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DEVELOPMENT AND QUALITY ASSESSMENT OF FLAVORED PROBIOTIC ACIDOPHILUS MILK

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ABSTRACT

Functional foods containing probiotic bacteria (lactic acid bacteria and bifidobacteria) are getting popularity in the world, due to tremendous health benefits conferred by these bacteria. However, the total viable count of bacteria in the final product and the sensory attributes of the product are of higher importance for its consumer acceptability. In this study, flavored (strawberry, pineapple, and mango) probiotic acidophilus milk (from buffalo) using probiotic starter culture *(Lactobacillus acidophilus)* was prepared and its microbiological, physicochemical and sensory quality studies were carried out up to 6 days of storage. A slight increase in acidity of the milk was observed after 6 days of storage resulting in a decrease of pH (from pH 4.5 to 4.3). Total viable count of *L. acidophilus* bacteria was decreased after 6 days of storage due to increase in acidity but it was still within acceptable range ($>10^6$). Sensory evaluation data shows that the quality of sensory attributes (color, taste, aroma, appearance and overall acceptability) was slightly decreased after 6 days of storage but still had considerable acceptability.

Key words: Functional foods, Probiotics, *Lactobacillus Acidophilus*, Acidophilus milk, Sensory evaluation, Keeping quality, Buffalo milk

INTRODUCTION

In recent years, there has been an increasing trend about incorporation of the health promoting bacterial species; *Lactobacillus acidophilus* and *Bifidobacterium longum*, into fermented milk products. In fact, these bacteria having prescribed population of viable cells, when administered orally as supplement or through food products impart a variety of beneficial health effects (Azlin *et al*., 1997; Anderson and Gilliland 1999; Andrade and Borges 2009).

A number of *Lactobacillus acidophilus* strains are used in the processing of various dairy products such as acidophilus yoghurt and sweet acidophilus milk. The nutritional and therapeutic benefits derived through the consumption of dairy products containing viable *Lactobacillus acidophilus* as a food or supplement have been the focus of studies for the last two decades (Walker and Gilliland, 1993; Salminen *et al*. 1996; Azlin *et al.* 1997; Anderson and Gilliland 1999; Andrade and Borges 2009;). However, the production of high-quality fermented milk products containing these probiotic bacteria is a major challenge (Yeung *et al*., 2002) due to particular attributes of the bacteria such as rapid acid production from lactose and development of suitable quantities of the volatile compounds such as diacetyl and acetaldehyde etc. These compounds must not be over or under produced nor should they be accompanied by off-flavoring compounds. A lot of information has been accumulated relating to starter culture for milk fermentation (Marshall, 1987) and now even the efforts are going on to use the mixed cultures of bacterial species to produce specific flavor.

Although significant attention has been placed towards the organoleptic characteristics of the product, enriched with probiotic bacteria but still most of the publications concerning probiotic bacteria have not paid much attention on the consumer acceptability and preference of the finished product. For enhancing the consumption of functional probiotic products, the consumer satisfaction must be achieved keeping in view the cost effectiveness and health perspectives (Hilde *et al*., 2003).

Presently in Pakistan none of the dairy company is producing value added flavored acidophilus milk. Keeping in mind the need for value added products and the beneficial and , flavored (strawberry, pineapple, and mango) probiotic acidophilus milk (from buffalo) using probiotic starter culture *(Lactobacillus acidophilus)* was prepared for its microbiological, physicochemical and sensory quality studies up to 6 days of storage. pH was decreased as a consequence of increase acidity in milk after 6 days of storage. Total viable count of *L. acidophilus* bacteria was also decreased although within acceptable range ($>10^6$). Similarly, satisfactory results were found regarding sensory attributes (color, taste, aroma, appearance and overall acceptability) that showed slight decrease after 6 days of storage similar to the findings of Lehtoranta (2013).

MATERIALS AND METHODS

Procurement of Lactobacillus acidophilus Culture: Probiotic *Lactobacillus acidophilus* culture used in this study was procured from starter culture collection center (Danisco, Denmark). All the reagents and glass ware sterilized either by autoclaving or hot air oven for each set of experiment.

Probiotic Acidophilus Milk Development: The fresh whole Buffalo milk was obtained from Dairy Animal Training and Research Center (DAT&RC), UVAS, Ravi campus, Pattoki. Milk was cooled and kept at 4±1°C before transportation to the laboratory. The milk was subjected to standardization at 3.5% fat and 8.5% SNF. The standardized milk was pasteurized in 500 ml quantities at 72 °C for 15 minutes in a water bath to kill pathogenic microorganism and was then cooled to 4 ± 1 °C in an ice bath.

The pasteurized and standardized milk samples (200 ml) for fermentation were equilibrated for one hour at the fermentation temperature (40ºC) in a water bath before inoculation with the starter cultures. The freeze-dried culture used for fermentation of milk was activated according to the recommendations of suppliers and was grown in milk at 40 ± 1ºC and then maintained at 4 ± 1ºC.

Preliminary studies were carried out to optimize the conditions of culture concentration used for fermentation of milk. For this purpose, milk samples (200ml) were inoculated with overnight active probiotic culture of *Lactobacillus acidophilus* at different concentrations ranging from 1-5%. It was filled into clean plastic containers (250ml) and incubated in a shaker water bath at different temperatures; 30 °C, 35 °C and 40 °C for different time; 04 and 08 hours. The aim of preliminary studies was to find out the best combination of culture concentration with temperature and incubation time for product development. It was observed that by increasing temperature, time for incubation and concentration of culture, the rate of fermentation was increased. With 1% culture incubated at 40°C for 40 minutes a good gel with pH 4.4 was observed. This combination was then used for further study. Mango, strawberry and pineapple flavors of Danisco Company purchased from the local supplier were incorporated in cooled pasteurized milk to produce flavored probiotic acidophilus milk. The addition of flavors did not alter the pH of milk which was 6.6±0.1. The flavored probiotic acidophilus milk obtained was cooled and stored at 4±1°C for six days. The microbiological, physicochemical and sensory evaluation of the product at day 1 and day 6 was carried to study the acceptability of the product.

Sensory Evaluation: Prepared flavored probiotic acidophilus milk was subjected to sensory evaluation by a trained panel of 10 judges. The panelists had previous experience in dairy products evaluation. The panel comprised of post graduate students and faculty members of Department of Dairy Technology, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki. The evaluation of the product was carried out on appearance, taste, color, flavor and overall acceptability on a 9-point hedonic scale (9 = like very much; 1 = dislike very much) (Peryam *et al*., 1952). Sensory evaluation performa along with the consent form to participate in sensory evaluation was prepared and distributed to the panelists. The sensory evaluation of flavored probiotic acidophilus milk was conducted at day-1 of its development and at day-6 after its storage. All the evaluations were carried out at room temperature in the Department of Dairy Technology, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki.

Physicochemical Analysis (Shelf Life Study): The flavored probiotic acidophilus milk prepared was evaluated regularly for titratable acidity (expressed as lactic acid %) and pH during its storage up to six days with one day interval. The titratable acidity was determined by titrating 09 ml of flavored probiotic acidophilus milk with 0.1 N NaOH to the phenolphthalein end point. The pH was determined by inserting a pH probe (inoLab pH720, WTW, Weilheim, Germany) directly into a homogenized sample of the flavored probiotic acidophilus milk. Between samples, the electrode was rinsed with distilled water and wiped with lint free tissue.

Microbiological Analysis (Total Viable Count of probiotics): In order to claim a product to be probiotic the viability of probiotic bacteria is of primary importance. The acidophilus product was evaluated at day-1 and day-6 of storage for its total viable number of *Lactobacillus acidophilus* using MRS agar (Oxoid, UK). One ml of sample was taken and diluted with 9 ml of normal saline solution (v/v) and then serial dilutions were prepared using 1:10 dilution technique. 1ml aliquot from different dilutions (10^{-3} and 10^{-4}) was used to check the total viable count per ml on MRS agar media.

Statistical Analysis:

Sensory evaluation was carried on hedonic scale (Peryam *et al*., 1952). Viability of bacteria in flavored milk was noticed at day 1 and day 6. The data collected on pH, acidity and sensory evaluation for different treatment groups of flavored (strawberry, pineapple, and mango) probiotic acidophilus milk was subjected to analysis of variance (ANOVA) and comparison was made for difference of acidity among various treatments with respect to storage through Duncan's Multiple Range (DMR) test with a probability $P \leq 0.05$ (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Optimization and Standardization of Conditions for Acidophilus Milk development: For determining the better conditions for the development of the product a preliminary study was conducted, where culture concentration (1 – 5%), temperature (30 – 40°C) and incubation time (04 – 8h) was varied. All the three parameters accelerated the rate of fermentation, which was observed in the form of increased acidity and pH of the final product. These parameters affected the consistency of the gel, as well as physicochemical and microbiological quality. Overall, keeping in mind the curd consistency, pH, acidity and its sensorial attributes like aroma, taste, texture and overall acceptability, 1% culture concentration incubated in milk at 40 °C for 04hrs similar to the procedure followed by Hekmat *et al*. (2013) and it was found to be the best possible combination of variables and thus used for further studies. It resulted in a good gel with final pH of 4.4 coinciding with the work Aprodu (2012). In fact the pH 4.4 is around the iso-electric pH of the milk proteins and help in efficient aggregation of these proteins molecules. These results are also corroborated with findings of Salwa *et al.,* (2000) for pH as there is an inverse relationship between acidity and pH. Zeynab *et al.,* 2010 incorporated same culture concentrations (1% v/v) in research study for development of functional synbiotic acidophilus milk.

Determination of keeping quality of Acidophilus milk: The average titratable acidity during the course of six days for mango flavor was found as 0.86^{a}, that differ non-significantly (P>0.05) from titrate able acidity of pineapple (0.87^{a}) and significantly (P<0.005) from titrate able acidity of strawberry flavour (0.85^{b}). The results of analysis of variance of pH with treatment (P=0.1313), and interaction (P=0.078) were statistically non-significant while the results with storage (P=0.00) were statistically significant. The mean pH values of all the treatments decreased momentously with storage interval. No effect on acidity as a result of different flavouring was observed. The increase of acidity may be attributed to the production of lactic acid as a result of microbial fermentation of lactose (Tamine and Robinson, 2004). These results were also correlates with findings for pH which also decreased with storage period. Increased production of lactic acid decreases the pH of the product is in agreement with the results of Salwa *et al.,* (2000). The pH of all the flavoured milk samples at 1^{st} day was around 4.5 but was continuously decreased during storage. After 6 days of storage the pH in all the samples was around 4.32. This decrease in pH is related to the production of lactic acid. There was slight variation in the overall acidity and pH of flavoured probiotic acidophilus milk after 6 days of storage. The results of analysis of variance of acidity with treatment (P=0.00) and storage (P=0.00) were statistically non-significant while the results with interaction (P=0.5463) were statistically significant.

Total Viable Bacterial Count: In order to claim a product to be probiotic the viability of the organism is of primary importance. The acidophilus milk was evaluated at day-1 and day-6 of storage (Table 1) for total viable count of *Lactobacillus acidophilus* per ml. It is important to maintain the viability of probiotic microorganisms until the products are consumed in order to ensure delivery of sufficient number of live microorganisms. According to international standards, the total viable count in a probiotic product must be at least 10^5 /g (Robinson, 1987) at the time of consumption. In fact, the beneficial effects of *Lactobacillus acidophilus* can be expected only when ingesting significant count of viable cells which then can colonize the human gut (Ishibashi and Shimamura, 1993). Average values of total viable count for *Lactobacillus acidophilus* observed in this work were $2.50x10^6$, $3.60x10^6$ and $2.87x10^6$ for mango, pineapple and strawberry flavor milk respectively at Day-1 of the development of flavored probiotic acidophilus milk (Table 1). However a slight decrease in total viable count of product from day-1 to day-6 was observed. Average values of total viable count for mango, pineapple and strawberry flavored probiotic acidophilus milk at day 6 were $1.50x10^6$, $3x10^6$ and $2.50x10^6$, respectively (Table 1). The results of analysis of variance of total viable count with treatment (P=0.0459) and with storage (P=0.0063) were statistically significant while the results with interaction (P=0.3112), were statistically non-significant. These results are in agreement with the results of Shah *et al*. (1995) who also observed a decrease of total viable count in samples of commercial yoghurt. In fact increase in acidity has lethal effect on bacterial population. However, the total viable count was under acceptable range even after 6 days of storage indicating good keeping quality up to 6 days.

Sensory evaluation of Acidophilus Milk: Color serves as a preliminary parameter for the acceptance of food and indicates the fitness of milk products for consumption. Table 2 shows that the Acidophilus milk with different flavors was rated about 7.8 by the panelists, which is quite reasonable for a product. This rating was decreased to about 6.6 after 6 days of storage. At both days, the difference of color among three different milk samples was very slight.

Flavor means an overall integrated perception of taste and aroma associated with the product (Meilgaard *et al*., 2007). The flavor for mango, pineapple and strawberry flavored milk was rated as 7.6 ± 1.1, 7.4 ± 1 and 8.0 ± 0.7 respectively. After 6 days of storage the rating was decreased to 6.6 ± 0.6, 5.8 ± 0.9 and 6.3 ± 1 respectively (Table 2).

Overall acceptability is based on multiple organoleptic quality parameters i.e. color, flavor, texture etc. and shows the accumulative perception and acceptance by the panelists. Table 2 shows that the overall acceptability was around 7.7 in mango and pineapple flavored milk; however it was slightly higher (8.2) in strawberry flavored milk. The overall acceptability was decreased after 6 days of storage and was around 6.3. The overall acceptability after 6 days of storage is considered still reasonable for a fermented dairy product.

Table 1. Total Viable Count of Lactobacillus Acidophilus in Flavored Probiotic Acidophilus Milk.

	Flavor					
	Mango		**Pineapple**		**Strawberry**	
Interval	Day-1	Day-6	Day-1	Day-6	Day-1	Day-6
Total viable count	$2.50x10^6$	$1.50x10^6$	$3.60x10^6$	$3x10^6$	$2.87x10^6$	$2.50x10^6$

Table 2. Summary of Sensory Evaluation Scores of Flavored Probiotic Acidophilus Milk Conducted at Day-1 (a) and Day-6 (b).

a)

Attributes	**Flavor**		
AAW	**Mango**	**Pineapple**	**Strawberry**
Color	7.8 ±0.92	8.0 ±1.05	7.5 ±1.08
Flavor	7.6 ±1.074	7.4 ±0.96	8.0 ±0.66
Over Acceptability	7.6 ±1.26	7.8 ±0.78	8.2 ±0.78

b)

Attributes	**Flavor**		
	Mango	**Pineapple**	**Strawberry**
Color	6.4 ± 0.84	6.7 ±0.95	6.8 ± 1.13
Flavor	6.6 ±0.69	5.8 ±1.032	6.3 ±1.05
Over Acceptability	6.9 ±0.57	5.9 ±0.88	6.1 ±0.74

A 9-point hedonic rating scale (9 = excellent; 1 = extremely poor) was used for sensory evaluation.

Conclusion: The probiotic Acidophilus milk produced with different flavors in this study has been seen to have higher overall acceptability. The overall acceptability was slightly decreased after 6 days of storage. The total viable count was within the acceptable range up to 6 days of storage without affecting deleteriously other physicochemical parameters. Keeping in view all above results, the production of flavored Acidophilus milk at commercial level is highly recommended.

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