PAPAIN HYDROLYSATES OF GOAT LIVER AS NATURAL ANTIOXIDANTS

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I. INTRODUCTION

Lipid oxidation is a major cause of food spoilage and deterioration of its nutritional, sensory quality and safety [1]. Synthetic antioxidants commonly used poses potential health risks with toxic and carcinogenic effects [2].

Long chain or complex proteins are encrypted however the small subunits of proteins having 2 and 20 amino acids residues known to exhibit functional bioactivity such as antimicrobial, ACE inhibition, antithrombotic, and antioxidant [3]. The *in-vitro* enzymatic digestion of food proteins can generate designer peptides with known functional efficiency whereas gastrointestinal breakdown of native protein do not generate uniform quality.

Edible animal offals are known to possess high quantity of quality proteins. Liver is the largest edible nutritive gland in animals and weighs nearly 0.5 kg in goat. Its nutrient density exceeds that of muscle meats and 100 grams of liver provides 25 grams of high-quality protein, [4] hence it is a suitable substrate for generation high-value functional protein hydrolysates. The present study is conducted to compare the efficiency of the different enzymes to generate antioxidant peptides.

II. MATERIALS AND METHODS

Process protocol was standardized for hydrolysis of goat liver using four enzymes viz. Papain, Trypsin, Alcalase and α-Chymotrypsin and their efficiencies were compared. Degree of hydrolysis (DH) was worked out for efficient recovery of protein hydrolysate at different reaction times (0, 2, 4, 6 h). Time hydrolysates were compared for its antioxidant activity on the basis of oxidative stability parameters viz. TBARS [5], 2, 2 diphenyl-1-picrylhydrazyl (DPPH) [6] and Ferric reducing antioxidant power (FRAP) [7]. The selected time hydrolysate was fractionated (<1 kDa, 1-3 kDa, 3-5 kDa, 5-10 kDa and >10 kDa) through ultrafiltration using MWCO membranes. Thereafter, each fraction was subjected to elucidate antioxidant activity in goat meat emulsion. The emulsion was evaluated for oxidative stability on alternate days for 6 days at refrigeration temperature (4±1ºC). SPSS ver. 22 was used for statistical analysis of variance (ANOVA) and Duncan's multiple range test.

III. RESULTS AND DISCUSSION

Degree of hydrolysis % (DH) increased with the increasing reaction time, irrespective of enzyme and measured highest at 6h for papain amongst all test enzymes (Figure 1). It might be due to both endo and exo peptidase activity of papain. The lower values for α-Chymotrypsin might be due to its high selectivity for proteins with aromatic amino acids at amide side.

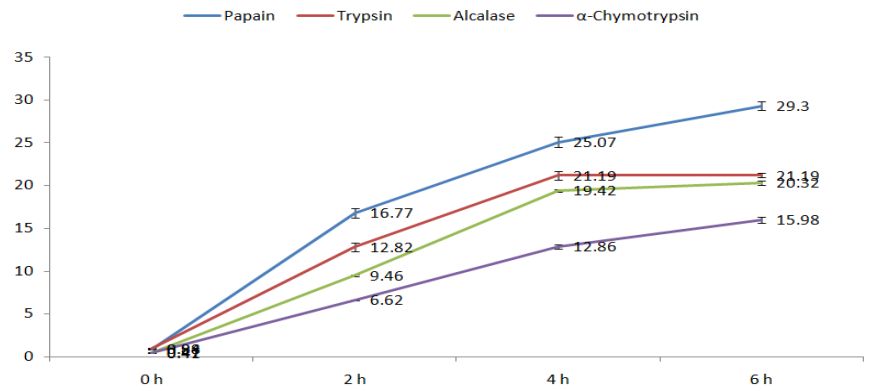

Figure 1. Degree of hydrolysis of goat liver protein hydrolysates with different proteases at time intervals

Table 1 Antioxidant activity of goat liver protein hydrolysate fractions (Mean±SE)*

MW Range	LP6	LT6	LA6	LC6	LP6	LT6	LA6	LC6
	DPPH (% Inhibition)				FRAP (mM Equivalent to $FeSO_4.7H_2O$)			
<1 kDa	53.26 ± 0.31^{Ed}	42.25 ± 0.21^{Dc}	39.89 ± 0.41^{Eb}	37.40 ± 0.16^{Da}	79.58 ± 0.20^{Dc}	54.39 ± 0.60^{Eb}	39.77 ± 0.21^{Ea}	38.23 ± 0.25^{Ea}
1-3 kDa	50.65 ± 0.16^{Db}	33.93 ± 0.26^{Ca}	33.65 ± 0.39^{Da}	33.02 ± 0.65^{Ca}	75.19 ± 0.24^{Cd}	48.23 ± 0.32^{Dc}	35.15 ± 0.27^{Db}	28.99 ± 0.58^{Da}
3-5 kDa	46.25 ± 0.32^{Cd}	32.73 ± 0.34^{Cc}	29.02 ± 0.54^{Cb}	27.53 ± 0.39^{Ba}	75.16 ± 0.26^{Cd}	35.15 ± 0.31^{Cc}	27.45 ± 0.16^{Cb}	14.37 ± 0.42^{Ca}
5-10 kDa	34.93 ± 0.18^{Bd}	27.90 ± 0.05^{Bc}	24.92 ± 0.42^{Bb}	10.46 ± 0.62^{Aa}	41.31 ± 0.20^{Bc}	26.83 ± 0.29^{Bb}	25.29 ± 0.28^{Bb}	8.67 ± 0.09^{Ba}
>10 kDa	26.19 ± 0.29^{Ac}	22.77 ± 0.32^{Ab}	9.48 ± 0.19^{Aa}	9.62 ± 0.11^{Aa}	19.67 ± 0.18^{Ab}	7.13 ± 0.23^{Ab}	5.59 ± 0.01^{Aa}	5.05 ± 0.04^{Aa}

(n=9),*Values bearing same superscripts row-wise (small alphabets) and column-wise (capital alphabets) do not differ significantly ($P<0.05$). LP6: Liver hydrolyzed with Papain for 6 h; LT6: Liver hydrolyzed with Trypsin for 6 h; LA6: Liver hydrolyzed with Alcalase for 6 h; LC6: Liver hydrolyzed with Chymotrypsin for 6h.

Antioxidant activity of the fractions increased with decreasing MW and size of peptides and <1 kDa papain fraction exhibited highest DPPH (53.26±0.31%), and FRAP (79.58±0.20 mM) amongst all the treatments (Table 1). Similar observations were recorded by Je et al. (2009) with tuna and Di Bernardinia et al. (2011) with the bovine liver hydrolysate fractions [8]. The selected fraction was incorporated in goat meat emulsion@ 0.03 T-1,0.06 T-2,0.09% T-3 and compared with blank (control, C-1) and goat emulsion with permitted level of BHT (synthetic antioxidant @ 0.1%, C-2) for oxidative stability. TBARS value was recorded significantly ($P<0.05$) lower in T-3 compared to blank control on 6th day of storage.

VI. CONCLUSION

Results concluded that the 0.09% concentration of <1kDa fraction extracted out of 6h papain hydrolysate from liver proteins can be successfully utilized as an effective replacement of synthetic antioxidant for the stability of goat emulsion under aerobic packaging at refrigeration temperature.

REFERENCES

[1] Antolovich, M., Prenzler, P.D., Patsalides, E., McDonald, S. & Robards, K. (2002). Methods for testing antioxidant activity. Analyst 127:183-198.

[2] Sakanaka, S., Tachibana, Y., Ishihara, N. & Raj Juneja, L. (2005). Antioxidant properties of casein calcium peptides and their effects on lipid oxidation in beef homogenates. Food Chem 53:464-468.

[3] Ohba, R., Deguchi, T., Kishikawa, M., Arsyad, F., Morimura, S. & Kida, K. (2003). Physiological functions of enzymatic hydrolysates of collagen or keratin contained in livestock and fish waste. Food Sci Techn Res 9(1):91-93.

[4] Romans, J.R., Costello, W.J., Carlson, C.W., Greaser, M.L. & Jones, K.W. (2001). Meat we Eat; Packing house by-products", Interstate Publishers, Inc./Danville, Illinois, USA, pp. 284.

[5] Witte, V. C., Krause, G. F. & Bailey, M. E. (1970). A new extraction method for determining 2-Thiobarbituric acid values of pork beef during storage. Journal of Food Science 35: 582-85.

[6] Brand-Williams, W., Cuvelier, M. E., & Berset, C., (1995). Use of a free radical method to evaluate antioxidant activity. LWT- Food Science and Technology 28: 25-30.

[7] Benzie, I. F. F., & Strain, J. J., (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Annals Biochemistry 239: 70-77.

[8] Di Bernardinia, R., Raie, D.K., Boltonb, D., Kerryc, J., O'Neill, E., Mullena, A.M., Harnedyd, P. & Hayese, M. (2011). Isolation, purification and characterization of antioxidant peptidic fractions from a bovine liver sarcoplasmic protein thermolysin hydrolysate. Peptides 32:388-400.