RECOVERY AND UTILISATION OF BY-PRODUCT PROTEIN OF MEAT INDUSTRY. PROCESS OPTIMIZATION USING RESPONSE-SURFACE METHODOLOGY.

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SUMMARY

Enzymic hydrolysis of food proteins generally results in profound changes in functional properties of the proteins treated. However, the reaction needs to be carefully controlled to avoid undesirable proteolysis resulting in bitter peptides and loss of desirable functionality. Varying hydrolysis degree led to a bland soluble protein hydrolysate suitable as ingredient in food products.

Introduction

Bone is probably the largest single by-product from a meat animal, varying from 8-12% of the live weight, depending on the species. The large amount of residual meat clinging to the bones is a potentially valuable protein source (Duerr and Earle, 1973), and is at present discarded or downgraded in manufacturing feeds and fertilisers. Enzymic hydrolysis represents a valid method for recovering proteins from meat by-products.

Enzymic hydrolysis of crushed bones (18% proteins) by an endo-protease, NEUTRASE 0.5L from *Bacillus subtilis* (NOVO Industry) was investigated in a batch reactor, in order to modelize the complex enzymic reaction and optimize the extraction via an empirical model. The influence of different degrees of hydrolysis on the functional properties was also examinated in order to obtain a bland soluble protein hydrolysate suitable as ingredient in food products for human consumption.

Materials and methods

Materials :

Fresh food grade bones (18% protein ; 11.5% fat ; 42.4% dry matter), taken from the production line of a local salting plant were milled at 4°C to an average size of ca. 1 cm, and was subject to the action of the enzyme according to the flow sheet (Fig 1). Enzyme inactivation was done by heat treatment with live steam injection. After screening the reaction mixture to discard the bone fraction, the fat layer and the sludge were removed by decanter centrifugation at 2°C and the soluble phase was freeze-dried and stored at -30°C.

Process control :

The extent of proteolysis was controlled throughout the process by the pH-stat (Adler-Nissen, 1976), osmometer and conductivity methods (correlation calibration : $R^2 = 0.99$).

Response Surface Methodology (RSM) was applied to quantitavely determine the effect of the experimental parameters on different yields and hydrolysis degree : temperature (40-60°C), pH (5.7-7.5), enzyme concentration (20-80 g kg^{-1} protein), hydrolysis time (30-180 mn) and protein concentration (60-90 g kg^{-1} mixture). The optimal level for each variable was determined using a Doehlert experimental design (Doehlert, 1970) leading to an efficiency of 92.7%. This matrix allows a number of distinct levels for each of the parameters according to the experimental constraints (5 levels for temperature ; 7 levels for pH, enzyme concentration and time ; 3 levels for protein concentration).

Nitrogen solubility index (NSI) of the hydrolysates was determined in a 1% protein dispersion over a pH ranging from 2 to 9. Solutions were centrifuged for 15 mn at 28 000 g. The supernatant was analysed for nitrogen content by the Kjeldahl procedure and NSI was calculated as the ratio (soluble N% / total N%).

Emulsifying capacity (EC) was determined using a procedure close to Vuillemard *et al.* (1990). Results were expressed as the amount of oil emulsified minus a blank, divided by the amount of protein.

Emulsifying activity index (EAI) of the hydrolysates was determined according to the turbidimetric procedure of Pearce and Kinsella (1978).

Foaming capacity and stability of 1% protein hydrolysate were determined at pH 7 by measuring the volume of foam under constant air flow rate (100 ml/min) for 1 min in the glass column (G3 fritted disk ; 2.4x30 cm).

Data analysis : Data were computed using the N.E.M.R.O.D. program including ANOVA and canonical analysis.

Results and discussion

The hydrolysis process performed in a batch reactor was optimized by using R.S.M. (34 experiments). Optimal levels for each variable (pH, temperature, enzyme, protein concentration) were determined using a Doehlert matrix. Each response (h_k) may be represented by a quadratic equation :

Where h_k is the dependent variable ; X_i are the coded independent variables ; b_{ki} are linear coefficients, b_{ij} are second order interaction coefficients ; and b_{kii} are quadratic coefficients. From the analysis of the surface contours (Fig.2), it comes out that enzyme amount and hydrolysis duration are prevailing factors on the yield and hydrolysis degree. Protein concentration over 8% in the batch teactor leads to a possible inhibition effect (homogenization, mineral amount...).

In the pH range investigated, changes in the responses were not significant. Consequently, pH may not be controlled during the hydrolysis reaction according to the results obtained by Sorensen and Rasmussen (1989) and Surowka and Fik (1992). In view of the economical constraints, optimal conditions were etablished considering the five parameters. Protein yields from the enzyme treatment as a function of the process parameters and vary in the range 50 to 80% of the extractable protein. The hydrolysis degree (DH) was used to describe the process ; in fact, such a parameter is presumed to be related to the functional properties of the resulting hydrolysates. DH and corrected osmolality as a function of time during the proteolytic process may be represented as curves of hydrolysis as shown on Fig. 3. Five Meat Bone Hydrolysates with different hydrolysis degree were obtained by varying either the enzyme/substrate ratio (a, b, c) or hydrolysis time (a, e, d) and were used to determine the functional properties of these resulting products. The variety of reaction conditions allows a range of protein hydrolysates to be formed, but with the important parameter that the degree of hydrolysis be kept below 10% in order that the products do not become bitter. The taste problem with bitterness due to intrinsic factors is frequently met in protein hydrolysates. It is in association with the presence of hydrophobic peptides and the amino-acid composition of the substrate. (Ney, 1972).

The hydrolysis process is continuously monitored and ended at different degrees of hydrolysis avoiding the development of bitterness (the resulting peptides are still large enough to conceal a part of buried hydrophobic amino-acids). The amino acid composition of the hydrolysates (expressed as $g.16g^{-1}$nitrogen) shows a high amount of amino-acid such as glycine (13.33), proline (8.28), hydroxyproline (6.44) and glutamic acid (11.00), improving the taste by masking effect according to Stainley (1981), and suggests that Neutrase has a great capacity to attack the collagenous tissues present in the substrate.

Protein functionality depends to a great extent on the amount of soluble protein present in the system (Kinsella, 1976). The influence of DH on solubility of Meat Bone Hydrolysates is indicated on Fig 4. All the hydrolysates over the pH range from 2 to 9, show a solubility greater than 75%, as pronounced as the DH is increased. The solubility is strongly dependent on prior treatment (heating, protein concentration, hydrolysis modification) and several environmental conditions, such as temperature, pH and the presence of other components. The high solubility of these products may have improved the usefulness of this protein extract in food.

Fig 5 shows the influence of the hydrolysis degree on the decrease of EC. (e.g. a maximal emulsifying capacity could be selected at 0.15% protein concentration for DH = 4%). The different hydrolysates exhibit a good EAI which could be improved by proteolysis (173 m^2g^{-1} at DH = 4.0, 224 at DH = 5 and 228 at DH = 8.2 m^2g^{-1}) but low foaming properties (respectively when stability decreases with DH increasing). These poor values are most probably due to the presence of fat and marrow compounds in the products.

Conclusion

The complex interactions between protein substrate and enzyme properties in relation to the different parameters (pH, temperature, protein and enzyme concentrations, time) involved in the enzymic hydrolysis process are a difficult undertaking. R.S.M. has been used to control the extent of the Meat Bone Proteins hydrolysis allowing the process optimization (the scale-up effect on a 300 liters batch reactor has been carried out with a similar experimental plan including mainly the temperature, time and enzyme concentration effects.
Considering chemical composition and functional properties, hydrolysates from Meat Bone Proteins would seem

to have future applications as ingredient in food products while reducing wastes.

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