

Optimization and scaling up bioactive peptides production from fish discards

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Introduction

The European Commission Common Fisheries Policy (CFP) introduced a discard ban in 2013 which stated that all catches of species subjected to catch quotas and/or Minimum Conservation Reference Size (MCRS) would have to be landed and counted against quota, but at the same time prevented the use of catches under MCRS for direct human consumption [1].

The WaSeaBi project's [2] goal is to identify obstacles to better utilisation of aquatic resources. This can be accomplished by creating sorting technologies, storage options, and decision-making tools that can guarantee an effective, sustainable supply system for by-catch materials as well as for solid and liquid side-streams from aquaculture, fisheries, and the aquatic processing industries, which then go to biorefining operations. This may enable those raw materials to be turned into commercial goods.

Undersized hake (*Merluccius merluccius*) was used as model of fish discard from the Bay of Biscay to produce protein hydrolysates with bioactivities. These by-catches, along with other fish side streams, were previously used as raw material for the production of fishmeal and fish oil but appropriate handling may allow their use in more valuable uses.

In a previous work seven enzymes, with different enzymatic activity, were tested to produce protein hydrolysates: a broad-spectrum endo-proteases (P), an endo-protease of the serine type (A), a trypsin specific protease (T), a chymotrypsin like protease (C), a blend of endo- and exo-peptidases (F) and a glutamic acid specific protease (G). Products obtained with enzyme A resulted in the most promising results in terms of yield and bioactivity.

In this work, the use of enzyme "A" for the production of bioactive products from undersized hake was optimized and the process scaled up.

Material and methods

Laboratory scale hydrolysis tests were carried out on a Symphony 7100 Bathless Dissolution Distek apparatus (Distek Inc., North Brunswick, NJ, USA), with temperature, time, and stir speed being controlled and monitored.

A final volume of 500 mL, 70 °C process temperature and pH 9 were used in all the experiments issued from a previous optimization. The hydrolysis process was followed by a 15-minute heat treatment at 95 °C to inactivate the enzymes, sieving of the reactor content to separate the bones, and centrifugation (2650 ×g; 15 min; ambient temperature) to separate 3 different layers, from the top to the bottom 1) oil fraction, 2) aqueous fraction that contains protein hydrolysate and 3) solid undigested pellet.

All fractions were evaluated for their protein concentration (Kjeldahl method), solid content (dry mater at 105 °C until constant weight) and ash (incineration at 700 °C until constant weight).

The protein hydrolysates were then freeze dried for their evaluation in the bioactivity test. Antioxidant activity (AO, g TEAC) of the samples were assessed by ABTS method, the antihypertensive capacity was evaluated by the angiotensin converting enzyme inhibition assay (ACE % at 1 mg/mL).

Process parameters were optimized via Box–Behnken Design (BBD) composed of three factors and three levels to fit a second-order model. The selected variables or factors for the hydrolysis process were enzyme/protein ratio (A, 0.5-1.25-2 %), initial solid % (B, 50-57.5-65 %) and time (C, 2-4-6 h), and their influence in Protein Extraction Yield (PEY, %), Degree of hydrolysis (DH, %), AO and ACE were analysed.

Results were expressed as a second-order polynomial equation. The statistical analysis of the model was performed using ANOVA (analysis of variance) with the Statgraphics software (Statgraphics Centurion XVI software package, 16.2.04 version; Statgraphics Technologies, Inc., The Plains, VA, USA). When their probability (p value) was lower than 0.05, factors were considered significant. The adequacy of the model was determined by the coefficients of determination (R^2), adjusted R^2 and lack of fit test. This software was also used to perform the response surfaces.

Process scale-up was performed in a 150 litres stirred reactor reproducing the most favourable process conditions to assess the products reproducibility at larger scale.

Results and discussion

Regarding process results obtained during the optimisation process, protein extraction yield varied from 29.36 and 46.47 % and the ANOVA analysis showed that only 2 variables have a significant effect on the results within the studied limits, the % of solids (with a negative effect) and the enzyme concentration (with a positive effect). The degree of hydrolysis of extracted proteins values varies between 4.32 and 12.64 % and only the process time of hydrolysis showed a sensitive response within the studied range with a positive effect.

Concerning bioactivities, antioxidant activity resulted in values between 125-157 mg TEAC/g protein, and two factors had a significant effect, the enzyme concentration that increased the results, while the combination of enzyme concentration and time showed a negative effect. For the antihypertensive activity, only reaction time showed a significant negative correlation.

To optimize the process different objectives were evaluated getting different results. If the process focusses on protein yield extraction, resulting model indicates that process conditions must be set at Solids: 50,0 %; Time: 2.3 h; Enzyme 1.95 %. However, if we focus on bioactivities maximization of both antioxidant and antihypertensive activities, we get similar results: Solids: 50.0 %; Time: 2.0 h; Enzyme 2.0 % (Figure 1). Therefore, one must decide between maximum peptide yield or maximise their bioactivity. To address this issue the total antioxidant compounds (AO tot) and antihypertensive compounds (ACE tot) extraction yield were evaluated and optimised leading to same process conditions, so that one could obtain the best hydrolysis results in terms of higher bioactivity and bioactive compounds yields.

These condition where used to produce bioactive peptides at pilot level and to compare the values predicted by the model with those obtained at pilot scale. Resulting pilot products had slightly lower protein yield and antioxidant capacity and higher antihypertensive activity, however all the results were within the predicted model deviation and therefore there were no statistical significant differences (Figure 2).

Conclusions

Undersized hake is a potential source of bioactive peptides with antioxidant and antihypertensive activity. Design of experiment allowed to select best operation conditions to maximise bioactivities and peptide yields. However, resulting conditions differ and the definition of total antioxidant and antihypertensive yield allowed to maximise the overall process. Pilot trial in the optimized conditions led to a result in accordance with the selected model which confirms the technical feasibility of the process at industrial scale. As a conclusion, the production of bioactive peptides stands out as a promising solution for these fish discards.

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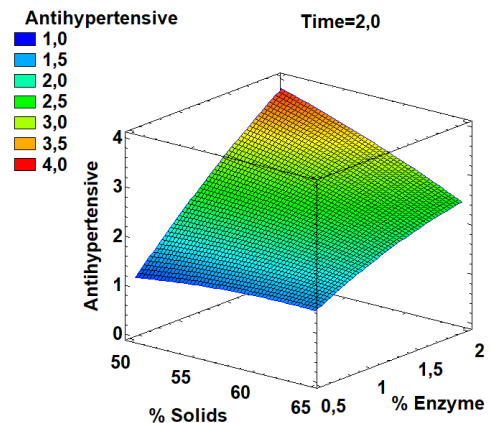


Figure 1: Hydrolysis yields

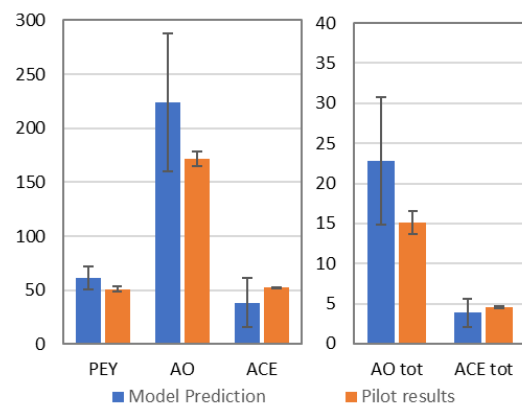


Figure 2: Comparison between model predicted and pilot hydrolysis results.