

**NATURE OF
GREEN OR OFFCOLOR CONDITION
IN PRECOOKED YELLOWFIN TUNA**



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Fish and Wildlife Service

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ABSTRACT

Preliminary results from the analytical use of spectral reflectance in the study of the "greening" condition in precooked yellowfin tuna are reported. It was found possible to predict tendency to greening from laboratory precooking. The preliminary conclusions were that color changes in tuna are the results of oxidative changes in the hemoglobin and myoglobin pigments of the flesh, and that differences in color, including greening, result from differences in the concentration of these pigment derivatives. A relatively higher concentration of methemoglobin or metmyoglobin in raw fish flesh seems to be indicative of a tendency to greening on precooking.

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The color of foodstuffs is often an important factor governing their acceptance or rejection by the processor and consumer. An interesting example of the influence of color in this regard is the "greening" of the flesh of certain tuna that becomes evident on precooking prior to canning. The exploration of the fishery resources of the central Pacific by the Pacific Oceanic Fishery Investigations of the U. S. Fish and Wildlife Service, and the use of the longline to catch large deep-swimming yellowfin tuna, Neothunnus macropterus (Temminck and Schlegel), have drawn attention to this particular color problem because of the relatively large percentage of tuna prone to "greening" in the catch made by this type of gear.

The Japanese have long recognized the problem, particularly in their winter catch of albacore, Germo alalunga (Bonnaterre), in the North Pacific and have given some study to its cause. The conclusions reached as a result of their investigations are that greenness is associated with low oil content, with low weight-to-length relationship, and is a physiological condition unrelated to freshness. Some of these investigators feel that the wasting effects of the struggles of the fish endeavoring to free themselves from the hook in the longline method of fishing have much to do with the discoloration subsequently encountered (Miyachi 1950).

More recently certain Japanese investigators have reported a correlation between the greening of tuna and the concentration of a flesh pigment spectrophotometrically identified by them as myoglobin. They also claim to be able to predict the occurrence of greening with 85 percent certainty by visual examination of the raw meat (Matsuzaka and Takahashi MS.).

It is the purpose of this report to summarize the conclusions reached as a result of a preliminary investigation on this problem conducted at the University of Hawaii during 1955-56 under contract No. 14-19-008-2318 with the Pacific Oceanic Fishery Investigations. We are indebted to Mr. Fred Jermann of Hawaiian Tuna Packers, Ltd., Honolulu, for assistance in preparing samples in the cannery

and for other services that have contributed to the study. Thanks are also due to Dr. Albert L. Tester, Director, and Mr. Garth I. Murphy, Assistant Director of the Pacific Oceanic Fishery Investigations, and to other members of the staff for help and suggestions in this work.

COLOR IN FOODS

The problem of the definition and specification of color is a highly complicated one, the detailed discussion of which would be out of place here. Suffice it to say that physiological as well as physical factors are involved. In other words, the "properties" of the observer must be considered as well as the spectral distribution at the source of color. Many systems (C.I.E., Munsell, U.S.C., for example) have been set up and many instruments devised to facilitate an objective definition of color. It has been recognized, however, that the spectrophotometric curve, the plot of reflected or transmitted intensity versus wave-length, is "the most unambiguous specification of color that can be obtained," and that the spectrophotometer is the basic instrument in the standardization of color (Mackinney and Chichester 1954). Recognition should be made of the limitations of such curves in the evaluation of color as it will appear to the eye since perceived color can be greatly altered by slight variations in spectral curves, and two different curves may give the same color response to the eye. In addition to the dominant wave-length (hue), the factors of whiteness-purity (chroma) and photometric brightness (value) are also to be considered in color specification.

THE COLOR OF NORMAL AND GREEN TUNA

In studying the color problem in tuna flesh it was necessary to establish objectively the normal color of desirable or acceptable meat. Accordingly precooked meat judged to be of good quality was selected and designated as normal. In order to define objectively the color of this meat, it was decided to measure its reflectance over the visible range of

wave-lengths of light (400 to 700 millimicrons) using the Beckman DU spectrophotometer with reflectance attachment. To obtain a diffuse or matt surface, and eliminate factors such as gloss and directional color, the meat was forced through a 16-mesh stainless steel screen and packed heaping full into a 1 1/4-inch aluminum planchet. A glass plate was pressed firmly on to the meat until a uniform and smooth surface was produced. The filled planchet was introduced into the reflectance attachment of the spectrophotometer and measurement was made in comparison with a pure high-fired alumina disk covered by a similar glass plate.

A typical series of such normal spectral reflection curves is given in figure 1. The reproducibility of the individual readings was within an average of five-tenths percent reflectance unit for tuna flesh, although for color stable materials, such as mixtures of powdered colored glasses, no variation was discernible. Reflectance curves measured on replicate samples from the same region of the fish loin gave an average precision of 1.5 percent reflectance units. Reflection from cooked tuna meat is generally high, being highest in the red end. The existence of the Soret absorption at about 410 millimicrons points to heme protein pigments as the source of color. It should be noted that absorption "peaks" lead to decreased reflectance, and so appear as troughs on the percent reflectance curves used in this report.

No attempt was made to fix the specification of this color on Munsell or similar systems since comparison, rather than standardization, was involved in the study. The complicating factor of oxidative browning was present, and caused a general lowering in reflectance with time after cooking (fig. 1a). At times a heightening of color (increased reflectance) was noted on frozen storage in metal foil wrapping (fig. 1b), but this may have been due to unusual sample variation. Further work to eliminate or control this additional browning variable is in process.

Samples similarly judged by experts to be green were prepared and measured. A typical reflectance curve is shown in figure 2 with a non-browned normal curve shown for comparison. It will be observed that curves for green and normal are somewhat similar save for a general decrease in brightness and the indication of a pigment absorbing in the blue (440-500 millimicrons) wave-lengths for the green meat. It seems anomalous that the perceived effect is an apparent green since less blue-green reflection is involved. The effect may be due to the higher proportionate red-to-blue reflectance for normal than for green tuna, since the ratio of reflectance of red (640 millimicrons) to blue (410 millimicrons) was 4.7 for normal and 3.5 for green flesh. The effect can be emphasized by reference to figure 3, which shows the reflectance curve of tuna meat that has been soaked in a green dye solution (malachite green)

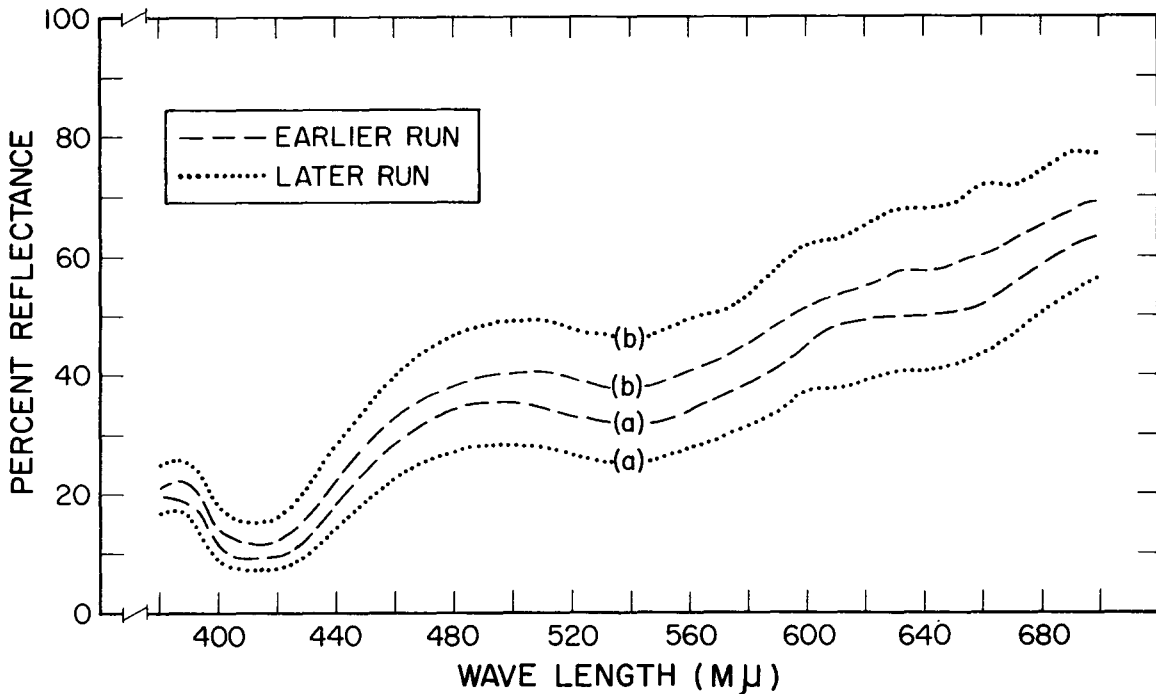


Figure 1. --Reflectance of normal cooked tuna meat: (a) an unwrapped sample in which reflectance decreased with time, (b) a metal foil wrapped sample in which reflectance increased with time.

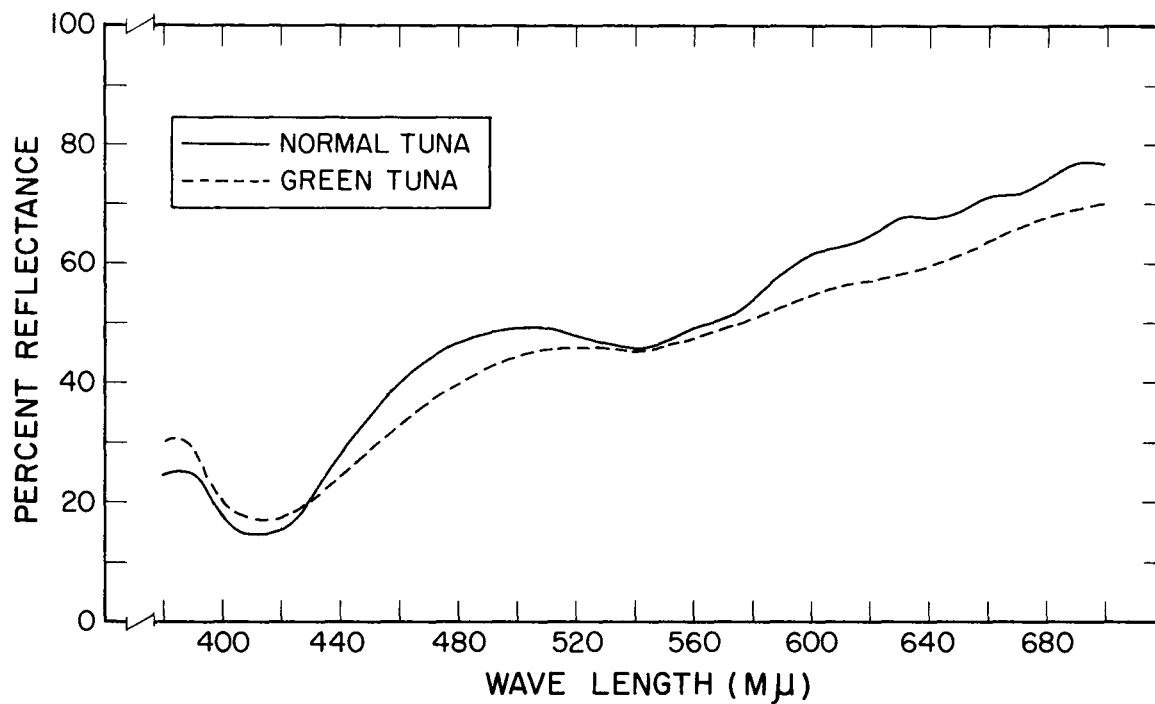


Figure 2.--Comparative reflectance of normal (solid line) and green (dashed line) tuna.

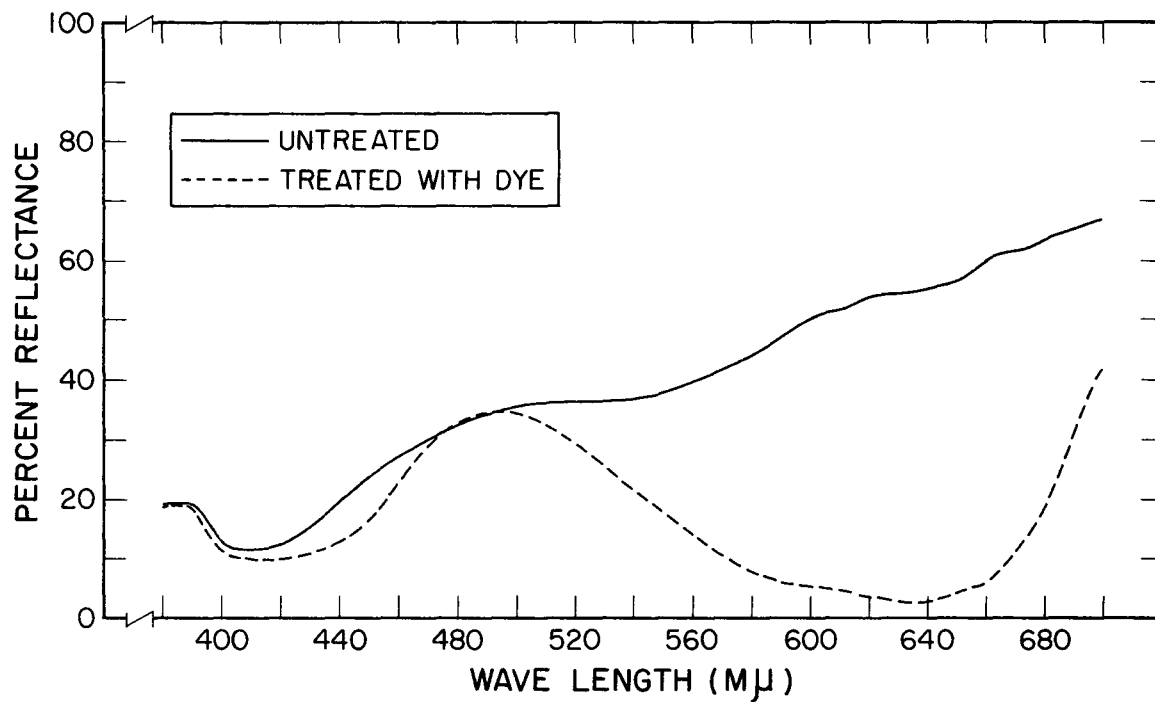


Figure 3.--Reflectance of cooked tuna meat with malachite green dye added and without green dye added.

in comparison with an untreated sample. An increased absorption in the red end of the spectrum is noted due to the pigment, with normal or less than normal reflection in the blue-green region (400-500 millimicrons). Similarly the slightly greater absorption in the red noted for green tuna meat may be the source of the green effect.

Similar reflectance studies were made on raw tuna whose color behavior on cooking was known (fig. 4). It will be noted that, for the few samples tested thus far, there is some correlation between known greenness and the reflectance of the raw flesh. A tendency toward normal color is shown by a higher proportionate reflectance in the red end of the spectrum (light "pinkness") in uncooked samples. The relationship is rather tenuous, therefore further experiments are needed to establish its existence with certainty.

LABORATORY COOKING METHOD

In order to simplify the magnitude of the cannery procedures necessary to produce greening, laboratory cooking was attempted. It was found that the color of 1-inch cubes of meat cooked in a pressure cooker at 102°C. indicated whether or not the fish from which they were taken had any tendency to turn green

under commercial methods. A 10-minute cooking time was sufficient to show this tendency; longer periods gave an increased darkening (browning?) of the meat. Spectrophotometric reflectance curves, which summarize the color difference produced by laboratory and commercial methods of cooking can be seen in figure 5. The green produced by the laboratory method was not as intense as that produced by commercial methods, probably because of concomitant browning. It was also noted that normal cooked flesh was less desirable in color than that produced commercially for the same reason. It was necessary therefore, in making comparisons between green and normal flesh, to be certain that the cooking methods corresponded.

THE NATURE OF THE PIGMENT IN TUNA FLESH

From what has been noted thus far, it is evident that the understanding of the processes that cause greening in tuna flesh must await an understanding of the nature of the pigment involved. It has been pointed out that the existence of the Soret absorption peak (410 millimicrons) in the reflectance curves is indicative of the presence of a heme protein, however, this still leaves the question of the exact nature of the heme compound unsettled. It would be expected that myoglobin, the hemoglobin found

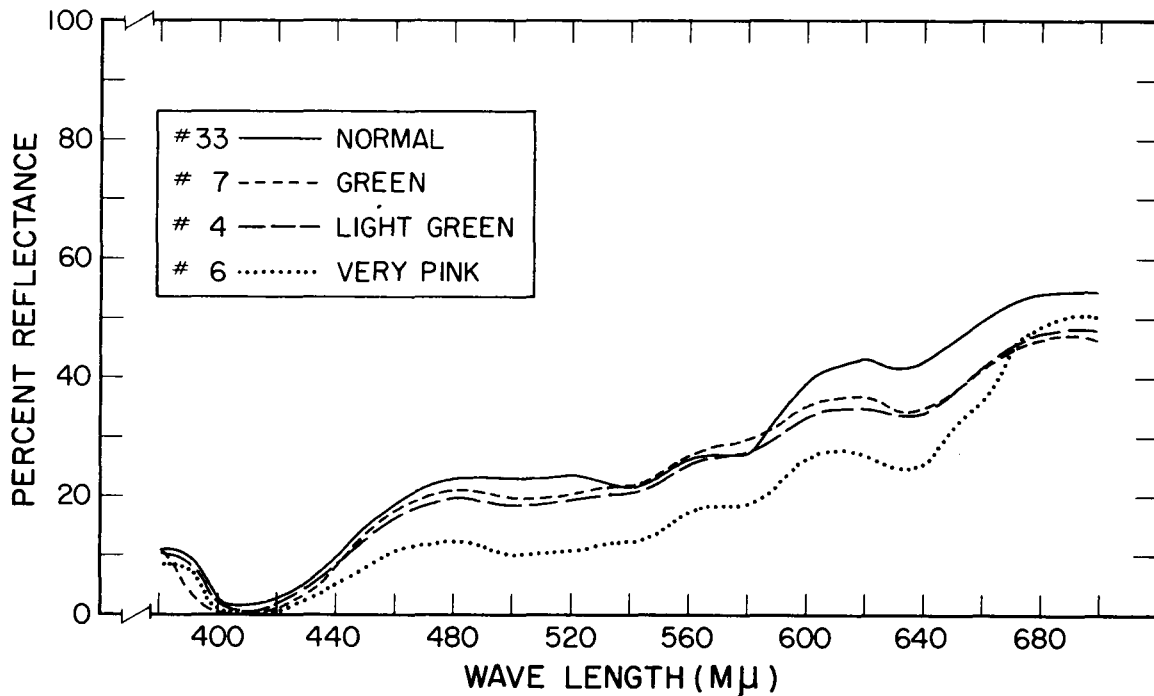


Figure 4. --Reflectance of raw tuna meat of known color behavior on cooking.

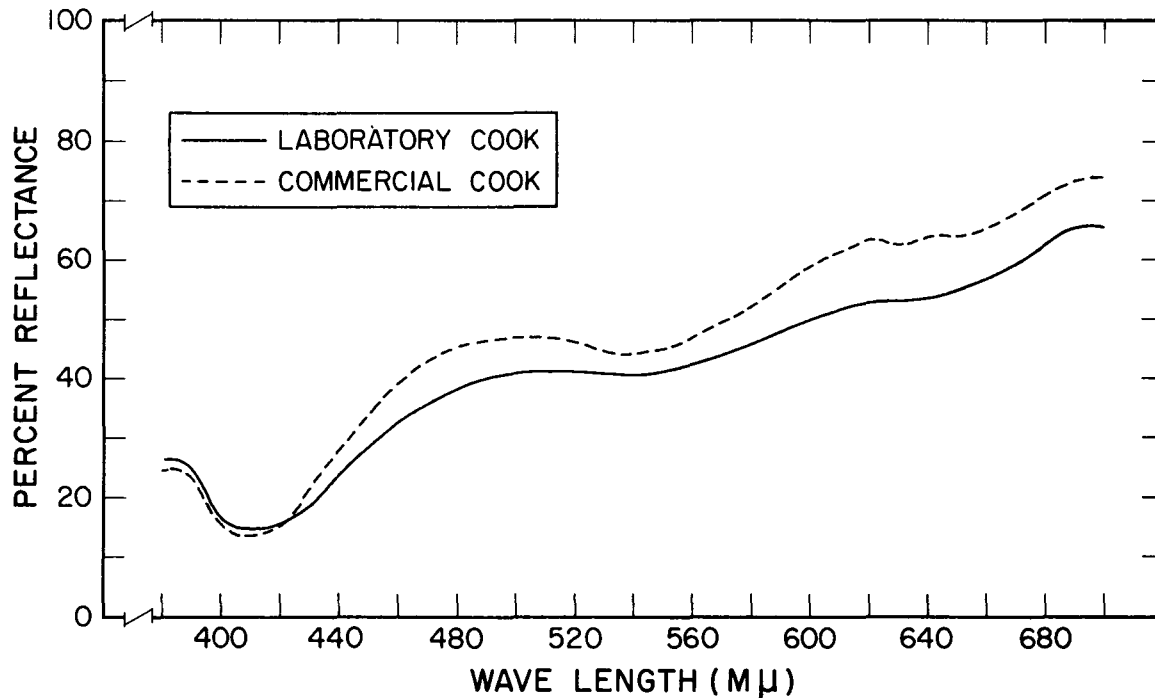


Figure 5.--Reflectance of cooked normal tuna prepared in the laboratory and commercially at Hawaiian Tuna Packers, Ltd.

in meat tissue, would be present, and this, in fact, has been reported in albacore by Matsuzaka and Takahashi (MS.). Moreover, myoglobin has been reported in the red blood meat of fish flesh by other investigators (Mondovi and Antonini 1955). In the species of tuna (yellowfin), under investigation in this study, the evidence seems to point to similar pigments or their derivatives being involved, despite the fact that earlier studies of the properties of the pigment led us to suspect a peroxidase-like heme protein.

Part of the work on this project involved an attempt to extract pigments from tuna flesh. A futile effort was made to obtain green substances of the verdohemochrome type from the green meat. In the case of raw meat it was found possible with aqueous or dilute alcoholic solvents to obtain an extract that contained much of the pigment of the meat. The final simplified procedure settled on was essentially that of Husaini et al. (1950), and involved blending and extraction with a dilute carbonate solution. Other solvents, such as alcohol and glacial acetic acid, extracted pigments which seemed to be altered products (probably by denaturation or cleavage) of the pigments present in the flesh. No success was achieved in extracting pigments from cooked meats.

Close examination of the spectrophotometric curves of the pigment extracted from raw flesh

shows it to be the ferric oxidized form of hemoglobin or myoglobin (methemoglobin or metmyoglobin). This is easily reducible to the ferrous form. Further work is under way through derivative formation to establish whether hemo- or myoglobin is involved, with the greater probability favoring the latter, judging from the findings of the Japanese workers.

CHANGES IN PIGMENT PRODUCING GREENING

In order to elucidate the mechanism or reason for greening, attempts were made to fractionate the pigmented mixture extracted from green and normal raw tuna flesh. Use was made of ammonium sulfate fractionation and filter paper electrophoresis, both without success to date. Extraction studies seem to show the presence, or the extractability, of a larger amount of methemoglobin or metmyoglobin from green meat than from normal. Reflectance studies also are in agreement with this since a lesser degree of reflectance seems to be indicated for the average green than for the average unbrowned normal tuna flesh, showing a higher concentration of the absorber or pigment. Certain normal samples do show an abnormal pinkness with high pigment concentration (e.g., No. 6, fig. 4). Further work is under way to separate more positively the factors of browning and greening, and to show the relationship, if any, between the two.

It is interesting to examine the reflectance curves with the possibility of qualitatively and, in a relative way, quantitatively making an estimate of the pigments present. It would be expected that the reflectance curves would resemble the well-known absorption curves for the heme pigments that might be involved, and examination reveals this to be the case.

Raw Meat (fig. 4). Two conditions will be noted, first, a normal type of curve with a variation in over-all position (brightness) due to variation in the amount of pigment present (compare curves for samples No. 6 and No. 33, fig. 4), and second, curves of a different shape (compare curves No. 4 and No. 7 with curve No. 33, fig. 4). Disregarding the Soret peak (about 410 millimicrons), other "peaks" (actually depressions in the curve) can be detected at 540 and 580 millimicrons, indicative of oxyhemoglobin or oxymyoglobin, and at 500 and 630 millimicrons, indicative of methemo- or metmyoglobin. If comparison is made with normal flesh (No. 33), it will be noted that the concentration of the oxy-compound is about the same in green and normal (No. 4 and No. 7, and No. 33). A higher concentration of the methemoglobin or metmyoglobin is indicated by reduced percent reflectance for the green tuna, however. The over-all condition seems to be, then, that similar pigments are present in green and normal raw flesh, with a higher concentration of oxidized methemoglobin or metmyoglobin in the green.

Various experiments were tried in an attempt to artificially produce color changes in tuna flesh. It was found that soaking raw normal flesh in 0.3 percent hydrogen peroxide solution imparted greenness after cooking. Also, soaking raw incipient green flesh in reducing agents such as ascorbic acid improved the color of the green meat after cooking. These effects are what would be expected from the normal reactions of hemo- or myoglobin pigments with hydrogen peroxide, or with reducing agents.

Cooked Meat (fig. 2). On cooking, the meat exhibits similar variations in the reflectance curves as noted for raw flesh (fig. 4). The curves reveal maximum absorption at about 540 and 575 millimicrons, characteristic of denatured globin hemichrome. Absorption maxima are also noted at about 650 millimicrons and for green fish a significant flattening in the region of 440 to 490 millimicrons, probably also due to absorption. Neither of these latter regions of absorption can readily be connected with common pigments of the hemo-myoglobin type, or their derivatives.

By way of a further check on these observations, samples of the same cooked meat were reduced with dithionite and the reflectance curves were examined (fig. 6). Save for the abnormally pink sample (No. 6), all samples were found to have about the same reflectance. A pink color was induced in the green meat on reduction. A

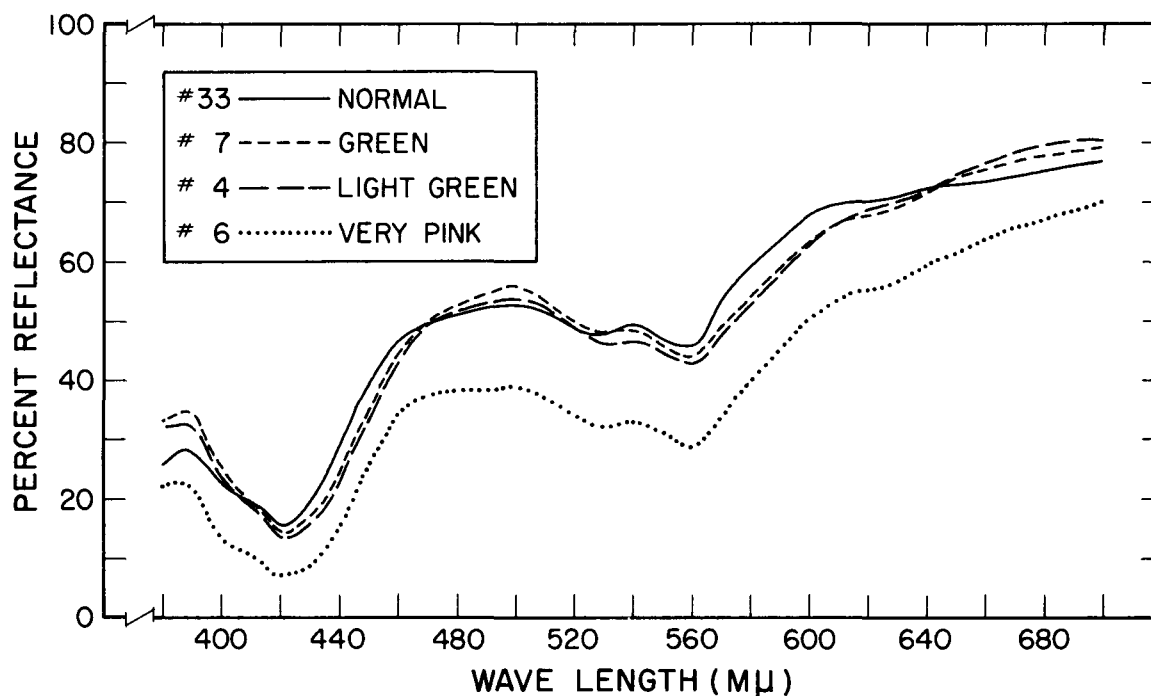


Figure 6. --Reflectance of reduced cooked tuna of various colors in the unreduced state.

Table 1.--Types and relative heme pigment content in raw and cooked tuna. Basic heme pigment is assumed to be myoglobin

	Oxy-myoglobin	Met-myoglobin	Denatured globin	
			hemichrome	hemochrome
	<u>Raw Tuna</u>		<u>Cooked Tuna</u>	
Normal	++	+	+	++
Green	++	++	++	++

+ = low concentration

++ = higher concentration

significant slight absorption of unknown source was noted at about 480 millimicrons for normal flesh that was present to but a slight extent, or absent, in the curves for green meat. The prominent absorption maxima at 530 and 560 millimicrons are the peaks characteristic of denatured globin hemochrome, the reduced form of the hemichrome pigment noted in the other cooked samples. The results of these studies on the reflectance curves for green and normal, and the pigments that are indicated as being present, are summarized in table 1.

It was noted that a copious quantity of yellow-colored leach liquor was obtained during the process of cooking tuna flesh in the laboratory. Also it was found that a deeper pigmentation was present in the leachate from the normal tuna than that from the green, indicating some correlation. The leached material was susceptible to oxidation with oxygen and reduction with dithionite, but gave no characterizing absorption curve. Attempts to purify by column chromatography were inconclusive. Laboratory cooking of normal and green tuna flesh in contact with leach liquor from normal or green tuna had no effect on the color of the samples.

CONCLUSIONS

From the above investigations we have reached the tentative conclusions that (1) the color changes, including greening, that take place in tuna flesh on cooking are the result of reactions of the normal pigments of the flesh (hemo- or myoglobin or their derivatives) with other ingredients of the flesh or with the environment, and (2) at this stage of the work we are inclined to the belief that differences between normal and green flesh lie chiefly in the differences in concentration of certain of the common heme pigment derivatives. Some evidence also exists for a low concentration of additional pigments in cooked green meat, which may be unusual hemo- or myoglobin derivatives. Browning and greening seem to be manifestations of oxidation of the heme

protein pigments. How these pigment factors are related to the physiological or physical condition of the fish flesh remains to be investigated.

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