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Methionine Oxidation in Commercially and Experimentally Produced Fish Meals

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ABSTRACT

The amounts of methionine oxidized to methionine sulphoxide were determined in 36 fish meals produced in 1988 and in 86 fish meals produced in 1975.

The 1988 meals were of three quality grades based on the freshness of the raw material. Total methionine (methionine plus methionine sulphoxide) and unoxidized methionine were slightly higher in the meals of the best quality than in the two other quality grades.

The 1975 meals were dried either directly by flame or indirectly by steam. The raw material was unpreserved or preserved with a mixture of sodium nitrite and formalin, and the meals were either protected by addition of an antioxidant or unprotected. There were no significant effects of type of drier or of antioxidant protection on the degree of methionine oxidation whereas there was significantly less oxidized methione in meals from preserved raw material. The various treatments showed little effect on the content of total methionine.

In model experiments with fish fillet meals, no obvious effects of oxidizing atmospheres and additions of unsaturated fat (cod liver oil) and trimethylamine oxide (TMAO) on methionine oxidation were found. During storage for 1–2 years a slow oxidation of methionine took place.

INTRODUCTION

The methods used in fish meal production are continually developed and improved. Practically all Norwegian fish meals are now produced from

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unpreserved raw material. They are dried indirectly in steam-heated driers and antioxidant (ethoxyquin) is added after drying. Three quality grades are produced, based mainly on the freshness of the raw material as measured by the amount of total volatile nitrogen (TVN).

Some 10–15 years ago the situation was radically different. When the raw material had to be stored before production it was preserved with a solution of formalin and sodium nitrite (V65: 150 ml 40% (w/v) formalin + 37·5 g $NaNO_2$ per litre). Drying was either direct by flame or indirect by steam and the addition of antioxidant was variable.

One reason for this change in production procedures was that the fur animal and the fish farming industries demanded fish meals from unpreserved raw material. Fish meal is a major ingredient in dry feed for farmed cold water fish reared in Norway. Unpreserved raw material is also required for production of fish meal used by humans as a health food (FPC—fish protein concentrate). During the first 6 months of 1989, 15 tons of FPC have been sold in Norway. FPC is also used to some extent by humanitarian organisations in their programs in developing countries.

In fish meal, usually 6–12% of the methionine has been found to be present as methionine sulphoxide (Slump & Schreuder, 1973; Njaa, 1980; Aksnes, 1984; Haaland *et al.*, 1989) but higher values have also been indicated (Gjøen & Njaa, 1977). Feed composition data and published amino acid requirements (NRC, 1981) indicate that the sulphur-containing amino acids may be first limiting for the protein utilization in farmed fish. It is therefore of interest to obtain information on the degree of methionine oxidation in fish meals produced by the procedures used today.

In the present paper, data for total and unoxidized methionine of commercial fish meals from 1988 are compared with data from the 1975 production. Results obtained with model fish meals produced from fish fillets in laboratory experiments are also recorded.

MATERIALS AND METHODS

Commercial meals

The samples were obtained from the Norwegian Herring Meal Control. The 1988 meals were all produced from unpreserved raw material and antioxidant (ethoxyquin) was added after steam drying. For LT-grade (low-temperature drying), TVN <50 mg/100 g raw material (or about 20 mg/g total N) is required. The NSM-grade (Norseamink) requires TVN <90 mg/100 g (or about 35 mg/g total N). LT- and NSM-meals are used in dry feeds for fish and for fur animals. When TVN >90 mg/100 g, raw material meals of STD-(standard) quality are produced.

Of the 1975 samples, 29 were produced from V65-preserved and 57 from unpreserved raw material, 44 were dried by flame and 42 by steam, and antioxidant was added to 47 and not added to 39.

Model meals

Effects of different atmospheres after drying

Ground fillets of four fish species, herring, saithe, salmon and halibut, were freeze-dried together in separate aluminium pans.

In five runs of the drier air, O_2, CO_2, N_2 and air, respectively, were introduced to the drying compartment after the drying was completed. The atmospheres were kept for 1 h before the portions were taken out, soaked in hexane overnight, filtered, air-dried and finely ground. The meals were analyzed 2 weeks after they were produced and again after storage at room temperature for 16 months.

Addition of cod liver oil and trimethylamine oxide

To one 1 kg portion of ground fillets of saithe, 50 g cod liver oil (clo) were added; another portion was without this addition. Two-hundred-and-fifty gram samples of each of these portions were mixed with graded amounts of trimethylamine oxide hydrochloride (TMAO · HCl) corresponding to 0, 28, 56 and 112 mg TMAO-N per 100 g fillet. Each sample was divided in two (125 g each). One of these was freeze-dried immediately and one was kept for 3 days at room temperature before freeze-drying. The 16 samples so obtained were ground and analyzed shortly afterwards and then again after storage at room temperature for about 2 years.

Chemical analyses

Protein (N × 6·25) was determined after a micro-Kjeldahl digestion at 370°C (Crooke & Simpson, 1971).

Unoxidized and total methionine were determined in $Ba(OH)_2$-hydrolysates according to Njaa (1980).

All analyses were run in duplicate.

RESULTS AND DISCUSSION

Commercial meals

The meals produced in 1988 and in 1975 had similar protein contents. Mean values and ranges were 719 (619–746) and 703 (672–745) g/kg, respectively. Mean total (*T*) and unoxidized (*U*) methionine over all samples were also similar (Tables 1 and 2).

TABLE 1
Total (T), Unoxidized (U) (mg/g protein) and Fraction of Unoxidized (U/T) Methionine in 36 Fish Meals Produced in 1988 and Analysed early in 1989

Meal	*All*	*LT*	*NSM*	*STD*	
n	36	12	12	12	
T	30·6	$31·6^a$	$29·8^b$	$30·2^b$	*
SD	0·96	0·73	0·80	1·00	
U	27·7	$29·3^c$	$27·0^d$	$26·8^d$	*
SD	1·38	0·45	1·18	0·76	
U/T	0·908	$0·927^e$	$0·907^e$	$0·889^e$	NS
SD	0·037 8	0·022 9	0·053 3	0·021 8	

The fish meals were of three quality grades: LT, TVN < 50 mg/100 g raw material; NSM, TVN < 90 mg/100 g raw material; STD, TVN > 90 mg/100 g raw material.
Significant difference is denoted by different superscript letters.
* $p < 0·001$.
NS, not significant.

The 1988 samples showed low degrees of methionine oxidation (Table 1). The concentrations of both total and unoxidized methionine were slightly, but significantly, higher in the LT-samples than in the NSM- and STD-samples. There were no significant differences in total and unoxidised methionine contents of the latter two meals. The degree of methionine oxidation as indicated by the U/T-values was generally low and not significantly different between the three grades of meals. All except two NSM-samples had U/T-values less than 0·85, corresponding to 15% oxidation of the methionine. Thus, the storage of the raw material and/or the higher temperatures used with the NSM- and STD-samples may have resulted in a small loss of methionine. Previously it has been found that whole herring showed a slight decrease in total methionine during anaerobic storage at 2°C for 21 days (Haaland *et al.*, 1988). Experiments with rats and chicks have shown that protein sources with much higher degrees of methionine oxidation are utilized practically as well as protein sources with low degree of oxidation (Slump & Schreuder, 1973; Sjøberg & Bostrøm, 1977; Gjøen & Njaa, 1977; Haaland *et al.*, 1989). There is thus no reason to expect that the low degree of oxidation found will affect the quality of these protein sources in feeds for fish and fur animals.

The 1975 results give more information as to which factors, i.e. type of drier (S = steam, F = flame), preservation ($\pm P$) and antioxidant ($\pm A$) might influence methionine oxidation. It must be stated, though, that the samples are from different reduction plants using widely different techniques. One of

TABLE 2

(a) Total (*T*), Unoxidized (*U*) (mg/g protein) and Fraction of Unoxidized (*U/T*) Methionine in 86 Fish Meals Produced and Analysed in 1975, Dried Directly by Flame (*F*) or Indirectly by Steam (*S*), With or Without Preservation of the Raw Material (*P*+/−) and With or Without Added Antioxidant (*A*+/−)

n	86	8	9	19	12	15	15	8
Drier		*S*	*F*	*S*	*F*	*F*	*S*	*F*
P		+	+	−	+	−	−	−
A		+	+	−	−	+	+	−
T	31·2	32·0	30·8	29·6	31·1	30·5	33·6	32·0
SD	2·04	1·70	0·66	1·31	1·18	1·94	2·05	1·07
U	26·1	29·5	27·9	25·5	26·6	24·3	26·4	24·2
SD	2·41	1·92	0·78	1·44	0·90	3·45	1·66	1·38
U/T	0·834	0·921	0·908	0·861	0·851	0·799	0·787	0·757
SD	0·077 0	0·028 8	0·019 8	0·036 6	0·028 1	0·102 2	0·074 0	0·045 0

(b) The Significance of *t*-Values for the Comparisons of *U/T* Values between Two and Two Groups. The Values are Ranked with the Highest at the Top and to the Left. The Significance Levels Relate to the Positive Differences between the Values in the Lefthand Row and those in the Horizontal Lines

	F++	*S*−−	*F*+−	*F*−+	*S*−+	*F*−−
S++	NS*sf*	***	***	***	***p	***
F++		***	***a	***p	***	***
S−−			NS	*	***a	***sf
F+−				NS	**	***p
F−+					NS*sf*	NSa
S−+						NS

NS, not significant; * $p < 0{\cdot}05$; ** $p < 0{\cdot}01$; *** $p < 0{\cdot}001$.
sf, effect of drier.
p, effect of preservation.
a, effect of antioxidant.

the eight possible combinations of these factors was not represented: preserved raw material dried by steam and without added antioxidant (*S*+−). When the samples were grouped according to the seven combinations of the three variables (Table 2), there were obvious differences between the groups, especially for unoxidized methionine (*U*) and for the ratio unoxidized over total methionine (*U/T*). The mean values for *U/T* showed degrees of methionine oxidation between 8 and 25%. Twenty one comparisons between two and two groups were made; the significance of the *t*-values for these comparisons are indicated in Table 2(b).

When only one variable is considered, steam drying was better than flame drying only with unpreserved raw material with no antioxidant added (*S*−−

versus $F--$). Antioxidant addition gave lower degrees of oxidation with preserved raw material dried by flame ($F++$ versus $F+-$) but not with unpreserved raw material dried by steam ($S--$ versus $S-+$). Preservation of the raw material gave less oxidation than unpreserved raw material with both types of drier ($S+-$ versus $S-+$, $F++$ versus $F-+$, $F+-$ versus $F--$).

Thus, in this material, the preservation of the raw material seems to protect methionine against oxidation better than ethoxyquin addition. There was no clearcut effect of the type of drier. The conclusions drawn are the same as may be drawn when all results are treated together in two and two categories. The 29 samples from preserved raw material showed a significantly lower degree of methionine oxidation than the 57 samples from unpreserved raw material. Between all samples treated with antioxidant and those not treated (47 versus 39) and between those dried by steam and those dried by flame (42 versus 44) the differences were not significant. Whether the reason for the effect of V65 preservation is that it has antioxidant properties, or the reason resides in its bacteriostatic and bacteriocide properties, cannot be answered.

Even if the effect of antioxidant in this material was not very clear, it has been shown earlier that protection of the fat by ethoxyquin also protected against methionine oxidation (Gulbrandsen *et al.*, 1983). Preservation (V65) showed no clear effect, but antioxidant was added only just before drying and had not had time to have any effect in the raw material.

Model meals

Several factors affecting oxidation have been studied (Slump & Schreuder, 1973; Aksnes, 1984, review). The aims of the present experiments with model fish fillet meals were to study whether methionine was especially vulnerable to oxidation by atmospheric oxygen during a short time after freeze-drying (Table 3) and whether added clo and/or TMAO would influence the rate of oxidation (Table 4). Clo contains unsaturated (oxidizing) fatty acids and unpublished results showed that heating of TMAO and methionine, together, resulted in some loss of the latter.

The results with atmospheres of O_2, air, N_2 and CO_2 added after freeze-drying of fillets from four different fish species did not show any significant difference between air and O_2 on the one hand and the inert atmospheres on the other. After 16 months of storage, methionine oxidation was more pronounced in herring fillets (about 30%) than in saithe, salmon and halibut (2–15%), probably because the residual fat was more agressive than in the other samples.

The effects of added clo and of TMAO alone and in combinations showed

TABLE 3

Total (*T*), Unoxidized (*U*) (mg/g protein) and Fraction of Unoxidized (*U*/*T*) Methionine in Fish Meals Produced from Fillets of Herring, Saithe, Salmon and Halibut and Exposed to Different Atmospheres in the Freeze-Drier after the Drying
(The samples were analysed after 2 weeks and 16 months storage)

Mean over all atmospheres

	n	*T*		*U*		*U*/*T*	
		2 weeks	*16 months*	*2 weeks*	*16 months*	*2 weeks*	*16 months*
Air	4	35·8	32·8	30·3	27·4	0·861	0·836
O_2	4	36·7	34·6	33·6	30·8	0·915	0·882
N_2	4	35·8	33·0	31·5	28·9	0·907	0·873
CO_2	4	36·2	34·6	31·8	29·8	0·877	0·862
Air	4	33·6	32·3	32·5	28·7	0·961	0·890

Mean over all fish species

	n	*T*		*U*		*U*/*T*	
		2 weeks	*16 months*	*2 weeks*	*16 months*	*2 weeks*	*16 months*
Herring	5	35·8	34·0	31·4	23·7	0·877	0·697
Saithe	5	34·0	30·8	31·3	30·1	0·920	0·978
Salmon	5	35·9	34·0	31·8	30·4	0·888	0·858
Halibut	5	36·7	35·1	33·6	33·5	0·945	0·899

TABLE 4

Total (*T*), Unoxidized (*U*) (mg/g protein) and Fraction of Unoxidized (*U*/*T*) Methionine in Fish Meals Produced from Saithe Fillets Without or With added Cod Liver Oil (CLO−/CLO+) and Without (TMAO-0) or With (TMAO-28, TMAO-56, TMAO-112) added TMAO·HCl (added at levels corresponding to 0, 28, 56 and 112 mg TMAO-N/100 g fillet), Freeze-Dried after 0 or 3 days (FD-0/FD-3) and Analyzed when Produced (Fresh) and after 2 years

	n	*T*	*U*	*U*/*T*
	32	31·7 ± 1·2	28·7 ± 1·8	0·905 ± 0·051
CLO−–CLO+	16/16	−0·8 *	0·2	0·027 ***
FD-0–FD-3	16/16	0·5 *	0·02	0·014 *
Fresh–2 years	16/16	0·7 *	3·3 ***	0·086 ***
TMAO-0	8	32·7	29·1	0·890
TMAO-28	8	31·3 (***)	28·8 *	0·921
TMAO-56	8	31·6	28·7	0·921
TMAO-112	8	31·1	28·0	0·901

* $p < 0{\cdot}05$.
*** $p < 0{\cdot}001$.
(***) linear component of treatment sum of squares significant ($p < 0{\cdot}001$).

unexpectedly that there was somewhat higher total methionine in samples with added clo than in samples without. When TMAO was added, there was a small but significant linear component of the treatment sum of squares showing a tendency for TMAO to react with methionine. The tendency was also evident with unoxidized methionine but not with the ratio U/T. Thus, it is possible that TMAO affected a small degree of methionine destruction rather than oxidize methionine to methionine sulphoxide.

The results indicate that when fish meals are stored for a long time, a high degree of methionine oxidation may be found. The oxidation is a slow process, but the exact time taken for methionine to oxidize has not been investigated. It will probably depend upon the type of residual fat and the time taken to exhaust added or natural antioxidants.

In fish meals produced by modern techniques, little oxidation takes place.

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