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A Comparative Study of Different Inulin Levels on Quality Parameters of Synbiotic Misti Dahi

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Inulin (prebiotic) is used as mean of providing texture, stabilizing foam, improving mouth feel in fermented dairy products, desserts, bakery products and infant formula. Inulin is introduced to synbiotic misti dahi in different concentrations to observe inulin effect on physicochemical, sensory, microbiological and textural properties of misti dahi. Fresh raw buffalo milk was standardized to 3% fat divided into two batches, control and treatment T1, T2, T3 and T4 with 2.5%, 5%, 7.5% and 10% inulin respectively. Acidity, whey separation, viscosity, lightness, redness, yellowness, firmness, stickiness, flavor, body texture, overall acceptability were determined. Acidity, pH, firmness and stickiness increases with increase in inulin level whereas whey separation, flavor, body texture and overall acceptability and microbiological analysis decreased with increase in inulin level. Hence on the basis of above results it is concluded that synbiotic misti dahi with 2.5% inulin showed acceptable results among all treatments and quality parameters of synbiotic misti dahi decreased with increasing inulin concentration.

Key words: Inulin, Synbiotic, Misti dahi, Color, Firmness, Quality parameters.

Dahi is traditional fermented milk product of Indian subcontinent and consumed in different forms as *Lassi, Shrikand, Misti dahi, kadhi* etc. Dahi is considered as a method of increasing the shelf life of milk. Dahi has been elevated to dessert by sweetening it. The sweetened variety of dahi is popularly known as misti dahi. Misti dahi has creamish to light brown color, firm consistency smooth texture and pleasant aroma. Sweet dahi is the product obtained by fermentation carried out by *Lactobacillus lactis ssp lactis, L.lactis ssp cremoris, L. lactis biovar diacetylactis* and *Leuconastoc mesenteroides ssp.* For making sour dahi, in addition to these cultures *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are used. Because of pleasant caramel and sour taste cherished by all age groups and availability of technology for industrial manufacture[1], misti dahi is now sold in various parts of country by leading brand owners[2].

Probiotic bacteria are live microbial strains that, when applied in adequate doses beneficially affect the host by improving its intestinal microbial balance. Probiotic strains used in commercial products might affect the oral ecology by specifically preventing the adherence of other bacteria and by modifying the protein composition of salivary pellicle[3]. The increased hygiene measure in food manufacturing plants and restaurants have resulted in human being exposed to as one millionth probiotic organisms to which their ancestors were exposed[4]. The major group of bacteria that constitute probiotics are lactic acid bacteria(LAB), microbial species other than LAB like *Bacillus subtilis, Propionobacterium s*sp and yeast have been accepted and used as probiotics.[5] In addition to directly introducing live probiotics to the colon

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through dietary supplementation, another approach to increase the number of beneficial bacteria in intestine is prebiotics. Prebiotics are non-digestible carbohydrates that supply a source of fermentable sugar for beneficial bacteria only in colon. Most commonly used prebiotics include lacitol, lactulose, tagatose and variety of oligosaccharides, fructo-oligosaccharides (FOS), fructans, fibers, gums etc. Inulin increases the beneficial *Lactobacillus* and *Bifidobacterium* and suppresses the number of bacteroides and *Candida ssp*. Inulin influence intestinal function by increasing stool frequency particularly in constipated patients, increasing stool weight and decreases stool pH which has been linked to suppression of the production of putrefactive substances in colon.[6]

Inulin reportedly decreased serum triglycerides and blood cholesterol level in hypercholesterolemic patients. Inulin has been termed "prebiotics" because they are non-digestible food ingredients that selectively stimulate growth and/or activity of a number of potentially health-stimulating intestinal bacteria.[6] They are often used in combination with "probiotics" or live bacteria that are added to the host's diet to promote health. The combinations of pre and probiotics that promote the growth of existing strains of beneficial bacteria in the colon, inulin improves survival, implantation and growth of newly added probiotic strains. Synbiotic are defined as a mixture of probiotic and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in Gastro Intestinal Tract, by selectively stimulating the growth of a limited number of health promoting bacteria, and thus improving host welfare.[6] In recent times, probiotics have been marketed as dietary supplements in the form of tablets, capsules and freeze dried preparations.[7] The market of synbiotic food category continues to expand.

MATERIALS AND METHODS

Raw material and ingredients

Milk

Fresh raw buffalo whole milk and skimmed milk were procured from the experimental dairy of National Dairy Research Institute.

Starter culture

Lactococcus lactis subsp *lactis* NCDC, *Lactobacillus delbrueckii* subsp *bulgaricus* NCDC, *Lactobacillus acidophilus* NCDC, *Streptococcus thermophiles* NCDC were obtained from National Collection of Dairy Cultures Karnal India, all these four cultures were mixed to form mixed starter culture which was used to produce synbiotic misti dahi.

Prepration of misti dahi

Buffalo whole milk was standardized to 3% fat, total solid of 13.5%. Skim milk and skim milk powder was used to standardize whole milk. Sugar was added at the rate of 10%. Milk was subjected to heat treatment (60-65ºC). Homogenization was carried out in two-stage homogenizer (GOMA homogenizer) with 1000 and 500 psi pressure at first and second stage. Milk was separated into two batches control and treatment; inulin was added to test sample or treatment. Both the batches were allowed to heat treatment at 90ºC. Milk was allowed to cool at room temperature. The starter culture (mixed culture) was added at the rate of 2% to both the batches. Then milk was transferred into 100ml beakers. Incubation was carried out for 18 hours at 37ºC. Without disturbing the set product, beakers were transferred to refrigerated storage (6-8ºC). The method was described by Ghosh and Rajorhia8 which was modified for the production of misti dahi.

Analysis of misti dahi

In the present study quality of synbiotic misti dahi has been assessed by physico-chemical properties, sensory attributes, microbiological evaluation, and texture measurement.

Physico-chemical parameters

pH

Misti dahi sample from refrigerated storage was allowed to come down at room temperature (25ºC). The pH of the sample was measured by digital pH meter after calibrating it with pH 7.0 standard buffer.

Titratable acidity

The titratable acidity in terms of percent lactic acid was determined. 10 g of sample was weighed, 10ml of distilled water was added into it, and 0.5% of phenolphthalein indicator was added. Mixture was mixed properly and was titrated against Noah with continuous stirring till the pink color appears.

Acidity was calculated as follows:

$$\text{Titratable acidity} = \frac{N_{NaoH} \times 9 \times V_{NaoH}}{WT}$$

Where,
N_{NaoH}= normality of NaoH
V_{NaoH}= volume of NaoH used
Wt = weight of the sample taken.

Whey separation

Whey separation syneresis was determined following drainage method as described by Chawla and Balachandran.[9] In this case, after about 16 h of storage under refrigeration, the set misti dahi beakers were taken out and tempered at 25ºC for 2 h. with the help of spatula the contents of beaker are emptied straight away into a glass funnel (12cm diameter) with a what man No.1 filter paper. The funnel was placed on graduated measuring cylinder with 100ml capacity.[10] The funnel with cylinder was kept in incubator (30ºC). The quantity of whey collected after 2 h of drainage is considered as syneresis and expressed as percent whey separated.

Viscosity

For measuring the viscosity, the samples were tempered to 25°C and stirred gently 20 minutes in clockwise and anticlockwise direction using a spatula.[10] Samples were then filled into a concentric rotational viscometer (viscostar plus, FUNGILAB) supplied by Barcelona Spain. Viscosity was measured using a 2-2 measuring system with a shear rate of 50 s^{-1} as per the procedure described in supplier's manual.

Rheology

Texture measurement

Texture attributes such as firmness and stickiness were determined by back extrusion method by using a texture analyzer, TA-XT2i (M/s stable micro systems, UK) fitted with a 25-kg load cell and was calibrated with a 5- kg standard dead weight prior to use.10 For determining the textural attributes, the beakers were tempered to 20ºC for 2 h prior to analysis. The probe (P/25) was penetrated upto 10mm (20% compression) into set misti dahi at a crosshead speed of 1.0mm/s. The probe displaced the material by compression followed by back extrusion, so that the fluid flowed through the concentric annular space. All measurements were done in triplicate.

Sensory analysis

All samples were evaluated for sensory attributes such as color and appearance, body and texture, flavor and overall acceptability on a nine-point hedonic scale (9 for liking extremely and 1 for disliking extremely) by the panel of nine judges.

Microbiological analysis

For determining microbiological count misti dahi samples were serially diluted and plated with various dilutions. *Lactobacillus* count and *Streptococcus thermophilus* count was observed on MRS and M-17 agar respectively. Initial and final counts were determined by pour plating method.

Color

The surface color of the set misti dahi was measured using a "colorflex" colorimeter supplied by Hunter lab (Hunter associates laboratory, Reston, Virginia, USA) along with the software (version 4.10). Before the test, the instrument was calibrated with standard black and white tiles as specified by the manufacturer. The light source was dual xenon flash lamp. Data were received through the software in terms of *L*(lightness), ranging from 0(black) to 100(white), *a*(redness), ranging from +60(red) to -60 (green), and *b*(yellowness), ranging from+60 to -60 (blue) values.10 About 16 h of storage at refrigerated temperature (6-8ºC), the cups were removed from the refrigerator and tempered at 25ºC for 2 h. later the contents of beakers were loosened from the sides with the help of spatula and the contents were inverted and transferred into the sample container attached with the color flex instrument. During transfer care was taken to avoid breaking of gel/curd. Three reading were taken for each sample.

RESULTS AND DISCUSSION

The set misti dahi was analyzed for physico-chemical, sensory, textural and microbiological analysis. The results of effect of different levels of inulin on synbiotic misti dahi are presented in Table 1.It was observed that pH and acidity values increased as inulin level was increased from control to treatment. Control, T1 and T2 showed acceptable pH for dahi i.e. less than 5. Rao and Dastur[11] stated that during the preparation of dahi, pH of milk drops to 5. A good

dahi has a pH 4.6-5.0. T3 and T4 showed pH more than 5. Good dahi should have acidity not more than 1. Control, T1 and T2 acidity values were less than 1, but T3 and T4 showed acidity values more than 1. The increased acidity among T3 and T4 may be due to increased acid production. Syneresis or whey separation is regarded as a defect on the surface of dahi and other set-style fermented dairy products. The syneresis value decreased with increase in inulin concentration. Antunes *et al.*[12] also reported that fat-free set yoghurt showed considerable wheying off when not stabilize by other ingredients. This may be explained by the fact that fat globules get entrapped physically within the gel network resulting in increased number of pores within the gel matrix occupied by fat globules, thus leading to prolonged gelation and more difficult whey drainage. Also fat has role in gel structure formation due to development of multiple interactions between fat globules, whey proteins, and casein micelles.[13] Viscosity value of misti dahi increased with the increase in inulin concentration. As whey separation decreases, viscosity among treatments increased.

Table 1. Effect of different levels of inulin on physico-chemical parameters pH, Titratable acidity, syneresis and viscosity

Sample	Incubation time (h)	Incubation temp ºC	pH Avg±Sd	Titratable acidity Avg±Sd	Syneresis Avg±Sd	Viscosity Avg±Sd
Control	18	37	4.85±0.09	0.84±0.15	13.2±1.16	1039.2±19.4
T1	18	37	4.91±0.06	0.85±0.09	11.2±2.16	1250.6±25.19
T2	18	37	4.96±0.05	0.87±0.89	9.4±1.51	1367.3±23.16
T3	18	37	5.56	1.08	8	1457.2±22.43
T4	18	37	5.43	1.12	7	1545.4±20.1

Table 2. Effect of different levels of inulin on sensory evaluation of synbiotic misti dahi

Sample	Incubation Time (h)	Incubation temp (°C)	Sensory			
			Colour and appearance Avg±Sd	Body and texture Avg±Sd	Flavour acceptability Avg±Sd	Overall Avg±Sd
Control	18	37	7.8±0.27	7.68±0.42	7.82±0.60	7.9±0.68
T1	18	37	7.94±0.27	7.64±0.51	7.82±0.50	7.9±0.89
T2	18	37	7.7±0.43	7.62±0.66	7.82±0.7	7.8±0.89
T3	18	37	7.5	5.6	5.6	6.1
T4	18	37	7.5	5.6	5.6	5.6

Table 3. Effect of different levels of inulin on color of synbiotic misti dahi

Sample	Incubation time (h)	Incubation temperature (°C)	Color		
			Lightness Avg±Sd	Redness Avg±Sd	Yellowness Avg±Sd
Control	18	37	76.23±0.01	2.12±0.20	18.19±0.13
T1	18	37	76.65±0.29	1.98±0.40	18.32±0.18
T2	18	37	76.68±0.43	2.03±0.36	18.36±0.20
T3	18	37	76.23	1.56	18.11
T4	18	37	76.24	2.03	18.15

Scores for all the sensory attributes were decreased with the increase in inulin concentration mentioned in Table 2. As inulin level increased color and appearance scores slightly increased from Control to T1, but decreased from T2 to T4 i.e. 7.7 to 7.5 respectively. The body and texture scores decreased from T2 to T4 i.e. 7.62 to 5.6. Increase in inulin level also affected flavor score, which got decreased from T2 to T4 i.e. 7.82 to 5.6. Overall acceptability decreased among T2, T3 and T4 treatments. Therefore overall acceptability decreased among T2, T3 and T4.

The increase in inulin level among the various treatments did not show any significant difference in lightness values as mentioned in table-3.Lightness value increased till T2 and then decreased from T2 to T4. Lightness value ranged from 76.23 to 76.68 among the different treatments, confirming that misti dahi has relatively dark color compared with standard white color (Lightness=100). Whiteness in fluid milk results from the presence of colloidal particles, such as milk and fat globules and casein micelles, capable of scattering light in visible spectrum.[14] The lower Lightness values of misti dahi than milk could be due to added caramel in the product, which is known to provide color and specific aroma to foods[15]. The redness and yellowness values of misti dahi were observed to be on the positive side, indicating that product was slightly reddish yellow.

A typical force-deformation curve of "back extrusion" test of misti dahi was studied. Back extrusion test is well suited to the gels because it is not affected by free whey on the surface of the samples.[16] Firmness value i.e., the peak force obtained during penetration of the probe revealed that increase in inulin level increased the firmness value from Control to T4 i.e., 1.76N to 2.05N respectively. Similar pattern was observed with the stickiness i.e. the negative peak force obtained during the removal of the probe, among the treatments as mentioned in table-4. Increase in stickiness was observed from control to T4. The increased stickiness in T2 could be due to increased lactose content resulted from increased MSNF content.

The viable count of micro-organisms was obtained on MRS and M-17 agar by pour plate

Table 4. Effect of different levels of inulin on rheological parameters of misti dahi

Sample	Incubation Time (h)	Incubation Temperature (°C)	Rheology			
			Firmness(N) Avg±Sd	Work of Adhesion (N.s) Avg±Sd	Work of ShearN.s) Avg±Sd	Stickiness (N) Avg±Sd
Control	18	37	1.76±0.02	-240.4±1.79	13.6±2.2	-0.64±0.1
T1	18	37	1.77±0.01	-236±1.26	20.64±0.5	-0.65±0.09
T2	18	37	1.84±0.1	-222.1±1.84	21.22±0.5	-0.66±0.01
T3	18	37	1.95	-220.1	21.65	-0.63
T4	18	37	2.05	-219.1	22.1	-0.60

Table 5. Effect of different levels of inulin on microbiological quality of synbiotic misti dahi

Sample	Incubation Time (h)	Incubation Temp. (°C)	Microbial count			
			Initial count (cfu)		Final count (cfu)	
			MRS	M-17	MRS	M-17
Control	18	37	9.30±0.60	10.3±2.03	9.97±0.61	11.21±0.6
T1	18	37	9.30±0.60	10.3±2.03	10.01±0.57	11.98±0.6
T2	18	37	9.30±0.60	10.3±2.03	9.76±0.84	9.84±0.59
T3	18	37	9.30±0.60	10.3±2.03	9.24	9.21
T4	18	37	9.30±0.60	10.3±2.03	9.20	9.19

technique. Results showed increase in final count of MRS and M-17 agar from control to T2 and then decrease from T2 to T4 which reveals the quality of misti dahi improved with the addition of inulin upto 2.5% and quality decreased with the increase in inulin concentration mentioned in Table 5.

CONCLUSION

Misti dahi is popular fermented dairy product of India cherished by all age groups. The present study was carried out with an aim to develop synbiotic misti dahi with acceptable inulin concentration. On the basis of present result, it can be concluded that control and T1 (2.5%) showed highly acceptable results and the quality of symbiotic misti dahi decreased with increase in inulin concentration.

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