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EFFECTS OF SOME ANTISEPTICS ON RATE OF ANTARCTIC KRILL AUTOPROTEOLYSIS

WPŁYW NIEKTÓRYCH ANTYSEPTYKÓW NA SZYBKOŚĆ AUTOPROTEOLIZY BIAŁEK KRYLA ANTARKTYCZNEGO

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Intensity of autoproteolysis in Antarctic krill (*Euphausia superba*) in the presence of some antiseptics was studied. The rate of autoproteolysis was assessed from release of amino and non-protein nitrogen in 100 g raw protein.

INTRODUCTION

In the recent years there have been many attempts to find appropriate methods of preserving and processing Antarctic krill as a source of protein for consumption. Owing to the high activity of its proteolytic enzymes, (Fik, 1977, 1979; Galas et al., 1970; Kubota and Sakai, 1978; Matumoto et al., 1978; Seki et al., 1977), krill can be utilised in production of protein concentrates for animal feeds and human consumption by means of enzymatic processing (Kołakowski et al., 1979; Fik et al., 1980). Enzymatic hydrolysis is often carried out in the presence of substances inhibiting growth of bacterial microflora. Introduction of such substances to the hydrolysed materials results most often in an altered proteolysis intensity. The present work was therefore aimed at assessing the influence of some antiseptics (ethanol, chloroform, NaCl, toluene) on krill proteolysis rate.

METHODS AND EXPERIMENTAL MATERIALS

MATERIALS

Antarctic krill (*Euphausia superba* Dana) caught in March 1978 by RV "Profesor Bogucki" was used in assays. The frozen and packed raw material was initially stored in the vessel holds at about −30°C and then, after delivery to Szczecin, in a freezer at −25°C. The assays were commenced after a 9-month frozen storage. Each time a 250–500 g krill sample was taken and ground when frozen in a meat grinder. The mince obtained was thoroughly mixed, thawed, and sub-sampled for autoproteolysis rate and chemical composition assays.

In order to determine the influence of antiseptics on the rate of autoproteolysis, three replicates of 10 g ground krill and 10 cm^3 distilled water each were placed in conical flasks with or without a suitable amount of an antiseptic. The antiseptics were added in amounts making up 5, 10, and 20% of the hydrolysate. The whole content of a flask was thoroughly mixed; tightly stoppered flasks were placed in a incubator at 50°C for a strictly determined heating time (0, 1, 2, and 4 hours). Proteolysis was carried out at a physiological pH value amounting to 7.2. After the hydrolysis was over, 10 cm^3 of 10% trichloroacetic acid (TCA) were added to each flask; their contents thoroughly mixed, the flasks were left for 30 minutes at room temperature in order to precipitate the proteins. The samples were filtered on dry blotting paper filters; amino and non-protein nitrogen were determined in the filtrates. The autoproteolysis rate was assessed from release of amino and non-protein N per 100 g raw protein.

Amino N was determined with the method of Pope and Stevens (1939), while the Kjeldahl method was used for non-protein N determinations (Krauze et al., 1962). The basic chemical composition of the materials used in the assays was determined with commonly applied analytical techniques (Krauze et al., 1962).

DISCUSSION OF RESULTS

The chemical composition of the krill analysed is presented in Table 1. The materials were found to differ slightly in their composition from the batch of krill delivered by the First Polish Antarctic Expedition (Fik, 1979). The present materials contained less fat and water, while showing a higher (by 2%) content of protein.

The results obtained in the present work demonstrate unequivocally that krill without any antiseptic added had the highest autoproteolysis rate. The 5 and 10% additions of NaCl resulted in a slightly increased activity of the proteolytic enzymes at the beginning of hydrolysis (1 hour) as evidenced mainly by the release of amino N. After a longer time of hydrolysis, however, the salt concentrations mentioned resulted in a lowered proteinases activity.

Ethanol was found to inhibit the krill proteolysis most (Fig. 1). The increased percentage of this antiseptic in the hydrolysed samples brought about a stronger decrease in the release of amino and non-protein N. The addition of 5, 10, 15, and 20% (w/v) of

Table 1

Chemical composition of krill used in assays

Component	Percentage	Standard deviation s
Protein	15.87	0.03
Water	77.21	0.08
Fat	3.53	0.02
Ash	3.39	0.03

ethanol to the ground krill resulted in the release, after 1 hour, of about 63, 49, 45, and 45% of amino N and about 60, 47, 43, and 36% of non-protein N, respectively, compared to the amounts released during autoproteolysis without any antiseptic (control sample). After 2 hours of hydrolysis the respective percentages were 77,69,56, and 49% for amino N and 78, 71, 61, and 55% for non-protein N, while after 4 hours the respective percentages amounted to 74, 63, 53, and 44% of amino N and 60, 58, 51, and 45% of nonprotein N.

20% ethanol was found to affect the proteolysis intensity in a particularly strong manner.

Chloroform was found to suppress the krill proteolysis intensity more than did NaCl and toluene but less than NaCl and ethanol. Addition of 5 and 10% of chloroform, while causing no reduction in release of amino N after 1 hour compared to the control, did reduce non-protein N release very considerably (Fig. 2). It was presumably an effect of differential influences of the antiseptics in question on activities of various proteolytic enzymes comprising the krill endogenous proteinase complex. However, after a prolonged enzymatic hydrolysis of krill proteins (2 and 4 hours) in the presence of the above-mentioned concentrations of the antiseptics, a considerable reduction in the release of low-molecule nitrogen compounds took place. After 2 hours of hydrolysis, the release of amino N dropped to 25–35% depending on a chloroform concentration, and to 31–47% after 4 hours, compared to the release in the controls. The reduction in non-protein N release under the same conditions was 17–31% and 42–56%, respectively.

Owing to its insignificant influence on activity of some enzymes, toluene is an antiseptic commonly used in biochemical assays. In the present work the antiseptic was found to exert a weaker effect on krill proteolysis rate than did ethanol and chloroform (except from the proteolysis during the first hour), and a stronger one than NaCl (Fig. 3). The inhibiting action of toluene depended, to a certain degree, on its concentration in a sample under test, as was the case with other antiseptics, too. The duration of enzymatic hydrolysis was also relevant. Toluene may be used as antiseptic in krill autoproteolysis at concentrations up to 15%.

Of the antiseptics tested, the slightest changes in the autoproteolysis rate resulted from the addition of NaCl (Fig. 4). As mentioned above, in the initial stage of hydrolysis the

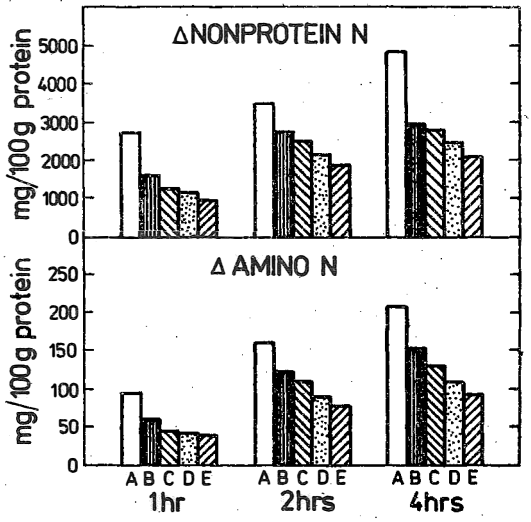

Fig. 1. Release of amino N and non-protein N during krill autoproteolysis with ethanol. A = control (no antiseptic); B, C, D, E = assays with 5, 10, 15, and 20% of antiseptic, respectively

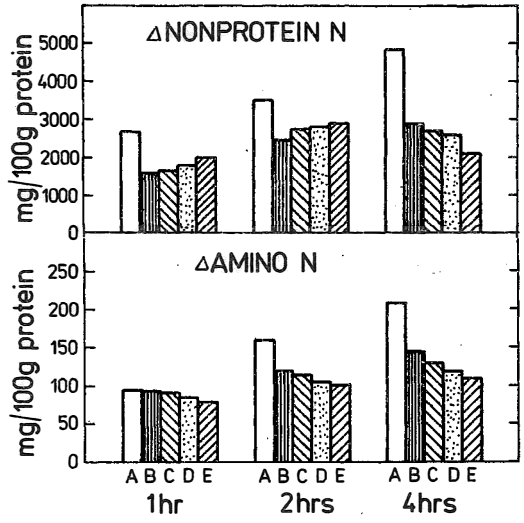

Fig. 2. Release of amino N and non-protein N during krill autoproteolysis with chloroform. For explanations see Fig. 1

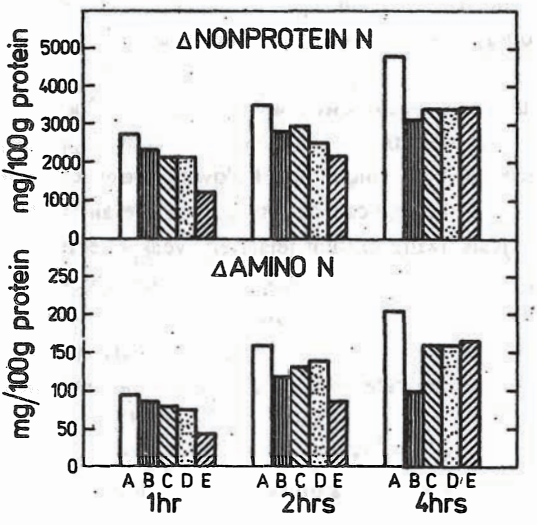

Fig. 3. Release of amino N and non-protein N during krill autoproteolysis with toluene. For explanations see Fig. 1.

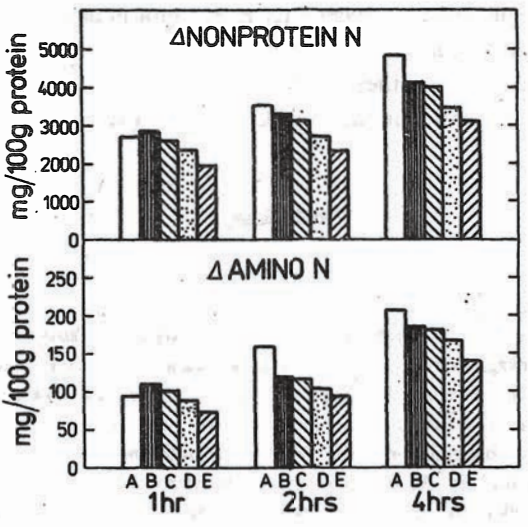

Fig. 4. Release of amino N and non-protein N during krill autoproteolysis with NaCl. For explanations see Fig. 1

antiseptic tended even to accelerate the action of krill proteolytic enzymes. After 2 hours of autoproteolysis, the NaCl solutions used were found to decrease the amino N release by 25 to 40% and the release of non-protein N decreased by 7 to 33%; after 4 hours under the same conditions of hydrolysis, the respective release values dropped by 11–32% and 15–36%.

To sum up, all the antiseptics tested were found to reduce the krill autoproteolysis rate. With respect to this inhibiting action towards krill endogenous proteolytic enzymes, the compounds tested can be arranged in a following order: ethanol>chloroform>toluene> NaCl. The latter and toluene can be regarded as the antiseptics most suitable for an industrial krill proteolysis owing to their relatively weak effects on enzymes. Ethanol and chloroform, while considerably reducing the proteolysis rate, will extend the duration of protein hydrolysis. However, for a very short time of hydrolysis, also chloroform and ethanol can be used as antiseptics, the former being particularly applicable for hydrolysates and feed concentrates. Ethanol, owing to its enzyme-inhibiting properties, was used to preserve krill by the Japanese (Uchi, 1978). Moreover, further studies on effects of other antiseptic substances on krill proteolytic enzyme activities seem purposeful in order to assess a possibility of their application in proteolysis and/or preservation of krill.

CONCLUSIONS

1. The maximum autoproteolysis rate was recorded in krill without any antiseptic added. Antiseptics reduce the protein hydrolysis intensity, their inhibiting influence increasing with their growing concentration. Ethanol inhibits the proteolytic enzymes activity to the highest degree.
2. NaCl and toluene, owing to their relatively weak effects on krill proteinases activity, can be applied as antiseptics in industrial krill proteolysis.

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Streszczenie

Zbadano wpływ alkoholu etylowego, chloroformu, toluenu i NaCl na szybkość autoproteolizy białek kryla antarktycznego. O szybkości hydrolizy sądzono na podstawie przyrostów azotu aminowego i niebiałkowego na 100 g białka surowego po 1, 2 i 4 godzinach inkubacji w temp. 50°C i pH 7,2.

Stwierdzono, że najwyższą szybkością autoproteolizy charakteryzował się kryl bez dodatku antyseptyków. Wszystkie badane antyseptyki wpływały na zmniejszenie szybkości autoproteolizy tego surowca. Inhibicja proteinaz wzrastała w miarę zwiększania się stężenia antyseptyków. Pod względem działania inhibującego można je uszeregować następująco: alkohol etylowy>chloroform> toluen > NaCl. Sól kuchenna i toluen, ze względu na stosunkowo niewielki wpływ na aktywność pro-

teinaz, mogą być stosowane w charakterze antyseptyków przy proteolizie przemysłowej surowca kryłowego. Natomiast zastosowanie jako antyseptyków alkoholu etylowego i chloroformu powodować będzie wydłużenie czasu hydrolizy białek.

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ВЛИЯНИЕ НЕКОТОРЫХ АНТИСЕПТИКОВ НА СКОРОСТЬ АВТОПРОТЕОЛИЗА БЕЛКОВ АНТАРКТИЧЕСКОГО КРИЛЯ

Р е з ю м е

Исследовали влияние этилового спирта, хлороформа, тоулена и соли (NaCl) на скорость автопротеолиза белков антарктического криля. Скорость гидролиза определяли на основании прироста аминового и небелкового азота на 100 гр сырого белка после 1, 2 и 4-х часовой инкубации при температуре 50°С и pH 7,2.

Обнаружили, что наибольшую скорость автопротеолиза имел криль без добавления антисептических средств. Все исследуемые антисептики влияли на уменьшение скорости автопротеолиза этого сырья. Ингибиция протеиназ возрастала по мере увеличения доз антисептиков. Принимая во внимание ингибирирующие свойства, можно их расположить в следующем порядке: этиловый алкоголь, хлороформ, тоулен, NaCl. Поваренная соль и тоулен в связи с относительно небольшим влиянием на активность протеиназ могут применятся в характере антисептиков при заводском протеолизе крильевого сырья.

Применение же в характере антисептиков этилового алкоголя и хлороформа удлинняет время гидролиза белков.

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