF**	**dry matter Method : DIN 38414-S2**		
(a)	dry residue		97.13	%
A7158	**GF**	**PCDD/F ~ 17 congeneres ~ food / feed Method : AIR DF 100**		
(a)	2,3,7,8 TetraCDD		< 0.01	ng/kg MC12%
(a)	1,2,3,7,8 PentaCDD		< 0.01	ng/kg MC12%
(a)	1,2,3,4,7,8 HexaCDD		< 0.02	ng/kg MC12%
(a)	1,2,3,6,7,8 HexaCDD		< 0.02	ng/kg MC12%
(a)	1,2,3,7,8,9 HexaCDD		< 0.02	ng/kg MC12%
(a)	1,2,3,4,6,7,8 HeptaCDD		< 0.17	ng/kg MC12%
(a)	OctaCDD		< 0.55	ng/kg MC12%
(a)	2,3,7,8 TetraCDF		< 0.02	ng/kg MC12%
(a)	1,2,3,7,8 PentaCDF		< 0.02	ng/kg MC12%
(a)	2,3,4,7,8 PentaCDF		< 0.02	ng/kg MC12%
(a)	1,2,3,4,7,8 HexaCDF		< 0.02	ng/kg MC12%
(a)	1,2,3,6,7,8 HexaCDF		< 0.02	ng/kg MC12%
(a)	1,2,3,7,8,9 HexaCDF		< 0.02	ng/kg MC12%
(a)	2,3,4,6,7,8 HexaCDF		< 0.02	ng/kg MC12%
(a)	1,2,3,4,6,7,8 HeptaCDF		< 0.03	ng/kg MC12%
(a)	1,2,3,4,7,8,9 HeptaCDF		< 0.03	ng/kg MC12%
(a)	OctaCDF		< 0.16	ng/kg MC12%
(a)	WHO(1998) PCDD/F TEQ excl. LOQ		ND	ng/kg MC12%
(a)	WHO(1998) PCDD/F TEQ incl. LOQ		0.056	ng/kg MC12%

Dioxin-Like PCBs			Results	(uncertainty)
A7347	**GF**	**PCB ~ dioxin-like / 12 WHO ~ food / feed Method : AIR DF 100**		
(a)	PCB 77		< 0.73	ng/kg MC12%
(a)	PCB 81		< 0.15	ng/kg MC12%
(a)	PCB 105		< 1.52	ng/kg MC12%
(a)	PCB 114		< 0.34	ng/kg MC12%
(a)	PCB 118		< 5.70	ng/kg MC12%
(a)	PCB 123		< 0.46	ng/kg MC12%
(a)	PCB 126		< 0.19	ng/kg MC12%
(a)	PCB 156		< 1.88	ng/kg MC12%
(a)	PCB 157		< 0.33	ng/kg MC12%
(a)	PCB 167		< 0.73	ng/kg MC12%
(a)	PCB 169		< 0.73	ng/kg MC12%
(a)	PCB 189		< 0.48	ng/kg MC12%
(a)	WHO(1998) PCB TEQ excl. LOQ		ND	ng/kg MC12%
(a)	WHO(1998) PCB TEQ incl. LOQ		0.029	ng/kg MC12%

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Dioxin-Like PCBs		Results (uncertainty)	
GF004	**GF WHO-PCDD/F+PCB TEQ**		
(a)	WHO(1998) PCDD/F+PCB TEQ excl. LOQ	ND	ng/kg MC12%
(a)	WHO(1998) PCDD/F+PCB TEQ incl. LOQ	0.085	ng/kg MC12%

Dioxins and PCB TEQ with WHO 2005 TEF		Results (uncertainty)	
A7158	**GF PCDD/F ~ 17 congeneres ~ food / feed Method : AIR DF 100**		
(a)	WHO(2005) PCDD/F TEQ excl. LOQ	ND	ng/kg MC12%
(a)	WHO(2005) PCDD/F TEQ incl. LOQ	0.052	ng/kg MC12%
A7347	**GF PCB ~ dioxin-like / 12 WHO ~ food / feed Method : AIR DF 100**		
(a)	WHO(2005) PCB TEQ excl. LOQ	ND	ng/kg MC12%
(a)	WHO(2005) PCB TEQ incl. LOQ	0.042	ng/kg MC12%
GF004	**GF WHO-PCDD/F+PCB TEQ**		
(a)	WHO(2005) PCDD/F+PCB TEQ excl. LOQ	ND	ng/kg MC12%
(a)	WHO(2005) PCDD/F+PCB TEQ incl. LOQ	0.094	ng/kg MC12%

SIGNATURE (b) (6)

Marie Jaillais
lytical Services Manager

Report electronically validated by Marie Jaillais

EXPLANATORY NOTE

The analysis are identified by a five digit code, their description is available on request.
This document can only be reproduced in full ; it only concerns the submitted sample. Results have been obtained and reported in accordance with our general sales conditions available on request.
In order to state whether the sample complies or not with the specifications of the product, the uncertainty of the result has been taken into account.

The tests identified by the two letters code JK are performed in laboratory Eurofins Analytik GmbH, Wiertz Eggert Jörissen. The symbol (a) identified the tests performed under accreditation DIN EN ISO/IEC 17025:2005 D PL 14251 01 00
The tests identified by the two letters code GF are performed in laboratory Eurofins GfA Gmbh Hamburg. The symbol (a) identified the tests performed under accreditation DIN EN ISO/IEC 17025:2005 D PL 14199 01 00
The tests identified by the two letters code AA are performed in laboratory Eurofins Analytics France.

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10.2 Attachment 2

The iidea Company

Tlaquepaque, Jalisco, México. Sep 08, 2011

To whom it may concern,

This letter is to certify that our Inulin Premium (Inufib) has very low concentrations of saponins and terpenes according with an external analysis.

The External Laboratory concluded that the concentrations of this compounds are below of 0.1 ppm.

Sincerely,

The iidea Company
— Premium Agave Quality Products —
QUALITY
ASSURANCE

(b) (6)

Ma. del Carmen Jiménez F.
Quality Manager

Periférico Sur No. 7750
Santa María Tequepexpan
C.P. 45601, Tlaquepaque, Jal. Mx.
Tel. 3003 4450

www.iidea.com.mx

10.3 Attachment 3

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AVFNOMA DE
MEXICO

FACULTAD DE QUÍMICA - UNAM
UNIDAD DE SERVICIOS DE APOYO A LA INVESTIGACIÓN
JEFATURA

U.S.A.I.

FQUI/USAI/468/2011

Asunto: informe.

To whom it may concern:

This letter is to certify that one sample of Powder Inulin Premium (Inufib) identified with number lot 4NIPP11064 , was analyzed for the detection of saponins and terpenes using a method of extraction developed internally by our laboratory combined with a validated method for the analysis of organic compounds by GC/MS Clave: **PT-USAI-FQ-EM-001.**

Extraction procedure

To 5g of the sample 15 ml of hexane where added and submitted to sonication for 15 min. The suspension was filtered and the extract was evaporated under a nitrogen current until reducing the volume to 1mL.
From the extract 1 ml was injected to the GC/MS system.

GC/MS conditions

The sample was analyzed in GC/MS system from Agilent using a GC 5890 and a MS 5973 with a 5% phenyl-methyl silicon capillary column 30 m long, 250 µm of inner diameter and a film thickness of 0.25 µm. The oven started at 50°C kept at that temperature for 1 min and then it was programmed at 7°C/min rate up to 300°C and maintained for 5 min. The injection was performed under the split mode with a split ratio of 30:1. The carrier gas was Helium (99.999% PRAXAIR).
The mass spectrometer conditions where under Electron Impact Ionization at 70 eV and 300 µA using the scan mode and performing the scan function from 33 to 550 amu.

Although the analysis was not a quantitative determination (lack of standards) our laboratory routinely works with samples that require very low concentration determinations. Our system usually works on the ppb level (analysis of phthalates with a detection limit 7ppb). Because the sample was analyzed under these same conditions it is possible to assume that if the compounds ecogenin or ecognin were present in the sample it's concentration would be under 7ppb.

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U.S.A.I.

Our laboratory has performed in the past a lot of analysis of natural products (plant extracts) so we do have experience on the mass spectra interpretation of saponins. When we searched the chromatogram looking for mass spectra that might resemble the spectra of of ecogenin or ecognin we couldn't find one that even resembles these molecules.

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, México D. F., October 18, 2011.

(b) (6)

M. C. HUMBERTO GOMEZ RUIZ
JEFE DE LA UNIDAD

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10.4 Attachment 4

INFORME DE RESULTADOS

DETERMINACION DE LA DISTRIBUCION DE CARBOHIDRATOS EN SEIS MUESTRAS DE FRUCTANOS DE AGAVE POR CROMATOGRAFIA DE EXCLUSIÓN

INFORME TÉCNICO

PRESENTADO A:

The iidea Company
Domicilio Fiscal: Periferico Sur No. 7750
Colonia: Santa María Tequepexpan, 45601
Ciudad: Tlaquepaque
Estado: Jalisco, México
TEL.: 01 (33) 30034450
Correo electrónico: German Zarate <german.zarate@iidea.com.mx>

RECIBÍ DE CONFORMIDAD:

_____
NOMBRE Y FIRMA

FECHA: _____

ELABORADO POR: UNIDAD DE BIOTECNOLOGÍA INDUSTRIAL

Dra. Rosa María Camacho Ruíz

Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C.
Avenida Normalistas 800 Guadalajara, Jalisco, 44270 México A.P. 2-191
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Identificación de las muestras:

Seis muestras de Fructanos de agave identificados con los números de lote:

1) I16 021516 LIQUIDA
2) 4IIPP15355 POLVO
3) 4IIPP115171 POLVO
4) 4IIPP16036 POLVO
5) 4IIPP16042 POLVO
6) 4IIPP16046 POLVO

Muestreo: Realizado por el cliente

Métodos utilizados:

Distribución de tamaños de los polisacáridos por cromatografía de exclusión por peso molecular. Se utilizó una columna Waters Ultrahydrogel DP*, fase móvil: agua, 0.3 ml/min. Se utilizaron estándares de diferentes pesos moleculares.

Determinación de glucosa, fructosa y sacarosa: Por Cromatografía de Líquidos de Alta Resolución (HPLC), con columna Aminex 42-C, fase móvil: agua, 0.35 mL/min. Se utilizaron estándares de glucosa, fructosa y sacarosa grado reactivo (Sigma-Aldrich), con curva de calibración de 0 a 10 g/L.

CONTENIDO DEL INFORME:

Objetivos:

1. Determinar el contenido de glucosa, fructosa y sacarosa por HPLC.
2. Determinar la distribución de tamaños de los polisacáridos presentes en muestras de fructanos de agave.

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Resultados:

1. Se determinó el contenido de glucosa fructosa y sacarosa por HPLC, los resultados se muestran en la siguiente tabla:

Lote	Contenido % (en base humeda)			
		Sacarosa	Glucosa	Fructosa
411PP15355	***Promedio***	**0.74**	**0.08**	**10.94**
	Desv.std	0.05	0.01	0.17
411PP15171	***Promedio***	**0.42**	**0.28**	**6.35**
	Desv.std	0.05	0.05	0.06
411PP16036	***Promedio***	**1.11**	**0.96**	**8.78**
	Desv.std	0.06	0.04	0.36
411PP16042	***Promedio***	**0.97**	**0.29**	**7.17**
	Desv.std	0.20	0.02	0.27
411PP16046	***Promedio***	**1.36**	**1.02**	**8.45**
	Desv.std	0.45	0.10	0.09
I16 021516	***Promedio***	**0.89**	**0.10**	**7.80**
	Desv.std	0.27	0.01	0.19

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2. Se determinó la distribución de tamaños por cromatografía de exclusión de alta resolución (HP-SEC) encontrando los siguientes resultados:

lote		Mn	Mw	GPn	GPw	PD	Fruc GP>10	Fruc GP<10
		g/mol	g/mol				%	%
	promedio	3657.57	4271.26	22.45	26.24	1.17	91.82	8.18
411PP15355	*desviación std*	112.11	96.58	0.69	0.60	0.01	1.68	1.68
	promedio	3320.74	4057.04	20.37	24.92	1.22	83.90	16.10
411PP15171	*desviación std*	7.22	6.62	0.04	0.04	0.00	0.14	0.14
	promedio	2796.99	3578.59	17.14	21.97	1.28	73.94	26.06
411PP16036	*desviación std*	0.84	4.35	0.01	0.03	0.00	0.20	0.20
	promedio	3362.62	4091.77	20.63	25.13	1.22	84.77	15.23
411PP16042	*desviación std*	105.57	87.20	0.65	0.54	0.01	2.19	2.19
	promedio	2752.50	3542.95	16.87	21.75	1.29	72.80	27.20
411PP16046	*desviación std*	6.42	0.55	0.04	0.00	0.00	0.06	0.06
	promedio	3388.19	4052.09	20.79	24.89	1.20	87.08	12.92
I16 021516	*desviación std*	1.55	3.22	0.01	0.02	0.00	0.02	0.02

GP: grado de polimerización, Fruc: fructanos, Mw: peso molecular promedio en masa, Mn: peso molecular promedio en número, PD: polidispersidad. Glucosa, Fructosa y Sacarosa no se consideran en el cálculo de éstos parámetros.

Se anexan histogramas con distribución de tamaños de cada muestra.

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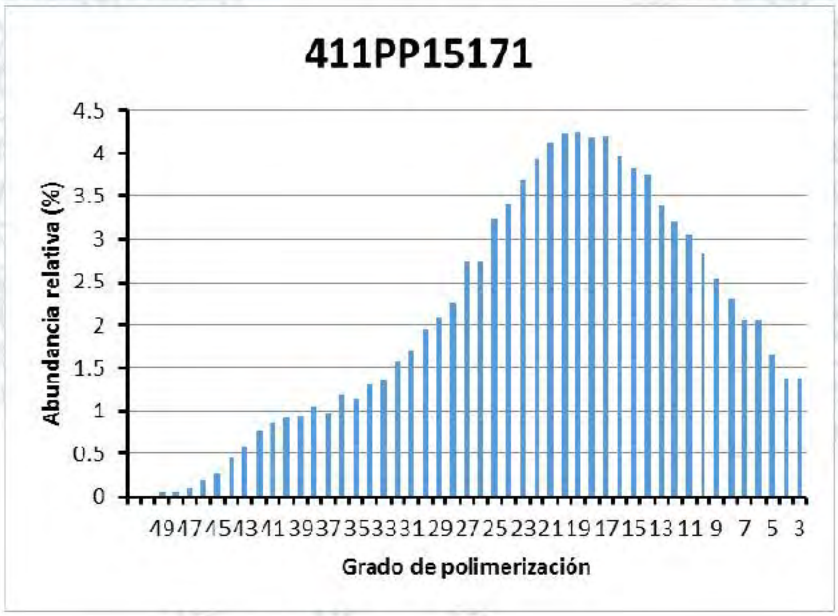

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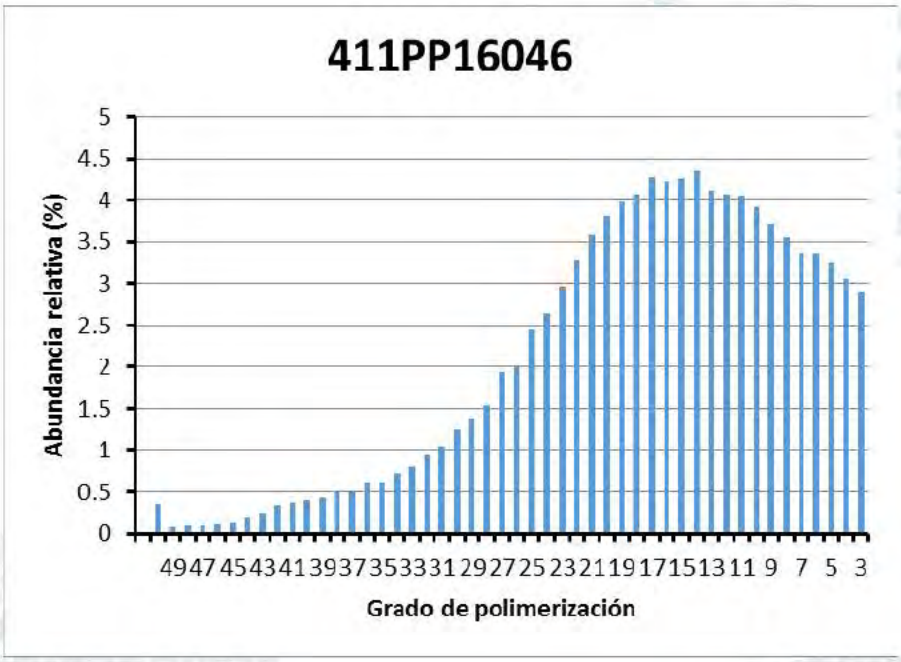

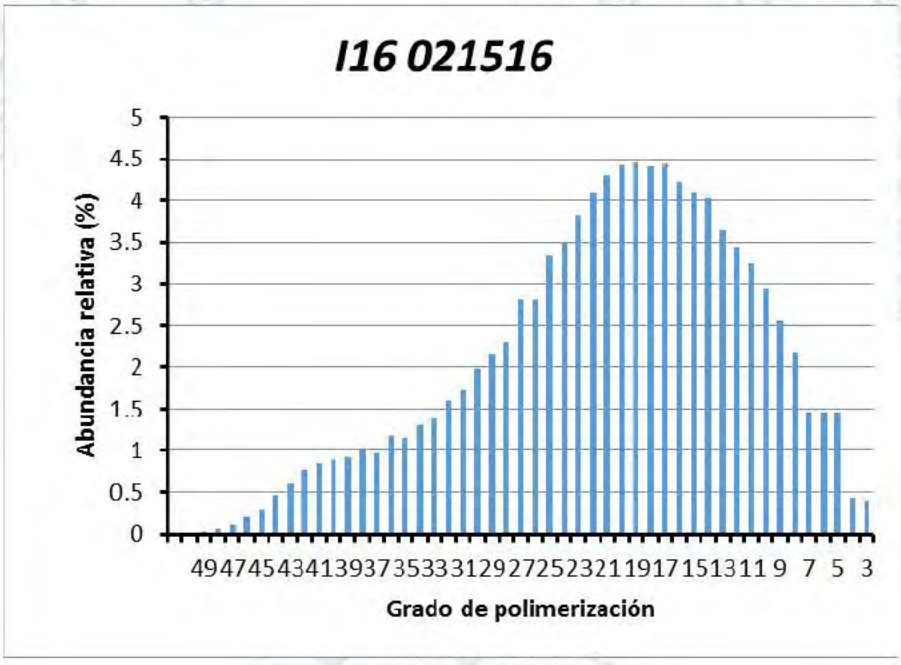

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(b) (6)

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10.5 Attachment 5

Bolsas Filtrantes Selladas *Accufit* de Nylon

Las bolsas filtrantes *Accufit Welded* de nylon de Filtration Systems son bolsas de superficie grado absoluto, específicamente fabricadas para altos contenidos de sólidos. Son una excelente opción para clarificar agua, químicos, pinturas, resinas, recubrimientos y pegamentos. Son filtros que no liberan ningún tipo de fibra y que además pueden soportar altas temperaturas.

La media filtrante que atrapa los contaminantes en la superficie del filtro o bien en alguna parte de la tortuosa matriz de fibras por la que atraviesa el fluido, está diseñada con una alta precisión en el micraje especificado y está integrada por varias capas ultrasónicamente selladas y laminadas con la tecnología patentada de Filtration Systems, todas ellas integradas a un cuello de zero bypass.
Estas bolsas son muy útiles para altos flujos y ofrecen una pérdida de presión sumamente baja. Estos filtros de superficie son lo opuesto de un filtro de profundidad ya que la mayor parte de los contaminantes son retenidos en la superficie de las capas filtrantes. Esta característica les permite atrapar grandes concentraciones de sólidos sin perder su permeabilidad, es decir, el flujo no disminuye en gran medida aunque se hayan atrapado grandes concentraciones de contaminantes.

Una de las grandes características que hacen único a este filtro, es que puede ser modificado para satisfacer una amplia variedad de condiciones de filtración de líquidos; Por ejemplo, un gran rendimiento combinado con una retención de sólidos en micrajes pequeños.

Datos Técnicos

Media Filtrante	Nylon N6
Cuello	Nylon Zero-Bypass
Acabado	Ultrasónicamente Selladas y Laminadas
No. de Capas	Múltiples. De 2 a 12
Cambio Recomendado	30 psid
Temperatura Máxima	180 °C
Caída Máxima de Presión	30 psi
Dirección del Flujo	De Adentro hacia Afuera
Micrajes	Absolutos
FDA	Aprobadas para Alimentos y Bebidas
Presentación	Empacadas Individualmente
Medidas	7”x16”, 7”x32”, 4”x14” y 4”x24”

Diseño

Tecnología

Rendimiento

Aplicaciones

- Pinturas
- Adhesivos
- Resinas
- Emulsiones
- Recubrimientos
- Ceras
- Derivados de Petróleo
- Tintas
- Aceites
- Solventes
- Agua
- Químicos

Filtration Systems

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SCB-27-001

10.6 Attachment 6

HACCP PLAN – AGAVE INULIN

1.0 PURPOSE

To establish uniform guidelines to assure the administration, implementation and maintenance of Hazard Analysis Critical Control Point Program to reduce or prevent food safety hazards, so that only safe products of the highest quality are produced

2.0 SCOPE

The HACCP plan applies to processes and departments involved in the manufacturing of Agave inulin. All employees assigned to these areas shall follow the steps in-process to ensure safe product is manufactured and delivered to the customer.

3.0 RESPONSIBILITIES

It is the responsibility of the safety team leader and the operations director to enforce the monitoring process required for this HACCP plan, and that training has occurred for all responsible individuals assigned to Production.

It is the responsibility of the safety team leader to ensure training has been provided and to audit the HACCP plan.

4.0 OVERVIEW OF PREREQUISITE PROGRAMS

There are nine (9) key prerequisite programs that are essential for an adequate and effective HACCP plan. IIDEA has incorporated these key programs into the day-to-day operation of the facility.

4.1 Sanitation Program:

The sanitation program includes a Master Cleaning Schedule. This schedule encompasses the entire plant, including the exterior of the building. All items on the schedule are to be completed on a specified day, week and month, and verified by the HACCP Coordinator for completeness. All sanitation employees receive training before starting this function. The sanitation program can be found in the Production office

4.2 Good Manufacturing Practices (GMPs):

The GMP program includes daily GMPs to be followed. The audit used encompasses the requirements of AIB Consolidated Standards for Food Safety. All employees and contractors are trained on the requirements of GMPs, and documentation of such is maintained on file. The plant has a strict “no glass” policy. Hard plastic is also monitored. The GMP program is maintained by Quality Assurance.

HACCP PLAN – AGAVE INULIN

4.3 Pest Control Program:

All pest control activities are performed by an outside contractor. Bait stations are used outside the building. Traps are used internally. The plant has a record of all contractor visits.

4.4 Chemical Control Program:

The chemical control program dictates that all chemicals are stored in secure locations separate from production areas. Access to these chemicals is limited to authorized personnel. MSDS books are located in several areas where the chemicals are commonly used.

4.5 Supplier control:

IIDEA follows a procedure for selection, evaluation and audit of authorized suppliers. All raw materials, packaging and finished goods transports are inspected. We also ratify our analytical methods with those of our suppliers.

4.6 Recall/Traceability Program:

All products manufactured and packaged by IIDEA are coded and identified for ease of recall in the event of a food safety issue. A mock recall procedure is performed at least once a year.

4.7 Quality System

IIDEA works according to a quality management system based in the SQF 2000 code. We have also deveoped, identified and follow our raw material specifications, packaging specifications and finished product specifications.

4.8 Production Teams

All products are manufactured according to standard opperating procedures, including methods for the verification and validation of our critical control points; metal detectors and instrument calibration.

4.9 Reception, Storage and Shipping

Finished products, raw materials, packaging materials and chemicals are stored according to good storage practices. Finished products are shipped according to good transport practices.

HACCP PLAN – AGAVE INULIN

5.0 HACCP TEAM

POSITION	NAME
Quality Manager HACCP Coordinator	Ma. del Carmen Jiménez F.
Operation Director	Alberto Vázquez Beltrán
HACCP Assistant	Violeta Gutiérrez Prado
Quality Control Chief	Carolina de los Santos González
Human Resources	Sergio Robles Muñoz
Maintenance	Adrián Pineda García
Supply chain Director	Miguel Esparza Ramirez
Purchasing	José Parrilla González
Traceability Chief	Blanca Magaña Bautista
Biosecurity	José A. Romero Méndez

6.0 PRODUCT DESCRIPTION

6.1 Dry Inulin:

6.1.1 Physical Chemical Properties

Humidity:	0.5 – 4.0%
pH:	4.0 – 6.0
Density:	0.6 – 0.8 g/ml
Color:	White, yellowish fine powder
Storage stability:	Stable, hygroscopic
Taste:	Slighlty sweet

6.1.2 Product Specifications

Ash content:	< 5.0 %
Dry matter:	≥ 98.0 % carbohydrates
Composition:	≥ 88.0 % Inulin
	≤ 10.0 % Fructose
	≤ 3.5 % Glucose
	≤ 2.0 % disaccharides
	Mesofilic: ≤ 2,500 UFC/g
Microbiological	Coliform: ≤ 10 UFC/g
Contaminats:	Yeast and molds: ≤ 100 UFC/g

6.1.3 Intended Use:

As a bulking agent or ingredient in a great variety of foods and the cosmetic industry.

HACCP PLAN – AGAVE INULIN

6.1.4 Packing
25 kg bag
30 kg bag

6.1.5 Shelf Life
3 years from manufacturing date

6.2 Liquid Inulin

6.2.1 Physical Chemical Properties

Humidity:	27 – 31%
Concentration:	69º – 73º Brix
pH:	3.5 – 6.0
Density:	1.34 – 1.36 g/ml
Color:	300 – 1000 icumsa
Storage stability:	Stable, hygroscopic
Taste:	Slighlty sweet

6.2.2 Product Specifications:

Ash content:	< 0.7 %
Dry matter:	≥ 98.0 % carbohydrates
Composition:	≥ 80.0 % Inulin
	≤ 15.0 % Fructose
	≤ 5.0 % Glucose
	≤ 2.0 % disaccharides
	Mesofilic: ≤ 2,500 UFC/g
Microbiological	Coliform: ≤ 10 UFC/g
Contaminats:	Yeast and molds: ≤ 100 UFC/g

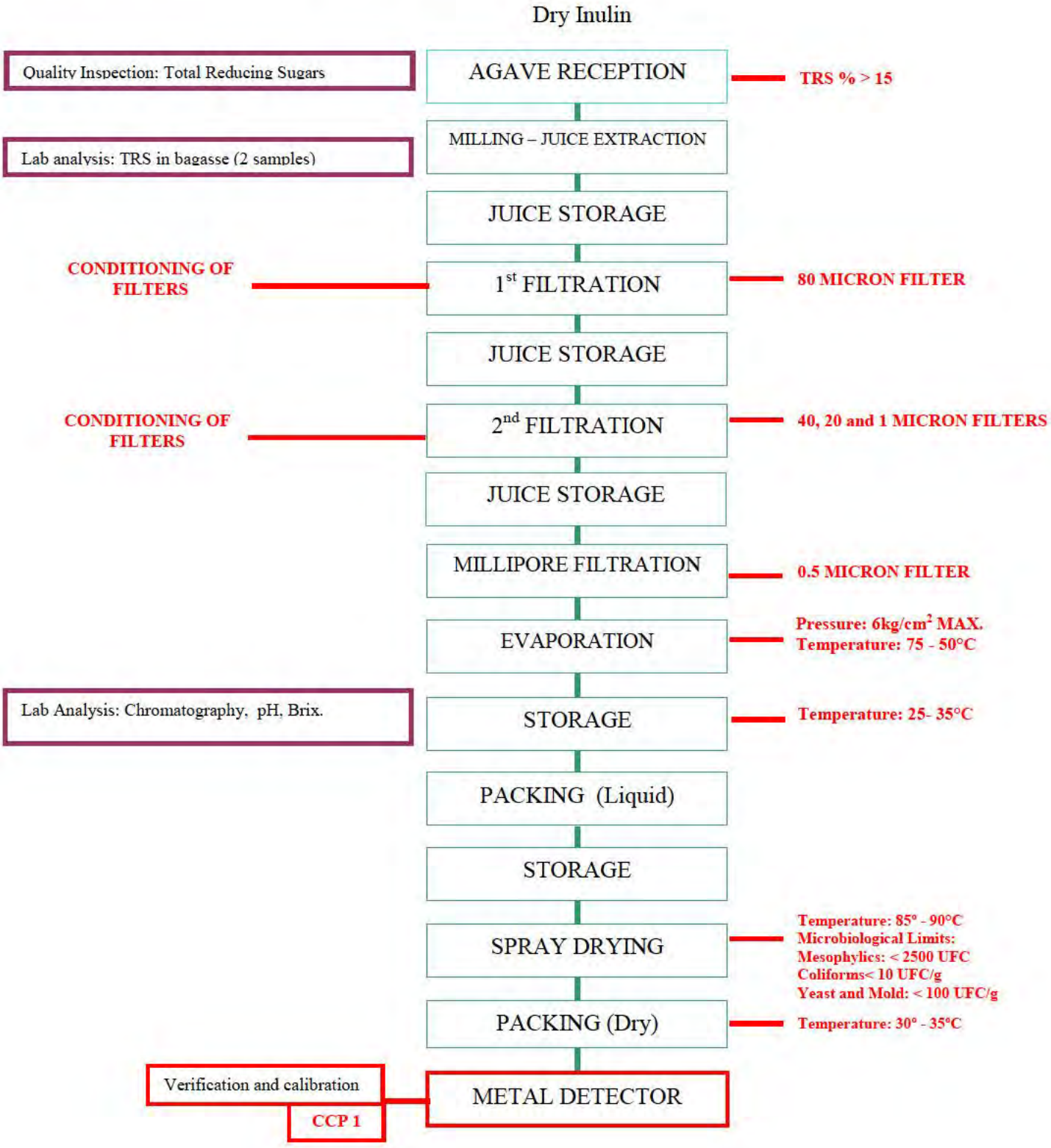
6.2.3 Intended Use
As a bulking agent or ingredient in a great variety of foods and the cosmetic industry

6.2.4 Packing
IBC, drums.

6.2.5 Shelf life
3 months from manufacturing date.

HACCP PLAN – AGAVE INULIN

7.0 FLOW DIAGRAM



iidea

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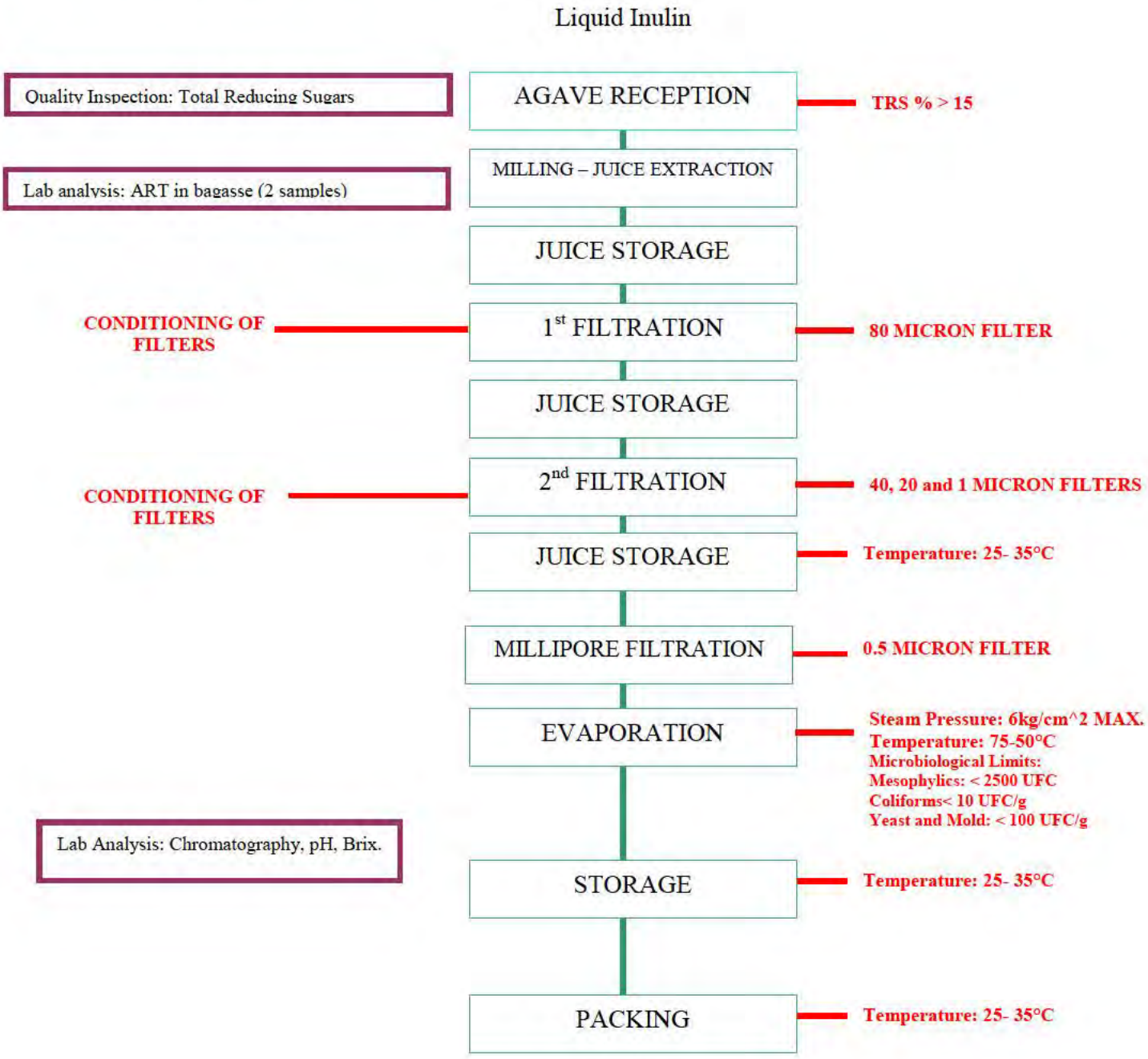
HACCP PLAN – AGAVE INULIN



HACCP PLAN – AGAVE INULIN

8.0 PROCESS DESCRIPTION

7.1 Dry Inulin

Step	Process	Description
1	Agave Reception	Quality control department checks the appereance of the product and determine the total reducing sugars percentage (TRS %) taking a representative sample of 5 out of every 200 agave "piñas" from each lot. The agave is then released for its unloading if the TRS% value is above 15%. The lot is rejected otherwise.
2	Milling – Juice Extraction	Agave "piñas" are led to a conveyour that transports them into a mill and a series of extractors. The product is sieved and squeezed. The juice falls into tubs while the resulting bagasse is separated and transported into a container.
3	Juice Storage	Extracted juice is pumped from the tubs through a series of pipelines and into storage tanks of "raw" juice.
4	1st filtration	Juice is filtrated by means of a press filter to eliminate suspended solids (media size: 80 micron)
5	Juice Storage	Once filtered, the juice is conducted to storage tanks until enough product is stored to continue to the next process
6	2nd filtration	The juice passes through a series of press filters using a filter aid (perlite) to eliminate suspended solids. Media sizes: 40, 20 and 1 micron.
7	Juice Storage	Once filtered the juice is conducted to storage tanks until enough product is stored to continue to the next process
8	Millipore filtration	The juice passes through a third filter to eliminate suspended solids and microorganisms. Media size: 0.5 micron.
9	Evaporation	The juice is concentrated using a triple effect evaporator until it reaches a concentration between 60º and 65º Brix. Using a steam pressure of 6 kg/cm^2 and a temperature between 75 and 50ºC
10	Storage	Once concentrated, the product is conducted to storage tanks until enough is stored to continue to the next process.
11	Packing (liquid)	The product is bottled in containers
12	Storage	The product is drained in tanks until enough is

		stored to continue to the next process.
13	Spray Drying	The product passes through a spray drier, that operates at a maximum temperature between 85º and 90ºC
14	Packing	The dried product is conducted through a series of pipelines into a hopper and then it is bagged and sealed.
15	Metal detector	The product is validated for metal residues.

7.2 Liquid Inulin

Step	Process	Description
1	Agave Reception	Quality control department checks the appereance of the product and determine the total reducing sugars percentage (TRS %) taking a representative sample of 5 out of every 200 agave "piñas" from each lot. The agave is then released for its unloading if the TRS% value is above 15%. The lot is rejected otherwise.
2	Milling – Juice Extraction	Agave "piñas" are led to a conveyour that transports them into a mill and a series of extractors. The product is sieved and squeezed. The juice falls into tubs while the resulting bagasse is separated and transported into a container.
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7	Juice Storage	Once filtered the juice is conducted to storage tanks until enough product is stored to continue to the next process
8	Millipore filtration	The juice passes through a third filter to eliminate suspended solids and microorganisms. Media size: 0.5 micron.
9	Evaporation	The juice is concentrated using a triple effect evaporator until it reaches a concentration between

		65º and 70º Brix. Using a steam pressure of 6 kg/cm2 and a temperature between 75º and 50ºC
10	Storage	Once filtered, the product is conducted to storage tanks until enough is stored to continue to the next process.
11	Packing	The product is bottled in different size containers according to our clients needs.

8.0 INGREDIENT HAZARD ANALYSIS

List all ingredients used in the product process, or plant	Identify known hazards	Likely Risk (likehood & severity) H = high, M = medium, L = low		Basis for the decision	Identify Prerequisite Programs or process steps to reduce or eliminate known hazards
		Likehood	Severity		
Bag	B – N/A				
	C – N/A				
	P – Foreign Matter	L	L	Storage, transport and manufacturing conditions	Transport verification procedure, Supplier approval and evaluation
IBC and others	B – N/A				
	C – N/A				
	P – Foreign Matter	L	L	Storage, transport and manufacturing conditions	Transport verification procedure, Supplier approval and evaluation
Filter Aid	B – N/A				
	C – N/A				
	P – Foreign Matter	L	L	Storage, transport and manufacturing conditions	Certificate of analysis of the product and supplier guarantee
Agave	B - Salmonella and coliforms	L	H	Raw materials comes from organic fields	Supplier control, good agricultural practices
	C – Pesticides	L	H	Raw materials comes from organic fields	Supplier control, good agricultural practices
	P - Foreign Matter	H	L	Process conditions	Visual inspection in the milling area, filtration process

Industrializadora Integral del Agave SA de CV

HACCP PLAN – AGAVE INULIN

(1) List each process step from the Process Flow Diagram. (For Receiving only, bring forward each Ingredient Hazard Analysis that was determined to be a critical Ingredient.)	(2) Does this ingredient or process step INTRODUCE a potential food safety hazard. Identify here. (Be as specific as possible when listing the hazard)	(3) Is this hazard CONTROLLED by a Prerequisite Program or process step? If YES, identify the Program or process. If a Prerequisite program or process is identified, do not complete Columns 4-6 and go to next process step. If NO, go to Column 4	(4) Is this hazard ELIMINITAED by a subsequent (later) process step? If YES, this step is NOT a CCP. Identify the subsequent process step in Column 5 and proceed to the next process step. If the hazard is eliminated at this step (no subsequent elimination step) enter NO and go to Column 6 and assign a CCP number.	(5) Identify the last process step that will eliminate the potential hazard (Example: metal detector, filter, etc.)	(6) Assign a CCP number when the answer in Column 4 is NO. Otherwise leave blank.
Agave reception	B - Salmonella and coliforms	Millipore Filtration, Supplier control, good agricultural practices			
	C - Pesticides	Organic Certificate of fields			
	P - Foreign Matter	1st Filtration			
Milling - Juice Extraction	B - Salmonella and coliforms	Millipore Filtration, GMP's			
	C - Lubricant oil	Maintenance, GMP's			
	P - Foreign Matter	1st Filtration			
Juice Storage	B – Salmonella and coliforms	Millipore Filtration, GMP's			
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P - Foreign Matter	1st Filtration			
1st Filtration	B – Salmonella and coliforms	Millipore Filtration, GMP's			
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P - Foreign Matter	2nd Filtration			
Juice Storage	B – Salmonella and coliforms	Millipore Filtration, GMP's			
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P - Foreign Matter	2nd Filtration			

HACCP PLAN – AGAVE INULIN

2nd Filtration	B – Salmonella and coliforms	Millipore Filtration, GMP's			
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P - Foreign Matter	Millipore Filtration			
Juice Storage	B – Salmonella and coliforms	Millipore Filtration, GMP's			
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P - Foreign Matter	Millipore Filtration			
Millipore Filtration	B - Salmonella and coliforms	Evaporation, GMP's			
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P – Metal Residues	Metal Detector			
Evaporation	B - Salmonella and coliforms	Spray Drying, GMP's			
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P – Metal Residues	Metal Detector			
Storage	B - Salmonella and coliforms	Spray Drying, GMP's			
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P – Metal Residues	Metal Detector			
Packing (liquid)	B - Salmonella and coliforms	Spray Drying, GMP's			
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P – Metal Residues	Metal Detector			

HACCP PLAN – AGAVE INULIN

Storage	B - Salmonella and coliforms	Spray Drying, GMP's			
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P – Metal Residues	Metal Detector			
Spray Drying	B – N/A				
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P – Metal Residues	Metal Detector			
Packing (dry)	B – N/A				
	C - Sanitation Products	GMP's, Sanitation program, Chemical control program			
	P – Metal Residues	Metal Detector			
Metal Detector	B – N/A				
	C – N/A				
	P - Metal residues	No	No		**CCP 1**

HACCP PLAN – AGAVE INULIN

9.0 HACCP MASTER PLAN

# PCC	PCC	Hazard	Critical limits	Monitoring		Corrective Action	Verification	Records
1	Metal Detector	Metal residues	7 mm	*What*	Metal residues	When a product with metal is detected the line must be stopped and it should be tested again. If the detection is confirmed, the product will be segregated and disposed of according to the non-conformity procedure.	Quality Analyst must verified and register the correct operation of metal detector every startup and end of the production.	Metal detector control (FREN05) Certificate of validation of metal detector (annual)
				How	Validate with contaminants 0.8 mm Fe, 0.8 mm N Fe, 1.2 mm SS			
				When	Every two hours			
				Who	Operator			

HACCP PLAN – AGAVE INULIN

10.0 DEFINITIONS

Hazard Analysis Critical Control Point (HACCP) – Assessment of the process to identify any reasonable potential hazards associated with the process, or rework.

Critical Control Point (CCP) – Any step where significant hazards can be controlled to prevent, eliminate, or be reduced to acceptable levels.

Biological Hazard – Source of hazard in relationship to bacterial pathogens that may result in personal injury.

Chemical Hazard – Uncontrolled use or application of chemicals that may result in personal injury.

Physical Hazard – Any potentially harmful extraneous matter not normally found in finished product.

11.0 CHANGE CONTROL

Version	Approval date	Modification	Approbed by
01	August 31, 2010	Initial Version	Food Safety leader
02	March 10, 2011	Revision and actualization	HACCP Coordinator

12.0 APPROVALS

(b) (6)	(b) (6)
Carolina De los Santos Quality Control Chief	Ma. del Carmen Jiménez Quality Manager
Review	Approve

13.0 DISTRIBUTION LIST

#CC	Responsible	Signature	Date
08	Quality Control Chief	(b) (6)	10 - 03 - 11

10.7 Attachment 7

2010 - 2011

CERTIFICATE OF REGISTRATION

This certifies that:

Industrializadora Integral Del Agave, S.A. de C.V.
Periferico Sur 7750, Colonia Santa Maria Tequepexpan
Tlaquepaque, Jalisco C.P 45601
Mexico

is registered with the U.S. Food and Drug Administration pursuant to section 305 of the United States Public Health Security and Bioterrorism Preparedness and Response Act of 2002, P.L. 107-188, such registration having been verified as currently effective on the date hereof by Registrar Corp.

U.S. FDA Registration No.: **13439186334**

U.S. Registration Agent: **Registrar Corp**
144 Research Drive, Hampton, Virginia, 23666, USA
Telephone: +1-757-224-0177 • Fax: +1-757-224-0179

This certificate affirms that the above stated facility is registered with the U.S. Food and Drug Administration pursuant to section 305 of the U.S. Public Health Security and Bioterrorism Preparedness and Response Act of 2002, P.L. 107-188, such registration having been verified as effective by Registrar Corp as of the date hereof, and Registrar Corp will confirm that such registration remains effective upon request and presentation of this certificate until the expiration of one year from the date hereof, unless terminated after issuance of this certificate. Registrar Corp makes no other representations or warranties, nor does this certificate make any representations or warranties to any person or entity other than the named certificate holder, for whose sole benefit it is issued. Registrar Corp assumes no liability to any person or entity in connection with the foregoing. The U.S. Food and Drug Administration does not issue a certificate of registration, nor does the U.S. Food and Drug Administration recognize a certificate of registration. Registrar Corp is not affiliated with the U.S. Food and Drug Administration.

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Russell K. Statman
Executive Director
Registrar Corp
Dated: October 28, 2010

10.8 Attachment 8

Global Standards certification

Certificate of Compliance

Industrializadora Integral del Agave, S.A. de C.V.

Periférico Sur 7750, Sta. María Tequepexpan
C.P. 45601 Tlaquepaque, Jalisco. México.

Has successfully implemented and passed the Certification Assesment and found its Hazard Analysis and Critical Control Points System in compliance with the requirements detailed bellow:

HACCP / Good Manufacturing Practices (GMP)

Scope: Manufacturing, Storage, Packaging and Sale of Agave Syrup.

Certificate Number:	**GSCHACCPMX-109**
Initial Registration Date:	**June 23, 2010**
Registration Date:	**June 23, 2010**
Registration Period:	**June 23, 2010 to June 22, 2013**
Certification Scheme:	**Single Site**

(b) (6)

Executive Director

Global Standards HACCP COMPLIANCE CERTIFICATE

Global Standards COMPANY CERTIFICATE

Global Standards, S.C. Pedro Moreno 1677 Piso 4 -3 Col. Americana C.P. 44160 Guadalajara, Jalisco. México.

adding-value to your business

10.9 Attachment 9

SILLIKER®
Food Safety & Quality Solutions

Audit Recognition

Industrializadora Integral del Agave SA de CV (IIDEA): Tlaquepaque, Jalisco, Mexico

Completed a
Silliker Good Manufacturing Practices and Food Safety Systems Audit
With a score of

94.2%

2/21/2011	(b) (6)	(b) (6)
Audit Date	Division Vice President	Chief Scientific Officer

With over 40 years of experience and part of the Mérieux Alliance group of companies,
Silliker provides services that help assure food safety and nutrition worldwide.

10.10 Attachment 10

Industrializadora Integral del Agave SA de CV

TECHNICAL DATA SHEET

PRODUCT: POWDER INULIN PREMIUM

SECTION 1: PRODUCER DATA	
Name of the company:	Industrializadora Integral del Agave SA de CV
Address:	Av. Periférico Sur 7750, Tlaquepaque Jalisco, México.
Telephone:	+52 (33) 3003-4450

SECTION 2: MICROBIOLOGIC CHARACTERISTICS AND PHYSICAL-CHEMICAL DATA	
Appearance	White powder
Aroma	Neutral
Moisture	0.5 – 4.0 %
pH (1%)	4.0 – 6.0
Ash	Máx. 5.0 %
Inulin:	88.0 – 94.0%
Fructose:	3.0 – 10.0 %
Glucose:	Máx. 3.5 %
Sucrose:	Máx. 2.0%
Carbohydrates Total:	98.0 – 100.0 %
Other carbohydrates:	Máx. 6.0%
Mesofilics:	Máx. 2,500 UFC
Coliforms:	Máx. 10 UFC
Yeast and Mold:	Máx. 100 UFC

SECTION 3: HAZARDOUS INGREDIENTS	
List:	1. The product does not contain any hazardous ingredient or substance.

SECTION 4: RISK OF FIRE OR EXPLOSION	
Method of Fire extinction:	Chemical dust, CO_2 preferably
Cautions:	Do not expose the product to temperatures higher than 300°F
Ignition Temperature:	300°F

SECTION 5: REACTIVITY	
Stability:	Stable
Incompatible Materials:	Strong Oxidants, flames.
Dangerous decompositions per component:	N/A
Conditions to avoid:	Do not overheat; reduce heat if the product begins to produce smoke.

Industrializadora Integral del Agave SA de CV

TECHNICAL DATA SHEET

PRODUCT: POWDER INULIN PREMIUM

SECTION 6: HEALTH RISK	
Routes: inhalation - N/A	cutaneous – N/A ingestion – No hazardous
Cancerigenous characteristics:	No
Exposure Health Damage Symptoms:	None
Exposure General medical conditions:	None
Applicable First Aid Procedures:	N/A

SECTION 7: PERSONAL PROTECTION	
Respiratory Protection:	Not necessary under normal use and handling. If dispersion of dust in the air using mouth covers.
Ocular Protection:	Use protection glasses on spilling cases to avoid splashing. If there is ocular contact, wash abundantly with clean water.
Hygiene Requirements:	Handle the product under the Good Manufacturing Practices and /or specific food regulations.

SECTION 8: USE AND HANDLING CAUTIONS	
In case of Spilling:	Do not step on the product, you may slip. Wash the area with water, once clean dry the surface.
Disposal handling:	Consult local regulations on disposal handling of food products.
Storage and Handling:	Handle the product with caution, avoid spilling. Store the product in cool places at room temperature; avoid overheating, highly hygroscopic product.
Shelf Life	3 years

10.11 Attachment 11

Analyses	Laboratory	Turnaround time
Nutritional Labelling Total fat Total Dietary Fiber Energy values according to EC 90/496 Energy values according to EC 2008/100 Moisture Ash Proteins Carbohydrates content Fatty extraction Fatty acid composition Fatty acids in 100 g product calculation Sodium Extraction for HPLC / IC sugar analyses Sugar profile	Eurofins Analytics France	5 weeks
Fructans		
PCB, Dioxine		
Heavy metals Arsenic Lead Mercury Cadmium	Silliker	20 working days
Pesticides		
Shelf life studies	Silliker	30 days - 2 months

10.12 Attachment 12

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a Mérieux NutriSciences Company

AC-F-007-3 Mexico City Unit

Company: **INDUSTRIALIZADORA INTEGRAL DEL AGAVE, S.A. DE C.V.**
Adress: Periférico Sur 7750, Santa María Tequepexpan, Tlaquepaque Jalisco, C.P. 45601
At'n: Carolina de los Santos

REF (S.A.) 49339
Arrival date: 18/03/11
Analysis date: 22/03/11
Deliver date: 03/05/11

ANALYSIS REPORT

SAMPLE (S) DESCRIPTION *Sample(s) received in this lab for its study*

1.- AGAVE INULIN POWDER, CODE NUMBER: 4NIPP10338, MATRIX SAMPLE: RAW MATERIAL

I.- ATOMIC ABSORPTION ANALYSIS

Analysis	Result (s)	Method
Arsenic (mcg/kg)	Not detected	NOM-117-SSA1-1994
Cadmium (mg/kg)	Not detected	NOM-117-SSA1-1994
Mercury mcg/kg)	Not detected	NOM-117-SSA1-1994
Lead (mg/kg)	Not detected	NOM-117-SSA1-1994

[redacted]

I.Q.P. Fernando Cruz Cortès
Chemistry Supervisor

I.Q. Fabián A. Gómez Martínez
Laboratory Director

FCC/BSCM

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1.- AGAVE INULIN POWDER, CODE NUMBER: 4NIPP10338, MATRIX SAMPLE: RAW MATERIAL

I.- GAS CROMATOGRAPHY ANALYSIS

Analysis	Result (s)	Analysis	Result (s)	Method
3-keto Carbofuran	Not detected	Butachlor	Not detected	Pesticide Analytical Manual Vol. 1
Aldicarb	Not detected	Captafol (Difolatan)	Not detected	
Aldicarb Sulfone	Not detected	Captan	Not detected	
Alidicarb sulfoxide	Not detected	Carfentrazone-ethyl	Not detected	
Aminocarb	Not detected	Clordane	Not detected	
Bendiocarb	Not detected	Cyanazine	Not detected	
Bufencarb	Not detected	Cyfluthrin	Not detected	
Carbaryl	Not detected	Cypermethrin	Not detected	
Carbofuran	Not detected	Cyproconazole	Not detected	
Ethiofencarb	Not detected	Chlordane	Not detected	
Fenobucarb	Not detected	Chlordimeform	Not detected	
Methiocarb	Not detected	Chlorfenapyr	Not detected	
Methiocarb sulfone	Not detected	Chlorfenson	Not detected	
Methiocarb sulfoxide	Not detected	Chlornitrofen	Not detected	
Methomyl	Not detected	Chlorobenzilate	Not detected	
Oxamyl	Not detected	Chloroneb	Not detected	
Propoxur	Not detected	Chlorothalonil	Not detected	
Thiodicarb	Not detected	Chloroxuron	Not detected	
Acetamiprid	Not detected	Chlorpropham	Not detected	
Alachlor	Not detected	Dactal (DCPA)	Not detected	
Aldrin	Not detected	d-BHC (d-HCH)	Not detected	
alpha-BCH- (a-HCH)	Not detected	DCPA (Dacthal)	Not detected	
Anilazine	Not detected	DDD	Not detected	
b-BHC (b-HCH)	Not detected	DDE	Not detected	
Benzoylprop-ethyl	Not detected	DDT	Not detected	
BHC, alpha	Not detected	Deltamethrin	Not detected	
BHC, beta	Not detected	Diclobutrazol	Not detected	
BHC, delta	Not detected	Dicofol	Not detected	
Bifenox	Not detected	Dichlobenil	Not detected	
Bifenthrin	Not detected	Dichlofluanid	Not detected	
Boscalid	Not detected	Dichlone	Not detected	
Bromacil	Not detected	Dieldrin	Not detected	

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ANALYSIS REPORT

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1.- AGAVE INULIN POWDER, CODE NUMBER: 4NIPP10338, MATRIX SAMPLE: RAW MATERIAL

I.- GAS CROMATOGRAPHY ANALYSIS

Analysis	Result (s)	Analysis	Result (s)	Method
Diethatyl-ethyl	Not detected	Mirex	Not detected	Pesticide Analytical Manual Vol. 1
Dimethachlor	Not detected	Monolinuron	Not detected	
Dimethomorph	Not detected	Myclobutanil	Not detected	
Endosulfan I	Not detected	Nitrofen	Not detected	
Endosulfan II	Not detected	Nuarimol	Not detected	
Endosulfan Sulfate	Not detected	Ofurace	Not detected	
Endrin	Not detected	Oxadiazon	Not detected	
Esfenvalerate	Not detected	Oxadixyl	Not detected	
Etaconazole	Not detected	Oxyfluorfen	Not detected	
Ethylan	Not detected	Paclobutrazol	Not detected	
Fenarimol	Not detected	PCNB (Quintozene)	Not detected	
Fenazquin	Not detected	Penconazole	Not detected	
Fenbuconazole	Not detected	Pentachloroaniline	Not detected	
Fenhexamid	Not detected	Permethrin	Not detected	
Fenson	Not detected	Perthane	Not detected	
Fenvalerate	Not detected	PP-DDE	Not detected	
Flucythrinate	Not detected	PP-DDT	Not detected	
Folpet	Not detected	Procymidone	Not detected	
Fuchloralin	Not detected	Prochloraz	Not detected	
Hepta epóxido	Not detected	Pronamide	Not detected	
Heptachlor	Not detected	Propachlor	Not detected	
Heptachlor epoxide	Not detected	Propanil	Not detected	
Hexaconazole	Not detected	Propiconazole	Not detected	
Hexachlorobenzene	Not detected	Pyraclostrobin	Not detected	
Imazalil	Not detected	Pyridaben	Not detected	
Indoxacarb	Not detected	Pyrifenox	Not detected	
Iprodione	Not detected	Quinoxyfen	Not detected	
Lambda-Cyhalothrin	Not detected	Simazine	Not detected	
Lindane	Not detected	Simetryn	Not detected	
Linuron	Not detected	Tebuconazole	Not detected	
Methoxychlor	Not detected	Terbacil	Not detected	
Metolachlor	Not detected			

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Chemistry Supervisor

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Laboratory Director

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I.- GAS CROMATOGRAPHY ANALYSIS

Analysis	Result (s)	Analysis	Result (s)	Method
Tetradifon	Not detected	Diazinon	Not detected	Pesticide Analytical Manual Vol. 1
Thiobencarb	Not detected	Dicrotophos	Not detected	
Thiodan I	Not detected	Dichlorvos / DDVP Vapona	Not detected	
Thiodan II	Not detected	Dimethoate	Not detected	
Tolylfluanid	Not detected	Dioxathion	Not detected	
Toxaphene	Not detected	Disulfoton (Dy-siston)	Not detected	
Tralomethrin	Not detected	Disulfoton-sulfone	Not detected	
Triadimenol	Not detected	Edifenphos	Not detected	
Triflumizole	Not detected	Ethion (Nialate)	Not detected	
Vegadex	Not detected	Etrimfos	Not detected	
Vinclozolin	Not detected	Fenamiphos	Not detected	
Acephate	Not detected	Fenitrothion	Not detected	
Azinphos-ethyl	Not detected	Fensulfothion	Not detected	
Azinphos-methyl	Not detected	Fenthion	Not detected	
Bensulide	Not detected	Fonofos	Not detected	
Bromophos	Not detected	Forate (Thimet)	Not detected	
Bromophos-ethyl	Not detected	Formothion	Not detected	
Cadusafos	Not detected	Guthion	Not detected	
Carbophenothion OA	Not detected	Heptenophos	Not detected	
Carbophenotion	Not detected	Iprobenfos	Not detected	
Coumaphos	Not detected	Isazophos	Not detected	
Crotoxyphos	Not detected	Isofenphos	Not detected	
Cyanophos	Not detected	Leptophos	Not detected	
Chlorfenvinphos	Not detected	Malaoxon	Not detected	
Chlorpyrifos	Not detected	Malathion (Cithion)	Not detected	
Chlorpyrifos-methyl	Not detected	Metasystox-R	Not detected	
Chlorthiophos	Not detected	Methacrifos	Not detected	
DEF	Not detected	Methamidophos	Not detected	
Demeton-o	Not detected	Methidathion	Not detected	
Demeton-s	Not detected	Methyl Trithion	Not detected	
Demeton-s-sulfone	Not detected	Mevinfos	Not detected	
Dialifos	Not detected	Mimethoate	Not detected	

[redacted]

I.Q.P. Fernando Cruz Cortès
Chemistry Supervisor

[redacted]

I.Q. Fabián A. Gómez Martínez
Laboratory Director

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AC-F-007-3 Mexico City Unit

Company: **INDUSTRIALIZADORA INTEGRAL DEL AGAVE, S.A. DE C.V.**
Adress: Perifèrico Sur 7750, Santa Marìa Tequepexpan, Tlaquepaque Jalisco, C.P. 45601
At'n: Carolina de los Santos

REF (S.A.) 49339
Arrival date: 18/03/11
Analysis date: 22/03/11
Deliver date: 03/05/11

ANALYSIS REPORT

SAMPLE (S) DESCRIPTION *Sample(s) received in this lab for its study*

1.- AGAVE INULIN POWDER, CODE NUMBER: 4NIPP10338, MATRIX SAMPLE: RAW MATERIAL

I.- GAS CROMATOGRAPHY ANALYSIS

Analysis	Result (s)	Analysis	Result (s)	Method
Monocrotophos	Not detected	Ametryn	Not detected	Pesticide Analytical Manual Vol. 1
M-parathion	Not detected	Amitraz	Not detected	
Naled	Not detected	Atrazine	Not detected	
Omethoate	Not detected	Azoxystrobin	Not detected	
Oxidemeton Methyl	Not detected	Benalaxyl	Not detected	
Parathion	Not detected	Benefin	Not detected	
Parathion-methyl	Not detected	Benfluralin	Not detected	
Phenthoate	Not detected	Biphenyl	Not detected	
Phorate	Not detected	Bitertanol	Not detected	
Phosalone	Not detected	Bromopropylate	Not detected	
Phosfolan	Not detected	Bupirimate	Not detected	
Phosmet	Not detected	Buprofezin	Not detected	
Phosphamidon	Not detected	Carbetamide	Not detected	
Piperophos	Not detected	Carbosulfan	Not detected	
Pirimiphos	Not detected	Cycloate	Not detected	
Pirimiphos-methyl	Not detected	Cyprodinil	Not detected	
Propetamphos	Not detected	Chlorfenapyr	Not detected	
Prothiofos	Not detected	Chlozolinate	Not detected	
Prothoate	Not detected	Dimethametryn	Not detected	
Pyrazophos	Not detected	Dimetoato (Cygon)	Not detected	
Pyridaphenthion	Not detected	Diphenylamine	Not detected	
Quinalphos	Not detected	EPN	Not detected	
Ronnel	Not detected	EPTC	Not detected	
Sulprofos	Not detected	Ethalfluralin	Not detected	
Terbufos	Not detected	Ethirimol	Not detected	
Tetrachlorvinphos	Not detected	Ethofumesate	Not detected	
Thiometon	Not detected	Ethoprop	Not detected	
Thionazin	Not detected	Ethoxyquin	Not detected	
Toclofos-methyl	Not detected	Fenamidone	Not detected	
Triazophos	Not detected	Fenpropathrin	Not detected	
Acrinathrin	Not detected	Fenpropimorph	Not detected	
Ametrine	Not detected	Fipronil	Not detected	

I.Q.P. Fernando Cruz Cortès
Chemistry Supervisor

I.Q. Fabián A. Gómez Martínez
Laboratory Director

FGM/FCC/BSCM

Any commentary or complains about our service please report it to fax 26-14-11-42 to the Attention of the Laboratory Director
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Company: **INDUSTRIALIZADORA INTEGRAL DEL AGAVE, S.A. DE C.V.**
Adress: Perifèrico Sur 7750, Santa Marìa Tequepexpan, Tlaquepaque Jalisco, C.P. 45601
At'n: Carolina de los Santos

REF (S.A.) 49339
Arrival date: 18/03/11
Analysis date: 22/03/11
Deliver date: 03/05/11

ANALYSIS REPORT

SAMPLE (S) DESCRIPTION *Sample(s) received in this lab for its study*

1.- AGAVE INULIN POWDER, CODE NUMBER: 4NIPP1[illegible]338, MATRIX SAMPLE: RAW MATERIAL

I.- GAS CROMATOGRAPHY ANALYSIS

Analysis	Result (s)	Analysis	Result (s)	Method
Fludioxonil	Not detected	Promecarb	Not detected	Pesticide Analytical Manual Vol. 1
Flurochloridone	Not detected	Prometon	Not detected	
Fluzilazole	Not detected	Prometryn	Not detected	
Furalaxyl	Not detected	Propargite	Not detected	
Iprodione	Not detected	Propham	Not detected	
Kresoxim-methyl	Not detected	Pymetrozine	Not detected	
Linuron	Not detected	Pyrethrins	Not detected	
Mecarbam	Not detected	Pyrifenox	Not detected	
Metalaxyl-M (Mefenoxam)	Not detected	Pyrimethanil	Not detected	
Metazachlor	Not detected	Pyriproxyfen	Not detected	
Methoprotryn	Not detected	Quinomethionate	Not detected	
Metribuzin	Not detected	Tebufenpyrad	Not detected	
Mexacarbate	Not detected	Tecnazene	Not detected	
Napropamine (Devrinol)	Not detected	Tefluthrin	Not detected	
Norflurazon	Not detected	Thiabendazole	Not detected	
o-Phenylphenol	Not detected	Tolyfluanid	Not detected	
Oryzalin	Not detected	Triadimefon	Not detected	
Pendimethalin	Not detected	Trifloxystrobin	Not detected	
Pirimicarb	Not detected	Trifluralin	Not detected	
Profluralin	Not detected	[illegible]	[illegible]	

I.Q.P. Fernando Cruz Cortès
Chemistry Supervisor

I.Q. Fabián A. Gómez Martínez
Laboratory Director

FGM/FCC/BSCM

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10.13 Attachment 13

Industrializadora Integral del Agave SA de CV

Methods of Analysis

We hereby confirm the methods of analysis and the official references used in the Agave Inulin production process:

Internal Analyses:

Assay	*IIDEA Document Number*	*Official Reference*
FRUCTOSE	MALB-02	NMX-FF-110-SCFI-2008
GLUCOSE	MALB-02	NMX-FF-110-SCFI-2008
SUCROSE	MALB-02	NMX-FF-110-SCFI-2008
INULIN	MALB-02	NMX-FF-110-SCFI-2008
OTHER CARBOHYDRATES	MALB-02	NMX-FF-110-SCFI-2008
TOTAL COUNT OF MESOPHYLIC AEROBIC MICROORGANISMS	MALB-02	NOM-092-SSA1-1994
COLIFORM MICROORGANISMS	MALB-02	NOM-112-SSA1-1994
YEAST AND MOLDS	MALB-02	NOM-111-SSA1-1994
HUMIDITY	MALB-02	NMX-F-591-SCFI-2010
pH	MALB-02	NMX-F-317-S-1978
FOREIGN MATTER	MALB-02	NMX-F-591-SCFI-2010
ASHES (% dry matter)	MALB-02	NMX-F-607-NORMEX
TOTAL REDUCING SUGARS (%)	MALB-02	NMX-V-006-NORMEX-2005
DEGREES BRIX	MALB-02	NMX-F-103-NORMEX-2009

External Analyses:

Assay	*Official Reference*
SALMONELLA	NOM-114-SSA1-1994
HEAVY METALS	NOM-117-SSA1-1997

(b) (6)

Sincerely,

(b) (6)

Benjamín Hijar Sollero
Quality Assurance

10.14 Attachment 14

GRUPO CENCON CENTRO DE CONTROL S.A. DE C.V.

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DESARROLLO DE PRODUCTOS • INDUSTRIA QUÍMICA EN GENERAL

FORMAT MPA-F-032A-01-0

ANÁLISIS

Microbiología:
Cuenta Bacteriana
Grupo Coliforme
Salmonella
Estafilococos
Estreptococos
E. Coli
Hongos
Levaduras
V. Cholerae
Anaerobios
Otros

Fisicoquímicos:
Bromatólogicos
Minerales
Vitaminas
Aditivos
Aflatoxinas
Materia Extraña
Otros

Instrumentales:
Cromatografía de Gases
Cromatografía de Líquidos
Absorción Atómica
Espectrofotometría
Infrarrojo
Aminogramas
Otros

Aguas:
Bacteriológicos
Fisicoquímicos
Aguas Residuales

Asesorías:
Control de Calidad
Inspecciones Sanitarias
Auditorias de Calidad
Desarrollo de Productos
Investigación Aplicada
Estudios Especiales
Restaurantes y Comedores
FDA Registrar
Unidades de Verificación

Otros Análisis:
Biodegradabilidad
Biodisponibilidad

TEST REPORT

REF: 1820-11
January 21th, 2011
O. T. 1827
Sample 1/6
Page 1/1

INDUSTRIALIZADORA INTEGRAL
DEL AGAVE, S.A. DE C.V.
PERIFÉRICO SUR No. 7750
SANTA MARÍA TEQUEPEXPAN
TLAQUEPAQUE, JAL, C.P. 45601

"INULINA EN POLVO LOTE: 4NIPP103365"

ASSAY	ANALYTICAL RESULTS
Account of aerobes mesophyll	150* UFC/g
Total coliforms	Less than 10* UFC/g
Fungi	Less than 10* UFC/g
Yeast	Less than 10* UFC/g
Salmonella sp (in 25g)	Absence (Negative)

* Estimated Value

ASSAY	METHOD REFERENCE
Account of aerobes mesophyll	NOM-092-SSA1-1994 this essay we realize in agar for standard account incubated for 48 h to 35ºC.
Total coliforms	NOM-113-SSA1-1994 this essay we realize in agar bile and violet red incubated to 35ºC for 24 ± 2 h.
Fungi	NOM-111-SSA1-1994 this essay we realize in agar potato dextrose acidified incubated for 25 ºC ± 1 ºC for 5 days.
Yeast	
Salmonella sp	NOM-114-SSA1-1994

GRUPO CENCON
(b) (6)

I.A. NALLELY HERNANDEZ ALVAREZ
GENERAL MANAGER
Q.F.B. LUCILA TRIGUEROS DÍAZ
CHEMICAL ANALYST OF MICROBIOLOGY

*jger

LOS RESULTADOS REPORTADOS AMPARAN ÚNICAMENTE LA MUESTRA ANALIZADA
Y NO NECESARIAMENTE EL LOTE QUE REPRESENTA

Av. Galileo Galilei 4299 Fracc. Arboledas C.P. 45070 Guadalajara, Jalisco, México **www.cencon.com.mx**
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FORMAT MPA-F-032A-01-0

TEST REPORT

REF: 1820-11
January 21th, 2011
O. T. 1827
Sample 2/6
Page 1/1

ANÁLISIS

Microbiología:
Cuenta Bacteriana
Grupo Coliforme
Salmonella
Estafilococos
Estreptococos
E. Coli
Hongos
Levaduras
V. Cholerae
Anaerobios
Otros

Fisicoquímicos:
Bromatólogicos
Minerales
Vitaminas
Aditivos
Aflatoxinas
Materia Extraña
Otros

Instrumentales:
Cromatografía de Gases
Cromatografía de Líquidos
Absorción Atómica
Espectrofotometría
Infrarrojo
Aminogramas
Otros

Aguas:
Bacteriológicos
Fisicoquímicos
Aguas Residuales

Asesorías:
Control de Calidad
Inspecciones Sanitarias
Auditorías de Calidad
Desarrollo de Productos
Investigación Aplicada
Estudios Especiales
Restaurantes y Comedores
FDA Registrar
Unidades de Verificación

Otros Análisis:
Biodegradabilidad
Biodisponibilidad

INDUSTRIALIZADORA INTEGRAL
DEL AGAVE, S.A. DE C.V.
PERIFÉRICO SUR No. 7750
SANTA MARÍA TEQUEPEXPAN
TLAQUEPAQUE, JAL, C.P. 45601

"INULINA EN POLVO LOTE: 4NIPP11004"

ASSAY	ANALYTICAL RESULTS
Account of aerobes mesophyll	90* UFC/g
Total coliforms	Less than 10* UFC/g
Fungi	Less than 10* UFC/g
Yeast	Less than 10* UFC/g
Salmonella sp (in 25g)	Absence (Negative)

* Estimated Value

ASSAY	METHOD REFERENCE
Account of aerobes mesophyll	NOM-092-SSA1-1994 this essay we realize in agar for standard account incubated for 48 h to 35°C.
Total coliforms	NOM-113-SSA1-1994 this essay we realize in agar bile and violet red incubated to 35°C for 24 ± 2 h.
Fungi	NOM-111-SSA1-1994 this essay we realize in agar potato dextrose acidified incubated for 25 °C ± 1 °C for 5 days.
Yeast	

GRUPO CENCON
(b) (6)

I.A. NALLELY HERNANDEZ ALVAREZ
GENERAL MANAGER
Q.F.B. LUCILA TRIGUEROS DÍAZ
CHEMICAL ANALYST OF MICROBIOLOGY

*jger

LOS RESULTADOS REPORTADOS AMPARAN ÚNICAMENTE LA MUESTRA ANALIZADA
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The iidea Company
Premium ga e Qua ty Products

PRODUCT: ***ORGANIC AGAVE POWDER INULIN 100% BLUE WEBBER***

AMOUNT	***Samples***
LOT:	***4NIPP11020***
PACK DATA	***20-ene-11***
USE BEFORE	***20-ene-14***

CERTIFICATE CHEMICAL ANALYSIS

ASSAY		***SPECIFICATIONS***	***METHOD***
APPEARENCE	CREAMY WHITE FINE POWDER		*ORGANOLEPTIC ANALYSIS*
FRUCTOSE	*4.62*	***3,0 - 10,0%***	*METHOD HPLC HP 1100 - HP 1200*
GLUCOSE	*0.71*	***Max. 3,5 %***	*METHOD HPLC HP 1100 - HP 1200*
SACCHAROSE	*1.18*	***Max. 2,0%***	*METHOD HPLC HP 1100 - HP 1200*
INULIN	*91.47*	***88,0 - 94,0 %***	*METHOD HPLC HP 1100 - HP 1200*
OTHER CARBOHYDRATES	*1.15*	***Max. 6,0 %***	*METHOD HPLC HP 1100 - HP 1200*
TOTAL CARBOHYDRATES	*99.13*	***Min. 98,0 %***	*METHOD HPLC HP 1100 - HP 1200*

ANALYZE

(b) (6)

I.Q. Carolina De Los Santos González.

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INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V

QUALITY ASSURANCE

INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION

Date 20/01/2011
FOLIO 003/11

Lot ***4NIPP11020***

Amount Samples

Analysis	Results	Specifications	Units	Method	
Total Count	13	Max. 2,500	CFU/g	AOAC 2002.07	NOM-092-SSA1 *
Coliform	< 10	Max. 10	CFU/g	AOAC 2005.03	NOM-113-SSA1*
Yeast	< 10	Max. 100	CFU/g	AOAC 2002.11	NOM-111-SSA1*
Mold	< 10	Max. 100	CFU/g	AOAC 2002.11	NOM-111-SSA1*
Salmonella	Absent	Absent	in 25 gr.	-	NOM-114-SSA1*

* Mexican regulations

(b) (6)

I.Q. Carolina De Los Santos González.
Analyze

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PRODUCT: **ORGANIC AGAVE POWDER INULIN 100% BLUE WEBBER**

AMOUNT	**Samples**
LOT:	**4NIPP10271**
PACK DATA	**28-sep-10**
USE BEFORE	**28-sep-13**

CERTIFICATE CHEMICAL ANALYSIS

ASSAY		SPECIFICATIONS	METHOD
APPEARENCE	CREAMY WHITE FINE POWDER		ORGANOLEPTIC ANALYSIS
FRUCTOSE	5.66	**3,0 - 10,0%**	METHOD HPLC HP 1100 - HP 1200
GLUCOSE	0.60	**Max. 3,5 %**	METHOD HPLC HP 1100 - HP 1200
SACCHAROSE	0.69	**Max. 2,0%**	METHOD HPLC HP 1100 - HP 1200
INULIN	90.98	**88,0 - 94,0 %**	METHOD HPLC HP 1100 - HP 1200
OTHER CARBOHYDRATES	1.09	**Max. 6,0 %**	METHOD HPLC HP 1100 - HP 1200
TOTAL CARBOHYDRATES	99.02	**Min. 98,0 %**	METHOD HPLC HP 1100 - HP 1200

ANALYZE

(b) (6)

I.Q. Carolina De Los Santos González.

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INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V

ASEGURAMIENTO DE LA CALIDAD

INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION

Date 28/09/2010
FOLIO 006/10

Lot ***4NIPP10271***

Amount Samples

Analysis	Results	Specifications	Units	Method	
Total Count	260	Max. 2,500	UFC/g	AOAC 2002.07	NOM-092-SSA1 *
Coliform	< 10	Max. 10	UFC/g	AOAC 2005.03	NOM-113-SSA1*
Yeast	< 10	Max. 100	UFC/g	AOAC 2002.11	NOM-111-SSA1*
Mold	< 10	Max. 100	UFC/g	AOAC 2002.11	NOM-111-SSA1*
Salmonella	Absent	Absent	in 25 gr.	-	NOM-114-SSA1*

* Mexican regulations

(b) (6)

I.Q. Carolina De Los Santos González.
Analyze

Periférico Sur No. 7750
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The iidea Company
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PRODUCT: **ORGANIC AGAVE POWDER INULIN 100% BLUE WEBBER**

AMOUNT	**Samples**
LOT:	**4NIPP10041**
PACK DATA	**10-feb-10**
USE BEFORE	**10-feb-13**

CERTIFICATE CHEMICAL ANALYSIS

ASSAY		ESPECIFICATIONS	METHOD
APPEARENCE	CREAMY WHITE FINE POWDER		ORGANOLEPTIC ANALYSIS
FRUCTOSE	4.73	3,0 - 10,0%	METHOD HPLC HP 1100 - HP 1200
GLUCOSE	2.32	Max. 3,5 %	METHOD HPLC HP 1100 - HP 1200
SACCHAROSE	0.93	Max. 2,0%	METHOD HPLC HP 1100 - HP 1200
INULIN	90.00	88,0 - 94,0 %	METHOD HPLC HP 1100 - HP 1200
OTHER CARBOHYDRATES	1.91	Max. 6,0 %	METHOD HPLC HP 1100 - HP 1200
TOTAL CARBOHYDRATES	99.89	Min. 98,0 %	METHOD HPLC HP 1100 - HP 1200

ANALYZE

(b) (6)

I.Q. Carolina De Los Santos González.

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INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V

QUALITY ASSURANCE

INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION

Date 10/02/2010
FOLIO 003/10

Lot ***4NIPP10041***

Amount Samples

Analysis	Results	Specifications	Units	Method	
Total Count	10	Max. 2,500	UFC/g	AOAC 2002.07	NOM-092-SSA1 *
Coliform	< 10	Max. 10	UFC/g	AOAC 2005.03	NOM-113-SSA1*
Yeast	< 10	Max. 100	UFC/g	AOAC 2002.11	NOM-111-SSA1*
Mold	< 10	Max. 100	UFC/g	AOAC 2002.11	NOM-111-SSA1*
Salmonella	Absent	Absent	in 25 gr.	-	NOM-114-SSA1*

* Mexican regulations

(b) (6)

I.Q. Carolina De Los Santos González.
Analyze

Periférico Sur No. 7750
Santa María Tequepexpan
C.P. 45601, Tlaquepaque, Jal., Mx.
Tel. 3003 4450

The iidea Company
Premium ga e Qua ty Products

PRODUCT: **ORGANIC AGAVE POWDER INULIN 100% BLUE WEBBER** **P**

AMOUNT	**Samples**
LOT:	**4NIPP11023**
PACK DATA	**23-ene-11**
USE BEFORE	**23-ene-14**

CERTIFICATE CHEMICAL ANALYSIS

ASSAY		SPECIFICATIONS	METHOD
APPEARENCE	CREAMY WHITE FINE POWDER		ORGANOLEPTIC ANALYSIS
FRUCTOSE	4.96	**3,0 - 10,0%**	METHOD HPLC HP 1100 - HP 1200
GLUCOSE	0.45	**Max. 3,5 %**	METHOD HPLC HP 1100 - HP 1200
SACCHAROSE	0.60	**Max. 2,0%**	METHOD HPLC HP 1100 - HP 1200
INULIN	91.98	**88,0 - 94,0 %**	METHOD HPLC HP 1100 - HP 1200
OTHER CARBOHYDRATES	1.03	**Max. 6,0 %**	METHOD HPLC HP 1100 - HP 1200
TOTAL CARBOHYDRATES	99.02	**Min. 98,0 %**	METHOD HPLC HP 1100 - HP 1200

ANALYZE

(b) (6)

I.Q. Carolina De Los Santos González.

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The iidea Company
Premium Agave Quality Products

INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V

QUALITY ASSURANCE

INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION

Date 23/01/2011
FOLIO 005/11

Lot ***4NIPP11023***

Amount Samples

Analysis	Results	Specifications	Units	Method	
Total Count	10	Max. 2,500	CFU/g	AOAC 2002.07	NOM-092-SSA1 *
Coliform	< 10	Max. 10	CFU/g	AOAC 2005.03	NOM-113-SSA1*
Yeast	< 10	Max. 100	CFU/g	AOAC 2002.11	NOM-111-SSA1*
Mold	< 10	Max. 100	CFU/g	AOAC 2002.11	NOM-111-SSA1*
Salmonella	Absent	Absent	in 25 gr.	-	NOM-114-SSA1*

* Mexican regulations

(b) (6)

I.Q. Carolina De Los Santos González.
Analyze

Periférico Sur No. 7750
Santa María Tequepexpan
C.P. 45601, Tlaquepaque, Jal., Mx.
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10.15 Attachment 15

The iidea Company
Premium ga e Qua ty Products

PRODUCT: **ORGANIC AGAVE LIQUID INULIN 100% BLUE WEBBER**

AMOUNT	**Samples**
LOT:	**IL110111**
PACK DATA	**11-ene-11**
USE BEFORE	**11-abr-11**

CERTIFICATE CHEMICAL ANALYSIS

ASSAY		METHOD
APPEARENCE	LIGHT AMBER	ORGANOLEPTIC ANALYSIS
pH	4.10	NMX-FF-110-SCFI
BRIX	60.4	NMX-FF-110-SCFI
FRUCTOSE	4.54	METHOD HPLC HP 1100 - HP 1200
GLUCOSE	1.65	METHOD HPLC HP 1100 - HP 1200
SACCHAROSE	1.11	METHOD HPLC HP 1100 - HP 1200
INULIN	90.00	METHOD HPLC HP 1100 - HP 1200
OTHER CARBOHYDRATES	1.62	METHOD HPLC HP 1100 - HP 1200
CARBOHYDRATES TOTAL	98.92	METHOD HPLC HP 1100 - HP 1200

ANALYZE

(b) (6)

I.Q. Carolina De Los Santos González.

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INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V

ASEGURAMIENTO DE LA CALIDAD

INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION

Date 11/01/2011
FOLIO 001/11

Lot ***IL110111***

Amount Samples

Analysis	Results	Units	Method	
Total Count	359	CFU/g	AOAC 2002.07	NOM-092-SSA1 *
Coliform	< 10	CFU/g	AOAC 2005.03	NOM-113-SSA1*
Yeast	< 10	CFU/g	AOAC 2002.11	NOM-111-SSA1*
Mold	< 10	CFU/g	AOAC 2002.11	NOM-111-SSA1*

(b) (6)

I.Q. Carolina De Los Santos González.
Analyze

Periférico Sur No. 7750
Santa María Tequepexpan
C.P. 45601, Tlaquepaque, Jal., Mx.
Tel. 3003 4450

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The iidea Company
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PRODUCT:	**ORGANIC AGAVE LIQUID INULIN 100% BLUE WEBBER**
AMOUNT	**Samples**
LOT:	**IL140111**
PACK DATA	**14-ene-11**
USE BEFORE	**14-abr-11**

CERTIFICATE CHEMICAL ANALYSIS

ASSAY		METHOD
APPEARENCE	LIGHT AMBER	ORGANOLEPTIC ANALYSIS
pH	4.00	NMX-FF-110-SCFI
BRIX	63.2	NMX-FF-110-SCFI
FRUCTOSE	3.13	METHOD HPLC HP 1100 - HP 1200
GLUCOSE	1.57	METHOD HPLC HP 1100 - HP 1200
SACCHAROSE	1.09	METHOD HPLC HP 1100 - HP 1200
INULIN	89.89	METHOD HPLC HP 1100 - HP 1200
OTHER CARBOHYDRATES	2.59	METHOD HPLC HP 1100 - HP 1200
CARBOHYDRATES TOTAL	98.27	METHOD HPLC HP 1100 - HP 1200

ANALYZE

(b) (6)

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INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V

ASEGURAMIENTO DE LA CALIDAD

INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION

Date 14/01/2011
FOLIO 002/11

Lot ***IL140111***

Amount Samples

Analysis	Results	Units	Method	
Total Count	338	CFU/g	AOAC 2002.07	NOM-092-SSA1 *
Coliform	< 10	CFU/g	AOAC 2005.03	NOM-113-SSA1*
Yeast	< 10	CFU/g	AOAC 2002.11	NOM-111-SSA1*
Mold	< 10	CFU/g	AOAC 2002.11	NOM-111-SSA1*

(b) (6)

I.Q. Carolina De Los Santos González.
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The iidea Company
Premium ga e Qua ty Products

PRODUCT:	**ORGANIC AGAVE LIQUID INULIN 100% BLUE WEBBER**
AMOUNT	**Samples**
LOT:	**IL150111**
PACK DATA	**15-ene-11**
USE BEFORE	**15-abr-11**

CERTIFICATE CHEMICAL ANALYSIS

ASSAY		METHOD
APPEARENCE	LIGHT AMBER	ORGANOLEPTIC ANALYSIS
pH	4.05	NMX-FF-110-SCFI
BRIX	60.4	NMX-FF-110-SCFI
FRUCTOSE	4.59	METHOD HPLC HP 1100 - HP 1200
GLUCOSE	0.50	METHOD HPLC HP 1100 - HP 1200
SACCHAROSE	0.62	METHOD HPLC HP 1100 - HP 1200
INULIN	89.63	METHOD HPLC HP 1100 - HP 1200
OTHER CARBOHYDRATES	2.70	METHOD HPLC HP 1100 - HP 1200
CARBOHYDRATES TOTAL	98.04	METHOD HPLC HP 1100 - HP 1200

ANALYZE

(b) (6)

I.Q. Carolina De Los Santos González.

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INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. De C.V

ASEGURAMIENTO DE LA CALIDAD

INTERNAL REPORT OF MICROBIOLOGICAL VERIFICATION

Date 15/01/2011
FOLIO 003/11

Lot ***IL150111***

Amount Samples

Analysis	Results	Units	Method	
Total Count	414	CFU/g	AOAC 2002.07	NOM-092-SSA1 *
Coliform	< 10	CFU/g	AOAC 2005.03	NOM-113-SSA1*
Yeast	< 10	CFU/g	AOAC 2002.11	NOM-111-SSA1*
Mold	< 10	CFU/g	AOAC 2002.11	NOM-111-SSA1*

(b) (6)

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Analyze

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10.18 Attachment 16

SA- 49372

Shelf life Study

"AGAVE INULIN POWDER"

Prepared for:

INDUSTRIALIZADORA INTEGRAL DEL AGAVE S.A. de C.V.

Periférico Sur 7750. Santa Maria Tequepexpan, Tlaquepaque Jalisco
C.P: 45601 Tel.: 33 30 03 45 56

Ing. Carolina de los Santos González

May de 2011

Silliker México, S.A. de C.V.

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1.- OBJETIVE

- ✓ Estimate agave inulin powder shelf life under ambient temperature (19-25 º C) and stressed temperatures of 35 º C and 45 º C, using sensory attributes loss as detrimental indicators,
- ✓ Determine the behavior of moisture as physical-chemical indicators.
- ✓ Initial microbiological analysis for Salmonella and E. coli analysis are also done
- ✓ Initial and final analysis for total coliformes, total plate count, yeasts and molds are also included.

2.- MATERIAL AND METHODS

Samples were identified individually in their commercial presentation (kraft packaging). They were placed in temperature controlled chambers: ambient temperature (19-25 º C, in a cool, dry and free from sunlight), 35 º C and 45 º C. Temperatures were recorded with a calibrated C- hygrometer / thermometer (Mod 10-95 Digital 355119-020).

- ❖ The moisture analysis was performed by Mexican Official Standard N0M-116-SSA1-1994.
- ❖ The total plate count was performed by Mexican Official Standard NOM-092-SSA1-1994
- ❖ Total Coliformes analysis was performed by Mexican Official Standard NOM-113-SSA1-1994
- ❖ The analysis of molds and yeasts was performed by Mexican Official Standard NOM-111-SSA1-1994
- ❖ The analysis of E. coli was performed using the method in CCAYAC-004-M-2006.
- ❖ Salmonella analysis was performed by Mexican Official Standard NOM-114-SSA1-1994

Sensory performance was conducted with seven trained panelists (trained to perceive different deterioration degrees when compared to an original sample), testing was for a 32 days period, using a structured 10 points scale "SENSORY SCALE LEVELS":

Level 10.0-8.0: Characteristic. The product has the taste, smell and original appearance as the initial sample or reference
Level 7.9–6.0: Acceptable. The product has undergone just perceptible changes in taste, smell and appearance, without being disagreeable.
Level 5.9-4.0: Marginal. The product has undergone slightly changes in taste or odor (slightly rancid or bitter), and / or color significantly different from the original.
Level 3.9-0.0: Unacceptable. The product has undergone noticeable changes in taste or odor (rancid or bitter), and color is significantly different from the original.

Sensory attributes identified as part of the customer's needs were valuated as:

- Appearance
- Color
- Odor
- Flavor
- Fluidity
- Rancidity

3 RESULTS

3.1 Sensory analysis

Sensory average scores are shown in the following table, taking into consideration time (days) and sensory attributes losses

Table 1. Panelists average results at different temperatures: ambient (19 - 25 ° C), 35 ° C and 45 ° C

	AMBIENT						35ºC						45ºC					
Days	Appearance	Color	Odor	Flavor	Fluidity	Rancidity	Appearance	Color	Odor	Flavor	Fluidity	Rancidity	Appearance	Color	Odor	Flavor	Fluidity	Rancidity
22-Mar	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
1-Apr	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	9.8	10.0	9.9	10.0	9.8	9.9	9.6	9.7	9.8	10.0
8-Apr	10.0	10.0	10.0	10.0	10.0	10.0	9.9	9.7	9.7	9.9	10.0	10.0	9.7	9.4	9.6	9.7	9.3	9.9
15-Apr	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.7	9.7	9.4	9.4	9.6	9 3	9.4	9.4
26-Apr	9.7	9.7	9.6	9.6	9.6	9.7	9.7	9.2	9.5	9.3	9.5	9.5	9.3	8.4	9.0	8.7	9.0	9.1

Note, Day one corresponds to a "fresh" reference, therefore the highest score ("10") is given. The "fresh" product represent o the best alternative presented by the client

Sensory loss (sensory level log) in relation to time (days) is done trough a first-order kinetics, which consists of evaluating the detrimental loss (deterioration as a Log Y) versus time:

$$Log_{10}\, Y = (m * t) + I \qquad \text{equation (1)}$$

Where:

Y: is sensory loss (according to the hedonic scale sensory levels) based on a Log 10 scale.
m: is the slope
t: is the time
I: is the intercept

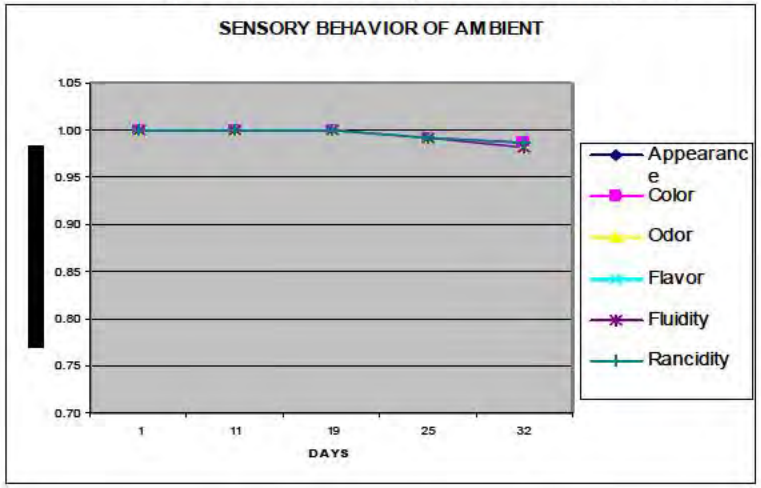
From the log Y values table 2 is obtained.

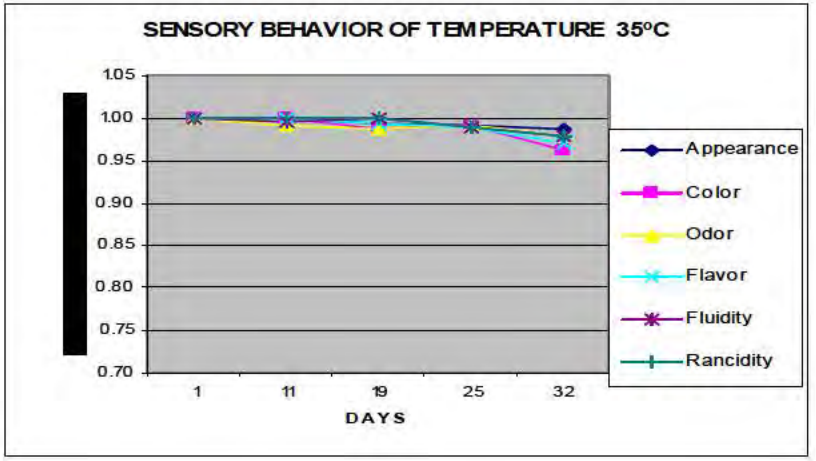
Table2. Sensory analysis log at different temperatures: ambient, 35, 45 ° C
Slope values (m), intercept (I)

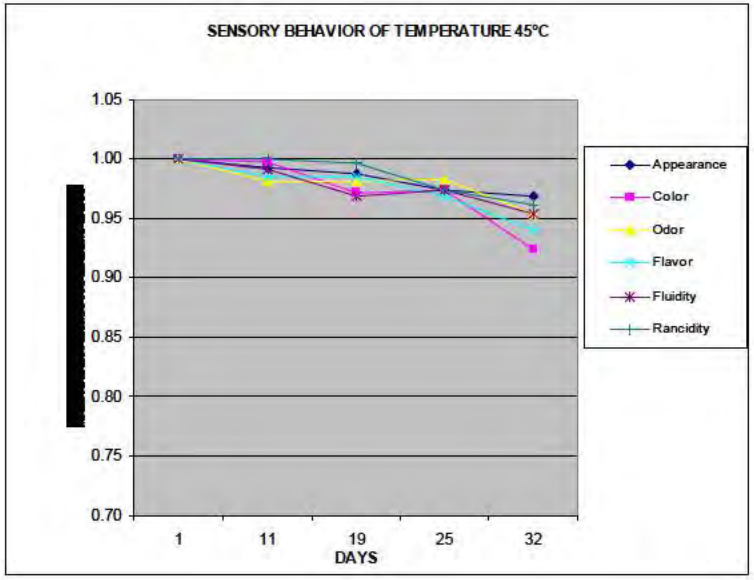
	AMBIENTE						35ºC						45ºC					
Days	Appearance	Color	Odor	Flavor	Fluidity	Rancidity	Appearance	Color	Odor	Flavor	Fluidity	Rancidity	Appearance	Color	Odor	Flavor	Fluidity	Rancidity
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1 00	1.00	1.00	1.00	1 00	1.00	1.00	1.00
11	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	0 99	1.00	0.99	1.00	0 98	0.99	0.99	1.00
19	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.99	0.99	0.99	1 00	1.00	0.99	0.97	0 98	0.99	0.97	1.00
25	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0 99	0.99	0.97	0.97	0 98	0.97	0.97	0.97
32	0.99	0.99	0.98	0.98	0.98	0.99	0.99	0.96	0.98	0.97	0 98	0.98	0.97	0.92	0 95	0.94	0.95	0.96
m	-0.0004	-0.0004	-0.0006	-0.0006	-0.0006	-0.0004	-0.0004	-0.0011	-0.0006	-0.0009	-0.0006	-0.0007	-0.0011	-0.0022	-0.0012	-0.0017	-0.0014	-0.0013
Int	1.0032	1.0032	1.0047	1.0047	1.0047	1 0032	1.0022	1.0071	0.9999	1.0063	1.0033	1.0055	1.0030	1.0127	1.0006	1.0066	1.0030	1.0092
R2	0.7209	0.7209	0.6925	0.6925	0.6925	0.7209	0.9134	0.6989	0.7892	0.7607	0.6685	0.7051	0.9463	0.7988	0.7655	0.8516	0.9202	0.7790

Table 2 presents the decline of sensory attributes at different temperatures (Figures 1, 2 and 3).

Figures1, 2 and 3 integrates the sensory loss, time (days) and temperature (ambient (19-25 ° C), 35 and 45 ° C







Hedonic level 7 was established as the acceptable sensory loss limit; Level 7.9–6.0 is within the acceptance range. The product has undergone just perceptible changes in taste, smell and appearance, without being disagreeable.

From Equation 1, Shelf life time (VU) is predicted when the level 7 is reached (Sensory level 7 was previously set). The Log10 Y represents a Shelf life (VU) in days at the selected temperature. For each temperature (room temperature (19-25 ° C), 35 ° C and 45 ° C) a particular shelf life time (VU) can be predicted for a sensory loss for a7 level (log of 7 is Log10 Y = 0.8450):

$$VU\ days = t = \frac{(Log_{10}\ Y) - I}{m} \qquad \textit{equation (2)}$$

Table 3 expresses the shelf life in days for a level 7, where the product has undergone any change in taste, smell and original appearance, without being disagreeable, The greatest loss is highlighted for the attribute which reaches first the detrimental 7 value

Table 3 Attribute summary relationship for sensory losses by temperature group (Level 7)

	AMB ENTE						35ºC						45ºC					
	Appearance	Color	Odor	Flavor	Fluidity	Rancidity	Appearance	Color	Odor	Flavor	Fluidity	Rancidity	Appearance	Color	Odor	Flavor	Fluidity	Rancidity
Shelf life VU(7)	374	374	275	275	275	374	359	151	261	180	250	231	148	75	131	93	109	125
Months	12.48	12.48	9.15	9.15	9.15	12.48	11.98	5.02	8.71	6.01	8.33	7.69	4.95	2.49	4.37	3.09	3.64	4.17

Limiting factors are highlighted in red

The interaction of sensory loss (days to reach a deterioration to a level 7) in relation to temperature, is obtained by Equation 3 and fig. 4, the equation that defines these changes is:

Log 10 (decay time) = (m * T) + I equation (3)

m: is the slope
T: is the temperature
I: is the intercept

Integrating the sensory loss for each temperature from table 3 and equation 3, the sensory loss is now associated with temperature (Table 4)

Table 4. - Projected stability (days) under different temperatures: room temperature (19 - 25 ° C), 35 and 45 ° C for a deterioration level of "7".

Appearance			Color			Odor			Flavor			Fluidity			Rancidity		
Tem	Days	log10 Days	Tem	Days	log10 Days	Tem	Days	log10 Days	Tem	Days	log10 Days	Tem	Days	log10 Days	Tem	Days	log10 Days
25	374	2.5733	25	374	2.5733	25	275	2.4387	25	275	2.4387	25	275	2.4387	25	374	2.5733
35	359	2.5555	35	151	2.1782	35	261	2.4174	35	180	2.2561	35	250	2.3976	35	231	2.3631
45	148	2.1714	45	75	1.8732	45	131	2.1178	45	93	1.9664	45	109	2.0383	45	125	2.0976
m	-0.020		m	-0.035		m	-0.016		m	-0.024		m	-0.020		m	-0.024	
Int	3.137		Int	3.433		Int	2.886		Int	3.047		Int	2.992		Int	3.177	
R2	0.783		R2	0.995		R2	0.800		R2	0.983		R2	0.826		R2	0.996	

Note: Tem = temperature, m = slope, int. = intersection

Figure 4. Behavior for a Level of "7": shelf live days (stability expressed as "log days") in relation to temperature

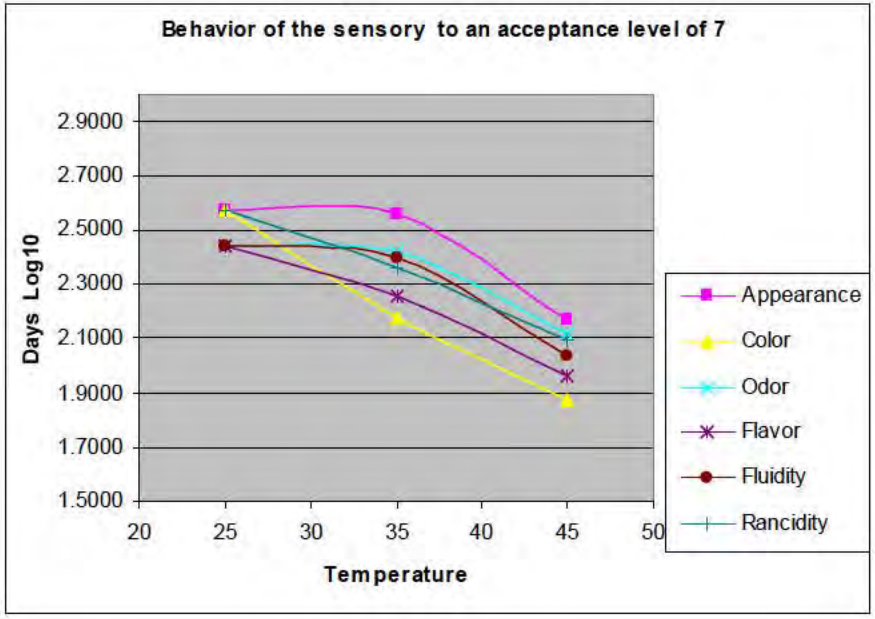
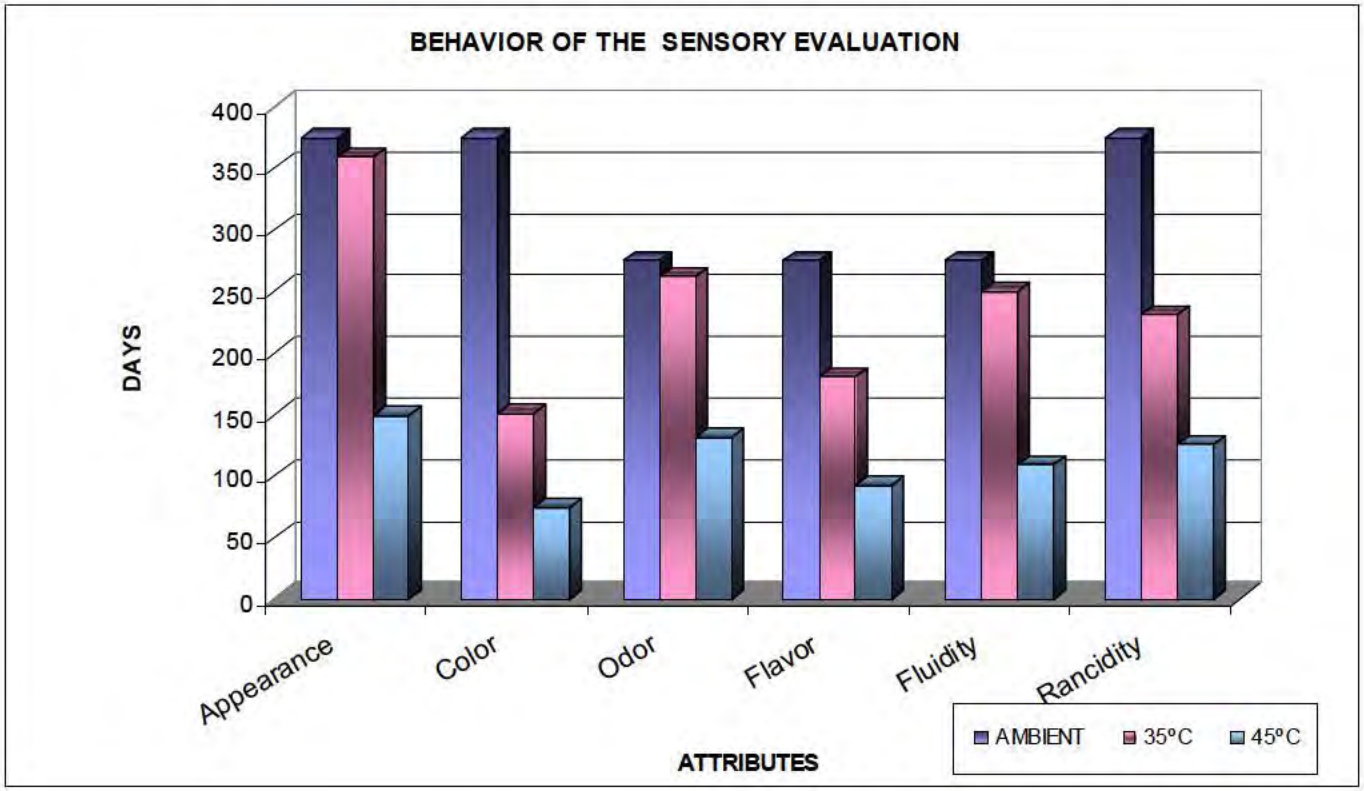
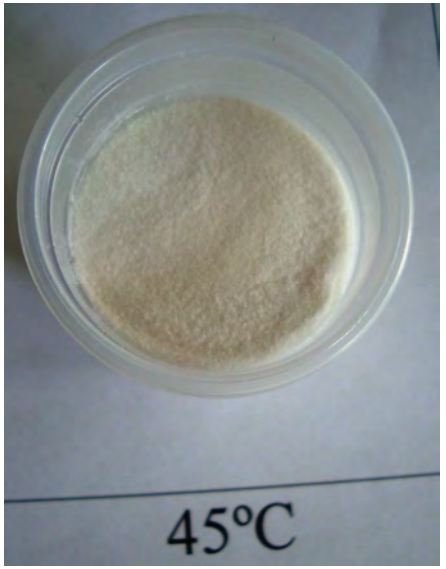


Table 5 and Figure 5: Projected Days to reach a sensory loss of "7"

	Appearance	Color	Odor	Flavor	Fluidity	Rancidity
Ambient	436	436	508	514	426	504
35 °C	538	150	158	282	274	191
45 °C	226	393	80	61	116	76



PHOTOS:



3.2 Moisture behavior:

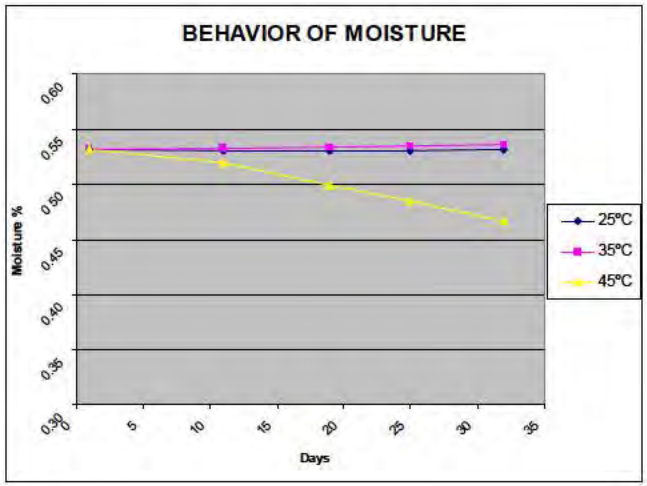
Moisture changes are summarized accordingly to storage time (days) at different temperatures and applying the same sequence of analysis.

Table 6. Behavior of moisture % about the days at different temperature

Moisture %			
Days	25ºC	35ºC	45ºC
1	3.40	3.40	3.40
11	3.39	3.41	3.31
19	3.39	3.42	3.16
25	3.39	3.43	3.06
32	3.40	3.44	2.93

Moisture %			
Days	25ºC	35ºC	45ºC
1	0.53	0.53	0.53
11	0.53	0.53	0.52
19	0.53	0.53	0.50
25	0.53	0.54	0.49
32	0.53	0.54	0.47
m	0.0000	0.0002	-0.0021
Int	0.5308	0.5311	0.5380
R2	0.0069	0.9907	0.9772

Figure 7 represents behavior of moisture changes. Its integration with days and temperatures



3.3 MICROBIAL CHANGES

Microbial changes are presented in table 8,

MICROBIAL ANALYSIS				
	ROOM TEMPERATURE	ROOM TEMPERATURE	35 °C	45°C
	INITIAL	FINAL	FINAL	FINAL
Total coliformes	<10 CFU/g			
E. coli	<3 MPN			
Yeast	<10 CFU/ g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Total plate count	<10 ev CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Molds	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Salmonella	Absent/ 25g			

ev= estimated value

4. - CONCLUSIONS

Taking into account the selected scale to estimate changes up to a level 7 (The product has undergone any change in taste, smell and original appearance, without being disagreeable), general considerations are:

The samples at room temperature have a shelf life of 275 days, where the limiting attributes are: odor, flavor and fluidity.

At 35 ° C the estimated shelf life is 151 days; limiting attribute are color and odor.

At 45 ° C a similar change were observed in most of the limiting attributes of: color and flavor, with 75 days of shelf life, followed by taste.

Attributes behave differently at different detrimental speeds; odor, color and flavor are consistently the limiting attributes associated to temperature. Panelists comments are: odor changes and perceived less intense, in particular at 45 ° C.

The determination of moisture initial is of 3.4 %, this ratio decreases upon time, final at 2.93 %, at 32 days and a 45 C, Does not affect the fluidity of the product

Microbiological testing implies a stable product.

As summary, the product has a shelf life of 275 days at ambient temperature (19-25 º C), when it reached the sensory value of "7"

5.0 BIBLIOGRAPHY

- Taoukis P.S, Labuza T. P, Saguy I.S. 2001. Kinetics of Food Deterioration and shelf life Prediction. The Handbook of Food Engineering Practice. CRC PRESS. Chapter 10.
- Internal method (VU-002-2)

Elaborated by
I.A. Celia Ortigoza Hernández
Sensory Analysis Coordinator

Reviewed by
Dr. Pedro Valle Vega
Technical Director

10.19 Attachment 17

The iidea Company

Tlaquepaque, Jalisco, México. September 14, 2011

To whom it may concern,

By this mean we assure that the shelf life of Inufib is 3 years. We determined it having as reference other similar products , like powder dextrose.

We protect the product with two polyethylene bags of 200 caliber and three paper kraft bags to avoid humidity absorption.

The results sent by an external laboratory show that the shelf life is less than 3 years, however the methodology used by them was a sensorial analysis. They realized microbiological analysis to the sample and it does not present changes with time.

Based in IIDEA's experience, the chemical composition in the product does not change with time.

Sincerely,

The iidea Company
— Premium Agave Quality Products —
QUALITY ASSURANCE

(b) (6)

Ma. del Carmen Jiménez F.
Quality Manager

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SUBMISSION END