PRODUCTION OF ANTIMICROBIAL AND ANTI-INFLAMMATORY PEPTIDES FROM SUSTAINABLE PROTEIN SOURCES AS INGREDIENTS FOR AQUACULTURE DIETS

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INTRODUCTION & AIM

- Protein is an essential compound for larval and juvenile rearing in aquaculture facilities, making up 55 – 60% of dry weight of the diets.
- Protein hydrolysates provide short-chain peptides [500 – 3000 Da] and free amino acids, which improve digestibility and dietary intake of aquaculture diets.
- Specific peptide sequences exert biological activities of interest for aquaculture production, such as antimicrobial, antioxidant or anti-inflammatory. Antioxidant compounds can restrain lipid oxidation in aquafeeds, where proteins are blended with fish oil, highly prone to lipid oxidation. Antimicrobial and inflammation-modulating peptides avoid the use of antibiotics and help withstand stress conditions (e.g. high rearing density, changes in water temperature) related to fish farming.

- This work aims to produce enzymatically **protein hydrolysates with potential antimicrobial, anti-inflammatory, and antioxidant activities** from insect (*Tenebrio molitor*) meal and sunflower (*Healianthus annuus*) meal. These hydrolysates can be incorporated as **functional supplements** into **aquaculture diets**.

METHODOLOGY

- Two hydrolysates at degree of hydrolysis (DH) 5% and 10% were produced from insect and sunflower seed meals, employing a mixture 1:1 of subtilisin and trypsin at 1% enzyme-to-protein ratio, pH 8 and 50ºC.
- *In vitro* antioxidant activity of the hydrolysates was evaluated by their DPPH scavenging and ferrous ion chelating capacities. The hydrolysates were tested for their inhibitory activity against several fish pathogen strains (e.g. *P. damselae, T. maritimum, V. anguillarum, L. aquatica, S. phocae*). Finally, their *in vitro* anti-inflammatory activity was assessed by their inhibitory activity against phospholipase A_2 (PLA_2) and Cyclooxigenase-2 (COX-2)

Fig. 1. Experimental set-up. (A) Automatic titrator with pH probe and acid/alkali burette; (B) Water bath; (C) Batch jacketed reactor with mechanical stirring

RESULTS

- As shown in Fig. 2, in vitro antioxidant activity improved or remained constant with increasing DH for both hydrolysates. Sunflower seed hydrolysates presented high inhibitory activity (IC50 < 1 mg/mL) against free radicals and metal ions.
- As for the anti-inflammatory activity (Fig. 3), sunflower hydrolysates at DH 10% displayed the highest inhibition against PLA_2, with an average IC30 value of 15 mg/mL.

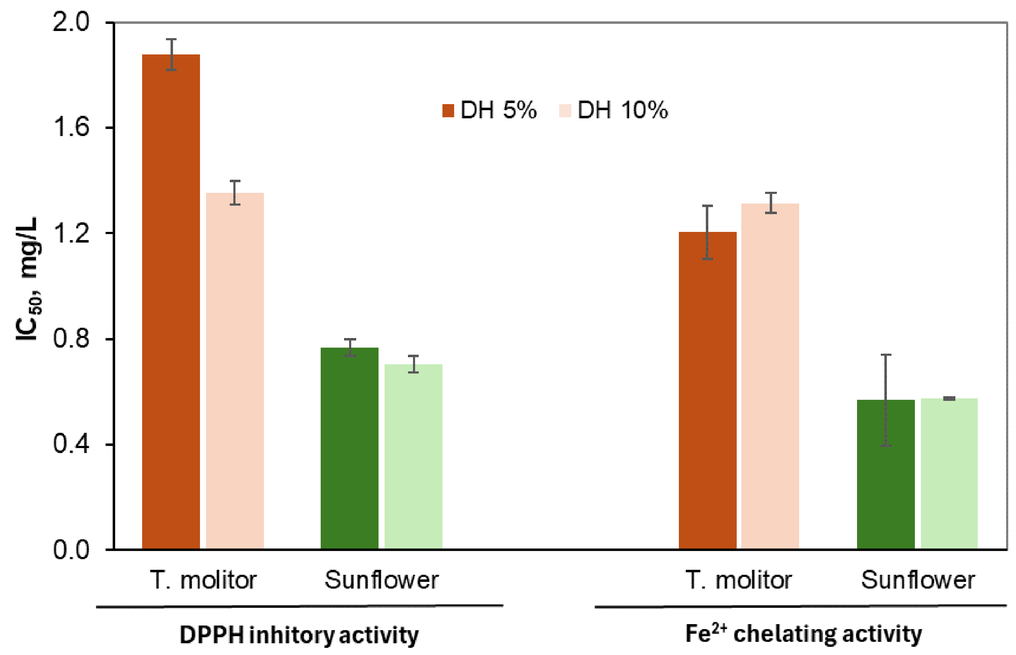

Fig. 2. Inhibitory activity of the protein hydrolysates against phospholipase A_2

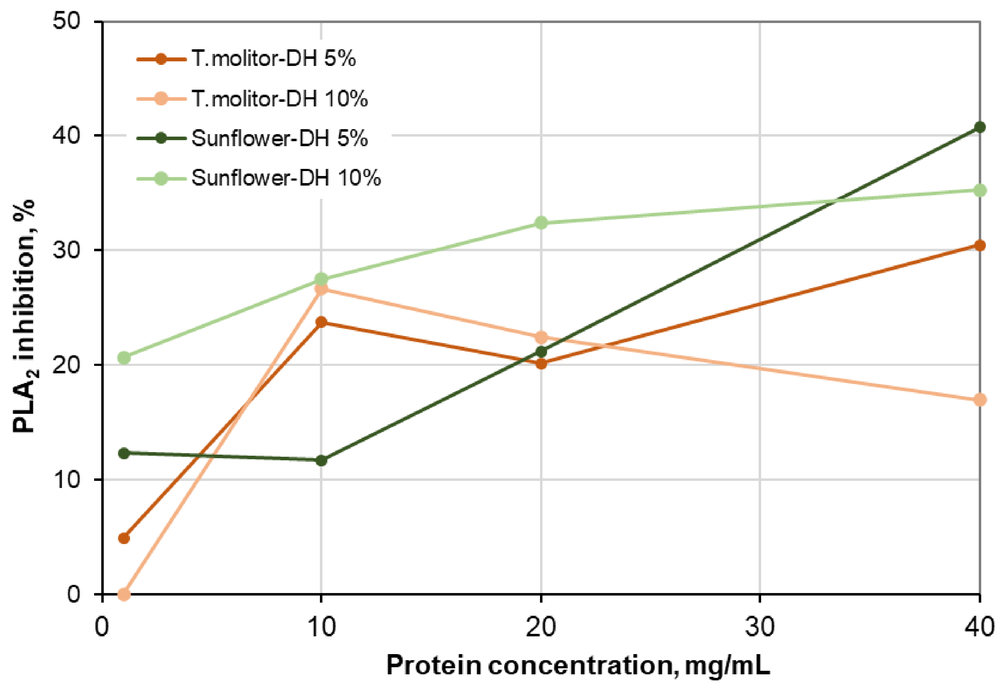

Fig. 3. Inhibitory activity of the protein hydrolysates against phospholipase A_2

CONCLUSIONS

- Fish oil is highly prone to lipid oxidation due to its high content of polyunsaturated fatty acids. The supplementation of aquafeeds with sunflower hydrolysates at DH 10% can restrain lipid oxidation, avoiding the use of synthetic compounds.
- Moreover, sunflower hydrolysates at DH 10% displayed in vitro modulation on inflammatory response against environmental conditions, which is desired to enhance immune response of reared fish.

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