

## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
ISSN Online: 2617-4707  
IJABR 2025; SP-9(1): 690-697  
[www.biochemjournal.com](http://www.biochemjournal.com)  
Received: 26-10-2024  
Accepted: 27-11-2024

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## Effect of differential heat treatments on biofunctional properties of fermented goat milk

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**DOI:** <https://doi.org/10.33545/26174693.2025.v9.i1Si.3591>

### Abstract

This study explores the effect of different heat treatments (72 °C/15s, 85 °C/10min, and 121 °C/15min) on the biofunctional properties of fermented goat milk, with a focus on protein profile, antioxidant, antimicrobial, and antihypertensive activities. The protein profiles were analyzed using SDS-PAGE, revealing that fermentation and heat treatments significantly impacted the milk proteins. Caseins showed reduced intensity over the 21-day storage period, with the most degradation observed in samples heated to 121 °C. Whey proteins, such as  $\beta$ -lacto globulin ( $\beta$ -Lg), were more susceptible to heat-induced degradation, particularly at 121 °C. Antioxidant activity, measured through radical scavenging activity (RSA), increased progressively throughout the storage period for all heat treatments, with the highest RSA observed in milk heated to 121 °C. The RSA increased from 45.36% to 64.63% for 72 °C/15s, 43.25% to 61.78% for 85 °C/10min, and 51.18% to 70.20% for 121 °C/15min. Antimicrobial testing revealed that fermented milk heated at 85 °C exhibited significant antibacterial activity against *E. coli*, but no activity was detected against *B. cereus*, *S. aureus*, or *S. enterica*. Scanning electron microscopy showed that antimicrobial peptides from the milk disrupted the bacterial membrane. Additionally, the ACE inhibitory activity, a marker for antihypertensive potential, increased with fermentation, with the highest activity recorded at 85 °C/10min, reaching up to 71.68% by day 14, after which a slight decrease was observed. These results suggest that heat treatment and fermentation improve the biofunctional properties of goat milk, particularly enhancing antioxidant and antihypertensive activities, with potential applications in developing hypoallergenic and functional dairy products.

**Keywords:** Goat milk, Fermentation, ACE, RSA, SDS-PAGE

### 1. Introduction

Goat milk has gained recognition as a unique and valuable dairy product, often referred to as hypoallergenic milk due to its lower levels of  $\alpha$ -S1 casein, a protein associated with allergic reactions in cow milk. Its slightly reduced lactose content compared to cow milk makes it a suitable alternative for individuals with lactose intolerance. Additionally, goat milk boasts several technological advantages over cow milk, including smaller fat globules for smoother texture, softer gel products due to reduced  $\alpha$ -S1 casein, higher water-holding capacity, and lower viscosity (Mwenze, 2015) <sup>[15]</sup>.

The protein composition of goat milk includes caseins ( $\kappa$ ,  $\beta$ ,  $\alpha$ -S1,  $\alpha$ -S2, and  $\gamma$ -caseins) and whey proteins such as  $\beta$ -lacto globulin,  $\alpha$ -lactalbumin, serum albumin, and immunoglobulins (Tzboula-Clarke, 2003) <sup>[21]</sup>. Among these,  $\beta$ -casein constitutes a significant portion, identified through electrophoretic mobility (Park, 2006) <sup>[17]</sup>. With an average protein level of 3.8% (higher than cow milk but lower than sheep milk), goat milk's protein content is influenced by factors such as breed, lactation stage, and environmental conditions. Caseins, including  $\beta$ -CN,  $\alpha$ -S1 CN,  $\alpha$ -S2 CN, and  $\kappa$ -CN, make up a substantial portion of goat milk's protein profile, with distinctive distributions compared to bovine milk.

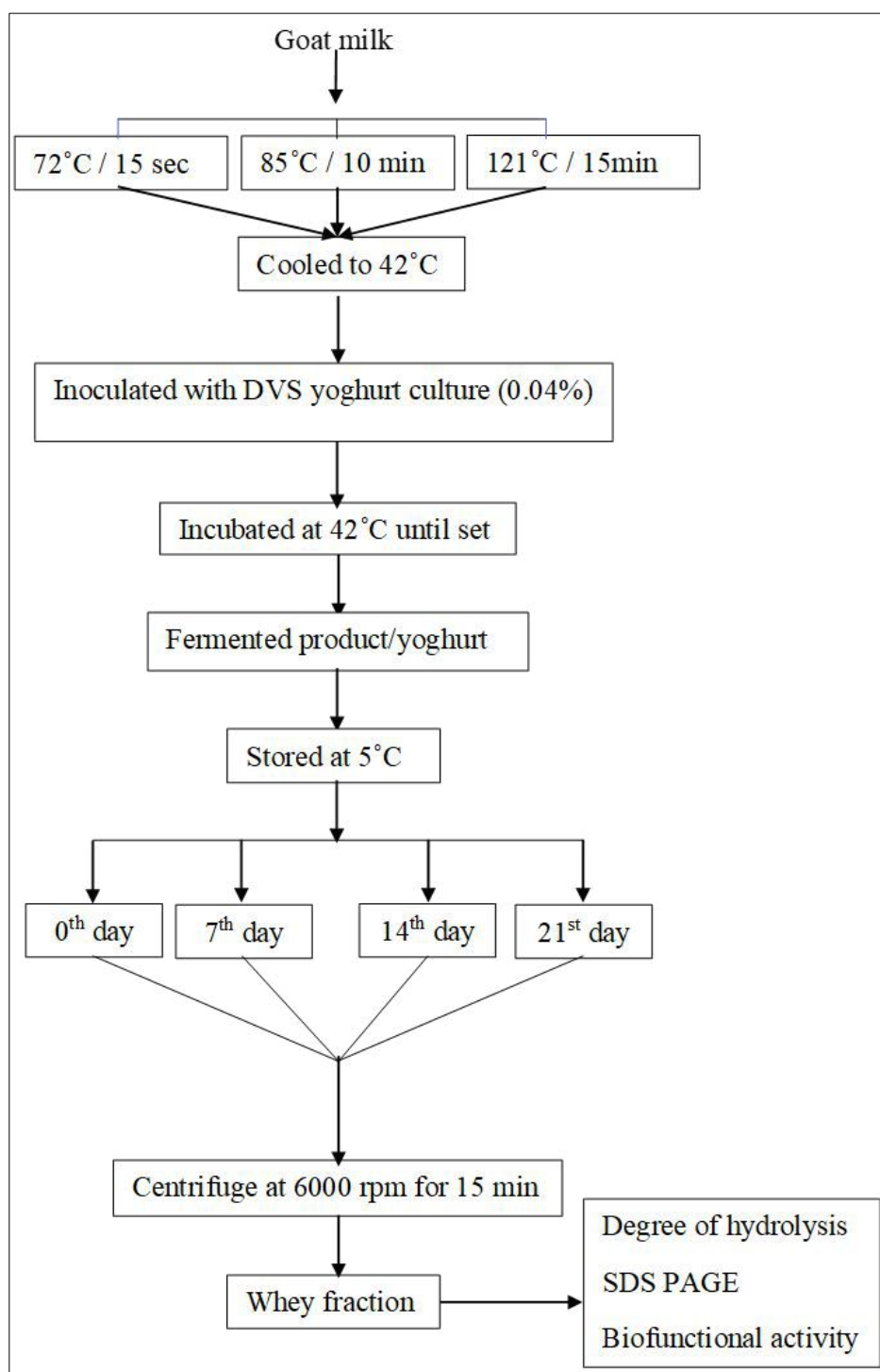
Fermented goat milk products offer numerous health benefits, including anti-allergenic, probiotic, and anti-carcinogenic properties. Fermentation enhances the therapeutic and nutritional qualities of goat milk while mitigating its distinct "goaty" flavor, improving its appeal to consumers (Slacanac *et al.*, 2010) <sup>[19]</sup>. The fermentation process also generates bioactive peptides, which exhibit significant biofunctional properties such as antihypertensive, antimicrobial, and antioxidative effects.

Bioactive peptides are typically produced through microbial fermentation or enzymatic hydrolysis, utilizing lactic acid bacteria (LAB) like *Lactococcus lactis*, *Lactobacillus helveticus*, and *Lb. delbrueckii* ssp. *bulgaricus*. These bacteria possess proteolytic systems capable of releasing bioactive oligopeptides and are cost-effective, safe, and efficient sources of proteolytic enzymes (Agyei *et al.*, 2011) <sup>[1]</sup>.

Goat milk's biofunctional properties have been extensively studied. Its antihypertensive activity, mediated by peptides that inhibit angiotensin-converting enzyme (ACE), offers a natural alternative to commercial ACE inhibitors without adverse side effects. Studies have shown that goat milk protein hydrolysates and kefir reduce blood pressure and ACE activity effectively (Geerlings *et al.*, 2006; Brunser *et al.*, 2007) <sup>[10, 3]</sup>. Additionally, goat milk peptides exhibit

antimicrobial activity, with lactoferrin and other proteins showing bacteriostatic and bactericidal effects against pathogens (Dionysius and Milne, 1997) <sup>[9]</sup>. The antioxidative properties of goat milk peptides, derived from caseins through fermentation or enzymatic hydrolysis, have been demonstrated to scavenge free radicals, chelate iron, and inhibit lipid peroxidation, thereby enhancing the shelf life and nutritional value of dairy products (Ahmed *et al.*, 2015; Gobba *et al.*, 2014) <sup>[2, 11]</sup>.

This study aims to evaluate the effects of differential heat treatments on the biofunctional properties of biopeptides in fermented goat milk. By examining changes in antihypertensive, antimicrobial, and antioxidative activities, this research seeks to contribute to the growing body of knowledge on optimizing the functional and therapeutic potential of goat milk-derived products.



## 2. Materials and Methods

### 2.1 Milk sample

Fresh pooled goat milk sample was collected from Government Goat Farm, Parassala, Trivandrum, and Kerala. The farm maintains the pure Malabari goat breed. Pooled milk was immediately transported to the laboratory.

### 2.2 Starter Culture

Yoghurt culture procured from Danisco (YO MIX 883 LYO 50 DCU) was used for the study, which was available in freeze-dried form. It is a blend of defined strains of thermophilic lactic acid bacteria (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*) for direct vat inoculation of milk, milkbase, and other food application.

### 2.3 Pathogenic organisms

The four bacterial pathogenic strains were used in this study. Organisms along with their source are as follows: *Bacillus cereus* ATCC 10876, *Escherichia coli* NCIM 2685, *Salmonella enterica* ATCC 6017, and *Staphylococcus aureus* ATCC 25923.

### 2.4 Packaging cups

The packaging cups made of polypropylene having 120 ml capacity with lids, purchased from the local market were used. These were cleaned with a detergent solution and immersed in hot water at 80 °C for 10 min and cooled to room temperature before use.

### 2.5 Sample preparation

Fresh pooled goat milk sample collected was divided into three lots. The three were subjected to three different time-temperature combinations: Treatment A: 72 °C for 15 sec, Treatment B: 85 °C for 10 min, and Treatment C: 121 °C for 15 min, then rapidly cooled to 42 °C. After inoculation with yogurt culture, the samples were packed in sterile plastic cups and incubated at 42 °C for 4-5 hours. Following incubation, the samples were stored at 5 °C for 21 days. Analyses were performed at specific intervals (0, 7, 14, and 21 days) to evaluate physicochemical properties, degree of hydrolysis, antioxidant and antimicrobial activities, and protein profiles. The flow chart for the preparation of samples is given below.

### 2.6 Protein fractionation

The samples containing protein were analyzed by SDS-PAGE under reducing conditions according to Laemmli (1970). The electrophoresis was performed in Orange Mini Dual Vertical Gel Electrophoresis Unit using 12.5 percent acrylamide separating gel and 4 percent acrylamide stacking gel.

### 2.7 Antioxidant Activity (DPPH)

The free radical scavenging activity of WSPE (Water Soluble Peptide Extract) was measured by the DPPH method with a slight modification (Zou *et al.*, 2014; Sravani, 2018) [24, 20].

### 2.8 Antibacterial activity

An agar well diffusion assay was performed to assess the inhibitory activity of WSPE against target strains (*Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, and

*Staphylococcus aureus*) as described by Vieira *et al.*, (2014) [22] with some modifications.

### 2.9 Anti-hypertensive activity

The samples were evaluated for the presence of ACE inhibitory activity. The assay of ACE-inhibitory activity based on the liberation of hippuric acid from hippuryl-L-histidyl-L-leucine (HHL) was catalyzed by ACE and measured by the method of Cushman (1971) [8].

### 2.10 Statistical Analysis

All experiments were carried out in triplicates and the mean values were tabulated. Differences between samples concerning bioactivity were tested using a two-way analysis of variance (GLM procedure), and means of samples were compared using Duncan's Multiple Range test (SPSS 29 static analysis, IBM).

## 3. Result and Discussion

### 3.1 Profiling of milk proteins

SDS-PAGE was utilized in this study to analyze milk protein profiles and the alterations caused by fermentation in milk subjected to different heat treatments. Fermented milk samples were separated into casein and whey fractions by centrifugation at 5000g for 30 minutes at 5 °C, and the clear whey was used for protein profiling. The protein profiles of fermented milk were studied across heat treatments and storage periods (0, 7, 14, and 21 days at 4 °C) to understand the effects of heat and storage, as shown in Figures 1. Whey was diluted with sample buffer in a 1:1 ratio before analysis. The changes in the protein bands of the fermented milk made from different heat treatments and stored for 0, 7, 14, and 21 days at 4 °C are presented in figure 1. As in Figure A, all of the four samples show typical milk-like protein characteristics, the most abundant proteins being  $\alpha$ -lactalbumin ( $\alpha$ -La),  $\beta$ -lacto globulin ( $\beta$ -Lg),  $\kappa$ -casein ( $\kappa$ -CN),  $\beta$ -casein ( $\beta$ -CN),  $\alpha$ -casein ( $\alpha$ -CN), and bovine serum albumin. The band intensity of  $\kappa$ -CN,  $\beta$ -CN, and  $\alpha$ -CN of the fermented milk visibly reduced from day 1 to day 21, while the electrophoretic bands referring to  $\alpha$ -La and  $\beta$ -Lg were essentially stable in all the samples. On the other hand, as anticipated, the use of heat treatments in Sample B resulted in a reduction in the intensity of the protein bands. Between 0 and 21 days of storage, casein showed no discernible modifications. On the other hand, from the 14<sup>th</sup> to the 21<sup>st</sup> day of storage,  $\beta$ -Lg displayed a sharp reduction. Furthermore, it is obvious that heating to 121 °C, induced more intensive degradation of casein and whey protein the  $\beta$ -Lg band completely disappeared.

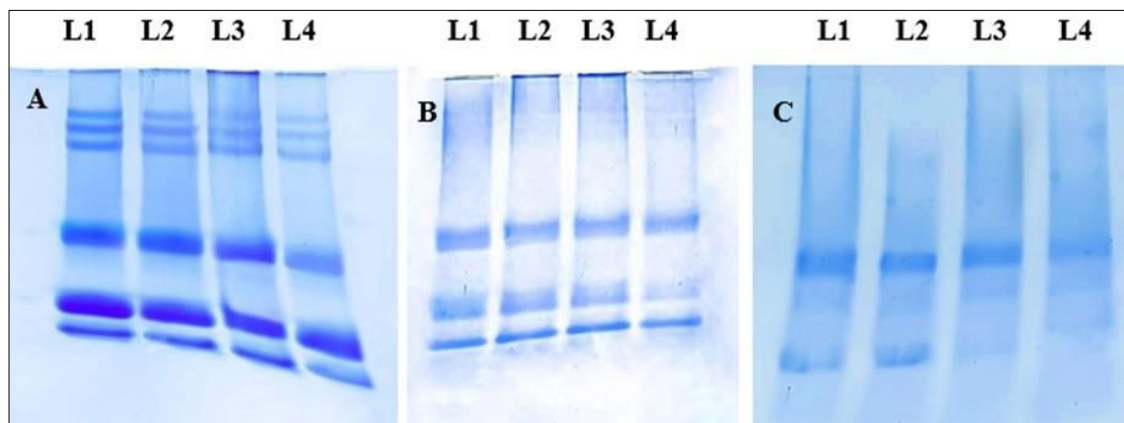
The electrophoretic profiles revealed that the whey protein bands were clearly visible in samples heated to 72 °C/15 seconds, with a thick band observed around 25 kDa. This band is likely a casein- $\beta$ -lacto globulin complex, as reported by Curlej *et al.* (2022). Heat treatments at 85 °C/10 minutes caused a moderate reduction in protein band intensity, while 121 °C/15 minutes resulted in pronounced degradation of both casein and whey proteins. Casein exhibited minimal changes until day 14, but  $\beta$ -Lg showed a sharp reduction between days 14 and 21, highlighting its poorer thermal stability and increased hydrolysis susceptibility compared to  $\alpha$ -La.

These findings are consistent with earlier studies, such as those by Savijoki *et al.* (2006) [18], which attributed casein degradation to the proteolytic activity of lactic acid bacteria.

The greater hydrolysis of  $\beta$ -Lg compared to  $\alpha$ -La may be due to lactic acid bacteria's stronger hydrolytic capacity and the denaturation of  $\beta$ -Lg during heat treatment, which unfolds its conformational structure, making it more susceptible to enzymatic breakdown. Bu *et al.* (2010) [4] reported similar outcomes, indicating that yogurt starter cultures effectively hydrolyze  $\beta$ -Lg.

The combination of heat treatment and fermentation significantly influenced whey protein hydrolysis, reducing

antigenicity. Lactalbumin and lactoglobulin, known milk allergens, were substantially degraded, as shown by the diminished antigenicity of fermented milk sterilized at 110 °C for 10 minutes, which reportedly reduces antigenicity by 99% (Jedrychowski and Wroblewska, 1999) [13]. These findings underscore the potential for developing hypoallergenic milk products with enhanced sensory properties through tailored heat and fermentation processes.



A: 72 °C/15sec B: 85 °C/10min C: 121 °C/15min. L1-0<sup>th</sup>, L2-7<sup>th</sup>, L3-14<sup>th</sup>, L4-21<sup>st</sup> day

**Fig 1:** Electrophoretic pattern of fermented goat milk heated to different temperatures during the storage period

### 3.2 Antioxidant activity

The antioxidant activity of fermented milk was evaluated over a storage period of 21 days at a refrigerated

temperature of 5±2 °C, with measurements taken at 7-day intervals. The results are presented in Table 1.

**Table 1:** RSA of differentially heated fermented goat milk

| Temperature   | 0 <sup>th</sup> day        | 7 <sup>th</sup> day        | 14 <sup>th</sup> day        | 21 <sup>st</sup> day       | F-Value  |
|---------------|----------------------------|----------------------------|-----------------------------|----------------------------|----------|
| 72 °C/15se    | 52.366±0.567 <sup>ax</sup> | 57.376±0.435 <sup>ax</sup> | 62.093±0.300 <sup>ay</sup>  | 64.633±0.650 <sup>ay</sup> | 67.799*  |
| 85 °C /10min  | 50.235±0.890 <sup>ax</sup> | 54.780±0.720 <sup>ax</sup> | 59.583±0.612 <sup>aby</sup> | 61.783±0.699 <sup>ay</sup> | 14.861*  |
| 121 °C /15min | 59.890±0.673 <sup>bx</sup> | 61.196±0.625 <sup>bx</sup> | 65.640±0.190 <sup>by</sup>  | 70.200±0.608 <sup>bz</sup> | 144.324* |
| F value       | 1.145 *                    | 2.024 *                    | 8.382 *                     | 10.836 *                   |          |

Figures are mean ± standard deviation of triplicate analysis. \*-Significant at five percent level ( $p < 0.05$ ).<sup>ns</sup>-non-Significant ( $p > 0.05$ ). A-C: means with different superscripts vary significantly within a column. X-Z: means with different superscripts vary significantly within a row.

The data in Table 1 indicate that radical scavenging activity (RSA%) increased progressively throughout the storage period. A statistically significant difference ( $p < 0.05$ ) was observed in RSA% between the samples analysed on the 0<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> days for all three treatments. The most notable increase occurred between the 0<sup>th</sup> and 21<sup>st</sup> days. Among the treatments, the sample initially heated to 121 °C/15 minutes exhibited the highest RSA%, with the difference being statistically significant ( $p < 0.05$ ) compared to the other two treatments in this study, a comparison of the antioxidant potential between unfermented and fermented milk samples showed a significant increase in antioxidant activity due to fermentation. This change was already noticeable on the 0<sup>th</sup> day of fermentation. The radical scavenging activity (RSA) of milk heated to 72 °C for 15 seconds increased from 45.36% to 52.36%, while at 85 °C for 10 minutes it rose from 43.25% to 50.23%, and at 121 °C for 15 minutes, it increased from 51.18% to 59.89%. Over 21 days of storage, the antioxidant activity further improved to 64.63%, 61.78%, and 70.20% for the respective heat treatments. Significant differences in antioxidant activity were observed both between heat treatments and across storage days. Milk initially heated to 121 °C for 15

minutes exhibited the highest antioxidant potential. Nandhini *et al.* (2012) [16] assessed the antioxidant activity of goat milk fermented with *Lactobacillus plantarum* using the DPPH assay and reported 61.7% RSA. Natural antioxidants in milk, such as  $\alpha$ -tocopherol, carotenoids, conjugated linoleic acid, casein, and lactoferrin in whey, contribute to its antioxidant properties (Calligaris *et al.*, 2004) [5]. Additionally, the Maillard reaction products, known for their antioxidant effects, may explain the increase in antioxidant activity during later stages of heating. According to Chen *et al.* (2003) [6], the higher scavenging capacity of fermented milk is likely related to milk proteins. It is well-established that milk proteins treated with digestive enzymes or fermented with lactic acid bacteria can generate peptides with potential biological activity.

### 3.3 Antimicrobial activity

The antibacterial activity of fermented milk was evaluated during refrigerated storage at 5 °C for up to 21 days, with analyses conducted at intervals of 0 (after four hours of incubation at 42 °C), 7, 14, and 21 days. The fermented milk was centrifuged at 10,000 rpm for 30 minutes to isolate the water-soluble peptide fraction (WSPF), which was then

freeze-dried and stored at -20 °C until analysis. Antibacterial activity was assessed against four pathogens: *B. cereus* (ATCC 10876), *E. coli* (NCIM 2685), *S. enterica* (ATCC 6017), and *S. aureus* (ATCC 25923). To prevent precipitation, the WSPF was adjusted to pH 7.4 before

testing. Results are shown in Table 2. Additionally, morphological changes in *E. coli* (NCIM 2685) were observed using Scanning Electron Microscopy, as described by Zhao *et al.* (2015) [23].

**Table 2:** Antimicrobial activity of differentially heated fermented goat milk

| Pathogens                    | Samples      | 0 <sup>th</sup> | 7 <sup>th</sup> | 14 <sup>th</sup> | 21 <sup>st</sup> |
|------------------------------|--------------|-----------------|-----------------|------------------|------------------|
| <i>Bacillus cereus</i>       | 72 °C/15sec  | Abs.            | Abs.            | Abs.             | Abs.             |
|                              | 85 °C/10min  | Abs.            | Abs.            | Abs.             | Abs.             |
|                              | 121 °C/15min | Abs.            | Abs.            | Abs.             | Abs.             |
| <i>Escherichia coli</i>      | 72 °C/15sec  | Abs.            | Abs.            | Abs.             | Abs.             |
|                              | 85 °C/10min  | 12±2.00mm*      | 17±0.55mm*      | 21±0.55mm*       | 14±0.433mm*      |
|                              | 121 °C/15min | Abs.            | Abs.            | Abs.             | Abs.             |
| <i>Staphylococcus aureus</i> | 72 °C/15sec  | Abs.            | Abs.            | Abs.             | Abs.             |
|                              | 85 °C/10min  | Abs.            | Abs.            | Abs.             | Abs.             |
|                              | 121 °C/15min | Abs.            | Abs.            | Abs.             | Abs.             |
| <i>Salmonella enterica</i>   | 72 °C/15sec  | Abs.            | Abs.            | Abs.             | Abs.             |
|                              | 85 °C/10min  | Abs.            | Abs.            | Abs.             | Abs.             |
|                              | 121 °C/15min | Abs.            | Abs.            | Abs.             | Abs.             |

\*Zone of inhibition includes 6mm well diameter as well. Figures are mean ± standard deviation of triplicate analysis.

From Figure 2, it is evident that the WSPF obtained from fermented milk initially heated to 85 °C/10 minutes exhibited antimicrobial activity against *E. coli* NCIM 2685. The zone of inhibition (average of triplicate analyses) measured 12 mm, 17 mm, 21 mm, and 14 mm on the 0<sup>th</sup>,

7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> days, respectively, with the highest inhibitory activity observed on the 14<sup>th</sup> day. However, no zones of inhibition were observed against *B. cereus* ATCC 10876, *S. enterica* ATCC 6017, or *S. aureus* ATCC 25923 throughout the 21-day storage period.

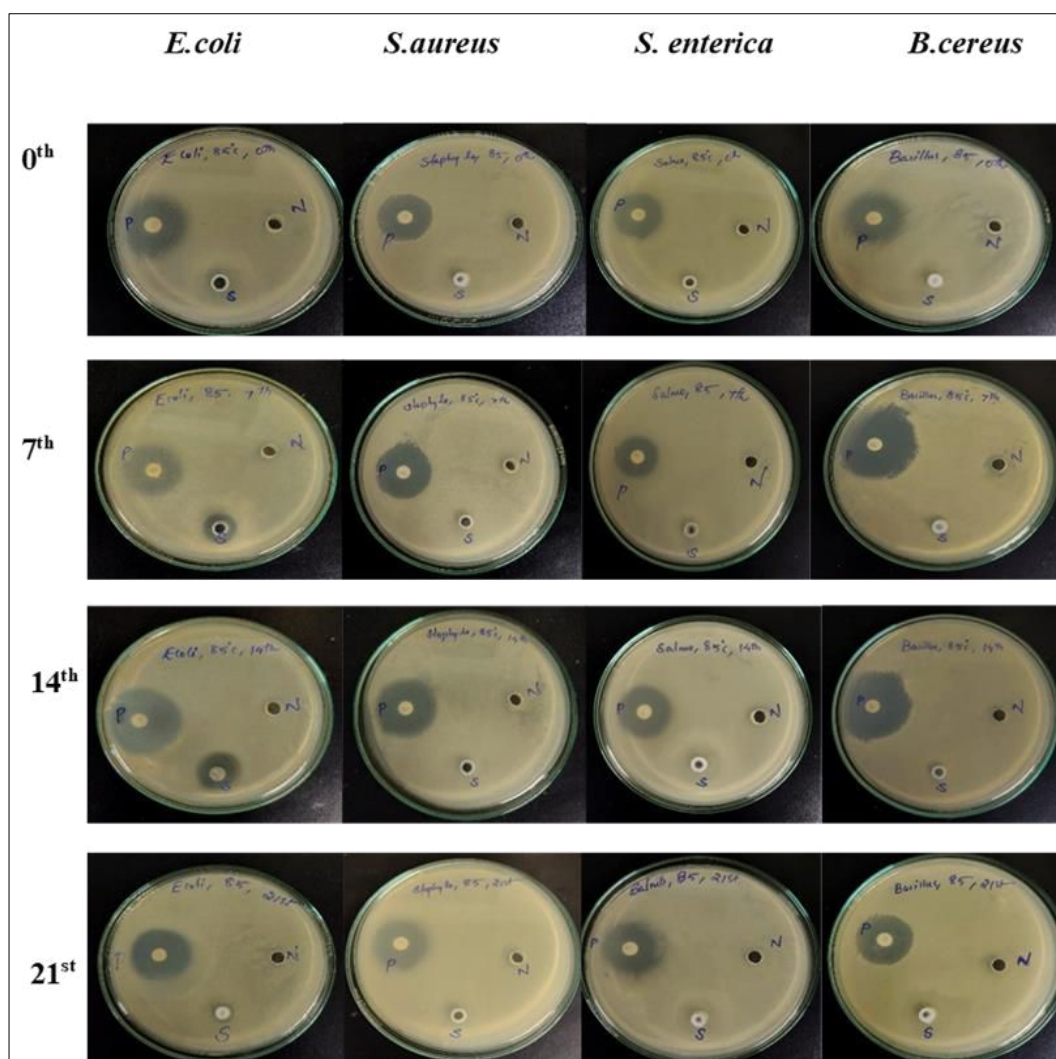


**Fig 2:** Antibacterial activity of the WSPF of fermented milk made from goat milk heated to 85 °C/10min against different pathogens during cold storage (inhibition zone in mm). P-positive control, N-negative control, S-sample

Further, Scanning Electron Microscopy (SEM) was utilized to examine the morphology of *E. coli* NCIM 2685 treated with WSPF (from the 7<sup>th</sup> and 14<sup>th</sup> days of fermented milk heated to 85 °C/10 minutes). The results revealed that the

peptides possessed membrane-lytic antimicrobial properties (Figure 3). The treated samples showed no intact bacterial cells, and fibrous material, likely resulting from cell lysis, leakage, and detritus, was observed.

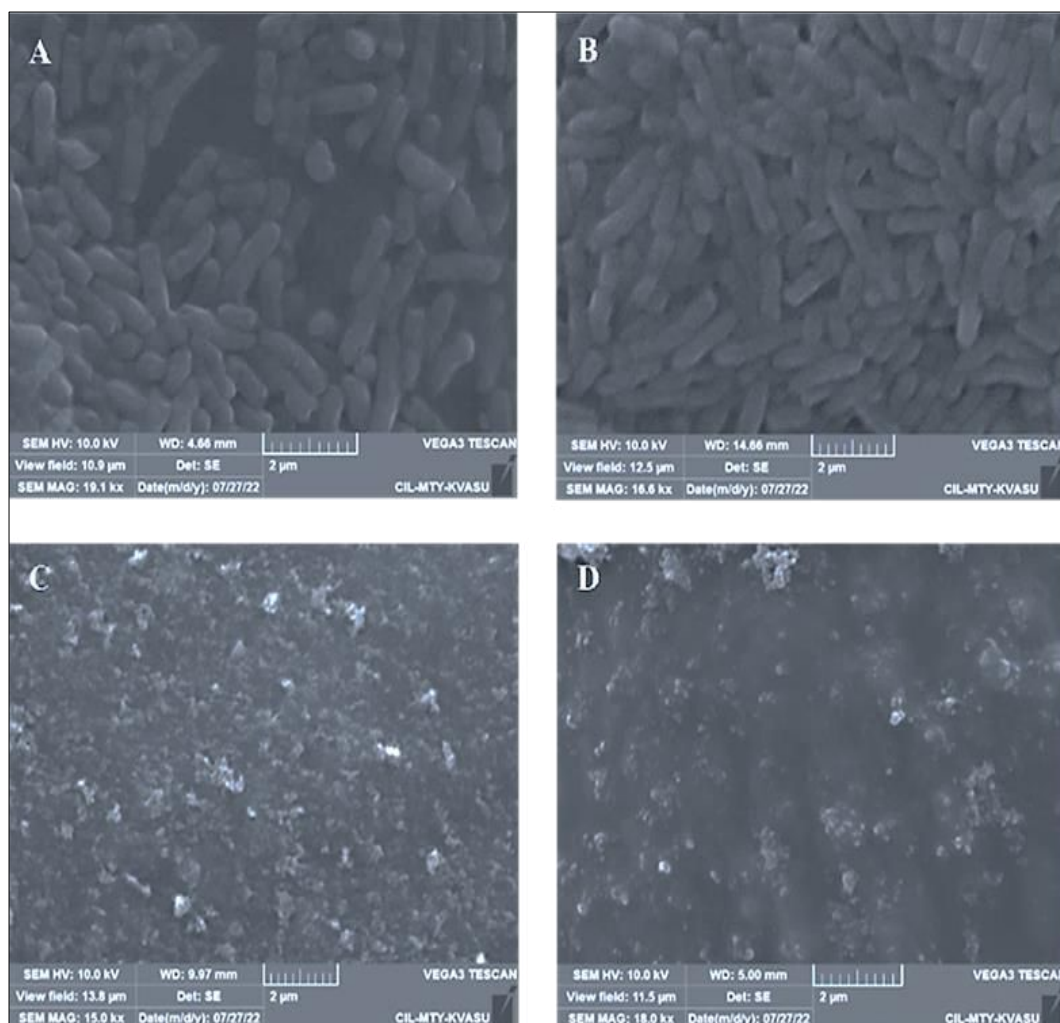


**Fig 3:** Scanning electron micrographs showing morphological changes of *Escherichia coli* (A, B-untreated control cells; C-cells treated with WSPE of 7<sup>th</sup> day fermented milk made from goat milk heated to 85 °C/10min; D-cells treated with WSPE of 14<sup>th</sup> day fermented milk made from goat milk heated to 85 °C/10min) induced by treating for 6 hours at 37°C with WSPEs.

Reported antimicrobial activity in goat milk fermented with *Lactobacillus* strains, effective against *E. coli*, *B. cereus*, *S. typhi*, and *E. faecalis*. However, in our study, antimicrobial activity was observed only against *E. coli*, likely due to differences in goat breed, lactation stage, and the pathogens used. SEM analysis of *E. coli* treated with WSPE from milk fermented at 85 °C for 10 minutes revealed no intact bacterial cells, but the appearance of fibrous material, likely resulting from cell lysis. This suggests that the antimicrobial peptides may have membrane-lytic properties, leading to cell death. Peptides, especially low molecular weight ones generated during fermentation, can disrupt microbial

membrane integrity or translocate across membranes to act on internal targets. The antimicrobial peptides likely interact with lipopolysaccharides or lipoteichoic acids on the bacterial membrane through electrostatic attraction, disrupting membrane integrity. This interaction could displace essential divalent cations like  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , causing membrane deformation and leading to cell death.

### 3.5 Anti-hypertensive activity

The antihypertensive activity of the milk samples was determined by the ACE inhibitory activity and the percentage inhibition obtained is illustrated in Table 3.

**Table 3:** ACE Inhibition of differentially heated fermented goat milk

| Temperature  | 0 <sup>th</sup> day        | 7 <sup>th</sup> day        | 14 <sup>th</sup> day       | 21 <sup>st</sup> day       | F-Value  |
|--------------|----------------------------|----------------------------|----------------------------|----------------------------|----------|
| 72 °C/15sec  | 31.490±0.550 <sup>ax</sup> | 43.026±0.154 <sup>ay</sup> | 62.073±0.214 <sup>az</sup> | 47.400±0.556 <sup>ay</sup> | 140.398* |
| 85 °C/10min  | 42.660±0.645 <sup>bx</sup> | 57.760±0.732 <sup>by</sup> | 71.68±0.650 <sup>cz</sup>  | 57.920±0.258 <sup>by</sup> | 112.411* |
| 121 °C/15min | 36.900±0.550 <sup>ax</sup> | 43.486±0.682 <sup>ay</sup> | 55.556±0.560 <sup>bz</sup> | 47.146±0.816 <sup>ay</sup> | 42.721*  |
| F-Value      | 31.202*                    | 94.727*                    | 25.017*                    | 53.716*                    |          |

Figures are mean ± standard deviation of triplicate analysis. \*-Significant at five percent level ( $p < 0.05$ ).<sup>ns</sup>-non-Significant ( $p > 0.05$ ). A-C: means with different superscripts vary significantly within a column, X-Z: means with different superscripts vary significantly within a row.

From the table, it could be concluded that ACE inhibitory activity increased from 0<sup>th</sup> to 14<sup>th</sup> day, but a slight decrease was found on the 21<sup>st</sup> day. A similar trend was seen in all three treatments.

In this study, fermentation markedly enhanced the ACE inhibitory activity of milk compared to unfermented samples. This improvement was evident from the 0<sup>th</sup> day of fermentation, with activity increasing from 28.66% to 31.49% for milk heated to 72 °C/15 seconds, from 35.47% to 42.66% for milk heated to 85 °C/10 minutes, and from 30.23% to 36.99% for milk heated to 121 °C/15 minutes. During 21 days of storage, ACE inhibitory activity steadily increased until the 14<sup>th</sup> day, reaching 62.07%, 71.68%, and 55.55% for the respective treatments. However, a slight decline was observed on the 21<sup>st</sup> day. Milk treated at 85 °C/10 minutes consistently demonstrated the highest inhibitory activity throughout the storage period. The increase in activity is attributed to the proteolytic action of LAB during fermentation, while the subsequent decline may be due to psychrotrophic microbial growth during prolonged refrigerated storage. These findings align with Nandhini *et al.* (2012) [16], who reported an ACE inhibitory activity of 88.96% in goat milk fermented with *Lactobacillus plantarum*.

#### 4. Conclusion

In conclusion, this study demonstrates the significant impact of differential heat treatments and fermentation on the biofunctional properties of goat milk. The results indicate that heat treatment, combined with fermentation, enhances the antioxidant, antimicrobial, and antihypertensive activities of milk, with the highest improvements observed in samples heated to 121 °C for 15 minutes. SDS-PAGE profiling revealed the degradation of casein and whey proteins, especially  $\beta$ -lacto globulin, over the storage period, with heating to higher temperatures leading to more pronounced protein degradation. The antioxidant activity increased progressively during fermentation and storage, with 121 °C treatment exhibiting the highest radical scavenging activity. Additionally, antimicrobial activity was observed in samples heated to 85 °C, particularly against *Escherichia coli*, and the antimicrobial peptides appeared to possess membrane-lytic properties. The ACE inhibitory activity also showed a notable increase during fermentation, indicating potential for developing functional dairy products with antihypertensive benefits. These findings highlight the potential of fermented and heat-treated goat milk as a functional food with improved bioactive properties, offering health benefits such as enhanced antioxidant, antimicrobial, and antihypertensive activities.

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