Production of biofertilizers from tuna cooking waters through membrane nanofiltration and enzymatic hydrolysis.

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Introduction

Brine as side-stream is one of the main environmental problems in the wastewater generation in different food industry processes, as it is for the tuna canning sector. The treatment of brines is a universal challenge due to its operation complexity and cost issues. More than 70% of the tuna caught in the world is canned or otherwise prepared or preserved. Spain leads Europe's production of canned seafood with more than 343,000 tonnes of product weight produced, valued at €1.5 billion. The Iberian country also produces 70% of the canned tuna processed in Europe. The ratio of spent brine used per unit of canned tuna is around 1-1.5 kg of brine/kg canned tuna. So that, the estimation for annual production of spent brine generation is around 300,000-500,000 tonnes. As the typical disposals of brine are discharges to the sea and is the management jointly with the rest of process side streams through wastewater treatment plants, and due to the high salinity, it causes, consequently, problems of inhibition in the effluent treatment, and poor performance in the final effluents. However, these brines are rich in valuable proteins.

On the other hand, fertilizer consumption in Europe increases annually by 3% to cover increasing agricultural demand. Most of the fertilisers used in Europe are imported, more than 3 million t are imported annually into the EU since 2015 (Fertilizers Europe, 2019). Several research studies have reported the benefits of protein hydrolysates as bio stimulants on the physiology of different plant crops (Colla G. et al. 2015). In this context, this study focuses on the concentration and desalting of the cooking brine of tuna and further enzymatic hydrolysis to produce free amino acids-based hydrolysate to be further used as an ingredient for the formulation of a new bio stimulant.

Materials and methods

Tuna spent cooking brine was kindly provided by the company Hijos de José Serrats, S.A. (Bermeo, Spain).

First, the cooking brine was passed through a 500 µm stainless steel sieve to remove the big particles to not damage the membranes of the nanofiltration equipment. Then, the brine was subject of up to three subsequent concentration and dilution steps to remove the salt while preserving the protein concentration using a nanofiltration (NF) unit (model 2-016-6-PV, from TIA, Bollene, France) and a membrane with a pore size of 150 and 300 Da (model DK2540C30, LENNTECH, Delfgauw, NL). The objective was to reduce the salt content enough to avoid the salting effect that can reduce the activity of the enzymes and below the 3% dry matter basis threshold recommended for its use as fertiliser. The progress of the concentration and desalting was monitored continuously through ºBrix measurement and its correlation with the dry matter, previously established.

For the hydrolysis process two endo- and two exo- commercial proteases were combined with the aim to produce the maximum yield in free amino acids. The hydrolysis was performed in duplicates in batches of 500 mL in a Sell Symphony 7100 Bathless Dissolution equipment (Distek Inc., North Brunswick, NJ, USA) at pH and temperature conditions as recommended by the provider. Time was set up at 6 h and enzyme dose at 1.8% with respect to the protein content. The exo- and endoproteases were used also alone as a control.

Dry matter content was determined by drying them at 100 °C until reaching constant weight (method 934.01). Crude protein content was determined by Kjeldahl methodology (method 955.04). Ash content was determined by heating samples at 500 ºC for 24 hours and then at 700 ºC for 2 hours. Free amino acid (FAA) content and profile were determined by high performance liquid chromatography with diode array detection (RP-HPLC). NaCl content of the permeates and retentates was determined with the volumetric dosing of chloride ions (Volhard's method) by titration with $AgNO_3$.

Results and discussion

The first trial was done with a preconcentrated brine which composition was: Dry matter 24.47 ± 0.01, ash 8.95 ± 0.05, NaCl 7.58 ± 0.01, protein 16.17 ± 0.13, fat 0.14 ± 0.04, FAA (%) 0.57 ± 0.01. The salt content was reduced

drastically from 7.58% to 0.25% after two dilutions and two cycles of diafiltration. The protein content after 2 DF cycles was 10.55 ± 0.03%.

The characterization of the liquid phase of the 12 batches of hydrolysates showed similar protein content and dry matter. The maximum yield in FAA was 12.79% (FAA/total protein), obtained with two of the endo and exoproteases combined. In the bibliography, similar results of free amino acids contents were obtained after hydrolysis of cooking juice of tuna (around 7 mmol of free amino acids/100 mL of cooking water) (Jao et al., 2002).

NaCl can either affect irreversibly proteins initially present in the brine or can affect proteins of the brine and/or enzymes during the hydrolysis process. Above a certain concentration of salt (1 M of NaCl), the phenomenon of salting-out occurs, which leads to protein aggregation, new chemical bonds get formed in the proteins, and changes in their conformation that can lead to their denaturation. Thus, certain enzyme activity can be reduced more than 50% with only 0.5% of NaCl (Vannabun et al., 2014). To test those hypotheses, a new trial was similarly planned with an extra diafiltration cycle (x3) to reduce the NaCl content. The brine of the second trial had the following composition: Dry matter 12.35 ± 0.11, ash 8.60 ± 0.17, NaCl 8.31 ± 0.01, protein 3.52 ± 0.00, fat 0.18 ± 0.07, FAA 0.49 ± 0.02. The protein content after 3 DF cycles was 15.86 ± 0.02% and the NaCl content 0.004 ± 0.001%.

Then, each hydrolysis test was repeated with about 250 g of concentrate obtained after DF3, and 250 g of water. All the enzyme combinations gave higher yield than in the first trial, achieving a 18.46% FAA yield with a concentration of FAA in the final product of 17% (DM basis), however, still lower than expected, which could be due to the denaturation and aggregation of the proteins after the thermal treatment in a salted medium.

It was also observed that the chloride content measured in the permeates was systematically higher than in the concentrates of the correspondent DF cycle. The pH of the brine measured during the nanofiltration varied between 6.05 to 6.37 while the isoelectric point of the tuna proteins was around pH 5.5. NF membranes are also usually charged. As a result, Na^+ could be retained in the concentrate affecting the ionic strength of the medium. The observed ash content in the final concentrates of 1.00 ± 0.00 and 1.14 ± 0.15% supports this idea.

Conclusions

A protein hydrolysate with FAA was obtained from tuna cooking brines that could be used as fertiliser. At the same time, the nano filtered brine from the first concentration step could be reused in the cooking process. Meanwhile, further research is needed to see if the FAA yield can be improved. Due the high variability in the composition of the brines studied in different companies, it is expected that the concentration degree in the NF/DF step as well as in the final hydrolysate will have to be adjusted to each case, affecting basically the yield in the final product.

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