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Effect of Copper, Iron and Hemin on Lipid Oxidation in Fish Flesh Homogenate

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Abstract

The acceleration of lipid oxidation by Cu^{++}, Fe^{++}, Fe^{+++} and hemin was determined in the homogenate sample of Alaska pollack and mackerel oil.

The oxygen absorption in the homogenate was measured with a Beckman Fieldlab model 1008 oxygen analyzer.

The relative activity of these metal ions and hemin determined at 40°C was as follows: Fe^{++}>Hemin>Cu^{++}>Fe^{+++}.

The activation energy of the homogenate-oxygen absorption at the temperature between 25° and 5°C, have shown that Fe^{++} and hemin had a higher prooxidant activity than Fe^{+++} and Cu^{++}.

Introduction

Fish lipid, in general, is subject to oxidative deterioration because it consists of highly unsaturated fatty acids.

It has been generally known that lipid oxidation is affected by temperatures and/or by metal ions, and their influences differ as a function of experimental conditions.

In non-aqueous systems, Ke et al.[1] found that Fe^{++} and Cu^{++} accelerated the oxidation of lipids prepared from mackerel skin and meat. El-Zeany et al.[2] found that Cu^{++} and Fe^{+++} had no effect on the autoxidative changes of methyl esters of polyunsaturated fatty acids prepared from cod liver oil mixed with egg-albumin.

On the other hand, in aqueous systems, Saunders et al.[3] found that Fe^{+++}, Fe^{++} and Cu^{++} accelerated the oxidation of methyl linoleate which was emulsified with dodecyl sulfate, and that there were no predominant differences between Fe^{++} and Fe^{+++} in their catalytic actions. Kendrick et al.,[4] using some heme compounds such as hemin, catalase, metmyoglobin, cytochrome *c* and methemoglobin, found that only low concentrations of these added compounds would accelerate the oxidation of methyl linoleate. Nagayama et al.,[5] in their experiments on the effects of temperatures on lipid oxidation with mackerel tissue homogenate, indicated that lipid oxidation could not be perfectly inhibited even at 0°C.

The present investigation is a study of the effects of temperature and concentrations of Cu^{++}, Fe^{++}, Fe^{+++} and hemin on the acceleration of lipid oxidation in fish homogenate.

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Materials and Methods

Preparation of blended fish muscle Fish muscle homogenate was prepared from the frozen gutted Alaska pollack (*Theragra chalcogramma*, PALLAS) caught from the Northern Pacific Ocean. The gutted fish were cut into very small pieces and were blended, stirring with cold water for 1 min, then allowed to stand for 10 min before discarding the supernatant fraction. The washing process was repeated two times more and the final leached muscle, obtained by centrifugal dehydration, put into a polyethylene bag and immediately stored at –20°C.

Preparation of substrate homogenate The frozen leached muscle of Alaska pollack, 1 g, was homogenized with 10 g of distilled water. Mackerel oil, 0.1 g, was added to the homogenate and mixed thoroughly with an Ultra-Turrax homogenizer for 90 sec in three 30-sec periods separated by 10-sec intervals to reduce foaming.

Measurement of oxygen absorption Nineteen ml of the substrate homogenate was pipetted into a 2.5 i.d.×11 cm length glass tube and placed in a water bath at 40°C. A Beckman Fieldlab model 1008 oxygen analyzer sensor was inserted into the glass tube, which was stirred magnetically. The recorder was started immediately after the desired amounts of copper, ferrous, and ferric salts and hemin, dissolved in 1 ml, were added to the tube. The rate of oxygen absorption was determined from the linear slope of the recording and expressed as a reciprocal of time until 50% of oxygen was absorbed.

Metal salt solutions The metal salt solutions tested were $CuSO_4$, $Cu(NO_3)_2$, $CuCl_2$, $FeSO_4$, $Fe_2(SO_4)_3$ and $FeCl_3$. These salts (Analytical Reagent Grade) were purchased from Wako Pure Chem. Ind. Ltd., and hemin (bovine crystalline type) was obtained from Sigma Chemical Co. Hemin was solubilized with a few drops of 10% KOH solution and diluted with distilled deionized water. All of these salts and hemin were dissolved and diluted with distilled deionized water to prepare the solutions which had the desired concentration of the metal ion in each 1 ml. Fe content in the hemin solutions was measured using atomic absorption spectroscopy.

Results and Discussion

Saunders et al.[3] have pointed out that the acceleration of lipid oxidation by metal salts give different effects depending upon the emulsifiers and buffers used, and no emulsifiers or buffers were used in the present experiment.

Effect of concentration of added metal ion on the oxygen absorption The comparative effects of various concentration of added Cu, Fe and hemin on the oxygen absorption by the substrate homogenate is shown in Fig. 1-A, B, C and D, respectively. As indicated in Fig. 1-A, it is apparent that the oxygen absorption by the homogenate is progressively accelerated by adding any of three Cu salts up to 25 ppm. This result is similar to that reported by MacLean et al.[6] when they measured the effect of Cu^{++} concentration on the development of rancidity, as indicated by TBA value.

Fig. 1-B shows that the rate of oxygen absorption increased in proportion to the increased concentration of added Fe^{+++} up to about 100–120 ppm.

However, the addition of concentrations higher than that level resulted in an antioxidative action rather than the accelerative action.

In the case of addition of Fe^{++}, the rate of oxygen absorption was accelerated considerably at low concentrations in comparison with Fe^{+++} (Fig. 1–C). The rate of oxygen absorption induced by the addition of hemin Fe increased proportionally up to 2.3 ppm (Fig. 1–D). This increase is in accord with the results described by Kendrick et al.[4] using a linoleic acid phosphate buffer emulsion. Kendrick et al.[4] pointed out that hemin showed a pro-oxidative action when added up to 100 times the amount of linoleic acid, but it showed an anti-oxidative action when added above that level. Hence, the appreciable pro-oxidative action of hemin observed in this study, is probably due to 1/10 level of the maximum used by Kendrick et al.[4]

The oxygen absorption by the substrate homogenates were calculated as μmoles/min/ppm of each added metal ion and tabulated in Table 1. The results indicated that the rates of oxygen absorption by the substrate homogenates induced by addition of Fe^{++} and hemin were about 100–1000 times as high as those of Fe^{+++} and Cu^{++}.

Effect of temperature on metal induced oxidation To determine the effect of temperature on pro-oxidative activities of the metal ions, the rate of oxygen absorption by the substrate homogenate were measured at the temperature from 5° to 40°C (Fig. 2). The Arrhenius plots Cu^{++} and Fe^{+++} gave a negative linear correlation with tem-

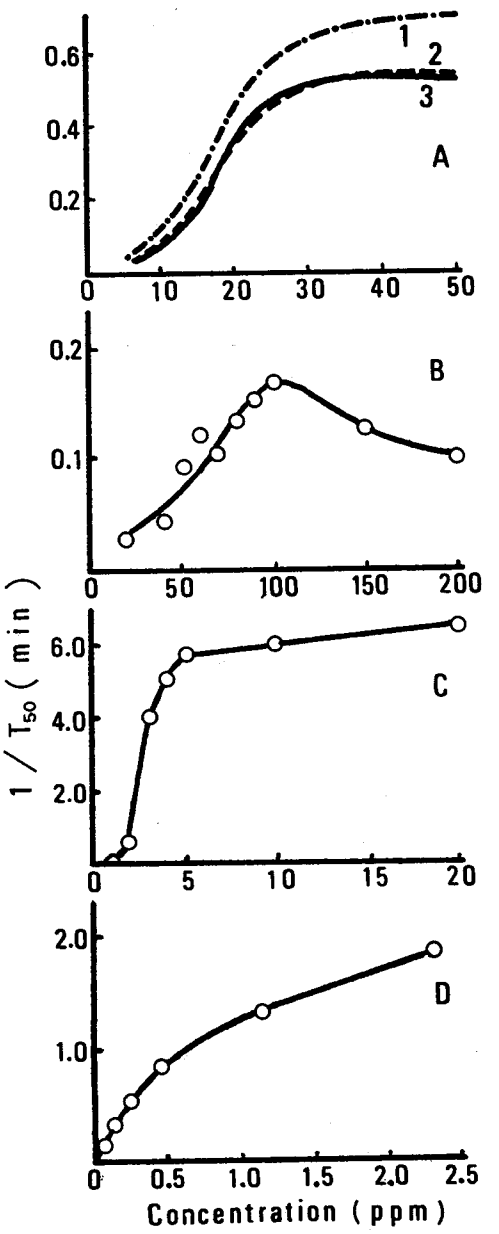

Fig. 1 Effect of the concentration of A: Cu^{++} (1, chloride; 2, nitrate; 3, sulfate), B: Fe^{+++}, C: Fe^{++} and D: Hemin on the rate of oxygen absorption in the homogenate at 40°C

Table 1. *Effect of Cu, Fe and Hemin on the rate of oxygen absorption in the homogenate at 40°C*

Metal	Oxygen absorption μmole/min/ppm
$CuSO_4$	36 $\times 10^{-3}$
$Cu(NO_3)_2$	40 $\times 10^{-3}$
$CuCl_2$	56 $\times 10^{-3}$
$Fe_2(SO_4)_3$	3.3 $\times 10^{-3}$
$FeCl_3$	3.5 $\times 10^{-3}$
$FeSO_4$	2.9
Hemin	2.5

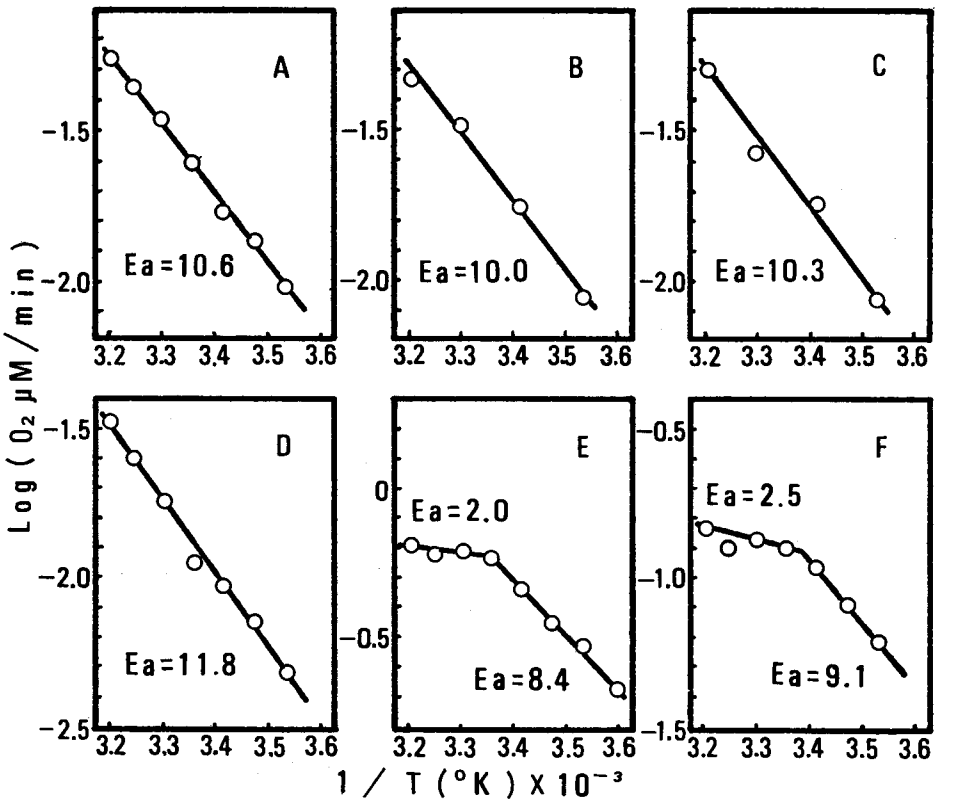

Fig. 2 Effect of temperature on the rate of oxygen absorption in the homogenate A: $CuSO_4$, B: $CuCl_2$, C: $Cu(NO_3)_2$, D: $Fe_2(SO_4)_3$, E: $FeSO_4$, F: Hemin

perature. However, in the plots referring to Fe^{++} and hemin, there is a characteristic change in slope at 25°C. Calculating the activation energies at 25°C and below from the slopes in Fig. 2, Fe^{++}, hemin, Cu^{++} and Fe^{+++} are 8.4, 9.1, 10.6 and 11.8 Kcal, respectively. Since Fe^{++} and hemin are comparatively independent of

temperature, it is thought that the pro-oxidative actions of these materials could not be ignored even at low temperatures.

The contents of Fe and Cu in leached fish muscles of Pacific fluke and flounder, prepared by the same procedure as previously described, were measured with atomic absorption spectroscopy. Fe was abundant as compared with Cu in all three leached muscles, but is especially high in Alaska pollack. Cu was contained ranging from 0.3 to 0.4 ppm in the three leached muscles (Table 2).

Table 2. *Content of Fe and Cu in the leached muscle*

	Content (ppm)		
	Alaska pollack	Pacific fluke	Flounder
Fe	12.7	4.37	3.73
Cu	0.39	0.29	0.27

It has been shown that the relative effectiveness of the metals in absorbing oxygen, when added to the substrate homogenate, was of the following decreasing order: Fe^{++}, hemin, Cu^{++} and Fe^{+++} (Table 1), and that Fe^{++} and hemin were not so dependent on temperature as compared with Cu^{++} and Fe^{+++} (Fig. 2). Hence, these results suggest that it is essential to be concerned about the accelerative activities of the metals especially heme Fe in the tissue that will produce rancidity. This will occur even though the fish muscle is kept from contamination with other metals during industrial processes and storage.

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