TECHNOLOGY AND EXPLORATORY FISHING SESSION

TUESDAY—NOVEMBER 16

Chairman—H. C. DIEHL, *Director, Refrigeration Research Foundation, Colorado Springs, Colorado.*

Further Experiments On the Control of Menanosis or "Black Spot" On Shrimp[1]

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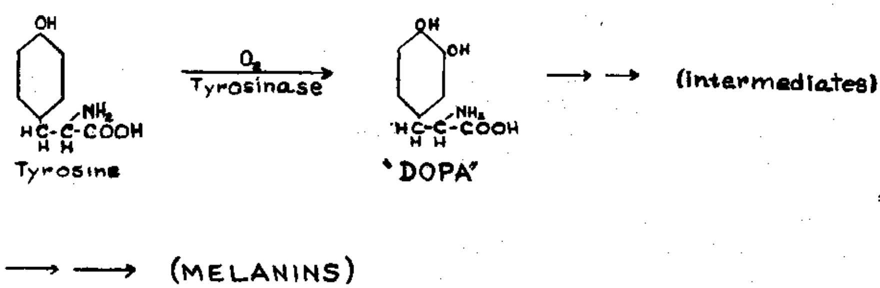
At the Fourth Annual Session of the Gulf and Caribbean Fisheries Institute the authors showed rather conclusively that "black spot" formation is catalyzed by an enzyme, tyrosinase, in which certain chromogens of the phenol amine type, resulting from the breakdown of protein, are converted to dark brown or black substances which are called melanins. The chemical change is as follows:



You will note that three substances are necessary for melanin or black spot formation: (a) a suitable substrate, in this case tyrosine or dihydroxyphenyl alanine (DOPA); (b) molecular oxygen or air; and (c) the enzyme tyrosinase, which is a complex of copper with protein. If any one of these substances is absent the formation of melanin is impaired or completely prevented. Knowing something of the chemical changes which result in black spot development should enable us to attack the problem of its practical prevention more intelligently. A list of a few substances which, in theory at least, should prevent melanin formation are:

1. Substances which combine with copper: thiourea, cysteine, glutathione, hydrogen sulfide, carbon monoxide and hydrogen cyanide.

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2. Metals which compete with copper for union with the proteins: mercury, gold and silver.

3. Sustances which delay melanin formation: tween detergents, and changes in pH or acidity.

4. Substances which compete for the available oxygen or reducing substances: ascorbic acid, sodium sulfite, and sodium bisulfite.

While the above list is by no means exhaustive, it still offers a large number of compounds which could be used for preventing melanin formation were it not for the fact that the treated shrimp tissue is ultimately used as human food. The majority of these substances are toxic and thus could not be used. Then, too, there has to be considered the economic aspect involved in treating shrimp with chemicals. Naturally, the most desirable chemicals for study of problems of this type would be the so-called physiological anti-oxidants such as ascorbic acid, vitamin E, or some of the B vitamins, but these are too expensive at present to be employed in sufficient quantities in chemical ices.

Taking into consideration the economic as well as the physiological aspect, a study was made of the efficacy of certain substances for prevention of black spot. A preliminary experiment was designed to determine the effectiveness of various solutions as inhibitors of black spot formation. Shrimp tails were dipped in various solutions and placed in aluminum containers, which were packed in ice. The chemicals were classified according to the degree of protection against black spot development and were rated in the following order:

Single strength lemon juice; 0.1 per cent sodium bisulfite; 0.1 per cent ascorbic acid; 0.1 per cent sodium sulfite; ascorbic acid-citric acid 8-92 mixture; 0.1 per cent citric acid; 0.1 per cent tartaric acid.

On the basis of the above experiment, it was decided to evaluate further the two most effective, namely, lemon juice and sodium bisulfite. Nedvidek has reported that dipping fish and shrimp in lemon juice for either fresh or frozen storage resulted in prevention of rancidity and retention of flavors and color far beyond what could be expected from its citric acid and ascorbic acid content. He explains this enhanced action by the presence of bioflavonoids in the lemon juice.

Approximately three grams of shrimp shells were placed in 50 ml. beakers. Then 26 ml. of solution was added to each beaker, the beakers were covered with watch glasses and stored at 40°F. At regular intervals observations were made on changes in the color of the solutions and the development of black spot on the shells. All solutions contained 96 parts per million (p.p.m.) "DOPA" to insure ample supply of substrate for melanin formation. The series of lemon juice solutions were made by dilution of Exchange No. 309 Calemona Concentrated Lemon Juice*. This juice was a 5.5 to 1 concentrate and the concentrations are expressed on the basis of normal strength lemon juice. For the sodium bisulfite solutions a 1000 p.p.m. concentration was diluted to the various solutions used. The data are summarized in Table 1. Sodium bisulfite at a concentration of 125 p.p.m. was as effective as one-half normal strength lemon juice in preventing black spot on shells in the presence of "DOPA". In general, the samples stored under lemon juice had a much better odor than those stored under the bisulfite solution. Lemon juice solu-

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* Kindly furnished by California Fruit Growers Exchange, Carona, California.

TABLE 1

The Effect of Various Concentrations of Lemon Juice and Sodium Bisulphate in the Presence of Dopa on Shrimp Shell Melanosis

All solutions contained DOPA at 96 PPM concentration

Solution and Concentration		Hours of Storage 16	24	48	72	96	120
Lemon Juice							
0.5	Normal Strength	0	0	0	0	0	0
0.2	" "	0	0	*	*	*	*
0.1	" "	*	**	***	***	***	***
0.05	" "	*	**	***	****	****	****
0.02	" "	**	***	***	****	****	****
0.01	" "	**	***	****	****		
0.005	" "	****	****	****	****		
0.0033	" "	****	****	****	****		
0.0025	" "	****	****	****	****		
Sodium Bisulfite							
1000.0 PPM		0	0	0	0	0	0
500.0 PPM		0	0	0	0	0	0
250.0 PPM		0	0	0	0	0	0
125.0 PPM		0	0	0	0	0	0
75.0 PPM		*	*	*	*	*	*
37.5 PPM		*	*	***	***	***	***
18.8 PPM		**	**	***	***	***	***
9.4 PPM		****	****	****	****		

0 no change in color of solution or of shells.
* solution slightly pink, beginning formation of black spots on shells.
** solution dark pink, shells had few black spots.
*** solution brown, several black spots on shells.
**** solution black—large number of black spots on shells.

tions, in concentration as low as 0.02 (1/50) normal strength lemon juice, had no odor after 96 hours at 40°F.

At the same time as the above experiments were being carried out, experiments were also conducted on the value of various chemical ices for preservation of freshly caught brown shrimp *(Penaeus aztecus)* and prevention of black spot. It was previously shown that shrimp packed in ice containing 0.25 percent (2500 p.p.m.) sodium bisulfite or sodium sulfite were protected almost perfectly from melanin formation for 14 days (Bailey and Fieger, 1954). These shrimp were completely odorless during the first 12 days of the storage period and maintained their natural colors. Processing methods similar to those employed by commercial canners and housewives, however, failed to remove all of the sulfite from shrimp which had been packed in 0.25 percent sodium bisulfite ice for five days. In an attempt to preserve shrimp adequately, prevent melanin formation and obtain a cooked product essentially free from sulfite, lower concentrations of sodium bisulfite were used in packing ices. In the first of two such experiments the following ices were used: commercial ice, sodium bisulfite ice (100 p.p.m. concentration), and ascorbic-citric mixture (8 parts ascorbic acid 92 parts citric acid 1000 p.p.m. concentration). The latter two ices were made by freezing small increments of the solutions in stainless steel pans in a plate freezer at —55°F. In this way, blocks of ice were made of quite uniform concentration. In order to obviate further the differences in concentration in various regions of the

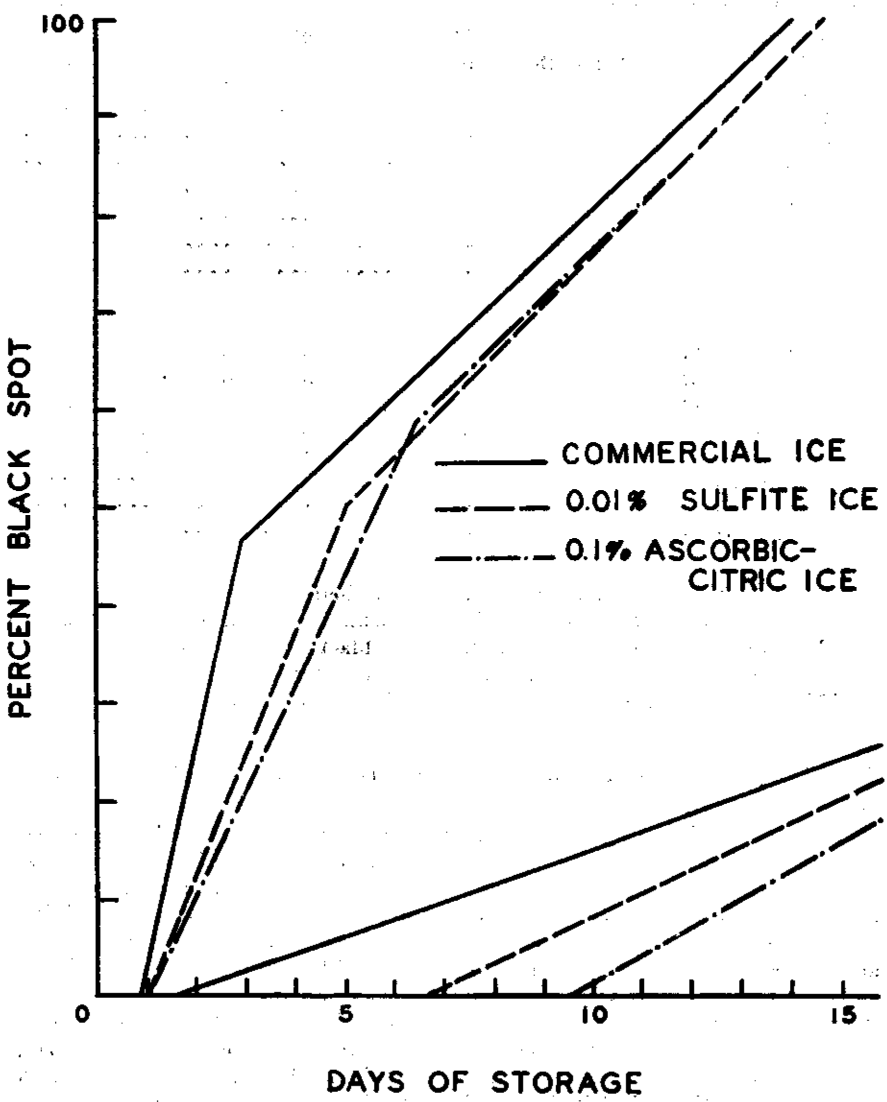

FIG. 1

blocks, the ice was shaved at right angles to the layers in a Snow King Ice Shaver. A sample of between 50 and 65 shrimp were removed daily from the three different types of ices, examined, and classified according to the severity of the black spots. Those containing only a few barely detectable black spots

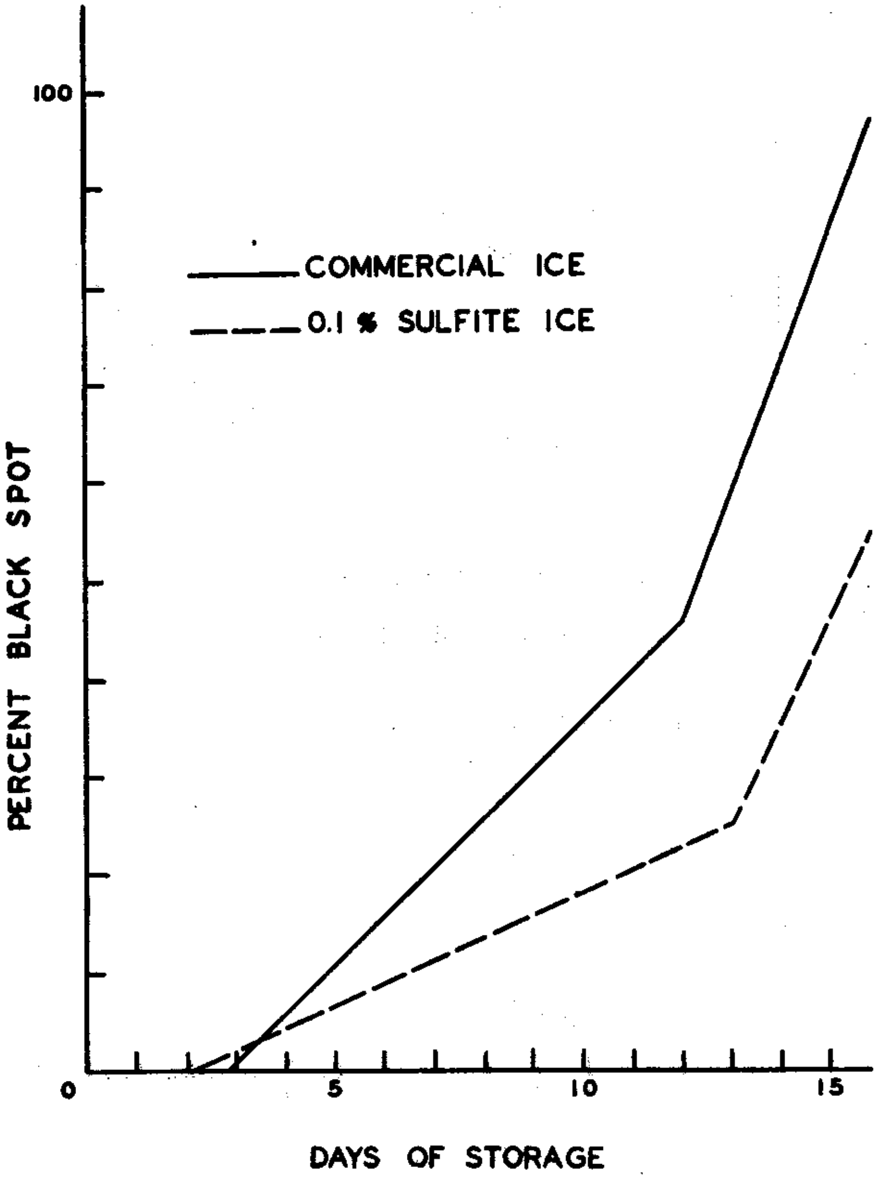

FIG. 2

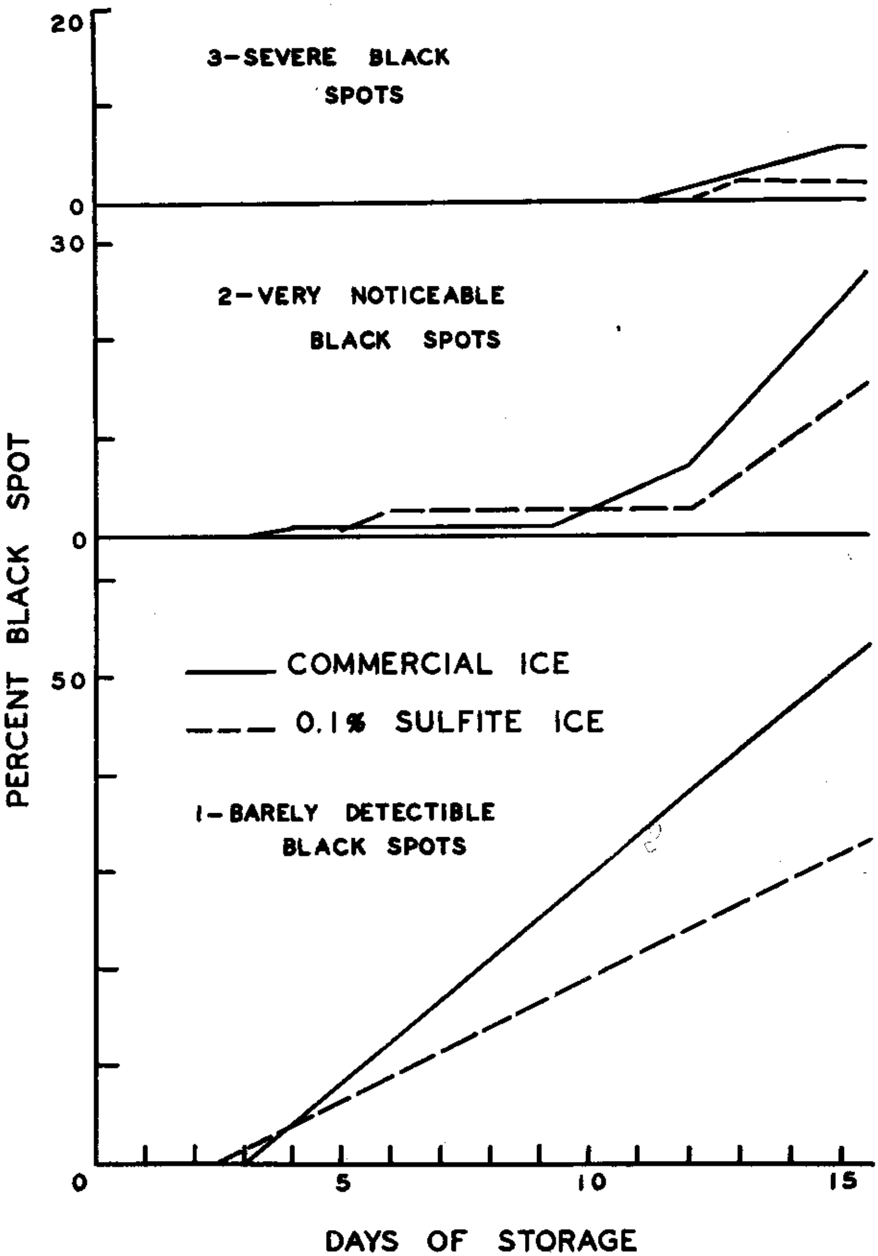
20
3-SEVERE BLACK SPOTS
0
30
2-VERY NOTICEABLE BLACK SPOTS
0
PERCENT BLACK SPOT
50
COMMERCIAL ICE
0.1% SULFITE ICE
1-BARELY DETECTIBLE BLACK SPOTS
0
5
10
15
DAYS OF STORAGE

FIG. 3

were separated from the ones containing severe black spots and the percent of each type was recorded. The incidence of these two classes of black spot is shown in Figure 1. The three upper curves represent the former condition and the three lower represent the latter condition.

The shrimp in ice containing ascorbic acid-citric acid mixture and those in

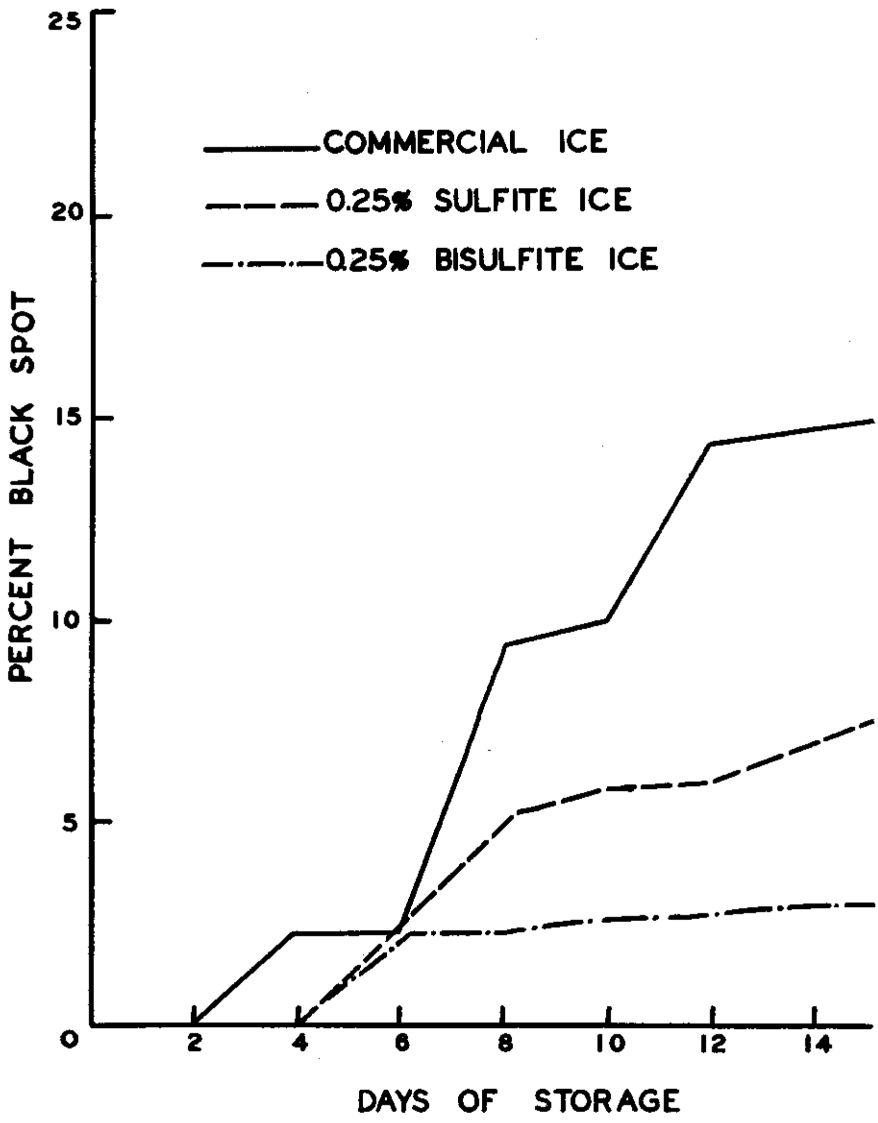

FIG. 4

ice containing sodium bisulfite had barely detectable black spots during the first five days of storage, and throughout the storage period had fewer severe black spots and were of slightly better general appearance than the controls. The shrimp stored in these ices were slightly firmer and had less slime through the first 10 days of storage in comparison with those stored in commercial ice. The sulfite treated shrimp contained only trace quantities of residual sulfite after washing and cooking. Neither of the chemical ices, at the concentrations used in this experiment, were sufficiently effective in the prevention of black spot to warrant their use commercially.

In the second experiment the procedure was modified somewhat by packing the shrimp in stainless steel baskets (4x4½x5 inches). Into each basket was placed 1¼ to 1½ pounds of headed, thoroughly washed shrimp. This resulted in a layer of shrimp 3½ to 4 inches in thickness. Sixteen baskets (two layers of eight baskets each) were packed in commercial ice and 1000 p.p.m. sodium bisulfite ice. One basket was removed each day and the number and intensity of the black spots were observed. The shrimp used were brown shrimp *(Penaeus aztecus)* mixed with a few white shrimp *(Penaeus setiferus.)*

The results of this experiment are shown in Figures 2 and 3. Figure 2 shows the percentage of shrimp having black spots of all degrees and intensity, during the 15 day storage period. It can be seen that the sodium bisulfite greatly retarded black spot formation. Figure 3 is a breakdown as to severity of the black spots that were formed in the shrimp during storage. When the black spots are classified as to: 1.—barely detectable, 2.—very noticeable, and 3.—severe, it was noted that in each instance the sulfite showed some degree of protection against melanin formation. There was an appreciable difference between the quality of the shrimp stored under pure ice and those stored under sulfite ice; not only were the black spots less severe in the sulfite treated shrimp but in general, they also had a better appearance, were firmer, and had less odor.

The ice containing 1000 p.p.m. sodium bisulfite did not preserve the shrimp as well nor prevent black spot development to as great an extent as did the 2500 p.p.m. sodium bisulfite ice reported previously (Bailey and Fieger, 1954) (Figure 4), but the sulfite content of the former shrimp was considerably less than that of shrimp packed in the latter ice, (Table 2). Shrimp packed in 1000 p.p.m. ice for various periods still contained small quantities of sulfite even after washing or cooking, but washing and cooking by a procedure similar to that employed by shrimp canners removed virtually all of the sulfite present After these shrimp had been cooked and canned (dry packed) there was 0.8 mg. of sulfite in 100 grams of the shrimp. The sulfite content of the shrimp was also decreased by canning in six per cent brine.

Discussion:

Sodium bisulfite when used at optimum concentration in storage ices affords good protection against melanin formation on shrimp shell. This protection is brought about by four possible mechanisms: First, it sequesters some of the cupric ions associated with the enzyme tyrosinase; second, it reduces the number of microorganisms on the shrimp surface, thereby decreasing protein breakdown; third, it prevents oxidation of tyrosine and dihydroxyphenyl alanine by acting as an anti-oxidant; and fourth, it reduces some of the oxidized products, thus delaying melanin formation. It is very economical when used in ice at a concentration of 1000 p.p.m., and practically all residual sulfite can be removed by commercial processing of the shrimp. Although higher concentrations of sodium bisulfite depress melanin formation for longer periods of time, there are several disadvantages in using this chemical in storage ices. Present commercial processes for manufacturing ice may result in localized highly concentrated sulfite, and such areas when left in contact with shrimp tissue for long periods of time cause it to change to an opaque white. These areas turn light brown when cooked and are much tougher than normal cooked tissue. The sulfite in such areas on shrimp is readily detectable by individuals sensitive to it.

Ascorbic acid also functions as an anti-oxidant and the citric acid lowers the pH, so both of these chemicals have a synergic effect in slowing down the formation of melanin.

Lemon juice preserves shrimp quite well at relatively high concentrations and also depresses black spot formation, but at the present time is too costly at optimum levels of concentration for use in commercial storage ices. A ton of ice containing lemon juice of 1/50 normal strength would cost $4.75 to $5.00 more than commercial ice. The ascorbic acid-citric acid mixture when used at 1000 p.p.m. concentration in ice would increase the cost by $2.65 per ton, while sodium bisulfite at the same concentration would increase the cost about $0.50 per ton.

Summary:

The inhibiting effects of various substances on shrimp melanosis was studied. Several different chemicals when used in this regard as dips for shrimp tails prior to their ice storage in aluminum containers were rated in the following order: single strength lemon juice: 0.1 percent sodium bisulfite; 0.1 percent ascorbic acid; 0.1 percent sodium sulfite; 0.1 percent ascorbic acid-citric acid (8-92) mixture; 0.1 percent citric acid; and finally 0.1 percent tartaric acid.

Further studies on the inhibitory effects of lemon juice and sodium bisulfite

TABLE 2

SULFITE CONTENT OF SHRIMP STORED FOR VARIOUS PERIODS IN 0.1% SODIUM BISULFITE ICE

Days of Storage	Treatment	Sulfite (mg/100 gm. shrimp)
10	Cooked Tap Water Peeled	3.4
10	Shelled: Washed (Uncooked)	2.4
10	Shelled: Cooked in 6% Brine	2.8
13	Shelled: Cooked in 6% Brine	8.1
16	Peeled: Washed: Blanched in 6% Brine (Dry Packed)	0.8

revealed that the former retarded melanin formation on shrimp shells in the presence of 96 p.p.m. dihydroxyphenylalanine for 120 hours, when used in concentrations as low as 0.2 percent of normal strength. The latter substance, when similarly tested, protected the shells perfectly when used in concentrations as low as 125 p.p.m. Seventy-five p.p.m. sodium bisulfite protected the shells from melanin formation almost as well as did the 0.2 percent normal strength lemon juice.

Melanosis retardation effects of various chemical ices were also studied by comparing shrimp stored in these ices with those stored in commercial ice. These ices, in order of the degree of protection against melanosis are as follows: 1000 p.p.m. sodium bisulfite ice; 1000 p.p.m. ascorbic acid-citric acid (8-92) mixture ice; and 100 p.p.m. sodium bisulfite ice. All three chemical ices showed some degree of protection against melanosis and resulted in shrimp of firmer texture, better appearance and less off odors than those stored in commercial ice.

Peeling, thorough washing and cooking reduces the sulfite content of shrimp packed with 1000 p.p.m. sodium bisulfite ice to practically negligible levels.

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Más Experimentos Sobre el Control de la "Melanosis" o "Mancha Negra" en los Camarones

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Abstracto

Se hicieron estudios sobre varias substancias químicas en solución, en forma de immersiones y hielos de varias concentraciones, para determinar su valor en la prevención del desarrollo de la "mancha negra" en los camarones. Se discute el mecanismo de la acción de estas substancias. También se discuten las ventajas y desventajas, tanto como los problemas económicos relacionados con el uso de las dichas substancias.