Changes in chemical and biological properties of yogurt prepared with concentrations of Fructooligosaccharide

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Abstract
This study was conducted to test the effect of adding different concentrations of Fructooligosaccharides (FOS) to yogurt production and to study the effect of the product on the rheological and chemical properties of the yogurt produced using starter bacteria Lactobacillus plantarum and storage at 1-5°C for 1 to 14 days. It was found that adding Fructooligosaccharides to the fermenter with Lactobacillus plantarum bacteria led to clear changes compared to the control treatment based on estimating the logarithm of the total number of bacteria Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus plantarum, coliform bacteria, yeasts and molds which increased significantly at the expense of starter bacteria. It was observed in treatment T1 that starter bacteria alone led to a significant increase in bacterial counts, especially in the presence of FOS at the lowest concentration of 3%. It also increased at 5% FSO, which did not differ significantly from that recorded using the highest concentration of 7% FSO. The number of Streptococcus thermophilus bacteria in the fermenter on the fourteenth day decreased for all treatments. In general, treating the yogurt fermenter with FOS always led to a reduction and suppression of coliform bacteria, yeasts and molds with an absolute fever period exceeding 10 days, regardless of the concentration used.

Keywords: food products, healthy yogurt, spoilage bacteria, yeasts

Introduction

Fructooligosaccharides (FOS) is hydrolyzed by saliva and digestive enzymes. Meanwhile, it can tolerate intestinal conditions due to the formation of glycosidic bonds, and is fermented in the intestine by anaerobic bacteria, which contributes to enhancing the bioavailability of dietary fiber used as a low-calorie value. FOS main source are some plants such as soybeans, artichokes and asparagus. it is used as a natural compound in the food and pharmaceutical industries, and these compounds are extracted from fruits and vegetables (Kherade, et al., 2021). Yogurt is a dairy product with high nutritional value and easy digestion and is a rich source of essential nutrients, vitamins and minerals. The nutritional composition of yogurt can vary according to the strains of the starter used in fermentation and the type of milk used (whole milk, low-fat milk or skimmed milk), and the source of milk (cow's milk, buffalo milk or goat's milk), and the sweeteners or fruits added before and/or during the fermentation process (Weerathilake et al., 2019).

The probiotics have been widely spread for therapeutic purposes to obtain health benefits began to depend on the type, class, and functional and therapeutic properties of the probiotic used (Pannerchelvan, et al 2024). Many studies have shown that the preventive and therapeutic roles of probiotics, such as their role in preventing allergic diseases, treating diarrhea, alleviating symptoms of lactose tolerance, treating colon, lowering cholesterol, and preventing cancer (Mehra et al, 2022; ; Najim and Mohsen, 2022). It was also noted that probiotics contribute to maintaining the intestinal membranes, improving lactose metabolism, reducing the appearance of inflammation, activating the immune system against gastrointestinal infections (Raheem et al, 2021), and strengthening the immune system in the body in all its forms (Hasan, et al 2022; ; Najm and Mohsen, 2024).

In a study, it was shown that lactic acid bacteria in general can produce a wide range of biologically active peptides during the fermentation process. This always came with antibacterial, anti-inflammatory, anticoagulant and immune system stimulant activities (Nourmohammadi, et al., 2019; Muhialdin et al., 2021) explained that some active peptides produced by some types of lactic bacteria can suppress the proliferation of viruses that cause respiratory infections. And that many of the peptides produced by lactic acid bacteria can stimulate the production of small protein molecules known as immunoglobulins such as (Y-IFN or B-IFN). This study, therefor, was conducted to determine the effect of adding different concentrations of Fructooligosaccharides (Fos) to yogurt production and to study the effect of the product on the rheological and chemical properties.

Materials and methods

Starters and bacterial isolates were used for lactic fermentation in producing yogurt from French Regelet nonfat dried milk. The strains of starter bacteria Streptococcus Salivarius Subsp thermophilus, Lactobacillus delbrueckii Subsp bulgaricus produced by the Italian company SACCO were used in the manufacture of lactic fermentation treatments with the use of *L. plantarum* bacteria that were activated in the laboratory.

Culture media

The solid MRS Agar medium was prepared according to the instructions of the producing company using the method of Holt et al. (1994) and was used to calculate the logarithm of the live numbers of starter bacteria. The liquid MRS Broth medium was also used to ensure that the medium was free of oxygen in the activation of the probiotics. The solid potato dextrose agar medium was also prepared to estimate the total count of yeasts and molds. MRS-vancomycine medium was prepared according to the method described by Ong and Shah (2009). MacConkey Agar was used to count coliform bacteria. M17 Agar was used to calculate the logarithm of the live count of Streptococcus thermophilus.

Preparation of lactic ferment medium was by adding bacterial isolates and starter culture according to the method described by Tamime and Robinson (1999). The milk was divided into five equal groups, the first was left as control treatment C, treatments T1 fermented with starter culture only, T2, T3 and T4 were prepared by adding starter culture L. plantarum and different proportions of FOS (3, 5, 7) % 100 ml milk respectively. The samples were mixed by an electric mixer to mix them well, the milk was placed at a temperature of 90 °C for a period of 10 minutes, then the milk was cooled to a temperature of 42 °C, then all treatments were inoculated with starter bacteria (thermophiles), Lactobacillus delbrueckii Subsp bulgaricus, Streptococcus salivarius Subsp thermophilus) after activation and directly added in the quantity indicated by the manufacturer, the product was filled in 120 ml plastic containers and incubated at 42 ± 2 °C until coagulation was completed, the containers were kept in the refrigerator for tests after (1, 3, 7, 14) days from the manufacturing process. (Pohlentz et al., 2022).

As for the experiment treatments, yogurt fermentation medium treatments included treated with starter bacteria (Lactobacillus delbrueckii Subsp bulgaricus and Streptococcus salivarius Subsp thermophilus) only TC, adding the starter and 1 ml of L. plantarum bacteria

T1, same T1 treatment beside medium supplemented with FOS at levels of 3%, 5% or 7% for the treatments T2, T3 and T4, respectively.

The experimental parameters included chemical indicators where Total acidity was estimated by titration according to the method (A.O.A.C. 2008) and pH was estimated by pH meter at 25°C directly (Shafiee 2010). The parameters also included taking data on biological characteristics where the total count of starter bacteria Streptococcus salivarius Subsp thermophilus was estimated and Lactobacillus delbruecii ssp. Bulgaricus was estimated according to the method described by Talon et al. (2002). Estimation was also performed for the Lactobacillus plantarum bacteria in MRS (de Man Rogosa Sharp) medium (Hi-Media, Mumbai, India) as a selective chemical agent that supports the optimal growth of "lactic acid bacteria" according to the method described by Isa 2017. Also, the total count of Tolal Coliform bacteria was estimated on MacConkey medium (Divya, 2016). Yeasts and molds were also determined on Potato Dextrose Agar (APHA, 1984).

Results and discussion

The results (Table 1) show that the total acidity value was the lowest in the control treatment 0.82% compared to higher levels ranging from 0.84 to 0.87% in the treatments added to FOS, which increased with increasing concentration. On the other hand, the total acidity values differed significantly according to the storage period after the treatment, which increased from 0.86% after 3 days to 0.92% after 14 days of storage. The results of Table (2) indicated that the pH values of the yogurt for the control treatment recorded a higher rate than the yogurt treatments treated with FOS concentrations immediately after manufacturing, which continued in the same vein after three days of storage. In general, after 7 or 14 days of storage, the control treatment remained the highest significantly in pH, which recorded 4.45, with a significant difference from the FOS treatments, which recorded values that did not decrease below 4.24 and did not increase above 4.39.

Table1. Acidity of yogurt enriched with different concentrations of FOS

Treatments	Day 1	Day 3	Day 7	Day 14	LSD
TC	0.82	0.83	0.85	0.86	0.01
T1	0.84	0.86	0.88	0.92	0.02
T2	0.84	0.87	0.89	0.91	0.01

T3		**0.85**	**0.89**	**0.91**	**0.94**	**0.03**
T4		**0.87**	**0.88**	**0.92**	**1.01**	**0.06**
LSD (P≤0.05)	**Treat.**	**0.03**	**0.02**	**0.04**	**0.09**	
	Interaction			**0.12**		

Values are means of three replications, were treatments included yogurt prepared using the starter bacteria only TC, adding the starter and 1 ml of L. plantarum bacteria T1, same T1 treatment beside medium supplemented with (FOS) at levels of 3%, 5% or 7% for the treatments T2, T3 and T4, respectively.

Table1. The pH values in yogurt prepared with adding different concentrations of FOS

Treatments		**Day 1**	**Day 3**	**Day 7**	**Day 14**	**LSD (P≤0.05)**
CT		**4.77**	**4.71**	**4.65**	**4.54**	**0.21**
T1		**4.63**	**4.69**	**4.52**	**4.39**	**0.17**
T2		**4.65**	**4.58**	**4.47**	**4.38**	**0.11**
T3		**4.45**	**4.43**	**4.41**	**4.34**	**0.09**
T4		**4.44**	**4.42**	**4.39**	**4.24**	**0.19**
LSD (P≤0.05)	**Treat.**	**0.21**	**0.19**	**0.11**	**0.13**	
	Interaction			**0.33**		

Values are means of three replications, were treatments included yogurt prepared using the starter bacteria only TC, adding the starter and 1 ml of L. plantarum bacteria T1, same T1 treatment beside medium supplemented with (FOS) at levels of 3%, 5% or 7% for the treatments T2, T3 and T4, respectively.

The continuous decrease in pH during the cold storage period is attributed to the continued activity of starter bacteria and bacteria added to the product during storage. The results are in agreement with Mani-Lopez et al. (2014) who indicated that the decrease in pH in yogurt is attributed to the actual activity of starter bacteria used in the preparation of yogurt.

As for the effect of treatments on the biological properties of the product, it was noted that adding FOS at higher concentrations often led to higher numbers of *L. bulgaracuse* and *S. thermophilus* bacteria with increasing number of days after treatment, while it was noted that the numbers of bacteria for the same two species decreased clearly after 14 days of treatment. On the other hand, L. plantarum increased after 14 days of treatment to higher numbers, especially in yogurt treated with concentrations higher than fos 7%. The results of the same table (3) show that FOS added to the fermentation medium always showed properties against the growth of coliform bacteria, yeasts and molds, which did not appear at all in yogurt treatments with FOS at higher concentrations of 5% and 7%, but were recorded after 14 days of treatment in very low numbers that did not exceed 1 and 2 logarithmic cycles at fos at concentrations of 5% and 7%, respectively.

Table3. Effect of yogurt prepared with adding different concentrations of FOS on microbial populations

Treatment	Days	C	T1	T2	T3	T4
L. bulgaracuse	1st day	12.75	12.82	12.65	12.53	12.47
	3rd day	11.34	11.01	12.41	13.11	12.87
	7th day	10.58	11.35	12.33	13.64	13.92
	14th day	9.01	10.4	11.86	12.41	12.01
S.thermophlius	1st day	9.77	9.45	9.76	9.78	9.8
	3rd day	9.23	6.53	9.57	9.75	9.63
	7th day	8.5	9.11	9.24	9.73	9.55
	14th day	7.16	8.17	9.03	8.42	9
L. plantarum	1st day	-	10.49	11.83	11.96	12.61
	3rd day	-	9.32	11.47	11.42	12.5
	7th day	-	8.68	11.18	11.63	12.52
	14th day	-	8.45	9.42	11.37	12.77
Coliform bacteria	1st day	0	0	0	0	0
	3rd day	0	0	0	0	0
	7th day	1.65	0	0	0	0
	14th day	1.71	0	0	0	0
Yeasts and molds	1st day	0	0	0	0	0

	3rd day	**0**	**0**	**0**	**0**	**0**
	7th day	**4.32**	**1.03**	**1**	**0**	**0**
	14th day	**4.59**	**1.21**	**1.11**	**2**	**1**

Values are means of three replications, were treatments included yogurt prepared using the starter bacteria only TC, adding the starter and 1 ml of L. plantarum bacteria T1, same T1 treatment beside medium supplemented with (FOS) at levels of 3%, 5% or 7% for the treatments T2, T3 and T4, respectively.

The difference in the number of bacteria and their gradual decrease may be due to the effect of the manufacturing method on the growth of microorganisms in the *L. plantarum* starter. This is what was indicated by Chouglani et al. (2008) Sanders (2006) that the difference in the number of bacteria is due to the method of manufacturing the yogurt and the type of milk used, and the decrease in bacterial colonies may also be due to the development of acidity in the yogurt after the storage period. The results showed the absence of colonic bacteria growth for all treatments and the control treatment immediately after manufacturing, while bacteria increased in the control treatment and did not appear in that treatment in FOS after 7 days of manufacturing and storage. This indicates that colonic bacteria were not present in all samples of manufactured yogurt, and is also due to the health practices followed during manufacturing and storage (Nawar, 2010).

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