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Compositional Changes in Molecular Species of Fish Muscle Phosphatidylcholine during Frozen Storage

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Abstract

Compositional changes in phosphatidylcholine molecular species of mackerel and Alaska pollack muscle during frozen storage were studied.

During storage, the molecular species of (20:5) (20:5), (20:5) (22:6) and (22:6) (22:6) drastically decreased in Alaska pollack, in contrast to mackerel which exhibited a relative increase in the amount of the same molecular species.

Organoleptic and chemical changes observed in taste, flavors and other properties of fish during frozen storage are of great commercial importance. Changes in lipids often make frozen fish less acceptable. Though there is no doubt that the development of oxidative rancidity is one of the main problems with fatty fish that are rich in neutral lipids,[1] changes in phospholipids also effect the quality of fish especially when it has been stored under low temperature[1,2] conditions. The susceptibility of attack from hydrolytic enzymes or oxygen is considered to be effected not only by the fatty acid composition, but also by the molecular species of the phospholipids.[3] Therefore, the compositional changes of molecular species of a representative phospholipid, phosphatidylcholine (PC), were examined.

Experimental

Materials

Fish used for this research were mackerel (*Scomber japonicus*) caught off the coast of Hachinohe, Japan, in May 1984 and Alaska pollack (*Theraga chalcogramma*) from Uchiura Bay, Hokkaido, Japan in Dec. 1981.

The mean body weight of five mackerel examined was 446 g and that of Alaska pollack was 610 g.

Methods

The dorsal muscle of mackerel was collected and separated into dark muscle (DM) and white muscle (WM). Dorsal muscle of Alaska pollack was also collected but it was not separated into DM and WM since the amount of DM was negligible. Muscles from both fish were chopped into small pieces with a kitchen knife and were packed in polyethylene bags. These bags were stored in a freezer at −20°C for 6 months and 9 months. Total lipid was extracted from the sample according to the method of Bligh-Dyer. Lipid composition was determined by the densitometric

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method (Ozumor Densitometer model 82, Tokyo) after charring the thin layer chromatographic plate at 150-160°C. The spray reagent used for thin layer chromatography was 3% copper acetate in 8% phosphoric acid.[4] The developing solvents used for thin layer chromatography were n-hexane/ethyl ether/acetic acid (80 : 20 : 0.5, v/v) for nonpolar lipids and chloroform/methanol/acetic acid/water (25 : 15 : 4 : 2, v/v) for polar lipids. Purification and identification of PC molecular species were done in the same manner as previously reported.[5]

All the molecular species analyzed were calculated as mg/100 g muscle for the principal component analysis (PCA).[6]

Results and Discussion

Lipid compositional changes in frozen fish stored at −20°C is shown in Fig. 1. The amount of free fatty acid (FFA) increased both in mackerel and Alaska pollack during the 6 months storage while all other lipid components decreased. After 9 months storage, all the lipid components, including FFA, decreased though the relative amount of FFA increased in mackerel DM. The drastic decrease in total lipids is considered to be mostly caused by the decrease in triglyceride in the case of mackerel and PC was considered to be responsible in the case of Alaska pollack.

From an idealistic viewpoint, changes in molecular species of all the lipid classes should be studied, but unfortunately, the methodology and theory in determining the molecular species composition of marine sources has been established only for PC and phosphatidylethanolamine.[3] Compositional change of the PC molecular species during frozen storage was investigated and the result is shown in Fig. 2 as high performance liquid chromatograms (HPLC). Outstanding differences in the chromatographic patterns were observed between mackerel and Alaska
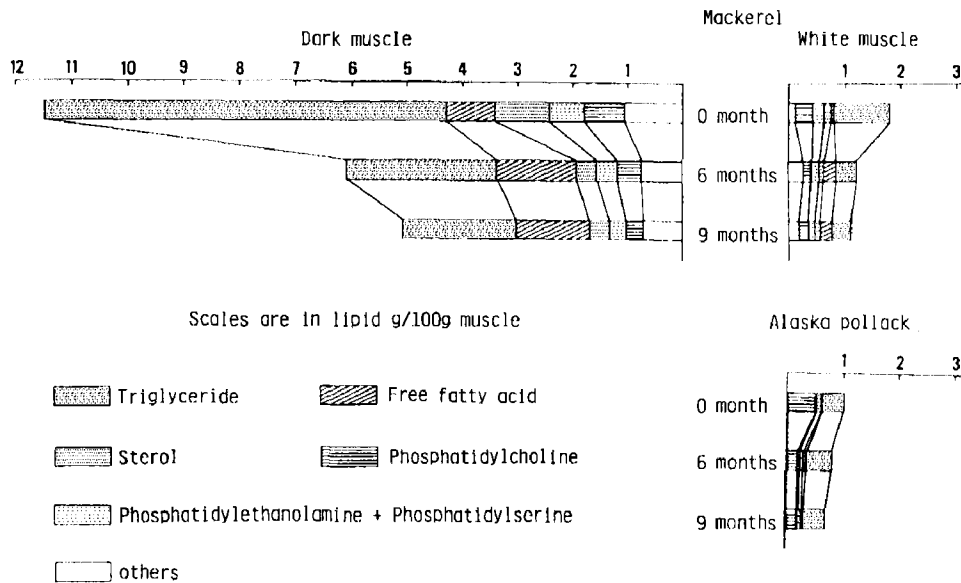

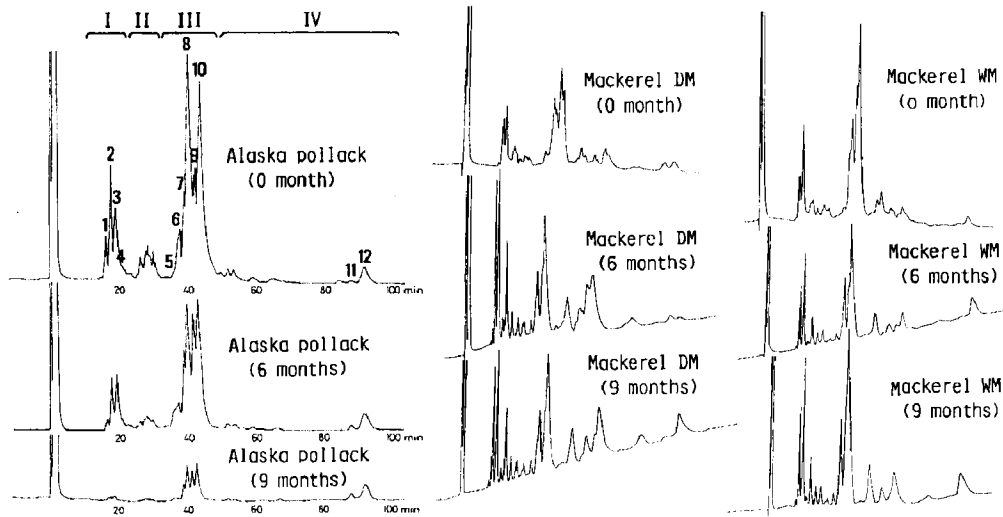Fig. 1. Lipid compositional change in fish flesh during frozen storage at −20°C.

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Fig. 2. Changes in HPLC chromatographic patterns of fish muscle PC molecular species during fronzen storge at -20°C.

I : Groups composed of highly unsaturated fatty acids, that is, 1 : (20 : 5) (20 : 5), 2 : (20 : 5) (22 : 6), 3 : (22 : 6) (22 : 6) and 4 : (20 : 4) (22 : 6).

III : Groups composed of highly unsaturated fatty acids in combination with generally found fatty acids, that is, 5 : (18 : 1) (20 : 5), 6 : (18 : 1) (22 : 6), 7 : (20 : 5) (16 : 0), 8 : (16 : 0) (20 : 5), 9 : (22 : 6) (16 : 0) and 10 : (16 : 0) (22 : 6).

II & IV : Others. 11 : (18 : 1) (16 : 0) and 12 : (16 : 0) (18 : 1).

pollack. The molecular species that belongs to group I i.e. (20 : 5) (20 : 5), (20 : 5) (22 : 6) and (22 : 6) (22 : 6), drastically dercased in Alaska pollack during storage contrasting with mackerel which exhibited a relative increase in the amount of molecular species that belongs to group I. Molecular species of group IV relatively increased in both fish though the composition of molecular species in group IV is quite different for both fish. (16 : 0) (18 : 1) was the representative molecular species of group IV in Alaska pollack while (16 : 0) (22 : 5), (16 : 0) (20 : 4), (17 : 1) (22 : 6), (18 : 0) (20 : 5) and (18 : 0) (22 : 6) were the representatives of group IV in mackerel.

Results of PCA of the compositional change in PC molecular species are illustrated in figures 3 and 4. Contribution of this PCA was 85% up to the second principal component. (16 : 0) (22 : 6) was observed to be the molecular species closest to the first principal component as shown in these figures. Eigenvectors of (16 : 0) (18 : 1) and (22 : 6) (22 : 6) appeared to have a large angle against the axis of the first principal component in both figures when compared with other molecular species. This suggests that the decrease in the amounts of (16 : 0) (18 : 1) and (22 : 6) (22 : 6) are small compared to other molecular species. It has been pointed out that 22 : 6 is the fatty acid most susceptible to oxidation.[1,2] But the results obtained in this study show that the effect of (22 : 6) (22 : 6) on the decrease in the amount of PC is smaller than that of (16 : 0) (22 : 6), especially in mackerel, even though these molecular species are composed of the same component, i.e. 22 : 6. Ohshima et al. had carefully removed the surface portion of the frozen stored sample before the lipid extraction in order to concentrate their discussion on enzymatic hydrolysis of
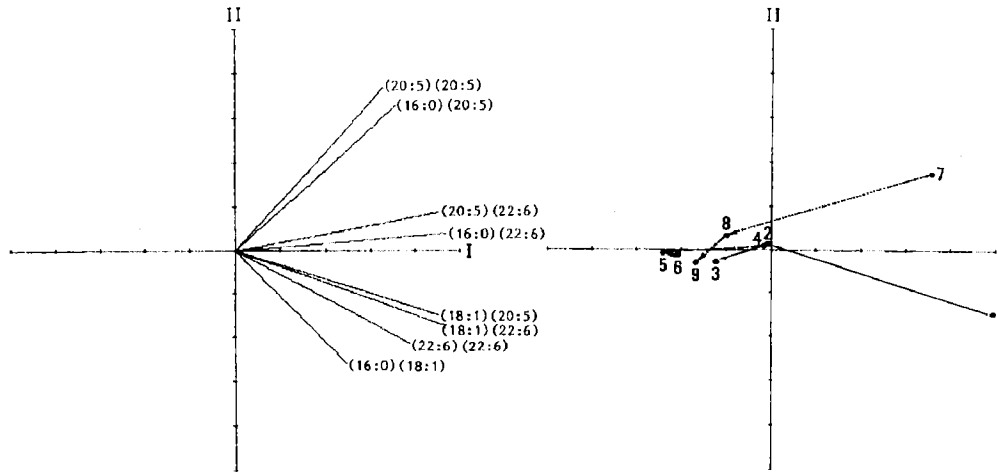
Fig. 3. Plots of principal loadings of the frozen samples and eigenvectors of the PC molecular species displayed on the first and second principal component plane on PCA.
—— Mackeral DM, - - - - - - Mackerel WM, ······ Alaska pollack.
I : First principal component, II : Second principal component.
1 : Mackerel DM (0 month), 2 : Mackerel DM (6 months), 3 : Mackerel DM (9 months), 4 : Mackerel WM (0 month), 5 : Mackerel WM (6 months), 6 : Mackerel WM (9 months), 7 : Alaska pollack (0 month), 8 : Alaska pollack (6 months), 9 : Alaska pollack (9 months).
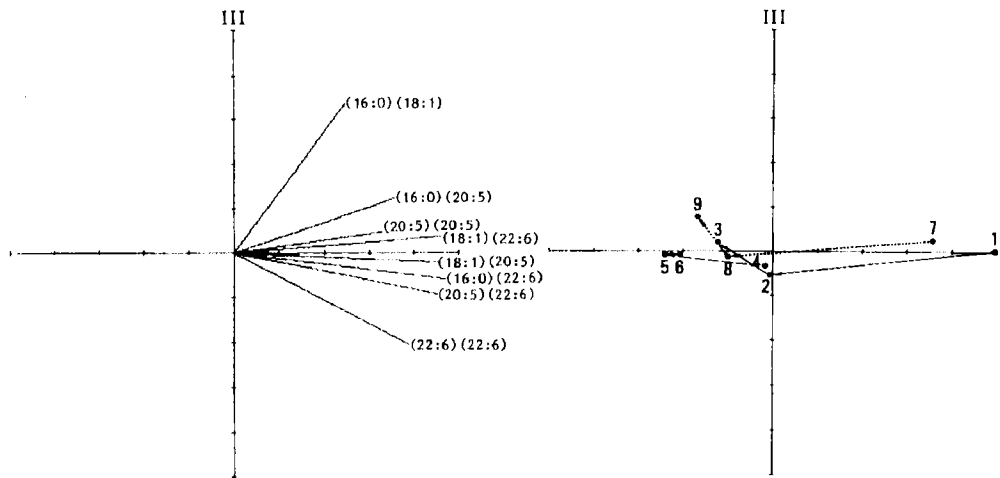

Fig. 4. Plots of principal loadings of the frozen samples and eigenvectors of the PC molecular species displayed on the first and third principal component plane on PCA.
I : First principal component, III : Third principal component.
Symbols and numbers are the same as in Fig. 3.

phospholipid.[7,8] In their discussion it was demonstrated that, both in skipjack and cod muscle, the relative percentage of $(22:6)(22:6)$ increased. This coincides with the HPLC chromatograms of mackerel shown in Fig. 2 which illustrate the relative increase in the amount of $(22:6)(22:6)$ and differ from that of Alaska pollack. In

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