Investigation of total phenolic content and antioxidant activity of fish protein hydrolysates obtained from marine fish

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ABSTRACT

The antioxidant activity of the partially hydrolysed proteins (PHPs) was studied after solubilising them in three different media - HEPES buffer, ammonium acetate buffer and distilled water and later testing them in specific bioassays. Their functional activity is assessed by biochemical assays such as Phosphomolybdenum method to estimate the total antioxidant capacity (TAC) and Folin Ciocalteu to examine the total phenolics content (TPC). All PHPs exhibited antioxidant activity and presence of phenolics content. Overall solubilization in HEPES buffer yielded higher TPC levels and antioxidant activity for all the hydrolysates in comparison to any other media. The highest antioxidant activity was found for peptide mix 2, a soluble fish protein hydrolysate, in combination with the HEPES buffer (121.38 ± 2.76 mM AA/g DW sample) where ascorbic acid (AA) was the standard with which the antioxidant activity was compared with. The highest phenolic content was found for peptide mix 3 (198.63 ± 0.17 mg GAE/g DW sample) where gallic acid (GA) was the standard the phenolic content was compared with. The goal of this study is to evaluate the biochemical potential of these hydrolysates and fortify their role as a functional aqua feed ingredient.

ANY QUESTIONS?
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INTRODUCTION

Fishmeal has been the traditional feed in fish diet ever since the advancement of aquaculture throughout the world. It is the preferred protein supplement but due to the rapidly increasing demand for fish oil and fish meal, alternate protein options are being constantly tested for a more balanced approach in terms of sustainability and efficacy. The substantial volume of raw material discarded every year by fish processing industries all around the world could be processed and potentially utilized as a valuable nutritional source in the form of fish protein hydrolysates (FPH). Inclusion of FPH in fish diet could potentially enhance the growth rate, feed efficiency, immunity and disease resistance of fish.

Fish are subjected to an extensive range of habitats where they encounter all kinds of contaminants that may induce oxidative stress. The link between reactive oxygen species (ROS) in living organisms and several pathological processes was established years ago. However, the innate antioxidant defence system might not be sufficient to maintain the levels of oxidative compounds. Several studies are being conducted to investigate the potential of marine resources for the development of antioxidative compounds. Peptides derived from FPH have been reported to exhibit high antioxidative activity in *in vivo* studies [1,2].

In this study, the potential bioactivity of protein hydrolysates in the form of their antioxidant activity will be evaluated. The overall objective is to develop a nutritional, functional and safety profile for fish protein hydrolysates for their role as suitable aquafeed.

METHODS AND MATERIALS

The total phenolic content of the hydrolyzed protein was determined by the Folin-Ciocalteu colorimetric assay [3].
The total antioxidant capacity of the extracts was determined by the Phosphomolybdenum method [4].
In addition to distilled water, an acidic (ammonium acetate) and alkaline (HEPES) buffer were chosen as media for the peptide mix.

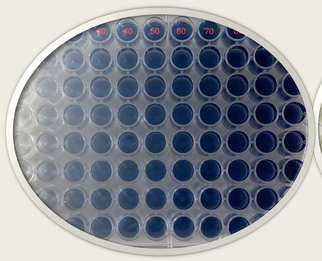


Figure 1. 96 well plate assay using Gallic Acid as standard for Folin Ciocalteu method

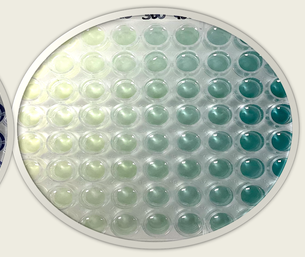


Figure 2. 96 well plate assay using Ascorbic Acid as standard for Phosphomolybdenum method

RESULTS

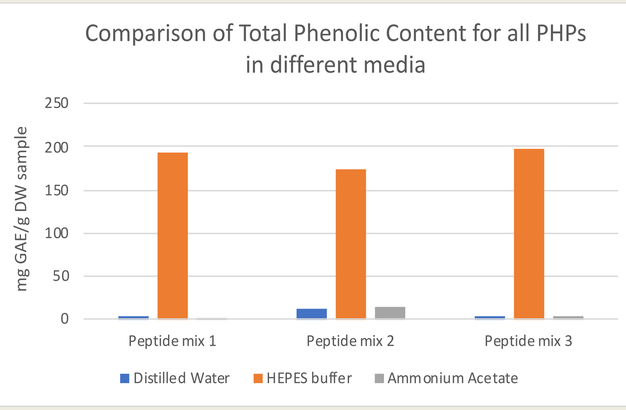
The total phenolic content (TPC) of the samples was quantified and expressed as Gallic Acid Equivalent (GAE) from a calibration curve; y=0.0068x (R^2=0.9997).

The results indicate that all three peptide mixes had some phenolic content present in them, but the highest amount was exhibited when the mixes were solubilized in HEPES buffer. The highest phenolic content was found in peptide mix 3 (198.63 ± 0.17 mg GAE/g DW sample) solubilized in HEPES buffer.

As for the total antioxidant capacity, the samples were quantified and expressed as Ascorbic Acid Equivalent (AAE) from a calibration curve; y = 0.0004x-0.0001 (R^2= 0.9993).

The results indicate high antioxidant capacity for all three peptide mixes, but the highest for Peptide mix 2 (121.38 ± 2.76 mM AA/g DW sample) solubilized in HEPES buffer.

Chart 1. The total phenolic content of protein hydrolysates in different media



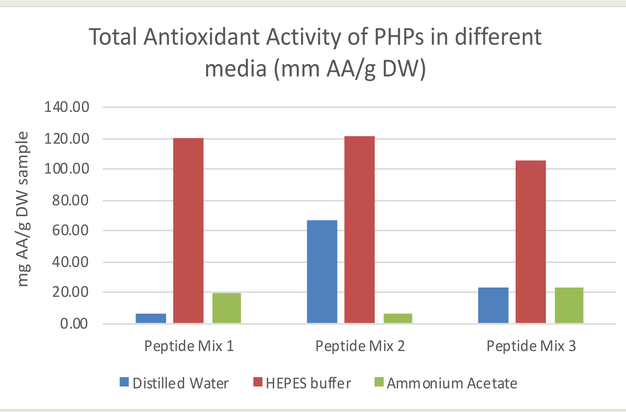


Chart 2. Total Antioxidant Activity of fish protein hydrolysates in different media.

DISCUSSION

In terms of evaluating the functional characterisation of our extracts, we focussed on assessing their bioactivity in the form of their antioxidant capacity. We also focussed on the solubility of our extracts in different media and their effect on their bioactivity. We chose an acidic (ammonium acetate) and alkaline (HEPES) buffer each as media for the proteins along with distilled water.

It is evident from our results that the overall solubilization in HEPES buffer yields higher TPC levels as compared to other media which is in good correlation with findings that signify HEPES buffer to be the best solvent for soluble protein yields [5].

In terms of the antioxidant activity, peptide mix 2 exhibited the highest antioxidant potential in most buffers, but especially in the HEPES. This assay supports the former observation that solubilization in HEPES buffer yields a higher bioactivity.

CONCLUSIONS

Hydrolyzed and concentrated fish proteins were found to have natural phenolic compounds. In terms of their antioxidant activity, the Total Antioxidant Capacity data demonstrated that fish protein hydrolysates are potent antioxidants, and the phenolic compounds present in them could be one of the main contributors to this activity.

In conclusion, it is possible to employ fish protein hydrolysates as a part of fish meal to serve as good sources of antioxidants in the process of enhancing the defense mechanism of fish.

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