Properties of sweetened Indian yogurt (mishti dohi) as affected by added tryptic whey protein hydrolysate

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Abstract Utilization of Indian sweetened yogurt (colloquially termed as Mishti Dohi), as vehicle for ACE inhibition and antioxidant activity, by added tryptic whey protein hydrolysate (TWPH) (@ 1, 2, 3 % v/milk), was attempted. Yogurt with 3 % TWPH exhibited non-significant ($p>0.05$) difference for sensory attributes; but for body & texture; and maximum biofunctional properties, electing it for storage study (5 ± 1 °C). Flavor and body & texture scores registered significant ($p<0.05$) decline under 14 days storage. ACE inhibition and antioxidant activity of control increased by 47.95 and 13.18 % and of experimental 24.58 and 13.43 %, correspondingly. Acidity rose to 1.18 % LA. Control samples conveyed 18.07 % and experimental of 20.77 % escalation for wheying-off. Tyrosine value was $27.04 \mu\text{g.mL}^{-1}$. Among rheological attributes, firmness, quantified by texture analyzer TA-XT2i, dropped ($p<0.05$), due to decrease of gel rigidity whereas work of adhesion revealed non-significant difference ($p>0.05$), throughout.

Keywords Yogurt · Whey protein hydrolysate · Physico-chemical · Rheological · ACE · Antioxidant

Introduction

The revivification of whey proteins, erstwhile exasperation to the dairy industry, into abundance of techno-functional and biofunctional potentials has unfurled prospects for research and benefits for the human health. Upon subjecting whey proteins to controlled hydrolysis, they often liberate

some sequences of amino acids detaching them from their mother chain. These liberated amino acid sequences impend to modulate certain biological processes within the human biological system and are predominantly recognized as biologically active, or bioactive, peptides. The health benefits related to antihypertension, anticancer, immunomodulation, opioid, mineral binding, antioxidative function, etc. offered by these peptides depend on the released sequence of amino acids. Hypotensive competency of bioactive peptides for preventing conversion of angiotensin - I to angiotensin - II by inhibiting angiotensin - I converting enzyme (ACE) results in a depression in blood pressure. The whey protein hydrolysates can prevent oxidation initiated by free radicals (Kamau and Lu 2010). The function of free radicals against ails as aging, cancer, inflammation etc. is on records (Ames et al. 1993). The quest for natural antioxidants is also drawing research interest.

Fermented milk products, are not only an excellent source of generation of such peptides but are effective carriers as well. Mishti dohi—a popular Indian variety of sweetened yoghurt—is produced in large quantities, both in organised as well as un-organised dairy sector. Technology for industrial production of mishti dohi is also available (Ghosh and Rajorhia 1990; Rao and Solanki 2007). This creamish light brown colored fermented product of set consistency, smooth texture, and pleasant aroma (Raju and Pal 2009) is usually cherished across all age palates. Available literature on mishti dohi is, however, scanty. Incorporation of a hydrolysate having ACE inhibition activity into mishti dohi would help the consumers to derive health benefits beyond basic nutrition. An attempt was, therefore, made to incorporate tryptic whey protein hydrolysate (TWPH) at the rate of 0 (control), 1, 2 and 3 %, volume ratio of milk, in mishti dohi and study the sensory and biofunctional properties of the resultant product and also some of its physical and rheological characteristics during storage at 5 ± 1 °C.

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Materials and methods

Whey protein concentrate (WPC) and enzymes

WPC was procured from Modern Dairies Pvt. Ltd. and stored in airtight container till further use. As per manufacturer's report, WPC had moisture 3.76 %, bulk density 0.38, acidity 1.26 % LA, pH 6.76, fat 4.66 %, protein 69.56 %, ash 3.89 %, heat stability at 66 °C and solubility index 0.25. The enzyme trypsin was obtained from M/s Sigma (USA).

Milk

Fresh raw whole milk of buffalo and skim milk were procured from the Experimental Dairy of the National Dairy Research Institute (NDRI), Karnal.

Starter culture

The mixed starter culture NCDC 263 (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) was obtained from the National Collection of Dairy Cultures, NDRI, Karnal.

Cane sugar

Cane sugar (Grade C) was obtained from the local market.

Caramel

Caramel in powdered form (P-III-190) (E 150 c) was obtained from M/s Alvin Chemicals (India) Pvt. Ltd., Ahmednagar, Maharashtra (India).

Chemicals

Analytical grade reagents and chemicals from reputed companies were used for chemical analysis.

Preparation of hydrolysate

Fresh reconstitute was prepared from the WPC to 5 % protein concentration in distilled water and heated to 65 °C for 10 min. The hydrolysis conditions viz. pH, temperature and time, were kept as 8.0, 37 °C and 8 h, respectively, based on earlier studies. The flow sheet for the hydrolysis process is presented in Fig. 1. Effective generation and identification of the ACE inhibitory and antioxidant peptides in such hydrolysate, has been reported by Chatterjee et al. (2014).

Preparation of mishti dohi

Flow given by Ghosh and Rajorhia (1990) was slightly modified by Raju and Pal (2009) for the production of mishti dohi

Fig. 1 Flow sheet for the preparation of whey protein hydrolysate using trypsin

Fig. 2 Schematic diagram for preparation of yogurt (mishti dohi) with TWPH

(Fig. 2). In our trials we introduced a slight modification to the latter. Modification introduced by Raju and Pal included use of skimmed milk powder instead of condensed skimmed milk for standardization and addition of caramel whereas modification included at our end was homogenization prior to addition of sugar. Fresh raw milk from buffalo was adjusted to 6 % milk fat using fresh raw skimmed milk from buffalo and the MSNF content was adjusted to 9.5 %. The milk was heated to 60–65 °C, homogenized using 1000 and 500 psi pressures in a two stage homogenizer (M/S. Goma Engineers, Mumbai), added with 14 % cane sugar, 0.1 % caramel and 3 % TWPH, v/v (for final selection) pasteurised at 90 °C for 10 min. and cooled to 41 °C followed by addition of 2 %, w/v, starter culture and filling into pre-sterilized 100 mL standard cups (M/s Tarsons Products Pvt. Ltd., Kolkata). Incubation was carried out at 41±1 °C for about 6 h till the titratable acidity reached 1 % lactic acid. Thereafter, without disturbing the set product, cups were delicately transferred to refrigerated storage (5–6 °C). All the trials were carried out in triplicate.

ACE inhibitory and antioxidant activity of mishti dohi

Mishti dohi (50 mL) was centrifuged at 15,000 g for 30 min for each experiment. The supernatant was filtered separately through Whatman No.1 filter paper. The collected sample was treated as under:

Determination of ACE inhibitory activity

The assay for the ACE inhibitory activity was based on the liberation of hippuric acid from hippuryl-L-histidyl-L-leucine

(HHL) catalyzed by ACE and measured by the method of Cushman and Cheung (1971).

The extent of inhibition was calculated as follows:

$$\% \text{ACE inhibition} = \left[\frac{(B-A)}{B} \cdot \{(B-C)\}^{-1} \right] * 100 \quad (1)$$

Where:

- A the absorbance in the presence of ACE and ACE inhibitory component
- B the absorbance without ACE inhibitory component, and
- C the absorbance without ACE.

Determination of antioxidant activity

Determination of antioxidant activity of the hydrolysate was based on DPPH (2, 2 diphenyl - 1 -picryl hydrazyl) method. DPPH from M/s Sigma Aldrich, was dissolved in ethyl alcohol (8 mg.100 mL⁻¹). This was added to 0.2 mL of the sample and shaken well, incubated for 30 min at room temperature (30 °C) and centrifuged at 3000 g for 10 min. The supernatant (0.5 mL) was added with 1.5 mL ethyl alcohol and the absorbance of the solution was measured at 517 nm against methanol using spectrophotometer. For blank determination, 0.2 mL of distilled water was used instead of the sample. The results were expressed as:

$$\% \text{DPPH scavenging activity} = \left\{ \frac{(\text{Absorbance of blank} - \text{Absorbance of sample})}{\text{Absorbance of blank}} \right\} * 100 \quad (2)$$

Titrateable acidity

The titrateable acidity (% lactic acid) was determined following the method as given by AOAC (1975) for cheese.

Viscosity

Samples were initially tempered at 20 °C and thereafter were given gentle stirring for 20 times in clockwise and anti-clockwise direction using spatula as an agitator. The mass was filled into a concentric rotational viscometer (Contraves Rheomat RM 108 ER) supplied by M/s Metler-Toledo, Switzerland (Plate 7). A 2-2 measuring system with shear rate of 50 S⁻¹ was used to quantify viscosity, as per the procedure described in the supplier's manual.

Wheyng-off

The wheyng-off in mishti dohi was determined by the drainage method of Yazci et al. (1997) with modification. After 16 h of storage under refrigeration, the set mishti dohi cups were taken out and tempered at 25 °C for 2 h. The complete contents of the cup (weighing approximately 50.0 g) were emptied onto a 120-mesh SS screen placed over a funnel in a graduated cylinder of 100 mL. The set-up was kept in the room maintained at 25 °C. Wheyng-off (mL) was expressed as the volume of exudates collected after 2 h.

Proteolysis

The extent of proteolysis in the storage samples of mishti dohi was determined by estimating the tyrosine value as per the

method of Hull (1947) with slight modification. Five grams of mishti dohi sample was weighed and taken in clean dry test tube, added with 10 mL of distilled water followed by addition of 10 mL of 0.72 Normal TCA. The test tubes were then stoppered, shaken vigorously and incubated at 37 °C for 10 min. The precipitated proteins were filtered through Whatman No. 42 filter paper. To 5 mL of the TCA soluble filtrate, 3 mL of distilled water, 10 mL of the sodium bicarbonate reagent followed by 3 mL of Folin's phenol reagent was added, mixed well and incubated at 37 °C for 10 min for color development. The intensity of the color was measured at 650 nm by using a single beam spectrophotometer. A stock solution was prepared by dissolving 100 mg of L-tyrosine in a minimum quantity of 0.1 N NaOH and made up the volume to 500 mL with distilled water. Five mL of this solution was then made up to 50 mL with distilled water to give a tyrosine concentration of 100 µg.5 mL⁻¹. Likewise a series of dilution was made to get range of concentration of tyrosine from 100 to 1000 µg.5 mL⁻¹. Five mL of aliquots of the tyrosine standards were used for developing the color. The standard curve with regression equation ($y=0.0152x+0.0215$, $R^2=0.99854$) was obtained using the absorbances against their tyrosine concentration (µg.5 mL⁻¹).

Compression test

Back extrusion technique in a texture analyzer TA-XT2i (M/s Stable Micro Systems, UK) was employed for measuring firmness and stickiness. A 25 kg load cell was fitted to the texture analyzer and was calibrated with 5 kg standard dead weight. For determination of textural attributes, the setting of the mishti dohi was performed in pre-sterilized 100 mL cups (7 cm height; 5.5 cm top and 4.5 cm bottom diameter) (M/s Tarsons Products Pvt. Ltd., Kolkata) filled up to 5 cm height and incubated. Tempering of the cups was done at 25 °C for 2 h prior to analysis. For determination of the gel force, the probe (A/BE 35) (Plate-g) was made to penetrate up to 10 mm (20 % compression) into the set mishti dohi at a crosshead speed of 1.0 mm.s⁻¹. From the consequential force-time curves, the force for compression i.e., firmness (Newton), reflected by the first substantial discontinuity in the curve resulting from the penetration of the plunger in mishti dohi through the 10 mm distance travel and the breaking distance, the negative peak force (Newton) through retraction of probe i.e., stickiness, shear force (Newton.s) and adhesiveness (Newton.s) were estimated using the Texture Expert Exceed software (v 2.55). A minimum of five replicates per sample was run.

Sensory analysis

Mishti dohi cups (100 mL) were drawn from the refrigerator just before sensory evaluation. Sensory attributes tested were

Table 1 Mean scores for sensory attributes of mishti dohi

Samples	Sensory attributes			
	Flavor	Acidity	Color and appearance	Body and texture
A	41.55 ^a ±1.37	7.32 ^a ±1.12	8.32 ^a ±0.46	28.14 ^a ±0.64
B	40.46 ^a ±2.02	7.32 ^a ±1.12	8.32 ^a ±0.41	27.68 ^a ±0.68
C	40.36 ^a ±1.03	7.46 ^a ±0.79	8.31 ^a ±0.50	26.09 ^b ±3.25
D	40.18 ^a ±1.40	7.41 ^a ±1.34	8.30 ^a ±0.60	25.96 ^b ±1.37

^{ab} Means with different letter column-wise are significantly different ($p<0.05$)

flavor, body & texture, color and appearance, and acidity with corresponding maximum scores being 45, 30, 10 and 10, respectively. The evaluators were a panel of 11 members from the faculty of Dairy Technology, Dairy Chemistry and Animal Biochemistry Division of NDRI, Karnal. Though the panelists were not separately trained for the study, they were dairy professionals with adequate knowledge about the sensory evaluation methods and attributes.

Statistical analysis

Statistical analysis was done using SAS enterprise guide 5.1 and Microsoft Excel 2010.

Results and discussion

Sensory evaluation for selection of optimum level of TWPH

Prepared TWPH was added with the milk at three different levels viz. 1, 2 and 3 % (volume ratio of milk) to assess the palate acceptance and tolerance owing to the addition of hydrolysate. The results of the sensory evaluation are indicated in Table 1 where samples categorized as A, B, C and D corresponds to control sample, trailed by samples of 1, 2 and 3 % hydrolysate added mishti dohi, respectively.

Flavor and, color and appearance scores exhibited tolerance and palate acceptance on account of hydrolysate addition

Table 2 Mean scores for biofunctional properties of mishti dohi

Sample	Bio-functional properties	
	% ACE inhibition	% Antioxidant activity
A	30.25 ^c ±1.78	17.62 ^b ±2.64
B	33.11 ^c ±1.95	17.64 ^b ±3.19
C	43.17 ^b ±2.13	19.92 ^{ab} ±2.04
D	57.19 ^a ±1.53	23.32 ^a ±1.42

^{abc} Means with different letter column-wise are significantly different ($p<0.05$)

Table 3 Mean scores for sensory parameters of mishti dohi during storage at 5 ± 1 °C

Days	Flavor		Acidity		Color and appearance		Body and texture	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
0	41.18 ^{aA} ±0.98	40.27 ^{aA} ±2.83	7.73 ^{aA} ±0.72	7.73 ^{aA} ±0.91	7.73 ^{aA} ±0.61	8.23 ^{aA} ±0.82	28.05 ^{aA} ±1.01	28.09 ^{aA} ±1.66
7	37.82 ^{aB} ±3.63	39.27 ^{aA} ±4.08	7.46 ^{aA} ±1.15	7.37 ^{aA} ±1.09	8.32 ^{aA} ±1.03	8.32 ^{aA} ±1.68	27.64 ^{aA} ±1.29	26.14 ^{bB} ±1.45
14	36.46 ^{aB} ±3.24	33.18 ^{bB} ±2.96	7.32 ^{aA} ±1.12	6.46 ^{aB} ±0.96	8.23 ^{aA} ±0.61	8.18 ^{aA} ±0.87	27.59 ^{aA} ±0.80	25.23 ^{bB} ±2.62

^{ab} Means in a row for a property with different letter are significantly different ($p < 0.05$)

^{AB} Means in a column for a property different letter are significantly different ($p < 0.05$)

for all levels. Acidity revealed irregular pattern of sensory scores, i.e., an initial static score (7.32) within control sample and sample B, then slender rise in sample C (from 7.32 to 7.46) which declined in sample D (7.46 to 7.41) with $p > 0.05$ for all, Body and texture scores revealed non-significant ($p > 0.05$) decrease with increase in TWPB level in the product. However for body and texture, though sample C (26.09) and D (25.96) did not register any statistical prominence ($p > 0.05$) within themselves, they were ($p < 0.05$) in contrast to control sample and sample B.

Evaluation of biofunctional attributes through initial screening for level of hydrolysate addition

It is evident from Table 2 that mishti dohi prepared by using 3 % level of TWPB exhibited highest and control lowest biofunctional properties in terms of both, % ACE inhibition as well as % antioxidant activity. Difference in % ACE inhibition values of sample C and D were statistically significant ($p < 0.05$) within themselves as well as against control sample and sample B. As of % antioxidant activity, sample D was significantly different ($p < 0.05$) from control sample and B but not C. The results indicated that addition of 3 % TWPB during the preparation of mishti dohi produced higher biofunctional values in terms of % ACE inhibition and % antioxidant activity than all other levels of TWPB and was, therefore, selected for carrying out storage studies.

Effect of addition of TWPB on sensory attributes of mishti dohi during storage

The effect of addition of 3 % TWPB on the sensory attributes of mishti dohi is presented in Table 3. Flavor score of control differed significantly ($p < 0.05$) from those of experimental samples between day 0 and 7 and also between day 0 and 14. Flavor score of 14th day experimental sample also differed significantly ($p < 0.05$) from that of control sample of same day, the latter showing higher scores. This might be due to the increase in acidity or bitterness produced by the peptides, given that ACE inhibitory peptides are mostly low molecular weight and hydrophobic (Pihlanto-Leppala et al. 1998) and

also are bitter (Saha and Hayashi 2001). With relation to time, rise of bitterness and thus drop of flavor scores may be due to proteolytic activity causing increase in tyrosine and thus rendering bitterness. Masking of yogurt flavor by bitterness has been suggested by Misirlilar et al. (2012), too. There were significant ($p < 0.05$) differences in body and texture scores within the experimental samples between day 0 and 7 as well as day 7 and 14. The difference in the body and texture scores may possibly be attributed to feeble gel and thus reduced viscosity due to addition of hydrolysate that might have resulted in higher proteolysis. The persistent drop in viscosity of yogurts due to addition of both casein and whey protein hydrolysates, individually, have been reported by Sodini et al. (2005) and Sodini et al. (2002) also.

Effect of addition of TWPB on biofunctional attributes of mishti dohi during storage

Table 4 reflects that % ACE inhibition was significantly ($p < 0.05$) different for both the samples within the groups as well as days. This might be due to generation of bioactive peptides, including some ACE inhibitor peptides, in the samples during storage as a result of proteolysis. The present findings agree well with the observations of Donkor et al. (2007) and Amirdivani and Baba (2011). In current study, a rise of 47.95 % was recorded for % ACE inhibitory activity in

Table 4 Effect on biofunctional properties of mishti dohi during storage at 5 ± 1 °C

Storage intervals	Bio-functional properties			
	% ACE inhibition		% antioxidant activity	
	Control	Experimental	Control	Experimental
0	30.53 ^{aC} ±3.80	54.52 ^{bB} ±4.04	17.30 ^{aA} ±1.53	23.68 ^{aA} ±4.16
7	37.92 ^{aB} ±3.06	60.69 ^{bB} ±2.65	19.21 ^{aA} ±2.65	25.85 ^{aA} ±4.04
14	45.17 ^{aA} ±2.52	67.92 ^{bA} ±2.65	19.58 ^{aA} ±1.00	26.86 ^{aA} ±4.73

^{ab} Means in a row for a property with different letter are significantly different ($p < 0.05$)

^{ABC} Means in a column for a property with different letter are significantly different ($p < 0.05$)

Table 5 Some physico-chemical properties of TWPH added mishti dohi during storage at 5 ± 1 °C

Days	Acidity (% LA)		Viscosity (Pa.s)		Wheyng-off (mL)		Tyrosine value ($\mu\text{g.mL}^{-1}$)	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
0	1.12 ^{aA} ±0.01	1.13 ^{aA} ±0.01	0.95 ^{aA} ±0.02	0.98 ^{bA} ±0.02	28.22 ^{aA} ±0.49	28.67 ^{aA} ±0.34	20.93 ^{aA} ±0.35	21.08 ^{aA} ±0.48
7	1.16 ^{aB} ±0.01	1.18 ^{bB} ±0.01	0.91 ^{aB} ±0.06	0.94 ^{bB} ±0.05	31.33 ^{aB} ±0.77	32.94 ^{aB} ±0.45	21.73 ^{aA} ±0.35	24.49 ^{bB} ±0.37
14	1.19 ^{aC} ±0.01	1.19 ^{aC} ±0.02	0.88 ^{aC} ±0.05	0.92 ^{bC} ±0.02	34.44 ^{aC} ±0.70	36.18 ^{aC} ±0.56	24.13 ^{aB} ±0.27	27.04 ^{bC} ±0.31

^{ab} Means in a row for a property with different letter are significantly different ($p < 0.05$)

^{ABC} Means in a column for a property with different letter are significantly different ($p < 0.05$)

control samples, over the storage period. The experimental sample registered an increase of 24.58 %, which might be due to the addition of hydrolysate. Control sample showed an increase of 13.10 % and experimental sample 13.43 % in antioxidant activity during storage. However, antioxidant activity of control and experimental samples did not reveal any significant differences between themselves during the entire period of storage. While Amirdivani and Baba (2011) reported an increase of 33.6 % in antioxidant activity, Singh et al. (2013) noticed a decrease in such activity during storage of hydrolysate-incorporated *dahi*. Presence of sugar in mishti dohi might be the reason for its lower antioxidant activity.

Effect of TWPH addition on physico-chemical attributes of mishti dohi upon storage

The average values for some physico-chemical properties of mishti dohi added with 3 % of TWPH are presented in Table 5. Acidity of experimental sample on 7th day (1.18 % LA) was significantly ($p < 0.05$) lower than that of control (1.16 % LA). No significant variation in acidity of control and experimental samples was noticed in the remaining storage period, which might be due to post acidification (Donkor et al. 2007; Raju and Pal 2009). Since the trend for acidity was similar in control and experimental samples, effect of TWPH on acidity could be termed as insignificant. Shekhar et al. (2012) reported that buffering capacity of milk solids disturbs the relationship between pH and acidity. The present findings contradict the observations of Donkor et al. (2007), who

noticed increase in pH in yoghurt up to 12 h and decrease thereafter during refrigerated storage. Viscosity of mishti dohi exhibited significant ($p < 0.05$) variation between control and experimental samples during the entire period of storage. There was a decline in viscosity from 0.95 to 0.88 Pa.s in control and from 0.98 to 0.92 Pa.s in experimental samples between day 0 and 14. Difference in viscosity in the freshly prepared control and experimental samples could be due to the difference in protein content in the products (Abu-Jdayil and Mohameed 2002). The present findings agree well with the report of Sodini et al. (2005) and Raju (2009), but contradict the observations of Ross-Murphy (1990), Dave and Shah (1998) and Abu-Jdayil and Mohameed (2002).

Wheyng-off or syneresis is associated to the unsteadiness of the gel grid and its reduced capacity to entangle all the serum phase (Lucey and Singh 1997). Table 5 suggests that for both, control and experimental mishti dohi, wheyng-off was not impacted significantly ($p < 0.05$) by treatment but by storage period. Values of wheyng-off depict an upsurge of gel framework instability with values rising from 28.22 to 34.44 and 28.67 to 36.18 mL for control and experimental mishti dohi, respectively. The works of Raju (2009) on mishti dohi, Salvador and Fiszman (2004) on whole and skimmed milk flavored set-type yoghurt and Olson and Aryana (2008) on yoghurt during storage corroborate, but Matumoto-Pintro et al. (2011) contradict the present findings on wheyng-off in mishti dohi. The increase in wheyng-off during storage could be due to the unstable and fragile gel arrangement in the product (Lucey et al. 1998).

Table 6 Rheological properties of TWPH added mishti dohi during storage at 5 ± 1 °C

Days	Firmness (Newton)		Adhesiveness (Newton.s)		Shear force (Newton.s)		Stickiness (Newton)	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
0	2.29 ^{aA} ±0.14	2.15 ^{aA} ±0.07	-2.71 ^{aA} ±0.33	-2.38 ^{aA} ±0.14	18.92 ^{aA} ±0.65	17.55 ^{bA} ±0.87	-0.43 ^{bA} ±0.03	-0.38 ^{aA} ±0.02
7	2.02 ^{aB} ±0.07	2.03 ^{aB} ±0.05	-2.41 ^{aA} ±0.21	-2.38 ^{aA} ±0.18	16.70 ^{aB} ±0.97	16.30 ^{aB} ±0.79	-0.38 ^{aB} ±0.01	-0.37 ^{aA} ±0.03
14	1.97 ^{aB} ±0.07	1.93 ^{aB} ±0.14	-2.50 ^{aA} ±0.33	-2.23 ^{aA} ±0.18	16.15 ^{aB} ±0.50	15.92 ^{aB} ±0.64	-0.37 ^{aB} ±0.02	-0.37 ^{aA} ±0.01

^a Means with different letter are significantly different ($p < 0.05$)

^{AB} Means in a column for a property with different letter are significantly different ($p < 0.05$)

Proteolysis, as manifested by tyrosine values, is the breakdown of large and complex proteins into smaller and simpler peptides. Given that starter culture contained *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, proteolysis was substantially high in both the samples. The proteolytic activity of *Streptococcus thermophilus* over that of *Lactobacillus delbrueckii* ssp. *bulgaricus* and of *Lactobacillus delbrueckii* ssp. *bulgaricus* over *Lactobacillus acidophilus* and *Bifidobacterium* spp. in yoghurt have been shown greater by Shihata and Shah (2000). Tyrosine value of control and experimental samples revealed significant ($p < 0.05$) variation on day 7 and 14. The tyrosine value of experimental sample differed significantly ($p < 0.05$) between day 0 and 7 and also between day 7 and 14, but those of control differed significantly ($p < 0.05$) only between day 7 and 14. Rejection of mishti dohi samples due to bitterness after 14 days by the judges might be related to the tyrosine value. Asperger (1977) and Guzel-Seydim et al. (2005), however, reported that perceptible bitterness in fermented milk products is experienced only when the tyrosine content exceeds the threshold level of 0.5 mg.mL^{-1} , which is much higher than the tyrosine content estimated in mishti dohi in the present study during storage.

Effect of TWPH addition on rheological attributes of mishti dohi upon storage

There were non-significant differences in the values of firmness, adhesiveness and shear force between control and experimental samples on day 0, 7 and 14 (Table 6). However, the firmness, shear force and stickiness values of control differed significantly ($p < 0.05$) on day 7 from those of day 0, but not from those values obtained on day 14. Hardness is defined as the maximum force required to penetrate a food while shear force is the extent of force necessary to achieve shearing process thereby indicative of the struggle made a sample during the penetration by the probe. Stickiness on the other hand is a feeling perceivable to tongue and palate (Adhikari et al. 2001). The values for adhesiveness of control and experimental samples did not change significantly during the entire period of storage. Similarly the stickiness values of experimental samples also did not reveal any significant variation between days of storage. This could be due to the presence of cane sugar in the product which might have impeded the development of gel structure by inhibiting strand formation and protein-protein interaction (Rawson and Marshall 1997).

Conclusion

ACE inhibitory and antioxidant activity of mishti dohi incorporated with different levels of whey protein hydrolysates, obtained by hydrolysing it with trypsin enzyme, were studied.

A level of 3 %, v/v, of TWPH was most acceptable to the judges. During storage at $5 \pm 1^\circ \text{C}$, the product, however, remained acceptable only up to 14 days and thereafter was rejected by the judges mainly due to bitterness. In general, ACE inhibitory and antioxidant property of control and treated samples of mishti dohi increased during storage. However, significant difference was observed in case of % ACE inhibition only and not antioxidant property, indicating that addition of 3 % TWPH may not be beneficial to improve antioxidant properties of mishti dohi but can enhance the % ACE inhibition.

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