

Histamine Formation and Honeycombing During Decomposition of Skipjack Tuna, *Katsuwonus pelamis*, at Elevated Temperatures

HILMER A. FRANK, DERRICK H. YOSHINAGA, and WAI-KIT NIP

Introduction

Most fish decompose rapidly unless some method of preservation is instituted soon after they are caught. One major cause of decomposition is the varied reservoir of spoilage bacteria contained by the fish and present in the environment. An extensive literature is available on the distribution and classification of the spoilage organisms as well as the natural microflora of many kinds of marine fish (Griffiths, 1937; Tomiyasu and Zenitani, 1957; Shewan, 1961, 1962; Shewan and Hobbs, 1967; Shewan, 1977). Considerable interest has been directed toward the decomposition of scombroid fish (family Scombridae), particularly in mackerel, sardines, and several types of tuna where spoilage is associated with high levels of histamine in the fish (Williams, 1954; Hillig, 1956a;

Tomiyasu and Zenitani, 1957; Kimata, 1961; Arnold and Brown, 1978).

Fresh tuna and other scombroid fish are essentially devoid of histamine (Geiger et al., 1944; Geiger, 1948; Hardy and Smith, 1976; Fernandez-Salguero and Mackie, 1979) but they contain substantial amounts of free histidine, exceeding 1 g per 100 g of skipjack tuna tissue (Lukton and Olcott, 1958). It is generally agreed that the histamine found in scombroid fish is formed by bacteria that can decarboxylate histidine (Shifrine et al., 1959; Kimata, 1961; Ferencik, 1970; Edmunds and Eitenmiller, 1975; Taylor et al., 1977; Arnold and Brown, 1978; Omura et al., 1978; Taylor et al., 1979). A variety of such bacteria, particularly Enterobacteriaceae, recovered from spoiled scombroid fish are considered to be responsible for their decomposition and the elevated histamine observed (Geiger, 1955; Mossel, 1968; Lerke et al., 1978; Arnold and Brown, 1978).

Because histamine is heat stable, some workers have suggested that the histamine content would be suitable as a quantitative index of prior microbial spoilage in canned tuna (Geiger, 1944; Williams, 1954; Ferencik et al., 1961; Ienistea, 1973). At present, histamine evaluation is used voluntarily as a rou-

tine quality control procedure by most of the tuna canning industry (Lieber and Taylor, 1978).

Many other indices have been employed to measure spoilage in fish (Tomiyasu and Zenitani, 1957; Lassen, 1965; Martin et al., 1978), including sensory assessments (Shewan et al., 1953; Burt et al., 1975), the formation of compounds such as volatile acids (Hillig, 1954, 1956a), trimethylamine (Farber and Lerke, 1961; Martin et al., 1978), hypoxanthine (Burt, 1977; Martin et al., 1978), volatile reducing substances (Farber and Lerke, 1961) and ethanol (Lerke and Huck, 1977) and high bacterial counts (Tomiyasu and Zenitani, 1957; Farber and Lerke, 1961; Lerke et al., 1965; Martin et al., 1978). For a number of years the tuna canning industry also has used honeycomb formation, a condition caused by the breakdown of connective tissue (Hillig, 1956b; Otsu, 1957; Lassen, 1965; Tanikawa, 1971; Finch and Courtney, 1976), as an index of decomposition.

The present investigation concerns the relationship between histamine formation and decomposition in skipjack tuna, *Katsuwonus pelamis*, and is part of a broader study of spoilage in tuna caught near the Hawaiian Islands. This study shows that the optimum temperature for histamine formation in skipjack tuna is 37.8°C (100°F), and that the optimum for honeycombing is 32.2°C (90°F). Moreover, histamine formation is dependent upon microbial activity, but honeycombing can occur in the presence of antibiotics that are inhibitory to microbial growth.

ABSTRACT—Decomposition was studied in skipjack tuna, *Katsuwonus pelamis*, caught in Hawaiian waters. Fresh skipjack tuna tissue was practically devoid of histamine (about 0.1 mg/100 g tuna), but this compound formed readily when whole fish were incubated at moderate and elevated temperatures. Histamine formation was optimum at 37.8°C (100°F) and was dependent upon microbial activity. Honeycombing, a condition characterized by the destruction of connective tissue, was evaluated in incubated skipjack tuna and had an optimum temperature of 32.2°C (90°F). Honeycomb formation occurred in the presence of antibiotics that inhibited microbial activity and histamine formation.

The authors are with the Department of Food Science and Human Nutrition, University of Hawaii, Honolulu, HI 96822. This article is Journal Series No. 2568 of the Hawaii Institute of Tropical Agriculture and Human Resources.

Materials and Methods

Fish

Skipjack tuna, each weighing about 1.8-2.3 kg (4-5 pounds), were caught in nearby ocean waters (mean temperature about 75°F or 24°C), delivered live to the National Marine Fisheries Service research facility at Kewalo Basin on Oahu, Hawaii, and held for about 12-18 hours in storage tanks fed by recirculating fresh seawater. The fish were removed from the tanks, allowed to expire by being kept for 5-10 minutes in ice-chilled seawater, and transported in crushed ice to the Department of Food Science and Human Nutrition at the University of Hawaii for use in experiments. Normally about 1 hour elapsed between expiration of the fish and the initiation of laboratory incubations. These precautions prevented any undesired postmortem spoilage changes during handling before incubation.

Incubation

The fish were placed in separate polyethylene bags containing 4-5 l of filtered fresh seawater and held for the desired time in a temperature-controlled water bath.

Precooking

Following incubation, the tuna were eviscerated and decapitated, and each side was cut into sections (Fig. 1). The number of sections obtained depended upon size; fish weighing 1.8-2.3 kg (4-5 pounds) yielded 5 sections, and smaller fish could be divided into 3-4 sections. The sectioned fish were given a low-pressure steam precook of 15 minutes at 104.4°C (220°F) in a home-style pressure cooker. In commercial processing, the purpose of precooking is to coagulate the flesh protein to aid removal of the skin and bones during cleaning and to facilitate cutting the loins into pieces suitable for canning (Lassen, 1965; Bacon, 1971; Finch and Courtney, 1976).

Honeycombing

After precooking, the tuna were cooled thoroughly, and honeycomb formation was evaluated by two experienced individuals using a five-point scale based on the degree and distribu-

tion of honeycombing throughout the fish (Table 1). Honeycombing ratings represent mean evaluation scores taken from duplicate experiments for each set of conditions tested. Honeycombing is seen mainly as pitted sponge-like deposits between loins and often as indentations of the loin surfaces. In cases of advanced honeycombing, the connective tissue appears vacuolated, and transverse sections of the loin resemble a vacant honeycomb (Hillig, 1956b; Otsu, 1957).

Table 1.—Scale for quantitative evaluation of honeycombing in skipjack tuna.

Score ¹	Description
0	No honeycombing in any part of fish
1	Very slight honeycombing in belly, dorsal line or blood meat but not in loins
2	Slight honeycombing in belly, dorsal line, blood meat and in parts of anterior loins
3	Moderate honeycombing in belly, entire dorsal line, blood meat and some loin sections
4	Moderate-to-extensive honeycombing in belly, entire dorsal line and blood meat, and moderate honeycombing in all loins
5	Extensive honeycombing in all parts of fish, including the loins. Connective tissue has a sponge-like appearance

¹Intermediate scores (e.g., 0.5, 1.5, etc.) can be assigned where appropriate.

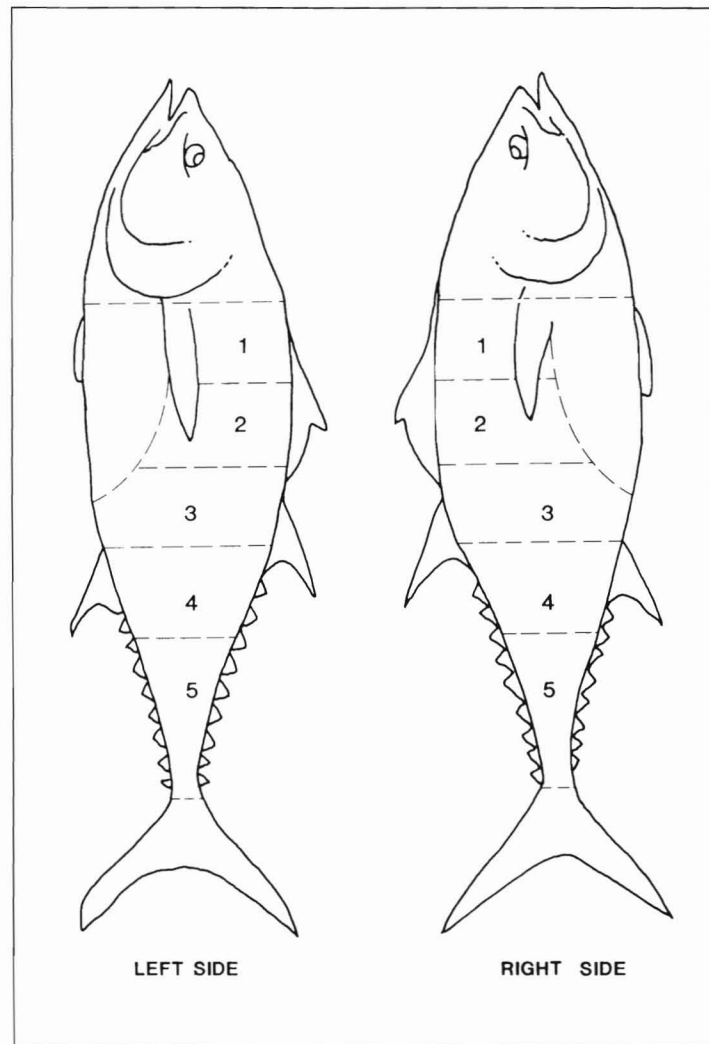


Figure 1.—Numbering scheme for identification of fish sections.

Histamine Content of Tuna

Precooked fish sections were cleaned, comminuted twice in a household meat grinder and analyzed for histamine as described by Staruszkiewicz et al. (1977) and in AOAC (1975) (Sections 18.060-18.062). Ten g of each section were extracted with methanol, passed through an anion exchange resin to remove interfering compounds, derivatized with *o*-phthalaldehyde, and the histamine measured fluorometrically. The histamine content reported for each section of fish represents the mean value of eight estimations taken from duplicate determinations by two technicians for both sides of the section.

Because histamine levels were not uniform for all sections of the same fish (see Tables 3, 4), it was difficult sometimes to compare tuna that had been given different treatments. However, this problem was overcome by considering each fish as a single system whose histamine load could be expressed by its composite histamine content. Composite histamine concentrations (mg/100 g tuna) reported for some fish (see Tables 4, 7) are calculated as $\frac{\text{total amount (mg) of histamine present in all fish sections}}{\text{total weight (g) of all fish sections}} \times 100$.

Microbiological Examination

Microbial counts were made with tissue taken from tuna section 2. Samples were diluted with sterile 0.1 percent peptone (BBL) and inoculated on trypticase soy agar (BBL); colonies were counted after incubation for 2-3 days at 37°C (98.6°F). Gram stains of tuna tissue and seawater incubation liquid also were examined microscopically for the presence of microorganisms.

Antibiotics

The inhibitory effect of antibiotics on histamine formation and honeycombing was studied by incubating tuna in seawater containing 160 units per ml of Penicillin G (Calbiochem¹, Los Angeles) and 0.1 mg per ml Tetracycline hydro-

chloride (A. H. Robbins, Richmond, Va.). Fish were incubated for 24 hours at 35°C (95°F), a temperature that is intermediate between the optima for histamine formation and honeycombing in skipjack tuna.

Preliminary experiments revealed that slight histamine formation occurred in areas of the fish that had not been reached by antibiotics, especially in section 1. To facilitate penetration of the antibiotics to all tuna tissue, the gills and viscera were removed and the fish severed between sections 2 and 3. Each piece was put in a separate polyethylene bag containing seawater plus antibiotics and placed in the water bath. After incubation for 24 hours at 35°C (95°F), analyses were conducted for histamine, honeycombing, and the presence of microorganisms.

Results

Histamine Formation

Fresh skipjack tuna contained essentially no free histamine; in most cases only about 0.1 mg/100 g was present in any fish section (Table 2).

Table 3 shows the effect of incubation temperature on histamine formation in skipjack tuna. Very little histamine had formed in 24 hours at 21.1°C (70°F) and below, but at 23.9°C (75°F) a small amount of histamine was present throughout most of the fish, with a substantial level (74.5 mg/100 g) in the anteriormost section 1. The optimum temperature for histamine production was 37.8°C (100°F) where levels of 472 to 643 mg/100 g were found in all sections. At higher temperatures, histamine formation decreased, and at 43.3°C (110°F) and above the tuna tissue underwent extensive deterioration, but very little histamine was present.

A characteristic pattern of histamine distribution was observed in this study. The earliest indication of histamine occurred in fish section 1, and the histamine level remained highest in that section throughout incubation. The other fish sections had less histamine, arranged in a gradually decreasing gradient toward the posterior end of the fish. The belly flaps (not included in Tables 2 and 3) were exceptions to this gradient and

Table 2.—Histamine content of fresh skipjack tuna.

Trial no.	Histamine (mg/100 g tuna) and fish section number ¹				
	1	2	3	4	5
1	0.06	0.07	0.06	0.08	0.09
2	0.12	0.11	0.09	0.12	0.06
3	0.11	0.10	0.10	0.10	0.07
4	0.14	0.17	0.08	0.09	0.13
5	0.23	0.17	0.19	0.11	0.09
Average	0.13	0.12	0.10	0.10	0.09

¹From Figure 1

Table 3.—Histamine content of skipjack tuna incubated 24 hours at various temperatures.

Temp.		Histamine (mg/100 g tuna) and fish section number ¹				
°C	°F	1	2	3	4	5
15.6	60	0.21	0.18	0.17	0.18	0.15
21.1	70	2.80	1.05	0.96	0.99	0.84
23.9	75	74.5	6.50	3.25	2.46	4.95
26.7	80	102	6.99	1.59	4.51	2.90
29.4	85	114	72.5	56.0	52.2	71.5
32.2	90	248	— ²	41.3	— ²	45.2
35.0	95	369	241	84.4	59.1	59.6
37.8	100	643	540	472	479	534
40.6	105	354	236	— ³	185	255
43.3	110	1.47 ⁴				
48.9	120	0.62 ⁴				

¹From Figure 1

²Very small fish; only 3 sections.

³Small fish; 4 sections.

⁴Sectioning was not possible because the fish disintegrated during incubation. Histamine estimations were made from composites of each side of the fish.

usually had histamine levels that were nearly as high as in section 1.

Table 4 shows the rates of histamine formation during incubation at the optimum temperature, 37.8°C (100°F). A lag period of 6-12 hours elapsed before significant histamine production was detected in section 1; at 18 hours considerable histamine was found in most sections, with greater concentrations occurring in the anterior part of the fish. After 24 hours the histamine level was very high throughout the fish, ranging from 261 to 481 mg/100 g in each section. Assessment of histamine formation from composite histamine values given in the righthand column of Table 4 also shows an initial 12-hour lag and the changing production rate that followed. Therefore, composite histamine values will be used below to express histamine formation in terms of the entire fish (see Table 7).

¹Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 4.—Histamine formation in skipjack tuna incubated at 37.8°C (100°F).

Time (h)	Histamine (mg/100 g tuna) and fish section number ¹					Composite histamine ² (mg/100 g tuna)
	1	2	3 (hours)	4	5	
0	0.13	0.12	0.10	0.10	0.09	0.11
6	0.47	0.43	0.51	0.52	0.55	0.50
12	42.1	6.13	3.69	3.91	6.72	15.1
18	213	49.5	21.1	20.7	31.7	67.3
24	481	367	424	261	289	343

¹From Figure 1.

²Composite histamine = $\frac{\text{Sum of histamine in all sections (mg)}}{\text{Total weight of fish sections (g)}} \times 100$.

Samples of loin tissue taken from tuna incubated 18 and 24 hours (Table 4) had total microbial counts of 2.5×10^5 and 2.8×10^5 /g, respectively. Cultures isolated from tuna incubated for 24 hours were mainly gram-negative, facultatively anaerobic rods (Yoshinaga, 1979) and will be the subject of another manuscript.

Honeycombing

Table 5 shows the effect of temperature on honeycombing in skipjack tuna. Honeycomb formation was negligible after 24 hours at temperatures below 26.7°C (80°F), but it increased as higher incubation temperatures were used and was optimum at about 32.2°C (90°F). At 43.3°C (110°F) and above, disintegration of the fish tissue prevented quantitative evaluation of honeycombing.

Table 6 shows the rate of honeycombing at the optimum temperature, 32.2°C (90°F). Honeycombing proceeded without any apparent lag and increased gradually until 22.5 hours when appreciable decomposition was noted. At 30 hours, breakdown of the connective tissue was widespread, and at 37.5 hours destruction was complete.

Effect of Antibiotics

Table 7 shows the effect of penicillin and tetracycline on honeycombing and histamine formation in skipjack tuna held at 35°C (95°F). When an intact fish was incubated for 24 hours, ample histamine (164 mg/100 g) was formed and moderate honeycombing (score = 3.0) occurred. After removal of the gills and viscera, the histamine level was re-

duced by about one-half (to 75.6 mg/100 g) but honeycombing was unaffected (score = 3.5). However, when uptake of the antibiotics was improved by cutting the fish between sections 2 and 3, histamine production was essentially insignificant (1.62 mg/100 g). Honeycombing, on the other hand, was fairly extensive (score = 3.5-4.0) after 24 hours and apparently not inhibited by the antibiotics.

Microbial counts from the loin tissue and microscopic examination of the seawater showed that few microorganisms were present, and that microbial growth had not occurred in the presence of the antibiotics.

Discussion

To study the relationship between histamine formation and decomposition it was necessary to determine how much histamine was present initially in fresh skipjack tuna. This study confirms previous observations that fresh scombroid fish contain very little free histamine (Geiger et al., 1944; Hillig, 1956a; Ferencik, 1970).

The effect of temperature on histamine formation in scombroid fish has been discussed in a number of publications (Kimata, 1961; Ienistea, 1973; Edmunds and Eitenmiller, 1975; Arnold and Brown, 1978; Lerke et al., 1978; Fernandez-Salguero and Mackie, 1979) but with little agreement on minimum, maximum, and optimum temperatures. Two major reasons for the inconsistencies are that different species of fish were studied and that variations occurred in fish handling before incubation. In this study we were fortunate to

Table 5.—Honeycomb formation in skipjack tuna incubated 24 hours at various temperatures.

Temperature		Honey-combing score ¹	Temperature		Honey-combing score ¹
°C	°F		°C	°F	
15.6	60	0	35.0	95	3.0-4.0
21.1	70	0.5	37.8	100	3.0
23.9	75	0.5	40.6	105	1.5
26.7	80	2.0	43.3	110	— ²
29.4	85	1.5-2.0	48.9	120	— ²
32.2	90	4.0			

¹See Table 1

²Fish disintegrated; honeycomb evaluation impossible.

Table 6.—Honeycomb formation in skipjack tuna incubated at 32.2°C (90°F).

Time in hours	Honey-combing score ¹	Time in hours	Honey-combing score ¹
0	0	22.5	4.0-4.5
7.5	1.0-1.5	30	4.5-5.0
15	1.5-2.0	37.5	5.0

¹See Table 1

Table 7.—Effect of antibiotics on honeycombing and histamine formation in skipjack tuna incubated 24 hours at 35°C (95°F).

Treatment	Anti-biotics ¹	Composite histamine ² (mg/100 g tuna)	Honey-combing ³ score
Whole fish	None	164	3.0
Fish eviscerated and degilled	None	75.6	3.5
Fish eviscerated, degilled and separated between sections 2 and 3	Yes	1.62	3.5-4.0

¹Penicillin, 160 units/ml; tetracycline hydrochloride, 0.1 mg/ml.

²Composite histamine = $\frac{\text{Sum of histamine in all sections (mg)}}{\text{Total weight of fish sections (g)}} \times 100$.

³See Table 1

have an ample supply of live skipjack tuna available for experiments. Hence, the entire postmortem thermal history of each fish was known, and it was possible to study the effect of temperature under carefully controlled conditions.

Our study shows that the optimum temperature for histamine production in skipjack tuna is 37.8°C (100°F) and that, as others have reported, the hista-

mine is not uniformly distributed throughout the fish (Hillig, 1956a; Ienistea, 1973; Lerke et al., 1978). Inhibition by penicillin and tetracycline observed in this study also supports the widely held view that histidine decarboxylating bacteria are responsible for the presence of histamine in scombroid fish (Geiger et al., 1944; Tomiyasu and Zenitani, 1957; Shifrine et al., 1959; Kimata, 1961; Ferencik, 1970; Ienistea, 1973; Edmunds and Eitenmiller, 1975; Taylor et al., 1977; Arnold and Brown, 1978; Omura et al., 1978; Fernandez-Salguero and Mackie, 1979; Taylor et al., 1979) and that histamine is not formed physiologically by fish tissue under aseptic conditions (Geiger et al., 1944; Geiger, 1955; Ferencik, 1970; Fernandez-Salguero and Mackie, 1979).

The postmortem temperatures of decomposing scombroid fish usually are moderate; hence, honeycombing and histamine formation generally develop at slower-than-optimal rates under normal conditions. Occasionally, however, fish temperatures can become high, particularly in warm tropical waters and during prolonged exposure on the hot deck of the fishing vessel. In these instances, shorter times would be required for spoilage to become extensive.

Whether or not specific levels of histamine can be used to measure decomposition or serve as indices of quality in canned tuna has been discussed for a long time (Geiger, 1944; Hillig, 1954; Williams, 1954; Hillig, 1956a; Arnold and Brown, 1978). However, additional data showing the relationship of histamine level to reliable measures of tuna quality will be necessary before such quality standards are possible.

The results given in this study relative to honeycombing are significant for a number of reasons. First, this study has shown that the optimum temperature for honeycombing in skipjack tuna is slightly lower than it is for histamine formation. Second, incubations employing antibiotics showed that honeycombing could occur when microbial inhibitors were present, suggesting that breakdown of connective tissue collagen may result from proteolytic enzymes in the fish tissue. Because many degrada-

tive enzymes have been found in the lysosomes of other animals, it is tempting to speculate that collagenolytic enzymes in the lysosomes of skipjack tuna may be responsible for honeycombing. Considerable investigation would be needed to identify these enzymes and to determine their location. Moreover, it is possible that some of the proteolytic microorganisms present in scombroid fish may contribute to honeycombing under natural conditions. Third, we have employed a quantitative scale to evaluate honeycombing to compare the effect of different variables (e.g., temperature, time, etc.) on destruction of the connective tissue. We anticipate that changes can be made to improve the accuracy and usefulness of this scale for skipjack tuna as well as other tuna that are subject to honeycombing.

Acknowledgments

This investigation was supported by Contract 03-6-208-35369 from the National Marine Fisheries Service, NOAA. We thank Carlene M. Char and Pam K. Goto for their expert technical assistance.

Literature Cited

- AOAC. 1975. Official Methods of Analysis. 12th ed., p. 315-317. Assoc. Off. Anal. Chem., Wash., D.C.
- Arnold, S. H., and W. D. Brown. 1978. Histamine (?) toxicity from fish products. *Adv. Food Res.* 24:113-154.
- Bacon, E. K. 1971. Quality control of ingredients used in tuna canning. In R. Kreuzer (editor), *Fish inspection and quality control*, p. 92-96. Fishing News (Books) Ltd., London.
- Burt, J. R. 1977. Hypoxanthine: a biochemical index of fish quality. *Process Biochem.* 12(1):32-35.
- _____, D. M. Gibson, A. C. Jason, and H. R. Sanders. 1975. Comparison of methods of freshness assessment of wet fish. Part I. Sensory assessments of boxed experimental fish. *J. Food Technol.* 10:645-656.
- Edmunds, W. J., and R. R. Eitenmiller. 1975. Effect of storage time and temperature on histamine content and histidine decarboxylase activity of aquatic species. *J. Food Sci.* 40:516-519.
- Farber, L., and P. Lerke. 1961. Studies on the evaluation of freshness and on the estimation of the storage life of raw fishery products. *Food Technol.* 15(4):191-196.
- Ferencik, M. 1970. Formation of histamine during bacterial decarboxylation of histidine in the flesh of some marine fishes. *J. Hyg., Epidemiol., Microbiol., Immunol.* 14:52-60.
- _____, V. Kremery, and J. Kriska. 1961. Fish poisoning caused by histamine. *J. Hyg., Epidemiol., Microbiol., Immunol.* 5:341-348.

- Fernandez-Salguero, J., and I. M. Mackie. 1979. Histidine metabolism in mackerel (*Scomber scombrus*). Studies on histidine decarboxylase activity and histamine formation during storage of flesh and liver under sterile and non-sterile conditions. *J. Food Technol.* 14:131-139.
- Finch, R., and G. Courtney. 1976. The tuna industry. In M. E. Stansby (editor), *Industrial fishery technology*, p. 91-109. Robert E. Krieger Publ. Co., Inc., Huntington, N.Y.
- Geiger, E. 1944. Histamine content of unprocessed and canned fish. A tentative method for quantitative determination of spoilage. *Food Res.* 9:293-297.
- _____. 1948. On the mechanism of histamine formation. *Arch. Biochem.* 17:391-395.
- _____. 1955. Role of histamine in poisoning with spoiled fish. *Science (Wash., D.C.)* 121:865-866.
- _____, G. Courtney, and G. Schnakenberg. 1944. The content and formation of histamine in fish muscle. *Arch. Biochem.* 3:311-319.
- Griffiths, F. P. 1937. A review of the bacteriology of fresh marine fishery products. *Food Res.* 2:121-134.
- Hardy, R., and J. G. M. Smith. 1976. The storage of mackerel (*Scomber scombrus*). Development of histamine and rancidity. *J. Sci. Food Agric.* 27:595-599.
- Hillig, F. 1954. Individual volatile acids, succinic acid, and histamine as indices of decomposition in Atlantic "little tuna" (*Euthynnus alleteratus*). *J. Assoc. Off. Agric. Chem.* 37:927-931.
- _____. 1956a. Volatile acids, succinic acid, and histamine as indices of decomposition in tuna. *J. Assoc. Off. Agric. Chem.* 39:773-800.
- _____. 1956b. Note on honeycombing in decomposed tuna. *J. Assoc. Off. Agric. Chem.* 39:1015-1016.
- Ienistea, C. 1973. Significance and detection of histamine in food. In B. C. Hobbs and J. H. B. Christian (editors), *The microbiological safety of food*, p. 327-343. Acad. Press, Lond.
- Kimata, M. 1961. The histamine problem. In G. Borgstrom (editor), *Fish as food*, Vol. I. Production, biochemistry, and microbiology, p. 329-352. Acad. Press, N.Y.
- Lassen, S. 1965. Tuna canning and the preservation of the raw material through brine refrigeration. In G. Borgstrom (editor), *Fish as food*, Vol. IV. Processing, part 2, p. 207-245. Acad. Press, N.Y.
- Lerke, P. A., and R. W. Huck. 1977. Objective determination of canned tuna quality: identification of ethanol as a potentially useful index. *J. Food Sci.* 42:755-758.
- _____, R. Adams, and L. Farber. 1965. Bacteriology of spoilage of fish muscle. III. Characterization of spoilers. *Appl. Microbiol.* 13:625-630.
- _____, S. B. Werner, S. L. Taylor, and L. S. Guthertz. 1978. Scombroid poisoning. Report of an outbreak. *West. J. Med.* 129:381-386.
- Lieber, E. R., and S. L. Taylor. 1978. Thin-layer chromatographic screening methods for histamine in tuna fish. *J. Chromatogr.* 153:143-152.
- Lukton, A., and H. S. Olcott. 1958. Content of free imidazole compounds in the muscle tissue of aquatic animals. *Food Res.* 23:611-618.
- Martin, R. E., R. J. H. Gray, and M. D. Pier-

- son. 1978. Quality assessment of fresh fish and the role of the naturally occurring microflora. *Food Technol.* 43(5):188-192, 198.
- Mossel, D. A. A. 1968. Bacterial toxins of uncertain oral pathogenicity. In H. D. Graham (editor), *The safety of foods*, p. 168-182. Avi Publ. Co., Inc., Westport, Conn.
- Omura, Y., R. J. Price, and H. S. Olcott. 1978. Histamine-forming bacteria isolated from spoiled skipjack tuna and jack mackerel. *J. Food Sci.* 43:1779-1781.
- Otsu, T. 1957. Development of "honeycombing" in Hawaiian skipjack tuna. *Commer. Fish. Rev.* 19:1-8.
- Shewan, J. M. 1961. The microbiology of seawater fish. In G. Borgstrom (editor), *Fish as food*, Vol. I. Production, biochemistry, and microbiology, p. 487-560. Acad. Press, N.Y.
- _____. 1962. The bacteriology of fresh and spoiling fish and some related chemical changes. In J. Hawthorn and J. M. Leitch (editors), *Recent advances in food science*, Vol. I. Commodities, p. 167-193. Butterworths, Lond.
- _____. 1977. The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In P. Sutcliffe and J. Disney (editors), *Proc. Conf. Handling, Processing and Marketing of Trop. Fish*, p. 51-66. Tropical Products Institute, Lond.
- _____, and G. Hobbs. 1967. The bacteriology of fish spoilage and preservation. *Prog. Ind. Microbiol.* 6:169-208.
- _____, R. G. MacIntosh, C. G. Tucker, and A. S. C. Ehrenberg. 1953. The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. *J. Sci. Food Agric.* 4:283-298.
- Shifrine, M., L. E. Ousterhout, C. R. Grau, and R. H. Vaughn. 1959. Toxicity to chicks of histamine formed during microbial spoilage of tuna. *Appl. Microbiol.* 7:45-50.
- Staruszkiewicz, W. F., Jr., E. M. Waldron, and J. F. Bond. 1977. Fluorometric determination of histamine in tuna: development of method. *J. Assoc. Off. Anal. Chem.* 60:1125-1130.
- Tanikawa, E. 1971. Marine products in Japan—size, technology and research. Koseisha-Koseikaku Company, Tokyo, 507 p.
- Taylor, S. L., E. R. Lieber, and M. Leatherwood. 1978. A survey of histamine levels in commercially processed scombroid fish products. *J. Food Qual.* 1:393-397.
- _____, L. S. Guthertz, M. Leatherwood, and E. R. Lieber. 1979. Histamine production by *Klebsiella pneumoniae* and an incident of scombroid fish poisoning. *Appl. Env. Microbiol.* 37:274-278.
- Tomiyasu, Y., and B. Zenitani. 1957. Spoilage of fish and its preservation by chemical agents. *Adv. Food Res.* 7:41-82.
- Williams, D. W. 1954. Report on chemical indices of decomposition in fish (histamine). *J. Assoc. Off. Agric. Chem.* 37:567-572.
- Yoshinaga, D. H. 1979. Microbial formation of histamine in skipjack tuna (*Katsuwonus pelamis*). Master's Thesis, University of Hawaii, Honolulu, 52 p.