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Effect of Blackgram (Phaseolus Mungo) Husk on Microbial, Physiochemical and Sensory Attributes of Synbiotic Yogurt

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Abstract: ***The present study introduces the potential of black gram husk as a prebiotic in yogurt. Black gram husk in the concentration of 0.5% - 2% was supplemented to probiotic yogurt containing Lactobacillus casei NCIM No. 2364 and Lactobacillus fermentum NCIM No. 2165. Evaluation of the microbial, physicochemical and sensory properties of the probiotic and synbiotic yogurt samples was carried out. The samples were examined for bacterial counts, pH value, total titratable acidity (TTA), syneresis and sensory properties over a period of 28 days of cold storage. At the end of the storage, the bacterial counts observed in probiotic yogurt and 2% synbiotic yogurt were 6.4 log CFU mL^{-1} and 8.15 log CFU mL^{-1} respectively. The pH values of the yogurts were found to decrease with an increase in the concentration of husk. The sensitivity to syneresis was significantly higher in case of synbiotic yogurts than in probiotic yogurt. The overall sensory evaluation for texture, flavor and color of the yogurt samples revealed that yogurt containing 1% husk was the best.***

Keywords: Black Gram husk, Synbiotic yogurt, Lactobacillus casei, and Lactobacillus fermentum

1. Introduction

A synbiotic is a supplement that contains both a prebiotic and a probiotic that work together to improve the "friendly flora" of the human intestine. Fermented milks (yogurt and kefir) are considered to be true synbiotic products, since they supply the live bacteria and the food they need to survive. Synbiotic foods have been reported to possess many important biological activities and health-promoting properties, including anti-atherosclerotic, anti-carcinogenic, anti-adipogenic, immune enhancing, anti-oxidative, hypotensive, anti-diarrheal, anti-allergic and anti-inflammatory effects. [1]

Prebiotics are typically non digestible food ingredients (i.e., soluble dietary fibres) that are resistant to human digestive enzymes but serve as food for probiotics to promote their growth and activity. Current sources of prebiotics include cereals, such as wheat and barley, soybeans, chicory, sago starch [2], *Gigantochloa Levis* (Buluh beting) shoots [1] and some fruits and vegetables. Cereal grain oligosaccharides function as prebiotics by increasing levels of beneficial bacteria in the large bowel thereby improving gut health. In addition to the prebiotic potential of cereal oligosaccharides, they have also been shown to possess antioxidant activity owing to their bound phenolic acids [3].

Black gram (*Phaseolus mungo*) is grown all over India, mainly in the monsoon season, the main areas of production being Madhya Pradesh, Uttar Pradesh, Punjab, Maharashtra, West Bengal, Andhra Pradesh and Karnataka, corresponding to the generation of a high amount of husks. Black gram is a part of human food consumption and is well known for its functional properties and the husks are exclusively utilized as a potential animal feed. Many feed industries in various parts of the country have been producing and marketing different types of compound animal feeds using the husks. The husks are a rich source of dietary fibers and oligosaccharides which function as prebiotics by increasing levels of beneficial bacteria in the large bowel thereby improving gut health. Currently significant research is being carried out internationally on the investigation of prebiotic effect of brans and husks in fermented milk products. However, to date, such research remains scanty in India and in particular in Karnataka, exploring the prebiotic potential of husks to improve the quality of fermented food products.

The state of Karnataka is blessed with rich milk-producing potential too, being the second largest milk producer in the co-operative sector after Gujarat, a favorable fact to develop a range of fermented synbiotic milk products. Although studies have been conducted on the prebiotic effects of whole grain wheat cereal [4], triticale bran [3][14], lentils [14] and their husks as prebiotic sources, to the best of our knowledge, absolutely no literature is available on the prebiotic effects of black gram husk. The aim of this study was to determine the effect of black gram husk as prebiotic and to evaluate the various physicochemical and sensory properties of the synbiotic yogurt.

2. Materials and Methods

2.1. Husk Preparation

Black Gram husk was obtained from Pulse Mill, Gulbarga and ground to a particle size of 1.0 mm using a waring blender and stored in sealed plastic bags for further use.

2.2 .Microbial cultures

Yogurt starters cultures *Lactobacillus bulgaricus* NCIM No.2671 and *Streptococcus thermophilus* NCIM No. 2904 as well as probiotic bacteria, *Lactobacillus casei* NCIM No. 2364 and *Lactobacillus fermentum* NCIM No. 2165 were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India All microbial cultures were maintained on MRS (deMan, Rogosa and Sharpe) medium (pH 6.5) containing (g L^{-1}) Proteose peptone 10, Beef Extract 10,Yeast extract 5, Dextrose 20, Ammonium citrate 2, Sodium acetate 5, Magnesium sulphate 0.1,Manganese sulphate 0.05, Dipotassium phosphate 2, Tween 80 1 and Agar 15.

2.3 Preparation of synbiotic yogurt

Milk samples were standardized by adding skim milk powder to achieve 16% total SNF (solids not fat) content, pasteurized (15 min. at 85°C) transferred into sterile containers, and cooled to 42°C [5]. Black Gram Husk was incorporated in the processed milk at varying concentration ranging from 0.5% - 2.5% with an increment of 0.5% in order to determine the maximum amount of husk that could be added to milk without disrupting fermentation. Control or probiotic yogurt was prepared without supplementation of husk. The inoculum standardization for yogurt preparation was performed as per the method of [3] wherein equal volumes of each bacterial culture with equal concentrations (6.7 log CFU mL^{-1}) were added to yogurt, such that the total volume of bacteria present constituted 4 mL of the 100 mL final sample volume. Yogurt samples were prepared and incubated at 42ºC until completion of fermentation at pH 4.5 [6] and stored at 4°C until further use. All the experiments were performed independently in triplicates and the results given here are the mean of three values.

2.4 Microbiological Analysis

Cell counts were carried out once in 7 days for a period of 28 days using MRS agar following serial dilution and colony counts were converted to log CFU mL^{-1} [5].

2.5 pH and Total Titratable Acidity (TTA)

The p^H and TTA of yogurt samples were measured as per the method of [14]. The samples were analyzed once in 7 days for a period of 28 days.

2.6 Determination of syneresis

Syneresis was determined as follows: 5 mL of yogurt were centrifuged at 5000 rpm for 20 min (Eppendorf 5430R) and the whey that accumulated after 1 min was measured. Syneresis (%) was expressed as volume of drained whey per 100 mL yogurt [7].

2.7 Sensory Evaluation

Sensory evaluation was conducted using a ten member (five males and five females) panel. The subjects selected were in the age group of 18-45 years. The samples were served in plastic cups with a clean spoon and the ingredients of the samples were clearly revealed to the subjects before tastings. Tastings were conducted between 10 am- 1 pm. The sensory attributes were texture, flavor and color. A 1–4 point scale was used [8]. The acceptability values were scored on 4 (very good), 3 (good), 2 (moderate), and 1 (bad). Sensory values with statistically significant differences ($P \leq 0.05$) between the various treatments were determined and mean comparison was performed using Fisher's protected Least Significant Difference test.

3. Results and Discussion

3.1 Effect of husk on yogurt production

Supplementation of husk at 2.5% resulted in over-fermentation of yogurt affecting the consistency and texture of the product. Stable, consistent and smooth yogurts were obtained in the range of 0.5% -2% husks. Hence further experiments were carried out by preparing yogurts in the said range.

3.2 Microbiological Analysis

Figure 1 depicts the variations in the microbial counts of probiotic and synbiotic yogurt samples during refrigeration over a period of 28 days. The microbial count in all the samples irrespective of the presence of prebiotic source was found to increase on day 1. The probiotic yogurt showed a count of 7.8 log CFU mL^{-1} as compared to 8.5 log CFU mL^{-1} observed in synbiotic yogurt with 2% husk. There was no increase in the bacterial counts by day 7 in any of the synbiotic yogurt samples, however in the probiotic yogurt, the bacterial count dropped from 7.8 log CFU mL^{-1} to 7.4 log CFU mL^{-1}. The steady fall in the microbial viability was observed on day 14 and the decline remained so thereafter in all the yogurt samples up to day 28. The decrease in bacterial growth is a result of the reduced amount of sugars remaining in the yogurt, leaving bacteria with far less nutrients to consume and promote growth [3]. Synbiotic yogurt samples demonstrated significantly higher bacterial counts throughout the storage period in comparison to control (probiotic) sample. At the end of the storage, the bacterial counts observed in probiotic yogurt and 2% synbiotic yogurt were 6.4 log CFU mL^{-1} and 8.15 log CFU mL^{-1} respectively. Bacterial counts were comparatively higher in 2% synbiotic yogurt than in 0.5% synbiotic yogurt throughout the storage period, thus revealing that increasing the concentration of husk from 0.5 % - 2 % increases bacterial viability. [9] suggested that a range of 6 to 8 log CFU mL^{-1} is the recommended level of viable probiotic bacteria that should remain at the end of the cold storage period.

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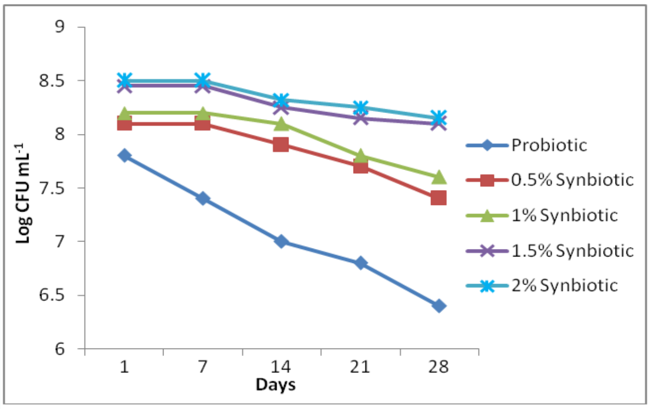

Figure 1: Microbial viability profile of synbiotic and probiotic during storage.

Some important factors affecting the survivability of probiotics in fermented dairy products are culture conditions, the used specific strain, final acidity, inoculation level, fermentation time and the nutrients [10]. Various reports on higher survivability of probiotic bacteria in the presence of prebiotics in yogurt have been presented [11]. Studies on green lentils as prebiotics [14] demonstrated that lentils selectively enhanced the number of probiotic bacteria in yogurt in the initial stages of storage and maintained overall microbial counts (starter cultures and probiotics) over a 28-day storage period. [3] reported the use of triticale bran as a prebiotic in yogurt. The authors demonstrated that by day 7, the number of bacteria greatly increased in yogurt samples containing triticale bran and maintained higher viable bacteria counts at the end of the cold storage period, in comparison to controls. Increased microbial viability of probiotic bacteria has also been reported in aloe vera fortified yogurt [12].

3.3 pH and Total Titratable Acidity (TTA)

At the end of fermentation the pH gradually decreased to 4.5. Figure 2 shows the variations in pH value of probiotic and synbiotic yogurt samples during refrigeration over a period of 28 days. The pH of the yogurt was comparatively lower in synbiotic samples than in probiotic sample. The variation trend was descending with increasing concentration of prebiotic and it declined significantly during the 4 week storage. Figure 2 depicts that bacteria are significantly more active in the presence of husk increasing the acidity and thereby lowering the pH. The lowest pH value of 3.7 was recorded at 28th day in synbiotic yogurt with 2% husk. These decreases might be attributed to the utilization of residual carbohydrates by viable microorganism and production of lactic acid, small amount of CO_2 and formic acid from lactose. Similar findings have been reported by [12] [13].

Figure 3 depicts the variations in the TTA (% lactic acid) profile of probiotic and synbiotic yogurt samples during refrigeration over a period of 28 days. There was a sharp increase in the TTA levels in all the synbiotic yogurt samples, thus depicting the production of lactic acid in the presence of husk. The ascending trend of TTA corresponds to the sharp decline in the p^H values of the yogurt samples during storage. Similar findings have been reported by [3] [13] [14].

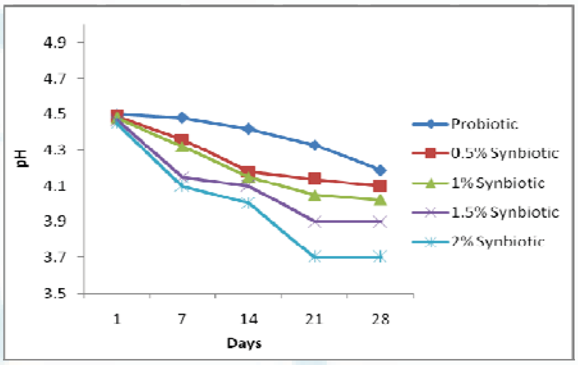

Figure 2: pH profile of synbiotic and probiotic yogurts during storage

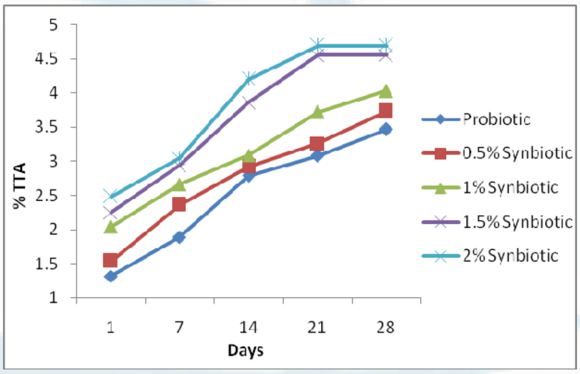

Figure 3: TTA profile of synbiotic and probiotic yogurts during storage

3.4 Syneresis

The results of syneresis measurement of yogurt samples during storage are presented in figure 4. The results revealed that syneresis increased with the storage time over a period of 28 days as evident in the figure 4. The initial value of syneresis for probiotic yogurt was found to be 23% on day 1, which after storage of 28 days reached to 30%. The sensitivity to syneresis was significantly higher in case of synbiotic yogurts than in probiotic yogurt which was associated with the acidity. The highest syneresis value of 36 % was observed in 2% synbiotic yogurt on day 28. Our findings are in confirmation with those of previous workers suggesting that the rate of syneresis is directly related with the acidity [12]. The similar ascending trend in syneresis in yogurt samples has been reported by [12] [13] [15]. The use of compounds such as gelatin, pectin, starch and prebiotics has been suggested to reduce syneresis ([16]. On the contrary, the results of some studies have suggested that using prebiotics may reduce syneresis percentage.

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3.5 Sensory Evaluation

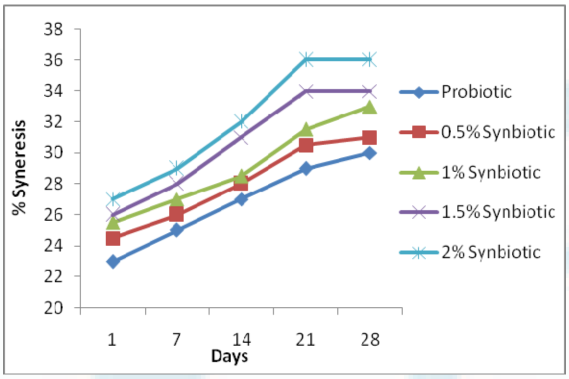

Figure 4: Syneresis profile of synbiotic and probiotic yogurts during storage

There was no significant difference (p>0.05) in the scores for texture among all of the yogurt samples on day 1 and day 7 as depicted in table 1. The texture score of yogurt with 2 % husk was significantly different (p<0.05) from other samples on day 14. The score was lowest in the sample with 2% husk as compared to other samples on day 14 and remained consistently lower thereafter till the end of refrigeration. The highest and lowest texture scores were recorded for samples containing 1% and 2% husk respectively on day 28.

Table 1: Sensory evaluation of synbiotic and probiotic yogurts during storage

	Texture*					***Flavor****					***Color****				
	1d	**7d**	**14d**	**21d**	**28d**	**1d**	**7d**	**14d**	**21d**	**28d**	**1d**	**7d**	**14d**	**21d**	**28d**
Probiotic	4^{a}	4:00 AM	3.8^{a}	3.3^{c}	3.0^{d}	3.9^{a}	3.6^{b}	2.9^{d}	2.7^{e}	2.2^{g}	4:00 AM	4:00 AM	4:00 AM	3.7^{b}	3.5^{b}
0.5% Synbiotic	4:00 AM	4:00 AM	3.9^{a}	3.7^{b}	3.3^{c}	3.8^{a}	3.3^{c}	3.6^{b}	3.1^{d}	3.0^{d}	4:00 AM	4:00 AM	4:00 AM	3.7^{b}	3.5^{b}
1% Synbiotic	4:00 AM	4:00 AM	4:00 AM	3.9^{a}	3.5^{b}	4:00 AM	3.5^{b}	3.4^{c}	3.2^{c}	3.2^{c}	4:00 AM	3.8^{a}	3.6^{b}	3.6^{b}	3.5^{b}
1.5% Synbiotic	3.9^{a}	3.8^{a}	3.5^{b}	3.2^{c}	2.4^{f}	3.6^{b}	3.2^{c}	2.6^{e}	2.4^{f}	2.1^{g}	4:00 AM	3.8^{a}	3.6^{b}	3.4^{c}	3.0^{d}
2% Synbiotic	3.9^{a}	3.8^{a}	3.4^{c}	3.1^{d}	2.2^{g}	3.6^{b}	3.1^{d}	2.5^{f}	2.1^{g}	1.9^{h}	4:00 AM	3.5^{b}	3.3^{c}	3.0^{d}	2.8^{e}

* Values are means and Values followed by the same letter are not significantly different according to Fisher's protected least significant difference test (*P* = 0.05)

There was no significant difference (p>0.05) in the flavor scores of probiotic and synbiotic yogurts with 0.5% and 1% husk on day 1, however, a significant difference (p<0.05) in flavor scores between these samples and samples containing 1.5 % and 2% husk was noted. The highest flavor score was recorded for sample containing 1% husk throughout the storage period. By the end of the storage period the flavor score was significantly reduced in sample containing 2% husk. High color scores were recorded for probiotic yogurt samples and samples containing 0.5% and 1% husk as compared to the other two samples. There was no significant difference (p>0.05) between the color scores of probiotic yogurt and yogurt with 0.5% husk throughout the storage period. Also no significant difference (p>0.05) was recorded in the color scores between the samples containing 1% and 1.5% husk by day 14 as depicted in table 1. However the lowest color score was recorded for sample containing 2 % husk on day 28. In the present study, the overall sensory evaluation of the test samples revealed that yogurt containing 1% husk was the best.

Studies on probiotic yogurt supplemented with various prebiotics were conducted by [17] using a 9-point facial hedonic scale. The authors reported that a mean score between one and three indicated that the sample product was well accepted. Probiotic yogurt containing onions, garlic and sweet potato received a score of 1.6 ± 0.84; banana and honey was 2.5 ± 1.72; and leafy greens, onions and garlic was 2.6 ± 1.54. Samples containing beans, 4.4 ± 1.99, and plantains, 5.3 ± 2.56 were not well accepted. Sensory evaluation of yogurt supplemented with Lactulose, Inulin and Oligofructose as prebiotics revealed that the highest taste and texture scores were related to the sample which had Inulin [13]. [18] conducted the sensory evaluation of the synbiotic yogurt supplemented with varied concentrations of inulin and reported that the synbiotic yogurt containing 2% inulin displayed the best organoleptic qualities. In related works, [19] reported that in a fermented frozen yogurt, the balance of flavoring systems may be significantly affected by varying levels of organic compounds. Additionally, they reported that acidity was the most important attribute, in terms of perceived flavors.

4. Future Scope

Genetic engineering and other approaches are being used to enhance the beneficial effects of probiotic microbes. Supplementation of promising strains of probiotic organisms may offer exciting solutions for reducing the problem of high cholesterol levels in human beings. However, extensive research is required to screen the potent probiotic strains and their evaluation for the effective management of good and bad cholesterol in the body and the sustainability of the desired results. The combination of probiotics and prebiotics significantly reduces the serum cholesterol level and that can be used as an alternative remedy for hypercholesterolemic problems without any side effects to the consumers (Marimuthu Anandharaj et al., 2014).

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