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Bioaccessibility of novel bioactive peptides from the body mucus of the Lusitanian Toadfish *Halobatrachus didactylus* using an *in vitro* digestion model

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Introduction

The bioprospection of marine resources for drug discovery is receiving increasing attention (1). Adverse marine environmental conditions lead organisms to develop a repertoire of bioactive molecules for survival (1). Mucus, acting as a first line of defense against pathogens (2), is known to protect fish from the surrounding environment. Our previous studies have already demonstrated the bioactive potential of body mucus from the Lusitanian toadfish *Halobatrachus didactylus* (3). LC-MS/MS was used to identify the potential peptides within the mucus peptide fraction, selecting them based on *in silico* predictions of their bioactivities. Key bioactivities, such as antioxidant, antihypertensive and antidiabetic were identified by bioinformatics tools, facilitating their broader application into pharmaceutical and nutraceutical products.

Methodology

Peptidomics

ID	Sequence
HdVLPN	VYPFPGPLPN
HdLPN	PFPGPLPN

Antioxidant potential

Bioinformatics
BIOPEP A*
VYPFPGPLPN
0.100
PFPGPLPN
Not detected

ORAC assay
VYPFPGPLPN
1.51±0.07 µmol Eq. Trolox/ mg peptide
PFPGPLPN
0.20±0.02 µmol Eq. Trolox/ mg peptide

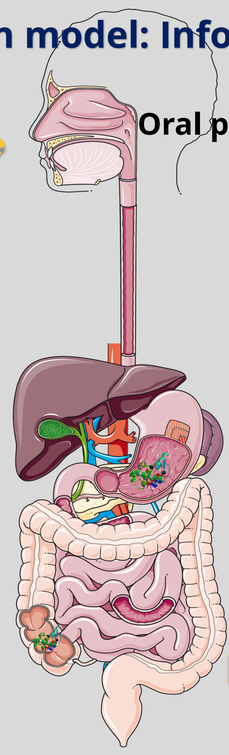
Bioactive Peptides

Mucus

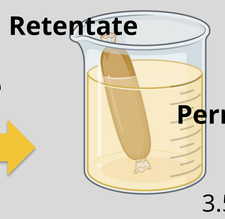
*The "A" represents the frequency of bioactive fragments in a protein, categorized by various bioactivity types predicted by BIOPEP-UWM

Digestion model: Infogest 2.0

HdVLPN 1 mg/mL
HdLPN 1.15 mg/mL



Dialysis



Biochemical assays

- Protein quantification (BCA).
- ORAC assay adapted from (4).
- Angiotensin-I converting enzyme inhibitory activity (iACE) assay from (5).

SE-HPLC analysis

- Size exclusion chromatography methodology from (3).

Aim

Assess the capacity of the novel bioactive peptides to resist the gastrointestinal tract and cross the intestinal epithelial barrier.

Results

Antioxidant and iACE activity

The antioxidant activity of the two digested peptides, in both permeate and retentate forms, was comparable to the control (which utilized water in place of peptides); similar results were obtained for their antihypertensive activity in the permeate form. The results for both bioactivities showed no significant differences when comparing the digested peptides' retentate and permeate forms with control. This suggests enzymatic hydrolysis during digestion degrades peptide bioactivity, as they showed antioxidant activity before digestion, and no subsequent activation or potentiation of antihypertensive activity.

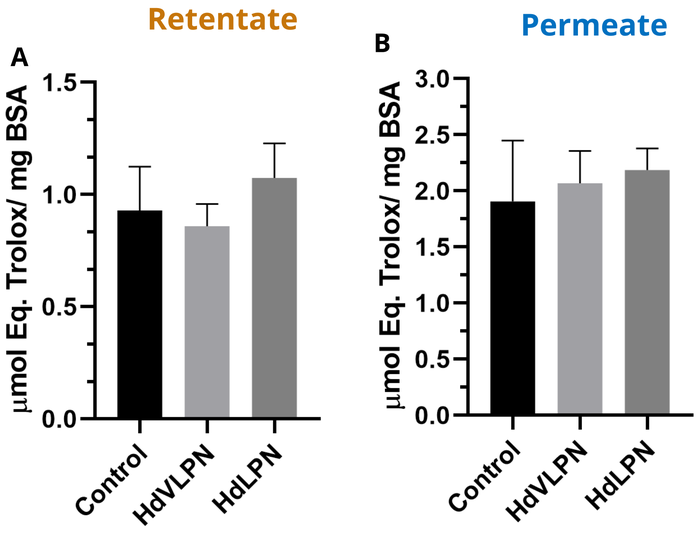


Figure 1. ORAC antioxidant activity at final phase of peptides digestion and dialysis **A** Retentate and **B** Permeate.

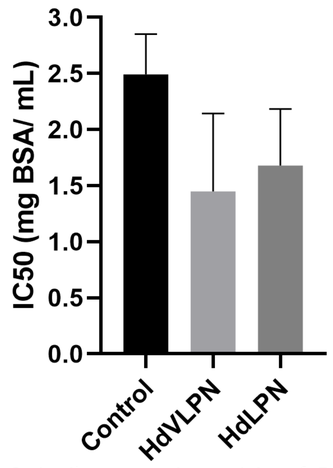


Figure 2. Antihypertensive activity of 50% inhibition of ACE at final phase of peptides digestion Permeate.

SE-HPLC

Size exclusion chromatography was utilized to evaluate the protein-peptide profile of both the retentate and permeate forms, comparing them with the control. Chromatogram analysis revealed similarities with the control for both the peptides HdVLPN and HdLPN retentate and permeate forms. Consequently, no discernible alterations, such as fractionation of the peptides, were observed in the chromatograms within the retentate and permeate forms.

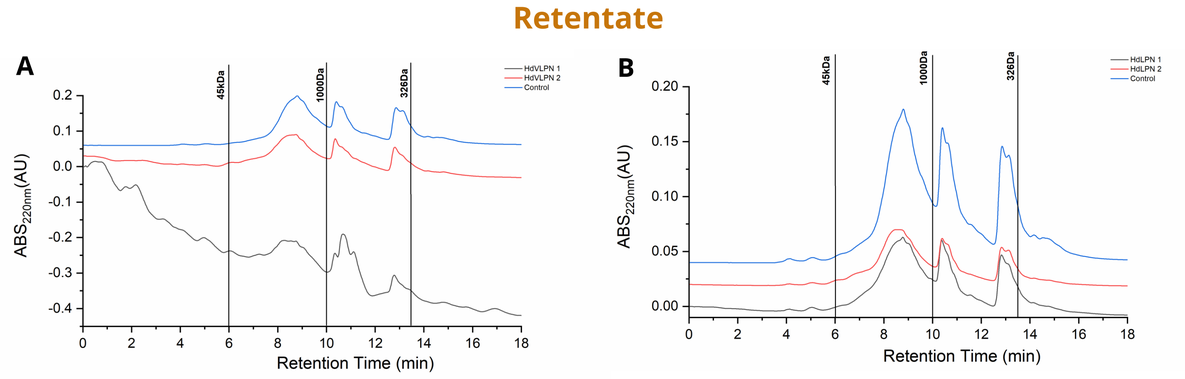


Figure 3. SEC chromatograms of retentate forms of peptides comparing with control: **A** HdVLPN and **B** HdLPN.

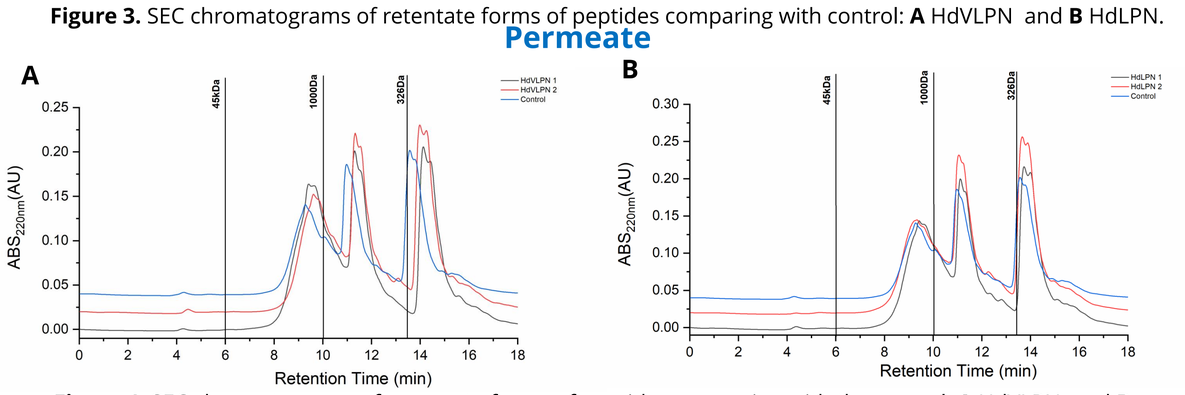


Figure 4. SEC chromatograms of permeate forms of peptides comparing with the control: **A** HdVLPN and **B** HdLPN.

Conclusion

The antioxidant and antihypertensive activities of the digested peptides from the Lusitanian Toadfish's mucus did not show a significant deviation from the control samples, suggesting that enzymatic hydrolysis during digestion does not enhance these activities. Furthermore, size exclusion chromatography was unable to provide any insights regarding the peptide profiles post-digestion. To conclude, these results emphasize the need for advanced analytical techniques like mass spectrometry to unravel the complexities of peptide digestion. Future research should consider developing protective strategies, such as encapsulation, to preserve the bioactive peptides' functionality for pharmaceutical and nutraceutical applications (6).

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