Biodegradation (2011) 22:287–295
DOI 10.1007/s10532-010-9398-0

ORIGINAL PAPER

In vitro antioxidant and antibacterial properties of hydrolysed proteins of delimed tannery fleshings: comparison of acid hydrolysis and fermentation methods

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Received: 20 December 2009 / Accepted: 22 July 2010 / Published online: 31 July 2010

Abstract Proteins in delimed tannery fleshings were fermentatively hydrolysed using *Enterococcus faecium* NCIM5335 and also hydrolysed using mild organic acids (formic acid and propionic acid). The liquor portion containing hydrolysed proteins was spray dried, in both the cases, to obtain a powder. The spray dried powder was evaluated for in vitro antioxidant activities with respect to scavenging different free radicals and antibacterial properties against nine different pathogens. Fermentation and acid hydrolysates scavenged 83 and 75.3% of 2,2-azino-bis-3-ethyl-benzthiazoline-6-sulphonic acid (ABTS) radicals, respectively, at a protein concentration of 0.25 mg. Further, fermentation hydrolysate showed higher 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of 59% as compared to 56% scavenging by acid hydrolysate at a protein concentration of 5 mg. Acid hydrolysate exhibited lesser (82.3%) peroxy radical scavenging compared to hydrolysate from fermentation (88.2%) at a protein concentration of 10 mg. However, acid hydrolysate exhibited higher (89.2%) superoxide anion scavenging while its fermentation counterpart showed lower activity (85.4%) at 2.5 mg hydrolysate protein. Well as superoxide anion scavenging properties. All the in vitro antioxidant properties exhibited dose dependency. Fermentation hydrolysate exhibited maximum antagonistic activity against *Salmonella typhi* FB231, from among host of pathogens evaluated. Both the hydrolysates have potential to be ingredients in animal feeds and can help reduce oxidative stress in the animals.

Keywords Tannery fleshings · Fermentation · Acid hydrolysis · Antioxidative · Antibacterial

Abbreviations

AAPH	2,2′-azobis(2-methyl–propionamide)dihydrochloride
ABTS	2,2′-azino-bis-3-ethyl-benzthiazoline-6-sulphonicacid
AH	Acid hydrolysate
BHI	Brain heart infusion agar
CFU	Colony forming units
DMW	De-mineralized water
DPPH	2,2′-diphenyl-1-picrylhydrazyl
GRAS	Generally recognized as safe
LAB	Lactic acid bacteria
LFH	LAB fermented hydrolysate
MRS Agar	de Mann Rogosa and Sharpe agar
PGR	Pyrogallol red
TF	Tannery fleshings
WWB	Wet weight basis

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Introduction

Tanneries generate highly polluting solid wastes that include raw hide trimming, limed animal fleshings, green animal fleshings, hide splitting, and chrome shaving and also emanate liquid waste containing variable amounts of proteins. Both solid as well as liquid wastes from tannery are currently not utilized and lead to pollution problems. Processing about 100 tonnes of hides daily produces about 30 tonnes of solid waste each day and creates serious ecological problems (Simeonova and Dalev 1997). The tanneries are confronted with two options for disposal of solid wastes they generate viz. minimize the quantity of waste generated or maximize the return on byproducts (Alexander et al. 1991). However, recovery of value added products from solid waste is a largely neglected area of research till recently. However, in the recent years several ways of utilization of these tannery fleshings (TF) have been documented that include their usefulness in biofuel production (Colak et al. 2005), as potential animal feed ingredients (Amit et al. 2009a, b, c) and as substrates for enzyme production (Kumar et al. 2008).

With regard to utilization of delimed TF, our research group has already optimized the conditions for fermentation using *Enterococcus faecium* NCIM5335, a native lactic acid bacteria (LAB) isolated from TF (Amit et al. 2010a) and acid hydrolysis using a mixture of organic acids (Amit et al. 2009b). Both acid hydrolysis and lactic acid fermentation are eco-friendly methods for the recovery of biomolecules from TF. The advantage of milder organic acids is that they are generally recognized as safe (GRAS) (FDA 2009). LAB is added to silage mainly as probiotic ingredients with an intention to improve animal performance (Bernardeau et al. 2006).

Effective methods for treatment of wastes generated from food industries including meat (Arvanitoyannis and Ladas 2008) and fish (Arvanitoyannis and Kassaveti 2008) along with applications of such reclaimed wastes have been recently reviewed (Arvanitoyannis 2007). In addition, The protein hydrolysates obtained from wastes/by-products of animal and fish processing industry are reported to exhibit bioactivities like antioxidative (Wu et al. 2004, Sachindra and Bhaskar 2008), antihypertensive (Suetsuna et al. 2004) and immunomodulatory properties (LeBlanc et al. 2002, Tsuruki et al. 2003). In recent years there is a growing interest in antioxidant properties of natural source such as protein due to the potential health hazards of certain synthetic antioxidants (Song et al. 2008). During fermentation or acid silaging, TF proteins (that are rich in collagen) are hydrolysed forming a liquor and an insoluble residue. The hydrolysed protein rich liquor is expected to show beneficial biofunctionalities like antioxidant and antibacterial properties. In this regard, the objective of our study was to evaluate the in vitro antioxidant and antibacterial properties of hydrolysates obtained by fermentation and acid hydrolysis of TF.

Materials and methods

Materials

Chemicals and bacterial strains

Wet limed TF from sheep and goat skins were procured from a tannery based at Bangalore, Karnataka, India and this material was used in the preparation of delimed TF. 2,2′-diphenyl-1-picrylhydrazyl (DPPH), pyrogallol red, 2,2′-azobis (2-methyl–propionamide) dihydrochloride (AAPH), 2,2′-azinobis-3-ethyl-benzothizoline-6sulphonate (ABTS), peroxidase and pyrogallol red (PGR) were purchased from Sigma–Aldrich Chemie (Steinheim, Germany). All microbiological media were procured from M/s Hi-Media, Mumbai, India. All other solvents and chemicals were of analytical grade unless otherwise mentioned.

The pathogenic strains used in the study for analyzing antibacterial activity were from the Institute culture collection and included *Aeromonas hydrophila* B445, *Bacillus cereus* F4433, *Staphylococcus aureus* FR1722, *Salmonella paratyphi* FB254, *Salmonella typh*i FB231, *Listeria innocua* FB21, *Listeria murreyi* FB69, *Listeria monocytogenes* ScottA, *Yersinia enterocolitica* MTCC859. These strains were stored at −20°C in brain heart infusion (BHI) broth containing 20% (v/v) glycerol. Cultures were propagated twice in fresh BHI medium before they were used. The cultivation of these strains was performed aerobically.

Methods

General

Proximate composition of fresh and delimed TF was determined as per AOAC (1995). Protein measurements were carried out by Kjeldahl method using Kjeltec protein analyzer (Foss Analytica AB, Sweden). The pH measurements were accomplished by directly immersing the combined glass calomel electrode into the sample using pH meter (Cyberscan 1000, Eutech, Singapore).

Deliming of wet TF

Deliming of TF was accomplished by the procedure established by our group earlier (Bhaskar et al. 2007a). Briefly, minced limed TF were dispersed in potable water at 1:10 (w/v) containing hydrogen peroxide (H_2O_2; 0.1% v/v of wash water). The material was allowed to stand for 30 min at room temperature (27 ± 2°C) with occasional stirring before draining the liquid, H_2O_2 treatment was repeated again and water drained after 30 min of standing time. H_2O_2 treated minced TF was then treated with 0.2 N HCl prepared in potable water (1:10 v/v) containing H_2O_2 (0.1% v/v of wash water). The material was allowed to stand for 30 min before draining solution to collect the solids. The treatment with water containing HCl and H_2O_2 was repeated again. The solids obtained after draining the treated water were termed as delimed TF.

Acid hydrolysis of TF

Acid hydrolysis was carried as per the method standardized previously (Amit et al., 2009b). Briefly, delimed TF was steam cooked for 15 min, cooled to room temperature (30 ± 2°C) and mixed with 20% acid mixture (propionic acid : formic acid 1:1 v/v) and salt (2%). The resulting mixture was allowed to stand for 9 days at room temperature. After 9 days the mixture was extracted three times with demineralised water (DMW; 1:1 w/v), the extracts were pooled and then spray dried. Spray drying was accomplished by a Spray Drier (Labplant SD05, LP Technologies, UK) with inlet temperature of 150°C, outlet temperature 90°C and a flow rate of 70 ml per minute. The spray dried powder was referred to as acid hydrolysate (AH).

Fermentation of delimed TF

Enterococcus faecium NCIM5335 (Genbank #FJ418568), a native LAB isolated from TF by our group (Amit et al. 2009a) was employed for fermentation experiments. The LAB culture was grown in 100 ml of MRS-Broth for 24 h at 37 ± 1°C in a shaking incubator (Technico Ltd, Chennai, India) set at 120 rpm. The cells were harvested by centrifugation (C31 Cooling centrifuge, Remi-India, Mumbai, India) at 7000 × *g* for 10 min followed by washing and centrifuging the harvested cells twice with sterile physiological saline. The washed cells were resuspended in physiological saline (100 ml) and used as the inoculum. The biomass in the inoculum was about 8 log cfu/ml.

Fermentation of delimed TF was carried out under conditions optimized by our group earlier (Amit et al. 2010a). Briefly, delimed TF was steam cooked (88–92°C) for 15 min and cooled to room temperature. The material was then mixed with equal proportion of distilled water (w/v), 2% (w/w) NaCl, 17.5% dextrose (w/w) and 12.5% inoculum (v/w) of LAB. This was mixed well and incubated at 37°C for 96 h at 150 rpm. The fermented TF was extracted three times with demineralised water (DMW; 1:1 w/v; 3X). All the extracts were pooled together and spray dried to collect the powder which was referred to as LAB fermented hydrolysate (LFH).

Antioxidant properties of protein hydrolysate

Preparation of protein hydrolysate AH and LFH were dissolved (50 mg/ml) in double distilled water and homogenized followed by centrifugation at 7000 × *g* for 15 min. The supernatant was collected and filtered through Whatman No.1 filter paper and protein content in the filtrate was estimated by the method of Lowry et al. (1951). This filtrate was used for assaying various antioxidant activities.

Antioxidant properties

DPPH radical scavenging activity The DPPH radical scavenging capacity of both AH and LFH samples were determined by the method previously

described in Ganesan et al. (2008). Briefly, 2.0 ml of 0.16 mM DPPH solution (in methanol) were added to the test tube containing different concentration of sample and made up to 2 ml with distilled water. The mixture was vortexed and kept at room temperature for 30 min in the dark. Sample blank was prepared by replacing DPPH with methanol. The absorbance of all the sample solutions was measured at 517 nm. Methanol along with DPPH served as the control. The scavenging activity (%) was calculated by using the formula:

$$\text{Scavenging activity}(\%) = \left[1 - \left\{\left(A_{\text{sample}} - A_{\text{sample blank}}\right)/A_{\text{control}}\right\}\right] \times 100.$$

Reducing power Reducing power of the samples was determined by the method as described in Ganesan et al. (2008). Briefly, different concentration of sample (made up to 0.5 ml with distilled water) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). Reaction mixture was incubated at 50°C for 20 min. After incubation, 2.5 ml of trichloroacetic acid (10%) was added and centrifuged at 8000 rpm for 10 min. From the upper layer, 2.5 ml solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%). Absorbance of all the sample solutions was measured at 700 nm. Increased absorbance indicates increased reducing power.

Peroxyl radical scavenging activity Peroxyl radical scavenging activity was measured by inhibition of PGR oxidation by the peroxyl radical generated from AAPH (Lopez-Alarcon and Lissi 2005). 1.0 ml of 60 mM PGR was mixed with 0.1 ml of sample and 0.015 ml of 600 mM AAPH and incubated at 37°C for 2 h in a water bath. The reaction mixture was chilled immediately after incubation and the absorbance was measured at 540 nm. For controls, sample buffer was added instead of sample extract and for blank AAPH was replaced with sample buffer. The scavenging rate was calculated as follows

$$\text{Scavenging } \% = 100 - \left[\left(100/\left(A_{\text{control}} - A_{\text{control test}}\right)\right) \times \left(A_{\text{sample blank}} - A_{\text{sample test}}\right)\right].$$

ABTS Radical scavenging activity ABTS radical scavenging activity of the sample was carried out as published in Sachindra and Bhaskar (2008). ABTS radical solution was prepared by mixing 5 ml of ready to use ABTS solution with 100 ml acetate buffer (0.05 M, pH 4.5) and 5 units of peroxidase and incubating for 15 h at 37°C. ABTS (1.9 ml) was mixed with 0.1 ml sample and incubated at 37°C for 1 h. Control was prepared by adding 0.1 ml of distilled water instead of sample. For sample blank buffer was added instead of ABTS. Scavenging activity was calculated as follows.

$$\text{Scavenging}\,\% = \left[1 - \left(A_{\text{sample}} - A_{\text{sample blank}}\right)/A_{\text{control}}\right] \times 100.$$

Super oxide scavenging activity Super oxide scavenging property of both the samples was determined by the method as explained in Heo et al. (2005). To 0.3 ml of sample solution of different concentration, 2.6 ml of 50 mM phosphate buffer (pH 8.2) was added and to this 90 µl of freshly prepared 3 mM pyrogallol dissolved in 10 mM HCl was added. Then the absorbance was measured from 0 min to 10th min at 325 nm. 0.3 ml of distilled water along with 2.6 ml phosphate buffer (50 mM and pH 8.2) served as blank.

Antibacterial properties of protein hydrolysate

Preparation of protein hydrolysate AH and LFH were dissolved (50 mg/ml) in double distilled water and homogenized followed by centrifugation at 7000×*g* for 15 min. The supernatant was collected and filtered through Whatman no.1 filter paper and protein content in the filtrate was estimated by the method of Lowry et al. (1951).The filtrate was filter sterilized with 0.22 µm pore size filter and used for antimicrobial assay.

Antibacterial studies The antibacterial activity of both AH and LFH was assayed by the agar-well diffusion method (Geis et al. 1983) under aerobic conditions. Pre-poured agar (nutrient agar) media plates were overlaid with BHI soft agar and inoculated with freshly grown pathogenic bacteria. *The pathogenic strain used were, Aeromonas hydrophila* B445, *Bacillus cereus* F4433, *Staphylococcus aureus* FR1722, *Salmonella paratyphi* FB254, *Salmonella typh*i FB231, *Listeria innocua* FB21, *Listeria murreyi* FB69, *Listeria monocytogenes* ScottA, and *Yersinia enterocolitica* MTCC859. In the agar plates wells of 5 mm diameter were cut using a sterile cork

borer. The wells were filled with 50 µl of each of samples at different concentrations and incubated for 18–24 h at 37°C. The antagonistic zones were detected at the end of incubation period and diameter of zone of inhibition was measured in mm.

Results and discussion

The results of proximate composition and pH (Table 1) of limed TF, delimed TF and hydrolysates produced from the delimed TF are in conformity with our previously published works (Amit et al. 2009a, b, 2010a) as the work was carried out using the same large batch of delimed TF. The cooked delimed TF had a protein content of 7.1% (wwb) and was hydrolysed under optimized condition either by a mixture of organic acids (propionic acid: formic acid 1:1) or by fermentation using LAB (*E. faecium* NCIM5335). Hydrolysis of protein involves major structural changes where the protein molecule is gradually cleaved into smaller peptide units and as hydrolysis increases the solubility of hydrolysed protein also increases (Kristinssons and Rasco 2000). The antioxidant and antibacterial properties of AH and LFH are presented below.

Antioxidant activity

The major in vitro antioxidant properties analysed included DPPH radical scavenging, ABTS radical scavenging, Peroxyl radical scavenging and superoxides scavenging along with the reducing power assay. DPPH has been used extensively as a free radical to evaluate reducing substance and is a useful reagent for investigating the free radical scavenging activities of protein hydrolysates (Sachindra and Bhaskar 2008, Amit et al. 2009a). DPPH radical scavenging activities (%) of LFH and AH with different concentration of the hydrolysate has been shown in Fig. 1a. The effective concentration for 50% scavenging activity (EC_{50}) of the hydrolysate was determined, which indicated that for 50% scavenging of DPPH radical requires protein concentration in the hydrolysate to be of 3.5 mg and 4.2 mg for LFH and AH, respectively. Scavenging activity of both the samples showed dose dependency as the activity increased with increasing concentration of the hydrolysate. Positive control α-tocopherol showed DPPH radical scavenging activity of 95% at 1 mg concentration.

The ABTS radical scavenging activity of both LFH and AH was stronger than DPPH radical scavenging activity with 83% and 75.3% scavenging, respectively, at 0.25 mg concentration of protein (Fig. 1b). The effective concentration to scavenge 50% of the radicals (EC_{50}) for ABTS radical

Table 1 pH and proximate composition of fresh, delimed and hydrolysed tannery fleshings (TF)

Parameters	Fresh TF	Delimed TF	Hydrolysed TF	
			LFH	AH
pH	12.2 ± 0.2	7.0 ± 0.2	–	–
Moisture (%)	70.4 ± 2.5	80.9 ± 2.1	2.9 ± 0.4	3.38 ± 0.4
Protein (%)	5.8 ± 1.7	7.1 ± 0.6	8.7 ± 1.9*,[a]	11.7 ± 2.1*,[a]
Fat (%)	7.2 ± 0.7	9.1 ± 0.8	8.1 ± 1.7*	1.8 ± 1.3*

All the values are on wet weight basis except those marked with asterisk

* Values are on dry weight basis

[a] Nitrogen content of the material

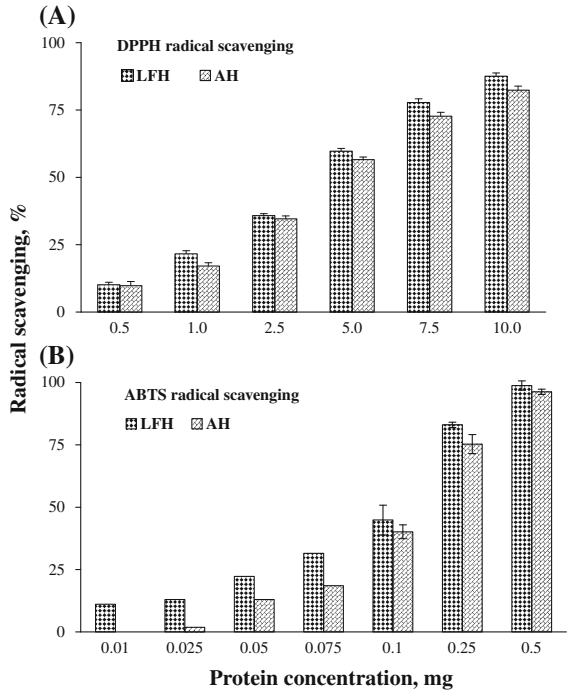

Fig. 1 **a** DPPH radical scavenging and **b** ABTS radical scavenging activity of LAB fermented hydrolysate (*LFH*) and acid hydrolysate (*AH*)

scavenging activity was as low as 0.11 mg for LFH and 0.13 mg for AH. Both ABTS and DPPH radical scavenging activity indicated marginally higher activity in case of LFH than AH. Antioxidative compounds have been shown to scavenge ABTS radicals at higher level compared to DPPH radicals (Sachindra and Bhaskar 2008). Many antioxidant peptide sequence identified have been reported to contain hydrophobic amino acid residues, especially valine or leucine, at the N-terminus of peptides apart from proline, histidine and tyrosine in the sequence (Song et al. 2008). Previous studies by our group have indicated (Bhaskar et al. 2007a; Amit et al. 2009a, b, c), that both AH and LFH have all these amino acids, except histidine, in considerably higher concentrations. This possibly explains the higher antioxidant activity observed in case of AH and LFH.

Peroxyl radical are extremely reactive oxidizing radicals that can react with other radicals and lipid hydroperoxides (Cheeseman and Slater 1993). Both LFH and AH showed higher scavenging activity toward peroxyl radical and the scavenging was dose dependent (Fig. 2a). At a concentration equivalent to 5 mg hydrolysate protein, LFH and AH showed 60 and 56.5% peroxyl radical scavenging, respectively. Higher peroxyl radical scavenging shown by these hydrolysates indicate the fact that both these hydrolysates have hydrogen donating (scavenging of DPPH and ABTS radicals) and chain breaking (sulfur containing amino acid like cystine) (scavenging of peroxide radical) antioxidant properties.

Unlike DPPH, ABTS and peroxyl radical scavenging activities superoxide radical scavenging activity (Fig. 2b) and reducing power assay (Fig. 3) showed higher activity for AH than LFH. The reducing power ability of both LFH and AH is associated with the presence of reductones that are reported to be terminators of free radical chain reaction (Ganesan et al. 2008). Reducing power of both LFH and AH indicated dose dependency (Fig. 3), wherein absorbance was found to increase with the increase in concentration of the hydrolysate. The reducing power of AH (0.5 mg) and LFH (0.5 mg) was comparable to that of the positive control (α-tocopherol; 0.25 mg). In general, superoxide anion radical is converted in organisms to hydrogen peroxide by the enzyme superoxide dismutase. In the absence of transition metal ions, H_2O_2 is fairly stable. However, hydroxyl radicals can be

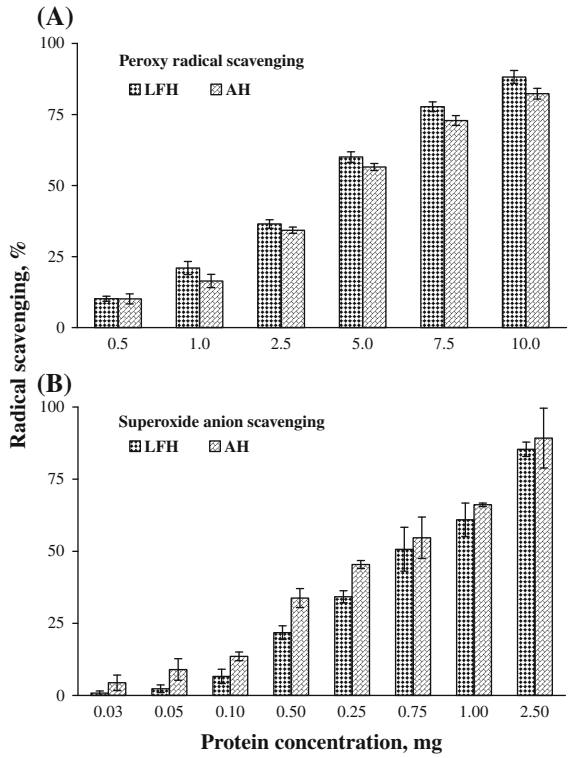

Fig. 2 **a** Peroxy radical scavenging and **b** Superoxide anion scavenging activity of LAB fermented hydrolysate (*LFH*) and acid hydrolysate (*AH*)

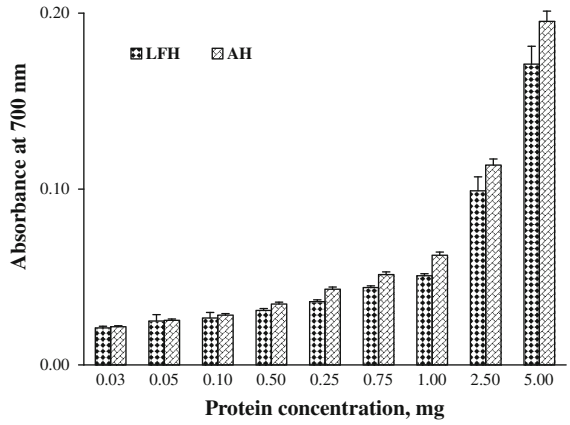

Fig. 3 Reducing power of LAB fermented hydrolysate (*LFH*) and acid hydrolysate (*AH*)

formed by the reaction of superoxide with H_2O_2 in the presence of metal ions, usually iron and copper (Macdonald et al. 2003). At concentrations equivalent to 1 mg hydrolysate protein, LFH showed 60.9%

scavenging as against higher activity of 66.1% by AH (Fig. 2b). The EC_{50} values were as low as 0.73 mg hydrolysate protein for LFH and an even lower 0.45 mg AH. The superoxide anion scavenging activity of both the samples (LFH and AH) as observed in this work is indicative of the fact that both these products can be beneficial in decreasing the possible toxicity of superoxide anions.

Overall, as observed in this study, both AH and LFH have antioxidative properties of varying degrees against different free radicals. They can be very useful in reducing the oxidative stress as they exhibit different degrees of scavenging against different free radicals. It has been widely accepted that the activities of putative antioxidants are due to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (Diplock 1997). This possibly explains the reasons why different in vitro assays may not correlate among each other for the same compound.

LAB are known to afford several beneficial effects and several species have been explored for their potential as probiotic in humans as well as livestock populations. Recently lactic acid fermentation has gained more attention in recovering biomolecules from different types of waste. LAB are applied for recovering proteins (Amit et al. 2009a), lipids (Amit et al. 2010b), carotenoid and chitin (Bhaskar et al. 2007b; Sachindra and Bhaskar 2008) from waste simultaneously adding more value to the product.

Antibacterial activity

The LFH and AH exhibited strong inhibitory activity against both Gram positive (*B. cereus* F4433, *S. aureus* FR1722) and Gram negative bacteria (*S. paratyphi* FB254, *S. typhi* FB231, *L. murreyi* FB69, and *Y. enterocolitica* MTCC859) (Table 2). The hydrolysates also exhibited dose dependency wherein the inhibition zone increased with increase in hydrolysate protein concentration. The antibacterial activity of 0.88 mg hydrolysate protein (both LFH and AH) was comparable to that of the positive control (amphicillin; 1 mg) for all the pathogens except *S. aureus* FR1722. Protein hydrolysates produced by enzymatic hydrolysis have shown to exhibit antagonistic properties against several bacterial pathogens. In this sense Kawai et al. (2007) have reported antagonistic property of bovine lactoferrin hydrolysate against mastitis pathogens. Similarly, hydrolysates of food proteins by gastrointestinal proteases have also been shown to be antibacterial as well as immunostimulatory in nature (Gediminas et al. 2006). The results of our studies corroborate well with antibacterial properties reported for distantly similar hydrolysates as mentioned above. In general, antibacterial properties in the fermented product can be either due to bacteriocin produced by LAB or by the peptide produced on hydrolysis of protein but in case of acid hydrolysis this is only due to the hydrolysed protein. Although we expected the antibacterial spectrum of the fermentation liquor to be the same as that of the culture used for fermentation (i.e., *E. faecium*

Table 2 Antimicrobial activity (diameter of zone of inhibition in mm) of lyophilized acid hydrolysate (AH) and LAB fermented hydrolysate (LFH) against different bacterial pathogens

	Control[a]	Concentration of LFH (mg)					Concentration of AH (mg)				
		0.88	0.7	0.52	0.32	0.17	0.88	0.7	0.52	0.32	0.17
B. cereus F4433	31	29	25	23	24	22	32	3	28	27	22
S. aureus FR1722	31	11	8	6	8	7	17	14	9	ND	ND
S. paratyphi FB254	30	29	24	26	25	24	26	25	25	25	20
S. typhi FB231	42	35	38	37	33	38	36	36	35	33	31
L. murreyi FB69	38	32	32	31	32	25	34	35	36	29	30
Y. enterocolitica MTCC859	26	26	26	24	23	22	26	25	24	22	22

LFH LAB fermented hydrolysate, *AH* acid hydrolysate, *ND* not detected

[a] Amphicillin (1 mg) is taken as control

HAB01) (Amit et al. 2009a), the antibacterial spectrum of LFH did not correlate with that of LAB used. Thus, it was concluded that the antibacterial spectrum of LFH was mainly due to the peptides that were present rather than the strain used.

Conclusions

Fermentation using LAB and hydrolysis using mild organic acids converts TF to products having biofunctionalities like antioxidant and antimicrobial properties. Both these products from TF exhibited close to 90% scavenging of different free radicals depending on type of free radical and concentration of protein hydroysate. The fact that EC50 values of protein hydrolysate is very low (0.11–3.5 mg) for all the radical scavenging activities and reducing power depending on type of radicals. Also, both products exhibited considerable antibacterial properties. These products have potential as ingredients in animal feeds as protein source and also help in reducing the oxidative stress related changes in the farmed animals owing to their inherent biofunctionalities. Bioconversion of limed TF to delimed TF and subsequently into hydrolysates would not only be an economical proposition to the tannery operations but also would help minimize the disposal problems faced by the tanneries. Further, both AH and LFH need to be evaluated for their in vivo biofunctionalities to know their exact effects when incorporated into livestock or aquaculture feeds.

Acknowledgments This study is supported by funding from Council of Scientific and Industrial Research (CSIR) through the network program (NWP-044) on Zero Emission Research Initiatives (ZERI). We thankfully acknowledge Masood Ahmed of M/s Mysore Super Reptiles Corporation, Bangalore, Karnataka, India for the gratis supply of limed TF. Authors thank Prakash V, Director, CFTRI, for encouragement and permission to publish this study.

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