Sensus America Inc.
Princeton Corporate Plaza
1 Deer Park Drive, Suite J
Monmouth Junction, NJ 08852

Sept/12/2007

Mr. Mark Bradley
Program Manager
National Organic Program
USDA/AMS/TMP/NOP
1400 Independence Ave., SW
Room 4008-So.
Ag Stop0268
Washington, DC 20250

Dear Mr. Bradley,

Enclosed is a petition regarding the inclusion of the non-organically produced agricultural substance "Chicory Root Extract" onto the National List section 205.606. I am the contact of Sensus America Inc. and can be reached as follows:

Connie (Ying-Pi) Lin, Ph.D.
Applications Manager
Sensus America Inc.
Princeton Corporate Plaza
1 Deer Park Drive, Suite J
Monmouth Junction, NJ 08852
Tel: (646)452-6146
E-mail: connie.lin@sensus.us

If you have any questions, please don't hesitate to contact me.

We appreciate your consideration of our request.

Sincerely,

Connie (Ying-Pi) Lin

Connie (Ying-Pi) Lin, Ph.D.
Applications Manager
Sensus America Inc.

Copy #1

From:

SENSUS

Connie (Ying-Pi) Lin, PhD
Applications Manager

Sensus America LLC

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TO:

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Program Manager
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Applications Manager
Sensus America Inc.

Petition to be included on the National List
"Chicory Root Extract Documented for Digestive Health"

Item A

1. Category

Non-organic agricultural substances allowed in or on processed products labeled as "organic" §205.606.

2. Justification for this category

The petitioned substance is chicory root extract including inulin and oligofructose documented for digestive health. The inulin is extracted from the roots of chicory plant (*Cichorium intybus*) with hot water. Partial enzymatic hydrolysis of the chicory inulin yields the oligofructose. The process is analogous to extracting sugar from the roots of sugar beets and creating maltodextrin from corn starch.

Item B

1. The common name of the substance.

Chicory root extract documented for digestive health has trade names as "Frutafit®" and "Frutalose®" inulin/oligofructose from Sensus, which can be declared as 'Chicory Root Extract', 'Chicory Root Fiber', 'Inulin', and 'Oligofructose'. This substance consists of fructose (F) units linked by β(2-1) bonds with mostly one terminal glucose molecule (G). Inulin derived from chicory root is composed of a mixture of oligomers and polymers (GFn; $2 \leq n \leq 60$). Oligofructose yielded from chicory inulin is composed of both GFn-type and Fn-type oligosaccharides, usually with less than 10 fructose units.

2. The manufacturer.

Sensus, headquarters and production facilities are located in the Netherlands. The address of the U.S. division of Sensus is as follows: Sensus America Inc. Princeton Corporate Plaza, 1 Deer Park Drive, Suite J. Monmouth Junction, NJ 08852.

3. The intended or current use of the substance.

Chicory root extract documented for digestive health (Frutafit® and Frutalose® inulin/oligofructose from Sensus) is an agricultural substance which has GRAS food ingredient status (see GRAS Notice No. 000118 in Appendix 1). The substance is also approved for use in non-standardized meat applications by the

USDA, and use in animal feed and pet food products by the FDA/CVM [see Notice of Association of American Feed Control Officials (AAFCO) in Appendix 2]. It is used to provide soluble prebiotic fiber, texture and consistency to the food and feed products (e.g., nutritional bars, dairy products, baked goods, beverages, baby foods, pet foods, etc.). The substance is also Kosher and Halal certified to satisfy the market requirements.

4. The handling activities for which the substance will be used and its mode of action.

Chicory root extract documented for digestive health (Frutafit® and Frutalose® inulin/oligofructose from Sensus) is a soluble prebiotic fiber inclusion. It is available in both powder and liquid forms. As a powder, it can be blended to other dry ingredients, or directly added into agitated aqueous phase (e.g., water). As a liquid, it can be directly incorporated into product formulations.

5. The source of the substance and a detailed description of its manufacturing or processing procedures.

The substance is produced from the root of natural chicory plant *(Cichorium intybus)*. A general process for isolation of inulin and production of partially enzymatically hydrolyzed inulin materials (i.e., oligofructose) is attached (see Production Process of Frutafit® and Frutalose® inulin/oligofructose from Sensus in Appendix 3). The washed roots are sliced and fed into a scalding apparatus that renders the plant cell walls semi-permeable. After scalding, the chicory root slices are fed into a diffusion tower, where inulin is extracted with hot water in a continuous countercurrent process. The juice emerging from this process is further purified and concentrated. Spray drying of the concentrated juice results in a powdered inulin product. The juice can be further enzymatically converted into oligofructose, which is highly soluble, and can be commercially available in both powder and liquid forms.

6. A summary of any available previous reviews of the petitioned substance by state or private certification programs or other organizations.

Chicory root extract documented for digestive health (Frutafit® and Frutalose® inulin/oligofructose from Sensus) has been reviewed and certified by the following organizations:

~Approval as GRAS Food Ingredient in the U.S. (issued by the FDA, see GRAS Notice No. GRN 000118 in Appendix 1)
~Approval of use in animal feed and pet food products in the U.S. [issued by the Association of American Feed Control Officials (AAFCO), see Notice T60.106 in Appendix 2]

~Approval as Traditional Fiber in Canada (issued by the Canadian Food Inspection Agency, see section 6.8.1: Dietary Fiber in Appendix 4)
~Approval for "healthy colon" claim in the Netherlands (issued by the Nutrition Center of the Netherlands, see Dutch Health Claim in Appendix 5)
~Approval for bifidigenic claims in France (issued by the French Food Safety Agency, AFSSA, Agence Francaise de Sécuritë Sanitaire des Aliments, see Advice of 20 April 2005 in Appendix 6)
~Kosher certificate (issued by Circle K-Rabbi Don Yoel Levy, Kashrath Administrator in Appendix 7)
~Halal certificate (issued by Halal Correct Certification in Appendix 8)

7. Information regarding EPA, FDA, and State regulatory authority registrations.

Status in the U.S.: Chicory root extract documented for digestive health (Frutafit® and Frutalose® inulin/oligofructose from Sensus) was granted as GRAS food ingredient by the FDA in 2002 (see GRAS Notice No. GRN 000118 in Appendix 1). Therefore, it is a food ingredient, not an additive.

Status in Canada: Frutafit® inulin from Sensus was permitted as "Fiber Source" in both regular foods and meal replacement by the Health Canada in 2006. It was classified as "Traditional Fiber Source" (see Canadian Food Inspection Agency section 6.8.1: Dietary Fiber in Appendix 4).

Status in the European Union: Although chicory inulin and oligofructose do not appear on Annex VI. of EEC2092/1991 as an approved non-organic ingredient, a request has been filed with Belgian authorities in 1997. In this request, the European Committee of Inulin Producers (CEFI, Comité Européen des Fabricants d'Inuline;) confirmed that non of its members can produce organic inulin or oligofructose from chicory (see Sensus's letter in Appendix 9).

8. The Chemical Abstract Service (CAS) numbers of the substance and labels of products that contains the petitioned substance.

Component	*CAS Registration Number*
Chicory root extract (Inulin/Oligofructose)	9005-80-5
Sucrose	57-50-1
Glucose	50-99-7
Fructose	57-48-7

A sample label from an organic frozen dessert containing chicory root extract as an ingredient is provided in Appendix 10.

9. The substance's physical properties and chemical mode of action including:

(a) chemical interactions with other substances, especially substances used in organic production;

Chicory root extract documented for digestive health (Frutafit® and Frutalose® inulin/oligofructose from Sensus) is used in organic handling, not organic production. It is a natural mixture of β 2,1-linked fructose chains, bound to a terminal glucose. As a non-digestible carbohydrate, it is used as a prebiotic soluble fiber inclusion in food and feed processing and a selective source of energy by beneficial bacteria (i.e., probiotics) in the guts of humans and animals. This action favorably affects the growth and activity of probiotics for the benefit of digestive health.

(b) toxicity and environmental persistence;

Chicory root extract documented for digestive health (Frutafit® and Frutalose® inulin/oligofructose from Sensus) is GRAS food ingredient when used in human food in accordance with good manufacturing practice. The substance is fermented by beneficial colonic bacteria. It can also be metabolized by many plant species as a natural reserve carbohydrate, and utilized by other organisms in nature, such as molds. Therefore, it does not persist in the environment.

(c) environmental impacts from its use or manufacture;

The production process of this substance involves water extraction of raw chicory roots to extract the inulin, which can represent up to 70% of the dry matter in the roots. The extracted root pulp waste and other process byproducts are used in animal feed or for other agricultural purposes.

(d) effects on human health;

The positive effects of chicory root extract documented for digestive health (Frutafit® and Frutalose® inulin/oligofructose from Sensus) have been the subject of many review articles over the past fifteen years. Especially, its prebiotic/bifidogenic properties to promote digestive health are well backed by scientific evidence. Chicory root extract (inulin/ oligofructose) stimulates the Bifidobacteria and Lactobacilli in the large intestine, whereas pathogenic strains are significantly reduced. Together this results in a healthier balance of the colonic flora. A healthy colon supports the natural defense systems of humans and therefore plays a major role in well-being. A sampling of publications regarding

prebiotic/bifidogenic effects of chicory root extract (Inulin/Oligofructose) for digestive health can be found in Appendix 11.

(e) effects on soil organisms, crops, or livestock.

Chicory root extract documented for digestive health (Frutafit® and Frutalose® inulin/oligofructose from Sensus) is used in handling, not production. Many microorganisms can utilize the carbohydrate of chicory root extract. Monogastric animals cannot digest chicory root extract, which accounts for its favorable effect on beneficial colonic microorganisms (i.e., the "prebiotic/bifidogenic" effect for digestive health).

10. Safety information about the substance.

As aforementioned, chicory root extract documented for digestive health (Frutafit® and Frutalose® inulin/oligofructose from Sensus) is a safe substance, which was granted GRAS food ingredient status by the FDA in 2002 (see GRAS Notice No. GRN 000118 in Appendix 1). No other commercial sources of inulin or oligofructose have been certified as GRAS food ingredient. MSDS of the Frutafit® and Frutalose® inulin/oligofructose from Sensus can be found in Appendix 12.

11. Comprehensive research reviews and research bibliographies, including reviews and bibliographies which present contrast positions.

Organic inulin and oligofructose are commercially available from Jerusalem artichoke and agave. However, none of these organic sources of inulin or oligofructose have been extensively investigated on their prebiotic/bifidogenic properties. Only inulin and oligofructose derived from chicory roots have strong scientific evidence to back up the digestive health claims based on their prebiotic/ bifidogenic properties. This is clearly illustrated by an advice from the French Food Safety Agency (AFSSA, Agence Francaise de Sécuritë Sanitaire des Aliments) where "chicory inulin is prebiotic at a dosage of 5 g/day" is mentioned (see Advice of 20 April 2005 issued by the AFSSA in Appendix 6).

In the Netherlands, a panel of independent experts concluded that chicory root extract (inulin and oligofructose) is prebiotic/bifidogenic which contributes to digestive health. Frutafit® inulin was approved for a "healthy colon" claim issued by the Nutrition Center of the Netherlands in 2002 (see Dutch 'Healthy Colon' claim in Appendix 5). Breads (brand name: Vitaalbrood flora) with this health claim were first sold through Ahold-owned Albert Heijn supermarkets across the Netherlands. Research suggests that 5 g of Frutafit® inulin a day stimulates growth of helpful gut bacteria, which leads to a healthy digestive system [see a sampling of articles regarding chicory root extract (inulin/oligofructose) documented for digestive health in Appendix 11]. Three slices of the bread per day supports a well-balanced gut

flora composition and colonic function by selectively stimulating the growth of Bifidobacteria. Nowadays, chicory root extract (inulin/oligofructose) has been widely used by major food companies in the world to make digestive health (prebiotic/bidogenic) claims. For example, Weetabix's bars & cereals (brand name: Alpen light bars and Weetaflakes cereal) and Warburton's bread (brand name: "Healthy Inside") in UK. In Asia and South America, Nestle's baby food products with Prebio 1, a unique blend of chicory inulin and oligofructose to facilitate feeding tolerance throughout the colon, are very popular.

The organic form of chicory inulin and oligofructose are not available on an industrial scale because that they can not be produced "organically" in an economically feasible way. For the economical production of organic chicory root extract (inulin/oligofructose), it would be necessary to stop a high capacity production (e.g., hundred tons of roots per day in each plant), clean the equipment and run on organically grown roots for a few days. Only by this way, we can separate normal production from organic production. However, this would be practically impossible due to high turnover costs to stop the production activities (see Sensus's letter on why organic chicory inulin and oligofructose can not be made on an industrial scale in Appendix 9).

European Regulation 2092/1991 on organic foods allows the use of non-organic products from agricultural origin up to a mixture of 5% as far as they are included in the list of Annex VI.C. Although chicory inulin and oligofructose are not included in that list, a request to include chicory inulin and oligofructose in the Annex VI.C. was directed to the Belgian Authorities by the European Committee of Inulin Producers CEFI (Comité Européen des Fabricants d'Inuline) in 1997. In this request, CEFI confirmed that none of its members can produce organic inulin or oligofructose from chicory. In June 2006, CEFI again submitted a request to the Belgian Authorities (Ministerie van de Vlaamse Gemeeschap) to amend Annex VI.C of EEC 2092/91 with the addition of chicory inulin and oligofructose. In April 2007, CEFI sent additional information to the Belgian Authorities to further distinguish between inulin and oligofructose derived from chicory and Jerusalem artichoke. Their differences in chemical structure, physical properties, technical features in foodstuff and nutritional benefits were clearly outlined.

12. A "Petition Justification Statement" which provides justification for inclusion of a non-organically produced agricultural substance onto the National List.

Chicory root extract documented for digestive health (Frutafit® and Frutalose® inulin/oligofructose from Sensus) falls under the category of §205.606., which encompasses non-organically produced agricultural products allowed as ingredients in or on processed products labeled as "organic". There are currently no organic equivalents of the product available. The product is not synthetic, being efficiently produced from natural extraction processes. Chicory root extract is well recognized as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of intestinal bacteria associated with health and well-being. Despite the fact that organic inulin and oligofructose

from sources other than chicory are available, there is no proper evidence for those organic inulin or oligofructose to make substantiated digestive health claims on food products. If chicory root extract (inulin/oligofructose) documented for digestive health can not be used in organic products, it will hamper development of organic products with substantiated digestive health claims based on inulin or oligofructose. Therefore, chicory root extract should be included on the National list, as it provides a valuable source of prebiotic soluble fiber that specifically feeds the beneficial probiotic microflora in the guts of humans and animals.

Besides providing digestive health, chicory root extract (Frutafit® and Frutalose® inulin/oligofructose from Sensus) also has been widely used in a variety of regular and organic foods, feeds and beverages, leading to other health, nutrition and functional benefits (see table below). It is thus a very important ingredient for food and feed products labeled as 'organic'. If chicory root extract documented for digestive health is not allowed to be listed in the National List, it would cause significant business disruption for organic food and feed producers.

Table

Health, Nutrition and Functional Benefits of Chicory Root Extract (Inulin/Oligofructose)

Colon & Bone Health	Weight Management	Texture Improvement	Taste Improvement
• Prebiotic effect (digestive health) • SCFAs* from fermentation • Fiber effect • Mineral absorption	• Calorie reduction • Fat replacement • Sugar replacement • Low GI • Satiety	• Gelling agent • Water binding • Mouthfeel • Humectant • Bulking agent	• Synergy with HIS** • Masks aftertaste of HIS** • Fruit taste enhancement

* SCFAs: Short Chain Fatty Acids (e.g., acetate, butyrate, propionate)

** HIS: High Intensity Sweeteners (e.g., aspartame, sucralose, Ace-K)

Contents of the Appendices

Appendix 1

GRAS Notice No. GRN 000118 issued by the FDA-

Approval of Chicory Root Extract (Frutafit® and Frutalose® Inulin/Oligofructose from Sensus) as GRAS Food Ingredient

U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
May 5, 2003

Agency Response Letter
GRAS Notice No. GRN 000118

Claire L. Kruger, Ph.D.
Environ
4350 North Fairfax Drive
Suite 300
Arlington, Virginia, 22203

Re: GRAS Notice No. GRN 000118

Dear Dr. Kruger:

The Food and Drug Administration (FDA) is responding to the notice, dated November 4, 2002, that you submitted on behalf of Imperial Sensus, LLC (Imperial Sensus) in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on November 7, 2002, filed it on November 14, 2002, and designated it as GRAS Notice No. GRN 000118.

The subject of the notice is inulin from the root of the chicory plant (*Cichorium intybus*). The notice informs FDA of the view of Imperial Sensus that inulin is GRAS, through scientific procedures, for use in food in general, including meat and poultry products, as a bulking agent. Imperial Sensus lists 43 proposed food categories that would contain inulin at varying use levels. Based on these proposed uses, Imperial Sensus estimates that dietary intake of inulin at the 90th percentile level would be approximately 6 grams per day for infants less than one year of age, approximately 15 grams per day for infants one year of age, and approximately 20 grams per day for the general population (i.e., two years of age and older).

Imperial Sensus' notice includes the findings of a panel of individuals who evaluated the data and information that serve as the basis for Imperial Sensus' GRAS determination. Imperial Sensus considers these individuals to be qualified by scientific training and experience to evaluate the safety of substances added to food.

Imperial Sensus describes published information about inulin, which is a polysaccharide that occurs naturally in plants such as chicory, Jerusalem artichokes, some cereal grains, and onions. It contains a chain of fructose units that are linked in a beta 2-1 configuration, usually with a single terminal glucose molecule. The degree of polymerization (DP) (i.e., the number of fructose units in the chain) ranges from two to greater than 60, depending on the plant source, the time of harvest and the duration and conditions of post-harvest storage.

Imperial Sensus describes published information about other "fructan" polysaccharides - i.e., polysaccharides that contain a chain of fructose units. These fructan polysaccharides include fructooligosaccharide (FOS; DP ranging from three to five) and oligofructoses (DP ranging from two to twenty). Because the fructose units in inulin, FOS, and oligofructoses all contain the characteristic beta 2-1 linkage, Imperial Sensus assesses the safety of inulin using data and information regarding all three of these fructan polysaccharides.

Imperial Sensus describes the manufacture of inulin, which is extracted from the root of the chicory plant. The harvested roots are washed via a process similar to that used in the sugar beet industry. The washed roots are sliced and fed into a scalding apparatus that renders the plant cell walls semi-permeable. After scalding, the chicory root slices are fed into a diffusion tower, where inulin is extracted in a continuous countercurrent process. The raw inulin juice emerging from this process is cooled and further purified and concentrated. Spray drying of this concentrated juice results in a final concentration of greater than 95 percent dry matter. Imperial Sensus provides specifications for this spray dried product.

Imperial Sensus describes published information about the presence of inulin as the energy reserve in a number of plants consumed as food world-wide, including chicory, dahlia, Jerusalem artichoke, murnong, and yacon. Imperial Sensus notes that these sources of inulin have been consumed either as dietary staples or as sustenance crops in times of hardship. Imperial Sensus describes published information about approximately two dozen inulin-containing food sources, including the percent of inulin contained in the edible part of the plant. Imperial Sensus notes that the inulin content of edible plants ranges from less than one percent (e.g., in many cereal grains) to more than 20 percent (e.g., in Jerusalem artichokes and other tubers). Imperial Sensus also cites a publication regarding the consumption of Jerusalem artichokes by some populations as a substitute for white potatoes and estimates that consumption of inulin by these populations may have reached 25 to 32 grams per day. In the U.S., the most commonly consumed inulin-containing foods are cereal grains, bananas, garlic, onions, and tomatoes. Based on published reports, Imperial Sensus estimates that the average intake of inulin and oligofructose in the U.S. is 2.6 grams per day, primarily from consumption of wheat and onions. In Europe, Imperial Sensus estimates that the intake of inulin and oligofructose may be as high as 10 grams per day.

Imperial Sensus cites published information to support its view that the beta 2-1 linkage between fructose units of inulin is largely resistant to digestion by mammalian digestive enzymes of the small intestine and that ingested inulin will pass largely intact to the colon, where it is subject to fermentation by the resident microbial flora. 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 either 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.

Imperial Sensus describes results from published *in vitro* genetic toxicity studies with a commercially available fructooligosaccharide and concludes that these studies demonstrate that this fructooligosaccharide lacks any significant genotoxic potential. Imperial Sensus also discusses published human studies and reviews related to human tolerance of inulin and related fructans and concludes that regular consumption of 40 to 70 grams per day of inulin by healthy adults appears to result in no significant adverse effects, especially when the consumption is divided over the course of the day.

Based on the information provided by Imperial Sensus, as well as other information available to FDA, the agency has no questions at this time regarding Imperial Sensus' conclusion that inulin is GRAS under the

intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of inulin. As always, it is the continuing responsibility of Imperial Sensus to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in the notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Potential Labeling Issues

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (FFDCA), a food is misbranded if its labeling is false or misleading in any particular. Section 403(r) of the FFDCA lays out the statutory framework for a health claim. In describing the intended use of inulin and in describing the information that Imperial Sensus relies on to conclude that inulin is GRAS under the conditions of its intended use, Imperial Sensus raises potential labeling issues under these provisions of the FFDCA. These labeling issues consist of Imperial Sensus' assertion that inulin has a variety of physiological effects that Imperial Sensus views as beneficial. If products that contain inulin bear any claims about such benefits on the label or in labeling, such claims are the purview of the Office of Nutritional Products, Labeling, and Dietary Supplements (ONPLDS) in the Center for Food Safety and Applied Nutrition (CFSAN). The Office of Food Additive Safety (OFAS) neither consulted with ONPLDS on these labeling issues nor evaluated the information in Imperial Sensus' notice to determine whether it would support any claims made about inulin on the label or in labeling.

Use in Meat and Poultry Products

During its evaluation of GRN 000118, FDA consulted with the Labeling and Consumer Protection Staff of the Food Safety and Inspection Service (FSIS) of the USDA. Under the Federal Meat Inspection Act and the Poultry Products Inspection Act, FSIS is responsible for determining the efficacy and suitability of food ingredients in meat and poultry products as well as prescribing safe conditions of use. Suitability relates to the effectiveness of the ingredient in performing the intended purpose of use and the assurance that the conditions of use will not result in an adulterated product, or one that misleads consumers.

FSIS has previously evaluated the use of inulin as a water binder, emulsifier, stabilizer and texturizer at a level between two and five percent in processed meat food products.[(1)] FSIS advised that, based on the current use of inulin in the production of meat products, FSIS would not object to the proposed use of inulin in non-standardized meat or poultry products at four percent of the total product formula. In regard to the use of inulin in standardized meat and poultry products, FSIS is currently in the process of completing rulemaking that would permit the use of any safe and suitable binder in the production of meat and poultry products where standards of identity and other Federal regulations already permit the addition of binders, e.g., hot dogs. Until this rulemaking is complete, inulin cannot be used to formulate meat and poultry products with a standard of identity in Title 9 of the Code of Federal Regulations, parts 319 and 381.

Sincerely,
/s/

Alan M. Rulis, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

cc: Dr. Robert Post, Director
Labeling and Consumer Protection Staff
Office of Policy, Program Development and Evaluation
Food Safety and Inspection Service
1400 Independence Ave., SW, Suite 602, Annex
Washington, DC 20250-3700

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(1)In a letter dated May 9, 1997, from FSIS to FDA, FSIS requested consultation with FDA regarding a request, from Imperial-Suiker Unie, that FSIS advise Imperial-Suiker Unie of the acceptability of the use of inulin as a water binder, emulsifier, stabilizer and texturizer at a level between 2 and 5 per cent in processed meat food products. In a letter dated May 14, 1999, FDA informed FSIS that FDA had completed its evaluation of the information submitted by Imperial-Suiker Unie as well as other information available to the agency. Based on its evaluation, FDA determined that, at that time, the agency would not challenge Imperial-Suiker Unie's conclusion that inulin is GRAS under the proposed conditions of use.

Food Ingredients and Packaging | Summary of all GRAS Notices

Foods Home | FDA Home | Search/Subject Index | Disclaimers & Privacy Policy | Accessibility/Help

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Hypertext updated by amd/lah/pmg/rxm on 2003-JUN-18

Appendix 2

Notice (T60.106) issued by the Association of American Feed Control Officials (AAFCO)-

Approval of Inulin/Oligofructose for use in animal feed and pet food products

Please Select: **Secure Site:** Enter

A. Approved moving the following Tentative Definitions to Official status:

1) **T48.30 Liquified Corn Product**

2) **T48.32 Corn Germ Dehydrated**

3) **T60.98 L-Carnitine**

B. Approved the following new Tentative Definitions:

1) **T57.164 Chromium L-Methionine Complex** is the stable, water-soluble monohydrochloride complex containing one molar equivalent of chromium (III) and three molar equivalents of L-methionine generally expressed as $(C_5N_{10}NO_2S)_3Cr(III)HCl$. It is to be used as a supplemental source of chromium in swine diets not to supply more than 400 ppb of chromium to the diet. Minimum chromium content from chromium-L-methionine complex must be specified.

2) **T60.106 Inulin** is a polysaccharide product obtained from plant sources such as chicory (*Cichorium intybus L.*), agave (*Agave azul tequilana*), and Jerusalem artichoke (Helianthus tuberosus) by hot water extraction. It is intended as a source of soluble, fermentable fiber. It must contain not less than 90% inulin. It may contain products of partially hydrolyzed inulin.

3) **T60.107 Mixed Feed Nuts** are the residue of the normal packaging and processing for human consumption of shelled tree nut and peanut products. This residue shall consist of broken, small, shriveled and cull edible tree nuts or peanuts of two or more kinds and shall be suitable for animal consumption. If salt has been added during processing, a guarantee must be made for maximum sodium.

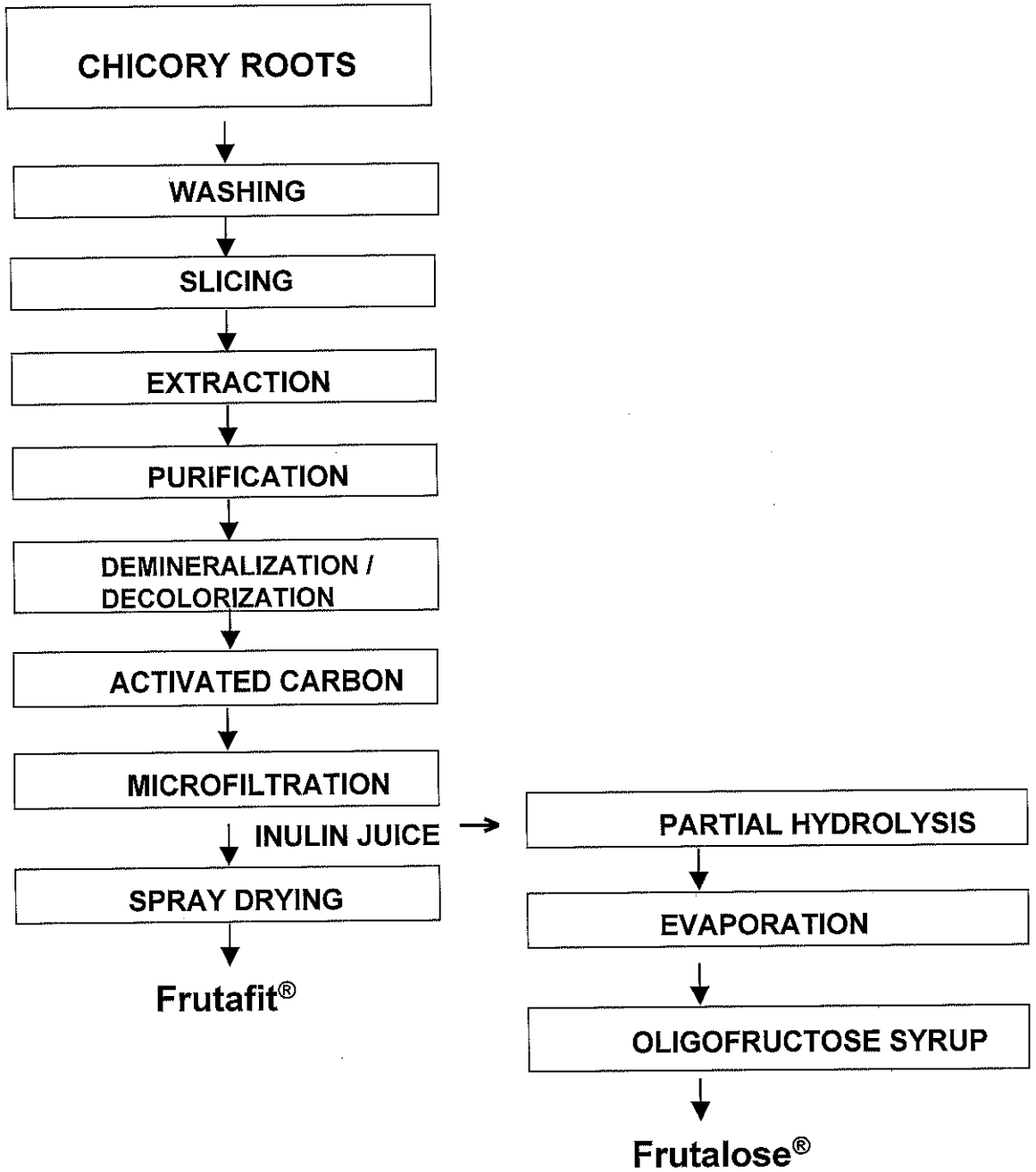
4) ***T60.108 Salvage Pet Food** is a product resulting from pet food manufacturing. This product may consist of, but is not limited to, start-up and over-run product, unfinished pet food, pet food fines and other product not suitable for packaging for retail sale. If it contains, or may contain, any material identified by 21 CFR 589.2000 as prohibited from use in the feed of ruminant animals, or if it is no longer accompanied by a detailed label listing all of the ingredients in the salvage pet food, the label must contain the precautionary statement "Do not feed to cattle or other ruminants". It shall be free of foreign materials harmful to animals, and must be suitable for the purpose for which it is being marketed. (* The asterisk indicates that this ingredient may be subject to 21 CFR 589.2000.)

5) ***T60.109 Distressed Pet Food** is a product resulting from pet food distribution, but which is no longer available for retail sale. This product may be pet food in, but not limited to, dented cans, torn bags, product past its sell-by date, or returned product that is suitable for use in feed. It may consist of a single formula, still in the original packaging, or a variety of formulas commingled into one bulk container and containing none of the original packaging or labeling. If it contains, or may contain, any material identified by 21 CFR 589.2000 as prohibited from use in the feed of ruminant animals, or if it is no longer accompanied by a detailed label listing all of the ingredients in the distressed product,

Appendix 3

Production Process of Chicory Root Extract

(Frutafit® and Frutalose® Inulin/Oligofructose from Sensus)



PRODUCTION SCHEME OF CHICORY ROOT INULIN/OLIGOFRUCTOSE (FRUTAFIT® AND FRUTALOSE®)

Appendix 4

Section 6.8.1: Dietary Fiber issued by the Canadian Food Inspection Agency-

Approval of Frutafit® Inulin from Sensus as 'Traditional Fiber' in Canada

Sodium content is based upon the total sodium present in the food regardless of the origin of the nutrient. Unlike most other mineral nutrients, sodium does not have a Recommended Daily Intake. Calculation of the % Daily Value is based on the Reference Standard value of 2400 mg [table to B.01.001.1(2)].

6.7 Potassium

Like sodium, potassium content is based upon the total potassium present in the food and does not have a Recommended Daily Intake. The % Daily Value is calculated by using the Reference Standard of 3500 mg [table to B.01.001.1(2)].

6.8 Carbohydrates

For labelling purposes, the total amount of declared carbohydrates must include sugars (e.g., monosaccharides such as glucose, and disaccharides such as sucrose), starch, dietary fibre, sugar alcohols (e.g., isomalt, lactitol, maltitol, maltitol syrup, mannitol, sorbitol, sorbitol syrup, xylitol, erythritol), glycerol and polydextrose.

The amount of carbohydrate may be determined by subtracting the content of protein, fat, ash and moisture from the weight of the product.

6.8.1 Dietary Fibre

"Dietary fibre are the endogenous components of plant material in the diet which are resistant to digestion by enzymes produced by humans. They are predominantly non-starch polysaccharides and lignin and may include, in addition, associated substances "(Health and Welfare Canada, 1985). There are two types of fibre: soluble, which will dissolve in water, and insoluble, which will not dissolve in water. The total fibre content of most plant foods consists of both types in varying amounts.

Some sources of insoluble fibre include wheat bran, some vegetables and whole grains. Some sources of soluble fibre include oats, barley, nuts, seeds, beans, lentils, and some fruits and vegetables.

The amount of dietary fibre is one of the 13 core nutrients that must be declared in the Nutrition Facts table [item 10 of the table to B.01.401]. The amount of both soluble fibre and insoluble fibre may be separately declared as additional information [item 10 and 11 in the table to B.01.402].

Novel fibre (or a novel fibre source) is a food that has been manufactured to be a source of dietary fibre, **and**:

(a) has not traditionally been used for human consumption to any significant extent; or

(b) has been chemically processed (e.g., oxidized) or physically processed (e.g., very finely ground) so as to modify the properties of the fibre; or

(c) has been highly concentrated from its plant source.

This definition was recommended by the Expert Advisory Committee on Dietary Fibre, 1985, reporting to Health Canada.

The **safety** of novel fibre sources must be established before they may be used as

ingredients in foods. As well, the physiological **efficacy** of novel fibre sources as dietary fibre must be established before they may be claimed to be a source of dietary fibre in foods. If the novel fibre source has **not** been tested for efficacy, it is considered an unproven novel fibre. If safe, it may be used in foods but it cannot be claimed to be a source of dietary fibre.

If a novel fibre source has been reviewed by the Health Products and Food Branch of Health Canada and found acceptable, either as an ingredient only (safety demonstrated) or as a dietary fibre source (safety and efficacy demonstrated), the manufacturer will receive a "letter of no objection". The letter will indicate any restriction on the use of the novel fibre source. These "letters of no objection" are specific to the brand of the fibre source that was reviewed, unless otherwise specified.

Manufacturers who are considering the use of novel fibre sources and require further guidance are advised to contact the Health Products and Food Branch, Health Canada.

In the case of ingredients manufactured to be sources of dietary fibre, such as novel fibre sources, the **common name of the fibre ingredient in the list of ingredients** should include:

- the **name** of the plant which is the origin of the fibre; and
- the **specific part** of that plant.

The term "**fibre**" may be included as part of the common name, if appropriate (e.g., the product is 90 percent fibre).

The amount of **dietary fibre from novel fibre sources must not be included as part of the total dietary fibre declaration** in the Nutrition Facts table **unless**:

- proof of efficacy as dietary fibre in the same type of food has been shown through clinical testing to the satisfaction of the Health Canada, and
- a letter of no objection has been issued by Health Canada. *Reference:* Health Canada's Food Directorate Guideline No. 9, "Guideline Concerning the Safety and Physiological Effects of Novel Fibre Sources and Food Products Containing Them," revised November, 1994.

All novel fibre foods must be reviewed by Health Canada in order for them to be considered a fibre source. This includes novel fibres which may have already been considered acceptable as a food or food ingredient, but which have not been previously promoted as a source of fibre, have not been traditionally used at higher levels and/or have not been used or added for the previously approved purpose.

Some examples of **novel fibres not currently recognized as food ingredients or fibre sources** include:

- fibre that has not traditionally been used for human consumption to any significant extent, such as cane sugar stalks, cocoa bean hulls, oat hulls, mucopolysaccharides (e.g., chitin) from shells of shellfish, and wheat straw;
- fibre that has been chemically processed, (e.g., oxidized), or physically processed (e.g., very finely ground), so as to modify the properties of the

fibre, such as bleached oat hulls, finely ground wheat bran, bleached pea hulls (seed coats), and bleached wheat straw; and

- fibre that has been highly concentrated from its plant source, such as beta-glucans from barley and oats.

Examples of **food additives not *currently* recognized as fibre sources or ingredients** include:

- pectin
- carrageenan
- guar gum
- methylcellulose, carboxymethylcellulose, microcrystalline cellulose, etc.
- wood cellulose (powdered cellulose) [Use is currently allowed under an Interim Marketing Authorization.]

Dietary Fibre - Summary of Sources, Acceptability and Labelling Table 6-12 (Source: Health Products and Food Branch (HPFB) of Health Canada. revised October 2002, subject to change)

Name of Fibre (see note a)	Ingredient Name	Classification of Ingredient as Fibre Source		Acceptable Ingredient?	Fibre Labelling: Regular Foods (see note c)	Fibre Labelling: Meal Replacements (see note d)	
		Traditional	Novel		Include amount in dietary fibre label declaration? Claim permitted? - see items 41, 42, 43, 44 of table following B.01.513	Include amount in dietary fibre label declaration?	Claim Permitted Including "Source of Fibre"?
Apple pomace *Treetop* brand	Apple pomace powder/ Poudre de tourteaux de pommes		✓	Yes	No	No	No
Corn bran by traditional milling (less than/equal to 65% total fibre)	Corn bran/ Son de maïs	✓		Yes	Yes	Yes	No
Corn bran at greater than 65% total fibre	Corn bran/ Son de maïs		✓	Yes	No	No	No
Mustard bran	Mustard bran/ Son de moutarde		✓	Yes but only In condimental amounts	No	No	No
Inulin from chicory root (*Orafti® inulin* - Quadra Chemicals) (*Frutafit® Inulin* - Sensus America) *Metamucil® FibreSure* (Procter & Gamble) Cargill's Oliggo-	Chicory root inulin	✓		Yes	Yes	Yes	Yes

Fiber™ inulin							
Oat bran ≥ 13 % total dietary fibre, ≥ 30% of fibre as soluble fibre, and ≤ 12% moisture	Oat bran/ Son d'avoine	✓		Yes	Yes	No	No
Oat hulls - ground, bleached *Canadian Harvest® Oat Fiber 300-58* (Opta® Food Ingredients)	Oat hull fibre/ Fibres de bale d'avoine		✓	Yes in grain and bakery products at levels that provide a source of fibre (see note b) and in bar-type meal replacements	Yes	Yes	No
Pea Hull Fibres *Hi Fi Lite* & *Centara* (Nutri-Pea Limited) *Exlite Coarse* (Parrheim Foods) *Ground pea hull fibre* (Best Cooking Pulses)	Ground pea hull fibre/ Fibre de cosses de pois moulue		✓	Yes	Yes but only in bakery products and cereals *Centara and BCP may also be used in meat products where a filler/binder is permitted	No	No
Psyllium seed husk	Ground psyllium fibre/ Fibre de psyllium moulue		✓	Yes but only if individual products submitted to / accepted by HPFB	Yes (if accepted)	No	No
Rice bran *Fiberice* (Farmers Rice Cooperative)	Rice bran/ Son de riz		✓	Yes	No	No	No
Soy cotyledon *Fibrim* *300, 1000, 1010, 1250, 1250, 1255, 1450*, and *2000* by Protein Technologies International	Ground soy cotyledon fibre/ Fibre de cotylédon de soya moulue		✓	Yes	Yes	No	No
Sugar beet fibre, *Fibrex* (Delta Fibre Foods) (> 0.125 mm)	Ground sugar beet fibre/ Fibre de betterave à sucre moulue		✓	Yes	Yes but only in bakery products at less than or equal to 7%	No	No
Wheat bran, coarse (>0.75 mm)	Wheat bran/ Son de blé	✓		Yes	Yes Claim for regularity if a reasonable daily intake provides 7 g of fibre from coarse wheat	Yes	Yes if a serving contains 7 g of fibre from coarse

Appendix 5

Dutch 'Healthy Colon' claim in Bread issued by the Nutrition Center of the Netherlands

Summary of the document supporting

Dutch Health Claim

Frutafit Inulin in Bread

The Netherlands Nutrition Board,

Ministry of Health, Den Haag

February 2002

Assessment Report

The undersigned:
Prof dr ir F.M. Rombouts (chairman), Wageningen University
Prof dr ir P.A. van den Brandt, Maastricht University
Dr F.M. Nagengast, University Hospital Nijmegen St. Radboud
Prof dr ir G.J. Schaafsma, Wageningen University/ TNO Voeding, Food and Health, Zeist

have been appointed by the Netherlands Nutrition Centre and the Applicant **Bakkerij Veenhuis BV, subsidiary of Borgesius Holding, Stadskanaal (supported by Sensus, a subsidiary of Royal Cosun, Breda)** to assess the scientific evidence for the following health benefit:

Product: **Vitaalbrood® flora**

Health Benefit: **Consumption of three slices of Vitaalbrood® flora per day supports a well-balanced gut flora composition and colonic function by selectively stimulating the growth of *Bifidobacterium*. Vitaalbrood® flora contains at least 5 g Frutafit®-inulin per 100 gram.**

In Dutch:
Consumptie van drie sneden Vitaalbrood® flora per dag ondersteunt een evenwichtige samenstelling van de darmflora en een goede dikke darmfunctie door selectieve groeistimulatie van *Bifidobacterium*. Vitaalbrood® flora bevat ten minste 5 g Frutafit®-inuline per 100 gram.

The undersigned have reached their decision in accordance with the Code of Practice for Assessing the scientific evidence for health benefits stated in health claims on food and drink products 1998. The Applicant submitted a high quality dossier, has co-operated fully in this assessment and has provided the undersigned with all the information requested.

The decision of the undersigned reads:

There is **sufficient scientific evidence**, as referred to in the Code of Practice, for the Health Benefit described above.

This assessment is based on the available data from the scientific literature and confidential data provided by the Applicant. The panel's decision is based in part on the following publications:

- Alberts DS, Martinez ME, Roe DJ Guillen-Rodriguez JM, Marshall JR, van Leeuwen JB, Reid ME, Ritenbaugh C, Vargas PA, Bhattacharyya AB, Earnest DL, Sampliner RE. Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. Phoenix Colon Cancer Prevention Physicians' Network. N Engl J Med 2000; 342: 1156-62.
- Bouhnik Y, Vahedi K, Ackhour L, Attar A, Salafati J, Pochart P, Marteau P, Flourie B, Bornet F, Rambaud JC. Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. J Nutr 1999; 129: 113-6.
- Brighenti F, Casiraghi MC, Canzi E, Ferrari A. Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. Eur J Clin Nutr 1999; 53(9): 726-33.
- Causey JL, Feirtag JM, Gallaher DD, Tungland BC, Slavin JJ. Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholersterolemic men. Nutr Res 2000; 20: 191-201.
- Cummings JH, Macfarlane GT. Role of intestinal bacteria in nutrient metabolism. Clinical Nutrition 1997; 16: 3-11.
- Cummings JH, Macfarlane GT, Englyst HN. Prebiotic digestion and fermentation. Am J Clin Nutr 2001; 73(2 Suppl): 415-20S.
- Fooks LJ, Gibson GR. In vitro investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens. FEMS Microbiol Ecol 2002; 39: 67-75.
- Fuchs CS, Giovannucci EL, Colditz GA, Hunter DJ, Stampfer MJ, Rosner B, Speizer FE, Willett WC. Dietary fiber and the risk of colorectal cancer and adenoma in women. N Engl J Med 1999; 340: 169-76.
- Gibson GR, Beatty ER, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 1995; 108(4): 975-82.
- Gibson GR, Wang X. Regulatory effects of bifidobacteria on the growth of other colonic bacteria. J Appl Bacteriol 1994; 77:412-20.
- Goldin BR. Intestinal microflora: metabolism of drugs and carcinogens. Ann Med 1990; 22(1): 43-8.
- Hopkins MJ, Sharp R, MacFarlane GT. Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. Gut 2001; 48: 198-205.
- Kleessen B, Sykura B, Zunft HJ, Blaut M. Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. Am J Clin Nutr 1997; 65(5): 1397-402.
- Kruse HP, Kleessen B, Blaut M. Effects of inulin on faecal bifidobacteria in human subjects. Br J Nutr 1999; 82(5): 375-82.
- Liu S, Buring JE, Sesso HD, Rimm EB, Willett WC, Manson JE. A prospective study of dietary fiber intake and risk of cardiovascular disease among women. J Am Coll Cardiol 2002; 39(1): 49-56.
- Mitsuoka, T. Bifidobacteria and their role in human health. J Ind Microbiol 1990; 6: 263-8.
- Rao VA. The prebiotic properties of oligofructose at low intake levels. Nutr Res 2001; 21: 843-48.
- Roberfroid MB. Prebiotics: preferential substrates for specific germs? Am J Clin Nutr 2001; 73(2 Suppl): 406S-9S.
- Roberfroid M, Van Loo JAE, Gibson GR. The bifidogenic nature of chicory inulin and its hydrolysis products. J Nutr 1998; 128: 11-9.

- Schatzkin A, Lanza E, Corle D, Lance P, Iber F, Caan B, Shike M, Weissfeld J, Burt R, Cooper MR, Kikendall JW, Cahill J. Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. Polyp Prevention Trial Study Group. N Engl J Med 2000; 342: 1149-55.
- Terry P, Giovanucci E, Michels KB, Bergkvist L, Hansen H, Holmberg L, Wolk A. Fruit, vegetables, dietary fiber, and risk of colorectal cancer. J Natl Cancer Inst 2001; 93: 525-33.
- Tuohy KM, Finlay RK, Wynne AG, Gibson GR. A human volunteer study on the prebiotic effects of HP-inulin – Faecal bacteria enumerated using fluorescent in situ hybridisation. Anaerobe 2001[a]; 7: 113-8.
- Tuohy KM, Kolida S, Lustenberger AM, Gibson GR. The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides – a human volunteer study. Br J Nutr 2001[b]; 86(3): 341-8.
- Van Loo J, Cummings J, Delzenne N, Englyst H, Franck A, Hopkins M, Kok N, Macfarlane G, Newton D, Quigley M, Roberfroid M, van Vliet T, van den Heuvel E. Functional food properties of non-digestible oligosaccharides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). Br J Nutr 1999; 81(2): 121-32. Comment in: Br J Nutr. 1999; 81(2): 83-4; 82(1): 75-6; 82(4): 329.
- Van Loo J, Coussement P, de Leenheer L, Hoebregs H, Smits G. On the presence of inulin and oligofructose as natural ingredients in the western diet. Crit Rev Food Sci Nutr 1995; 35(6): 525-52.

The **panel's decisions are motivated as follows:**

The panel assessed the quality of the scientific evidence using criteria listed in the Code of Practice Health Benefits 1998. In order to reach final conclusions on the health benefit of Vitaalbrood® flora, the panel has sought answers to the following questions:

1. *What is the panel's judgement of the completeness of the dossier and the interpretation of scientific literature?*

   - The applicant has carefully interpreted and correctly presented the scientific literature on the bifidobacteria stimulating (bifidogenic) effect of inulin.

2. *Are there sufficient data to sustain a bifidogenic effect of Frutafit®-inulin that supports a healthy gut flora composition and colonic function?*
- *What is the effective dose?*
- *Does this bifidogenic effect imply a relevant health benefit to the consumer?*

   Inulin and other fructo-oligosaccharides (FOS) are characterized by _(2,1) bonds of fructose units, with a variable degree of polymerisation (DP). Due to these _(2,1) bonds inulin is not or hardly digestible by digestive enzymes in the human small intestine, but it is fermented to short chain fatty acids (SCFA) and gases by bacteria in the colon, especially *Bifidobacterium*. Inulin has been classified as a prebioticum. A prebiotic effect has been defined as the capacity to selectively stimulate the growth and activity of specific species of bacteria in the gut, usually bifidobacteria and lactobacilli, with benefits to health. Inulin is naturally present in wheat, onions, leeks and garlic. In the western diet the intake is estimated to range from 1 to 10 g per day. In southern European countries the consumption is about twice as high as in the Benelux (Van Loo et al. 1995). Frutafit®-inulin is derived from chicory roots. The average DP varies between 20 and 25.

The bifidogenic effect of inulin has been consistently shown, in animal and *in vitro* studies and more important in controlled trials with human beings (Roberfroid et al. 1998; Van Loo et al. 1999):

- A significant increase in numbers of bifidobacteria in faeces has been observed in a series of controlled human studies using variable amounts of inulin or FOS for 1-9 weeks (e.g. Brighenti et al. 1999; Causey et al. 2000; Kruse et al (1999); Rao 2001; Tuohy et al. 2001[a]). In some controlled studies, there was a concomitant decrease in numbers of potential harmful bacteria, such as *Clostridium* species and *Enterococcus* (Gibson et al. 1995; Kleessen et al. 1997). In other studies no such changes were observed or other bacterial groups were not monitored during the trial.
- In one study (Bouhnik et al. 1999) a dose response relation between the amounts of FOS, varying between 0 to 20 g/day, and the growth of bifidobacteria was found, but other investigators conclude that there is no straightforward dose-response effect (Roberfroid 2001). In studies with inulin or FOS the increase in counts of bifidobacteria in human faeces appeared to be correlated with the initial number of these bacteria before the trial, irrespective of the dose of inulin or FOS (Roberfroid et al. 1998; Tuohy et al. 2001[b]). Thus, consumption of extra inulin may not result in an additional growth of bifidobacteria in individuals with already high numbers of these bacteria in their colonic microflora.

An extra daily dose of about 5 g inulin provides a considerable part of a total daily intake of 5-20 g inulin. Based on various controlled studies it is reasonable to assume that this amount of inulin selectively stimulates the growth of bifidobacteria, especially in those individuals with relatively low numbers of bifidobacteria in their colonic microflora.

Bifidobacteria are generally assumed to be health promoting. The question remains what are the health benefits of having a colonic flora in which bifidobacteria predominate? Also, as the Applicant indicated the general population to be the target group, the question is whether there is a health benefit for all age groups:

- The hypothesis that the number of Bifidobacteria is a marker of a healthy colonic microflora has been postulated by Mitsuoka. Reduction or disappearance of bifidobacteria in the human intestine, as occurs with various diseases and in conditions of emotional stress, aging, administration of antibiotics etc., would indicate an "unhealthy" state (Mitsuoka 1990). Bifidobacteria are the most important organisms in infants, breast babies having higher numbers of *Bifidobacterium* than bottle-fed children. In the faeces of healthy children and adults bifidobacteria constitute 5 to 10% of the total flora (Mitsuoka 1990). In elderly persons the number of bifidobacteria is decreased. Reductions in these organisms in the large bowel may be related to increased disease risk in elderly people (Hopkins et al. 2001). Within the general adult population (except elderly and diseased persons) faecal counts of bifidobacteria are estimated to vary between 10^8 and 10^{11} colony forming units per gram faeces (Bouhnik et al. 1999; Goldin 1990; Hopkins et al. 2001; Kruse et al. 1999). Due to technical problems in measuring types and numbers of bacteria more detailed data on variations in numbers of bifidobacteria in different age groups are not available. Recent hybridization detection techniques have a higher sensitivity and specificity than the traditional plate counting methods. In future it might be possible to detect smaller changes in the composition and metabolic activity of the colonic microflora that might be nutritionally relevant.
- It must be concluded that it has not been proven beyond any reasonable doubt that the number of bifidobacteria is a marker of a healthy colonic microflora. However,

bifidobacteria have a role in controlling the pH of the large intestine through the production of lactic and acetic acids and other short chain fatty acids (SCFA) that are formed as a result of interactions with other bacteria species. SCFA exert several metabolic effects (Cummings and MacFarlane 1997). SCFA induce a low pH, which might restrict the growth of potential pathogens. *In vitro* studies and studies with various animal species indicate that higher numbers of *Bifidobacterium* increase colonization resistance. Also, acetate and lactate may have an anti-microbial effect beyond this effect of lowering the pH (Fooks and Gibson 2002). The growth of biomass and the osmotic effect due to the production of lactic acid and SCFA, increase peristaltic movements, enhance faecal bulking and affect bowel habit. For instance, inulin has been shown to be mildly laxative in habitually constipated patients (Kleessen et al. 1997).

- As inulin is a dietary fibre component, it has been postulated that inulin might have beneficial fibre effects, especially a contribution to a protective role in colon cancer and cardiovascular diseases. There is some preliminary evidence from animal studies that inulin may affect carcinogenesis and lipid metabolism. However, results from human trials are inconclusive. Also, it must be kept in mind that results of recent prospective studies do not confirm dietary fibre relationships with colon cancer (Fuchs et al. 1999) and cardiovascular diseases. Terry et al. (2001) found no association between colorectal cancer risk and consumption of cereal fibre, but individuals who consume very low amounts of fruit and vegetables have the greatest risk of colorectal cancer. Also, in intervention studies (Alberts et al. 2000; Schatzkin et al. 2000) high fibre diets did not influence the recurrence of colorectal adenomas. Liu et al. (2002) report a statistically not significant association between fibre intake and risk of cardiovascular disease (after adjusting for confounding factors).

Considering the available data, it is concluded that inulin stimulates bifidobacterial growth and fermentation, which can beneficially affect bowel habit. For individuals with already high numbers of bifidobacteria there may be no additional bifidogenic effect, but fermentation of extra inulin still produces higher levels of short chain fatty acids that are potentially beneficial. *In vitro* and animal experiments indicate that fermentation of inulin leads to increased colonization resistance. Data on effects on blood lipids, in relation to cardiovascular diseases prevention, and on reduction of carcinogenesis are inconclusive.

3. *Are there sufficient data to sustain the health benefit of Vitaalbrood® flora containing Frutafit®-inulin, taking into account the stability of inulin in the matrix of Vitaalbrood® flora and the availability of inulin for fermentation by the large bowel microflora? If so,*

- *Does a realistic use of Vitaalbrood® flora provide an effective dose of inulin?*
- *Are there any possible side effects that may counteract the beneficial effect?*

- The bifidogenic effect has not directly been shown in studies with Vitaalbrood® flora. However, it is anticipated that due to the solid matrix of bread the inulin gradually becomes available, which may have a prolonged effect. Thus, taking into account that there are no reasons to doubt the availability and the stability of inulin, the panel concludes that indirect evidence of the efficacy of Frutafit®-inulin is sufficient. Also, a bifidogenic effect was shown in a study with comparable products like breakfast cereals or biscuits (Brighenti et al. 1999).
- Three slices of Vitaalbrood® flora per day provide 5.3 g inulin. Three slices present a realistic daily use of bread. It is not known whether the bifidogenic effect may be different when the Vitaalbrood® flora is eaten in one meal or spread in 2 or 3 meals.
- Controlled studies in humans have shown that the intake of inuline up to 15-20 g per day does not induce serious adverse side effects, but can induce in some individuals a

mild to moderate intestinal discomfort, such as flatulence and a bloated feeling. It is calculated that men aged between 19-22 years, the group with the highest consumption of wholemeal bread, would not exceed a consumption of 20 g inulin per day. Depending on the food matrix and individual sensitivity, intestinal discomfort can be induced by gas formation and osmotic pressure (due to SCFA and lactic acid), resulting from bacterial metabolism.

4. *What is the mechanism of action?*

- Bifidobacteria produce specific enzymes required for the fermentation of inulin. The consistently observed prebiotic potential of inulin-type fructans may be ascribed to their linear chains composed of _(2,1) linked fructose molecules. From experiments with pure cultures it appears that bifidobacteria are the most efficient species in fermenting inulin and FOS (Roberfroid 2001).
- The main fermentation products of bifidobacteria are acetic acid and lactic acid. Butyrate and propionate are also formed, indicating that bacterial populations other than bifidobacteria also benefit. It is suggested that bifidobacteria have an inhibitory effect on other bacteria species, related to the production of acids and a consequent lowering of gut pH to levels below those at which other bacteria are able to effectively compete. However, also other mechanisms of inhibition may be involved (Gibson and Wang 1994). An inhibitory effect on other bacteria has been confirmed in some controlled studies (Gibson et al. 1995; Kleessen et al. 1997).

Conclusion

It is reasonable to assume that an extra daily dose of about 5 g inulin provided by three slices of Vitaalbrood® flora has a bifidogenic effect, which can beneficially affect bowel habit. For individuals with already high numbers of bifidobacteria there may be no additional bifidogenic effect, but fermentation of extra inulin still produces higher levels of short chain fatty acids that are potentially beneficial. *In vitro* and animal experiments indicate that fermentation of inulin leads to increased colonization resistance. Data on effects on blood lipids, in relation to cardiovascular disease prevention, and on reduction of carcinogenesis are inconclusive. Vitaalbrood® flora may cause mild side effects (flatulence and a bloated feeling) in individuals who are sensitive to gastrointestinal discomfort.
Consumers should be clearly instructed on the use of Vitaalbrood® flora within the context of the Dietary Guidelines. The importance of a healthy lifestyle should be underlined.

As it is useful to keep the dossier on the efficacy of Vitaalbrood up to date, the panel advises to collect data on the long-term nutritional implications for individuals in all age groups who routinely consume this bread.

The members of the panel

Prof dr ir F.M. Rombouts (chairman)
Prof dr ir P.A. van den Brandt
Dr F.M. Nagengast
Prof dr ir G.J. Schaafsma

Appendix 6

Bifidigenic claims for chicory inulin issued by the French Food Safety Agency (AFSSA, Agence Francaise de Sécuritë Sanitaire des Aliments- Advice of 20 April 2005)

afssa
AGENCE FRANÇAISE
DE SECURITE SANITAIRE
DES ALIMENTS

LE DIRECTEUR GÉNÉRAL

Maisons-Alfort, le 20 avril 2005

AVIS

de l'Agence française de sécurité sanitaire des aliments relatif à l'évaluation des allégations nutritionnelles concernant l'effet bifidogène de l'inuline sur la flore intestinale humaine : « L'inuline native de chicorée est bifidogène (stimulation de la croissance des bifidobactéries intestinales) à un dosage quotidien de 5 g/j. », « L'inuline native de chicorée stimule la croissance des bifidobactéries intestinales à un dosage quotidien de 5 g/j », « L'inuline native de chicorée est prébiotique à un dosage quotidien de 5 g/j » et « L'inuline native de chicorée à un dosage quotidien de 5 g/j aide à maintenir une flore intestinale saine dans le côlon »

Par courrier reçu le 26 octobre 2004, l'Agence française de sécurité sanitaire des aliments (Afssa) a été saisie le 20 octobre 2004 par la Direction générale de la concurrence, de la consommation et de la répression des fraudes, d'une demande d'évaluation des allégations nutritionnelles concernant l'effet bifidogène de l'inuline sur la flore intestinale humaine : « L'inuline native de chicorée est bifidogène (stimulation de la croissance des bifidobactéries intestinales) à un dosage quotidien de 5 g/j. », « L'inuline native de chicorée stimule la croissance des bifidobactéries intestinales à un dosage quotidien de 5 g/j », « L'inuline native de chicorée est prébiotique à un dosage quotidien de 5 g/j » et « L'inuline native de chicorée à un dosage quotidien de 5 g/j aide à maintenir une flore intestinale saine dans le côlon ».

Après consultation du Comité d'experts spécialisé « Nutrition humaine » réuni le 27 janvier 2005, l'Afssa rend l'avis suivant :

Considérant que l'inuline est un polymère constitué de molécules de fructose de degré de polymérisation compris entre 10 et 60 ; que l'inuline proposée par le pétitionnaire est extraite de la chicorée ; que l'effet bifidogène est défini par l'augmentation du niveau de population et/ou de l'activité des bifidobactéries totales[1] ; que l'Afssa a reconnu dans son avis du 22 décembre 2000 (saisine 2000-SA-0134) que la consommation de 9 g d'inuline par jour a un effet bifidogène ; que la demande concerne l'évaluation de l'effet bifidogène de l'inuline pour une consommation quotidienne de 5 g ainsi que 4 allégations ; que pour argumenter sa demande, le pétitionnaire se base sur deux études originales menées *in vitro* et *in vivo* ;

Considérant que l'étude *in vitro* a été réalisée avec un système qui permet de simuler la digestion et la fermentation colique chez l'humain, après inoculation avec un mélange d'échantillons fécaux de 3 volontaires sains ; que la fermentation in vitro a été réalisée pendant 5 semaines, d'une part en présence de l'inuline, d'autre part avec un témoin (amidon) ; qu'un accroissement de la population de bifidobactéries est observé ; que toutefois la méthodologie suivie quant au mode d'évaluation de l'effet de l'inuline sur la flore lactique est critiquable, et ne permet pas de conclure à un effet : l'inuline est introduite dans le réacteur au niveau « colique » en substitution à de l'amidon dont la nature n'est pas indiquée et il n'est pas possible de savoir si cet amidon est fermenté ou non dans le réacteur ;

Considérant que l'étude in vivo a été réalisée sur 39 volontaires sains répartis en deux groupes : 20 sujets dans le groupe ayant consommé 2 fois/j un sachet de 2,5 g d'inuline dilué dans un verre d'eau et 19 dans le groupe ayant pris un placebo ; que l'étude est contrôlée, randomisée, parallèle en double aveugle contre placebo ; que les concentrations initiales de bifidobactéries ne

[1] Rapport Afssa relatif aux « Effets des prébiotiques et probiotiques sur la flore et l'immunité de l'homme adulte » - Février 2005.

27-31, avenue du Général Leclerc
BP 19, 94701
Maisons-Alfort cedex
Tel 01 49 77 13 50
Fax 01 49 77 26 13
www.afssa.fr

sont pas significativement différentes entre les deux groupes (7,7±0,3 log cfu/g de selles fraîches dans le groupe inuline contre 8,2±0,2 log cfu/g ww pour le groupe placebo) ; que l'étude a consisté en une analyse de la flore intestinale, des métabolites issus de la fermentation ainsi que de la fréquence et la consistance des selles ; qu'après deux semaines d'expérimentation, les résultats de l'étude montrent une augmentation significative des concentrations en bifidobactéries dans le groupe inuline (8,7±0,3 log cfu/g ww) ; qu'en revanche, l'augmentation observée dans le groupe placebo n'est pas significative (8,6±0,2 log cfu/g ww) ; que la méthodologie de l'étude est conforme aux lignes directrices présentées dans le rapport Afssa[1] indiquant que « les tests statistiques doivent être effectués sur une population définie a priori en début d'essai et non a posteriori en fin d'essai » ; que l'effet bifidogène de l'inuline pour une consommation de 5 g/j est vérifié ;

Considérant qu'en conséquence, les allégations « L'inuline native de chicorée est bifidogène (stimulation de la croissance des bifidobactéries intestinales) à un dosage quotidien de 5 g/j. », « L'inuline native de chicorée stimule la croissance des bifidobactéries intestinales à un dosage quotidien de 5 g/j. », « L'inuline native de chicorée est prébiotique à un dosage quotidien de 5 g/j. », sont scientifiquement fondées ; que d'après le rapport Afssa[1], « il n'est pas possible de définir un bon profil de flore », que par extension, « une flore saine » n'est pas non plus définissable ; qu'en conséquence, l'allégation « L'inuline native de chicorée à un dosage quotidien de 5 g/j aide à maintenir une flore intestinale saine dans le côlon » n'est pas acceptable,

L'Afssa considère que :

- La consommation quotidienne de 5 g d'inuline native de chicorée est bifidogène ;
- Les allégations « L'inuline native de chicorée est bifidogène (stimulation de la croissance des bifidobactéries intestinales) à un dosage quotidien de 5 g/j. », « L'inuline native de chicorée stimule la croissance des bifidobactéries intestinales à un dosage quotidien de 5 g/j. », « L'inuline native de chicorée est prébiotique à un dosage quotidien de 5 g/j » sont scientifiquement validées ;
- L'allégation « L'inuline native de chicorée à un dosage quotidien de 5 g/j aide à maintenir une flore intestinale saine dans le côlon » n'est pas acceptable.

Martin HIRSCH

Appendix 7

Kosher certificate of Frutafit® and Frutalose® Inulin/Oligofructose from Sensus issued by the Circle K-Rabbi Don Yoel Levy, Kashrath Administrator

בס"ד

KOSHER CERTIFICATE

KC# 976329-1
28 Elul, 5767
September 11, 2007

KOSHER CERTIFICATION
Rabbi Don Yoel Levy
Kashruth Administrator

SENSUS
P.O. BOX 13081 ROOSENDAAL
NL-4700 BH HOLLAND
TEL 011 31 165 582 500
FAX 011 31 165 567 796

The following products sold by SENSUS are certified Kosher with the listed restrictions.

Name	K-ID	Status	Restriction	Size
Fibrelite	GVL-FRCQ	Pareve	Ⓚ SYMBOL	
Frutafit CLR	DGP-LNJM	Pareve	Ⓚ SYMBOL	
Frutafit HD	SFK-KNLL	Pareve	Ⓚ SYMBOL	
Frutafit IQ	XLT-GBMX	Pareve	Ⓚ SYMBOL	
Frutafit TEX!	TFM-JFTB	Pareve	Ⓚ SYMBOL	
Frutalose L60	ZMM-MKWM	Pareve	Ⓚ SYMBOL	
Frutalose L85	PGD-TTJJ	Pareve	LETTER FROM RABBI-BULK	TANKERS
Frutalose L85	WHM-NLTV	Pareve	Ⓚ SYMBOL	
Frutalose L92	KFG-NBLG	Pareve	Ⓚ SYMBOL	
Frutalose LF92	SXB-TPGL	Pareve	Ⓚ SYMBOL	

This certificate is VALID UNTIL September 30, 2008

Verify authenticity by entering K-ID at www.digitalkosher.com

Powered by: K-ID DIGITALKOSHER.COM

RABBI DON YOEL LEVY, Kashruth Administrator

Appendix 8

Halal certificate of Frutafit® and Frutalose® Inulin/Oligofructose from Sensus issued by the Halal Correct Certification

Halal Correct ® الأمانة الإسلامية

International Halal Correct Certification

Halal Certificate شهادة حلال

No: 070103-SSN

تشهد مؤسسة الأمانة الإسلامية، بأن المنتجات المذكورة أسفله، التي تنتجها شركة:

SENSUS located in the Netherlands

Halal Correct Certification ® أنها حلال، وقد تمت مراقبتها من طرف

☆☆☆☆☆

We declare, Hereby, that the below mentioned products of
SENSUS located in the Netherlands
Are Halal; prepared by the rules of Halal Correct Certification ®

☆☆☆☆☆

Product name:	Frutafit®-Inulin/Fructo-oligosaccharides
Product name:	Frutalose® -Fructo-oligosaccharides

العينة الخاصة والمراقبة
Inspection department
Halal Correct Certification
Mr. Ben Ali Salah

مؤسسة الأمانة الإسلامية لمراقبة اللحوم والأغذية
Organization Halal Service Benelux
On behalf of the governing board
Imam Galal A.Amer

Valid until December 2007

This certificate is exclusively delivered for products with a Product Halal declaration.
We are not responsible for products without Halal Correct certificate.
Only original certificates accepted - لا يقبل إلا بالأصل

Appendix 9

Sensus's letter on why organic Chicory Root Extract (Inulin/Oligofructose) can not be made on an industrial scale

Sensus

Borchwerf 3
4704 RG ROOSENDAAL
P.O. Box 1308
4700 BH ROOSENDAAL
The Netherlands

Telephone +31 165 582 500
Telefax +31 165 567 796

National Organic Standards Board
Attn. Mr. Robert Pooler, Agricultural and
Marketing Specialist
USDA/AMS/TM/NOP
Washington, D.C.
Unites States of America

Roosendaal, 26 July 2007

Re. : Organic inulin and oligofructose from chicory
File :
Our reference : JvW/DM

Dear Sir,

With this statement we want to confirm and clarify that inulin and oligofructose from chicory roots cannot be produced "organically" in an economically feasible way. All production plants (see the Addendum) operate at a high processing capacity of a few hundred tons of roots every day in each plant. For the economical production of organic inulin or oligofructose it would be necessary to stop production, clean the equipment and run on organically grown roots for a few days. Only this way we can separate normal production from organic production. This would be practically impossible due to the high turnover costs to stop the production activities for at least one day. We also want to stress that even running a production plant for only a few days would require large amounts of organically grown chicory roots which are also not easily available. Therefore Sensus does not produce these ingredients "organically". We also confirm that none of the other producers of inulin and oligofructose from chicory produce organic inulin or oligofructose. This means that organic inulin and oligofructose from chicory roots are not available in the marketplace.

The addition of non-organic ingredients to foods up to a maximum level of 5 % and still call these foods "organic" is allowed according to European Regulation CEE 2092/91 provided the non-organic ingredients are included in Annex VI.C of the aforementioned Regulation. Inulin or oligofructose are not mentioned in this Annex.

In 1997 a request to include inulin and oligofructose in the Annex was directed to the Belgian Authorities by CEFI (European Confederation of Inulin Producers). In this request CEFI confirmed that none of its members produce organic inulin or oligofructose.
In June 2006 CEFI again submitted a request to the Belgian Authorities (Ministerie van de Vlaamse Gemeenschap) to amend Annex VI.C of EEC 2092/91 with the addition of inulin and oligofructose. In April 2007, CEFI submitted additional information to the Belgian Authorities in order to show the important differences of inulin and oligofructose from chicory and from Jerusalem artichoke. Differences in chemical structure, physical properties technical features in

foodstuffs and nutritional benefits were clearly outlined. Up till now we have not yet received an answer from the authorities.
We want to emphasize here that especially for the bifidogenic, prebiotic properties the evidence for inulin and oligofructose from chicory is very strong. Indeed, based on the scientific evidence the French authorities has lend approval to prebiotic claims , whereas in the Netherlands also a panel of independent experts concluded that inulin and oligofructose were bifidogenic and this can contribute to gut health. Such claims are not possible for inulin or oligofructose from other sources, such as from Jerusalem artichokes.
Also for other physiological effects there is much more and stronger evidence for inulin and oligofructose form chicory than from other sources.
This implies that despite the fact that organic inulin and oligofructose from sources other than chicory is available, this cannot be used to make substantiated claims on food products as the proper evidence for the claims for inulin from e.g. Jerusalem artichoke is not available. This will hamper development of organic products with substantiated claims based on inulin or oligofructose.

Yours faithfully,

Sensus

J. van Wessem
Managing Director

Addendum

The following additional information may be relevant to evaluate this application.

1. Chicory (*Cichorium intybus*) is grown in Belgium, the Netherlands and the north of France, and in Chile. The crop grown in these areas serves as a raw material for:
a. production of inulin and oligofructose and
b. production of roasted chicory as a coffee substitute.
The total area grown in these regions is about 15000 h (delivering about 600.000 t of roots), and by far the most of this is used as raw material for production of inulin and oligofructose.
The first production of inulin and oligofructose from chicory took place in 1985 in Belgium, and the area has grown steadily since then. Until 2005 chicory was also used as a raw material for the production of fructose syrups. This production has ceased completely as a consequence of the changes in EU sugar regulations.

2. There are three suppliers of inulin and oligofructose from chicory:
a. Sensus (with a production plant in Roosendaal, Netherlands);
b. Orafti (with two production sites in Oreye, Belgium and in Chile) and
c. Cosucra (with a plant in Warcoing, Belgium)
Production data are confidential but can be estimated to be in total about 50.000 t of inulin/oligofructose in 2006 in Europe. This estimation is based on the growth area of chicory and the estimated yield of inulin per ha.

3. The temperate climate in the growth region has so far not led to production stops or destruction of crops by pests or adverse weather conditions.

4. The stable political conditions in the EU have prevented restrictions in production and supplies.

Appendix 10

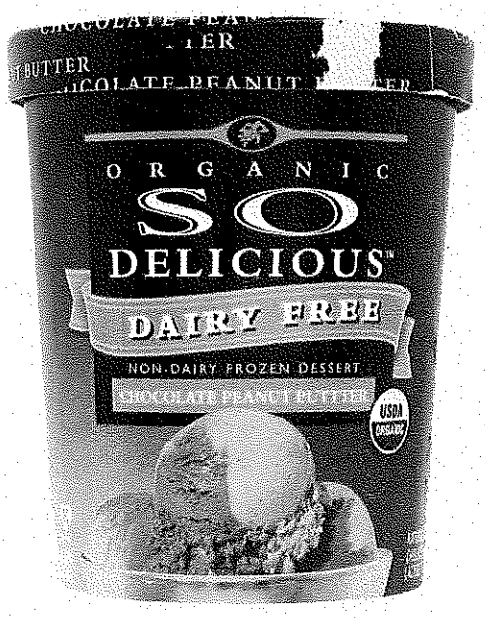
A sample label of an organic frozen dessert containing Chicory Root Extract

gnpd
part of Mintel Group

MINTEL

Organic Non-Dairy Frozen Dessert

Record ID:	10250084
Company:	Turtle Mountain
Brand:	So Delicious
Category:	Desserts & Ice Cream
Sub-Category:	Take Home Ice Cream
Country:	USA
Date Published:	31 Jan 2006
Launch Type:	New Product
Price in local currency:	4.19
Price in Euros:	3.48



Product Description

So Delicious Organic Non-Dairy Frozen Dessert is available in a Chocolate Peanut Butter flavor. The product is USDA-certified organic.

Product Analysis

Package Type:	Tub
Package Material:	Board
Pack Size:	946.00
New Product Count:	1
Storage:	Frozen
Bar Code:	744473720644
Distribution (US records only):	National
Distribution Type (US records only):	Supermarket

Product Variants

Product Variant	Flavours	Positioning Claims
—	Chocolate (unspecified), Peanut Butter	Organic

Ingredients & Nutrition

Ingredients: Organic soymilk (filtered water, organic soybeans, organic dehydrated cane juice, organic brown rice syrup and/or organic tapioca syrup, organic peanuts (roasted in organic peanut oil, salt), organic soybean oil and/or organic, safflower oil, organic cocoa (processed with alkali), chicory root extract, vanillia extract, carob bean gum, non-GMO potato sugar, guar gum, carrageenan, natural flavors

Nutrition: Serving size 1/2 cup (81g), servings per container 8, caloires 140, calories from fat 40, total fat 4.5g, saturated fat 1g, trans fat 0g, cholesterol 0mg, sodium 60mg, total carbohydrate 23g, dietary fiber 2g, sugars 13g, protein 2g, vitamin A 0%, vitamin C 0%, calcium 0%, iron 4%

Appendix 11

A sampling of articles regarding Prebiotic/Bifidogenic effects of Chicory Root Extract (Inulin/Oligofructose) documented for Digestive Health

A Sampling of Research Articles Regarding Prebiotic/Bifidogenic Effects of Chicory Root Extract (Inulin/Oligofructose) Documented for Digestive Health

A Summary Table

Reference (Author, Year)	Substrate	Dose, Duration Subjects (Age)	Results	Reference (Title, Journal)
Gibson et al., 1995	Chicory Oligofructose	15 g/d, 15 days 8 adults (21-48 y)	Significantly increased bifidobacteria, from $10^{8.8}$ to $10^{9.5}$ ($p<0.01$). Lactobacilli tend to increase. Significantly reduced bacteroides ($p<0.01$), clostridia (($p<0.05$) and fusobacteria ($p<0.01$).	Selective Stimulation of Bifidobacteria in the Human Colon by Oligofructose and Inulin (Gastroenterology 108, P 975-982)
Menne et al., 2000	Chicory Oligofructose	8 g/d, 5 weeks	Significant increase in bifidobacteria, from $10^{8.6}$ to $10^{9.6}$ ($p<0.01$). Total anaerobes are unchanged. Lactobacilli tend to increase, Coliforms and Clostridium perfringens tend to decrease.	Fn-type chicory inulin hydrolysate has a prebiotic effect in humans (J Nutr 130, P1197-1199)
Rao, 2001	Chicory Oligofructose	5 g/d, 3 weeks 8 adults (24-48 y)	Close to 1 $\log_{10}$ cycle increase in bifidobacteria ($p<0.001$	The prebiotic properties of oligofructose at low intake levels (Nutr Res 21, P843-848
Kolida et al., 2007	Chicory Inulin	5 and 8 g/d, 2 weeks 30 adults (19-35 y)	Bifidobacteria increase	A double-blind placebo controlled study to establish the bifidogenic dose of inulin in healthy humans (Eur J Clin Nutr doi: 10.1038/ sj.ejcn.1602636 P1-7
Kim et al., 2007	Chicory Inulin	1.5 (± 0.3) g/d, 3 weeks, 14 babies (12.6 weeks ± 6.4 weeks)	Increase in *Bifidobacterium* and *Lactobacillus* in the faeces of formula-fed babies	Supplementation of infant formula with native inulin has a prebiotic effect in formula-fed babies [Asia Pac J Clin Nutr 16 (1): P172-177
Bouhnik et al., 2007	Chicory Inulin	5 g/d, 8 weeks 20 adults (20-58 y)	Bifidobacteria increa (x 12)	Prolonged administration of low-dose inulin stimulates bifidobacteria growth in humans (accepted for publication in Nutr Res)

FYS-34

GASTROENTEROLOGY 1995;108:975–982

Selective Stimulation of Bifidobacteria in the Human Colon by Oligofructose and Inulin

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Medical Research Council, Dunn Clinical Nutrition Centre, Cambridge, England

Background/Aims: Oligofructose and inulin are naturally occurring indigestible carbohydrates. In vitro they selectively stimulate the growth of species of *Bifidobacterium*, a genus of bacteria considered beneficial to health. This study was designed to determine their effects on the large bowel microflora and colonic function in vivo. *Methods:* Eight subjects participated in a 45-day study during which they ate controlled diets. For the middle 15 days, 15 g · day^{-1} oligofructose was substituted for 15 g · day^{-1} sucrose. Four of these subjects went on to a further period with 15 g · day^{-1} inulin. Bowel habit, transit time, stool composition, breath H_2 and CH_4, and the predominant genera of colonic bacteria were measured. *Results:* Both oligofructose and inulin significantly increased bifidobacteria from 8.8 to 9.5 $\log_{10}$ g stool^{-1} and 9.2 to 10.1 $\log_{10}$ g stool^{-1}, respectively, whereas bacteroides, clostridia, and fusobacteria decreased when subjects were fed oligofructose, and gram-positive cocci decreased when subjects were fed inulin. Total bacterial counts were unchanged. Fecal wet and dry matter, nitrogen, and energy excretion increased with both substrates, as did breath H_2. Little change in fecal short-chain fatty acids and breath CH_4 was observed. *Conclusions:* A 15-g · day^{-1} dietary addition of oligofructose or inulin led to *Bifidobacterium* becoming the numerically predominant genus in feces. Thus, small changes in diet can alter the balance of colonic bacteria towards a potentially healthier microflora.

The human large intestine contains a substantial and diverse population of bacteria that is important to human health. This predominantly anaerobic microflora is able to salvage energy for the host through the bacterial fermentation of undigested carbohydrates and protein to short-chain fatty acids,[1] which are then absorbed. The gut microbiota may also synthesize vitamins,[2] protect against invasive species that are often pathogenic,[3,4] and possibly contribute to the economy of essential amino acids in humans.[5,6] However, not all intestinal bacteria are beneficial to health, and a long-established concept is that of beneficial and harmful species.[7] Beneficial genera include *Bifidobacterium* and *Lactobacillus*, both of which are saccharolytic, whereas species such as *Clostridium perfringens* and *Escherichia coli* are considered detrimental.[8,9]

Bifidobacteria are the numerically predominant bacterial genus in the feces of breast-fed infants. It is believed that this may contribute to the protection that breast feeding provides against gut infections.[10–12] Most adults also carry bifidobacteria in their colons[12,13] but in lower numbers than in breast-fed infants. Because of their potentially beneficial properties, there have been attempts to increase their relative proportion in the adult colon. One method has been the feeding of probiotic microorganisms to introduce more bifidobacteria into the bowel. Bifidobacteria administered in this way are able to pass through the terminal ileum[14] and are detected in feces at about $10^{8.8}$ g^{-1}.[15] However, they rapidly disappear from feces when oral dosing ceases.

When administered by mouth, bifidobacteria can alter fecal bacterial enzyme activities,[16] reduce antibiotic-induced side effects,[17] inhibit 2-amino-3-methylimidazo[4,5-*f*] quinoline–induced mammary and liver tumors in rats,[18] and reduce 1,2-dimethylhydrazine induced colonic carcinogenesis in mice in conjunction with oligofructose.[19] They may be partly responsible for colonization resistance, which the resident microflora offers against invading pathogens,[20,21] and bifidobacteria stimulate the immune system towards certain tumors[22,23] and bacterial invasion.[21,24]

What controls the growth of bifidobacteria in the gut? In breast milk, the "bifidus factor" is a glycoprotein containing glucose, galactose, fucose, and *N*-acetyl glucosamine.[12] In uncontrolled studies of elderly people administered 8 g · day^{-1} of a fructooligosaccharide (Neosugar; Meija Seika, Tokyo, Japan), bifidobacterial numbers increased in feces, as did total anaerobes.[25,26] Using in vitro cultures of human fecal bacteria, we have shown that two structurally similar carbohydrates, oligofructose (OF) and inulin, selectively stimulate bifidobacterial growth while maintaining potential pathogens such as

Abbreviations used in this paper: OF, oligofructose; PYG, peptone yeast glucose.

0016-5085/95/$3.00

E. coli and clostridia at low levels.[27] Moreover, in defined coculture experiments, various species of bifidobacteria inhibited the growth of *E. coli* and *C. perfringens*. This effect was caused by the secretion of an inhibitory substance that was independent of changes in the culture pH. Plating experiments showed that this antimicrobial substance variably suppressed species belonging to the genera *Salmonella, Listeria, Campylobacter,* and *Shigella,* as well as *Vibrio cholerae.*[28]

To determine whether the addition of OF or inulin to a normal diet can lead to changes in the gut microflora, we fed these carbohydrates for 15 days to 8 healthy volunteers under controlled dietary conditions, substituting them for 15 $g \cdot day^{-1}$ sucrose in the basal diet. The effect of this dietary change on the major genera of fecal bacteria and the colonic function, including stool output and composition and breath H_2 and CH_4, was measured.

Materials and Methods

Subjects

Eight healthy volunteers (7 men and 1 woman) with a mean age of 33.6 years (range, 21–48 years) and a mean body mass index of 22.4 (range, 18.7–25.4) were used in the study. All subjects underwent a medical examination, were healthy, and had not taken antibiotics for at least 3 months before the commencement of the study. Written consent was obtained from each person, and the protocol was approved by the Medical Research Council Dunn Nutrition Ethical Committee. As part of the initial assessment, a stool sample was collected from the volunteers for bacteriological analyses. All had an initial viable count of bifidobacteria in the range of 10^8–10^9 g wet wt $feces^{-1}$.

Oligofructose and Inulin

Oligofructose was the oligosaccharide fraction of Raftilose (Orafti, Tienen, Belgium). It is composed of molecules of the GFn and Fn type [G, glucose; F, fructose; n, number of fructose moieties linked by β (2,1) linkages in a ratio of about 2:1], with n being between 2 and 6, with an average degree of polymerization of 4. Inulin was the oligosaccharide fraction of Raftiline (Orafti), which was obtained by the extraction of chicory roots. It is composed of molecules of the GFn type, with n ranging from 2 to 60 and an average degree of polymerization of 10.

Diet and Experimental Design

The study was conducted using controlled diets with each subject fed for 15 days. The protocol included an initial few days to settle into the Unit's routine on a free diet and then 45 days that were divided into 15 days of initial control, 15 days when 15 $g \cdot day^{-1}$ sucrose was replaced with 15 $g \cdot day^{-1}$ OF, and finally 15 days of a second control period (15 $g \cdot day^{-1}$ sucrose). This was then followed by 5 days on a free diet for stool collections to complete fecal marker recoveries. Four of the subjects went on to participate in another 25-day study, comprising the same control sucrose diet for 10 days, with 15 $g \cdot day^{-1}$ sucrose being substituted by 15 $g \cdot day^{-1}$ inulin during a further 15 days, and again followed by 5 days of stool collections to recover all markers. Subjects lived in the metabolic suite of the Medical Research Council Dunn Clinical Nutrition Centre. No food other than the diets provided or any alcohol were allowed. Subjects' normal energy requirements were assessed before the study from height, weight, and food records. The diets were weight maintaining.

Three 1-day menus comprising normal foods were designed and fed in rotation to the subjects throughout the study. The basal diet provided 9 MJ energy and had the following composition[29]: protein, 63 g; fat, 93 g (polyunsaturated/saturated fat ratio, 0.19); starch, 142 g (resistant starch, 4.7 g[30]); sugars, 124 g; and nonstarch polysaccharides, 16.4 g.[31] Energy intakes were adjusted to meet individual needs by adding 1-MJ increments of the same composition as the basal diet to that diet. Energy intakes ranged from 9 to 15 $MJ \cdot day^{-1}$. Five grams of sucrose, OF, and inulin were administered with breakfast in a free form, and the other 10 g was incorporated into biscuits. Volunteers kept a daily diary in which to record their weight, times of radiopaque markers taken, stools passed, and any unusual events such as diarrhea and flatulence.

Collection and Analysis of Breath

End expiratory breath samples were collected in duplicate using a dedicated sampling device[32] together with a room air sample at 7 PM and 10 PM on days 10, 13, and 15 of each experimental period, except for during the control sucrose period before inulin, when breath samples were collected on days 8 and 10. Hydrogen was measured using an exhaled hydrogen monitor (GMI, Renfrew, Scotland) that was adjusted to detect to 0.1 ppm and was calibrated daily using a 52-ppm standard. Methane was measured using a Pye 104 gas chromatograph[32] (Pye Unicam; Cambridge, Cambridgeshire, England) that was calibrated with 4.8- and 48-ppm CH_4 standards. Breath CH_4 was reported as the concentration in the sample less that in room air. All samples were analyzed within 24 hours of collection and corrected for H_2 losses during storage using previously obtained correction factors for the syringes.

Stool Collection and Estimation of Intestinal Transit Time and Balance Markers

All stools passed were collected and, apart from those required for bacteriologic analyses, were immediately weighed and frozen at −20°C. During the last 6 days of each dietary period, three small subsamples of well-mixed stool were obtained within 1 hour of defecation for immediate bacteriologic analyses, and another sample was frozen for short chain fatty acid measurements. Mean transit time was measured using the continuous marker method.[33] Volunteers were given 10 radiopaque shapes with each meal (30 per day), and marker type was changed every 15 days with diet. All stools were x-

rayed before any sampling, and the markers in each stool were counted. The mean transit time was calculated, and marker excretion was used to correct stool weights, nitrogen, energy, and ash outputs so that results represented a complete 5-day collection period.[34]

Diet and Fecal Analysis

All stools from the last 5 days of each dietary period were pooled, weighed, and lyophilized to constant weight. The resultant dry sample was milled in a centrifugal mill and used for fecal analyses. Total nitrogen was measured using an automated Kjeldahl procedure, energy was determined by an adiabatic bomb calorimeter, and ash was quantified at 500°C after an initial burning off of organic residues over a flame. Fecal subsamples for short-chain fatty acid analysis were defrosted at 4°C, mixed, and 1 g was diluted to 10 mL and centrifuged. One milliliter of the supernatant was then acidified and extracted into diethyl ether over ice,[35] along with three calibration standards. 2-Methyl-valerate was used as an internal standard. The ether was dried with fused calcium chloride, and 0.5 µL ether was injected onto a Pye 204 gas liquid chromatograph fitted with a flame ionization detector and a 25-m BP21-fused silica capillary column (inside diameter, 0.53 mm) (S.G.E. Australia, Ringwood, Victoria, Australia). The column was held at 100°C with helium as the carrier gas at 11 psi of inlet pressure. There was linearity of extraction and recovery for all short-chain fatty acid analysis. All samples were analyzed in duplicate.

Bacterial Enumeration and Identification

All bacteriologic analyses of feces were performed within 1 hour of defecation. Five grams of fresh stool was homogenized using 50 mL of anaerobic 0.1 mol/L sodium phosphate buffer (pH 7.0) to provide a 10% (wt/vol) fecal slurry. The slurry was sieved (aperture 250 µm), and 1 mL was diluted serially in half-strength Wilkins Chalgren anaerobic broth (Oxoid, Basingstoke, Hampshire, England) in an anaerobic cabinet ($H_2/CO_2/N_2$, 10:10:80). Plates were inoculated in triplicate using selective media for the enumeration of total anaerobes, total aerobes, coliforms, gram-positive cocci, bifidobacteria, bacteroides, fusobacteria, lactobacilli, and clostridia, and plates were incubated aerobically or anaerobically as appropriate.[35–39]

After incubation, individual colonies were removed from the media plates and subcultured into peptone yeast glucose (PYG) broth.[35] Bacteria were then characterized to genus level on the basis of colonial appearance, gram reaction, spore production, cell morphology, and fermentation end-product formation. Bifidobacteria were further identified by the production of acetate and lactate in PYG broth and a positive detection of fructose-6-phosphate phosphoketolase activity in crude cell extracts.[39]

Statistical Analyses

Systat version 5.2.1 was used for all statistical analyses. Diet and fecal results were analyzed using paired Student's *t* tests and presented as mean (SEM), and breath and short chain fatty acids results were analyzed by analysis of variance (ANOVA) and presented as SEM. Before statistical analysis of bacteriologic results, the normality of a representative set of data was checked by means of the Kolmogorov–Smirnov test. Bacterial counts were logarithmized to fit a normal distribution, and the normality of the data was confirmed by Box and Whisker plots of the logged counts. ANOVA was used to show the effect of the diet on bacteriologic analyses. To confirm unequivocally statistical results of the bacterial count data, the analysis was repeated by means of the nonparametric Kruskal–Wallis test, which does not assume normality of the distribution. Bacteriologic results are presented as mean values (SD).

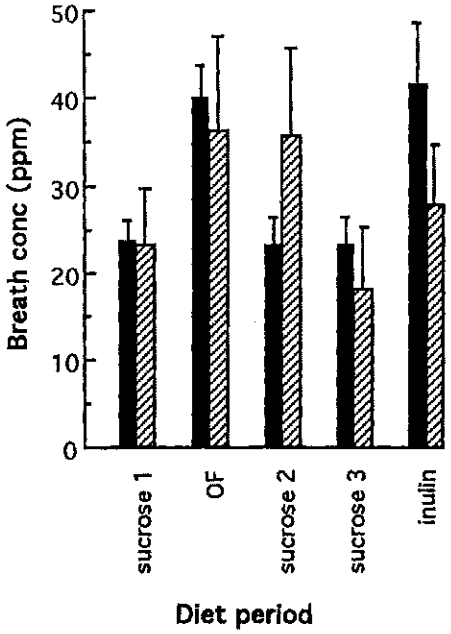

Figure 1. Mean breath H_2 (■) and CH_4 (▨) concentrations in 8 subjects with 15 g·day^{-1} of sucrose, OF, or inulin added.

Results

All 8 subjects completed the controlled diet study without any periods of ill health or abnormal bowel habit and maintained their weight. One subject complained of flatulence intermittently throughout, and 1 complained of flatulence and abdominal pain during the OF feeding period.

Breath H_2 and CH_4

The inclusion of OF in the diet resulted in a significant increase ($P = 0.001$) in breath H_2 concentration to reach 40.1 ± 3.6 ppm compared with the first and second control sucrose periods of 23.8 ± 2.0 ppm and 23.5 ± 2.9 ppm (Figure 1). This increase occurred for all the subjects. Only 3 of the 8 subjects had detect-

Table 1. Bowel Habit, Fecal Composition, and Apparent Digestibility of Nitrogen, Energy, and Ash

	Sucrose 1	OF	Sucrose 2
Stools (*per 5 days*)	4.1 ± 0.52	5.3 ± 0.41[a]	5.3 ± 0.53[a]
Mean transit time (*h*)	51.5 ± 8.65	53.8 ± 9.74	56.6 ± 12.2
Dry matter (%)	22.4 ± 1.97	22.2 ± 1.84	23.5 ± 1.76
Corrected wet wt ($g \cdot day^{-1}$)	135.8 ± 22.8	154.1 ± 22.9	131.3 ± 19.5[b]
Corrected dry wt ($g \cdot day^{-1}$)	27.8 ± 2.79	32.2 ± 3.39	29.0 ± 3.43
Nitrogen density (*% dry matter*)	5.49 ± 0.22	5.76 ± 0.18	5.38 ± 0.21[b]
Nitrogen excreted ($g \cdot day^{-1}$)	1.51 ± 0.12	1.83 ± 0.17[a]	1.55 ± 0.19[b]
Energy density ($kJ \cdot g$ *dry matter*$^{-1}$)	21.5 ± 0.50	21.5 ± 0.53	21.9 ± 0.57[a]
Energy excreted ($kJ \cdot day^{-1}$)	596.6 ± 56.4	695.6 ± 78.3	639.9 ± 83.3
Ash density (*% dry matter*)	14.1 ± 0.78	12.9 ± 0.73[a]	14.1 ± 0.74[b]
Ash excreted ($g \cdot day^{-1}$)	3.87 ± 0.30	4.02 ± 0.17	3.94 ± 0.22
Dry matter digestibility (%)	96.2 ± 0.34	95.7 ± 0.37	96.1 ± 0.44
Nitrogen digestibility (%)	91.9 ± 0.75	90.1 ± 0.92[a]	91.6 ± 1.06[b]
Energy digestibility (%)	96.2 ± 0.40	95.6 ± 0.49	95.9 ± 0.59
Ash digestibility (%)	80.3 ± 1.73	79.2 ± 2.04	79.7 ± 2.20

NOTE. Values are expressed as mean ± SEM.
[a]Significantly different from sucrose 1.
[b]Significantly different from OF ($P < 0.05$).

able CH_4 in breath during the study period, and these subjects showed no obvious relationship between OF intake and breath CH_4 concentration (Figure 1) (23.5 ± 6.2 ppm and 35.4 ± 10.1 ppm for sucrose 1 and 2, respectively; 36.2 ± 10.7 ppm for OF).

When subjects were fed inulin, a similar significant increase in breath H_2 was observed (from 23.4 ± 3.11 ppm to 41.5 ± 6.94 ppm; $P = 0.031$). Of the 4 subjects who received inulin, 3 were methanogenic, and breath CH_4 increased in 2 of these 3 but decreased in the third, resulting in an increase, which was not significant in mean breath CH_4, from 18.0 ± 7.22 ppm to 27.7 ± 6.72 ppm.

Bowel Habit, Stool Composition, and Digestibility

Both OF and inulin increased stool frequency, the excretion of wet and dry matter, nitrogen, and energy (Tables 1 and 2), with increases in wet matter and nitrogen excretion being significant when subjects were fed OF. Moisture content was unaffected, and there was an increase in transit time that was not significant throughout all diet periods, suggesting a slight underlying constipating effect of the basal diet. Ash excretion also increased when subjects were fed inulin, although changes were not significant. When considering only the results from the 4 subjects who participated in both OF and inulin diet periods (Table 2), the inclusion of inulin resulted in a larger increase in fecal wet and dry weight than did OF (34% and 23% vs. 14% and 12%); however, it also slowed mean transit time more than OF did.

All measured components of the diet were highly digestible (Table 1), and the inclusion of OF had very little effect on apparent digestibility, except a very small but significant decrease in apparent nitrogen digestibility compared with both the sucrose diet periods ($P < 0.05$).

Total short-chain fatty acids in feces (Table 3) averaged between 111 and 131 $mmol \cdot kg^{-1}$ for each dietary pe-

Table 2. OF and Inulin Fecal Results for the 4 Subjects Who Participated in Both Studies

	OF			Inulin	
	Sucrose 1	OF	Sucrose 2	Sucrose 3	Inulin
Stools (*per 5 days*)	3.3 ± 0.85	4.5 ± 0.65[a]	4.5 ± 0.87[a]	3.8 ± 0.63	4.3 ± 0.63
Mean transit time (*h*)	55.9 ± 14.7	56.4 ± 17.3	60.7 ± 22.0	51.1 ± 7.73	57.1 ± 18.3
Dry matter (%)	25.0 ± 3.4	24.1 ± 3.6	24.1 ± 3.5[a]	24.6 ± 1.9	23.5 ± 2.6
Corrected wet wt ($g \cdot day^{-1}$)	107.1 ± 22.4	121.9 ± 18.0	104.8 ± 24.4	92.4 ± 12.6	123.4 ± 24.0
Corrected dry wt ($g \cdot day^{-1}$)	24.5 ± 2.7	27.4 ± 1.3	22.8 ± 2.7	22.2 ± 1.9	27.3 ± 3.3
Nitrogen density (*% dry matter*)	5.71 ± 0.10	6.11 ± 0.09[a]	5.59 ± 0.17[b]	5.88 ± 0.25	5.81 ± 0.26
Nitrogen excretion ($g \cdot day^{-1}$)	1.41 ± 0.17	1.68 ± 0.10	1.29 ± 0.19	1.31 ± 0.16	1.56 ± 0.14
Energy density ($kJ \cdot g$ *dry matter*$^{-1}$)	21.3 ± 0.13	21.4 ± 0.36	21.9 ± 0.03[a]	21.0 ± 0.17	20.8 ± 0.28
Energy excretion ($kJ \cdot day^{-1}$)	523 ± 56	585 ± 19	497 ± 60[a,b]	466 ± 42	565 ± 64
Ash density (*% dry matter*)	14.4 ± 1.16	13.0 ± 0.83[a]	14.9 ± 0.90[b]	14.9 ± 0.39	14.0 ± 0.89
Ash excretion ($g \cdot day^{-1}$)	3.44 ± 0.15	3.55 ± 0.11	3.33 ± 0.25	3.28 ± 0.21	3.74 ± 0.33

NOTE. Values are expressed as mean ± SEM; n = 4.
[a]Significantly different from sucrose 1.
[b]Significantly different from OF ($P < 0.05$).

Table 3. Fecal Short-Chain Fatty Acid Concentrations and Molar Ratios During Three Diet Periods

	Sucrose 1 (n = 15)	OF (n = 16)	Sucrose 2 (n = 16)
Acetate	80.2 ± 9.22	76.8 ± 6.77	64.3 ± 5.64
Propionate	23.5 ± 3.04	23.0 ± 2.68	21.8 ± 2.44
Isobutyrate	2.09 ± 0.198	2.10 ± 0.234	2.46 ± 0.299
Butyrate	19.3 ± 2.30	18.5 ± 2.20	16.0 ± 2.00
Isovalerate	2.92 ± 0.331	2.98 ± 0.426	3.67 ± 0.501
Valerate	2.33 ± 0.304	2.67 ± 0.390	2.46 ± 0.264
Caproate	0.81 ± 0.247	0.92 ± 0.285	0.52 ± 0.170[a]
Acetate	60.4 ± 1.59	60.4 ± 1.36	58.1 ± 1.13
Propionate	17.9 ± 1.38	17.8 ± 1.26	19.0 ± 1.26
Isobutyrate	1.92 ± 0.27	1.90 ± 0.27	2.43 ± 0.30
Butyrate	14.5 ± 0.66	14.1 ± 0.93	13.9 ± 0.80
Isovalerate	2.72 ± 0.43	2.75 ± 0.46	3.66 ± 0.51
Valerate	1.92 ± 0.23	2.17 ± 0.26	2.27 ± 0.26
Caproate	0.72 ± 0.21	0.80 ± 0.23	0.58 ± 0.17[a,b]

NOTE. Two fecal samples were collected on separate days from each of 8 subjects (1 sample was unobtainable). Values are in millimoles per kilogram of feces and are expressed as mean ± SEM.
[a]Significantly different from OF ($P < 0.008$).
[b]Significantly different from sucrose 1 ($P = 0.034$).

riod. No changes were observed in fecal short-chain fatty acid concentrations during any of the dietary periods, except for a minor decrease in caproate during the second sucrose period ($P = 0.008$). Similarly, molar ratios (Table 3) were unaltered, again with the exception of caproate ($P = 0.002$).

Stool Bacteriology

The addition of both OF and inulin had little effect on total viable counts of aerobes or anaerobes. However, for both carbohydrates, bifidobacterial counts (Table 4) were significantly higher than during the sucrose periods ($P < 0.01$). This increase was observed in 7 of the 8 volunteers when they were fed OF and all 4 subjects who were fed inulin. Numbers of bifidobacteria declined significantly ($P < 0.01$) when OF was withdrawn, showing that the increase was directly attributable to the addition of OF to the diet and not to some underlying change in the microflora from becoming a resident at the Dunn Clinical Nutrition Centre on a controlled diet.

A significant decrease in counts of bacteroides ($P < 0.01$) was observed during the OF diet period with the result that bifidobacteria became the numerically predominant species in 7 of the 8 volunteers. Although bacteroides numbers did not change when subjects were fed inulin, bifidobacteria still became the predominant species in 3 of the 4 volunteers.

Clostridia and fusobacteria both declined significantly when subjects were fed OF ($P < 0.05$ and $P < 0.01$). In most of the volunteers, subsequent ingestion of sucrose was required for a relatively long period (>13 days) for counts of fusobacteria to recover to levels detected during the initial control period. Thus, viable counts in the second sucrose period were significantly lower ($P < 0.05$) than those recorded in the first sucrose period (Table 4). Counts of fusobacteria and clostridia were unchanged by the addition of inulin, although counts of gram-positive cocci decreased significantly ($P < 0.001$). In all the volunteers, numbers of lactobacilli were not affected by OF, but inulin increased lactobacilli counts, although not significantly ($P = 0.075$). Counts of coliforms were unaffected by any dietary addition (Table 4).

Discussion

Although the resident bacteria of the colon depend on exogenous (dietary) substrates to a large extent for energy and growth, particularly nondigested carbohy-

Table 4. Mean Viable Bacterial Counts From Three Fecal Samples With Either 15 g·day^{-1} of Sucrose, OF, or Inulin Added to a Controlled Diet

Bacteria	Sucrose 1 (n = 8)	OF (n = 8)	Sucrose 2 (n = 8)	Sucrose 3 (n = 4)	Inulin (n = 4)
Total aerobes	6.4 ± 1.3	6.2 ± 1.0	6.3 ± 0.9	6.7 ± 0.88	6.7 ± 1.0
Coliforms	6.0 ± 1.2	5.9 ± 0.7	5.8 ± 1.0	6.3 ± 1.2	6.2 ± 1.4
Gram-positive cocci	5.8 ± 1.0	5.8 ± 0.9	5.5 ± 0.8	6.0 ± 0.32	5.5 ± 0.27[d]
Total anaerobes	9.9 ± 1.4	10.2 ± 0.9	10.3 ± 0.8	10.6 ± 0.22	10.7 ± 0.25
Bifidobacteria	8.8 ± 0.5	9.5 ± 0.7[a]	8.9 ± 0.9[b]	9.2 ± 0.46	10.1 ± 0.44[d]
Bacteroides	9.4 ± 0.8	8.8 ± 1.1[a]	8.9 ± 0.9[c]	9.7 ± 0.47	9.8 ± 0.50
Fusobacteria	8.5 ± 0.7	7.7 ± 0.9[a]	8.1 ± 0.8[c]	8.8 ± 0.44	8.9 ± 0.62
Clostridia	8.0 ± 1.2	7.5 ± 0.9[c]	7.7 ± 0.7	8.3 ± 0.54	8.1 ± 0.72
Lactobacilli	6.8 ± 1.2	7.0 ± 1.4	7.1 ± 1.0	6.0 ± 1.1	6.3 ± 0.76

NOTE. Values are in $\log_{10}$ grams wet weight of feces^{-1} and are expressed as mean ± SD.
[a]Significantly different from sucrose 1 ($P < 0.01$).
[b]Significantly different from OF ($P < 0.01$).
[c]Significantly different from sucrose 1 ($P < 0.05$).
[d]Significantly different from sucrose 3 ($P = 0.0002$).

drates, it has proved experimentally difficult to produce consistent changes by dietary manipulation. The colon seems to be a self-regulating environment. The advent of "dietary fiber" approximately 20 years ago led to a number of studies that aimed to show beneficial effects on the microflora, but most failed to show any significant change.[40,41] Some alterations in fecal bacterial enzyme activity have been noted[42,43] but no consistent changes in bacterial numbers. Similar findings emerge from studies of meat[44] and fat[45] on the microflora. An increase in overall numbers of bacteria in the colon with additional fermentable carbohydrate in the diet is apparent from studies of fecal biomass excretion,[46] but the balance of groups seems difficult to change by dietary means alone. Although specific effects of diet on the microflora are rarely observed, we have recently shown that the growth of sulfate-reducing bacteria could be stimulated by adding sulfate to the diet.[47]

The present study has shown that a small alteration in diet, namely, the substitution of 15 $g \cdot day^{-1}$ sucrose by 15 $g \cdot day^{-1}$ OF or inulin, can lead to significant changes in the balance of the constituent microflora in the large intestine. Despite the relatively insensitive nature of bacteriologic techniques for enumerating microorganisms in feces and wide individual variation, bifidobacteria increased significantly when subjects were fed OF and inulin, whereas numbers of potential pathogens decreased with OF. These observations confirm results from in vitro studies[27,48,49] that showed that the stimulation of bifidobacterial growth was relatively specific to OF and the related carbohydrate inulin. In vivo studies with other fermentable carbohydrates have failed to show a bifidogenic effect. It is believed that bifidobacteria have relatively high amounts of β-fructosidase that is selective for β1-2 glycosidic bonds present in fructooligosaccharides.[50] Subsequent transport mechanisms and rates of hydrolysis may also be faster. After oligosaccharide hydrolysis, monomers then serve as an efficient growth substrate for the bifidus pathway of hexose fermentation.[51] In addition, the inhibitory effects of bifidobacterial growth on other colonic organisms[28] are likely to assist in the competitive influence that occurred with OF and inulin.

OF is a lower-molecular-weight version of inulin, and the two forms exist in plants such as artichokes, chicory, onions, leeks, garlic, asparagus, with smaller amounts in many cereals. Inulins are mostly linear polymers of fructose with glucose as the terminal sugar. OF, usually DP 2–5, is produced commercially in one of two ways: either by partial hydrolysis using endoglycosidases, e.g., Raftilose, which is made from chicory inulin, or by synthesis from sucrose using fungal fructofuronosidase, e.g., Neosugar.[25] OF is almost certainly not digested in the human small intestine, although final proof of this is still lacking.[52] Its recovery from the small intestine of rats is approximately the same as that of an unabsorbed marker, polyethylene glycol.[53] OF is not hydrolyzed when incubated with human salivary enzymes or rat pancreatic homogenate,[25] and oral dosing of OF does not affect blood glucose in humans.[25,54,55] Fermentation in the large bowel is most probable. In vitro incubation of ^{14}C-labeled OF with the cecal contents from rats showed 66% of the label appearing as short-chain fatty acids, with some being metabolized to CO_2 or incorporated into the biomass.[56] In the same study, feeding OF to conventional rats showed rapid fermentation of OF, whereas germ-free animals delayed excretion of label for many hours, and substantial amounts appeared in feces. Breath H_2 studies in humans have indicated that the majority of OF is fermented.[54] These oligosaccharides cannot be detected in feces even after inulin feeding.[57] In the present studies, a clear increase in breath H_2 was observed during OF and inulin feeding, although changes in breath CH_4 were more erratic in the 3 methanogenic subjects.

Previous studies in humans have shown that OF had a modifying effect on stool frequency, reducing functional constipation[58] and relieving both constipation and loose stools.[55] In our study, stool frequency increased when OF was included in the diet. However, the increase persisted after OF was replaced by sucrose, suggesting that this effect was not mediated via the presence of a fermentable substrate in the colon but more likely some other factor, such as a change in the composition of the colonic microflora. Increases (14% and 34%) in daily stool output were observed when OF and inulin were introduced into the diet. In this respect, they are acting like any other indigestible carbohydrate, such as nonstarch polysaccharides[59] and resistant starch,[60] which produce a laxative effect through fermentation. In terms of the magnitude of the effect determined in the present study (1.3 g and 2.0 g increase in stool weight per gram in subjects fed OF and inulin, respectively), this is less than that observed with sources of nonstarch polysaccharides, such as bran (5.4 g) and fruit and vegetables (4.7 g), but similar to that produced by rapidly fermented nonstarch polysaccharides, such as pectin (1.2 g).[59] The increase in fecal output is likely to be attributable to an increase in biomass that is confirmed by the significant increase in nitrogen excretion observed for both substrates. The additional 0.32 g of N excreted is equivalent to approximately 5 g of bacterial solids,[61] and at the moisture content of the stools in the present study, this amount is the equivalent of 20–25 g of wet stool. This is almost exactly the increase observed (Tables 1 and 2) in stool wet weight output.

Fifteen grams of OF has a gross energy of about 240 kJ. During the OF dietary period, about 77 $kJ \cdot day^{-1}$ additional fecal energy was excreted, which suggested that although fermentation of OF resulted in some increased excretion of energy, much of the energy provided became available through the absorption of short-chain fatty acids. Although there was a larger increase in fecal energy excreted when subjects were fed inulin (about 99 $kJ \cdot day^{-1}$), energy from this increase was still available through fermentation. The fact that all subjects maintained their weight when 15 g of sucrose was substituted by OF or inulin confirms that, at this level of inclusion, they had little effect on total metabolizable energy. Previous studies have shown that feeding subjects OF increases fecal mineral losses[62]; however, in this study, OF had no effect on total minerals (ash) excreted, although inulin feeding did result in an increase in excreted minerals that was not significant. In general, OF and inulin had very little effect on bowel habit and fecal composition other than increasing output. The most important effects were those on the colonic microflora.

Both OF and inulin acted as selective bifidogenic factors by increasing their numbers in absolute terms and as a proportion of total anaerobes, thus resulting in *Bifidobacterium* becoming the predominant bacterial genus. Are the changes which these oligosaccharides induce in the colonic microflora of any benefit to health? Bifidobacteria can represent up to 95% of the total gut microflora of breast-fed infants compared with only 25% in adults.[12,13] This is believed to be a principal reason why such infants suffer relatively less gastrointestinal infection than bottle-fed infants. Although bifidobacteria have been implicated in some opportunistic infections,[63] they are generally regarded as benign and even beneficial. They stimulate immune function, particularly against malignant cells, and produce vitamins of the B group, and perhaps most importantly, we have shown in vitro that they suppress the growth of pathogenic species.[28] In this present study, numbers of bacteroides, clostridia, and fusobacteria were significantly decreased when subjects were fed OF. All of these may be pathogenic, e.g., through their proteolytic capabilities and toxin production.

In conclusion, this study has shown that a relatively straightforward dietary manipulation may alter the composition of the gut bacteria to produce a potentially healthier community.

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Fn-type Chicory Inulin Hydrolysate Has a Prebiotic Effect in Humans

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ABSTRACT The partial enzymatic hydrolysis of chicory inulin (GFn; $2 \leq n \leq 60$) yields an oligofructose preparation that is composed of both GFn-type and Fn-type oligosaccharides ($2 \leq n \leq 7$; $2 \leq m \leq 7$), where G is glucose, F is fructose, and n is the number of $\beta(2\rightarrow1)$ bound fructose moieties. Human studies have shown that feeding GFn-type oligomers significantly modifies the composition of the fecal microflora especially by increasing the number of bifidobacteria. The experiments reported here were used to test the hypothesis that the Fn-type molecules have the same property. During a controlled feeding study, 8 volunteers (5 females and 3 males) consumed 8 g/d of an Fn-rich product for up to 5 wk. Fecal samples were collected and analyzed for total anaerobes, bifidobacteria, lactobacilli, bacteroides, coliforms and *Clostridium perfringens*. Both 2 and 5 wk of oligofructose feeding resulted in a selective increase in bifidobacteria ($P < 0.01$). In addition, a daily intake of 8 g of the Fn-type oligofructose preparation reduced fecal pH and caused little intestinal discomfort. J. Nutr. 130: 1197–1199, 2000.

KEY WORDS: • prebiotics • inulin • oligofructose • humans

The colon, along with its bacterial microflora, is an important organ that provides a great variety of functions, such as digestion, fermentation, metabolic, immunological and protective functions, as well as detoxifying functions, that are essential to the whole organism (Cummings 1997). Proliferation of bifidobacteria in fecal microflora, a surrogate marker for the colonic microbiota, has been associated with several beneficial effects. A dietary approach aimed at improving the composition of the fecal microflora by supplying substrates that allow selective proliferation of such indigenous bacteria, the prebiotic approach, has been proposed (Gibson and Roberfroid 1995) and validated in different human studies using different nondigestible oligosaccharides (Gibson et al. 1999). In particular, it has been shown that the consumption of chicory inulin or its partial hydrolysate (oligofructose), a mixture of $\beta(2\rightarrow1)$ bound GFn-type (glucosyl-[fructosyl]n-1-fructose) and $\beta(2\rightarrow1)$ bound Fn-type ([fructosyl]n-1-fructose) species (De Leenheer and Hoebregs 1994), significantly modifies the composition of the human fecal flora in such a way that bifidobacteria become numerically predominant (Roberfroid et al. 1998, Van Loo et al. 1999). Native chicory inulin is composed of >99% of the GFn-type species ($2 \leq n \leq 60$), but the oligofructose preparation, which is produced from inulin by partial enzymatic hydrolysis, is a mixture of both GFn[2] ($2 \leq n \leq 7$) and Fn ($2 \leq n \leq 7$)-type molecules [where G is glucose, F is fructose and n is the number of $\beta(2\rightarrow1)$ bound fructose moieties] which also occur naturally in plant foods such as banana, garlic, onion, salsify, asparagus, leek, wheat, chicory, etc. (Van Loo et al. 1995).

The objective of the present study was to test the hypothesis that, like the GFn-type, the Fn-type chicory oligofructose preparation selectively stimulates the growth of fecal bifidobacteria in humans. The protocol for the human study was very similar to recently published studies in terms of number of volunteers (8–12), protocol and bacteriological methodologies employed (Buddington et al. 1996, Gibson et al. 1995, Kleessen et al. 1997, Williams et al. 1994).

MATERIALS AND METHODS

Chemicals. All chemicals used in this study were of the purest grade available and were purchased from Merck (Darmstadt, Germany), Oxoid (Basingstoke, United Kingdom) or Sigma (St. Louis, MO).

Study food. The Fn-type-rich chicory oligofructose preparation was provided by ORAFTI (Tienen, Belgium) as Raftilose® L60, which is produced by partial enzymatic hydrolysis of a refined hot-water extract of chicory roots (i.e., inulin). It is available as an aqueous syrup containing 750 g/kg dry matter composed of 75 (10%) glucose + fructose, 225 g (30%) sucrose and 450 g (60%) oligofructose [with 45 g (10%) GFn-type and 405 g (90%) Fn-type]. The product used in the experiments was of food-grade quality.

Volunteers. The study protocol was approved by the ad hoc ethical committee of the University (UCL-Brussels, Belgium) and complies with the Helsinki declaration of 1975 as revised in 1983. No history of gastrointestinal disease and no use of gastrointestinal or antibiotic medications for at least 3 mo prior to and during the trials were the inclusion criteria. Human subjects who participated in the trial were five women and three men aged between 20 and 50 y having a body mass index between 19 and 25 kg/m^2, and between 18 and 24 kg/m^2, respectively. Subjects gave written consent to participate in the study.

Protocol for the human study. The eight volunteers participated in the experiment, which lasted for 7 wk divided into three successive periods: i) control, a period of 2 wk, during which the volunteers were all given a controlled diet without any addition of oligofructose; ii) treatment 1, a first treatment period of 2 wk, during which the controlled diet was supplemented with 8 g/d of chicory oligofructose; iii) treatment 2, a second treatment period of 3 wk, during which the volunteers consumed their usual home-cooked diet to which they added 8 g/d of chicory oligofructose. The chicory oligofructose (Raftilose® L60) composed of 90% Fn-type and 10% GFn-type molecules was incorporated into orange juice, various desserts (puddings, creams and fruit mousses), cakes and biscuits that were part of the

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[2] Abbreviations used: cfu, colony-forming units; GFn, G is glucose, F is fructose, and n is number of $\beta(2\rightarrow1)$ fructose moieties; Fn, F is fructose, and n is number of $\beta(2\rightarrow1)$ fructose moieties.

food consumed by the volunteers during the day, in such quantities as to provide a total daily intake of 8 g of chicory oligofructose, of which 90% (7.2 g) was pure Fn-type.

Feeding a controlled diet during periods 1 and 2 was intended to minimize the interindividual variations in food intakes that could have influenced the composition of the fecal microflora independent of oligofructose intake.

During these two periods, the volunteers were required to visit a central restaurant, where they had access to a buffet (breakfast and lunch) and were given a vacuum-sealed dinner to consume at home. These meals were prepared so as to minimize the consumption of naturally oligofructose/inulin-rich products (Van Loo et al. 1995) like onions, leeks, bananas, artichokes and wheat, as well as yogurts and fermented milk products. During these two periods, the foods given to the volunteers were very similar, except for the intake of chicory oligofructose (8 g/d) during period 2. During period 3, the volunteers were asked to consume their usual home-cooked meals but still excluding oligofructose/inulin-rich food products and fermented dairy products.

As in other studies on the bifidogenic effect of fructans (Buddington et al. 1996, Gibson et al. 1995, Kleessen et al. 1997, Williams et al. 1994), each volunteer acted as his/her own control and no separate placebo group was included. Using such a protocol avoids a cross-over design in which the length of the wash-out interval is often difficult to evaluate precisely.

Sample collection. Fresh stools were collected: sample 1 (last day of wk 2) at the end of the control period; sample 2 (last day of wk 4) at the end of the treatment 1 period; and sample 3 (last day of wk 7) at the end of the treatment 2 period.

During both the control and treatment 1 periods, the volunteers were requested to complete a daily well-being questionnaire, providing information about possible digestive discomfort (cramps, bloating, flatulence, soft stools or diarrhea) as well as frequency and appearance of stools.

Protocol for bacteriological analyses (Beerens 1991, Gibson et al. 1995). All stool samples (minimum weight 20 g) were processed anaerobically (desk-type home-made anaerobic glove-box containing an atmosphere of H_2, CO_2 and N_2, 10:10:80) within 60 min after defecation. Samples were weighed and then homogenized in 0.1 mol/L (pH 7) phosphate buffer to obtain a 100 g/L fecal suspension. Serial dilutions were prepared using half-strength Peptone water (Oxoid), the samples (0.1 mL) were inoculated onto agar medium specific for the growth of total anaerobes (Wilkins-Chalgren anaerobic agar), bifidobacteria (*Clostridia* agar supplemented with 0.0125 g/L iodoacetic acid, 0.02 g/L nalidixic acid, 0.05 g/L kanamycin, 0.009 g/L polymyxin, 0.025 g/L triphenyltetrazolium chloride), lactobacilli (Rogosa), coliforms (MacConkey #3), bacteroides (BMS supplemented with 5 g/L glucose, 0.5 g/L ammonium sulfate, 0.01g/L nalidixic acid and 0.003 g/L vancomycin) and *Clostridium perfringens* (Tryptose Sulfite Cycloserine Agar Base or TSC supplemented with fluorcult).

Anaerobic incubations (in duplicate) for colony development took place in anaerobic jars containing Anaerocult A (Merck). For each fecal sample, a count was made of viable colony-forming units (cfu) of total anaerobes after incubation at 37°C for 4 d, bifidobacteria (4 d), bacteroides (4 d), lactobacilli (3 d), coliforms (1 d) and clostridia (1 d). After incubation, individual colonies were removed from the plates and subcultured into peptone/yeast/glucose broth. Bacteria were characterized to genus level on the basis of colony appearance, Gram's reaction and cell morphology. Presumptive culture identities were confirmed through colony morphotype, microscopic characteristics and limited biochemical tests (Gibson et al. 1995).

Statistical analysis. The nonparametric Friedman test was used after logarithmic transformation of the data. This test, made by order of rank (rank averages) was chosen because it permits comparison of several mean values of nonindependent observations, which is the case in this study, where comparable samples were all taken from the same volunteers but during different feeding periods. Results were statistically analyzed on the basis of: i) a global comparison of mean values to identify differences between the three feeding periods and ii) paired comparisons to search for differences between periods. The significance threshold was set at 5% ($P < 0.05$).

TABLE 1

Effect of feeding 8 g/day chicory oligofructose (of which 7.2 g was Fn-type molecules) on the logarithm (log_{10}) of the number of the colony-forming units (cfu) of major bacteria in fresh fecal samples of male and female volunteers fed either a controlled (treatment 1) or a home cooked-diet (treatment 2)[1]

	Timing of microbiological analyses		
	Before treatment	After 2 weeks	After 5 weeks
Bacteria	Control value	Treatment 1	Treatment 2
Total anaerobes	10.3 ± 0.6	10.1 ± 0.5	10.4 ± 0.4
Bifidobacteria	8.6 ± 0.5	9.6 ± 0.3*	9.4 ± 0.6*
Lactobacilli	5.7 ± 1.0	6.0 ± 1.5	6.4 ± 0.7
Bacteroides	8.9 ± 0.2	8.8 ± 0.2	9.2 ± 0.7
Coliforms	7.0 ± 1.3	6.6 ± 1.6	6.5 ± 1.2
C. perfringens	3.5 ± 1.2	3.2 ± 1.0	3.2 ± 0.8

[1] Values are means ± SD, $n = 8$.
* Significantly different from before treatment.

RESULTS AND DISCUSSION

The key criterion for a prebiotic effect is the demonstration of the selective stimulation of growth of one particular, or a limited number of, potentially beneficial bacteria in the complex fecal microbiota following the consumption of a particular food. Data should demonstrate that the number (e.g. expressed as log_{10} cfu/g of feces) of bacteria in that particular population increased significantly, while the others did not change or even decreased (Gibson and Roberfroid 1995, Gibson et al. 1999, Roberfroid et al. 1998).

Table 1 reports the values of the total numbers of cfu (expressed as log_{10} cfu/g of feces) for the various bacteria analyzed in the feces of the eight volunteers fed a diet with and without chicory oligofructose. A global analysis of the different values reveals that the daily intake of 8 g of oligosaccharides did not significantly ($P > 0.05$) modify the counts of total anaerobes, lactobacilli, bacteroides, coliforms or C. *perfringens*, but it did significantly ($P < 0.01$) increase the counts of bifidobacteria.

The paired comparisons reveal that: i) at the end of the treatment 1 period, after eating a control diet supplemented with 8 g/d chicory oligofructose (of which 7.2 g was Fn-type molecules) for 2 wk, the number of bifidobacteria in feces had increased significantly ($P < 0.01$) compared to the end of the control period; ii) at the end of the treatment 2 period, after eating the usual home-cooked diet supplemented with 8 g/d chicory oligofructose (of which 7.2 g was Fn-type molecules) for an additional period of 3 wk, the number of bifidobacteria in feces were still significantly ($P < 0.01$) higher than at the end of the control period but not significantly different from the counts at the end of the treatment 1 period.

These data thus demonstrate that, as is the case with GFn-type oligofructose (Gibson et al. 1995, Roberfroid et al. 1998, Van Loo et al. 1999), a preparation of chicory oligofructose containing 90% of Fn-type molecules selectively stimulates the growth of colonic bifidobacteria in human volunteers, as evidenced by the increase in fecal number. Furthermore, the data demonstrate the selectivity of that

stimulation of growth, thus confirming the prebiotic nature of chicory Fn-type oligofructose.

At the end of the treatment 1 and treatment 2 periods, the fecal pH in all the volunteers had dropped by ~1 pH unit compared to the end of the control period. Such an effect is best explained by a change in colonic fermentation and confirms previous observations both in vitro (Wang and Gibson 1993) and in vivo (Gibson et al. 1995, Kleessen et al. 1997). The present study was not specifically designed to quantify changes in gut function parameters. However, when analyzing answers to the well-being questionnaires recorded during the control period vs. the treatment 1 period, changes in stool frequency (+ 12%) as well as in the appearance (softer) and the amount (evaluated qualitatively as "more than usual") of stools showed a tendency to confirm the bulking effect reported by Gibson et al. (1995) and by Den Hond et al. (1997). Moreover, an analysis of the intestinal side-effects associated with the meals during the periods of chicory oligofructose intake, as reported on the acceptability forms, revealed that from a total of 224 meals (8 volunteers receiving 2 meals/day for 2 wk), only six "mild" complaints were reported. These included one case of increased flatulence, three cases of intestinal distension and two cases of cramps in the intestine. It can be stated that the consumption of 8 g/d chicory oligofructose (of which 7.2 g was Fn-type molecules) is therefore not likely to cause significant intestinal discomfort.

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The prebiotic properties of oligofructose at low intake levels

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Abstract

Oligofructose and inulin, which are increasingly used in human food preparations, are now recognised as important prebiotic agents influencing the microbial composition of the gastrointestinal tract of the host. The specific objective of this study was to investigate the effect of ingesting a low dose of oligofructose (5 g/day) by healthy human subjects on the faecal microflora, especially bifidobacteria, and to compare it with the ingestion of a placebo (sucrose). In a placebo-controlled study design, faecal samples were collected in the morning from 8 healthy human subjects, who were not on any medication, and immediately enumerated for bifidobacteria, Bacteroides, coliforms, total anaerobes and total aerobes. Subjects first took sucrose (placebo) daily (5 g) for 3 weeks with their normal diet except for known sources of oligofructose and inulin and subsequently were administered oligofructose (5 g) daily for 3 weeks. Faecal samples were collected after 11 days and after 3 weeks. At 2 weeks post ingestion of oligofructose, another set of faecal samples was taken.

All samples were subjected to immediate microbial enumeration. Ingestion of sucrose (5 g/day) was without effect on all faecal bacteria enumerated, whereas consumption of oligofructose (5 g/day) for 11 days resulted in close to one log cycle increase in bifidobacteria numbers. No further increase was observed after the next 10 days. At 2 weeks after termination of oligofructose ingestion, bifidobacteria numbers had decreased to almost that of the period before treatment. Increases in numbers of Bacteroides and total anaerobic bacteria but not in aerobic bacteria also occurred.

Keywords: Prebiotic agents; Oligofructose; Inulin; Gastrointestinal tract; Bifidobacteria; Bacteroides

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1. Introduction

Oligofructose and inulin are non-digestible carbohydrates that are being used increasingly in food preparations for human consumption [1]. Recent studies have identified several beneficial attributes of oligofructose and inulin. These include management of constipation [2], improvement of the composition of the intestinal flora by promoting a saccharolytic, and suppressing a proteolytic metabolism with bifidobacteria and lactic acid bacteria as main indicator organisms [3–5], stimulating calcium absorption from the food [6–8], modulating lipid metabolism [9–11] and prevention of cancer [12–14]. They are now being recognized as important prebiotic agents influencing the microbiological composition of the gastrointestinal tract and the health of the host [15].

Most of the studies pertaining to the physiological role of these non-digestible carbohydrates have been conducted at relatively high concentrations (8–40 g per day). However, there is a growing interest in using these compounds as a substrate for the selective growth of beneficial gastrointestinal bacteria such as the bifidobacteria. Fermentation studies in vitro have demonstrated that oligofructose and inulin are efficient substrates for the growth of most strains of bifidobacteria compared to glucose [16,17]. There is also strong evidence that oligofructose and inulin selectively stimulate the growth of bifidobacteria in vivo.

The purpose of this study was to investigate the effect of ingesting a low dose of oligofructose (5 g/day of RAFTILOSE® P95), which is closer to a feasible daily intake by healthy human subjects, on the faecal microflora, especially bifidobacteria, and to compare it with the ingestion of a placebo (sucrose).

2. Subjects and methods

Subjects were recruited via advertisements on the university campus and by contacting participants in our previous studies. Only subjects who were healthy and had normal bowel movements and were not on any medication, including antibiotics, were asked to come to the faecal collecting facilities of the Department in the morning to collect faecal samples. Of the 8 subjects, four were male and 4 were female. Their average age was 28 y (range 24–48) and the average weight and body mass index (BMI) were respectively 64.8 kg and 23.8 kg/m^2. Immediately following collection, samples were enumerated for bifidobacteria and other bacteria. After faecal sample collections, subjects were provided with sufficient numbers of 5 g packs of sucrose (placebo) to last 3 weeks and instructed to ingest 1 pack each morning mixed in the beverage of their choice. Subjects were requested to maintain their normal lifestyle pattern during the course of the study and were instructed to avoid consuming onions, garlic and other known sources of oligofructose and inulin. A symptoms diary was kept by each subject during this period. At the end of 3 weeks, subjects returned to the Department and collected a faecal sample, which was enumerated immediately for the presence of bifidobacteria, Bacteriodes, coliforms, total anaerobes and total aerobes. Subjects were then given sufficient numbers of packs (5 g) of oligofructose (RAFTILOSE® P95, ORAFTI, Tienen, Belgium) with adjusted sweet taste (aspartame 2.97 g per kg oligofructose) and comparable granulometry and color so as to mimic the placebo, to last 3 weeks and

Table 1
Media and incubation parameters for faecal microflora profile

Microorganisms	Media and incubation parameters
Total anaerobes	Schaedler agar; anaerobic* ; 37°C; 72 h
Total aerobes	Schaedler agar; aerobic; 37°C; 72 h
Bacteroides	K-V laked blood agar; anaerobic; 37°C; 72 h
Bifidobacteria	MRS agar + Cys-HCl; anaerobic; 37°C; 72 h
Coliforms	MacConkey agar; aerobic; 37°C; 18 h

* Anaerobic jar, Anaerocult "C" pack.

instructed to follow the procedures used before with sucrose. Faecal samples were collected after 11 days on the oligofructose and again at the end of 3 weeks. Subjects also provided another faecal sample 2 weeks post ingestion of oligofructose. All samples were subjected to microbial enumeration immediately following collection.

Microbial enumeration of faecal samples was carried out immediately after collection by weighing into sterile anaerobic media followed by serial dilution. Aliquots of the diluted samples were then plated under anaerobic conditions on selective media and incubated adequately. The media and the incubation parameters used for faecal microflora profiling are shown in Table 1. The colonies growing on the selective agars were identified on the basis of their morphological characteristics, including colony margins, form, elevation and color. Bifidobacteria growing anaerobically on the MRS agar for 72 h at 37°C had characteristically convex, circular, cream-colored colonies with entire margins. On the other hand Bacteroides resistant to neomycin in the agar had convex, punctiform, mucoid colonies with entire margins. Colony identification was previously confirmed and established through microscopic observations and glucose metabolism patterns. Colonies were counted at the end of the incubation periods and expressed as Log_{10} CFU/g wet faeces.

For statistical analyses, zero-time faecal microbiological values were compared against values from faecal samples obtained after ingesting sucrose for 21 days and oligofructose for 11 and 21 days, and 14 days after termination of the treatment. Student's t test was used for statistical treatment of the data in order to compare the results.

3. Results

Ingestion of sucrose (5 g/day) for 3 weeks had no effect on the numbers of all fecal bacteria enumerated, whereas consumption of oligofructose (5 g/day) for 11 days resulted in close to one log cycle increase in the number of bifidobacteria. Ingestion of oligofructose for another 10 days did not result in further increase in the numbers of bifidobacteria but 2 weeks after the termination of oligofructose intake bifidobacteria numbers decreased to slightly above that of the period prior to treatment (Table 2).

Oligofructose ingestion also yielded an increase in numbers of Bacteroides. Values for coliform bacteria were difficult to interpret due to large standard deviations although ingesting oligofructose for 11 days clearly gave the lowest numbers of coliform bacteria. No

Table 2
Effect of sucrose and oligofructose on the composition of faecal microflora

Measuremnets (Statistical significance)	Sucrose 21 days	Oligofructose 11 days	Oligofructose 21 days	Post-Oligofructose
Total anaerobes	$9.97^{a} \pm 0.27$	$10.60^{b} \pm 0.22$***	$10.55^{b} \pm 0.17$***	$10.10^{a} \pm 0.32$
Total aerobes	$8.30^{a} \pm 0.52$	$8.32^{a} \pm 0.85$	$8.86^{a} \pm 0.80$	$8.33^{a} \pm 0.38$
Bifidobacteria	$8.85^{a} \pm 0.45$	$9.80^{b} \pm 0.40$(***	$9.77^{b} \pm 0.24$***	$9.11^{a} \pm 0.35$
Bacteroides	$9.75^{a} \pm 0.40$	$10.41^{b} \pm 0.29$**	$10.15^{b} \pm 0.28$*	$9.76^{a} \pm 0.14$
Coliforms	$6.72^{a} \pm 1.34$	$5.99^{a} \pm 1.17$	$6.93^{a} \pm 2.21$	$6.53^{a} \pm 0.33$

Figures on the same row with a different suffix are significantly different.
Bacteria are expressed as CFU per gram fresh faecal material.
Results are presented as mean ± SD.
Statistical significance compared to sucrose (21 d) data (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).

change in total aerobes was observed upon ingestion of either sucrose or oligofructose but numbers of anaerobic bacteria showed increases over zero time values with oligofructose and greater increases than with sucrose. Ratios of anaerobes to aerobes among the different groups were quite similar, ranging between 1.57 and 2.07. These data show a selective stimulation of certain groups of bacteria (Bifidobacteria). Statistical comparisons of the bifidogenic effects of oligofructose in humans are presented in Table 2, showing significant differences between sucrose and oligofructose treatment after 11 and 21 days in bifidobacteria and also Bacteroides and total anaerobes.

4. Discussion and conclusion

The data presented confirm that a dose of oligofructose, close to the minimum effect level deduced by means of a meta-analysis [18], still has a significant bifidogenic effect. Also, when the increase in bifidobacteria was plotted against the initial Bifidobacterium count of the individual volunteers, it is observed that the data match very well with the results of the latter authors (Fig. 1). This confirms the hypothesis that the initial count of bifidobacteria and not just the dose of oligofructose is the influential factor in determining the relative increase in bifidobacteria.

The reduction in Bacteroides counts as observed by Gibson et al. [3] was not reproduced here. A possible explanation might be that the latter study used a higher dose (15 g/day) of oligofructose. As a consequence there was a higher concomitant production of metabolites (end product of anaerobic intestinal oligofructose fermentation is SCFA and lactic acid), which is an important factor in the process of colonisation resistance.

In this low-dose study, a selective stimulation of the growth of certain groups of bacteria (bifidobacteria) was demonstrated, thus confirming the prebiotic properties of oligofructose. Whereas certain groups of bacteria (e.g. bifidobacteria) significantly increased in numbers, other groups of counted bacteria remained at the same levels (e.g. coliforms, the group of total aerobes). These data are in line with those reported by Buddington et al. [5], who

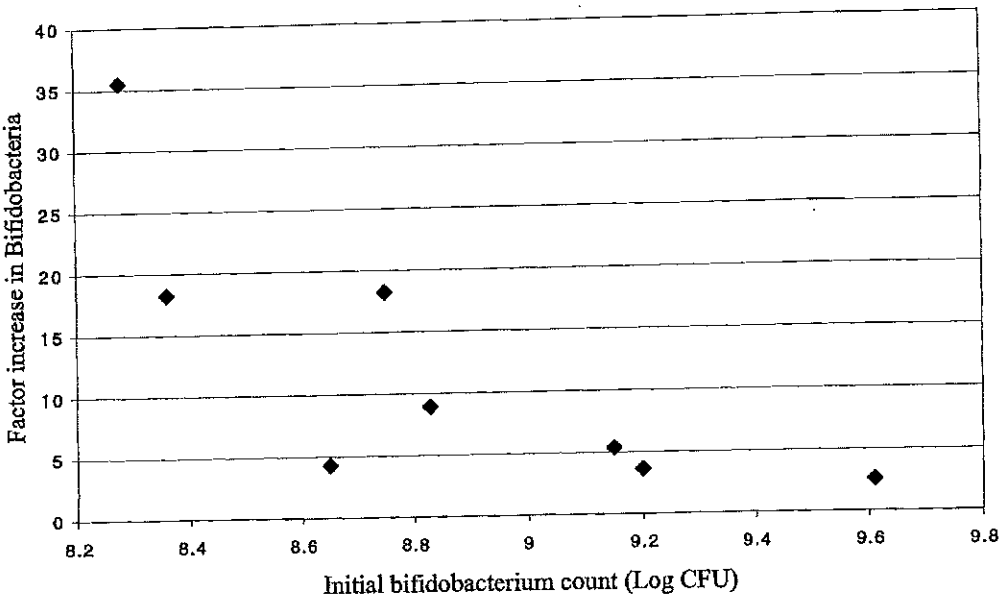

Fig. 1. Increase in bifidobacteria as a function of initial Bifidobacterium count, demonstrates that even at low intake levels of oligofructose, the initial counts are determining factor for the prebiotic effect.

observed in a study with 12 volunteers, that with 4 g per day of an oligofructose obtained by enzymatic synthesis, during a period of 3 weeks, the bifidobacteria numbers increased significantly, whereas the total aerobes and enterobacteria were less affected. This observation additionally confirms that, with respect to bifidogenic potential, there is no difference between oligofructose obtained by partial enzymatic hydrolysis (containing fructan chains ending with a glucose moiety as well as fructan chains ending with a fructose moiety) and oligofructose obtained by enzymatic synthesis from sucrose [19]. It is clear that the β(2-1) bond linking the fructose moieties in the oligofructose chains is central in the non-digestibility as well as in the bifidogenic properties of oligofructose.

As a conclusion it can be stated that present study confirms the prebiotic effect of oligofructose at low, nutritionally feasible, intake levels. The results were in line with published results and interpretations.

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ORIGINAL ARTICLE

A double-blind placebo-controlled study to establish the bifidogenic dose of inulin in healthy humans

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Objective: To evaluate the bifidogenic efficacy of two inulin doses in healthy human adults.
Design: A double-blind, placebo-controlled, crossover human study.
Setting: Food Microbial Sciences Unit, The University of Reading, Reading, UK.
Subjects: Thirty healthy volunteers, 15 men, 15 women (age range 19–35).
Interventions: Subjects consumed a chocolate drink containing placebo (maltodextrin, 8 g/day), 5 g/day inulin and 8 g/day inulin for a 2-week treatment period. Each treatment was followed by a 1-week washout at the end of which volunteers progressed to the next treatment. Faecal samples were obtained at the start of the study (baseline) and at the end of each treatment and washout period. Fluorescent *in situ* hybridization was used to monitor populations of *Bifidobacterium* genus, *Bacteroides – Prevotella*, *Lactobacillus – Enterococcus* and *Clostridium perfringens – histolyticum* subgroup.
Results: Bifidobacterial levels increased significantly upon ingestion of both the low ($9.78 \pm 0.29 \log_{10}$ cells/g faeces, $P<0.05$) and the high inulin dose ($9.79 \pm 0.38 \log_{10}$ cells/g faeces, $P=0.05$) compared to placebo ($9.64 \pm 0.23 \log_{10}$ cells/g faeces).
Conclusions: Both inulin doses exhibited a bifidogenic effect but a higher volunteer percentage responded to the high dose. A dose response effect was not observed but the magnitude of increase in bifidobacteria levels depended on their initial numbers. The higher the initial concentrations the smaller was the increase upon ingestion of the active treatments.
Sponsorship: Financial support for the completion of this project was provided by Sensus (Roosendaal, The Netherlands).
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Keywords: inulin; FISH; prebiotic; colon; *Bifidobacterium*

Introduction

Fructooligosaccharides (FOS) and inulin are polymers of D-fructose joined by $\beta(2\text{–}1)$ bonds with an $\alpha(1\text{–}2)$ linked D-glucose at the terminal end of the molecule. Molecules with a degree of polymerization (DP) between 3 and 10 are referred to as oligofructose or FOS and those with a DP between 3 and 65 are known as inulin. Inulin occurs naturally in a range of plants such as chicory, onion, garlic, Jerusalem artichoke, tomato and banana and, as such, it is a part of everyday human diet. According to the US Department of Agriculture 1994–1995 continuing survey of food intakes by individuals, the average daily intake of naturally occurring inulin and oligofructose was 2.6 and 2.5 g, respectively (Moshfegh *et al.*, 1999). European populations are estimated to consume between 2 and 10 g of inulin per day (Van Loo *et al.*, 1995).

Inulin is not hydrolyzed by digestive enzymes in the upper gastrointestinal tract and reaches the colon intact, where it is then selectively fermented by bifidobacteria. Bifidobacteria have long been regarded among the beneficial members of the human gut microflora. The bifidobacterial dominated gut microbiota of breast fed infants has been associated with improved health benefits (Campbell and Jones, 1996; Vanderhoof and Young, 1998). High numbers of bifidobacteria are also perceived as beneficial for adult health. Bifidobacteria have been shown to inhibit growth of pathogenic bacteria, modulate the immune system, produce digestive enzymes, repress the activities of rotaviruses and restore microbial integrity of the gut microbiota following antibiotic therapy (Bernet *et al.* 1993; Gibson and Wang, 1994; Saavedra *et al.* 1994; Collins and Gibson, 1999; McCracken and Gaskins, 1999).

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The health benefits of bifidobacteria have resulted in research focusing upon the promotion of their growth and activity in the colon. Previous human feeding studies have demonstrated the bifidogenic nature of both inulin and oligofructose. A variety of doses have been thus far reported effective in increasing faecal bifidobacteria *in vivo*, ranging from 4 to 40 g/day (Williams *et al.*, 1994; Gibson *et al.*, 1995; Buddington *et al.*, 1996; Kleessen *et al.*, 1997; Bouhnik *et al.*, 1999; Kruse *et al.*, 1999; Den Hond *et al.*, 2000; Tuohy *et al.*, 2001a; Bouhnik *et al.*, 2004). Although microbial culture techniques were used in the majority of studies, results reported thus far on the prebiotic efficacy of inulin and oligofructose are in agreement with studies employing molecular methodologies of monitoring the prebiotic effect on the bacterial microflora (Tuohy *et al.*, 2001a, b).

It is evident from the above that inulin and oligofructose exhibit bifidogenic efficacy at various daily intakes in healthy humans. Although the safety of inulin ingestion cannot be disputed, it is essential that its efficacy at low daily doses be defined. Results obtained thus far from a multitude of different studies cannot be directly compared as different methodologies, population groups as well as different types of inulin have been used. High daily doses of inulin could result in manifestation of adverse effects such as increased flatulence, through non-selective fermentation by the gut microflora. It is important to establish whether an increase in the daily intake of inulin correlates with an increased bifidogenic effect.

The aim of this study was to establish the minimum daily dose of inulin required to stimulate the numbers of faecal bifidobacteria in healthy humans, without the manifestation of gastrointestinal side effects.

Methods

Subjects

Thirty healthy human volunteers (15 male, 15 female, average age 26.5±3.1 years) participated. Written consent was obtained from all participants and the study protocol was approved by the Ethics and Research Committee of the University of Reading.

Pre-trial assessment

Volunteers were assessed for good health and selected on basis of adherence to exclusion and inclusion criteria.

Eligibility criteria

Inclusion criteria for the participation in the study were: signed consent form, age 18–50 years inclusive, body mass index 20–30 inclusive, good general health and absence of gastrointestinal disorders (chronic constipation, diarrhoea, inflammatory bowel disease, irritable bowel syndrome or other chronic gastrointestinal complaints) as determined through a medical questionnaire.

Volunteers with a history of physical/mental disease, major surgery, severe allergy, abnormal drug reaction and drug/alcohol abuse were excluded. Volunteers were also excluded if pregnant, lactating or planning pregnancy. Volunteers taking pro/pre/synbiotics, drugs active on intestinal motility, or laxatives of any class, within 4 weeks before the study or antibiotics within 6 months before the study were also excluded.

Requirements for diet and medication during the study

Subjects were instructed not to consume any additional prebiotics, probiotics or synbiotics. They were also asked not to take any antibiotics or drugs that could affect gastric motility. Any medication taken was recorded in diaries. Volunteers were instructed not to alter their usual diet or fluid intake during the trial period.

Treatments

The trial was conducted in a crossover design between two active (high and low inulin dose) and a placebo treatments.

Volunteers consumed each treatment for a 14-day period, which was followed by a 7-day washout. All volunteers consumed the same treatment during each experimental period.

Treatments were supplied by Sensus (Roosendaal, Netherlands) and were colour coded so as to be blind to the investigators as well as the volunteers (Table 1). Each treatment was delivered as one 29 g chocolate powder sachet per day to be dissolved in warm water. Test sachets containing chocolate powder along with inulin, average DP 9–10 (Frutafit IQ, Sensus, Roosendaal, Netherlands) or placebo (maltodextrin) were administered to the volunteers at the start of each 14-day treatment period. Volunteers were asked to drink one chocolate powder sachet per day and were free to consume it at any time during the day.

Faecal sample preparation

Freshly voided faecal samples were collected in sterile plastic pots and were processed within 10 min of collection. Samples were diluted 1 in 10 (w/w) with phosphate-buffered saline (PBS, 0.1 M; pH 7.0) and mixed in a Stomacher® 400 (Seward, Norfolk, UK) for 2 min at normal speed.

Bacterial enumeration

Oligonucleotide probes. Synthetic oligonucleotide probes targeting specific regions of the 16S rRNA molecule, labelled with the fluorescent dye Cy3, were utilized for the enumeration of *Bifidobacterium* genus (Bif164, Langendijk *et al.*, 1995), *C. perfringens – histolyticum* subgroup (His150, Franks *et al.*, 1998), *Bacteroides – Prevotella* (Bac303, Manz *et al.*,

Table 1 Study outline and nutritional information of the 3 treatments ingested throughout study duration

Treatment 1 (placebo)	*Washout 1*	*Treatment 2 (5 g/day inulin)*	*Washout 2*	*Treatment 3 (8 g/day inulin)*
Days 1–14 1 sachet/day Stool sample on day 1 (baseline) diary for GI symptoms	Days 15–22 No treatment Stool sample at day 22 GI symptom diary	Days 23–37 1 sachet/day Stool sample on day 37 (treatment 2) GI symptom diary	Days 38–45 No treatment Stool sample on day 45 GI symptom diary	Days 46–60 1 sachet/day Stool sample on day 60 (treatment) GI symptom diary
Treatment composition and nutritional information 8 g maltodextrin substituting for the inulin content of the active treatments.		5 g inulin, 3 g maltodextrin. All other ingredients identical to high active treatment		8 g inulin, sucrose, skimmed milk powder, lactose, skimmed cocoa powder (9%), vegetable fat, stabilisers (E339, xanthan gum), salt and aroma.

Each treatment was delivered as 29 g of chocolate powder/day. The sequence the treatments were administered was: placebo, 5 g/day inulin, 8 g/day inulin. Formulae nutritional value per 100 g: energy 410 kcal, protein 12 g, carbohydrates 70 g of which sugars 69 g, sodium 0.4 g, fat 8.5 g of which saturated 8.0 g and monounsaturated 0.5 g, dietary fibre 4.0 g.

1996) and *Lactobacillus – Enterococcus* (Lab158, Harmsen *et al.*, 1999). All probes were provided by MWG-Biotech (London, UK). Total cell counts were achieved by adding the nucleic acid stain 4,6-diamidino-2-phenylindole to the hybridization mixture. Labelled cells were visualized using fluorescent microscopy. The sequences and specific hybridization conditions for each molecular probe are presented in Table 2.

Fluorescent in situ hybridization. The method was carried out as previously described by Rycroft *et al.* (2001) and Tuohy *et al.* (2001b).

Samples were fixed overnight (4°C) in 4% (w/v) paraformaldehyde. Fixed cells were centrifuged at 15 000*g* for 5 min and washed twice in 1 ml filtered PBS. The washed cells were resuspended in 150 μl PBS and stored in ethanol (1/1 v/v) at −20°C.

Following overnight hybridization with each probe, the fixed cells were washed and vacuum filtered (2 μm polycarbonate isopore membrane filter, Millipore UK Ltd, Watford, UK). They were then mounted onto a glass slide with 20μl of slowfade to prevent fading of fluorescence (Molecular Probes, Leiden, The Netherlands). The fluorescent cells were enumerated using the Fluor 100 lens (Eclipse 400 epifluorescent microscope, Nikon, Kingston upon Thames, UK). Fifteen different random fields of view were counted on each slide.

Gastrointestinal symptoms and stool characteristics

Volunteers were asked to keep diaries throughout the study to record stool frequency (bowel movements per day) and consistency (constipation, hard, formed or diarrhoea) on a daily basis. Abdominal pain, stomach or intestinal bloating and flatulence were also recorded as none, mild, moderate or severe and were given a numerical score ranging from 0 for none, up to 3 for severe symptoms. Data obtained from volunteer diaries were used to determine gastrointestinal tolerance and symptoms throughout the study.

Table 2 Probe sequences and hybridization/washing temperatures

Probe name	*Sequence*	*Hybridization/ washing temperature (°C)*
Bac303	5′-CCAATGTGGGGGACCTT-3′	45
Bif164	5′-CATCCGGCATTACCACCC-3′	50
Chis150	3′-TTTCCYTCTAATTATGGCGTATT-5′	50
Lab158	5′-GGTATTAGCA(T/C)CTGTTTCCA-3′	50

Bac303 is specific for *Bacteroides – Prevotella*, Bif 164 for *Bifidobacterium* genus, Chis 150 for *Clostridium perfringens histolyticum* subgroup and Lab158 for *Lactobacillus – Enterococcus*.

Statistical analysis

The primary efficacy variable was considered to be the change in bacterial numbers from baseline to the end of each treatment for each bacterial species and total bacterial count. All bacterial concentrations were expressed in $\log_{10}$cells/g faeces.

Paired *t*-tests were used after logarithmic transformation to determine if there was a statistically significant difference between placebo and each of the two treatments. The differences between the treatments were estimated with 95% confidence intervals.

A correlation analysis was performed to determine whether there the magnitude of change in bacterial levels depended on initial bacterial concentrations. The null hypothesis was that the change in bacterial levels after treatment did not depend on initial bacterial levels. The critical value of the correlation coefficient ($r_{critical}$) for 28 degrees of freedom (df = n-2 for a two-tailed test, where n is the number of observations) was $r_{critical} = 0.361$ at 95% confidence intervals. Where the absolute calculated value of r was greater than the value of $r_{critical}$ the null hypothesis was rejected the alternative hypothesis was accepted.

Results

Faecal bacterial populations present in 30 volunteers throughout the study period are presented in Table 3. Changes in total faecal bacteria numbers and the levels of four of the numerically significant and functionally important bacterial populations in human faeces, namely *Bifidobacterium*, *Bacteroides – Prevotella*, *C. perfringens – histolyticum* subgroup and *Lactobacillus – Enterococcus*, were monitored using fluorescent *in situ* hybridization (FISH) and are expressed in $\log_{10}$ cells/g faeces. Bacterial numbers were obtained at baseline (start of trial), at the end of treatment 1 (8 g/day maltodextrin-placebo), end of washout 1, end of treatment 2 (5 g/day inulin, 3 g/day maltodextrin), end of washout 2 and at the end of treatment 3 (8 g/day inulin). Bacterial levels at the end of each treatment period were compared to their respective baseline and statistical significance of the results evaluated using the paired *t*-test at 95% confidence intervals.

At baseline, bacteroides and bifidobacteria were the numerically predominant bacterial populations in faeces ($10.06 \pm 0.24 \log_{10}$ cells/g faeces and $9.61 \pm 0.31 \log_{10}$ cells/g faeces, respectively), whereas *C. perfringens – histolyticum* subgroup and *Lactobacilli – Enterococci* existed at lower levels ($8.66 \pm 0.38 \log_{10}$ cells/g faeces and $9.22 \pm 0.22 \log_{10}$ cells/g faeces, respectively).

Bifidobacteria did not exhibit any statistically significant change upon ingestion of the placebo sachets (8 g/day maltodextrin) with respect to baseline levels.

A significant increase was observed in bifidobacteria at the end of the second treatment (5 g/day inulin, 3 g/day maltodextrin) with respect to placebo ($P<0.05$), washout 1 ($P=0.01$) and baseline ($P<0.01$) levels. Overall, 20 of the 30 volunteers responded to inulin supplementation at 5 g/day. The average initial *Bifidobacterium* levels were generally lower ($9.53 \pm 0.25 \log_{10}$ cells/g faeces) in volunteers that responded to the treatment as compared to mean *Bifidobacterium* levels in volunteers that did not respond to treatment ($9.76 \pm 0.30 \log_{10}$ cells/g faeces, $P=0.05$). There was a negative correlation between initial levels and the magnitude of increase in bifidobacterial numbers at the end of the low inulin dose treatment ($r=-0.523$, $P=0.010$).

Similarly, the higher inulin dose (8 g/day), also resulted in a statistically significant increase in bifidobacterial levels with respect to washout 2 ($P<0.05$), placebo ($P=0.05$) and baseline ($P=0.01$) numbers. Twenty-five volunteers responded to the high inulin treatment. The average initial bifidobacterial levels in the volunteers that responded to the higher dose were lower ($9.62 \pm 0.32 \log_{10}$ cells/g faeces) than those of non-responders ($9.81 \pm 0.30 \log_{10}$ cells/g faeces), ($P=0.05$). Seven of the volunteers that did not respond to the low-dose treatment responded to the high dose, whereas three volunteers did not respond to any of the treatments. Two of the volunteers that exhibited an increase in bifidobacteria with the low-dose treatment did not respond to the high inulin dose and only exhibited small decreases in bifidobacterial levels. Again, there was a negative correlation between initial levels and the magnitude of increase in faecal bifidobacteria ($r=-0.438$, $P<0.05$).

C. perfringens – histolyticum subgroup exhibited no significant increase upon ingestion of the placebo treatment with respect to baseline levels. Numbers showed a significant increase at the end of washout 1 as compared to levels at the end of the placebo treatment ($P<0.01$). Twenty-five volunteers exhibited increased *C. perfringens – histolyticum* subgroup levels. Their numbers stabilized hereafter and there was no significant change at the end of the low-dose treatment compared to washout 1 levels. There was a significant decrease in *C. perfringens – histolyticum* subgroup at the end of the high-dose treatment with respect to levels at the end of washout 2 ($P<0.01$). Overall, they decreased in 25 out of 30 volunteers but were still significantly higher than baseline and placebo levels ($P<0.001$).

Table 3 Mean bacterial counts obtained through FISH enumeration, expressed in $\log_{10}$cells/g faeces, ±s.d.

	Total cells		Bifidobacterium		Bacteroides – Prevotella		Clostridium perfringens – hystolyticum *subgroup*		Lactobacillus – Enterococcus	
	Mean	*±s.d.*	*Mean*	*±s.d.*	*Mean*	*±s.d.*	*Mean*	*±s.d.*	*Mean*	*±s.d.*
Baseline	10.61	0.30	9.61	0.31	10.06	0.24	8.66	0.38	9.22	0.22
Treatment 1	10.69	0.20	9.64	0.23	10.15	0.14	8.68	0.49	9.66	0.31
Washout 1	10.70	0.21	9.61	0.29	10.24	0.14	9.36*	0.56	9.71	0.18
Treatment 2	10.62	0.19	9.78‡	0.29	10.15	0.17	9.51*	0.22	9.49†	0.32
Washout 2	10.61	0.22	9.65	0.32	10.22	0.18	9.60*	0.19	9.09	0.21
Treatment 3	10.61	0.26	9.79€	0.38	10.08	0.24	9.34†*	0.28	9.10	0.24

Abbreviations: FISH, fluorescent *in situ* hybridization; s.d., standard deviation.
Samples were obtained at the start of the study (baseline), end of treatment 1 (placebo), end of washout 1, end of treatment 2 (5 g/day inulin), end of washout 2 and at the end of treatment 3 (8 g/day inulin).
‡Significant increase with respect to baseline ($P<0.01$), placebo ($P<0.05$) and washout levels ($P=0.01$).
€Significant increase with respect to baseline ($P=0.01$), placebo ($P=0.05$) and washout levels ($P<0.05$).
*Significant increase with respect to baseline levels ($P<0.001$).
†Significant decrease with respect to washout levels ($P<005$).

Lactobacillus – Enterococcus increased significantly during the placebo treatment with respect to baseline levels ($P<0.01$) in 29 volunteers, remained stable during washout 1 and did not return to baseline. Numbers at the end of washout 1 were still significantly higher than baseline ($P<0.01$). At the end of the low inulin dose treatment, *Lactobacillus – Enterococcus* significantly decreased with respect to washout 1 ($P<0.05$). At the end of washout 2, they returned to pretreatment levels. No significant change was observed between washout 2 and treatment 3 numbers.

Total bacteria and bacteroides numbers did not exhibit any significant variation throughout the duration of the trial.

Gastrointestinal symptoms

Results from the volunteer diaries are summarized in Table 4. The higher the score the higher was the severity of the gastrointestinal symptom.

Although there was a wide range of responses noted by the volunteers with respect to the frequency and severity of gastrointestinal symptoms the only statistically significant change observed was an increase in stool number ($P=0.029$), intestinal bloating ($P=0.011$) and flatulence ($P<0.001$) upon ingestion of the low-dose inulin treatment with respect to washout levels. The only significant effect of the placebo treatment was an increase in abdominal pain ($P=0.014$). Volunteers were also asked to record stool consistency as hard, formed or soft throughout the trial duration. Despite the wide range of responses given by different volunteers, there were no significant differences between the different treatments with the majority reporting formed stools.

Discussion

The aim of this study was to determine the bifidogenic efficacy of two doses of inulin formulated as chocolate powder drinks, in a double-blind, placebo-controlled, cross-over study of 30 healthy volunteers. The trial was structured as three, 2-week test periods during which volunteers consumed a placebo chocolate drink, a low dose (5 g/day) and a high-dose (8 g/day) inulin chocolate drink. The first two test periods were followed by a 1-week washout to avoid the effect of one treatment being carried over to the next. FISH was used to determine populations of faecal *Bifidobacterium* genus, *Lactobacillus – Enterococcus*, *Bacteroides – Prevotella* and *C. perfringens – hystolyticum* subgroup.

A wide variation in bacterial levels as well as magnitude of response on microflora was observed between the 30 volunteers participating in the trial, upon ingestion of the three different treatments. This was expected as composition of the bacterial microflora varies greatly among different individuals both quantitatively and qualitatively (Tuohy *et al.*, 2001b) resulting in a different response to the study treatments.

The placebo treatment had no significant effect upon any of the bacterial groups enumerated for, apart from *Lactobacillus – Enterococcus*, which increased significantly. This effect was retained during washout 1 and levels gradually decreased at the end of the low inulin treatment to reach approximately baseline numbers at the end of washout 2 and the 8 g/day treatment. As all treatments had identical formulations apart from the active ingredients, it can be assumed that the stimulatory effect on *Lactobacillus – Enterococcus* was due to the maltodextrin content of the chocolate powder (Engfer *et al.*, 2000). The maltodextrin used in this study (DP = 50) may have not been accessible to amylase digestion due to the poor solubility of the placebo chocolate drink. The low-dose inulin treatment delivered 3 g/day maltodextrin along with 5 g/day inulin, that sustained *Lactobacillus – Enterococcus* at the end of this treatment at levels significantly higher than baseline concentrations ($P=0.001$) despite a significant decrease with respect to washout 1.

Although the placebo treatment had no significant effect on *C. perfringens – hystolyticum* subgroup, numbers significantly increased during washout 1 and remained significantly higher than baseline levels throughout the study. Ingestion of active treatments did not suppress *C. perfringens*

Table 4 Summary of data obtained from diaries completed by 30 volunteers during the three treatment and two washout periods of the trial

	Gastrointestinal symptoms							
	Stool number		*Abdominal pain*		*Intestinal bloating*		*Flatulence*	
	Mean	*±s.d.*	*Mean*	*±s.d.*	*Mean*	*±s.d.*	*Mean*	*±s.d.*
Placebo	1.478	0.674	0.143[¥]	0.165	0.337	0.452	0.672	0.567
Washout 1	1.428	0.888	0.079	0.181	0.251	0.412	0.685	0.603
5 g/day inulin	1.508*	0.633	0.175	0.295	0.418**	0.532	0.942[§]	0.672
Washout 2	1.379	0.567	0.121	0.288	0.159	0.344	0.544	0.558
8 g/day inulin	1.461	0.7345	0.154	0.224	0.363	0.566	0.733	0.655

*Statistically significant increase in stool number ($P=0.029$) compared to washout.
**Statistically significant increase in intestinal bloating ($P=0.011$) compared to washout.
[§]Statistically significant increase in flatulence ($P<0.001$) during the low dose treatment.
[¥]Statistically significant increase in abdominal pain ($P=0.001$) compared to washout.

– *histolyticum* subgroup to baseline/placebo numbers. Apart from certain restrictions concerning the intake of probiotic and prebiotic containing foods, this was otherwise a free-living human study and participants were allowed to ingest their normal diets. As such, changes in the faecal microflora due to seasonal or other factors not related with the intake of the test drinks could be avoided. This may also, in part, account for the increase in *Lactobacillus – Enterococcus* levels observed during the placebo period.

The effect on bifidobacteria populations was clearer. Both the low and the high doses had a statistically significant stimulatory effect on the faecal bifidobacterial microbiota. Although more volunteers responded to the higher dose, the bifidogenic effect was approximately the same with both treatments and no dose–response relationship was observed. Nevertheless, a negative correlation existed between initial faecal concentrations of bifidobacteria and the magnitude of increase during both active treatments. The higher the initial numbers of bifidobacteria the smaller was the bifidogenic effect. The same effect has been observed in previous studies (Roberfroid *et al.*, 1998; Tuohy *et al.*, 2001b) and suggests that in cases where initial numbers of bifidobacteria are sufficiently high, a marked bifidogenic effect may not be observed. The majority of volunteers participating in this study had high initial levels of bifidobacteria. There may have been a more apparent dose effect if a study population with lower initial bifidobacteria levels had been investigated but the target group chosen here was the general healthy population. The fact that both inulin doses promoted bifidobacterial growth is of importance as it is essential to establish the efficacy of inulin to exert a bifidogenic effect on the gut microflora at lower doses. When excess fermentable oligosaccharides are delivered to the colon side effects such as bloating and flatulence may occur. If the fermentation capacity of saccharolytic organisms in the colon is saturated, the excess substrate will be available to organisms that may generate gas (unlike the bifidobacteria). As previously mentioned, tolerance to inulin varies among individuals (Tuohy *et al.*, 2001a). In general, both active treatments were well accepted by the human volunteers and all subjects completed the study. There was a significant increase in stool frequency, intestinal bloating and abdominal pain during the low-dose treatment that was not observed during the higher dose. This suggests that recruits may adapt to inulin or that volunteers may become accustomed to experiencing the symptoms, which become less noticeable. On the other hand, during the higher inulin dose supplementation, *C. perfringens – histolyticum* subgroup levels exhibited a statistically significant decrease with respect to the low dose ($P<0.05$). However, as previously mentioned clostridia levels did not return to baseline/placebo and gastrointestinal symptoms were slightly but not significantly elevated with respect to placebo. Bifidobacteria do not produce gas during carbohydrate fermentation, whereas clostridia are prolific gas producers. This may explain why the high dose was better tolerated.

The most important attribute of a prebiotic, along with resistance to digestion in the upper gastrointestinal tract, is a stimulation of beneficial bacterial populations in the colon, with the main target being bifidobacteria. The anatomy of the human colon makes it largely inaccessible to routine sampling and, although a compromise, the use of faeces as an indication of the composition of colonic luminal content provides invaluable insight on the bacterial population changes. The fact that increased faecal bifidobacteria levels were observed shows that inulin ingestion promoted their growth in the gut.

The bifidogenic efficacy of inulin at 5 and 8 g/day formulated in a processed food product was investigated and demonstrated *in vivo* in 30 healthy human volunteers in this study. Previous studies by Tuohy *et al.* (2001b) established the bifidogenicity of prebiotic biscuits, but no attempt was made to investigate the efficacy of different prebiotic doses. Moreover, here, monitoring of bacterial populations throughout the human trial was performed using FISH, a molecular based method that accurately identifies and quantifies bacterial populations *in situ*. Although the technique was developed several years ago and has proven efficiency, the majority of studies investigating the prebiotic effect of inulin have employed bacterial culture methods to monitor bacterial changes. The lack of selectivity of such methods impedes the accurate evaluation of prebiotic efficacy. Indeed, two recent studies on the bifidogenic efficacy of long chain inulin report conflicting results. At 8 g/day long chain inulin was found to effect a significant bifidogenic effect when bacterial changes were monitored through FISH (Tuohy *et al.*, 2001a) but not at 10 g/day when bacterial culture was used (Bouhnik *et al.*, 2004). The latter result may have occurred because of the use of culture-based technologies and their lack of selectivity.

In conclusion, inulin exerted a bifidogenic effect in healthy human adults at doses of 5 and 8 g/day. In light of both doses proving effective, the higher dose may be preferable as no significant side effects were noted and a higher percentage of the study population responded to the treatment.

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Original Article

Supplementation of infant formula with native inulin has a prebiotic effect in formula-fed babies

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Objectives: In this study we investigated the effects of native inulin in formula-fed babies. The influence of inulin on the microbial composition, pH, consistency and amount of faeces, and on frequency of defecation was assessed. Methods: In this study a daily dosage of 0.25 g/kg/d was used: 3 weeks of inulin consumption were followed by 3 weeks without or vice versa. The study group consisted of 14 babies with an average age of 12.6 weeks (± 6.4 weeks) and the average intake of inulin was 1.5 (± 0.3) g/d. Results: The consumption of inulin increased the content of *Bifidobacterium* and *Lactobacillus* in the faeces of formula-fed babies, without affecting the number of *Bacteroides* or the total anaerobic count. With inulin there was a trend for stools to become softer and for the amount of faeces to increase significantly. Frequency of defecation was not affected by the consumption of inulin. No adverse effects were reported during the periods of inulin consumption. Conclusions: We conclude that, with native inulin, a prebiotic effect can be observed in formula-fed babies. Inulin may therefore be a useful ingredient in infant formulae.

Key Words: inulin, babies, colonic microbiota, infant formula, prebiotic

Introduction

Inulins are β- (2,1)-fructans that occur as a reserve carbohydrate in many plants. [1] As such we consume these components in moderate amounts with our daily diet. They are found in vegetables such as wheat, onions and leeks and in fruits such as bananas. Inulins are also available as a food ingredient and are used in a wide variety of food products. Many applications of inulins are based on the prebiotic features of these fructans: they are able to stimulate growth of bifidobacteria in the human colon, thereby possibly providing health benefits to the host.[2] This property may also be useful in baby food formulae, which serve to replace mother's milk.

Next to lactose (68 g/l), fat (37 g/l) and protein (10-12 g/l) human milk also contains 10 – 20 g/l of oligosaccharides. These oligosaccharides have a complex molecular structure and consist of a variety of sugars. Galactose, glucose, fucose, N-acetylglucosamine and sialic acid are the constituents of these oligosaccharides.[3] This rich diversity is unique to human milk. They are not digested in the babies' gastrointestinal tract [4] and a variety of functions are attributed to them, including prebiotic activity and anti-infective action.[5] Other factors such as the presence of lactoferrin and lysozyme and the low buffer capacity also play a role in the prebiotic properties of human breast milk.[6]

Distinctive differences in intestinal microbiota between breast fed babies and bottle fed ones have been reported [7,8,9]: *Bifidobacterium* and *Lactobacillus* are the main species found in breast-fed babies, while formula-fed babies have an adult-type flora with a predominance of *Streptcoccus*, *Enterobacteriaceae* and *Bacteroides*. One of the purported beneficial properties of *Bifidobacterium* and *Lactobacillus* is activation of the immune system [10,11] and it has been suggested that the fact that bottle fed babies' death rate and infection of contagious diseases is significantly higher than that of breast-fed babies [12] is related to this difference. Obviously these differences in microbial composition are caused by differences in the composition of human breast milk and formula milk.

In bovine milk the oligosaccharides described above are absent, meaning that baby formulae based on cow's milk lack these prebiotic properties. In order to improve the functionality of baby formulae attempts are made to improve the nutritional features by including prebiotic oligosaccharides [e.g. 13,14,15] or probiotic bacteria.[e.g. 16]

For the prebiotics applied so far much attention has been paid to oligosaccharides derived from sucrose and inulin (fructo-oligosaccharides[17]) or lactose (galacto-oligosaccharides), or mixtures thereof.[13] Native inulin (a natural polydisperse mixture of fructan polymers derived from chicory roots) has not been tested in baby formulae thus far. In this study we investigated the effects of inulin on the microbial composition of the faecal microbiota and stools of formula-fed babies.

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Methods & Materials

Subjects and experimental design

The subjects were 14 infants (male: 10, female: 4) living in the orphanage of the Civic Children's Hospital (Seoul, Korea) aged on average 12.4 weeks (see Table 1). All were bottle fed before the weaning period and in good health.

The subjects were randomly assigned to either Group 1 (7 subjects, 5 male, 2 female), which started with the inulin treatment, or Group 2 (7 subjects, 5 male, 2 female), which started with the control treatment (formula without native inulin). For the inulin treatment the subjects were fed milk formula containing inulin (0.25 g/kg/d) three times a day for three weeks; the control treatment consisted of the same formula without the inulin supplement. Appropriate amounts of inulin were mixed with the powder formula. After 3 weeks the treatment was switched; there was no washout period between the treatments. Faecal samples were collected as described below after 3 weeks of treatment and after 6 weeks of the reverse treatment.

Native inulin (Frutafit® IQ) was supplied by Sensus (Roosendaal, Netherlands). The composition of the powdered formula is given in Table 2.

The study protocol was approved by the Medical Ethical Committee of the hospital. The hospital received a financial compensation for contributing to this study.

Analysis

Faeces collection

Faeces was collected from the subject's diaper just after a bowel movement, mixed with sterilized phosphate buffer (10 %, w/w, 0.2 M sodium phosphate, pH 7.0), frozen and stored until further analysis.

Analysis of microbial composition of faeces

The total numbers of anaerobic bacteria, *Bifidobacterium, Lactobacillus*, and *Bacteroides* were determined by plate counting. A sample of frozen faeces (0.5 g) was homogenized in 4.5ml sterilized 0.2 M sodium phosphate buffer, pH 7.0. This homogenate was further diluted as appropriate for inoculating the various media. BL agar (Difco C220-17, USA) was used for total anaerobic bacteria, BS agar medium (*Bifidus* selective agar medium) added to BL agar with antibiotic for *Bifidobacteria*, M-LBS agar medium *(Lactobacillus* selective agar medium), and NBGT agar medium for *Bacteroides*. The composition of the various media is found in detail elsewhere [18]. Each diluted solution was spread on the selective agar medium and cultivated anaerobically at 37 ℃ for 72 hours.

Examination of bowel parameters

The amount of faeces and frequency of defecation were recorded at a daily basis by the hospital nurses. The amount of the excreta was determined by weighing the diapers and subtracting the weight of an empty diaper. Consistency of the faeces was scored on a scale from 1-4, in which 1 was watery, 2 was muddy, 3 was clay-like and 4 was hard pellets. The records of the hospital also tracked data on the babies' health situation during the trial.

Table 1. Characteristics of the subjects

Parameter	Average (range)	SD
Age (wks)	12.6 (5-24)	6.4
Initial weight (g)	5006 (3750-6960)	1169
Final weight (g)	5926 (4630-7640)	1019
Weight gain (g)	9 21 (170-1920)	604
Growth (g/wk)	153 (28-320)	101
Inulin intake (g/d)	1.5 (1.0-1.75)	0.3

The average data and standard deviation were calculated for all 14 subjects. Final weight and weight gain were measured after 6 weeks.

Table 2. Composition of baby food formula.

Component	Per 100 g
Fat (g)	27.0
Linoleic acid (g)	3.5
γ-linolenic acid (mg)	14.0
DHA (mg)	70.0
Arachidonic acid (mg)	22.0
Protein (g)	12.2
Carbohydrate (g)	56.0
Oligosaccharides (g)†	1.5
Minerals (g)	1.52
Vitamin mix (mg)‡	94.4
Others components (mg)§	805.0
Energy content (kcal)	516.0

† A mixture of raffinose, galactooligosaccharide and fructooligosaccharide was used (each 500 mg per 100 g); ‡ Vitamin A (1700 IU), vitamin B1 (0.3 mg), vitamin B2 (0.6 mg), vitamin B6 (0.3 mg), vitamin B12 (2 μg), vitamin C (50 mg), vitamin D3 (380 IU), vitamin E (6 IU), nicotinic amide (5 mg), folic acid (100 μg), biotin (20 μg), pantothenic acid (3 mg), inositol (35 mg) and β-carotene (60 μg); § L-arginine (480 mg), L-cysteine (200 mg), taurine (30 mg), lactoferrin (80 mg), nucleotide (15 mg)

Statistical analysis

Mean and standard deviation were calculated for all variables. A t-test was used to determine statistical significance (two-sided; $p < 0.05$) between the treatments. The frequency and consistency of bowel movements were analyzed by the chi-square test.

Results

Growth

Table 1 shows the overall characteristics of the babies. No differences in growth were observed between the periods of inulin-treatment and the periods without inulin: average weight increase with inulin was 509 (± 372) g in 3 weeks, whereas without inulin this increase was 411 (± 394) g in 3 weeks (p=0.5051). Also there were no adverse effects (like diarrhoea) observed during the control and inulin treatments.

Microbial composition of faeces

The microbial composition of the faeces with and without inulin treatment is shown in Table 3. The content of total anaerobic bacteria and *Bacteroides* did not differ between the two treatments. A trend was found for the total number anaerobic bacteria increasing with inulin treatment (p=0.0618) and for *Bacteroides* no significance was reached (p=0.5224). A significant increase in *Bifido bacterium* sp. was found when the numbers of control treatment and inulin-treatment were compared (p=0.0163).

Table 3. Microbial composition of faeces

Bacteria	Inulin treatment	Control treatment
Total anaerobes	10.58 (0.224)	10.27 (0.344)
Bacteroides	9.51 (0.389)	9.40 (0.344)
Bifidobacterium	9.85 (0.523) *	9.22 (0.741)
Lactobacillus	9.09 (0.377) *	8.61 (0.741)

All data are expressed in log colony forming units per g of faecal matter as the average of 14 subjects with the standard deviation in parentheses. Data with * in the same row are significantly different ($p < 0.05$).

Table 4. Stool parameters

Parameter	Inulin treatment	Control treatment
pH	6.31 (0.34)	6.51 (0.49)
Consistency	1.8 (2.7)	2.4 (4.2)
Amount (g)	167 (80) **	75 (30)
Frequency	1.6 (2.4)	1.2 (1.6)

All data are given as the average of 14 measurements with the standard deviation in parentheses. Faecal pH and amount (in g) were measured as described, consistency was scored on a scale from 1-4 (watery to hard). Data with ** in the same row are significantly different ($p < 0.01$).

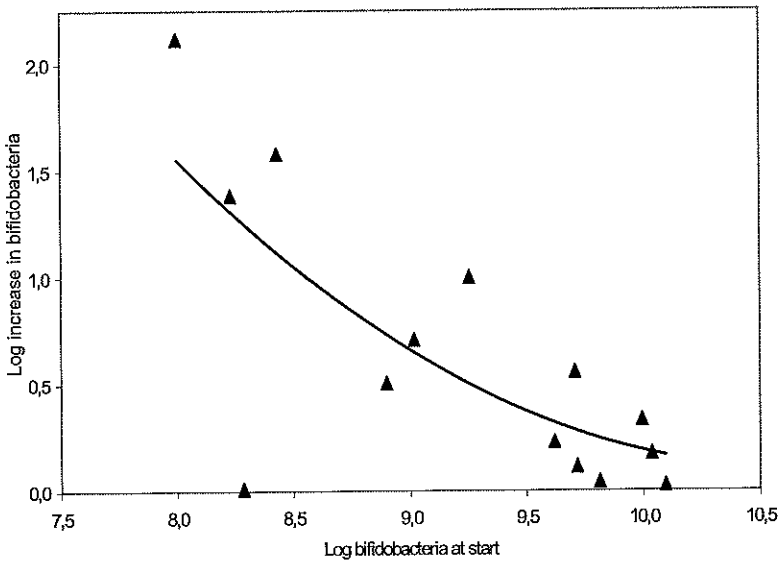

Figure 1. Increase in *Bifidobacterium* sp. in formula-fed babies as a function of the original number of *Bifidobacterium* sp. The faecal content of *Bifidobacterium* sp. was determined without and with inulin supplementation of the formula, and the logarithmic increase was calculated.

When the individual results were plotted against the original value of bifidobacteria, it was observed that, when the starting value was lower, a higher increase was found (Fig 1). It is also noteworthy that two subjects showed no response during inulin treatment (Fig 1).

For *Lactobacillus* sp. a significant increase during inulin-treatment was found ($p = 0.020$).

Effects on stools

Despite the large variations in the various parameters of bowel habit (as shown by the large standard deviations; see Table 4) some differences and trends could be discerned. The pH of the faeces decreased slightly during inulin treatment (from 6.51 ± 0.49 to 6.31 ± 0.34), but this decrease did not reach statistical significance ($p = 0.2206$).

Defecation frequency was increased slightly compared to control, from 1.2 (± 1.6) times per day to 1.6 (± 2.4) times per day during inulin treatment (p=0.1900), whereas faecal consistency became softer following 3 weeks of inulin-treatment. A trend towards a decreased consistency was found: it changed from an average 2.4 (± 4.2) to 1.8 (± 2.7; $p = 0.0584$). We found an increase in the amount of faeces: during inulin treatment the amount was 167 (± 80) g versus 75 (± 30) g during the control ($p = 0.0088$). The magnitude of the changes in stool parameters was not correlated with the magnitude of the changes in microbial composition of the faeces; for instance, changes in faecal consistency as described above were not correlated with the change in content of bifidobacteria or lactobacilli (data not shown).

Discussion

This study shows that supplementation with native inulin of a standard infant formula containing oligosaccharides has a prebiotic effect in formula-fed Korean babies: an increase of *Bifidobacterium* sp. and *Lactobacillus* sp. was found. At the same time there was no increase in *Bacteroides* sp. or in total anaerobic count. Plate counting techniques were used to assess changes in microbial composition of the faeces. Despite the potential shortcomings of these techniques the data clearly show prebiotic changes in this composition. The numbers of *Bifidobacterium*, *Lactobacillus* and *Bacteroides* which are found with other techniques (e.g. FISH or DGGE [e.g. 19]) do not deviate from

what we find in this study. In the study by Favier et al. [20] these genera were identified by molecular methods as the major constituents of an infant's faecal microbiota. Moreover, Boehm et al. [21] using plate counting, report similar numbers in microbial composition of the faecal flora of formula-fed babies.

The changes in microbial composition were accompanied by changes in faecal consistency and amount. Only the latter reached statistical significance, but with the trend observed for consistency the data are indications that inulin leads to enhanced fermentation in the colon. In this respect our data are not essentially different from the data found in adults.[22,23,24]

The dosage at which these effects were observed in babies seems lower than published for effects in adults. When compared on a body weight basis however, the difference is much less or even absent. For native inulin prebiotic effects in adults have been reported in the range from 0.2 g/kg/d [22] to 0.5 g/kg/d.[23]The dosage applied in our study (0.25 g/kg/d) is well within this range.

On the other hand, when compared with the dosages used by others in studies with babies, the dosage in the present study seems low. For instance, in their study with preterm infants Boehm et al. [15,21] used about 1 g/kg/d of the oligosaccharide mixture (a mixture of short chain galacto-oligosaccharide and long chain inulin, 9:1, w/w). Similar dosages were applied in other studies.[13,15] Low dosages of fructooligosaccharides were without effect on the faecal microbial composition.[17] An explanation may be that the subjects in our study already were being bottle-fed for a considerable time on a formula containing oligosaccharides. The total daily dosage is therefore higher than from the addition of the native inulin alone. It may also be that the chain length distribution of native inulin was suitable for a prebiotic effect. Boehm et al. [21] have shown that fructooligosaccharides alone do not exhibit a bifidogenic response [17] whereas a mixture of these oligosaccharides with long chain inulin does have such an effect. They suggested that the mixture has a chain length distribution more or less equal to that of the oligosaccharides in mother's milk. In line with this we suggest that the chain length distribution present in native inulin with a degree of polymerization ranging from 3 to 60 also favours a bifidogenic effect.

The dosage is also low when compared to mother's milk, which contains 10-20 g/l of oligosaccharides. Not all of these carbohydrates show bifidogenic properties; probably only the low molecular weight saccharides are responsible for this effect.[25] This implies that the daily prebiotic dosage obtained from mother's milk is lower than expected from the concentration alone.

The type of dose response curve (a higher bifidogenic response with a lower starting value of *Bifidobacterium* sp.) has also been reported for adults.[26,27] In adults it is also not uncommon to find individuals who do not respond to inulin consumption in terms of changes in faecal microbial composition.[28] Taken together it follows from our data that in formula-fed babies native inulin behaves as a prebiotic in much the same way as in the adult population.

Next to the bifidogenic effect we also found a clear positive effect on the faecal *Lactobacillus* content. For adults significant changes in this genus have not been reported [28,29], but in infants Boehm et al. [21] and Moro et al. [13] showed increases in *Lactobacillus* sp. similar to the present study. It may well be that the longer fructan chains as present in native inulin are responsible for this stimulatory effect on *Lactobacillus*.[30]

Both the increase in *Bifidobacterium* and *Lactobacillus* could have health benefits. With regard to the difference in *Bifidobacterium* composition in allergic and healthy infants [31] it could be interesting to study the effects of native inulin on the allergic response of infants. There are already indications that inulins may affect the immune system in a positive way.[30,32] Inulins may stimulate mineral absorption in infants just as they do in adults.[33]

In summary we conclude that the addition of native inulin to baby formula elicits a prebiotic response in formula-fed babies. The significant increase in stool amount and the trend for softer stool consistency due to inulin consumption may offer benefits to infants that suffer from constipation or hard stools. Native inulin may therefore help improve the babies' health. Further research is required to show the health benefits.

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Research Articles

Prolonged administration of low-dose inulin stimulates the growth of bifidobacteria in humans

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Abstract

The effect of low-dose inulin consumption on fecal bifidobacteria growth, microbial activity, and tolerance in healthy adults was investigated in a double-blind, randomized, placebo-controlled, parallel-group study. Thirty-nine healthy volunteers were randomly assigned to 2 groups and ingested 2.5 g inulin or placebo twice a day for 4 weeks (from week 2 to week 6). Fresh stools were collected after 2, 4, 6, and 8 weeks for fecal bacteria count and fecal bacterial enzymatic activity measurement. Tolerance was evaluated from a daily chart. In the inulin group, fecal bifidobacteria count increased ($P < .0001$), whereas no change was observed in the placebo group. *Lactobacillus* counts did not change in the inulin group and decreased in the placebo group ($P = .0004$). In the inulin group, a decrease in β-glucuronidase activity ($P = .001$) was found, which was negatively correlated with the level of *Bifidobacterium* ($P = .04$). Throughout the study, there was no change in fecal enterobacteria, pH, β-galactosidase activity, reductase activity, or short-chain fatty acid level in either of the groups. Excess flatus significantly increased in both groups (inulin, $P < .0001$; placebo, $P = .03$), but its intensity was very mild. Even at doses as low as 2.5 g twice a day, inulin can exert a prebiotic effect in healthy volunteers by stimulating bifidobacteria growth.

Keywords: Bifidobacterium; Human; Intestinal microflora; Inulin; Prebiotic; Randomized controlled trial

1. Introduction

The human large intestine contains a variety of bacterial genera, species, and strains, which are considered either beneficial (eg, *Bifidobacterium*, *Eubacterium*, and *Lactobacillus*) or detrimental (eg, *Clostridium* and *Veillonella*) to the host's health [1,2]. Although this generalization probably gives a too simplistic view of gut microbiology, it is a feasible working concept for the development of functional food components to modulate the composition of the colonic microbiota [3]. In this context, a prebiotic was defined as "a nondigestible 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 and thus improves host health" [4]. Several nondigestible oligosaccharides (fructans, galacto-oligosaccharides, xylo-oligosaccharides) have demonstrated their ability to stimulate the growth of bifidobacteria in animals [5] and in humans [6-8]. Moreover, according to a recent review, inulin is included in the short list of the currently available

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ingredients for which convincing evidence of a prebiotic effect has been reported [9]. Stimulation of bifidobacteria with relatively high doses of inulin ranging from 8 to 40 g/d has been repeatedly demonstrated in humans [10-14]. However, the effects of lower dosages of this particular ingredient on the intestinal flora have not yet been tested in humans. Recent in vitro data indicate that inulin has a clear prebiotic effect at a concentration corresponding to a human daily dose of only 5 g [15]. Therefore, the aim of the present study was to confirm this effect in healthy adults at a low daily dose of 5 g/d.

2. Methods and materials

2.1. Subjects and experimental design

Thirty-nine healthy volunteers, aged 20 to 58 years (mean, 33.9 years), participated in the study. None had any history of gastrointestinal disease except for appendectomy. No antibiotics or laxatives had been taken during the 2 months before the study. No other medication was allowed during the investigation period. The research protocol was approved by the institutional Human Ethics Committee of Saint Louis Hospital (Paris, France).

The volunteers were randomly assigned to 2 groups, 20 to the inulin group and 19 to the placebo group. Both groups were required to follow the treatment (inulin or placebo) in parallel. The basal period was from week 0 to week 2; then, for 4 weeks (from week 2 to week 6), the volunteers ingested a powder mixture containing 5 g inulin or placebo divided into 2 oral doses taken after breakfast and after dinner. In the washout period, from week 6 to week 8, the volunteers were allowed to return to their normal diet. The inulin (Fibruline Instant, Cosucra, Warcoing, Belgium) used was composed of linear chains of fructose units with one terminal glucose unit with a degree of polymerization ranging from 2 to 60 and with average polymerization of about 10. The placebo was composed of a blend of maltodextrins and sucrose.

To avoid bias that would mask the effects of the treatment, subjects were asked to limit consumption of products containing high levels of inulin or oligofructose (chicory, onion, asparagus, wheat, rye, triticale, and Jerusalem artichoke) and fermented dairy products containing viable bifidobacteria for the duration of the study [16]. These dietary restrictions started at week 0, that is, 14 days before the first stool recovery, with the aim of obtaining comparable basal levels of bifidobacteria in the 2 groups, and were continued during the last 2 weeks (from week 6 to week 8) for evaluation of the prolongation of the potential prebiotic effect.

A dietary inquiry was performed during four 2-day diet records in the basal period (from day 13 to day 14), after 2 (from day 27 to day 28) and 4 (from day 41 to day 42) weeks of product ingestion, and 2 weeks after treatment cessation (from day 55 to day 56) for calculation of total energy intake and the amount of carbohydrates, fiber, fat, and protein. This dietary inquiry was also used to verify that volunteers had strictly ingested the allowed diet. Tolerance was evaluated by using a daily chart where the symptoms (excess flatus, borborygmi, bloating, abdominal pain) were graded from 0 (no symptom) to 3 (severe symptoms). For each 2-week period, symptom intensity was obtained by adding each daily score with the following gradation: 0, no symptom; 14, mild symptom; 28, moderate symptom; 42, severe symptom. Frequency and consistency of stools were also noted, and diarrhea was defined as one or more liquid stools or more than 3 stools per day.

Stools were collected 4 times, on the first day before the treatment consumption started, that is, after 2 weeks of dietetic restriction (W_0), after 2 (W_2) and 4 (W_4) weeks of product ingestion, and 2 weeks after treatment cessation (W_6). They were collected in plastic containers under anaerobic conditions (Anaerocult A, Merck, Darmstadt, Germany), immediately stored at 4°C, and analyzed within 2 hours. Two aliquots of stool were frozen for further analysis of fecal bacterial enzymatic activities.

2.2. Laboratory analysis

The laboratory analyses were performed following the methods described previously [17]. An aliquot of stool was prepared and analyzed within 2 hours of elimination. The remaining stool samples were frozen at −20°C for later analysis. This fresh stool aliquot was diluted 10-fold (wt/vol) in an anaerobic solution (1/4-strength cysteinated Ringer diluents). After homogenization, the fecal slurry was diluted 10-fold up to 10^{-9} in the anaerobic solution. According to the chosen genera, selective media were inoculated: total anaerobic count (Wilkins-Chalgren agar) and bifidobacteria (Beerens medium), *Lactobacilli* (MRS agar), enterobacteria (McConkey agar), and *Bacteroides* (BBE agar). Plates were incubated aerobically or anaerobically as appropriate. The pH was measured by a pH meter on a fresh stool aliquot (Bioblock, Illkirch, France).

Enzyme activities were measured in fecal samples by using a thermoregulated anaerobic chamber (H_2, CO_2, N_2; 10:10:80), as previously described [18]. Five grams of each fecal sample stored at −20°C was introduced into the anaerobic chamber and immediately diluted 1:20 with prereduced phosphate-buffered saline (pH 6.7). β-Galactosidase and β-glucuronidase activities were measured by determining the rate of *p*-nitrophenol released from *p*-nitrophenyl glycosides. Azoreductase activity was determined by using amaranth (5 mmol/L) as substrate. Nitrate reductase was determined by the generation of nitrite. Nitroreductase was measured as described by Wise et al [19]. Protein concentration was determined in triplicate by the method of Lowry et al [20] at 1:500 fecal dilution in Na_2CO_3 (2%) and NaOH (0.1N). Bovine serum albumin was used as the standard.

The concentration of short-chain fatty acids (SCFAs) in fecal samples was analyzed in duplicate after water extraction of acidified samples using gas chromatography (Perkin-Elmer 1020 GC; Marseille, France) [18]. Total SCFAs were expressed as micromoles per gram of fecal sample.

2.3. Statistical analysis and determination of sample size

Fecal concentrations of bacteria were expressed as log colony-forming units per gram (cfu/g) wet weight. Enzyme activities were expressed as micromoles of metabolized substrate per minute and per gram of protein. Results were expressed as mean ± SEM. Comparison of bacterial concentrations, pH, fecal bacterial enzymatic activities, SCFAs, and dietary inquiries were performed by repeated-measures analysis of variance (ANOVA) to test the time effect and by using factorial ANOVAs for the comparison between treatments. Because a possible interaction between basal bifidobacteria counts and treatment response has been suggested previously [21], a 3-level independent variable (low, middle, and high basal counts) was added to the factorial ANOVA model. Symptoms experienced with inulin and placebo were compared by using repeated-measures ANOVAs.

Previous studies [22,23] have shown that the variability of the main criteria (fecal bifidobacteria counts, expressed in log cfu/g) in terms of SD was 1 unit. The relevant beneficial effect is a difference in 1 log cfu/g unit between treatment and placebo. Under these conditions, a 2-way formulation with α of .05 and power of .80, each treatment group has to be composed of 16 volunteers to show a difference of 1 log cfu/g in favor of the inulin group.

3. Results

Table 1 summarizes the bacterial counts and pH during the 3 periods in the 2 groups. In the basal period, basal bacterial counts were similar in both groups, except for lactobacilli, which were significantly lower in the inulin group ($P = .03$). The fecal bifidobacteria count significantly increased in the inulin group ($P < .0001$), whereas no change was observed in the placebo group.

When count variations were analyzed, the bifidobacteria count increased by 1.1 ± 0.2 log in the inulin group compared with 0.4 ± 0.2 log in the placebo group during the ingestion period ($P = .03$) (Fig. 1). The results were not modified by adjustment of the basal bifidobacteria count, defined as low (5.70 to 7.64, n = 13), medium (7.65 to 8.54, n = 13) and high (8.55 to 9.73, n = 13): the increase in bifidobacteria count was still significantly higher in the inulin than in the placebo group ($P = .045$).

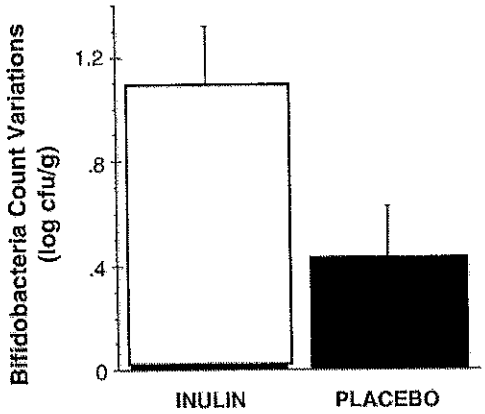

Fig. 1. Effect of 4-week ingestion (from week 2 to week 6) of 2.5 g inulin or placebo twice a day on bifidobacteria count variations in healthy volunteers ($P = .03$).

Two weeks after treatment finished (W_8), bifidobacteria counts were still significantly higher than during the basal period (W_2) in the inulin group, but not in the placebo group. *Lactobacillus* counts significantly decreased in the placebo group ($P = .0004$) and did not change in the inulin group.

Total anaerobe counts significantly increased in the inulin group ($P < .0001$) and in the placebo group ($P = .01$) during the study. Similarly, *Bacteroides* significantly increased in both inulin ($P = .0001$) and placebo ($P = .01$) groups. Neither enterobacteria nor the fecal pH changed in either group throughout the study.

The β-galactosidase activity remained unchanged in both groups throughout the study (Table 2). The β-glucuronidase activity significantly decreased during inulin ingestion ($P = .001$) but not in the placebo group ($P = .10$).

In the inulin group, the β-glucuronidase activity was negatively correlated with the level of *Bifidobacterium* ($P = .04$). In the 2 treatment groups, a low level of β-glucuronidase was related to a high level of *Bifidobacterium* ($P = .006$). There was no significant modification of

Table 1
Effects of 4-week ingestion (from week 2 to week 6) of 2.5 g inulin or placebo twice a day on the fecal bacterial counts (log cfu/g wet weights) in healthy volunteers

	Placebo (n = 19)					Inulin (n = 20)				
	Basal, W_2	Ingestion, W_4-W_6		Follow-up, W_8	P	Basal, W_2	Ingestion, W_4-W_6		Follow-up, W_8	P
Bifidobacteria	8.2 (0.2)	8.6 (0.2)	8.6 (0.3)	8.4 (0.3)	NS	7.7 (0.3)	8.7 (0.3)	9.0 (0.1)	8.8 (0.1)	.0001
Total anaerobes	9.5 (0.1)	9.7 (0.1)	10.0 (0.1)	9.9 (0.1)	.0001	9.1 (0.2)	9.7 (0.1)	10.0 (0.1)	10.0 (0.1)	.0001
Lactobacilli	6.3 (0.3)	5.8 (0.2)	4.9 (0.3)	5.4 (0.2)	.0004	5.4 (0.2)	5.4 (0.3)	5.4 (0.3)	5.2 (0.2)	NS
Bacteroides	7.5 (0.5)	8.8 (0.2)	8.8 (0.1)	8.8 (0.2)	.01	7.2 (0.4)	8.2 (0.3)	8.7 (0.2)	8.7 (0.1)	.0001
Enterobacteria	7.0 (0.3)	7.1 (0.3)	7.3 (0.2)	7.1 (0.2)	NS	6.4 (0.3)	6.2 (0.4)	6.4 (0.3)	6.8 (0.3)	NS
pH	6.5 (0.1)	6.6 (0.1)	6.5 (0.1)	6.4 (0.1)	NS	6.5 (0.1)	6.5 (0.1)	6.4 (0.1)	6.4 (0.1)	NS

Values are expressed as mean (SEM). W indicates week. Significantly different from the basal period ($P < .05$).

Table 2
Effects of 4-week ingestion (from week 2 to week 6) of 2.5 g inulin or placebo twice a day on fecal enzymatic activities and SCFAs in healthy volunteers

	Placebo (n = 19)				Inulin (n = 20)			
	Basal, W_2	Ingestion, W_4-W_6		Follow-up, W_8	Basal, W_2	Ingestion, W_4-W_6		Follow-up, W_8
β-Galactosidase (μmol/min per gram of protein)	58 (7)	65 (9)	61 (8)	59 (8)	78 (9)	64 (8)	74 (10)	58 (7)
β-Glucuronidase (μmol/min per gram of protein)	3.5 (0.5)	2.4 (0.4)	2.7 (0.3)	3.9 (0.7)	4.5 (0.5)	2.4 (0.4)*	2.5 (0.4)*	2.9 (0.5)*
Nitrate reductase (μmol/min per gram of protein)	0.11 (0.03)	0.07 (0.03)	0.15 (0.06)	0.07 (0.03)	0.1 (0.05)	0.08 (0.04)	0.05 (0.01)	0.06(0.03)
Nitroreductase (μmol/min per gram of protein)	0.02 (0.01)	0.03 (0.01)	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.01 (0.00)
Azoreductase (μmol/min per gram of protein)	0.18 (0.05)	0.12 (0.04)	0.10 (0.02)	0.10 (0.02)	0.10 (0.02)	0.07 (0.02)	0.09 (0.03)	0.08 (0.02)
Total SCFAs (μmol per gram of fecal sample)	87 (8)	83 (8)	103 (11)	98 (9)	97 (10)	95 (12)	109 (10)	93 (9)

Values are expressed as mean (SEM). W indicates week.
* $P < .05$, significantly different from the basal period.

nitrate reductase activity during the experiment in either group. Similarly, nitroreductase and azoreductase activities were not altered by inulin intake. Neither the concentration nor the profile of SCFAs changed in the 2 groups over the course of the study.

The results of the four 2-day diet queries are reported in Table 3. There was no significant difference in total energy intake or mean daily intakes of protein, fat, carbohydrates, or fiber between the 2 groups or within each group before or after treatment ingestion.

The digestive symptoms reported by the volunteers during the study are shown in Table 4. Flatus significantly increased in the inulin group ($P < .0001$) and in the placebo group ($P = .03$), but the intensity of these symptoms was very mild. The symptom frequency (number of days that symptoms >0 were recorded) was rather low (≤43% of the total ingestion period) and was not significantly different between the 2 groups. Bloating, borborygmi, abdominal pain, stool weight, and stool frequency were not significantly different in all periods. Diarrhea was not reported in any of the volunteers.

4. Discussion

In this placebo-controlled study, 4 weeks of ingestion of inulin at a low dose of 2.5 g twice a day was well tolerated and led to a significant increase in fecal bifidobacteria counts in healthy volunteers. The results confirm recent in vitro data showing that inulin has a clear prebiotic effect in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) at a concentration corresponding to a human daily dose of 5 g [15]. Other human intervention studies had previously found a bifidogenic effect of inulin, but all of them used higher doses of inulin, from 8 to 40 g/d, and a linear design without a control group [10-14]. Tuohy et al [10] showed that 8 g/d long-chain inulin for 2 weeks led to a significant increase in bifidobacteria in 10 adults. Using 9 g/d inulin for 4 weeks in 11 adults, Brighenti et al [12] found similar results. A direct comparison of inulin to fructo-oligosaccharides (GFn, $2 < n < 6$) in a human feeding trial demonstrated that inulin at a dose of 15 g/d had a similar prebiotic effect to that of fructo-oligosaccharides [11]. However, this study should be interpreted with caution because only 4 subjects were studied. In the other 2 studies, inulin was also bifidogenic, but administration started at doses of 20 g/d, thereafter increasing to 40 g/d [13,14].

In most studies evaluating the bifidogenicity of certain substrates, linear study designs have been used and measurements were made before and after treatment [8,11,24-26] without a control group. Our results indicate the importance of a placebo treatment to exclude the possible effects of time and unknown environmental factors. The effect of a control group was also underlined by Alles et al [26], as it changed their conclusion about bifidogenicity of the treatment tested:

Table 3
Mean daily intakes in the 2 groups of volunteers

	Inulin (n = 20)				Placebo (n = 19)			
	Basal, W_2	Ingestion, W_4-W_6		Follow-up, W_8	Basal, W_2	Ingestion, W_4-W_6		Follow-up, W_8
Energy (kJ)	7638 (473)	7672 (582)	6662 (348)	704 (385)	9193 (486)	7915 (507)	7902 (381)	7814 (436)
Protein (g)	77 (6)	69 (5)	67 (5)	80 (13)	74 (4)	68 (5)	68 (4)	76 (4)
Fat (g)	82 (7)	80 (8)	66 (6)	77 (6)	94 (7)	76 (6)	76 (4)	80 (4)
Protein (g)	77 (6)	69 (5)	67 (5)	80 (13)	74 (4)	68 (5)	68 (4)	76 (4)
Fat (g)	82 (7)	80 (8)	66 (6)	77 (6)	94 (7)	76 (6)	76 (4)	80 (4)
Carbohydrate (g)	181 (11)	196 (15)	196 (15)	169 (11)	238 (13)	215 (12)	215 (12)	196 (13)
Fibers (g)	15 (2)	13 (1)	14 (1)	14 (1)	18 (1)	18 (1)	15 (1)	17 (2)

Values are expressed as mean (SEM). W indicates week.

Table 4
Effect of 4-week ingestion (from week 2 to week 6) of 2.5 g inulin or placebo twice a day on digestive symptom intensity in healthy volunteers

	Inulin (n=20)					Placebo (n=19)				
	Basal, W_2	Ingestion, W_4-W_6		Follow-up, W_8	P	Basal, W_2	Ingestion, W_4-W_6		Follow-up, W_8	P
Excess flatus	4.0 (1.2)	7.8 (1.7)	8.1 (1.9)	4.0 (1.4)	.0001	4.4 (1.4)	6.5 (1.7)	5.2 (1.4)	3.8 (1.6)	.03
Borborygmi	1.8 (0.8)	2.0 (0.8)	1.4 (0.6)	1.4 (0.8)	NS	2.8 (1.1)	3.1 (1.3)	2.9 (1.2)	3.0 (1.5)	NS
Bloating	1.5 (0.7)	2.5 (1.1)	2.6 (1.0)	1.6 (0.8)	NS	3.8 (1.3)	4.9 (1.7)	4.3 (1.3)	3.1 (1.3)	NS
Abdominal pain	0.6 (0.3)	1.4 (0.8)	1.6 (0.7)	0.6 (0.3)	NS	1.9 (1.0)	2.9 (1.3)	2.5 (1.0)	1.9 (1.2)	NS
No. of stools per period (14 d)	17 (1.4)	16 (1.5)	16 (1.1)	15 (1.1)	NS	16 (1.1)	16 (1.2)	15 (1.1)	16 (1.3)	NS

Values are expressed as mean (SEM). Symptom intensity was noted as follows: 0 = no symptom; 14 = mild symptom; 28 = moderate symptom; 42 = severe symptom. W indicates week.

transgalacto-oligosaccharides were found to be bifidogenic on linear analysis, but this effect was not different from the one observed in the placebo group.

One of the hypothetical confounding factors when interpreting a bifidobacteria-level increase is the initial level of bifidobacteria, as shown by us (Y. Bouhnik, unpublished data) and others [21,27]. We confirmed that a low basal bifidobacteria count is significantly associated with an increase after treatment.

It has been shown that fecal bacterial β-glucuronidase activity increases in patients on a high-meat diet and that this enzyme could act to increase the amount of substances, such as carcinogens, within the colonic lumen [28]. This enzyme may play a role in the metabolic activation of procarcinogens and deconjugation processes in the colonic lumen [29]. Our results corroborate those from studies with probiotics, which have shown that increasing the proportion of the fecal flora represented by bifidobacteria is associated with lower activity of reductive enzymes [30-33]. However, Kleessen et al [13] were unable to show changes in β-glucuronidase activity with long-chain inulin consumption at a dose range of 20 to 40 g/d for 19 days. The reciprocal shifts in activities of β-glucuronidase and densities of bifidobacteria during supplementation with inulin can be caused by a combination of diet-induced shifts in the species composition of the fecal flora and in the metabolic characteristics of individual species or strains. Results similar to ours have been reported in an in vitro simulation of the human intestinal microbial ecology [34].

In a previous study, we compared the effect on fecal bifidobacteria of prolonged ingestion of exogenous bifidobacteria administered as fermented milk with or without the addition of inulin at the rate of 18 g/d [22]. We observed that the ingestion of bifidus-fermented milk itself, without the addition of inulin, significantly increased fecal bifidobacteria. As a result, no further increase was shown with the addition of inulin. However, in this study, 2 weeks after stopping the ingestion of bifidus-fermented milk, subjects who consumed inulin-supplemented bifidus-fermented milk had significantly higher fecal bifidobacteria than subjects who ingested unsupplemented bifidus-fermented milk. These results support the conclusion that inulin is able to sustain a higher level of bifidobacteria for longer periods after its cessation, as we found in the present study.

We did not find any differences in fecal pH and SCFAs during the treatment periods. This effect was desirable because a decrease in colonic pH might reduce the risk of developing colon cancer [35]. As in our previous studies [22,23], fecal pH did not change during the ingestion of oligosaccharides. However, fecal pH may not accurately reflect the pH in the colon, which depends on absorption of SCFAs and bicarbonate secretion [35,36]. Short-chain fatty acids are implicated in colonic physiology and, among them, butyrate arising from microbial fermentation is important for energy metabolism and normal development of colonic epithelial cells and has a mainly protective role in relation to colonic disease [37]. In vivo, the study of SCFAs is difficult and relies mostly on determination of the concentrations in feces. This approach, however, is limited as far as SCFAs are absorbed by the colon epithelium. It is thus not surprising that little has been learned from it about the fermentation of prebiotics. In the 3 comprehensive human studies that have been published, neither inulin nor oligofructose at doses ranging from 4 to 40 g/d produced any significant change in the concentration or molar ratios of fecal SCFAs [11,13,38]. In vitro, however, at a concentration corresponding to a human daily dose of 5 g, inulin significantly increased the total concentration of SCFAs in the 3 compartments of the SHIME large intestinal model, with a shift toward higher proportions of propionate and butyrate and lower proportions of acetate [15].

Symptoms related to gas production in the gut are widely reported in human prebiotic feeding studies but remain very mild at recommended intakes [11]. In our study, excess flatus was significantly more intense (but not significantly more frequent) during inulin compared with placebo ingestion, but this symptom was very mild. Few studies have evaluated the digestive tolerance of inulin. At a higher dose of 14 g/d, inulin led to a significant increase in flatus, bloating, borborygmi, and abdominal pain in a group of 64 women taking inulin in a double-blind crossover study over 4-week periods; 12% of the volunteers considered the flatulence severe and unacceptable, and no adaptation in symptoms occurred over time [39].

Although increased stool weight and frequency have been described previously, especially for constipated volunteers at daily inulin doses of 15 g or higher [11,13], the absence of such increase in the present study might be explained by the relatively low dose used and/or the already high average initial number of stools (>1/d) in both the inulin and the placebo groups.

In conclusion, under our experimental conditions, this study showed that even at a low dose of 2.5 g twice a day, inulin can exert prebiotic effects such as stimulation of bifidobacteria and decrease in β-glucuronidase activity, which could be perceived as potentially beneficial for the host.

Acknowledgment

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Appendix 12

MSDS for Chicory Root Extract (Frutafit® and Frutalose® Inulin/Oligofructose from Sensus)

Material Safety Data Sheet

1. IDENTIFICATION

Name of the product: FRUTAFIT® types
Codes N°: HD, IQ, TEX!, CLR
Use: Broad spectrum of inulin and oligosaccharides
Supplier: Sensus America, LLC, Princeton Corp. Plaza,
One Deer Park Drive, Suite J,
Monmouth Jct., NJ 08852 USA
Phone: 646-452-6140 Fax: 646-452-6150
Producer: SENSUS Operations C.V., Roosendaal (NL)

2. COMPOSITION/INFORMATION ON INGREDIENTS

Inulin, Fructo-oligosaccharide, Oligofructose, Polyfructose
100% natural vegetable material.

3. HAZARD IDENTIFICATION

Ingestion : Not limited by ADI-value. Excessive intake may cause flatulence.
Inhalation : No known health risk. Inhaling of large quantities of powder may affect the ability to breathe.
Eye contact : Irritation may occur.
Skin contact : No known health risks.

4. FIRST AID MEASURES

Ingestion : No action required.
Inhalation : After inhalation of large quantities, keep the airways open and contact a doctor.
Eye contact : Rinse with excess water.
Skin contact : Wash off with water.

5 FIRE FIGHTING MEASURES

Fire and explosion : Minimum ignition temperature : 360° C (680°F). Min. Ignition Energy (MIE) : 154 mJ <= MIE <= 381 mJ
Fire fighting procedures : none. Extinguish with water.

6. PROCEDURES IN CASE OF ACCIDENTAL SPILLAGE

Steps to be taken in case material is released or spilled : Wipe up and wash down with water.

7. HANDLING AND STORAGE

Precautions to be taken in handling and storing : Store at cool to normal ambient temperatures, in air- and watertight package, in dry surroundings to avoid water uptake (slightly hygroscopic substance).
Other precautions: None

8. EXPOSURE CONTROL AND PERSONEL PROTECTION

Respiratory protection : None, or simple inhalation mask to protect against inhalation of dust. **Other protective clothing or equipment :** Gloves (for reason of hygiene).

9. PHYSICAL AND CHEMICAL PROPERTIES (ND).

Description :	White powder, odorless, neutral to slightly sweet taste.
Melting point :	N/A
Bulk density :	+/- 0.5 kg/L
Solubility in water :	100 g/L at 25°C (77°F)
Percentage Moisture by Weight:	<=5%

CONTINUED

SENSUS
Sensus America LLC
Princeton Corporate Plaza
1 Deer Park Drive, Suite J
Monmouth Junction
NJ 08852, USA
t. 646.452.6140 f. 646.452.6150
www.sensus.us
e-mail: contact@sensus.us

10. STABILITY AND REACTIVITY.

Stability: Stable
Reactivity : Inert
Incompatibilities (materials to avoid) : None
Melting /decomposition temp : 180°C (356°F) ()
Polymerisation : N/A

11. TOXICOLOGICAL INFORMATION

See paragraph III

12. ECOLOGICAL INFORMATION

Environmental information : Inulin has no known negative environmental effects. Because inulin and derivates are natural polysaccharides, it is easily biological degradable.

13. WASTE DISPOSAL CONSIDERATIONS.

Instructions for removal : Remove in accordance with local rules and regulations. In general dry material can be put on a dumping ground. Wet material can be disposed by the sewer, if diluted to prevent blockage.

14. TRANSPORTATION ADVISE

Instructions for transport : Product is non hazardous. No special precautions required for transport.

15. REGULATORY INFORMATION AND LABELLING INFORMATION

AAFCO:
Products approved by FDA/CVM. Pending AAFCO listing
Food ingredient status :
In EU, Nordic countries, Switzerland, Israel, Australia, New-Zealand, Japan, Canada : No E-number
USA : GRAS-status
Use at libitum in all food categories
Dietary fiber status:
In Italy, Belgium, Scandinavian Countries, Switzerland, the Netherlands, France, Japan, Other Asian countries.
In the USA and Canada, the FDA and Health Canada are dealing with this issue and the dietary fiber status for all Frutafit inulin is expected.
Caloric value:
The national regulations of some European countries, together with the available results of scientific calculations on the caloric value for Frutafit-inulin (1.5 kcal/g) results in the following overview :

Country	Caloric value
Italy, Switzerland, Nordic Countries	0 kcal / gram
Belgium, the Netherlands	1 kcal / gram
Denmark, France, Finland	2 kcal / gram
Other countries	unregulated

16. OTHER INFORMATION

Other information :
More information can be obtained from the supplying or producing partner companies.

Warning: The information contained herein is based on the present state of our knowledge and does not therefore guarantee certain properties. Recipients of our products must take responsibility for observing existing laws and regulations. This data sheet does not exempt in any case the customer/user to know and respect the regulations linked to his activity in their entirely and prerequisite cautions to take. Regulatory prescriptions and precautions mentioned in this sheet do not have to be considered as exhaustive. Their objective is to help the customer/user to respect safety requirements he has to conform to. Frutafit® products are refined industrial food products intended to professionals who have to verify, before using them, by any acceptable means, that they are suitable technically and regulatory for the intended purpose.

Date first issue : 02.10.2001
Version n° : 3
Revision Date : 01.22.2003

Material Safety Data Sheet
Frutalose®

Effective : 05•2006

Section I Product Identification

Substance :	Oligofructose syrup
Trade Names/Synonyms :	Frutalose/oligofructose
Chemical family :	carbohydrate - fructan
Average molecular weight :	± 900
Formula :	$C_6H_{12}O_6 - [C_6H_{10}O_5]n$; n aver. 9(range $2 \leq n \leq 10$)
Cas Nr.	9005-80-5

Section II Hazardous Ingredients

Info product :	Food ingredient
Component :	Oligofructose
Percent :	L90 ≥ 90% on dry matter

Section III Health Hazard Data

Ingestion :	Not limited by ADI-value. Excessive intake may cause flatulence.
Inhalation :	No known health risks. Inhaling of large quantities of liquids may affect the ability to breathe.
Eye contact :	No known health risks.
Skin contact :	Rinse with excess water. No known health risks. Wash off with water.

Section IV First Aid

Ingestion :	No action required
Inhalation :	After inhalation of large quantities, keep the airways open and contact a doctor
Eye contact :	Rinse with excess of clean Water or eye-wash solution for at least 15 minutes
Skin contact :	Wash off with water

Section V Fire and Explosion Hazard Data

Fire and explosion :	not relevant
Fire fighting procedures :	none

Section VI Accidental release

Steps to be taken in case material is released or spilled: wipe up and wash down with water

Section VII Precautions for Safe Handling and Use

Precautions to be taken in handling and storing :	store at cool temperatures (5-15°C), in its original, closed package.
Other precautions :	none

SENSUS
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e-mail: contact@sensus.us

Section VIII Control Measures

Respiratory protection :	none
Other protective clothing or equipment :	gloves (for reasons of hygiene)

Section IX Physical / Chemical Characteristics

Description :	Clear syrup
Smell :	Neutral
Taste :	Neutral, slightly sweet
Melting point :	NA
Bulk density :	± 1.38 kg/liter
Solubility in water :	in any quantity
Perc. Moisture by weight:	24 - 27%

Section X Reactivity Data

Reactivity :	inert
Incompatibilities (Materials to avoid):	none
Melting/decomposition temp:	NA
Polymerisation :	NA

Section XI Toxicological Information

Effect after ingestion, inhalation, eye/skin contact:	irritation is unlikely to develop.
Constituents which are carcinogenic :	none to our knowledge
LD 50 :	ND

Section XII Environmental Information

Environmental information :
Fructooligosaccharides have no known negative environmental effects. Because fructooligosaccharides are natural carbohydrates, they are easily biological degradable.

Section XIII Instructions for removal

Instructions for removal :
Remove in accordance with local, state or national legislation. In general material can be disposed by the sewer.

Section XIV Instructions for transport

Instructions for transport :
Product is non hazardous. No special precautions required for transport.

Section XV Legal status

EU countries :	ingredient (no E-number)
USA:	GRAS-status

Use *ad libitum* in all food categories

Hazard Symbol :	none required
Risk phrases :	none required
Safety phrases :	none required

Section XVI Other Information

For further information contact Sensus America
p: 646 452-6140 *f:* 646 452-6150

NA = not applicable ND = no data

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