Protein recovery animal by-products from rendering plants and fleshings for biostimulant applications in agriculture

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INTRODUCTION

According to the European Fat Processors and Renderers Association (EFPRA), approximately 60% of each meat-producing animal is currently processed into food for human consumption, and the remaining 40% becomes animal by-products (ABPs).

Every year, 17 million tonnes of animal by-products (ABPs) are managed in Europe, of which 328 million are head of cattle and 6 billion are poultry slaughtered in slaughterhouses, amounting to 3.7 million tonnes of animal protein and 2.85 million tonnes of animal fat. From these ABPs, only EFPRA members produce 6 million tonnes of rendered products, and the water output of the process, which is almost 65% by weight, must be treated for safe return to the environment (EFPRA, 2022).

ABPs must be conveniently managed in compliance with European Regulation (EC) No 1069/2009 (ABP Regulation), which allows different applications for these by-products, depending on their risk category. In practice, while Category 3 (low risk) by-products such as liver, viscera, bone, intestines, and other by-products produced in slaughterhouses are mainly intended for animal feed, Category 2 (medium risk) by-products are mainly derived to energy recovery (last route recommended by the EU's Circular Economy strategy) or disposed of in authorised landfills, despite the fact that this biowaste is rich in valuable proteins as biopolymers.

According to their level of risk, ABPs should be processed while subjected to a sterilisation treatment. Processed animal proteins (PAPs), which are commonly transformed in rendering plants, are dried to obtain meat and bone meals (MBM). These drying processes for MBM production imply significant economic and environmental costs and produce a high volume of protein-containing water condensate, known as wastewater from MBM, the protein of which could potentially enhance the properties of bioproducts after being recovered.

Currently, the main industrial methodology for protein recovery is thermal hydrolysis. This process consists of several steps with a high water requirement and high energy consumption and negative environmental impacts, due to the acid or alkaline pre-treatment (process repeated more than 20 times for conditioning) required due to the hieratical fibrous structure that makes proteins insoluble in water. After the pre-treatment or conditioning process, the extraction stage to obtain protein biopolymers is assessed by a thermal process (60-85 ◦C, 5–8 h) with high energy consumption.

In order to implement a more sustainable and versatile process for different slaughterhouse raw materials such as greaves, wastewater rich in protein from PAP process, and fleshings, a process based on enzymatic hydrolysis was developed to obtain the protein biopolymer from Category 3 and 2 ABPs for industrial applications as a biostimulant.

MATERIALS AND METHODS

In this work three different types of raw material were recovered: Category 2 processed animal proteins (PAPs), Category 3 wastewater, and fleshing.

Category 2 PAPs originate from pig carcases previously pressure-sterilised through method 1, according to Regulation (EC) No 1069/2009 with 50 mm particle size, 133 °C for at least 20 minutes without interruption at an absolute pressure of at least 3 bar.

Category 3 wastewater is the condensed (protein-rich) water produced from the drying of Category 3ABPs to produce meat and bone meals (MBM). The sterilisation method used for Cat 3 ABPs is method 5 with 20 mm particle size, at 80ºC, for 120 minutes. Once the sterilisation has been conducted, those ABPs are ready to be utilised in PAPs.

Fleshings, a solid by-product from tanneries, are part of the hypodermis, before it is separated from the skin or hide, which undergo a treatment with an intense alkali solution of lime ($Ca(OH)_2$) and sodium sulphide (Na2S) to ensure hair and wool removal (unhairing process).

Due to the diversity in composition of the raw materials used in this work, it was necessary to carry out a conditioning step to improve the efficiency of the enzymatic hydrolysis process to be carried out on the animal by-products. Enzymatic processes are efficient processes that do not require severe conditions of temperature or pH. Consequently, there is no racemisation of amino acids, and they are sustainable bioprocesses. Nevertheless, enzymatic hydrolysis requires an important control of temperature, pH and hydrolysis time to obtain optimal enzyme activity. For this reason, different enzymes with endo- and exo-proteolytic activity working at acidic (6), neutral (7) and alkaline (8-11) pH were used to evaluate their degree of hydrolysis in the different by-products evaluated. In addition, the different bioprocess parameters were optimised. Finally, protein hydrolysates obtained were evaluated in terms of yield, total and free amino acids, and organic nitrogen content.

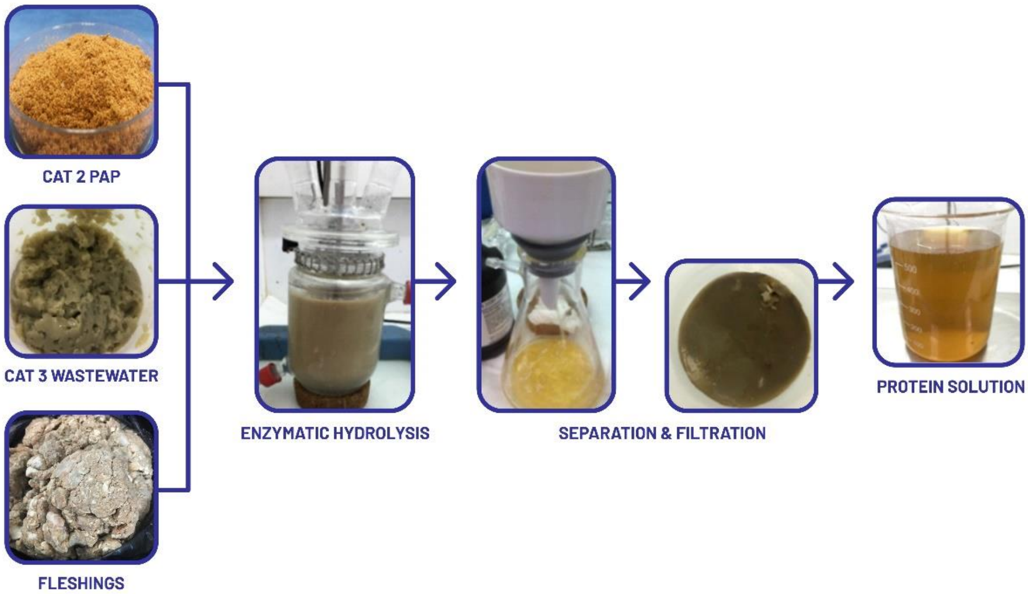

Figure 1. Process to obtain hydrolysed collagen from category 2 and 3.

RESULTS AND DISCUSSION

Different types of conditioning were evaluated depending on the animal by-products (ABPs) used for protein recovery with the aim of achieving an adequate content in fat, soaps and ashes for an efficient enzymatic hydrolysis. After the selection of the conditioning stage of the different ABPs, the enzymatic hydrolysis parameters were optimised and protein yields in the 78-97% range were obtained depending on the ABPs used.

The protein hydrolysates with the best properties for application as a biostimulant based on free amino acids were selected and it was verified that protein hydrolysates comply with the requirements established according to European Regulation 1009/2019 in terms of total and free amino acid content, organic nitrogen, etc.

In vitro germination bioassays were conducted in Petri dishes according to ISO standard 16086-2 2012 in order to evaluate and validate the potential of the protein hydrolysates obtained to stimulate plant growth with satisfactory results. These proved an improvement in Chinese cabbage (Brassica rapa pekinensis) and lettuce (Lactuca Sativa) seeds growth by 17-39% with the optimal biostimulant dilution concentration in the range of 0.05-0.2% depending on the protein hydrolysate used, as shown in table 1.

Table 1. Comparison of the parameters corresponding to the different protein hydrolysates obtained with each animal by-product used.

Parameter	Biostimulant obtained from Cat 2 PAP	Biostimulant obtained from Cat 3 wastewater	Biostimulant obtained from Fleshings
Protein yield (%)	87.50	97.22	78.18
Total nitrogen (%)	7.43	7.54	8.05
Ammonia nitrogen (%)	0.22	0.625	0.625
Organic nitrogen (%)	5.13	7.41	7.41
Urea nitrogen (%)	0.008	0.05	0.05
Total amino acids (% in dry matter)	70.68	60.72	68.06
Free amino acids (%) in solution	3.53	5.09	4.67
Dry matter (%)	57.52	56.74	67.25
Organic matter (%)	53.38	55.85	77.85
Ashes (% in dry matter)	7.8	7.3	10.5
Density (kg/L)	1.24	1.23	1.25
pH	5.6	5.7	5.7

Subsequently, the process developed on a laboratory scale was scaled up to a 5 m^3 reactor, in which it was validated that the process is efficient to be used on an industrial scale.

CONCLUSIONS

The bioprocess described in this work shows the versatility and robustness of enzymatic hydrolysis to recover the protein present in various animal by-products with a very different composition in all cases, such as wastewater from processes rich in proteins, greaves and fleshings. Furthermore, it shows how decisive the selection of an adequate conditioning stage is for the animal by-product to be suitable for efficient enzymatic hydrolysis with protein yields in the 78-97% range depending on the animal by-product used.

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REFERENCES

Pérez-Aguilar, H.; Lacruz-Asaro, M.; Ruzafa-Silvestre, C; Arán-Ais, F. 2023. Protein recovery from wastewater animal processing by-products of rendering plants for biostimulant applications in agriculture. Sustainable Chemistry and Pharmacy 32, 101009.

Pérez-Aguilar, H., Lacruz-Asaro, M., Arán-Ais, F., 2022. Towards a circular bioeconomy: High added value protein recovery and recycling from animal processing by-products. Sustainable Chemistry and Pharmacy 28, 100667.

Bada, J.L., 1984. In Vivo racemization in mammalian proteins, in: Methods in Enzymology, Posttranslational Modifications Part A. Academic Press, pp. 98–115.

Brandelli, A., Sala, L., Kalil, S.J., 2015. Microbial enzymes for bioconversion of poultry waste into added-value products. Food Research International, Byproducts from agri-food industry: new strategies for their revalorization 73, 3–12.

Chuck-Hernández, C., Ozuna, C., 2019. Chapter 5 - Protein Isolates from Meat Processing By-Products, in: Galanakis, C.M. (Ed.), Proteins: Sustainable Source, Processing and Applications. Academic Press, pp. 131–162.

Garcia, R.A., Pyle, D.J., Piazza, G.J., Wen, Z., 2011. Hydrolysis of Animal Protein Meals for Improved Utility in Non-Feed Applications. Applied Engineering in Agriculture 27, 269.

Garcia, R.A., Phillips, J.G., 2009. Physical distribution and characteristics of meat and bone meal protein: Physical distribution and characteristics of meat and bone meal protein. J. Sci. Food Agric. 89, 329–336.

Gómez-Guillén, M.C., Giménez, L.B., López-Caballero, M.E., Montero, M.P., 2011. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocolloids.

Galanakis, C.M., 2015. Food Waste Recovery: Processing Technologies, Industrial Techniques, and Applications. Academic Press.

Kvenvolden, K.A., Peterson, E., Wehmiller, J., Hare, P.E., 1973. Racemization of amino acids in marine sediments determined by gas chromatography. Geochimica et Cosmochimica Acta 37, 2215–2225.

Pérez-Limiñana, M.A., Sánchez-Navarro, M.M., Escoto-Palacios, M.J., Arán-Aís, F., Orgilés-Barceló, C., 2016. Influence of the Extraction Temperature on the Properties of Biopolymers Obtained from Tannery Wastes. j renew mater 4, 3–8.

Pituello, C., Ambrosini S., Varanini Z., Pandolfini T., Zamboni A., Povolo C., Agnolon F., Franco E., Cinzia M. & Neresini M. 2022. Animal-Derived Hydrolyzed Protein and Its Biostimulant Effects. Biostimulants: Exploring Sources and Applications. Plant Life and Environment Dynamics. Springer, Singapore.

Povolo, C., Avolio R., Doria E., Marra A, Neresini M., 2022. Development and validation of an analytical method to ensure quality requirements of hydrolysed proteins intended for agricultural use as biostimulants. Talanta Open 5.

Publications [WWW Document], n.d. EFPRA. URL https://efpra.eu/publications/ (accessed 10.5.22).

Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation), 2009, OJ L. (accessed 05.3.21)