Safety and Benefits of Fructooligosaccharides as Food Ingredients

Addition of fructooligosaccharides to plain, unsweetened yogurt improves taste and is considered by the authors to be generally recognized as safe

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□ FRUCTOOLIGOSACCHARIDES are naturally occurring sugars that can have beneficial effects as food ingredients. This article will discuss their safety and their use to improve the flavor of unsweetened lowfat yogurt.

Fructooligosaccharides

Fructooligosaccharides (FOS) are 1^F-(1-β-fructofuranosyl)$_{n-1}$ sucrose oligomers, where n may vary from 2 to 4 (Oku et al., 1984). They consist of sucrose molecules to which one, two, or three additional fructose units have been added by a β-(2-1)-glycosidic linkage to the fructose unit of sucrose (Fig. 1; Clevenger et al., 1988).

Abbreviated as GF_2 (1-kestose), GF_3 (nystose), and GF_4 (1^F-β-fructofuranosylnystose), these common sugars are found in a variety of edible plants (Fig. 2) that have been used as human and animal food sources for many years, including banana, barley, garlic, honey, onion, rye, brown sugar, tomato, asparagus root, Jerusalem artichoke, wheat, and triticale (Fishbein et al., 1988; Kamen, 1992).

Natural levels of FOS (as 1-kestose) have been evaluated in several foods (NET, 1990). Table 1 presents the total daily consumption of FOS from all sources, estimated by multiplying the concentration of FOS in those foods by the average daily consumption rate for each, derived using data from the Environmental Protection Agency's Dietary Risk Evaluation System (EPA, 1984). Consumption estimates used in this analysis were year-round average consumption rates, with an assumed average body weight of 58.9 kg. Water content values were derived from U. S. Dept. of Agriculture consumption data (USDA, 1975). Wet-weight FOS levels reported in Table 1 represent the midpoint of the range of reported analytical values. Based on these data, average daily consumption of FOS from natural sources is estimated to be approximately 13.7 mg/kg/day or 806 mg/day.

Commercially produced FOS (marketed under the brand name Nutraflora™ by Golden Technologies, Inc., Westminster, Colo.) are 0.4–0.6 times as sweet as sucrose. They are produced by the action of a fungal (*Aspergillus niger*) β-fructofuranosidase on sucrose, and are no different from FOS found naturally in plants. The commercial product is a mixture of GF_2, GF_3, GF_4, sucrose, glucose, and fructose.

The isolation and development of FOS were first reported by Hidaka (1983). The manufacturing procedures employed are similar to those used for common food ingredients, such as maltose and dextrose syrup. The enzyme reaction is followed by decloration, filtration, desalting, and concentration processes. In-process quality controls are used to eliminate or reduce any contaminants that could occur during the manufacturing process. High-performance liquid chromatography is used to determine the composition of the final product. *A. niger* is a common naturally occurring, nonpathogenic organism with an extensive history of safe use in the food industry (21 CFR 173.120, 173.280, 184.1318; Pelczar and Reid, 1972).

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Safety

In Japan, FOS are considered food, not food ingredients, and are found in more than 500 food products, resulting in significant daily consumption. In addition, FOS are currently used as a feed additive in poultry in the United States and Japan.

Numerous in-vitro and in-vivo studies have been conducted to evaluate the potential toxicity of FOS to animals and man. In-vitro animal toxicology studies, including a microbial reverse mutation assay (Ames assay), a mammalian gene mutation assay, and an unscheduled DNA synthesis assay were conducted (Clevenger et al., 1988). Results provided no evidence that FOS possessed any genotoxic potential. Subchronic and chronic toxicity and

carcinogenicity studies in rats revealed no significant adverse effects at doses up to 2,170 mg/kg/day (Tokunaga et al., 1986; Clevenger et al., 1988). The no-observed-effect level (NOEL) for chronic administration of FOS is therefore 2,170 mg/kg/day. The only effect noted was the occurrence of soft stools or diarrhea after ingestion of large quantities of FOS (more than 5% in the diet of rats), but this is common to oligosaccharides of poor digestibility found in the everyday human and animal diet.

Numerous feed-efficiency studies in male broiler chicks revealed no adverse effects related to feed supplementation with FOS, and feed efficiency was enhanced (Fishbein et al., 1988). These results are consistent with studies in other species where FOS have a positive effect on the gut flora and health outcomes of piglets (Fukuyasu, 1986) and dogs and cats (Ogata, 1986).

FOS are resistant to digestion by mammalian α-amylase, sucrase, and maltase. They are therefore nondigestible by humans but can be utilized by select Gram-positive organisms, such as bifidobacteria (Fishbein et al., 1988). In-vitro studies indicate that FOS are not hydrolyzable by rat digestive tract enzymes taken from the pancreas and duodenum and that very little, if any, hydrolysis is accomplished in the small intestine (Oku et al., 1984; Tsuji et al., 1986). In-vivo studies in the rat suggest that in addition to being nondigestible, FOS may possess some dietary fiber–like function, since they cause a decline in the hydrolysis of sucrose and maltose (Oku et al., 1984).

FOS have been shown to suppress the formation of putrefactive products (noxious substances formed by some intestinal microorganisms) in rats (Hidaka et al., 1986) and humans. Confirmation of the nondigestibility of FOS in the internal organs of the rat was obtained in studies using radioactive FOS administered intravenously; more than 97% recovery of unmetabolized FOS was found in urine (Oku et al., 1984).

In-vitro tests using FOS components and human salivary enzymes revealed little hydrolytic activity, while in-vitro anaerobic fermentation using human stool and radioactive FOS revealed almost 90% recovery after 8 hr (Hidaka et al., 1986). However, recent in-vivo studies indicate that FOS undergo bacterial fermentation to metabolizable products in the lower intestine (Hidaka et al., 1986; Tokunaga et al., 1989; Hosoya et al., 1988).

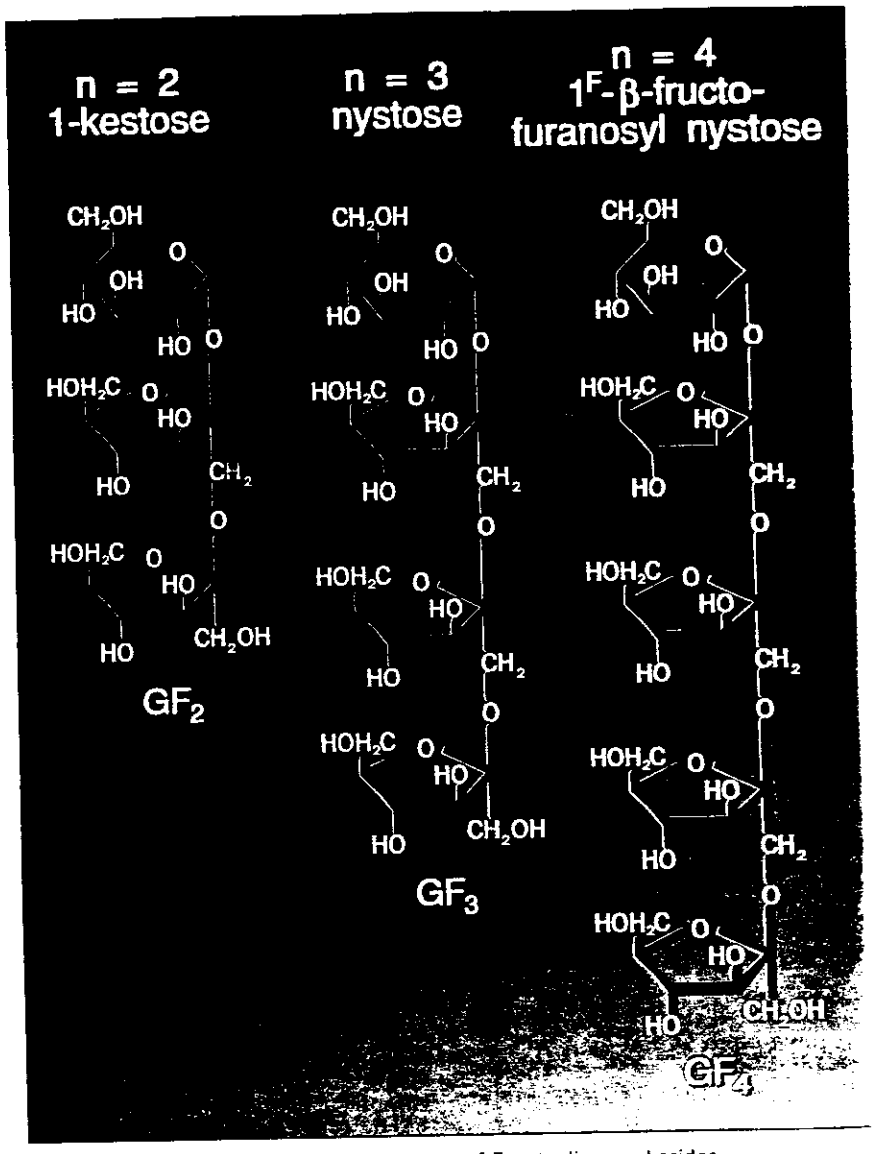

Fig. 1—**Molecular Structure of Fructooligosaccharides**

FOS are utilized by bifidobacteria from the human intestinal tract in vitro (Hidaka et al., 1986). Human clinical studies (Hidaka et al., 1986) in Japan revealed that FOS were selectively utilized by bifidobacteria; the authors found that this activity improved intestinal flora, relieved constipation, improved blood lipids, and suppressed the production of putrefactive substances. A dose response was noted in one study in which bifidobacteria increased with increasing consumption of FOS. Because FOS are difficult to digest, ingesting a large volume may result in diarrhea, as is the case with many other oligosaccharides. However, Hata and Nakajima (1985) examined the relationship between FOS and intestinal symptoms in human volunteers and found that the minimum dose of FOS required to induce diarrhea was 44 g for men and 49 g for women when FOS were added to food.

Beneficial Effects

Yogurt is recognized as a "healthy food" which contains viable benefi-

cial bacteria, including *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. There is an increased interest in yogurt which also contains cultures of *Lactobacillus acidophilus* and *Bifidobacterium infantis*. Tests were conducted to determine the effect of the addition of FOS to plain, unsweetened yogurt on sensory parameters, yogurt cultures, and pH.

Sensory testing was conducted using a consumer panel of 30 people. The panelists rated four different yogurt samples. The commercial FOS mixture containing 0.5% FOS was added or not added to a standard culture yogurt (containing *L. bulgaricus* and *S. thermophilus*) and to a yogurt preparation containing the above bacteria plus *L. acidophilus* and *B. infantis*.

Panelists were served paired samples of the yogurt, one set with the two organisms, the other set with four organisms. Each pair consisted of a sample with and without the FOS mixture. The yogurt was served in 2-oz containers, and the panelists were instructed to evaluate and rate each sample, and compare each pair of samples. Sensory analyses used a 10-point scale and included aroma (milk and sour aroma); appearance (firmness, glossiness, creaminess, and separation); texture (firmness, creaminess, chalkiness, mouth coating); flavor (sweetness, sour/tart, sour/vinegar, milkiness, buttery taste, fermented taste, mildness, lingering aftertaste); and overall acceptance (10 like extremely, 1 not like at all).

Consumer preference data are presented in Tables 2–4 and demonstrate that addition of the FOS mixture improved plain, unsweetened yogurt. The standard two-organism yogurt containing the FOS mixture was preferred over the sample without it. The former was identified as being creamier in appearance and having a less chalky and more creamy texture. The yogurt containing the FOS mixture was sweeter, with a less sour/fermented taste and aftertaste.

Results of sensory testing with the four-organism yogurt also indicated a slight preference for the FOS-enhanced yogurt. The FOS-containing yogurt was noted to be glossier and less separated in appearance, firmer in texture, less chalky, and sweeter.

Fig. 2—**Fructooligosaccharides** *are naturally occurring sugars found in fruits and vegetables*

Table 1—**Concentration and Consumption of FOS in Foods**

Food	Water content (%)	Daily consumption of food (g/kg/day) Dry-weight	Daily consumption of food (g/kg/day) Wet-weight	Wet-weight FOS (%)	Wet-weight average daily consumption of FOS g/kg/day	Wet-weight average daily consumption of FOS mg/day
Banana	76	0.224	0.933	0.30	2.80×10^{-3}	164.95
Barley	11	0.057	0.064	0.15	9.66×10^{-5}	5.69
Garlic	61	0.001	0.002	0.60	1.17×10^{-5}	0.69
Honey	17	0.015	0.018	0.75	1.38×10^{-4}	8.11
Onion	89	0.002	0.018	0.23	4.00×10^{-5}	2.36
Rye	11	0.004	0.005	0.50	2.26×10^{-5}	1.33
Brown sugar	2	0.011	0.011	0.30	3.22×10^{-5}	1.90
Tomato	93	0.492	7.029	0.15	1.05×10^{-2}	620.99
					Total 1.37×10^{-2}	806.02

Tests of the functionality and stability of 0.5% FOS mixture in plain, unsweetened yogurt over the shelf life of the product (42 days) revealed the following: (1) no significant differences in viable microbial counts were observed as a result of the addition of FOS; (2) no significant differences were observed in pH between test and control yogurts over the product shelf life; (3) FOS did not degrade over the shelf life of the yogurt, as demonstrated by HPLC analyses; and (4) FOS-containing yogurt retained flavor acceptability over the expected shelf life of the product (Golden Technologies, 1992).

Additional beneficial characteristics associated with FOS include its low caloric utilization by man, reported to be 1.5 kcal/g (Hosoya et al., 1988). Health effects/benefits reported to be associated with FOS consumption include production of volatile fatty acids (Hidaka et al., 1986), increase in bifidobacteria and other beneficial microorganisms in

Table 2—**FOS Effect on Aroma, Appearance, and Texture of Plain, Lowfat Yogurt**

No. of yogurt organisms[a]	Contains 0.5 % FOS?	Aroma[b]		Appearance[b]				Texture[b]			
		Milk	Sour	Firm	Glossy	Creamy	Separated	Firm	Creamy	Chalky	Coats mouth
2	No	3.56	3.96	6.70	7.33	6.23	5.46	5.85	6.12	5.19	5.08
	Yes	3.41	3.39	6.54	7.36	6.93	4.68	5.21	6.57	3.93	5.04
4	No	3.00	3.70	6.67	7.07	6.30	6.74	6.18	7.00	3.86	5.93
	Yes	3.65	3.89	6.71	7.68	6.36	6.29	6.61	6.11	3.61	4.71

[a]2-culture bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) or 4-culture bacteria (*L. bulgaricus, S. thermophilus, Lactobacillus acidophilus,* and *Bifidobacterium infantis*)

[b]Scale of 1-10; e.g., 1 = no aroma, 10 = strong aroma; 1 = not at all firm, 10 = very firm

the intestine (McKellar and Modler, 1989; Mitsuoka et al., 1987), lowering of intestinal pH (Hidaka et al., 1986), and decreased production of putrefactive substances in the intestine (Hidaka et al., 1986). Reported secondary health benefits associated with the effect of FOS on intestinal microflora include a reduction in constipation (Sano, 1986; Takahashi, 1986), amelioration of antibiotic-associated diarrhea, and a reduction in serum triglycerides and cholesterol (Mitsuoka, 1986).

GRAS Analysis

The criteria for affirmation that a substance is GRAS are set forth in federal regulations found in 21 CFR 170.30. They are GRAS based on both history of use prior to 1958 and scientific procedures, which are defined by the Food and Drug Administration to include "those human, animal, analytical and other scientific studies, whether published or unpublished, appropriate to establish the safety of the substance" (21 CFR 170.3 (h)).

The criterion by which the safety of a food ingredient is judged is that "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use" (21 CFR 170.3(i)). Self-determination or classification of any substance as GRAS without a formal petition to FDA can be made by any group of qualified experts (Rodricks and Jackson, 1992).

We conducted a safety evaluation of the potential use of FOS as a flavor enhancer/sweetener in plain, unsweetened yogurt.

Three factors are typically considered in determining safety:

1. The probable consumption of the substance and any substance formed in or on food because of its uses.
2. The cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substances in such diet.
3. Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

Using these factors, we established an acceptable daily intake (ADI), which represents the maximum amount of the additive that can safely be consumed on a daily basis for a lifetime. FDA has specified that an ADI is established by application of an appropriate safety factor (100 to 1) to the NOEL identified in the most sensitive animal species studied.

• **ADI.** There is widespread and common knowledge of the natural occurrence and consumption of FOS as human and animal food prior to 1958. Numerous studies have been conducted in animals and humans, with little evidence of adverse effects associated with FOS consumption. Most food ingredients are evaluated on the basis of animal studies only. The extensive human experience with FOS as a constituent of the normal diet allows consideration of an ADI without the use of safety factors typically applied to animal or limited human data. This is consistent with FDA's regulation concerning safety factors (21 CFR 170.22), which gives FDA the scientific flexibility to conclude that no safety factor is necessary. Furthermore, we feel it is appropriate to compare the estimated daily intake (EDI) of FOS to the total daily consumption of FOS in the average American diet.

Generally, an additional use of a naturally occurring product in the American diet which results in less than a doubling of background daily consumption is not considered to be a significant safety concern (Irausquin, 1989; Vanderveen, 1988), and there is no reason to specify a numerical ADI or apply safety factors. As presented in Table 1, the daily intake of FOS from common food items has been estimated to be approximately 806 mg/day. Therefore, the use of FOS in yogurt at levels that would not result in a doubling of this daily intake would raise little safety concern.

• **EDI.** The following consumption data are based on menu census data (MRCA, 1992). The yogurt consumption data reported included eaters only and was compiled over a 14-day survey period. The average and 90th-percentile nonfrozen, ready-to-eat yogurt consumption data indicated portion sizes of 41.4 and 98.3 g/day, respectively. Estimates of daily FOS intake from addition of 0.5% FOS to yogurt are as follows:

Average: 41.4 g of yogurt/day
× 0.005 g of FOS/g of yogurt
= 0.207 g of FOS/day

90th percentile: 98.3 g/day
× 0.005 g/g = 0.492 g/day

Clearly, the estimated average (207 mg/day or 3.5 mg/kg/day) and 90th percentile (492 mg/day or 8.2 mg/kg/day) daily intake levels of FOS (for a 60-kg adult) from con-

Table 3—**FOS Effect on Flavor of Plain, Lowfat Yogurt**

No. of yogurt organisms	Contains 0.5% FOS?	Flavor[a]							
		Sweet	Sour/Tart	Sour/Vinegar	Milk	Buttery	Fermented	Mild	Aftertaste
2	No	2.57	6.18	4.36	4.26	2.93	3.67	5.29	5.00
	Yes	3.61	5.29	3.96	4.75	3.25	3.15	5.54	4.68
4	No	2.68	4.52	3.36	5.22	3.59	2.96	6.25	4.96
	Yes	3.21	4.59	3.32	5.30	3.50	3.19	6.11	4.96

[a]Scale of 1-10; e.g., 1 = not at all sweet, 10 = very sweet

sumption of yogurt containing 0.5% FOS are less than double the average daily consumption of FOS from common everyday food sources (806 mg/day or 13.7 mg/kg/day).

In addition, the daily intake levels are far below the NOEL in the most sensitive animal species (2.170 mg/kg/day) and levels found to produce adverse effects (soft stools/diarrhea) in humans (44 g) and animals (> 5% in the diet).

FOS are naturally occurring food components which have an established, acceptable background in food. They are food components of very low acute and chronic toxicity and do not represent a hazard to health. Therefore, the preparation of FOS from natural products (sucrose and *A. niger*) and its use as a flavor enhancer/sweetener in plain, unsweetened yogurt at a concentration of 0.5% can, by our independent determination, be considered generally recognized as safe.

Table 4—**FOS Effect on Overall Acceptability** *of plain, lowfat yogurt*

No. of yogurt organisms	Contains 0.5% FOS?	Overall preference[a]
2	No	3.57
	Yes	4.39
4	No	3.96
	Yes	4.04

[a]Scale of 1-10; 1 = not like at all, 10 = like extremely

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