Effect of banana (Musa paradisiaca) pseudostem juice on proteolytic and lipolytic changes in cow milk (Musa paradisiaca) during storage

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ABSTRACT
Proteolytic and lipolytic changes in cow milk were studied after addition of banana pseudostem juice (BPJ) for preservation. Raw cow milk added with 0.3 % (v/v) BPJ had a shelf life of 6 h at 30± 2°C unlike control sample (without BPJ) which kept well for 4 h only under identical condition during storage. The proteolytic changes in raw cow milk due to addition of BPJ was studied by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and fatty acid composition was determined by Gas Liquid Chromatography (GLC) technique. The electrophoretic profile of casein of both control and BPJ treated cow milk samples exhibited casein band of molecular weight between 29 KD and 43 KD. The concentration of saturated and unsaturated fatty acids was found to be 65.97% and 29.66% in cow milk. No significant change in the electrophoretic profile of BPJ treated samples was observed during the study when compared with that of untreated milk samples. Non-significant differences in the contents of saturated and unsaturated fatty acids as well as in the individual fatty acid were observed in raw cow milk preserved with BPJ for 6 h at 30± 2°C. Individual fatty acid present in cow milk also did not exhibit any significant change among themselves during storage. It is concluded that addition of 0.3 % (v/v) BPJ did not cause any significant change in the fat and protein quality of cow milk when stored at 30± 2°C for 6 h as electrophoretic and fatty acid profile is concerned.

Key words : BPJ, Chromatography, Cow milk, Electrophoresis, Milk, Lipolytic, Proteolytic.

INTRODUCTION
Milk is considered as most nutritious and perishable food but it is also a good medium for the growth of microorganisms. Special measures and considerations are necessary to ensure the quality of milk at the end user. In many parts of rural India, even in other countries, this is not possible for lack of proper infrastructural facilities. High ambient temperature prevailing in the tropical areas often compounded the problem. Successful preservation of milk at rural farmer’s level, therefore, requires utmost care. Addition of banana pseudostem juice (BPJ) to milk to increase its shelf life during transportation is a common practice among rural dairy farmers in West Bengal. Historically BPJ has been used to treat urinary tract infection, high blood pressure, kidney and gall bladder stone (SampathKumar et al., 2012). Banana pseudostem juice contain tannic acid as main constituents which was proven to have antimicrobial property (Scalbert, 1991; Chung et al., 1998). Ray (2008) noticed that banana pseudostem juice can enhance the shelf life of raw milk at different temperatures.

MATERIALS AND METHODS
Collection of milk: Holstein x Haryana cross breed cow milk was procured from the milk producers in the local Haringhata block in the district of Nadia, West Bengal during April when the average temperature ranged between 30-35°C. The animals from which milk samples were collected were fed on green grass and leaves grown in the pasture.

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The average contents of fat and SNF in cow milk were 3.62 ± 0.20 and 9.14±0.15% respectively. Milk samples were transported to the laboratory in a sterile polyethylene bottle immediately after addition of BPJ in milk after milking.

**Extraction and addition of banana pseudostem juice to milk:** BPJ was extracted following the procedure described by Biswas (2004) and Bharti (2005) with slight modification. Pseudostem of Martaman (Musa paradisiaca) variety of banana plant was chopped into small pieces, placed into a hand driven mechanical juicer and subjected to a high pressure in the juicer to obtain BPJ through an outlet located at the bottom of the juicer. The collected juice was filtered through a Whatman filter paper No.40. The average pH and tannic acid content in BPJ were 6.3±0.10 and 12.4±0.12 g/l, respectively. The average concentration of Zn, Mn, Mg, Ca and Na(expressed in mg/l) in BPJ were 0.8±0.01, 0.78±0.01, 26.1±1.02, 89.5±1.10 and 90±0.45, respectively while the content of Fe was 2.45±0.03 g/l. The level of 0.3% (v/v) BPJ juice for addition to milk was selected on the basis of the report of Ray (2008).

Fresh cow milk procured from local milk producers was separately added with BPJ at 0.3% (v/v) level immediately after procurement of milk and the treated milk samples along with control samples were kept at 30±2°C for 6 h.

**Chemical analysis of banana pseudostem juice:** Tannic acid content of the BPJ was estimated spectrophotometrically following the process described by Tinkilic and et al. (1992). Fe, Zn, Cu, Mn and Mg concentration of milk fat samples were determined by Gas Liquid Chromatography (GLC) technique after 0 and 4 h for control and 6h for BPJ treated samples. The samples were converted into methyl esters following the method described by Christie (1992). Fatty acid methyl esters of milkfat were prepared by the sealed tube following the method given by (Alonso et al., 1999). About 40-50 g of milk fat was weighed in glass ampoule and added with double volume of 0.2N sodium methoxide. The ampoule was heat sealed and kept in an oven at 80°C for esterification of fatty acids till the formation of a uniform layer. Prior to analysis, an ampoule was broken and sample injected without further treatment. GLC analysis was carried out in a double column gas chromatography unit (Model: Aimil –Nucon 5700, Japan) fitted with FID, temperature control modules and single pen recorder Ann.scribe. A 5 ft x1/4 inch glass column was packed with DMSC-80-100 (support) and DEGS –15% (liquid phase) followed by injection of the sample at 70°C. The temperature was increased at the rate of 4°C /min till it reached 200°C. Nitrogen was used as carrier gas and the temperature of injection port and detector was 220°C.

**Identification of fatty acid peaks and calculation of peak area:** Fatty acid peaks were identified by chromatographic standard mixture of methyl esters of known fatty acids. The peak areas were measured by planimeter having zero setting device and polar compensation with optical tracer followed by calculation of relative percentage of area (Alonso et al., 1999).
Table 1. Effect of addition of BPJ to cow milk on the fatty acid composition (mean ± S.E) during storage at 30±2°C

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Control sample at 0 h</th>
<th>Control sample after 4 h</th>
<th>Treated sample after 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4:0</td>
<td>3.70±0.1*</td>
<td>3.68±0.1*</td>
<td>3.65±0.1*</td>
</tr>
<tr>
<td>C6:0</td>
<td>2.11±0.2*</td>
<td>2.07±0.2*</td>
<td>2.10±0.2*</td>
</tr>
<tr>
<td>C8:0</td>
<td>1.14±0.1*</td>
<td>1.16±0.2*</td>
<td>1.12±0.1*</td>
</tr>
<tr>
<td>C10:0</td>
<td>2.61±0.03*</td>
<td>2.59±0.4*</td>
<td>2.59±0.2*</td>
</tr>
<tr>
<td>C12:0</td>
<td>2.72±0.03*</td>
<td>2.72±0.4*</td>
<td>2.70±0.2*</td>
</tr>
<tr>
<td>C14:0</td>
<td>11.93±1.5*</td>
<td>11.90±1.6*</td>
<td>11.92±1.3*</td>
</tr>
<tr>
<td>C14:1</td>
<td>2.12±0.2*</td>
<td>2.14±0.2*</td>
<td>2.12±0.1*</td>
</tr>
<tr>
<td>C16:0</td>
<td>29.85±0.1*</td>
<td>29.90±0.2*</td>
<td>29.92±0.1*</td>
</tr>
<tr>
<td>C16:1</td>
<td>2.14±0.3*</td>
<td>2.15±0.2*</td>
<td>2.14±0.2*</td>
</tr>
<tr>
<td>C17:0</td>
<td>1.34±0.1*</td>
<td>1.30±0.1*</td>
<td>1.32±0.1*</td>
</tr>
<tr>
<td>C18:0</td>
<td>10.57±0.5*</td>
<td>10.51±0.6*</td>
<td>10.52±0.3*</td>
</tr>
<tr>
<td>C18:1</td>
<td>23.47±2.1*</td>
<td>23.35±2.2*</td>
<td>23.42±1.98*</td>
</tr>
<tr>
<td>C18:2</td>
<td>1.35±0.2*</td>
<td>1.35±0.2*</td>
<td>1.35±0.2*</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.58±0.01*</td>
<td>0.56±0.01*</td>
<td>0.57±0.01*</td>
</tr>
</tbody>
</table>

Average of five (5) replicates

Means having different superscripts within a row vary significantly (P<0.05)

Statistical analysis

The results, expressed as mean ± standard error of mean (SEM), were analyzed by one- way analysis of variance (ANOVA) to test the significance of difference among different fatty acids using the SPSS version 11.0.1 (2001) for windows software package (SPSS Inc., Chicago, IL, USA) followed by Duncan’s (1957) multiple range test.

RESULTS AND DISCUSSION

Based on titratable acidity (% lactic acid), clot-on- boiling test and alcohol test, the shelf life of control samples and treated samples (containing 0.3% v/v of BPJ) of cow milk was found to be 4 and 6 h, respectively during storage at 30±2°C. The identity and relative proportions of different fatty acids from the peaks as depicted by the chromatograms after 0 and 4 h for control and 6 h for treated cow milk are presented in Table 1.

The PAGE pattern of cow milk is presented in Figure 1. Casein profile of control milk samples as well as 0.3% (v/v) BPJ treated samples were studied by employing Vertical Rod Polyacrylamide Gel Electrophoresis technique. Electrophoretic profile of control cow milk samples kept at 30±2°C was determined after 4 h of storage whereas electrophoretic profile of 0.3% (v/v) BPJ treated samples were determined after 6 h of storage. Five standard markers were used in both cases to compare the result.

It was observed that both control and BPJ treated cow milk whole casein were resolved into two bands. The electrophoretic pattern revealed the appearance of two low molecular weight components in the milk samples when compared with the standard marker samples having five (5) different molecular weight (97.4KD, 68KD, 43 KD, 29 KD and 14.3KD in downward decreasing order).

The proportion of saturated and unsaturated fatty acids in fresh cow milk were 65.97% and 29.66% respectively (Table 1). Proportion of saturated and unsaturated fatty acids decreased non-significantly to 65.84% and 29.41% respectively in BPJ treated cow milk after 6 h of storage. Palmitic acid (C16:0) was found to be highest among the saturated fatty acids with an average concentration of 29.85% in cow milk. Non-significant increase in palmitic acid content to 29.92% in BPJ treated cow milk sample was observed after 6 h of storage. Among the unsaturated fatty acids proportion of oleic acid (C18:1) was found to be highest cow milk with an average concentration of 23.47%. The chromatograms for identification and calculation of peak areas have been depicted in Figure 2 and 3, for control cow milk and BPJ treated cow milk respectively. No significant variation in the concentrations of other saturated and unsaturated fatty acid were observed in cow milk treated with 0.3% v/v BPJ after 6 h of storage at 30±2°C.
Preservation of quality of raw milk from the producer farmer’s level to the processing plant always creates problem in the tropical countries like India. In a country like India, where milk production points are located in remote areas, availability of thermal and chemical technologies are very scare and cost involving. There has been existing lot scope of using different herbs and plants to preserve and extend the shelf life of milk in remote areas without affecting the quality of milk. Biswas (2004) reported the use of banana pseudostem juice in fishery and agriculture as a very good antimicrobial agent. Bharti (2005) reported the use of banana pseudostem juice in preservation of milk. Sanyal (2013) reported that environmental condition (both macro and micro), animal production system, animal breed, type of breeding rearing procedure of animals, type of feed for animals, pastures influence the characteristics of milk and the preservation process. But there has always been a chance of deterioration of nutritional quality of milk when an external agent is added to milk for preservation. In this study, the effect of addition of banana pseudostem juice on electrophoretic and fatty acid profile of cow milk, collected from Holstein x Haryana cross breed cow, during the month of April and fed on green grass and leaves, was reported.

The composition of BPJ in the present study agrees well with that reported by Biswas (2004). The increase in shelf life of cow milk treated with 0.3% v/v BPJ up to 6h of storage at 30±2°C proves that banana pseudostem juice may well be used to preserve raw milk at rural level where no other alternative methods of preservation are available. This finding agrees well with that reported by Bharti (2005).

The SDS-PAGE pattern of isolated protein revealed that there was no change in the electrophoretic pattern of 0.3% (v/v) banana pseudostem juice treated cow milk casein as compared to control cow milk casein after 6 h of storage at 30±2°C. The proportion of saturated and unsaturated fatty acid in control and BPJ treated cow milk and the fatty acid profile of control and BPJ treated cow milk that are presented in Table 1 and 2 revealed that proportion of saturated and unsaturated fatty acid as well as individual fatty acids did not show any statistically significant change in concentrations after 6 h of storage at 30±2°C. The results could not be suitably compared due to lack of published literature. Addition of 0.3% v/v BPJ to cow milk did not seem to affect significantly the quality of fat suggesting that banana pseudostem juice may safely be added to raw milk to preserve it without deteriorating the quality of milk.

CONCLUSION

The present study was focused on the effect of addition of banana pseudostem juice on the electrophoretic and fatty acid profile of cow milk. It could be concluded from the present study that the keeping quality of cow milk can be enhanced up to 6 h at 30±2°C through incorporation of 0.3% v/v BPJ which did not cause any significant proteolytic, lipolytic and oxidative change in the raw cow milk. However in depth studies on other biochemical parameters need to be carried out before arriving at any final conclusion on safety of such BPJ added milk from human health point of view.

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