

Collagen determination in fish skin: development of a flow analysis system for quantification of hydroxyproline

<u>Maria M. P. Melo</u>, Ezequiel R. Coscueta, Manuela E. Pintado, Raquel B. R. Mesquita, António O. S. S. Rangel*

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327 4169-005, Porto, Portugal **arangel@ucp.pt*

Collagen is a protein with various applications, namely in the food area. It has valuable properties, since it is a polymer with weak antigenicity, low toxicity, and high nutritional value, among other features [1]. Its extraction from mammalian sources, i.e., bovines, is decreasing due to health and environmental problems and, therefore, fish have become a good alternative for collagen resources [2].

One way to quantify the collagen present in fish skin, in order to obtain high-value fractions, is the determination of hydroxyproline (HYP), an amino acid highly present in collagen [1]. The determination of HYP from fish skin requires the hydrolysis of a skin section, to break collagen in its amino acids and the HYP value quantified is compared to the amount present in pure collagen, studied previously (38 µg of HYP per mg of pure collagen).

The quantification of HYP is based on its oxidation combined with the reaction with DAB (dimethylaminobenzaldehyde) that forms a chromophore-coloured product. The HYP can then be correlated with the spectrophotometric measurement of this coloured product. A batchwise approach was performed to study the best reaction conditions, namely different reagents, heating times and proportions.

The main goal of this work is to develop an automated flow injection analysis (FIA) method, to miniaturize the determination of HYP. Several operation parameters like flow rates, number of channels, tube diameters and lengths of reactors will be studied to optimize the developed FIA method.



Fig.1. Scheme of collagen break into hydroxyproline, through a hydrolysis process; adapted from Cissel et al [3].

Acknowledgments

M.M.P.Melo thanks for the grant POCI-01-0247-FEDER-049636_BI. This work is a result of the project "FISHCOLBOOSTER - Development of collagen peptides from fish in an integrated system to obtain high-value fractions for human consumption, aquaculture and cosmetics" supported by the European Regional Development Fund (FEDER) through Programa Operacional Competitividade e Internacionalização (POCI).

References

- [1] C. G. Sotelo, M. Blanco Comesaña, P. Ramos Ariza, R. I. Pérez-Martín, *Journal of Aquatic Food Product Technology*, vol. 25, no. 3, pp. 388–399, 2016
- S. Benjakul, S. Nalinanon, and F. Shahidi, Food Biochemistry and Food Processing: Second Edition, John Wiley & Sons, Ltd, pp. 365–387, 2012,
- [3] D. D. Cissell, J. M. Link, J. C. Hu, and K. A. Athanasiou, *Tissue Engineering Part C: Methods*, vol. 23, no. 4, pp. 243–250, 2017.