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CHEMICAL COMPOSITION AND VARIATION IN SOME PARAMETERS DURING STORAGE OF 8 FORMIC ACID SILAGES PREPARED FROM CAPELIN

By

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ABSTRACT

Eight formic acid silages were prepared from the same batch of frozen capelin. Before ensiling, portions were given varying treatments which resulted in different chemical characteristics of the raw materials ensiled. The silages were stored for one year and samples were taken for chemical analyses at intervals.

The parameters analysed were dry matter, protein, fat, ash, water soluble and TCA soluble nitrogen, TMA N, DMA N, TMAO N, NH_3 N, amide N, free fatty acids, peroxide value, anisidine value, iodine value and biogenic amines. Further, counting of living bacteria and of mold were performed.

Several parameters varied with the length of storage time , and regression equations relating the parameters to the logarithm of the storage time were calculated.

The parameters were considered as quality criteria for fish silage. Only total volatile nitrogen (TVN) and biogenic amines seemed to be promising in this connection. Among the amines tyramine was the most promising as it was found to occur in the silages in about the same amounts as were present in the raw materials.

Total volatile nitrogen (TVN) and tyramine may be used primarily to assess whether the raw material was fresh at the time of ensiling. Of these tyramine would seem to be best suited.

The degree of autolysis may be of importance in assessing the age of a silage. The two measures for soluble nitrogen seemed to reach a plateau at about 3 months storage. Thus a better criterion for the degree of autolysis is required.

If routine control should be based on amine analyses more rapid methods are required.

INTRODUCTION

Quality criteria are needed for the evaluation of fish silage intended for use in feeds for fish and fur animals (Espe et al., 1989; Haaland and Njaa 1990). In

this communication data on the chemical composition and on the variation of chemical parameters in 8 silages prepared from frozen capelin and stored for one year, are presented. By pretreatments of the frozen capelin before ensiling, the raw materials used showed various chemical characteristics. The experiment was a joint project by the Central Laboratory and the Institute of Nutrition, both of the Norwegian Directorate of Fisheries.

MATERIALS AND METHODS

Raw materials

Capelin *(Mallotus villosus)* caught off the the coast of Iceland in February 1988 frozen in blocks (20 kg) was used in the experiment. Eight blocks were used, one for each of 8 silages. The blocks were thawn over night in plastic bags at 10-15°C. After thawing three blocks were kept at 10-12°C (MT) for 3,5 and 7 days, respectively. Three other blocks were kept at 2°C (KJ) for 5,10 and 14 days. After these periods the fish was minced and samples of the raw materials were taken before ensiling. The timing was such that all silages were prepared on the same day (day no. 1).

Silages

The minced fish was mixed with potassium-sorbate (2g/kg) and ethoxyquin (EMQ-RALUQUIN, Roche 66%) (250 mg/kg) before formic acid (85%) was added (22g/kg). The silages were identified as MT3, MT5, MT7, KJ5, KJ10 and KJ14. To MT7 and KJ14 2 and 4g extra formic acid respectively, were added on day no. 2 to bring pH to 4.2 or below. In addition to these 6 silages, one was prepared from thawn capelin without prestorage (FRS) and one from thawn capelin steamed for 2hrs at 90°C before ensiling (VB). The symbols used for identification and the various treatments are summarized in Table 1.

Table 1. Description of the formic acid silages prepared from frozen capelin after thawing.

FRS:	Ensiled directly after mincing.
MT3:	Kept at 10 - 12° for 3 days, minced, ensiled.
MT5:	Kept at 10 - 12° for 5 days, minced, ensiled.
MT7:	Kept at 10 - 12° for 7 days, minced, ensiled.
KJ5:	Kept at 2° for 5 days, minced, ensiled.
KJ10:	Kept at 2° for 10 days, minced, ensiled.
KJ14:	Kept at 2° for 14 days, minced, ensiled.

Sampling

The silages were stored in 30 l plastic containers at 10-12°C for one year. During the first week they were stirred daily, later only when samples were taken. Samples were taken on the first day (Day 1) and on day numbers 2, 5, 8, 15, 31 (1 month), 43 (6 weeks), 91 (3 months), 181 (6 months), 271 (9 months) and 361 (12 months). The samples were stored frozen prior to analysis. At each sampling pH values were recorded for all the silages and found to be within 4.0 ± 0.2.

Analyses

The parameters analysed are listed in Table 2. The methods used were those in routine use at the Central Laboratory, (Anon 1974 and 1984) and at the Institute of Nutrition. The latter methods were described by Espe et al., (1989) and Haaland and Njaa (1989 a and b) and Haaland et al., (1990).

RESULTS

In Table 2 are given the results obtained with the 8 raw materials used for ensiling.

Complete proximate analyses of the silages were performed after storage for 1 week (Day 8) and for 3, 6, 9 and 12 months. Mean values are given in Table 3. For comparison results for the raw materials obtained from Table 2 are given in parentheses.

Generally the results showed that dry matter, protein, fat and ash were lower in the silages than in the raw materials. Among the silages dry matter (DM) was significantly lower in FRS, KJ10 and KJ14 than in the others ($p<0.001$). DM in the 1 week old silage was lower than in the silages stored for 3 months or more ($p<0.001$). For protein there were no significant differences neither between length of storage time, nor between silages.

In Table 4 is given a comparison of the protein content in the silages as determined in the two laboratories. For each silage there were 11 protein determinations at each laboratory, 154 determinations altogether. The results were treated in an analysis of variance with laboratories, storage time and silages as the three variables. The analysis showed a highly significant difference between laboratories ($p<0.001$) and no significant differences between storage time and silages. Also there were no significant interactions. The mean difference between laboratories was 4.4g protein per kg silage, for the individual silages the range was from 2.8 to 5.4 g per kg silage (Table 4).

Table 2. Chemical analyses of the raw materials ensiled.

	FRS	MT3	MT5	MT7	KJ5	KJ10	KJ14	VB
DM g/kg	268	265	272	275	258	266	264	288
Prot. g/kg	136	141	143	151	143	145	142	155
Fat g/kg	108	111	110	121	107	113	111	118
Ash g/kg	19	19	22	23	20	19	10	21
W.s. % of total N	50.9	69.5	61.5	57.6	58.7	62.1	73.9	36.8
TCA's % of total N	18	22	28	35	23	30	39	19
TVN % of total N	1.4	3.6	6.8	9.1	4.5	5.7	10.3	1.1
TMA N % of total N	0.2	2.0	2.4	2.5	2.3	2.0	2.7	0.25
DMA N % of total N	0.01	0.02	0.03	0.04	0.02	0.04	0.05	0.06
TMAO N % of total N	2.2	0.5	0.14	0.19	0.31	0.08	0.25	2.0
NH_3 N % of total N	0.5	0.8	2.5	4.2	0.9	2.8	5.0	0.8
Amide N % of total N	5.2	4.8	3.6	3.2	5.0	3.5	2.7	4.9
FFA % of fat	1.9	3.1	4.8	7.7	3.1	5.0	6.3	2.5
Peroxide value	0.8	0.9	1.5	0.5	0.8	0.3	0.3	2.1
Anisidine value	5.0	10.5	3.9	4.0	10.5	5.7	3.2	5.7
Iodine value	113	111.3	112	115	109.2	114.0	113.0	110.0
Putrescine mg/g prot.	-	-	1.6	4.4	0.3	1.3	4.5	
Cadaverine mg/g prot.	-	-	7.9	13.4		8.6	15.7	
Histamine mg/g prot.	-	-	0.3	1.4	-	0.3	2.8	
Tyramine mg/g prot.	-	-	2.6	5.8	-	2.9	5.8	
Phenethylamine mg/g prot.	-	-	0.3	1.3	0.5	2.4		
Bacteria	9000	51×10^3	9.5×10^6	76×10^6	70×10^3	40×10^6	80×10^6	-
Mold	700	3.7×10^3	19×10^3	45×10^4	860	2×10^5	75×10^5	-

Table 3. Proximate analyses of 8 silages from capelin stored for 1 week, and for 3, 6, 9 and 12 months. Mean values over time. (DM Dry matter g/kg; Protein, Fat and Ash g/kg DM).

	FRS	MT3	MT5	MT7	KJ5	KJ10	KJ14	VB
DM	274.6(268)*	279.4(265)	282.2(272)	279.6(275)	279.8(258)	273.0(266)	272.8(264)	297.0(288)
Protein	507.5(507)	497.2(532)	505.0(549)	502.0(549)	499.0(554)	514.2(538)	517.0(538)	517.0(553)
Fat	382.4(403)	372.0(419)	375.0(404)	391.0(440)	381.8(415)	385.2(425)	390.0(420)	403.4(410)
Ash	70.6(70.9)	72.8(71.7)	77.2(80.9)	81.6(83.6)	71.4(71.4)	68.8(71.4)	73.2(75.7)	72.0(72.9)

* Values in parentheses calculated from the data in Table 2.

Table 4. Mean protein contents (SEM) in the silage (g/kg) analysed 11 times during the experiment.

	Central Laboratory	Institute of Nutrition
FRS	139.7 ± 1.2	142.4 ± 1.4
MT3	138.0 ± 1.7	143.4 ± 1.2
MT5	138.4 ± 1.3	143.6 ± 1.1
MT7	138.9 ± 1.5	143.0 ± 1.1
KJ5	137.9 ± 0.9	142.7 ± 1.1
KJ10	138.3 ± 1.2	142.4 ± 1.1
KJ14	138.7 ± 1.1	144.1 ± 1.0
All7	138.6 ± 0.5	143.0 ± 0.4
VB	150.1 ± 2.0	154.4 ± 1.8

SEM = Standard error of the mean.

Most of the other parameters varied with time. The time scale was from day 1 to day 361. It was found practical to use a logarithmic time scale and to give the results for the various parameters as linear regressions of the parameter (y) on the log day number (x). The regression coefficient indicates how fast the parameter changes with time, doubling of the day number corresponds to a change in the parameter of 0.3 times the regression coefficient.

Regression equations for DM and protein on log day number are given in Table 5. DM increased significantly with time of storage at rates from 0.9 to 1.5 g/kg silage for each doubling of storage time. For protein there was no significant effect of storage time. These findings agree with the results referred to in Table 3. The increase in DM was probably due to water being bound when peptide and glyceride bonds were broken during autolysis (Espe et al., 1989). Thus lower protein, fat and ash contents on a dry matter basis would be expected in the silages than in the raw materials. This is borne out in most of the cases listed in Table 3. However, protein content was unaffected by storage time in the data referred to in Table 3 and 5. There is no obvious explanation for this.

The regression equations for water soluble and for TCA soluble nitrogen (Table 5) show that they increased with time but at different rates. Water soluble nitrogen showed rates between 3.7 and 5.5 percentage units for each doubling of the storage time, the corresponding rates for TCA (10% w/v trichloro acetic acid) soluble nitrogen were between 5.9 and 7.5 percentages units. The equations were based on observations up to 9 months for water soluble and up to 12 months for TCA soluble nitrogen. The rates of change were somewhat higher if only observations up to 3 months were included:

Rates were 4.5-6.6, and 6.9-9.6, respectively. The analyses of variance showed, however, no significant differences between silages at any of these times. Water soluble protein did not distinguish clearly between the raw materials, in contrast to TCA soluble protein (Table 2). Silage which was prepared from steamed capelin (VB) showed very low rates of change in either type of soluble nitrogen, rates were 1.1 and 1.3 percentage units.

Table 5. Regression equations for (y): dry matter (DM g/kg), protein (g/kg), water soluble protein (Ws % of total N) and TCA soluble nitrogen (TCAs % of total N) on (x): logday number, and results obtained on day 1.

			r*	Day 1
FRS	DM	y = 3.05x+ 266	+ 0.699	271
	Protein	y = 3.43x+ 532	- 0.182	523
	W.s	y =16.4 x+ 56	+ 0.978	56
	TCA's	y =24.9 x+ 35	+ 0.930	30
MT3	DM	y = 5.53x+ 262	+ 0.875	267
	Protein	y =-5.09x+ 539	- 0.373	539
	W.s	y =17.6 x+ 53	+ 0.941	49
	TCA's	y =23.1 x+ 37	+ 0.935	31
MT5	DM	y = 5.23x+ 269	+ 0.898	272
	Protein	y =-3.63x+ 525	- 0.293	533
	W.s	y =14.0 x+ 56	+ 0.973	54
	TCA's	y =19.8 x+ 40	+ 0.940	35
MT7	DM	y = 3.98x+ 265	+ 0.695	267
	Protein	y = 2.32x+ 525	+ 0.179	532
	W.s	y =14.4 x+ 55	+ 0.983	56
	TCA's	y =18.5 x+ 41	+ 0.939	37
KJ5	DM	y = 4.38x+ 265	+ 0.800	265
	Protein	y = 0.50x+ 525	+ 0.039	532
	W.s	y =13.9 x+ 58	+ 0.966	59
	TCA's	y =21.6 x+ 39	+ 0.951	35
KJ10	DM	y = 4.84x+ 259	+ 0.892	260
	Protein	y =-0.61x+ 533	- 0.058	542
	W.s	y =15.9 x+ 54	+ 0.982	53
	TCA's	y =21.8 x+ 38	+ 0.942	34
KJ14	DM	y = 5.12x+ 257	+ 0.918	260
	Protein	y =-7.27x+ 555	- 0.457	558
	W.s	y =12.2 x+ 61	+ 0.920	56
	TCA's	y =20.0 x+ 41	+ 0.971	37
VB	DM	y = 4.50x+ 283	+ 0.670	286
	Protein	y =-6.54x+ 543	+ 0.259	535
	W.s	y = 3.6 x+ 32	+ 0.615	31
	TCA's	y = 4.1 x+ 21	+ 0.782	19

* r = correlation coefficient

Table 6. Regression equations for (y): TVN, $NH_3 \cdot N$ and amide N (% of total N) on(x): log day number. (TVN and $NH_3 \cdot N$ on day 1 to day 361 (12 months) (n = 10), amide · N from day 2 to day 271 (n = 8))

			r	Day 1
FRS	TVN	y = 0.823x + 0.371	0.923	0.6
	$NH_3 \cdot N$	y = 1.033x + 0.006	0.967	0.4
	Amide · N	y = -0.841x + 5.55	-0.935	0.5*
MT3	TVN	y = 0.787x + 0.695	0.952	0.9
	$NH_3 \cdot N$	y = 1.049x + 0.273	0.942	0.8
	Amide · N	y = -1.056x + 5.78	-0.947	5.3*
MT5	TVN	y = 0.530x + 3.947	0.915	3.9
	$NH_3 \cdot N$	y = 0.914x + 0.173	0.872	2.2
	Amide · N	y = -0.624x + 4.26	-0.841	3.8*
MT7	TVN	y = 0.590x + 7.459	0.785	7.3
	$NH_3 \cdot N$	y = 0.866x + 3.610	0.820	4.1
	Amide · N	y = -0.842x + 3.830	-0.841	3.2*
KJ5	TVN	y = 0.739x + 0.754	0.846	1.0
	$NH_3 \cdot N$	y = 0.808x + 0.650	0.945	1.0
	Amide · N	y = -0.700x + 5.24	-0.812	4.8
KJ10	TVN	y = 0.757x + 5.228	0.815	5.1
	$NH_3 \cdot N$	y = 0.827x + 2.270	0.946	2.4
	Amide · N	y = -0.715x + 4.040	-0.836	3.7*
KJ14	TVN	y = 0.655x + 9.298	0.911	9.0
	$NH_3 \cdot N$	y = 0.812x + 4.310	0.803	4.1
	Amide · N	y = -0.688x + 3.320	-0.747	3.1*
VB	TVN	y = 0.330x + 1.071	0.163	1.1
	$NH_3 \cdot N$	y = 0.218x + 6.90	0.860	0.8
	Amide · N	y = -0.114x + 5.54	-0.464	5.7*

* Day 2

Table 7. Levels of TMA N and TMAO N (% of total N) in raw materials (R) and silages after storage for 1, 3, 6, 9, and 12 months.

	FRS		MT3		MT5		MT7		KJ5		KJ10		KJ14		VB	
	TMA	TMAO	TMA	TMAO	TMA	TMAO	TMA	TMAO	TMA	TMAO	TMA	TMAO	TMA	TMAO	TMA	TMAO
R.	0.2	2.0	2.0	0.5	2.4	0.2	2.5	0.2	2.3	0.3	2.0	0.1	2.7	0.3	0.2	2.0
Day 1	0	2.5	0.1	2.3	1.5	0.1	2.6	0.1	0.1	2.6	2.1	0.6	2.9	0.1	0.2	2.1
1 month	0	2.4	0	2.5	1.6	0	2.7	0	0.1	2.3	2.2	0	3.0	0	0.3	2.3
3 months	0.1	2.3	0.3	2.1	1.6	0	2.6	0	0.1	2.4	2.3	0.3	3.0	0	0.3	2.0
6 months	0.1	2.0	0.2	2.5	1.5	0	2.3	0	0.2	2.3	2.1	0.4	3.0	0	0.2	2.0
9 months	0.2	2.0	0.1	2.5	1.4	0.1	2.5	0.1	0.2	2.6	2.2	0.6	2.9	0.1	0.2	2.1
12 months	0.1	2.0	0	2.1	1.2	0	2.4	0	0.1	2.2	1.9	0.4	2.8	0	0.2	2.0

Table 8. Regression equations for (y): free fatty acids (FFA) in percent of total fat, on (x): log day number, and results obtained on day 1.

			Day 1
FRS	$y = 3.0x + 6.6$	$r = 0.867$	7.3
MT3	$y = 3.0x + 5.5$	$r = 0.969$	5.5
MT5	$y = 2.8x + 5.3$	$r = 0.984$	5.6
MT7	$y = 2.7x + 8.0$	$r = 0.942$	8.4
KJ5	$y = 3.0x + 6.1$	$r = 0.985$	6.0
KJ10	$y = 2.8x + 6.7$	$r = 0.926$	7.4
KJ14	$y = 2.4x + 9.0$	$r = 0.890$	10.0
VB	$y = 1.8x + 4.4$	$r = 0.811$	4.0

Total volatile nitrogen (TVN), trimethylamine N (TMA N), dimethylamine N (DMA N) and trimethylamineoxide N (TMAO N) are commonly used parameters for evaluation of fish raw material quality. It is assumed that DMA N is negligible and that for practical purposes TVN comprises ammonia-N (NH_3 N) and TMA N. In the present experiment DMA N ranged from 0.01 to 0.1% of total protein. The NH_3 N quantities were also determined directly.

Table 2 shows that TVN and NH_3 N increased in the raw material the longer the capelin was kept at 12-15°C before ensiling. They also continued to increase in the stored silages (Table 6). TVN increased at a slightly lower rate (1.6-2.5 mg/g total N) than NH3 N (2.4-3.1 mg/g) for each doubling of the storage time. This difference may be related to the different techniques used in the two laboratories. In the VB silage both parameters varied at very low rates (0.1 and 0.6 mg/g). The variations in TMAO N, TMA N, and DMA N in the silages were not related to the time of storage, indicating that their formation (TMA N and DMA N) or reduction (TMAO N) were arrested in the acid medium of the silages (Table 7). Thus the increase in TVN during storage is mainly due to ammonia formation.

It has been shown previously that the extra amount of ammonia liberated from silages when they undergo mild hydrolysis with 2M HCl decreases nearly in parallel with the increase in free ammonia in silages stored over long time (Haaland and Njaa, 1988). The difference between the total amount of ammonia N in the HCl hydrolysates and the amount of free ammonia N is termed amide N, or labile amide N. This assumes that the ammonia formed in the silages during storage derives from the amide groups of glutamine and asparagine.

In Table 6 the regression equations relating amide N to log daynumber are given together with the equations for TVN and NH_3 N. It is seen that the

Table 9. Peroxide-, Anisidine- and Iodine-values in fat extracted from the silages stored for 1 week, and for 3, 6 and 9 months.

	FRS	MT3	MT5	MT7	KJ5	KJ10	KJ14	VB
7 days								
Peroxide value	0.9	0.7	0.7	0	1.1	0.5	0	2.1
Anisidine value	0.3	2.4	2.1	0	1.2	2.7	0.8	4.7
Iodine value	112.6	120.4	116.8	118.0	120.5	118.3	118.2	108.9
3 months								
Peroxide value	0	0	0.4	0	0	0	0	0
Anisidine value	0	2.9	4.6	2.0	2.1	2.2	1.7	15.1
Iodine value.....	126.0	125.8	123.0	122.4	126.0	122.2	122.4	118.7
6 months								
Peroxide value	1.0	0.6	0.5	0	1.0	0.5	0	1.5
Anisidine value	6.5	7.5	7.8	3.7	6.7	5.7	0.5	18.8
Iodine value	126.7	125.4	125.0	122.3	125.7	123.7	122.8	118.3
9 months								
Peroxide value	2.1	1.0	1.0	4.7	1.6	1.1	1.1	1.4
Anisidine value	10.3	7.8	8.7	5.3	8.4	6.9	3.0	17.2
Iodine value	n.d*	n.d	n.d	n.d	n.d	n.d	n.d	n.d

*nd = not determined

Table 10. Cadaverine (Cad) and Tyramine (Tyr) in silages, fresh (day 1) and stored for 3, 6, 9 and 12 months (mg/g protein).

	MT5		MT7		KJ10		KJ14	
	Cad	Tyr	Cad	Tyr	Cad	Tyr	Cad	Tyr
Day 1		1.2	16.3	5.1	5.5	1.6	14.	5.2
1 month		1.1		4.6	6.3	1.7	9.2	5.3
3 months	3.5	1.0	10.5	4.5	4.6	1.5	12.3	4.7
6 months	7.0	1.3	22.7	6.0	4.5	1.6	22.0	6.0
9 months	7.1	1.3	14.4	4.6	-	1.8	15.8	6.8
12 months	2.2	1.2	11.8	4.5	4.4	1.9	13.3	6.3

three regression coefficients are similar, indicating that amide N was reduced slightly slower than NH_3 N increased. This is in accordance with previous results (Haaland and Njaa, 1988).

Regression equations for percentage free fatty acids of total lipid (FFA) against log day number are given in Table 8. The rate of change ranged between 0.7 and 0.9 percentage units for each doubling of the storage time. The lowest rate was for KJ14. The reason for this was probably that there was already 10% FFA in the raw material at the time of ensiling. This is also reflected in the constant term in the regression equation. The degree of fat oxidation in the silages were estimated by determining the peroxide value, the anisidine number and the iodine value. The results from samples taken after 1 week, 3, 6 and 9 months are given in Table 9. Only the anisidine value seemed to increase with the time of storage, but it is difficult to draw any conclusion as to the relation to the ensiling conditions.

From Table 2 it is seen that amines were present in the raw materials for the silages MT5, MT7, KJ10 and KJ14. In Table 10 the contents of cadaverine and tyramine in the silages are listed. When the amounts found are compared with the amounts present in the raw materials, it is indicated that there was no further production of amines, even when the silages were stored for one year. There are analytical difficulties involved in the amine analysis, with our method more so with the cadaverine determination than with tyramine.

The bacteria and mold counts given in Table 2 varied between the raw materials as would be expected. Count after storage for 1 week and 3 months showed maximum values of 1000 for bacteria and 200 for mold.

DISCUSSION

Fish silage from fresh fish or fish offal liquefy during storage due to endogenous proteolytic enzymes which hydrolyse the protein to short peptides and free amino acids (Raa and Gildberg, 1976, Hall et al., 1985; Stone and Hardy, 1986) When the raw material is cooked before the addition of acid, the silage will have a porridge-like consistence because autolysis is arrested (McBride et al., 1961; Tatterson and Windsor 1974; Wood et al., 1985).

Fish silage may partly substitute for fish meal and fish oil in feeds for farmed fish and fur animals. Two qualities of fish meal are accepted for such feeds, LT (*L*ow *t*emperature) meal and NSM (*N*orsea *m*ink) meal. For both qualities it is required that they are produced from fresh unpreserved raw material, and the criteria for freshness are maximum 40 and 90 mg TVN per 100g raw material, respectively. The requirement for LT-meal quality was recently adjusted from 50 to 40 mg TVN per 100 g. It is reasonable to use the same criteria for fish and fish offal used in silage production. As the water content in raw material and silage may vary, it is practical to express the freshness criteria as TVN relative to total nitrogen. In the present material with a protein content of about 140g/kg the criteria would thus be maximum 1.8 and 4.0%, respectively. With these criteria only the raw materials for FRS and VB would be accepted according to LT requirements, and MT3 for the NSM requirement (Table 2). However, lower values for TVN were found in the silages on day 1 than in the raw materials (Table 6). It is likely that these values are better indicators of the raw material freshness than those given in Table 2 as the samples used for analysis of the raw materials and of the silages were frozen for later analysis.

Bacterial reduction of TMAO was stopped by the low pH in the silages. When raw material and silage samples were thawn to be analysed, further bacterial breakdown of TMAO in the raw material may have taken place. In fact, in the silages FRS, MT3, KJ5 and KJ10 the TMAO N was higher on day 1 than in the raw materials (Table 7). It may therefore be concluded that the silages FRS, MT3, KJ5 and VB were prepared from raw materials meeting the LT-meal criterion for freshness. As shown in Table 6 TVN increased during storage. It may be inferred from the equations that the 2% level of TVN would be reached for FRS, MT3 and KJ5 between 6 weeks and 3 months storage time. This was also borne out in the actual analyses. For VB it would take 21 months before TVN reached 2% of the total nitrogen, and it would take years for FRS, MT3 and KJ5 to reach the 4% level.

It is concluded that silages showing up to 2% of total N as TVN were produced from raw materials meeting the freshness criterion for LT-meal production. As there is a continuous liberation of NH3 N even at low temperatures (Table 6, and Haaland and Njaa, 1989 a and b) the TVN level

may increase above 2% during relatively short storage periods. Therefore, TVN is not a good criterion for raw material freshness. Levels of TVN approaching 4% of total N may either mean that the silages were freshly prepared from raw material just meeting the NSM-meal freshness criterion (MT5, Table 6), or they were produced from a fresh raw material and stored for a long time.

The parameters measured in this experiment were not well suited to evaluate the age of the silages. Water soluble protein and TCA soluble protein varied and tended to approach constant levels between 1 month and 3 months of storage. Free fatty acids were represented by rather similar regression equations, but particularly FRS, MT3 and KJ5, those from the best quality raw materials, showed rather great differences between FFA measured in the raw materials (Table 2) and in the silages on the first day (Table 8).

It is possible that determination of free α-amino groups would be of value to more critically determine to what degree and at what rate pepide bonds were broken during the storage time. Determination of ninhydrin active substances was tested but as NH_3 N and amines react with ninhydrin, no conclusive results were obtained. A method specific for α-amino groups was not available.

Cadaverine which is formed by decarboxylation of lysine, was recently introduced as a quality criterion for LT-meal. The requirements are maximum 1 g cadaverine/kg meal i.e. 1.5 g/kg protein, and a minimum content of protein of 680 g/kg (Anon 1990). The amount of cadaverine present in the raw material is recovered in the meal in contrast to TVN.

In the present experiment putrescine, cadaverine, histamine,tyramine and phenethylamine were considered as possible criteria for the quality of silage raw material. Histidine, the parent amino acid for histamine varies so much between fish species that histamine was considered to be of little use. Putrescine, which is formed from arginine over ornithine is not an end product as it is also used in the syntheses of spermine and spermidine. The amounts found of phenethylamine are small and may be difficult to use as a criterion for that reason.

In Table 2 are listed the analyses of amines in the raw materials, and in Table 10 are given the analyses of cadaverine and tyramine in the silages where they were found. In our hands the analysis of tyramine was easier and more reproducable than the analysis of cadaverine. The concentrations of tyramine were practically the same during the 12 months of storage in the four silages found to contain this amine. The concentrations in day 1 samples are considered to give a better indication of the contents in the raw material for the same reason as mentioned for TVN and NH_3 N. The results also

indicated that cadaverine was constant within silages, the reason for the greater variation is believed to be due to difficulties in the evaluation of the chromatograms. The requirement that LT-meal to contain maximum 1g cadaverine/kg is equivalent to about 1.5g/kg protein or about 2.5% of the lysine. Assuming that decarboxylations of lysine and of tyrosine are proportional, 0.6 g tyramine/kg protein is suggested as a maximum in raw materials of LT-meal quality, and also for high quality silage.

At present amine analyses are tedious so more rapid methods are required if such analyses are to be used in the evaluation of feed ingredients for fish and fur animals. Of the methods studied in this experiment only cadaverine and tyramine determinations seem promissing. Based on the suggested maximum value of 0.6 g tyramine/kg protein only the raw materials for FRS, MT3 and KJ5 would be accepted. Tyramine was suggested to be a better criterion for the quality of vacuum packed meat than cadaverine (Edwards et al., 1987).

Silage VB from cooked raw material showed low values for most of the parameters evaluated, except for peroxide value and anisidine value. It is however difficult to include these as quality criteria as experience shows very great variation between laboratories (Pettersen et al., 1989).

CONCLUSIONS

Of the parameters considered as possible quality criteria for formic acid silages of fish and fish offal only total volatile nitrogen (TVN) and either of the amines cadaverine or tyramine seem to be useful for the evaluation of raw material. For the amines it must be agreed upon maximum values accepted.

TVN values below 1.8 - 2.0% of total N indicate that the raw material used meet the requirement for the production of LTmeal. Similary, values below 4% of total N indicate raw material requirement for NSM meal production. However, values exceeding these values may be due to long time storage of the silages as there is a continuous increase in TVN during storage. Criteria for estimation of the age of the silages, and for the degree of autolysis are lacking.

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