Optimization of the autolysis of rainbow trout viscera for amino acid release using response surface methodology

Haizea Domínguez, Bruno Iñarra, Carlos Bald

Food Research, AZTI, Basque Research and Technology Alliance (BRTA), Derio, 48160, Bizkaia, Spain
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Presenting author email: hdominguez@azti.es

Introduction

Aquaculture has grown exponentially during the last decades, even overcoming traditional fishing in volume since 2012 (Iñarra *et al*, 2018). The rise of the production of fish involves the rise of fish by-products, that in case of being disposed could suppose an environmental risk. Fish viscera are part of the by-products used to produce fishmeal and are the 10-18 % of the whole fish weight.

Fish silage or acid autolysis is commonly used in areas with high fisheries rates and consists of liquefaction and stabilization of minced fish at room temperature, normally adding formic acid until reaching a pH between 3.5 and 4.5 to prevent microbial growth. Hydrolysis of proteins occurs thanks to the endogenous acid proteases that are located at the fish viscera, which enable to get low molecular weight peptides and amino acids (Toppe *et al*, 2018). The resulting protein hydrolysates could be used as fertilisers. However, silage can take several days to achieve a high hydrolysis degree. In this work, autolysis has been carried out simulating the conditions of enzymatic hydrolysis but only working with the endogenous enzymes from fish viscera with the aim of accelerating a typical silage to get free amino acids while saving costs derived from the use of commercial enzymes.

Materials and methods

Fish viscera came from cultured rainbow trout (*Oncorhynchus mykiss*) and composition of both raw material and hydrolysate were determined. Previously to the hydrolysis, viscera were minced and defatted by decantation at room temperature to avoid the inactivation of native enzymes.

Lab-scale hydrolysis was carried out for 7 hours and the conditions for the hydrolysis were stablished based on the information by Vannabun *et al* (2014) regarding endogenous enzymes of fish. After the hydrolysis, hydrolysates were heated at 90 ºC for 15 minutes to inactivate the enzymes and centrifuged (4737 r.c.f., 15 min) to separate the solubilized protein from the remaining oil and non-digested protein.

Dry matter content was determined by drying them at 105 °C until reaching constant weight (method 934.01, AOAC). Crude protein content was determined by Kjeldahl methodology (method 955.04, AOAC). 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 was determined by high performance liquid chromatography with diode array detection (RP-HPLC).

Statgraphics Centurion XVI software was used to carry out the experimental design as well as the statistical analysis. Optimisation experiments of the autolysis conditions were established by employing the response surface methodology (RSM) with a Box-Behnken experimental design. A factorial design was generated considering 3 factors (percentage of water in the sample, pH and temperature) and 3 levels (Table 1). The design resulted in 15 experiments, including the central point in triplicate, which was established from preliminary experiments.

Table 1. Experimental conditions established to optimize the accelerated silage.

Independent variables	Factor levels		
	-1	**0**	**+1**
pH	6	7	8
Temperature (ºC)	40	50	60
% Water added to the sample	0	25	50

Results and discussion

Results were analysed with ANOVA and factors were considered significant with a p-value less than 0.05. pH (0.024) and the combination of pH and percentage of added water (0.047) resulted to be significant factors to obtain the highest degree of hydrolysis, yield of FAAs from viscera and yield of FAAs from total protein.

The optimum conditions according to the RSM (Figure 1) were: pH 8, temperature 40 ºC and 6.85 % of added water to the sample, with the following predicted results: 68.8 % degree of hydrolysis (% FAA / % protein), yield of 5.0 % FAAs from viscera, and yield of 62.8 % FAAs from total protein.

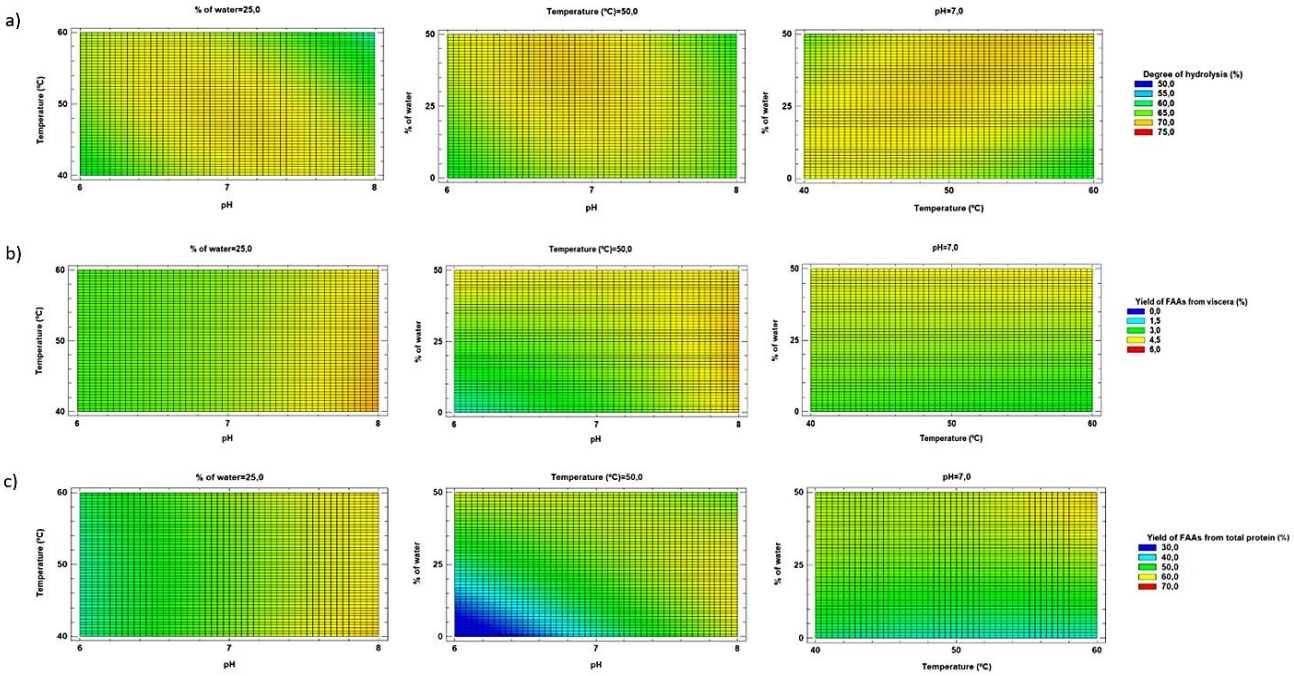

Figure 1. Response surface plots considering three variables (pH, temperature, and percentage of added water to the sample) to obtain the maximum of a) degree of hydrolysis, b) yield of FAAs from viscera, and c) yield of FAAs from total protein.

Comparing results with the acid silage from previous experiments, the degree of hydrolysis achieved is lower but the yield of FAAs from total protein in the raw material is higher (Table 2) in the optimized autolysis probably due the obtained higher yield in protein in the liquid phase.

Table 2. Comparison of results between acid silage and optimized autolysis (predicted).

	Optimized autolysis	7-day acid silage
Degree of hydrolysis (% FAA / % protein)	68.8	75.7 ± 0.5
Yield of FAAs from 100 g viscera (%)	5.0	3.2 ± 0.02
Yield of FAAs from total protein (%)	62.8	52.5 ± 0.0

Conclusions

It is concluded that alkaline endogenous proteases have the highest activity to solubilise protein and hydrolyse it, being the pH the most influential factor during autolysis alongside water added to the sample. It was also verified that RSM is a valuable tool to find optimum conditions for hydrolysis of fish viscera and to predict results, and that the autolysis can improve the results from the acid silage.

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