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Storage Stability of Cow and Caprine Milk Dahi Incorporated with β- Casein Bioactive Peptides

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A B S T R A C T

Dahi was prepared using cow and caprine milk and the experiment was carried out to assess the quality Dahi during storage from two distinct species of milk. The cow samples were collected from Dairy farm, KVAFSU, Hebbal, Bengaluru and caprine milk samples were collected from Sinchana goat and sheep farm, Marenahalli village (Bengaluru Rural District) and Yashodhavana Goat Farm (Mysuru) were used. The Dahi prepared were incorporated with different level of Bioactive peptides (BAPs) to studied their antibacterial and antioxidative role during storage as a index for storage stability. The Dahi prepared from cow was used as a control and the caprine milk Dahi was incorporated with different level of β-casein hydrolysed Bioactive Peptides (BAP's) were 1.0, 1.5 and 2.0 per cent respectively. The prepared Dahi samples were used to evaluate their quality of the Dahi were physical, chemical and microbiological parameters. It was found that the caprine milk Dahi incorporated with β-casein hydrolysates of BAPs were optimised to the level of 1.5 per cent were found statistically significant ($p<0.05$) with control and other samples. It extends their shelf life up to 4 days compare to control was 3days at room temperature 27º C).

Introduction

The livestock sector has emerged as vital sector for ensuring a more inclusive and sustainable agricultural system in India. The livestock sector of India is one of the largest in the world. The milk production of our nation was provisionally estimated was 176.35 million tonnes during in 2017-18. The goat is one of the main contributors of dairy and it produces about more than 2 per cent of the world's total annual milk supply. India has witnessed an increasing trend of goat milk production with a growth rate of 3.82 per cent during 2015 – 16 (www.nddb.org). Caprine milk fat have smaller fat globules, it is easier to digest than cow milk. Further, the exact composition varies according to many factors like breeds, individual animal, lactation, health condition, environmental condition etc. (Yadav *et al.*, 2016). Caprine milk and its products are highly nutritious, health benefits

and widely consumed in many other parts of the world. Therefore, awareness about advantage of consumption of goat milk should be popularized in India so that production and utilization of goat milk could be enhanced. Dahi is a fermented Indian dairy product consumed by larger section of peoples throughout the country, either as a part of daily diet or as refreshing beverage. About 7 per cent of total milk production is used for Dahi making. Dahi is produced by using mixed mesophilic cultures of *Lactococcus lactis ssp lactis, L. lactis ssp cremoris, L.lactis ssp. diacetylactis* along with *Lecunostoc* species and lactose fermenting yeasts. Dahi is well recognized for their therapeutic properties, particularly in curing gastrointestinal disorders (Mudgal and Prajapati, 2017). The enzymatic hydrolysis of casein obtained by urea filtration produced bioactive peptides (BAPs) plays important role in metabolic regulation. The released peptides can be easily incorporated in to fermented milk products to perform many vital physiological function such as anti-hypertensive, anti-oxidant, anti-cancer, anti-microbial, opiod activities, anti-oxidative and immunomodulatory (Naik *et al*., 2013). Further, incorporation of hydrolysate enhanced the growth of *S. thermophilus* and increases the acidity, as a result of that reducing the fermentation time, and growth of probiotic bacteria. Further there was survival of probiotic bacteria was improved when milk was added with hydrolysate (0.25 to 4 g L^{-1}). The sensory evaluation of the goat milk with/without transglutaminase (TGase, at ranges of 0–4 units/g protein), TGase treated Labneh (a concentrated type of yogurt) the overall scores of the sensory properties showed that significant differences were observed among samples for appearance, brightness, texture, odour, flavour and consistence ($P < 0.05$). The lowest overall score was detected for control labneh with value 12.45 while the highest overall score was detected for 2 U TGase treated labneh sample (Aloˇglua and Oner, 2013). Plain dahi was in good condition up to 3 days of storage period and banana juice based dahi was up to 2 days only at room temperature (Kamruzzaman *et al*., 2002).

Many works have been done in different countries on quality of dahi prepared from different species milk especially on cow and buffalo where as limited work has been made on caprine milk dahi and available data was also scanty. Hence, an attempt has been made to bring new changes in the product by incorporating bioactive peptides in fermented product may enhances the physiological, biological and therapeutical values in the human diet.

Materials and Methods

Milk samples

The indigenous and exotic caprine breed milk samples were collected from Sinchana goat and sheep farm, Marenahalli village (Bengalru Rural Dist) and Yashodhavana Goat Farm (Mysuru) and cow milk samples were collected from Dairy Farm, Hebbal, KVAFSU Bengaluru.

Starter culture

The mixed starter culture consisting of *Lactococcus lactis* ssp. *lactis*, *L. lactis* ssp *cremoris*, *L. lactis* ssp *diacetylactis* along with *Leuconostoc* species were procured from the NDRI, Audugodi, Bengaluru.

Trypsin enzymes

2000 IU (Loba Chemicals Bovine Pancreas) a commercially available enzyme used for the hydrolysis of casein to obtain higher degree of hydrolysis (Enzyme substrate ratio of 1: 25).

Fractionation of casein

The fractionation of casein from acid whole casein (wet) was followed as per procedure of Hipp *et al*., (1952) on the basis of differential solubility in urea solution. Fractionation of whole casein from 6.6 M urea to 4.63M urea yields a precipitate of α_s-casein and β- casein is obtained by further dilution of the supernatant to 1.7 M urea at pH of 4.7.

Preparation of casein fraction hydrolysates

The β-casein (β-CN) fractions of caprine milk was dispersed separately in distilled water at 40^0 C to give a 5 per cent (w/v) protein concentration and the pH of the solutions was adjusted to optimum as that of the enzymes using 0.1N NaOH. The enzyme trypsin (10 mg of enzyme/5 g of protein) at pH 8.0 and temperature 40^0 C was maintained. Enzymatic hydrolysis of casein fractions was carried out at an enzyme- substrate (E: S) ratio of 1:25 (Nagamani, 2013).

Degree of hydrolysis

Degree of hydrolysis (DH) was determined by the pH stat method (Mcdonagh and Fitzgerald., 1998) with slight modification in temperature and strength of alkali used to keep the pH constant during hydrolysis.

Degree of Hydrolysis (DH) = B × Nb × 1/αs × 1/Mp × 1 / htot × 100

Where,
B= Base consumption in ml
Nb = Normality of Base (alkali)
Mp = Mass of protein in gram (N × fN)
htot = Total number of peptide bonds in protein substrate (meq/g of protein for casein; htot = 8.2)
α = Average degree of dissociation of α - NH_2 groups 1/α factor was considered.

$$\alpha s = \frac{10^{pH-pKa}}{1+10^{pH-pKa}}$$

For casein pKa = 7.45 at pH 7.5, 1/α = 1.89.

Isolation of Bio-active peptides (BAPs)

The BAPs were isolated from β- casein fractions by adapting the method of Fitz Gerald (1998) which is based on the principle that BAPs are soluble at pH 4.6 and aggregated with divalent cat-ion such as calcium at neutral pH of 7.0. BAPs obtained by ethanol extraction were dried overnight in an oven maintained at a temperature of 70 ± 1^0 C and stored at 4^0 C before use. Hydrolysates of casein fractions were subjected for centrifugation (3000 rpm/10 min). The obtained supernatant was adjusted to pH 7.0 then 1 per cent calcium chloride was added and kept for 1h. Ethanol (50 per cent v/v) was added to the supernatant to yield BAPs of β-casein fractions. The quantification of BAPs from casein fraction was carried out by adapting the method suggested by Bradford (1976).

Statistical analysis

Experimental data obtained in the study was analyzed by Randomized column block design as per the method described by Snedecor and Cochran (1983) to test for 'F' values to know the statistical significance. Critical Difference (CD) value was calculated to determine whether the treatment means were similar or not. The analysis was done using SPSS software package and MS Excel 2007.

Results and Discussion

Sensory characteristics of caprine milk Dahi incorporated with β –casein BAPs stored at room temperature (30 ± 1 °C)

Caprine milk dahi incorporated with 1.5 per cent β –casein BAPs showed the score of 8.49,

8.36 and 7.93 for colour and appearance 8.64, 8.54 and 8.23 for body and texture and 8.14, 8.10 and 8.05 for flavour and 8.48, 8.39 and 8.0 for overall acceptability at room temperature was presented in Table 2. The score of caprine milk Dahi flavour was statistically significant (P≤0.05) with the flavour of cow milk Dahi. These values are well within the range that was obtained by earlier workers Kamruzzaman *et al.*, (2002) observed the organoleptic quality of Dahi for 3 days stored at room temperature and also Nikil Mahabalashetti, (2017) reported that the sensory quality of control and developed coconut and kiwi fruit pulp enriched Dahi stored at room temperature (30±1^0 C) was unacceptable on 4th day of storage.

Chemical quality of caprine milk Dahi incorporated with β-casein BAPs during storage at room temperature (30 ±1^0 C)

The pH and acidity (%LA) of β-CN BAPs incorporated at 1.5 per cent was 5.0 and 0.69 per cent lactic acid (Table 1). There was a highly significant difference (P≤0.05) between the pH and acidity of caprine milk Dahi and cow Dahi. Similar findings was made by Manmatha (2014) that the pH and acidity of caprine milk yoghurt was 4.8 and 1.05 per cent lactic acid respectively.

Free Fatty Acids and Soluble Nitrogen content of caprine milk Dahi incorporated with β-casein BAPs during storage at room temperature (30±1^{0}C)

The development of Free Fatty Acid (FFA) value of oleic acid content β-casein at 1.5 per cent BAPs level of caprine milk dahi (Table 3) were 7.3, 10.74, 11.30 and 13.26 respectively and soluble nitrogen content was 0.09, 0.10, 0.17 for 1st, 2nd and 3rd day. The cow milk dahi sample found higher soluble nitrogen content and lower FFA value. Incorporated BAPs have extended their shelf life to 4th day stored at room temperature (30±1^{0}C). The results were agreed with Manmatha (2014) FFA value was 13.02 per cent and soluble nitrogen 0.26 per cent for caprine milk yoghurt.

Table.1 Chemical quality of caprine milk Dahi incorporated with β-casein BAPs during storage at 30±1°C

Sample	Fresh		1st Day		2nd Day		3rd Day	
	pH	**Acidity (% LA)**	**pH**	**Acidity (% LA)**	**pH**	**Acidity (% LA)**	**pH**	**Acidity (% LA)**
C	5.2^a	0.68^a	5.0^a	0.78^a	4.9^b	1.12^b	-	-
C1	5.0^a	0.71^a	4.9^a	0.79^a	4.8^b	1.15^b		
C2	5.1^a	0.69^a	5.0^a	0.76^a	4.9^b	1.10^b		
C3	5.0^a	0.67^a	5.0^a	0.69^a	4.9^b	0.70^a	4.8^b	1.11^b
C4	4.9^a	0.72^a	4.8^a	0.78^a	4.7^b	1.12^b	-	-
CD (P ≤0.05)	0.032	0.042	0.032	0.051	0.036	0.024	-	-

- All values are average of three trials
- Similar superscripts indicates non-significance at the corresponding critical difference on a row
  - C_0: Control cow milk Dahi
  - C_1: Caprine milk Dahi
  - C_2: Dahi incorporated with β-casein BAPs at 1.0 per cent level
  - C_3: Dahi incorporated with β-casein BAPs at 1.5 per cent level

C_4: Dahi incorporated with β-casein BAPs at 2.0 per cent level

Table.2 Sensory attributes of caprine milk Dahi incorporated with β-casein BAPs during storage at 30±1°C

Product	Colour & Appearance				Body & Texture				Flavour				Overall acceptability			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
C	8.54^{a}	8.10^{a}	Not acceptable		8.72^{a}	7.92^{b}	Not acceptable		8.58^{a}	8.12^{a}	Not acceptable	-	8.46^{a}	7.92^{b}	Not acceptable	-
C1	8.32^{b}	7.58^{b}			8.35^{b}	7.78^{b}			7.87^{b}	7.76^{b}		-	8.36^{b}	7.59^{b}		-
C2	8.36^{b}	7.60^{b}			8.32^{b}	7.82^{b}			7.84^{a}	7.80^{b}		-	8.32^{b}	7.49^{b}		-
C3	8.49^{a}	8.36^{a}	7.93^{b}	NA	8.64^{a}	8.54^{a}	8.23	NA	8.14^{a}	8.10^{a}	8.05	NA	8.48^{a}	8.39^{a}	8.0	NA
C4	8.36^{b}	7.35^{b}	NA		8.34^{a}	7.89^{b}	NA		7.89^{b}	7.98^{b}	NA		8.36^{a}	7.59^{b}	NA	-
CD (P≤0.05)	0.48	0.56	-	-	0.82	0.036	-	-	0.048	0.052	-	-	0.39	0.028	-	-

- All values are average of three trials
- Similar superscripts indicates non-significance at the corresponding critical difference on a row
- NA – Not acceptable
  - C0: Control cow milk Dahi
  - C1: Caprine milk Dahi
  - C2: Dahi incorporated with β-casein BAPs at 1.0 per cent level
  - C3: Dahi incorporated with β-casein BAPs at 1.5 per cent level
  - C4: Dahi incorporated with β-casein BAPs at 2.0 per cent level

Table.3 Microbiological quality of caprine milk Dahi incorporated with β -casein BAPs during storage at 30 ±1^0 C

Products	Fresh		1st Day		2rd Day		3rd Day		4th Day	
	Coli	Yeast	Coli	Yeast	Coli	Yeast	Coli	Yeast	Coli	Yeast
C	Nil	Nil	Nil	Nil	Nil	Nil	Not acceptable		-	-
C1	Nil	Nil	Nil	Nil	Nil	Nil			-	-
C2	Nil	Nil	Nil	Nil	Nil	Nil			-	-
C3	Nil	Nil	Nil	Nil	Nil	Nil	Nil		Not acceptable	
C4	Nil	Nil	Nil	Nil	Nil	Nil	Not acceptable			-
Co-efficient	0	0	0	0	0	0	-		-	-
t-value	0	0	0	0	0	0	-		-	-

- All the values are average of three trials
- Similar superscripts indicates non-significance at the corresponding critical difference on a row
  - $C_{0:}$ Control cow milk Dahi
  - C_1: Caprine milk Dahi
  - C_2: Dahi incorporated with β-casein BAPs at 1.0 per cent level
  - C_3: Dahi incorporated with β-casein BAPs at 1.5 per cent level
  - C_4: Dahi incorporated with β-casein BAPs at 2.0 per cent level

Table.4 Free fatty acids and soluble nitrogen content of caprine milk Dahi incorporated with β-casein BAPs during storage at 30±1°C

Products	Storage days						
	1		**2**		**3**		**4**
	FFA (% oleic acid)	**Soluble nitrogen (%)**	**FFA (% oleic acid)**	**Soluble nitrogen (%)**	**FFA (% oleic acid)**	**Soluble nitrogen (%)**	
C	5.20[a]	0.09[a]	11.50[b]	0.20[b]	Not acceptable		Not acceptable
C1	7.8[a]	0.14[a]	11.60[b]	0.17[b]			
C2	7.6[a]	0.12[a]	11.58[b]	0.15[b]			
C3	7.3[a]	0.09[a]	10.74[b]	0.10[b]	11.30[b]	0.17[b]	
C4	7.6[a]	0.13[a]	11.54[b]	0.14[b]	Not acceptable		
CD (P≤0.05)	0.036	0.027	0.056	0.078	-	-	

- All the values are average of three trials
- Similar superscripts indicates non-significance at the corresponding critical difference on a row
  - C0: Control cow milk Dahi
  - C1: Caprine milk Dahi
  - C2: Dahi incorporated with β-casein BAPs at 1.0 per cent level
  - C3: Dahi incorporated with β-casein BAPs at 1.5 per cent level
  - C4: Dahi incorporated with β-casein BAPs at 2.0 per cent level

Microbiological quality of caprine milk Dahi incorporated with β -casein BAPs during storage at 30 ±1^0 C

The microbiological quality of caprine milk Dahi incorporated with β-casein BAPs during storage at room temperature (30 ±1^0 C) was presented in Table 4. The coliform and yeast & molds counts are absent throughout the storage period of Dahi. The control showed the shelf of 3 days where as 1.5 per cent BAPs incorporated Dahi showed the shelf of 4 days. Similar observation was made by Dardashti *et al.,* (2001), and Manmatha (2014) and Tamime and Robinson, (1985) this may be due the slower lactic fermentation, lesser acidity development, variation in buffering activities and may presence of antibacterial and antioxidative action of BAPs.

In conclusion, the sensory score of cow and caprine milk Dahi of all the samples were decreased during storage period at room temperature. There was significant difference ($p \leq 0.05$) on all sensory attributes of all the samples with respect to control. C3 sample (1.5 % $\beta_{\text{-CN}}$ BAPs) secured significantly higher score than other samples this is mainly because it retained the freshness of product. The development of acidity, release of free fatty acids and soluble nitrogen content were slow found in C3 sample compare to control and other samples. The microbiological examination of Dahi reveals that the coliforms were absent during storage. All samples were unacceptable at the end of 3rd day of storage due to surface discolouration, higher level of synergies and development of off-flavour, hence they are considered as unacceptable whereas C3 sample extends their shelf life up to 4th day of storage under refrigeration (30±1 ^{0} C). This may be due to slower acidity development, delay in release of free fatty acids, action of antibacterial and anti oxidative activity of BAPs.

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