SE-HPLC and RP-HPLC as powerful tools for analyzing the gastrointestinal delivery of collagen hydrolysates obtained from codfish skins using chitosan-TPP hydrogels

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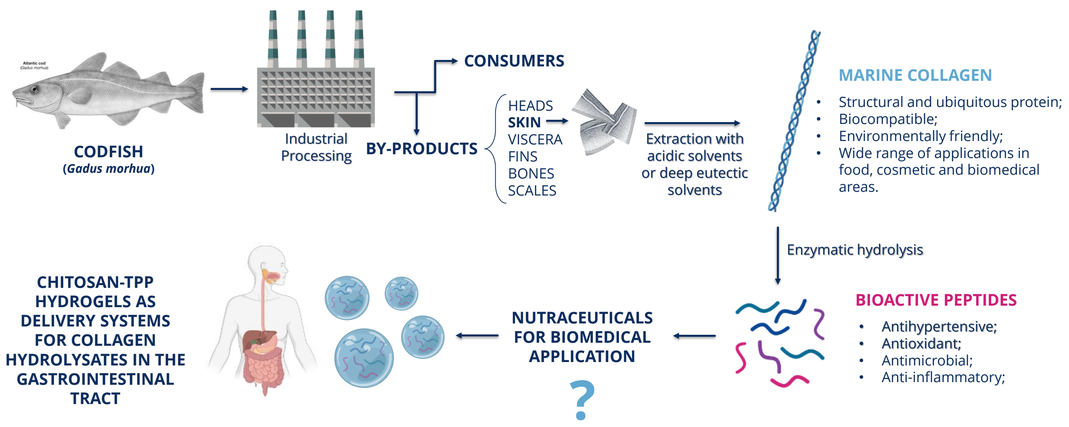
Introduction



Objectives

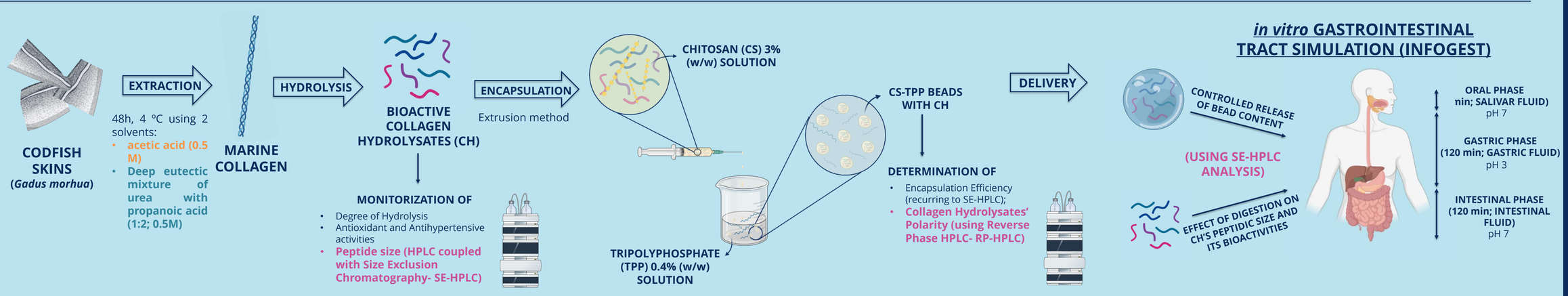
I. Characterize the molecular size distribution profile of enzymatically produced bioactive hydrolysates of marine collagens.

II. Characterize and quantify the incorporation of these hydrolysates into chitosan hydrogels.

III. Characterize the release of the peptides in the hydrogels across the gastrointestinal tract.

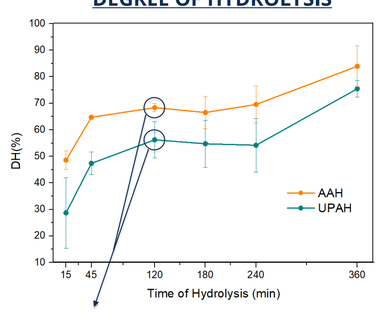
IV. Characterize the stability of the peptides and their bioactivities along the gastrointestinal tract.

Methods



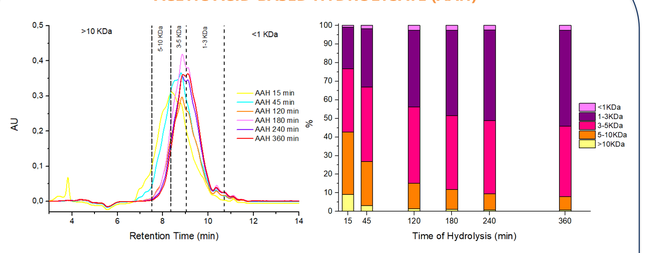
Results: Production of bioactive collagen hydrolysates

DEGREE OF HYDROLYSIS

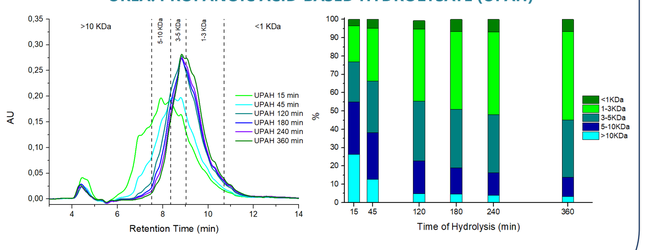


SELECTED TIME OF HYDROLYSIS

ACETIC ACID-BASED HYDROLYSATE (AAH)



UREA: PROPANOIC ACID-BASED HYDROLYSATE (UPAH)



SE-HPLC: PEPTIDE SIZE DISTRIBUTION

BIOACTIVITIES
(at 120 min)

Antioxidant

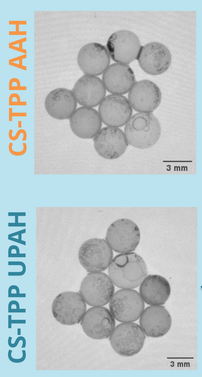
AAH 324 ± 11 µmol TE/ g protein
UPAH 149 ± 7 µmol TE/ g protein

Antihypertensive

AAH 30 % of ACE inhibition
UPAH 39 % of ACE inhibition

- Major hydrolysis occurs up until **120 min** (selected time);
- 1-3 kDa and 3-5 kDa are the predominant fractions, being **1-3 kDa** and <1 kDa reported as the most bioactive peptide fraction;
- Both AAH and UPAH samples showed similar peptide size distributions.

Results: Production and Delivery of CS-TPP hydrogels



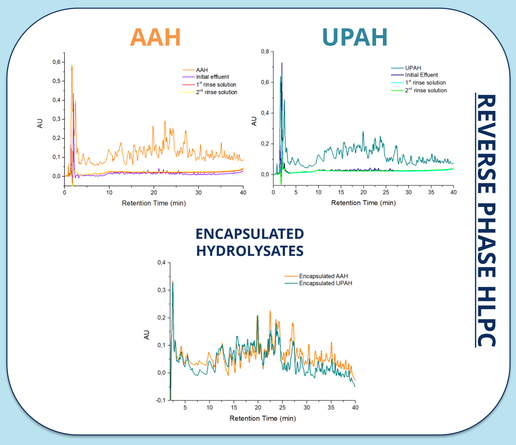
ENCAPSULATION EFFICIENCY(%)
(CALCULATED WITH SE-HPLC AREAS)

38.3%

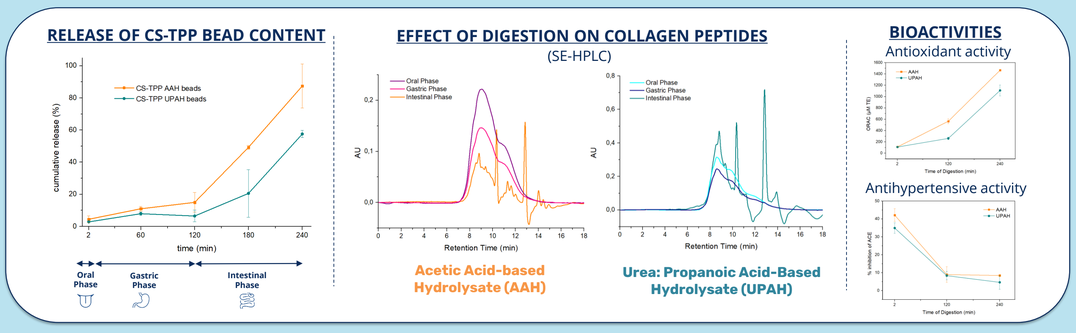
39.3%

WHY?

- **HYDROGEL PROPERTIES:**
  - CS and TPP concentrations;
  - pH;
  - Ionic strength.
- **HYDROLYSATE PROPERTIES:**
  - Molecular Weight;
  - **Peptide Polarity.**



GASTROINTESTINAL TRACT SIMULATION



Conclusions

- Both samples (AAH and UPAH) produced bioactive hydrolysates with an identical peptide size distribution, being 1-3 kDa one of the most prevalent fractions;
- The amount of encapsulated collagen peptides was relatively low and similar in both AAH and UPAH, probably due to heterogeneous peptide size and polarity;
- CS-TPP hydrogels are adequate vehicles for sustained collagen peptide delivery in the gastrointestinal tract, since major peptide fraction was released in the intestinal phase;
- SE-HPLC and RP-HPLC are very useful tools for the analysis of collagen hydrolysates and their properties. On the other hand, it was important to complement this analysis with a monitorization of the bioactivities, since some changes imperceptible to chromatography can give noticeable changes in the bioactive properties.

Acknowledgments

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