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Research Article

Bioactivities of *Gaddi* Goat Milk Whey Protein Hydrolysates Processed with Papain

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Abstract

Gaddi goat milk whey protein fractions were hydrolysed using proteolytic enzyme- papain, and further subjected to *in vitro* digestion with gastric and intestinal digestion enzymes- pepsin, trypsin and pancreatin. Bioactive peptides obtained subsequently enzymatic hydrolysis and *in vitro* digested hydrolysates were centrifuged and supernatants exploited for characterization by SDS-PAGE, antioxidant, antihypertensive and antimicrobial activities. SDS-PAGE exhibited that *Gaddi* goat whey proteins degraded subsequently *in vitro* digestion of hydrolysates prepared with papain. OPA activity was noted highest (29.51 ± 0.18 mg/ml) in overnight *in vitro* digested *Gaddi* goat whey protein hydrolysates prepared with papain P1 (1:100) as compare to *Gaddi* goat whey protein digested hydrolysates prepared with papain P2 (0.01:100) and P3 (0.005:100). FRAP antioxidant values were noted higher (38.26 ± 0.06 mg/100ml) in *Gaddi* goat whey hydrolysates prepared with P1. DPPH antioxidant activity increases with time during enzymatic hydrolysis. DPPH antioxidant activity was observed higher (27.59 ± 0.53 %) after 4 h of enzymatic hydrolysis of *Gaddi* goat whey with P1. Dry dot TLC showed that DPPH activity remains stable afterwards overnight of spray in *Gaddi* goat whey hydrolysates prepared with varied concentration of papain. Antihypertensive activity was noted highest (72.70 ± 0.76 %) in overnight *in vitro* digested *Gaddi* goat whey protein hydrolysates prepared with P1. Antimicrobial activity was observed in digested samples of *Gaddi* goat whey protein hydrolysates generated with papain against *Rhodococcus equi*. Among the three concentrations of papain (P1, P2 and P3) used for the present study, antioxidant and antihypertensive activities were found highest in *Gaddi* goat whey protein hydrolysates prepared with enzyme-papain (P1), indicating likely application as bioactive and functional components in various food preparations.

Keywords: *Gaddi* Goat; Whey Proteins; Enzymatic Hydrolysis; Antioxidant Activity; Antihypertensive Activity; Antimicrobial Activity

Introduction

Milk is nature's most complete food, provides basic nutritional requirements and bioactive components, and impart a wide range of additional health benefits to both the neonate and the adult. Milk is an important source of complex proteins, enzymes, and peptides of diverse biological activities [1]. 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 [1-3]. Milk and milk products are nutritious food items containing numerous essential nutrients such as oleic acid, conjugated linoleic acid, omega-3 fatty acid, vitamins, minerals and bioactive compounds such as antioxidants [4].

Whey protein, a by-product recognized as valuable food ingredient with important nutritional and functional properties is gaining acceptance as functional food ingredient. Hydrolysis of whey protein is known to release bioactive peptides that exhibit a number of physiological properties and enable them to revelation antioxidative, antihypertensive, antimicrobial, opoid, and antithrombotic activities [5].

Additionally, sheep/goat whey protein improved the antioxidant defence of EA.hy926 cells by activating antioxidant enzymes via the nuclear factor (erythroid-derived 2)- like2/antioxidant response element (Nrf2/ARE) signalling pathway [6]. In another study, sheep/goat whey protein improved the redox status of blood and tissues in an *in vivo* rat's model [7]. Furthermore, it improved the antioxidant ability of athletes and hence, could potentially be used as a performance-enhancing substance [8,9]. Enzymatic hydrolysis is the most widely used technology to produce bioactive peptides, due to mild processing conditions, easily controlled reaction and minimal formation of by-products [10]. Enzymatic hydrolysis of milk proteins modifies the bio-functional properties of hydrolysates depending on the enzyme and hydrolysis conditions. Recently, bioactive peptides or cryptides has become the theme of scientific research due to their multifunctional properties [11,12]. Bioactive peptides have garnered huge scientific interest because of their multifunctional biological activities such as antioxidative, antimicrobial, antihypertensive, anticancer, antidiabetic, and immunomodulatory effect [13].

These peptides are the isolated protein fragments that are synthesized from the hydrolysis of whey proteins, which are mostly composed of 2-20 AAs residue per molecule [14,15]. Milk protein hydrolysates and peptides derived from caseins and major whey proteins can enhance immune cell functions measured by lymphocyte proliferation, antibody synthesis, and cytokine regulation [16].

Digestive enzymes (pepsin, trypsin and chymotrypsin) and enzymes of animal, microbial, or plant origin (papain, alcalase, flavourzyme, pronase, ficin, thermocylin and neutrase) are used to break large polypeptides into specific small peptides that contain 2-20 amino acid units with molecular weights ranging from 500 to 1800 Da, and their activity depends upon amino acids composition and sequence [17-20]. The hydrolysis of whey proteins with trypsin produces hydrolysates exhibiting good ACE- inhibitory activity. These ACE inhibitors regulate the peripheral blood pressure which ultimately reduces the oxygen demand from the human heart [21]. Antimicrobial activity of the milk protein hydrolysates has been identified [22], which include CPPs, CMPs, GMPs, and whey-based peptide lactoferricin (derived from lactoferrin), lactoferrampin [23]. and αs1 and αs2-casein [24].

The advantage of enzyme hydrolysis is that the nature and extent of treatment can be controlled due to the inherent specificity of the different proteases. The process of releasing peptides from casein and whey proteins occurs in three ways: through digestive enzymes, through the fermentation process or through the action of proteolytic enzymes, obtained from microorganisms or plants [25-28]. The plant enzymes are preferred over enzymes of microbial origin mainly due to safety, lack of pathogenicity and other adverse effects [29,30].

Mann., *et al*. [31] demonstrated antioxidant activity of flavoured milk, enriched with whey protein hydrolysate. Whey protein concentrate is hydrolysed using proteolytic enzymes. The antioxidant activity of all hydrolysates is higher than the output whey protein. One of the most important aspects of goat milk is its higher content of short chain fatty acids [32,33]. Medium chain triglycerides in goat milk are also present in higher concentration as compared to cow milk, thereby reducing the synthesis of endogenous cholesterol [34]. Goat milk is rich in protein, lipids, carbohydrates, vitamins, minerals, and other micronutrients. Studies have reported that goat milk has small fat particles, low allergenicity, easy digestion and absorption, greater biological value than cow's milk; thus, it can be a potential substitute for infant formula production [35].

Material and Methods

Collection of milk sample

Gaddi goat milk samples were procured from local areas. pH of fresh samples was noted and processed for further analyses.

Separation of whey from milk

Gaddi goat milk was processed to separate the whey [1]. The pH of milk was adjusted to 4.0 with 4M HCL followed by centrifugation at 5000 rpm for 30 minutes. Supernatant was collected and again pH of the supernatant was adjusted to 7.0 using 40% NaOH. It was centrifuged at 5000 rpm for 30 minutes. Whey (supernatant) was collected and allowed for drying by lyophilisation. 15g of obtained whey was dissolved in PBS pH 7.0 (15%) by stirring on magnetic stirrer.

Preparation of *Gaddi* goat milk whey protein hydrolysates

pH of the 15% whey was adjusted to 7.0 (using 40 % NaOH / 4M HCL whichever required). *Gaddi* goat milk whey was divided into 4 parts. One part was used as control whey (W). Other were labelled as P1, P2, and P3. To these three portions enzyme Pa-

pain (w/v) was added separately at an enzyme-to-substrate ratio of 1:100 (P1-1g/100ml), 0.01:100 (P2- 10mg/100ml), 0.005:100 (P3- 5mg/100ml), respectively.

In vitro digestion of *Gaddi* goat milk whey protein hydrolysates

In vitro digestion of *Gaddi* goat milk whey protein hydrolysates prepared with papain was done by the method as described by Parrot., *et al*. (2003) [36] with modifications. A two-step process was used to simulate gastric and intestinal digestion of *Gaddi* goat milk whey protein hydrolysates of papain using the *in vitro* enzymatic digestion with pepsin, trypsin and pancreatin. Undigested and digested samples were centrifuged at 10000 rpm for 10 minutes and supernatants were stored at -20°C for analyses. All the estimations including SDS protein profile, OPA [37], FRAP [38], DPPH [39], antihypertensive [40] and antimicrobial activities [41] were done in supernatants.

Statistical analysis

All experiments were performed in triplicate and the data were presented as means and standard error of means. The statistical analysis was done by using SAS 9.2 statistical package and a P-value of 0.05 ($p < 0.05$) was considered statistically significant.

Results and Discussion

SDS-PAGE protein profile in *Gaddi* goat milk whey protein hydrolysates and *in vitro* digested hydrolysates generated with papain

Protein profiles of *Gaddi* goat milk whey protein hydrolysates generated with papain showed almost degradation of whey proteins in hydrolysates prepared with P1 as shown in figure 1A. SDS-PAGE also showed complete degradation of major whey protein fractions after overnight *in vitro* digested papain treated *Gaddi* goat whey protein hydrolysates prepared with P1 as shown in figure 1C and 1D. However complete degradation was not evident for P2 and P3. These results indicated that papain hydrolysis caused the protein structure changed, leading to complete or partial hydrolysis of protein subunits and production of small peptides [42].

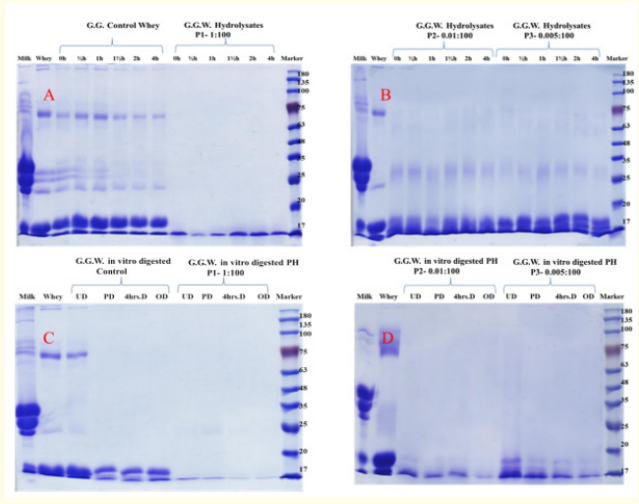

Figure 1: A, B- *Gaddi* goat milk whey (G.G.W.) protein hydrolysates generated with papain; C, D- *In vitro* digested *Gaddi* goat milk whey protein hydrolysates generated with papain.

OPA activity in *Gaddi* goat milk whey hydrolysates and *in vitro* digested hydrolysates generated with papain

OPA activity increases with time during enzymatic hydrolysis with papain. OPA activity was observed higher (26.76 ± 0.15 mg/ml) after 4 h of enzymatic hydrolysis of *Gaddi* goat whey with papain (P1) as depicted in table 1. Further, *in vitro* digestion enhances the OPA activity of *Gaddi* goat whey hydrolysates prepared with papain. OPA activity was noted highest (29.51 ± 0.18 mg/ml) in overnight *in vitro* digested *Gaddi* goat whey protein hydrolysates prepared with papain (P1) as shown in table 2. The presence of low molecular weight proteins in SDS-PAGE could also be confirmed with OPA activity. These results were consistent with protein profiles of hydrolysates.

S. No.	Time of collection	OPA (mg/ml hydrolysed sample) equivalent Tryptone			
		Gaddi goat milk whey hydrolysates of papain			
		Control	P1 (1:100)	P2 (0.01:100)	P3(0.005:100)
1	0 hour	$5.05^{Ad} \pm 0.06$	$23.79^{DCa} \pm 0.10$	$14.13^{Cb} \pm 0.04$	$10.74^{Cc} \pm 0.08$
2	½ hour	$5.02^{Ad} \pm 0.03$	$24.12^{Ca} \pm 0.08$	$14.32^{Cb} \pm 0.03$	$10.96^{Cc} \pm 0.03$
3	1 hour	$5.19^{Ad} \pm 0.04$	$24.64^{Ca} \pm 0.13$	$14.38^{Cb} \pm 0.03$	$10.98^{Cc} \pm 0.01$
4	1½ hour	$5.20^{Ad} \pm 0.03$	$24.89^{BCa} \pm 0.18$	$16.26^{Bb} \pm 0.09$	$12.01^{Bc} \pm 0.06$
5	2 hour	$5.39^{Ad} \pm 0.06$	$25.51^{Ba} \pm 0.08$	$17.72^{Ab} \pm 0.04$	$12.32^{Bc} \pm 0.04$
6	4 hour	$5.55^{Ad} \pm 0.02$	$26.76^{Aa} \pm 0.15$	$18.32^{Ab} \pm 0.05$	$14.54^{Ac} \pm 0.03$

Table 1: OPA activity in *Gaddi* goat milk whey hydrolysates generated with papain.

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 whey hydrolysates of Papain			
		Control	P1 (1:100)	P2 (0.01:100)	P3 (0.005:100)
1	UD	$5.34^{Cd} \pm 0.15$	$24.07^{Ca} \pm 0.18$	$14.22^{Db} \pm 0.05$	$10.84^{Cc} \pm 0.02$
2	PD	$5.50^{CBd} \pm 0.05$	$25.05^{Ca} \pm 0.10$	$16.81^{Cb} \pm 0.03$	$12.52^{Bc} \pm 0.06$
3	4hrs.D	$6.36^{Bd} \pm 0.05$	$27.56^{Ba} \pm 0.08$	$18.35^{Bb} \pm 0.03$	$12.72^{Bc} \pm 0.09$
4	OD	$7.39^{Ad} \pm 0.06$	$29.51^{Aa} \pm 0.18$	$19.39^{Ab} \pm 0.08$	$14.98^{Ac} \pm 0.03$

Table 2: OPA activity in *Gaddi* goat milk whey *in vitro* digested 1-hour hydrolysates generated with papain.

Capital letters represent significant P-value ($p < 0.05$) within column whereas small letters represent significant P-value ($p < 0.05$) within row. (UD- Undigested, PD- Pepsin digested, 4hrs. D- 4hours digested, OD-Overnight digested)

FRAP antioxidant activity in *Gaddi* goat milk whey hydrolysates and *in vitro* digested hydrolysates generated with papain

FRAP antioxidant activities were noted higher in 4hour hydrolysates of *Gaddi* goat whey protein hydrolysates prepared with papain. FRAP antioxidant values were noted higher (38.26 ± 0.06 mg/100ml) in *Gaddi* goat whey hydrolysates prepared with papain (P1) than whey hydrolysates prepared with papain (P2 and P3) as depicted in table 3. *In vitro* digestion further enhances the antioxidant activities after overnight digestion of *Gaddi* goat whey protein hydrolysates prepared with papain than the corresponding undigested hydrolysates. However, in *Gaddi* goat whey hydrolysates, FRAP antioxidant activities were found to be maximum ($38.62^{Aa} \pm 0.12$ mg/100ml) in overnight digested hydrolysates prepared with P1 as shown in table 4.

S. No.	Time of collection	FRAP (mg/100ml hydrolysed sample) equivalent $FeSO_4$			
		Gaddi goat milk whey hydrolysates of papain			
		Control	P1 (1:100)	P2 (0.01:100)	P3 (0.005:100)
1	0 hour	$14.43^{Bc} \pm 0.09$	$33.63^{Da} \pm 0.12$	$16.04^{Cb} \pm 0.09$	$16.07^{Cb} \pm 0.24$
2	½ hour	$14.20^{Bc} \pm 0.03$	$35.35^{Ca} \pm 0.11$	$15.89^{Db} \pm 0.06$	$16.69^{Cb} \pm 0.09$
3	1 hour	$14.94^{Bc} \pm 0.18$	$36.18^{Ca} \pm 0.12$	$16.66^{Bb} \pm 0.12$	$16.96^{CBb} \pm 0.18$
4	1½ hour	$14.46^{Bc} \pm 0.12$	$37.19^{Ba} \pm 0.06$	$16.84^{Bb} \pm 0.12$	$17.79^{BAb} \pm 0.12$
5	2 hour	$14.43^{Bd} \pm 0.21$	$38.02^{Aa} \pm 0.12$	$16.96^{BCc} \pm 0.18$	$18.20^{Ab} \pm 0.11$
6	4 hour	$15.18^{Ad} \pm 0.18$	$38.26^{Aa} \pm 0.06$	$17.22^{ABc} \pm 0.15$	$18.44^{Ab} \pm 0.06$

Table 3: FRAP antioxidant activity in *Gaddi* goat milk whey hydrolysates generated with papain.

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 whey hydrolysates of Papain			
		Control	P1 (1:100)	P2 (0.01:100)	P3 (0.005:100)
1	UD	$15.23^{Bc} \pm 0.12$	$36.30^{Ca} \pm 0.18$	$15.92^{Bbc} \pm 0.21$	$16.42^{Bb} \pm 0.12$
2	PD	$15.47^{Bc} \pm 0.12$	$36.84^{CBa} \pm 0.12$	$16.69^{Bb} \pm 0.09$	$17.16^{Bb} \pm 0.09$
3	4hrs.D	$15.92^{ABc} \pm 0.21$	$37.34^{Ba} \pm 0.21$	$17.43^{Ab} \pm 0.12$	$18.32^{Ab} \pm 0.18$
4	OD	$16.66^{Ad} \pm 0.11$	$38.62^{Aa} \pm 0.12$	$18.44^{Ac} \pm 0.13$	$19.09^{Ab} \pm 0.17$

Table 4: FRAP antioxidant activity in *Gaddi* goat milk whey *in vitro* digested 1-hour hydrolysates generated with papain.

Capital letters represent significant P-value ($p < 0.05$) within column whereas small letters represent significant P-value ($p < 0.05$) within row.

The reduction of the Fe3+ to the ferrous form may be due to pervasive 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 [43,44].

DPPH antioxidant activity in *Gaddi* goat milk whey hydrolysates and *in vitro* digested hydrolysates generated with papain

DPPH antioxidant activity increases with time during enzymatic hydrolysis. DPPH antioxidant activity was observed higher (27.59 ± 0.53 %) after 4 h of enzymatic hydrolysis of *Gaddi* goat whey with papain (P1) in comparison to 4 h hydrolysates prepared with papain (P2 and P3) as shown in table 5. DPPH antioxidant activity was found to be increased after overnight *in vitro* digestion corresponding to undigested hydrolysates prepared with papain. However, DPPH antioxidant activity was noted highest (31.47^{Aa} ± 0.29 %) in pepsin digested *Gaddi* goat whey protein hydrolysates prepared with papain (P1) as depicted in table 6.

S. No.	Time of collection	DPPH (% inhibition)			
		Gaddi goat milk whey hydrolysates of papain			
		Control	P1 (1:100)	P2 (0.01:100)	P3 (0.005:100)
1	0 hour	7.94^{Cb} ± 0.29	23.47^{Da} ± 0.18	8.53^{Fb} ± 0.29	8.06^{Eb} ± 0.29
2	½ hour	8.59^{Cc} ± 0.59	24.41^{Ca} ± 0.18	9.65^{Eb} ± 0.35	9.65^{Db} ± 0.35
3	1 hour	8.94^{Cc} ± 0.12	25.47^{Ba} ± 0.29	10.76^{Db} ± 0.41	10.53^{Cb} ± 0.18
4	1½ hour	10.24^{Bc} ± 0.12	25.59^{Ba} ± 0.28	13.29^{Cb} ± 0.35	13.00^{Bb} ± 0.65
5	2 hour	11.53^{Ad} ± 0.35	26.29^{Ba} ± 0.29	14.76^{Bb} ± 0.40	13.59^{ABc} ± 0.06
6	4 hour	11.76^{Ad} ± 0.59	27.59^{Aa} ± 0.53	15.82^{Ab} ± 0.53	14.35^{Ac} ± 0.35

Table 5: DPPH antioxidant activity in *Gaddi* goat milk whey hydrolysates generated with papain.

S. No.	Samples	DPPH (% inhibition)			
		In vitro digested *Gaddi* goat milk whey hydrolysates of papain			
		Control	P1 (1:100)	P2 (0.01:100)	P3 (0.005:100)
1	UD	8.76^{Dc} ± 0.65	26.00^{Ca} ± 0.12	11.76^{Db} ± 0.24	11.18^{Db} ± 0.47
2	PD	16.94^{Ac} ± 0.59	31.47^{Aa} ± 0.29	18.47^{Ab} ± 0.23	17.12^{Ac} ± 0.41
3	4hrs.D	12.82^{Cc} ± 0.12	27.71^{Ba} ± 0.41	14.35^{Cb} ± 0.35	13.18^{Cc} ± 0.35
4	OD	13.94^{Bc} ± 0.41	28.24^{Ba} ± 0.24	16.12^{Bb} ± 0.25	14.94^{Bc} ± 0.24

Table 6: DPPH antioxidant activity in *Gaddi* goat milk whey *in vitro* digested 1-hour hydrolysates generated with papain.

Capital letters represent significant P-value ($p < 0.05$) within column whereas small letters represent significant P-value ($p < 0.05$) within row.

Qualitative analysis of DPPH antioxidant activity by dry dot TLC in *Gaddi* goat milk whey hydrolysates and *in vitro* digested hydrolysates generated with papain

Dry dot TLC analysis showed the presence of DPPH radical scavenging activity in hydrolysates and *in vitro* digested hydrolysates of *Gaddi* goat whey protein prepared with papain. 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 whey hydrolysates prepared with papain as shown in figure 2. DPPH spray indicating that milk has both slow and fast reacting antioxidants as reported previously [1].

Antihypertensive (ACE) activity in *Gaddi* goat milk whey *in vitro* digested hydrolysates generated with papain

Antihypertensive activity was found to be increased after overnight *in vitro* digestion corresponding to undigested hydrolysates prepared with papain. Antihypertensive activity was noted highest (72.70^{Aa} ± 0.76 %) in overnight *in vitro* digested *Gaddi* goat whey

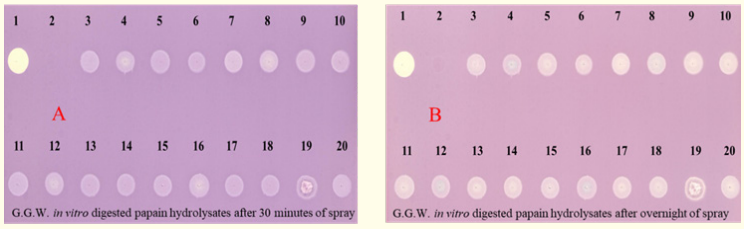

Figure 2: DPPH TLC in *Gaddi* goat milk whey *in vitro* digested hydrolysates generated with papain.

1-Ascorbic acid (+ve control), 2- Methanol (-ve control), 3- UD (Undigested control whey hydrolysate), 4- PD (30 minutes pepsin digested control whey hydrolysate), 5- 4 hrs.D (4 hours tyrpsin and pancreatin digested control whey hydrolysate), 6- OD (Overnight tyrpsin and pancreatin digested control whey hydrolysate), 7- UD (Undigested whey P1 hydrolysate), 8- PD (Pepsin digested whey P1 hydrolysate), 9- 4 hrs.D (4 hours tyrpsin and pancreatin digested whey P1 hydrolysate) , 10- OD (Overnight tyrpsin and pancreatin digested whey P1 hydrolysate), 11- UD (Undigested whey P2 hydrolysate), 12- PD (Pepsin digested whey P2 hydrolysate), 13- 4 hrs.D (4 hours tyrpsin and pancreatin digested whey P2 hydrolysate) , 14- OD (Overnight tyrpsin and pancreatin digested whey P2 hydrolysate), 15- UD (Undigested whey P3 hydrolysate), 16- PD (Pepsin digested whey P3 hydrolysate), 17- 4 hrs.D (4 hours tyrpsin and pancreatin digested whey P3 hydrolysate) , 18- OD (Overnight tyrpsin and pancreatin digested whey P3 hydrolysate), 19- *Gaddi* goat milk, 20- *Gaddi* goat milk whey.

protein hydrolysates prepared with papain (P1) as shown in figure 3. An increased antihypertensive activity in overnight *in vitro* digested *Gaddi* goat whey protein hydrolysates generated with papain may possess more potent antihypertensive peptides. Moreover, herbal supplemented milk had reported to better antihypertensive and antimicrobial activities Based on the results described above, we surmise that herbal supplemented fermented milk had better antihypertensive activity [45].

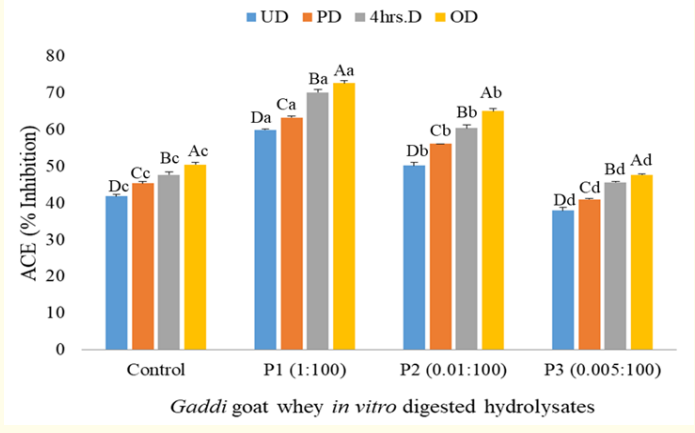

Figure 3: ACE activity in *Gaddi* goat milk whey *in vitro* digested 1-hour hydrolysates generated with papain.

Antimicrobial activity of *in vitro* digested *Gaddi* goat milk whey hydrolysates generated with papain against different cultures by disc diffusion method

Antimicrobial activity of *in vitro* digested *Gaddi* goat milk whey protein hydrolysates was studied against various microorganisms viz., *Escherichia coli, Staphylococcus aureus, Shigella flexneri, Rhodococcus equi* and *Bacillus cereus*. Antimicrobial activity was observed in digested samples of *Gaddi* goat whey hydrolysates generated with papain against *Bacillus cereus* and *Rhodococcus equi* Antimicrobial activity was noted maximum (14 mm) in 4h digested hydrolysates of *Gaddi* goat whey protein, prepared with papain (P2) against *Rhodococcus equi*. Maximum antimicrobial activity against *Rhodococcus equi* may be due to production of specific AMPs in *in vitro* digested *Gaddi* goat milk whey hydrolysates generated with papain [41,46].

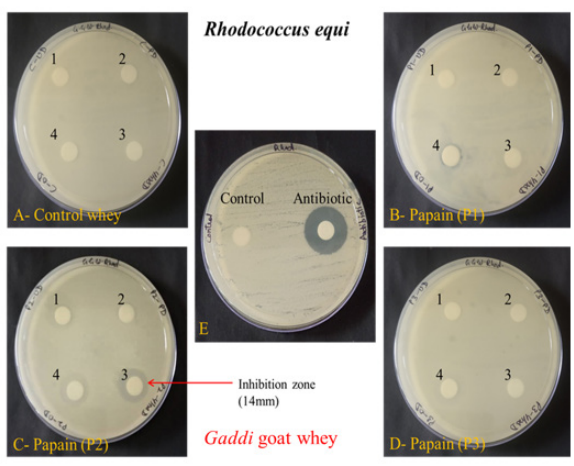

Figure 4: Antimicrobial activity in A- *Gaddi* goat goat milk whey *in vitro* digested 1-hour hydrolysates generated with papain against *Rhodococcus equi*.

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

Conclusion

It is revealed from the present study that the major whey proteins present in *Gaddi* goat milk were observed to be degraded during enzymatic hydrolysis and further whey proteins were found to be degraded completely after *in vitro* digestion of hydrolysates prepared with enzyme papain. *Gaddi* goat whey protein hydrolysates prepared with papain (P1) exhibited higher antioxidant activities. *Gaddi* goat whey *in vitro* digested protein hydrolysates prepared with papain showed highest antimicrobial activity against *Rhodococcus equi*.

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