ELECTROPHORETIC CHARACTERIZATION OF CASEIN OF REFRIGERATED COW AND BUFFALO MILK PRESERVED WITH BANANA PSEUDOSTEM JUICE

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ABSTRACT

Cow milk, a highly nutritious food item, is amenable to quality degradation due to lipolysis and proteolysis, as a sequel to the growth of spoilage microorganisms, particularly under ambient condition in tropical countries, with consequential loss of aesthetic appeal and product output. Prescription antibiotics used for milk preservation have many undesirable side effects on the health of consumers, besides bolstering development of antibiotic resistant strains of bacteria. This paper has tried to elucidate the effect of treatment of raw cow milk and buffalo milk stored under refrigeration with Banana (Musa paridasiaca) Pseudostem Juice (BPJ), a natural antimicrobial agent on the electrophoretic profile of whole casein of milk, since BPJ can checkmate proteolysis with consequential alteration of molecular mass, on which no study has been done earlier. Fresh cow milk and buffalo milk, procured from local milk producers were treated with 0.3% (v/v) BPJ, and were kept under refrigeration at 7±2°C for 5 days and 4 days respectively, while BPJ untreated (control) samples of cow and buffalo milks were kept at same temperature for 3 days and 2 days respectively. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) profile of control and BPJ treated cow milk samples were determined after 3 days and 5 days respectively, whereas the same was done for buffalo milk samples after 2 days and 4 days respectively. The SDS-PAGE pattern revealed that the whole caseins of BPJ treated cow and buffalo milk samples resolved in to two bands of low molecular weight components ranging between 29 kD and 43 kD. The SDS-PAGE pattern of isolated whole caseins of BPJ treated cow milk and buffalo milk did not exhibit any deviation from control cow and buffalo milk caseins. It is concluded that addition of BPJ (0.3%, v/v) to cow milk and buffalo milk did not alter the molecular configuration of milk casein under refrigerated (7±2°C) preservation for 5 days and 4 days respectively due to its inhibitory effect on proteolysis.

KEY WORDS

Banana Pseudostem Juice, Electrophoresis, Milk Casein

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INTRODUCTION

Cow milk is a highly nutritious food item, containing a variety of nutrients such as fats, proteins, carbohydrates, minerals, vitamins, and water, but extremely perishable, since it also confers an excellent medium for the growth of spoilage microorganisms, particularly under ambient condition in tropical countries, leading to loss of aesthetic appeal and product yield, especially cheese (Prescott, 1999; Anderson et al., 2011; Ramos et al., 2015).

Spoilage behavior is attributed to increased lipolysis as well as proteolysis. Proteolysis, which is the focus of this study is caused by higher production of protease due to microbial action causing off-flavour of milk and milk products, besides reducing the alpha-, beta- and kappa-casein fractions with consequent alteration of their molecular masses, and leading to industrial imbroglio due to decrease in cheese output (Deeth et al., 2002; Santos et al., 2003; Zhanq et al., 2013; Ramos et al., 2015).

A common practice, adopted by rural dairy farmers of West Bengal and Bangladesh to preserve raw milk is addition of Banana (Musa paridasiaca) Pseudostem Juice (BPJ) to check spoilage during transportation under ambient condition due to non-availability of transport vehicles with freezer facility. The authenticity of this practice has been scientifically validated, as BPJ has proven antimicrobial properties, presumably due to its high tannin content (Duke, 1985; Scalbert, 1991; Chung et al., 1998; Ray, 2008; Ray and Ghatak, 2013; Ray et al., 2015).

Earlier studies (Ray et al., 2015) have indicated no-change in the lipid profile of raw cow milk and buffalo milk stored with BPJ at ambient temperature (30 ± 2°C). However, there is no study on proteolytic changes and molecular characteristics of milk casein of the milk stored with BPJ as preservative, under refrigeration, since rural households in West Bengal and Bangladesh generally store BPJ added milk in refrigerator for preservation. However, an earlier work has revealed that some protein fractions in semi-hard cheese samples during Mucor induced ripening were degraded into peptides of low molecular weights (Zhanq et al., 2013).

The present study was conducted to evaluate the effect of addition of BPJ (0.3%, v/v) on the shelf-life and electrophoretic profile of whole casein under refrigerated (7±2°C) storage.
MATERIALS AND METHODS

Collection of milk: Fresh cow and buffalo milk samples were procured from the local milk producers.

Extraction of Banana Pseudostem juice: Banana pseudostem juice (BPJ) was extracted, following the procedure described by Biswas (2004) and Bharti (2005) with slight modification. Martaman variety of banana (Musa paradisiaca) plant pseudostem was chopped into small pieces, and put into a hand driven mechanical juicer. The pseudostem pieces were subjected to high pressure in the juicer and the juice coming out of the pseudostem was collected, and then filtered by Whatman filter paper no. 40 to obtain a clean juice. Banana pseudostem juice was added to milk at the rate of 0.3% (v/v) as per Ray (2008).

Preservation of milk: Banana pseudostem juice 0.3% (v/v) treated cow and buffalo milk samples were kept under refrigeration at 7±2 °C for 5 days and 4 days, respectively (Ray, 2008). Control samples of cow and buffalo milks were also kept under same temperature for 3 days and 2 days respectively. PAGE profile of control and treated cow milk samples were determined after 3 days and 5 days respectively, whereas the same was done for buffalo milk samples after 2 days and 4 days respectively.

Preparation of Casein: Casein was obtained by adding 10% acetic acid to milk adjusting the pH at 4.6. Milk samples were diluted with distilled water at 1:1 ratio and warmed at 35°C before addition of acid. Coagulum was filtered with Whatman no. 42 filter paper, washed with water, alcohol, petroleum ether, and dried subsequently. One (1) mg of each dried sample of casein was dissolved in 500µl sample buffer and applied in to the lanes of PAGE.

Determination of PAGE profile of milk casein: Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) of cow and buffalo milk casein was carried out according to the method of Laemmli (1970). A GENEI make RODGEL electrophoretic apparatus equipped with power pack for maintaining constant voltage was used to determine the electrophoretic pattern. Bangalore Genei make standard medium range protein markers of five different molecular weights (14.3 kD, 29 kD, 43 kD, 68 kD, 97.4 kD) were used to compare the electrophoretic profile of milk casein samples.

Stacking Gel was prepared by using Tris HCl (pH6.8), 10% w/v SDS, acrylamide/BIS acryl amide (30%, 0.8%, w/v), Ammonium persulfate (10% w/v) and distilled water. Separating Gel was prepared by using acrylamide/BIS acryl
amide (30%, 0.8%, w/v), 1.5 M TRIS (pH 8.8), 10% w/v SDS, 10% w/v Ammonium persulfate and distilled water. Staining solution was prepared by using amido black, glacial acetic acid, methanol and distilled water, whereas destaining solution was prepared by using methanol, acetic acid and distilled water. Gel preparation was completed within 20-30 minutes.

20 µl of marker and 40 µl of each sample were applied in to the lane by micropipette. Gel tubes were fixed vertically between upper and lower electrode of the electrophoresis apparatus and subjected to constant electric supply of 9 mA for 3 hours after which gel tubes were taken out. The gel was stained overnight in Comassie Brilliant Blue solution and was destained by repeated

Figure-1. (I) Electrophoregram of milk protein: (A) standard marker. (B) untreated cow milk. (C) banana pseudostem juice treated cow milk. (D) untreated buffalo milk. (E) banana pseudostem juice treated buffalo milk. (II) Bands (1 and 2) represent casein band of molecular weights between 29 kD and 43 kD.
rinsing in the destaining solution till the separated components became prominent. After de staining, gels were analyzed under Gel Documentation System (BIORAD make).

**RESULTS AND DISCUSSION**

The electrophoretic (SDS-PAGE) behavior of cow and buffalo milk samples is presented in Figure-1. It revealed the appearance of two low molecular weight caseins in untreated cow milk as well as buffalo milk, so also in 0.3% (v/v) banana pseudostem juice (BPJ) treated cow milk and buffalo milk ranging between 29 kD and 43 kD, as compared to five molecular weight components (97.4 kD, 68 kD, 43 kD, 29 kD and 14.3 kD) in downward decreasing order, in the standard marker samples.

The SDS-PAGE pattern of isolated whole caseins of BPJ treated cow milk and buffalo milk did not exhibit any deviation from control cow and buffalo caseins. Non availability of data on the effect of banana pseudostem juice on the PAGE pattern of cow and buffalo milk caseins made it difficult to compare the results of the present finding. However, the report of Kumar and Mathur (1989) regarding negligible changes in various nitrogen fractions during preservation of buffalo milk under lactoperoxidase system was in accordance with the present finding.

**CONCLUSION**

It is concluded that addition of BPJ (0.3%, v/v) did not alter the molecular configuration of milk casein of raw cow milk and buffalo milk stored under refrigeration (7±2°C) for 5 days and 4 days respectively due to its proteolysis inhibitory effect.

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