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Hydrolysis of Proteins from *Liza subviridis*

S.Y. YU AND R. AHMAD

Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
43400 UPM Serdang, Selangor.

Abstract

Proteins from *Liza subviridis*, a marine fish, were hydrolyzed using Alcalase 2.5L. The parameters studied were pH, temperature, ratio of enzyme to substrate concentration, and substrate concentration.

It was observed that the optimum conditions for the hydrolysis were pH 9 at 40°C, using an enzyme to substrate ratio of 16 Au/kg and a substrate concentration of 5%.

Introduction

Proteolytic modification of food proteins is a means for improving the use of this valuable food source. For decades, autolysates of fish have provided added flavor and nutrients to the cuisines of Southeast Asia (McIver et al. 1982). Among the uses of modified protein by chemical and enzymic hydrolysis are : to improve nutritional characteristics, retard deterioration, impart texture, increase or decrease solubility, add foaming or coagulation properties, add emulsifying capacity, prevent undesired interactions, remove off-flavors or odors, and remove toxic or inhibitory ingredients (Lahl and Grindstaff 1989; Feeney 1986; Yu and Tan 1990).

The functional potential of fish hydrolysates has been studied by Miller and Groninger (1976) and Hoyle and Merrit (1994). Hydrolysis has been employed as a possible means of upgrading the use of underutilized fish by Ghosh et al. (1991), Lewis (1981), Gildberg et al. (1989), Skorupa and Sikorski (1993) and Yu and Tan (1990), and as a method of recovering protein from processing by-products (Tarky et al. 1973; Jacobsen and Rasmussen 1984). Its potential for accelerating the fermentation process of fish sauce has been ascertained from the work of Beddows et al. (1976), Beddows and Ardeshir (1979) in 'budu' fish sauce, and Raksakulthai et al. (1986) in fish sauce from capelin. Recent technologies in fish processing such as mechanical deboning has made it possible to use bony and small fish, where before such traits would have been a hindrance to their utilization.

In Malaysia, where such fish products as 'budu' and 'keropok' (fish crackers) are widely accepted, the potential exists for the use of fish hydrolysate in the production of novel products or its incorporation in food products to upgrade functional properties without the inherent fishy odor.

L. subviridis is found among trash fish which are almost always converted to fishmeal. The use of *L. subviridis* as a substrate for hydrolysis will improve the use of this low-value fish.

This paper describes the hydrolysis of the proteins of *L. subviridis* using the enzyme Alcalase 2.5L

Materials and Methods

Raw Materials

Alcalase 2.5L, a commercial protease isolated from the bacteria *Bacillus licheniformis*, was a gift from Novo Industries A/S, Denmark. The declared activity of the enzyme was 2.5Au/g. One Au is equal to 430 International Units at 30°C (Adler-Nissen 1986).

Fresh *L. subviridis* were obtained from a local supplier and transported to the laboratory in ice. The fishes were degutted, deboned and washed in chilled water before they were frozen for storage at -20°C until further use.

The chemicals used were of analytical grade. The hydrochloric acid, petroleum ether and copper sulphate were from BDH (UK), the sulphuric acid, sodium hydroxide and selenium dioxide from Merck (Germany), the boric acid from R & M Chemicals (UK) and the sodium sulphate was from May and Baker (UK).

Proximate Analyses

Protein contents were determined using the Kjeldhal distillation technique (Pearson 1973). The digest was distilled using 10ml of 40% NaOH and titrated with 0.05N H_2SO_4. The amount of sample used was 0.1g-0.15g. The conversion factor used was 6.25.

Crude fat content was determined by extracting fat from a previously-dried sample using the Soxhlet method, with extraction time of 8 hours on an electrothermal unit (Gerhardt, Germany). Petroleum ether was used as the solvent. The fat content was calculated on a dry sample basis (Pearson 1973).

Ash content was determined by placing dried samples into a furnace set at 450-500°C until a whitish residue was obtained. The ash content was taken as a percentage of the dried sample (Pearson 1973).

Moisture content of the fish protein was determined by placing the sample in an oven at 106°C until a constant weight was obtained. The moisture content was calculated as a percentage of the dried sample (Pearson 1973).

The hydrolysis process

Hydrolysis was carried out using the pH-Stat method of Adler-Nissen (1986). The fish mince was homogenized (Braun no. 4249, Germany) with an appropriate amount of water at a low speed for 1 min. The enzyme in solution was added to the equilibrated fish slurry and hydrolysis carried out with continuous stirring. Equilibration of pH was done by adding 1N HCl or 3N NaOH and the temperature of the mixture was maintained by the use of a waterbath (Polytherm Type PY2, US). pH values were monitored using an

Orion 420 A2 Bench Top pH-meter (U.S) with an automated temperature-compensated electrode.

The hydrolysis parameters used were based on literature (Adler-Nissen 1986), where for Alcalase, the optimum conditions using soya isolate is S=8%, E/S=12 Au/kg, pH 9.0 and a temperature of 50°C. From these settings, the parameters were varied in turn to find the optimum conditions for *L. subviridis*.

Degree of hydrolysis

The total number of peptide bonds of fish protein was taken as 8.6 meq/g as reported by Lalasidis et al. (1978).

The approximate value of pk of the amino acids of polypeptides is 7.5 at 25°C (Steinhardt and Beychock 1964). Using the Gibbs-Helmholtz equation, the pk value at 50°C can be calculated as approximately 7. This means that the -NH2 groups are fully titrated and the equivalents of peptide bonds cleaved are equal to the equivalents of base consumed. Using the calculated values of $1/\alpha$ where α is the average degree of dissociation of the -NH2 groups (based on temperature)(Adler-Nissen 1986), the DH (degree of hydrolysis) is calculated directly from the base consumption in a pH stat hydrolysis technique using the following equation :

$$DH = B \times Nb \times 1/ \times 1/MP \times 1/h\ tot. \times 100\%$$

where,

B = base consumption in mL
Nb = normality of base (NaOH)
α = average degree of dissociation of the -NH2 groups (based on temperature) Adler-Nissen, 1986
MP = mass of protein (N X 6.25) in g
$h_{tot.}$ = total number of peptide bonds in the protein substrate

Hydrolysis parameters

Four process conditions were varied to ascertain optimum parameters for hydrolysis. These conditions were pH, temperature, enzyme to substrate ratio, and substrate concentration.

The pH values used were 7, 8, 9 and 10. The three temperatures tested were 30, 40 and 50°C while the enzyme to substrate ratios used were 12, 16 and 20 Au/kg. The three substrate concentrations used were 5, 6 and 7%. The data were statistically analyzed using Duncan's multiple range test using SAS software (SAS Institute Inc., North Carolina, U.S).

Results and Discussion

L. subviridis has 4.7% of crude fat, a protein content of 19.3%, an ash content of 1.9% and a moisture content of 77%.

Temperature effects on the hydrolysis of *L. subviridis* was carried out at 30, 40 and 50°C (Figure 1). At 30°C, the hydrolysis was significantly lower (at 5% confidence level) from that of 40°C and 50°C. At 40 and 50°C, the

hydrolyses curves were not significantly different at the 5% level of confidence, which meant that a working temperature of 40°C would be a more economical choice.

The enzyme Alcalase is basically an alkaline enzyme, although it has been known to work as effectively at a low pH of 6.3 (Jacobsen and Lykke-Rasmussen 1984). The effect of pH on the hydrolysis was studied at pH 7, 8,

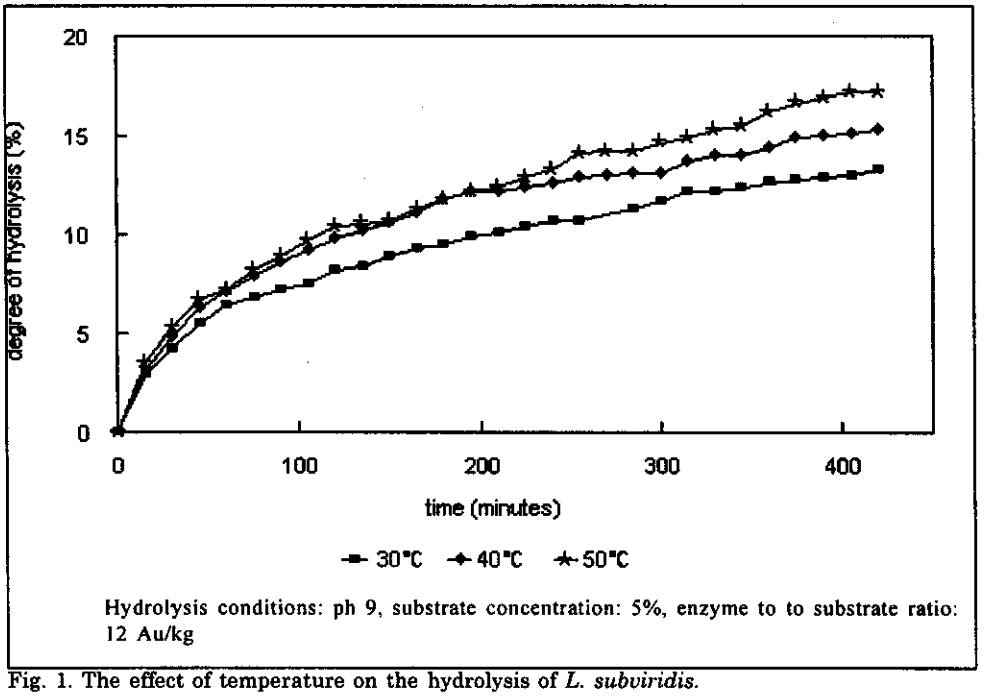

Fig. 1. The effect of temperature on the hydrolysis of *L. subviridis*.

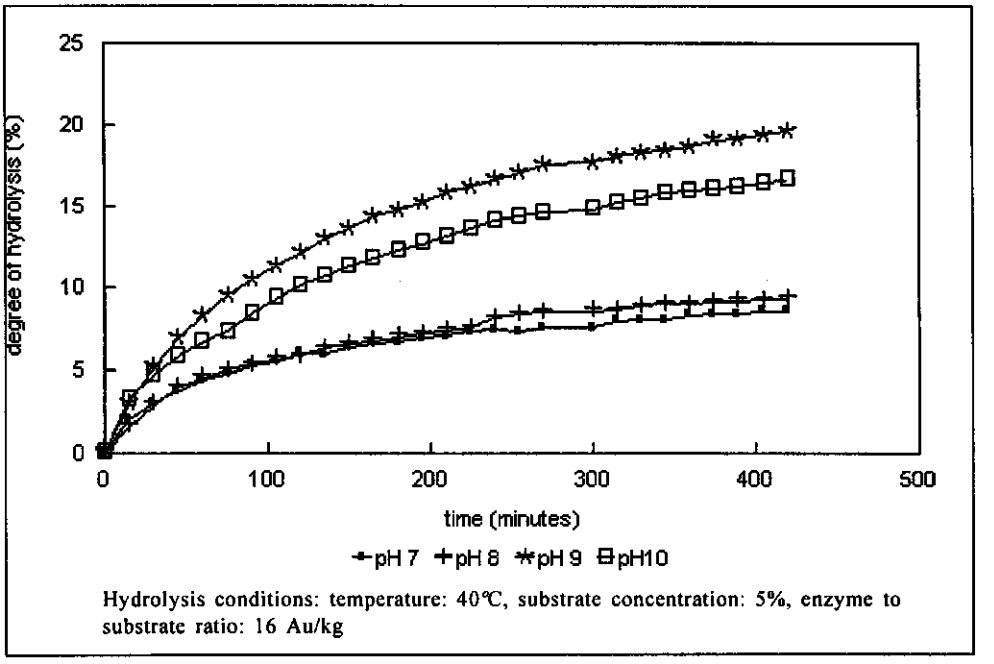

Fig. 2. The effect of pH on the hydrolysis of *L. subviridis*.

9 and 10 (Figure 2). pH 9.0 gave a hydrolysis that was significantly higher at the 5% level than that of the other pHs, including the higher pH of 10. This observation could be typical of many enzyme-catalyzed reactions where pH could cause changes in the reactants, among them being the possible denaturation of the protein structure of the enzyme or the disturbance of the ionic character of the substrates which, in turn, could affect the ability of the substrate to bind to the enzyme (Boikess et al. 1986).

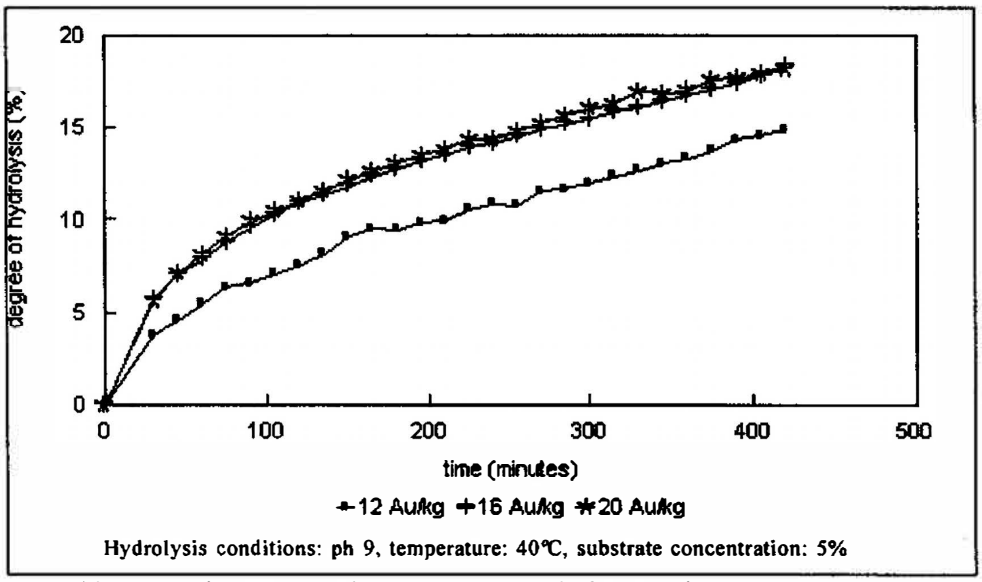

Fig. 3. The effect of enzyme to substrate ratio on the hydrolysis of *L. subviridis*.

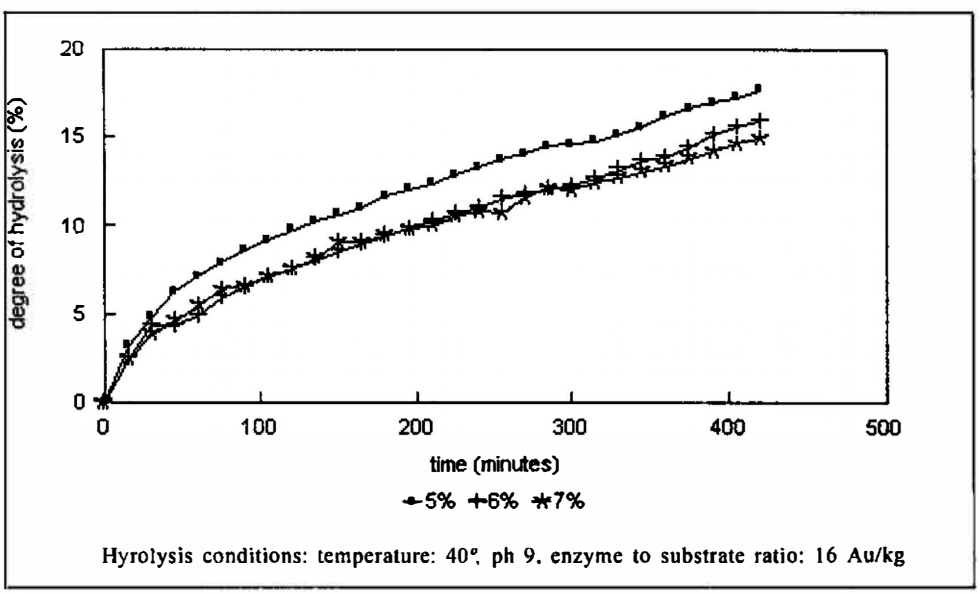

Fig. 4. The effect of substrate concentration on the hydrolysis of *L. subviridis*.

The hydrolysis profile of *L. subviridis*, as shown in Figure 3, was similar for enzyme to substrate ratios of 16 and 20 Au/kg but were both significantly different at the 5% level from that of 12 Au/kg. Hence, the lower ratio of 16 Au/kg was chosen for further hydrolysis work.

The substrate concentration of the hydrolysis mixture was constrained by the thickness of the fish slurry which became too thick at more than 5% level for effective stirring. This is likely to be due to the good gelling capability of fish protein. Stirring was difficult only at the initial stage. However, once the enzyme started to hydrolyze the protein, the slurry became thinner and easier to stir. Figure 4 shows the effect of the three substrate concentrations on the hydrolysis profile of *L. subviridis*. The profiles showed that the hydrolysis at the three substrate concentrations were not significantly different at 5% level of confidence.

Conclusion

Preliminary studies on the hydrolysis of *L. subviridis* using the enzyme Alcalase 2.5L show that the optimum parameters for further hydrolysis work are pH 9, a temperature of 40°C and an enzyme to substrate ratio of 16 Au/kg at a substrate level of 5%.

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