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Chemical composition of dahi prepared from whole Murrah buffalo milk using *Lactobacillus plantarum* CRD 2 and *Lactobacillus rhamnosus* CRD 9 probiotics cultures

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Abstract

The present experiment was carried out at ICAR-National Dairy Research Institute, Karnal (Haryana) to study the chemical composition of Dahi prepared from whole Murrah buffalo milk using *Lactobacillus plantarum* CRD 2 and *Lactobacillus rhamnosus* CRD 9 probiotics cultures. Whole Murrah Buffalo milk was collected from Livestock Research Centre, National Dairy Research Institute, Karnal (Haryana). Milk samples were scanned for the determination of quantity of total solid, fat, protein, lactose, solid not fat (SNF) present in milk sample and found that the percent fat, protein, lactose, solid not fat (SNF), total milk solids (TS), acidity and pH in milk were 7.85 ± 0.09 , 3.78 ± 0.07 , 5.17 ± 0.06 , 9.69 ± 0.10 , 17.55 ± 0.11 , 0.15 ± 0.08 and 6.74 ± 0.05 respectively. A freeze-dried pure culture of *Lactobacillus plantarum* CRD 2 and *Lactobacillus rhamnosus* CRD 9 procured from the Synbiotic functional foods laboratory number-107, Dairy Microbiology Division, National Dairy Research Institute, Karnal (Haryana). Purity and morphology of cultures were determined by Gram's and negative staining. Both the cultures were activated in MRS broth and it was reported that broth contain 10^8 cfu/ml. One loop of inoculums from this broth used to ferment milk i.e. Dahi as source of starter culture. Starter culture was added at the rate of 1% and found that the percent fat, protein, lactose, solid not fat (SNF), Total milk solids (TS), Acidity and pH in fermented milk (Dahi) were 7.88 ± 0.11 , 3.83 ± 0.08 , 4.18 ± 0.12 , 9.62 ± 0.14 , 7.58 ± 0.21 , 0.69 ± 0.09 and 4.39 ± 0.03 for *Lactobacillus Plantarum* CRD 2 while 7.82 ± 0.14 , 3.81 ± 0.06 , 4.11 ± 0.17 , 9.64 ± 0.19 , 17.53 ± 0.14 , 0.72 ± 0.06 and 4.31 ± 0.07 for *Lactobacillus rhamnosus* CRD 9 respectively.

Keywords: Chemical composition, dahi, Murrah Buffalo, Milk, *Lactobacillus plantarum* CRD 2, *Lactobacillus rhamnosus* CRD 9, probiotics

Introduction

India leads in global milk production with 163.7 million tonnes (Singh, 2017) ^[19] and with respect to this major portion i.e. 76.19 million tonnes of milk (49%) is mainly contributed by the buffaloes (DAHDF, 2016) ^[6]. Fermented foods having great importance as they provide and preserve the large amount of nutritious foods in a wide diversity of flavours, aromas and textures which ultimately enrich the human diet and the Dahi is one of them. Dahi is the traditional Indian fermented milk product, prepared from cow milk, goat milk and sometimes also from buffalo milk and it is widely consumed all over the country. It is the mainly product of lactic acid fermentation of milk and it is quite easy and simplest way of preserving milk for human consumption and development of different product for further consumption. Dahi having different therapeutic application in different diseases such as digestive disorder which property mainly absent in the milk. An estimated 50 to 55 % of the milk produced in India is converted into a variety of traditional milk products, using processes such as coagulation, desiccation and fermentation (Swapna and Chavannavar, 2013) ^[20]. Chakraborty (1998) ^[5] reported that about 7% of the total milk produced is converted to Dahi for consumption. Currently, dairy industry in the country is showing keen interest in large scale production of various indigenous milk products including Dahi and with respect to this production of Dahi in organized sector had been taken a big leap and is expected to grow further and further. But the traditional cultures for Dahi are not well defined and it contains various strains of Lactic Acid Bacteria.

In traditional Dahi preparation, a small portion of product containing microbes of previous fermentation (back slopping) is generally added to milk after boiling then cooling to room temperature. However, production of Dahi with defined single strain culture of *Lactobacillus* or a combination of cultures containing *Lactobacillus* and *Lactococcus* have reported in production of good quality product. For the preparation of any type of Dahi desirable cultures should possess good acidification property which resulted in the stability and shelf life of final product. The availability of high quality starter cultures will be helpful for commercial scale production of good quality Dahi and preparation of Dahi using selected, specified and well defined probiotics cultures is new approach. Probiotics; are “live microorganism which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002) [8]. The fermented milk products are probiotics that contains viable lactic acid bacteria (LAB) and its metabolic by-products act as an antioxidant, immune modulator and antimicrobial agents (Ramasamy and Suyambulingam, 2015) [15]. With the advent of health foods, Dahi is valued for controlling the growth of intestinal bacteria and incurring intestinal diseases like constipation, diarrhoea and dysentery with effectivity in lowering blood cholesterol level (Akter *et al.*, 2010) [2]. The Dahi prepared from the buffalo milk sometimes called as buffalo curd and that product is popular throughout the Indian subcontinent. Buffalo milk is traditionally better than cow milk due to its higher fat content making a thicker yogurt mass (Kristbergsson *et al.*, 2016) [11]. Also research studies indicated that Buffalo curd has a higher nutritional value of protein, fat, lactose, minerals and vitamins. Currently research findings have been lacking on the quality of Dahi prepared from whole buffalo milk also, information on preparation of Dahi using different species and strains of different bacteria is also scanty. Hence an attempt was made to judge the quality of Dahi prepared from whole Murrah buffalo milk using *Lactobacillus plantarum* CRD 2 and *Lactobacillus rhamnosus* CRD 9 probiotics cultures.

Materials and Methods

The experiment was conducted at National Dairy Research Institute, Karnal, (Haryana). Whole Murrah buffalo milk was used for the preparation of Dahi. Whole Murrah Buffalo milk was collected from Livestock Research Centre, National Dairy Research Institute, Karnal (Haryana). Milk sample was scanned for the determination of quantity of total solid, fat, protein, lactose, solid not fat (SNF) present in milk sample. For that 30 ml of milk sample was taken in sample bottle at room temperature and loaded in Milk scanner (An ISO 9001:2008 Co. Nuline) and recorded the readings. A freeze-

dried pure culture of *Lactobacillus plantarum* CRD 2 and *Lactobacillus rhamnosus* CRD 9 procured from the Synbiotic functional foods laboratory number-107, Dairy Microbiology Division, National Dairy Research Institute, Karnal. Purity and morphology of cultures were determined by Gram's and negative staining (Moyes *et al.*, 2009) [13]. Both the cultures were activated in MRS broth and it was reported that broth contain 10^8 cfu/ml. One loop of inoculums from this broth used to ferment milk i.e. Dahi as source of starter culture. Collected whole buffalo milk sample was heated to 85°C for 10 minutes while stirring thoroughly with the help of stirrer. Then milk pan was taken out from the heater and allowed to cool down at 37°C . Starter culture was added at the rate of 1%. After inoculation, it was kept undisturbed in the incubator at a temperature of 37°C until proper and complete settlement. It took 6-7 hours for the complete settlement. The Dahi samples were taken out from the incubator and analyzed for fat, protein, lactose, SNF and total solids as per standard protocol of bureau of Indian Standards (BIS), pH was determined electrochemically with a pH meter as per the method described in IS: SP 18 (Part XI, 1981) [10]. The pH meter was first calibrated using standard buffers of pH 4.0, 9.2 and 7.0 at $20\pm 0.1^\circ\text{C}$. Titratable acidity was determined by the procedure described in IS: 1479, Part I, ISI (1960) [9] for that 10 gm of the sample was taken in a beaker and titrated against 0.1 N NaOH using phenolphthalein as an indicator till the pH of sample reached ~ 7 . The titratable acidity was expressed as percent lactic acid.

$$\text{Acidity (\% Lactic acid)} = 9 \text{ N V/X}$$

N = Normality of NaOH

V = Volume of NaOH used (mL)

X = Amount of sample taken (gm)

Flow Diagram illustrating the preparation of Dahi from whole Murrah Buffalo milk using *Lactobacillus plantarum* CRD 2 and *Lactobacillus rhamnosus* CRD 9 Probiotics Cultures

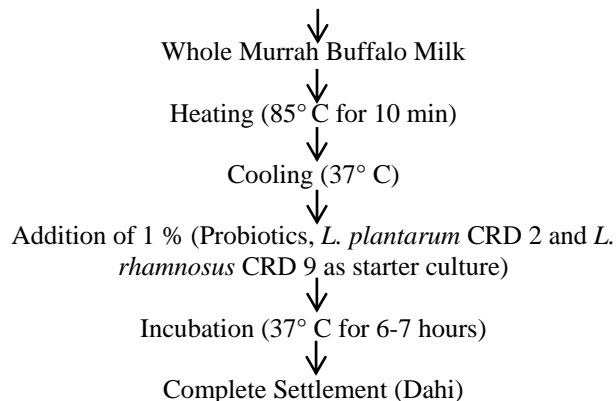




Plate 1: Preparation of Dahi from whole Murrah Buffalo milk using *Lactobacillus plantarum* CRD 2 and *Lactobacillus rhamnosus* CRD 9 Probiotics Cultures

Results and Discussion

Chemical composition (%) of Milk

The taste, appearance and colour of the buffalo milk sample were normal. The chemical composition (%) of milk was presented in Table- 1. The percent fat, protein, lactose, solid not fat (SNF), Total milk solids (TS), Acidity and pH in milk were 7.85 ± 0.09 , 3.78 ± 0.07 , 5.17 ± 0.06 , 9.69 ± 0.10 , 17.55 ± 0.11 , 0.15 ± 0.08 and 6.74 ± 0.05 respectively. These chemical composition values of buffalo milk were in similar lines with those reported by Saini (2012) [17] and Kumar (2014) [12] who reported these values for Murrah buffalo milk. The values for fat and SNF percent in milk were also similar to the values reported by Sharma *et al.* (1980) [18] and Dubey *et al.* (1997) [7] who similarly reported for Murrah buffalo milk.

Table 1: Chemical composition (%) of milk

S. No.	Constituents	(%)
1	Fat	7.85 ± 0.09
2	Protein	3.78 ± 0.07
3	Lactose	5.17 ± 0.06
4	Solid Not Fat (SNF)	9.69 ± 0.10
5	Total Milk Solids (TS)	17.55 ± 0.11
6	Acidity	0.15 ± 0.08
7	pH	6.74 ± 0.05

Chemical composition (%) of fermented milk (Dahi)

The chemical composition (%) of fermented milk (Dahi) prepared from whole Murrah buffalo milk using two different probiotic cultures i.e. *Lactobacillus plantarum* CRD 2 and *Lactobacillus rhamnosus* CRD 9 was presented in Table- 2. The percent fat, protein, lactose, solid not fat (SNF), Total milk solids (TS), Acidity and pH in fermented milk (Dahi) were 7.88 ± 0.11 , 3.83 ± 0.08 , 4.18 ± 0.12 , 9.62 ± 0.14 , 7.58 ± 0.21 , 0.69 ± 0.09 and 4.39 ± 0.03 for *L. Plantarum* CRD 2 while 7.82 ± 0.14 , 3.81 ± 0.06 , 4.11 ± 0.17 , 9.64 ± 0.19 , 17.53 ± 0.14 , 0.72 ± 0.06 and 4.31 ± 0.07 for *L. rhamnosus* CRD 9 respectively. In this study the chemical composition values of Dahi prepared from buffalo milk were in accordance with the normal range found in different literatures. Akin *et al.* (1995) [1] reported that buffalo milk Dahi contained 9.08% fat while Nahar *et al.* (2007) [14] similarly reported that Dahi prepared from buffalo milk contained 10.85% fat. With respect to the protein content of Dahi the results of this experiment are contradictory with the findings of Ali (1998) [3] and Nahar *et al.* (2007) [14] whom found that buffalo milk Dahi contained 5.1% and 5.13% protein, respectively. Lactose content of Dahi is slightly lower than that of the milk because of the some part of the lactose is mainly converted into the lactic acid due to the action of the *L. Plantarum* CRD 2 and *L. rhamnosus* CRD 9 bacteria. In present experiment the results of the SNF and Total solids content in the Dahi were similar

to the milk composition because no addition of any sugar in Dahi as Ray *et al.* (1972) [16] reported that variation in TS content of Dahi could be attributed due to addition of sugar in different concentration. Dahi prepared from *L. rhamnosus* CRD 9 had slightly higher values for acidity content i.e. 0.72 ± 0.06 than the Dahi prepared from *L. Plantarum* CRD 2 i.e. 0.69 ± 0.09 , these results of the acidity content of Dahi are in agreement with the findings reported by the Nahar *et al.* (2007) [14] while contradictory with the findings of Cardoso *et al.* (1991) [4]. The average pH values of Dahi prepared from buffalo milk in present experiment were slightly lower than that of reported by Nahar *et al.* (2007) [14].

Table 2: Chemical composition (%) of fermented milk (Dahi)

S. No.	Constituents (%)	<i>L. Plantarum</i> CRD 2	<i>L. rhamnosus</i> CRD 9
1	Fat	7.88 ± 0.11	7.82 ± 0.14
2	Protein	3.83 ± 0.09	3.81 ± 0.06
3	Lactose	4.18 ± 0.12	4.11 ± 0.17
4	Solid Not Fat (SNF)	9.62 ± 0.14	9.64 ± 0.19
5	Total Milk Solids (TS)	17.58 ± 0.21	17.53 ± 0.14
6	Acidity	0.69 ± 0.09	0.72 ± 0.06
7	PH	4.39 ± 0.03	4.31 ± 0.07

Conclusions

From the findings of this research study, it might be concluded that Dahi prepared from the whole Murrah buffalo milk using two different probiotic cultures i.e. *Lactobacillus plantarum* CRD 2 and *Lactobacillus rhamnosus* CRD 9 produced better quality Dahi with better nutrients composition. So, utilization of whole buffalo milk for Dahi preparation is advocated. Also the introduction of *Lactobacillus plantarum* CRD 2 and *Lactobacillus rhamnosus* CRD 9 as the selected, specific and defined strains of probiotics cultures for Dahi preparation is new approach and can be used for further study over the traditional cultures.

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