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Physicochemical, microbiological and antioxidant property of traditionally prepared Misti Dahi sold in West Bengal

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75 Misti Dahi samples from different districts of West Bengal were collected randomly and analysed for physicochemical, microbiological quality and antioxidant activity. Significant variations in the composition of the Dahi samples were observed. The average fat, protein, lactose, ash, total solids, pH and titratable acidity content of Dahi samples were found to be 3.94±0.55%, 3.7±0.032%, 4.39±0.035%, 0.66±0.012%, 12.82±0.087%, 3.48±0.045 and 1.16±0.008% lactic acid, respectively. Hydroxy methyl furfural (HMF) value, peroxide value (PV) and tyrosine value (TV) of the market Dahi samples were found to be 1.03±0.04604 µmole/L, 0.344±0.009 meq /kg of fat and 0.276±0.008 mg of tyrosine/mL respectively and varied significantly. Baudouin test which is an indicator of adulteration of Dahi with Vanaspati showed positive results for 48% of the samples. Average coliform and yeast &mould count were found to be 4.37±0.21 log cfu/g and 3.54±0.11 log cfu/g, respectively. Antioxidant activity was measured by ABTS and DPPH method. All Misti Dahi samples showed good antioxidant activity and varied significantly ($p<0.05$).

Keywords: Antioxidant, ABTS, DPPH, Misti Dahi, Traditional, Quality

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Dahi is a popular fermented traditional dairy products of India. It is a lactic fermented product produced from cow or buffalo milk[1]. Dahi poses various health benefits like improvement of lactose intolerance, risk reduction of certain cancers, anticholesterolaemic properties, genital and urinary tract infection prevention[2]. Besides this, Dahi is recommended for the treatment of dysentery, dyspepsia and other gastrointestinal disorders[3]. Being a highly nutritious food, Dahi also improves the appetite with the help of lactic acid bacteria by stimulating the B and T cells of macrophages[4,5]. Dahi is a good source of vital nutrients like calcium, phosphorus which reduces bone problems and it plays an important role inthe digestive tract being a pre-digested food[1,6]. Nowadays probiotic cultures like *Streptococcus thermophillus, L. bulgaricus, Bifidobacterium* are used for manufacturing probiotic Dahi[7].

Commercially, Dahi is prepared by standardization of milk followed by pasteurization, inoculation of the milk with specified cultures, incubating the batch at specified temperature and finally preserving it in chilled condition[8]. The traditional process is mainly followed in the rural sweet shops where it is usually prepared in earthen pot by inoculation with previous culture and kept at room temperature[5].

One of the most popular dairy products of West Bengal is Misti Dahi[9]. Traditionally it is mainly packed in earthen pots[10]. It is mainly sweetened and thickened with palm jaggery, cane jiggery and cane sugar[9]. In West Bengal, several sweet shops, vendors and households prepare Misti Dahi and sell it to the consumers. The lactic fermentation of milk produces different bioactive peptides and amino acids which exhibits different biological activities like inhibition of the angiotensin -converting enzyme[11,12], immune activity[13] and antioxidant activity[14]. Common consumers are not aware of these health benefits of Misti Dahi and unknowingly consuming these bioactive peptides which possess this biological activity in their guts. The present study was aimed to evaluate the physicochemical, microbiological and

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antioxidant property of the Misti Dahi samples collected from different districts of West Bengal. The aim of determining the antioxidant property of Misti Dahi was to validate the potential health benefits of this popular traditional fermented dairy product. The study will also help to make the product a potential candidate for Geographical Indication (GI) registration.

Materials and Methods

Collection of samples

75 Misti Dahi samples were collected from 25 different locations comprising different districts of West Bengal from March to June 2019. Each location was represented by 3 samples collected from three different shops. All samples were collected in sterilized glass bottles and kept in an insulated box (temperature was maintained with Ice gel pack) and brought to the laboratory. Collected samples were then kept in the refrigerator at 4±2°C for further analysis. Samples collected from different locations of West Bengal are indicated in this study by A to Y location wise.

Physico-chemical analysis of Misti Dahi samples

Collected Misti Dahi samples were analysed for acidity, fat, protein, ash, total solid, lactose, pH, HMF value, peroxide value and tyrosine value.

A method as described in AOAC (1996)[15] was followed to determine the acidity of Dahi samples. 25 mL distilled water was added in 10 g Dahi sample which was then titrated against 0.1 (N) NaOH using phenolphthalein indicator for determination of acidity. The Fat content of Dahi samples were determined by the Rose Gottlieb Method described in AOAC(1990)[16]. Fat content was determined by taking 10 g of Dahi with the addition of 1 mL ammonia and 10 mL concentrated HCl which was then followed by a fat extraction process using petroleum ether and diethyl ether. Determination of Protein, Ash, Total Solid, Lactose, pH of Dahi samples were done by specified processes described in AOAC(1996)[15]. The Protein content of Dahi was determined using conventional Micro-Kjeldahl digestion and distillation procedure as per the method described in AOAC (1996). AOAC (1996) copper reduction method was followed for detrmining the lactose content of Dahi samples. 1 g Dahi sample was taken for determining both the protein and lactose content. Peroxide value (PV) was determined following the method as given in BIS (1981)[1]. Fat was extracted from 5 g Dahi sample by Rose-Gotleibb method for determination of peroxide value. Potassium iodide and a mixture of glacial acetic acid and chloroform (2:1) were added in the extracted fat which was followed by boiling for 30 seconds. Subsequently potassium iodide was added in the solution, washed with water and titrated against 0.002 (N) sodium thio sulphate solution for determining the peroxide value of Dahi. Tyrosine value (TV) was estimated using the method described by Juffs (1973)[17]. 24% TCA (Tri carboxylic acid) solution was added in 5 g Dahi sample for determination of tyrosine value. The mixture was then held for 15 min at room temperature, subsequently filtered and added with alkali reagent. 0.2 mL 0.67 (N) folin-ciocalteau was added and subsequently kept for 45 min for colour development, the absorbance of which was then measured at 680 nm using a spectrophotometer. Method described by Bandyopadhaya *et al.*,was used to determine Hydroxy Methyl Furfural (HMF) value of Misti Dahi samples. HMF value was determined using a spectrophotometric method where 5 mL oxalic acid was added in 10 g Dahi sample followed by addition of TCA acid after boing and cooling. 0.5 M thio bar butyric acid was added in the filtrate taken from the mixed sample and the absorbance of the sample was determined at 450 nm after keeping in water bath at 40^{o}C for 30 min. The Presence of Vanaspati (vegetable oil containing sesame oil) in Dahi was determined by the Baudouin test as described in BIS (1981)[1] with slight modification. 5 mL HCl and 0.4 mL furfural solution were added in 10 g dahi sample to determine the presence of Vanaspati in Dahi. The appearance of pink or red colour after shaking the mixture for 2 min and subsequent separation confirmed the presence of Vanaspati in the sample.

Micrbiologicalanalysis of Misti Dahi samples

Misti Dahi samples were analysed for coliform and yeast and mould. Coliform count enumeration was done by using Violet Red Bile Agar (VRBA) and yeast & mould count was determined by using Potato Dextrose Agar (PDA) following "Standard Methods for examination of Dairy Products" by APHA (1967)[19]. Coliform count was measured by transferring 1 g of sample in a sterile petridish followed by addition of 15-20 mL of selected media. Typical pink colonies were counted for determining coliform count after incubating the petri dish at 37^{o}C for 24-48 h. For yeast and mould count 15 mL of melted and cooled media was added in 1 mL diluted sample followed by adjusting the pH to 3.5% with 10% sterile tartaric

acid. After solidification the plates were incubated at 22°C for 3-5 days and subsequently the developed yeast and mouldcolonies were counted.

Determination of antioxidant activity of Misti Dahi samples

Antioxidant activity of Misti Dahi samples available in different districts of West Bengal was also evaluated. Antioxidant activity of market Misti Dahi samples was determined by ABTS [2, 2'-azinobis (3 ethyl benzothiazoline)-6-sulfonic acid) and DPPH (2, 2 diphenyl-1-picryl hydrazyl) assay.

Preparation of supernatants for antioxidative activity

100 g of Misti Dahi samples were taken in the centrifuge tubes and then centrifuged for 30 min (3000 r.p.m), filtered (using Whatman no. 40) aseptically in sterile test tubes for analysis.

Measurement of antioxidant activity by ABTS method

Total antioxidant activity was assayed using the spectrophotometric method suggested by Re *et al.*, (1999)[20]. ABTS solution was prepared by dissolving 80 mg ABTS in 10 mL of double-distilled water. The ABTS radical cation was produced by the reaction between 2.45 mM potassium persulphate and 7 mM ABTS in 1:1 ratio which was then stored in dark condition at room temperature for 12-16 h.1 mL ABTS stock solution was diluted with methanol to produce ABTS working solution until gave an absorbance of 0.70±0.02 at 734 nm. ABTS working solution (3.9 mL) was mixed with 0.1 mL of appropriately diluted sample and kept at room temperature for 5 min. Absorbance was recorded at 734 nm using a UV- visible double beam spectrophotometer. Methanol was used as a blank. The standard Trolox curve against ABTS is given in Figure 1. ABTS free radical inhibition activity (%) was calculated using the formula given below:

ABTS free radical inhibition activity (%) =(Abs $_{blank}$ – Abs $_{sample}$) x 100/ Abs $_{blank}$ where Abs $_{blank}$ is the absorbance of the blank sample and Abs $_{sample}$ is the absorbance of the sample. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC), i.e., µM Trolox/100 g dry weight (DW).

Measurement of antioxidative activity by DPPH method

DPPH (2,2 diphenyl-1-picryl hydrazyl) radical based antioxidant activity of supernatant of Dahi samples were determined following the method of y Brand-williams *et al.*, (1995)[21]. DPPH methanolic solution was prepared by dissolving 3.95 mg DPPH in 100 mL methanol. 12.5 mg of Trolox (6-hydroxy, 2,5,7,8 tetramethyl chroman-2-carboxylic acid) was dissolved in 10 mL of methanol and then diluted with distilled water to get final concentration between 100-1000 µM. Appropriate dilution of sample solution (0.1 mL) was mixed with 2.9 mL of freshly prepared DPPH working solution in a 10 mL test tube. The contents were vortexed and incubated for 30 min in a dark room at 37°C by covering the test tube with an aluminium made foil. Solution absorbance was measured at 517 nm against methanol using a spectrophotometer. 0.1 mL methanol was taken instead of sample for blank determination. Trolox standard curve against DPPH is given in Figure 2. The scavenging activity of the sample was expressed by the percentage of scavenged DPPH radicals in the above assay system, calculated by the following equation.

DPPH free radical inhibition activity (%) = (Abs $_{blank}$ – Abs $_{sample)}$ x 100 / Abs $_{blank}$

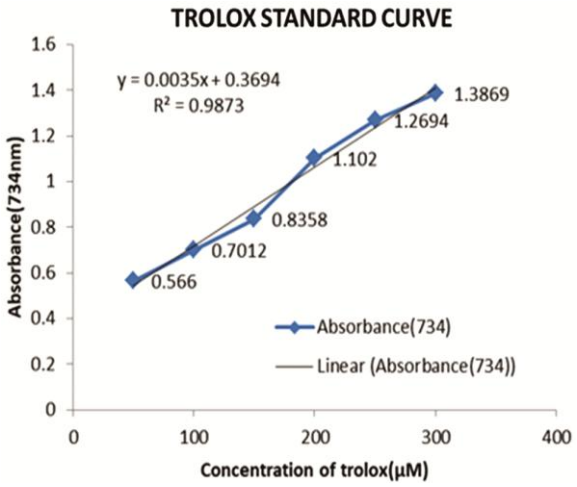

Fig. 1 — Standard curve of Trolox (ABTS)

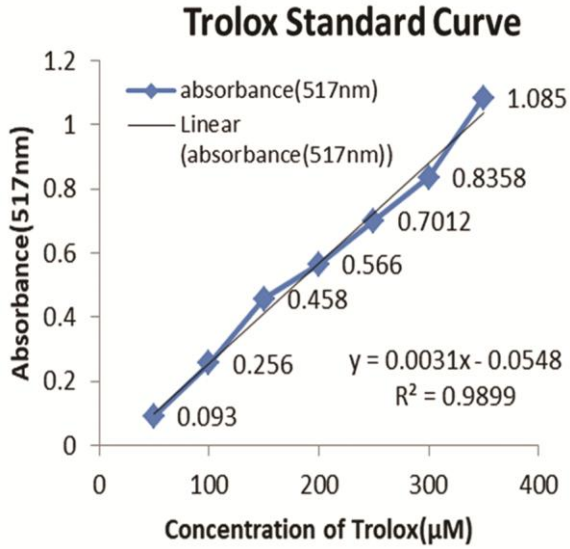

Fig. 2 — Standard curve of Trolox (DPPH)

Calculation of IC_{50} and Trolox equivalent antioxidant capacity

IC_{50} was calculated for each analytical sample using the following procedure:

Inhibition activity (y) was plotted against the concentration of sample (x) at all seven points and the corresponding regression line (y= ax+b) was drawn. The DPPH radical scavenging activity of the samples was expressed as the Trolox Equivalent antioxidant capacity (TEAC). TEAC was calculated using the following equation:

TEAC = IC_{50} of Trolox (µg/mL)/ IC_{50} of sample (µg/mL)

A high TEAC value represents a high DPPH radical scavenging activity. The IC_{50} value of the samples collected from each location and IC_{50} value of Trolox were measured on the same day.

Statistical analysis

The statistical analysis of the data obtained during investigation was done for meaningful interpretation of the results. Statistical software IBM SPSS statistics 20.0[22] was used for statistical analysis of the results. The least significant difference at ($p<0.05$) was used to determine significant differences between means from all the treatments.

Results and Discussion

Physico-chemical quality of Misti Dahi samples

The physicochemical properties of Misti Dahi samples collected from different parts of West Bengal are given in Table 1.

The maximum and minimum fat content was found in location C and T, respectively with a fat percentage of 4.63±0.033 and 3.03±0.033. The average fat content of Misti Dahi samples was observed to be 3.94±0.055%. Fat content varied significantly ($p<0.05$) among the samples. This result is lower than that reported by other researchers[23,24], who observed the fat content of sour Dahi samples in India ranged from 4-8% whereas the result is supported by other

Table 1 — Some physico-chemical properties of market Misti Dahi sample

Area	Fat	T.S.	Protein	Lactose	Ash	T.A.	pH	HMF (µmole/L)	PV(me/kg of fat)	TV (mg/mL)
A	3.66± 0.033^{ghi}	11.81± 0.143^{lm}	3.61± .080^{bcdef}	4.27± 0.123^{e}	0.73± .023^{b}	1.24± .018^{bc}	3.21± .086^{fg}	0.76± 0.006^{h}	0.32± 0.010^{g}	0.34± 0.006^{bc}
B	4.13± 0.033^{ef}	12.37± 0.184^{ghi}	3.54± .020^{cdef}	4.48± .070^{abcde}	0.56± .013de	1.11± .033^{ghij}	3.93± .040^{bc}	0.94± 0.006^{f}	0.19± 0.006^{j}	0.17± 0.006^{hi}
C	4.63± 0.033^{a}	12.62± 0.037^{efgh}	3.59± .043^{cdef}	4.6± .040^{abcde}	0.73± .010^{b}	1.17± .012^{defg}	3.45± .028de	0.66± 0.013^{i}	0.23± 0.006^{i}	0.37± 0.008^{a}
D	4.26±0.066^{de}	13.71± 0.107^{b}	3.62± .020^{bcdef}	4.4± .100^{abcde}	0.66± .020^{c}	1.18± .006^{bcdef}	3.07± .050^{gh}	0.76± 0.008^{h}	0.30± 0.003^{g}	0.33± 0.003^{c}
E	4.13±0.033^{ef}	14.32± 0.193^{a}	3.74± .143^{bcd}	4.56± .150^{abcde}	0.53± .006^{ef}	1.24± .013^{b}	2.99± .013^{h}	1.65± 0.015^{b}	0.43± 0.010^{c}	0.36± 0.010^{ab}
F	4.06± 0.066^{f}	12.76± 0.070^{efg}	4.24± .040^{a}	4.68± .046^{abc}	0.46± .013^{g}	1.19± .026^{bcdef}	3.24± .013^{f}	1.85± 0.016^{a}	0.36± 0.000^{ef}	0.19± 0.006^{h}
G	4.36± 0.033^{cd}	12.77± 0.069^{efg}	3.66± .000^{bcde}	4.48± .113^{abcde}	0.75± .006^{b}	1.03± .040^{k}	3.94± .026^{bc}	1.19± 0.003^{c}	0.26± 0.003^{h}	0.27± 0.003^{ef}
H	3.56± 0.033^{hi}	11.48± 0.228^{m}	3.75± .096^{bcd}	4.27± .060^{e}	0.65± .003^{c}	1.1± .040^{ij}	3.49± .012de	0.52± 0.006^{k}	0.37± 0.008^{ef}	0.22± 0.006^{g}
I	4.23±0.033^{de}	12.49± 0.078^{fgh}	3.48± .036^{defg}	4.36± .106^{abcde}	0.66± .023^{c}	1.1± .020^{ij}	3.95± .036^{bc}	0.87± 0.006^{g}	0.46± 0.010^{b}	0.31± 0.005^{d}
J	3.26±0.066^{j}	12.51± 0.251^{efgh}	3.37± .043^{fg}	4.32± .120^{cde}	0.64± .013^{c}	1.11± .017^{ghij}	4.04± .036^{ab}	1.03± 0.010^{e}	0.26± 0.010^{hi}	0.28± 0.003^{e}
K	3.7± 0.057^{gh}	12.93± 0.052^{de}	3.59± .130^{cdef}	4.74± .033^{a}	0.58± .003^{d}	1.16± .006^{efghi}	3.6± .020^{d}	0.74±0.006^{h}	0.31± 0.000^{g}	0.22± 0.003^{g}
L	3.13± 0.033^{jk}	12.57± 0.033^{efgh}	3.65± .096^{bcde}	4.67± .056^{abcd}	0.5± .010^{fg}	1.16± .014^{efgh}	3.43± .010de	1.02± 0.013^{e}	0.25± 0.014^{hi}	0.18± 0.006^{hi}
M	4.46± 0.066^{bc}	12.87± 0.043^{def}	4.15± .016^{a}	4.60± .043^{abcde}	0.65± .020^{c}	1.13± .013^{fghij}	3.03± .013^{h}	0.89± 0.000^{g}	0.25± 0.013^{hi}	0.26± 0.006^{f}
N	4.3± 0.057^{d}	12.94± 0.090^{de}	3.36± .113^{fg}	3.81± .060^{f}	0.53± .013^{ef}	1.22± .016^{bcde}	2.99± .016^{h}	0.97± 0.006^{f}	0.35± 0.003^{f}	0.34± 0.006^{bc}
O	3.53± 0.033^{i}	12.26± 0.188^{ijk}	3.72± .133^{bcd}	4.50±.243^{abcde}	0.73± .023^{b}	1.17± .010^{defgh}	3.32± .063^{ef}	1.85± 0.006^{a}	0.47± 0.003^{b}	0.16± 0.006^{i}

(*Contd*.)

Table 1 — Some physico-chemical properties of market Misti Dahi sample

Area	Fat	T.S.	Protein	Lactose	Ash	T.A.	pH	HMF (μmole/L)	PV(me/kg of fat)	TV (mg/mL)
P	4.53± 0.033^{ab}	13.25± 0.098^{cd}	3.65±.040^{bcde}	4.32± .170^{cde}	0.84± .010^{a}	1.09± .006^{j}	3.81± .160^{c}	0.57± 0.013^{j}	0.38± 0.006^{de}	0.28± 0.003^{e}
Q	3.7± 0.0577^{gh}	12.69± 0.037^{efg}	3.42±.080^{efg}	4.33± .023^{bcde}	0.66± .013^{c}	1.39± .006^{a}	3.53± .010^{d}	0.86± 0.013^{g}	0.55± 0.006^{a}	0.33± 0.006^{c}
R	4.33± 0.033^{cd}	14.34±0.187^{a}	3.77± .040^{bc}	4.71± .023^{ab}	0.66± .013^{c}	1.18± .033^{bcdef}	3.04± .023^{h}	1.16± 0.006^{d}	0.29± 0.006^{g}	0.21± 0.003^{g}
S	3.73± 0.033^{g}	12.68±0.116^{efg}	4.19± .176^{a}	3.83± .123^{f}	0.73± .023^{b}	1.18± 0.00^{cdef}	3.52± .033^{d}	0.95±0.006^{f}	0.40± 0.006^{d}	0.18± 0.006^{hi}
T	3.03± 0.033^{k}	12.04± 0.080^{jkl}	3.76± .110^{bc}	4.41± .123^{abcde}	0.77± .016^{b}	1.11± .030^{hij}	4.06± .043^{ab}	0.77± 0.006^{h}	0.38± 0.006^{de}	0.36± 0.003^{ab}
U	4.6± 0.057^{ab}	12.59± 0.020^{efgh}	4.14± .011^{a}	4.60± .043^{abcde}	0.65± .020^{c}	1.13± .013^{fghij}	3.03± .013^{h}	0.66± 0.013^{i}	0.23± 0.006^{i}	0.37± 0.008^{a}
V	4.33± 0.033^{cd}	13.54± 0.073^{bc}	3.25±.003^{g}	3.81±.060^{f}	0.53±.013^{ef}	1.23± .006^{bcd}	2.96± .013^{h}	0.76± 0.008^{h}	0.30± 0.003^{g}	0.33± 0.003^{c}
W	4.13± 0.033^{ef}	14.32± 0.193^{a}	3.86±.003^{b}	4.50±.243^{abcde}	0.73±.023^{b}	1.16± .006^{efgh}	3.42± .015de	1.65± 0.015^{b}	0.43± 0.010^{c}	0.36± 0.010^{ab}
X	3.73± 0.033^{g}	12.83± 0.015^{ef}	3.67±.035^{bcde}	4.32±.170^{cde}	0.84± .010^{a}	1.10± .008^{hij}	3.81± .160^{c}	1.85± 0.016^{a}	0.36± 0.000^{ef}	0.19± 0.006^{h}
Y	3.13± 0.033^{jk}	11.94± 0.016^{kl}	3.65±.006^{bcde}	4.28±.003de	0.77± .008^{b}	1.09± .010^{j}	4.13± .008^{a}	0.95± 0.006^{f}	0.40± 0.006^{d}	0.18± 0.006^{hi}
Mean± S.E	3.94± 0.055	12.82± 0.087	3.7± 0.032	4.39± 0.035	0.66± 0.012	1.16± 0.008	3.48± 0.045	1.03± 0.04	0.34± 0.009	0.27± 0.008

S.E = standard error

All the values are expressed in % (w/w)

No. of samples-75, Values are the mean of three replicates with SE; different superscripts within a column differ significantly ($p<0.05$)

researchers[25,26]. The lower fat content could be attributed to the use of crossbred cows' milk, low- fat content milk or most importantly use of skimmed milk powder for the preparation of Dahi.

The highest amount of protein content was 4.24±.040% found in location F whereas the lowest protein content of 3.25±.003% was found in location V. The average protein content of Misti Dahi samples was observed to be 3.7±.032%. The protein content of Misti Dahi samples showed a significant difference ($p<0.05$) among different locations. The average protein content of Misti Dahi samples was found higher when compared to the report of other researchers[25-27]. Higher protein content in the Misti Dahi samples was mainly attributed to the addition of skim milk powder in milk during the processing of some samples. It was reported that the addition of non-fat dry milk and vegetable oil improves the protein content of curd[25]. The values found in this study are similar to the protein values obtained by another researcher[28].

The maximum and minimum ash content found in the Misti Dahi samples were 0.84±.010% and 0.46±.013%, respectively. The maximum value was found in location P and X simultaneously whereas the minimum ash content was found in location L. The average ash content of Misti Dahi samples was observed to be 0.66±0.012%. The mean values of ash differ significantly ($p<0.05$) among the samples. The ash content gives an idea of mineral content in the sample. The findings are following the results documented by other researchers[26,28]. The average ash content is lesser than the observed data as reported by other researchers[23,25].

Misti Dahi sample collected from location R exhibited the highest Total Solid (TS) content with a value of 14.34±0.187% whereas the lowest TS content was 11.48±0.228% found in location H. The average TS content of Misti Dahi samples was observed to be 12.82±0.087%. The range varied significantly ($p<0.05$) among the Dahi samples. This might be due to the use of non-standardized milk, different milk sources and fermentation in the earthenware vessel. Moreover, adulteration and storage duration also affect the amount of TS content in Dahi[26]. The TS content found in this study is more than the findings of some workers[25,27,29] but is similar to the findings of other workers[23,26].

The average lactose content of Misti Dahi samples was found to be 4.39±0.035% with a maximum value of 4.71±.023% found in location R and the minimum value of 3.81±.060% found in location N and V simultaneously. Significant variation ($p<0.05$) in lactose

content between the samples of different localities was observed. The lactose content found in Misti Dahi samples is lower than the lactose content reported by other workers[25], who observed average lactose content of 4.83±0.25%[24] in the Misti Dahi samples. Another worker[30] reported a wide variation in lactose content (4.21-8.23%) for Dahi samples marketed in Kolkata as compared to the lactose content found in the present study. Differences in lactose content are mainly attributed to the milk type, breed of cow and number of microorganisms present in Dahi.

The pH of the Misti Dahi samples collected from different areas varied significantly ($p<0.05$).The highest pH value of Dahi samples was 4.13±.008 observed in location Y and the lowest pH value was 2.96±.013 observed in location V. The average pH of Misti Dahi samples was found to be 3.48±0.045 which was lower than the pH of Dahi samples reported by other workers[23,24,26]. The nature of the starter culture used, longer incubation time during processing and storage conditions might be the contributing factors of higher pH in the Misti Dahi samples.

Titratable acidity of different samples also showed significant ($p<0.05$) differences. The maximum Titratable acidity of Misti Dahi samples was 1.39±.006% Lactic Acid (LA) obtained from location Q and minimum Titratable acidity was 1.03±.040% LA obtained from location G. The average titratable acidity of Dahi samples was found to be 1.16±0.008% of LA, which did not conform to the minimum standard prescribed by BIS (1981)[1]. The result found was also higher than the results reported by other workers[24-26]. Higher titratable acidity in the Misti Dahi samples might be due to the type of starter culture used and the days of storage of Misti Dahi samples before collection.

HMF value, peroxide value and tyrosine value of Misti Dahi samples collected from different locations of West Bengal were determined to assess the extent of heating during processing, sugar content, degree of oxidation in the product and degree of proteolysis during storage for different storage conditions with variations in starter culture used for the preparation of Misti Dahi. The results are demonstrated in Table 1. Maximum HMF value of 1.85±0.016 µ mole/L in the Misti Dahi samples was obtained from location F and location X simultaneously whereas the minimum HMF value of 0.52±0.006 µ mole/L was found in location H. The average HMF content of Misti Dahi samples was found to be 1.03±0.04 µ mole/L. HMF content of Dahi samples showed significant variations ($p<0.05$). HMF content mainly varies with the temperature during processing and with the age of the product. The higher the temperature is during processing, the higher will be the HMF content. Significant variations in HMF value among the Misti Dahi samples might be due to the differences in temperature of heating of milk before processing. No previous work was reported on the HMF content of market Dahi samples.

Location Q exhibited the maximum peroxide value of 0.55±0.006 milli equivalent/kg of fat and the minimum value was exhibited by location B which was 0.19±0.006 milli equivalent/kg of fat. The average peroxide value of Dahi samples was found to be 0.344±0.009 milli equivalent/kg of fat. Peroxide value of Misti Dahi samples showed significant differences ($p<0.05$). Peroxide value indicates the extent of oxidative deterioration of lipid in any food product Peroxide value mainly varies with the age of the product. The higher peroxide value of Misti Dahi samples indicates improper storage conditions and longer shelf life of the product. The results could not be adequately compared due to the lack of published literature on the peroxide value of market available Dahi samples.

Misti Dahi samples collected from location C and location U simultaneously showed the highest tyrosine value of 0.37±0.008 mg of tyrosine/ml and the lowest tyrosine value obtained from location O is 0.16±0.006. Tyrosine value of Dahi samples showed significant differences ($p<0.05$). The average tyrosine value of Dahi samples was observed to be 0.276±0.008 mg of tyrosine/mL. The higher tyrosine value of the Misti Dahi samples resulting from a higher degree of proteolysis may be attributed to the higher processing temperatures that causes the unfolding of protein molecules thereby creating a larger surface area or sites of action of bacterial enzymes. The tyrosine value of the market Dahi samples was not reported earlier.

Baudouin test was performed for all the Dahi samples for detecting the presence of Vanaspati which was added to increase the total solid content as a cheap source of fat. Out of 75 samples, 36 samples of Dahi were tested positive. The result implies that 48% of Dahi manufactured in the unorganized sector adulterated with Vanaspati. The result agrees well with the report of the previous worker[25].

Micrbiological quality of Dahi samples

Misti Dahi samples were analysed for coliform and yeast & mould count the results of which are given in Table 2.

Coliform count showed significant differences ($p<0.05$) among the Misti Dahi samples collected from different locations. The maximum coliform count of4.75 ± 0.00^{a} log cfu/g was found in location Q as compared to the minimum coliform count of 3.30 ± 0.00^{k} log cfu/g found in location D. The average coliform count of the Misti Dahi samples was 4.37±0.21 log cfu/g. None of the Misti Dahi samples conformed to the maximum prescribed limit of coliform count given by BIS (1981)[1]. Significant differences ($p<0.05$) were also observed in the Yeast & mould count of Misty Dahi samples. The maximum Yeast & Mould count was 5.4 ± 0.00^{a} log cfu/g in location A whereas the minimum count found was 3.30 ± 0.00^{o} log cfu/g in location G. The average Yeast & Mould count of Misti Dahi samples was 3.54±0.11 log cfu/g. None of the samples collected from different sweet shops conformed to the maximum prescribed limit suggested by BIS (1981)[1] for coliform and Yeast & Mould count. The result found is higher than the result obtained by other workers[24,25]. Coliform and Yeast & mould count indicates hygiene of processing operation and storage condition and has an important effect on sensory quality and storage life of the product[31]. Improper handling of raw materials during processing and poor storage conditions are mainly responsible for higher coliform and Yeast & Mould count in Misti Dahi samples.

Table 2 — Some Microbiological properties of market misti Dahi samples

Location	Coliform count (log cfu/g)	Yeast and Mould count (log cfu/g)
A	3.84 ± 0.00^{j}	5.4 ± 0.00^{a}
B	4.44 ± 0.00^{defg}	3.41 ± 0.05^{n}
C	4.53 ± 0.00^{bcde}	3.56 ± 0.04^{l}
D	3.30 ± 0.00^{k}	5.04 ± 0.00^{c}
E	4.54 ± 0.00^{abcde}	5.30 ± 0.00^{b}
F	3.90 ± 0.00^{j}	4.66 ± 0.00^{f}
G	4.42 ± 0.00^{defg}	3.30 ± 0.00^{o}
H	4.50 ± 0.00^{cdef}	3.60 ± 0.00^{kl}
I	4.32 ± 0.00^{fghi}	4.80 ± 0.00^{d}
J	4.67 ± 0.00^{abc}	3.84 ± 0.00^{h}
K	4.19 ± 0.00^{hi}	4.74 ± 0.00^{e}
L	4.68 ± 0.00^{abc}	3.69 ± 0.00^{j}
M	4.55 ± 0.00^{abcde}	$3.88\pm.01^{h}$
N	4.49 ± 0.00^{cdef}	3.47 ± 0.00^{m}
O	4.61 ± 0.00^{abcd}	3.63 ± 0.032^{k}
P	4.57 ± 0.00^{abcde}	3.56 ± 0.04^{l}
Q	4.75 ± 0.00^{a}	3.84 ± 0.00^{h}
R	4.65 ± 0.00^{abc}	3.69 ± 0.00^{j}
S	4.72 ± 0.00^{ab}	3.60 ± 0.00^{kl}
T	4.67 ± 0.00^{abc}	3.77 ± 0.00^{i}
U	4.27 ± 0.01^{ghi}	4.83 ± 0.00^{d}
V	4.13 ± 0.23^{hi}	4.14 ± 0.00^{g}
W	4.37 ± 0.21^{efgh}	3.77 ± 0.00^{i}
X	4.67 ± 0.00^{abc}	3.60 ± 0.00^{kl}
Y	3.74 ± 0.04^{j}	4.71 ± 0.00^{e}
Mean± S.E	**4.37±0.21**	**3.54±0.11**

S.E = standard error

No. of samples-75, Values are the mean of three replicates with SE; different superscripts within a column differ significantly ($p<0.05$)

Antioxidant activity of Misti Dahi Samples

All Misti Dahi samples tested in this study showed good antioxidant activity. Antioxidant activity of Misti Dahi samples was determined by ABTS and DPPH method and is given in Table 3. TEAC value determined by ABTS method ranged between

Table 3 — Antioxidant properties of market misti Dahi samples

Location	ABTS TEAC (μM)	DPPH TEAC (μM)
A	206.55 ± 4.04^{a}	336 ± 9.19^{a}
B	127.44 ± 1.06^{h}	198 ± 2.00^{fg}
C	165.889 ± 1.86^{cd}	$213.77\pm.222^{c}$
D	162.44 ± 2.16^{de}	194.88 ± 1.12^{g}
E	130.88 ± 4.05^{g}	176.11 ± 1.05^{h}
F	202.44 ± 0.222^{a}	$269.77\pm.222^{b}$
G	136 ± 3.50^{fg}	$150.66\pm.333^{jk}$
H	165.77 ± 3.44^{cd}	$210.55\pm.111^{cd}$
I	105.33 ± 2.96^{i}	149.33 ± 1.01^{k}
J	126.77 ± 1.06^{h}	157.44 ± 1.06^{i}
K	167.66 ± 1.00^{bcd}	$192.55\pm.400^{g}$
L	127.55 ± 1.11^{h}	$149.22\pm.777^{k}$
M	$72.99\pm.333^{k}$	$118.44\pm.111^{m}$
N	169.44 ± 0.111^{bc}	$206.33\pm.333^{de}$
O	135.88 ± 0.444^{fg}	$150.44\pm.293^{jk}$
P	106.33 ± 0.192^{b}	$147.44\pm.111^{k}$
Q	139 ± 0.333^{h}	$140.22\pm.400^{l}$
R	88.55 ± 0.293^{j}	$147.44\pm.111^{k}$
S	136.11 ± 0.400^{fg}	156.33 ± 1.17^{ij}
T	172.44 ± 0.293^{b}	$151.77\pm.111^{ij}$
U	128.44 ± 0.111^{h}	198 ± 2.00^{fg}
V	164.11 ± 1.31^{cde}	$213.77\pm.222^{c}$
W	158.22 ± 0.293^{e}	194.88 ± 1.12^{g}
X	130.88 ± 4.05^{gh}	176.11 ± 1.05^{h}
Y	132.22 ± 0.587^{gh}	$201.88\pm.293^{ef}$
Mean±S.E	142.33±1.264	184.05±1.837

No. of samples-75, Values are the mean of three replicates with SE different superscripts within a column differ significantly ($p<0.05$) S.E=Standard Error, TEAC – Trolox Equivalent Antioxidan Capacity studied at 734 nm and 517 nm for ABTS and DPPI respectively

72.99±.333to 206.55±4.04 µM Trolox/100 g DW with an average value of 142.33±1.264 µM Trolox/100 g DW. Radical scavenging activity in terms of TEAC value determined by DPPH method was found to be ranged between 118.44±.111 to 336±9.19 with an average value of 184.05±1.837. The corresponding samples collected from different locations showing different antioxidative activity has been given in Figure 3 and 4. Antioxidant activity of Misti Dahi samples determined both by ABTS and DPPH method varies significantly ($p<0.05$). Antioxidant activity of fermented milk like Dahi, yoghurt etc. is influenced by the formation of different bioactive peptides from milk proteins through proteolysis and the relationship has been reported by many workers[14,32,33]. Nishino *et al*. [34] reported that protein peptides produced in fermented milk are generally responsible for enhanced radical scavenging activity. Significant differences found in the antioxidant activity of Misti Dahi samples might be due to the use of different non-descriptive strain of starter cultures in different areas of West Bengal. The presence of antioxidant activity in Misti Dahi samples suggests that they could be used as a natural antioxidant supplement for improving human health. The results could not be suitably compared due to the lack of published literature on the antioxidant activity of market available Dahi samples.

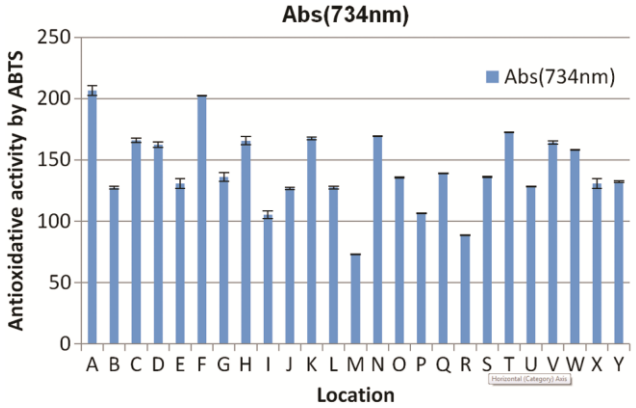

Fig. 3 — Antioxidant activity of Misti Dahi by ABTS

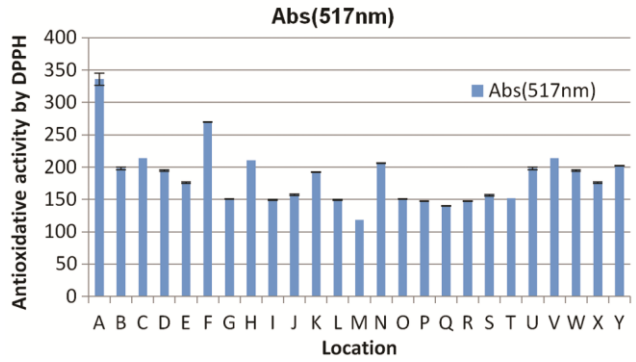

Fig. 4 — Antioxidant activity of Misti Dahi by DPPH method

Conclusion

The present study attempted to find out the physico-chemical, microbiological and antioxidative characteristics of Misti Dahi, a popular traditional fermented milk product of West Bengal. Wide variation in the physico-chemical and microbiological quality of market samples of Misti Dahi collected from different districts of West Bengal was observed. The variations in physico-chemical and microbiological quality could be attributed to the difference in the type of milk, temperature and duration of incubation, starter cultures type, total solid content of milk, heat treatment during concentration and conditions of handling and storage. The results showed that the Misti Dahi samples had strong antioxidant activity. Consumption of Misti Dahi can provide a good source of antioxidant part from the nutrients present in it and therefore they may have the potential for use as a functional food for the benefit of human health. There is also a need to standardize the manufacturing method for exploiting commercial benefit with the adoption of hygienic practices to get a uniform physico- chemical and microbiological quality conforming to the FSSAI standard.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Rimli Chatterjee gathered and analysed the research data, Pinaki Ranjan Ray conceptualised, supervised the work, edited the research paper, Chandrakanta Sen analysed the research data and wrote the research paper, Surajit Mandal supervised the research work.

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