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EFFECT OF TEMPERATURE ON THE AUTOLYSIS OF CAPELIN SILAGES STORED FOR ONE YEAR

By

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ABSTRACT

Conventional formic acid silage was prepared from frozen capelin, stored at pH 3.8–4.0 for twelve months at 2, 20 and 37 °C, and analysed at intervals. Soluble N rapidly attained a maximum of 90% of total N when stored at 20 and 37 °C; at 2 °C the increase was slower and reached a lower maximum. Ninhydrin-reactive substances (ammonia and α-amino N) increased during the first months and stabilized at higher levels at the two higher temperatures than at the lower. The amino acid composition remained constant. TVN (total volatile nitrogen) and ammonia-N increased during the whole period, though faster during the first week. Amide-N decreased at a corresponding rate. Ammonia-N was below 3.5% of total N. The results are discussed relative to literature.

INTRODUCTION

Ensiling whole fish or fish offal with formic acid is an alternative to fish meal production. Fish silage is used as a feed component in feeds for farmed fish and for fur animals. Formic acid silages of whole fish or fish offal liquefy fast due to autolysis and the rate is greatly influenced by temperature. Silage is produced throughout the year and the storage temperature may vary widely under practical conditions. The nutritional quality of the silage may depend on the degree of autolysis and the products formed. We have found that the increase in total volatile nitrogen (TVN) in properly produced silage is due mainly to ammonia derived from the amide groups of glutamine and asparagine (Haaland and Njaa, 1988). Therefore it may be assumed that given time the same final amount of ammonia as well of soluble nitrogen would be formed regardless of storage temperature.

The present experiment was set up to test these assumptions. Silages from whole capelin were stored for one year at three temperatures, 2 °C (winter temperature), 20 °C (summer temperature) and 37 °C (extreme high temperature), and analysed at intervals.

MATERIALS AND METHODS

Capelin *(Mallotus villosus)* caught off Iceland in January 1987 was transported on ice to Austevoll Fiskeindustri A/S, Western Norway, where it was frozen. After a short thawing time, the fish was minced in a meat grinder. Thereafter 2 g potassium sorbate, 0.2 g ethoxyquin (Raluquin, 66–68.5%) and 22 g formic acid (85%) were blended in per kg mince according to commercial practice. The silage was then divided into three portions which were stored in plastic buckets with tight lids at 2 °C, 20 °C (ambient room temperature) and at 37 °C, respectively. Samples were taken from the minced capelin and from the silages at the start of the experiment and after storage for 1, 4, 7 and 14 days and 1, 2, 3, 4, 5, 6, 9 and 12 months. The silages were stirred daily for the first two weeks and thereafter when samples were taken. The samples were stored at –18 °C until analysis.

Analytical methods

Nitrogen was determined essentially as described by Crooke and Simpson (1971) after digestion at 375 °C. Protein = N · 6.25. Dry weight was determined after drying at 105 °C for 20 hours (until constant weight).

TCA-soluble N (non-protein N) was determined in 10% trichloro-acetic-acid extracts. Total volatile N (TVN), ammonia-N and amide-N (the difference in NH_3-N before and after hydrolysis in 2M HCl) were determined as described by Haaland and Njaa (1988). Ninhydrin-positive extractives in 0.02M phosphate buffer relative to total in 6M HCl-hydrolysates were analysed by the method by Moore and Stein (1954).

Amino acid compositions were determined on a Kontron Amino Acid Analyzer Liquimat III after hydrolysing in 6M HCl. Tryptophan was determined in $Ba(OH)_2$ hydrolysates according to Sachse (1981).

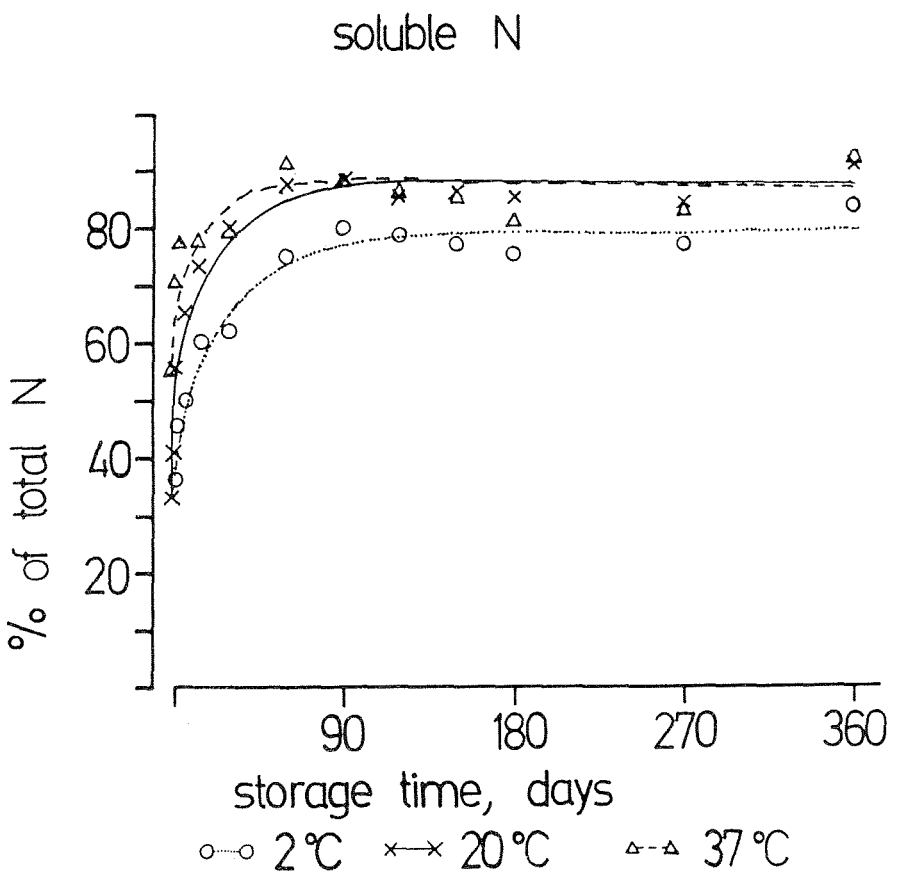
RESULTS AND DISCUSSION

Silage is usually prepared from fresh raw material but freezing of fish before ensiling has been found not to affect autolysis (Gildberg and Raa, 1977; Haard et al., 1985).

The pH-values in the silages were stable at 3.8–4.0 throughout the experiment. The 37 °C silage was quite liquid after one day, the 20 °C silage less so, while the 2 °C silage was more like porridge. During storage all became more or less liquid but there were differences between them throughout the experiment. The protein content varied little, the mean of all samples was 124 g protein/kg silage (SD ± 3.4). Mean dry matter was 291 g/kg (SD ± 7.1) and no increase with time was found.

In the minced capelin before ensiling 13% of total N was TCA-soluble and 10% of total ninhydrin-reactive substances were extracted in phosphate buffer. There were 47 mg amide-N, 8 mg NH_3-N and 13 mg TVN per gram total N. The low TVN-value indicates a fresh raw material.

Fig. 1. Soluble N.



Three capelin silages, produced from the same raw material and stored for 12 months at 2 °C, 20 °C and 37 °C.

TCA-soluble N (Fig. 1) increased from 30 to 80% of total N in about 7 days in the 20 °C- and 37 °C-silages while the corresponding increase took 3 months in the 2 °C-silage. The two former attained a maximum of 85–90% whereas the maximum was somewhat lower, 75–80%, for the last one. 90% soluble N was found in a herring silage stored at room temperature (Espe et al., 1989).

Raa and Gildberg (1976) in short time experiments with cod viscera silage found that the rate of autolysis was significantly faster at 27 °C than at 17 °C. Tatterson and Windsor (1974) working with sprat silages, also found a slower increase in soluble N at 2 °C than at 23 °C, and the former attained a lower maximum than the latter. In white fish offal silages the same authors

Fig. 2. Ninhydrin reactive substances.

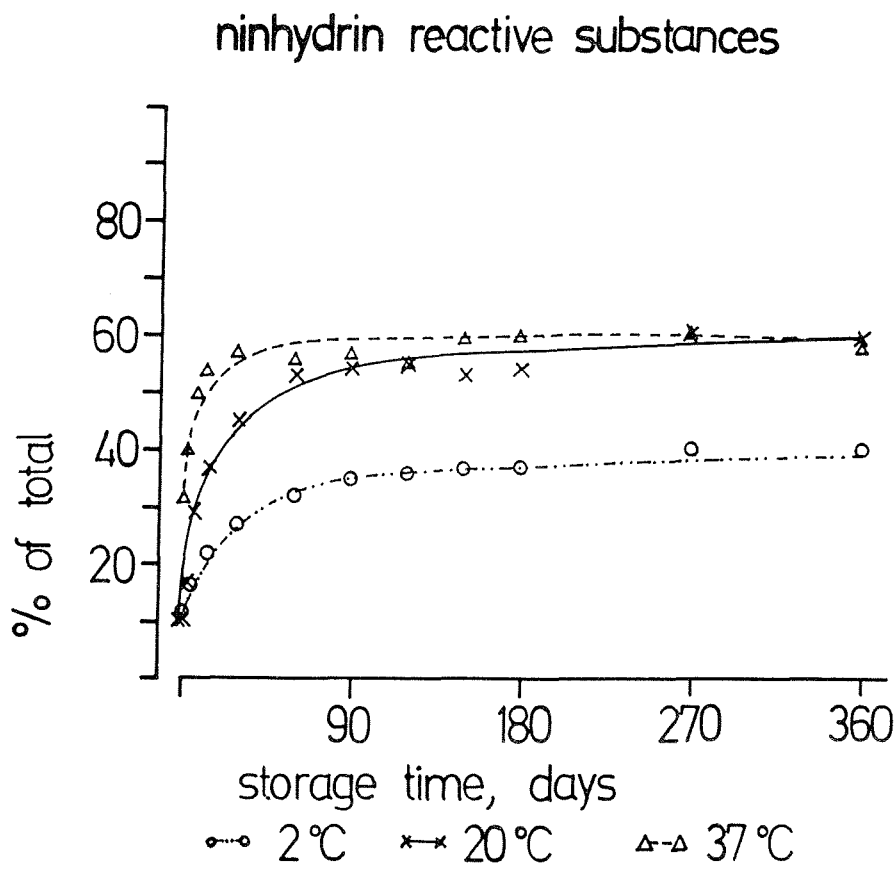


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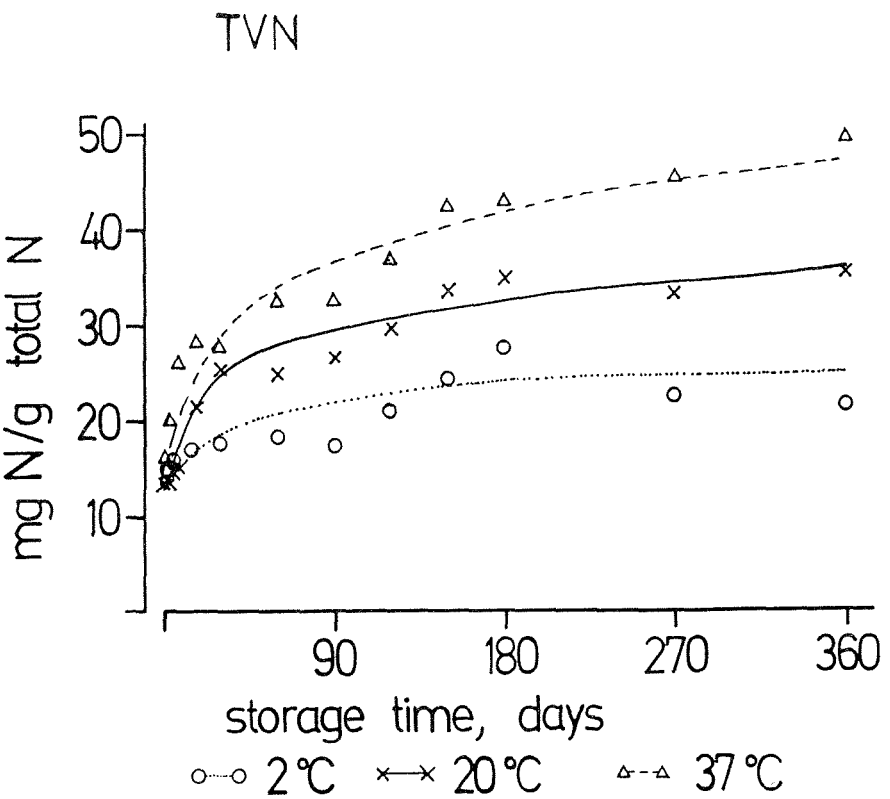
Fig. 3. Total volatile nitrogen.



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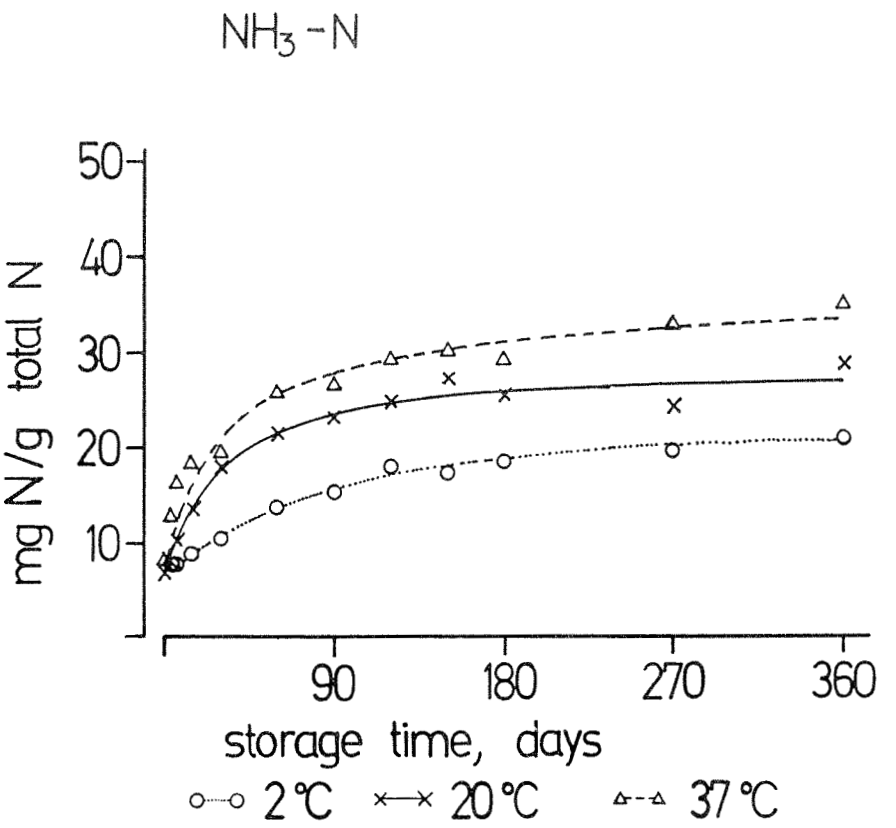
found that the increase was slower at the lower temperature but both silages stabilized at 75–80% soluble protein suggesting that the type of raw material influences the rate of autolysis.

Backhoff (1976) found that while soluble N attained a maximum of 83% of total N in a silage of mixed gut and fish flesh, individual maxima in gut silage and flesh silage were about 77% and 20%, respectively. Similar results was reported by Haard et al. (1985).

Ninhydrin-reactive extractives (ammonia and α-amino-N) (Fig. 2) increased during the first months and stabilized at 50–60% of total ninhydrin-reactive extractives for the 37 °C- and the 20 °C-silages and at 30–40% for the 2 °C silage. Espe et al., (1989) reported a stable level at about 40% in

Fig. 4. Ammonia-N.



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a herring silage after 2 months at room temperature whereas Backhoff (1976) found a maximum in TCA-extracts of around 25% of total N within 7 days at 30 °C in silage when the gut fraction was included.

In contrast to the values for TCA-soluble N and for ninhydrin-reactive substances which in the present silages both stabilized within 1–3 months storage time, TVN (Fig. 3) and NH_3-N (Fig. 4) increased during the whole period, though at a decreasing rate. The increase in TVN was due to the increase in NH_3-N as the difference between the two values remained fairly constant. The amount of NH_3 released at 37 °C was almost twice the amount liberated at 2 °C, and at 20 °C somewhere in between. After one year the silages contained respectively 33, 20 and 28 mg NH_3-N per gram total N.

Backhoff (1976) and Gildberg and Raa (1977) also reported that ammonia increased in stable silages during storage. Amide-N (in glutamine and asparagine) (Fig. 5) decreased in the 20 °C- and 37 °C silages and less pronounced in the 2 °C silage, and since the sum of NH_3-N and amide-N also remained fairly constant at 55–60 mg/g total N, hydrolysis of the amide groups account for most of the ammonia formed during storage (Haaland and Njaa, 1988).

The amino acid composition analysed at zero time and at 3 months showed no significant change (Table 1). This is in accordance with results from, among others, Espe et al., (1989), though it has also been reported that some amino acids may be susceptible to decomposition (reviewed by Raa and Gildberg, 1982).

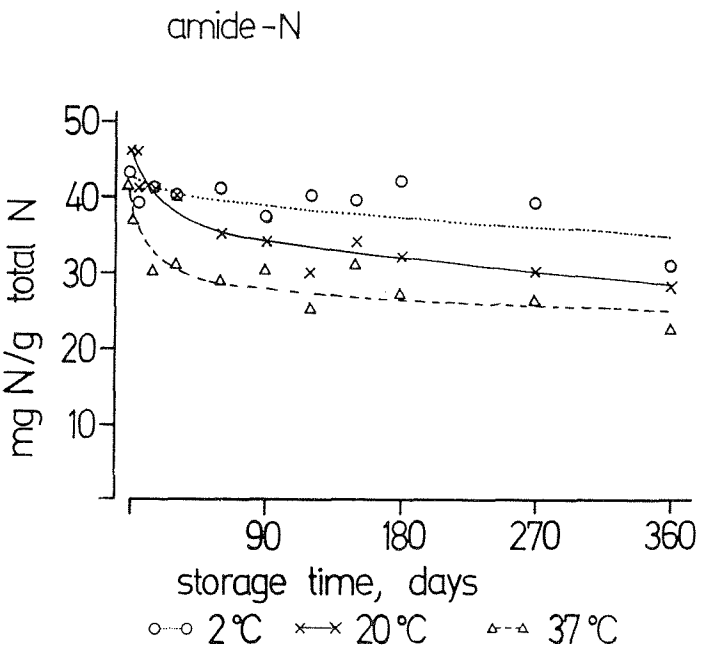
The overall results of the storage experiment showed an influence of temperature both on the degree of autolysis reached after one year of storage and on the degree of hydrolysis of the amide groups. The undissolved fraction which always remains in a silage, was smaller in the silages stored at high temperatures than in the silage at 2 °C. The ammonia released reached only half the value at 2 °C to that at 37 °C.

Table 1. Amino acid composition (mg/g protein) in three capelin silages, produced from the same raw material and stored for 1 day and for 3 months at three different temperatures.

	1 day			3 months		
	2 °C	20 °C	37 °C	2 °C	20 °C	37 °C
PROTEIN (g/kg wet weight)	126	124	128	120	120	122
AMINO ACIDS						
Aspartic acid	103	99	95	104	93	97
Threonine	51	49	46	52	46	48
Serine	49	48	45	50	45	46
Glutamic acid	154	147	140	153	136	142
Glycine	60	58	55	60	55	56
Alanine	65	62	60	66	60	61
Valine	49	46	45	52	50	52
Methionine	28	28	27	26	26	27
Isoleucine	39	38	37	42	42	43
Leucine	81	77	73	84	76	78
Tyrosine	38	36	35	39	36	36
Phenylalanine	40	38	37	41	37	39
Lysine	91	88	86	94	88	90
Histidine	22	21	21	23	21	22
Arginine	62	60	58	63	59	60
Tryptophan	n.a.[1]	10	n.a.	n.a.	10	n.a.

[1] not analysed.

Fig. 5. Amide-N.



Three capelin silages, produced from the same raw material and stored for 12 months at 2 °C, 20 °C and 37 °C.

ACKNOWLEDGEMENTS

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