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EFFECTS OF pH, TEMPERATURE, AND TIME OF HEATING ON AUTOPROTEOLYSIS RATE IN BLUE WHITING, *MICROMESISTIUS POUTASSOU* (RISSO, 1826), MEAT

WPŁYW pH, TEMEPRATURY I CZASU OGRZEWANIA NA SZYBKOŚĆ AUTOPROTEOLIZY MIĘSA BŁĘKITKA *MICROMESISTIUS POUTASSOU* (RISSO, 1826)

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Effects of pH, temperature, and time of incubation on intensity of autoproteolysis in blue whiting, *Micromesistius poutassou*, meat were studied. The autoproteolysis rate was assessed from the release of amino nitrogen, nonprotein nitrogen, and peptides.

INTRODUCTION

The worldwide protein deficiency, search for protein reserves as well as a growing crisis in raw materials supply for the fishing industry have made it imperative to utilise the accessible food resources as much as possible. It was for this reason that a growing interest in blue whiting has in recent years been evident in our fisheries. Until recently, the species had benn treated as a by-catch due to its small size and doubtful consumptive utility. The wide distribution of the species in the North-East and South Atlantic and its large concentrations point out to considerable potential in terms of catches (Salmonowicz, 1978). In this context studies on biology, stock assessment and technological utility are under way.

Table 1

Basic chemical composition of blue whiting meat

Component	% content	Standard Deviation S
Protein	18.20	0.041
Fat	0.73	0.031
Water	80.30	0.197
Ash	1.04	0.067

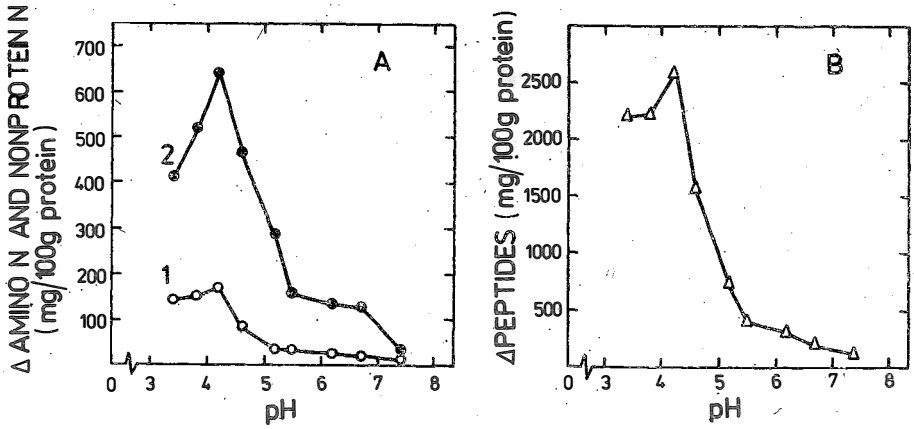

Fig. 1. Effect of blue whiting meat autoproteolysis pH at 40°C on release of amino N and non-protein N (A) and peptides (B). 1 = increase in amino N; 2 = increase in non-protein N.

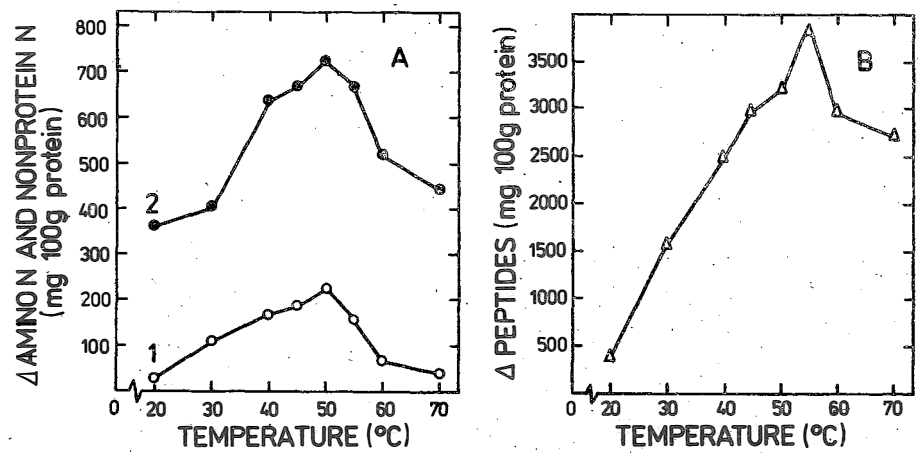

Fig. 2. Effect of blue whiting meat autoproteolysis temperature at pH 4.2 on release of amino N, non-protein N, and peptides
For explanations see Fig. 1

The present work was aimed at determining optimal conditions for blue whiting meat autoproteolysis with a particular reference to pH, temperature, and time of heating. Knowledge on endogenous proteolytic activity towards the native proteins under different pH and temperature is necessary both for preservation and processing of fish.

METHODS AND EXPERIMENTAL MATERIALS

Materials

Materials to be studied comprised carcasses of blue whiting *Micromesistius poutassou* (Risso) caught by MT "Rekin" in June 1978 from the North-East Atlantic and stored frozen for 5 months at −25°C. Each time an 0.5–1 kg sample was taken and left for 14 hours at 0–4°C for thawing. The fish meat after filleting was ground in a meat grinder. A thoroughly mixed ground meat was used in assays.

Methods

In order to assess the autoproteolysis rate, 10 g samples of ground meat(in three replicates each for every temperature, pH, and time value chosen), previously adjusted to a definite pH by means of 0.025–0.25 N HCl and 0.025–0.25 N NaOH were incubated. Hydrolysis was terminated after a predetermined time by adding enough trichloroacetic acid (TCA) to obtain the final concentration of 5%. The sample was then mixed and left for 30 minutes at room temperature, after which non-hydrolysed solids were separated and contents of amino and non-protein N as well as peptides determined in hydrolysates. At the same time control samples (autoproteolysis at time O) were prepared. The autoproteolysis rate was expressed as the amounts of amino N, non-protein N, and peptides released per 100 g raw protein. Effects of pH and temperature were studied after 2-hr heating, while effects of time were assessed at optimum pH (4.2) and temperature (50°C).

Contents of amino N, non-protein N, and peptides were determined using the techniques of Pope and Stevens (1939), Kjeldahl (Krauze et al., 1962), and burette technique (Mejbaum-Katzenellenbogen and Mochnacka, 1968), respectively. The basic chemical composition of the blue whiting meat was determined by means of commonly used analytical techniques (Krauze et al., 1962).

DISCUSSION OF RESULTS

The basic chemical composition of the blue whiting meat used in the assays is presented in Table 1.

When the effect of pH on autoproteolysis was studied in terms of the amino N, non-protein N, and peptides release (Fig. 1), a single peak at pH of about 4.2 was

revealed. The curves depicting the dynamics of the release of amino N, non-protein N, and peptides are similar. From the pH values of 3.4–4.2 on, a slow increase in each nitrogen fraction is observed, the non-protein N increase being somewhat more rapid. Owing to a gradual reduction in the activity of proteolytic enzymes, changes in pH from the optimum to alkaline brought aobut a decrease in the release of all the nitrogen fractions studied, particularly with respect to amino N. At pH 4.6 about 50% of amino N, 61% of peptides, and 72% of non protein N were released compared to their amounts at the optimal pH, the respective percentage release at pH 5.5 being 18, 24, and 24% for each nitrogen fraction studied. The autoproteolysis proceeded particularly slowly at pH 7.4. The analysis of nitrogen fractions release shows that – similarly to the case with sardine (Fik and Valencia Mecola, 1981) – the optimal activity of the blue whiting muscle proteinases is confined to a narrow pH range, the optimum values being almost identical for the two species.

Effects of temperature on protein hydrolysis was assessed at a pH close to the optimum. The results are presented in Fig. 2. The maximum activity of muscle proteolytic enzymes was recorded at about 50°C. Within the temperature range of 20–45°C an almost linear increase in the nitrogen fractions took place, the increase being rather slow in amino N and rapid in peptides. The dynamics of non-protein N release appeared differently. Within 20–30°C a slight increase was observed, followed by at first large increments up to 40°C, to be then reduced all the way up to 50°C. A temperature rise from 50 to 60°C brought about a rapid decrease in the autoproteolysis rate resulting most probably from a partial denaturation of enzyme proteins. Thus at 60°C 31, 72, and 76% of amino N, non-protein N, and peptides were released, respectively, compared to

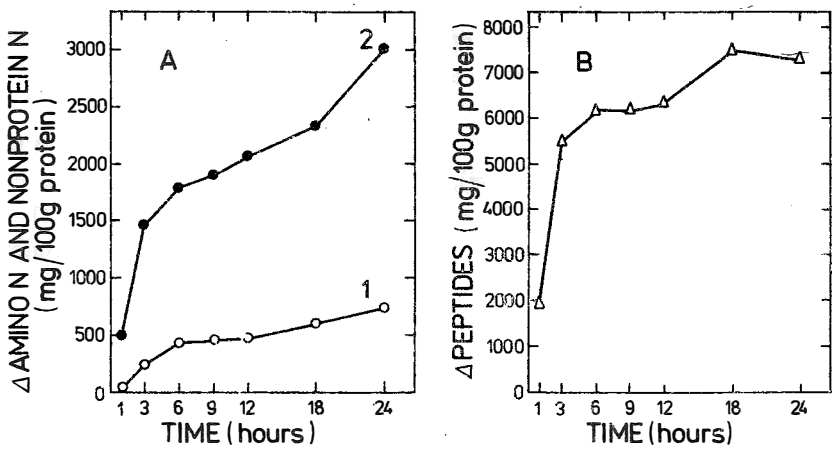

Fig. 3. Effect of blue whiting meat autoproteolysis time under optimum conditions (pH 4.2; 50°C) on release of amino N, non-protein N, and peptides
For explanations see Fig. 1

the percentages at 50°C, while at 70°C the respective percentages were 18, 62, and 69%. These data point out to a narrow temperature range in which the muscle enzymes will exhibit their highest proteolytic activity. The highest peptides content at 55°C and their considerable release within 55–70°C may have resulted from a weaker denaturation effect of higher temperatures on endoproteinases compared to exoproteinases contained in the blue whiting meat.

When studying the autoproteolysis rate as affected by the duration of heating (1–24 hours), three clearly marked stages of increase in the contents of low-molecular nitrogen compounds soluble in TCA were observed (Fig. 3). During the initial 6 hours of hydrolysis (stage 1) large increases in amino N, non-protein N, and peptides were recorded; then, within 6–12 hours for amino N and peptides and 6–18 hours for non-protein N (stage 2), gradual increases could be seen, and – at stage 3 – a return to large increases in the fractions discussed was recorded. The accumulation of peptides over 18–24 hours of proteolysis showed, a certain tendency of approaching the state of equilibrium. Autoproteolysis carried out under optimal conditions resulted in an increase of non-protein N accumulation being 5–7 times that of amino N, which could have a bearing on yields of each fraction obtained from the same substrate.

When the autoproteolysis in blue whiting was compared to that in sardine (Fik and Valencia Mecola, 1981), the first was shown to release much less amino N and almost the same amount of non-protein N as the other. Thus the activity of the blue whiting meat endogenous proteolytic enzymes towards the native proteins cannot be regarded as high. A possible utilisation of the raw material under study in the production of hydrolysates and protein concentrates assisted by an enzymatic technique will call for an accelerated proteolysis effected by an addition of proteolytic enzymes or other substances of a high enzymatic activity (Fik. 1979).

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WPŁYW pH, TEMPERATURY I CZASU OGRZEWANIA NA SZYBKOŚĆ AUTOPROTEOLIZY MIĘSA BŁĘKITKA

Streszczenie

Przebadano wpływ pH, temperatury i czasu inkubacji na szybkość hydrolizy białek mięsa błękitka. Intensywność autoproteolizy oceniano na podstawie przyrostów azotu aminowego, azotu niebiałkowego i peptydów na 100 g białka surowego.

Maksymalną aktywność enzymów proteolitycznych tkanki mięśniowej błękitka stwierdzono w pH ok. 4,2 i temp. ok. 50°C. Badając szybkość autoproteolizy w czasie 1–24 godzin wykazano wyraźny 3-etapowy charakter przyrostów niskocząsteczkowych substancji azotowych rozpuszczalnych w kwasie trójchlorooctowym. Proces proteolizy w warunkach optymalnych (w pH 4,2 i temp. 50°C) powodował 5–7-krotnie szybsze narastanie azotu niebiałkowego niż aminowego, co może wpływać na wydajność różnych frakcji uzyskanych z tego surowca. Stwierdzono stosunkowo niską aktywność enzymów proteolitycznych badanego surowca względem własnych białek. W związku z tym zastosowanie mięsa błękitka do ewentualnej produkcji hydrolizatów i koncentratów białkowych metodą enzymatyczną wymagać będzie dodatku preparatów enzymatycznych dla szybszej hydrolizy.

ВЛИЯНИЕ pH, ТЕМПЕРАТУРЫ И ВРЕМЕНИ ПОДОГРЕВА НА СКОРОСТЬ АВТОПРОТЕОЛИЗА МЯСА ПУТАССУ

Р е з ю м е

Исследовали влияние pH, температуры и времени инкубации на скорость гидролиза белков мяса путассу. Интенсивность автопротеолиза оценивали на основании прироста аминового азота, небелкового азота и пептидов на 100 гр белкового сырья.

Максимальная активность энзимов протеолитической мышечной ткани путассу была обнаружена при pH ок. 4,2 и темп. ок. 50°C. Исследуя скорость автопротеолиза в течение 1–24 часов доказали отчетливый трехэтапный характер прироста низкомолекулярных азотных веществ растворящихся в трихлоруксусной кислоте. Процесс протеолиза в оптимальных условиях (при pH 4,2 и темп. 50°C) вызывал 5–7 кратное возрастание скорости прироста небелкового

азота, чем аминового азота, что может влиять на результативность различных фракции полученных из этого сырья. Обнаружили относительно низкую активность протеолитических энзимов исследуемого сырья в отношении собственных белков.

В связи с этим применение мяса путассу для предполагаемой продукции белковых гидролизатов и концентратов энзиматическим методом вызовёт необходимость добавления энзиматических препаратов для более быстрого гидролиза.

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