Table for Estimating Histamine Formation in Skipjack Tuna, *Katsuwonus pelamis*, at Low Nonfreezing Temperatures

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Introduction

Histamine is not present in the muscle tissue of fresh fish but can be found in some marine fish that have decomposed or have been involved in food poisoning incidents (Kimata, 1961; Arnold and Brown, 1978; Omura et al., 1978; Frank et al., 1981; Behling and Taylor, 1982; Yoshinaga and Frank, 1982; Frank et al., 1983; Frank, 1985a, b; Frank et al., 1985; Taylor, 1986). Histamine is produced through bacterial decarboxylation of the free histidine present in scombroid fish (Kimata, 1961; Arnold and Brown, 1978; Omura et al., 1978; Arnold et al., 1980; Yoshinaga and Frank, 1982; Frank and Yoshinaga, 1984; Frank, 1985a; Taylor, 1986) and other species (Taylor, 1986), including the dolphin or mahimahi, *Coryphaena hippurus*, (Frank et al., 1985).

For many years the tuna canning industry used honeycomb formation as a simple, rapid criterion for determining

ABSTRACT—Histamine levels were measured in fresh, whole skipjack tuna, Katsuwonus pelamis, *that had been incubated at 30°, 40°, and 50°F, and an equation was derived from the data to describe the relationship between histamine formation and incubation time at low nonfreezing temperatures. Values obtained with this equation were used to make a table showing how long it would take for the development of specific histamine levels at several temperatures ranging from 30° to 60°F. The table could be used to estimate decomposition during cold storage of skipjack tuna before the fish are frozen on commercial fishing vessels.*

whether decomposition is present (Frank et al., 1984). However, during the past several decades the industry, as well as various agencies, have employed histamine as a quantitative indicator of the loss in quality through bacterial spoilage (Frank et al., 1981, 1983, 1985). Other microbial end products have also been suggested as indicators of decomposition in tuna, including volatile acids, trimethylamine, hypoxanthine, volatile reducing substances, ethanol, and particularly putrescine and cadaverine (Mietz and Karmas, 1977; Ritchie and Mackie, 1980; Frank et al., 1981; Staruszkiewicz and Bond, 1981; Frank et al., 1985), but to date none has been used as widely as histamine to measure deterioration in quality. In 1982 the U.S. Food and Drug Administration (FDA) established histamine levels above 50 mg per 100 g canned tuna as a potential health hazard and 20 mg as an indication of substantial prior decomposition (Food and Drug Administration, 1982). These levels have been used as the basis for FDA regulatory actions.

Numerous histidine decarboxylating bacteria have been isolated from decomposed and fresh scombroid fish and mahimahi, but these have been mainly mesophilic members of Enterobacteriaceae and others belonging to *Vibrio*, *Lactobacillus*, and *Clostridium perfringens* (Arnold and Brown, 1978; Omura et al, 1978; Behling and Taylor, 1982; Yoshinaga and Frank, 1982; Frank et al., 1985; Frank, 1985a; Taylor, 1986). However, only a few studies are available on histamine production at low temperatures in fish or in cultures of psychrotrophic bacteria. Histamine formation in

fish has been observed in the range of 32°-50°F, but results have varied. Behling and Taylor (1982) reported that *Klebsiella pneumoniae* produced histamine slowly at 44.6°F in a medium containing histidine. Frank and Yoshinaga (1984) observed histidine decarboxylation at 39°F by whole cell suspensions of *K. pneumoniae* that had been grown at 100°F. Okuzumi et al. (1981) isolated a group of unidentified halophilic, psychrophilic, histamine-forming bacteria (called N-group bacteria) from mackerel homogenates at 41°F. N-group bacteria are regarded as part of the normal microflora of marine fish (Okuzumi et al., 1982) and have been isolated from several fresh histidine-containing fish (Okuzumi et al., 1984). Frank et al. (1985) reported that decarboxylation of histidine, ornithine, and lysine was comparable among cultures of mesophilic and psychrotrophic bacteria isolated from mahimahi. Nonetheless, they concluded that cadaverine and putrescine might be less suitable as indices of decomposition because of the low substrate levels of lysine and ornithine in mahimahi. Baranowski (1985), who reported slight urocanic acid production from histidine at 50°F by 58 of 166 psychrotrophic bacteria isolated from mahimahi, suggested that urocanic acid might be an alternative to histamine as a quality index at low temperatures.

On commercial purse seiners, the fish are brought on board the vessel, cooled in a well containing refrigerated seawater

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(RSW) at 28°-30°F, frozen in a brine and stored at 15°-20°F in dry wells until the boat is unloaded at the cannery. Depending on how quickly the fish are cooled, the process may provide opportunities for microbial growth, especially by psychrotrophic bacteria. Individual large fish cool slowly, and a large load of fish placed simultaneously in the hold also cools slowly, particularly at the geometric center. Thus, the fish in a well may take several days to reach the temperature of the RSW (30°F). Also, freezing may be delayed when there are not enough fish to fill the hold, and the fish are kept unfrozen for periods ranging from a few days to 3 weeks until more are added (Behling and Taylor, 1982). The end result is that psychrotrophic bacteria could cause spoilage that may be accompanied by histamine formation. Needless to say, fish on boats that employ icing only to lower the fish temperature would be particularly vulnerable to spoilage by psychrotrophic bacteria.

Earlier we prepared a nomograph for estimating histamine production in skipjack tuna at warm temperatures (Frank et al., 1983) based on a study of controlled decomposition in incubated fresh fish. However, the original equation for the nomograph inferred that bacterial histamine production ceased at 32°F, whereas Ratkowsky et al. (1982, 1983) have shown that the rate of bacterial growth is dependent on the difference between the ambient temperature and T_o, a hypothetical temperature that is intrinsic to the organism. Consequently, the equation for the nomograph was modified to include this concept, and a revised nomograph was prepared accordingly (Frank, 1985b).

This paper includes a table for estimating histamine formation at low nonfreezing temperatures (30°-60°F), also based on an equation derived from a study of controlled decomposition in incubated fresh skipjack tuna, and including the T_o concept. This table could be used in the fishing industry to determine the extent of spoilage during the handling of tuna at low nonfreezing temperatures.

Materials and Methods

Fish

Live skipjack tuna, weighing about 4-5 pounds (1.8-2.3 kg) each, were obtained locally and handled as described previously (Frank et al., 1981) to assure freshness of the fish.

Incubation

Individual fish were placed in separate polyethylene bags containing 4-5 liters of filtered fresh seawater and incubated for the desired times at 30°, 40°, or 50°F as described by Frank et al. (1981). Each incubation was run in duplicate.

Histamine

The histamine content in each fish was measured by the AOAC fluorometric method as described by Frank et al. (1983). The mean of the composite histamine levels for both sides was used as the histamine level of each fish.

Results and Discussion

Equation for Histamine Formation at Low Temperatures

Table 1 shows the effect of incubation time on histamine formation in skipjack tuna over the range of 30°-50°F, a relationship that can be expressed mathematically by the power function equation

$$H = a \cdot t^x \cdot F^y, \qquad (1)$$

where

H = histamine (mg/100 g tuna),
t = time in days,
F = incubation temperature (°F), and
a, x, and y = constants.

Previously (Frank, 1985b), the power function equation for mesophilic spoilage of skipjack tuna was adapted to include T_o, a hypothetical temperature that is the theoretical minimum for the process under consideration but is not the minimum growth temperature (McMeekin and Olley, 1986). In this paper, Equation (1) was adapted to include T_o for psychrotrophic spoilage, and a modified equation was obtained in which

$$H = a \cdot t^c \cdot (F - F_o)^b, \qquad (2)$$

where

$F_o = T_o$ (Ratkowsky et al., 1982, 1983), °F, and
a, b, c = constants.

The data in Table 1 were fitted by the method of least squares (Draper and Smith, 1966), and Equation (2) was converted to the following equation which accounted for 49.4 percent of the variance:

$$H = 1.927 \cdot 10^{-7} \cdot (F - F_o)^{4.436} \cdot$$
$$e^{(0.086857)t}, \qquad (3)$$

or

Table 1.—Histamine formation in skipjack tuna at low temperatures[1].

Temperature and time (days)	Histamine (mg/100 g tuna)		
	Trial 1	Trial 2	Mean
30°F			
0			0.11[2]
7	0.16	0.28	0.22
14	0.30	0.45	0.38
21	0.09	0.29	0.24
28	0.13	0.14	0.14
35	0.20	0.44	0.32
42	1.93	3.09	2.01
40°F			
0			0.11[2]
4	0.58	0.71	0.65
8	0.51	0.60	0.56
14	1.83	9.85	5.84
20	16.4	17.7	17.1
24	7.12	7.46	7.29
50°F			
0			0.11[2]
3	0.31	0.47	0.39
6	0.53	0.56	0.55
9	2.86	4.72	3.79
12	11.5	22.3	16.9

[1]Fresh, whole fish were placed in separate polyethylene bags containing seawater, incubated at the times and temperatures shown, and analyzed for histamine.
[2]Six fish. All other values were obtained from duplicate fish incubated at times and temperatures indicated.

$$H = 1.927 \cdot 10^{-7} \cdot (F - 12.92)^{4.436} \cdot$$

$$(1.0907)^t \qquad (3a)$$

where

$e = 2.71828$ (base of natural logarithms), and

$F_o = 12.92°F$ (calculated from the "square root" equation of Ratkowsky et al. (1982, 1983) after plotting log H vs. log t and setting 2.5 mg histamine per 100 g tuna as the spoilage level); this value is typical of those reported for other types of psychrotrophic spoilage (Ratkowsky et al., 1982).

Equation (3a) was used to calculate the incubation periods needed for accumulation of different levels of histamine in skipjack tuna at temperatures in the range of 30°-60°F, as shown in Table 2.

Use of Table 2

The effect of lowering the fish temperature on the rate of psychrotrophic microbial spoilage is illustrated in Table 2. For example, production of 1 mg histamine per 100 g tuna would take only 1 day at 45°F, a typical food refrigerator temperature, but would take 33 days at 30°F.

Obviously, histamine formation would be negligible when the hold of the fishing vessel is filled rapidly and the fish are frozen immediately after being caught. However, when the volume of fish is too small to fill the hold, the fish may be kept unfrozen for as long as 3 weeks (Behling and Taylor, 1982), and a decision would be needed eventually on when to freeze the catch to prevent additional deterioration. The ability to estimate histamine production through knowing the fish temperature and incubation time would be useful in dealing with the problems stemming from long-term storage at low temperatures. Thermocouples can be used to measure the fish temperature during storage and the histamine level for a given time period can be determined from Table 2. Intermediate values that do not appear in Table 2 can be calculated by interpolation.

Table 2.—Effect of incubation time and temperature on histamine formation in skipjack tuna.

Histamine, mg/100 g tuna	Days required to produce indicated histamine level[1] at various temperatures						
	30°F	35°F	40°F	45°F	50°F	55°F	60°F
0.5	25	12	2	< 1			
1.0	33	20	10	1			
2.5	40	27	17	8	1		
5	52	38	28	19	12	6	
10	60	46	36	27	20	14	8
20	65	51	41	32	25	19	13
50	78	64	55	45	38	32	26
100	86	73	63	54	46	40	34
500	104	91	81	72	65	58	52

[1]Calculated from text Equation (3a):

$$H = 1.927 \cdot 10^{-7} \cdot (F - 12.92)^{4.436} \cdot (1.0907)^t,$$

where H = histamine (mg/100 g tuna), F = temperature (°F), and t = incubation time in days.

Temperature Function Integrators

Temperature function integration is based on the cardinal role of temperature in affecting microbial growth and the rate of food spoilage. Temperature function integrators (McMeekin and Olley, 1986) are commercially available electronic devices that can continuously accumulate measurements of the changing temperatures in a product during storage. When the relationship is known between incubation conditions (time and temperature) and the spoilage rate in a food, the circuitry of these instruments can be calibrated to integrate the measurements into an expression that represents the total deterioration in that product. Since this expression also can be stated as an equivalent storage time at some reference temperature, the shelf life remaining in the food can be predicted for any known combination of time and temperature.

Temperature function integrators can be adapted for estimating spoilage in fish (Olley and McMeekin, 1985). The mathematical model reported previously for mesophilic histamine formation in skipjack tuna (Frank, 1985b) could be employed in estimating spoilage at moderate temperatures when fish are being cooled

slowly, e.g., at the center of a hold that has been filled rapidly. Likewise, the model described above for psychrotrophic histamine formation could be used to monitor spoilage during prolonged holding at low nonfreezing temperatures on fishing vessels. However, application of these models to histamine formation in other histidine-containing fish should be done with caution. Because various species of marine fish have different histidine levels (Lukton and Olcott, 1958; Hibiki and Simidu, 1959; Suyama and Yoshizawa, 1973) as well as dissimilar microfloras, the rates and levels of histamine formation can be expected to differ from those observed in skipjack tuna.

McMeekin and Olley (1986) have discussed the uses and limitations of several methods for monitoring spoilage in foods. For example, a chemical indicator can be combined with an enzyme-substrate system to measure the cumulative effect of time and temperature, but the chemical indicator must have the same temperature characteristics as the spoilage being monitored.

Other Considerations

Deterioration of tuna following frozen storage has not been studied thoroughly.

Nevertheless, histamine formation could occur during the handling of fish in preparation for canning. In particular, histamine production by psychrotrophic bacteria is possible during the thawing process because of the prolonged periods involved (Baranowski and Pan, 1985; Pan, 1985). Furthermore, since the fish have not been eviscerated before freezing, a variety of intestinal bacteria are present that could contribute to decomposition and the accumulation of histamine (Olley et al., 1985).

Acknowledgments

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