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## The use of subcritical water extraction for the recovery of protein from sardine residues

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Nowadays, due to the growing world population, we face sustainability issues caused by animal protein shortage and environmental impact of animal protein-based industries [1]. This rose the need of full usage of high-quality protein sources, capable of being produced on a commercial scale and minimizing environmental impact. The design of extraction methodologies to recover protein from underused biomasses, using sustainable approaches, emerges to create value-added ingredients for food and feed applications, easing these sustainability problems.

The present work aimed to recover and solubilize protein-rich fractions from sardine heads residues, using subcritical water extraction. Extractions were performed at temperatures ranging from 150 to 250 °C. Further, a sequential treatment with two steps was also tested: the first one at 120 °C, to recover soluble protein, followed by a second one at 180 °C, to hydrolyse and solubilise the remaining insoluble protein. The protein fractions were analysed for their lipid, protein and ash contents, and their potential antioxidant activity with colorimetric assays.

For protein quantification, sardine residues' samples were freeze-dried and analysed by Kjeldahl and the protein solubilized fractions were analysed by Bradford. The ash content was obtained with a muffle furnace and the lipid content by the Bligh and Dyer method.

The protein solubilization of the fractions and their protein content increased with the increase of temperature. Moreover, the colorimetric assays revealed that all samples presented antioxidant activities, being the 250 °C sample the one presenting the highest antioxidant activity. Further, the sequential process allowed the recovery of two fractions with different functionalities.

Taken together, the use of subcritical water extraction has proved to be an efficient and sustainable method to recover and solubilize protein from sardine residues, and that these residues have great potential to be included in food and feed formulation as a protein-rich and bioactive fraction.

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