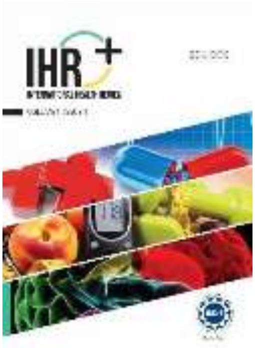

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Evaluation of Physicochemical Properties and Sensory Attributes of Galactooligosaccharides (GOS) Enriched Cottage Cheese

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Abstract

Galactooligosaccharides (GOS) mimic the role of dietary fibres and are known to offer several health benefits, especially those relating to heart and gastrointestinal health. Glycosidation of lactose in milk can be utilized to produce GOS in cottage cheese. The current study was conducted to evaluate the sensory as well as physicochemical properties of GOS containing cottage cheese. For this purpose, transgalactosylated milk was used to obtain casein after it was curdled using citric acid. Subsequently, the sensory evaluation of organoleptic properties was done by ten panellists on the 0, 4th, 8th, and 12th day of storage. The sensory characteristics during the shelf-life study did not show a significant difference. The prebiotic cheese was sweeter and softer as compared to the control cheese. Additionally, the prebiotic cheese was ranked higher in the overall sensory characteristics score than the control cheese. Based on the findings, we suggest that GOS containing cheese as well as other food products should be commercially produced since they would be a valuable healthy addition to the diet.

Keywords: functional foods, galagtooligosaccharides, gastrointestinal health, prebiotics, probiotics,

Introduction

Biotechnology is an emerging field. Its application on microbes, metabolites and enzymes has accelerated in the food chain/the production and lifespan of food items in various aspects. It can be used for bio-

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preservation of wholesome food items, bio-conversion of ingredients, maintenance of quality, and preparation of edible, healthy and safe food as well as novel food production. Cheese is an edible enriched dairy product used all over the world due to its health properties or nutraceutical abilities. It contains protein, health-friendly probiotics, and bioavailable products, such as bacteriocins, ɣ-aminobutyric acid, exopolysaccharides, vitamins, healthy fatty acids, and organic acids [1]. These biologically active composites of cheese are good for health due to their nutraceutical abilities, such as antimicrobial activities, antioxidant immune-modulatory activities, anti-proliferative activities, and anti-inflammatory activities [2]. Oligosaccharides enhanced cheese raises the probiotic environment in the gastrointestinal tract by introducing beneficial gut microorganisms [3]. Prebiotics play an essential role in managing acute as well as chronic diseases through alteration of gut microbiota. Galactooligosaccharides (GOS) constitute an array of prebiotics produced from lactose (milk sugar) through the mechanistic reaction of trans-galactosylation. Physiologically, GOS act similar to dietary fibres and are known to improve gastrointestinal problems [4], lower cholesterol levels, lower blood pressure, and enhance the growth of beneficial microorganisms in the gastrointestinal tract [5]. The commercial-scale production of GOS involves an enzymatic reaction, which uses β-Galactosidase to add galactose units to lactose to increase the chain length. Many products are utilizing prebiotics and are gaining popularity in the market due to its health benefits and organoleptic properties. In addition to the preventive effects, reduced lactose contents of milk and milk products after the formation of GOS is useful for lactose-intolerant people partaking in therapeutic diets [6]. The distinct physiological and physiochemical capabilities of GOS are different due to the formulization level in blends containing oligosaccharides, un-reacted lactose, and monosaccharide [7]. The current study was conducted to longitudinally assess the sensory properties of prebiotic cottage cheese during several days of storage. Subsequently, these properties were compared with control cottage cheese properties. The main aim of this research was to test the sensory appeal and assess the consumer acceptability of prebiotic enriched cottage cheese for possible commercial production purposes.

Materials and Methods

Research Setting

The study was conducted in a Laboratory situated at the Institute of Food Science and Technology, Faculty of Allied Health Sciences, University of Lahore.

Transgalactosylation in Lactose Solution

The enzyme (β galactosidase from Kluyveromyces lactis by Sigma) was exported from Denmark. A mixture of 600mM lactose was prepared in a 50mM NaPO4 buffer having a pH of 6.5 and 1mM MgCl2 was added per ml. Three samples of 5ml of lactose solution were taken in a falcon tube. Enzyme concentrations of 10 µl, 15 µl, and 20 µl (0.52 units, 0.78units and 1.04 units respectively) were added to the lactose solution to start the transgalactosylation process. The reaction was carried out for 3 hours at 37° C, 45°C, and 50° C with gentle agitation of 130 rpm. A sample of 400 µl was taken from each falcon tube at 0 min, 15 min, 30 min, 1hr, 2hr, and 3hr. The taken samples were dipped in boiling water for 5 minutes to deactivate the enzyme and were stored at 4^{o} C to run a Thin Layer Chromatography (TLC) analysis.

Process Optimization of Transgalactosylation in Milk

The milk was purchased from the local market and its pasteurization was carried out at 63^{o}C for 30 minutes. The pasteurized milk sample was stored to conduct a physic-chemical analysis. A milk sample of 5ml was taken in a falcon tube to optimize the condition for enzyme activity. 10µl, 15µl and 20µl of the enzyme was added in the $1^{st,}$ 2^{nd} and 3^{rd} sample at 37° C, 45° C, and 50° C, respectively. 400µl sample was taken at 15min, 30min, 1hr, 2hr, and 3hr. These samples were heated at 90^{o}C by dipping the Eppendorf in the boiling water for 5 min. This was done to denature the enzymes. Seventy percent acetone of the same quantity was added to have the precipitation of protein. Centrifugation was carried out at 12000rpm for 15min. After the centrifugation, two layers were formed. The uppermost transparent layer was separated in another Eppendorf. The enzyme was denatured by heating at 90^{o}C. Subsequently, TLC was performed using Yakult oligomer (as standard) and lactose.

Buffers and Solutions

Use of Buffer to Check Activity of Enzyme and TLC

50mM solution of NaHPO4
600mM solution of Lactose

The buffer was used to prepare the lactose solution, in which 6.5 pH was regulated. This regulation helps measure the performance of the enzyme.

Thin Layer Chromatography Running Buffer

The 2:3:3:2 ratio of water, n-propanol, ethanol, and n-butanol was applied to conduct a running buffer.

Staining of TLC

The solution of 10% (w/v) thymol with 5% v/v H_2SO_4 in ethanol was used. The solution of glucose, galactose, and lactose of 20g/l was run on the TLC plate along with samples taken from the trials.

Plating of TLC

Reference samples, samples gained during the process of transgalactosylation, and 1.5µl of the reference samples were used at the silica gel plate. The 2:3:3:2 ratio of water, n-propanol, ethanol, and n-butanol was applied to conduct a running buffer so that samples accomplished at upper side of TLC plate. The showering of solution of 10% (w/v) thymol with 5% v/v H_2SO_4 in ethanol was done on a TLC plate. The 110 °C treatment in a hot air oven was used to clear the sugar stains.

The Large-Scale Process of Transgalactosylation of Milk

From the local market, a total of 10 litres of buffalo milk was purchased. It was pasteurized at 45°C to kill all pathogens. For the process of transgalactosylation, optimized condition for the milk i.e. 10ml enzyme at 45°C for 3 hrs was done and milk temperature was stabilized at 45°C with continuous stirring. Finally, milk was treated to denature the whole of the enzyme at 90°C.

Cottage Cheese Development

The transgalactosylated milk was placed into a vat. The processed milk was heated up to boiling temperature. At this temperature, citric acid @ 1g/l was added. The mixture was set to rest for 5 min without heating. Due to the citric acid, there was a curd formation by coagulation of casein. The formulated curd was cut by a sharp blade cutter. The whey was removed from the curd and then was packed in proper packaging and stored at a temperature less than 4°C.

Activity of β-galactosidase

An o-nitro-phenyl-β-D-galactopyranoside (oNPG) standard assay was applied to find the status of β-galactosidase. The substrate was lactose that proceed as standard assay which was done at 30°C. The enzyme sample of 20µl in 480 µl of oNPG 22mM in a buffer of sodium phosphate was used at the start with pH 6.5. The change in the colour of oMPG confirms the activation of the enzyme [8].

Sensory Evaluation

The sensory evaluation for the parameters, namely appearance, taste/flavour and, overall acceptability, was done using a 9-point hedonic scale [9]. The expert panel consisted of ten members who were trained in the sensory evaluation of food products. The panellists were blindfolded so they could objectively and independently evaluate the organoleptic properties of cheese samples (control and prebiotic cheese). The sensory evaluation was repeated under the same settings by the same experts on the 1st, 4th, 8^{th,} and 12th day of cheese production to evaluate the sensory appeal during storage of cheese using standard AOAC methods [10].

Results

A minor effect of fermentation and starter culture conditions on the prebiotic milk having GOS was identified and determined as shown in Figure 1.

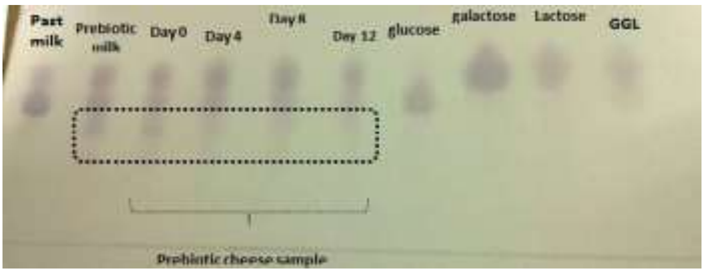

Figure 1. It Displays a TLC Plate of Pasteurized, Prebiotic Milk, and Prebiotic Cottage Cheese on Day 1, 4, 8, and 12, Respectively against the Standard

The panellist evaluation of the sensory characteristics of the product gained during various periods of storage is shown in Table 1. The overall acceptability of prebiotic cheese was maximum during day 4, while that of control cottage cheese was maximum on the day of production. The sensory characteristics did not show a significant difference when compared with the stored cheese over a period of 12 days for the two types of cheese tested.

Table 1. Sensory Evaluation of Control Cottage Cheese and Prebiotic Cottage Cheese During Shelf Life Study

Sensory attributes	Type of cottage cheese	0 Day Mean±SD	4 day Mean±SD	8 Day Mean±SD	12 Day Mean±SD
Color	Control Cottage cheese	12.4a±1.38	10.7a±1.45	10.35a±2.1	9.98a±1.6
	Prebiotic Cottage cheese	11.66a±1.43	11.69a±1.41	9.42a±2.46	9.19a±1.77
Taste	Control Cottage cheese	10.15b±1.02	12.03a±1.5	7.16a±3.93	8.76a±1.78
	Prebiotic Cottage cheese	12.59a±1.49	12.19a±1.42	11.34a±4.18	11.35a±2.26
Consistency	Control Cottage cheese	10.5a±1.23	11.7a±1.3	9.05a±2.98	7.36a±1.8
	Prebiotic Cottage cheese	12.9a±1.18	11.9a±1.3	7.83a±3.21	8.38a±1.84
Overall acceptability	Control Cottage cheese	12.32a±1.4	11.7a±1.41	10.11a±2.5	10.4a±1.56
	Prebiotic Cottage cheese	10.78a±1.3	11.9a±1.6	12.54a±2.30	10.71a±1.95

The same alphabets in a row show that the difference was statistically non-significant.

Table 2 shows that certain aspects of the prebiotic cottage cheese were more appealing than normal cottage cheese. This was due to the effect of GOS on the physical state of cottage cheese. According to the findings obtained from the data, the cheese taste was more acceptable than the control cheese due to the enhanced sweetness caused by the prebiotics. The consistency of the prebiotic cottage cheese was better than the control because of the prebiotics. Hence, the developed cheese was more admissible as compared to the control due to its improved characteristics.

The mean $\pm$ *SD* was used for the evaluation of the data. For the pasteurized and prebiotic milk, the data of Physico-chemical analysis was evaluated using *t*-test; whereas, for control and Prebiotic cheese, data of

Physico-chemical and sensory attributes, during the study of shelf life, was analyzed through 2-way analysis of variance (ANOVA) using SPSS (ver. 21). The Duncan Multiple Range (DMR) significant difference test was used to analyze the level of significance among the means at $p \geq 0.05$.

Table 2. Analysis of Variance of Sensory Characteristics

SOV	Df	SS	MS	F	p-Value
Colour					
Treatments	1	2.9645	2.9645	0.9931523	.3222^{NS}
Days	3	77.943	25.981	8.7040276	.0001***
Error	75	223.8705	2.98494		
Total	79	304.778			
Taste					
Treatments	1	11.4244	11.4244	1.4786941	.2270^{NS}
Days	3	219.973	73.324333	11.805623	.0000
Error	75	465.8225	6.2109667		
Total	79	694.64			
Consistency					
Treatments	1	7.260125	7.260125	1.85551	.1772
Days	3	76.804375	25.601458	6.5431051	.0005
Error	75	293.455375	3.9127383		
Total	79	377.519875			
Overall acceptability					
Treatments	1	2.738	2.738	0.8394756	.3625 ns
Days	3	48.587	16.195667	4.9656197	.0034
Error	75	244.617	3.26156		
Total	79	295.942			

Discussion

Prebiotics were first defined as 'non-digestible food ingredients which beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health [11]. As the archaeological evidence reveals, prebiotics have long been a part of the human diet, and for some areas and time-periods, the amount of prebiotics consumed far exceed those currently consumed by modern populations [12]. The synthesis of GOS from lactose, using β-galactosidases, has been extensively investigated over the last 50 years due to the functional prebiotic properties of GOS [13]. The scope in GOS preparation has enhanced since its introduction in Japanese legislation concerning foods for definite health application. Cottage cheese is a crucial milk product made by curdling milk protein using citric acid. GOS is increasingly used in food products because it provides various health and physiochemical benefits. The definite physiological and physicochemical properties of GOS change marginally depending on the polymerization level in the blend of un-reacted lactose, oligosaccharides, and the generated monosaccharide. Physical properties of GOS are quite outstanding [14].

Galacto-oligosaccharides are uncoloured, soluble in water, possess ideal viscosity, and enhance the taste of cheese. These prebiotics can be balanced at pH 2 by keeping the temperature stable at 37°C. and stored for a number of months that can be embattled for nonrefrigerated liquid drinks, meantime with the likes of linkages of single type constituents have protection in environment which is acidic. The prebiotics are kept at 160° C for 10 mins at pH 7. Next, it is treated for 10 min at 120°C with pH 3 or for 10 min at 100°C with pH 2. hence have broad scope of food applications. Their taste is 0.3 to 0.6 times sweeter than sucrose so it can be used in many sweet food items to enhance food flavours [15].

The current study was conducted to evaluate the organoleptic characteristics of the prebiotic cottage cheese in order to determine whether it can be incorporated into diets, owing to its wide array of health benefits. Next, the organoleptic properties of prebiotic cottage cheese were evaluated to ascertain its appearance, taste, texture, and overall acceptability by an expert panel comprising ten members. It was determined that the colour of cottage cheese, having no GOS, was less yellowish as compared to prebiotic cottage cheese, which has GOS. The remarks of the expert panel also suggested that the darker colour of the prebiotic cheese was due to the Maillard reaction as well as the high temperature. The prebiotic cottage cheese resembled cheddar cheese in colour. Additionally, it was determined that the statistical change in colour during the storage period of 8 days was not significant (Table 1).

The sensory evaluation showed that the prebiotic cottage cheese was tastier and slightly sweeter. The sweet taste developed in milk during transgalactosylation. The texture of prebiotic cheese was more compact and softer as compared to the controlled sample. During the next sensory evaluation, cottage cheese having no GOS got harder and stiffer when it was stored at 4^{o}C. Thus, due to its texture and taste, prebiotic cottage cheese was more accepted than the control sample (Table 2).

Better score for the texture and taste is becuause of the more thick and sweetened because of the presence of prebiotics [16]. During lactose transgalactosylation, lactose is transformed and GOS are formed. Afterwards, glucose and galactose are formed. The circumstance of self-sweeting can be done so that it lessens the charge on sweetening the dairy products [17]. Previously, diverse policies such as corn fiber use, inulin, and polydextrose mixing that advance prebiotic milk products with enhanced sensory attributes [18].

Probiotic cottage cheese is being produced in Pakistan; however, prebiotic cottage cheese has never been produced. The most important challenge with the application of probiotic cultures in the production of functional foods is their viability during the processing stage. Probiotic microorganisms must also be technologically appropriate so they can be incorporated into food items to preserve both the efficacy and viability of food products throughout the production and storage period. In contrast, prebiotics are easier to handle because they are comparatively more pH resistant and temperature tolerant than probiotics. Therefore, the usage of prebiotic enriched cheese seems to be a viable option to enhance the functional properties of food products. Such prebiotic foods have their application in therapeutic as well as preventive field of nutrition science.

Conclusion

It is concluded that GOS containing cottage cheese has satisfactory sensory characteristics, making it an acceptable, healthy option to be introduced in diets. Based on the findings of the study, we suggest that GOS containing cheese as well as other food products should be commercially produced so that the general population can take advantage of its natural benefits.

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