Optimization of the process parameters for the preparation of synbiotic yoghurt-cheese

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Abstract

Attempts were made to prepare synbiotic yoghurt-cheese having functional attributes of both prebiotics and probiotics. Firstly, the conditions for the preparation of prebiotic yoghurt were optimized in order to determine the optimal level of prebiotic ingredients - raftiline (the fat replacer), raftilose (the sugar replacer), oat fiber and honey as an enricher and combinations thereof; by means of sensory evaluation. Incorporation of raftiline, raftilose, honey @ 4, 4 and 3 percent respectively, and combinations of oat fiber with raftiline or raftilose (0.5%+4%) and honey (0.5%+3%) in pre-standardized low fat (0.5% fat and 11 % SNF) buffalo milk resulted in acceptable yoghurt. The yoghurt-cheese was then made by centrifuging the set yoghurt at pre-optimized centrifugation conditions (9000 rpm for 15 min). In order to prepare synbiotic yoghurt-cheese, optimized level of prebiotic ingredients/enricher and the combinations thereof were added to standardized milk and the fermentation was carried out by different combinations of probiotic cultures namely *L. acidophilus* with yoghurt culture (C_2, 1:1:1), *B. bifidum* with yoghurt starter (C_3, 1:1:1) and combination of *L. acidophilus* and *B. bifidum* with yoghurt culture (C_4, 1:1:1:1) taking yoghurt culture as control (C_1, 1:1). Each culture combination was added @ 3% and incubation was carried out at 42±1 ^{0}C. It was concluded on the basis of physico-chemical and sensory evaluation that a quite acceptable quality synbiotic yoghurt-cheese could be prepared using C_3 culture combination with raftiline, raftilose and combinations of oat fiber with raftiline, raftilose and honey.

Keywords: Synbiotic yoghurt-cheese**,** *Lactobacillus acidophilus*, *Bifidobacter bifidum*, raftiline, raftilose.

1. Introduction

With the rapid development of low fat and no fat products demanded by calorie conscious society, new growth areas for cultured milk products are opening. The fat and / or sugar are replaced by hydrocolloids, starches and functional dietary fibers in order to achieve desirable functional and quality attributes. Among cultured milk products, yoghurt and yoghurt products are gaining market day by day [1,2]**.** Yoghurt is an acidic fermented milk product produced thermophilic lactic acid bacteria (*Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus salivarius* sub sp. *thermophilus.* The various forms of yoghurt available in the market are frozen yoghurt, dried yoghurt, smoked yoghurt, strained yoghurt, yoghurt butter, yoghurt-cheese apart from the flavored, low-calorie and carbonated types of yoghurt. The processes of producing variations in yoghurt essentially add to shelflife, novelty and do help in meeting specific demands of today's consumer.

The concentrated yoghurt, formed by lactic acid coagulation of milk followed by contraction of the milk protein, which is collected together with fat, and other milk components retained in curd produced is called as yoghurt-cheese or labneh [3]. As a result of concentration by whey removal, it has a consistency resembling cultured cream [4]. It is usually consumed with bread as a part of a main meal in some countries of Middle East [5,6,7,8] and widely eaten as a sandwich spread specially for breakfast and supper meals [9,10]. It also seems to be suitable vehicle for development of functional food, which have a relevant effect on the well-being, health or cause reduction in disease risk. Functional claims have already and will in the future lead to new concepts in nutrition. Examples of such new concepts are prebiotics and synbiotics, colonic foods and bifidogenic factors [11]. Probiotics are technically defined as live microbial food ingredients that have a beneficial effect on human health. Prebiotics are non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in colon that can improve the host health [11]. When prebiotics are used in combination with probiotics or live bacteria, the resultant has synergistic effects, referred to as 'synbiotics'. This is because in addition to the action of probiotics that promote the growth of existing strains of beneficial bacteria in the colon, prebiotics such as inulin and oligofructose also act to improve the survival, implantation and growth of newly added probiotics strains [12].

Yoghurt-cheese bears good potential to be used in manufacture of low fat spread, low fat processed cheese, dressing and cheesecakes from milk. By incorporation of variations in terms of flavors, it could be easily used as dairy spread, high protein dietetic preparation. Such fermented dairy food with innovative use would feed the wide range of nutritional and organoleptic qualities being demanded by consumers of all ages, health status and

cultures. Very scanty information is available on incorporation of prebiotics and probiotics in yoghurt-cheese. In view of the above, the present investigation was undertaken to develop process for the preparation of synbiotic yoghurt-cheese from low fat buffalo milk. Specific objectives of the study were to study the effect of addition of prebiotic ingredients and/or enrichers and use of probiotic cultures on the physico-chemical and sensory attributes of yoghurt-cheese.

2. Materials and methods

2.1. Source of materials

Buffalo skim milk was obtained from Live Stock Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal. The freeze-dried cultures of *Lactobacillus delbrueckii* sub sp. *bulgaricus-RTS, Streptococcus salivarius* sub sp. *thermophilus-74, Lactobacillus acidophilus-13, Bifidobacterium bifidum*-NCDC-255 were obtained from National Culture Collection Centre, National Dairy Research Institute (NDRI), Karnal, Haryana. Inulin (raftiline), the fat replacer and oligofructose (Raftilose), the sugar replacer, were obtained by M/s ORAFTI-Belgium Active Food Ingredients, Belgium. Oat fiber was obtained from M/s Clarico-FPC (India) Pvt. Ltd, Mumbai. Other ingredients were purchased from local market. The chemicals and media used in the present investigation were of Analytical Reagent (AR) grade.

2.2. Activation of starter cultures

The freeze-dried cultures were activated according to the recommendations of suppliers.

2.3. Preparation of yoghurt-cheese

Yoghurt-cheese was prepared from the yoghurt, which was prepared as per the method described by Tamime and Robinson, (1988) [8], with some modifications in standardization and heat treatment of milk. The buffalo milk was standardized to 0.5 % fat and 11.0 per cent SNF by adding 2 per cent skim milk powder. The milk was heated in glass bottles at 4 psi for 10 min in a laboratory autoclave as suggested by Mothey, (2001) [2].The milk was then cooled to about 45±1 ^{0}C and inoculated by 3 per cent starter cultures. The inoculated milk was incubated at 42±1 ^{0}C for 4 to 4.5 hours or until an acidity of 1.2±0.1 percent as lactic acid and pH 4.4±0.1 was attained. The yoghurt samples thus prepared were cooled to 5 ^{0}C and stored for 2 hours in order to have a cold induced firm coagulum. The coagulum was centrifuged in a laboratory centrifuge (R24, REMI Instruments, and Mumbai, India) at 9000 rpm for 15 minutes. The speed and time of centrifugation was optimized by conducting preliminary trials so as to obtain maximum cheese yield. The cheese curd settled down in the bottom of the centrifuge tubes and a clear whey layer from the top was decanted. The cheese was transferred to high-density polyethylene cups, covered with aluminum foil and stored under refrigeration at 5±1 ^{0}C till further use.

2.4. Optimization of prebiotic/enriching ingredients level in yoghurt-cheese

Raftiline and raftilose were added separately to the standardized milk @ 2.5, 3.0, 3.5, 4.0 and 4.5%, whereas the attempted levels of oat and honey were 0.5, 1.0 and 1.5% and 2.0, 2.5, 3.0 and 3.5%, respectively. The optimized levels of prebiotic/enriching ingredient was decided on the basis of evaluating the yoghurt prepared from standardized milk after adding prebiotic ingredients/enricher for sensory scores for overall acceptability. The optimized level of raftiline, raftilose and honey were combined with 0.25, 0.5 and 0.75% of oat fiber and the products obtained were also subjected to sensory analysis to obtain the optimal level of combined ingredients.

2.5. Preparation of synbiotic yoghurt-cheese

Synbiotic yoghurt was prepared after adding optimized levels of prebiotic ingredients and/or enricher to standardized milk before heating. The milk was fermented using following four combinations of bacterial cultures:

- *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius subsp. thermophilus* =1:1 (Control, C_1)
- *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius subsp. thermophilus*: L*actobacillus acidophilus* = 1:1:1 (C_2)
- *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius subsp. thermophilus*: *Bifidobacterium bifidum* =1:1:1 (C_3)
- *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius subsp. thermophilus*: *Lactobacillus acidophilus: Bifidobacterium bifidum* =1:1:1:1 (C_4)

The synbiotic yoghurt-cheese was obtained after centrifuging the above yoghurt samples and were analysed for physico-chemical and sensory characteristics.

2.6. Physico-chemical Analyses

Yoghurt-cheese was analysed for moisture, protein, fat, ash and titratable acidity (as % lactic acid) by AOAC (1984) method [13]. The carbohydrate content was calculated by difference. pH of yoghurt-cheese was directly measured using a digital pH meter (Electronics Corporation of India Limited, Hyderabad after diluting the sample 10 times with glass distilled.

2.7. Sensory Evaluation

The yoghurt and yoghurt-cheese samples were evaluated for their sensory characteristics namely color, flavor, body and texture and over all acceptability using semi-trained panel comprising of 10 panelists drawn from faculty members and post graduate scholars of Department of Food Science and Technology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal. The panelists were asked to record their observations on the sensory sheet based on 9 Point Hedonic Scale.

3. Results

3.1. Preparation of yoghurt-cheese

Yoghurt-cheese was prepared with the (modification in standardization and heat treatment of milk) method described by Tamime and Robinson, (1988) [8].The yoghurt samples prepared were cooled to 5 ^{0}C and stored for 2 hours in order to have a cold induced firm coagulum. The cheese curd and a clear whey layer were separated and decanted by centrifugation. The cheese was transferred to high-density polyethylene cups, covered with aluminum foil and stored under refrigeration at 5±1 ^{0}C till further use.

3.2. Optimization of prebiotic/enriching ingredients level in yoghurt-cheese

The levels of prebiotic/enriching ingredients in milk for yoghurt preparation for preparation of yoghurt-cheese were optimized on the basis of sensory quality of yoghurt. It was found that the colour of yoghurt remained unaffected by adding raftiline and raftilose to the extent of 4.5 percent. However, the addition of oat fiber and honey significantly (P≤0.01) reduced the sensory score for colour of yoghurt **(Table 1)**.

Table 1: Effect of prebiotic ingredients/enricher and storage period on sensory scores for colour of yoghurt-cheese

Prebiotic ingredient in yoghurt-cheese (M)	**Sensory score for colour**				
	Storage period in days (S)				
	0	7	14	21	28
Control	8.216	8.216	7.785	7.618	7.466
Raftiline	8.216	8.126	7.838	7.694	7.488
Raftilose	8.211	8.211	7.827	7.688	7.489
Oat fiber	7.877	7.877	7.655	7.283	7.050
Honey#	8.055	8.055	7.811	7.672	7.427
Raftiline + Oat fiber	8.172	8.172	7.816	7.622	7.472
Raftilose + Oat fiber	8.172	8.033	8.033	7.622	7.472
Honey + Oat fiber	7.977	7.977	7.638	7.422	7.250
Mean	8.110	8.095	7.800	7.578	7.389

The sensory scores for flavour, texture and overall acceptability of yoghurt increased by adding raftiline, raftilose to the extent of 4 percent and oat fiber and honey to the extent of 1 and 3 per cent, respectively. Addition of either 4 percent raftiline or raftilose or 3 percent honey along with 0.5 percent oat fiber gave yoghurt of significantly (P≤0.01) higher sensory scores with respect to flavour, texture and overall acceptability **(Fig 1)**, however, the scores for colour decreased.

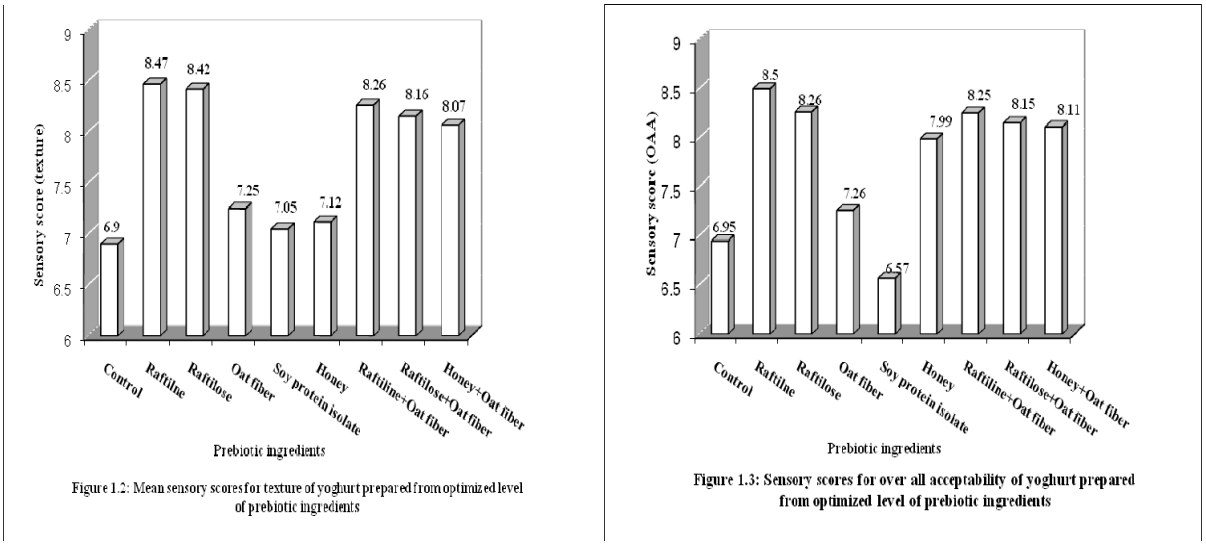

Fig 1: The sensory scores for flavour, texture and overall acceptability of yoghurt.

3.3. Optimizations of centrifugation of yoghurt

Preliminary trials were conducted using yoghurt prepared by traditional yoghurt-cultures to optimize the speed of centrifugation for obtaining maximum cheese yield with minimum possible loses of viable cells in whey. The maximum cheese yield with minimum losses of starter cells in whey was obtained by centrifugation at 9000 rpm for 15 min.

3.4. Physico-chemical composition of synbiotic yoghurt-cheese

The yoghurt-cheese samples prepared with or without addition of prebiotic/enriching ingredients using 4 different combinations of starter cultures were analysed for proximate principles, pH and titratable acidity. The results are presented in **(Fig 2)**. On statistical analysis of data, it was revealed that only prebiotic ingredients or enricher affected the level of proximate principles in the yoghurt-cheese significantly ($P \leq 0.01$). The type of starter cultures had no effect on the proximate composition. The pH and titratable acidity, however, were significantly ($P \leq 0.01$) affected by the type of culture combination and no significant effect of prebiotic/enriching ingredients could be observed on pH or titratable acidity.

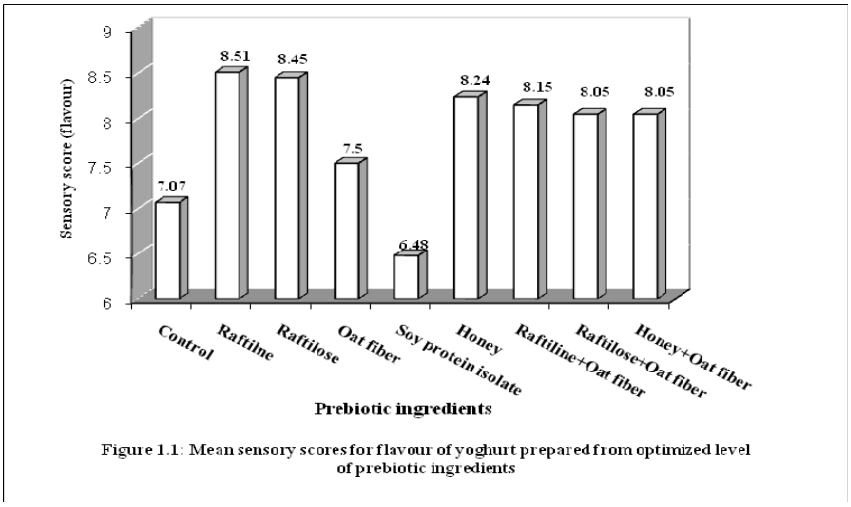

Fig 2: Effect of bacterial culture combination and incubation time on Physico-chemical composition of synbiotic yoghurt-cheese

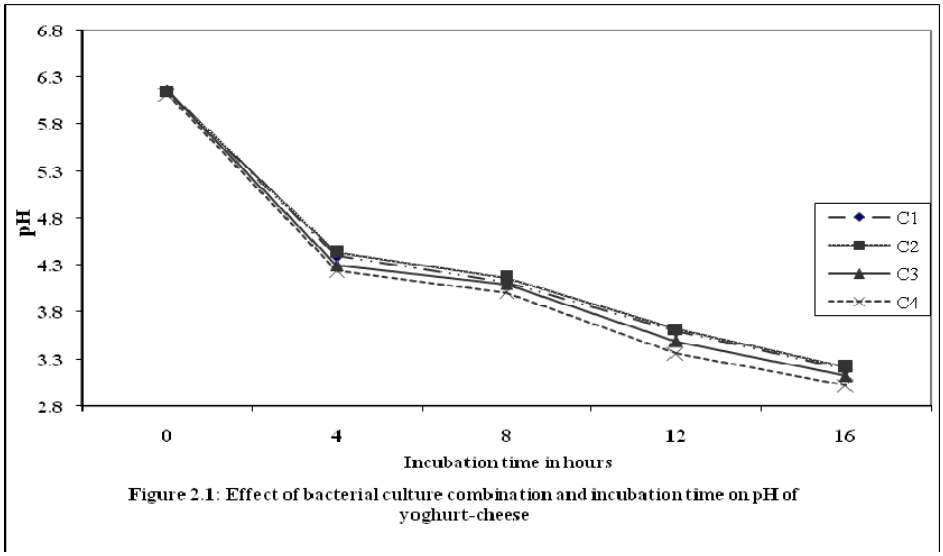

Figure 2.1: Effect of bacterial culture combination and incubation time on pH of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1), C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1) , C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1) ,*C4=L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

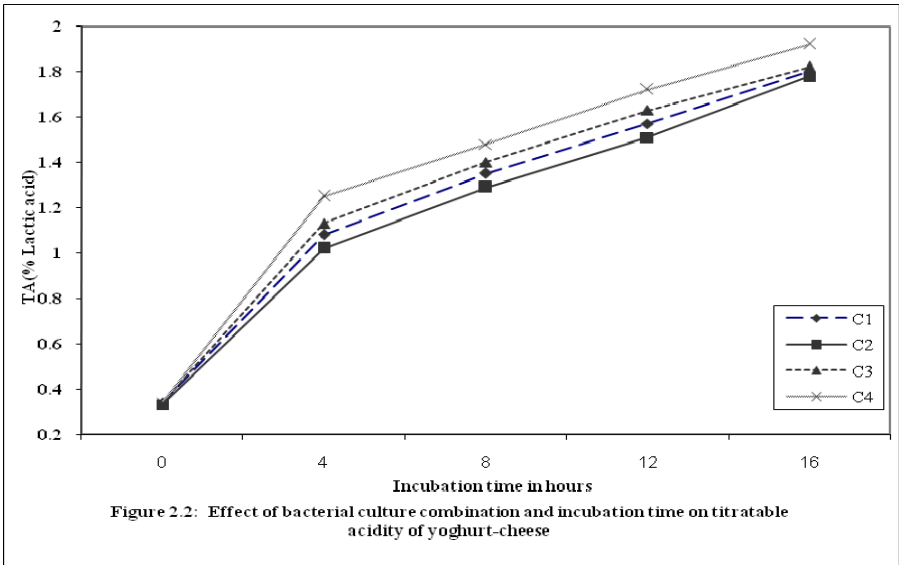

Figure 2.2: Effect of bacterial culture combination and incubation time on titratable acidity of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1), C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1), C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1) C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

3.5. Sensory quality of synbiotic yoghurt

All the yoghurt-cheese samples prepared in the investigation were also subjected to sensory evaluation with respect to colour, flavour, texture and overall acceptability. The results obtained are presented in **(Fig 3)**. The colour and texture of cheese remained unaffected by the type of culture used for their preparation. However, the flavour and overall acceptability were found to depend upon the type of culture significantly ($P \leq 0.01$). Among the prebiotic ingredients, raftiline and raftilose had no effect on colour of yoghurt-cheese. But other ingredients and their combinations caused a significant decrease in the sensory scores for colour. The sensory scores for flavour, texture and overall acceptability increased significantly by adding optimized levels of prebiotic ingredients/enricher, except sensory score for flavour which was reduced on addition of oat fiber.

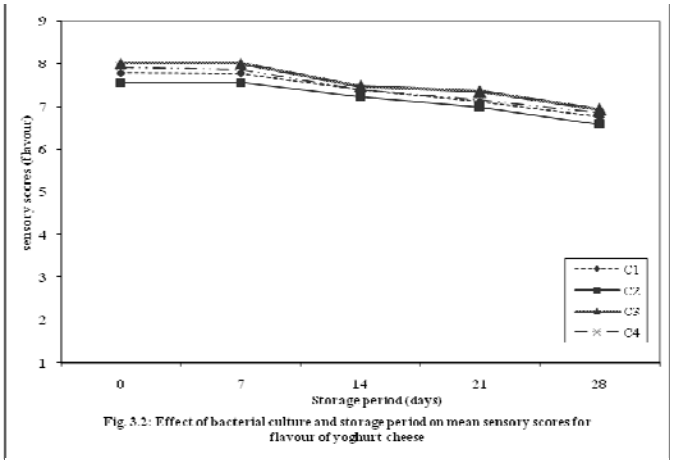

Fig. 3.2: Effect of bacterial culture and storage period on mean sensory scores for flavour of yoghurt cheese

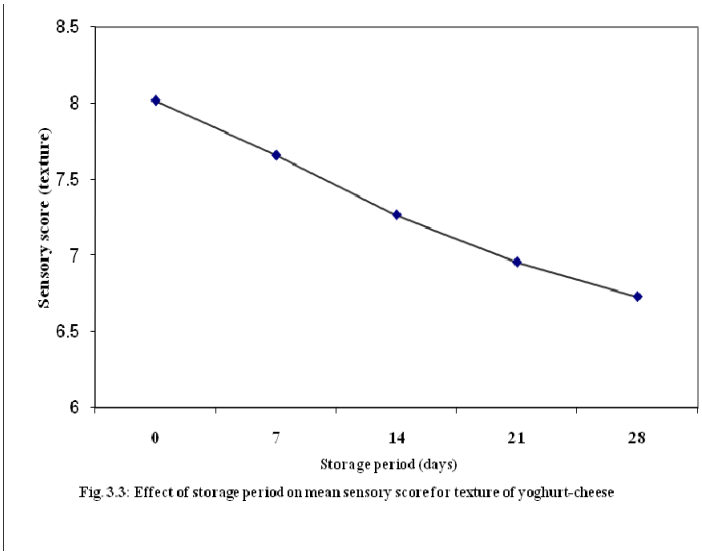

Fig. 3.3: Effect of storage period on mean sensory score for texture of yoghurt-cheese

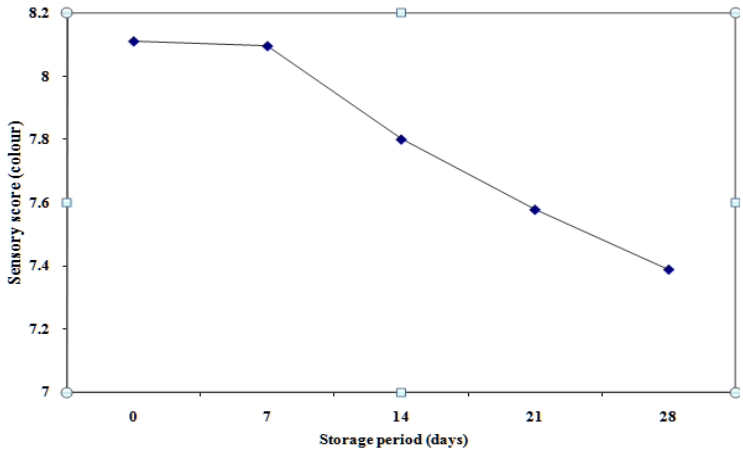

Fig. 3.1: Effect of storage period on mean sensory score for colour of yoghurt-cheese

Fig 3: Effect of bacterial culture combination and incubation time on mean sensory score yoghurt-cheese

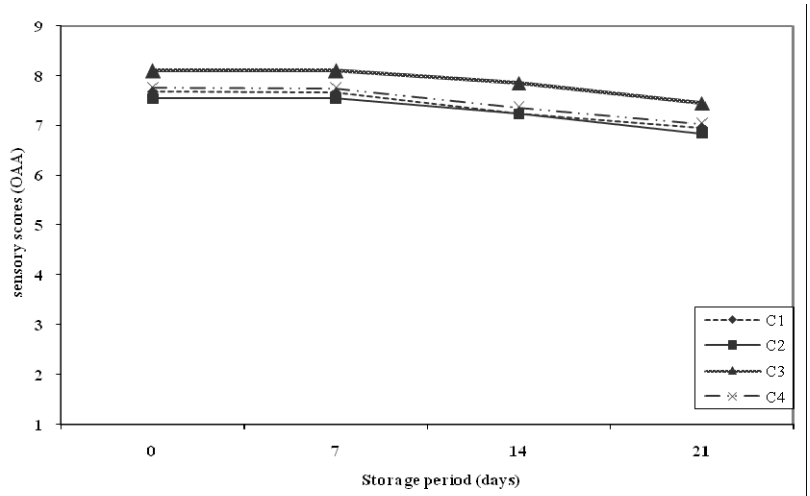

Fig.3.4: Effect of bacterial culture and storage period on mean sensory scores for overall acceptability (OAA) of yoghurt-cheese

C1= *L. bulgaricus*+ *S.thermophilus* (1:1), C2= *L.bulgaricus*+*S.thermophilus*+ *L. acidophilus* (1:1:1), C3= *L. bulgaricus*+ *S.thermophilus*+*B.bifidum* (1:1:1), C4=*L.bulgaricus*+*S.thermophilus*+ *L. acidophilus*+*B.bifidum* (1:1:1:1)

4. Discussion

Studies were conducted to prepare yoghurt-cheese using low fat buffalo milk in which raftiline (a fat replacer), raftilose (a sugar replacer), oat fiber and honey, an enricher were added to improve the quality of low fat yoghurt-cheese. The levels of these prebiotic ingredients were optimized by sensory evaluation of yoghurt prepared after addition of these prebiotic/enriching ingredients. The probiotic cultures, namely *L. acidophilus* and *B. bifidum* were combined with traditional yoghurt cultures and the attempts were made to produce symbiotic yoghurt-cheese using prebiotic/enriching ingredients along with probiotic cultures.

Addition of 4 per cent raftiline or raftilose gave significantly best product determined on the basis on sensory attributes namely colour, flavour, texture and overall acceptability **(Table 1)**. The colour of yoghurt remained unaffected by addition of different levels of raftiline or raftilose. However, flavour, texture and overall acceptability of yoghurt improved by increased the level of raftiline or raftilose in low fat milk upto 4 per cent. But higher levels of raftiline or raftilose addition resulted in a significantly ($P \leq 0.01$) inferior product with respect to colour, flavour, texture and overall acceptability. Amiri (2001) observed that sensory scores for overall acceptability of cow skim milk yoghurt was improved by incorporating raftiline or raftilose to the extent of 3.25 per cent and study optimized the level of raftiline and raftilose using response surface methodology [14]. But in the present investigation, the addition of 4 per cent raftiline/raftilose gave product of excellent sensory characteristics. These differences may be attributed to the type of milk used in the two investigations.

Addition of oat fiber to low fat milk significantly reduced the sensory scores for colour and flavour of yoghurt, however, the scores for texture and overall acceptability improved significantly ($P \leq 0.01$) by adding 1 per cent oat fiber **(Table 1)**. But addition of oat fiber at 0.5 per cent level combination with optimized levels of raftiline or raftilose gave a significantly superior product with respect to all the sensory attributes **(Fig 1)**. Incorporation of honey as an enricher to low fat milk reduced the sensory scores of yoghurt prepared significantly ($P \leq 0.01$) probably due to the fact that colour of honey masked the original colour of yoghurt. The flavour and overall acceptability of yoghurt improved by incorporating honey to the extent of 3 per cent. No significant effect of honey addition on texture was observed **(Table 1)**. When honey was added along with 0.5 per cent oat fiber, the sensory scores were also improved significantly for all attributes as compared to control **(Fig 1)**.

The pH and titratable acidity of yoghurt-cheese were affected by the type of culture combination used for fermentation of milk **(Fig 2)**. However, the prebiotic ingredients added to milk had non-significant effect on pH and titratable acidity of yoghurt-cheese. The pH and titratable acidity had an inverse relationship. The pH of yoghurt-cheese in the present investigation ranged between 4.29 to 4.45. The lowest pH was obtained in the yoghurt-cheese samples prepared by using C_4 combination of starter cultures followed by C_2, C_1, and C_3 cultures. This indicated that *L. acidophilus* has an ability to reduce pH of yoghurt-cheese to a greater extent as compared to *Bifidobacterium bifidum*. But when both the probiotic cultures were used along with traditional cultures, they showed a synergistic effect. Amiri (2001) also reported that *Bifidobacterium bifidum* in combination with yoghurt starters gave a slightly higher pH in yoghurt as compared to *L. acidophilus*, thus resembles the findings of present investigation. Samona *et al.* (1996) noted a synergistic effect of probiotic culture when added to yoghurt culture for fermentation [15]. The results of present investigation are in confirmation with above findings as the lowest pH was observed by C_4 culture, which had both probiotic

bacteria. The Titratable acidity of yoghurt-cheese samples ranged between 1.1 to 1.9 percent as lactic acid. The highest titratable acidity was obtained in C_4 culture followed by C_2, C_1 and C_3 which indicates an inverse relationship between pH and titratable acidity. El-Samragy (1997) reported a higher range of titratable acidity of 1.16 to 2.5 per cent as lactic acid in labneh, a product resembling yoghurt-cheese [16]. The higher acidity values in the experiment were probably obtained due to use of traditional method of labneh preparation, which involved longer time for whey removal by hanging yoghurt in cloth bags.

Sensory analysis: The cheese samples prepared from milk containing prebiotic/enriching ingredient differed significantly ($P \leq 0.01$) with respect to colour, flavour, texture and overall acceptability **(Fig 3)**. However, the effect of type of culture on the sensory score of colour and texture of yoghurt-cheese samples were non-significant. But the sensory scores for flavour and overall acceptability differed significantly ($P \leq 0.01$) by using different cultures. The overall acceptability of yoghurt-cheese was found maximum when C_3 culture was used for fermentation, followed by C_4, C_1 and C_2. This indicated that *Bifidobacterium bifidum* gave significant superior product than *L. acidophilus* with respect to overall acceptability of the product. These findings are also in agreement with the findings of Amiri (2001). She observed no change in colour of yoghurt samples prepared using different cultures. She further reported that *Bifidobacterium bifidum* gave yoghurt that was rated higher for sensory quality followed by *L. acidophilus.* However, in the present investigation, the yoghurt-cheese prepared by using *L. acidophilus* along with yoghurt culture was found to score lowest due to the typical smell in the product.

5. Conclusion

It was concluded that yoghurt-cheese could be developed as a novel low fat dairy spread or high protein dietetic product. By incorporation of prebiotics and /or probiotics, a newer functional dairy food can be developed. In the present study, quite acceptable synbiotic yoghurt-cheese could be prepared after adding prebiotic fibers namely raftiline (inulin), raftilose (oligofructose) singly and combining oat fiber with raftiline, raftilose or honey and employing *Bifidobacterium bifidum* with yoghurt starters for fermentation.

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