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Original Article

THE PREBIOTIC EFFECTS OF MIXED SWEETENER CONTAINING POLYDEXTROSE AND OLIGOFRUCTOSE SUBSTITUTED SUGAR IN DIET

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Abstract

Prebiotics are non-digestable food ingredients, made of carbohydrates targeting human colonic microflora. In the present study, the prebiotic potential of oligofructose (OF) and polydextrose (PD) in mixed sweetener (MS) in cake was investigated in healthy male volunteers. MS included PD (40.9 %) and OF (20%). Aim of this study was to investigate the tolerable amount of MS and to evaluate the prebiotic effects of MS ingestion. This study was conducted in two steps. In the first step of the study, gastro-intestinal system symptoms of the volunteers were examined during four weeks and the tolerable amount of MS was detected. In the second step the prebiotic effects of tolerable dosage of MS (12 g/d) ingestion was investigated. At the end of the placebo and test period, faecal samples were analysed. Flatus was more frequent and intense in volunteers consuming MS_{48} than the other groups and MS ingestion affected fecal weight in all groups. MS ingestion increased the amount of Bifidobacteria, Lactobacillus and total anaerobes (except for Clostridium) and decreased all aerobes. However these changes were not statistically significant ($p>0.05$). MS consumption decreased the amount of all aerobes, but only the reduction in the number of Staphylococcus was statistically significant compared to placebo period ($p<0.01$). As a result, 12g/d consumption of MS generated prebiotic effects in colon of healthy volunteers.

Keywords: *Functional foods, prebiotic effects, bifidobacteria.*

Şeker Yerine Kullanılan Polidekstroz ve Oligofruktoz İçeren Karışım Tatlandırıcının Prebiyotik Etkilerinin Değerlendirilmesi

Prebiyotikler, insanlarda bağırsak florasını hedefleyen ve sindirilemeyen karbonhidrat yapısındaki besin bileşenleridir. Bu çalışmada, oligofruktoz (OF) ve polidekstroz (PD)'dan hazırlanmış karışım tatlandırıcının (KT), bağırsak florası üzerindeki prebiyotik etkileri incelenmiştir. KT, % 40.9 oranında PD ve %20 oranında OF içermektedir. Çalışmaya gönüllü ve sağlıklı erkek bireyler alınmıştır. Çalışma iki aşamada yürütülmüştür. Birinci aşamada, gönüllülerin dört hafta süreyle gastro-intestinal sistem şikâyetleri değerlendirilerek tolerans dozu belirlenmiştir. İkinci aşamada, KT'nın en iyi tolere edilebilen dozu (12 g/gün)'nun prebiyotik etkileri belirlenmiştir. Plasebo ve test periyodlarının sonunda dışkı örneklerinde bakteriyel analizler yapılmıştır. Gaz şikâyeti olanların sayısı ve şiddeti, KT_{48} grubunda, diğer gruplardan daha fazla olmuş ve karışım tatlandırıcı tüketimi tüm doz gruplarında dışkı ağırlığını etkilemiştir. KT tüketimiyle dışkıda görülen Bifidobacteria, Lactobacillus, Bacteroides ve total anaerob bakterilerin sayıları artarken, Clostridium sayısı azalmış, fakat bu azalma istatistiksel açıdan önemli bulunmamıştır ($p>0.05$). Aerob bakterilerin tümü KT tüketimiyle azalmıştır. Sadece Staphylococcus sayısında görülen azalma, istatistiksel olarak önemli bulunmuştur ($p<0.01$). Sonuç olarak, 12 g/gün KT tüketimi sağlıklı gönüllülerde kolonda prebiyotik etkiler göstermiştir.

Anahtar kelimeler: *Fonksiyonel besinler, prebiyotik etki, bifidobakteri.*

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INTRODUCTION

Usage of sugar substitutes initiated in Turkey with the sweeteners having intense sweet taste such as aspartame, asesulfame K, and saccharine. However, in food industry, sugar, besides its taste, has also other important functions such as providing volume, consistency, and crystallization. The sweeteners providing intense sweet taste cannot actually provide these characteristics. Therefore prebiotics have been widely preferred, since they contain fewer calories and have positive effects on health besides having functional characteristics similar to that of sugar.

The product development suggested the use of prebiotics in combination with other sweeteners, which perform the distinct functions of sugar. In the present study, to assure a better quality product, a mixture composed of two low energy components (i.e. aspartame and asesulfame K), providing intense sweet taste, a sugar alcohol (i.e. maltitol) providing volume and two low energy prebiotics (i.e. oligofructose (OF) and polydextrose (PD) providing volume and fat taste was prepared.

The effects of prebiotics on improving health and protecting diseases are supported by the projects backed by the government, with private sector participation in developed countries (1).

Since the effects of prebiotic sweeteners and their particular tolerable dosages have been investigated separately in the literature, the level and amount of prebiotic effects of such a mixed product is unknown.

In this study, we aimed to determine the tolerable dose and the prebiotic effects in the colon of a mixed sweetener (MS) developed as the sugar substitute, that includes oligofructose (OF) and polydextrose (PD).

EXPERIMENTAL

Subjects

Thirty five healthy male volunteers, at the age of 19-30 years, non-alcoholic, non-smoker with Body Mass Index (BMI) of 20-25 Kg/m^2, were enrolled in the study. They had no gastro-intestinal system (GIS) illness, and lactose intolerance. They had been warned not to take any antibiotics, laxatives or pro/prebiotic food in the three months before the beginning of the study. Onion, garlic, leeks, etc. were completely excluded from the diet of the volunteers and bread was limited to a certain amount (200 g/d).

The properties of sweeteners and the composition of cakes

The commercial name of the product studied is FIBERSWEET. Composition of this sweetener is shown in Table 1.

Table 1. Composition of mixed sweetener

Ingredients	%
Oligofructose (Raftiline)	20.0
Polidexstrose (Raftilose)	40.9
Poliols	34.5
Other sugars (glucose, sucrose)	2.0
Total carbohydrate	97.4
Aspartame	0.08
Asesulfame K	0.09
Sodium	0.7
Neohesperidine	0.013
Water	1.1
Silicone dioxide	0.6

Experimental design

This study was conducted in two steps. In the first step, we investigated the tolerable dosages of MS and in the second step; we determined the prebiotic effects on the colon by tolerable dosage 12g/d of MS.

First Step: The aim of this step was to determine the effects of the mixed sweetener (MS) including PD and OF on the weight of feces, defecation frequency and the GIS symptoms with increasing dosage over a short period. The effects of three different dosages (MS_{12}, MS_{24} and MS_{48}) of the MS, including OF and PD, on the symptoms of GIS were determined by a single-blind study. Each dosage of MS was completed to 48 g. with addition of sugar. The total amount of sweetener was determined to be equal to 12 % of the dieting individuals' average daily energy intake (1600 kcal/d). The reason for this was to keep the total amount of sweetener used for providing taste in the diet at a fixed level. In the period of $Test_{1-2,}$ depending on the MS dosage, subjects were provided 4 mid meals with portions of test cake (TC) or test cake (TC) + placebo cake (PC); on the other hand, in the periods of $Placebo_1$ and $Placebo_2$, the PC with sugar but no sweetener was used (Table 2 and 3). The gastro-intestinal system symptoms of the individuals and the severity of these symptoms were reported and the MS dosage with the lowest symptoms was determined as 'the tolerable dosage'.

The first step of the experiment continued during the overall period of four weeks and each period lasted for one week. It was ensured that the individuals reported daily the GIS symptoms they faced, the degree of the severity of these symptoms (1 weak, 2 moderate, 3 severe) and viscosity of their feces (1 diarrhoea form, 2 soft, 3 normal, 4 hard, 5 constipation form) during this step.

Table 2. General plan of first step

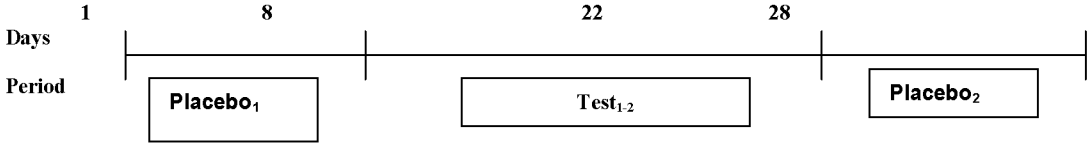


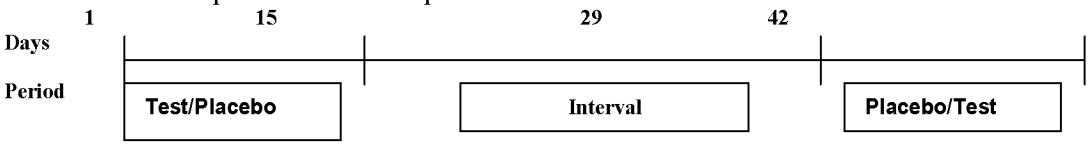
Table 3. Cake consumption in the first step of the experiment.

Experimental Periods	Cake consumption MS_{12} (n=9)	MS_{24} (n=9)	MS_{48} (n=9)
$Placebo_1$	**4 slice PC**	**4 slice PC**	**4 slice PC**
$Test_{1-2}$	**3 slice PC** + **1 slice TC**	**2 slice PC** + **2 slice TC**	**4 slice TC**
$Placebo_2$	**4 slice PC**	**4 slice PC**	**4 slice PC**

* Each of test cakes contains 12 g MS, each of placebo cakes contains 12 g sucrose.

Second step: The aim of this step was examining the prebiotic effects of the tolerable amount of the mixed sweetener (12 g/d) determined in the first step on the colon. The prebiotic effects of the cake prepared with the tolerable dosage (12 g/d) on the colon was investigated on 8 individuals through a placebo (sugar) controlled single-blind cross-over design. Eight individuals were randomly divided into two equal groups, where one of the group was given placebo cake (containing 12 g of sugar) while the other group was given test cake (containing 12 g MS) during two weeks. After a two-week-interval, during the last two weeks of the experiment, the group given placebo cake previously was given test cake, and the group consuming test cake previously was given placebo cake (Table 4).

Table 4. General plan of second step



The microbiological analyses of the feces samples from the participants was carried out on the specified days (Table 5).

Table 5. Sampling time of feces samples

	(n=4)	(n=4)	Experiment period (day)	**Days (consecutive 3 feces samples)**
Mixed sweeteners dosage (g/day)	**MS_{12}**	**MS_{12}**		
First period	**Test**	**Placebo**	**1-14**	4-5-6-7 11-12-13-14
Second period	**Interval**	**Interval**	**15-28**	18-19-20-21 25-26-27-28
Third period	**Placebo**	**Test**	**29-42**	32-33-34-35 39-40-41-42

Faecal culture

The microbiological analyses were performed at the pharmaceutical microbiology laboratory in the Faculty of Pharmacy of Gazi University. The feces samples collected in special sterile containers were analyzed immediately because of the sensitivity of anaerobic bacteria to oxygen. The feces samples were homogenized using a mechanical mixer, afterwards weighed on a digital scales sensitive to 0.01g (2.5g), then were diluted 10 fold with pepton water (peptone, sistein, glucose) which is selective to anaerobes and then were filtered through a micro filter (0.22μm), finally they were spreaded as 20μL on the appropriate media. Beeren's agar (2), Wilkins-Chalgren Agar (Oxoid), Rogosa and MRS Agar (Merck), Reinforced Clostridial Agar and MRS Agar (Merck) were used for the isolation of *Bifidobacteria, Bacteroides*, *Lactobacillus* and *Clostridium*, respectively. In addition, Mac Conkey Agar (Merck) was used for isolation of *Escherichia coli, Klebsiella* and *Enterobacter*, and Triptic Soy Agar (Merck) supplemented with blood was used for the isolation of *Streptococcus* and *Staphylococcus*. Anaerobe bacteria were incubated with Anaerocult A at 37 °C under the anaerobic conditions (Gas Pack System; Oxoid) for 5 days, and facultative anaerobe bacteria were incubated at 37 °C for 2 days. After incubation, the identification of bacteria were made through their colony morphologies and gram staining and other conventional biochemical tests (nitrate reduction, acid formation from glucose, indole formation, catalase test) (3). Each bacterial colony was counted and then calculated according to the formula for per gram of feces, as follows: N (CFU/gr) = X.1/V.D (Mean colony forming unit (CFU). 1/Spreading volume (ml). Dilution factor (4).

Statistical analyses

Statistical analyses of the data were performed with SPSS 12.0 for Windows and GraphPad Prism 4.0.

In studying the effects of increasing dosage of the mixed sweetener over a short period of time on gastro-intestinal system, Friedman Two Way ANOVA non-parametric test was used to find out the differences within the groups according to the weeks in all tables, and Kruskal-Wallis One Way ANOVA tests were used to determine the differences between the groups.

When examining the prebiotic effects of the mixed sweetener with the amount of the tolerable dosage determined in the first step (12 g/d) on the colon, Sample Paired t test was applied for determining the differences between the number of bacteria and the means of pH levels in the feces of the test and the placebo groups. The differences were accepted as significant at $p<0.01$ and <0.05.

Ethics

This study was certified by the Committee of Clinic and Medicine Investigations of Local Ethics of the Faculty of Medicine of Gazi University on 13.02.2004 with the register number of 2004/176.

RESULTS

The consumption of MS affected the weight of feces. In MS_{12} group the weight of feces increased in the first week of MS consumption and decreased when MS consumption was over. In MS_{24} the weight of feces increased in the second week of MS consumption and remained at high level when MS consumption was over. In MS_{24} group the weight of feces which was high in the beginning (222g/d), decreased in the first week of MS consumption and tended to increase in the second week. This increase continued when MS consumption was over. However, the differences in the weight of feces of all dosage groups were not found significant according to the periods (Table 6).

Table 6. The average weight of the feces depending on the mixed sweetener dosage in the periods

		Fecal weight (g/day)		
		MS_{12} (n=9)	MS_{24} (n=9)	MS_{48} (n=9)
Week	Period	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
1	$Placebo_1$	165.8 ± 86.69	159.9 ± 54.87	222.1 ± 48.31
2	$Test_1$	180.7 ± 96.33	146.7 ± 50.48	167.6 ± 20.58
3	$Test_2$	222.3 ± 96.37	176.1 ± 80.71	189.0 ± 53.76
4	$Placebo_2$	213.0 ±109.52	185.5 ± 55.00	203.4 ± 66.73
p*		>0.05	>0.05	>0.05

* Friedman Two Way ANOVA

The comparison of average points of GIS symptoms in the individuals within and between the groups depending on the MS dosage ($\bar{X} \pm SD$) is shown at Table 7.

The complaints of abdominal cramps were increased and reached to the highest level in MS_{24} and MS_{48} groups at $Test_1$ period when the consumption of MS started. The severity of complains tended to decrease during the $Test_2$ period. The most severe abdominal cramps were seen in the group which consumed 24g/d MS (0.42 ± 0.48 points) during the $Test_2$ period and followed by the group that consumed 48g/d MS (0.26 ± 0.45 points). When the consumption of MS was terminated ($Placebo_2$), the severity of abdominal cramps reached almost to beginning levels ($Placebo_1$) in all three dosage groups. The complaints of cramps in the $Test_2$ period exhibited significant differences between the groups depending on the dosage of MS ($p<005$). This difference was found between MS_{12} (0.04 ± 0.10) and MS_{24} (0.39 ± 0.61) groups.

In all dosage groups, the severity of flatulence increased at the $Test_1$ period when MS consumption started, and decreased during the $Test_2$ and the $Placebo_2$ periods compared to the $Placebo_1$ period. The group with the most severe complaints of flatulence was the group who consumed 48g/d MS (1.30 ± 0.62 points).

The severity of complaints of abdominal pain did not exhibit significant differences according to the weeks neither within groups nor between groups ($p>0.05$).

The differences in the severity of complaints of burping in all three groups according to weeks were not found significant ($p>0.05$) neither within nor between groups.

Even in the MS_{48} group, the severity of the flatulence complaint appeared to be between light and moderate. While the viscosity of feces was close to the normal in the period of the $Placebo_1$, in all 3 groups, it had a tendency to become softer with consumption of MS (Table 7).

The severity of complaints of abdominal grumbling in all three dosage groups continued as being higher than the initial level even after switching from the consumption of MS to consumption of placebo ($Placebo_2$) (0.44 ± 0.51, 0.6 ± 0.62, 0.28 ± 0.32, respectively). In the MS_{24} and MS_{48} groups, the differences in the points of complaints of abdominal grumbling occurred according to the periods were not found significant ($p>0.05$), neither within nor between groups.

The viscosity of feces in all groups and during all weeks was found as soft (2 points) and normal (3 points). Fecal viscosity is soft when the point is close to 2 and normal when it is close to 3. In all three groups the fecal viscosity, which was close to normal in the beginning ($Placebo_1$), became softer with consumption of MS ($Test_1$). With MS consumption, in the MS_{24} and the MS_{48} groups the fecal viscosity was detected as softer than in the MS_{12} groups. When the consumption of MS was terminated ($Test_2$) and placebo consumption started ($Placebo_2$), the viscosity of feces started to increase and tended to be normal. However, the viscosity of feces became neither in diarrhoea form nor in constipation form in none of the groups. The difference

in the viscosity of feces within and between groups was not statistically significant ($p>0.05$) (Table 7) according to the weeks.

Daily intake of 12 g MS, which kept the GIS symptoms at the lowest level and increased the weight of feces the most, was determined to be the tolerable dosage of the MS.

At the second step, where the effect of MS consumption on the number of bacteria in the feces was investigated, it was found that, lactic acid bacteria (*Bifidobacteria* and *Lactobacillus*) increased in number compared to placebo consumed period. However, the difference between the number of *Bifidobacteria* and *Lactobacillus* in the test and placebo periods was not found to be significant ($p>0.05$). All the pathogen bacteria, except the *Bacteroides*, were reduced in the period when MS was consumed compared to placebo consumed period. However, while the difference between the numbers of *Clostridium, E.coli, Klebsiella* and *Streptococcus* wasn't found significant in terms of periods ($p>0.05$), the difference in the number of *Staphylococcus* turned out to be significant ($p<0.01$) (Table 8).

Table 7. The comparison of average points of GIS symptoms within and between the groups depending on the mixed sweetener dosage ($\bar{X} \pm SD$)

Point[2] ($\bar{X} \pm SD$)						
	Periods					Differences between
GIS Symptoms	Placebo$_1$	Test$_1$	Test$_2$	Placebo$_2$		the
Grup[1]	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	p*	periods
Abdominal Cramp						
MS_{12}	0.06 ± 0.14	0.06 ± 0.14	0.04 ± 0.10	0.07 ± 0.16	>0.05	-
MS_{24}	0.17 ± 0.19	0.42 ± 0.48	0.39 ± 0.61	0.20 ± 0.30	>0.05	-
MS_{48}	0.11 ± 0.22	0.26 ± 0.45	0.11 ± 0.23	0.09 ± 0.28	>0.05	-
p**	>0.05	>0.05	<0.05	>0.05		
Difference between groups	-	-	12-24	-		
Abdominal flatulence						
MS_{12}	0.39 ± 0.29	0.65 ± 0.38	0.55 ± 0.41	0.39 ± 0.41	>0.05	-
MS_{24}	0.57 ± 0.51	1.04 ± 0.61	1.01 ± 0.66	0.79 ± 0.52	<0.01	a, b
MS_{48}	0.22 ± 0.23	1.30 ± 0.62	1.09 ± 0.65	0.44 ±0.40	<0.01	a, b, c
p**	>0.05	>0.05	>0.05	>0.05		-
Abdominal pain						
MS_{12}	0.17 ± 0.30	0.30 ± 0.28	0.17 ± 0.21	0.11 ± 0.24	>0.05	-
MS_{24}	0.31 ± 0.40	0.41 ± 0.50	0.52 ± 0.58	0.33 ± 0.39	>0.05	-
MS_{48}	0.06 ± 0.10	0.25 ± 0.50	0.14 ± 0.22	0.09 ± 0.15	>0.05	-
p**	>0.05	>0.05	>0.05	>0.05		
Burping						
MS_{12}	0.15 ± 0.19	0.30 ± 0.28	0.28 ± 0.23	0.23 ± 0.32	>0.05	-
MS_{24}	0.42 ± 0.37	0.34 ± 0.38	0.52 ± 0.69	0.47 ± 0.80	>0.05	-
MS_{48}	0.09 ± 0.10	0.19 ± 0.20	0.33 ± 0.29	0.26 ± 0.34	>0.05	-
p**	>0.05	>0.05	>0.05	>0.05		
Abdominal grumbling						
MS_{12}	0.23 ± 0.17	0.55 ± 0.25	0.42 ± 0.22	0.44 ± 0.51	<0.05	a
MS_{24}	0.52 ± 0.51	0.60 ± 0.74	0.68 ± 0.77	0.63 ± 0.62	>0.05	-
MS_{48}	0.14 ± 0.18	0.55 ± 0.74	0.44 ± 0.47	0.28 ± 0.32	>0.05	-
p**	>0.05	>0.05	>0.05	>0.05		
Feces viscosity[3]						
MS_{12}	2.91 ± 0.46	2.86 ± 0.41	2.86 ± 0.60	2.94 ± 0.71	>0.05	-
MS_{24}	2.98 ± 0.59	2.58 ± 0.63	2.67 ± 0.42	2.73 ± 0.22	>0,05	-
MS_{48}	2.82 ± 0.37	2.61 ± 0.37	2.69 ± 0.41	2.80 ± 0.34	>0.05	-
p**	>0.05	>0.05	>0.05	>0.05		

[1] n=9, each dosage groups, * *Friedman Two Way ANOVA,* ** *Kruskal-Wallis One-Way ANOVA*

[2] point: 1 light, 2 moderate, 3 severe

[3] Feces viscosity: 1 diarrhoea form, 2 soft, 3 normal, 4 hard, 5 constipation form

a: placebo$_1$-test$_1$, b: placebo$_1$-test$_2$, c: test$_1$-placebo$_2$

Table 8. Average number of bacteria (log_{10} CFU/g in feces during Test and Placebo ingestion periods (n=8)

	Number of bacteria (log_{10} CFU/g)					
	Test		Placebo	t*	SD	p
	$\bar{X} \pm SD$	Δ	$\bar{X} \pm SD$			
Anaerob						
Bifidobacteria	7.341 ± 0.065	+0.07	7.271 ± 0.100	2.2580	7	>0.05
Lactobacillus	7.525 ± 0.090	+0.10	7.409 ± 0.128	2.1340	7	>0.05
Bacteroides	7.461 ± 0.195	+0.04	7.424 ± 0.205	0.3619	7	>0.05
Clostridium	7.438 ± 0.123	-0.04	7.482 ± 0.094	0.7348	3	>0.05
Total anaerob	8.015 ± 0.08	+0.03	7.980 ± 0.110	0.9270	7	>0.05
Aerob						
E.coli	7.320 ± 0.320	-0.04	7.359 ± 0.230	0.4277	7	>0.05
Klebsiella	6.941 ± 0.272	-0.27	7.210 ± 0.116	1.3000	4	>0.05
Staphylococcus	6.628 ± 0.182	-0.33	6.958 ± 0.398	22.330	2	<0.01
Enterobacter	6.720 ± 0.169	-0.32	7.040 ± 0.496	0.8402	3	>0.05
Streptococcus	6.820 ± 0.124	-0.17	6.991 ± 0.302	0.5338	2	>0.05
Total aerob	7.640 ± 0.223	-0.08	7.790 ± 0.075	1.6610	7	>0.05

* paired sample t test, Δ: changing

DISCUSSION

The effects of prebiotic sweeteners on health have been examined separately in the previous studies. In this study, the tolerable dosage of a mixed sweetener (MS), which is made of low energy components providing intense sweet taste (aspartame and asesulfame K), sugar alcohols providing volume (maltitol) and low energy prebiotics providing both volume and fat taste-sweet taste (oligofructose and polydextrose) in order to assure a better quality product, was determined and the prebiotic effects of this dosage on colon were investigated by microbiological analyses in feces samples.

In a study of Cumming et al.(5) the increase of feces weight by 18g/d with 15g FOS (fructo-oligosaccharides) showed similarity with our finding of the increase feces weight by 16g/d with MS dosage of 24 g which contains approximately 15g/d prebiotic.

In another study by Achour et al.(6) , with 30g/d PD, the weight of feces showed an increase of 36g/d. MS dosage of 48g containing approximately 30g/d prebiotic in total was not found to cause such an increase, on the contrary, it caused a decrease by an amount of 33g/d. On the other hand, in the study of Jie et al.(7) , the increase in the weight of feces was consistent to the increase in PD dosage. In the two earlier studies performed on humans, it has been demonstrated that taking the prebiotic did not affect the weight of feces (8, 9).

In the human studies driven by prebiotics, undesired symptoms, especially flatulence, were observed (5,10). Moreover, abdominal pain, stress, cramp and similar complaints were also encountered in some of the studies (11, 12). With the use of 15g/d FOS, a significant number of the individuals have had complaints of abdominal pain, burping, stress and flatulence; however the severity of these complaints were not over moderate level (13, 14). It has been found that consumption between 5 and 20g/d of FOS caused an important increase in flatulence and burping complaints (10, 12). It has also been observed that consumption of 20g SCFOS per day caused extremely high flatulence complaint [15] and that 6.9% of the individuals who consumed biscuits including FOS (6.6g) and guargam (3.4g) had abdominal pain at a light level and 7.28% of these at a moderate level (12). It has been found that consumption of 10g SCFOS caused flatulence and grumbling complaints in 10% of the individuals, and it caused complaints of excessive flatulence, grumbling and swelling in 20-30% (11). In another study (13), where 10 volunteers have been eaten 15g/d FOS for 12 days, the volunteers have had more severe ache,

spasm, flatulence and swelling in abdomen than the groups who consumed sucrose. Even after 12 days, there has been no adaptation; but the complaints were stated to be at moderate level. Similar observations were obtained in our study. In the MS_{48} group, more GIS complaints than those in the MS_{12} group were observed. Depending on the increase in the dosage, GIS complaints were observed to increase. All of these complaints were moderate or severe (Table 7).

In the present study, softening in the viscosity of feces was observed especially in the MS_{24} and MS_{48} groups. After the consumption of MS was terminated, viscosity of feces started to become close to normal (Table 7). In another study, it has been observed that taking inulin solved the constipation problem in 9 out of 10 people and caused sickness and flatulence in stomach only in a few patients (16). Hidaka et al. (17) have found that the consumption of 8g of OF or FOS per day during 14 days rendered the viscosity of feces normal in the people who suffer from constipation at moderate or severe levels.

Various studies have showed that tolerable dosage of prebiotics varied between 4-12.5g/d (11, 18). In this study, the MS dosage of 12g/d containing OF and PD was chosen as the tolerable dosage, since it caused the lowest GIS symptoms.

Bifidogenic effects of prebiotics related to dosage have been mentioned in many studies carried out in vivo and in vitro conditions.

In most of the studies, it has been observed that the number of *Bifidobacteria* and *Lactobacillus* in feces increased depending on the prebiotic dosage. Gibson et al. have indicated (14) that the number of *Bifidobacteria* and *Lactobacillus* was increased with the consumption of 15g/d OF. It has been determined by Hidaka et al. that (17) by using 8g/d OF and by Mitsuako et al.(18) by using 8g/d FOS, the number of *Bifidobacteria* has increased 10 fold. In another study by Hidaka et al. (19) with the same dosage of OF, it was found that the number of *Bifidobacteria*, in comparison to the groups taking sucrose increased significantly. In another study (20) it has been found that taking OF at a lower dosage (5g/d) increased the number of *Bifidobacteria* at approximately 1 log cycle. Williams et al. (21) and Buddington et al. (22) have reported that a dosage of 4g OF per day increased the number of *Bifidobacteria*. In another study, it has been determined that consumption of biscuits containing 6.6g FOS and 3.4g guargum increased the number of *Bifidobacteria* at 0.47 $\log_{10}$ CFU/g level (12). It has been observed that consumption of 8 and 12g PD per day increased the number of *Bifidobacteria* and *Lactobacillus* by a considerable level amount (7). Reading et al. (23) have indicated that taking even 1g/d OF increased the number of *Bifidobacteria* while, with the rise of the OF dosage to 2g/d, the number of *Bifidobacteria* increased substantially. It has been finally determined that the best bifidogenic effects were realised when OF was given at a dosage of 4g per day (15, 23).

In this study, where microbial changes on colon resulted from consuming 12 g/d of MS including OF and PD compared to sucrose consumption for 14 days were analysed, an increase in the number of *Bifidobacteria* and *Lactobacillus* in feces have been observed as in several previous studies ($p>0.05$). However, this increase did not appear to be as high as the levels previously reported. The reason is that 12g/d MS dosage included 4.9g PD and 2.4g OF (7.1g prebiotic in total); i.e. the dosage of OF was lower than the dosage used in the previos studies. In spite of this fact, the increase in the numbers of *Bifidobacteria* and *Lactobacillus* is noteworthy.

In the studies, it is observed that the number of fecal *Bacteroides* changed depending on the prebiotic dosage. In the study of Gibson et al.(14), the number of *Bacteroides* decreased significantly with 15g/d OF. In the group taking 8g OF per day, a rapid decline in the number of *Bacteriodes* has also been observed [19]. As for the studies done by Williams et al. (21) with 4g OF dosage per day, the number of *Bacteroides* has decreased. It has been also found that taking 8 and 12g PD per day decreased the number of *Bacteroides*. There are also studies indicating an increase in the number of *Bacteroides*. In the study of Reading et al. (23) 5 different OF dosages

(1,2,4,6 and 8g/d) were employed under in vitro conditions , and they have observed an increase in the number of *Bacteroides* in all dosages. In another study [19] an increase in the number of *Bacteroides* has been observed in the group taking OF at low dosage (5g/d). In our study, an increase in the number of *Bacteroides* at 0.04 $\log_{10}$ CFU/g level was found ($p>0.05$). This was probably due to the differences in the dosage of the prebiotic and period of time employed.

Although Gibson et al. (14) found no change in the number of gram-positive cocci , Reading et al. [23] have reported an increase in the number of gram-positive cocci with 5 different OF dosages (1,2,4,6, and 8g/d) under in vitro conditions. In our study, a decrease in the number of *Staphylococcus* and *Streptococcus* gram-positive cocci was observed. Especially the decrease in the number of *Staphylococcus* was found to be significant ($p<0.01$).

In the studies carried out by Williams et al. [21] with 4g/d OF dosage, there was a decrease in the number of *Fusobacteria* and *Clostridium*. In the study done by Gibson et al. (14) with 15g OF, the number of *Clostridium* and *Fusobacteria* decreased at a considerable degree. Moreover, in the studies done by Hidaka et al (17) with OF and by Mitsuoka et al. [18] with FOS, it has been found that consumption of 8g OF or FOS per day for 14 days decreased the number of *Clostridium*. It has also been determined that consumption of 15g/d PD per day decreased the number of *C.perfringens (*24).

Buddington et al. (22) found that the number of total anaerobes increased and the number of total aerobes did not change when 4g/d OF was used. Bouhnik et al. (25) have determined that consumption of FOS did not affect the number of total anaerobes. Nevertheless, MS consumption decreased the number of total aerobes in our study albeit statistically insignificantly ($p>0.05$) (Table 8).

CONCLUSIONS

When this study is compared with other studies, the differences between amounts of change in bacteria are probably due to the low prebiotic dosage and the fact that MS included OF and PD in combination. OF and PD that existed in the components of the mixed sweetener, whose prebiotic effects had been examined separately previously, did not exert synergetic effects over a short period of time.

The prebiotic effects of the MS, which has been in the markets for almost a year in Turkey, were demonstrated in this study. To reveal the prebiotic effects of these products, their continuous and regular consumption is required. The most distinctive characteristic of MS, whose components include 60% soluble diet residues, is its different technological characteristic besides its prebiotic characteristics. MS has technological properties such as having 60% less calories than sugar, giving consistency like sucrose and endurance to heat. It is possible to benefit from the prebiotic effect and low calories of these sweeteners without abandoning the taste of our food products.

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REFERENCES

1. **Dye, L., Blundel, J.** "Functionals foods, psychological and behavioral functions" *British J. Nutrition.* 88(2),18-211, **2002**.

2. **Beerens, H.** "An elective and selective medium for Bifidobacterium spp" *Lett. Appl. Microbiol.* 11,155-157, **1990**.

3. **Baron, EJ., Finegold, SM.** Bailey&Scott's Diagnostic Microbiology. The CV Mosby Company, 8. Edition, pp.523-525, **1990**.

4. **Flickinger, EA., Hatch, TF., Wofford, RC., Grieshop, CM., Murray, SM., Fahey, GC.** "In vitro fermentation properties of selected FOS-containing vegetables and in vivo colonic microbial population are affected by the diets of healthy human infants" *J. Nutr.* 132,2188-2194, **2002**.

5. **Cummings, JH., Macfarlane, GT., Eneglyst, HN.** "Prebiotic digestion and fermentation" *Am. J. Clin. Nutr.* 73 (suppl),415-20S, **2001**.

6. **Achour, L., Flourie, B., Briet, F., Pellier, P., Marteau, P., Rambaud, JC.** "Gastrointestinal effects and energy value of PD in healthy non-obese men" *Am. J. Clin. Nutr.* 59,1362-8,**1994**.

7. **Jie, Z., Bang-yao, L., Ming-jie, X., Hai-wei, L.** "Studies on the effect of PD intake on physiologic functions in Chinese people" *Am. J. Clin. Nutr.* 72(6),1503-1509, **2000**.

8. **Alles, MS., Hautvast, JG., Nagengast, FM., Hartemink, R., Van Laere, KM., Jansen, JB.** "Fate of FOS in the human intestine" *Br. J. Nutr.* 76(2),211-221, **1996**.

9. **Bouhnik, Y., Flourie, B., D'Agay-Abensour, L., Pochart, P., Gramet, G., Durand, M., Rambaud, J.C.** "Administration of transgalacto-oligosaccharides increases faecal *Bifidobacteria* and modifies colonic fermantation metabolism in healthy humans" *J. Nutr.* 127, 444-448, **1997**.

10. **Luo, J., Rizkalla, S.W., Alamovitch, C., Boussairi, A., Blayo, A.** "Chronic consumption of short chain fructooligosaccharides by health subjects decreased basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism". *Am. J. Clin. Nutr.* 63,639-645, **1996**.

11. **Briet, F., Achour, L., Flourie, B., Beaugerie, L., Pellier, P., Franchisseur, C.** "Symptomatic response to varying levels of FOS consumed occasionally or regularly" *Eur. J. Clin. Nutr.* 49,501-507, **1995**.

12. **Tuohy, KM., Kolida, S., Lustenberger, AM., Gibson, GR.** "The prebiotic effects of biscuits containing partially hydrolyzed guargum and FOS a human volunteer study" *Br. J. Nutr.* 86,341-348, **2001**.

13. **Stone-Dorshow, T., Levitt, MD.** "Gaseous response to ingestion of a poorly absorbed FOS sweetener" *Am. J. Clin. Nutr.* 46,61-65, **1987**.

14. **Gibson, GR., Beatty, ER., Wang, W., Cummings, JH.** "Selective stimulation of Bifidobacteria in the human colon by OF and inulin" *Gastroenterology.* 108,975-982, **1995**.

15. **Bouhnik, Y., Vahedi, K., Achour, L., Attan, A., Salfati, J.** "SCFOS administration dose dependently increases fecal Bifidobacteria in healthy humans" *J. Nutr.* 129,113-116, **1999**.

16. **Kleessen, B., Sykura, B., Zunft, HJ., Blaut, M.** "Effects of inulin and lactose on faecal microflora, microbial activity and bowel habit in elderly constipated persons" *Am. J. Clin. Nutr.* 65,1397-1402, **1997**.

17. **Hidaka, H., Eida, T., Takizana, T., Tokunaga, T., Toshiro, Y.** "Effect of FOS on intestinal flora and human health" *Bifidobacteria Microflora.* 5,37-50, **1986**.

18. **Mitsuoka, T., Hidaka, H., Eida, T.** "Effect of FOS on intestinal flora" *Die Nahrung.* 31,426-436, **1987**.

19. **Hidaka, H., Tashiro, Y., Eida, T.** "Proliferation of Bifidobacteria by oligosaccharides and their useful effect on human health" *Bifidobacteria Microflora,* 10,65-79, **1991**.

20. **Rao, VA.** "The prebiotics properties of OF at low intake levels" *Nutr. Res.,* 21,843-848, **2001**.

21. **Williams, C., Witherly, S., Buddington, R.** "Influence of dietary neosugar on selected bacterial groups of the fecal microbiota" *Microb. Ecol. Health. Dis.* 7,91-97, **1993**.

22. **Buddington, RK., Williams, CH., Chen, S., Witherly, SA.** "Dietary supplementes of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects" *Am. J. Clin. Nutr.,* 63,709-716, **1996**.

23. **Reading, S., Aramendi, S., Gibson, G., Mc Cartney, D.** An in vitro investigation of prebiotic effect. Profibre, Lisbon, Portugal, **1998**.

24. **Endo, K., Kumemura, M., Nakamura, K., Fujisawa, T., Suzuki, K., Benno, Y., Mitsuoka, T.** "Effect of high cholesterol diet and PD supplementation on the microflora, bacterial enzyme activity, putrefactive products, volatile fatty acid (VFA) profile, weight, and pH of the feces in healthy volunteers" *Bifidobacteria Microflora.*,10 (1), 53-64, **1991**.

25. **Bouhnik, Y., Flourie, B., Andrieux, C., Bisetti, N., Briet, F., Ramboud, JC.** "Effects of bifidobacterium fermented milk ingestion with or without inulin on colonic bifidobacteria and enzymatic activities in healthy human" *Eur. J. Clin. Nutr.,* 50 ,269-273, **1996**.

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