



Original Research

Effect of Oat Flour Addition on the Viability of Probiotic Micro-organisms During Refrigerated Storage of Synbiotic Milk Drink

Praveen Kumar Tiwari*, Shakeel Asgar, Sudhir Uprit, Manorama Chauhan, Kushal Kumar Sandey and Nitin W. Shinde

College of Dairy Science and Food Technology, CGKV, Raipur, Chhattisgarh, INDIA

*Corresponding author: praveen.tiwari0611@gmail.com

Rec. Date:	Apr 30, 2018 02:06
Accept Date:	Sep 10, 2018 16:55
DOI	10.5455/ijlr.20180430020654

Abstract

Oats and probiotics have been long known for providing health benefits. An attempt has been made to prepare the synbiotic milk drink by adding oat flour in different concentrations (0.5 %, 1.0 % and 1.5 %) with *L. casei* 019 and *L. acidophilus* 014 as a probiotic microorganisms. The several works that were carried on probiotic bacteria in food systems have proved a low viability during storage period. This work presents some research whose main purpose is the improvement of the behaviour and functionality of the added probiotic strains by adding oat flour. At the end of the storage period of 21 days, the probiotic count in the samples T_0 , T_1 , T_2 and T_3 were found to be 5.78, 5.95, 7.04 and 7.08 \log_{10} cfu/ml respectively. The milk drink samples (T_2) 1 % and (T_3) 1.5 % had maintained the desirable number of probiotic microorganisms in the product.

Key words: Coliform Count, Oat, Probiotics, Synbiotics, Total Viable Count, Yeast and Mold Count

How to cite: Tiwari, P., Asgar, S., Uprit, S., Chauhan, M., Sandey, K., & Shinde, N. (2019). Effect of Oat Flour Addition on the Viability of Probiotic Microorganisms during Refrigerated Storage of Synbiotic Milk Drink. International Journal of Livestock Research, 9(1), 66-73. doi: 10.5455/ijlr.20180430020654

Introduction

The word 'probiotic' is derived from the Greek language 'pro bios' which means 'for life'. Probiotics are defined as "live microorganisms which on ingestion in certain numbers, exert health benefits beyond basic nutrition" (Guarner and Schaafsma, 1998). When the milk or milk products are added with these probiotics micro-organisms, they contributes in improvement of health and provide numerous health benefits. The lactic acid bacteria (LAB) are the most common probiotic micro-organisms that is mostly used as human probiotic micro-organisms. In LAB, *Lactobacillus* and *Bifidobacterium* constitutes the most frequently used genera (Holzapfel and Schillinger, 2002), either singly or in combination, constitutes a significant proportion of probiotic cultures in nutritional supplements, pharmaceuticals and functional foods (Piano et



al., 2006). The applications of probiotic bacteria in food products are increasing due to their potential health benefits mainly through maintenance of normal intestinal microflora, protection against gastrointestinal pathogens (D'Aimmo *et al.*, 2007), enhancement of the immune system (Gilliland, 1990), reduction of serum cholesterol level, blood pressure and anti-carcinogenic activity (Rasic, 2003), improved utilization of nutrients and improved nutritional value of food (Lourens-Hattingh and Viljoen, 2001). Therapeutic applications of probiotics include prevention of infantile diarrhea, urogenital diseases, osteoporosis, food allergy and atopic diseases; reduction of antibody-induced diarrhea; alleviation of constipation and hypercholesterolemia; control of inflammatory bowel diseases; and protection against colon and bladder cancer (Mattila-Sandholm *et al.*, 2002; Salminen, 1996; Venturi *et al.*, 1999).

From the past few decades, a significant success has been achieved in the development of dairy based probiotic products such as fermented milks, ice cream, various types of cheeses, baby food, milk powder, frozen dairy desserts, whey based beverages, sour cream, buttermilk, normal and flavored milk (Mortazavian *et al.*, 2012). It is also be consumed as live probiotic cells in many nutraceutical products. Probiotic food products only provide significant health benefits when consumed in adequate amounts of 10^6 - 10^7 cfu/ml of viable microorganisms count. The approximate quantity of probiotic products consumed regularly for desired health benefits should be 100 g/day in order to deliver required amount of probiotic microorganisms (Karimi *et al.*, 2011). The efficacy of added probiotic bacteria in food systems depends largely on dose level and their viability must be maintained throughout storage period, and they must survive and colonize easily in the gut environment (Gustaw *et al.*, 2011). For improving these features of probiotic bacteria, probiotic products should be supplemented with non-digestible food ingredients i.e. prebiotics that beneficially affect the host by selectively stimulating the growth of the bacteria in the colon. The product obtained after probiotic microorganisms and prebiotic substrates is termed as synbiotic product. This combination of probiotics and prebiotics can improve the survival of probiotic organism. Cereals are one of the most suitable components for the production of food products containing a probiotic microorganisms and may also have prebiotic properties due to the presence of non-digestible components (Gupta *et al.*, 2010). Oats, unlike other cereals have received considerable interest in recent years as a delivery vehicles for probiotics due to their high content of soluble and insoluble fibres. Oats are an excellent source of soluble fibre β -glucan and the content of β -glucan in oats is in the range of 3-7 % (Skendi *et al.*, 2003). These oats due to the presence of nutrients shows the high ferment ability upon applying probiotic lactic acid bacteria.

Materials and Methods

Bacterial Strains

Two probiotic cultures *i.e.* *Lactobacillus casei* 19 and *Lactobacillus acidophilus* 14 have been utilized in the preparation of the milk drink. The cultures were obtained in freeze dried form from the National

Collection of Dairy Cultures (NCDC), National Dairy Research Institute (NDRI), Karnal, Haryana, India. The cultures were maintained by sub-culturing into sterilised skim milk and stored at 4°C.

Oat Substrate

Oats ground into fine oat flour were used as a substrate in different concentrations (0.5, 1.0 and 1.5%). The slurry of oat flour was prepared in small quantity of milk then added to prevent formation of lumps in the milk.

Synbiotic Milk Drink Production

The synbiotic milk drink was prepared from fully pasteurized and homogenized milk with 1.5 % fat and 9 % SNF. The milk was pre-heated at 35-45°C. Sugar was added @ 7% w/v followed by addition of oat flour in different concentrations of 0.5 % (T₁), 1.0 % (T₂) and 1.5 % (T₃) and control (T₀) was prepared by without addition of oat flour. Sterilization was carried out in the conical flask by putting cotton plug as a capping material to maintain hygienic condition of the product and for attaining sterility, temperature is maintained at 121°C for about 15 min. After sterilization, milk is allowed to cool in atmospheric conditions to 37°C for inoculation of probiotic cultures (*L. acidophilus* and *L. casei* in 1:1 ratio). The inoculated milk was transferred to clean, pre-sanitized plastic bottles and then incubated for 2 h at 37°C. The synbiotic milk drink was stored at refrigerated temperature.

Microbial Analysis

The microbial analysis of synbiotic milk drink was done for 21 days in the interval of every 7 days to determine to viability of probiotic microorganisms (APHA, 1992) during storage. The total viable count (TVC), coliform count and Yeast and mold count were also determined during storage period (APHA, 1992).

Statistical Analysis

Data were subjected to analysis of variance for a one factor completely randomized design (CRD) and for two factor factorial completely randomized design (FCRD) procedure at a significance level of 0.05. (Gupta and Kapoor, 2007).

Results and Discussion

Effect on Microbial Quality in Synbiotic Milk Drink Samples during Refrigerated Storage for 21 Days

The influence of the refrigerated storage on microbial quality of control and experimental samples of synbiotic milk drink were analysed for probiotic count, yeast and mold count, coliform count and total viable count (TVC) during storage period of 21 days in the interval of every 7 days and is expressed in

log₁₀cfu/ml. Microbial population in the food products is of utmost importance when it is related to the safety of the product. The study of microbial population in the samples of synbiotic milk drink supplemented with oats was of primary importance to produce safe and good quality products. Each microorganisms can be either beneficial or harmful to the consumer. Hence, the microorganisms involved in the preparation of the synbiotic milk drink and their microbial load must be known so as to decide the safety and quality of the product. In the control and experimental samples of synbiotic milk drink, its probiotic count, coliform count, yeast and mold count and total viable count (TVC) were determined and shown in Table 1.

Table 1: Effect of addition of oat flour on the microbial population (in log cfu/ml) of synbiotic milk drink samples during refrigerated storage (Mean ±SE)

T	Probiotic count				Coliform count				Yeast and mold count				Total viable count			
	0	7	14	21	0	7	14	21	0	7	14	21	0	7	14	21
T ₀	8.79 ± 0.189 ^a	7.3 ± 0.137 ^b	6.96 ± 0.134 ^c	5.78 ± 0.124 ^d	-	-	-	-	-	-	-	-	8.80 ± 0.121 ^a	7.31 ± 0.076 ^b	6.98 ± 0.100 ^c	5.78 ± 0.064 ^d
T ₁	8.82 ± 0.086 ^a	7.6 ± 0.11 ^b	7.02 ± 0.11 ^c	5.95 ± 0.119 ^d	-	-	-	-	-	-	-	-	8.84 ± 0.118 ^a	7.61 ± 0.073 ^b	7.03 ± 0.093 ^c	5.96 ± 0.086 ^d
T ₂	8.88 ± 0.11 ^a	7.7 ± 0.147 ^b	7.49 ± 0.099 ^c	7.04 ± 0.091 ^d	-	-	-	-	-	-	-	-	8.90 ± 0.093 ^a	7.72 ± 0.088 ^b	7.51 ± 0.051 ^c	7.05 ± 0.090 ^d
T ₃	8.92 ± 0.104 ^a	7.7 ± 0.091 ^b	7.56 ± 0.063 ^c	7.08 ± 0.163 ^d	-	-	-	-	-	-	-	-	8.94 ± 0.365 ^a	7.72 ± 0.096 ^b	7.58 ± 0.090 ^c	7.11 ± 0.103 ^d

Changes in the Probiotic Count during Storage of 21 Days

The probiotic count in the synbiotic milk drink represents the microbial load of *Lactobacillus casei* 19 and *Lactobacillus acidophilus* 14 in the product. The effect of addition of oat flour on the probiotic count of synbiotic milk drink during storage period of 21 days is presented in the Table 1 and graphically in Fig.1.

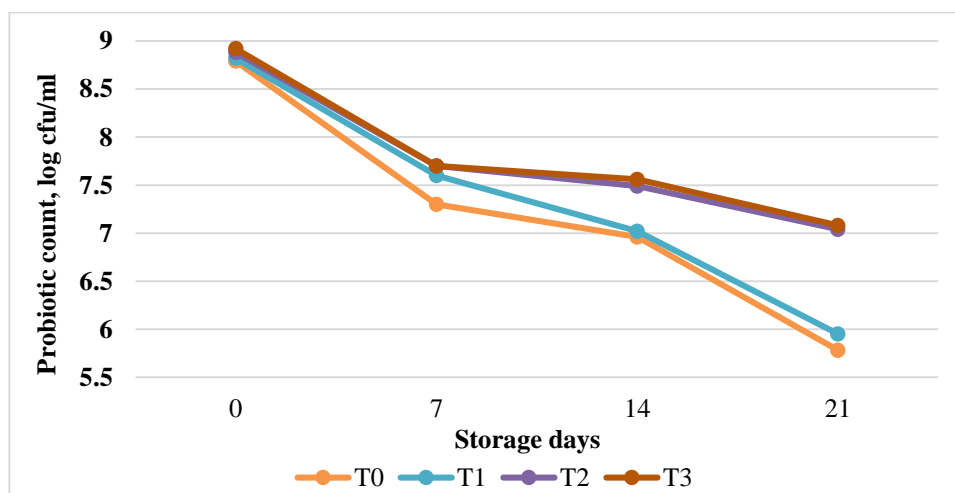


Fig. 1: Effect of addition of oat flour on probiotic count of synbiotic milk drink samples during storage at refrigerated temperature

The probiotic count of all the samples in fresh synbiotic milk drink were determined and found to have the lowest probiotic count of 8.79 log cfu/ml in T₀, whereas in the experimental samples the probiotic count increased from 8.82 log cfu/ml in T₁, 8.88 log cfu/ml in T₂ and found highest in T₃ with the probiotic count of 8.92 log cfu/ml. For providing health benefits, the minimum probiotic count in the products when ingested must contain a minimum of 6-7 log cfu/ml in the final product (Shah, 2007). The results of probiotic count in synbiotic milk drink was fulfilling the minimum required dosage in the probiotic products. Based on existing standards and from a health point of view, it is very important that probiotic microorganisms retain their viability and functional activity throughout the shelf life of product. The *L. casei* 19 and *L. acidophilus* 14 were added as a probiotic culture in the symbiotic milk drink. The probiotic count of synbiotic milk drink in the samples T₀, T₁, T₂ and T₃ at 0th day were 8.79, 8.82, 8.88 and 8.92 log₁₀ cfu/ml respectively. Whereas the corresponding values of probiotic count for the samples T₀, T₁, T₂ and T₃ at the end of storage period (21 days) were found to be 5.78, 5.95, 7.04 and 7.08 log₁₀ cfu/ml respectively. The experimental samples T₂ and T₃ of synbiotic milk drink had maintained the minimum number of probiotic microorganisms that must be present in the product to exert health benefits, whereas the probiotic count in samples T₀ and T₁ were below the minimum probiotic count in the product at the end of storage period. The statistical analysis showed that the probiotic count in the synbiotic milk drink samples were decreased significantly (p<0.05) during storage period. The above results revealed that the addition of oat flour above 1.0% level maintained the minimum probiotic count in the product during storage. The probiotic count in the product remained ≥ 7.0 log₁₀ cfu/ml even after 21 days of storage period in T₂ and T₃ which are considered sufficient to exert health beneficial effects. The shelf life of the synbiotic milk drink samples T₂ and T₃ were estimated to be 21 days under refrigerated storage.

The probiotic count in the synbiotic milk drink was decreased during storage period. The decrease in the count of probiotic microorganisms during storage may be due to the decrease in the viability of probiotic microorganisms in the synbiotic milk drink samples. The decrease in the viability of probiotic microorganisms in the milk drink is due to a number of factors including H₂O₂ produced by starter bacteria, oxygen content, pH, storage environment and concentration of metabolites such as lactic and acetic acids (Akalin *et al.*, 2007). Yadav *et al.* (2007) reported for decrease in the probiotic count of dahi during storage period. Bolin *et al.* (1998) analyzed the viability of *L. acidophilus* strains in milk cultures in refrigeration conditions (7°C) and showed that the number of bacteria significantly decreased during storage (35 days). The mean difference between initial count (7.97 log cfu/ml) and end count (6.21 log cfu/ml) was 1.76 log cfu/ml (Bolin *et al.*, 1998). Dave and Shah, (1997) studied the viability of bacteria from commercial starter cultures during yoghurt manufacture and storage. The count of *L. acidophilus* was in the range of 1.8-3.8×10⁷cfu/g after incubation but decreased during storage and recommended level of 10⁶ cells was

maintained only for 20-25 days. Nighswonger *et al.* (1996) reported for decrease in the viability of *L. casei* and *L. acidophilus* in buttermilk and yoghurt during refrigerated storage.

Changes in the Total Viable Count (TVC) of Synbiotic Milk Drink during Storage

The total viable count (TVC) of the synbiotic milk drink represents the total microbial load in the product and is presented in the Table 1 and graphically in Fig. 2. The TVC in the fresh product was found to have the lowest microbial count of 8.80 log₁₀ cfu/ml in control (T₀) sample, whereas in the experimental samples the microbial count increased from 8.84 log₁₀ cfu/ml in T₁, 8.90 log₁₀ cfu/ml in T₂ and found highest in T₃ with the total viable count of 8.94 log₁₀ cfu/ml. During storage period, the TVC was significantly (p<0.05) decreased in the samples T₀, T₁, T₂ and T₃ and were found to be 5.78, 5.96, 7.05 and 7.11 log₁₀ cfu/ml respectively. There was no major difference found between the probiotic count and TVC of the synbiotic milk drink samples, as the probiotic products contains only probiotic micro-organisms.

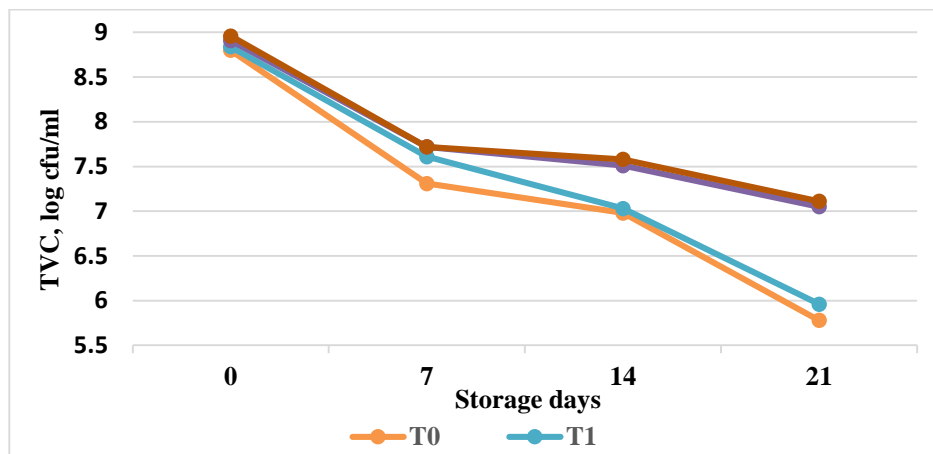


Fig. 2: Effect of addition of oat flour on total viable count of synbiotic milk drink samples during storage at refrigerated temperature

The TVC count in the synbiotic milk drink samples were decreased as the storage period proceeds. The total viable count in the fresh synbiotic milk drink was higher in experimental samples T₂ and T₃ containing 1.0 % and 1.5 % oat flour. The oat flour addition to synbiotic milk drink protects the microbial population due to its prebiotic effect. Tesfaye, (2013) prepared bio-yoghurt by adding oat flour and reported that the microbial population were decreased at the end of storage period of 21 days.

Coliform Count of Synbiotic Milk Drink during Storage

The Table 1 shows that the no coliform was observed in the fresh samples of synbiotic milk drink supplemented with oats after 2 h of incubation. The absence of coliforms indicated that the synbiotic milk drink was prepared under hygienic conditions. The absence of coliform in the synbiotic milk drink samples revealed that all samples have undergone the better sterilization treatment and also the probiotic culture

that was added in the product does not contain any coliforms which ultimately resulted in the absence of coliform in the final product. During storage of 21 days, no coliform growth was found in any of the milk drink samples. Hence, the synbiotic milk drink samples were safe for consumption until 21 days of storage period. Kale *et al.* (2011) prepared value added dahi with the addition of oat flour and reported for the absence of coliform in the control as well as in the experimental samples during storage period.

Yeast and Mold Count of Synbiotic Milk Drink during Storage

The Table 1 shows the yeast and molds count in the fresh samples of synbiotic milk drinks. The yeasts and molds growth were not detected in all the samples of fresh synbiotic milk drinks. Yeasts and molds may get entry into the product from atmosphere, utensils and human hands under natural conditions. Yeasts and molds in the milk based products produces gassiness and flavour defects associated with lipolysis of milk fat. The experimental samples of synbiotic milk drink showed the absence of yeast and mold growth even after 21 days of storage period at refrigeration temperature. Hence, the samples of synbiotic milk drink added with oat flour were safe for consumption until 21 days as it is preserved at refrigeration temperature.

Conclusion

In the synbiotic milk drink, the addition of oat flour had maintained the minimum level of probiotic microorganisms that must be present in the product for providing health benefits. During storage, the samples T₂ and T₃ had maintained the recommended level of probiotic microorganisms of 7.0 log cfu/ml in T₂ and T₃ samples during storage. Coliform count and yeast and mold count were neither found in the fresh nor in the stored samples of synbiotic milk drinks. It is concluded that the synbiotic milk drink prepared by adding oat flour above 1.0 % have the ability to maintain the viability of probiotic microorganisms in the fresh as well as in stored product.

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