Fructooligosaccharides

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
fructooligosaccharides
oligofructose
oligofructan
fructan polysaccharides

Other Names:
Short-chain fructooligosaccharides (scFOS)
FOS

Trade Names:
Neosugar, NutraFlora®, Meioligo®, Actilight®

CAS Number:
308066-66-2

Other Codes:
None

Summary of Petitioned Use

Fructooligosaccharides (FOS) are currently included on the National List of Allowed and Prohibited Substances (hereafter referred to as the National List) as nonorganically-produced ingredients in or on processed products labeled as "organic" when the products are not commercially available in organic form (7 CFR 205.606). In organic processing/handling, FOS are used as soluble prebiotic fiber ingredients in food products. FOS are considered prebiotic food ingredients because they are included in food products as sources of energy for probiotic bacteria residing in the gut of humans, but are not used as nutrient sources directly for humans (Sangeetha et al., 2005). As prebiotic food ingredients, FOS are intended to benefit human health by increasing growth and activity of probiotic bacteria. FOS are incorporated into milk products, cakes, biscuits, cookies, crackers, yogurt, ice creams, soup, and hard candy, among other foods (Roberfroid, 2007; Sangeetha et al., 2005; U.S. FDA, 2000).

Characterization of Petitioned Substance

Composition of the Substance:
FOS are short-chain sugars composed of a single glucose molecule (a six-carbon sugar) bonded to two, three, or four additional fructose (five-carbon sugar) molecules (U.S. FDA, 2000). FOS and other fructan sugars are considered indigestible sugars, but can serve as prebiotics or nutrition for microflora in the digestive system (Ophardt, 2003; Roberfroid, 2007). FOS 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 FDA Generally Recognized as Safe (GRAS) notice for FOS, GRN 000044, states that FOS may not be completely indigestible; approximately 89 percent of the ingested FOS passes to the digestive tract, while the balance of the mass is hydrolyzed (broken down) by stomach acid and absorbed into the body as fructose and glucose (U.S. FDA, 2000).

The complex fructan sugars that make up FOS 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). Kestose, nystose, and fructosyl nystose are also referred to as GF2, GF3, and GF4, respectively. These complex sugars are referred to as FOS when they are present together as a mixture (Silva et al., 2013). Molecular structures of kestose, nystose, and fructosyl nystose (also called fructofuranosyl nystose) are pictured in Figure 1.

FOS were originally derived from inulin, a type of dietary fiber that is found in many foods and is most often extracted from chicory (*Cichorium intybus*) (Coussement, 1999; Roberfroid, 2007). FOS are typically produced synthetically through fermentation of sucrose by a group of enzymes called fructofuranosidases, which are isolated from the fungal species *Aspergillus japonicus* as well as other *Aureobasidium* and *Penicillium* species (Mussatto et al., 2009; Sangeetha et al., 2005; Tymczyszyn et al., 2014). The FDA GRAS notification for FOS by GTC Nutrition Co. (U.S. FDA, 2000) describes using the enzyme β-fructofuranosidase from *Aspergillus japonicus* for FOS production. The β-fructofuranosidase enzyme breaks the sucrose molecules into glucose and fructose and then transfers 1–3 fructose molecules to a glucose-fructose chain to create one of the FOS complex sugars kestose, nystose, or fructosyl nystose (U.S. FDA, 2000).

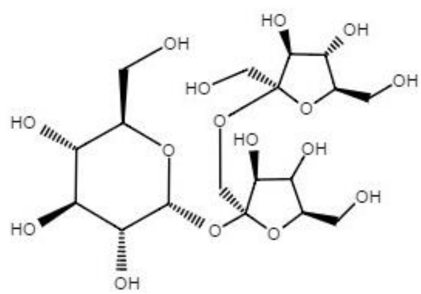	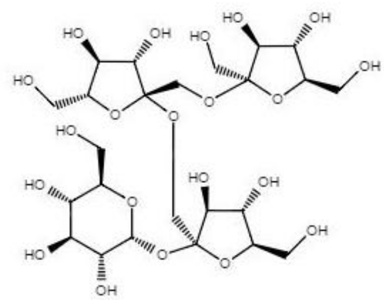	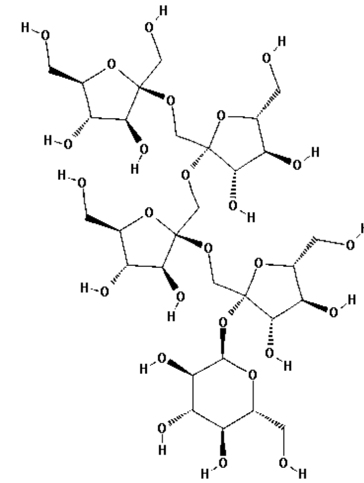
Kestose	**Nystose**	**Fructosyl Nystose (fructofuranosyl nystose)**
GF2: glucose – fructose – fructose	GF3: glucose – fructose – fructose – fructose	GF4: glucose – fructose – fructose – fructose fructose

Figure 1: Chemical Structures of FOS Components (NLM, 2012)

Source or Origin of the Substance:

Inulin is a natural carbohydrate present in a number of vegetables and fruits that can be processed to release FOS (Tymczyszyn et al., 2014). Inulin was first discovered by isolation from the roots of the elecampane plant (*Inula helenium*) (Coussement, 1999). Inulin is found in food plants such as bananas, asparagus, Jerusalem artichoke, garlic, onion, chicory, wheat, and rye (Coussement, 1999; Kowalchik and Hylton, 1998; Morris and Morris, 2012). FOS are not naturally available from unprocessed foods, but must be released from inulin through partial hydrolysis or chemical breakdown by reaction with water (Coussement, 1999; Tymczyszyn et al., 2014). Chicory is the most commonly used vegetable source for the industrial production of inulin (Roberfroid, 2007). FOS can be produced from inulin by the inulinase enzyme, which breaks down inulin via enzymatic hydrolysis—a process by which enzymes facilitate breakdown using elements of water (Roberfroid, 2007). 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, 2007).

Industrially-produced FOS can also be synthesized from sucrose, a sugar that is a combination of glucose and fructose (Sangeetha et al., 2005; Tymczyszyn et al., 2014). In this method, FOS are derived from sucrose by enzymatic synthesis using the enzyme β-fructofuranosidase, a type of fructosyl transferase (FTase) enzyme (Sangeetha et al., 2005; Tymczyszyn et al., 2014) that can be extracted from *Aspergillus japonicus* (Mussatto et al., 2009; Sangeetha et al., 2005; U.S. FDA, 2000). Specifically, the β-fructofuranosidase enzyme removes the fructose molecules from sucrose and then transfers up to three fructose molecules to another sucrose molecule to create one of the FOS complex sugars: kestose, nystose, or fructosyl nystose (U.S. FDA, 2000; Tymczyszyn et al., 2014).

Properties of the Substance:

FOS are odorless, white to cream colored solids with a neutral to slightly sweet taste. Molecular weights of FOS components range from 504.43–828.72 g/mol as shown in Table 1 (NLM, 2012; Olesen and Gudmand-Hoyer, 2000; Spectrum Chemical, 2009). The solubility of FOS in water is 100 g/L at 25 °C (Spectrum Chemical, 2009). FOS are mixtures of the complex sugars kestose, nystose, and fructosyl nystose (Silva et al., 2013). The sugars all have the same molecular base—sucrose—with the addition of 1–3 fructose molecules attached to the sucrose.

Table 1: Molecular Weights and Molecular Formulas of FOS Molecules (NLM, 2012)

FOS Molecule	CAS Number	Molecular Weight (g/mol)	Molecular Formula
Kestose	470-69-9	504.43	$C_{18}H_{32}O_{16}$
Nystose	13133-07-8	666.58	$C_{24}H_{42}O_{21}$
Fructosyl nystose	59432-60-9	828.72	$C_{30}H_{52}O_{26}$

Specific Uses of the Substance:

FOS are added to foods as nondigestible carbohydrates and selective energy sources for species of probiotic bacteria in the gut. The majority of FOS are moved to the lower digestive tract undigested, but a small proportion (about 11 percent) of the FOS may be hydrolyzed by stomach acid and then absorbed as glucose and fructose (U.S. FDA, 2000). The ultimate goal of using FOS in food products is to increase the growth and activity of probiotic bacteria in the lower digestive tract for the benefit of human health (Roberfroid, 2007; Sangeetha et al., 2005; Sheu et al., 2013; Tymczyszyn et al., 2014).

FOS are also incorporated into foods (e.g., jams, hard candies, ice cream) as noncaloric sweeteners or to improve the taste or texture of foods (e.g., ice cream, yogurt) (Sangeetha et al., 2005; Tymczyszyn et al., 2014). In general, oligosaccharides are incorporated in foods as sources of dietary fiber to aid in digestion and to regularize bowel function (Roberfroid, 2007; Sangeetha et al., 2005). Human and animal studies have shown that inulin-type fructans, including FOS, can increase the bioavailability of calcium and magnesium and absorption of calcium leading to increased bone density (Coxam, 2007; Roberfroid, 2007). Human and animal studies have also shown that consumption of inulin-type fructans such as FOS 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). Other human and animal studies have observed beneficial effects of FOS for diabetes control and improved lipid metabolism (Sangeetha et al., 2005). Some animal studies have reported an association between FOS consumption and colon cancer prevention. FOS consumption by experimental animals has resulted in improved defense from gut pathogens for chickens, pigs, rats, and mice (Sangeetha et al., 2005).

Approved Legal Uses of the Substance:

FOS are currently included on the National List as nonorganically-produced agricultural products allowed as ingredients in or on processed products labeled as "organic" (7 CFR 205.606). The listing further states that items listed at 205.606 may be used as ingredients in or processed products labeled as "organic" only when products are not commercially available in organic form.

In 2000, GTC Nutrition submitted a notification to the U.S. FDA for FOS to be considered GRAS (U.S. FDA, 2000). The notice was reviewed by the FDA and the Agency concluded that they had "no questions at this time regarding GTC Nutrition's conclusion that fructooligosaccharide is GRAS under the intended conditions of use." According to the FDA's response to the notice, the Agency has not made its own determination of the GRAS status of FOS, but has relied on the conclusions of GTC put forth in the GRAS notification (U.S. FDA, 2000). Inulin-oligofructose enriched is considered GRAS as reported in U.S. FDA notification 00118 (U.S. FDA, 2003).

Action of the Substance:

FOS are considered "prebiotics," meaning that they are incorporated into food products to serve as energy sources for bacteria in the large intestine (Kleesen et al., 1997; Morris and Morris, 2012; Roberfroid, 2007; Santos and Maugeri, 2007). FOS are mostly fermented in the large intestine by beneficial bacteria and are completely used as a microbial food source aside from the portion that is digested in the stomach (about 11 percent) (Morris and Morris 2012; Tymczyszyn et al., 2014). FOS are not digestible 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 FOS molecules—specifically the positioning of the alcohol group (–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 2 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).

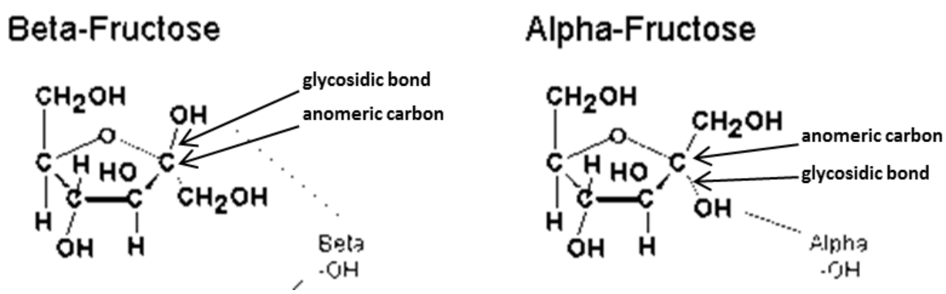

Figure 2: Alpha- and Beta-Fructose Conformations (Ophardt, 2003)

Combinations of the Substance:

FOS can be extracted from inulin, a carbohydrate found in numerous foods. Inulin is also a component of another commonly used prebiotic compound: oligofructose-enriched inulin. "Inulin-oligofructose enriched" is included on the National List as a nonorganically-produced agricultural product allowed as an ingredient in or on processed products labeled as "organic" (7 CFR 205.606). Oligofructose is another name for FOS, so "inulin-oligofructose enriched" is a combination of inulin and FOS.

FOS are added to foods for prebiotic nutritive purposes and are also used as thickening and sweetening agents. No information was found on the addition of other substances to FOS. When FOS are produced by enzymatic synthesis from sucrose, the end product may contain 45% or more of glucose, fructose, and sucrose that did not react with the enzymes (Sangeetha et al., 2005; Tymczyszyn et al., 2014). The FOS solution must then be purified to remove the additional sugars, which can be accomplished by using filtration with zeolite (a porous mineral commonly used as a filter medium) and activated carbon (Tymczyszyn et al., 2014). High-content FOS (greater than 98%) can be produced using mixed enzyme systems to produce a higher concentration of FOS and to remove the residual glucose (Sangeetha et al., 2005; Tymczyszyn et al., 2014). These mixed systems include glucose oxidase and catalase enzymes derived from fungi and yeast as well as calcium carbonate to maintain a pH of 5.5 during the enzymatic processes (Sangeetha et al., 2005). An additional byproduct of the production method is calcium gluconate, which is precipitated out of the solution from the reaction of calcium carbonate with gluconic acid (Sheu et al., 2001, as cited in Sangeetha et al., 2005). The gluconic acid is generated from the enzymatic reaction of glucose and glucose oxidase (Sheu et al., 2001, as cited in Sangeetha et al., 2005).

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 *Bifidobacterium* 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 these 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). FOS were considered GRAS by the U.S. FDA in 2000 and were added to the National List in 2007. The functional foods market, which includes prebiotics such as FOS, 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). FOS are incorporated as prebiotics into many different types of food such as yogurt, milk, and breads. 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:

FOS are allowed for use as nonorganically-produced ingredients in or on processed products labeled as "organic" when FOS are not commercially available in organic form (7 CFR 205.606). A similar substance, inulin-oligofructose enriched, is also allowed for use as a nonorganically-produced ingredient in or on processed products labeled as "organic" (7 CFR 205.606). FOS and inulin-oligofructose enriched are not described in the OFPA or the USDA Final Rule.

International:

Health Canada

FOS are not officially recognized as dietary fiber sources by Health Canada due to the fact that the fiber policy has not been updated since 1997 (Health Canada, 2012). Health Canada notes that dietary fiber types that are not officially recognized, including FOS, may be included as safe food ingredients and used on the market in food products. To accomplish this, manufacturers of those food ingredients must submit a petition to Health Canada for approval, supported by clinical data and expert opinions (Health Canada, 2012).

International Federation of Organic Agriculture Movements (IFOAM)

FOS are not listed specifically in the 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).

Japan Ministry of Health, Labour, and Welfare (MHLW)

FOS are listed in the Japan Ministry of Health, Labour and Welfare (MHLW) Food for Specified Health Uses (FOSHU) as "oligosaccharides." FOS are listed in 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. 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).

FOS are not specifically listed in:

- Canadian General Standards Board Permitted Substances List;
- CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling, and Marketing of Organically Produced foods (GL 32-1999)
- European Economic Community (EEC) Council Regulation, EC No. 834/2007 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.

Evaluation Question #1: 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)).

FOS are produced industrially from one of two carbohydrate sources: inulin or sucrose. Inulin is extracted from the roots of the chicory plant (*Cichorium intybus*) by shredding the roots, treating them with hot water, and then juicing them (Coussement, 1999; De Leenheer, 1996; Frippiat et al., 2010; Gibson et al., 1994; Roberfroid, 2007; Singh and Singh, 2010; U.S. FDA, 2003). FOS are obtained from the resulting inulin by hydrolysis using the enzyme inulinase, which is extracted from an enzyme complex (carbohydrase) found in the fungus *Aspergillus niger* (Coussement, 1999; Morris and Morris, 2012; Sangeetha et al., 2005; Tymczyszyn et al., 2014). This method of FOS production is not as common as the production of FOS from sucrose, which described below.

As discussed in the Source or Origin of the Substance section, FOS are most commonly produced from sucrose using enzymes from *Aspergillus japonicus*. Production of FOS from sucrose occurs through fermentation by *A. japonicus* by action of the β-fructofuranosidase enzyme (Sangeetha et al., 2005; Sheu et al., 2013; Tymczyszyn et al., 2014; U.S. FDA, 2000). The *A. japonicus* cells must be immobilized for production of high-purity FOS, which can be accomplished by creating beads of the *A. japonicus* culture suspended in calcium alginate, an immobilizer (Sheu et al., 2013). During fermentation, the β-fructofuranosidase enzyme within the *A. japonicus* cells hydrolyzes (breaks) the sucrose molecules into glucose and fructose and then transfers fructose molecules to an existing glucose-fructose chain to create one of the FOS complex sugars: kestose, nystose, or fructosyl nystose (U.S. FDA, 2000).

In addition to the feedstocks described above, other chemical and physical inputs are used to produce FOS. Heat is used to speed up enzymatic reactions. The pH of enzyme reactions is controlled to enable the enzymes to produce the most efficient conversion of sucrose to FOS (Sangeetha et al., 2002; Sangeetha et al., 2005). Adjustment of pH is accomplished using hydrochloric acid (a strong acid) or sodium hydroxide (a strong base); potassium phosphate is also used for pH control (Sangeetha et al., 2005; Sheu et al., 2013).

Production of FOS from sucrose by the enzymatic processes described above is somewhat inefficient. According to one report, this reaction produces an FOS yield of approximately 55% with residual glucose, fructose, and sucrose comprising the remainder of the solution (Tymczyszyn et al., 2014). One method of optimizing FOS production from sucrose is through filtration of the sugar solution using packed-bed columns of zeolite and activated carbon, which removes the monosaccharides (single molecule sugars) glucose and fructose (Tymczyszyn et al., 2014).

Another method of increasing the purity of FOS production is to use a series of tanks as reactors through which the sugar solution is circulated (Sheu et al., 2013). In this tanks-in-series system, immobilized *A. japonicus* and an additional yeast species, *Pichia heimii*, are used to ferment unreacted glucose in the solution (Sheu et al., 2013). The *P. heimii* yeast metabolizes the glucose into ethanol and the remaining sucrose is completely converted to FOS, yielding a purity of 98.2% FOS with ethanol as the main byproduct (Sheu et al., 2013). An alternative approach is to use additional enzymes (e.g., glucose oxidase and catalase) to further remove glucose from the FOS solution (Sangeetha et al., 2005; Tymczyszyn et al., 2014).

<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.

FOS are produced using a process that uses enzymes, heat, filters, and pH stabilizers to transform sucrose to create glucose-fructose chains of lengths between 2 and 4 fructose units. Sucrose may be obtained from a natural agricultural product (e.g., sugar cane or sugar beets), but the production methods reviewed do not mention the source of sucrose (Sangeetha et al., 2002; Sangeetha et al., 2005; Sheu et al., 2013). The enzymatic reactions that convert sucrose to FOS do not occur in nature, but the fermentation process is a natural process performed by fungi and yeasts.

Fermentation of sucrose by *A. japonicus* is generally inefficient, yielding approximately 55% FOS (Tymczyszyn et al., 2014). As described previously, higher purity FOS solutions can be achieved by several methods: filtration, enzyme extraction, or mixed culture fermentation with the yeast *P. heimii* to increase the purity of the FOS solution. Each of these methods introduces additional chemical or physical agents to the production process.

In the case of filtration, zeolite and/or activated carbon are used as physical separation methods to remove glucose and fructose from the FOS solution (Tymczyszyn et al., 2014). The filtration is possible because glucose and fructose are smaller molecules than the FOS molecules. Filtration does not chemically alter the FOS and does not involve additional inputs to the solution, but rather refines the existing FOS solution.

The enzyme extraction method uses the enzymes glucose oxidase and catalase from *A. niger* to remove remaining glucose from the FOS solution (Sangeetha et al., 2005). The glucose oxidase enzyme converts glucose to gluconic acid, which must then be precipitated out (a chemical change that causes the compound to become insoluble), made possible by the addition of calcium carbonate to create calcium gluconate (Sangeetha et al., 2005). The resulting solution contains more than 90% FOS by weight, with glucose, sucrose, and a "small amount" of calcium gluconate remaining (Sangeetha et al., 2005).

Mixed culture fermentation uses immobilized *A. japonicus* and *P. heimii* in a circulating reactor to ferment unreacted glucose in the solution. The *P. heimii* yeast metabolizes the glucose into ethanol and the remaining sucrose is completely converted to FOS. Immobilization requires the addition of calcium alginate, and yeast extract is used as a nutrient source for the immobilized cells (Sheu et al., 2013). The process produces an FOS solution of more than 98% purity, with an additional byproduct, ethanol (ethyl alcohol) (Sheu et al., 2013). The ethyl alcohol may be removed from the solution by distillation (Sheu et al., 2013).

According to the "baseline criteria" included in an NOSB recommendation to the NOP (NOSB, 2013), ancillary substances are *intentionally added* to petitioned substances. There are no ancillary substances intentionally included in the FOS formulations as described in the petition, and no ancillary substances are intentionally added to the FOS products in the selected high-purity FOS fermentation.

Additional components may remain in the FOS solution after the purification steps described above. Depending on the production method and method of refinement, glucose, sucrose, calcium gluconate, glucose oxidase enzyme, catalase enzyme, or ethyl alcohol may be present in the FOS solution in small amounts. All of these additional components fit the definition of processing aids (defined at 7 CFR 205.2), in that they are added to the food for processing and do not have a technical or functional effect in the food. The amounts of these remaining substances may vary, but the general approach in producing FOS is to purify the FOS solution and thereby limit the amount of processing aids that remain.

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