ENZYMATIC HYDROLYSIS OF HIDE 'FLESHING' MEAT: CHARACTERISATION AND OPTIMIZATION

C. Anzani[1*], T. Tedeschi[1], A. Dossena[1] and S. Sforza[1]

[1]Food and Drug Department, University of Parma, Italy.

*cecilia.anzani@studenti.unipr.it/Cecilia.Anzani@teagasc.ie

Abstract –The meat portion directly attached to bovine hides (fleshing meat) is a meat industry by-product. It has the potential to become a new source of proteins. Therefore the aim is to recover fleshing meat proteins, by enzymatic hydrolysis, in order to revalorize it as a possible food or feed product. The proteolytic activity of several commercial enzymes was tested and the resultant hydrolysates were characterized. The results demonstrated that papain and Alcalase were efficient in hydrolyzing collagen, which is the main protein in fleshing meat. The optimum conditions for the hydrolysis were determined by the response surface method (RSM) followed by a scaling-up to 5L volume.

Key Words – by-products, beef hide, fleshing, protein hydrolysates, scale-up, response surface methodology.

I. INTRODUCTION

The meat sector generates a substantial impact on the environment in terms of land occupation, carbon footprint and food wastage. Hence it is imperative that we use all parts of the animal in an optimal and efficient manner. Beef meat accounts for only 44% of total live animal weight with the highest production of low value products and waste material being generated in the abattoir. In particular the specific waste index of the cow is 0.56 [1]. Nowadays meat industries try to add value to by-products through the generation of innovative food and non-food products [2]. Therefore fleshing meat could be a suitable substrate for recovery of usable free amino acids in spite of the techno-functional properties. Enzyme hydrolysis offers a fast and gentle alternative to other mechanical or chemical treatments. The ability of the enzyme to hydrolyze proteins to produce free amino acids and short peptides allows the nitrogen to be more soluble and easily recovered and purified; for this reason fleshing meat hydrolysates have the potential to become a new source of valuable proteins.

II. MATERIALS AND METHODS

Samples, made of fleshing meat from cow, calf and steer, were provided by Inalca Industria Alimentare Carni SpA (Castelnuovo Rangone, Italy) and stored at -20°C until use. Moisture, protein and crude fat content were determined following standard A.O.A.C. (2002) procedures. Five gram samples of fleshing were hydrolyzed for 16h with common commercial enzymes derived from vegetal (papain), bacterial (Alcalase, dispase) or animal sources (pepsin, trypsin, pancreatin). Hydrolysates were centrifuged after enzyme deactivitation. Kjeldahl analysis was performed on pellet and supernatant fractions. According to Nollet (2004) [3], total and free amino acids profile was determined by high performance liquid chromatography with fluorescence detector (HPLC-FLD). The peptide profile was measured using LTQ-orbitrap and the degree of the hydrolysis by o-phthalaldehyde (OPA)-method [4]. The experimental design for the optimization was settled and the analysis of the data obtained through the response surface method (RSM), was accomplished using the STATISTICA software (Statsoft, 1999). Following the optimum conditions, a scale-up reaction in a 5 L reactor was executed.

III. RESULTS AND DISCUSSION

Proximate analysis of fleshing is presented in Table 1. These differences, between the fleshings of animals with different ages, derived from the liming treatment which altered the fat and protein content in the residual fleshing [5]. The total amino acid analysis reveals that the percentage distribution of total amino acids in the three different fleshing is similar; in particular there is a great amount of Hyp, Gly and Pro, which indicates the high amount of collagen present in these samples. Alcalase and papain were the most efficient releasing in solution 97% of the proteins originally present according to the analyses with Kjeldahl method. The other enzymes were clearly less efficient, with trypsin and pancreatin giving slightly better results than pepsin and dispase (38%, 25%, and 18%, 18% respectively).

Table 1 Percentage of moisture, protein and fat in beef, calf and steer fleshing.

	Cow Fleshing	Steer Fleshing	Calf Fleshing
	average	average	average
% moisture	80 ± 3	34 ± 1	47 ± 1
% proteins	20 ± 3	8.8 ± 0.8	12 ± 2
% fat	1.6 ± 0.4	54 ± 2	39 ± 3

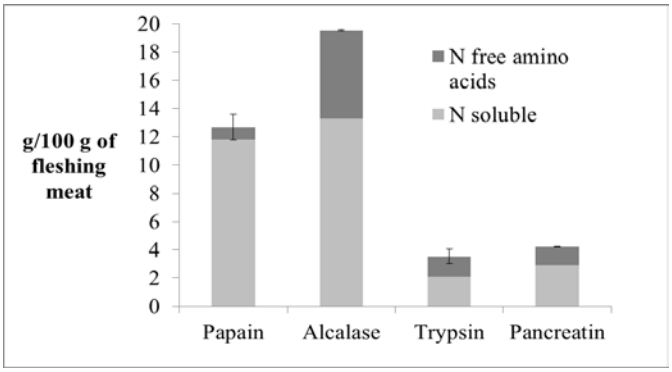

Fig.1 TAA and FAA in the supernatant after hydrolysis:

Alcalase and papain hydrolysis displayed similar efficiencies in solubilisation of the collagen proteins. However they gave rise to hydrolysates with differing distributions of the nitrogen substances (Figure 1): Alcalase produces a larger amount of free amino acids and small peptides, whereas papain generates less free amino acids and longer peptides (figure 1). An experimental designed was established, based on RSM, to further optimise hydrolysis using Alcalase. The influence of volume and % of enzyme on the hydrolysis by Alcalase was evaluated. The desirability profile indicated that an optimum solubilised proteins could be achieved from both calf and steer fleshing with a volume of 28.3 mL and 0.54% of Alcalase. A larger scale experiment was conducted following the optimised hydrolysis conditions. After 2 hours the calf fleshing was completely hydrolysed, otherwise the steer fleshing needed 8 hours to be entirely hydrolysed. On the other hand the kinetic profile of DH has the same curve and trend, the maximum %DH is 25.

CONCLUSION

Alcalase hydrolysis of beef fleshing was effective at generating large amount of free amino acids. Conditions of hydrolysis were optimized and validated. The results obtained by the experimental design showed the optimum condition to have a complete digestion of fleshing, taking into account the variability of the raw material in terms of protein, fat and moisture contents. In particular this procedure need at least 10 hour, therefore it is could be a possible way to recover fleshing through hydrolysates which could have the potential to be used as supplement in the food industry.

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REFERENCES

1. Russ, W. Meyer-Pittroff, R. (2004). Utilizing Waste Products from the Food Production and Processing Industries Critical Reviews in Food Science and Nutrition 44:57–62.
2. Toldrá, F., Aristoy, C. M., Mora, L., Reig, M. (2012). Innovations in value-addition of edible meat by-products. Meat Science 92: 290–296.
3. Nollet, L. M. L. (2004). Handbook of food analysis physical characterization and nutrient analysis, second ed. Marcel Dekker, Inc.
4. Spellman, D., McEvoy, E., O'Cuinn, G., FitzGerald, R.J. (2003). Proteinase and exopeptidase hydrolysis of whey protein: Comparison of the TNBS, OPA and pH stat methods for quantification of degree of hydrolysis. International Dairy Journal 13(6): 447–453.
5. Sundar, V. J., Gnanamani, A., Muralidharan, C., Chandrababu, N. K., & Mandal, A. B. (2011). Recovery and utilization of proteinous waste of leather making: a review. Review in Environmental Science Biotechnology, 10, 151-163.