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BIOCHEMICAL CHANGES IN BULK-STORED CAPELIN

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ABSTRACT

In capelin bulk-stored under industrial conditions trimethylamine oxide is relatively rapidly reduced to trimethylamine; this process represents a first step in the bacterial spoilage of the fish. Ammonia production sets in after a lag period, presumably due to the activity of special types of bacteria delayed in their development compared to those causing the reduction of trimethylamine oxide. Amino acids and not the other nitrogen extractives of the fish seem to be the main source of the NH_3 copiously produced upon prolonged storage, the hydrophilic rather than the hydrophobic amino acids being most readily decomposed. Acetic acid is a main fermentation product in the fish mass. Butyric and propionic acids accumulate at the later stages of the storage.

INTRODUCTION

Capelin (*Mallotus villosus*) is a major raw material for the Norwegian, Icelandic and Russian fish meal industry, with annual catches of about 2 mill. tons over the past 10 years. During the fishing seasons the capelin is stored in large tanks (1000–5000 tons) prior to processing. The capelin is very fragile and easily mashed upon mechanical handling. Anaerobiosis develops rapidly in the capelin mass due to bacterial activity. We reported earlier on the use of extracts of herring, incubated anaerobically and inoculated with pure cultures of fish spoilage bacteria, as a model system for the study of biochemical changes taking place in bulk-stored fish (STRØM and LARSEN, 1979; STRØM et al., 1979). In the present paper we report on the biochemical changes taking place in capelin bulk-stored under actual industrial conditions.

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MATERIALS AND METHODS

Storage and sampling

The capelin was caught in February in the open sea north of Norway and transferred from the fishing vessel to 150 m^3 storage tanks at the Norwegian Herring Meal and Oil Research Institute's Experimental Station, Honningsvåg, Norway, about 24 hours after capture. The temperature of the fish mass was 3–6°C throughout the storage period. Samples were drawn from the centre of the tank at intervals with the sampler described by UTVIK (1967).

Viable counts

Liquid strained from samples was serially diluted in a solution containing 0.25% yeast extract (Oxoid), 1% NaCl, at pH 7.0, and aliquots plated on the meat extract-peptone-agar medium given by SHEWAN et al. (1960). Viable counts were registered after 3–4 days incubation at 13°C in air.

Chemical analysis

Capelin mass from two different catches were sampled and analysed. The samples from the first catch were strained, protein precipitated from the liquid with trichloroacetic acid, perchloric acid or picric acid, and in the resulting extracts trimethylamine oxide (TMAO), trimethylamine (TMA), ammonia, free amino acids, creatine, nucleotides, nucleosides and purines were determined as specified by KJOSBAKKEN and LARSEN (1981). The samples from the second catch were homogenized and perchloric acid extracts made as described by KJOSBAKKEN and LARSEN (1981). In the latter extracts volatile fatty acids were determined by gas chromatography as described by STRØM and LARSEN (1979); lactic acid was determined according to BARKER (1957).

RESULTS

Growth of bacteria and changes in TMAO, TMA and ammonia in the bulk-stored capelin mass from the first catch are illustrated in Fig. 1. TMAO was relatively rapidly converted and corresponding amounts of TMA accumulated. Ammonia production was clearly delayed compared to the TMA production, and set out at a high rate only after TMAO was completely reduced and the bacterial count had reached its maximum.

Changes in the contents of the free amino acids in the liquid strained from the bulk-stored capelin mass are shown in Fig. 2. The amino acids could be grouped characteristically according to these variations. Alanine, leucine, valine, isoleucine, phenylalanine and methionine, i.e. the typical hydrophobic amino acids, all increased throughout the storage period (Fig. 2A). Glutamic

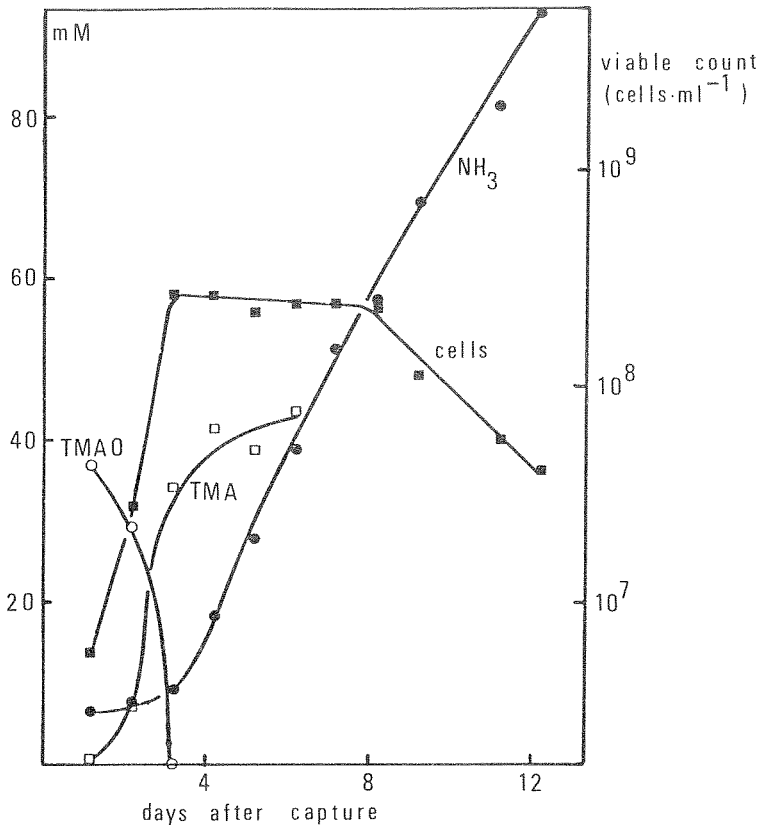

Fig. 1. Bacterial growth (cells per ml) and changes in mM trimethylamine oxide (TMAO), trimethylamine (TMA) and ammonia in capelin bulk-stored industrially at 3–6°C, as measured in liquid strained from the capelin mass.

acid, glycine and threonine increased during the first part of the storage period (5–7 days), then decreased. A rather striking accumulation of γ-aminobutyric acid took place during the second part of the storage period (Fig. 2B). Serine decreased very rapidly. Also arginine, aspartic acid, tyrosine, lysine and histidine decreased but with a lag period; the lag was most pronounced for lysine and histidine. Proline displayed a fluctuating pattern (Figs. 2C and D).

Taurine, anserine, β-alanine and creatine, were also determined in the liquid strained from the bulk-stored capelin mass. The contents of taurine (about 9.5 mM), anserine (about 3.0 mM) and β-alanine (about 0.4 mM) did not change significantly during the 12 days storage period. Creatine, however, decreased slowly from about 18 mM to about 11 mM after 12 days.

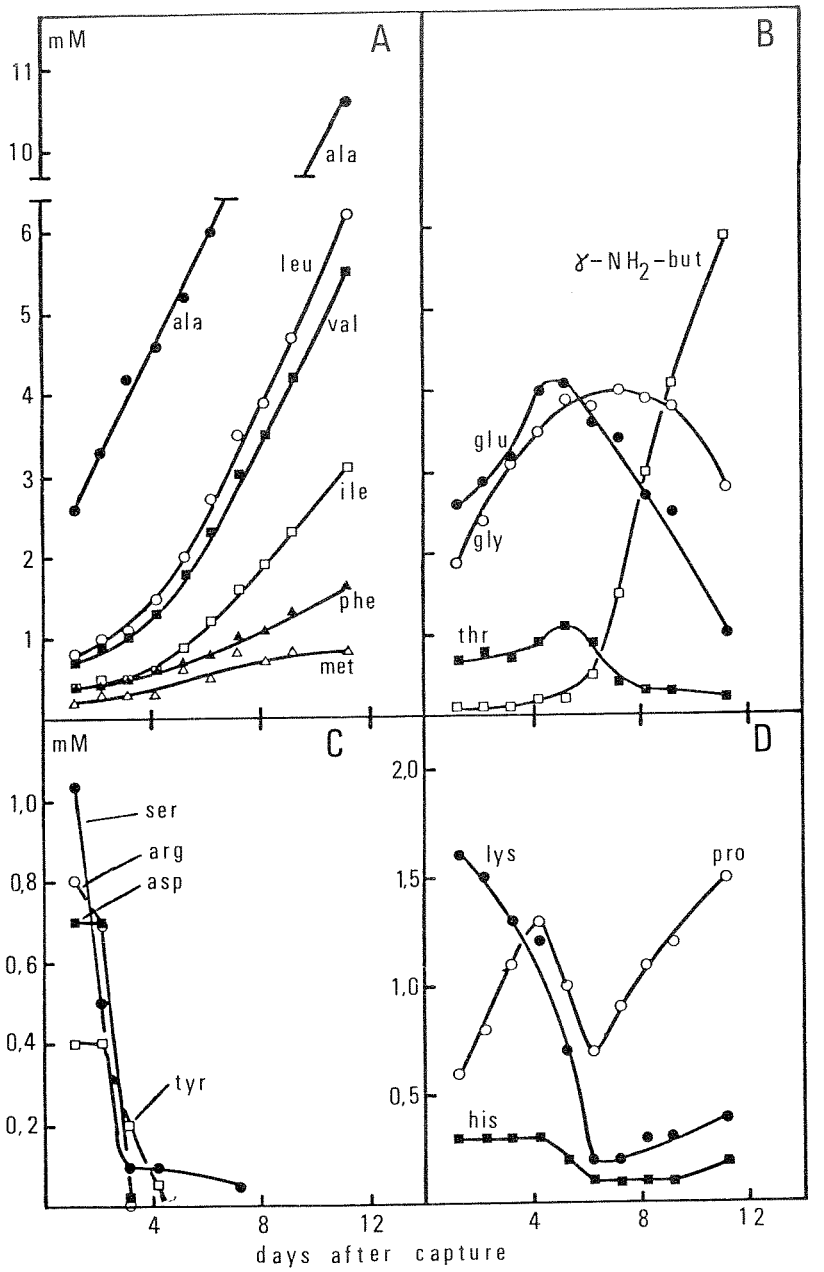

Fig. 2. Changes in free amino acids (in mM) in capelin bulk-stored industrially at 3–6°C, as measured in liquid strained from the capelin mass. γ-NH_2-but.: gamma-aminobutyric acid.

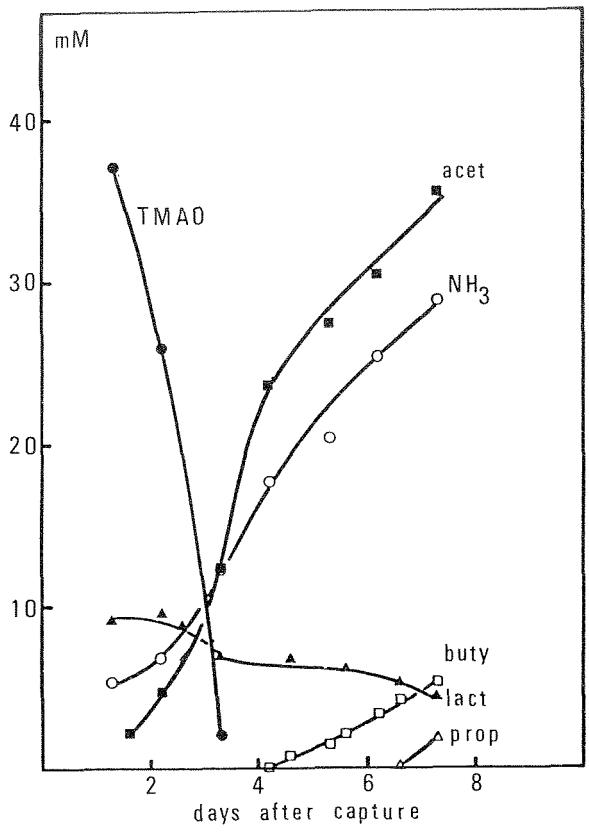

Fig. 3. Changes in volatile fatty acids, lactic acid, trimethylamine oxide (TMAO) and ammonia (in mM) in capelin bulk-stored industrially at 3–6°C, as measured in homogenized samples of the fish mass.
acet: acetic acid; prop: propionic acid;
buty: butyric acid; lact: lactic acid.

The only nucleotide and nucleoside present in noticeable amounts in the liquid strained from the capelin mass, were inosine monophosphate and inosine. These stem from ATP which is rapidly broken down by endogenous enzymatic activity (SHAW and BOTTA, 1975 a, b). The inosine monophosphate and inosine were further reduced to trace amounts after 3 days of storage. A corresponding amount of hypoxanthine accumulated, and stayed constant at a level of about 2.7 mM during the rest of the storage period of 12 days.

The capelin of the second catch was assayed for TMAO, ammonia, volatile fatty acids and lactic acid during a storage period of 7 days. The results are given in Fig. 3. The production of ammonia again showed a lag compared to TMAO conversion although less conspicuous than in the case of

the first catch. Acetic acid was the dominating volatile fatty acid formed in the fish mass. After 4 days butyric acid and after 7 days propionic acid started to accumulate. Lactic acid which is present in considerable quantities (9.2 mM) at an early stage of the storage as a result of glycolysis, was degraded only slowly and reached 4.4 mM after 7 days.

DISCUSSION

We reported earlier on the biochemical changes taking place in fish (herring) extracts when incubated anaerobically with pure cultures of 3 different types of bacteria, all considered to be typical «fish spoilers» (STRØM and LARSEN, 1979; STRØM et al., 1979). These organisms, strains of *Proteus*, *Enterobacter* and *Aeromonas*, developed readily in the fish extract at the expense of a fermentation process in which the dominating substrates were carbohydrate and, in 2 of the 3 cases, lactic acid. The substrates were converted dominantly to acetic acid and CO_2, concomitantly with a reduction of TMAO to TMA. The latter compound is a typical product of fish spoilage. The only amino acid decomposed by all 3 organisms was serine; one of the organisms (*Enterobacter* sp.) decomposed threonine in addition. Only little NH_3 was formed by the 3 organisms in the fish extract. The amount accumulating (1 mM) could be accounted for by the degradation of serine (and of threonine). The 3 «fish spoilers» thus seemed unable to produce NH_3 from the other nitrogenous compounds present in the fish extract in notable amounts, including the other amino acids, or, for that matter, to develop at the expense of other constituents of the fish extract than those mentioned.

In the present experiments where the spoilage process occurred under the influence of a «natural» mixed biota of bacteria and under actual industrial conditions, a reduction of TMAO to TMA took place at a relatively early stage of the storage, and this process coincided with a strong increase in the bacterial viable count (Fig. 1). Only little NH_3 was produced, and the changes taking place during this early period thus seemed to correspond to those reported for the pure cultures of «fish spoilers» growing anaerobically in fish extract (STRØM and LARSEN, 1979).

Freshly caught capelin contains a small amount of NH_3 (about 5 mM, KJOSBAKKEN and LARSEN, 1981), presumably originating from ATP by endogenous deamination (TARR, 1966). It is well known that upon subsequent bulk-storage under industrial conditions additional NH_3 is formed in copious amounts (MJELDE and URDAHL, 1974). In the two experiments of storage we report on in the present paper there was a characteristic lag of 2–3 days before the major NH_3-production set in (Figs. 2 and 3), presumably due to the activity of special types of bacteria that seemed somewhat delayed in their

development compared to those causing the reduction of TMAO to TMA (KJOSBAKKEN et al., 1983). NH_3 production thus seems to represent a second stage in the spoilage process, following, or to some extent overlapping, TMAO reduction.

During the period of NH_3 production the viable count observed was fairly constant at first, and decreased towards the end (Fig. 1). It may well be that the method for viable count, which is in common use for the estimation of bacteria in spoiling fish, does not give reliable figures for the number of active bacteria in the anaerobic fish mass.

The contents of the various free amino acids varied in characteristic patterns during the storage period. The increase in the hydrophobic amino acids (Fig. 1A) is reasonably explained by a proteolytic activity in the fish mass and by a slower, or possibly no, decomposition of the liberated amino acids. The early increase in glutamic acid, glycine and threonine, followed by a decrease at the later stage (Fig. 2B), is reasonably explained by proteolytic liberation accompanied by a somewhat delayed decomposition as a result of the development of a secondary biota of amino acid decomposing bacteria. Serine was decomposed rapidly at a very early stage (Fig. 2C) concomitantly with the reduction of TMAO in accordance with the values reported for the earlier pure culture studies (STRØM and LARSEN, 1979). The other amino acids showed a lag period in their conversion pattern (Figs. 2C and D) supporting the notion of their decomposition by a secondary biota of bacteria.

The various nitrogenous compounds assayed represent close to 100% of the nitrogen extractives of the fish mass (KJOSBAKKEN and LARSEN, 1981). Except for the amino acids these compounds changed very little during the period of observation, whereas NH_3 increased strikingly. It may therefore be concluded that the bulk of the NH_3 originated from amino acids, and mainly from the hydrophilic amino acids continously supplied by proteolysis. Glutamic acid represented a special case since its disappearance was accompanied by an accumulation of γ-aminobutyric acid (Fig. 2B).

In contrast to acetic acid, butyric and propionic acids accumulated in the fish mass at the later stage of storage (Fig. 3). Also this observation points to special types of bacteria delayed in their development.

ACKNOWLEDGEMENT

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