Inulin-oligofructose Enriched

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
oligofructose-enriched inulin
oligofructose only: fructose oligosaccharide, fructooligosaccharides

Other Names:
easily fermentable inulin (EFI)
hardly fermentable inulin (HFI)

Trade Names:
BENEO®™ Synergy 1 Inulin/OFS Powder
Raftiline® (inulin)
Fibrulose® (inulin)
Orafti® (inulin)
Raftilose® (oligofructose)
NutraFlora® (oligofructose)
Osteoboost™ (inulin-oligofructose)
Inulin FOS (inulin-oligofructose)

CAS Number:
9005-80-5

Other Codes:
EC number: 232-684-3
MDL number: MFCD00131407

Summary of Petitioned Use

Inulin-oligofructose enriched (IOE) is currently included on the National List of Allowed and Prohibited Substances (hereafter referred to as the National List) as a nonorganically-produced agricultural product that can be used as an ingredient in or on processed products labeled as "organic" when the substance is not commercially available in organic form (7 CFR 205.606). In organic processing and handling, IOE is used as an ingredient in foods, specifically yogurt, to improve calcium bioavailability and absorption, to serve as soluble dietary fiber or a prebiotic ingredient, and to enhance the texture and consistency of the food.

Characterization of Petitioned Substance

Composition of the Substance:

IOE is made up of two carbohydrates—inulin and oligofructose—that are included in a group of dietary fiber compounds called fructans (Kaur and Gupta, 2002). Inulin is a naturally-occurring carbohydrate found in the roots of chicory (*Cichorium intybus*) and many other food plants, and oligofructose is derived from inulin (Coussement, 1999; Roberfroid, 2007; Singh and Singh, 2010; U.S. FDA, 2003). To extract the oligofructose portion of IOE, inulin must be further refined.

Inulin is a polymer chain of multiple fructose molecules with a glucose molecule at one end. The length of the fructose chain of inulin can range from 2–60 fructose molecules (Frippiat et al., 2010). "Native inulin" extracted from the fresh roots of the chicory plant has an average chain length of 10 to 20 fructose molecules (Flamm et al., 2001). Shorter inulin chains (2–10 fructose molecules) are also called easily fermentable inulin (EFI), and longer inulin chains (10–60 fructose molecules) are also referred to as hardly fermentable inulin (HFI) (Coussement, 1999; Frippiat et al., 2010; U.S. FDA, 2003). This report uses interchangeably the terms EFI for oligofructose, HFI for inulin, and IOE for inulin-oligofructose enriched. The ideal percentages of EFI and HFI in food to increase calcium absorption are approximately 45% EFI and 55% HFI (Frippiat et al., 2010). The molecular structure of generic inulin (also referred to as GFn) is pictured in Figure 1. The complex fructan sugars that make up oligofructose are called kestose (one glucose and two fructose molecules), nystose (one glucose and three fructose molecules), and fructosyl nystose (one glucose and four fructose molecules). These oligofructose polymers (also referred to as GF2, GF3, and GF4, respectively) are also shown in Figure 1.

Oligofructose is also used separately in organic handling and processing as a specific mixture of short-chain EFIs called fructooligosaccharides (Coussement, 1999; Eurofins, 2012; Roberfroid, 2007; Singh and Singh, 2010). The short-chain EFI molecules can be obtained by filtering the inulin solution or from the partial hydrolysis (i.e., breakdown by water) of inulin by the inulinase enzyme (Coussement, 1999; Roberfroid, 2007; Singh and Singh, 2010). The primary source of the inulinase enzyme for industrial production is the fungus *Aspergillus niger* (Coussement, 1999; Singh and Singh, 2010).

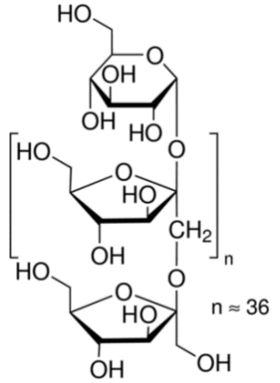
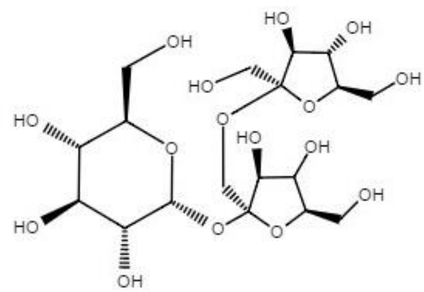
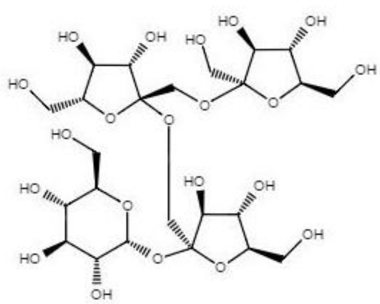
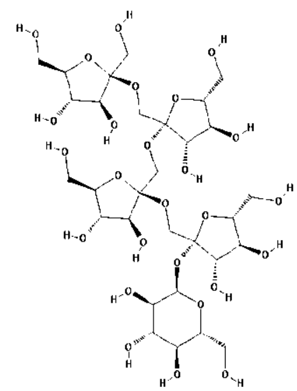
	
Generic Inulin	**Kestose**
GFn: glucose – fructose – n - fructose (n = up to 58 fructose units)	GF2: glucose – fructose – fructose
	
Nystose	**Fructosyl Nystose**
GF3: glucose – fructose – fructose – fructose	GF4: glucose – fructose – fructose – fructose – fructose

Figure 1: Chemical Structures of Inulin and Selected Oligofructose Sugars (NLM, 2012; Sigma-Aldrich, 2014)

IOE is considered dietary fiber and is mostly indigestible by human digestive enzymes due to its shape (relative to the shape of the digestive enzymes), but is digestible by microbes in the large intestine (Roberfroid, 2007). EFI is typically fermented in the proximal or ascending part of the large intestine whereas HFI is typically fermented in the transverse or descending part of the large intestine (Frippiat et al., 2010). For this reason, IOE can serve as a prebiotic, a nutrient source for microflora in the human digestive system (Ophardt, 2003; Roberfroid, 2007).

<u>Source or Origin of the Substance:</u>

IOE contains inulin and oligofructose, two carbohydrates found in many plant foods that function as dietary fiber (Coussement, 1999; Roberfroid, 2007). Chicory (*Cichorium intybus*) is a natural source of inulin and oligofructose and it is the most commonly used vegetable source for the industrial production of inulin (Coussement, 1999; Roberfroid, 2007). Oligofructose can also be produced from the enzyme synthesis of sucrose as is most common with the production of fructooligosaccharides, a group of short-chain oligofructose compounds (Sangeetha et al., 2005). Inulin was first discovered by isolation from the roots of the elecampane plant (*Inula helenium*). In addition to chicory, inulin is found in food plants such as garlic, onion, bananas, Jerusalem artichoke, asparagus, wheat, and rye (Coussement, 1999; Kowalchik and Hylton, 1998; Morris and Morris, 2012).

Production of IOE begins with washed and shredded chicory roots, which are treated with hot water, juiced, and filtered to remove the raw inulin (Frippiat et al., 2010; Gibson et al., 1994). The raw inulin is purified by treatment with lime (calcium hydroxide), carbonated, and filtered to remove the short-chain inulin and simple sugars (Gibson et al., 1994). A portion of the inulin is then spray-dried while the remaining portion is treated with enzymes to convert the inulin to oligofructose (Gibson et al., 1994).

EFI is produced from inulin by filtration and use of the enzyme inulinase (Singh and Singh, 2010). Filtration removes any short-chain oligofructose molecules from the raw inulin extract before treatment with the inulinase enzyme (Frippiat et al., 2010; Singh and Singh, 2010). The inulinase enzyme is naturally occurring in several species of fungi, including *Aspergillus niger, Aspergillus japonicus, Fusarium oxysporum,* and *Aureobasidium pullulans* (Coussement, 1999; Santos and Maugeri, 2006; Singh and Singh, 2010). Inulinase breaks down inulin through the process of enzymatic hydrolysis—a process by which enzymes facilitate the breakdown using water (Roberfroid, 2007).

<u>Properties of the Substance:</u>
IOE is an odorless, white to cream-colored solid, with a neutral to slightly sweet taste and a molecular weight ranging from 504.43–9908.84 g/mol, depending on the specific numbers of fructose chain units in the sample (NLM, 2012; Spectrum Chemical, 2009). The water solubility of oligosaccharides with 10 or fewer fructose units is 120 g/L at 25 °C, while the solubility of inulin with fructose chains between 10 and 60 units is 25 g/L at 25 °C (Spectrum Chemical, 2009).

IOE is included in a larger class of chemicals called fructans, which includes inulin from chicory, oligosaccharides from chicory-derived inulin, and synthetically-produced oligosaccharides derived from sucrose (Roberfroid, 2007). IOE is a mixture of inulin with oligosaccharides, the properties of which are listed in Table 1 for selected IOE compounds and fructooligosaccharides (NLM, 2012; Silva et al., 2013). Molecular weight, molecular formula, CAS number, and water solubility for these compounds are also provided in Table 1. The sugars all have the same molecular base (sucrose) with the addition of 1–59 fructose molecules attached to the terminal sucrose, resulting in sugar chains of 2–60 fructose molecules. Figure 2 shows a schematic diagram of fructans and some of its carbohydrate subgroups that are discussed in this report.

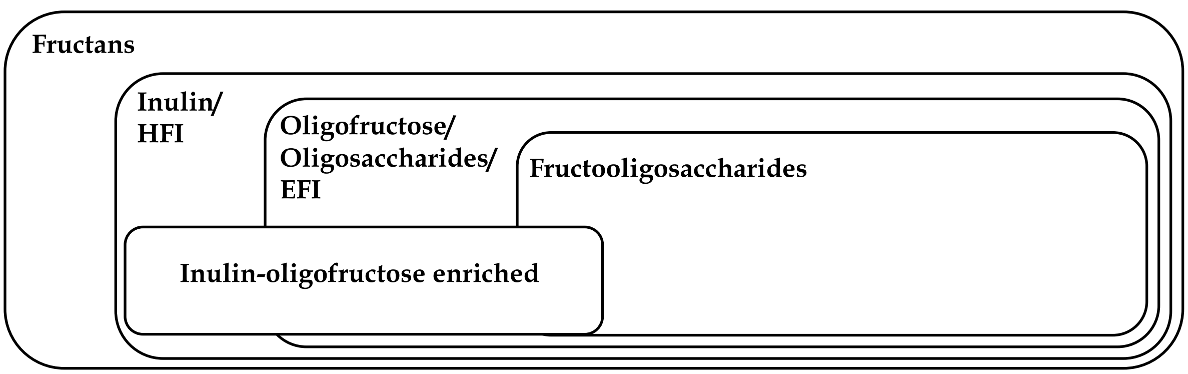


Figure 2: Schematic Diagram of Fructans and Selected Carbohydrate Subgroups

Table 1: Properties of Selected Oligofructose Compounds and Inulin (NLM, 2012; Ranawana, 2008)

Compound	CAS Number	Fructose Chain Units	Molecular Weight (g/mol)	Molecular Formula	Water Solubility (g/L, 25 °C)
Kestose	470-69-9	2	504.43	$C_{18}H_{32}O_{16}$	120
Nystose	13133-07-8	3	666.58	$C_{24}H_{42}O_{21}$	
Fructosyl nystose	59432-60-9	4	828.72	$C_{30}H_{52}O_{26}$	
GF10	not available	10	1801.59	$C_{66}H_{112}O_{56}$	25
GF60	not available	60	9908.84	$C_{366}H_{612}O_{306}$	

Specific Uses of the Substance:

IOE is added to yogurt (primarily) and other foods to: (1) improve calcium bioavailability and absorption, (2) serve as a nondigestible soluble dietary fiber, and (3) enhance the food's texture and consistency. As a dietary fiber, IOE is moved to the lower digestive tract undigested and serves as a nutrient source for beneficial bacteria in the gut (Roberfroid, 2007; Sangeetha et al., 2005; Sheu et al., 2013; Tymczyszyn et al., 2014). When mixed in yogurt, IOE improves its texture and consistency and acts as a food source for *Lactobacillus* bacteria in the yogurt (Pimentel et al., 2012). The *Lactobacillus* bacteria consume IOE in the yogurt and produce exopolysaccharides that act as stabilizers and thickeners in the yogurt (Pimentel et al., 2012).

IOE is incorporated into foods (e.g., jams, hard candies, ice cream) as a noncaloric sweetener or to improve the taste or texture of foods (e.g., ice cream, yogurt) (Pimentel et al., 2012; Ranawana, 2008; Sangeetha et al., 2005; Tymczyszyn et al., 2014). In general, oligosaccharides are incorporated in foods as dietary fiber to aid in digestion and to regularize bowel function (Ranawana, 2008; Roberfroid, 2007; Sangeetha et al., 2005). Human and animal studies have shown that inulin-type fructans such as IOE can increase in the bioavailability of calcium and magnesium and the absorption of calcium leading to increased bone density (Coxam, 2007; Roberfroid, 2007). Studies in humans and animals also show that consumption of inulin-type fructans such as IOE is associated with improved lipid homeostasis (balance) resulting in reduced triglyceridemia, a condition in which high levels of triglycerides (a type of fat) enter the bloodstream (Delzenne et al., 2002; Roberfroid, 2007). Animal studies have reported an association between IOE consumption and colon cancer prevention (Sangeetha et al., 2005). Some animal studies have observed decreased plasma cholesterol following low-level inulin exposure. Oligosaccharide consumption by experimental animals has resulted in improved defense from gut pathogens such as *Salmonella* for chickens, pigs, rats, and mice (Sangeetha et al., 2005).

Approved Legal Uses of the Substance:

The use of IOE in food products is regulated by U.S. FDA under the general category of "modified food starch" and the use of IOE in food products is under authority of 21 CFR 172.892. The U.S. FDA had no questions as to the GRAS status of inulin based on the use of inulin as reported in GRAS Notification 00118 (U.S. FDA, 2003). Fructooligosaccharides, a specific group of oligofructose sugars, are also considered GRAS as reported in GRAS Notification 000044 (U.S. FDA, 2000). Inulin (as CAS 9005-80-5) is exempt from the requirements of the EPA Chemical Data Reporting Rule and the Toxic Substances Control Act (TSCA) Chemical Data Reporting Requirements (40 CFR 711). As stated in the Summary of Petitioned Use, IOE is currently included on the National List as a nonorganically-produced agricultural product that can be used as an ingredient in or on processed products labeled as "organic," when the substance is not commercially available in organic form (7 CFR 205.606).

Action of the Substance:

As described above, IOE is incorporated into yogurt and other foods for three purposes: (1) to improve calcium bioavailability and absorption from the food; (2) to serve as a prebiotic energy source for bacteria in the large intestine; and (3) to enhance food texture and consistency (Kleesen et al., 1997; Morris and

Morris, 2012; Ranawana, 2008; Roberfroid, 2007; Santos and Maugeri, 2006). Calcium absorption in humans mainly occurs in the upper portion of the small intestine (Abrams et al., 2007). Calcium absorption may also occur in the colon (large intestine) after IOE exposure (Abrams et al., 2007). The most commonly proposed mechanism for increased calcium absorption is through production of lactic acid that occurs following fermentation of EFI in the ascending colon; the lactic acid lowers the pH of the colon causing an increase in the solubility of minerals such as calcium (Abrams et al., 2007; Ranawana, 2008). IOE is fermented in the large intestine by beneficial bacteria, such as *Bifidobacteria* and *Lactobacillus* species, and is completely used as a microbial food source (Morris and Morris 2012; Ranawana, 2008; Tymczyszyn et al., 2014).

The reason for the selective metabolism of IOE in humans is that they are mostly indigestible by human digestive enzymes due to their shape relative to the shape of the digestive enzymes, but are digestible by microbes in the large intestine (Roberfroid, 2007). The shape of the fructose portion of the oligosaccharide molecules—specifically the positioning of the alcohol group (abbreviated –OH) of the glycosidic bond on the fructose molecule—helps to dictate whether the sugar will be digested in the large or small intestine (Roberfroid, 2007). The alpha- and beta- fructose molecular conformations and the locations of the glycosidic and anomeric bonds are illustrated in Figure 3 below. Inulin-type fructans with the –OH in the beta (β) position of the glycosidic bond will resist digestion by enzymes in the small intestine (Roberfroid, 2007).

In yogurt, IOE is added to improve texture and storage stability. Specifically, when inulin and other fructan sugars such as IOE are added to the yogurt, they reinforce bonding between different components of the food (Pimentel et al., 2012). Probiotic bacteria, such as *Lactobacillus* species, ferment fructan sugars while in the yogurt and produce exopolysaccharides that can also act as stabilizers and thickeners (Pimentel et al., 2012). Inulin may cause syneresis (i.e., release of whey from the yogurt suspension) in the yogurt, so corrections in its formulation, such as the addition of different *Lactobacillus* species, may be needed (Pimentel et al., 2012).

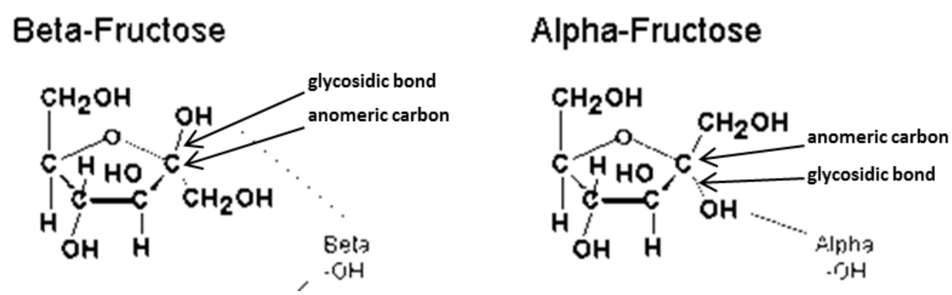


Figure 3. Alpha- and Beta-Fructose Conformations (Ophardt, 2003)

<u>Combinations of the Substance:</u>

Inulin is extracted from chicory to yield oligofructose (also called easily fermentable inulin [EFI]) and long-chain inulin (also called hardly fermentable inulin [HFI]) that are combined to form IOE. Oligofructose is also another name for a commonly-used prebiotic compound: fructooligosaccharides (FOS). “Fructooligosaccharides” are listed on the National List at 7 CFR 205.606 and are allowed for use in organic foods under the same restrictions applicable to IOE at 7 CFR 205.606.

IOE is added to foods as a prebiotic and is also used as a thickener and sweetening agent. No information was found on the addition of other substances to IOE. Based on the criteria for ancillary substances from NOSB, no ancillary substances are intentionally included in the IOE formulations. When IOE is produced from inulin, the end product may contain up to 20% glucose, fructose, and sucrose (Frippiat et al., 2010). The concentrations of these sugars can be reduced by altering the manufacturing process, which can reduce the simple sugar concentration to 2% by weight (Frippiat et al., 2010). Chromatography or other filtration methods can be used to remove residual simple sugars (Niness, 1999).

Status

Historic Use:

Research on prebiotics has been conducted since approximately 1954, soon after which lactulose was recognized as a "bifidus factor" (promoting the growth of a Bifidobacteria strain) in 1957 (Tymczyszyn et al., 2014). In the 1970s and 1980s, Japanese researchers discovered several oligosaccharides that were "bifidus factors," leading to increased interest in and additional study of similar compounds and their effect on intestinal microbiota (Tymczyszyn et al., 2014). The term "prebiotic" was used much later, around 1995 (Tymczyszyn et al., 2014). Prebiotics are defined as nondigestible food components that benefit the host (person eating them) by causing growth in populations of specific bacteria in the lower digestive tract to the ultimate benefit of the health of the host (Coussement, 1999; Roberfroid, 2007; Sangeetha et al., 2005; Sheu et al., 2013; Tymczyszyn et al., 2014).

IOE were considered GRAS by the U.S. FDA in 2003 and were added to the National List in 2007 (U.S. FDA, 2003). The functional foods market, which includes prebiotics such as IOE, has experienced 10–15 percent growth in the past 10 years and is expected to grow from $70 million (2008) to $200 million by 2015 (Tymczyszyn et al., 2014). Prebiotics are used in many different types of food such as yogurt, breads, and milk. They are also used as noncaloric sweeteners in products such as jams, candies, and ice cream (Sangeetha et al., 2005; Tymczyszyn et al., 2014).

Organic Foods Production Act, USDA Final Rule:

IOE is allowed for use as nonorganically-produced agricultural products used as ingredients in or on processed products labeled as "organic" as listed at 7 CFR 205.606. As specified in the regulations, nonorganic IOE may only be used when the product is not commercially available in organic form.

International:

International Federation of Organic Movements (IFOAM)

IOE is not listed in the International Federation of Organic Movements (IFOAM) Norms for Organic Production and Processing (IFOAM, 2012). The IFOAM Norms state that organically-processed products must be made from organic ingredients, and preparations of microorganisms and enzymes for use in food processing must gain approval from the control body or certifier before use. Genetically-engineered microorganisms and their products are not allowed according to IFOAM Norms (IFOAM, 2012).

Canadian General Standards Board (CGSB)

The Canadian General Standards Board (CGSB) does not list inulin on its Permitted Substances List (CGSB, 2011). CGSB does discuss enzymes in organic processing and permits "any preparations of enzymes normally used in food processing derived from edible, non-toxic plants, non-pathogenic fungi or non-pathogenic bacteria" (CGSB, 2011).

CODEX Alimentarius Commission

IOE is not listed in the CODEX Alimentarius Commission Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (CODEX, 2013). The CODEX guidelines state that "any preparation of micro-organisms and enzymes normally used in food processing, with the exception of micro-organisms genetically engineered/modified or enzymes derived from genetic engineering" is allowed (CODEX, 2013).

Japan Ministry of Health, Labour and Welfare (MHLW)

IOE is not specifically listed in the Japan Ministry of Health, Labour and Welfare (MHLW) Food for Specified Health Uses (FOSHU) although there is a listing for "oligosaccharides." Oligosaccharides are listed on the Approved FOSHU products list and classified as "foods to modify gastrointestinal conditions." The FOSHU is a list of foods and ingredients that have a health function and are officially approved to claim certain physiological effects on the body (Japan MHLW, undated). In order to be listed as FOSHU, a food must be assessed for safety by the Food Safety Commission and reviewed for its effectiveness in attaining given health functions by the Council on Pharmaceutical Affairs and Food Sanitation (Japan MHLW, undated).

IOE is not specifically listed in:

- European Economic Community (EEC) Council Regulation, EC No. 834/2008 and 889/2008
- Japan Agricultural Standard for Organic Production

Evaluation Questions for Substances to be used in Organic Handling

Note: This is a limited-scope Technical Evaluation Report that includes Evaluation Questions #1 and #2 only, as requested by the NOSB.

<u>Evaluation Question #1:</u> Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

IOE is produced through physical and chemical processes using the roots of the chicory plant (*Cichorium intybus*), which are shredded and treated with hot water and then juiced to extract inulin (Coussement, 1999; De Leenheer, 1996; Frippiat et al., 2010; Gibson et al., 1994; Roberfroid, 2007; Singh and Singh, 2010; U.S. FDA, 2003). The shredding makes the inulin more available from the roots and the heat makes the long inulin molecules more soluble in water. The inulin extract is purified using lime (calcium hydroxide) and carbonation, leaving behind various precipitates, proteins, peptides, and colloids that may be used as a soil amendment by farmers (De Leenheer, 1996). The inulin extract is filtered first using ion exchange using inputs of ammonia and sulfuric acid; the liquid is then filtered again using activated carbon (De Leenheer, 1996). Byproducts of this process are ammonium sulfate and potassium sulfate, which are precipitated out of solution and sold as conventional (i.e., not for use in organic agriculture) fertilizer (De Leenheer, 1996).

The extracted inulin polymers range in chain length from 2–60 units. The shortest polymers range from 2–10 fructose units and are called EFI or oligofructose. The longer polymers range from 10–60 units and are called HFI. If insufficient amounts of EFI are present, the HFI is treated with inulinase enzyme from *Aspergillus niger* although other fungal species can also be used (Coussement, 1999; Santos and Maugeri, 2006; Singh and Singh, 2010). Inulinase breaks down inulin into shorter polymer chains through the process of enzymatic hydrolysis (i.e., breakdown by the combined action of the water and enzyme) (Roberfroid, 2007). The hydrolysis is a chemical change, and although the change occurs using an enzyme from a fungus (the inulinase in *Aspergillus niger*), this is not a process that occurs in nature. Both EFI and HFI are spray-dried for improved storage and stability and are then combined before packaging (De Leenheer, 1996).

<u>Evaluation Question #2:</u> Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)). Discuss whether the petitioned substance is derived from an agricultural source.

Inulin is a naturally-occurring carbohydrate found in chicory and other plant sources, which are agricultural products. In its final, purified form, the extracted inulin is very similar to the inulin within the plant tissue. However, the plant material must be shredded, soaked in hot water, and pressed so that the inulin polysaccharides can be released from the plant material. A similar process is used in the manufacturing of sugar from sugar cane or sugar beets. The physical processes of heat, pressure, and filtration are required to release the inulin from the plant material.

Inulin must be further refined to produce short-chain oligosaccharides (i.e., EFI) from the long-chain oligosaccharides (i.e., HFI). EFI is produced through enzymatic hydrolysis using the inulinase enzyme, a product of fermentation from the fungus *Aspergillus niger*. Enzymatic hydrolysis is required to break the longer inulin fructose chains into shorter chains of 2–10 units. Hydrolysis by inulinase is a naturally-occurring process in *Aspergillus niger*, but the specific hydrolysis of inulin by this pathway does not occur in nature.

The goal of IOE production is to extract EFI and HFI from the plant source without any additional materials remaining in the solution. Depending on the production method, additional monomeric or dimeric sugars (fructose, glucose, sucrose) may remain in the IOE solution at a concentration of up to 20% by weight, but typically are less than about 2% (Frippiat et al., 2010). These sugars are not added to the IOE, but are existing simple sugars in the plant material or are byproducts of inulin breakdown. The concentrations of simple sugars may be reduced through additional hydrolysis and filtration of the sugar solution (Frippiat et al., 2010; Niness, 1999).

According to the baseline criteria included in an NOSB recommendation to the NOP (NOSB, 2013), ancillary substances are *intentionally added* to petitioned substances. Based on these criteria, no ancillary substances are intentionally included in the IOE formulations.

Additional components are not expected to remain in the industrially-prepared IOE after filtration (Frippiat et al., 2010). Any processing aids are removed in favor of a pure IOE product. The amounts of these remaining substances may vary, but the general approach in producing IOE is to purify the IOE solution and thereby limit the amount of processing aids that remain.

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