Fisk.Dir. Skr., Ser. Ernæring, Vol. II: No. 4, s. 109–116 (1983)

OXIDATION OF FAT AND METHIONINE DURING STORAGE OF PROTECTED AND NON-PROTECTED FISH MEALS FROM MACKEREL

By
KNUT ERIK GULBRANDSEN[1]), ANDERS AKSNES[2])
and LEIF R. NJAA[2])

ABSTRACT

Four fish meals were produced from mackerel in a pilot plant. Before drying, the antioxidant ethoxyquin and the preserving agent sodium nitrite and formaldehyde, with possible antioxidant properties, were added to obtain one meal with no addition, one with antioxidant, one with preserving agent and one with both additions. The meals had 70% protein and 20% fat. The meals were stored for one year, and the oxidation of the residual fat was followed in extracts from monthly samples. The fat in the two antioxidant protected meals were stable towards oxidation as none of the analysed parameters changed during storage. The major oxygen uptake took place during the first five months in the two non-protected meals, as shown by increasing peroxide values and decreasing iodine values and % 22:6 of the fatty acids. During the seven remaining months of storage the fat oxidation continued at a new level as shown by decreasing peroxide values, increasing % free fatty acids and stable low values for iodine values and % 22:6. No effect of the preserving agent could be ascertained.

Non-oxidised and total methionine were determined in the monthly samples by the iodoplatinate method before and after reduction by $TiCl_3$. No change was found in the two protected meals, whereas non-oxidised methionine decreased steadily in the two non-protected meals, resulting after one year in 20% oxidised methionine (sulphoxide) in the meal without preserving agent, and 17% in the meal with preserving agent.

The biological availability of methionine was assayed in the samples stored for 10 months by a growth test with chicks. All four meals gave availabilities corresponding to the contents of total methionine, showing that oxidised methionine retained its biological value. Further were determined the tryptophan contents of the meals which were constant for the four meals and storage times. Available lysin was tested in the meals at the start and end of the storage by the dye binding capacity method. No change was seen in any of the four meals.

INTRODUCTION

Fish meal is a valuable admixture in feeds, having high contents of animal protein and of minerals. Fish meals are produced from pelagic fish species which normally contain 10 to 20% of fat. After production there is a residual

[1]) Norwegian Herring Oil and Meal Industry Research Institute, N–5033 Bergen, Norway
[2]) Institute of Nutrition, Directorate of Fisheries, N–5013 Bergen, Norway

fat content in the meals up to 12%. The fish fat is highly unsaturated, and will oxidise during production and storage of the meals, if these are not protected by the addition of an antioxidant. The sulphur containing, essential amino acid methionine is of particular interest as one of the nutritionally «limiting» amino acids in fish meals. Part of the methionine content of fish meal has been found to be present in the oxidized form, methionine sulphoxide, retaining its biological availability as methionine for the young rat (NJAA, 1962).

Autoxidation of fats may possibly be responsible for the oxidation of methionine to the sulphoxide, as shown in model experiments in which mixtures of methyl linoleate and casein were left to autoxidise (TANNENBAUM et al., 1969, CUQ et al., 1978). Radicals derived from oxidising fat components may well be involved in the oxidation of methionine (ROUBAL, 1971). Experiments with normal foods and feeds containing unsaturated fat seem not to have been reported.

The present investigation was based on mackerel meals produced in a pilot plant and stored for a year with and without the addition of an antioxidant. A report to the Norwegian Council for Fisheries Research (NJAA, 1977) gave values for commercial fish meals which pointed to a possible antioxidant effect of the preserving agent nitrite and formaldehyde. Therefore this admixture was also tested in the investigation. The mackerel was chosen because of its content of highly unsaturated fat (LAMBERTSEN, 1978). Mackerel is also high in selenium, (GABRIELSEN and OPSTVEDT, 1980) and the meals were further used in a study on the contents and biological availability of selenium of the stored meals (AKSNES et al., 1983).

MATERIALS AND METHODS

Production and storage of the fish meals

500 kg of mackerel caught in June 1980 were frozen when fresh and kept frozen until production in a pilot plant. Two batches, each of 250 kg were thawed overnight, chopped and heated to 90°, after which the cooked mass was pressed. The fat was separated from the stickwater, which after concentration in an evaporator was returned to the press cake. To one batch was added a preserving agent giving 0.6 g formaldehyde and 0.4 g sodium nitrite per kg dry matter. Both batches were then divided into two halves, to one of each was added the antioxidant ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethyl-quinoline) in a concentration of 0.4 per kg dry matter. The four batches were steam-dried, giving four mackerel meals: One with no additions, one with antioxidant, one with preserving agent and one with both additions. The fish meals were stored in plastic containers with tight lids at ambient temperatures in an unheated storage from September 1980 to September 1981. Samples were analysed before storage, and thereafter the meals were sampled each month.

Analytical methods

The proximate composition of the meals was determined by conventional methods. The residual fat in the meal samples was extracted with chloroform and methanol as described by HANSON and OLLEY (1963). Free fatty acids (FFA), iodine value and peroxide value were determined in the extracts by standard methods (DEUTSCHE EINHEITSMETHODEN, 1981). The content of the fatty acid docosahexaenoic acid (22:6) was determined by gas chromatography as described by OPSTVEDT et al. (1970). Dye binding capacity was determined in the meals as mmol «Acid Orange 12» bound per 100 g protein according to JACOBSEN et al. (1972). Tryptophan was determined according to SLUMP and SCHREUDER (1969). Methionine was determined colorimetrically with an iodoplatinate reagent in an automated apparatus after hydrolysis of the samples with barium hydroxide. Unoxidated methionine was determined before, and total methionine after reduction with titanium trichloride, giving methionine sulphoxide by difference. Details of the method are given by NJAA (1980).

The biological availability of methionine in the fish meal samples was assayed in a growth test with white Leghorn chicks, using a basal low methionine diet containing soybean meal. Graded additions of the meal to be tested were compared with graded amounts of crystalline DL-methionine. Details of the method with an extensive discussion of its merits are given by OPSTVEDT (1975) and OPSTVEDT and MUNDHEIM (1977). The contents of available methionine were evaluated statistically in a factorial twoway design, using the addition of antioxidant and preserving agent as the two factors.

RESULTS AND DISCUSSION

The mackerel used in the production contained 17.3% protein and 10.2% fat. Ammonia-nitrogen was measured to 0.5 g/kg, which is considered very low, indicating a fresh raw material.

Table 1 shows that the meals had about 20% of fat, about twice the amount found in commercial meals. The meals should therefore be parti-

Table 1. Protein and fat in four mackerel meals, produced with and without added antioxidant and preserving agent. (All values in g/kg dry matter).

Antioxidant	−	+	−	+
Preserving agent	−	−	+	+
Protein[1]	677	679	694	708
Fat[2]	204	220	184	187

[1] Analysed before storage, N (Kjeldahl) · 6.25.
[2] Analysed before storage, Soxhlet, diethylether.

Table 2. Analyses on a chloroform/methanol fat extract from four mackerel meals, stored for one year with and without added antioxidant and preserving agent.

Antioxidant	−				+				−				+			
Preserving agent	−				−				+				+			
Storage time, months	FFA[1]	PV[2]	IV[3]	22:6[4]	FFA	PV	IV	22:6	FFA	PV	IV	22:6	FFA	PV	IV	22:6
0	7.5	12	145	8.8	8.3	0	153	9.5	7.9	15	156	10.1	7.9	0	157	11.1
1	7.4	12	142	8.6	8.4	0	151	9.4	7.8	17	148	9.3	7.9	0	156	11.1
2	7.3	15	137	7.9	6.9	0	150	–	8.0	98	133	7.8	8.0	6	154	–
3	7.5	46	133	–	7.7	0	151	–	11.1	180	108	–	7.9	2	154	–
4	8.9	129	105	3.2	8.3	1	148	–	12.7	129	84	1.6	7.9	2	152	–
5	10.5	67	85	1.7	8.5	0	146	9.4	13.5	57	79	1.2	9.4	0	149	10.8
6	11.5	155	81	0.9	8.4	10	149	–	13.1	125	79	1.1	8.0	7	151	–
7	10.5	146	94	2.3	9.1	12	145	–	12.1	121	85	1.9	8.6	24	147	–
8	9.1	64	115	4.5	8.6	18	148	–	11.1	68	103	5.2	8.0	20	150	–
9	10.5	78	94	1.9	8.9	9	148	–	12.7	82	85	1.6	9.8	15	151	–
10	12.2	42	90	–	8.6	4	149	–	13.3	36	85	–	8.2	9	152	–
11	13.8	16	87	–	10.2	6	150	–	14.3	18	82	–	11.1	8	154	–
12	12.3	10	85	1.1	7.4	1	150	9.3	13.9	13	85	1.8	7.8	1	152	11.7

[1] FFA: free fatty acids, g/100 g by titration.
[2] PV: Peroxide value, milleq.0_2/kg.
[3] IV: Iodine value, Wijs, g J_2/100 g.
[4] 22:6: Docosahexaenoic acid, g/100 g of calc. peaks in GLC.

cularly prone to fat oxidation. Table 1 further shows that the meals from the first batch (without preserving agent, with/without antioxidant) held 1 to 2 percent less protein and 2–3% more fat than the meals from the second batch. The residual fat in the second production of fish meals was, however, somewhat more unsaturated (iodine value and percent 22:6, table 2).

Analytical values from the fat extracts during the twelve months' storage time of the meals are compiled in table 2. Contents of free fatty acids, and the most highly unsaturated fatty acid 22:6, together with peroxide and iodine values were determined to follow the course of oxidation of the residual fat in the meals. The added amount of ethoxyquin was clearly sufficient to protect the meals from oxidation, even at the very high fat level of 20%. The iodine values, measuring the unsaturation of the fat, remained at the original levels throughout the storage time, with 149±2.1 (S.D.) for the meal without preserving agent, and 152±2.8 (S.D.) for the meal with preserving agent. Correspondingly, percentages 22:6 were constant at 9.4 and 11%, respectively. Also, FFA remained constant at 8.5% in both meals, showing that no further hydrolysis of the fat had taken place during storage. The peroxide values, measuring the uptake to oxygen in the fat, showed slight increases during the months March to June.

A very different picture was seen in the two non-protected meals. Increased peroxide values and lower iodine values and % 22:6 relative to the antioxidant protected meals before storage showed that some fat oxidation had taken place already during the drying process. The major oxidation of the fats was completed within the first five months at which time the iodine value and percentage 22:6 had reached constant low values (IV. = 90±7.5 (S.D.) and 83±2.7 (S.D.) for the meal without and with preserving agent, respectively). The oxidation continued during the remainder of the storage time on a new level, with increasing levels of FFA and decreasing peroxide values, the latter having peak values at 6 months of storage. A break in the course of the analytical values occurred in May at 8 month's of storage. This was probably due to sampling from new containers used for the storage.

It was difficult to ascertain an effect of the addition of nitrite and formaldehyde to the meals. Changes in the peroxide value and the iodine value were steeper during the first four months in the meal with nitrite plus formaldehyde, but, as pointed out above, this meal also started with a more unsaturated fat.

The fall in non-oxidised methionine, together with the corresponding iodine values for the two meals without antioxidant, is illustrated in fig. 1. Results of the determinations of non-oxidised, total and biologically available methionine are given in table 3. A content of total methionine – determined after reduction of the sulphoxide with titanium trichloride – of 29 g/kg protein was found in the four meals. No effect on this value of either addition of antioxidant, preserving agent or time of storage were found. There was no

Table 3. Methionine content of four mackerel meals stored for one year with and without added antioxidant and preserving agent (all values in g/kg protein).

Antioxidant	−	+	−	+
Preserving agent	−	−	+	+
Total[1]	29.3	29.3	28.7	29.2
Non-oxidised				
2 first samples	27.7	–	28.2	–
2 last samples	22.2	–	23.4	–
Ave. of 13 monthly samples[2]	–	28.0	–	27.9
Percent ox. before storage	5.5	4.4	1.7	4.5
Percent ox. during storage	20	–	17	–
Available after 10 month's storage[3]	28.8	30.5	27.8	31.9
% avail. of total after 10 months' storage	97	100	96	103

[1] Average of 13 monthly samples, max. S.D. = ± 1.2.
[2] Max. S. D. = 0.7.
[3] Max. S.D. = 2.8 of replicate assays.

change during storage in the values for non-oxidised methionine in the two antioxidant protected meals. Thus, the addition of 0.4 g/kg of ethoxyquin fully protected these high-fat mackerel meals against oxidation of fat as well as of methionine. The average values were 4.5% lower than those of total methionine, and this difference probably corresponds to the amount of methionine sulphoxide present in the meals before storage.

The amount of non-oxidised methionine fell steadily during the year of storage in the two non-protected meals. After one year the values were reduced with 20 and 17 percent in the meals with and without preserving agent, respectively. Fig. 1 shows that the reduction in non-oxidised methionine was roughly parallel with the reduction in iodine value, i.e. the fatty acids and methionine in the meals were oxidised in parallel, possibly either methionine as a consequence of fatty acid oxidation or by the same mechanism. In the meal containing nitrite plus formaldehyde as a preserving agent the methionine oxidised at a somewhat higher rate during the first four months of storage than in the non-preserved meal, in correspondence with the fat oxidation rate. During the latter stage of oxidation, however, the methionine in the preserved meal oxidised at a slower rate than in the non-preserved, ending up after a year of storage at three percent less oxidised methionine. This may point to an effect of preserving agent in accordance with the results on commercial meals given by NJAA (1977). Further analyses of the meals after 18 and 24 months gave practically the same values for non-oxidised methionine as after 12 months of storage, showing that the

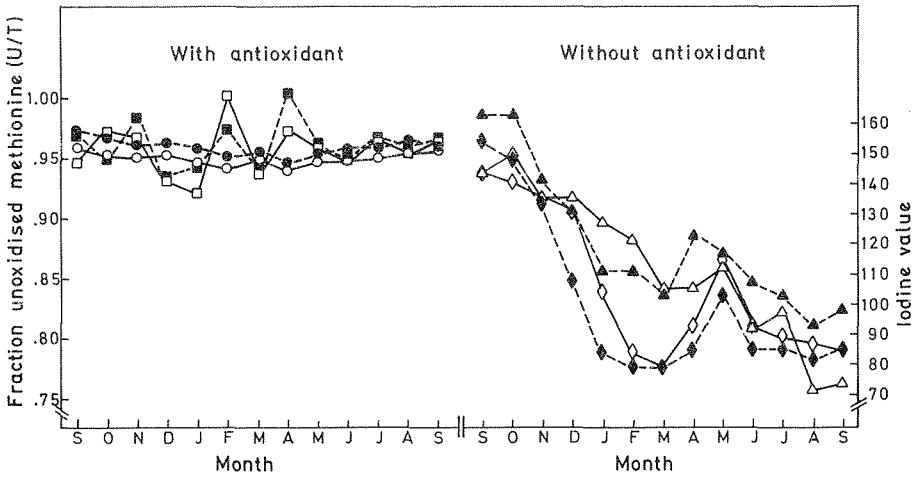

Fig. 1. Iodine number and fraction of total methionine in unoxidised form (U/T) analysed during one year of storage at ambient temperature.
—○— - - -●- - - Iodine number — ◇ — - - ◆ - - - -
—□— = = =■= = = U/T — △ — - - ▲ - - - -
Open symbols: With preserving agent.
Filled symbols: Without preserving agent.

methionine oxidation stopped when the fat was fully oxidised and the peroxide values had returned nearly to nil.

The biological availability of the methionine was assayed in the samples stored for 10 months. As seen from the values in table 3, the biological availability remained, within the reproducibility of the methods, equal to the total methionine contents in all four meals, thus confirming results reported by OPSTVEDT (1975). A tendency to lower availability of methionine in the non-protected meals may perhaps be suggested from the values in table 3.

The meals were also analysed for tryptophan. All monthly samples of the four meals gave the same value, 11 g/kg protein within a standard deviation of 0.7. As a test for available lysin, the meals were analysed for their dye binding capacity (JACOBSEN et al., 1972). A mean value of 100 mmol «Acid Orange 12» bound per 100 g protein was found for the four meals at the start of the storage period as well as after a year of storage. No effect was found for either the addition of antioxidant or preserving agent, nor for the time of storage.

ACKNOWLEDGEMENTS

Messrs. E. Nygård and A. Brodin from the Herring Oil and Meal Industry Research Institute, Bergen, are thanked for the analyses of the fat oxidation parameters. The study was supported in part by a grant from the Norwegian Fisheries Research Council.

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