to function by competing with proline for the available nitrosating species, the initially formed 1-nitrosoethoxyquin rearranging to 8-nitrosoethoxyquin prior to undergoing aerial oxidation to 8-nitroethoxyquin. The latter substance was isolated and positively identified from ethoxyquin treated bacons in amounts of up to 60 ppb.

Ethoxyquin, dihydroethoxyquin, and the compounds closely related to them are perhaps the most efficacious antinitrosamine agents in bacon discovered so far. Ethoxyquin is already an accepted additive in feed and some foods, and its use could probably be safely extended to other foods. However, our work clearly shows that in judging the safety of a food additive, we must not only consider the additive itself but also the mechanism by which it acts and the products which may be formed from it. Thus in the system, described above, we must not only consider the safety of ethoxyquin and its analogues, but also of the corresponding *N*-nitroso, 8-nitroso, and particularly the 8-nitro derivatives. This principle, although supposedly generally accepted, is not always applied with sufficient care and thoroughness.

Registry No. I, 16489-90-0; II, 97191-39-4; III, 66170-15-8; IV, 66170-14-7; V, 66053-07-4; VI, 120-15-0; VII, 66099-38-5; VIII, 66053-08-5; $NaNO_2$, 7632-00-0; NOCl, 2696-92-6; ethoxyquin, 91-53-2; 1-nitrosoethoxyquin, 71043-62-4; 8-nitrosoethoxyquin, 97191-40-7; 8-nitroethoxyquin, 71043-61-3.

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Changes in Free Amino Acids and Protein Denaturation of Fish Muscle during Frozen Storage

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Changes in quantity and composition of free amino acids and their relation to protein denaturation in the muscle of various fish during frozen storage at −20 °C were studied. Based on the measurements of extractability of 0.6 M KCl soluble proteins, extractability of actomyosin, Ca ATPase activity of actomyosin, and electrophoretic analysis, the muscle protein of frozen mackerel was the most unstable, then amberfish, mullet, and carp, respectively. Muscle having higher free amino nitrogen content also had greater protein denaturation. The predominant free amino acids in the muscle of frozen mackerel, amberfish, and mullet were histidine, lysine, alanine, and taurine, whereas proline, glycine, alanine, histidine, and taurine was abundant in carp. It was also found that the muscle protein with high levels of free histidine and lysine was relatively unstable, while that of fish muscle with high levels of free glycine, proline, and alanine was stable during frozen storage at −20 °C.

INTRODUCTION

Deterioration in the quality of fish muscle has frequently occurred during frozen storage, due to undesirable processes taking place in lipid and proteins (Sikorski, 1978, 1980). These changes in fish muscle are of great commercial importance since they determine the storage life of frozen seafoods. Deterioration in texture, flavor, and color is considered to be the most serious problems especially when both the poor freezing practices are employed and the quality of fish is inferior. The undesirable changes in flavor are thought to be due to the formation of low molecular weight compounds from lipid oxidation or protein degradation. The deteriorative changes in color and appearance are due to the irreversible changes that occurred in muscle proteins, protein-bound pigments, or in certain pigmented proteins (Matsumoto and Matsuda, 1967; Shenouda, 1980; Suzuki, 1981; Noguchi, 1982). However, undesirable changes in texture as a consequence of long term storage are a major consideration in grading the quality of frozen seafoods. It is also an unsolved problem for many fishery products. It is considered to be due to protein denaturation during frozen storage (Dyer, 1951; Sikorski et al., 1976; Sikorski, 1978, 1980; Matsumoto, 1979, 1980; Shenouda, 1980; Andou et al., 1979, 1980; Noguchi, 1982; Acton et al., 1983). The degree of protein denaturation is influenced by many factors such as treatments before freezing, state of rigor at the time of freezing, freezing rate, ultimate freezing temperature, storage temperature, and period, fluctuation of storage temperature, and thawing methods, etc.

After Dyer and his co-workers suggested that the lipids and their derivatives might be involved in protein denaturation during frozen storage of fish muscle (Dyer, 1951; Dyer and Morton, 1956; Dyer et al., 1956; Dyer and Dingle, 1961), many studies were carried out to investigate this

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possibility (Dyer and Fraser, 1959; Bligh, 1961; Hanson and Olley, 1965). The effect of fish lipid on protein denaturation during frozen storage varies according to the state of the lipid (Matsumoto, 1980; Shenouda, 1980; Andou et al., 1979, 1980, 1981a, 1981b). The role of intact lipid on the stability or instability of fish myofibrillar proteins has been vague. There were some evidences that their presence, particularly in biological systems, was essential and played a protective role for the proteins involved (Dyer, 1951; Simizu and Simizu, 1957; Dyer and Dingle, 1961; Love and Elerian, 1965; Akiba et al., 1967; Ikeda and Taguchi, 1967, 1968; Taguchi and Ikeda, 1968a, 1968b; Andou et al., 1979). Some evidences, particularly from model system studies, indicated that they had a detrimental effect on protein stability by forming lipoprotein complexes (Shenouda and Pigott, 1974, 1975a, 1975b, 1976; Shenouda, 1980).

Various factors involved in fish preservation not only influence the activity of proteolytic enzymes but bring about denaturation of muscle proteins and their interactions with other compounds originally present in the muscle (Sikorski, 1978, 1980). Many secondary changes in proteins, following the denaturation, are caused by the interaction of SH groups, both of protein and nonprotein origin. Aggregation of protein molecules can occur due to sulfhydryl–disulfide exchange, without net change in available SH groups (Buttkus, 1970). Some amino acids revealed protective effects on the stability of fish muscle proteins (Noguchi, 1974; Ooizumi et. al., 1982). However, evidences that some amino acids triggered the denaturation of muscle proteins during frozen storage were also observed in studies by Noguchi et al. (1970, 1971, 1974, 1975a, 1975b). The effect of free amino acids from protein degradation and the interaction between proteins and these free amino acids during frozen storage have not yet been studied, in spite of their potential importance in intensifying or activating the other deteriorative biochemical reactions. The present study aims to investigate the effect of free amino acids on protein denaturation so as to clarify the nature and mechanism of protein denaturation in frozen fish muscle.

MATERIALS AND METHODS

The fish used in this study included salt water fish—mackerel (*Scomber tapeinocephalus*) and amberfish (*Qecapterus marudasi*)—brackish fish–mullet (*Mugil cephalus, Linnaeus*), and fresh water fish—carp (*Cyprinus carpino, Linnaeus*). Mackerel (body weight, 450–600 g; length, 25–30 cm) and amberfish (body weight, 250–300 g; length, 20–25 cm) were purchased on the same day from a fish market in Northern Taiwan. They were harvested by the same fishing boat from the Pacific Ocean on Feb, 1982, and iced for about 24 h before studying. Mullet (body weight, 450–600 g; length, 20–30 cm) and carp (body weight, 400–500 g; length, 20–30 cm) were obtained from a commercial culture farm in Southern Taiwan. After being netted, the fish samples were kept alive and transported immediately to the laboratory. All fish were gutted and headed. After washing the carcases, they were frozen to a body temperature below −18 °C. These frozen samples were packed in polyethylene bags with five fish to each bag, and stored at −20 °C for 12 weeks. At definite time intervals, one bag of each group were removed and thawed by immersing in running water until the body temperature reached 0 °C. The body temperature was measured by using a thermocouple. Five freeze-thawed fish from each group were deboned, skinned, and pooled together. Three samples from each group were subjected to the following measurements.

Extraction of Free Amino Acids and Analyses of the Composition. The extraction of free amino acids was done according to Suyama (1974). The nitrogen content in these samples was determined by the micro-Kjeldahl method (Lang, 1958). The amino acid composition of these samples was measured with a JEOL LC-6AH Amino Acid Analyzer according to the procedure of Spackman et al. (1958).

Determination of the Protein Denaturation of Fish Muscle. The extractability of the 0.6 M KCl soluble proteins, extractability of actomyosin, Ca ATPase activity of actomyosin, and changes in the electrophoretic patterns of the 0.6 M KCl soluble proteins were determined for evaluating the extent of protein denaturation of fish muscle during frozen storage.

Extraction of 0.6 M KCl Soluble Proteins. To 10 g of fish meat was added 90 mL of chilled 0.6 M KCl solution and the solution homogenized with a Waring Blender subjoined with a baffle plate. Centrifugation was performed under 5000*g*, 0 °C, for 30 min. The supernatant was made to 300 mL with 0.6 M KCl solution. The concentration of protein was determined by the Biuret method modified by Umemoto (1966). Extractability of the 0.6 M KCl soluble proteins was expressed as milligrams of protein per gram of muscle.

Extraction of Actomyosin. The actomyosin was extracted according to Noguchi (1974). Concentration of actomyosin was determined by using the Biuret method modified by Umemoto (1966). Extractability of actomyosin was expressed as milligrams of actomyosin per gram of muscle.

Ca ATPase Activity of Actomyosin. To 1 mL of actomyosin solution (1–5 mg/mL), 0.5 mL of 0.5 M Tris-maleate buffer (pH 7.0), 2.2 mL of 2.0 M KCl, 0.5 mL of 0.1 M $CaCl_2$, 5.3 mL of distilled water, and a 0.5 mM Na_2 ATP solution (pH 7.0) were added in this order. After the addition of 5 mL of 15% trichloroacetic acid to stop the reaction, the rate of release of inorganic phosphate at 25 °C from the addition of ATP was determined according to Arai (1974). The specific Ca ATPase activity was shown as micromoles of inorganic phosphate released per milligram of actomyosin within a minute for the reaction at 25 °C. The total Ca ATPase activity was expressed as micromoles of phosphate released per minute from actomyosin solution extracted from 10 g of muscle.

Sodium Dodecyl Sulfate (SDS)–Polyacrylamide Gel Electrophoretic Analyses. For understanding the conformational changes of the salt-soluble proteins, the SDS–polyacrylamide gel electrophoretical analysis was monitored according to Weber and Osborn (1969). The 0.6 M KCl soluble proteins were incubated at 40 °C for 2 h in a solubilizing solution consisting of 0.01 M sodium phosphate buffer (pH 7.2), 1% SDS, 25% glycerin, and 2% mercaptoethanol. The solubilized protein solution was then dialyzed overnight at room temperature against 0.01 M sodium phosphate buffer containing 0.1% SDS, pH 7.2. On the top of the polyacrylamide gel, 0.02 mL of 0.05% bromophenol blue and 0.03 mL of the dialyzed protein sample were pipetted. After the electrophoretic run in 0.1% SDS–0.1 M sodium phosphate buffer (8 mA each gel), the gels were stained overnight with 0.12% Comassie blue–50% methanol–9.2% acetic acid solution. Destaining was done with immersing in a mixture of 50% methanol and 7.5% acetic acid for 8–10 h, as recommended by Seki (1974). The concentration of polyacrylamide in this study was 10%.

Distance scanning at the wavelength 585 nm, for which the staining solution has maximum absorbance, was em-

Table I. Effect of the Frozen Storage on the Extractability of 0.6 M KCl Soluble Proteins and Actomyosin (mg/g of Meat) of Mackerel, Amberfish, Carp, and Mullet Muscle[a-c]

weeks at −20 °C	mackerel		amberfish		carp		mullet	
	A	B	A	B	A	B	A	B
fresh	205.6 (100) a	109.9 (100) a	181.1 (100) a	116.6 (100) a	175.3 (100) a	92.0 (100) a	206.4 (100) a	135.9 (100) a
3	197.4 (95.8) b	107.7 (98.0) a	168.0 (92.8) b	105.8 (90.7) b	168.1 (95.9) b	89.2 (97.0) b	187.2 (90.7) b	114.4 (84.2) b
6	168.3 (81.9) c	88.2 (80.3) b	164.9 (91.1) b	94.5 (81.1) c	161.6 (92.2) c	78.8 (85.7) c	184.5 (89.4) b	96.9 (71.3) c
9	142.4 (69.3) d	58.2 (53.0) c	144.0 (79.5) c	83.5 (71.6) d	158.6 (90.5) c	70.5 (76.6) d	171.0 (82.9) c	74.9 (55.1) d
12	136.8 (66.5) d	50.5 (46.0) d	130.8 (72.2) d	74.9 (64.2) e	139.8 (79.7) d	65.0 (70.7) e	155.5 (75.3) d	69.4 (51.1) e

[a] A, extractability of 0.6 M KCl soluble proteins. B, extractability of actomyosin. [b] Values in the parenthesis are percentage values relative to the original one. [c] Values in the same column bearing unlike letters differ significantly ($P < 0.01$).

Table II. Effect of Frozen Storage on the Ca ATPase Activity of, Actomyosin of Mackerel, Amberfish, Carp, and Mullet Muscle[a-c]

week at 20 °C	mackerel	amberfish	carp	mullet
	Specific Ca ATPase Activity (Pi μmol/min/mg of Protein)			
fresh	0.172 (100) a	0.188 (100) a	0.241 (100) a	0.208 (100) a
3	0.098 (57) b	0.149 (79) b	0.146 (61) b	0.146 (70) b
6	0.059 (34) c	0.098 (52) c	0.109 (45) c	0.101 (49) c
9	0.050 (29) c	0.073 (39) d	0.098 (41) c	0.090 (43) c
12	0.057 (33) c	0.050 (27) e	0.073 (30) d	0.063 (30) d
	Total Ca ATPase Activity (Pi μmol/min/10 g of Meat)			
fresh	189.0 (100) a	219.2 (100) a	221.7 (100) a	282.7 (100) a
3	105.6 (56) b	157.6 (72) b	130.2 (59) b	167.0 (59) b
6	52.1 (28) c	92.6 (42) c	85.9 (39) c	97.9 (35) c
9	29.1 (15) d	61.0 (28) d	69.1 (31) d	67.4 (24) d
12	28.8 (15) d	37.5 (17) e	47.5 (21) e	43.7 (16) e

[a] Values in this table were the means from 3 determinations. [b] Values in the parenthesis are percentage values relative to the original one. [c] Values in the same column bearing unlike letters differ significantly ($P < 0.01$).

ployed to analyze the bands on the gel by using an UV–vis Microprocessor-Controlled Spectrophotometer System 2600 (Gilford Instrument).

Statistic Analyses. Duncan's multiple range test was used for statistical analyses.

RESULTS AND DISCUSSION

Protein Denaturation during Frozen Storage. Changes during frozen storage in the extractability of salt-soluble proteins and actomyosin of mackerel, amberfish, carp, and mullet are shown in Table I. Regardless of the species used in this study, the quantity of salt-extractable proteins decreased gradually during 12 weeks storage. The mackerel samples exhibited the highest insolubilization in salt-soluble proteins, followed by amberfish, mullet, and carp. The salt-soluble proteins decreased to 66.5, 72.2, 75.3, and 79.7% of the original values in these species, respectively, after 12 weeks storage at −20 °C.

Actomyosin is considered to be the protein group responsible for the gel strength, or ashi, of minced fish products. As shown in Table I, insolubilization of actomyosin was observed in all species. The highest actomyosin insolubilization was found to be the mackerel, then amberfish, mullet, and carp in decreasing order. Decrease in extractable actomyosin exhibited was almost parallel to that of salt-soluble proteins. However, extractable actomyosin was more affected by freezing and frozen storage than the salt-soluble proteins were. This is because the salt-soluble proteins extracted in this study consists of myofibrillar proteins and sarcoplasmic proteins, and the sarcoplasmic proteins do not undergo significant changes during frozen storage (Sikorski et al., 1976).

Changes in Ca ATPase activity of actomyosin during frozen storage are shown in Table II. Protein quality is considered to be more sensitively reflected by the enzymatic activity than the extractability. This is because small microstructural changes in protein molecules will cause alterations in enzymatic activity. As shown in Table II, severe loss in Ca ATPase activity was obtained in actomyosin of freeze-thawed mackerel after 6 weeks storage at −20 °C. The Ca ATPase activity of actomyosin in this species lost 66% of the original value after 6 weeks storage. Considering the loss in Ca ATPase activity of actomyosin, the inactivation rate was fastest in mackerel samples, followed by amberfish, mullet, and carp, respectively.

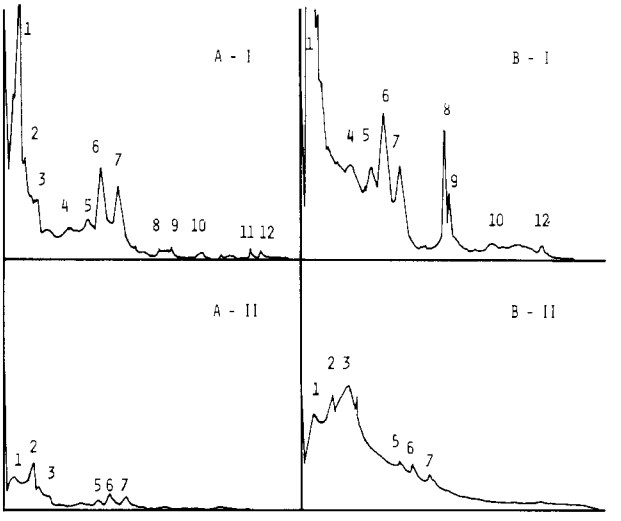

Figure 1. Changes in the electrophoretic profiles of 0.6 M KCl soluble proteins of mackerel (A) and amberfish (B) muscle during frozen storage at −20 °C (I, from fresh muscle; II, after 12 weeks storage).

The changes in electrophoretic separation of 0.6 M KCl soluble proteins of these species during frozen storage are expressed schematically in Figures 1 and 2. In these figures, peaks 1 and 6 are considered to be the myosin heavy chains and actin, respectively. The changes in electrophoretic separation pattern of salt-soluble proteins of mackerel and amberfish during 12 weeks storage are shown in Figure 1. Peaks 9, 11, and 12 are considered to be the light chains of myosin. After 12 weeks storage all the light chains disappeared, and a broad band was formed on the top of the gel, especially in the amberfish sample.

Table III. Changes in the Quantity of Free Amino Nitrogen (mg/g of Meat) of Mackerel, Amberfish, Carp, and Mullet Muscle during Frozen Storage at -20 °C[a]

species	weeks at -20 °C				
	0	3	6	9	12
mackerel	5.31 ± 0.08	5.78 ± 0.11	5.86 ± 0.11	7.46 ± 0.18	10.18 ± 0.14
amberfish	4.22 ± 0.10	5.03 ± 0.09	5.21 ± 0.06	6.18 ± 0.14	9.12 ± 0.13
carp	2.02 ± 0.04	2.89 ± 0.06	2.96 ± 0.10	3.67 ± 0.09	5.48 ± 0.11
mullet	2.98 ± 0.07	3.38 ± 0.11	3.53 ± 0.09	4.27 ± 0.13	6.70 ± 0.03

[a] Means ± SD. Values in this table were the means from 3 determinations.

Table IV. Changes in Free Amino Acids (μmol/100 g of Meat) of Mackerel, Amberfish, Mullet, and Carp Muscle during Frozen Storage at -20 °C[a]

amino acid	species							
	mackerel		amberfish		mullet		carp	
	A	B	A	B	A	B	A	B
Tau	414	480	468	425	599	585	615	690
Asp	4		trace		trace		5	
Thr	52	28	76	33	50	40	65	30
Ser	52	22	98	53	27	20	105	100
Glu	74	39	54	61	47	45	45	23
Pro	34	11	136	18	143	120	80	90
Gly	104	44	110	57	430	530	1915	1438
Ala	260	123	246	163	203	175	560	403
Val	44	24	58	24	23	25	40	28
$^1/_2$CyssCy	6	trace	4		13		15	
Met	6	13	8	11	7		30	8
Ile	28	13	32	11	13	15	30	15
Leu	48	20	60	18	23	20	50	28
Tyr	28	18	10	7	10		20	8
Phe	18	7	8	7	7		15	8
His	4334	4492	2014	3056	2314	1460	630	720
Lys	88	35	216		173		160	115
Arg	20		16		17		5	
ammonia	1138	1117	822	801	639	525	470	320

[a] A, measured at fresh state. B, measured after 12 weeks storage at -20 °C.

It suggests that interactions among the myosin light chains and other components occurred during frozen storage. Although the actin (peak 6) could still be observed after 12 weeks storage, the percentage of actin was relatively low in salt-soluble proteins. The electrophoretic separation of salt-soluble proteins from carp and mullet is shown in Figure 2. The myosin heavy chains and light chains, and actin of carp muscle proteins could clearly be observed even after 12 weeks storage at -20 °C, however, the prcentage of myosin heavy chains decreased. It is obvious that the protein denaturation in this species occurs mainly on the myosin heavy chains. As shown in Figure 2 part D, the band size of myosin heavy chains and actin decreased after 12 weeks storage. A broad band formed around 50 000–80 000 daltons in molecular weight. Apparently, dissociation of myosin heavy chains, and further interaction with low molecular weight components occurred in the salt-extractable proteins of frozen mullet.

According to the electrophoretic analyses, it is clear that the protein denaturation patterns vary depending on the fish species. Generally, actin is considered to be stable during frozen storage (Connell, 1960; Sikorski et al., 1976; Matsumoto, 1979, 1980; Shenouda, 1980). The actin in muscle proteins of carp showed very stable during frozen storage, based on the electrophoretic analyses, whereas that of mackerel, amberfish, and mullet was unstable.

From the above data, it is seen that the Ca ATPase activity of acomyosin of frozen fish muscle decreased much faster than the extractability of salt-soluble proteins and actomyosin. This might be due to the dissociation and/or interactions occurring on the heavy chains of the extractable myosin molecules.

Development of Free Amino Acids and Their Compositions during Frozen Storage. The corresponding

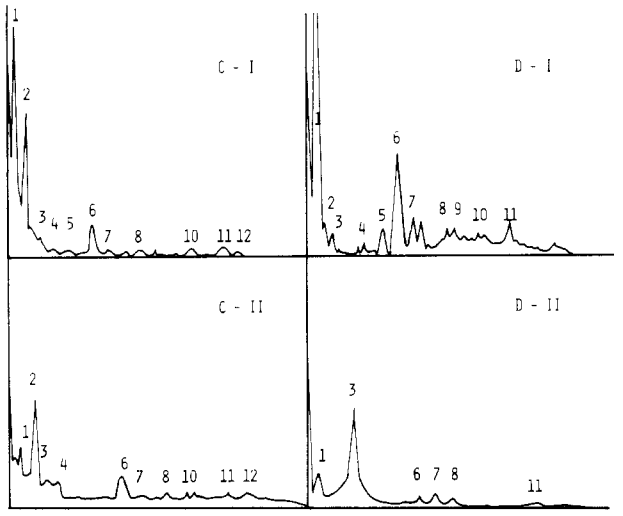

Figure 2. Changes in the electrophoretic profiles of 0.6 M KCl soluble proteins of carp (C) and mullet (D) muscle during frozen storage at -20 °C (I, from fresh muscle; II, after 12 weeks storage).

changes in the free amino nitrogen of these samples are given in Table III. The free amino nitrogen was quite high in fresh mackerel and amberfish muscle, while that of carp and mullet muscle was relatively low. The quantities of free amino nitrogen of all samples were almost twice the original values after 12 weeks storage at -20 °C. It appears that the quantity of free amino nitrogen in fish muscle is related to the instability of muscle proteins of frozen fish. The free amino nitrogen content of mackerel and amberfish, which is considered to be unstable during ice and frozen storage (Otake, 1979; Tsao et al., 1981), was 5.31 and 4.22 mg/g of meat, respectively, while that of carp and

mullet, which are thought to be relatively stable during frozen storage (Takashi, 1973; Arai, 1974; Jiang, 1977), was 2.02 and 2.98 mg/g of meat, respectively. The muscle proteins of carp were the most stable in this study, then mullet, amberfish, and mackerel in decreasing order. It is obvious that the higher the free amino nitrogen content, the less stable the muscle protein is during frozen storage.

Changes in composition of the free amino acids during frozen storage are given in Table IV. The predominant amino acids contained in extracted free amino acids were histidine, taurine, lysine, glycine, proline, and alanine. The ammonia content was also high in all species. No distinct changes in taurine content in all samples was observed during frozen storage. The content of free glycine was very high in carp and followed by mullet, amberfish, and mackerel. The free glycine content in muscle seems to be related to the stability of muscle proteins during frozen storage. The content of free glycine, alanine, and lysine in all samples slightly increased with the duration of storage. The content of free histidine was high in all samples. No distinct changes in histidine of all samples were observed during frozen storage. It seems that high levels of histidine and lysine, which are basic amino acids, are related to the instability of muscle proteins. The high content of free glycine, proline, and alanine is related to the stability of muscle in proteins during frozen storage at −20 °C.

In order to investigate the action of free amino acids on protein aggregation during frozen storage, the changes in composition of free amino acids may provide necessary information. The content of free glycine, alanine, taurine, proline, histidine, lysine, and ammonia of the fish muscle were originally high in fish muscle and during frozen storage. Under neutral condition, many charged functional groups, both positive and negative, are exposed outwardly on the protein molecules. The myofibrillar proteins show negative charge (Noguchi, 1974, 1982; Matsumoto, 1979). If compounds with a positive charge are present in the media, they are drawn toward the negatively charged sites on the protein molecules. The protein molecules will thus decrease in the net negative charge and this subsequently decreases the electrostatic repulsion force among protein molecules. Histidine and lysine with isoelectric points (PI) of 7.59 and 9.74, respectively, show net positive charge in fish muscle, since the pH value of fish muscle (5.8–6.5) is below the isoelectric point of these free amino acids. The absorption of these free amino acids on the protein molecules subsequently cause the protein aggregation. In the study on the control of the denaturation of frozen actomyosin suspended in 0.6 M KCl solution (pH 7.2), histidine showed a moderate inhibitory effect on protein denaturation (Noguchi, 1974). It is conjectured that the pH of the media of proteins would be the main factor affecting the interactions between free histidine and protein molecules.

Taurine, proline, glycine, and alanine, with isoelectric points of 5.12, 6.30, 5.97, and 6.02, respectively, indicate that the net charge is zero or slightly negative in fish muscle. Little effect on the electrostatic repulsion is concerned, when they are absorbed by protein molecules. However, interactions with positively or negatively charged functional groups on protein molecules might occur during frozen storage and lead to aggregate with inter- and intramolecular interactions among protein molecules. In order to clarify the mechanism of protein denaturation of frozen fish muscle, more detailed investigation about the types of interactions occurring between these free amino acids and protein molecules is necessary.

Registry No. ATPase, 9000-83-3.

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Evaluation of the Toxic Components of Toxaphene in Lake Michigan Lake Trout

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Lake trout from Lake Michigan were analyzed for residues of the insecticide toxaphene and two of the primary toxic constituents, toxicants A and B. Using various chromatographic techniques and mass spectrometric confirmation, we have identified these toxic congeners in Lake Michigan lake trout residues. Levels of toxicants A and B have been found to be roughly 1 order of magnitude or more less than the estimated total toxaphene residue. Since the environmentally derived toxaphene is extensively altered in comparison to the technical material, measurment of toxic congeners may be a more toxicologically relevant measure of toxaphene derived residues.

INTRODUCTION

Contaminant residues in Great Lakes fish are well documented and have been widely studied for a number of years (Schmitt et al., 1983). In the majority of cases the residues are derived from single components and represent a relatively simple task for quantitation and evaluation. Until recently, analysts have not had adequate techniques for assessment of residues derived from complex mixtures. With the development and routine usage of capillary column gas chromatography and capillary GC/mass spectrometry, a more complex variety of chemicals is being detected and analyzed at low concentrations.

One such complex mixture is the chlorinated hydrocarbon insecticide, toxaphene. Toxaphene is a chlorinated organic insecticide composed of over 177 components (Holmstead et al., 1974; Saleh, 1983) which, until recently, was the most widely used chlorinated pesticide in the United States. Recent studies of Great Lakes fish have shown high residue levels of chlorinated hydrocarbons which appear to be very similar to toxaphene (Schmitt et al., 1981, Rappe et al., 1979, Rice and Evans, 1984). Alterations in composition of the mixture prior to and during deposition as a residue creates difficulty for accurate quantitation and evaluation due to the lack of a suitable analytical standard (Musial and Uthe, 1983; Jansson et al., 1979; Wideqvist et al., 1984).

Since the changes which give rise to the residue pattern are so significant, semiquantitative residue levels of overall "toxaphene" may not accurately reflect the true toxicologic nature of the material being measured. Studies have shown that toxicity to various organisms is not distributed evenly among various fractions of technical toxaphene. Studies by Nelson and Matsumura (1975), Saleh et al. (1977), and Isensee et al. (1979) have shown that toxicity to invertebrates and fish can be attributed to several components, while mammalian toxicity is apparently more restricted. In addition, Harder et al. (1983) have shown that anaerobic sediment decomposition products are at least as toxic as technical material to two species of estuarine fish.

Because of the complexity of the technical mixture, very few of the components have been isolated and identified. Studies by Turner et al. (1977) and Saleh et al. (1977) contain excellent summaries of several chemical structures that have been isolated and their toxicities. It should be noted that only small quantities of a few of these compounds exist, or are available for routine analytical use.

In all of the studies to date, including those of Seiber et al. (1975) and Pollock and Kilgore (1980), two constituents of toxaphene have proven to be the most toxic to all organisms tested. Toxicant B (2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane, heptachlorobornane I) was originally isolated and identified by Casida et al. (1974). Toxicant A (a mixture of 2,2,5-*endo*,6-*exo*,8,9,9,10- and 2,2,5-*endo*,6-*exo*,8,8,9,10-octachlorobornanes) was isolated and identified by Turner et al. (1975) and Nelson and Matsumura (1975). These two components comprise approximately 8% of the toxaphene mixture as manufactured by the Hercules Chemical Co., Wilmington, Delaware (Casida et al., 1974; Saleh, 1983; Turner et al., 1975). This study was conducted to investigate the presence and potential use of quantitation of toxicants A and B as a more toxicologically meaningful measure of residues derived from toxaphene. Here, we report the presence and levels of these two constituents in lake trout from Lake Michigan.

EXPERIMENTAL SECTION

Materials. The lake trout examined in this study were obtained as incidental catch from commercial fishing nets approximately 5 miles south of Muskegon, MI, in Lake Michigan on Aug 6, 1982. Fish (50–60 cm total length) were kept on ice approximately 10 h before being frozen in individual plastic bags.

Methods. For analysis, fish were partially thawed and a sample of the "belly flap" region was removed. This region is rich in adipose tissue and was chosen since it is

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