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BREAKDOWN PRODUCTS OF TRIMETHYLAMINE-OXIDE IN AIRDRIED STOCKFISH. MEANS OF ENHANCING THE FORMATION OF FORMALDEHYDE AND DIMETHYLAMINE.

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ABSTRACT

Fish fillets contain trimethylamineoxide (TMAO) which is reduced to trimethylamine (TMA) during bacterial spoilage, but which also may be split into dimethylamine (DMA) and formaldehyde (FA) by an enzymatic prosess in the fish fillet. TMAO TMA, DMA and FA were determined in samples of fish fillets of the cod family in twelve experiments. Fillets with or without skin were used, and partly pretreated by one month's frozen storage and by irradiation. Samples were taken after cold storage from 0 to 6 weeks. Fillets were thereafter dried in a windtunnel and stored for 2 years.

It was found that a pre-freezing period as well as storage of the fillets with skin enhanced the formation of DMA and FA. This resulted in a later formation and lower levels of TMA. This effect reached a level where irradiation of the fillet gave no further decrease in the TMA-formation.

If FA and DMA were formed in proportional amounts, then part of the FA was not available for analysis. Low levels of FA were found in the dried and dry-stored fillets particularly. As FA is formed in the fillet during cold storage, it exerts a bacteriostatic effect in the fillet.

A treatment of fish fillets which enhances the formation of DMA and FA from TMAO should improve the keeping quality even in an airdried product.

INTRODUCTION

Trimethylamineoxide (TMAO) is found in marine fish fillets in concentrations of 20 to 120 mg TMAO-nitrogen per 100 g fillet. Up till now, little is known about the biochemistry of TMAO (RUITER, 1971). During bacterial spoilage of the fish, TMAO is reduced to trimethylamine (TMA). By reducing the effect of bacterial spoilage, it has been shown that TMAO is broken

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down to dimethylamine (DMA) and formaldehyde (FA) in proportional amounts by an enzyme in fish of the cod family, (AMANO and YAMADA, 1964). This formation may be enhanced by frozen storage of the fish (TOKUNAGA, 1970). Relations between the two types of breakdown reactions were studied by JEBSEN et al., (1972), JEBSEN, (1972) and UNDERDAL et al., (1973). They avoided bacterial spoilage by irradiation of the fish fillets. During these experiments it was found that storing the fillet with skin resulted in a further enhancement of the DMA/FA formation.

A formation of formaldehyde «in situ» in the fillet during storage should give a bacteriostatic effect. This should be of advantage for fish products needing long storage, such as airdried stockfish. The present paper reports analyses of FA, DMA, TMA and TMAO in fish fillets before and after airdrying and dry storage. Fillets were stored with or without skin, and were partly pretreated by irradiation and/or freezing. They were stored at 0°C up to 6 weeks, followed in part by airdrying at 30° and 2 years of storage of the dried and ground fish fillets.

MATERIAL AND METHODS

Samples and treatments. Small saithe were obtained alive at the local fish market and immediately filleted. Ling was used in one experiment. Series of fillets with or without skin were packed in portions of 500 g in plastic bags (Mylothene 58/3) using the vacuum packing technique.

Series of samples were frozen at ÷25°C and kept frozen for one month.

Series of frozen samples were irradiated with a dosis of 200 krad at the Institute of Atomic Energy, Kjeller, Norway. (JEBSEN et al., 1972). The irradiation was performed in the first week of the freezing period.

All series were stored at 0°C and samples taken out for analysis or further treatment at intervals between 0 and 6 weeks.

Series of fillet samples were dried in a wind tunnel of 0.35 m^2 cross section and a length of 1.5 m. The temperature was kept at 30°C and the relative humidity at 60%. The drying period was one week, after which the fillets had reached a moisture content of 7–8%.

The dried fillets were stored in closed plastic bags for 2 years at room temperature.

Methods of analysis. Formaldehyde was determined by distilling a sulfuric acid extract of the homogenized sample and reacting the distillate with chromotropic acid followed by colorimetry. A calibration curve was made by using hexamethylenetetramin as a source of FA, and fresh fish fillet as a blank (BREMANIS,, 1949, ANTONACOPOULIS, 1960). FA was calculated as mg per 100 g fresh fillet.

Dimethylamine was determined by distillation of a protein-free extract of the sample (100 g of fresh fillet, correspondingly less dried fillet), and complexing the distillate with an alkaline copper solution, followed by extraction and colorimetry, (DYER and MOUNSEY, 1945). A protein-free extract was made by adjusting the suspension to pH 5.2, adding colloidal $Fe(OH)_3$, heating to 70°C and filtering.

Trimethylamine was determined by the Conway microdifussion method. Details of the method are given by KJOSBAKKEN, (1970).

Trimethylamineoxide was determined by the microdiffusion method of Conway after reduction by $TiCl_3$ and correcting for volative bases (RONOLD and JAKOBSEN, 1947).

DMA, TMA and TMAO were calculated as mg N per 100 g original wet fillet, i.e. corrected for loss of moisture during drying.

RESULTS AND DISCUSSION

Results of the determinations of FA, DMA, TMA and TMAO in 12 experiments are given in Table 1. These experiments were done during the years 1973–1976 and the sampling times do not correspond throughout. The table shows that in some experiments there was no sampling at weeks 0, 3, 5 and 6. Parallel sets of fillets with and without skin were analysed for all experiments except for no. 1 and no. 6. Ling was used in experiment no. 6, whereas saithe was used in all the others.

Experiment 1 gives the normal picture of a fish fillet stored at 0°C without any pretreatment. Low levels of FA and DMA were found at all sampling times, where as TMA rose quickly after one week of storage and all TMAO had disappeared within 2 to 3 weeks of storage.

Experiment 2 shows the effect of one month of frozen storage as a pretreatment. JEBSEN (1974) found that the effect of prefreezing the fillets increased from 24 hours up to 6 weeks at ÷25°C. In all the present experiments, the pre-freezing time was set at one month. The levels of FA and DMA varied widely in this experiment, but were clearly higher than in experiment 1. High TMA values were found after 4 weeks of cold storage, i.e. the time before spoilage was doubled compared with expt. 1. TMAO disappeared after 3 weeks.

Experiment 3 shows the effect of storing the fillets with skin. FA and DMA increased substantially compared with the values from fillets without skin, TMA did not rise above 10 mg N/100 g, and TMAO was still present after 6 weeks. The ratio between FA (in mg/100 g fillet) and DMA (mg N/100 g) was 1.37 as an average for weeks 3, 5 and 6. The stoichiometric ratio between FA and DMA is 2.14, and therefore nearly half of the FA must have disappeared or was unavailable for determination.

Experiment 4 includes irradiation of the fillets. This treatment excludes bacterial spoilage. Only small levels of TMA were found, and 60% of the presumed original TMAO was still present in the last four sampling times. Less DMA was found in this experiment than in expt. 3. pointing to a specific effect of storing the fillets with skin. FA/DMA-ratio was 1.58, so that proportionally more FA was available in the fillets in this experiment.

Experiment 5 paralleled expt. 4 but the fillets were stored with skin. Again very low levels of TMA were found, but the TMAO-content fell to 30% of the original content, and DMA as well as FA increased substantially, compared with expt. 4. It must be concluded that the enzymatic formation of DMA and FA from TMAO is increased with the presence of skin. As these fillets were irradiated, the skin did not simply act as a protection against bacteria. A possible explanation is that the splitting enzyme is concentrated under the skin or in the dark muscle which is removed in part by the skinning prosess. The FA/DMA-ratio was 1.46, i.e. between those found in expts. 3 and 4.

Experiment 6 was done on fillets of ling, which evidently had a higher original level of TMAO, ca. 80 mg N/100 g. The drying prosess introduced in this expt. did not result in losses of the nitrogen bases, as seen in the column of sums. TMA was found at the usual low levels, and TMAO fell to about 50% of the original level, with corresponding increases in the contents of FA and DMA. Part of the FA must have been lost during the drying prosess, and the FA/DMA-ratio had fallen to 0.79.

Experiments 7 and 8. A storing time of 2 years of the dried product equalled out all differences. The analytical values given in expts. 7 and 8, Table 1, were nearly the same with and without skin for all sampling times. TMA had disappeared completely, and 70% of the original TMAO content was found as DMA. The ratio FA/DMA was down to 0.57 so that more than 70% of the theoretically formed FA had disappeared or was not available for analysis. A small effect of storing the fillets with skin may possibly be found in the somewhat higher DMA values and lower TMAO values for the last two sampling times in expt. 8 compared with expt. 7. The high levels of DMA in the two first sampling times compared with expt. 6 are difficult to explain, as they point to a further formation of DMA during the storage of the dried fillets. Nearly all the nitrogen bases originally present as TMAO are accounted for even after a storage time of 2 years of the dried product.

Experiments 9 and 10 give results on dried and stored fillets, with and without skin, prefrozen, but without irradiation. As a consequence, TMA increased from the third week, less in the samples with skin than in those without skin. Some of the TMA must have disappeared, as the sum of bases decreased from week 3. The high values for DMA in the first three samplings, about 60% of the original TMAO, correspond to those in expts. 7 and 8. The effect of drying and storage of the fillets can be seen when the

DMA values in expts. 9 and 10 are compared with those in expts. 2 and 3. The major effect was a substantial decrease in available FA. Another difference worth noting is the higher DMA-values in the first three weeks, mentioned above.

Experiments 11 and 12. The final experiments were based on dried and stored fillets, with and without skin but without any pretreatment. As in expt. 1, TMA increased from week 2 in fillets without skin, whereas the onset of TMA-formation was delayed until week 3 in the fillets with skin. Lower values for TMA as well as for the sum of N-bases points to a disappearence of TMA during the drying prosess and storage, corresponding to the results of expts. 9 and 10. Low values were found for FA and DMA in the latter samplings corresponding to expt. 1. However, higher values for FA and DMA were found in the two first samplings, corresponding to expts. 7 to 10, i.e. to all fillets which were stored after drying. This later formation of FA/DMA in samples of fillet with high TMAO (week 0, 1 and 2) could be explained by an increased enzyme activity during the drying prosess at 30°C. Expt. 6, however, giving results after drying of ling fillets, does not show correspondingly high initial values for FA/DMA. The ling used in expt. 6 were bigger fish than the saithe used in the other experiments, and the ling fillets were thicker, possibly resulting in a slower diffusion of substances needed for the reaction. If expt. 6 is comparable to the other experiments, then the high initial FA/DMA-values in expts. 7 to 12 must be an effect of the 2 years of storage after drying, and therefore a spontaneous reaction, not an enzyme activated reaction.

Conclusions. Table 2 gives averages of DMA and TMA in samples from week 3 to 6, shown as percentages of the sums for N-bases. This presentation points out some conclusions.

1. 5 sets of experiments were done on fillets with and without skin. Comparing the values in Table 2, we find an average increase of 50% in the DMA contents, and a decrease of 35% in the TMA contents, in fillets with skin relative to those without skin. Non-irradiated samples with skin gave delayed TMA formation of at least one week compared with samples without skin.
2. Comparison of the experiments 2–1, 9–11 and 10–12 shows the effect of a freezing period before the cold storage of the fillets. Freezing gave an increase of 150 to 300% in the DMA contents, and a decrease of 33–65% in the TMA contents.
3. Comparison of expts. 3 and 5 shows fairly similar results. Consequently, the combined effect of freezing and skin has avoided bacterial spoilage to such an extent that irradiation gave no further effect.

Table 1. The contents of formaldehyde (FA, mg/100 g fillet), and dimethylamine nitrogenbases (all in mg N/100 g wet fillet), in fish fillets after storage at 0° C

Weeks at 0°	FA	DMA	TMA	TMAO	SUM	FA	DMA	TMA	TMAO	SUM
	1. No treatment, ÷ skin.					2. Frozen, ÷ skin.				
0	0	1	0	36	37	—	—	—	—	—
1	5	5	11	27	43	20	11	3	36	50
2	10	6	32	4	42	—	—	—	—	—
3	5	8	33	0	41	29	18	4	31	53
4	8	8	32	0	40	11	—	31	2	—
5	4	8	40	0	48	9	14	35	0	49
6	3	8	37	0	45	10	11	45	0	56
	5. Irradiated, frozen, + skin.					6. Irradiated, frozen, dried ÷ skin (ling).				
0	—	—	—	—	—	15	12	3	64	79
1	33	19	3	27	49	20	11	2	68	81
2	—	—	—	—	—	22	23	4	61	88
3	45	30	3	15	48	33	40	0	43	83
4	46	—	2	14	—	27	28	7	35	70
5	34	27	4	18	49	21	39	0	39	78
6	49	31	9	12	52	—	—	—	—	—
	9. Frozen, dried, stored, ÷ skin.					10. Frozen, dried, stored, + skin.				
0	16	29	0	25	54	20	24	0	20	44
1	12	33	0	21	54	20	33	0	17	50
2	9	32	0	18	50	17	36	2	11	49
3	8	26	6	12	44	11	29	3	5	37
4	2	15	25	1	41	13	25	13	3	41
5	—	—	—	—	—	—	—	—	—	—
6	2	14	27	0	41	13	26	12	1	39

(DMA), trimethylamine (TMA), trimethylamineoxide (TMAO) and the sum of from 0 to 6 weeks, in 12 experiments of different treatment (see text).

FA	DMA	TMA	TMAO	SUM	FA	DMA	TMA	TMAO	SUM	Weeks at 0°
3. Frozen, + skin					4. Irradiated, frozen, ÷ skin.					
—	—	—	—	—	—	—	—	—	—	0
35	21	3	27	51	21	10	2	41	53	1
—	—	—	—	—	—	—	—	—	—	2
49	31	1	18	50	38	20	1	29	50	3
54	—	1	12	—	29	—	5	32	—	4
38	34	5	11	50	27	20	3	29	52	5
53	36	10	3	49	25	16	4	31	51	6
7. Irradiated, frozen, dried, stored, ÷ skin.					8. Irradiated, frozen, dried, stored, + skin.					
17	33	0	21	54	17	32	0	16	48	0
14	31	0	20	51	20	35	0	17	52	1
19	34	0	17	51	21	33	0	15	48	2
18	37	0	17	54	22	36	0	10	46	3
28	34	0	12	46	20	39	0	8	47	4
—	—	—	—	—	—	—	—	—	—	5
17	34	0	15	49	18	38	0	7	45	6
11. Dried, stored, ÷ skin.					12. Dried, stored, + skin.					
17	28	0	28	56	20	26	0	27	53	0
9	24	2	18	44	12	23	0	11	44	1
1	9	16	10	35	2	15	0	7	22	2
0	9	25	7	41	0	12	16	5	33	3
0	7	23	5	49	0	3	29	0	32	4
—	—	—	—	—	—	—	—	—	—	5
—	—	—	—	—	—	—	—	—	—	6

Table 2. The contents of DMA and TMA, as averages for all sampling times from week 3, given as percentages of the sum of N-bases.

Expt.	Treatment		DMA,%	TMA,%
1	No treatment	÷ skin	18,4	81,6
2	Frozen	÷ skin	27,2	54,6
3	»	+ skin	67,8	8,6
4	Frozen, irradiated	÷ skin	36,6	6,4
5	» »	+ skin	59,0	9,1
6	Frozen, irradiated, dried	÷ skin	46,3	3,0
7	Frozen, irradiated, dried, stored	÷ skin	70,5	0
8	» » » »	+ skin	81,9	0
9	Frozen, dried, stored	÷ skin	43,6	46,0
10	» » »	+ skin	68,4	23,9
11	Dried, stored	÷ skin	17,8	68,9
12	» »	+ skin	23,1	69,2

4. Table 2, expt. 1 shows a maximum formation of 82% of TMA from TMAO, the rest being DMA. Expt. 8 shows a maximum formation of 82% of DMA, the rest being TMAO.
5. Less than proportional quantities of FA were available for analysis compared with the DMA values. Maximum values of 60% of the theoretical amounts were found in expts. 3 and 5, whereas values down to 10% of theoretical amounts were found in dried and stored products. FA is formed continously during the cold storage and seem to give a bacteriostatic effect in the fillets even in such small quantities.

Any treatment of fish fillets which will enhance the enzymatic splitting of TMAO into DMA and FA should according to these experiments improve the keeping quality even in an airdried and stored product.

REFERENCES

AMANO, K. and YAMADA, K., 1964, Bull. Jap. Soc. Scient. Fish., *30*, 639–645.

ANTONACOPOULOS, N., 1960, Vorl. Mitt., Inst. für Fishverarbeitung, Bundesforsch. Inst. Fisherei, Hamburg.

BREMANIS, E., 1949, Z. Anal. Chem., *130*, 44–

DYER, W.J. and MOUNSEY, Y.A., 1945, J. Fish. Res. Bd., Can., *6*, 359–

JEBSEN, J.W., 1972, Ann. Rep., Dir. Fisheries, Bergen, Norway.

JEBSEN, J.W., 1974, Comm. de 5. Reunion, Ass. Techn. Ouest-Europ. Produits de la Peche, Nantes., France.

JEBSEN, J.W., LUNDE, G. and UNDERDAL, B., 1972, Fisk. Dir. Skr. Ser. Tekn. Unders., *5*, no. 11, 14 p.

KJOSBAKKEN, J., 1970, Rapp., Inst. for Tekn. Biokjemi, N.T.H., Trondheim.

RONOLD, O. A. and JAKOBSEN, F., 1947, J. Soc. Chem. Ind., *66*, 160–166.

RUITER, A. 1971, Voedingsmiddeltechnologie, *2*, no. 43, 1–10.

TOKUNAGA, T., 1970, Bull. Jap. Soc. Scient. Fish., *36*, 510–515.

UNDERDAL, B, JEBSEN, J.W. and LUNDE, G., 1973, Fisk. Dir. Skr. Ser. tekn. Unders., *5*, no. 14, 15 p.