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U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
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The Effect of Premortem Stress, Holding Temperatures, and Freezing on the Biochemistry and Quality of Skipjack Tuna

LADELL CRAWFORD

SEATTLE, WA
April 1972

National Marine Fisheries Service, Special Scientific Report--Fisheries Series

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The Effect of Premortem Stress, Holding Temperatures, and Freezing on the Biochemistry and Quality of Skipjack Tuna

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The Effects of Premortem Stress, Holding Temperatures, and Freezing on the Biochemistry and Quality of Skipjack Tuna

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ABSTRACT

This experiment was designed to determine if there were differences (biochemical and/or organoleptic) before and after canning rested and stressed skipjack tuna. The live fish were captured off Oahu and were placed in shoreside tanks in Honolulu, Hawaii. After having been under observation for 24 hr, the fish were sacrificed in a rested or stressed condition. Stress was induced by forcing fish to swim around a tank until they showed signs of exhaustion. The rested fish were kept in a separate tank and were agitated as little as possible before being sacrificed.

Some of the sacrificed tuna were canned immediately to serve as controls. Others were held in 32°, 60°, and 78° F seawater (SW) for 6 hr, and some were held in 78° F SW for 9 hr before canning. An equal number of fish from all treatments were brine frozen (for 20 hr), then thawed and canned. Sample wedges were taken before canning for measurements of glycolytic and purine degradation products. These measurements together with organoleptic evaluation were also determined on the canned product.

There were no commercially discernible differences between rested and stressed skipjack subjected to various time-temperature treatments. The relation of the measured biochemical parameters to the treatment of the fish and the subsequent relation to the quality of the canned product were studied. There were not sufficiently defined relations on which to base quality predictions.

INTRODUCTION

The canned sea food industries have been pioneers in the area of convenience foods. Canned tuna, a quarter billion dollar industry, has been and is the leader in this large and rapidly growing enterprise. This product contains 20 to 25% well-balanced protein with generous amounts of essential fatty acids, vitamins, and minerals. Canned tuna provides one of the cheapest sources per pound of quality protein.

Wider consumption of canned tuna and other fishery products could play an important role in reducing malnutrition found in many low-economic groups in the United States.

The Terminal Island Technology Laboratory was set up by the Bureau of Commercial Fisheries (now the National Marine Fisheries Service) to assist in solving some of the technical quality problems which the tuna industry had experienced for a number of years. The solving of these problems could result in a higher quality pack and increased yields. A small

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staff was assembled and the laboratory commenced operations early in 1966. (I was the research chemist in charge and later the Acting Laboratory Director.) This introduction gives an account of what has been accomplished prior to the current studies; the latter covers some aspects of premortem stress and postmortem changes in skipjack tuna and the effects of these changes on the quality of the canned product.

The General Problem

The tuna industry has experienced substantial losses of raw fish for many years. In 1966, the losses from the U.S. fleet rejected at the cannery for all causes was 1.98% of the landings (Alverson, 1967). This was lower for the temperate tunas, being 0.7% for bluefin and 0.4% for albacore. It was higher for the tropical tunas which are carried for longer times from distant waters, being 1.8% for yellowfin and 3.8% for skipjack. Prorating these figures for the same year, the total loss to the U.S. fleet was estimated to be over $1 million based on average prices during the period. This is the only year for which reliable loss figures have been computed; it is known that the losses vary considerably from year to year. The primary cause of losses is believed to be inadequate refrigeration on the boat, but other effects are caused by the length of time between catching and canning, which depends on the rate of catch delays at canneries, or auctions, or sometimes tie-ups due to price disagreements, etc. Reduction in losses during recent years has been achieved by some canners working with individual boats to identify the causes and to eliminate them.

In addition to direct losses, the tuna pack shows a considerable variation in quality. There is little doubt that, while some of this may be due to natural factors such as the dark color typical of very large fish, there is a considerable lowering of quality in a number of instances through inadequate refrigeration prior to arrival at the cannery (Finch, 1967a). In order to reduce losses and improve quality, it is important to identify which quality changes result from natural causes and which can be attributed to unsatisfactory handling. Methodology had to be established for the measurement of quality in tuna and the effects of refrigeration and some aspects of the chemistry of tuna in relation to quality of the canned product had to be examined.

Quality in Tuna

Since "quality" means many things to many people, it was important to define this term at the outset. The laboratory's tuna research program concentrated its attention on the quality of tuna as it appears in the can. It is canned tuna which is offered for sale and which competes on the shelf and in the kitchen for the consumer's dollar. So it is the quality of canned tuna, rather than that of the raw fish, which must form the basis for study. The apparent quality of the raw tuna as judged by appearance and odor is important, but it does not guarantee a good canned product. It is possible to have raw tuna of apparently high quality which shows only average quality when canned. It is also possible to find raw tuna which appears to be of poor quality to sensory judgment that makes an excellent canned product (Crawford and Finch, 1968).

So the questions are: First, what is quality? Second, how can it be measured? By quality the canner means those attributes or properties of canned tuna such as color and flavor which a consumer likes and which persuade her to continue to buy the product. In the absence of any comprehensive survey, it was deduced which of these attributes are important and what are their relative importance. It is known that preferences are different in different countries—probably based on traditional acceptance of local species. For instance, large bluefin, which may be regarded as too dark in color by the U.S. consumer, is acceptable in the Mediterranean, where it has been commonly caught for years. It is known that consistency in quality is important. A mixture of good and indifferent quality tuna in a can or even in different cans of the same lot produces a critical reaction when examined concurrently (Loewe, 1967).

There had been two main sources of information as to the consumers' likes and dislikes. One was the accumulated experience of canners and their distributors who have sold more than 210 million cases in the last 20 years;

their experience had formed the basis of scoring systems generally used by canners. The second was the letters of complaint received from consumers, which letters, although relatively few in number, reflect points which may be critical to acceptance.

Finch (1967a, 1967b) and Crawford et al. (1970) reported some of the difficulties in assessing the quality of tuna. It was pointed out that the literature to date on tuna technology offered very little useful information although they have reported various aspects of composition and changes in tuna. Finch and Crawford et al. also pointed out some of the factors that affect the quality of tuna:

Physical Characteristics: The size of the tuna is important because the larger the fish, the longer it takes to chill, freeze, and cook; these longer times may alter some quality parameters, such as color, scorch, and texture (Barrett et al., 1965). The differences in species contributes to differences in color: for example, albacore has less heme pigments than skipjack and is therefore naturally lighter in color.

Catching and Handling: The premortem conditions have long been known to effect the postmortem chemistry and quality of fish. Fish caught by methods that allow long periods of struggle and stress before death have low pH, glycogen content, etc., all of which affects the quality of the finished canned product.

Trawl-caught halibut produces a low pH that causes the edible flesh to appear "chalky" whereas longline halibut has a higher pH and less incidence of chalkiness (Spinelli, unpublished). Longline tuna often has a higher pH than seined tuna which leads to the formation of struvite (harmless glasslike crystals of magnesium ammonium phosphate that form in the can after processing). The canned products of seined fish, on the other hand, are generally darker, tougher in texture, flake more readily, and show more evidence of bruises, which appear as dark-pigmented "stains."

Other aspects that affect quality such as chilling, freezing, and thawing will be discussed later.

Based upon these factors and with the help of industry technologists, scales of quality have been worked out which can be used for judging the effects that various experimental conditions, such as different freezing methods, have on the canned product (Crawford and Finch, 1968; Crawford et al., 1969). A trained taste panel examines the sample and gives it a series of scores as shown in Table 1. (Sometimes in special tests other factors such as blood spots

Table 1.—Organoleptic scoring system for canned tuna. The can is opened and turned out carefully onto a white enamelled surface for examination.

Attribute	Description	Maximum score	Minimum score
Scorch	Darkening on the surface of the tuna in the headspace area.	5 = no scorch	1 = very severe scorch
Color	The tunalike color on the inside of the sample (average).	10 = white and bright	1 = extremely dark brown
Flakiness	The tendency of the flakes to separate.	10 = firm, not showing flakes	1 = falls apart completely
Firmness	The softness of the pieces when tested with a fork.	10 = very firm	1 = very soft
Fibers	The toughness of the tuna to chewing.	10 = very tough	1 = very tender
Odor	The amount of off odors present.	5 = no off odor	1 = very strong off odor
Flavor	The amount of off flavor present.	5 = no off flavor	1 = marked off flavor

are also scored.) These marks are not judgments of the panels' preferences, but assessments of how severe is the scorch, how light or dark is the color, and so on. This method has been experimented with and the techniques refined until scoring is reasonably consistent, although all subjective judgments of this kind are somewhat inconsistent. It should be noted that "workmanship," that is, qualities such as incomplete removal of skin or bone, are primarily measures of the canner's efficiency and so are not included in the panel's judgments.

Taste panels have their limitations, and it would be preferable to use instruments to measure quality for all purposes except acceptance tests, which can only be made with people. Such an approach, if successful, could be more precise and repeatable than a panel. It would eliminate the need for selection and training of personnel, and the inevitable person-to-person, day-to-day, even year-to-year variation which trained tasters show. Quality measurement by instruments has been developed successfully for some foods, although it is not possible to measure all the quality attributes in food with the same reliability. Methods for color and texture have been developed for many products, and it seemed likely at the outset of the program that they could be applied to tuna. Figure 1 shows that relation between panel color scores on canned tuna and reflectance measurements measured with a suitable instrument (Color Master) on the samples. Dr. Angela Little, a specialist in this area at the University of California, Berkeley, has investigated such objective tests that may be adaptable for use with canned tuna. A fairly simple method was developed which measured color with greater precision and consistency than could be shown by a taste panel (Little et al., 1969). Development of methods for the measurement of texture is also showing some promise, although these are likely to be more complicated, since texture is made up of several factors. Such methods would be helpful for research work and might also be used in routine quality control. Objective measurements of odor and flavor in foods generally have had a very limited success so far, and there is no present prospect of their useful application to canned tuna. However, there are indications that odor and flavor levels may be related to other properties which can be measured and which would enable their indirect assessment.

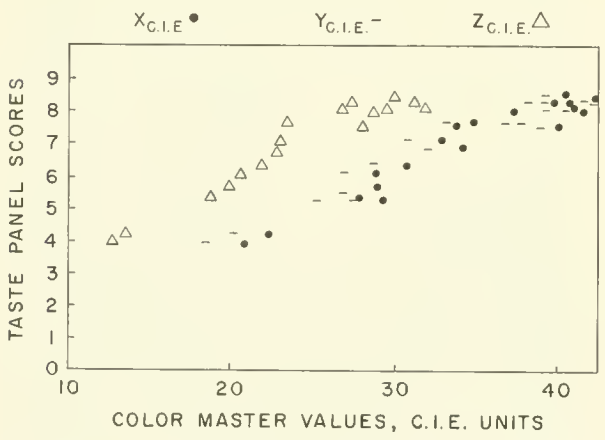

Figure 1.—A comparison of color master reflectance values in C.I.E. units to a taste panel's ratings of canned tuna.

Hence, it seems likely that in the not too distant future we may be able to dispense with the taste panel for some purposes and use the more precise—although possibly rather slower —methods of objective measurement for quality. While this approach will give sound and useful measures of such qualities as color and flavor, the inability to relate these qualities more closely to consumer preferences is a serious weakness. In other words, it can be determined how light or dark a sample may appear and how much off-flavor it may have, but it has to be determined by a national consumer survey as to how this influences the customer's buying judgment. Present restrictions upon the use of surveys by Federal agencies prevents their engaging in consumer surveys by which this relationship may be directly determined, and we must rely upon the indirect evidence for the time being.

Refrigeration of Tuna: Chilling and Freezing

The refrigeration requirements of the U.S. tuna fishery are unique among fisheries. The variable and often very high catch rate of fish, the high tropical water temperatures, the long distances from the canneries, and the considerable differences in size of the fish pose unusual

requirements. The growth and expansion of the fishery in the late 20's and early 30's forced the rapid development of the brine system we have today, which is in some ways one of the most efficient systems yet devised for freezing fish at sea. It consists essentially of a chilled seawater storage accumulation, followed by brine freezing, frozen holding (wet or dry) and thawing, all in the same well. Ideally operated, this system delivers fish to the cannery in good condition. However, in the variable operating circumstances encountered, a number of problems can arise which lead to loss and lowering of quality of all or parts of the load. These problems have been the subject of some study.

The experiments we have carried out so far have been exploratory in nature and aimed at locating which of the operating variables have the most influence on quality so that work might be concentrated in these areas. It has not been possible to carry out confirmatory studies in this time so that present conclusions must be regarded as tentative until repeated.

Fish at tropical and subtropical ocean temperatures deteriorate rapidly and have to be chilled to retard bacterial, enzymatic, and oxidative spoilage. As the temperature falls, so does the rate of deterioration and the nearer the temperature to the freezing point, the more stable is the tuna. (However, much lower freezing temperatures are needed to stop enzymatic and oxidative changes.) Advantage is taken of this fact in the chilling stage for raw tuna when the fish are put into 30° F seawater to cool before freezing, and in many cases to hold them until more fish are caught to fill the well. In order to maintain good quality fish, it is essential to chill it rapidly. Temperatures of less than 40° F are an absolute necessity. We have chilled whole tuna in 30° F seawater and held them at this temperature. Samples of albacore lasted 35 days before being spoiled to the point of rejection (Crawford and Finch, 1968), and samples of bluefin lasted 22 days with no apparent spoilage as shown by the organoleptic evaluation of canned samples (Crawford, unpublished). This contrasts remarkably with the normal time for which wet tuna may be held aboard a tuna vessel which is usually only 7 to 10 days before serious deterioration sets in. It was reasoned that the difference is probably due to various factors such as the relatively poorer cooling in commercial wells, especially when they are overpacked, resulting in a lower ratio of brine to fish, which may be as little as 1 to 5.5, and the consequent poor circulation. This not only limits effective cooling, but the accumulation of dirt, blood, and slime forms a perfect medium for the growth of the spoilage organisms contributing to the deterioration of the fish when temperatures are relatively high. It appears that poor brine circulation (and not the characteristics of the fish themselves) is the most important factor which limits the time for which tuna may be held in chilled storage and leads to quality deterioration when the tuna is held for longer periods. This is important because if correct, it means that improved chilling on vessels would enable tuna to remain in better condition after the same length of chill storage. Alternatively, under conditions of slow fishing, tuna might be held longer at the same temperature without spoilage—thus increasing the flexibility of the freezing system.

A further important point revealed by the chilling experiments was that fresh tuna, at least as harvested by present commercial methods, showed numerous blood flecks, spots, and prominent dark blood vessels on canning. After continued chill storage, blood appeared to diffuse into the surrounding tissues so that the blood vessels became progressively less evident (Crawford and Finch, 1968). This explains why prominent blood vessels are usually associated with albacore and bluefin which being caught relatively near to canneries are usually landed in a much shorter time after catching and before the blood has had time to diffuse. Another factor reported by Crawford and Finch was that fresh fish had a greater tendency to scorch, which diminished on chilled storage. This factor could be especially significant with institutional packs which are retorted for long times.

In U.S. commercial practice, the chilled tuna are frozen in brine to temperatures near 20° F to preserve them during transport. Then the brine is removed to avoid excessive salt penetration. This freezing operation takes place at a much slower rate than is usually regarded as satisfactory for freezing fish. For example, Crawford et al. (1969) found that a 50-lb. bluefin tuna took 170 hr to freeze to 20° F, in a

commercial load. (A rate of approximately 0.02 inches per hour.)

For comparison, the Codex Alimentarius proposed draft provisional standard for frozen gutted Pacific salmon specifies a minimum freezing rate of 0.25 inches per hour. Although there are some differences in the operating circumstances, a higher rate for freezing, or holding, at lower temperatures would improve either the yield (the number of cans of tuna per ton of raw tuna), the quality, or both.

Freshly caught yellowfin and skipjack were still-air frozen at sea, stored at temperatures of 14°, 8°, and —5° F for the time periods of approximately 30, 60, and 90 days, and then canned. These were compared with tuna of the same size and species caught in the same sets, which had been stored for approximately 30 days in a commercial brine well before canning. The quality of the quicker air frozen product showed some improvement over the brine frozen controls, although there was no remarkable difference in the quality between fish frozen and stored at different times and temperatures (Finch and Crawford, unpublished). These samples had been still-air frozen and the rate of freezing was fairly slow. Therefore another experiment was carried out in which yellowfin, skipjack, and bluefin were frozen very rapidly by immersion directly in liquid dichlorodifluoromethane (Freezant 12), (Crawford et al., 1969). The time taken to reach 0° F was measured and found to run from 3 hr for 6½-lb. skipjack to 7 hr for 52-lb. bluefin. The freezing rate conformed to the equation, $Z = 67.5\ W$ where Z is the time in minutes to freeze to 0° F and W is the weight in pounds. Some of the tuna were frozen immediately when taken aboard the vessel and some held in brine wells for 11 and 25 hr before freezing. After freezing, some of the fish were stored at 0° F and some at —50° F. On returning to the cannery, the fish were thawed in running seawater and canned at the same time as samples of fish from the same sets which had been frozen commercially in the vessel's brine wells. The fish which were frozen rapidly in Freezant 12 showed quality improvements over those frozen in the ship's well, especially in the case of the 16-lb. yellowfin, and in the case of skipjack which had been stored at —50° F after freezing. It was also noted that the blood spots and flecks were quite prominent in the rapidly frozen yellowfin and bluefin tuna as compared with the brine well controls, which showed little or none. This was the same effect we had found with the very fresh albacore and bluefin before brine or ice storage. It would be premature to come to a final conclusion on the basis of one experiment, but a first impression is that the improvement by rapid freezing produced a definite but fairly small quality increase over tuna which is properly brine frozen. It must be emphasized in relation to these results that so little is known about consumer reactions to higher quality that it is not possible at present to tell what level of improvements are needed to be significant to marketing.

Relation of Refrigeration Experiments to Present Practices

In looking at the present quality of the commercial pack in relation to the improvements obtained by better freezing methods, it is clear that there are two areas of potential upgrading. One is by improvement of the present refrigeration procedures to improve the poorer commercially landed tuna until it is equal in quality to the best that is brine frozen by present methods. The second is by improvement of the overall level of quality beyond this present point by the application of new methods. The first of these is likely to be more feasible for immediate exploration. While the important longer term prospects cannot be neglected, we should concentrate our activity upon learning more about the exact points of weakness and strength of brine freezing techniques so that we have a sound knowledge upon which to base improvements.

During a review period we made a start with both these activities. We worked out a specification for a small brine-freezing unit capable of being carried on the deck of a tunaboat. This is a simplified compact unit, designed for programmed chilling and freezing, so that a series of precise refrigeration circumstances can be studied in a manner similar to shipboard conditions. Once its operation has been worked out, more of these units could be put aboard commercial tuna vessels so that, with the cooperation of the engineers, there can be col-

lection of a regular supply of samples frozen in a series with accurately defined circumstances. In this way a detailed picture can be obtained of the effects of all phases of brine freezing annually. This will also help to overcome the problem of the considerable amount of time it takes the staff to obtain samples at sea. A preliminary study of quicker chilling and freezing on a large scale was made aboard a commercial tuna vessel, the MV *Westport* with the cooperation of its owner, National Marine Terminal Inc. of San Diego, whose staff and crews gave outstanding help. Two heat exchangers were installed on the *Westport* with a series of brine spreaders in two wells (Fig. 2). This system will enable brine to be cooled more rapidly than at present. A shakedown investigation revealed that this system has some merit.

One serious source of loss, damage, and poor refrigeration is the overpacking of wells. This not only puts pressure on the fish at the bottom but increases the ratio of fish to brine from a designed figure of around 3.5:1 to nearer 5.5:1. The passage of the brine is obstructed through many parts of the load, thus reducing the rate at which it can transfer heat from the fish to the coils. The effects are particularly pronounced with skipjack, which has a fragile skin and is smaller and softer, and so packs down more readily. The obvious answer to this is to fill less tuna into the wells. However, the economics of operation discourage the fisherman from doing this. Even if his loss of acceptable tuna is increased by overpacking, it is still not, as a rule, equal to the extra amount he packs in, so that the net turnout of his vessel is larger and his income greater. Thus he has no economic incentive to pack correctly. If a reduction in packing were to give substantially better quality and if as a result he were to receive a premium rate for his quality fish, he could afford to pack his wells lighter. At present, it is not clear whether the packer could recover the necessary

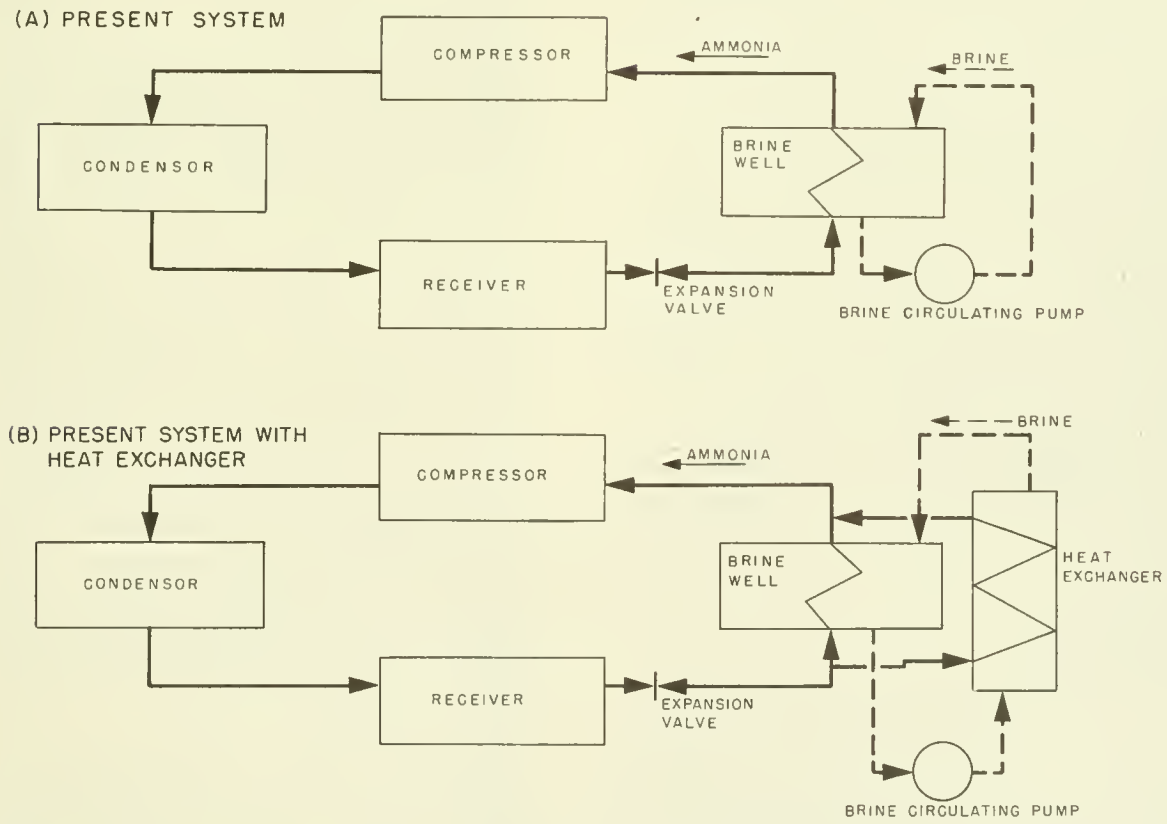

Figure 2.—Schematic diagram of brine immersian freezing system used for freezing tuna aboard commercial fishing vessels.

premium for his improved pack which he would need to reimburse the fishermen. In any case, the establishment of a basis for premium fish payment, whether it would be by rated load capacity of a well, by freezing records of a load, or by his visual or chemical inspection of the raw or canned fish, would be difficult. One alternative which might be considered in future vessels is design modification which removes the incentive to overpack.

Effect of Refrigeration on Yield

It has long been supposed that poor refrigeration, which can lead to the breakdown of proteins and to loss of water retention, should decrease the yield of canned tuna. Experiments made with the first brine system on the vessel, the *American Beauty*, showed better yields than were obtained on comparable loads from ice boats, although no records are now available (Mann, 1967). Measurements made on freezing brines have at time shown high nitrogen contents, demonstrating the loss of considerable amounts of protein. This would indicate that poor quality and poor yield go hand in hand and that conversely, procedures which improve quality would also increase yield. Therefore, some measurements of yield factors were included in our freezing experiments.

Accurate measurements of yield are notoriously difficult to make because of the big natural variations encountered, especially those involving the cleaning operation. For this reason, and because of the limited amounts of fish available in the experiments, the results should be regarded only as indicative.

We found that all the tuna collected at sea aboard the MV *Lois Seaver* (a sister ship to the MV *Westport*), which had been frozen and stored at temperatures of —5°, 8°, and 14° F, and then canned when landed, gave higher yields than the brine frozen controls. The increase was less than 1% in the case of 40-lb. yellowfin but nearly 8% in the case of 14-lb. skipjack (Finch and Crawford, unpublished).

On storing the tuna for an additional 30 and 60 days, at the same temperatures, the yield dropped. In the case of the yellowfin samples, it became less than the original brine frozen control samples, although the skipjack yield was still nearly 6% higher when canned 84 days after catching. There was no difference in yield among samples stored at —5°, 8°, or 14° F. With skipjack frozen rapidly in Freezant 12, there was a considerable increase in yield on the cooked fish. The overall yield after cleaning showed an increase of nearly 8%, but the yield for the fish which had been held in the well prior to freezing was less than that of the controls. Further experiments on a larger scale should be carried out in which the yield differences should be judged using considerably larger quantities of fish to obtain more reliable figures. This aspect is important because it offers a potential prospect of increased payment for better refrigerated fish since the canner may recover his costs from his greater case yield.

Chemistry of Raw and Canned Tuna

In addition to the direct work carried out in our tuna program, some work has been carried out on certain compounds which occur or are formed in tuna and which are connected with the quality. This serves two purposes: A thorough knowledge of the variations in the amounts of these compounds occurring in the different tunas, and the changes they undergo during catching, refrigeration, and processing, would be valuable in helping to influence them most favorably in the maintenance of quality. Secondly, there is a lack of reliable ways of measuring the quality of raw tuna. Successful development of such measures would not only be of value to research, but could also provide methods by which raw fish quality might be judged. None of the present freshness tests appear to have much application to tuna, and this may be in part because the requirements for the handling and processing of fish which are to be canned are likely to differ in some important ways from the requirements for fish to be used in other ways. A number of compounds in tuna have been studied and, of these, the following appear likely to have the most influence on quality.

Heme pigments, the principal color factors in tuna.—It has been noted that color is an important factor in canned tuna, perhaps the most important. Control of color to give a light, bright, consistent appearance would al-

low the up-grading of a good deal of the present pack. The natural pink color of canned tuna is due to substances known as denatured hemochromes and hemichromes which are formed during the precooking stage from the heme pigments of the tuna (Brown and Tappel, 1957). Two heme pigments occur naturally in tuna—myoglobin found in the muscle and hemoglobin in the blood. There is usually considerably more myoglobin in the "white" dorsal muscle of the tuna used for canning for human consumption. Both these compounds are normally deep red proteins which are concerned with oxygen transport in the living fish. (The role of myoglobin in oxygen transport has not been clearly elucidated.) They are closely similar to the red heme compounds which give the color of meat and perform similar functions in land animals. The amount of these compounds in tuna is believed to be the major factor controlling the intensity of the tuna color that is important commercially. Myoglobin comprises about 85% of the total heme pigments in the white muscle. Thus, albacore, the lightest colored of the tunas, contains an average of 0.15% of myoglobin in the white muscle; yellowfin, which is darker, 0.22%; and skipjack, usually considered the darkest of the tunas, 0.4%. The red meat of tuna (the highly vascular, hemoglobin-rich dorsal muscles canned for pet food) with its dark red-brown color contains 3% or more. The amounts of pigments present within each species also show some variation. It is not likely that much can be done to change the amount of these pigments present in tuna, but it seems possible that the condition of the pigment myoglobin, which is present in the largest amount, may be more important to the color in any one species. For instance, large yellowfin are generally darker than smaller yellowfin on cooking and canning, but the few figures available on different sized yellowfin seems to indicate that large yellowfin contain no more heme pigments than the small (Crawford et al., 1969). In this case then, the darker color may be altered by changing the storage condition in a way which favors a better color. Therefore, experiments were conducted on the occurrence of heme pigments in tuna, their state of oxidation and other changes to determine how they affect the color of the canned product. Figure 3 summarizes the changes in myoglobin from the time the tuna is caught and processed.

Derivatives of hemoglobin and myoglobin are formed in the precooking and canning process; a knowledge of the changes in which they are involved is important in understanding and influencing color development in the end product. Earlier work by Brown et al. (1958) and other investigators had shown that oxidation plays an important part in cooked color, the oxidized or met forms being brown as compared with the usual pink color of the oxy form. Also the phenomenon of "greening" undoubtedly involves the heme pigments. Grosjean et al. (1969) and Koizumi and Matsuura (1967) reported that "greening" resulted from the interaction of free cysteine, myoglobin, and TMAO. Accordingly these studies have been concerned with the stability to autoxidation of the heme pigments and their derivatives under the differing conditions of pH and temperature likely to occur in tunas being handled through regular commercial channels. The first step was the predation of pure myoglobins by ammonium sulfate fractionation and DEAE-cellulose chromatography from tuna muscle. To study oxidation, the purified yellowfin and skipjack metmyoglobins and hemoglobins were dissolved in a citric acid-phosphate buffer and reducted to the oxy form found in the fish. These solutions at various pHs from 5.7 to 6.3 were then exposed to oxygen at differing temperatures and the course of autoxidation followed visually by color change and by spectrophotometric measurements of the 580 mμ peak which is characteristic of the oxy form. The results showed that the heme pigments all oxidize more rapidly at lower pH, and the rates of oxidation are directly proportional to the hydrogen ion concentration. A reaction mechanism postulated was:

$$H^+ + MbO_2 \underset{K_2}{\overset{K_1}{\rightleftharpoons}} (Hmb)^+ + O_2 \quad \text{(rapid equilibrium)}$$

$$\tfrac{1}{2}O_2 + (HMb)^+ \xrightarrow{K_3} MetMb + \tfrac{1}{2}H_2O$$

Our notable point was that while the rates of oxidation decreased with temperature under the same conditions down to freezing, the reverse occurred with the frozen solutions. Thus

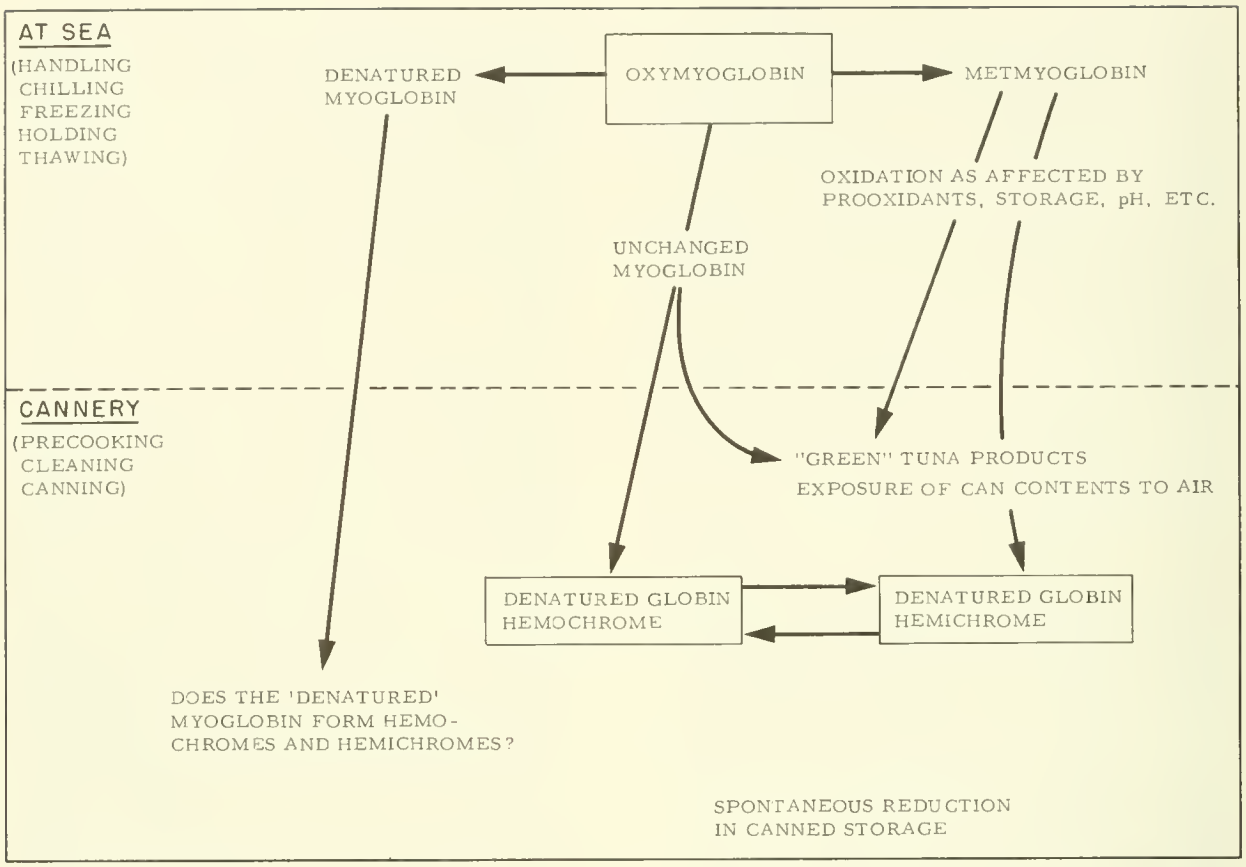

Figure 3.—Hypothetical diagram of the changes which tuna heme pigments may undergo in the course of catching and canning.

at pH 5.8, the following first order oxidation rates were found at different temperatures ($K \times 10^3$ hr^{-1}).

Oxidation Rates ($K \times 10^3$ hr^{-1}).

Temperature	*Yellowfin Mb*	*Bigeye Hb*
22° C	120	--
9° C	25	50 (10° C)
−2° C (not frozen)	5	5
−10° C (frozen)	40	175
−20° C (frozen)	75	--

A second stage was a study of the effect of the oxidation state of the heme pigments on their ability to form ferrohemochromes. A model system was developed using hemoproteins denatured by a detergent, Dupanol. The surprising result was that both the oxidized and the reduced pigments form the denatured hemichrome (oxidized form). However there is some doubt that this model system resembles closely the in vivo conditions, and better alternatives are being devised. Further stages of this work will concern the effect of denaturation of heme pigments on their ability to form hemochromses and the factors influencing the stability of the hemochromes.

Barrett et al. (1965) and our laboratory also indicated that the final color of canned tuna could be correlated with the amount of water-extractable heme pigments present before canning, and to a lesser extent with the shift of the Soret peak from 412 mμ for oxymoglobin to 406 mμ for metmyoglobin, the oxidized form. This methodology did not prove entirely satisfactory and was abandoned because the heme pigments undergo other independent complex chemical reactions, thus adding other variables.

Sugars, the secondary color factors in tuna. —In addition to the tuna color due to heme compounds, the appearance of tuna is also af-

fected by scorch and caramelization. Scorch is the tan to brown color seen on that surface of the canned tuna which is not covered with liquid (headspace) during the retort process. In most cases the commercial pack is randomly dropped into retort baskets and so the headspace scorch can occur in any position in the can. It is usually easy to identify. The second type of browning reaction, caramelization, is a general browning occurring throughout the whole of the tuna in the can and occurs usually only under the more severe conditions of retorting: at a low level it may only have the effect of generally dulling the appearance of the canned product. The former type of browning appears to be due to Maillard reactions (nonenzymic browning), which occur widely in food processing. This reaction is due to the interaction of a carbonyl compound, usually a reducing sugar such as glucose, fructose, or ribose, with amino compounds, usually proteins or amino acids. Such reactions have been described as occurring during the prolonged heating or during the drying of fish (Jones, 1957). Sometimes carbonyl compounds from fish oils and other sources may be involved.

We found that by adding increased amounts of glucose and ribose and some of their naturally occurring phosphorylated derivatives to tuna before canning, progressive scorching and caramelization could be developed. Small amounts of added sugars first produce a headspace scorch. As the amount of added sugar is increased, it becomes more intense and then an overall browning appears in the tissue. At higher concentrations, a bitter flavor is also noted. About three times as much glucose as ribose is required for the same scorch intensity, and the scorch increases with prolonged retorting, corresponding to the increased process time required for institutional packs. Replacement of the air in the headspace with nitrogen gives partial protection during long periods of retorting.

We have not made extensive measurements on tuna, but, in general, glucose is normally present in freshly caught fish and has been found at quite high levels in tuna (Crawford et al., 1970; Tarr, 1954; and Jones, 1957). The amounts of glucose present are likely to depend on various factors relating to the circumstances of catching. On storage the glucose decreases, and ribose, liberated during the last stage of the breakdown of the purine compounds, progressively increases. The ribose in tuna may decrease depending on the activity of the spoilage bacteria or other factors. In the case of tuna, there is likely to be some loss of sugars and soluble nitrogen compounds in the stickwater during the precook.

These general observations tie in with results on chill storage of albacore (Crawford and Finch, 1968). Fresh caught samples showed considerable scorch after canning, presumably due to high glucose content, although the sugar contents were not measured. In later samples the scorch was considerably less, but with the tuna canned during the final week of storage (between day 27 and day 35), scorch became more severe, presumably due to the increase in ribose. It is not clear at present how these observations can be turned to advantage except perhaps that sugar measurements on the raw fish may serve as another index of the quality to be expected after canning. It is quite possible that a more complete understanding of the way in which factors such as catch method, exhaustion of the fish, chilling, freezing, storage, and thawing conditions, affect the development of sugars may enable suggestions of control measures to reduce scorch and discoloration due to this cause. This would be especially valuable in the institutional pack which is increasing in importance to the industry, since the prolonged retort process makes it especially vulnerable to this kind of quality loss. The sugar content of skipjack tuna, and their relation to scorch, was therefore chosen as one of the studies to be included in this investigation.

Purine compounds.—In the living fish, energy for muscular action is provided by a series of high energy phosphate compounds. The most important is adenosine triphosphate (ATP) which is converted to adenosine monophosphate (AMP) as it gives up its energy during muscular contraction and then is rebuilt to ATP by creatine phosphate. When the fish dies these compounds go through a series of degradations to successively simpler compounds at rates which depend on the storage temperatures. The sequence of degradation is

presumed to be: ATP→ADP→AMP→Inosine Monophosphate→Inosine→Hypoxanthine. The intermediate compound, inosine monophosphate (IMP), and the end product, hypoxanthine, have been credited under some circumstances with affecting the flavor of fish. IMP has a sweet, flavor-enhancing effect, and hypoxanthine is said to contribute a bitter flavor. If this applies in the case of tuna under commercial storage conditions, it could lead to a deterioration from a pleasant to an unpleasant flavor as the fish ages. Investigations so far do not indicate that flavor changes in canned tuna are closely associated with the changes in purine compounds (Crawford and Finch, 1968). This may be because in the canning of tuna, other flavors, notably those relating to the degradation of the oils, and/or other oxidation degradations may dominate.

A more useful aspect of these changes is to provide measures of the storage history of the raw fish. Fortunately, the rates of degradation of purine compounds in tuna appear to be a good deal slower than in most other fish. Thus, in albacore held at 30° F, a progressive formation of hypoxanthine was recorded, and after 35 days a quarter of the purine compounds had completely degraded to form hypoxanthine (Crawford and Finch, 1968). This means that it should be possible to use the development of changes in hypoxanthine as an index of storage over a prolonged period of time. This is unlike many other fish in which the changes are essentially complete in a few days after which measurements of hypoxanthine cannot be used as a measure of continued storage. In the case of tuna, where storage temperature measurement in the well is very difficult and time consuming, measurement of hypoxanthine, which is quite simple, could provide an easier method to assess the time-temperature storage history and also the parallel changes in conditions before canning (Crawford, 1970). The natural fish-to-fish and other variations are likely to preclude this method from giving a precise indication of storage; however, it may well serve to classify tuna into broad categories of storage history such as excellent, good, fair, poor. This is especially interesting since the method lends itself to automation whereby large numbers of samples may be examined by one person.

Other factors.— We have looked at several other chemical or physical factors to see if they appear to relate to the quality of the canned product. It seems likely that the oil content of the fish muscle is related to texture, low oil content being associated with a tough dry texture and a high oil content with a soft tender texture. This was found to be true of raw fish muscle and also to some extent of the drained canned tuna in texture studies carried out by the University of Maryland.

We have attempted to apply the Torry cell fragility test to measure protein changes on storage. After a considerable number of experiments, we were able to modify the technique to give consistent results, but we have insufficient results so far to indicate whether this is likely to give results which correlate usefully with the storage history of the tuna. Other measurements we have made, such as the free fatty acid content of the extracted lipids, the nonprotein nitrogen, or the percentage of denatured protein offer little promise as indices of storage change.

The Effects of Premortem Stress and Postmortem Biochemical Changes on the Quality of Canned Skipjack Tuna

Analyses of the aforementioned experiments and a perusal of the literature indicated that a knowledge of the sugars and nucleotides in tuna may very well elucidate some of the mechanisms evolved in causing quality changes in tuna (especially with reference to color changes and the presence of scorch). Tarr (1969) reported that fish acclimated to warmer climates make use of the Embden-Meyerhof pathway while those acclimated to colder temperatures prefer the hexosemonophosphate shunt. Tarr also stated that almost all of the sugars result from the glycolytic pathway. It has been postulated that the tuna uses the white dorsal muscles only as an emergency supply of energy for rapid swimming while using the red muscle for the constant swimming typical of pelagic fish that do not have swim bladders to keep them afloat. Peterson (1970) agrees with this concept of a voluntary and involuntary musculature in skipjack tuna. He showed with electron micrographs that the red muscle is indeed more

heavily laden with fat globules than is the white muscle, suggesting that lipids are the primary source of energy in the red muscle. He also showed that mitochondria were very abundant in both the red and white muscle but more abundant in the red. Nagayama (1961) studied changes of some glycolytic intermediates to observe the phenomenon of browning in fish flesh. He reported that glucose-6-phosphate, fructose-6-phosphate, and fructose diphosphate produced browning but glucose-1-phosphate, AMP, and ATP did not. The browning reaction was said to occur through the loss of phosphate whose ion later catalyzed the freed sugars reaction with some amino group. In another series of papers, Nagayama suggested that hexose sugars were the limiting factors in browning of fresh fish but pentose sugars (ribose in particular) were later liberated during storage and contribute to browning.

Crawford et al (1970) simulated stress in skipjack tuna and compared some of the sugars in the white muscle of this fish to those of unstressed fish. Sugars in stressed and unstressed skipjack held at various temperatures were also compared, as were organoleptic qualities of the fish after processing and canning. It was concluded that there were differences in some of the chemical and organoleptic parameters measured; while there were some trends noted, there was no overwhelming evidence connecting the induced variables with the difference in quality of the final product. It was reasoned that such a complex experiment should be performed more than once if one is to draw any valid conclusions. The following study repeats the experiment of Crawford et al. (1970) with some added design features and measurements.

It is not the purpose of this study to elucidate the mechanism of the Embden-Meyerhof pathway in skipjack tuna although some comments will be made. Rather, this study will focus its attention on those conditions of stress, time, temperature as they affect the rate at which glycolysis proceeds and consequently the final level of sugars formed in the fish muscle. A wide range of temperatures was employed to see if glycolysis can be retarded or increased postmortem thus exercising some control over the degradation of products formed.

Live skipjack tuna were captured off the shores of Oahu and were placed in shoreside tanks. They were observed for a day or two to note their apparent health. Rested fish and exercised fish (induced stress) which served as controls were sacrificed and canned immediately while others were sacrificed and held in seawater at 78°, 60°, and 32° F for time intervals of 6 and/or 9 hr before canning. Some fish were also frozen after holding to note the effect of freezing. Additionally, some fish were frozen alive in 0° F refrigerated seawater (a condition which often occurs on a commercial tuna boat) and subsequently canned. A sample wedge for analyses of some autolytic degradation products was taken from all fish before canning. The organoleptic quality and some chemical analyses were determined on the canned products.

MATERIALS AND METHODS

Materials

The skipjack used in this experiment were caught by the Bureau of Commercial Fisheries MV *Charles H. Gilbert* off Honolulu, Hawaii, using live bait and barbless hooks. The fish were taken to Kewalo Basin and held alive in special tanks developed by the BCF Biological Laboratory (Honolulu). Only apparently healthy fish were used after 1 or 2 days of observation.

The 125 skipjack used in this experiment were utilized as follows:

A. 32° F study.
   1. Sacrificed (blow on the head) 5 "rested" fish, sampled for chemical analysis and canned immediately (rested controls).
   2. Sacrificed 5 "exercised" fish (induced stress by chasing fish at the limit of his swimming speed around a tank for about 45 min at which time signs of exhaustion appeared, e.g., slowed swimming speed and/or turning belly side up). These fish were sampled for chemical analysis and canned immediately (stressed controls).
   3. Sacrificed 10 rested and 10 stressed fish and held for 6 hr in 32° F circulating seawater. Sampled and

canned 5 from each group. The other 5 in each group were frozen in 10° F circulating brine for 20 hr, then thawed in running seawater (3 to 4 hr), sampled, and canned.

B. 60° F study.
Proceeded as in "A" except held fish in 60° F seawater for 6 hr.

C. 78° F study.
Proceeded as in "A" except held fish in 78° F seawater for 6 hr.
Note: Experiments B and C were performed on the same day so that the controls for these groups are the same.

D. 78° F study for 9 hr.
Proceeded as in "A" but held fish in 78° F seawater for 9 hr, rather than 6 hr.

E. The freezing of stressed and rested controls proceeded as in 1 and 2 of "A" except that they were frozen in 10° F brine for 20 hr before sampling and canning.

F. Live fish.
1. 5 fish were placed alive in 0° F brine for 20 hr.
2. The fish were then thawed, sampled and canned.

Sampling

The muscle sampling procedure has been described by Crawford and Finch (1968). Briefly, a 100-g wedge was removed from the dorsal side of the fish. This wedge was bounded by the first and fourth ray and just above the lateral line. This wedge was immediately frozen in liquid nitrogen and later packed in dry ice and shipped to the BCF laboratory on Terminal Island, Calif., and later the University of California in Berkeley for analyses.

Chemical Analyses

Four muscle wedges from each group of five were used as follows by the BCF Laboratory. The wedges were cut in half with one-half being returned to —90° C storage for reference. Twenty grams of the other half were ground cold with 50 ml of 0.6 N $HClO_4$ and then neutralized to pH 6.8 with 5N KOH. The potassium perchlorate precipate was allowed to settle and was removed from the extract in the cold. The extract was distributed into several vials and frozen and stored at —90° C. In this manner, no extract was thawed more than once when analysis was carried out.

Fructose (free) and fructose phosphate were estimated on the neutralized extract by the method of Roe (1934) and glucose phosphate by the method of Morris (1948), after separation on Dowex 1 × 8 (chloride) resin and elution with 2N HCl (Spinelli et al., 1964). The free sugars (glucose, fructose, and ribose) are not retained on the column and are collected as the extract is put over the column to remove the phosphorylated sugars, glucose phosphate, and fructose phosphate (nucleotides are also held on the column). Glucose phosphate and fructose phosphate were estimated by mixing 5 ml of the HCl wash in a 19-mm test tube with 10 ml of 0.2% Anthrone in 95% H_2SO_4; absorbance was read 10 min later at 620 mμ.

Fructose phosphate was determined on the same HCl wash from the column by mixing a 1-ml aliquot in a 19-mm test tube with 1 ml of 0.1% resorcinol in 95% absolute alcohol and 3 ml of 30% HCl. The test tube was heated for 8 min in an 80° C water bath, cooled and read at 490 mμ. This result was subtracted from the glucose-fructose phosphate estimation, leaving an estimation of glucose phosphate. Free fructose was determined by the Roe method. Ribose was determined by the method of Mejbaum (1939) after adjusting the extract to pH 11 and passing it over Dowex 1 × 8 columns to remove all ribose containing nucleotides, nucleosides, and phosphorylated sugars. A 3-ml aliquot of the resin treated extract was mixed in a glass stoppered test tube with 1% resorcinol in 0.1% $FeCl_3$ in concentrated HCl. The tubes were heated in boiling water for 30 min and cooled, and the absorbance was read at 670 mμ.

Free glucose, pyruvate, and lactate were determined enzymatically with commercial kits purchased from Calbiochem of Los Angeles, Calif. Total reducing sugars were estimated by the previously described method of Morris.

Hypoxanthine (Hx) was determined on the neutralized extract by the xanthine oxidase method of Spinelli et al. (1964). That is, the shift in optical density at 290 mμ was determined on a known amount of Hx treated with

xanthine oxidase followed by 1 min bubbling of air through the solution. The shift in optical density in the neutralized extracts when treated with xanthine oxidase was then compared to this value (0.08 OD = 1γ of Hx). An aliquot of the neutralized extract was read at 248 mμ, then shaken with Dowex 1 × 8 (chloride) resin to remove the nucleotides, and read again at the same wavelength (Jones and Murray, 1964). The difference in the readings was taken as an estimate of inosinemonophosphate (IMP) since this author and others have observed that IMP is the only nucleotide of consequence remaining shortly after death. The reading of the extract after resin treatment was taken as an estimation of inosine and Hx. The Hx was subtracted leaving an estimation of inosine.

Twenty grams of canned product from each fish was ground with 0.6N $HClO_4$ as described above. All analyses run above were determined on this extract in the same manner. All determinations on the raw tuna muscle were run in duplicate for each individual fish. The results for each fish in each treatment were averaged and are so reported. The determinations on the canned product were run in duplicate on the combined extracts for each treatment.

Organoleptic Evaluation

The taste panel description is given in Table 1 as used and reported previously by Crawford et al. (1968, 1969, 1970). The canned tuna samples were allowed to stand for at least 4 weeks before they were examined by an expert panel of judges. In this analytical panel, the judge was not asked to assess preference but to give a sensory description of the product.

RESULTS AND DISCUSSION

Glycolytic Degradation Products (Table 2)

Total reducing sugars, μM/g.—The total reducing sugars (TRS) determined on the raw muscle and canned product was calculated as μM/g of glucose. The content varied from 5 to 31 μM/g. The results from the different groups were as follows:

The rested controls that were sampled and canned immediately were higher in TRS content than the corresponding stressed controls for each treatment. However, when frozen, the stressed controls had a higher content.

Although this observation is consistent with the need for more energy during stress, no valid conclusions can be drawn from these observations because the test employed will determine glycogen content as well. Glycogen is not quantitatively extracted with the method used. However, this test does give an estimate of the total carbohydrate content and is used sometimes in canneries with some degree of success as a guideline in selecting raw material for 4-lb. institutional packs. These packs, because of their size, are retorted at high temperatures for long periods of time, thus giving full opportunity for browning reactions. Consideration of the scores for scorch in Table 4 would seem to invalidate this procedure since there was marked evidence of scorch in almost all cans for all treatments regardless of the TRS content. But, it may be reasoned that since the fish delivered to the canneries are usually several weeks old, there is probably very little glycogen remaining. (The temperature at which tuna is stored on the vessels, about 20° F, is not sufficient to stop glycolysis.) Therefore, the TRS content of this fish should contain very little glycogen and mostly carbohydrate that can be involved in browning reactions, e.g., glucose phosphate, fructose phosphate, glucose, and ribose.

The TRS content of the can, with only two exceptions, showed lower values than the corresponding raw material. This is consistent with the concept of sugar-amino reactions (Maillard reaction) in browning. The rested fish when frozen after holding showed a lower TRS content than the unfrozen for all temperatures including the controls. This is also true of the stressed fish except those held for 6 hr in 78° F refrigerated seawater (RSW) where the values are nearly the same. These observations indicate that freezing may retard glyconeogenesis and suggest that glyconeogenesis does indeed occur at least during these time intervals postmortem.

Table 2.—The effect of premortem stress, holding temperatures, and freezing on th

Treatment	[1]TRS		Free glucose		[2]G-PO_4		[3]F-PO_4	
	Raw	Can	Raw	Can	Raw	Can	Raw	Can
	μM/g	μM/g	μM/g	μM/g	μM/g	μM/g	μM/g	μM/g
Live fish, 0° brine ...	20.56	9.35	12.37	1.87	7.35	0.99	1.19	0.40
32° F-R-F	9.01	6.79	8.05	1.17	4.05	0.36	0.62	0.18
32° F-R-UF	21.28	18.70	8.67	0.93	8.50	0.63	4.98	0.23
32° F-control-R	20.08	16.78	14.62	1.03	10.66	0.48	1.86	0.18
32° F-S-F	9.80	7.99	10.08	1.11	6.58	0.36	0.83	0.28
32° F-S-UF	12.11	12.14	7.81	1.41	7.66	0.65	1.78	0.21
32° F-control-S	5.02	12.94	7.55	1.22	4.57	0.57	0.92	0.26
78° F-R-F	12.99	7.99	10.32	1.45	4.84	0.44	0.69	0.23
78° F-R-UF	14.35	11.75	10.89	1.28	7.52	0.72	1.97	0.30
78° F-control-R	19.37	16.14	13.74	1.03	5.70	0.88	6.29	0.30
78° F-S-F	10.28	6.95	11.19	1.41	5.33	1.42	0.65	0.40
78° F-S-UF	9.01	8.79	9.61	1.27	4.38	0.71	0.44	0.28
78° F-control-S	8.21	11.67	8.68	0.88	4.29	0.60	1.39	0.33
60° F-R-F	22.56	8.15	8.82	1.32	3.45	0.31	0.63	0.23
60° F-R-UF	31.08	20.13	7.27	1.11	15.91	0.89	5.89	0.23
60° and 78° F (9) ... control-R	22.16	19.98	9.99	1.33	6.68	0.74	0.76	0.31
60° F-S-F	16.10	6.79	9.85	1.53	5.15	0.66	0.64	0.20
60° F-S-UF	26.54	15.02	9.90	1.27	9.30	0.70	1.80	0.23
60° and 78° F (9) ... control-S	18.97	14.62	8.79	0.96	7.17	0.60	0.86	0.23
78° F (9)-R-F	13.15	5.82	10.60	1.43	5.82	0.43	0.57	0.18
78° F (9)-R-UF	16.98	7.83	11.70	1.33	6.60	0.49	0.73	0.20
78° F (9)-S-F	11.96	5.43	8.88	1.38	3.89	0.44	0.43	0.23
78° F (9)-S-UF	17.14	8.95	11.17	1.35	4.69	0.73	0.84	0.23
Frozen control-R	14.27	6.71	11.26	1.39	5.10	0.36	0.44	0.21
Frozen control-S	22.95	8.79	17.16	1.88	9.09	0.56	1.07	0.28

[1] TRS = Total reducing sugars calculated as μM/g of glucose.
[2] G-PO_4 = Glucosee phosphate calculated as μM/g of glucose.
[3] F-PO_4 = Fructose phosphate calculated as μM/g of fructose.

Glucose (free), μM/g.—The glucose content varied from 7.3 to 17.2 μM/g in the raw muscle. The rested controls (unfrozen) showed higher levels of glucose than did the stressed. Glucose seems to be used at a faster rate in the stressed fish. This is consistent with the need for more energy. However, the stressed controls when frozen show a higher level of glucose. This may indicate that gluconeogenesis occurs even at these frozen temperatures and that the stressed fish have more enzymes for this mechanism than do the rested fish in that the level of glucose in the stressed fish is by far the highest of all treatments (17.2 μM/g). The glucose contents of the rested controls are higher than that of the fish held in RSW and SW for all treatments. The glucose content of the rested fish is nearly the same for all treatments whether frozen or not after holding in RSW and SW. This seems to be temperature-dependent since the content is generally lower in fish held at 32° or 60° F and higher in those held at 78° F.

The glucose contents of the stressed controls are somewhat lower than that of the fish held in RSW and SW for all treatments. This is again consistent with the suggestion that gluconeogenesis may still occur postmortem under these conditions.

The glucose content of the corresponding canned products in all instances showed a marked decrease to less than 2 μM/g regardless of the level in the raw fish. This is not surprising since experiments reported earlier showed that glucose readily participates in browning reactions.

Glucose phosphate, μM/g. — The glucose phosphate (GP) content was calculated as μM/g of glucose. It varied from 3.5 to 15.9

glycolytic degradation products in the raw muscle and canned product of skipjack tuna.

Treatment	Free fructose		Free ribose		Pyruvic		Lactic	
	Raw	Can	Raw	Can	Raw	Can	Raw	Can
	$\mu M/g$	$\mu M/g$	$\mu M/g$	$\mu M/g$	$\mu M/g$	$\mu M/g$	$\mu M/g$	$\mu M/g$
Live fish, 0° brine	0.88	2.07	0.71	1.00	0.20	0.24	151.2	137.0
32° F-R-F	0.55	1.28	0.66	0.76	0.10	0.18	109.7	143.5
32° F-R-UF	0.81	2.22	0.50	0.90	0.07	0.27	125.9	116.2
32° F-control-R	1.16	2.50	0.53	1.14	0.16	0.33	117.7	99.3
32° F-S-F	0.56	1.29	0.66	0.76	0.08	0.19	128.3	131.0
32° F-S-UF	0.55	2.36	0.46	1.14	0.07	0.26	136.0	131.5
32° F-control-S	0.44	2.44	0.49	1.02	0.18	0.30	140.6	126.6
78° F-R-F	1.08	2.50	0.87	0.82	0.08	0.23	146.0	117.3
78° F-R-UF	1.28	1.96	0.79	0.87	0.09	0.24	142.9	131.0
78° F-control-R	1.51	1.53	0.85	1.02	0.16	0.32	114.4	122.8
78° F-S-F	1.16	2.11	1.10	0.76	0.08	0.25	122.7	135.4
78° F-S-UF	1.17	1.78	0.79	0.67	0.07	0.27	114.4	127.2
78° F-control-S	0.53	1.45	0.57	0.76	0.13	0.32	150.0	117.3
60° F-R-F	0.62	2.05	1.07	0.95	0.10	0.18	142.1	115.7
60° F-R-UF	1.22	2.00	1.37	1.32	0.09	0.27	130.3	116.3
60° and 78° F (9) control-R	0.80	2.22	0.68	1.34	0.10	0.27	132.5	124.4
60° F-S-F	0.87	1.73	0.96	0.83	0.11	0.24	144.6	118.4
60° F-S-UF	0.67	2.31	0.46	1.43	0.08	0.19	138.0	124.4
60° and 78° F (9) control-S	1.00	2.31	0.85	1.40	0.12	0.28	110.0	105.3
78° F (9)-R-F	1.36	2.21	1.12	0.83	0.08	0.21	152.0	128.3
78° F (9)-R-UF	1.37	1.82	1.21	0.71	0.09	0.19	156.5	109.2
78° F (9)-S-F	0.94	2.01	1.40	0.90	0.08	0.18	136.5	127.7
78° F (9)-S-UF	0.98	2.24	0.66	0.83	0.09	0.18	147.4	141.9
Frozen control-R	0.71	1.56	1.40	0.88	0.12	0.25	155.7	117.9
Frozen control-S	1.38	2.21	1.43	0.89	0.12	0.25	169.8	126.1

$\mu M/g$ in the raw muscle. In general, the GP content was higher in the unfrozen rested controls than in the stressed. The frozen controls had a higher content than the rested.

The GP content was higher in the unfrozen than in the frozen stressed and rested fish for all treatments except for the controls and for the stressed fish held for 6 hr in 78° F SW where the reverse was true. This observation seems to indicate that freezing may very well destroy or partially destroy the enzymes needed for the formation of GP.

The GP content of the canned products showed a marked decrease (with but one exception) to less than 1 $\mu M/g$ regardless of the content of the raw tissue. This indicates that GP is degraded and probably participates in browning reactions as previously reported.

Fructose phosphate, $\mu M/g$.—Fructose phosphate (FP) content varied from 0.4 to 6.3 $\mu M/g$ in the raw tissue. In general, the observations for this sugar are the same as those described for GP. The FP content of the can decreased to less than 0.5 $\mu M/g$, thus indicating degradation. It is probable that this compound participates in browning reactions. Some evidences of this have been obtained by Olcott (unpublished).

Free fructose, $\mu M/g$.—The free fructose content varied from 0.4 to 1.5 $\mu M/g$ in the raw muscle. It was surprising to find free fructose in that the author had not found measurable amounts of free fructose before. The significance of this discrepancy is not known.

Higher levels of free fructose were found in the canned product, perhaps resulting from the degradation of fructose phosphate.

Free ribose, μM/g.—The free ribose content varied from 0.5 to 1.4 μM/g in the raw muscle. The level in the fish held at higher temperatures tended to be slightly higher than the content of the fish held in 32° F SW. This small rise may be attributed to the breakdown of purine compounds.

The free ribose content of the canned fish was not significantly different from that of the raw muscle. Since ribose readily participates in browning reactions, this may seem surprising. However, it may be that such small amount of ribose cannot effectively compete with the much larger amounts of glucose and glucose phosphate in browning reactions.

Pyruvate and lactate, μM/g.—The pyruvate content was very consistent. It varied from 0.07 to 0.20 μM/g previously reported by Crawford et al. (1970). The content of the canned product was approximately the same.

The lactate content varied from 109 to 170 μM/g. These levels agree with the results reported by Crawford et al. (1970). However, Crawford also reported significantly lower lactate content in the control whereas the content of the controls in this experiment are not significantly different from those of the fish held for 6 or 9 hr in RSW or SW. The difference may lie in the fact that in the earlier experiment extracts were made immediately after sampling, but in this experiment the extracts were prepared after first freezing the sample wedges in liquid nitrogen.

There were no consistent differences in the lactate content of the stressed and rested fish. This may indicate that fatigue in the skipjack is not due to lack of NAD but rather may be due to the fish's inability to exchange oxygen and carbon dioxide fast enough across its gills (there was sufficient oxygen available in the tank at all times). There was a small decrease in lactate content in the canned product.

Purines and Purine Derivates (Table 3)

Inosinemonophosphate, μM/g. — The estimated inosinemonophosphate (IMP) content varied from 7.4 to 13.0 μM/g. With one exception, the unfrozen rested controls showed a lower IMP content than the stressed controls. However, the frozen rested and stressed controls show about the same content. The IMP content of the rested fish is higher than the stressed fish when held in 32° F RSW whether frozen or unfrozen. These results indicate that the stressed fish began with higher levels of high energy phosphorylated compounds but degraded to lower levels when held in 32° F RSW as expected. On the other hand, the IMP content of the rested fish showed no significant change when held at the same temperature (see total purines, Table 3). The same changes also occur in the fish held in 78° F SW for 6 hr, but both rested and stressed fish show a decreased content when held. Also, the unfrozen stressed fish has a higher IMP content than the frozen. These observations may be attributed to the generation of more enzymes for the synthesis of high energy phosphorylated compound to meet higher energy output during stress.

It is interesting to note that the skipjack held in 60° F RSW for 6 hr and in 78° F SW for 9 hr showed a higher IMP content than the fish held at 32° F or 78° F for 6 hr. This indicates that perhaps purines are being synthesized as well as being degraded at these temperatures. (See inosine content and total purines, Table 2). This is in sharp contrast to the results reported by Crawford et al. (1969) where IMP showed a marked degradation with time in skipjack (stressed) held for 30 hr at 30° F RSW.

The IMP content of the can varied from 10.0 to 13.4 μM/g and was fairly uniform for all treatments. Another point of interest is that the IMP content of the canned product of rested and stressed fish held in 32° F RSW and 78° F SW showed an increase whereas the fish in other treatments which had higher IMP levels before canning, show little change.

The author hesitates to speculate about what specific purine compound may have been synthesized but will point out that ATP, ADP, and AMP contents of fish are readily subject to degradation, especially in the presence of heat.

Inosine and hypoxanthine, μM/g.—The inosine (I) content (estimated), which results from the degradation of IMP, varied from 0.3 to 4.7 μM/g. In general, the I content increased with time and temperature, as expected. The contents of the can show a tendency to remain

Table 3.—The effect of premortem stress, holding temperatures and freezing on the purine nucleotide degradation products in the raw muscle and canned product of skipjack tuna.

Treatment	[1]Estimated IMP		Estimated inosine		[2]Hx		Total	
	Raw	Can	Raw	Can	Raw	Can	Raw	Can
	μM/g	μM/g	μM/g	μM/g	μM/g	μM/g	μM/g	μM/g
Live fish, 0° brine ..	11.22	11.24	1.40	1.92	--	0.17	12.62	13.33
32° F-R-F	10.85	11.52	1.15	1.96	--	0.19	12.00	13.48
32° F-R-UF	9.77	12.42	1.37	0.69	0.06	0.07	11.20	13.18
32° F-control-R	9.80	12.03	0.33	0.04	--	0.07	10.13	12.14
32° F-S-F	8.97	12.49	0.91	1.74	0.09	0.20	9.97	14.43
32° F-S-UF	8.61	11.20	0.63	1.45	0.05	0.10	9.29	12.75
32° F-control-S	12.98	11.63	0.35	0.87	--	0.08	13.32	12.58
78° F-R-F	7.46	11.74	1.33	1.81	0.14	0.24	8.93	13.74
78° F-R-UF	7.41	11.88	1.10	0.76	0.15	0.24	8.65	12.88
78° F-control-R	9.93	11.31	2.06	0.15	--	0.10	11.91	11.56
78° F-S-F	7.63	10.95	2.10	1.92	0.23	0.29	9.96	13.16
78° F-S-UF	11.22	11.74	2.31	1.60	0.13	0.24	13.65	13.58
78° F-control-S	12.05	13.03	1.28	0.40	--	0.09	13.33	13.52
60° F-R-F	10.46	10.69	2.61	1.63	0.10	0.25	13.17	12.57
60° F-R-UF	11.95	11.99	1.38	1.31	0.07	0.18	13.39	13.48
60° and 78° F (9)-control-R	12.20	11.85	1.13	1.23	--	0.11	13.33	13.19
60° F-S-F	10.24	10.05	3.20	3.08	0.16	0.34	13.60	13.47
60° F-S-UF	12.75	11.42	1.46	1.74	0.06	0.25	14.27	13.41
60° and 78° F (9)-control-S	11.89	11.78	0.35	1.16	--	0.11	12.24	13.05
78° F (9)-R-F	11.33	10.88	2.38	2.54	0.23	0.47	13.94	13.89
78° F (9)-R-UF	11.90	10.27	2.13	2.43	0.16	0.32	14.19	13.02
78° F (9)-S-F	9.34	10.02	3.85	3.08	0.38	0.56	13.56	13.66
78° F (9)-S-UF	10.11	10.88	3.67	2.61	0.37	0.47	14.13	13.96
Frozen control-R	10.20	13.39	4.20	0.25	0.12	0.31	14.51	13.95
Frozen control-S	10.57	10.48	4.67	3.37	0.07	0.30	15.30	14.15

[1] IMP = inosinemonophosphate.
[2] Hx = hypoxanthine.

at the same level as that of the raw product although there were in some instances slight increases and decreases.

The hypoxanthine (Hx) content, which results from the degradation of I, varied from 0.0 to 0.4 μM/g. In the canned product, the content varied from 0.1 to 0.6 μM/g. No attempts will be made to draw any conclusion from these very low levels, although there is an indication that the Hx content is time and temperature dependent.

Organoleptic Analysis (Table 4)

Scorch and color.—The most severe scorch was observed in the canned product of the fish that was frozen alive in 0° F brine, the stressed frozen controls, the frozen stressed and rested fish held in 32° F RSW, and the unfrozen stressed and rested skipjack held in 60° F RSW for 6 hr and 78° F SW for 9 hr, respectively. The least scorch was observed in the rested frozen and unfrozen fish held for 6 hr in 78° F SW. A moderate amount of scorch was noted for all other treatments. There are some parallels between severe scorch and poor color (dark). The best color (lightest) was observed in the rested unfrozen fish held for 6 hr in 32° F RSW and 78° F SW. All scores for the unfrozen controls were relatively uniform and showed fairly poor color. The poorest color was noted for the fish that were frozen alive, the frozen controls, the stressed frozen and unfrozen fish held for 6 hr in 60° F SW, and all of the fish held for 9 hr in 78° F SW regardless of treatment.

Firmness, flakiness.—Almost without exception, the unfrozen controls (rested and

Table 4.—The effects of premortem stress, holding temperatures, and freezing on the organoleptic quality of canned skipjack tuna.

Treatment	Score[1]						
	Scorch	Color	Odor	Flavor	Flakiness	Firmness	Fibers
Life fish, 0° brine	1.0	1.6	3.6	3.2	6.4	6.6	5.6
32° F-R-F-6 hr	1.0	4.5	2.8	4.5	5.8	6.5	7.3
32° F-R-UF-6 hr	2.8	5.6	3.6	3.4	4.4	6.2	6.6
Control, R	2.6	3.2	2.4	2.6	4.0	4.0	5.0
32° F-S-F-6 hr	1.4	4.2	3.0	2.8	6.6	6.6	6.4
32° F-S-UF-6 hr	2.6	3.8	2.6	3.0	3.4	3.4	6.2
Control, S	2.4	3.0	2.4	2.2	3.8	3.4	5.4
78° F-R-F-6 hr	3.8	4.0	4.6	3.4	6.2	6.2	5.4
78° F-R-UF-6 hr	4.3	6.0	3.5	3.3	6.3	5.3	7.3
Control, R	3.0	3.4	2.6	2.6	3.6	4.8	8.2
78° F-S-F-6 hr	3.0	4.8	2.8	2.4	5.2	7.2	6.8
78° F-S-UF-6 hr	3.0	3.3	2.3	2.0	5.3	5.7	6.3
Control, S	3.2	3.4	3.2	2.8	4.8	5.0	6.4
60° F-R-F-6 hr	3.2	3.2	3.6	3.2	6.0	7.2	7.2
60° F-R-UF-6 hr	3.2	3.0	3.4	3.6	5.4	4.8	7.4
Control, R	2.8	3.0	2.6	2.4	2.8	2.2	7.2
60° F-S-F-6 hr	3.4	1.4	3.2	2.4	5.6	6.8	4.8
60° F-S-UF-6 hr	1.8	2.2	1.8	2.2	3.4	4.8	6.0
Control, S	3.2	2.8	2.2	2.0	3.4	3.4	6.6
78° F-R-F-9 hr	2.2	2.0	2.8	2.8	7.2	6.4	6.6
78° F-R-UF-9 hr	1.6	2.6	3.2	3.6	5.4	6.0	5.6
Control, R	2.8	3.0	2.6	2.4	2.8	2.2	7.2
78° F-S-F-9 hr	3.0	2.2	3.0	2.6	7.6	7.0	4.6
78° F-S-UF-9 hr	2.8	2.8	3.6	2.8	7.2	6.8	5.4
Control, S	3.2	2.8	2.2	2.0	3.4	4.8	6.0
Freeze control, R	2.4	1.8	3.2	2.6	5.0	7.8	5.6
Frozen control, S	1.0	1.0	2.0	1.0	--	--	--

[1] See Table 1.

stressed) were less firm and more flaky (less cohesive) than any of the fish in other treatments. Nearly all of the fish that were frozen after holding were firmer and more cohesive in texture than the fish that were held and not frozen. The observation of the effects of freezing on flakiness and firmness more or less agrees with that of Crawford et al. (1970).

Fibers.—The fish held in 32° F RSW, 60° F RSW (rested), and 78° F SW for 6 hr showed a tendency to be fibrous (chewy). It is interesting to note that there is no agreement in the unfrozen controls, which show a wide range in this characteristic, with the rested controls being generally more fibrous.

Off odor and flavor.—There are some notable general trends in the development of off flavor and odor. However, the most significant observation is that the control fish (especially, the frozen stressed controls) were generally scored as having more off flavor and odor than the fish that was held at the various temperatures before canning. Presumably by definition, off odor and flavor are those attributes not typical or characteristic of fresh fish that has been properly handled and processed. Most assuredly, no commercial procedure could match the care and expediency achieved in the catching, handling, and processing of the controls in this experiment. Furthermore, since those fish (controls) presumably represent the epitome of freshness and good handling and processing and since the panel of expert judges was not considering preference, then one must conclude that:

> Fresh skipjack contains inherently objectionable flavors and odors in which case some pretreatment is necessary to achieve a product devoid of these adverse characteristics.

or:

> This panel and industry experts (Crawford et al. (1970), whose experience with skipjack of this degree of freshness was limited, may have been conditioned to accept standards of freshness less than that achieved here (heretofore, controls were captured at sea some distance from the cannery and frozen at some low temperature to preserve its freshness until such time as it could be processed upon arrival at the cannery).

In any case, these observations disagree with those reported by Crawford et al. (1970) where the controls (rested) received near perfect odor and flavor scores. It has been suggested by the Hawaiian Tuna Packers (Honolulu, Hawaii) that this phenomenon may be attributed to seasonal variations (winter- vs. summer-caught fish). Other conflicts in results with those reported by Crawford et al. (1970) tend to support this contention. This suggests that different quality parameters may be applied and that different handling procedures may be required for each seasonal catch, at least for the skipjack caught in Hawaiian waters.

In general, none of the treatments produced cans of skipjack that were of excellent overall quality. However, there were some packs that were of fairly poor overall quality. The packs that were of fairly poor overall quality were those held for 6 hr in 32° F RSW and 78° F SW (stressed frozen, and unfrozen) and the fish held for 9 hr in 78° F RSW (all treatments) and to some extent, the controls. The stressed fish show a tendency to have more off flavor and poor color than the rested for each treatment (except for the controls). However, the differences between the two are slight and are not sufficiently discernible to be of any commercial value. A fairly good overall quality pack was produced from the rested fish held at 32° F; the other packs of tuna were of fair quality.

CONCLUSIONS

Skipjack tuna were sacrificed in a rested and stressed condition. Some were canned immediately to serve as controls. Others were held in 32° F RSW, 60° F RSW, and 78° F SW for 6 hr, and some were held in 78° F SW for 9 hr before canning. Additionally, an equal number of the fish from all treatments were frozen (for 20 hr), then thawed, and canned. Samples were taken before and after canning for measurement of glycolytic and purine degradation products. Organoleptic evaluations were made on the canned product. This experiment was designed to show if there were any differences (biochemical or organoleptic) between stressed and rested skipjack tuna held at various temperatures for either 6 or 9 hr.

There were no commercially discernible differences between rested and stressed skipjack. The relation of biochemical components measured to the treatment (time and temperature) of the fish and the subsequent relation to the quality of the canned product were not sufficiently defined to use for quality predictions. Glucose, glucose phosphate, and fructose phosphate were some of the sugars measured and were found in relatively large quantities in the raw muscle of the fish (regardless of treatment). These sugars showed marked decreases in the canned product which suggest that they may have been involved in the browning reactions that were in evidence in the canned product. There was no apparent correlation between the amounts of sugars present and the degree of browning. There were no consistent differences between the lactic and pyruvic acid content of stressed and rested fish. Freezing had an apparent effect upon some of the glycolytic enzymes. Purine degradation products were estimated but no significant relations or correlations were established.

The organoleptic evaluation of the quality of the canned product revealed that none of the packs were of excellent overall quality. The fish held in 32° F RSW (rested) had fairly good overall quality. All other packs were of fair to poor quality. There was some evidence that the stressed fish may be of poorer general quality than the rested. However, this difference was not commercially significant.

The fact that there were only relatively small differences between rested and stressed fish may be due to the inability to capture a truly rested fish. The "thought" of capture may be sufficient to induce a stressful condition in the skipjack tuna. Capture and sacrifice by electronarcosis or powerful anesthetics might be used to study this further.

In view of the dynamic action of the enzymes in skipjack even for long periods of time postmortem, the difference between rested and stressed fish may indeed be only academic. In relation to commercial practice, this is certainly true. The lack of definite and repeatable correlation between biochemical parameters, treatment, and quality suggests that the chemical steady state of the live fish has not yet reached an equilibrium, at least under the conditions of time and temperature used in this experiment. A better correlation between biochemical parameters and quality might be achieved if fish are stored for longer periods of time. This is certainly true of the correlation between IMP and the overall quality of commercial packs of tuna (Crawford, unpublished). But then again, the question of the control of quality (and perhaps improvement of quality as well) by pretreatment and temperature control remains elusive. Since the author has witnessed far better quality of skipjack from commercial practice which had been stored for longer periods of time, it may be that good, longer term storage may be beneficial to quality in some instances. Perhaps the differences in quality of commercial packs result from longer periods of time and/or higher temperatures than those in this experiment. Or maybe the see-sawing of temperatures which takes place in a brine well when fresh-caught fish is added to previously chilled fish, contributes to freezing and thawing of fish which may have adverse effects upon the quality.

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