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Research Article

Effect of Thermal Processing on Antioxidant and Antimicrobial Activities in Different Milk Types

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Abstract

Present investigation was undertaken to study the effects of various thermal treatments on antioxidant and antimicrobial activities of milk fractions of indigenous cattle, Jersey cross-bred cattle, local non-Gaddi goats and Gaddi goats. The significant ($p < 0.05$) variation in total phenolic content (TPC) was observed among all the breeds and the significantly higher value was noted in Gaddi goat boiled milk. Thermal processing significantly ($p < 0.05$) enhanced TPC in boiled milk of indigenous and Jersey cross-bred cattle. Ferric ion reducing antioxidant power (FRAP) was significantly ($p < 0.05$) higher in local non-Gaddi goats fresh milk. Antimicrobial activity was detected only in indigenous cattle whey fractions, boiled milk and its whey fractions against *Escherichia coli* and *Staphylococcus aureus*. Maximum antimicrobial activity was observed in boiled whey protein fractions against *E. coli* and *S. aureus*. SDS-PAGE profiling of milk protein revealed that casein protein fractions were more heat-resistant as compared to whey protein fractions in all milk types. It is inferred that thermal processing of milk led to increased total phenol, antioxidant, and antimicrobial activities.

Keywords: Milk; Thermal Processing; Antioxidant Activity; Antimicrobial Activity; Protein Profile

Introduction

Milk is the secreted fluid of the mammary glands of female mammals. It contains the -various vital nutrients to sustain neonates' life. White or yellowish colour in milk is governed by scattering and absorption of light by fat globules and protein micelles [1]. Main composition of milk is water (87%-88%), the remaining part is total milk solids which include carbohydrates, fat, proteins and ash or minerals. However, the composition is not constant, the average percentage of milk component varies with animal species and breeds, season, feeds, stage of lactation and health and physiological status of a particular animal. Sometimes the composition might even change from day by day, depending on feeding and climate, but also during milking the first milk differs from the last milk drops [2]. Milk proteins are divided into two main groups: caseins and whey proteins. In addition to supply of amino acids, milk proteins are also important for their biological properties; in fact some peptide derived from milk digestion has possible regulatory effect on nutrient uptake postprandial hormone secretion, immune defense and neuroendocrine information transfer [3]. Casein proteins are not simple because of their micellar composition and common properties. However, casein proteins are easily separated from other milk proteins by acid precipitation at pH 4.6. This is because acidification causes the casein micelles to destabilize or aggregate by decreasing their electric charge to the isoelectric point [4].

Milk whey proteins have been demonstrated to have antioxidant properties and some authors have hypothesized their use in food industry as a natural conservant [5]. Antioxidant compounds are considered to be essential in diets or medical therapies for delaying aging and biological tissue deterioration [6]. Antioxidant peptides present in food chain play a major role in immune-system by preventing formation of free radicals or by scavenging free radicals and active oxygen species which induce oxidative damage to biomolecules and cause aging, cancer, heart disease, stroke, and arteriosclerosis. Antioxidants from natural sources are more superior to those produced chemically, because some synthetic antioxidants have been reported to be carcinogenic [7]. A number of bioactive peptides have been identified in milk protein hydrolysates and fermented dairy products [8].

Literature suggested that raw milk is highly favorable medium to sustain the growth of spoilage and pathogenic bacteria and other microbes. To destroy the unwanted bacteria, pasteurization, the process named after French Microbiologist Louis Pasteur, is used [9]. Heat treatments such as pasteurization, boiling or sterilization have direct influence on the nutritional, biological and functional properties of milk proteins. Information on nutritive and therapeutic values of hilly cattle and goat milk is scarce. Therefore, present investigation was carried to study the effect of thermal processing on therapeutic potential of locally available cattle and goats milk.

Materials and Methods

Sample collection

Milk samples of indigenous cattle, Jersey cross-bred cattle, Gaddi goats and local non-Gaddi goats were obtained from surrounding areas of Palampur (Himachal Pradesh, India). Milk samples were collected in 250ml sterilized container for analysis of bioactive potential. Milk samples were immediately transported in an ice box to the laboratory. The pH and titrable acidity (%) of milk samples were measured and then milk containers were stored in a freezer at -20°C until required. Before analysis, milk samples were allowed to thaw at room temperature for approximately 30 min.

Separation of casein and whey fraction

Separation of casein and whey from milk was carried out using the method described by [10] with slight modifications. Casein and whey fractions were separated from raw milk processed under different conditions *viz*., Direct milk (raw milk); Pasteurized at 63°C (Holder pasteurization): About 30ml of fresh milk was taken in a test tube and held in water bath at 63°C for half an hour; Pasteurized at 72°C (HTST): About 30ml of fresh milk was taken in test tube and kept in water bath at 72°C for 15sec.; Boiled milk: 30 ml of fresh milk was taken in test tube and heated for boiling temperature.

15ml of milk of each part was taken in a beaker and recorded the pH using pH meter. The whole casein was obtained by adjusting the pH to 4.0 (the isoelectric point of casein) using 1N HCl drop wise with constant stirring. Curdled milk was then centrifuged at 5000 rpm for 30 min. Upper layer of milk fat was removed with the help of spatula, and pellet obtained was labeled as casein fraction. Supernatant was collected in another beaker and its pH was adjusted up to 7.0 using 1N NaOH with constant stirring followed by centrifugation for 30min. at 5,000 rpm. Supernatant obtained after centrifugation was labeled as whey fraction. Both casein and whey fractions were stored at -20°C for further analysis.

Sample preparation and antioxidant extraction

Extraction of milk samples was carried out using the method described by [11]. The samples were placed at room temperature. Extraction was done by using 1ml of milk; whey fraction (obtained from 1ml milk) and casein fraction (obtained from 1ml milk) were taken in different conical flasks. Added 10ml of the solvent extract (One normal solution of HC1 (1 N)/95% ethanol (v/v, 15/85) was prepared and used as extraction solvent) to each flask. Contents were kept in orbital shaker for 1hr. at 120 rpm at 37°C followed by centrifugation at 5000 rpm for 15min. Pallet was discarded and supernatant was collected and labeled as sample extract, which was stored at -20°C for further estimation of TPC and FRAP.

Antioxidant activity

Total phenol content (TPC)

Antioxidant activity through TPC was determined according to the method of [11] with minor modifications. About 100 µl of extracted milk and its fractions was added to 0.4 ml distilled water and 0.5 ml diluted Folin-Ciocalteu reagent. Samples with the reagent were left for 5 min, and then 1 ml 7.5% sodium carbonate (w/v) was added. The absorbance was measured after 1 h at 765 nm using a spectrophotometer. Calibration curve of tannic acid was plotted to evaluate the activity capacity of the samples. Result was expressed as milligram of tannic acid equivalents per 100 gram of fresh sample (mg TA/100 g of FW).

Ferric reducing antioxidant power (FRAP)

FRAP assay was performed according to method of [11] with minor modifications. FRAP reagent was prepared fresh using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate, 16ml glacial acid made up to 1:1 with distilled water), 10 mM TPTZ (2,4,6-tris (2-pyridyl)-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in the ratio of 10:1:1 to give the working reagent. Approximately 200 µl of extracted fresh milk was added to 2 ml FRAP reagent, and the absorbance was measured at 595 nm - using a spectrophotometer after 30 min. Calibration curve of ferrous sulphate was set up to estimate the activity capacity of samples. Result was expressed as milligram of $FeSO_4$ equivalents per 100g of fresh samples (mg $FeSO_4$ /100g of FW).

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The method of [12] with minor modification was used to evaluate antioxidant activity through DPPH scavenging. To prepare the stock solution, 3.9 mg DPPH was dissolved in 100 ml methanol and kept in the dark for 1h. Approximately 100 µl of milk and its fractions was added to 2 ml DPPH solution. After 30 min. 2 ml chloroform was added, and centrifuged at 5000 rpm for 30 min. Absorbance was measured at 517 nm using a spectrophotometer. The percentage of DPPH scavenging activity was calculated as

Inhibition (%) = [(A blank - A sample)/A blank] × 100

Where A is the absorbance.

Protein profile by SDS-PAGE

The protein profile of milk samples was determined according to method of [13], using the Mini-PROTEAN Tetra-Cell system (Bio-Rad, Budapest, Hungary). Samples were mixed with sample buffer [Stacking gel buffer of pH 6.8, 10% (w/v) SDS, 20% (v/v) glycerol, ß-mercaptoethanol, Bromophenol Blue], heated at 95 °C for 90 sec. and then applied onto a discontinuous gel system consisting of a 5% stacking, and 12.5% resolving sodium dodecyl sulfate-polyacrylamide gel. The protein concentration was determined by the Lowry method [14] using bovine serum albumin as a standard. Pre-stained Protein Marker (14-205kDa, Sigma) was used as mass standard. Proteins bands on gel were stained with Coomassie Brilliant Blue.

Antimicrobial activity

Antimicrobial activities of various samples of milk were evaluated by disc diffusion method. A saturated solution of Nutrient agar was prepared in double distilled water and it was autoclaved for 15 min at 103kPa, and allowed to cool to approx. 40°C and then poured in petri-plates. After solidification and drying of Nutrient agar at room temperature, *Escherichia coli*, *Shigella flexneri*, *Rhodococcus equi*, *Staphylococcus aureus* and *Bacillus cereus* were spread by using standard spread plate technique. Sterile absorbent paper discs of size 10mm (HIMEDIA) were placed on the surface of Nutrient agar plate using a sterile forceps. Added 100µL of sample onto the disc and left undisturbed for 30min. Then the microbiological plates were incubated aerobically at 37°C for overnight. Zone of inhibition developed around discs was measured. The bigger the diameter of the inhibition zone, the more susceptible is the microorganism to the antimicrobial fraction applied on discs.

Milk and whey were directly loaded on the disc. Casein was completely dissolved in phosphate buffered saline (PBS) (0.1g of casein in 1ml PBS). After complete dissolution it was used to determine the antimicrobial activity of casein. Streptomycin sulphate (1mg/5ml of broth) was used as positive control, and nutrient broth served as negative control.

Statistical analysis

The statistical analysis was done by using SAS 9.2 statistical package. Results are presented as means and standard error of means. A *P-value* of 0.05 ($p < 0.05$) was considered statistically significant.

Results and Discussion

Antioxidant activity

The total antioxidant capacity of samples was evaluated using three different methods. TPC, DPPH as well as FRAP profile of different animal breeds milk under different thermal conditions has been presented in Tables 1, 2 and 3.

Total phenol content (TPC)

Phenolic compounds, being good sources of natural dietary antioxidants are found in noticeable amounts. Indeed, many of the phenolic metabolites in milk are derived from the feed and dietary ingredients. TPC in milk and its fraction of different breeds have been depicted in Table I. In this study, we found that the pheno-

lic content in milk was significantly ($p < 0.05$) affected by thermal treatments. Among different thermal treatments the phenolic content was significantly higher under boiling conditions in milk. The phenolic content was found to be highest in Gaddi goat milk and lowest in local non-Gaddi goat's milk (Table 1). The alleviated level of milk antioxidant properties is primarily due to the presence of sulfur containing amino acids such as cysteine as well as vitamins (Vitamins A and E), minerals (zinc, selenium) and carotenoids, These factors decrease the reactive oxygen species (ROS) level *via* upregulating the endogenous enzyme level such as superoxide dismutase, catalase and glutathione peroxidase [15]. Antioxidant activities of indigenous cattle [16] and Gaddi goat milk, whey and casein in different lactation stages [17] have already been reported. Gaddi goats are reared primarily for meat, fiber and milk by the native traditional "Gaddi" shepherds [18].

It is apparent from review of the literature that antioxidant capacity of the milk and dairy products is due to sulfur containing amino acids like cysteine, phosphate, vitamins A, E, carotenoids, Zn, Se, catalase, glutathione peroxidase, SOD, and milk peptides released as result of fermentation and cheese ripening [19]. Phytochemicals consumed through fodder or forage [7] or supplemented during fermentation of milk can enhance antioxidative activities [20].

Herbal supplemented indigenous cattle milk was found to possess enhanced ACE activity. Among different undigested milk samples containing native herbal species *viz*., harad (*Terminalia chebula*), baheda (*T. bellirica*), arjuna (*T. arjuna*) and amla (*Phyllanthus emblica*), the arjuna (*T. arjuna*) exhibited highest ACE-inhibitory activity [20].

Ferric reducing antioxidant power (FRAP)

FRAP assay, a simple, convenient and reproducible method is based on the reduction of a ferric tripyridyltriazine (TPTZ) complex to its ferrous form [21]. The ferrous ion chelating activities in milk and its fraction of different breeds were estimated and depicted in table 2. As observed in the present study, we found that Gaddi goat's milk is comparatively a rich source of FRAP activity. Moreover, FRAP was found to increase in boiled milk of all types. Similar DPPH and FRAP observations have been recorded by [11,22], where goat milk showed higher antioxidant potential as compared to cow milk.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH is a stable organic free radical. It is based on this principle, the reduction in the concentration of the DPPH solution in the presence of a hydrogen-donating antioxidant is allowed to monitor the decrease in its absorbance at a characteristic wavelength and lead to the formation of a non-radical form DPPH-H [23]. Significant differences in DPPH per cent inhibition were found under different thermal conditions among all the breeds ($p < 0.05$) (Table 3). Direct and indirect heat treatments facilitate the Millard reaction between the nucleophilic group of amino acids and carbonyl group of sugars leads to the formation of new antioxidant compounds which contribute for its antioxidant property [24]. Similar results were also observed by [25] where Ultra High Temperature (UHT) treated milk showed the higher antioxidative potential, which was confirmed by TPC, FRAP and DPPH assays. Increase in antioxidant potential may be due to the development of antioxidant compounds [25].

Antimicrobial activity

One of the primary functions of milk is to protect the health of neonates. Milk contains several peptides and proteins which exhibit antimicrobial property [26-28]. The antimicrobial activity in milk depends upon several factors such as peptides charge, molecular size, chemical conformational and hydrophobic properties. In some cases activities of antimicrobial peptides is enhanced by processing techniques such as fermentation and thermal treatments. Moreover, bacteria residing in human gastrointestinal tract in human can also produce antimicrobial peptides [29]. In a study, Ozturk, *et al.* [30] demonstrated that milk is good source of antimicrobial compound, the xanthine oxidase (XO). Xanthine oxidase inhibits the growth of pathogenic bacteria and reduces the generation of reactive oxygen species. In addition, its activity is preserved up to 100 per cent by high temperature short time pasteurization (HIST). Antimicrobial activity against *Staphylococcus aureus* was resulted in milk and whey fraction of indigenous cattle. In case of milk, antimicrobial activity was noticed only when milk was pasteurized at 72°C and at boiling temperature, in whey fraction antimicrobial activity was recorded in all the pasteurized treatments. In casein fraction, no activity was recorded against *Staphylococcus* (Plate 1). However, antibacterial activity in casein fraction has been reported by some other workers [31]. In our study, antimicrobial

S. No.	Sample	Indigenous Cattle	Cross-bred Cattle	Gaddi goat	Local non-Gaddi goat
	Milk				
1	Raw milk	33.17 ± 2.15^{c}	40.4 ± 1.80^{c}	49.29 ± 1.59^{a}	34.14 ± 1.16^{b}
2	Pasteurized milk at 63˚C	38.28 ± 1.99^{b}	41.42 ± 2.05^{c}	44.96 ± 1.56^{b}	34.18 ± 1.09^{b}
3	Pasteurized milk at 72˚C	40.3 ± 2.41^{a}	46.90 ± 2.15^{b}	44.59 ± 1.6^{b}	32.54 ± 1.52^{c}
4	Boiled milk	41.12 ± 2.35^{a}	48.73 ± 2.06^{a}	49.52 ± 1.83^{a}	36.01 ± 1.10^{a}
	Whey fraction of milk				
1	Fresh/Raw milk whey	13.47 ± 1.01^{a}	17.80 ± 1.58^{d}	7.00 ± 0.38^{c}	15.19 ± 0.72^{a}
2	Pasteurized milk whey (at 63˚C)	13.58 ± 1.06^{a}	19.18 ± 1.40^{c}	10.07 ± 0.25^{a}	13.62 ± 0.74^{d}
3	Pasteurized milk whey (at 72˚C)	13.51 ± 0.90^{a}	22.01 ± 1.46^{b}	5.07 ± 0.44^{d}	15.07 ± 0.65^{bc}
4	Boiled milk whey	10.07 ± 1.12^{b}	23.66 ± 1.92^{a}	8.28 ± 0.37^{b}	14.85 ± 0.70^{c}
	Casein fraction of milk				
1	Fresh/Raw milk casein	9.70 ± 1.89^{a}	8.81 ± 0.84^{b}	8.51 ± 0.25^{b}	19.03 ± 0.88^{a}
2	Pasteurized milk casein (at 63˚C)	9.78 ± 1.46^{a}	10.49 ± 0.41^{a}	7.95 ± 0.19^{c}	17.65 ± 0.85^{c}
3	Pasteurized milk casein (at 72˚C)	5.60 ± 1.51^{c}	8.69 ± 0.81^{b}	6.49 ± 0.27^{d}	18.28^{b} ± 0.83
4	Boiled milk casein	7.84 ± 1.54^{b}	10.11 ± 0.51^{a}	9.14 ± 0.19^{a}	17.91 ± 0.89^{c}

Table 1: Effect of thermal processing on TPC (mg TAE/100ml) in milk and its fraction of different breeds

Values with different superscripts with in groups are statistically significant (a, b, c, d = P < 0.05).

S. No.	Sample	Indigenous Cattle	Cross-bred Cattle	Gaddi goat	Local non-Gaddi goat
	Milk				
1	Raw milk	44.61 ± 2.19^{d}	48.98 ± 1.85^{c}	19.61 ± 0.58^{c}	65.55 ± 2.66^{a}
2	Pasteurized milk at 63˚C	47.89 ± 1.19^{c}	49.38 ± 1.81^{c}	25.00 ± 0.91^{a}	64.53 ± 2.33^{ab}
3	Pasteurized milk at 72˚C	52.50 ± 1.52^{b}	53.13 ± 1.93^{b}	18.13 ± 0.65^{d}	57.42 ± 2.35^{c}
4	Boiled milk	55.47 ± 2.18^{a}	51.26 ± 2.22^{a}	22.81 ± 0.90^{b}	63.13 ± 2.46^{b}
	Whey fraction of milk				
1	Fresh/Raw milk whey	33.67 ± 1.19^{a}	38.98 ± 1.88^{a}	16.48 ± 0.66^{a}	39.69 ± 1.50^{a}

2	Pasteurized milk whey (at 63˚C)	31.80 ± 1.25^{b}	32.73 ± 1.70^{c}	16.41 ± 0.59^{a}	35.47 ± 1.30^{c}
3	Pasteurized milk whey (at 72˚C)	26.19 ± 0.91^{c}	37.42 ± 1.72^{b}	15.78 ± 0.47^{b}	33.28 ± 1.48^{d}
4	Boiled milk whey	32.19 ± 1.20^{b}	37.66 ± 2.04^{a}	17.11 ± 0.63^{a}	37.42 ± 0.32^{b}
	Casein fraction of milk				
1	Fresh/Raw milk casein	34.38 ± 1.31^{a}	34.61 ± 1.64^{c}	25.30 ± 0.34^{a}	36.25 ± 1.18^{a}
2	Pasteurized milk casein (at 63˚C)	25.47 ± 0.90^{d}	32.34 ± 1.31^{d}	23.00 ± 0.84^{b}	26.72 ± 1.13^{c}
3	Pasteurized milk casein (at 72˚C)	29.06 ± 0.77^{c}	36.48 ± 1.54^{b}	21.50 ± 0.75^{c}	27.42 ± 0.99^{c}
4	Boiled milk casein	31.17 ± 0.71^{b}	38.13 ± 1.21^{a}	19.20 ± 0.37^{d}	32.27 ± 1.13^{b}

Table 2: Effect of thermal processing on FRAP (mg $Fe.SO_4$ equivalent/100ml) in milk and its fraction of different breeds.Values with different superscripts with in groups are statistically significant (a, b, c, d = P < 0.05). Table 1: Effect of thermal processing on TPC (mg TAE/100ml) in milk and its fraction of different breeds

Values with different superscripts with in groups are statistically significant (a, b, c, d = P < 0.05).

S. No.	Sample	Indigenous Cattle	Cross-bred Cattle	Gaddi goat	Local non-Gaddi goat
	Milk				
1	Raw milk	34.51 ± 2.75^{c}	26.45 ± 1.95^{b}	12.55 ± 1.28^{c}	6.63 ± 0.89^{c}
2	Pasteurized milk at 63˚C	34.34 ± 2.64^{c}	28.56 ± 1.96^{a}	16.50 ± 0.87^{a}	8.73 ± 0.94^{b}
3	Pasteurized milk at 72˚C	43.82 ± 2.43^{a}	25.50 ± 1.60^{b}	14.39 ± 1.02^{b}	12.51 ± 0.88^{a}
4	Boiled milk	36.94 ± 2.55^{b}	26.52 ± 2.34^{b}	14.86 ± 1.14^{b}	7.83 ± 0.87^{b}
	Whey fraction of milk				
1	Fresh/Raw milk whey	20.70 ± 1.71^{b}	17.54 ± 0.98^{NS}	12.470.76^{a}	6.42 ± 1.11^{c}
2	Pasteurized milk whey (at 63˚C)	21.85 ± 1.39^{ab}	16.73 ± 0.88^{NS}	12.67 ± 0.71^{a}	8.17 ± 1.06^{b}
3	Pasteurized milk whey (at 72˚C)	23.04 ± 1.23^{a}	17.01 ± 0.88^{NS}	10.87 ± 0.84^{b}	9.69 ± 1.11^{a}
4	Boiled milk whey	15.48 ± 1.23^{c}	17.30 ± 0.82^{NS}	12.34 ± 0.84^{a}	7.23 ± 0.98^{c}
	Casein fraction of milk				
1	Fresh/Raw milk casein	23.91 ± 0.71^{a}	10.29 ± 0.94a	9.75 ± 0.51^{b}	10.61 ± 0.76^{b}
2	Pasteurized milk casein (at 63˚C)	18.25 ± 0.49^{b}	9.94^{a} ± 0.96^{b}	11.43 ± 0.52^{a}	12.80 ± 0.74^{a}
3	Pasteurized milk casein (at 72˚C)	17.27 ± 0.65^{c}	9.06 ± 1.05^{b}	11.07 ± 0.53^{a}	12.37 ± 0.72^{a}
4	Boiled milk casein	19.10 ± 0.44^{b}	9.92 ± 0.82^{ab}	9.79 ± 0.45^{b}	10.52 ± 0.69^{b}

Table 3: Effect of thermal processing on DPPH (% inhibition) in milk and its fraction of different breeds.

Values with different superscripts with in groups are statistically significant (a, b, c, d = P < 0.05).

activity was observed under thermal treatments, as small amount of inactive antimicrobial peptides are present in raw milk and these peptides may become active after thermal treatments.

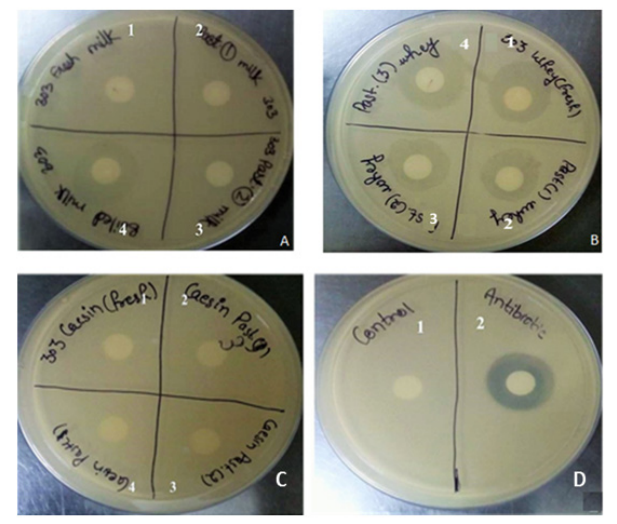

Plate 1: Effect of thermal treatments on antimicrobial activity in milk and its fractions of indigenous cattle against *E. coli*. A: 1- Fresh milk; 2 - Milk pasteurized at 63°C/30min.; 3- Milk pasteurized at 72°C/15sec.; 4- Boiled milk B: 1 - Fresh milk whey; 2- Whey obtained from milk pasteurized at 63°C/30min.; 3- Whey obtained from milk pasteurized at 72°C/15sec; 4- Whey obtained from boiled milk C: 1- Fresh milk casein; 2- Casein obtained from milk pasteurized at 63°C/30min.; 3- Casein obtained from milk pasteurized at 72°C/15sec.; 4- Boiled milk casein; D: Control. 1- -ve control *i.e.* Nutrient Broth, 2 +ve control - Streptomycin sulphate.

However, in Jersey cross-bred cattle, local non-Gaddi goats and Gaddi goats, no antimicrobial activities were found against indicator organisms cited ahead. Antibacterial activity in 100 µL milk fractions of indigenous cattle, Jersey cross-bred cattle, local non-Gaddi goats goat and Gaddi goat was recorded against different micro-organisms i.e. *Escherichia coli, Staphylococcus aureus*, *Rhodococcus equi*, *Shigella flexneri*, *Bacillus cereus*. The results of antimicrobial activity are depicted in figure 1 and also in plate 2.

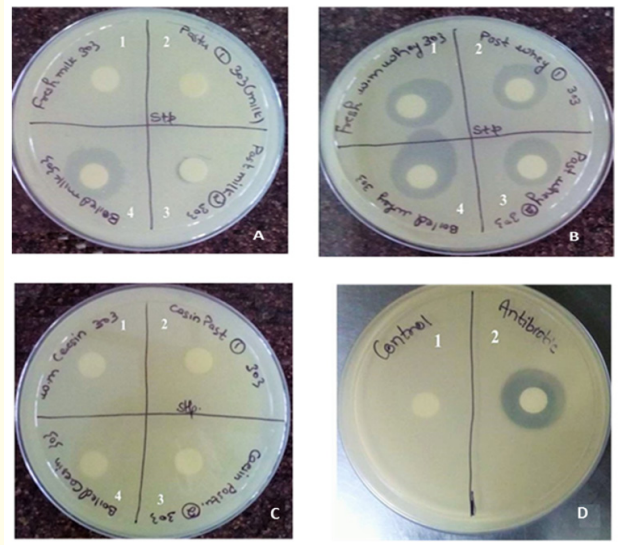

Plate 2: Effect of thermal treatments on antimicrobial activity in milk and its fractions of indigenous cattle against *S. aureus.* A: 1- Fresh milk; 2 - Milk pasteurized at 63°C/30min.; 3- Milk pasteurized at 72°C/15sec.; 4- Boiled milk B: 1 - Fresh milk whey; 2- Whey obtained from milk pasteurized at 63°C/30min.; 3- Whey obtained from milk pasteurized at 72°C/15sec; 4- Whey obtained from boiled milk C: 1- Fresh milk casein; 2- Casein obtained from milk pasteurized at 63°C/30min.; 3- Casein obtained from milk pasteurized at 72°C/15sec.; 4- Boiled milk casein; D: Control. 1- -ve control *i.e.* Nutrient Broth, 2 +ve control - Streptomycin sulphate.

Antimicrobial activity in milk is mainly resulted by whey proteins such as lactoferrin, lysozyme, immunoglobulins and lactoperoxidase. However, the content of these proteins may vary, depends upon the species and breeds [32,33]. In our study, antimicrobial activity against *E. coli* was observed in milk and whey fractions of indigenous cattle in all the thermal treatments *i.e.* pasteurization at 63°C, 72°C and boiling temperature, whereas whey fraction obtained from raw milk also showed the antimicrobial activity against *E. Coli*. However, no antimicrobial activity was noticed against *E. coli* with casein fraction. Kamel, *et al*. [34] have reported the similar findings, wherein lactobacillus species isolated from raw bovine milk could inhibit the growth of *staphylococcus aureus*.

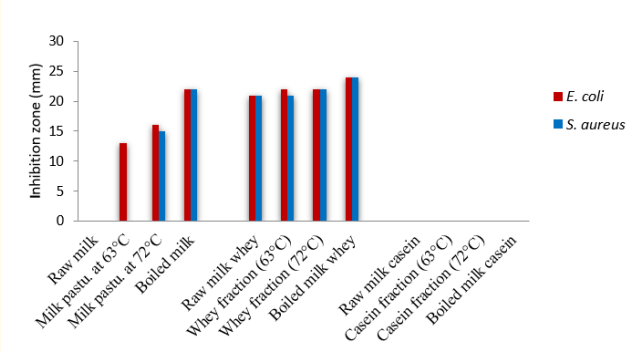

Figure 1: Antimicrobial activity of indigenous cattle milk and its fractions against *E. coli* and *Staphylococcus aureus.*

Protein profile by SDS-PAGE

SDS-PAGE of raw milk and its fractions under different temperature conditions was run in order to observe the changes induced by heat treatments on the milk protein patterns of indigenous cattle, Jersey cross-bred cattle, local non-Gaddi goats and Gaddi goat milk. The resulting SDS-PAGE showed the effect of pasteurization on the milk proteins of above breeds are shown in figure 1. These figures show the major difference in whey proteins when subjected to boiling temperature. Whey proteins undergo conformational changes when subjected to high temperature, especially the UHT [35] In present study, high molecular weight protein bands of whey protein disappeared under boiling conditions, which related to the results reported earlier [36], whereas in case of HTST (High Temperature Short Time) pasteurization and holder pasteurization, the resulting protein profile are similar to protein bands appeared in raw milk. In casein protein fraction, no effect of pasteurization was observed which indicates that casein proteins are more heat resistant as compared to the whey proteins. The whey proteins as found in milk are typical globular proteins with well-defined secondary and tertiary structures. In contrast to the highly stable caseins, the globular whey proteins retain their native conformations only within relatively limited temperature ranges. Exposing the whey proteins to boiling temperature resulted in denaturation and aggregation of the proteins. Other studies have also shown the effect of different thermal treatment on casein and whey proteins from raw milk. Under different thermal treatments whey proteins bands were changed from deep to shallow. At 65°C, protein profile of whey was similar to raw milk however, higher temperature showed denaturation in whey proteins. On other hand casein is thermally stable and leads to the formation of polymer in combination with whey proteins (αLa and βLg) [37,38]. (Figure 2)

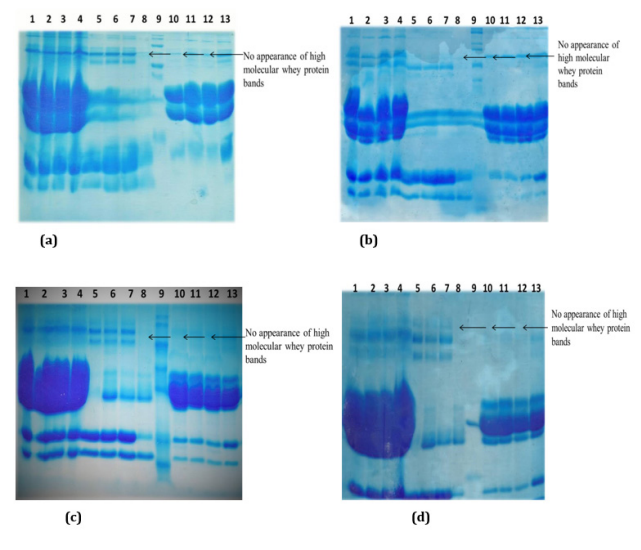

Figure 2: SDS-PAGE protein profile of milk proteins (whey and casein) from indigenous cattle (a), Jersey cross-bred cattle (b), local non-Gaddi goats (c) and Gaddi goats (d), before and after thermal processing. L1 - Whole milk; L2 - Whole milk pasteurized at 63°C/30 min.; L3 - Whole milk pasteurized at 72°C /30 min.; L4 - Boiled whole milk; L5 - Whey obtained from raw milk; L6 - Whey obtained from milk Pasteurized at 63°C; L7 - Whey obtained from milk Pasteurized at 72°C; L8 - Whey of Boiled milk; L9 - Marker Sigma; L10 - Casein obtained from whole milk (dissolved in PBS); L11 - Casein obtained from milk Pasteurized at 63°C (dissolved in PBS); L12 - Casein from milk Pasteurized at 72°C (dissolved in PBS); L13- Casein of Boiled milk (dissolved in PBS).

Conclusion

The present study provides preliminary data on the antioxidant activity in different animal milk under thermal processing. Results showed that the TPC exhibit higher value in Gaddi goats milk under boiling condition whereas the ferric ion reducing capacity was higher in Local non-Gaddi goats milk. Antimicrobial proteins naturally present in milk have the ability to kill and inhibit a certain

bacteria. The antibacterial properties of these proteins make them suitable for use in a variety of applications. The antimicrobial activity of milk and its fractions under different thermal conditions was determined in indigenous cattle, Jersey cross-bred cattle, local non-Gaddi goats and Gaddi goats. In indigenous cattle milk and its whey fraction, the activity was noticed only against *E. coli* and *S. aureus* and was increased after thermal treatment. However, no activity was observed in other milk types. No change was observed in protein content of milk after thermal treatment/pasteurization. High molecular weight whey proteins denatured under boiling conditions, whereas no effect of heat treatment was observed on casein protein fractions, implying that casein proteins are more heat resistant than whey proteins. The study may pave the way to the unearthing of milk-origin potent antioxidants and antimicrobials for future health and pharmaceuticals applications.

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