

## Studies on the Physico-Chemical and Microbiological Analysis of Plain Dahi of Assam (India)

Mona Deb and \*Dibyakanta Seth

Assistant Professor, Department of Food Engineering and Technology, Tezpur University, Tezpur-784028, Assam, India.

### Abstract

Dahi (Indian yoghurt), a popular fermented milk product of Eastern and North-Eastern part of India have lot of variations in the preparation methods. A market study was carried out to evaluate the quality characteristics of set dahi (curd) of Assam, India. Curd samples from different districts were collected randomly and analyzed for physico-chemical, microbiological and organoleptic properties. Physico-chemical analysis revealed that there was lot of variations in the constituents of dahi samples. The fat, protein, lactose, ash, total solid, pH, titratable acidity, syneresis were in the range of 2.45-3.60%, 2.66-3.69%, 4.12-4.73%, 0.48-0.74%, 12.38-18.55%, 4.11-5.05, 0.58-1.07% lactic acid and 28.09-38.57% respectively. The variations in the samples were significant at  $p < 0.05$ . The colour measuring indices L, a and b values ranged from 57.29-85.25, (0.33)-(1.69) and 1.90-2.39 respectively. Among these L value only showed significant changes. The microbiological studies showed that total plate count were within the maximum permissible limit. The total coliform counts were observed in some of the samples. Organoleptically, there were variations in the quality characteristics of dahi samples. It was concluded that due to variations in sources of raw milk, processing and storage conditions, uniformity in the quality cannot be maintained in traditional products.

**Keywords:** Plain dahi, Physico-chemical, Microbial, Organoleptic properties.

\*Corresponding Author:

Dibyakanta Seth

Email: [dibya05@tezu.ernet.in](mailto:dibya05@tezu.ernet.in)

Received: 19/04/2014

Revised: 10/05/2014

Accepted: 20/05/2014

### 1. Introduction

Dahi is a fermented dairy product of major importance in Indian subcontinent. From the time immemorial it is being used for its nutritive and therapeutic values (Aneja *et al.*, 2002). In Ayurveda, use of dahi is recommended for curing of ailments like dysentery, dyspepsia and other gastrointestinal disorder (Gandhi, 2010). It also improves appetite by stimulating B and T cells of macrophages by lactic acid bacteria (Meydani and Ha, 2000; Sinha and Sinha, 2000). Readily available form of all the nutrients is also present in dahi. It alleviates bone problems and a good form of predigested food (Patel and Singh, 2011). Bureau of Indian Standards (1980) defines dahi as a product obtained by lactic fermentation of cow or buffalo or mixed milk caused by single or mixed strains of lactic acid bacteria or by alcoholic fermentative yeasts. The preparation of dahi includes pasteurization of raw milk, standardization followed by inoculating the standardized milk with starter cultures

and incubation at about 37-42°C depending on the types of cultures used (Tamime and Robinson, 1999).

Traditionally, it is produced by boiling cow, buffalo or mixed milk fermented naturally or with previous day's dahi (Gandhi and Muralidhara, 1989). Because of the natural souring process, wide variations are seen in the quality of market dahi (Younus *et al.*, 2002). The starter cultures used in dahi are not definite and the quality of dahi varies according to the culture used (Masud *et al.*, 1991). There are reports of wide variations in the physico-chemical, microbial and sensory qualities of yoghurt and yoghurt like products in many countries (Younus *et al.*, 2002; Obi and Ikenebomeh, 2007; El-Bakri and El-Zubeir, 2009).

In India, the north-east states like Assam, Arunachal Pradesh, Meghalaya, Manipur etc. are known for their multi-ethnic, multi-cultural people in inhabiting here. Consequently, the food habits changes from one community to other (Baruah, 2009). Assam, in itself has a tradition of producing different fermented products and dahi is one among those products

prepared and consumed, though not in large volume. The total milk production in Assam was 0.79 million tons in the year 2010-2011 (NDDB, 2012). Marketing of milk and milk products in Assam is relatively insignificant. Traditional/unorganized sector dominates about 97% of the milk market (NIAM, 2012). Traditional knowledge for the preparation of dahi is followed in Assam even though a lot of advancements in research have been achieved worldwide. Dahi is prepared with or without using starter cultures popularly known as *Assamese curd*. Moreover, as bamboo is abundantly available in this area and its flavour is liked by people of Assam, this liking reflects in preparation of dahi by incubating inoculated milk inside the bamboo stem. Some people also prepare dahi by inoculating milk by earthworm extracts. The various methods of preparation give different sensory attributes and believed to have different physicochemical and microbiological characteristics. Keeping in view the ethnicity of preparation of dahi, a thorough study is of utmost importance in this region. Since, there has not been any documented market survey of *Assamese curd*; this study was designed to evaluate market dahi for physico-chemical, micro-biological and sensory properties.

## 2. Materials and Method

### 2.1 Sample Collection

Dahi were collected from six districts of Assam, India based on their topological and cultural variations viz. Guwahati, Tezpur, Tihu, Jorhat, Goalpara and Tinsukia. They were coded as GT, TZ, TH, JH, GL and TN respectively. Fresh *dahi* was collected from the vendors/shopkeepers and was brought to the University campus keeping the samples in ice box. It was ensured that the temperature variation was not remarkable in the product. Three samples from each location were collected. The samples were immediately analyzed in the laboratory of the Department of Food Engineering and Technology, Tezpur University, Assam.

### 2.2 Physico-Chemical Analysis

The pH and titratable acidity (% Lactic acid) were determined using standard methods as described by AOAC (1996). Digital pH meter (Eutech Instruments, Mumbai, India) with temperature sensor was used for pH measurement (Method No. 981.12) (Younus *et al.*, 2002). Fat was determined by Mojonnier method. Protein content was estimated from the total nitrogen present which was measured by micro-Kjeldahl method (IDF, 1993). Ash content was determined by AOAC (1996). Lactose was determined as per the method described in Bureau of Indian

Standards (BIS, 1981). Total solids were determined according to the modified method of AOAC (1996). Syneresis was measured as described by Peri *et al.* (1985), Raju and Pal (2009). The color of the dahi was determined using a High Performance Color Measuring Spectrophotometer (Hunter Lab, USA).

### 2.3 Microbiological Analysis

The microbiological analysis of samples was carried out for total viable count and coliform count using pour plate technique as described by Resubal (1977). Coliform counts were estimated using Eosin Methylene Blue agar (Merck) incubated at 37°C for 24 hours.

### 2.4 Sensory Analysis

The sensory characteristics like color, flavour, texture, taste and overall acceptability of dahi samples were analyzed using a nine point hedonic scale as described by Larmond (1977). A panel of six judges was selected from the department for the analysis.

### 2.5 Statistical Analysis

Significance differences ( $p < 0.05$ ) were statistically analyzed in single factor analysis of variance using the statistical software tool (SPSS-13, Chicago IL).

## 3. Results and Discussion

### 3.1 pH and Titratable Acidity

The pH and titratable acidity of dahi samples are shown in Table 1. The sample code TH showed the highest average pH of 5.05 ( $\pm 0.21$ ) while sample GT showed the lowest average pH of 4.11 ( $\pm 0.04$ ). The results of pH between different samples were significant at  $p < 0.05$ . The variation in pH might be due to methods of preparation such as incubation time and temperature, amount of starter culture added and the fermentation container. Titratable acidity of different samples also showed significant differences. Sample GL showed highest acidity of 1.07% ( $\pm 0.11$ ) while sample TH had the lowest mean acidity of 0.58% ( $\pm 0.13$ ). The pH and acidity values showed an inverse relation which is in line with the findings of Vahedi *et al.* (2008). So, it was interpreted that, the differences in the pH and acidity values among dahi samples were due to the fermentation conditions.

### 3.2 Fat

Fat content of the samples are enlisted in Table 1. The sample TZ showed the highest mean fat content of 3.60% ( $\pm 0.50$ ) and the lowest mean fat content was observed in sample GT having mean fat content of

2.45% ( $\pm 0.02$ ). The values of fat content were significantly different among the samples ( $p < 0.05$ ). The reason of high fat content in samples might be attributed to its preparation method of using bamboo stem as the fermenting medium. There are number of oils been identified in bamboo stem for example palmitic acid (16.5%), E-nerotidiol (10.2%) and E-indol (8.1%), (Takahashi *et al.*, 2009). Moreover, fat in dahi depends upon the initial fat content of milk and some level of volume reduction during boiling. The fat values agree with the findings of Inoue *et al.* (1998).

### 3.3 Protein

The protein content of the samples are enlisted in Table 1. The protein content of dahi samples showed significant differences ( $p < 0.05$ ). Sample TN showed highest mean protein value of 3.69 with a standard deviation of 0.18 while the lowest mean protein values was shown by sample JH (2.66) with a standard deviation of 0.44. Protein is influenced by the total solid content and milk reduction during boiling. Higher protein content than the standard composition was attributed to the addition of skim milk powder in some of the samples. It was reported that the addition of non-fat dry milk and vegetable oil improves the protein content of curd (Younus *et al.*, 2002). The values were similar to the protein values obtained by Obi and Ikenebomeh (2007).

### 3.4 Ash Content

The mean values of ash differ significantly ( $p < 0.05$ ) among the dahi samples. The ash content of samples is shown in Table 1. The highest value of ash was found in sample JH ( $0.74 \pm 0.02$ ) and lowest value of ash was found in sample GT ( $0.48 \pm 0.01$ ). The values of ash give an idea of mineral content in the sample. The findings are in accordance with the results documented by El-Bakri and El-Zubeir (2009).

### 3.5 Total Solids

The range of total solid varied from 18.55 to 12.38% among the dahi samples which are significant from one another at probability level of  $p < 0.05$ . The total solid content of the samples are enlisted in Table 1. Sample GL showed the highest value of 18.55% with a standard deviation of 0.01 and lowest value  $12.38 \pm 0.01$  was found in sample GT. The reasons of differences in total solids of dahi of different places might be due to the use of non-standardized milk, different milk sources and in case of dahi samples fermented in earthenware vessel as in case of sample TN which shows a mean value of 17.52 with a standard deviation of 0.03. Moreover, adulteration and storage duration also affects the amount of total solids in prepared dahi. The total solid found in Assamese dahi

is more than the findings of Younus *et al.* (2002) and El-Bakri and El-Zubeir (2009).

### 3.6 Lactose

Table 1 demonstrated the significant variations ( $p < 0.05$ ) in lactose content between the samples of different localities. These results are in line with findings of Davis and Mclachlan (2001). Highest and lowest lactose content was observed in the samples TN ( $4.41 \pm 0.16$ ) and GT ( $4.12 \pm 0.01$ ). Differences in lactose content are attributed due to milk type, breed of cow and number of microorganisms present in dahi.

### 3.7 Syneresis

There were significant differences in syneresis values of the dahi samples. The highest whey separation of 38.57% was shown by the sample GL with a standard deviation of 1.05 and the lowest value was observed in sample TN with the percent whey separation of 28.09% and standard deviation of 0.33 (Table 1). The differences in syneresis values are mostly attributed to the incubation condition, kind of microbial culture used, homogenization and the total solid of the milk. The findings are in alliance with Younus *et al.* (2002).

### 3.8 Color Parameters

L-value is a measure of whiteness in the product. The highest L-value of 85.25 was exhibited by sample TH with a standard deviation of 0.95 and lowest value was shown by sample TN of 57.29 with standard deviation of 0.30. The samples with more fat exhibited higher L-values. The samples were found to be differed significantly in lightness ( $p < 0.05$ ). This might be due to light scattering properties of fat particles. The a-values in almost all the samples were declined more towards greenness indicated by negative sign. There were no significant differences among the samples ( $p < 0.05$ ). The mean with standard deviation of a-values of samples GT, TZ, TH, JH, GL and TN were found to be  $-0.84 \pm 0.004$ ,  $-1.69 \pm 0.02$ ,  $-1.04 \pm 0.51$ ,  $-0.33 \pm 0.96$ ,  $-1.15 \pm 0.04$  and  $-1.07 \pm 0.01$  respectively. The b-values of dahi samples also showed non-significant ( $p > 0.05$ ) differences among the samples with highest value of  $2.39 \pm 0.02$  in the sample TZ and the lowest value of  $1.92 \pm 0.0$  in sample JH (Table 1).

### 3.9 Total Plate Count (TPC)

The total viable counts of the samples are presented in Table 2. The mean values of total viable counts different samples varied from 7.02 to  $7.98 \times 10^7$  CFU/ml. The difference in TPC among the samples was not significant at  $p < 0.05$ . It was interpreted that, the culture types and amount added indigenously for

Table 1: Physico-chemical analysis of plain dahi of different region of Assam (Mean±SD)

Sample Location	Guwahati (GT)	Tezpur (TZ)	Tihu (TH)	Jorhat (JH)	Goalpara (GL)	Tinsukia (TN)	CD-value
Fat (%)	2.45 <sup>ab</sup> ± 0.02	3.60 <sup>ac</sup> ± 0.50	3.54 <sup>ac</sup> ± 0.66	2.60 <sup>b</sup> ± 0.05	3.02 <sup>ab</sup> ± 0.05	3.33 <sup>ac</sup> ± 0.11	0.611
Protein (%)	3.42 <sup>ad</sup> ± 0.04	3.27 <sup>abd</sup> ± 0.43	2.96 <sup>bc</sup> ± 0.03	2.66 <sup>b</sup> ± 0.04	3.12 <sup>ac</sup> ± 0.02	3.69 <sup>d</sup> ± 0.18	0.429
Ash (%)	0.48 <sup>a</sup> ± 0.01	0.68 <sup>b</sup> ± 0.02	0.53 <sup>a</sup> ± 0.01	0.74 <sup>c</sup> ± 0.02	0.55 <sup>a</sup> ± 0.01	0.71 <sup>bc</sup> ± 0.01	0.093
Total Solids (%)	12.38 <sup>a</sup> ± 0.01	15.63 <sup>b</sup> ± 0.1	16.19 <sup>c</sup> ± 0.02	13.21 <sup>d</sup> ± 0.02	18.55 <sup>e</sup> ± 0.01	17.52 <sup>f</sup> ± 0.03	0.293
Lactose (%)	4.12 <sup>a</sup> ± 0.01	4.20 <sup>a</sup> ± 0.00	4.73 <sup>b</sup> ± 0.01	4.71 <sup>b</sup> ± 0.11	4.50 <sup>c</sup> ± 0.01	4.41 <sup>c</sup> ± 0.16	0.189
pH	4.11 <sup>a</sup> ± 0.04	4.29 <sup>a</sup> ± 0.02	5.05 <sup>b</sup> ± 0.21	4.87 <sup>b</sup> ± 0.17	4.72 <sup>c</sup> ± 0.09	4.65 <sup>ac</sup> ± 0.05	0.026
Titrateable acidity (% LA)	1.04 <sup>a</sup> ± 0.04	0.96 <sup>b</sup> ± 0.006	0.58 <sup>c</sup> ± 0.13	0.69 <sup>d</sup> ± 0.07	1.07 <sup>ac</sup> ± 0.11	0.71 <sup>e</sup> ± 0.28	0.340
Syneresis (%)	32.61 <sup>a</sup> ± 0.71	31.21 <sup>ab</sup> ± 2.52	34.33 <sup>a</sup> ± 0.411	28.86 <sup>bd</sup> ± 0.23	38.57 <sup>c</sup> ± 1.05	28.09 <sup>bd</sup> ± 0.33	4.210
L	76.187 <sup>ab</sup> ± 0.89	78.69 <sup>ab</sup> ± 4.74	85.25 <sup>c</sup> ± 0.95	69.06 <sup>d</sup> ± 2.341	72.27 <sup>ac</sup> ± 2.76	57.29 <sup>e</sup> ± 0.30	5.438
a	-0.84 ± 0.004	-1.69 ± 0.02	-1.04 ± 0.51	-0.33 ± 0.96	-1.15 ± 0.04	-1.07 ± 0.01	NS
b	2.29 ± 0.05	2.39 ± 0.02	1.90 ± 0.28	1.92 ± 0.01	2.16 ± 0.01	2.02 ± 0.01	NS

<sup>abcde</sup> values with at least one same superscript does not differ significantly; <sup>NS</sup> not significant; Columns with CD values are significant at  $p < 0.05$ ; L, a, b are CIE-Lab colour values

Table 2: Microbial analysis of plain dahi of different region of Assam (Mean±SD)

Samples	Total Plate Count (cfu/ml), (10 <sup>7</sup> )	Total Coliform Count (cfu/ml), (10 <sup>3</sup> )
Guwahati (GT)	7.68±0.01	2.32 <sup>a</sup> ±0.11
Tezpur (TZ)	7.23±0.01	Nil
Tihu (TH)	7.02±0.02	Nil
Jorhat (JH)	7.37±0.2	2.01 <sup>b</sup> ±0.01
Goalpara (GL)	7.98±0.01	3.2 <sup>c</sup> ±0.05
Tinsukia (TN)	7.66±0.06	1.75 <sup>d</sup> ±0.06
CD value	NS	0.103

<sup>abcd</sup> values with at least one same superscript does not differ significantly; <sup>NS</sup> not significant; Columns with CD values are significant at  $p < 0.05$

Table 3: Sensory data of plain dahi of different region of Assam (Mean±SD)

Samples	Colour	Flavour	Texture	Taste	Overall Acceptability
Guwahati (GT)	6.83±0.28	7.00 <sup>b</sup> ±0.5	7.41 <sup>ab</sup> ±0.31	6.50 <sup>ad</sup> ±0.5	6.83 <sup>ab</sup> ±0.62
Tezpur (TZ)	7.16±0.57	7.41 <sup>a</sup> ±0.38	8.00 <sup>b</sup> ±0.40	7.30 <sup>abd</sup> ±0.5	7.05 <sup>ab</sup> ±0.54
Tihu (TH)	7.00±0.5	7.66 <sup>a</sup> ±0.29	8.16 <sup>ba</sup> ±0.24	7.59 <sup>bcd</sup> ±0.14	8.33 <sup>c</sup> ±0.52
Jorhat (JH)	6.83±0.28	7.17 <sup>a</sup> ±0.29	7.41 <sup>bd</sup> ±0.31	7.08 <sup>acd</sup> ±0.14	7.00 <sup>b</sup> ±0.01
Goalpara (GL)	6.50±0.5	6.00 <sup>b</sup> ±0.5	6.61 <sup>de</sup> ±0.23	6.86 <sup>ad</sup> ±0.23	6.67 <sup>a</sup> ±.23
Tinsukia (TN)	7.00±0.86	5.73 <sup>b</sup> ±0.21	6.67 <sup>e</sup> ±0.23	6.67 <sup>ad</sup> ±0.28	5.50 <sup>d</sup> ±0.41
CD-value	NS	0.717	0.646	0.598	0.752

<sup>abcde</sup> values with at least one same superscript does not differ significantly; <sup>NS</sup> not significant; Columns with CD values are significant at  $p < 0.05$

preparation of dahi does not vary significantly even though there are heterogeneous mixture of lactic acid bacteria. The findings are in line with the observation of Younus *et al.* (2002).

### 3.10 Coliform Count

Coliform count is an indication of hygienity of dahi preparation. Mean coliform count in samples GT,

TZ, TH, JH, GL and TN were 2.32, nil, nil, 2.01, 3.2 and 1.75 respectively. The occurrence of coliform bacteria in dahi samples might be due to poor and unhygienic fermentation medium, improper handling and storage conditions. In addition to this, some samples were subjected to fermentation without being pasteurized i.e. raw milk was used for preparing curd. So this might be one of the reasons behind the

occurrence of coliform bacteria in dahi sample (Table 2).

### 3.11 Sensory Characteristics

The mean scores for sensory evaluation of dahi samples are presented in Table 3. The mean sensory score of colour of samples were in the range of 6.5 to 7.16. The highest score was of sample TZ and lowest value of sample GL with standard deviation of 0.50 and 0.57 respectively. The colour scores did not differ significantly ( $p < 0.05$ ). The mean scores for flavor ranged from 5.73 to 7.66 and the samples differed significantly ( $p < 0.05$ ). The texture of sample GL obtained lowest hedonic score of 6.61 and sample TH with highest mean score of 8.16. The differences were significant among all the curd samples. The taste of samples varied from each other so as its sensory scores. Sample TH had the highest mean score of 7.59 and the lowest value was of sample GT with 6.5 and standard deviation of 0.14 and 0.5 respectively. There was found

to be a significant difference in sensory score for taste ( $p < 0.5$ ). There was no significant variation in sensory scores for overall acceptability ( $p < 0.05$ ). The highest acceptability by panel members was for sample TH ( $8.33 \pm 0.52$ ). The least acceptability was for sample TN.

### 4. Conclusions

It was concluded from the study that, there were lot of variations in the physico-chemical, microbiological and organoleptic qualities of dahi traditionally produced in the Assam state of India. More hygienic practices are required to make the product safe from the harmful microorganisms like coliform. Uniformity in quality is a vital aspect as per the marketability of dahi product is concerned. Therefore, proper sensitization to local farmers on these aspects required to be addressed.

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