OPTIMIZATION OF THE ENZYMATIC HYDROLYSIS OF MECHANICALLY DEBONED CHICKEN MEAT: THE USE OF EXPERIMENTAL STATISTICAL DESIGN

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Abstract – This paper introduces the application of response surface methodology (RSM) for the optimization of processing parameters of the mechanically deboned meat (MDM) hydrolysis process. The hydrolysis time (tim) was 4 hours, the hydromodule (Hyd) was 1 and the enzyme to substrate ratio (E:S) 0.4364. With these parameters the protein recovery was about 90 %. The molecular weight distribution analysis shown that the smallest fragments were observed at 50 Da and the largest proteins at 45 kDa of molecular weight. Majority (approx.. 70%) of the proteins were around 10 kDa indicating that the hydrolysis treatments were rather mild. In future work, the functional properties of the protein hydrolysate will be analysed that work as a tool for planning potential food formulations. Beside, a sustainability of the process will be performed usinf LCA method.

Key Words –molecular weight distribution, optimal process parameters, response surface methodology

I. INTRODUCTION

The continuously increasing consumption of poultry meat and poultry products results in a substantial increase in production of mechanically deboned meat (MDM) as a by-product from poultry industry. At the same time, the use of protein hydrolysates in specific food formulations is of a remarkable industrial interest (Toldra et al., 2016). The aim of this study was to develop enzymatic processes to recover high quality protein from chicken MDM and thereof, enhance the utilization and value of this poultry industry by-product.

II. MATERIALS AND METHODS

Raw materials

Mechanically deboned chicken meat from the second stage of the process was obtained from HK Scan (Eura, Finland) and were stored in -20 ^{0}Cuntil use. Enzymewas obtained from MeatCo, the Netherlands.

Hydrolysis units and pocedures

The hydrolysis unit was a 30 L tank reactor equipped with a stirrer and a heating jacket. The optimum temperature for protease was 55 ^{0}C. The hydrolysis time varied between 1 and 4 hours, the liquid to solid ratio from 1 to 3 and the enzyme to substrate ratio from 0.05 to 0.5.

Analytical measurements

Protein content analysis

Protein contents in the hydrolysates were measured according to the Kjeldahl method using a N-factor of 6.25 in the calculations.

Molecular weight distribution

Protein hydrolysates produced in the optimum conditions were subjected to size exclusion chromatography in order to analyze the protein fragmentation during the hydrolysis treatments. Prior to the analysis hydrolysates were purified from non-proteinaceuos components with solid phase extraction using Sep-Pak C18 cartridges according to Pihlanto et al. (2008). Molecular weight distribution of the purified hydrolysates was analyzed with an UPLC method using an Acquity UPLC system (Waters Corporation, USA) equipped with an Acquity BEH125 SEC column, 1.7um particles, 4.6x150mm (Waters Corporation, USA). Proteins and peptides were eluted with 100 mM sodium phosphate buffer pH 6.8 with a flow rate of 0.3 ml/min.

Experimental design and optimization

Response surface methodology was used to determine the optimum conditions for protein hydrolyses and extraction from MDM meat, and for this aim MODDE 9.0 from Umetrics was used. To obtain the optimum extraction parameters, the effects of the following factors has been investigated: hydrolysis time (X_1), the liquid to solid ratio (hydromodule, X_2) and the enzyme to substrate ratio (E:S, X_3).The response function was measured as the protein content of the protein stock.

The complete design consisted of 17 runs. The design included 5 replicates in the center point to be able to evaluate the model validity and the experiments were carried out in a randomized order to avoid the unexplained variability in the response due to systematic error.

RESULTS AND DISCUSSION

Model fitting and refinement

The experimental data was fitted to a second-order polynomial equation, and the model was refined by the removal of non- significant terms.

The order of the importance of variables was as follows: Quadratic term of enzyme to substrate ratio (X_3^2) > Hydromodule (X_2) > hydromodule quadratic term (X_2^2) > enzyme to substrate ratio (X_3) > hydromodule and enzyme to substrate ratio interaction term (X_2* X_3) > time and enzyme to substrate ratio interaction term (X_1* X_3) >time (X_1).

The second order polynomial equation obtained was:

$Y=4{,}183X_1-13{,}75X_2+10{,}97X_3+11{,}83X_2^2-19{,}51X_3^2+8{,}22X_1X_2-8{,}99X_2X_3$

where X_1=time, X_2=hydromodule, X_3=E:S ratio

The model was significant at a confidence level of 95 %, and according to the parameters, adequate to determine the optimum process parameters.

Determination of the optimum process parameters

With the help of the model response surface plot was generated to evaluate the optimum processing parameters. The optimization plot is illustrated in Figure 3.

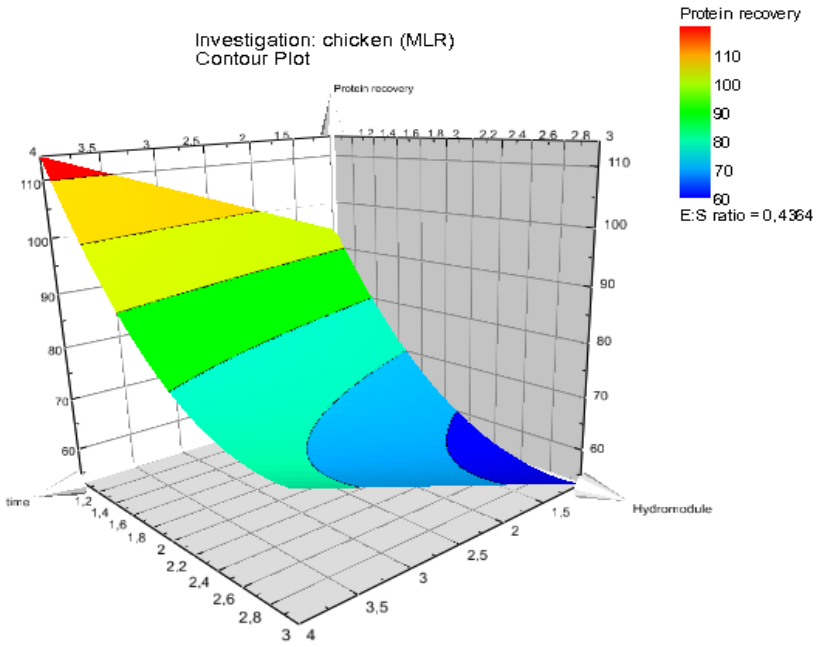

Figure 1 Response surface plot for the optimization

With the help of the optimization function of the MODDE software an extraction time of 4 hours, E:S=0.4364 and hydromodule of 1 were selected as the optimum parameters. Under these conditions the protein recovery was shown to be above 110 g/kg protein stock (yield approx. 90%). The model was verified by repeating the experiments under optimum process parameters two times and similar results were found for the protein recovery values.

Molecular weight distribution of the hydrolysates

Enzymatic hydrolysis treatments at the optimum process conditions produced protein fragments with a broad molecular weight range. The smallest fragments were observed at 50 Da and the largest proteins at 45 kDa of molecular weight. Majority (approx.. 70%) of the proteins were around 10 kDa indicating that the hydrolysis treatments were rather mild. .

III. CONCLUSION

The results indicated that response surface methodology was adequate to determine the optimum process parameters of the hydrolysis of the mechanically deboned chicken meat. As a next step the functional properties such as the water binding/water holding capacity and the solubility of the protein hydrolysate will be analysed. Beside, an organoleptic

evaluation of the protein stock will be performed to determine its applicability in potential food formulations. And at last, the sustainability of the production will be evaluated using LCA

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