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# OPTIMIZATION OF DIFFERENT LEVEL OF BIOACTIVE PEPTIDE FROM CAPRINE MILK $A_S$ - CASEIN FRACTION ($A_S$-CN) ON QUALITY OF DAHI

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***Abstract***

*Milk proteins are considered as power house of bioactive peptides. These bioactive peptides may be released in enzymatic hydrolysis(Trypsin 10 mg of enzyme/5 g of protein) at pH 8.0 and temperature $40^0$ C was of casein fractions was carried out at an enzyme- substrate (E: S) ratio of 1:25. 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. The optimum sensory score of caprine milk dahi incorporated with $\alpha_s$ –casein ($\alpha_s$ –CN) 1.5 per cent level bioactive peptides (BAPs) was found to be higher than control and viz., colour & appearance was 8.25, body & texture was 8.08, flavour was 8.00 and overall acceptability was 8.38. The flavour of caprine Dahi was significantly ($P \leq 0.05$) different with the control. The development of acidity and increase in pH was found to be slower in case of optimised (1.5% BAPs) The curd tension and synersis of caprine milk Dahi incorporated with 1.5 per cent BAPs of $\alpha_s$-casein was found to be 345.65 mm, 24 per cent respectively.*

***Key Words: Caprine Milk, Dahi Quality, Casein Hydrolysates, Bioactive Peptides, Optimization, Sensory, Chemical Curd Tension and Synersis.***

## Introduction

The use of caprine and bovine milk in cheese making is well known, but the production of fermented caprine milk via enzymatic hydrolysis of protein fraction incorporating into fermented product has not yet been developed, although many studies have highlighted the requirements for production of healthy food. There are different researchers have studied for incorporation of cow milk casein bioactive peptides into weaning food but the research on caprine milk proteins has not been explored in detailed study with respect to the role of Bioactive peptides which are released by enzymatic hydrolysis can be incorporated in to the fermented dairy products to enhance the health benefits as a functional food in human diet. 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 immune modulatory (Naik et al., 2013).

The whey proteins hydrolysate to optimize among various levels of casein phosphopeptides (CPPs) with 3 per cent incorporation in weaning resulted in superior quality weaning food and was found to be optimum (ShashiKumar., 2012). Similarly Nagamani (2013) also revealed that, the higher levels of incorporation of BAPs (10 per cent) of $\alpha_s$-casein in weaning foods showed extended shelf of the products. Hence it has increased the attention in Indian continent to popularise the caprine milk. In this context, the present investigation was aimed at optimization of caprine milk casein fraction hydrolysates of bioactive peptides in to fermented dairy product had been studied.

## 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)**: 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 $\alpha_s$-casein was obtained by further dilution of the supernatant to 1.7 M urea at pH of 4.7.

**Preparation of casein fraction Hydrolysates:** The $\alpha_s$-casein 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 ò Nb ò 1/αs ò 1/Mp ò 1 / htot ò 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)
$\alpha$ = Average degree of dissociation of $\alpha$ - $NH_2$ groups $1/\alpha$ factor was considered.

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

For casein pKa = 7.45 at pH 7.5, $1/\alpha = 1.89$.

**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 $70\pm1^0$ C and stored at $4^0$ C before use. 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**
The sensory scores pertaining to colour and appearance, body and texture, flavour and overall acceptability of dahi as judged by a five panel of judges during sensory evaluation of control and experimental dahi by incorporation of 1.0, 1.5 and 2.0 level of both caprine milk of $\alpha_s$- casein (-CN) Bioactive peptides ( BAPs) incorporated to dahi presented in Table 1.

The optimum sensory score of caprine milk dahi incorporated with BAPs with $\alpha_s$-CN 1.5 per cent level for colour & appearance was 8.25, body & texture was 8.08, flavour was 8.00 and overall acceptability was 8.38. The body and texture and flavour scored less than control. The obtained results in the present study are in agreement with the findings of Nahar et al., (2007) on goat milk dahi and Bozanic et al., (1998) prepared from goat milk had softer body and texture than cow milk Dahi .

**Table 1: Sensory Attributes of Caprine Milk Dahi Incorporated With $A_s$-Casein Baps**

| Level of BAPs incorporated in Dahi | Color & Appearance | Body & Texture | Flavour | Overall acceptability |
|---|---|---|---|---|
| C | $8.28^a$ | $8.14^a$ | $8.16^a$ | $8.40^a$ |
| C1 | $8.16^a$ | $8.02^b$ | $7.82^b$ | $8.12^a$ |
| C2 | 8.20b | 8.04 | 7.86 | 8.18b |
| $C_3$ | $8.25^a$ | $8.08^b$ | $8.00^b$ | $8.38^a$ |
| C4 | $8.18^a$ | $8.05^a$ | $7.88^b$ | $7.98^b$ |
| CD(P≤0.05) | 0.42 | 0.48 | 0.026 | 0.25 |

All values are average of three trials
Incubation: $37\pm1^0$ C

Similar superscript indicates non significance at the corresponding critical difference.

$C_0$ : Control cow milk Dahi
$C_1$: Caprine milk Dahi
$C_2$: Dahi incorporated with $\alpha_s$- casein BAPs at 1.0 per cent level
$C_3$: Dahi incorporated with $\alpha_s$- casein BAPs at 1.5 per cent level
$C_4$: Dahi incorporated with $\alpha_s$- casein BAPs at 2.0 per cent level

Acidity and pH of caprine milk Dahi incorporated with $\alpha_s$ - casein ($\alpha_s$ –CN) BAPs presented in Table 2. The pH and acidity (% LA) of $\alpha_s$-CN BAPs incorporated at 1.5 per cent was 5.0 and 0.69 per cent lactic acid similar to the findings of Manmatha, (2014) was also observed the pH and acidity of caprine milk yoghurt was 4.8 and 1.05 per cent lactic acid.

**Table 2: Chemical Quality of Caprine Milk Dahi Incorporated With $A_s$-Casein Baps**

| Product | Level of BAPs (%) | Acidity (LA%) | pH |
|---|---|---|---|
| **C0** | - | 0.68 | 5.2 |
| C1 | - | 0.72 | 5.0 |
| C2 | 1.0 | 0.71 | 5.1 |
| C3 | 1.5 | 0.69 | 5.0 |
| C4 | 2.0 | 0.73 | 4.9 |
| CD (0.05) | | 0.038 | 0.026 |

# All the values are average of three trials
LA: Lactic acid

$C_0$ : Control cow milk Dahi
C1: Caprine milk Dahi
C2: Dahi incorporated with $\alpha_s$-casein BAPs at 1.0 per cent level
C3: Dahi incorporated with $\alpha_s$-casein BAPs at 1.5 per cent level
C4: Dahi incorporated with $\alpha_s$-casein BAPs at 2.0 per cent level

The curd tension and synersis of caprine milk Dahi incorporated with 1.5 per cent BAPs of $\alpha_s$-casein is 345.65 mm, 24 per cent respectively (Table 3). Higher curd tension and synersis obtained from cow milk Dahi than caprine milk. Similar observation was done from Manmatha (2014) that the average penetration values and synersis of goat milk yoghurt.

**Table 3. Curd Tension and Syneresis of Caprine Milk Dahi**

| Parameters | Level | Curd Tension (mm/5 sec) | Syneresis (%) |
|---|---|---|---|
| Control | C0 | 349.50 | 28 |
| Unhydrolysed casein | C1 | 295.51 | 16 |
| $\alpha_s$-casein | C2 | 292.48 | 17 |
| | C3 | 345.65 | 24 |
| | C4 | 320.05 | 20 |
| CD (P ≤0.05) | | 0.034 | 0.026 |

# All the values are average of six trials.

Curd tension: expressed as penetration value
C0: Control cow milk Dahi
C1: Caprine milk Dahi
C2: Dahi incorporated with $\alpha_s$-casein BAPs at 1.0 per cent level
C3: Dahi incorporated with $\alpha_s$-casein BAPs at 1.5 per cent level
C4: Dahi incorporated with $\alpha_s$-casein BAPs at 2.0 per cent level

**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≤0.05) on all sensory attributes of all the samples with respect to control. C3 sample (1.5 % $\alpha_s$-CN BAPs) secured significantly higher score than other samples. The development of acidity and increase in pH was found to be slower. This may be due to slower acidity development, delay in release of free fatty acids, action of antibacterial and anti oxidative activity of BAPs. However, higher curd tension and synersis obtained from cow milk Dahi than caprine milk.

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