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Effects of Starter Culture and Types of Bamboo Tubes on Microbiological and Shelf Life Characteristics of Buffalo Milk *Banhor Chunga Doi*- an Ethnic Product of India

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ABSTRACT

Background: The present research was undertaken to study the effects of yoghurt culture and bamboo tubes on the microbiological and shelf life attributes of curd prepared from buffalo milk in bamboo tubes (popularly known as "*Banhor Chunga Doi*" in Assam) under room temperature.

Methods: *Banhor Chunga Doi* was prepared using *Jati* and *Jatie Makal* bamboo tubes. Starter culture was added and the tubes were kept in ambient room temperature. The shelf-life of the set products was ascertained by objective assessment of pH, titratable acidity and microbial load.

Result: Amongst the 4 treatment groups, the samples of B(JB) *i.e.*, *Banhor Chunga Doi* prepared from boiled and cooled buffalo milk and stored in tubes of *Jati* bamboo at room temperature for 5d showed the highest pH value of 4.01±0.011 with a corresponding least lactic acid content of 1.21±0.005%. Amongst the 2 control and 4 treatment groups, the highest mean TVC of 8.69 $\log_{10}$cfu/g±0.001 was enumerated in the samples of SC (JB) which might be due to the addition of the starter culture at the dose level of approximately 6.86 $\log_{10}$cfu/g in the beginning of the production process. The starter culture added samples [SC (JB) and SC (JMB)] showed an increasing TVC up to 3rd day of storage. Thereafter, the TVC of these samples decreased on 5th day. The lowest mean TVC of 4.38±0.003 $\log_{10}$cfu/g was noted in the samples of B (JMB) which is due to the thermal destruction of the contaminating microbes during boiling of the milk sample. The samples of the buffalo milk *Banhor Chunga Doi* that were not inoculated with the starter culture exhibited an increasing TVC from 1st to the 5th d of storage at room temperature. Over the storage period, none of the *Banhor Chunga Doi* samples either from the control or the treatment groups were found to possess coliform organisms, *Escherichia coli*, *Salmonella*, *Shigella*, *Staphylococcus aureus,* yeast and moulds. The shelf life of the end product was found to be good for consumption upto 5 days with gradual decrease in pH values and concomitant increase in titratable acidity in all the treatment groups.

Key words: Bamboo tubes, Buffalo milk, Microbiology, Shelf life, Starter culture.

INTRODUCTION

Fermented milk products have been an integral part of human diet because of its improved sensory, nutritional, prophylactic as well as therapeutic properties. Indigenous traditional knowledge is generally employed in preparation of vast majority of the fermented milk products and the background microbiota of the milk is mostly relied upon to bring about fermentative changes in the milk which fail to guarantee uniformity in the sensory properties and shelf-life of the product. The 'back slopping' technique is largely followed. Application of dairy starter cultures has the proven advantages of uniformity in products' quality, better shelf-life, improved sensory qualities and over and above significant improvement in safety indices of the final product.

Over the years, the people of Assam have developed an ITK for fermentation of buffalo milk in bamboo tubes. The product is popularly known as "*Banhor Chunga Doi*". It is prepared by fermenting fresh, raw buffalo milk in a bamboo tube capped with either banana leaves or paddy straw at room temperature for few days. Fermentation is mostly relied upon on the background microflora of the milk, of the bamboo tube as well as the microflora of the banana leaves/paddy straw used for capping the bamboo tubes. Spontaneous and uncontrolled fermentation takes place and therefore day to day variations in eating quality, safety indices and storage life of the product are common. Various types of locally available bamboo tubes, *viz, Jati* bamboo, *Jatie makal* bamboo and *Kako* bamboo tubes are used for *doi* production in Assam.

Shelf-life of *Banhor Chunga Doi* vary considerably due to uncontrolled production management. The types of

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bamboo may also have certain influence on the aroma and other eating quality attributes of the product. Since, addition of starter culture has been reported to enhance shelf-life of fermented milk with superior eating quality characteristics (Kiran *et al.,* 2012), it may be an approach towards value addition to employ dairy starter culture in preparation of *Banhor Chunga Doi* to ensure products' uniformity with superior sensory attributes, increased shelf-life and safety indices for better marketability of the product.

Literature survey, however, revealed scanty information on the use of starter culture in production of *Banhor Chunga Doi*. Therefore, in the present study an attempt was made to study the effect of dairy starter culture and types of bamboo tubes on the microbiological quality and shelf life of buffalo milk *Banhor Chunga Doi* under room temperature storage.

MATERIALS AND METHODS

The study was undertaken in the laboratories of the Department of Livestock Products Technology and in the All India Coordinated Research Project on Post-Harvest Engineering and Technology, College of Veterinary Science, Assam Agricultural University, Khanapara Campus, Guwahati during the period from August, 2018 to February, 2019.

Procurement of raw materials

Fresh raw buffalo's milk and *Jati* and *Jatie Makal* bamboo tubes for the study was procured from the local market. The sizes of the *Jati* and *Jatie Makal* bamboo tubes were 40 cm × 6 cm and 40 cm × 8 cm, respectively.

Starter culture

Yoghurt culture NCDC 144 (mixed culture of *Lactobacillus delbrueckeii* ssp. *bulgaricus* and *Streptococcus thermophilus*) obtained from the National Dairy Research Institute, Karnal, Haryana-132001 was used in the study. Freeze dried culture was inoculated in reconstituted and sterilised skim milk (12% w/v). Working cultures were maintained in skim milk, sub-cultured at weekly interval and the stock culture was maintained in nutrient agar slants. Stock culture was stored at (-) 20°C and sub-cultured at 3 month's interval. Active culture of sixteen to eighteen hour was prepared by inoculating the stock culture @ 2% in sterilised skim milk (12% w/v). Repeated sub-culturing, at least three times, was done before using it as starter culture for preparation of *Banhor Chunga Doi*.

Banhor Chunga Doi preparation

Banhor Chunga Doi was prepared by following the standard technique given by Aneja *et al*. (2002) with slight modification (APPENDIX I). For the purpose, *Jati* and *Jatie makal* bamboo tubes were used and filled with buffalo milk and allowed to set at room temperature.

Immediately after setting, the products were analysed for their microbiological quality. For shelf-life study, samples were kept at room temperature till spoilage. pH, acidity and microbial load were assessed on fresh product (1st day), 3rd and 5th day.

Treatment groups

The following treatment groups of *Banhor Chunga Doi* were prepared:

Control (JB)	Raw buffalo milk in *Jati* bamboo tube.
Control (JMB)	Raw buffalo milk in *Jatie makal* bamboo tube.
Boiled (JB)	Boiled and cooled buffalo milk in *Jati* bamboo tube.
Boiled (JMB)	Boiled and cooled buffalo milk in *Jatie makal* bamboo tube.
SC (JB) starter	Boiled and cooled buffalo milk+active culture of NCDC 144 [@ 1.5% (v/v)] in *Jati* bamboo tube.
SC (JMB)	Boiled and cooled buffalo milk + active starter culture of NCDC 144 [@ 1.5% (v/v)] in *Jatie makal* bamboo tube.

Microbiological analysis of *Banhor Chunga Doi* and bamboo tubes

Microbiological analysis of *Banhor Chunga Doi* samples were done by standard method (Harrigan and McCance, 1976). Total viable count, coliform count and counts for *Escherichia coli*, *Salmonella* and *Shigella*, *Staphylococcus aureus*, yeast and moulds were enumerated by using appropriate media (Plate Count Agar, Endo Agar, Salmonella-Shigella agar, Mannitol Salt Agar Base and in Rose Bengal Chloramphenicol Agar Base, respectively) and optimum incubation conditions. *Lactobacilli* of bamboo tubes was also enumerated in *Lactobacilli* MRS Agar.

Shelf-Life of *Banhor Chunga Doi*

The shelf-life of the set products was ascertained by objective assessment of pH using digital pH meter (Model 780, Metrohm, Switzerland), titratable acidity by following the method of Atherton and Newlander (1977) and microbial load (Total Viable Count, coliform, *Escherichia coli*, *Salmonella* and *Shigella*, *Staphylococcus aureus*, yeast and mould counts) was enumerated by standard techniques given by Harrigan and McCance (1976).

Statistical analysis

Experimental data obtained from the experiment were analysed by using Randomised Block Design (RBD) and ANOVA was done with the help of SAS 9.4.

RESULTS AND DISCUSSION

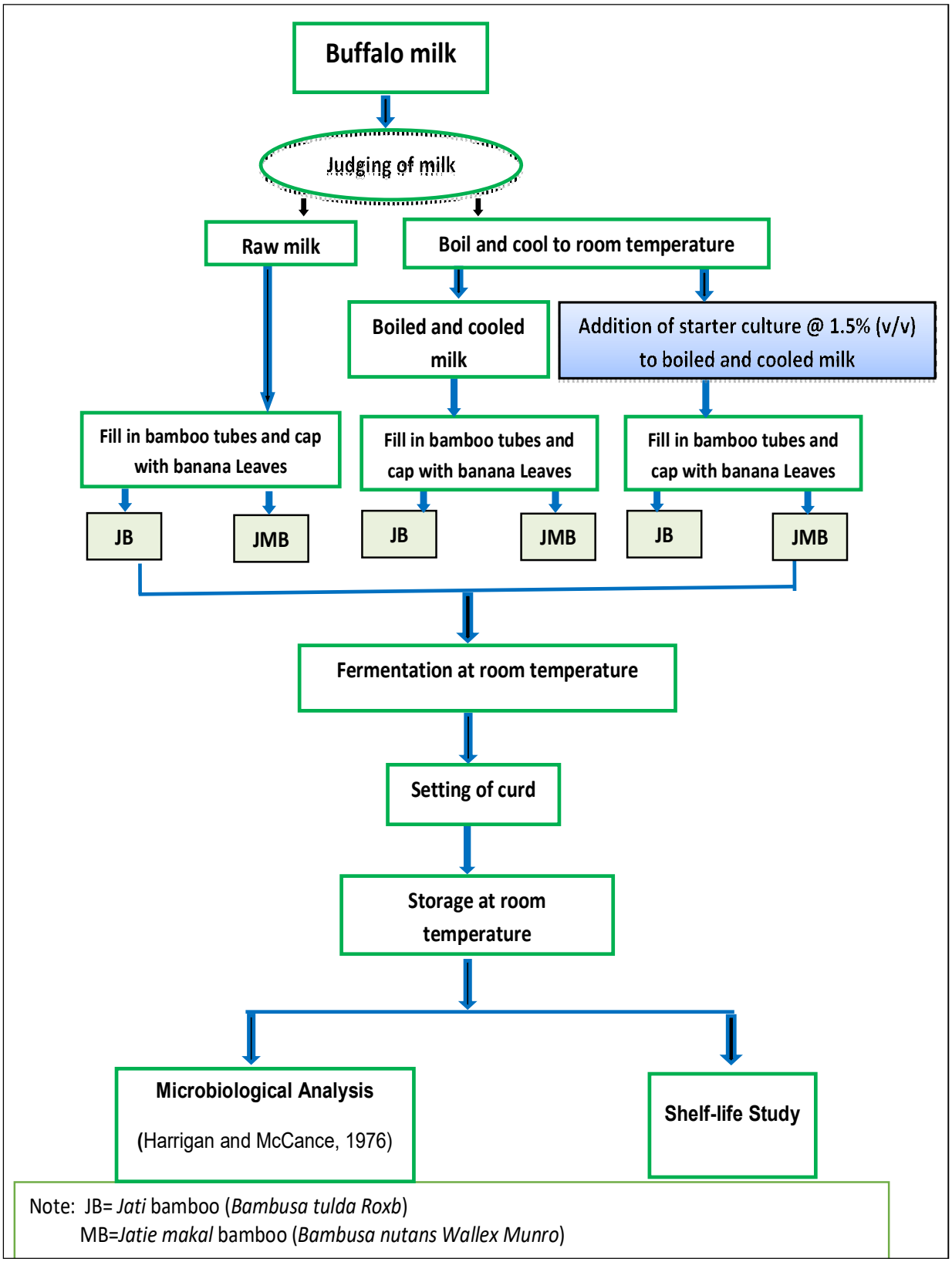
Enumeration of microorganism

Aerobic count of bamboo tubes

In the present study, nil lactobacilli were recorded in the bamboo tubes of all types (Table 1). However, some

Table 1: Total viable and lactobacilli count (Mean±Se) of bamboo tubes (Log_{10}cfu/Ml).

Bacteriological Count	*Jati* bamboo	*Jatie makal* bamboo	t-value
Total viable count	2.815±0.017	2.780±0.040	0.806
Lactobacilli count	0.00	0.00	0.00



Appendix 1: Technical programme of research work.

researchers have reported presence of LAB in bamboo tubes (Puspawati *et al*., 2018).

Shelf life

Shelf life of the finished products were judged by assessing their pH values, titratable acidity and microbiological counts on 1st, 3rd and 5th day of storage.

pH and titratable acidity

Mean pH values of all the treatment and control groups of *Banhor Chunga Doi* showed a decreasing trend during the entire storage period at ambient temperature. Ready products exhibited a variation in mean pH values between 4.58±0.01and 4.99±0.005 on 1st day which showed a further decrease till 5th day of storage (Table 2). Among the treatment groups, SC added (JMB) product showed the least pH of 3.33±0.13 on 5th day. Such decline in pH values of finished product during storage was also reported by Sokolinska *et al*. (2004), Han *et al.* (2012) and Karsheva *et al.* (2013). This decrease in pH values may be presumed to be due to continued lactic acid bacterial fermentation during storage (Shah, 2000; Kailasapathy, 2006) as was indicated by increasing TVC in treatment groups - boiled (JB and JMB) and SC added boiled (JMB) products. The decreasing trend in pH in the present study may not only be due entirely to LAB but also may be due to proteolytic bacteria present naturally in the bamboo tubes and banana leaves used for capping the tubes (Nurmiati *et al.,* 2018).

Analysis of variance (Table 3) of data on the effects of starter culture and types of bamboo on pH of *Banhor Chunga Doi* showed highly significant variations between treatments and days of storage while interaction effect between treatments and days of storage did not show any significant variation (P>0.05).

A gradual increase in the acid content from 1st to 3rd day could be noted in all the groups between 0.96±0.009 and 1.38±0.011 while it was not observed in control (JB) and SC added JMB products (Table 4). This might be attributed to the production of lactic acid from lactose of milk by LAB present in raw milk or by SC being added to boiled and cooled milk during the fermentation process (Fadela *et al.,* 2009). In general, boiled milk products prepared in both JB and JMB exhibited somewhat lesser acid content on 5th day (1.21±0.005 and 1.23±0.004) as compared to control and SC added JB and JMB products (Table 4). This might be due to the effect of boiling the milk

Table 3: Analysis of variance of data on effects of starter culture and types of bamboo on ph of *Banhor Chunga Doi.*

Source of variation	df	Mean square of dependent variable
Treatment	4	0.602991**
Day	2	10.87011**
Treatment × Day	10	0.042326
Error	20	0.000433

**Significant at P<0.01.

Table 2: Effects of starter culture and types of bamboo on ph (mean±se) of *Banhor chunga doi.*

Days	Treatments				
	C(JB)	C(JMB)	B(JB)	B(JMB)	SC(JB)	SC(JMB)
1	$_{c}4.91\pm0.005^{A}$	$_{c}4.83\pm0.007^{A}$	$_{b}4.99\pm0.005^{A}$	$_{b}4.94\pm0.009^{A}$	$_{c}4.82\pm0.010^{A}$	$_{b}4.58\pm0.010^{A}$
3	$_{b}4.45\pm0.010^{AB}$	$_{b}4.38\pm0.007^{AB}$	$_{b}4.71\pm0.010^{B}$	$_{b}4.65\pm0.011^{B}$	$_{b}4.36\pm0.011^{AB}$	$_{b}4.22\pm0.007^{A}$
5	$_{a}3.73\pm0.008^{AB}$	$_{a}3.49\pm0.011^{A}$	$_{a}4.01\pm0.011^{B}$	$_{a}3.98\pm0.007^{B}$	$_{a}3.45\pm0.010^{A}$	$_{a}3.33\pm0.13^{A}$

n=5.
Mean having different subscripts in the column (small letter) differ significantly (P<0.01)
Mean having different superscripts in the row (capital letter) differ significantly (P<0.01).

Table 4: Effects of starter culture and types of bamboo on titratable acidity (% lactic acid) (Mean ±SE) of *Banhor Chunga Doi*.

Days	Treatments				
	C(JB)	C(JMB)	B(JB)	B(JMB)	SC(JB)	SC(JMB)
1	$_{a}0.98\pm0.006^{A}$	$_{a}1.06\pm0.004^{B}$	$_{a}0.96\pm0.009^{A}$	$_{a}0.98\pm0.005^{A}$	$_{a}1.06\pm0.007^{B}$	$_{a}1.12\pm0.007^{C}$
3	$_{b}1.21\pm0.009^{B}$	$_{b}1.23\pm0.007^{BC}$	$_{b}1.09\pm0.004^{A}$	$_{b}1.11\pm0.004^{A}$	$_{b}1.24\pm0.009^{C}$	$_{b}1.38\pm0.011^{D}$
5	$_{c}1.28\pm0.007^{B}$	$_{c}1.39\pm0.010^{C}$	$_{c}1.21\pm0.005^{A}$	$_{c}1.23\pm0.004^{A}$	$_{c}1.48\pm0.007^{D}$	$_{c}1.50\pm0.003^{D}$

n=5

Mean having different subscripts in the column (small letter) differ significantly (P<0.01).

Mean having different superscripts in the row (capital letter) differ significantly (P<0.01).

Table 5: Analysis of variance of data on effects of starter culture and types of bamboo on titratable acidity (% lactic acid) of *Banhor Chunga Doi*.

Source of variation	df	Mean square of dependent variable
Treatment	5	0.136273**
Day	2	0.770881**
Treatment × Day	10	0.009205
Error	72	0.000231

**Significant at P<0.01.

before allowing it to undergo natural fermentation. These findings are in accordance to the findings of Metwally *et al*. (2011) who reported a reduction in bacterial count of buffalo milk by boiling, for 0.5 to 1 min, from 7.8×10^{9} to 2.26×10^{3}cfu/ml and 1.3×10^{3}cfu/ml, respectively. The titratable acidity of the finished product showed highly significant variations between treatments and days of storage while interaction effect between treatments and days of storage did not show any significant variation (P>0.05) (Table 5).

Microbiological analysis

A sharp increase in TVC of all the groups including the control groups was noted from 1st to the 3rd day of storage at ambient temperature between 4.380±0.003 and 9.332±0.004 (Table 6). Here after, irrespective of the bamboo types used, a decrease in TVC of both the control and SC added samples was recorded. This might be attributed to the fact that some of the organisms might have reached their stationary phage in their life cycle resulting in lesser count on 5th day of storage. On the other hand, boiled JB and JMB samples showed a gradual increasing trend in TVC from 1st to 5th day of storage at ambient temperature (Table 6). Such variation in TVC of *dahi* samples might be due to the metabolites produced by SC or by other natural fermenting LAB as well as their storage condition (Sivakumar and Kalaiarasu, 2010). Initial increase in TVC may be attributed to faster growth of LAB (Hamann and Marth, 1984). Effect of type of bamboo on TVC was also documented by Puspawati *et al.* (2018). Among the different bamboo tubes used, TVC of dadih samples prepared in dry petung bamboo samples, showed the maximum TVC of 9.4×10^{9} to 1.5×10^{10}cfu/g. In general, a higher TVC in SC added treatment groups during the entire storage period was noted which might be due to the addition of SC@6.86$\log_{10}$cfu/ml.

Analysis of variance (Table 7) of data on the effects of starter culture and types of bamboo on microbiological quality of *Banhor Chunga Doi* showed highly significant variations between treatment groups and days of storage at room temperature but interaction effect between treatments and days of storage did not show any significant variation (P>0.05)

Nil coliform, *E. coli*, *Salmonella*, *Shigella*, yeast and moulds in *Banhor Chunga Doi* samples indicate proper sanitary/ hygienic conditions followed during the production process as well as production of probable bacteriocin during

Table 6: Effects of starter culture and types of bamboo on total viable count (Mean ±Se) of *Banhor Chunga Doi* (log_{10}cfu/g) during storage (room temperature)*.

Days	Treatments					
	C(JB)	C(JMB)	B(JB)	B(JMB)	SC(JB)	SC(JMB)
1	$_{a}7.833\pm0.002^{D}$	$_{a}7.690\pm0.001^{C}$	$_{a}4.431\pm0.002^{B}$	$_{a}4.380\pm0.003^{A}$	$_{a}8.690\pm0.001^{F}$	$_{a}8.544\pm0.001^{E}$
3	$_{c}8.431\pm0.001^{D}$	$_{c}8.243\pm0.002^{C}$	$_{b}6.204\pm0.002^{B}$	$_{b}6.130\pm0.002^{A}$	$_{c}9.332\pm0.004^{F}$	$_{c}9.114\pm0.003^{E}$
5	$_{b}8.013\pm0.003^{D}$	$_{b}7.942\pm0.001^{C}$	$_{c}6.455\pm0.002^{B}$	$_{c}6.290\pm0.001^{A}$	$_{b}9.119\pm0.005^{F}$	$_{b}8.921\pm0.001^{E}$

Mean having different subscripts in the column (small letter) differ significantly (P<0.01).

Mean having different superscripts in the row (capital letter) differ significantly (P<0.01).

*Coliform, *E.coli*, *Salmonella*, *Shigella*, *Staphylococcus aureus,* yeast and molds, anaerobic spore count not be detected.

Table 7: Analysis of variance of data on effects of starter culture and types of bamboo on total viable count of *Banhor Chunga Doi* (log_{10}cfu/g) during storage (room temperature).

Source of Variation	df	Mean square of dependent variable
Treatment	5	36.34025**
Day	2	5.803846**
Treatment × Day	10	1.614118
Error	72	0.0000271

**Significant at P<0.01.

the fermentation process (Sivakumar and Kalaiarasu, 2010; Chowdhury *et al*., 2011; Igbabul *et al*., 2014).

Suggestion

Since non-availability of suitable commercial yoghurt culture in the region is a hindrance in transfer of the technology to the traditional producers of *Banhor Chunga Doi,* development of a repository of dairy starter cultures and commercial production of suitable yoghurt cultures in the region may be a boon to the traditional producers in production of uniform quality *Banhor Chunga Doi* with superior sensory-, microbiological- and safety indices.

CONCLUSION

From the study it could be concluded that good quality buffalo milk curd may be prepared by using *Jati* and *Jatie makal* bamboo tubes. The ITK on use of both *Jati* and *Jatie makal* bamboo tubes in preparation of *Banhor Chunga Doi* is validated. Boiling and cooling of the buffalo milk and addition of starter culture @1.5% (v/v) prior to filling in bamboo tubes add value to the finished product in terms of microbiological attributes. Buffalo milk *Banhor Chunga Doi* had a shelf-life of 5d at room temperature.

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Conflict of interest

All authors declare that they have no conflict of interest.

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