MEAT INDUSTRY BY-PRODUCT RECOVERY: GELATIN AND FILM FORMING EVALUATION

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Abstract – Beef meat is a primary source of energy and protein, however, it accounts for only 44% of live animal weight. The other 56% is composed of edible (e.g. offal) and non-edible (e.g. SRM) parts. There is increasing interest in examining these non-meat products with a view to extract or recover additional value from the meat processing chain. Bovine hide is one of such product and it is used for leather production. However some parts e.g. covering the legs are not suitable for this purpose but, being a protein rich material, may provide a feedstock for the generation of peptides with value as a nutritional or food ingredient product. The aim of this research was the optimization of the solubilisation and recovery of bovine hide proteins *via* enzymatic hydrolysis. The efficiency of Alcalase has been evaluated. The effect of hydrolysis time and water/substrate ratios on hydrolysis process was evaluated. Recovery yield (solubilisation), peptide profiles, gelling capacity and film forming ability have been evaluated.

Key Words – bovine hide, enzymatic hydrolysis, protein hydrolysates

I. INTRODUCTION

Both reduction and recovery of the food waste have become hot topics and a challenge for the modern society. Beef meat accounts for approximately 44% of the total live animal weight, with the high production of low value or waste material generated during slaughtering [1]. Hide is one example of a meat industry by-product and it is used as a raw material for leather production; however, some pieces of hide are not suitable for leather industry (too small, damaged, etc) and are disposed. For this research these hide trims, with neutral or negative value, have been employed for protein recovery in order to valorise them as new food or feed ingredient. Hide chemical composition is characterized by the presence of a large amount of collagen, a structural fibrous protein, which is often converted to gelatines by thermal denaturation and/or chemical degradation. The focus of this research is to examine the use of enzyme hydrolysis, as a means of recovering protein products from pieces of bovine hide. Alcalase was selected based on previous research, where its performance compared favourably with other commercial enzymes.

II. MATERIALS AND METHODS

The enzymatic reactions were carried out using two different water/substrate ratios (hide:water 1:2 and 1:3), in a reactor of 5 L capacity with a heating shell and agitator blade. In both experiments, pieces of hide were first washed and degreased twice for 30 minutes of stirring at 60°C. After degreasing, the hides were swollen in Na_2HPO_4 (10 mM, pH=7.5, T=60º) for 1 hour and then 1% of Alcalase from *Bacillus licheniformis* (Sigma-Aldrich) was added. Samples were collected every 30 min for 6 h and, boiled for 10 minutes after collection to deactivate the enzyme. The total protein content of the solution was estimated using a rapid Kjeldahl system (UDK 139 Semi-Automatic Kjeldahl Distillation Unit, VELP SCIENTIFICA, Milan, Italy). The degree of hydrolysis was determined, by the OPA (o-phthaldialdehyde) following the method as described by Spellman [2] with slight modifications. Peptide analysis was carried out using an LTQ-Orbitrap mass spectrometer, with peptide identification performed by Protein Discoverer software. 5 L of broths were obtained for both the water/substrate ratio after 3h and 6h of hydrolysis. After an ultra-filtration step using a cut-off of 3 KDa, permeate and retentate were analysed with SDS-PAGE and with SEC (size exclusion chromatography). Gelatines were obtained from the broths and the techno-functional and rheological properties were evaluated. The production of films was also tested.

III. RESULTS AND DISCUSSION

The kinetics profiles of the enzymatic hydrolysis were first studied by characterising the soluble protein content. The kinetic profile of the protein solubilisation showed similar trends regardless of the sample/water ratio employed, with a logarithmic increase of the solubilised nitrogen starting to flatten after 5-6 h. Protein yield (ie protein solubilised) was

90 and 93% when 1:2 and 1:3 ratios were employed respectively. Both, SDS-PAGE profile and the kinetic profile for the degree of hydrolysis, are illustrated in figures 1 and 2, respectively. Results indicated an extensive protein hydrolysis, reaching a degree of hydrolysis of 20% in both cases; which agrees with the higher yields obtained.

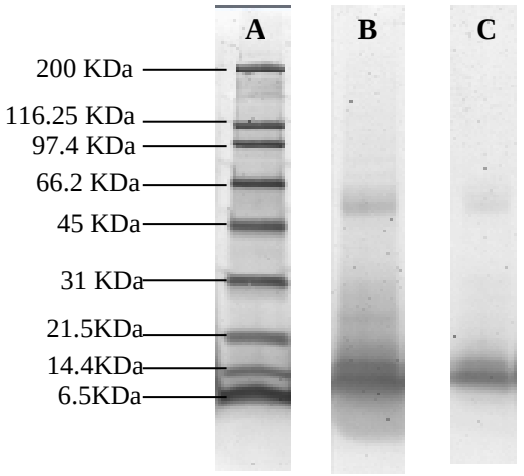

Figure 1. SDS-PAGE profile: A-standard marker, B-hydrolysate 1:2, C-hydrolysate 1:3

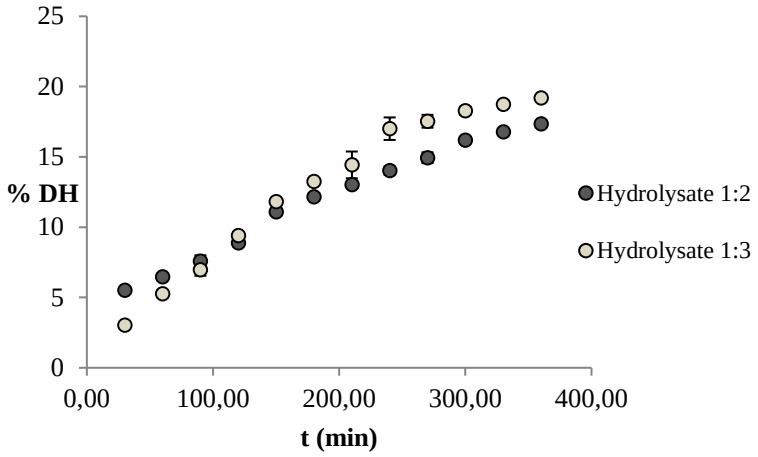

Figure 2. Kinetics profile of the degree of hydrolysis (%DH)

To further understand the mechanism of the proteolytic process, proteomic analyses were performed on the resulting peptide fraction. The LTQ-Orbitrap analysis of the peptides present in the hydrolysates indicated that they derived mainly from collagen, showing that Alcalase's efficiency in solubilising the hide is mostly due to its ability to degrade collagen. Both hydrolysates were tested for gelation properties by combining with agar, to promote the gelation process. The film forming ability of the recovered protein is under investigation.

IV. CONCLUSION

Enzymatic hydrolysis has been shown to be an effective way to recover peptides from bovine hide not suitable for leather industry. Alcalase turned out to be very efficient to hydrolysed bovine hides, because it degrades collagen releasing collagen-derived peptides. Testing of the techno-functional and the film forming properties of these peptides is underway.

ACKNOWLEDGEMENTS

This research has been carried out with the financial support by Cluster Agrifood Project 4 "Sustainability of the Italian food chain", (Project So.FI.A CTN01_00230_450760). INALCA Industria Alimentari Carni Spa is gratefully acknowledged for the samples supply.

"This work forms part of the ReValueProtein Research Project (Grant Award No. 11/F/043) supported by the Department of Agriculture, Food and the Marine (DAFM) under the National Development Plan2007–2013 funded by the Irish Government."

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