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Chairman—RICHARD T. WHITELEATHER, Assistant Chief, Branch of Commercial Fisheries, Fish & Wildlife Service. Washington, D.C.

Studies on Black Spotted Shrimp*

E. A. FIEGER. *Department of Agricultural Chemistry and Biochemistry, Louisiana Agricultural Experiment Station, Louisiana State University, Baton Rouge, Louisiana.*

IN THE PAST DECADE shrimp production has increased considerably through the use of larger boats which were able to extend the off-shore fishing areas. This fact has resulted in longer storage on the trawlers and a longer period of time between catching the shrimp and their ultimate consumption. New species of shrimp, furthermore, which are more susceptible to deterioration in refrigerated storage, are entering commercial channels. Because of these conditions, problems of proper refrigerated storage have arisen; one of the most important of these has been designated as black spot of shrimp (Fieger, Colmer & Alford, 1950; Fieger 1951).

Black spot is not peculiar to Louisiana-caught shrimp, for Idyll (1951) in a recent article described this condition in shrimp caught off the Florda Keys and designated it as black discoloration. L'Ecluse(a) mentioned the seriousness of black spot in shrimp caught in the Gulf of California and transported by truck to Los Angeles. From Australia, Empey,(a) Principal Research Officer of the Division of Food Preservation and Transport, reports some observations and preliminary work on a condition known as "black head" in incompletely cooked prawns (shrimp). Likewise, Chambionnat (n.d.) of the Official Chemical Laboratory of Morocco has studied the development of black discoloration of pink shrimp of Morocco, *Parapenaeus membraneus*, Risso or *Parapenaeus longirostrus*, Lucas. From these reports it is evident that black spot or black discoloration of shrimp is of world-wide distribution. From reports received, it appears that black spot is a more serious problem in Morocco and Australia than it is in the United States.

Since black spot or black discoloration has become of international importance to the shrimp industry in the past few years, this condition is described. Black spots develop in shrimp, both whole and headless, during refrigerated storage. With headless shrimp, the abdomen or "tail" may have one or several black spots or bands, usually at the base of shell segments or across the back of tail where shell segments overlap. In general, the discoloration first develops in the membrane which separates the tail flesh from the shell, especially in that area where the membrane connects together the two ends of overlapping shell segments.

When whole shrmip are stored black spot usually develops first in the

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(a) Personal communication to the author.

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head cavity so that the carapace or "head shell" becomes black. This is followed by discoloration of the antennule and rostrum or "spine" and the uropod or "tail fin." The crawling legs, or periopods, change color first at the joints and this change finally progresses over the entire leg, while the swimming legs or pleopods become black at the tip ends. When the condition becomes severe, the interior of the head becomes a black soft or mushy mass and the carapace becomes flexible instead of retaining its normal stiff consistency. In fully 75 per cent of the black spotted shrimp examined, the chitin or shell, has been punctured or eroded away. Since the edges of the pierced portion are smooth and not jagged it is believed that these holes in the shell result from chemical solution and not from mechanical injury. The liquid which exudes from the shrimp is black and is spoken of by shrimp fishermen as acid, but is in reality alkaline.

What causes this condition to develop? Knowing the cause it is reasonable to ask what can be done to prevent its development. As indicated above, several laboratories have become interested in this problem. A year ago last September J. A. Alford and the writer showed that black spot is not caused by microorganisms and does not result from chemical changes in the shrimp brought about by microbial activity. Rather, it was proved to be caused by an enzyme system which is present in the shrimp tissue. Quite independently, at about the same time, the Morocco group under the direction of Chambionnat came to the same conclusion. Their work came to the writer's attention last February.

It has been shown (Fieger, 1951) that air, or more specifically oxygen, is necessary for black spot development, by the following experiments. 1.—Whole shrimp, suspended in water in flasks closed either with a rubber stopper or a cotton plug, did not develop black spots after 125 hours storage in crushed ice. The odor of the shrimp was that of fresh shrimp, the liquid had a slight brown color. 2.—The same set-up as described above was used, but with air bubbling through the suspension of shrimp and water. After 75 hours, several shrimp had developed black spots and after 125 hours 90 per cent of 18 shrimp had black spots on their heads, 70 per cent had them on their tails. The liquid was black and the odor of the shrimp was not quite as good as that of fresh shrimp. 3.—When pure oxygen was bubbled through a set-up similar to that previously described, after 54 hours one shrimp had a black spot and at 125 hours 100 per cent of 18 shrimp had black spots on both sides of their heads, and all had black spots on their tails. The liquid was black and the odor was not quite as good as the controls.

These results proved that oxygen was required for black spot development and strongly suggested an oxidative enzyme was also involved. Many of the brown and black pigmentations found in nature, such as black feathers, wool and hair, and the brown color of freckles and sun-tan, the pigmentation of Addison's disease and of malignant tumors are caused by the oxidation of tissue substances to brown or black compounds. These colored substances are called melanin. In marine animals, the black liquid of the ink-sac of the cuttle fish contains tyrosinase (Gessard, 1903). Bhagvat and Richter (1938) found evidence of tyrosinase in the blood of the octopus. Pinhey (1930) has shown that the black discoloration of the spider crab and edible crab is caused by the action of the enzyme tyrosinase present in blood corpuscules upon compounds in its blood. To prove an oxidation enzyme or enzymes cause black spots to develop, the following experiments were performed.

Whole shrimp and headless shrimp were suspended in solutions containing dihydroxyphenylalanine ("dopa"), the containers were packed in ice and air was bubbled through the solution. Within six hours the liquid had turned black and black spots were well developed on all of the shrimp, in contrast to the 125 hours required to develop spots in the previously described experiments. Also, the shrimp suspended in the dopa solution had more intense or severe black spots than those suspended in water. Likewise, water-clear extracts of shrimp tissue turn black in the presence of dopa and air. Other dihydroxyphenyl derivatives besides dopa, such as catechol, hydroquinone and pyrogallol, are oxidized by shrimp tissue, the former two resulting in red solutions and red spots and the latter black solution and black spots. Of the monohydroxyphenyl derivatives, tyrosine and ortho-and meta-cresols are apparently only slightly oxidized, while para-cresol is rapidly oxidized by shrimp tissue and shrimp extracts. Since both types of hydroxyphenyl dervatives are oxidized the writer has tentatively designated the enzyme as tyrosinase in conformity with the suggestion of Lerner and Fitzpatrick (1950).

The following facts present further evidence that an enzyme is involved. When shrimp extracts are boiled for one minute over a free flame complete inactivation results, even in the presence of dihydroxyphenols. The optimum pH is 7.0, while a pH of 4.5 or lower prevents spot development. Chemical compounds which bind or unite with copper (oxidase enzymes are copper proteins), such as sodium cyanide, sodium azide, thiourea, cysteine, glutathione and sodium molybdate, completely prevent black spot development.

All the above evidence proves rather conclusively that an oxidase enzyme is responsible for black spot or black discoloration. Having shown that an enzyme, tyrosinase, is involved naturally raises the question of distribution of the enzyme in the shrimp. It is present in the head, in the fins, legs, and the shell and membrane of the tail. It is not present, however, in the tissue of the tail.

In continuing our investigations on this problem Mr. James Friloux, using the Barcroft Warburg manometric apparatus, has obtained evidence indicating a marked species difference in the amount of the enzyme tyrosinase present in the tail shell. Brown shrimp contains the highest amount, while white shrimp has the smallest quantity of the enzyme. This order of enzyme content checks well with known incidence of development of black spot in these species in commercial storage.

Since the black substance formed in the shrimp through the action of tyrosinase is undoubtedly melanin, it is suggested that the terms "black spot," "black discoloration" and "black head" be discarded, at least in the scientific literature, and in their stead that we use the term melanotic shrimp. The chemical changes which occur would then be known as melanogenesis and the black coloration as melanosis.

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Freezing Shrimp At Sea

H. E. CROWTHER, *Chief, Technological Section, Branch of Commercial Fisheries, Fish and Wildlife Service, Washington, D. C.*

ONE OF THE MAJOR PROJECTS in the Fish and Wildlife Service's present technological program is freezing fish at sea. By means of this research work it is hoped to determine whether it is possible to freeze fish in the round at sea and later at a shore plant defrost, fillet, package, and refreeze the fillets and still obtain a high quality product. Such a process, to be practical, must be workable not only from a technological and mechanical viewpoint but it must be economically sound. Experiments now in progress at Boston are providing information which will permit a definite recommendation to the fishing industry on the feasibility of the process.

Specifically. the main purpose of this paper is to describe briefly the work being done at Boston and to pass on to those interested in the shrimp industry the information gained thus far in fish freezing work which may be applicable to shrimp.

Present Refrigeration Methods Used on Shrimp Boats

Shrimp boats in the Gulf of Mexico may be divided into two general classes, according to their size and area of operation. The small boats, ranging in lengths from 25 to 55 feet concentrate on the shallow, inshore waters. The larger vessels from 50 to 80 feet in length fish the off-shore waters and can remain at sea for as long as 10 days. Many of the vessels in the 50 to 80 foot class and capable of fishing in any part of the Gulf and are often found in the distant Campeche area.

Crushed ice is practically the only means of refrigeration available to a majority of the shrimp boats. On long trips, such as those to Campeche, ice may be the item which determines the length of the trip. Fishing time on the grounds is limited because provision must be made for the 3 or 4 days required for the voyage to the plant, so fishing must cease when the ice supply reaches a low point. Travel to and from distant fishing grounds is not only costly but involves the chance of loss of the entire catch of shrimp through spoilage if delay occurs. One possibility of extending the length of the trip is to use some form of mechanical refrigeration to retard the melting of the ice or to replace the ice entirely as a refrigerant. Some boats have installed such equipment but they are few in number.

The most recent approach to solving the refrigeration problem is the use of freight boats or mother ships to pick up the shrimp from trawlers on the fishing grounds and freeze the headless shrimp at sea in 5 pound cartons. Several boats of this type are now in operation and apparently are having some