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Research Article

Bioactive Properties of *Gaddi* Goat Milk Casein Protein Hydrolysates Treated with Alcalase

Gorakh Mal*, Birbal Singh, Gauri Jairath, Rinku Sharma, Ajayta Rialch, Devi Gopinath and Suma N

ICAR-Indian Veterinary Research Institute, Regional Station, Palampur (HP), India

***Corresponding Author:** Gorakh Mal, ICAR-Indian Veterinary Research Institute, Regional Station, Palampur (HP), India.

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Abstract

Gaddi goat milk casein protein fractions were hydrolysed with proteolytic enzyme alcalase, and further subjected to *in vitro* digestion using gastric (pepsin) and intestinal (trypsin & pancreatin) enzymes. Bioactive peptides obtained after enzymatic hydrolysis and *in vitro* digestion of hydrolysates were centrifuged. Supernatants were used for protein profiling by SDS-PAGE and estimation of various antioxidant, antihypertensive and antimicrobial activities. SDS-PAGE revealed that major of the *Gaddi* goat casein proteins were degraded after enzymatic hydrolysis and, degraded completely after *in vitro* digestion. OPA activity was noted to be increased with enzymatic hydrolysis and further enhanced after *in vitro* digestion. OPA activity was observed highest (37.46 ± 0.26 mg/ml) in overnight *in vitro* digested casein hydrolysates generated with enzyme alacalase A1 (1:100). FRAP antioxidant activities were also noted highest (22.59 ± 0.18 mg/100ml) in overnight *in vitro* digested *Gaddi* goat casein protein hydrolysates generated with enzyme alcalase A1 (1:100) as compare to *Gaddi* goat casein *in vitro* digested hydrolysates prepared with alcalase A2 (0.01:100) and A3 (0.005:100). DPPH activity was observed higher in pepsin digested hydrolysates than the corresponding undigested hydrolysates and overnight *in vitro* digested hydrolysates. DPPH activity was noted maximum (36.76 ± 0.14 %) in pepsin digested *Gaddi* goat casein protein hydrolysates generated with enzyme alcalase (A1). Dry dot- TLC showed that DPPH antioxidant activity remains stable subsequently overnight in *Gaddi* goat casein hydrolysates and *in vitro* digested hydrolysates prepared with various concentrations of alcalase. Antihypertensive activity was noted highest (48.24 ± 0.35 %) in overnight *in vitro* digested *Gaddi* goat casein protein hydrolysates prepared with enzyme alcalase (A1). Antimicrobial activity was noted maximum (14 mm) in pepsin digested hydrolysates of *Gaddi* goat casein protein hydrolysates generated with enzyme alcalase (A1) against *Rhodococcus equi.* Among the three concentrations of alcalase (A1, A2 and A3) used in the present study, antioxidant, antihypertensive and antimicrobial activities were found to be highest in *Gaddi* goat casein protein hydrolysates prepared with enzyme alcalase (A1), indicating likely application as bioactive and purposeful components in various food preparations.

Keywords: *Gaddi* Goat; Casein Proteins; Enzymatic Hydrolysis; *In Vitro* Digestion; OPA Activity; Antioxidant Activity; Antihypertensive Activity; Antimicrobial Activity

Introduction

Among native goats, *Gaddi* goat also known as ''White Himalayan goat'' is predominantly a migratory and economically important multipurpose goat breed of high altitude in Northwestern Himalayan Region. They are reared primarily for meat, fiber, and milk by the native ''*Gaddi*'' shepherds [1]. Goat milk is highly nutritious and consumed in many nations; yet, the development of functional foods from goat milk has been slower than that of other types of milk [2].

Approximately 2% of the world's total annual milk supply comes from goats [3]. Certain goats are bred specifically to produce milk. Goat milk doesn't require homogenization because it naturally contains tiny, well emulsified fat globules that keep the cream suspended longer than cow's milk [4].

Despite the fact that cow milk accounts for the majority of dairy products consumed worldwide, goat milk ranks third (2.3%) (FAOSTAT, 2022a), and its production is significant economically in several nations [5,6]. New producers and investors have entered the market as a result of the goat products' appealing prices, particularly for milk [7]. Yogurt, cheese, fermented milk, and goat milk powder are only a few of the goods made from goat milk that are already on the market; the majority of them are made using refined processing techniques [6]. Furthermore, goat milk has special qualities because of its physiological and biochemical characteristics, which are frequently regarded as being far better than those of cow milk [8]. The protein content of goat milk is comparable to that of human milk, making it extremely nutrient-dense [9,10]. Goat milk's casein fraction is mostly made up of β-casein, which is followed in decreasing order by αs-casein and κ-casein. The primary component of the protein fraction in cow's milk is αs1-casein [11,12]. Goat milk has a much lower percentage of αs1-casein than cow milk, which is known to disrupt human digestion and result in the development of solid curds in the stomach [13-15]. The proteins found in goat milk exhibit superior digestibility and hypoallergenic characteristics [16]. The bioactivities associated with peptides derived from milk proteins cover a broad spectrum, including antihypertensive, antioxidant, and cholesterol-lowering effects [17,18].

Casein and whey proteins make up the majority of milk proteins; when they hydrolyze to varying degrees, they produce hydrolysates with distinct functional characteristics [19]. Goat milk proteins have demonstrated potential as a source for the creation of hydrolysates that display antioxidant properties. Recently, [20] generated antioxidant peptides through the enzymatic hydrolysis of goat milk microfiltration fractions utilizing subtilisin and trypsin enzymes that contain hydrophobic or positively charged amino acids at the C- terminus, respectively. Research has demonstrated that these characteristics are advantageous for promoting bioactivities [21,22]. Milk proteins serve as the primary source of bioactive peptides that are liberated through enzymatic hydrolysis during gastrointestinal passage or food processing. These peptides have been identified in dairy protein hydrolysates and have demonstrated properties such as opioid effects, immune modulation, antimicrobial activity, antithrombotic effects, growth stimulation, and antihypertensive properties [23,24]. Milk hydrolysis breaks down bigger protein molecules into smaller peptides and amino acids, making them easier to absorb and digest in the body [25]. This method can improve the sensory properties by changing taste, flavour, and texture [26]. The proteolysis of dairy raw materials yields bioactive peptides (BAP), which have antimicrobial, hypotensive, immunomodulatory, and antioxidant properties. These BAPs have great potential as ingredients in functional foods and therapeutic agents [27-30]. The selection of the substrate and enzyme or enzymes, the circumstances of the enzymatic reaction, and the characterization of the proteolysis products are all steps in the technological process of creating bioactive hydrolysates [31,32]. Alcalase is a protease enzyme produced by the bacterium *Bacillus licheniformis*. It is known in the dairy industry for its important function in milk protein hydrolysis [33]. Alcalase works best in alkaline circumstances, giving it an excellent choice for breaking down complicated protein structures into smaller peptides and amino acids [34].

The primary active peptides found in goat milk include one peptide derived from whey β-lactoglobulin, specifically the PEQSLACQCL fragment 113–122, along with two peptides sourced from caseins: ARHPHPHLSFM (fragment 96–106 κ-casein) and QSLV-

YPFTGPI (fragment 56–66 β-casein). These peptides exhibited significant ACE inhibitory activity, which is comparable to that of captopril, an ACE inhibitor, when assessed on a weight basis [35]. Owing to the significant genetic variability present in milk proteins, there exists a substantial opportunity to obtain bioactive peptides with diverse characteristics. Among various bioactive substances, antimicrobial peptides (AMPs) derived from milk are becoming increasingly appealing as safe additives that enhance the shelf life of minimally processed foods. These peptides exhibit a wide range of antagonistic effects against bacteria, fungi, viruses, and protozoans [36].

The present study was undertaken with the objective to study the effect of alcalase generated enzymatic hydrolysates for various bioactivities including antioxidant, antimicrobial and antihypertensive activities.

Material and Methods

Chemicals and reagents

Fine chemicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), orthophthaldialdehyde (OPA), angiotensin converting enzyme from rabbit lung (ACE) and molecular markers were purchased from Sigma- Aldrich (St. Louis, MO, USA). All enzymes such as alcalase (from *Bacillus liceniformis*), Pepsin (from porcine gastric mucosa), Trypsin (from porcine pancreas), and Pancreatin (from porcine pancreas) were also purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade. TLC silica gel F_{254} coated aluminium plates used for qualitative antioxidant activity were purchased from Merck KGaA (Germany).

Milk samples

Milk samples of *Gaddi* goat were procured from local areas of Palampur (H.P.). Milk Samples were collected in aseptic conditions and immediately transported in the ice box to the laboratory. pH of the fresh samples was noted and processed for further analyses.

Separation of casein from milk

The separation of casein from milk was carried out using the procedure outlined by [1]. A total of 15 g of the obtained casein was reconstituted in 100 ml of 0.1M PBS, pH 7.0 (15%) by stirring on a magnetic stirrer while heating to 50°C.

Preparation of *Gaddi* goat milk casein hydrolysates with enzyme alcalase

Gaddi goat milk casein protein hydrolysates were prepared following the method outlined by [37], with minor adjustments. The pH of the 15% casein was adjusted to 8 (by using either 40% NaOH or 4M HCL as needed). The *Gaddi* goat milk casein was divided into four parts, one as the control casein (C). The other three parts were designated as A1, A2, and A3. Each of these three portions had enzyme alcalase (w/v) added separately at enzyme-to-substrate ratios of 1:100, 0.01:100, and 0.005:100, respectively.

The samples were thoroughly mixed and placed in an orbital shaker at 50°C and 120 rpm. Aliquots were taken at various time points (0h- immediately after mixing, and then at ½h, 1h, 1½h, 2h, and 4h after incubation) as described by [1].

In vitro digestion of *Gaddi* goat milk casein hydrolysates generated with enzyme alcalase

A two-step process was used to simulate gastric and intestinal digestion of *Gaddi* goat casein 1 hour hydrolysates prepared with enzyme alcalase using the *in vitro* enzymatic digestion protocol outlined by [1] in the presence of enzymes: pepsin, trypsin and pancreatin.

After inhibition of enzymatic activity, samples were collected at every step such as undigested hydrolysates (UD), after 30 minutes of pepsin digestion (PD), after 4 hours of trypsin & pancrea-

tin digestion (4hrs.D), and after overnight digestion with trypsin & pancreatin (OD) followed by centrifugation at 10000 rpm for 10 minutes. Supernatants were collected and stored at -20°C for further analyses.

All the estimations, including SDS protein profile, OPA, FRAP, DPPH, antihypertensive and antimicrobial activities were carried out in the supernatants of undigested and *in vitro* digested hydrolysates generated with enzyme alcalase [1].

Statistical analysis

The statistical analysis was done by using SAS One way Anova. Results were presented as means and standard error of means. A P-value of 0.05 ($p<0.05$) was considered statistically significant.

Results and Discussion

Protein profile by SDS-PAGE in *Gaddi* goat milk casein hydrolysates and *in vitro* digested hydrolysates generated with enzyme alcalase

Protein profiles of *Gaddi* goat milk casein protein hydrolysates generated with alcalase showed the degradation of casein proteins completely just after adding alcalase (A1) as shown in figure 1A. Further, SDS-PAGE also showed complete degradation of major casein protein fractions after 4 hour of enzymatic hydrolysis in A2 and A3 (figure 1B). SDS-PAGE revealed almost degradation of major *Gaddi* goat casein protein fractions after overnight *in vitro* digestion hydrolysates generated with enzyme alcalase as shown in figure 1C and 1D.

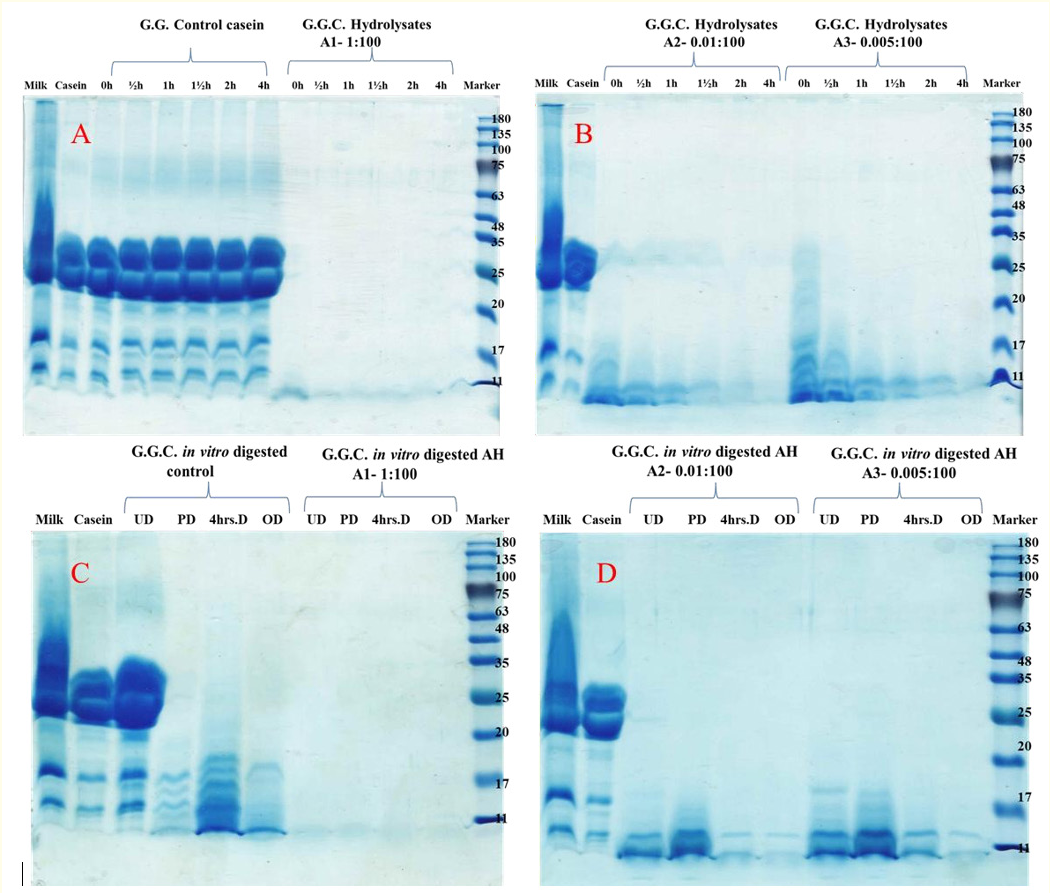

Figure 1: A, B- *Gaddi* goat milk casein protein hydrolysates; C, D- *Gaddi* goat milk casein protein *in vitro* digested hydrolysates generated with enzyme alcalase.

Comparable disappearance of casein protein bands within a few hours under enzymatic treatment has been documented for bovine [38] and camel caseins [39], corroborating the rapid susceptibility of caseins to typical hydrolysis conditions. *Gaddi* goat whey protein hydrolysates generated with papain have also observed comparable findings [1].

OPA activity in *Gaddi* goat milk casein protein hydrolysates and *in vitro* digested hydrolysates generated with enzyme alcalase

OPA activity increases with time during enzymatic hydrolysis with alcalase. OPA activity was observed higher in hydrolysates generated with higher concentration of enzyme (A1) than the other concentrations of enzyme used (A2 and A3). However, OPA activity was observed maximum (35.99 ± 0.33 mg/ml) after 4 h of enzymatic hydrolysis of *Gaddi* goat casein with enzyme alcalase A1 as depicted in table 1. Further, *in vitro* digestion enhances the OPA activity of *Gaddi* goat casein hydrolysates generated with alcalase than the corresponding undigested sample. OPA activity was noted maximum (37.46 ± 0.26 mg/ml) in overnight *in vitro* digested *Gaddi* goat casein protein hydrolysates prepared with alcalase (A1) as shown in table 2. The ready susceptibility of caseins to proteolysis is mechanistically expected because caseins behave as intrinsically disordered proteins with flexible, solvent-exposed backbones that provide numerous accessible cleavage sites [40].

S. No.	Time of collection	OPA (mg/ml hydrolysed sample) equivalent tryptone			
		Gaddi goat milk casein hydrolysates of alcalase			
		Control	A1 (1:100)	A2 (0.01:100)	A3 (0.005:100)
1	0 hour	6.78^{Ab} ± 0.09	25.20^{Da} ± 0.69	7.90^{Cb} ± 0.23	6.17^{Cb} ± 0.03
2	1 hour	7.18^{Ac} ± 0.10	32.69^{Ca} ± 0.18	12.02^{Bb} ± 0.32	11.03^{Bb} ± 0.04
3	2 hour	8.47^{Ad} ± 0.13	34.41^{Ba} ± 0.51	16.10^{Ab} ± 0.03	13.71^{Ac} ± 0.12
4	4 hour	7.55^{Ac} ± 0.48	35.99^{Aa} ± 0.33	17.13^{Ab} ± 0.17	16.88^{Ab} ± 0.09

Table 1: OPA activity in *Gaddi* goat milk casein protein hydrolysates generated with enzyme alcalase. Capital letters represent significant P-value ($p < 0.05$) within column whereas small letters represent significant P- value ($p < 0.05$) within row.

S. No.	Samples	OPA (mg/ml digested hydrolysate) equivalent tryptone			
		In vitro digested *Gaddi* goat milk casein hydrolysates of alcalase			
		Control	A1 (1:100)	A2 (0.01:100)	A3 (0.005:100)
1	UD	6.83^{Dd} ± 0.20	32.07^{Da} ± 0.10	12.60^{Cb} ± 0.48	10.28^{Cc} ± 0.43
2	PD	7.02^{Cd} ± 0.23	34.17^{Ca} ± 0.08	13.52^{Cb} ± 0.05	10.75^{Cc} ± 0.10
3	4hrs.D	9.53^{Bd} ± 0.47	35.58^{Ba} ± 0.26	14.79^{Bb} ± 0.27	12.97^{Bc} ± 0.29
4	OD	11.82^{Ac} ± 0.26	37.46^{Aa} ± 0.26	16.19^{Ab} ± 0.17	15.86^{Ab} ± 0.36

Table 2: OPA activity in *Gaddi* goat milk casein protein *in vitro* digested hydrolysates generated with enzyme alcalase. Capital letters represent significant P-value ($p < 0.05$) within column whereas small letters represent significant P-value ($p < 0.05$) within row.

FRAP antioxidant activity in *Gaddi* goat milk casein protein hydrolysates and *in vitro* digested hydrolysates generated with enzyme alcalase

FRAP antioxidant activities were noted higher in 4 hour hydrolysates of *Gaddi* goat casein protein hydrolysates prepared with alcalase. FRAP antioxidant values were noted higher (20.72 ± 0.27 mg/100ml) in *Gaddi* goat casein hydrolysates prepared with alcalase A1 than A2 and A3 after 4 hours of enzymatic hydrolysis as depicted in table 3. *In vitro* digestion further enhances the antioxidant activities after overnight digestion of *Gaddi* goat casein protein hydrolysates than the corresponding undigested hydrolysates. However, in *Gaddi* goat casein *in vitro* digested hydrolysates, FRAP antioxidant activities were found to be maximum (22.59 ± 0.18 mg/100ml) in overnight *in vitro* digested hydrolysates prepared with alcalase (A1) as shown in table 4. The reduction of the Fe3+ to the ferrous form may be due to ubiquitous antioxidant in hydrolysates with diverse nature. Antioxidant activity in various types of milk may be due to occurrence of aromatic amino acids, casein, lactoferrin in whey, and the microorganisms and their associated activities in milk [41,42].

S. No.	Time of collection	FRAP (mg/100ml hydrolysed sample) equivalent $FeSO_4$			
		Gaddi goat milk casein hydrolysates of alcalase			
		Control	A1 (1:100)	A2 (0.01:100)	A3 (0.005:100)
1	0 hour	$5.44^{Ac} \pm 0.06$	$15.23^{Ca} \pm 0.06$	$8.05^{Db} \pm 0.53$	$6.24^{Dc} \pm 0.03$
2	1 hour	$5.74^{Ad} \pm 0.30$	$18.85^{Ba} \pm 0.12$	$10.37^{Cb} \pm 0.12$	$9.24^{Cc} \pm 0.18$
3	2 hour	$5.83^{Ac} \pm 0.09$	$19.77^{ABa} \pm 0.03$	$12.89^{Bb} \pm 0.09$	$12.18^{Bb} \pm 0.15$
4	4 hour	$6.06^{Ad} \pm 0.06$	$20.72^{Aa} \pm 0.27$	$16.09^{Ab} \pm 0.39$	$14.70^{Ac} \pm 0.30$

Table 3: FRAP antioxidant activity in *Gaddi* goat milk casein protein hydrolysates generated with enzyme alcalase. Capital letters represent significant P-value ($p < 0.05$) within column whereas small letters represent significant P-value ($p < 0.05$) within row.

S. No.	Samples	FRAP (mg/100ml digested sample) equivalent $FeSO_4$			
		In vitro digested *Gaddi* goat milk casein hydrolysates of alcalase			
		Control	A1 (1:100)	A2 (0.01:100)	A3 (0.005:100)
1	UD	$5.65^{Cc} \pm 0.27$	$19.57^{Ca} \pm 0.12$	$10.96^{Db} \pm 0.30$	$10.61^{Cb} \pm 0.12$
2	PD	$6.24^{Cd} \pm 0.09$	$21.26^{Ba} \pm 0.15$	$16.39^{Bb} \pm 0.33$	$15.06^{Bc} \pm 0.18$
3	4hrs.D	$12.12^{Bc} \pm 0.09$	$21.50^{Ba} \pm 0.39$	$14.73^{Cb} \pm 0.03$	$14.23^{Bb} \pm 0.30$
4	OD	$15.12^{Ac} \pm 0.41$	$22.59^{ABa} \pm 0.18$	$17.96^{Ab} \pm 0.30$	$17.25^{Ab} \pm 0.06$

Table 4: FRAP antioxidant activity in *Gaddi* goat milk casein protein *in vitro* digested hydrolysates generated with enzyme alcalase. Capital letters represent significant P-value ($p<0.05$) within column whereas small letters represent significant P-value ($p<0.05$) within row.

DPPH antioxidant activity in *Gaddi* goat milk casein protein hydrolysates and *in vitro* digested hydrolysates generated with enzyme alcalase

DPPH antioxidant activity increases with time during enzymatic hydrolysis in *Gaddi* goat casein protein hydrolysates prepared with enzyme alcalase (A1) as shown in table 5. *In vitro* digestion further enhances the DPPH antioxidant activities after overnight digestion of *Gaddi* goat casein protein hydrolysates corresponding to undigested hydrolysates prepared with enzyme alcalase. Though, DPPH antioxidant activity was noted highest (36.76 ± 0.14 %) in pepsin digested *Gaddi* goat casein protein hydrolysates generated

S. No.	Time of collection	DPPH (% inhibition)			
		Gaddi goat milk casein hydrolysates of alcalase			
		Control	A1 (1:100)	A2 (0.01:100)	A3 (0.005:100)
1	0 hour	$25.70^{Aa} \pm 0.42$	$1.90^{Cc} \pm 0.35$	$4.35^{Ab} \pm 0.56$	$2.39^{Ac} \pm 0.14$
2	1 hour	$24.79^{Aa} \pm 0.35$	$5.27^{Bb} \pm 0.21$	$2.46^{Bc} \pm 0.07$	$2.46^{Ac} \pm 0.07$
3	2 hour	$25.28^{Aa} \pm 0.28$	$6.25^{Bb} \pm 0.49$	$2.67^{Bc} \pm 0.42$	$2.11^{Ac} \pm 0.28$
4	4 hour	$24.51^{Aa} \pm 0.21$	$7.44^{Ab} \pm 0.14$	$1.19^{Cc} \pm 0.35$	$1.76^{Ac} \pm 0.35$

Table 5: DPPH antioxidant activity in *Gaddi* goat milk casein protein hydrolysates generated with enzyme alcalase. Capital letters represent significant P-value ($p < 0.05$) within column whereas small letters represent significant P-value ($p < 0.05$) within row.

with enzyme alcalase (A1) as depicted in table 6. According to previous studies about the influence of DH (degree of hydrolysis) and molecular mass of peptides on antioxidant capacity, short peptides (<1500 Da) show higher antioxidant activity than higher molecular mass peptides [43].

Qualitative analysis of DPPH antioxidant activity by dry dot-TLC in *Gaddi* goat milk casein protein hydrolysates and *in vitro* digested hydrolysates generated with enzyme alcalase

S. No.	Samples	DPPH (% inhibition)			
		In vitro digested *Gaddi* goat milk casein hydrolysates of alcalase			
		Control	A1 (1:100)	A2 (0.01:100)	A3 (0.005:100)
1	UD	$23.94^{Da} \pm 0.28$	$6.20^{Cb} \pm 0.28$	$2.89^{Cc} \pm 0.07$	$2.96^{Dc} \pm 0.28$
2	PD	$33.45^{Ab} \pm 0.63$	$36.76^{Aa} \pm 0.14$	$22.61^{Ac} \pm 0.35$	$23.94^{Ac} \pm 0.28$
3	4hrs.D	$26.06^{CDa} \pm 0.42$	$7.89^{BCb} \pm 0.42$	$5.07^{Bc} \pm 0.56$	$8.80^{Cb} \pm 0.35$
4	OD	$28.31^{BCa} \pm 0.14$	$8.87^{Bcb} \pm 0.56$	$5.99^{Bd} \pm 0.07$	$11.48^{Bb} \pm 0.77$

Table 6: DPPH antioxidant activity in *Gaddi* goat milk casein protein *in vitro* digested hydrolysates generated with enzyme alcalase. Capital letters represent significant P-value ($p < 0.05$) within column whereas small letters represent significant P-value ($p < 0.05$) within row.

Dry dot-TLC analysis showed the presence of DPPH radical scavenging activity in hydrolysates and *in vitro* digested hydrolysates of *Gaddi* goat casein protein prepared with alcalase. The intensity of the spots increases with time which showed the increasing effect of antioxidant activity with time. DPPH activity remains stable after overnight of spray in *Gaddi* goat casein hydrolysates prepared with enzyme alcalase as shown in figure 2. These findings indicated that milk has both slow and fast reacting antioxidants as reported earlier [44].

Antihypertensive (ACE) activity in *Gaddi* goat milk casein protein *in vitro* digested hydrolysates generated with enzyme alcalase

Antihypertensive activity was found to be increased after overnight *in vitro* digestion corresponding to undigested hydrolysates prepared with enzyme alcalase. Antihypertensive activity was noted maximum (48.24 ± 0.35 %) in overnight *in vitro* digested *Gaddi* goat casein protein hydrolysates prepared with enzyme alcalase (A1) as shown in table 7/figure 3.

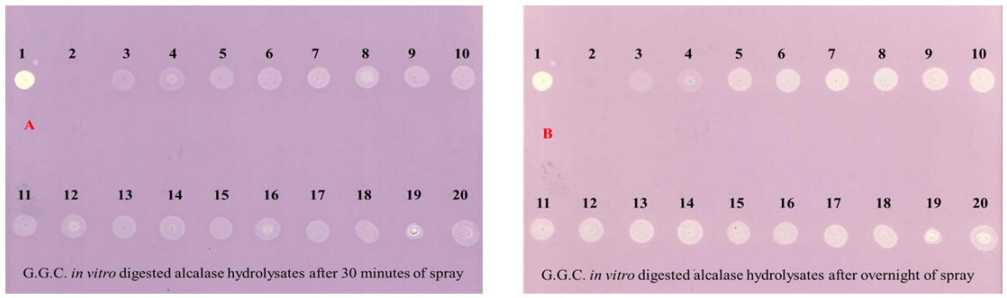

Figure 2: A, DPPH TLC in Gaddi goat milk casein protein *in vitro* digested hydrolysates generated with enzyme alcalase. 1- Ascorbic acid (+ve control), 2- Methanol (-ve control), 3- UD (Undigested control casein hydrolysate), 4- PD (30 minutes pepsin digested control casein hydrolysate), 5- 4 hrs.D (4 hours tyrpsin and pancreatin digested control casein hydrolysate), 6- OD (Overnight tyrpsin and pancreatin digested control casein hydrolysate), 7- UD (Undigested casein A1 hydrolysate), 8- PD (Pepsin digested casein A1 hydrolysate), 9- 4 hrs.D (4 hours tyrpsin and pancreatin digested casein A1 hydrolysate) , 10- OD (Overnight tyrpsin and pancreatin digested casein A1 hydrolysate), 11- UD (Undigested casein A2 hydrolysate), 12- PD (Pepsin digested casein A2 hydrolysate), 13- 4 hrs.D (4 hours tyrpsin and pancreatin digested casein A2 hydrolysate) , 14- OD (Overnight tyrpsin and pancreatin digested casein A2 hydrolysate), 15- UD (Undigested casein A3 hydrolysate), 16- PD (Pepsin digested casein A3 hydrolysate), 17- 4 hrs.D (4 hours tyrpsin and pancreatin digested casein A3 hydrolysate), 18- OD (Overnight tyrpsin and pancreatin digested casein A3 hydrolysate), 19- *Gaddi* goat milk, 20- *Gaddi* goat milk casein.

S. No.	Samples	ACE (% inhibition)			
		In vitro digested *Gaddi* goat milk casein hydrolysates of alcalase			
		Control	A1 (1:100)	A2 (0.01:100)	A3 (0.005:100)
1	UD	$14.44^{Dd} \pm 0.35$	$30.99^{Da} \pm 0.70$	$22.89^{Db} \pm 0.33$	$17.25^{Dc} \pm 0.35$
2	PD	$16.55^{Cd} \pm 0.35$	$38.73^{Ca} \pm 0.70$	$27.11^{Cb} \pm 0.35$	$21.83^{Cc} \pm 0.71$
3	4hrs.D	$21.83^{Bd} \pm 0.70$	$45.42^{Ba} \pm 0.34$	$31.34^{Bb} \pm 0.33$	$25.35^{Bc} \pm 0.70$
4	OD	$29.58^{Ac} \pm 0.71$	$48.24^{Aa} \pm 0.35$	$36.62^{Ab} \pm 0.70$	$29.23^{Ac} \pm 0.34$

Table 7: ACE activity in *Gaddi* goat milk casein protein *in vitro* digested hydrolysates generated with enzyme alcalase. Capital letters represent significant P-value ($p < 0.05$) within column whereas small letters represent significant P-value ($p < 0.05$) within row.

[20,45] reported that the goat milk protein hydrolysates prepared with alcalase have antioxidant and antibacterial activities, and contains ACE-inhibitory peptides, which can be effective at prevention and treatment of lifestyle diseases such as hypertension [46].

Antimicrobial activity in *in vitro* digested *Gaddi* goat milk casein protein hydrolysates generated with enzyme alcalase against different microbes

Antimicrobial activity was observed in digested samples of *Gaddi* goat casein protein hydrolysates generated with enzyme alcalase against *Bacillus cereus* and *Rhodococcus equi.* Maximum inhibition zone of 14 mm was observed in pepsin digested samples of *Gaddi* goat casein protein *in vitro* digested hydrolysates prepared with alcalase (A1) against *Rhodococcus equi* (figure 4) and against *Bacillus cereus,* antimicrobial activity (13 mm) was noted in overnight digested hydrolysates prepared with enzyme alcalase (A2). Antimicrobial activity of the milk protein hydrolysates has been identified [47], which include CPPs, CMPs, GMPs, and whey-based peptide lactoferricin (derived from lactoferrin), lactoferrampin [48] and αs1 and αs2-casein [49].

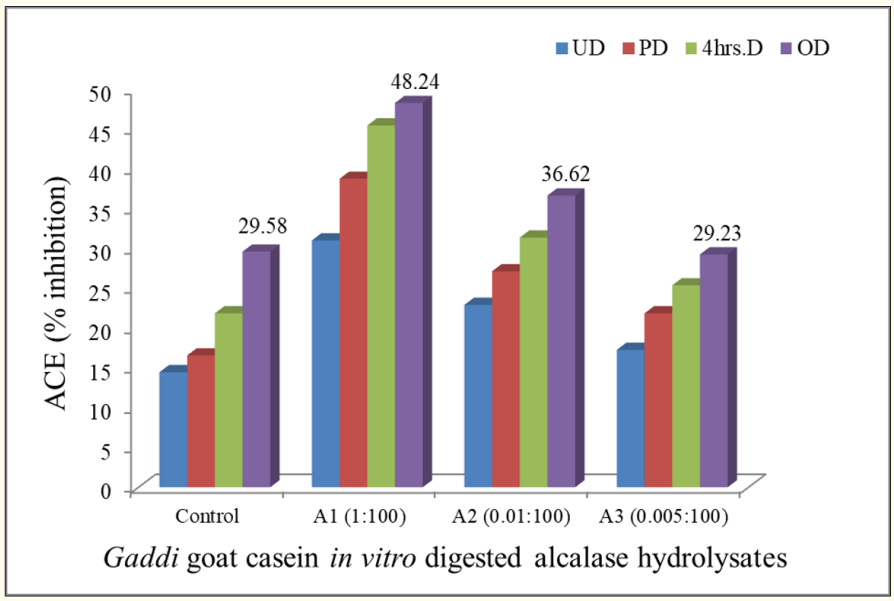

Figure 3: ACE activity in *Gaddi* goat milk casein *in vitro* digested hydrolysates generated with enzyme alcalase.

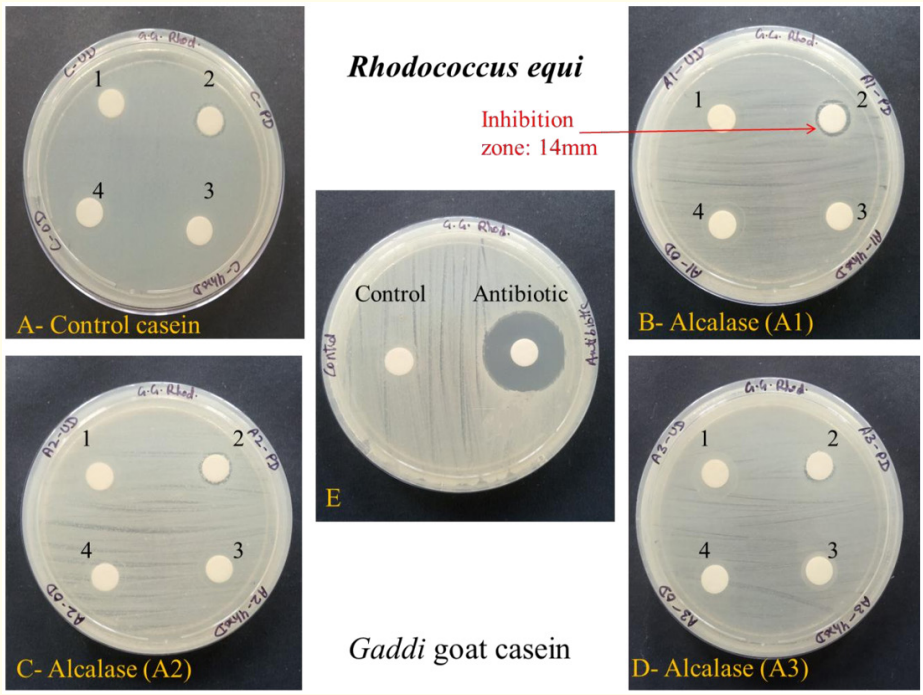

Figure 4: Antimicrobial activity in *Gaddi* goat milk casein *in vitro* digested hydrolysates generated with alcalase against *Rhodococcus equi*.

1-Undigested hydrolysate (UD), 2- Pepsin digested hydrolysates after 30 minutes (PD), 3- Trypsin & pancreatin digested hydrolysates after 4 hours (4hrs.D), 4- Trypsin & pancreatin digested hydrolysates after overnight (OD), Control- Nutrient broth as -ve control, Antibiotic- Streptomycin sulphate as +ve control.

Conclusion

It may be concluded from the present study that the major casein proteins present in *Gaddi* goat milk were observed to be degraded during enzymatic hydrolysis and after *in vitro* digestion of hydrolysates prepared with enzyme alcalase. *Gaddi* goat casein hydrolysates prepared with alcalase (A1) exhibited higher OPA activity and FRAP antioxidant activity which was further enhanced after *in vitro* digestion. Present study also revealed that *Gaddi* goat casein *in vitro* digested hydrolysates prepared with alcalase showed promising antimicrobial activity against *Rhodococcus equi* than other microorganisms used for this study. The casein protein fraction of

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