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# CONTENTS AND BIOLOGICAL AVAILABILITY OF SELENIUM IN OXIDISED AND PROTECTED FISH MEALS FROM MACKEREL

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## ABSTRACT

Four mackerel meals were produced in a pilot plant, one without protection, one with added ethoxyquin as an antioxidant, one with formaldehyde and sodium nitrite as a preserving agent with possible antioxidant properties and one with both additions. The meals were stored for 10 months and thereafter analysed by atomic absorption spectrophotometry for total selenium contents and for Se (VI) by difference between the graphite furnace method and the hydride generation method. Biological available selenium was determined in a chick assay based on Se depletion and a compared supplementation with the fish meals and graded levels of hydrogen selenite. Available selenium was measured by the glutathione peroxidase activity in the blood plasma.

The residual fat content of the two fish meals without antioxidant addition was extensively oxidised as shown by different analyses. The total selenium content found was 3.2 mg/kg dry matter (2.9–3.4) and the available selenium content was estimated to 1.4 mg/kg dry matter (1.3–1.6) corresponding to an availability of 45% (41–51). No effect of fat oxidation in the meals or protection by additions of antioxidant or preserving agent could be found. The contents of Se (VI) were determined to 4 to 10% of the total selenium contents.

## INTRODUCTION

Fish products are rich sources of selenium, and contents in the range of 0.6 to 6 mg/kg have been reported for fish meals (SCOTT and THOMPSON, 1971; EGAAS and BRÆKKAN, 1977; GABRIELSEN and OPSTVEDT, 1980 b). Roughage and grain produced in Norway have very low selenium contents (0.002–0.03 mg/kg), and fish meals give an important contribution to the selenium content of mixed feeds for farm animals (MATTSSON, 1980; FRØSLIE, 1980). Selenate, selenite, seleno-aminoacids and complexes of selenolipids and of mercury and selenium have been found in products of marine origin (LUNDE, 1972; CAPPON

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and SMITH, 1981). Widely varying and conflicting values have been reported for the biological availability of selenium in fish products as well as in other foods and feeds, but the reason for the varying biological availability relative to the total content is not clear. CANTOR et al. (1975) reported selenium availabilities of less than 25% in fish meals, whereas feeds of vegetable origin ranged from 60 to 90%. On the other hand, GABRIELSEN and OPSTVEDT (1980 b) reported selenium availability values between 32 and 60% in fish meals and less than 25% in vegetable products. Reasons for such conflicting results may be sought in the accuracy of the analytical methods for the low contents of selenium in the products studied, in the biological factors used for the determination of the availability, and further in the levels of such substances as tocopherols, methionine and antioxidants in the experimental diets.

UNDERWOOD (1977) pointed out that drying and storage of feed ingredients may influence the availability of selenium. GABRIELSEN and OPSTVEDT (1980 b) discussed the possibility that oxidation of fish meals may influence the selenium availability. The present paper reports on the contents and biological availability of selenium in oxidised and protected mackerel meals, produced in a pilot plant. The mackerel is one of the fish species exploited in Norway for the production of fish meal and oil. It is known to be rich in selenium (GABRIELSEN and OPSTVEDT, 1980 b) and also in unsaturated fat (LAMBERTSEN, 1978). The meals were also used in a study on the oxidation of fat and methionine during one year of storage of protected and non-protected fish meals (GULBRANDSEN et al., 1983).

## MATERIALS AND METHODS

The samples of mackerel meals used in the present study were available from a one-year storage investigation of antioxidant-protected and non-protected fish meals (GULBRANDSEN et al., 1983). The fish meals were produced from fresh frozen mackerel in a pilot plant. Five hundred kg cooked, chopped and pressed mackerel were divided into four equal portions which were treated in the following way before the steam drying process; 1: no addition, 2: addition of 0.4 g ethoxyquin per kg dry matter, 3: addition of a preserving agent giving 0.6 g formaldehyde and 0.4 g sodium nitrite per kg dry matter and 4: addition of both ethoxyquin and preserving agent in the same concentrations.

The fish meals were stored in plastic containers for 10 months at ambient temperatures in an unheated storage, and the oxidation of the residual fat was followed by the determination by conventional methods of peroxide values and iodine values in fat extracts. The fat content was determined by Soxhlet extraction with diethyl ether. Further was measured the decrease in the percentage of the fatty acid 22:6 in a lipid extract as determined by GLC.

Selenium contents were determined by atomic absorption spectrophotometry. The methods have been described in details elsewhere (JULSHAMN et al., 1982). 0.1 g of samples of fish meals were digested in 2 ml «suprapure» grade nitric acid and perchloric acid (9:1). The total selenium content was determined with a Perkin-Elmer 5000 AAS equipped with a HGA-76 graphite furnace (GFAA). A 20 microlitre aliquot was introduced into the graphite furnace by a Perkin-Elmer AS-1 Auto Sampling System. Conventional graphite tubes and 0.25% (w/v) of a nickel solution as a matrix modifier were used. Selenium as selenate is not detectable by the hydride generation technique (HGAA). Estimates of the selenate contents were therefore obtained by subtraction of the HGAA values for selenium from the GFAA values, representing the total selenium content. The hydride generation device was based on Perkin-Elmer MHS-20 electrothermal system. A 4% (w/v) solution of sodium borohydride in 1% aquous sodium hydroxide was used as the reducing and stabilizing agent. The dissociation temperature was 875°C. The overall reproducibility of the procedure (including sample preparation and acid digestion) was estimated by replicate analyses on a crustacean sample which gave a relative standard deviation of 2.5% for the HGAA-method. The accuracy of the two methods was evaluated by analysing two NBS materials by both methods. The results in table 1 show that both methods give a satisfactory accuracy. The detection limit was estimated to 20 microg/kg dry matter.

The determination of the biological availability of selenium was performed mainly as described by GABRIELSEN and OPSTVEDT (1980 a). One-day old white Leghorn chicks were fed for 9 days a deficient diet (30±10 microg/kg Se) based on single cell protein and soy bean meal and then for 10 days on the same diet supplement with graded levels (0, 0.03, 0.06, 0.09, 0.12 and 0.20 mg/kg) of selenium or of the mackerel meals stored for 10 months (table 2). Sodium hydrogen selenite ($NaHSeO_3$) was assumed to be 100% available and used as a reference material. Graded levels of $NaHSeO_3$ were given to groups of five chicks, whereas ten chicks were used per dose level of fish meals. Feed and water were given *ad libitum*. Blood samples were collected by heart puncture, and plasma obtained by centrifugation at 1000 × g for 5 min and

Table 1. Comparative analyses of selenium in two samples from National Bureau of Standards (Values in mg/kg dry matter ± standard deviation for n = 3).

| Sample | GFAA[1)] | HGAA[2)] | Cert. value |
|---|---|---|---|
| Oyster tissue (SRM 1566) | 2.07 ± 0.03 | 2.05 ± 0.05 | 2.1 ± 0.5 |
| Bovine liver (SRM 1577) | 1.11 ± 0.03 | 1.17 ± 0.18 | 1.1 ± 0.1 |

[1)] graphite furnace atomic absorption
[2)] hydride generation atomic absorption

Table 2. Composition of diets (g/kg diet).

| Diet | Basal | Supplemented | Test |
|---|---|---|---|
| *Variable portion* | | | |
| Toprina[1)] | 233 | 233 | 197 |
| Mackerel meal | – | – | 36 |
| Dextrinised potato starch | 355 | 355 – 347 | 355 |
| $NaHSeO_3$/Dextrine[2)] | – | 0 – 8 | – |
| *Constant portion in all diets* | | | |
| Soybean meal | | 203 | |
| Sunflower seed oil | | 66 | |
| Finely ground oat hulls | | 88 | |
| L-Arginine | | 2 | |
| DL-Methionine | | 2 | |
| $Ca_3(PO_4)_2$ | | 14 | |
| $CaCO_3$ | | 15 | |
| NaCl | | 2 | |
| Vitamins[3)] | | 10 | |
| Minerals[3)] | | 10 | |

1. Single cell protein British Petroleum, UK. *(Candida sp)*.
2. The $NaHSeO_3$/dextrine mixture contained 47.8 mg $NaHSeO_3$/kg dextrine, equivalent to 25 mg Se/kg dextrine.
3. Vitamins and minerals as descibed by GABRIELSEN and OPSTVEDT, (1980a).

frozen at –20°C until analysed. Blood plasma tested before and after freeze storage, showed the same glutathione peroxidase activity, indicating that there was no loss of activity during this storage.

The activity of Se-dependent glutathione peroxidase was measured as described by TAPPEL (1978) using hydrogen peroxide as substrate. The enzyme activity was measured as the decrease in the absorbance at 340 nm corresponding to the oxidation of NADPH, using a spectrophotometer (Zeiss PMQ II) equipped with a recorder. Further details are given in table 3. One unit of enzyme activity was defined as one micromol NADPH oxidised per minute. Three replicates were measured for each determination.

The decrease in the absorbance observed when plasma was absent in the reaction mixture, was subtracted in the calculations of enzyme activity. This usually accounted for 5–10% of the total decrease.

## RESULTS AND DISCUSSION

The pilot plant production of the mackerel meals followed to some extent the normal industrial production routine. The meals had ca. 70% protein and 20% fat, i.e. a higher content of highly unsaturated fat than commercial

Table 3. Assay conditions for glutathione peroxidase (the constituents are mentioned in the order of addition).

| | |
|---|---|
| Temperature | 37°C |
| Volume | 1 ml |
| Tris-HCl | 50 µmol pH 7.8 |
| EDTA | 0.1 µmol |
| NADPH | 0.1 µmol |
| Glutathione reductase (SIGMA) | 5 units |
| *Glutathione(reduced form)* | 0.25 µmol |
| Blood plasma | 50 µl |
| $H_2O_2$ | 0.20 µmol |

All solutions were deaerated before use and the glutathione solution was kept under nitrogen atmosphere.

Table 4. Analytical values regarding the fat oxidation of the mackerel meals at the time of production and after ten months of storage.

| Addition | Fat content[1] | Peroxide number[2] | | Iodine number[3] | | 22:6[4] | |
|---|---|---|---|---|---|---|---|
| | | before/ after storage | | before/ after storage | | before/ after storage | |
| None | 20.4 | 12 | 42 | 145 | 90 | 8.8 | 1.1 |
| Preserving agent | 18.4 | 15 | 36 | 156 | 85 | 10.1 | 1.8 |
| Antioxidant | 22.0 | 0 | 4 | 153 | 149 | 9.5 | 9.3 |
| Antioxidant + preserving agent | 18.7 | 0 | 9 | 157 | 152 | 11.1 | 11.7 |

[1] Soxhlet, diethyl ether, g/100 g dry matter, before storage.
[2] Millieq $O_2$/kg, $CH_3OH/CHCl_3$-extract.
[3] Wijs, g $J_2$/100 g, $CH_3OH/CHCl_3$-extract.
[4] Docosahexaenoic acid, g/100 g of calc. GLC peaks of fatty acids after 12 months.

meals. The meals were stored in plastic containers in an unheated storage under ambient temperatures during the months September to June. After ten months extensive oxidation was found in the two unprotected meals whereas only minor changes were found in the two meals containing ethoxyquin (table 4). The fat oxidation had taken place mainly during the first 4 to 6 months. Further details on the quality of the four experimental meals were given by GULBRANDSEN et al. (1983).

Methods for the determination of selenium in foods and feeds were compiled and discussed by HOFSOMMER and BIELIG (1981). Fluorimetry based on 2,3-diamino-naphtalene is the commonly used method. HOFFMAN et al. (1968) found a standard deviation of 26% at levels of 20 microg/kg for this methods. Neutron activation as an analytical method for selenium showed a

reproducibility of 10–15% at 1 mg/kg (LUNDE, 1968). GLC based on an organic derivative of selenium was used by CAPPON and SMITH (1982). Atomic absorption spectrophotometry either using the graphite furnace method or the hydride generation method was shown to be practicable and give good accuracy and high reproducibility (JULSHAMN et al., 1982). This method is particularly applicable as several elements may be measured in the same digest.

The total selenium contents in the four mackerel meals averaged 3.2 mg/kg dry matter with a S.D. of 7% (table 5). This is lower than the 6.2 mg/kg reported by GABRIELSEN and OPSTVEDT (1980 b), based on neutron activation analysis, but earlier values for Norwegian mackerel meals, also from neutron activation analyses, gave values ranging from 1.5 to 4.8 mg/kg (LUNDE, 1968; OPSTVEDT et al., 1970). The values found confirm that fish meal is a good source of selenium.

The assay for the biological activity of selenium is based on the rapid depletion of chicks on a diet of single cell protein, soy bean meal and potato starch (GABRIELSEN and OPSTVEDT, 1980 a). The plasma glutathione peroxidase activity was reduced to a value corresponding to less than 0.05 mg/kg selenium in the diet in 9 days (fig. 1 A). Using sodium hydrogen selenite as standard supplementation and measuring the enzyme activity as described by TAPPEL (1978), the standard curve shown in fig. 1 B was obtained. In this assay the four mackerel meals gave available contents of selenium relative to selenite of 1.3 to 1.6 mg/kg dry matter, corresponding to a biological availability of 45% (41–51%) (Table 5). Neither the contents of selenium nor the relative availability were statistically different in the four meals. Consequently the protection of the fish meals by the addition of antioxidant and/or preserving agent had no effect on the content or biological availability of selenium in the mackerel meals tested.

The values found are within the range reported earlier for mackerel meals. MILLER et al. (1972) found a selenium availability of 43% by measuring the retention of selenium in chicks and GABRIELSEN and OPSTVEDT (1980 b) reported 34% availability using a different assay for glutathione peroxidase and a different method for selenium determination. This low value may possibly be explained by the high total selenium content reported by these authors. CANTOR et al. (1975) reported availabilities only half of these values, but their results varied much with the level of selenium source in the assay diets. Table 5 further gives values for the relative amounts of selenates (Se–VI) found as the difference between the GFAA- and the HGAA-determination. These values were in the range 4–10%, again independent on the level of fat oxidation in the meals. As Se(IV) is more easily reduced than oxidised as compared with sulphur(IV), it is not surprising that fat oxidation did not influence the relative content of Se(VI) in the meals. The values given in table 5 are lower than the average reported by CAPPON and SMITH (1978, 1981,

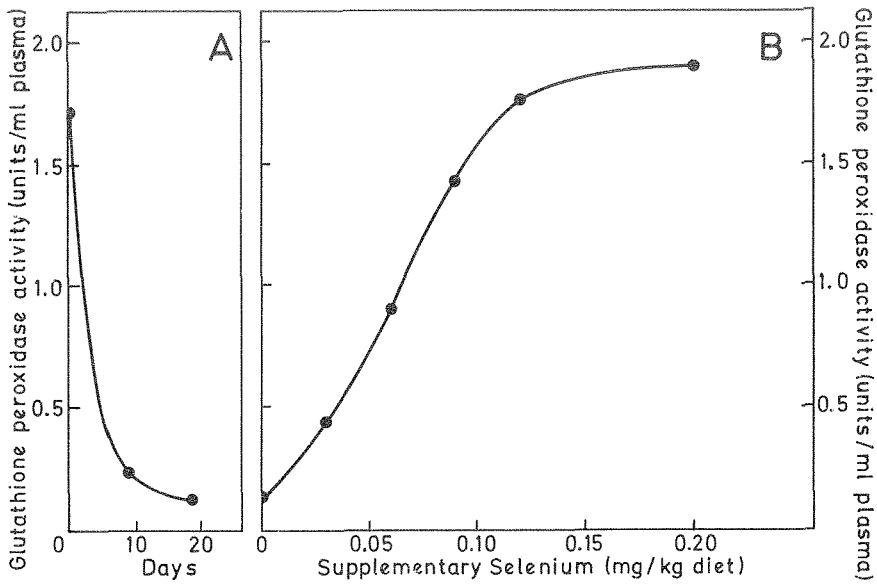


Fig. 1. Glutathione peroxidase activity in plasma of chicks. A: Selenium depletion of chicks fed the basal diet containing 30±19 µg total selenium per kg diet. B: Glutathione peroxidase activity in plasma of chicks fed for 10 days on diets containing graded levels of $NaHSeO_3$.

Table 5. Selenium contents and availabilities in mackerel meals with and without added antioxidant and preserving agent.

| Addition | Total Se mg/kg dry matter GFAA | Se (-II, 0, II, IV) mg/kg dry matter HGAA | Se (VI) by difference % of total | Available Se[1] mg/kg dry matter | Se-availability ($NaHSeO_3$ = 100%) % |
|---|---|---|---|---|---|
| None ..... | 3.16 | 2.83 | 10.4 | 1.30 | 41.1 |
| Preserving agent .. | 3.30 | 3.16 | 4.2 | 1.58 | 47.9 |
| Antioxidant ..... | 3.39 | 3.04 | 10.3 | 1.40 | 41.3 |
| Antioxidant + Preserving agent. | 2.86 | 2.65 | 7.3 | 1.45 | 50.7 |

[1] By interpolation from curve in fig. 1B.

1982), who found that Se(VI) ranged from 5 to 45% of the total selenium in different samples of marine origin.

As Cappon and Smith (1982) recently pointed out, very little is known about the availability of selenium in various compounds and oxidation stages. As the mackerel meals had Se(VI) contents of less than 10% of total selenium

and availabilities were lower than 50%, the main source of unavailable selenium must exist in lower oxidation stages. Part of the selenium in marine material is postulated to be complexed to mercury and BURK (1976) suggested that such complexes may explain some of the low availability of selenium. However, WRENCH and CAMPBELL (1981) found that selenium in marine species from unpolluted waters was almost completely incorporated in a protein fraction. LUNDE (1972) reported that selenium in marine fish may be partly bound to a lipid-soluble fraction. If lipid-selenium compounds are more available than selenite, this may explain the low values in fish products reported by CANTOR et al. (1975), as they extracted their samples with hexane to remove vitamin E. Whatever chemical forms of selenium are present in fish products, we may conclude that selenium in fish meals is stable against oxidation, as severe fat oxidation in the experimental meals did not affect the contents of total selenium or the biological availabilities of selenium.

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