Inhibitory Effect of Bigeye Tuna Meat Extract on Sodium Chloride-Catalyzed Oxidation of Linoleate

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(Accepted May 18, 1983)

Effect of the outer fraction of dialyzed water extract of bigeye tuna meat on NaCl-catalyzed oxidation of linoleate was examined using model system. Oxidation of linoleate was carried out at 25°C and Aw 0.84 and followed by determinations of remaining linoleate and POV.

Linoleate oxidation was markedly inhibited by meat fraction which is diffusive through a cellophane membrane, adsorptive on cation exchange resin, and soluble in 80% EtOH. The inhibitory effect of the meat fraction on linoleate oxidation depended upon the concentration but not the pH. Furthermore, HCl-hydrolysis of the meat fraction resulted in loss of inhibitory effect on linoleate oxidation.

From these results, amino acids and/or small molecular peptides such as anserine were considered to participate in the inhibitory effect of bigeye tuna meat extract on NaCl-catalyzed oxidation of linoleate.

It has been presumed that some fractions which act in conjugation with NaCl in accelerating lipid oxidation are present in the pork1) and fish meat.2) In the previous study,3) therefore, effect of meat extract of bigeye tuna on NaCl-catalyzed oxidation of linoleate was examined using model system. As a result, the inner fraction obtained by dialysis of meat extract of bigeye tuna accelerated NaCl-catalyzed oxidation of linoleate. Heme pigments such as myoglobin and hemoglobin in the extract seemed to act in conjugation with NaCl in accelerating linoleate oxidation. On the other hand, the outer fraction of dialyzed bigeye tuna meat extract was found to inhibit markedly NaCl-catalyzed oxidation of linoleate. From a practical point of view, it is of interest to elucidate the inhibitory effect of the outer fraction of meat extract of bigeye tuna on lipid oxidation.

This paper deals with the effects of various meat fractions of bigeye tuna on NaCl-catalyzed oxidation of linoleate.

Materials and Methods

Materials
Methyl linoleate and Avicel (microcrystalline cellulose) were obtained from Sigma and Funakoshi, respectively. Bigeye tuna Thunnus obesus was purchased from a local fish market.

Preparation and Dialysis of Water Extract
Preparation and dialysis of water extract of bigeye tuna meat were made in the same manner as in the previous study.3)

Fractionation by Ion-exchange Chromatography
Outer solution obtained by dialysis of water extract was fractionated using a column of Amberlite IR-120 (H+) resin. After addition of the sample, 100 ml of water were passed through the column, followed by 200 ml of 2 N NH4OH. The fractions eluted with water and with 2 N NH4OH were designated nR and R fractions, respectively. Both fractions were concentrated in a rotary evaporator and adjusted to pH 6.1.

Fractionation Based on Solubility in EtOH
R fraction was concentrated to dryness in a rotary evaporator and dissolved in a small amount of water. To the aqueous solution was added absolute EtOH with continuous stirring to give a final concentration of 80% and the resulting EtOH solution was allowed to stand overnight at 25°C. The precipitate formed was separated by filtration (80% EtOH insoluble fraction). The 80% EtOH solution was concentrated to dryness in a rotary evaporator and dissolved in a small amount of water. Similarly, to the aqueous solution was added absolute EtOH to give a final concentration of 90% and the 90% EtOH solution was allowed...
to stand overnight at 25°C. The precipitate formed was separated (80-90% EtOH soluble fraction). The 90% EtOH solution, after concentration, was designated 90% EtOH soluble fraction.

**HCl-hydrolysis**

80% EtOH soluble fraction of R fraction was hydrolyzed with 6 N HCl at 110°C for 24 h in a glass tubing sealed under vacuum.

**Model System for linoleate oxidation**

Model system was prepared in the same manner as in the previous study.3)

**Determinations of Peroxide Value, Linoleate, and NaCl**

Peroxide value (POV), linoleate content, and NaCl content in the model system were determined in the same manner as in the previous study.3)

**Results**

**Effects of R and nR Fractions**

Effects of R and nR fractions prepared from the outer solution of dialyzed water extract on the rate of NaCl-catalyzed oxidation of linoleate are shown in Figs. 1 and 2.

Percentage of remaining linoleate of the sample with addition of R fraction, eluted with 2 N NH₄OH from Amberlite IR-120 resin column, decreased slightly during storage, while that of the sample with addition of nR fraction, eluted with water from the same column, markedly. The rate of decrease in percentage of remaining linoleate of the sample with addition of nR fraction was almost the same as that of control sample with addition of NaCl only. These results coincided well with those obtained by POV determination (Fig. 2).

From these results, it is clear that the inhibitory effect of water extract of bigeye tuna meat on NaCl-catalyzed oxidation of linoleate is attributed to the fraction capable of being adsorbed on cation exchange resin.

**Effects of Fractions Separated Based on Solubility in EtOH**

Effects of 80% EtOH insoluble, 80-90% EtOH soluble, and 90% EtOH soluble fractions of R fraction on the rate of linoleate oxidation are shown in Fig. 3 and Table 1.

Percentage of remaining linoleate in the sample with addition of 90% EtOH soluble fraction remained almost unchanged during storage. Contrary to this, those in the samples containing either 80-90% EtOH soluble or 80% EtOH insoluble fractions decreased slowly; the decreas-
ing rate of the latter was faster than that of the former. These results coincided well with those obtained by POV determination, as shown in Table 1.

These results suggest that the meat constituents with relatively lower molecular weight are more effective in inhibition of NaCl-catalyzed oxidation of linoleate.

Effects of pH

Model systems of various pHs were prepared as follows: Thirty ml portions of 90% EtOH soluble fraction (equivalent to the extract obtained from 20 g of tuna meat) were mixed with 30 ml of 0.2 M phosphate buffer adjusted to desired pH. To the resulting solution were added 1 g of NaCl and 20 g of Avicel. After freeze-drying, the mixture was treated with linoleate in the same manner as in the previous paper. In the case of sample without addition of 90% EtOH soluble fraction, 30 ml of water was replaced with 30 ml of water.

The results obtained in the samples without addition of 90% EtOH soluble fraction are shown in Fig. 4 and Table 2. The decreasing rates of percentage of remaining linoleate in the samples were faster at the acid pH, though those in samples adjusted to pH 6.1 and 7.0 were almost the same in rate. On the other hand, as shown in Fig. 5,
slight changes in percentages of remaining linoleate occurred in the all samples with addition of bigeye tuna meat fraction (90% EtOH soluble fr.) at various pHs during storage at 25°C and Aw 0.84. ▲, pH 5.5, NaCl 3.84%, moisture contents 17.5-19.5%; ▲, pH 6.1, NaCl 3.58%, moisture contents 16.8-18.4%; ○, pH 7.0, NaCl 3.63%, moisture contents 17.9-19.6%; ●, pH 7.9, NaCl 3.68%, moisture contents 17.6-18.4%.

Table 3. Changes in POV of the samples with additions of bigeye tuna meat fraction (90% EtOH soluble fr.) at various pHs during storage at 25°C and Aw 0.84

<table>
<thead>
<tr>
<th>Additive</th>
<th>Storage, day</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl 3.84% pH 5.5</td>
<td>34*</td>
<td>119</td>
<td>226</td>
<td>391</td>
<td></td>
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<tr>
<td>NaCl 3.58% pH 6.1</td>
<td>23</td>
<td>65</td>
<td>99</td>
<td>257</td>
<td></td>
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<tr>
<td>NaCl 3.63% pH 7.0</td>
<td>19</td>
<td>53</td>
<td>77</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>NaCl 3.68% pH 7.9</td>
<td>20</td>
<td>48</td>
<td>66</td>
<td>126</td>
<td></td>
</tr>
</tbody>
</table>

* meq/kg

20 g of Avichel to 80% EtOH soluble fractions equivalent to the extracts obtained from 20, 13.4, 6.7, and 4.0 g of tuna meat. The results obtained are shown in Fig. 6.

Percentage of remaining linoleate in the sample containing 80% EtOH soluble fraction at highest concentration decreased rapidly after 3 days of storage when compared with those of 80-90% EtOH soluble and 90% EtOH soluble fractions which were shown in Fig. 3, though all samples contained the fractions equivalent to the extracts obtained from 20 g of tuna meat. This seems to be due to difference in composition of the extract of bigeye tuna used in these experiments. The decreasing rate of percentage of remaining linoleate in the sample decreased with decreasing concentration of extract added to the sample: The lowest decreasing rate was obtained with the sample containing the fraction equivalent to extract obtained from 6.7 g of tuna meat.

The effects of HCl-hydrolysis of the extract on
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linoleate oxidation are also shown in Fig. 6. HCl-hydrolysis caused the rapid decrease in percentage of remaining linoleate in the sample. These results suggest that low molecular peptides in the meat extract participate in inhibition of NaCl-catalyzed oxidation of linoleate under these experimental conditions.

Discussion

As mentioned above, bigeye tuna meat fractions which were effective in inhibition of NaCl-catalyzed oxidation of linoleate are diffusive through a cellophane membrane, adsorptive on cation exchange resin, and soluble in 80% EtOH. These are the properties of amino acids and low molecular peptides.

There have been many studies on antioxidant effects of amino acids,4-15 peptides,16-18 and partially hydrolyzed proteins19-25 so far. Some of them are found to be effective as antioxidant under certain conditions. From these facts, inhibitory effect of meat extract of bigeye tuna might be attributable mainly to amino acids and/or peptides.

According to Suyama and Yoshizawa26 who determined amino acid composition of meat extract of bigeye tuna, methionine,9,10 tryptophan,9 and proline4-13 which are known to have strong antioxidant effects as compared with other amino acids are contained at relatively low levels in the extract. On the other hand, histidine and anserine are contained at extremely high levels; contents of histidine and anserine are determined to be 745 mg and 817 mg/100 g, respectively. Histidine4-9 is known to act as pro- or antioxidant depending upon concentration and pH; there is a tendency to act as prooxidant at relatively high concentrations or low pH. However, inhibitory effect of meat extract of bigeye tuna on linoleate oxidation depended upon the concentration but not the pH (Figs. 5 and 6). The meat extract seemed to be different from histidine in the properties of antioxidant effect.

No information concerning antioxidant effects of anserine and carnosine has been available, except that of carnosine reported by Matsushita and Iwami.5-7. As mentioned above, the meat extract of bigeye tuna lost most of its inhibitory effect, when hydrolyzed with HCl. This strongly suggests that anserine is partly responsible for the inhibitory effect of bigeye tuna extract. Further study is necessary to elucidate the inhibitory effect of fish meat extracts on the linoleate oxidation.

The authors wish to thank Mr. M. Kohno and Mr. Y. Yamada for their assistance in the experimental work. This work was partly supported by a grant from the Ministry of Education.

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