ANTIMICROBIAL BIOACTIVITY OF ENZYMETICALLY HYDROLYZED GOAT BLOOD

D. Krofa[1]*, M.K. Chatli, N.Mehta, P. Kumar, O.P. Malav and R.V. Wagh
Department of Livestock Products Technology, GADVASU, Ludhiana, Punjab, India-141001
[1]*Department of Livestock Products Technology, CSKHPAU, Palampur, (H.P.), India-176062
*Corresponding author email: dkrofa@gmail.com

I. INTRODUCTION

Bioactive peptides are short amino acid sequences between 2 and 20 amino acid residues, generated by hydrolysis of proteins [1]. Bioactivities of peptides derived from food proteins include antimicrobial, ACE inhibitor, antithrombotic and antioxidant etc. [2]. The hydrolysis of intact proteins generates peptides with bioactivities potentially higher than its native protein [3]. Protein hydrolysis can occur either through gastrointestinal digestion, or via treatment with exogenous proteases, acid and alkali, microbial fermentation. Proteolytic hydrolysis is one of the most common methods used to generate hydrolysates containing bioactive peptides [4]. Animal blood, obtained during the slaughter of animals at the abattoir, is an important but under-utilized protein source. Conversion of blood into bioactive peptide hydrolysates could find applications in the nutraceutical and pharmaceutical industries, would improve the economic yield from animal agriculture and increase the worth of this low value offal.

II. MATERIALS AND METHODS

Four different enzymes (Papain, Trypsin, Alcalase and α-Chymotrypsin) were used to hydrolyze goat blood after standardizing the protocols for efficient hydrolysis. The concentration level, pH and temperature of tested proteases were identified in accordance with the available literature and preliminary trials. Four different reaction times (0, 2, 4, 6 h) were taken and the efficiency of recovered protein hydrolysates was compared on the basis of degree of hydrolysis (DH). Hydrolysates exhibiting best requisite characteristics for each enzyme were selected and fractionated viz. <1 kDa, 1-3 kDa, 3-5 kDa, 5-10 kDa and >10 kDa via ultrafiltration using molecular weight cut off (MWCO) membranes. Each fraction was assessed for antimicrobial activity tested against Gram positive *S. aureus* (MTCC No. 7443) and Gram negative *E. coli* (MTCC No. 2991). Statistical analysis was done by analysis of variance and Duncan's multiple range test using SPSS ver. 22.

III. RESULTS AND DISCUSSION

Gel diffusion technique was used to examine the antimicrobial effect of hydrolysate as well as its fractions. The diameter of zone of inhibition increased with the increasing reaction time, irrespective of type of enzyme and type of test organisms. The papain hydrolysates exhibited significantly ($P \leq 0.05$) greater inhibition zone followed by the Trypsin, Alcalase and α-Chymotrypsin hydrolysates. This is attributed to endo and exo peptidase activity of papain. . Antibacterial activity might be due to cationic and the hydrophobic peptides that interact with anionic components of the bacterial cell wall such as lipoteichoic acid of gram positive and lipopolysaccharide of gram negative bacteria, disrupting the cell wall, thus cell death. The papain hydrolysates collected after 6h hydrolysis recorded zone diameter as 17.16±0.20 and 18.01±0.17 for *E. coli* and *S. aureus* (Figure 1), respectively which were significantly ($P \leq 0.05$) higher than other hydrolysates. This might be due to higher percentage of lower molecular weight peptides. Similar observations documented for pepsin hydrolysates of bovine hemoglobin against *E. coli*, *L. innocua* and *M. luteus* [5].

All the peptidic fractions of papain also recorded significantly ($P \leq 0.05$) higher antimicrobial activity against both the test organisms than fractions of Trypsin, Alcalase and α-Chymotrypsin, respectively between the groups. Further, it was observed that the antimicrobial efficacy increased with the decrease in MW of peptides i.e. it was recorded highest for <1 kDa fraction and lowest for >10kDa, irrespective of the type of enzyme and type of bacteria. It might be due to higher penetrability of small size peptides in the cell wall. Blood papain hydrolysate fraction of <1 kDa exhibited the inhibition zones of 18.36±0.16 mm (Table-1) against *S. aureus* that was significantly ($P<0.05$) higher than all other fractions. Similar results were recorded against *E. coli* where papain

peptidic hydrolysate fractions were 30-45% higher than respective chymotrypsin fraction. Wessely-Szponder et al., (2010) also observed higher antimicrobial efficiency of low range peptides generated from porcine neutrophils [6].

Figure 1. Zone of inhibition by the blood hydrolysates of trypsin at 0, 2, 4 and 6 hours against *Staph. aureus*.

Table 1: Antimicrobial activity of different goat blood protein hydrolysate fractions (Mean±SE)*

MW Range	BP6	BT6	BA6	BC6	BP6	BT6	BA6	BC6
	Staphylococcus aureus				*Escherichia coli*			
<1 kDa	18.36±0.16^{Dc}	17.46±0.20^{Dc}	16.66±0.13^{Db}	12.46±0.39^{Ca}	15.49±0.11^{Cc}	15.43±0.12^{Dc}	14.00±0.21^{Db}	12.36±0.20^{Ea}
1-3 kDa	17.45±0.17^{Cc}	15.31±0.23^{Cb}	14.63±0.17^{Cb}	11.12±0.09^{Ba}	15.39±0.14^{Cc}	15.37±0.14^{Dc}	13.72±0.17^{Cb}	11.62±0.12^{Da}
3-5 kDa	16.43±0.19^{Bd}	14.63±0.25^{Bc}	12.50±0.24^{Bb}	10.98±0.20^{Ba}	15.23±0.18^{Cd}	14.18±0.20^{Cc}	12.97±0.19^{Bb}	10.64±0.17^{Ca}
5-10 kDa	13.14±0.11^{Ad}	12.50±0.24^{Ac}	11.68±0.23^{Ab}	10.48±0.20^{Ba}	13.26±0.21^{Bb}	13.19±0.11^{Bb}	9.89±0.23^{Aa}	9.37±0.16^{Ba}
>10 kDa	13.12±0.19^{Ad}	12.46±0.39^{Ac}	11.11±0.07^{Ab}	9.94±0.13^{Aa}	12.74±0.13^{Ad}	10.73±0.20^{Ac}	9.80±0.07^{Ab}	8.83±0.19^{Aa}

(n=9),*Values bearing same superscripts row-wise (small alphabets) and column-wise (capital alphabets) do not differ significantly (P<0.05). BP6: Blood hydrolyzed with Papain for 6 h; BT6: Blood hydrolyzed with Trypsin for 6 h; BA6: Blood hydrolyzed with Alcalase for 6 h; BC6: Blood hydrolyzed with Chymotrypsin for 6 h; (zone of inhibition values are in mm).

IV. CONCLUSIONS

Results concluded that high value can be added to the goat blood by it's successfully utilization as substrate for the generation of papain hydrolysates with antimicrobial properties. The antimicrobial efficacy of the hydrolysates increased with the increasing hydrolysis time upto 6 h and its <1 kDa fraction have highest antimicrobial efficacy against *S. aureus and E. coli*, respectively.

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