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### Vitamin D3 enrichment from fish waste: green extraction optimization and nutraceutical development

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In recent years, the vitamin D has become increasingly important for human health due to its crucial role in maintaining bone and neuromuscular system homeostasis<sup>1</sup>. However, vitamin D deficiency is nowadays a concerning issue, with approximately 40% of the population estimated to have insufficient levels<sup>2</sup>. The ability of fish to store large quantities of Vitamin D3 in their tissues, makes them an excellent dietary source of Vitamin D3 for humans. Considering the activities related to the fishing sector, including fishery, aquaculture and the fish processing industry, significant amounts of fish waste are daily produced, leading to a significant environmental impact<sup>3</sup>. These precious wastes are often discarded because they do not meet market specifications, but they can serve as an excellent natural substrate for producing Vitamin D3-rich extracts.

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The final goal is to use fish production waste as a substrate for the extraction, enrichment, characterization and formulation of Vitamin D3 nutraceuticals. An innovative approach is being tested to convert a pure waste into a valuable resource for the pharmaceutical and food industries. For this purpose, green and non-conventional techniques, such as Supercritical Fluid Extraction (SFE), has been tested to obtain vitamin D3-rich extracts. Extraction parameters were optimized using the Design of Experiment tool of XLSTAT software (version 2023.1.4.1408) in Box-Behnken design mode. Preliminary data showed the significant potential of supercritical carbon dioxide in extracting the lipophilic fraction from fish waste. The quantification of vitamin D3 and its precursor, 7-dehydrocholesterol, which is highly concentrated in fish, was performed using a high-performance liquid chromatography (HPLC) coupled with a diode array detector (DAD), through a specifically developed method. After the optimization, a formulation study based on microencapsulation followed by a bio-accessibility study will be conducted to obtain a nutraceutical to be tested in pharmacokinetics and bioequivalence in comparison with a standard Vitamin D supplement.

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