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Physicochemical characterization of fructooligosaccharides and evaluation of their suitability as a potential sweetener for diabetics

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Abstract—Fructooligosaccharides (FOSs) were prepared from sucrose using fungal fructosyl transferase (FTase) obtained from *Aspergillus oryzae* MTCC 5154. The resulting mixture consisted of glucose (28–30%), sucrose (18–20%) and fructooligosaccharides (50–54%) as indicated by HPLC analysis. Identification of oligomers present in the mixture of fructooligosaccharides was carried out using NMR spectroscopy and LC–MS. No compounds other than mono-, di-, tri-, tetra- and pentasaccharides were identified in the FOS mixture prepared using FTase. NMR and LC–MS spectra proved the absence of any toxic microbial metabolites of *Aspergillus* species in FOS thereby emphasizing its safe use as a food ingredient. Animal studies conducted on streptozotocin-induced diabetic rats suggested that the use of FOS as an alternative non-nutrient sweetener is without any adverse effects on various diabetes-related metabolic parameters. Despite the high free-sugar content associated with it, FOS did not further aggravate the hyperglycemia and glucosuria in diabetic animals, even at 10% levels. On the other hand, by virtue of its soluble fibre effect, it has even alleviated diabetic-related metabolic complications to a certain degree.

Keywords: *Aspergillus oryzae*; Fructooligosaccharides; Physicochemical characterization; Non-nutrient sweetener

1. Introduction

Fructooligosaccharides (FOSs) are non-digestible carbohydrates that possess interesting functional and physiological attributes like low sweetness, non-cariogenicity, low caloric value, prebiotic, hypolipidemic and hypocholestrolemic properties.[1] They also facilitate mineral absorption[2] and inhibit the growth of pathogenic bacteria in the colon.[3] Although FOSs are present in trace amounts in natural foods like onions, asparagus, wheat, banana, tomato and honey, commercial production is accomplished using microbial transferase like fructosyl transferase (FTase). Many fungal and bacterial strains are reported to produce FTase.[4] The structure and linkage of FOSs differ depending on the microbial source of FTase used for its production. They can be classified as inulin-type FOSs, which have a β-(2→1) linkage, such as 1-kestose, nystose and fructofuranosyl nystose, and neo-FOSs, which have a β-(2→6) linkage, such as neokestose. The authors have reported the production of FOSs using FTase from *Aspergillus oryzae* MTCC 5154 and the influence of various parameters involved in the process[5,6] and *Aspergillus pullulans* CFR 77.[7,8]

The physicochemical characteristics of any food ingredient have a significant impact on its applicability. The first objective of the present investigation was to characterize FOSs produced enzymatically using FTase from *A. oryzae* MTCC 5154. The next objective was to evaluate their efficiency of application as a sweetener for diabetics by conducting animal studies.

Diabetes mellitus is an endocrine disorder affecting millions of people worldwide and is becoming very

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Table 1. Composition of the basal synthetic diet

Component	g/10 kg
Vitaminized starch	100
Salt mixture	400
Cane sugar	1000
Casein	2100
Groundnut oil	990
Vitaminized oil	10
Corn starch	5400

common with changing lifestyles. Dietary intervention is one of the main therapies proposed in the case of type 2 diabetes patients, and hence non-digestible dietary fibres and polysaccharides are gaining importance for the treatment of diabetic subjects.[9,10] It is in this context that fructooligosaccharides (FOSs) may be considered as a non-nutritive sweetener.

Thus, the second objective of this investigation was to evaluate the efficacy of application of FOS as a sweetener for diabetics. This was carried out in an animal study involving streptozotocin-induced diabetic rats. In this study, we have examined the effect of dietary FOSs (at two dose levels, 5% and 10%) on diabetes-related metabolic parameters to evaluate the suitability of its use as a sweetener.

2. Experimental

2.1. Chemicals

All the chemicals used were of analytical grade. FOS standards, 1-kestose, 1-nystose and 1-fructofuranosyl nystose were from Wako Pure Chemical Industries, Ltd (Osaka, Japan, gift from Dr. Sosaku Ichikawa). Streptozotocin, glucose oxidase, horseradish peroxidase, *o*-dianisidine, albumin, Coomassie Brilliant Blue G-250, thiosemicarbazide, cadmium sulfate and diacetyl monoxime were from Sigma Chemical Co., USA. All other chemicals used were of analytical grade.

2.2. Fructooligosaccharide (FOS) production using FTase from *A. oryzae* MTCC 5154

FTase was produced by submerged fermentation using *A. oryzae* MTCC 5154. The medium and parameters

Table 2. ^{13}C chemical shifts (ppm) for FOS produced using FTase from *A. oryzae* MTCC 5154

Carbon atom	Chemical shifts (ppm)				
	Glucose	Fructose	Sucrose	GF_2	GF_3
α-D *Glucopyranoside*					
C-1	96.3 (β) (**1**) 92.9 (α) (**2**)	64.35 (**9**)	92.5 (**14**)	92.9 (**26**)	92.6 (**44**)
C-2	72.8 (**3**)	—	71.4 (**15**)	70.1 (**27**)	70.0 (**45**)
C-3	74.3 (**4**)	76.2 (**10**)	72.8 (**16**)	72.8 (**28**)	73.0 (**46**)
C-4	69.6 (**5**)	75.3 (**11**)	70.0 (**17**)	71.9 (**29**)	71.8 (**47**)
C-5	73.0 (α) (**6**) 76.3 (β) (**7**)	81.0 (**12**)	73.2 (**18**)	71.4 (**30**)	71.5 (**48**)
C-6	60.8 (**8**)	63.7 (**13**)	60.6 (**19**)	60.6 (**31**)	60.6 (**49**)
β-D *Fructofuranoside*					
C-1′			62.6 (**20**)	61.8 (**32**)	61.4 (**50**)
C-2′			104.0 (**21**)	103.6 (**33**)	103.6 (**51**)
C-3′			78.0 (**22**)	77.2 (**34**)	77.3 (**52**)
C-4′			74.4 (**23**)	74.9 (**35**)	74.8 (**53**)
C-5′			81.1 (**24**)	81.7 (**36**)	81.6 (**54**)
C-6′			62.7 (**25**)	61.2 (**37**)	61.2 (**55**)
β-D *Fructofuranoside*					
C-1″				61.1 (**38**)	60.9 (**56**)
C-2″				103.4 (**39**)	103.6 (**57**)
C-3″				77.1 (**40**)	77.1 (**58**)
C-4″				74.3 (**41**)	74.5 (**59**)
C-5″				81.5 (**42**)	81.5 (**60**)
C-6″				62.5 (**43**)	61.8 (**61**)
β-D *Fructofuranoside*					
C-1‴					60.85 (**62**)
C-2‴					103.4 (**63**)
C-3‴					76.8 (**64**)
C-4‴					74.3 (**65**)
C-5‴					81.4 (**66**)
C-6‴					62.6 (**66**)

Some of the C-2–C-5 signals of fructose units are interchangeable.

were as reported earlier.[6] FOS production was carried out by incubating 2.5 L of FTase with 7.5 L of the substrate (60°Bx sugar)[†] at 55 °C for 18 h. The reaction was terminated by raising the temperature to 85 °C for 15 min, and the samples were analyzed by HPLC (LC-6A, Shimadzu, Kyoto, Japan) with a refractive index detector using a polar bonded-phase column (Exsil NH_2, 4.6 mm × 25 cm, 5 μm) at room temperature. The mobile phase used was 80:20 acetonitrile–water at a flow rate of 1.0 mL/min. The retention times of the individual FOSs were compared with that of standards for identification and quantification. The lyophilized sample was used (Heto Drywinner DW3, Heto-Halten, Denmark) for determining the chemical structure.

2.3. Purification of FOS

Lyophilized FOS (1 g) was dissolved in 2 mL of triple-distilled water, centrifuged (4000 rpm for 10 min) and loaded to a BioGel P-2 (BioRad Laboratories, California, USA) gel-filtration column (100 × 1 cm). Elution was carried out using triple-distilled water at a flow rate of 5 mL h^{-1}, and 1-mL fractions were collected. The fractions were analyzed by HPLC.

2.4. Analytical procedures

2.4.1. NMR analysis. ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz on a Bruker DRX 500-MHz spectrometer (500.13 MHz proton and 125 MHz carbon frequencies). Proton and carbon 90° pulse widths were 12.25 and 10.5 μs, respectively. About 50 mg of the sample dissolved in D_2O was used for recording the spectra. Chemical shifts were expressed in ppm relative to the internal standard trimethylsilane (TMS).

Two-dimensional heteronuclear multiple quantum coherence transfer spectra (2D HMQCT) were recorded in the magnitude mode with the sinusoidal shaped *z* gradients of strengths 25.7, 15.42 and 20.56 G/cm in the ratio of 5:3:4 applied for a duration of 1 ms each with a gradient recovery delay of 100 μs to defocus unwanted coherences. The t_1 was incremented in 256 steps. The size of the computer memory used to accumulate the 2D data was 4 K. The spectra were processed using unshifted and π/4 shifted sine bell window function in F_1 and F_2 dimensions, respectively.

2.4.2. LC–MS analysis of purified oligomers of FOS. LC–MS was performed on a Bruker Esquire 3000 mass spectrometer (Bruker Instruments, Germany) connected to an Agilent 1100 HPLC system (Hewlett–Packard Company, Pennsylvania) fitted with an Exsil amino column (250 cm × 4.6 mm, 5 μm) at 25 °C with 80:20 acetonitrile–water as the mobile phase. Electrospray-ionization (ESI) of the sample was carried out in the positive-ionization mode at 51.0 psi nebulizer pressure, 10 L min^{-1} dry gas flow rate at 360 °C dry gas temperature. The detector used was an ion-trap analyzer.

2.5. Animal studies

Male Wistar rats (12 weeks old) weighing 150–160 g were used for the induction of diabetes by streptozotocin. Streptozotocin solution was prepared in fresh citrate buffer (0.1 M, pH 4.5) and injected intraperitoneally at a concentration of 60 mg/kg body weight, in a volume of 1 mL per rat. The animals were given 5% glucose solution for 24 h following streptozotocin injection to prevent initial drug-induced hypoglycemic mortality. Hyperglycemic rats (having at least two times the normal blood glucose levels) were sorted out after determining fasting blood glucose values, one week after streptozotocin administration. Three groups of hyperglycemic rats (10 per group) and in parallel, three groups of normal rats, were housed in individual stainless steel metabolic cages with free access to food and water.

The basal semisynthetic diet was of the composition shown in Table 1. The test material (FOS) was included at 5% and 10% levels, at the expense of corn starch. Consequently, there were six rat groups, Normal control, Normal FOS 5%, Normal FOS 10%, diabetic

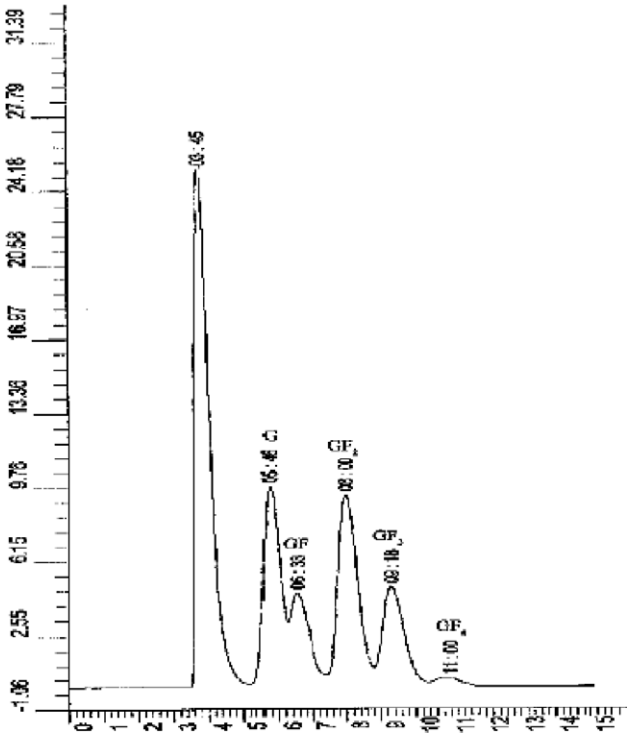

Figure 1. HPLC profile of the FOS mixture.

[†] °Bx = degrees Brix, a measurement of the mass ratio of sugar to water in a solution.

control, diabetic FOS 5%, and diabetic FOS 10%. The rats were maintained on these diets for a period of six weeks. The diet was given ad libitum. After induction of diabetes, rats were fed with two types of diet, containing 5% and 10% FOS, respectively. FOS is added replacing corn starch in each case. During the dietary regimen, fasting blood glucose levels were monitored at fortnightly intervals by collecting blood from the retro-orbital plexus. Body weights were recorded at weekly intervals. The amounts of glucose, protein, urea and creatinine excreted in the urine were monitored at weekly intervals in the 24-h urine samples collected. At the end of the feeding period, the animals were sacrificed, and blood samples were collected and used for the determination of plasma glucose, urea, albumin and creatinine.

2.6. Metabolic parameters

Plasma and urinary glucose was determined by the glucose oxidase method employing glucose oxidase, peroxidase and *o*-dianisidine.[11] Urinary protein was measured by dye-binding method described by Bradford,[12] while plasma albumin was also quantitated by a dye-binding method described by Gustafsson.[13] Urinary urea was assayed by the method of Wyeberga et al.,[14]

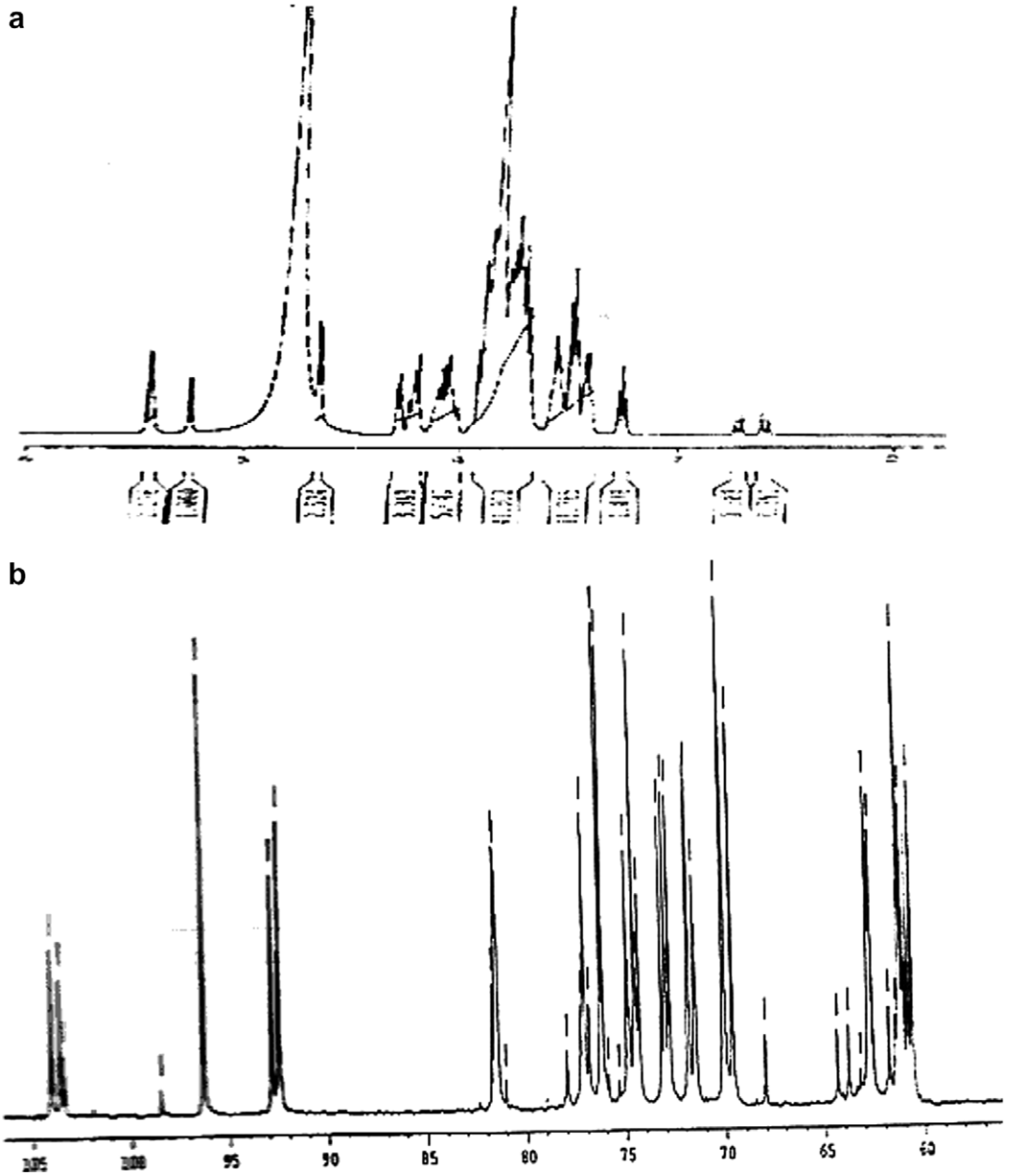

Figure 2. ^{1}H NMR spectra and ^{13}C NMR spectra of FOS (in D_2O) produced using FTase from *A. oryzae* MTCC 5154.

while creatinine was measured by the method of Folin and Wu.[15] Plasma urea was estimated by the method of Levine et al.[16]

2.7. Statistical analysis

Results were expressed as mean ± SEM, and comparisons between groups were made by means of an unpaired Student's *t*-test.[17] Differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Physicochemical characterization of FOS

FOS production was carried out using FTase obtained from A. oryzae MTCC 5154 as described previously.[18] FOS resulting from the experiment is a mixture of mono-, di-, tri-, tetra- and pentasaccharides as observed by HPLC analysis (Fig. 1).[18]

Studies on the proton and carbon chemical alignments of kestose (GF_2) have already been reported.[19,20] The chemical shift values of the ^{13}C NMR spectra of FOS are shown in Table 2. FOS used in the study was the lyophilized reaction product resulting from 18 h of enzymatic synthesis from sucrose using FTase. The sample was analyzed for structure by recording 2D HMQCT spectra, and the structures were assigned based on the literature information.[21–24] As shown in Figure 2a and b, the spectra clearly showed the presence of free glucose, a small amount of free fructose, unreacted sucrose, GF_2 (kestose) and GF_3 (nystose). The presence of GF_2 and GF_3 was clearly detected from both ^1H proton and ^{13}C spectral correlation observed in 2D HMQCT (Fig. 3). In GF_2 and GF_3, the linkage between glucose and fructose is 1→2 and α for the 1-linked glycosidic linkage and β for the (1→2)-linked glycosidic linkage of fructose.

Three kestoses are known so far. They are 1-kestose [β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside], 6-kestose [β-D-fructofuranosyl-(2→6)-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside] and neokestose [β-D-fructofuranosyl-(2→6)-α-D-fructofuranosyl–(1→2)-glucopyranoside]. The data clearly showed that 1-kestose [β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside (inulin type)] is the major product detected for GF_2. The six-linkage was not detected in any fructose unit of GF_3. Compared to GF_3, the concentration of GF_2 was found to be higher correlating with the HPLC data.[18] The NMR spectra showed less intense peaks, which merged with the noise, probably indicating small amounts of GF_4 (fructofuranosyl nystose).

The free glucose was present both in α and β forms as observed by the anomeric C-1 signals (96.3 ppm for β and 92.8 ppm for α). Unreacted sucrose was found to be present in substantial amount (12% by HPLC). In GF_2 and GF_3, the anomeric glucose linkage was observed at 92.8 ppm, indicating that the linkage is α. Correspondingly, the C-2′, C-2″ and C-2‴ signals observed for fructose were all observed around 103 ppm, indicating a β-linkage. The C-1 and C-6 signals of fructose were in the range of 60.9–61.8 ppm. All the fructose signals in both GF_2 and GF_3 were detected by NMR spectroscopy. From the 2D HMQCT spectrum, a few proton signals could also be detected (Table 3). Thus, NMR analysis conclusively proved the presence of GF_2 and GF_3 in FOS prepared using FTase produced by *A. oryzae* MTCC 5154.

The oligomers present in the FOS mixture were purified using column chromatography. The oligo-, di- and monosaccharides eluted sequentially based on their decreasing molecular weight. The different fractions containing pure kestose, nystose and fructofuranosyl nystose were analyzed by LC–MS. There have been many reports on the analysis of FOS by HPLC, GC–MS, NMR, etc.[21–24] In this work, attempts have been made to explore the application of LC–MS for the direct analysis of FOS. The MS results were obtained in the positive-ionization mode, using a sodium salt contained

Table 3. ^1H chemical shifts (ppm) for FOS produced using FTase from *A. oryzae* MTCC 5154

	Chemical shifts (ppm)			
	Glucose (free)	Fructose (free)	GF_2	GF_3
α-D *Glucopyranoside*				
H-1	5.24 (α) (**1**) 4.65 (β) (**2**)	3.72 (**6**)	5.43 (**9**)	5.41 (**18**)
H-2	3.78 (**3**)		3.49 (**10**)	3.42 (**19**)
H-3		4.23 (**7**)	3.75 (**11**)	3.72 (**20**)
H-4				
H-5	3.52 (**4**)		3.56 (**12**)	3.57 (**21**)
H-6	3.69 (**5**)	3.74 (**8**)	3.83 (**13**)	
β-D *Fructofuranoside*				
H-1′				
H-2′				
H-3′			3.48 (**14**)	3.43 (**22**)
H-4′				
H-5′			3.88 (**15**)	3.81 (**23**)
H-6′				3.86 (**24**)
β-D *Fructofuranoside*				
H-1″				3.76 (**25**)
H-2″				
H-3″			4.2 (**16**)	4.21 (**26**)
H-4″			4.05 (**17**)	
H-5″				
H-6″				
β-D *Fructofuranoside*				
H-1‴				
H-2‴				
H-3‴				
H-4‴				
H-5‴				
H-6‴				3.9 (**27**)

in the mobile phase to facilitate ionization. Hence, all the masses are presented as sodium adducts. Figure 4 shows the HPLC profile of purified 1-kestose with its mass spectrum in the inset. The signals at *m*/*z* 527.1 correspond to 1-kestose, and those at *m*/*z* 365.1 correspond to sucrose, whereas the minor signal at *m*/*z* 203.0 corresponds to glucose. The HPLC profile of purified nystose and its mass spectrum represented in Figure 5 shows a major signal of *m*/*z* 689.1. Figure 6 shows the mass spectrum of fructofuranosyl nystose corresponding to *m*/*z* 851.1. The actual masses of kestose, nystose and fructofuranosyl nystose were calculated as 504, 666 and 828, respectively.

3.2. Evaluation of the suitability of FOS as a sweetener in an animal study

While all the non-diabetic animals survived the duration of the experiment, considerable mortality was observed among the diabetic rats. Whereas 80% mortality was observed in the diabetic control group during the first fortnight of the experiment, the mortality was only 10% during this period in the FOS-fed group. Thus the survival rate was higher due to FOS feeding, especially at the 10% levels, while considering the mortality in subsequent weeks. There was a steady decline in the body weight in the diabetic control group during the six-week experimental period. Such a trend was not observed in either of the FOS-treated groups (Fig. 7).

FOS treatment did not adversely influence fasting blood sugar values in diabetic animals, in spite of the presence of free blood sugar (50%) associated with it, as judged by fasting blood glucose (FBG) values at fortnightly intervals (Fig. 8). This observation was also corroborated by the urinary glucose excretion pattern throughout the experimental duration (Fig. 9).

Increased protein catabolism is a characteristic feature of diabetes mellitus. Thus hypo-albuminemia (decreased titres of albumin in serum) should be expected during diabetes mellitus. Lower serum albumin levels (15%)

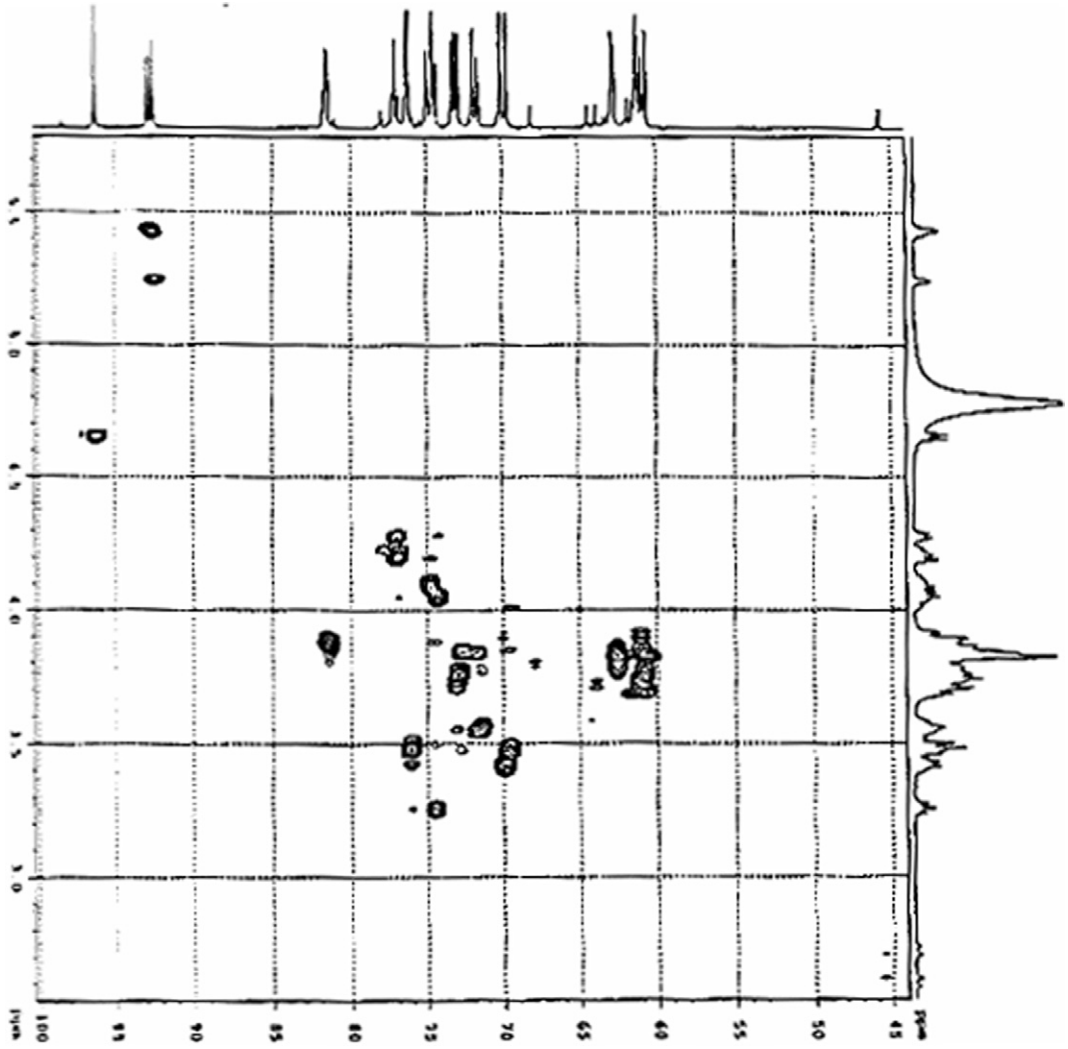

Figure 3. 2D HMQCT spectrum of FOS (in D_2O) produced using FTase from *A. oryzae* MTCC 5154.

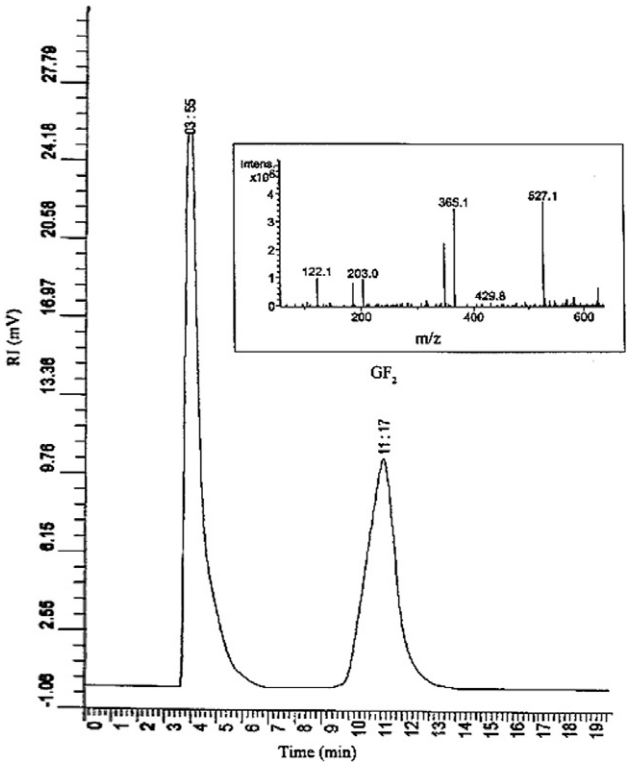

Figure 4. HPLC elution profile of purified 1-kestose. Inset: mass spectrum of 1-kestose.

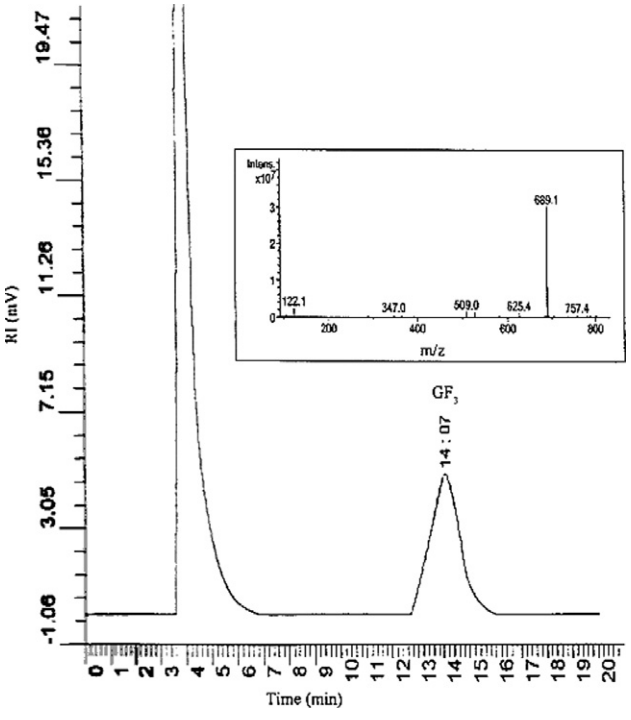

Figure 5. HPLC elution profile of purified nystose. Inset: mass spectrum of nystose.

were seen in diabetic control animals compared to their normal counterparts (Table 4). FOS treatment protected

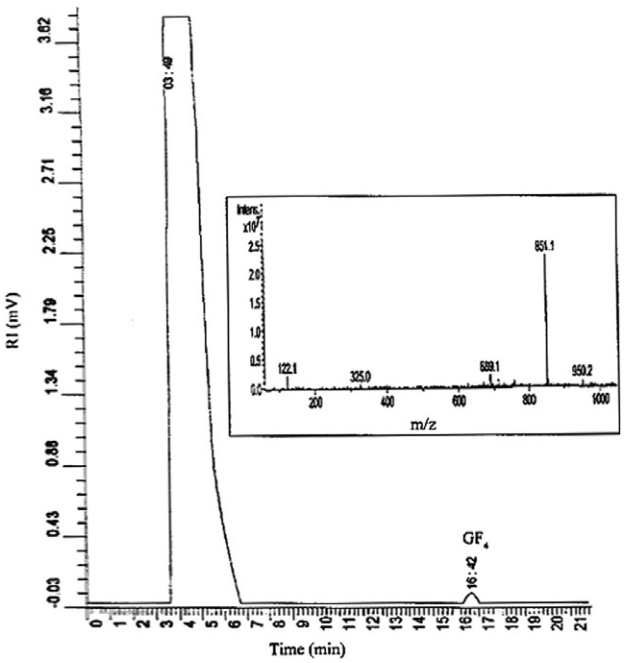

Figure 6. HPLC elution profile of purified fructofuranosyl nystose. Inset: mass spectrum of fructofuranosyl nystose.

this albumin destruction, as indicated by higher than normal serum albumin levels in diabetic rats.

Blood creatinine levels were higher in diabetic rats compared to normal animals, which is an indication of increased muscle wasting during this metabolic disease (Table 4). FOS treatment at either of the dietary levels significantly lowered blood creatinine concentration in diabetic rats. This indicates that FOS has countered the muscle wasting normally associated with diabetes mellitus, and this is in concurrence with the maintenance of body weight observed in FOS-fed rats. Proteinuria, which is an indicator of progressive kidney damage during diabetes, was considerably lowered by dietary FOS towards the end of the experimental period, especially at 10% levels (Fig. 10).

There have been a few studies on the effect of FOS on diabetes. The study by Yamashita et al.[25] demonstrated that the daily intake of fructooligosaccharides for fourteen days by type-2 diabetic patients caused an 8% decrease in fasting blood glucose concentrations, a 6% decrease in total cholesterol and a 10% decrease in LDL cholesterol. The authors claim that these observations of decrease in fasting blood glucose and serum total cholesterol levels were statistically significant. Several studies have been conducted in rats since, all showing that fructooligosaccharides lower serum triglycerides and total cholesterol as well as LDL and VLDL cholesterol.[26–29] Although fructooligosaccharides showed various effects on metabolism, the exact mechanism is not explained. Fructooligosaccharides may possibly bind to nutrients such as carbohydrates and lipids and to

the absorptive mucosal surface, and the absorption of the nutrients from the intestinal mucosa may be prevented. Since fructooligosaccharides alter the composition of bacterial flora in the intestine,[30] the digestion of carbohydrates and the metabolism of bile acids may be changed. Hence, absorption of carbohydrates and lipids will be reduced, resulting in lowering of blood glucose along with serum lipids.[25]

A study by Luo et al.[31] showed that chronic consumption of 20 g of fructooligosaccharides by healthy humans increased basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism

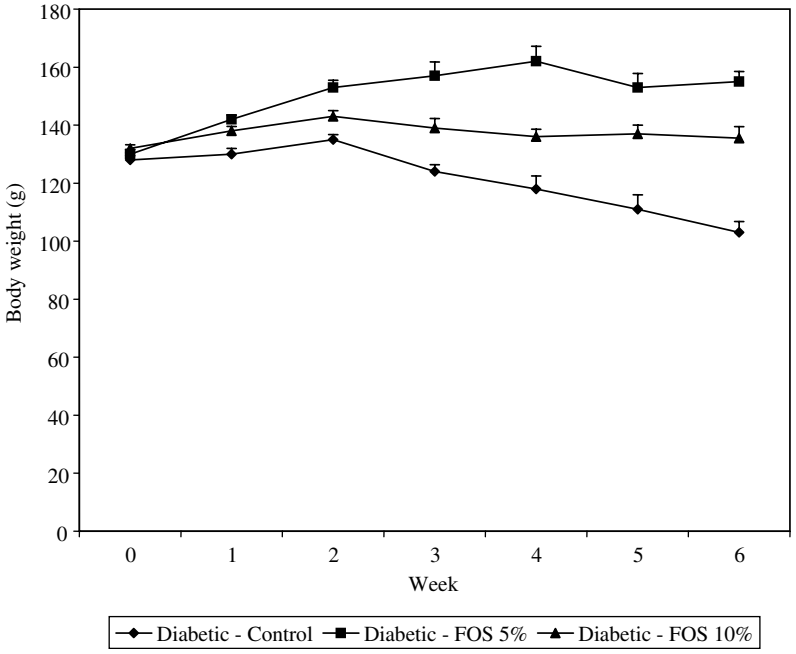

Figure 7. Body weight gain in diabetic rats maintained on FOS diet. Values are mean ± SEM of six animals in each group.

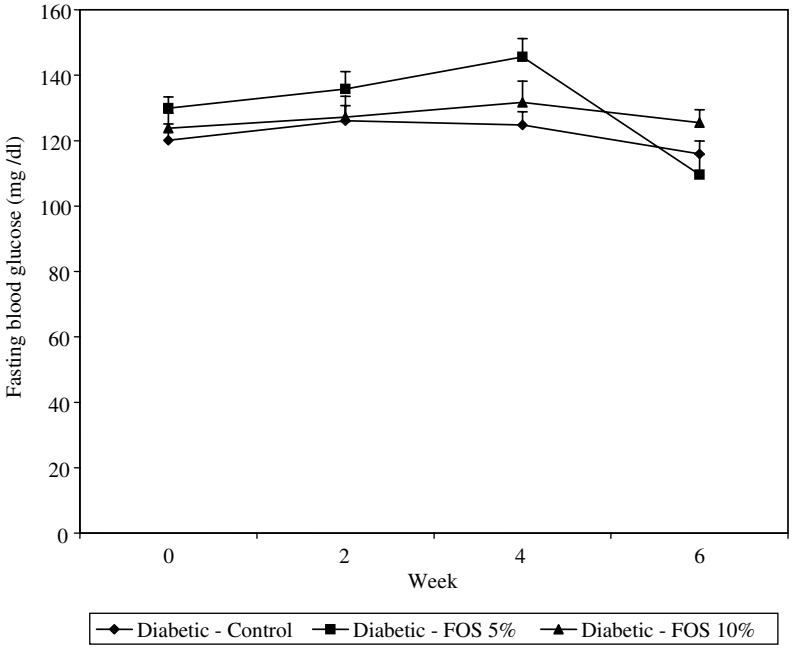

Figure 8. Blood glucose in diabetic rats maintained on FOS. Fasting blood glucose values in normal animals were 40–45 mg/dL. Values are mean ± SEM of six animals in each group.

or serum lipids. Pedersen et al.[32] studied the effect of inulin on blood lipids and did not find any changes in total cholesterol, HDL cholesterol, LDL cholesterol or triglyceride. Thus the effects of fructooligosaccharides on blood glucose and serum lipids are not clear. Alles et al.[33] have evidenced that consumption of fructooligosaccharides for three weeks by patients with type-2 diabetes does not favourably affect blood glucose and serum lipid concentrations. Even in our present study, we did not observe any major influence of FOS on blood glucose. The FOS preparation used in the present study was around 50% in terms of FOS, and the remainder consisted of free sugars. Despite the high free-sugar content associated with it, FOS did not further aggravate the hyperglycemia and glucosuria in diabetic animals even at 10% levels. This may be attributed to the fact that, FOS as soluble fibre can adsorb nutrients such as glucose, thus retarding their absorption. As a result, the glucose flux in the blood following a carbohydrate meal is retarded, and the amount of insulin needed by the body is lowered. By virtue of its soluble fibre effect, FOS has even alleviated diabetic-related metabolic complications to a certain degree. Thus the present study suggests that the use of FOS as an alternative sweetener is without any adverse effects.

In conclusion, identification of oligomers present in a mixture of fructooligosaccharides has been carried out using NMR spectroscopy and LC–MS. No compounds other than mono-, di-, tri-, tetra- and pentasaccharides were identified in the FOS mixture prepared using FTase produced by *A. oryzae* MTCC 5154. NMR and LC–MS spectra proved the absence of any toxic microbial metabolites in FOS, thereby emphasizing its safe use as a food ingredient. In this study, it was also shown that the intake of fructooligosaccharides for a period of six weeks did not adversely influence the sugar values in diabetic animals. FOS was also found to protect animals from the destruction of albumin. It also countered

Table 4. Blood albumin, urea and creatinine values in diabetic rats maintained on FOS diets for 6 weeks[a]

Animal group	Albumin (g/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Diabetes control	6.67 ± 0.37[b]	116.3 ± 5.20[a]	9.35 ± 0.63[b]
Diabetes FOS 5%	7.85 ± 0.38[c]	112.1 ± 6.85	5.92 ± 0.59[c]
Diabetes FOS 10%	8.66 ± 0.50[c]	110.6 ± 6.07	5.84 ± 0.71[c]
Normal control	7.84 ± 0.27	96.3 ± 3.24	5.75 ± 0.65
Normal FOS 5%	7.66 ± 0.13	92.0 ± 1.69	5.03 ± 0.49
Normal FOS 10%	8.67 ± 0.32	102.4 ± 1.67	5.42 ± 0.29

[a] Values are mean ± SEM of five rats in each group.
[b] Significantly different from normal control.
[c] Significantly different from diabetes control.

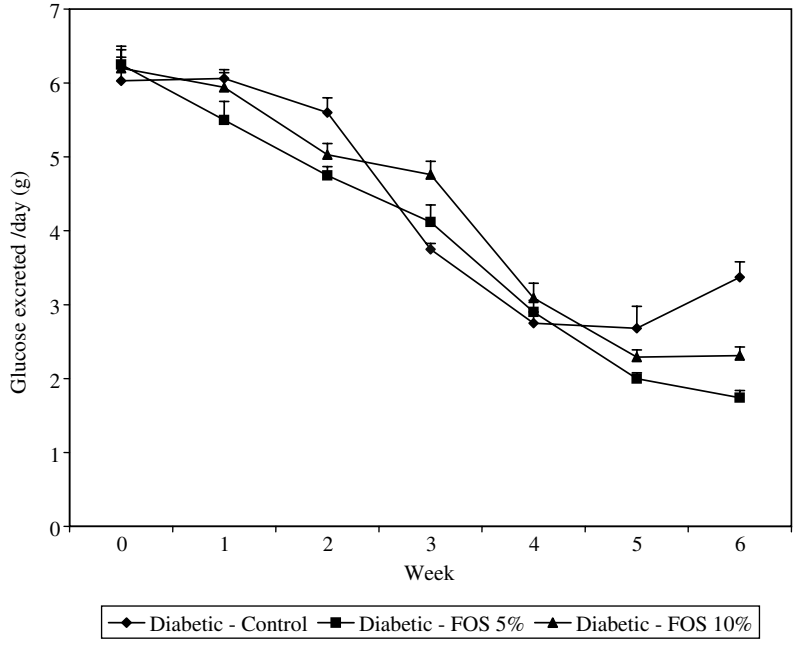

Figure 9. Urinary glucose excretion in diabetic rats maintained on FOS. Glucose excretions in normal animals were 0.3–0.5 mg/day. Values are mean ± SEM of six animals in each group.

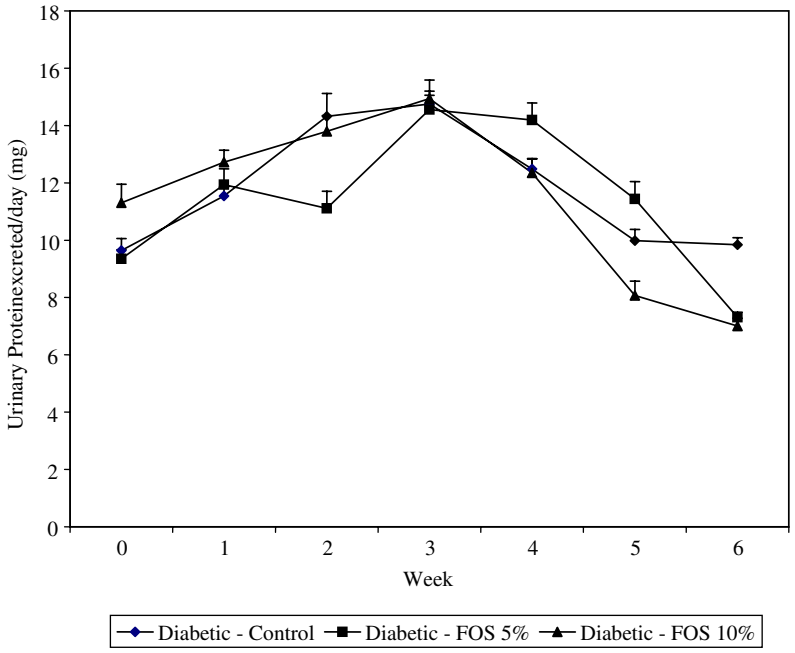

Figure 10. Urinary protein excretion in diabetic rats maintained on FOS. Protein excretions in normal animals were 2.5–3.5 mg/day. Values are mean ± SEM of six animals in each group.

the muscle wasting normally associated with diabetes mellitus, and which is in concurrence with the maintenance of body weight observed in FOS-fed rats.

Thus the present study on rats shows that FOS maybe used as a sweetener by diabetics or as an adjunct to dietary therapy with various beneficial effects on glucose and protein metabolism. However, in-depth studies are needed to assess the effect of daily intake of fructooligosaccharides by diabetics over a longer period of time.

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