真空凍結乾燥魚肉の脂質劣化に及ぼす関係湿度の影響

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Lipid Deteriorations of Freeze-Dried Fish Meats at Different Equilibrium Relative Humidities

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The rates of lipid deteriorations of freeze-dried big-eye tuna, a typical red muscle fish, and halibut, a typical white muscle fish, were studied as a function of moisture equilibrium relative humidity at 25°C.

At relative humidities of 0 and 11%, corresponding to below the monomolecular layer of water, the lipids of both fish underwent oxidation, as estimated by TBA value measurements. The rates of oxidation were faster in the big-eye tuna than in the halibut. Furthermore, the triglycerides of halibut were hydrolyzed enzymatically even at a relative humidity of 11%, though no hydrolysis of the triglycerides of big-eye tuna was detectable at the same relative humidity.

On the other hand, at higher relative humidities, such as 52 and 71%, neither fish underwent oxidative deterioration. However, the lipids were hydrolysed enzymatically during storage, with the exception of the phospholipids of big-eye tuna at 52% r.h. The hydrolyses of lipids were more significant in the halibut than in the big-eye tuna.

Most of the deteriorative changes in low and intermediate moisture foods during storage are caused by lipid oxidation, nonenzymatic browning, and enzymatic decompositions of various food constituents. It is well known that the rates of such deteriorative changes closely relate to the relative humidity of atmosphere, *i.e.* water activity, in which the food is stored. In general, as the water activity of the food system decreases, the rates of deteriorative changes also decrease or stop. However, the food exhibits highest stability to lipid oxidation when stored at the moisture content corresponding to just above the monomolecular layer of water.[1] At above and below this moisture level the rate of lipid oxidation increases. The triglycerides in the liquid state are hydrolyzed enzymatically even at the mositure content corresponding to below the monomolecular layer value, while the enzymatic phospholipid hydrolysis does not occur at the same moisture content[2,3].

Fish products are highly susceptible to oxidative rancidity, since the lipids of the products are rich in highly unsaturated fatty acids. However, no information has been available regarding the rate of lipid deterioration of fish meat stored at different relative humidities, with the exception of the report of MARTINEZ and LABUZA[4] for freeze-dried salmon meat.

In this study, the rates of lipid deteriorations of freeze-dried big-eye tuna, a typical red muscle fish, and halibut, a typical white muscle fish, were investigated as a function of moisture equilibrium relative humidity. From the results obtained it was pointed out that these two kinds of fish meats exhibit different patterns of lipid deterioration.

Experimental

Preparation of Samples

The muscles of big-eye tuna, *Thunnus obesus,* and halibut, *Hippoglossus stenolepis,* were freeze-dried. The skin and dark muscle were removed after freeze-drying and the ordinary muscles were ground into coarse powder in a mortar.

Storage Conditions of Samples

Fifteen g portions of the ground freeze-dried meat were spread in petri dishes (12 cm in diameter) which were held at 25°C in desiccators (22 cm in diameter) equilibrated to the relative humidities of 0, 11, 52, and 71% by means of phosphorus pentoxide and the saturated solutions of lithium chloride, magnesium nitrate, and strontium chloride[5], respectively. At appropriate intervals, the moisture content and thiobarbituric acid value (TBA value) of the samples were determined. The lipids were also extracted from the samples for analysis of fatty acid compositions.

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Determination of Moisture Content

Moisture content of the samples was determined using a Karl Fischer Moisture Automatic Titrator, Tsutsui Rikagaku Kikai Co., LTD.

TBA Value

TBA value of the samples was determined by the steam-distillation method described by SIDWELL *et al.*[6] For this purpose, 2 g of the samples were used.

Extraction of Lipids

The lipids were extracted from the samples after the method of FOLCH *et al.*[7]

Fractionation of Lipids

The total lipids (TL) were separated into polar (PL) and non-polar lipid fractions by column chromatography on Bio-Beads SX-2 using benzene as a solvent[8]. The non- polar lipid fraction was further separated into triglyceride (TG) and free fatty acid (FFA) fractions by column chromatography on Sephadex LH-20 using chloroform as a solvent[9]. Percentages of these lipid classes separated were calculated on the basis of their weights after drying in a vacuum desiccator.

Analysis of Fatty acid Composition of Lipids

Fatty acid composition of the lipids was analyzed by gas liquid chromatography after saponification and methylation in the usual manner. Gas liquid chromatographic conditions used were as follows; column dimension, 3 mm × 3 m; liquid phase, 15% diethylene glycol succinate; supporting material, Shimalite 60–80 mesh; column temperature, 195°C; carrier gas, nitrogen, 12 m*l*/min.

Results and Discussion

Water Sorption Isotherms

Water sorption isotherms of freeze-dried big-eye tuna and halibut meats were prepared at 25°C using desiccators equilibrated to various relative humidities by means of different concentrations of sulfuric acid. The results are shown in Fig. 1. The moisture contents of these samples corresponding to the monomolecular layer of water were calculated from the BET equation; 5.2 g H_2O/100g dry solids at 18.5% RH for big-eye tuna and 6.5 g H_2O/100 g dry solids at 21.0% RH for halibut. These values are essentially identical with 5 g H_2O/100 g dry solids at 19% RH for freeze-dried salmon reported by MARTINEZ and LABUZA[4].

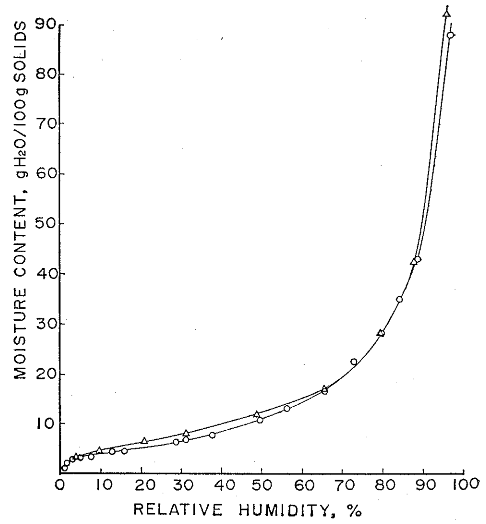

Fig. 1. Water sorption isotherms of freeze-dried big-eye tuna and halibut meats at 25°C. ○, big-eye tuna; △, halibut.

Changes in Moisture Contents during Storage

At appropriate intervals, moisture contents o the samples were determined and are shown in Fig. 2. At higher relative humidities such as 71 and 52%, the moisture contents of both samples became constant after 5 days for big-eye tuna and 7 days for halibut, while at lower relative humidity, slight decreases in the moisture contents continued for a period of more than 5 and 7 days. At 52% RH, the equilibrium moisture contents of big-eye tuna samples were lower than those of halibut. Similar differences in the moisture contents were

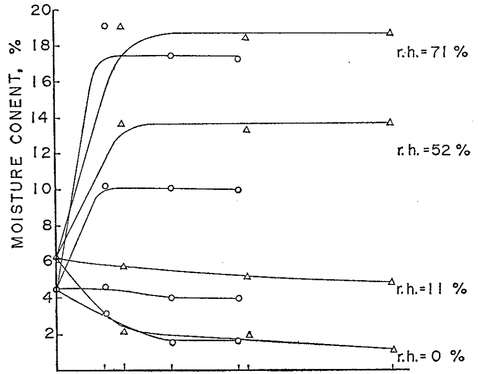

Fig. 2. Changes in the moisture contents of freeze-dried big-eye tuna and halibut meats during storage at different relative humidities indicated in the figure. ○, big-eye tuna; △, halibut.

found to a lesser extent between big-eye tuna and halibut at 71 and 11% RH.

TBA Values

Effect of relative humidities on TBA values of the samples during storage are shown in Fig. 3 for big-eye tuna and Fig. 4 for halibut. The TBA values of big-eye tuna increased at 0 and 11% RH but not at 52 and 71% RH. Similarly, the TBA values of halibut increased at lower relative humidities, though the rates of increase of TBA values were considerably slower than those in big-eye tuna.

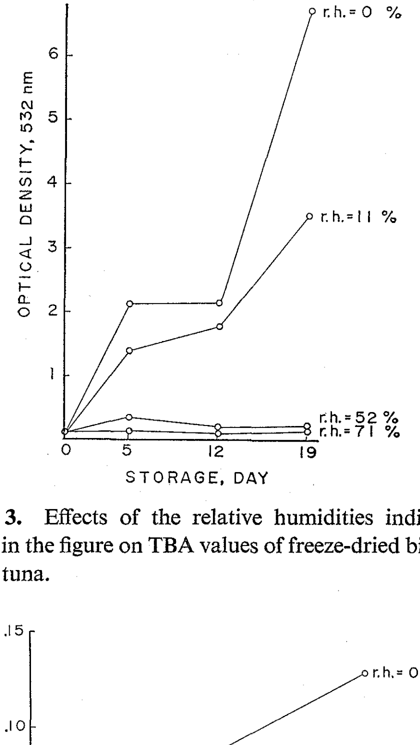

Fig. 3. Effects of the relative humidities indicated in the figure on TBA values of freeze-dried big-eye tuna.

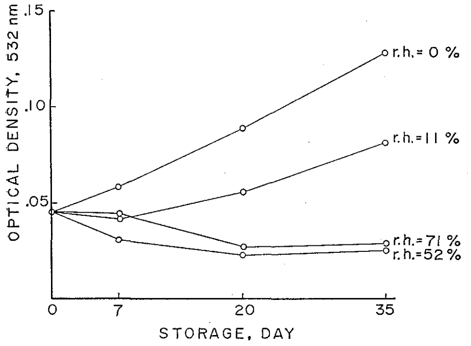

Fig. 4. Effects of the relative humidities indicated in the figure on TBA values of halibut.

These results indicate that freeze-dried big-eye tuna and halibut meats undergo oxidative deterioration when stored at the moisture levels corresponding to below the monomolecular layer of water, while both fish meats are protected from the oxidative deterioration by adsorped water at moisture contents corresponding to above the monomolecular layer value. The results obtained at the lower relative humidities coincide with those reported by MARTINEZ and LABUZA[4] for freeze-dried salmon. However, these workers did not examine the effect of water on lipid oxidation at higher relative humidity such as 71%. According to LABUZA *et al.*[10] who studied the oxidation of chicken system at different water activities, the rate of oxygen uptake of the chicken system was twice as fast at a water activity of 0.75 as at 0.1. A similar result has been reported on pork[11]. In the above studies, it has been postulated that the higher rate of oxygen uptake at high water activity is attributable to oxidation of lipids catalyzed by a trace amount of metal in the systems. These results differ from the results obtained with big-eye tuna and halibut meats in that oxidative deterioration did not occur at 71% RH. The following reasons for this might be considered: 1) the fish meats used here did not contain a metal catalyst at a concentration high enough to induce lipid oxidation and 2) the moisture contents of these fish samples were lower than those of the chicken and pork systems whose water activities were lowered by adding glycerol as a humectant. It has been known that in the intermediate moisture range the rate of lipid oxidation is faster in food of higher moisture content than in food of lower moisture content at the same water activity level[12,13].

Under the experimental conditions the lipids in halibut meat seem to be stable to autoxidation as compared with those in big-eye tuna meat.

Fatty Acid Compositions of Lipids

Fatty acid compositions of the lipids of samples are shown in Table 1. Both lipids are rich in highly unsaturated fatty acids characteristic of fish lipids.

Percentages and Fatty Acid Compositions of Lipid Classes

Big-eye tuna: The big-eye tuna meat used in this study contained 13.5% total lipid on dry basis consisting of about 75% TG, 20% PL, and 5% FFA. Effects of relative humidities on the percentages of PL, TG, and FFA of the extracted lipids are shown in Fig. 5.

At 0% RH, the TG fraction in the lipids of big-eye tuna decreased gradually accompanied by slight increases in the PL and FFA fractions up to 12 days of storage. Thereafter, the TG fraction decreased markedly while the PL and FFA

Table 1. Fatty acid compositions of the lipids of freeze-dried big-eye tuna and halibut meats (%).

Fatty acid	Big-eye tuna				Halibut			
	TL	PL	TG	FFA	TL	PL	TG	FFA
14:0	3.9	0.7	3.3	3.4	—	2.4	—	3.3
15:0	0.9	0.5	0.9	1.1	0.3	0.2	0.6	0.4
16:0	25.3	31.1	23.6	31.6	23.2	23.3	17.6	35.6
16:1	5.7	1.3	5.9	5.5	6.9	3.7	13.5	4.9
17:0	1.8	1.5	2.0	1.6	0.5	1.0	0.9	0.7
17:1	1.2	1.1	1.1	1.1	—	—	—	—
18:0	7.0	10.5	5.3	7.0	7.6	11.4	3.7	6.2
18:1	26.1	15.6	30.0	25.0	22.2	11.3	26.1	19.2
18:2	0.9	0.8	0.9	1.2	1.0	1.7	1.6	0.6
18:3	0.5	0.3	0.4	0.2	0.3	trace	1.1	0.3
19:0	0.6	0.4	0.6	0.2	—	—	—	—
20:0	0.5	0.1	0.4	0.1	—	—	—	—
20:1	3.2	0.9	3.3	2.4	6.8	2.4	11.1	4.2
20:2	0.3	0.2	0.3	0.2	1.8	0.9	3.0	0.6
20:4	3.7	5.6	2.7	2.6	1.8	3.1	2.9	1.2
20:5	4.5	3.6	5.1	3.7	9.9	17.3	7.7	10.1
22:1	—	—	—	—	2.5	—	4.4	1.2
22:4	0.4	0.4	0.2	0.1	—	—	—	—
22:5	0.9	1.9	1.0	0.8	2.3	1.9	2.3	1.0
22:6	12.5	23.6	12.8	12.2	13.0	19.3	3.4	10.5
Sat.*	40.0	44.8	36.1	45.0	31.6	38.3	22.8	46.2
Mon.*	36.2	18.9	40.3	34.0	38.4	17.4	55.1	29.5
Pol.*	23.7	36.4	23.4	21.0	30.1	44.2	22.0	24.3

* Sat., Saturated acid; Mon., Monoenoic acid; Pol., Polyenoic acid.

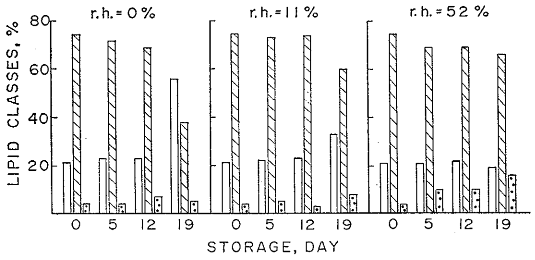

Fig. 5. Effects of the relative humidities indicated in the figure on the percentages of lipid classes in freeze-dried big-eye tuna. □, PL fractions; ▨, TG fraction; ⬚, FFA fraction.

fractions continued to increase, with a marked increase occuring in the PL fraction. A similar pattern of changes in percentages of lipid classes was found at 11% RH, though the changes in the TG and PL fractions were not so marked as those found at 0% RH. The marked increase in the PL fraction after 19 days of storage seemed to be due to some of the oxidized triglycerides which might be separated into the PL fraction by column chromatography on Bio-Beads SX-2. This appears to be supported by the fact that such a marked increase in the PL fraction did not occur in the big-eye tuna stored at 52% RH at which water exerted protective effect against oxidative deterioration of lipids. These results show that the TG fraction, in addition to the PL fraction, is oxidized markedly when big-eye tuna meat is stored at the moisture content corresponding to below the monomolecular layer of water.

On the other hand, at 52% RH, the TG fraction decreased during storage, while the FFA fraction increased. In general, formation of free fatty acids in fish products is considered to be due to either oxidative degradation of unsaturated fatty acids or enzymatic hydrolysis of lipids or both. The increase in the FFA fraction at 52% RH implies the enzymatic hydrolysis of the lipids since oxidative deterioration did not occur as estimated by TBA value. The triglycerides of the lipid may be hydrolyzed to liberate free fatty acids through the endogenous enzymatic system of the big-eye tuna.

Effects of relative humidities on the fatty acid compositions of the lipids during storage are shown in Figs. 6–9. The fatty acid compositions of lipids were expressed as relative amounts of three groups of fatty acids, saturated, monoenoic, and polyenoic acids.

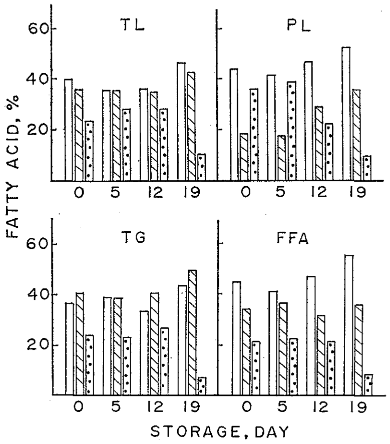

Fig. 6. Effect of the relative humidity of 0% on the fatty acid compositions of the lipid classes in freeze-dried big-eye tuna. ☐, saturated acid; ▨, monoenoic acid; ░, polyenoic acid.

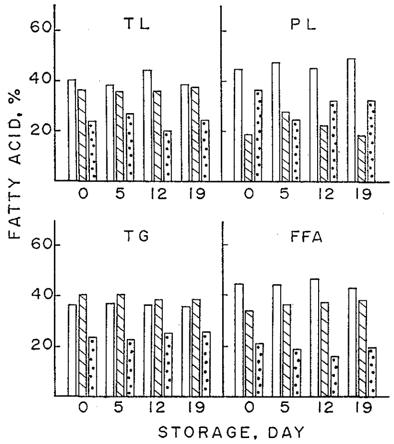

Fig. 8. Effect of the relative humidity of 52% on the fatty acid compositions of the lipid classes in freeze-dried big-eye tuna. See the legend of Fig. 6.

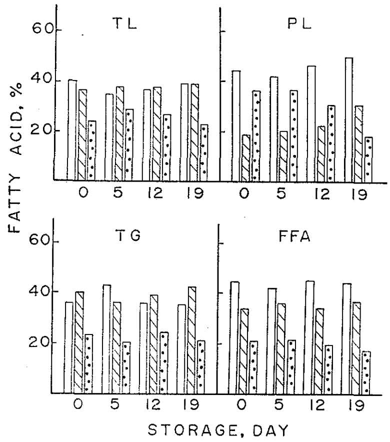

Fig. 7. Effect of the relative humidity of 11% on the fatty acid compositions of the lipid classes in freeze-dried big-eye tuna. See the legend of Fig. 6.

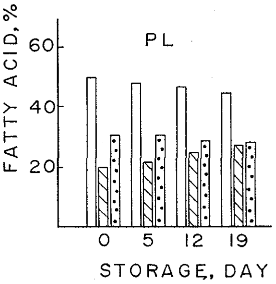

Fig. 9. Effect of the relative humidity of 71% on the fatty acid compositions of the polar lipid fraction in freeze-dried big-eye tuna. See the legend of Fig. 6.

At 0% RH, the polyenoic acid in the PL fraction decreased after 12 days of storage and those of the TG and FFA fractions after 19 days of storage, showing that lipid oxidation commenced with the polyenoic acid of PL fraction. Similarly, at 11% RH the polyenoic acid in these lipid classes decreased, but to a lesser extent.

At 52% RH, the percentage of polyenoic acid in the PL fraction varied irregularly during storage, as shown in Fig. 8. However, the monoenoic acid in the TG fraction gradually decreased with an accompanying slight increase in the polyenoic acid. The apparent incrcase in polyenoic acid content of TG fraction may be due to the decrease of monoenoic acid content. On the other hand, in the FFA fraction, the monoenoic acid increased gradually while polyenoic acid decreased. According to Brockerhoff *et al.*[14)], in the triglycerides of fish lipids the saturated and monoenoic acids such as $C_{18:1}$ distribute at high frequency on 1- and 3-positions of glycerol moiety and polyenoic acid such as $C_{22:6}$ on 2-position. That the monoenoic acid content decreased in the TG fraction and increased in the FFA fraction seems to be elucidated as a result of enzymatic hydrolysis of the triglycerides at 1- and 3-positions, though no examination for mono- and diglyceride formations was made. The enzyme involved in the hydrolysis of triglycerides is not derived from microorganisms because microorganisms can not grow at this relative humidity range.

From the results that the PL content remained almost unchanged during storage at 52% RH, as seen in Fig. 5, the phospholipids seemed not to be hydrolyzed at this relative humidity.

The freeze-dried big-eye tuna meat used in the experiment at 71% RH contained 6.2% total lipid on dry basis consisting of about 17% TG, 70% PL, and 13% FFA. Effect of relative humidity of 71% on the fatty acid composition of PL fraction, a major constituent of this lipid, are shown in Fig. 9. During storage the saturated acid and polyenoic acid decreased gradually and the monoenoic acid increased. The changes in fatty acid compositions may be due mainly to enzymatic hydrolysis of phospholipids but not to oxidative destruction of phospholipids because the TBA values of the samples did not increased during storage as mentioned before. If the saturated acids distribute in high frequency on the 1-position of glycerol moiety in the fish phospholipids as in the pork muscle phospholipids[15], then the decrease in saturated acid content of the PL fraction may be elucidated as a result of hydrolysis at the 1-position of glycerol in phospholipids. An alternative explanation for the decrease of saturated acid content in the PL fraction is enzymatic hydrolysis commencing specifically with the phospholipids containing saturated acids as constituent fatty acids.

Halibut meat: Halibut meat used in this study contained 5.5% total lipid on dry basis consisting of about 32% TG, 55% PL, and 13% FFA. Effects of relative humidities on the percentages of PL, TG, and FFA of extracted lipids are shown Fig. 10.

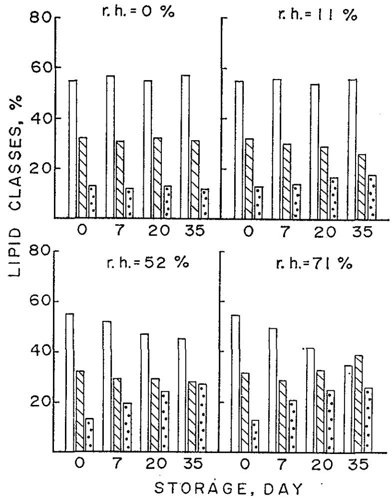

Fig. 10. Effects of the relative humidities indicated in the figure on the percentages of lipid classes in freeze-dried halibut. ☐, PL fraction; ▨, TG fraction; ⁙, FFA fraction.

At 0% RH, the percentages of the three classes of lipids remained almost unchanged during 35 days of storage. The lipids in halibut meat seem to be stable to oxidative and enzymatic degradations at this relative humidity.

On the other hand, at 11% RH, the TG fraction decreased gradually, the FFA fraction increased, and PL fraction remained unchanged, suggesting that the enzymatic hydrolysis of triglycerides occurred during storage. At 52% RH, though the samples did not undergo oxidative deterioration, as mentioned before, the PL and TG fractions decreased gradually and the FFA fraction increased markedly. At increased relative humidity of 71%, the decrease of the PL fraction and the increase of the FFA fraction became more notable than at 52% RH. At this relative humidity, the TG fraction increased markedly after 35 days of storage. These results suggest that the hydrolyses of lipids occur during storage at higher relative humidities.

In Figs. 11–14, effects of relative humidities on the fatty acid compositions of the lipids are shown. At 0% RH, the percentage of polyenoic acid in PL fraction decreased within 20 days of storage and, thereafter, increased up to the initial level. The results for 7 and 20 days of storage seem to be due to experimental error. The fatty acid compositions of TG and FFA fractions remained

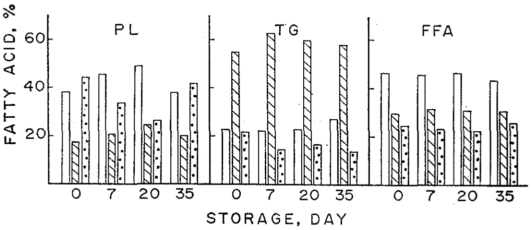

Fig. 11. Effect of the relative humidity of 0% on the fatty acid compositions of the lipid classes in freeze-dried halibut. See the legend of Fig. 6.

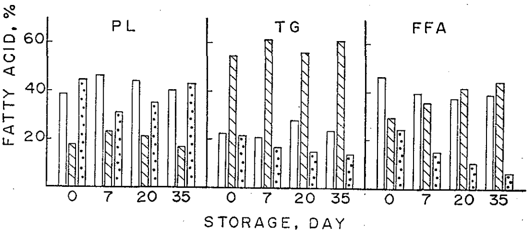

Fig. 12. Effect of the relative humidity of 11% on the fatty acid compositions of the lipid classes in freeze-dried halibut. See the legend of Fig. 6.

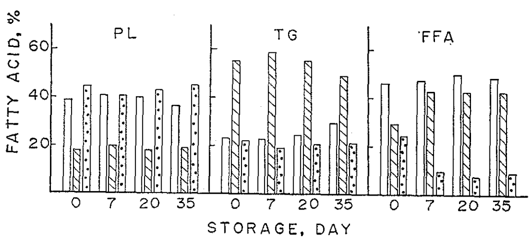

Fig. 13. Effect of the relative humidity of 52% on the fatty acid compositions of the lipid classes in freeze-dried halibut. See the legend of Fig. 6.

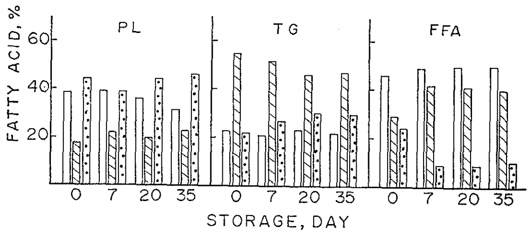

Fig. 14. Effect of the relative humidity of 71% on the fatty acid compositions of the lipid classes in freeze-dried halibut. See the legend of Fig. 6.

almost unchanged during 35 dayts of storage.

At 11% RH, marked changes in the fatty acid compositions of FFA fraction occurred, that is, the percentage of polyenoic acid decreased and that of monoenoic acid increased. The apparent decrease in polyenoic acid content may be due to increase of the monoenoic acid content because the oxidative deterioration of these samples, as estimated by TBA value, were negligible. On the basis of the facts that in the triglycerides of fish muscle lipids the saturated and monoenoic acids distribute in high frequency on 1- and 3-positions of glycerol moiety, as discussed before, and that the hydrolysis of triglycerides, differing from that of phospholipids, occurs even at the low relative humidity such as 10%[2,13,16], the increase of monoenoic acid content of FFA fraction may be attributed to the hydrolysis of triglycerides. Such hydrolysis of triglycerides was not found in big-eye tuna at this relative humidity. This may be because the equilibrium moisture contents of the big-eye tuna at 11% RH are slightly lower than those of the halibut at the same relative humidity or the enzyme system involved in the hydrolysis is more active in the halibut than in the big-eye tuna.

At 52 and 71% RH, the percentages of saturated acid in the PL fraction, monoenoic acid in the TG fraction, and polyenoic acid in the FFA fraction decreased during storage. The apparent decrease in polyenoic acid content of FFA fraction might be elucidated as a result of increases in the contents of saturated and monoenoic acid liberated from the PL and TG fractions through their enzymatic hydrolysis.

From these results, it is clear that the freeze-dried halibut meat undergoes enzymatic hydrolysis of both phospholipids and triglycerides when stored at higher relative humidity.

In short, the freeze-dried meats of big-eye tuna, a red muscle fish, and of halibut, a white muscle fish, undergo deterioration of lipids when stored at 25°C and this deterioration follows a different pattern depending on the equilibrium relative humidity at which the fish meats are stored.

At relative humidity corresponding to below the monomolecular layer of water, the lipids of both fish meats undergo oxidation and the rate of oxidation is faster in the big-eye tuna than in the halibut. Furthermore, the triglycerides of halibut meat are hydrolyzed enzymatically even at a relative humidity of 11%, though the hydrolysis of triglycerides of big-eye tuna is not detectable at the same relative humidity.

On the other hand, at higher relative humidities such as 52 and 71%, neither fish meats undergo oxidative deterioration. However, the lipids are hydrolyzed enzymatically during storage, with the exception of the phospholipids of big-eye tuna at 52% RH. The hydrolyses of lipids are emphasized in the halibut meat compared with the big-eye tuna meat.

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