Relationship Between Honeycombing and Collagen Breakdown in Skipjack Tuna, *Katsuwonus pelamis*

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ABSTRACT—Honeycombing, a condition that affects the connective tissue, was studied in skipjack tuna under controlled conditions over a wide range of temperatures. A numerical scale used to measure honeycomb formation was closely correlated with the amount of collagen solubilized during decomposition.

Introduction

Honeycombing, a spoilage condition that affects the connective tissue of tuna, appears after the fish have been given a low-pressure steam "precook" to coagulate the flesh protein which facilitates cleaning and cutting before canning (Lassen, 1965; Finch and Courtney, 1976). Honeycombing consists of irregular holes and pitted, sponge-like deposits between the loins (Arnold and Brown, 1978) and sometimes indentations of the loin surface. In extreme cases the connective tissue appears vacuolated and resembles a vacant honeycomb (Hillig, 1956; Otsu, 1957; Lassen, 1965; Tanikawa, 1971; Finch and Courtney, 1976; Frank et al., 1981).

Fresh tunas do not have honeycombing, but those exposed to warm temperatures for extended times can become honeycombed. Tunas that have been cooled properly after catching and refrigerated during storage do not become honeycombed (Otsu, 1957). Because other kinds of deterioration also occur at the same time, honeycombing has been associated with high levels of histamine (Williams, 1954; Finch and Courtney, 1976; Frank et al., 1981), quality deterioration (Hillig, 1956; Lassen, 1965), and scombroid poisoning in commercially canned tuna (Merson et al., 1974).

Screening for honeycombing is a simple way of detecting decomposition in tuna without resorting to time-consuming methods for measuring histamine. For many years the canning industry and regulatory agencies have considered honeycombing to be definite evidence of decomposition in tuna (Hillig, 1956) and acceptable as grounds for rejection of tuna shipments. Rejection of honeycombed tuna at the cleaning stage is considered responsible for the low level of histamine generally observed in commercially canned tuna (Finch and Courtney, 1976). Nevertheless, quantitative measurement of honeycombing has not been used to evaluate tuna decomposition in the canning industry. Since collagen is the major constituent in connective tissue of fish (Love et al., 1982), it is likely that honeycomb formation and collagen breakdown are related. In this investigation we studied the relationship between the amount of honeycombing and collagen breakdown in skipjack tuna, *Katsuwonus pelamis*, decomposed under controlled conditions at several temperatures between 30° and 90°F.

Materials and Methods

Fish

Skipjack tuna, weighing 4-5 pounds each, were caught in the ocean around Oahu, Hawaii, and kept alive until shortly before incubation, as described by Frank et al. (1981).

Incubation

Forty-one fish were studied and seven were used as untreated, zero-time controls. The remaining 34 tunas were put in separate polyethylene bags containing 4-5 l of filtered fresh seawater and incubated for the desired times at several temperatures: 12 were kept at 30°F, 8 at 40°F, 6 at 50°F, and 8 at 90°F. After incubation, each fish was eviscerated and decapitated, and the two sides were separated. The left side was used to measure honeycombing and the right side to determine collagen content.

Honeycomb Evaluation

The left side of the fish was given a "precook" of 15 minutes at 220°F in a home-style steam pressure cooker and cooled thoroughly. The precook fillet was evaluated by two experienced persons who rated each fish on a five-point scale (Table 1) based on the amount and distribution of honeycombing (Frank et al., 1981). The honeycomb rating for

Table 1.—Scale for evaluating honeycombing in skipjack tuna.

Honeycombing score[1]	Degree of honeycombing[2]
0	None
1	Very slight
2	Slight
3	Moderate
4	Moderate-to-extensive
5	Extensive

[1]Intermediate scores can be assigned where appropriate.
[2]Detailed description of honeycomb appearance for each score is given in Table 1 of Frank et al. (1981).

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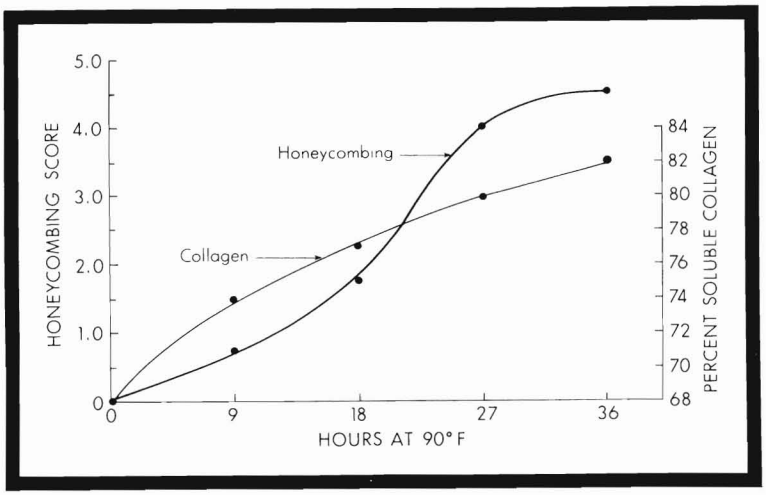

Figure 1.—Honeycomb formation and collagen breakdown during decomposition of skipjack tuna at 90° F. Each data point represents the mean value for two fish incubated for the time shown.

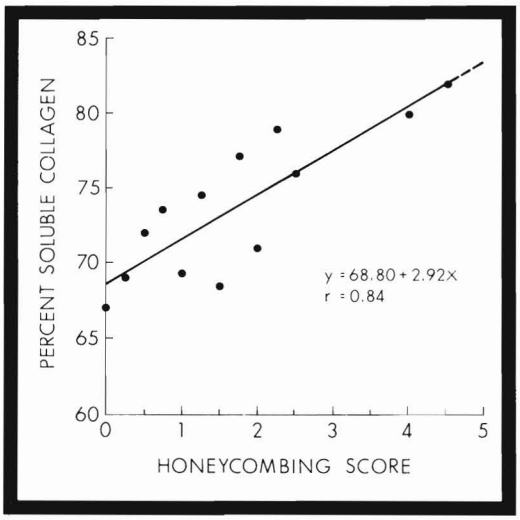

Figure 2.—Relationship between collagen breakdown and honeycomb formation during decomposition of skipjack tuna. Thirty-four fish were incubated for various times at 30°, 40°, 50°, or 90° F. Data points shown are based on results obtained from a minimum of two fish for nearly all cases. The correlation coefficient, r, was highly significant ($p < 0.01$).

each fish was the mean of the scores given by the two judges.

Collagen Determination

Collagen was measured in duplicate 5 g samples from the middle of the uncooked right loin. Each sample was freeze-dried, weighed, extracted with quarter-strength Ringer's solution, and centrifuged to separate the soluble and insoluble collagen fractions (Hill, 1966). Each fraction was hydrolyzed with 6N HCl for 3 hours at 212° F (Thompson and Thompson, 1968), decolorized with activated carbon (Sekoguchi et al., 1978), and its collagen estimated by the method of Woessner (1961) based on the amount of hydroxyproline present. The hydroxyproline portion of skipjack tuna collagen was measured in the insoluble fraction obtained from 140 g of fresh loin tissue by the procedure of Thompson and Thompson (1968). Percent soluble collagen was calculated from the total of soluble and insoluble collagen fractions obtained from each fish.

Statistical Analysis

The correlation between honeycombing score and percent soluble collagen was assessed by linear regression analysis on a Texas Instruments[1] TI-55 programmable calculator. The confidence interval for the slope of the regression line was determined with a t-test (Goldstein, 1964).

Results and Discussion

Collagen in Fresh Skipjack Tuna

Insoluble collagen contained 7.7 percent hydroxyproline; consequently, in the calculations used in this study, 1 mg of hydroxyproline was equivalent to 13 mg of collagen. Samples from loin and belly flap tissue of fresh fish had 68 percent soluble collagen, while the dark, so-called "blood meat" had 75 percent. Loin tissue had 4.34 mg of collagen per g wet weight and 72 percent moisture.

Honeycomb Formation and Collagen Breakdown

Previously we reported that the optimum temperature for honeycombing in skipjack tuna was about 90° F (Frank et al., 1981). Figure 1 shows that during decomposition of skipjack tuna at 90° F, honeycombing was nearly complete after 36 hours, and soluble collagen increased from 68 to 82 percent. At this stage the fish had deteriorated markedly, and the histamine content was 616 mg per 100 g.

Figure 2 shows the relationship between honeycombing and collagen solubilization during decomposition of skipjack tuna. For every increase of a single unit in honeycombing score, there was a corresponding increase of about 3 percent in collagen solubilized. Thus, honeycombing scores of 1, 2, 3, 4, and 5 corresponded to soluble collagen percentages of 68.6, 71.7, 74.7, 77.6, and 83.7, respectively (Fig. 2).

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

Further studies are needed to determine the cause of honeycombing in tuna. Solubilization of connective tissue collagen during decomposition may have resulted from collagen melting at elevated temperatures (Stryter, 1975) or from enzymatic activity originating in tuna lysosomes. Wu et al. (1981) reported that lysosomal glucosidases stimulated collagen breakdown in bovine connective tissue during the postmortem ageing process.

Significance of Results

This study shows that a numerical honeycombing scale described earlier (Frank et al., 1981) is correlated with the solubilization of collagen during decomposition of skipjack tuna. These observations suggest that a scale based on the breakdown of connective tissue could be used to measure quality loss. Furthermore, honeycombing appears to be more sensitive than histamine content as a criterion for spoilage in tuna. We have observed that decomposed skipjack tuna with honeycombing scores of about 1.5 invariably contained < 5 mg of histamine per 100 g. We believe that a honeycombing score of 1.5 (corresponding to 73.2 percent soluble collagen) could be employed as a decomposition threshold for skipjack tuna, and that fish with scores exceeding 1.5 can be considered spoiled and discarded. Decomposition thresholds for other types of tuna would require honeycombing studies similar to those reported here for skipjack tuna.

Acknowledgments

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