1973

Causes and Prevention of Discoloration Reactions in Breaded Shrimp.

Michael Walker Moody

Louisiana State University and Agricultural & Mechanical College

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IN BREADED SHRIMP

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirement for the degree of
Doctor of Philosophy

in
The Department of Food Science

by
Michael Walker Moody
B.S., Louisiana State University, 1970
M.S., Louisiana State University, 1971
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ABSTRACT

The causes of discoloration in breaded shrimp were determined, and methods for its prevention were developed. Experiments showed that pH is responsible for some types of discoloration reactions. The factors of (1) temperature during storage, (2) days on ice prior to breading, (3) length of time in storage, and (4) section of shrimp, i.e., the anterior end of the tail and the posterior end of the tail, were tested for their roles in affecting pH changes. Differences in pH were recognized within the same shrimp samples. The anterior end of the tail had a lower pH than the posterior end.

The following conditions were evaluated as factors which could influence or cause discoloration: (1) temperature of storage, (2) iron ions, (3) quality of the shrimp at the time of breading, and (4) length of time of storage. All were found to be highly significant. Various interactions between these factors were also studied.

An ascorbic acid-citric acid mixture was found to be effective in preventing discoloration, whereas sodium tripolyphosphate was found to be ineffective.

Volatile base production was evaluated in respect to time and temperature relationship, because of their
possible involvement in increasing the pH in the breading material.

Commercial conditions which are likely to cause discoloration during the manufacture of breaded shrimp are discussed, and recommendations are offered for its prevention. Severe financial losses may be avoided by following these suggestions.
INTRODUCTION

To the consumer, the visual appearance of a food product is extremely important when judging its acceptability. When a food product does not appear to be in the condition expected by the customer, it will usually be rejected, regardless of its nutritional quality. This is understandable from the knowledge that the sense of sight is of primary importance when judging what we eat or don't eat. The undesirable appearance of food products is often the result of unnatural colors produced by discoloration reactions.

When a consumer finds discoloration in breaded shrimp, he naturally rejects the product. The breaded shrimp may be in an edible state, but its discoloration causes it to appear spoiled or moldy, and therefore unacceptable.

Although millions of dollars have been lost by discoloration developing in breaded shrimp, relatively little work has been done in this field. By identifying the factors which are responsible for this problem and instituting actions that can be taken to inhibit these undesirable reactions, a product of acceptable organoleptic quality could be produced on a regular and predictable basis.
The purpose of these experiments was to evaluate the factors which are responsible for causing or influencing discolorations in breaded shrimp, and to test (1) an ascorbic acid-citric acid mix and (2) sodium tripolyphosphate, as possible discoloration inhibiting agents. A sight panel was used to determine the degree of discoloration due to various factors and to test the effectiveness of discoloration inhibiting agents. The role of pH and volatile bases in discoloration was also evaluated.
REVIEW OF THE LITERATURE

Discoloration in foods is the result of one or more physical and chemical processes whereby a food or its components undergo color changes which render it less acceptable to the consumer. Such discolorations in foods are widespread and diverse in nature. Usually they are very complex, chemically and microbiologically, and may be caused by inferior raw material, poor manufacturing practices, malprocessing, etc.

Seafoods are among the food products in which the phenomenon of discoloration occurs. Many environmental conditions are responsible for causing or influencing discoloration reactions. The following review discusses the types of seafood or seafood-related discolorations that have been recorded in the literature, and factors involved in this phenomenon are discussed in detail.

I. Discolorations Occurring in Seafoods

A common type of discoloration in seafood occurs in canned shrimp. Thompson (70) discussed this type of discoloration and reported that an agent within the flesh of the shrimp is responsible for initiation and production of the discoloration. The heat processing step is responsible
for accelerating the reaction. However, the author goes on to state that this type discoloration may be controlled by the addition of either citric acid or lemon juice. Thompson and Waters (71) found that low acid foods propagate reactions of this nature. Landgraf (47) stated that a high pH in canned shrimp would indeed cause discoloration and that the pH was directly related to the condition of the shrimp before it was put into the can. As the age of iced shrimp increases, so does the pH, and although the shrimp may visually appear to be in acceptable form, the pH may be relatively high. He also found that an important factor in this type of discoloration is the size of the shrimp, in that the smaller the shrimp size, the worse the discoloration. He found no significant increase in discoloration when the shrimp were peeled by hand as opposed to machine peeling. He concluded that the most significant factor in preventing graying in shrimp is canning a product of satisfactory quality.

"Greening" of tuna fish meat is another common type of seafood discoloration. Bito (9) stated that the discoloration of frozen tuna meat blocks could be related to the length of time it takes the tuna to go from -5°C to -1.5°C when thawing out. The length of time it takes the tuna meat to go through this critical temperature range is also very important, because the longer the time, the heavier the discoloration, Bito and Amano (10) state that sulfmyoglobin or sulfhaemyoglobin are major constituents
of the "green" tuna, and suggest that in order to prevent the discoloration, the fish should not be contaminated with bacteria before freezing. As with many other papers, it was stressed that industry must start out with a good product to avoid discoloration. Koizumi (43) did not think that sulfmyoglobin was a constituent of "green" tuna, because its absorption spectra did not agree with the absorption maxima of green tuna pigment. Dollar and others (17) found that fish which developed "green" meat usually had less total heme pigments, more ferriheme as opposed to ferroheme pigments, and higher peroxide content in the lipids. Their work indicated that factors such as pH and total reducing substances had little significance in causing greening discoloration in tuna. Work by Ono and Tawara (58) showed that tuna meat which had a tendency to turn green also had a weaker power of decolorizing a redox indicator. A relationship between green discoloration in tuna meat and the amount of trimethylamine oxide has been reported (3). This strongly indicates that freshness is a factor which influences this type of seafood greening. Yamagata and others (82) developed a method for determining trimethylamine oxide to assess greening in tuna. Yamagata and others (81) also showed a relationship between trimethylamine oxide and myoglobin content of the raw meat. They also experimented with the effects of cooking temperature and time to assess greening. Bito (8) studied the
effects of the partial pressure of oxygen on the discoloration of frozen tuna meat. This was done by using different packaging materials.

Brown and Tappel (12) studied the pink pigments produced in precooked and canned tuna. They found that a hemochrome is the causative agent in discoloration. A reducing substance, such as ascorbic acid, could be used to stabilize the pigment.

Thompson and Farragut (72) made a study of a green discoloration that occurs occasionally in breaded shrimp. They found that the discoloration was not soluble in organic solvents but was soluble in water, indicating that the pigment was inorganic in nature. Although they did not carry out an extensive microbiological examination of the green breaded shrimp samples, they concluded on the basis of their test that the problem was not caused by microorganisms. Their experimental results indicated that the green shrimp usually had a pH of about 7.8 and non-green shrimp had a pH of about 7.4. The authors were actually able to reproduce the greening in breaded shrimp by incorporating metal (iron) dust in the shrimp tissue. In their conclusions on green color production, they said that certain factors are necessary in order to produce greening in breaded shrimp. Metal dust must settle on the shrimp meat and be dissolved, the shrimp must have a high pH thus creating an ammonia environment, the shrimp has to go
through freezing and thawing cycles, and a sufficient amount of storage time. The authors believe ammonia reacts with iron, copper, nickel, and other metals to form complexes such as the following:

\[(\text{NH}_4)\text{Cr}((\text{SO}_4)_2\cdot12\text{H}_2\text{O})\]

\[\text{NH}_4\text{Cl} \cdot \text{NiCl} \cdot 6\text{H}_2\text{O}\]

\[(\text{NH}_4)_3\text{Fe}(\text{C}_2\text{O}_4)_3 \cdot 3\text{H}_2\text{O}\]

Recommendations to alleviate the problem during processing are as follows: (1) metal components should be complexed in the batter by use of chelating agents, (2) shrimp having a low pH should be used in processing, and (3) care should be taken in the temperature used to initially freeze, store and transport the product.

Sieling (65) said that the green, brown, yellow, red and black discolorations occurring in oysters can be attributed to several factors. He points out, for example, that some algae eaten by oysters will cause the oyster to take on the same color as the algae, while red pigmented organisms (naked dinoflagellates) can cause oysters to become red. This paper discussed trace metals in water which are capable of discoloration in oyster shells. Kimura (45) found that a green discoloration in oysters was due to plankton. Determinations made on the pigments showed that they were composed of chlorophyll and carotenoids, and that the intensity of the coloration depended upon the amount of chlorophyll a in the water. Osada (59)
found no relationship between the green pigment content and amount of Fe, Zn, Mg, and Cu in canned oysters. Osada (60) extracted green pigments from the entrails of canned oysters using 10% HCl in acetone. In his research, three green pigments giving negative bile test were isolated, and they were found not to be copper chelates.

Fujimoto, Maruyana, and Kaneda (25) found that a brown discoloration may occur during the storage of fish products. They blame carbonyl compounds formed from auto-oxidized oil with various amino acids. Koizumi, Kurobe, and Nonaka (44) said that browning which occurs in dried fish is related to aldehydes derived from oxidation products of fish oils. Nakamura and Kida (56) found that browning could be prevented in headless shrimp by immersing the shrimp in iced seawater containing 0.5% Na D-isoascorbate for one hour. According to the experimental procedures, the shrimp were then frozen overnight at -25°C, glazed with the above solution and then stored at -18°C to -23°C. Toyama and Miyoshi (74) found that shrimp dipped in 10% formalin retained its color very well for 5 months in the presence of an oxidant.

Agzhitova (1) found that a yellowing which occurs in fish is an indication of fat oxidation. This yellow discoloration in fish actually occurs twice in storage. Carotene was found to be responsible for initial yellowing with fish containing more than 10% fat; however, this
yellowing is not maintained throughout storage conditions. Yellowness occurs again during this same storage period, and it is thought to be a result of oxidation. He pointed out that although oxidation occurred in fish with less fat, there was no loss of flavor. Yakovleva (80) found a yellowing in the subcutaneous fat layer of fish which apparently was not related to oxidation. He noted that there was a relationship in the amount of carotene content in the fish and the degree of yellowing discoloration. He said that due to freezing and thermal processes, denaturation of the protein could occur and free the carotenoid pigment from the protein allowing the carotenoid material to become solubilized in the fat. According to Tseluiko (75) there was no relationship between "yellowing" and the oxidation spoilage of fat that occurs in freezing of fresh Atlantic hardtail and sardinops. The fish were glazed and were kept under constant temperature from -22°C to -24°C.

An article in Pacific Fisherman (2) refers to the "bluing" occurring in king crab meat. This discoloration process is due to the fact that the king crab contains a high percentage of copper in its blood which may be oxidized during processing to produce a blue-gray color. The article stated that from 10-15% of the total body weight of a king crab is blood. Inoue and Motohiro (37) isolated a blue colorant from blue meat. The substance had a high copper content and gave a positive hemocyanin-like
reaction. They concluded that the discoloration was due to a hemocyanin derivative. Inoue and Motohiro (38) stated that agglutination of the hemocytes of hemolymph in a plasma clot could be responsible for the blue coloration. Waters (78) found that the following factors did not affect the "bluing" of canned crab: (1) the method of vacuum processing, (2) the use of parchment liner, (3) the plant water in processing, (4) the use of dying and dead crabs, and (5) several metal ions. He did state, however, that iron was definitely involved in the discoloration. The use of citric acid at pH 6.5-6.8 prevented the formation of the "bluing," though ascorbic acid did not have any appreciable effect in preventing the discoloration.

Pedraja (61) reported another type of discoloration which occurs in shrimp. He stated that even after death, the flesh of the shrimp is still in a dynamic state and changes could occur as a result of this. He said that there are three basic pigments in shrimp: (1) haemocyanin, (2) the melanins, and (3) astaxathin, a carotenoid. Haemocyanin, a copper protein compound which occurs on the outside of the shrimp blood cell, could lead to discoloration under certain conditions. The copper may be freed from the compound and then become part of a reaction in the ionic state, which may be responsible for discoloration.
II. Factors Causing or Influencing Discoloration

Storage

Discoloration can be brought about by processing and storage conditions. Since breaded shrimp are usually held for considerable lengths of time before consumption, the importance of good storage conditions should be emphasized.

Faulkner and Watts (22) stated that precooked frozen shrimp kept under stressed storage conditions could have deteriorative changes in color, flavor and odor after 3 months. However, when the same type of shrimp were stored under optimum conditions, they could be stored up to 10 months without significant change. Similarly, Lane (49) stated that frozen seafoods' quality depends on time and temperature. The relationship seems to indicate that the lower the temperature of the product, the longer it can be kept in desirable form. Further, he said that two factors influence the quality of seafoods when temperatures fluctuate. The first factor is the amount of time over which the fluctuations occurred, and the second is the temperature range the product itself undergoes. He found that fluctuations over 0°F do more harm than temperatures below 0°F. Lane concludes that temperature changes have a cumulative effect on storage life. He said that the longer it takes to initially freeze a seafood product, the shorter will be the time the product can be stored. However, this
is not as critical as the temperature at which the products are held during the storage period.

Kelly and Dunnott (40) showed that fish fillets frozen at -7°C and then stored at -7°C did not deteriorate any faster than fish fillets frozen at -195°C and stored at -7°C, indicating that quality did vary at these freezing temperatures. Lane (48) studied the temperature fluctuations that a seafood product undergoes while being delivered to commercial outlets. He found that the average temperature of the truck was 7.8°F, the freezer warehouses was -8.0°F, the warehouse holding temperature was -10° to -5°F, the retail distribution trucks was 24.0°F and the retail freezer cabinets were 5.0°F. He stated that the areas that need the most improving in this chain are the local distribution carrier and the retail outlets. He indicated that the product should never be stored above the food liner in retail freezer cabinets.

**Microorganisms, pH, Volatile Bases, and Decomposition**

Shrimp is a highly perishable product. The condition under which this raw material is received for processing can influence the quality of the final product. It is of significance, then, to discuss the work of previous workers as far as the number of organisms found in shrimp products and factors which can cause increases and decreases in numbers of bacteria. Discoloration is
associated with decomposition of the shrimp. Since bacteria are responsible for a reasonable amount of breakdown, the following literature review is important for understanding the role that microorganisms play. It is especially important to keep in mind the time interval and condition of storage of the shrimp from time of death until breaded and frozen. This is a critical period of time when decomposition can occur to a varying degree depending upon handling methods. Factors such as pH and the concentration of compounds formed from decomposition, which influence pH, are discussed.

Luna (51) tested chemical and bacterial changes of shrimp at 5°C and 24°C over an extended period of time. He stated that decomposition and quality can be evaluated by total plate count, pH and total volatile bases. Harrison and Lee (31) found that the bacteriological count in raw Pacific shrimp ranged from $1.3 \times 10^6$ to $3.0 \times 10^6$ bacteria per gram. The most predominant type of bacteria present was *Acinetobacter-Moraxella*. Flavobacterium was next in predominance. Pseudomonas, gram positive cocci, and Bacillus species were other common microorganisms present.

Research done by Hoff and coworkers (33) discusses the effects of time and temperature upon the bacterial quality of shellfish. They found that there were no seasonal patterns in bacteriological changes in live shellfish.
Under storage conditions of live shellfish, there was very little pH change at 10°, 20°, and 27.5°C. In another paper by Hoff and others (34), the effects of shucked oysters stored in crushed ice and at more elevated temperatures was studied. Data collected by them showed that there was a poor relationship between the changes in pH and the changes in bacterial numbers.

Bailey and others (6) concluded from their work that a bacterial count of $10 \times 10^6$ per gram or higher appearing on headless, shell-on shrimp denotes products in a spoiled condition, speaking here primarily of iced shrimp. They go on to say that shrimp with a pH below 7.7 are considered to be prime quality, shrimp with a pH between 7.7 and 7.95 are not spoiled but are of poor quality, and shrimp with a pH higher than 7.95 are spoiled or near spoiled.

Findings in work by Dyer and coworkers (18) concluded that the spoilage of most fishery products occurs on the surface of these products by means of bacterial action, and the best way to detect this decomposition is by measuring the end products of these bacterial actions. They go on to say that simply measuring the pH of the product will give a good indication of the freshness. Their work also showed that fresh fish (on the surface) will have a pH range of 6.2 to 6.8 and that readings above this pH range are indicative of spoilage. The higher the
pH above 6.8, the more spoiled the product can be assumed to be.

A paper by Fieger and others (23) said that if shrimp are not iced properly after catching, the numbers of bacteria will increase rapidly. They showed that if shrimp had not been iced properly, a three-fold increase occurred in bacterial numbers after eleven days as compared to a properly iced sample. They also said that melanosis, a discoloration, can also occur if the shrimp are not iced properly. Green (30) showed that thirteen samples of freshly caught, headless, washed shrimp contained only $7.4 \times 10^3$ bacteria per gram but after 10 days on ice the samples numbers increased to $1.0 \times 10^6$ or more.

The numbers of bacteria in precooked frozen Chilean shrimp was studied by Virgilo et al. (77). They found that the numbers ranged from $1.0 \times 10^4$ to $1.0 \times 10^5$ organisms per gram. They pointed out that there was a relationship between the number of bacteria and the sanitary conditions under which the shrimp were produced.

Research carried out by Silverman and coworkers (66) tested for bacterial numbers in various frozen shrimp products. The numbers were higher in raw products as opposed to precooked products. The highest number was $9.4 \times 10^6$ found in peeled raw shrimp. The high number in raw breaded shrimp was $2.9 \times 10^6$ while the low was $5.2 \times 10^4$. 
Holmes and McCleskey (35) showed that peeled shrimp had a smaller population of bacteria than unpeeled shrimp, and that freezing the shrimp at -40°F caused the bacterial population to decrease more on peeled shrimp than unpeeled shrimp.

Consumer Reports (24) ran bacterial counts on breaded shrimp samples from various processors. They said that although bacteria are present in nearly all foods, a high plate count can be indicative of poor manufacturing practice. They found that most of the breaded shrimp samples they tested did not have satisfactory bacterial counts.

Kachikian and others (39) also studied the bacterial content of breaded shrimp. They found that there was a large difference in the numbers of bacteria in different samples. The lowest value was $2.25 \times 10^4$ bacteria per gram and the high value was $5.4 \times 10^7$ bacteria per gram. Bacterial numbers were less than $1.0 \times 10^6$ in 61% of the samples. He states that the difference in bacteria population is due to the process that headed shrimp have gone through.

In work done by Nickerson (57) it was found that bacterial numbers varied in frozen breaded shrimp. His findings ranged from $2.4 \times 10^4$ to $6.0 \times 10^6$ bacteria per gram. Of the 136 samples that they examined, 48% had more than $1.0 \times 10^6$ bacteria per gram.
Bacterial numbers in raw breaded shrimp were determined in research by Surkiewicz and coworkers (68). They found that 85% of the samples produced under a good sanitary environment had less than $1.0 \times 10^6$ bacteria per gram. In their work, it was demonstrated that the batter used in coating the shrimp is a good vehicle for bacterial contamination if it has not been treated properly. They showed that high bacterial counts were not present in batter which was chilled and discarded at frequent intervals. Counts ranged from $5.0 \times 10^3$ to $5.0 \times 10^5$ bacteria per gram. However, bacterial counts were sometimes over $1.0 \times 10^6$ in poorly cared for batter. Conclusions of the paper state that both the shrimp and the batter make excellent growth substrates for bacteria, so the processor should make every attempt to maintain good sanitary conditions. The point was made that there is a relationship between the bacterial count of the finished product and the sanitary conditions in companies that are involved in the production of breaded shrimp.

Findings by Campbell and Williams (14) indicate that shrimp can be held on ice for 16 days and still be of commercial value. However, they recommend that the shrimp should be used in less than 12 days of storage on ice.

Certain compounds, such as volatile bases, are released in shrimp as a result of spoilage and decomposition. These compounds are able to bring about chemical changes
within the breading of shrimp which in turn could influence
discoloration of the breading material. Gould and Peters
(29) said that volatile bases are composed of such organic
compounds as \( \text{NH}_3 \), TMA, and DMA.

Many researchers feel that volatile bases do not
reliably indicate early deterioration of quality (29).
Farber (20) found in his work that tests such as volatile
nitrogen compounds and pH were not significant in early
spoilage in tuna, California sardines and Pacific mackerel.
Moorjani and others (55) showed that total volatile bases
remained at the same level in fresh water fish of optimal
quality. However, they did increase as the quality de­
creased and increased to a significant degree when the fish
became spoiled. Using canned fish, Farber and Ferro (21)
showed that the total volatile nitrogen did not correlate
well with organoleptic judgments.

Heavy Metals

Traces of heavy metals in foods play an important
role in nutrition and are needed by the body for proper
function. However, they are many times responsible for
the poor keeping qualities of food products. Metal ions
have been found to be responsible for such undesirable ef­
facts as oxidation, rancidity, discoloration, taste, odor
and lowering of nutritional components of foods. Monier­
Williams (54) points out that when trace elements and com­
ponents in food react, discoloration may result.
Ikuta (36) showed that organisms like oysters, living in polluted water, may absorb metallic ions, such as copper, from the water. As an example, he states that oysters collected in factory-polluted water had a higher copper content than those in non-polluted waters.

According to Furia (26) discoloration, off flavors, and unacceptable odors can occur when organic components react with metals present in shellfish. These reactions can take place under processing and storage conditions. Kelco Company (1961), as cited in The Handbook of Food Additives (26), stated that shellfish usually contain high concentrations of heavy metals such as 100 ppm iron, 400 ppm copper and 600 ppm zinc.

Iron has been found to cause discoloration in many food products. For instance, the discoloration of canned pork involves iron (69) and the color of sake is increased when it contains iron ions (62).

A research publication by Schweigart and others (64) said that iron could cause discoloration in peanuts containing corn meal. However, it could be prevented by the addition of lactic acid.

It was found by Livingston and others (50) that iron and copper caused a darkening discoloration in apple, beet, pear and peach puree, and pear and squash puree, and that the condition of storage was a factor in the degree of discoloration.
Kibbel (41) said that calcium, magnesium, copper and iron are usually the ions responsible for discoloration in fruits and vegetables.

It was pointed out by Smith (67) that food products such as mushrooms, beans, maize, and peas can become discolored in the presence of iron and copper.

Williams (79) says that copper and iron are oxidizing catalysts and that only 1 part of copper, or 10 parts of iron, per 100 million of oil can cause undesirable characteristics.

Monier-Williams (54) stated that the form of the iron ion can have an effect upon the food product. He said that 0.7 ppm of ferrous iron could cause off flavor in milk in half an hour, but that it would take 60 ppm of ferric iron to produce the same effect after 24 hours. In this case, it is evident that the ferrous form is more reactive than the ferric iron. He said that discoloration in foods is caused by iron in two ways: (1) by precipitation as sulfide or (2) by combination with tannic acid.

A paper dealing with "greening" of breaded shrimp by Thompson and Farragut (72) explained that the ferrous ion was contained in both green and in non-green breaded shrimp. However, the breading did not contain as much ferrous ion before being applied to the shrimp.

Waters (78) found that both ferric and ferrous iron would cause about the same degree of "blue" discoloration in crab meat.
Timberlake (73) found that there was no difference in the reaction rate between ferrous and ferric iron in deterioration of black currant juice.

Monier-Williams (54) also discusses the presence of copper in foods. He says that copper is naturally found in high quantities in foods such as crustaceans and shellfish. The copper content in oysters can be as high as several hundred parts per millions due to industry waste.

Kitson and Strachan (42) studied the effects of heavy metals on candied cherries. Their findings showed that copper in amounts of 10 ppm or larger can cause discoloration.

Board and Haque (11) found that 0.5 ppm copper was enough to produce discoloration in canned ox tongues, and that 5 ppm gave a very dark black discoloration. They showed that sodium salt of EDTA, sodium hexametaphosphate, gluconic acid or histidine did not prevent discoloration. However, by soaking the tongues in 0.5% citric acid solution before processing, the discoloration was prevented slightly. They state that copper, even in small amounts in foods, can be harmful, since it can catalyze oxidative deterioration.
III. Prevention of Discoloration

Chelating agents are probably the most common and useful substances used in removing undesirable metallic ions from food. Chelating or sequestering agents usually "react with metals to form complexes which, depending on the stability of the metal complex, tend to alter the properties and effects of metal in a substrate" (26). The phenomenon of chelation is of a complex nature, but according to Furia (26) the following simple formula may be used to illustrate the mechanism involved:

$$M + L = ML$$

Where:  
- **M** = metal ion  
- **L** = ligand (sequestrant, chelating agent)  
- **ML** = metal complex

Organic compounds such as citric acid or inorganic compounds such as polyphosphate may be used as the ligand. Furia (26) states that two conditions must be met in order for chelation to occur: (1) the ligand configuration, both steric and electronic, as related to the metal to be chelated, must be compatible and (2) the environment of chelation must be favorable for the reaction to occur.
Ascorbic Acid and Citric Acid

The use of citric acid and ascorbic acid together often bring about a synergistic effect. As an example, the addition of citric acid has been found to be very valuable in frozen fruit because of its ability to inactivate trace metals which could destroy the ascorbic acid which in turn helps to prevent color and flavor changes (27). "Rusting" in oily fish and shrimp is prohibited by use of a citric acid-ascorbic acid mixture (27).

Gardner (27) points out the fact that because citric acid affords an acidic environment, less ascorbic acid can be used because of the increased stability.

According to Gardner (28) a buildup in hydrogen sulfide may occur in shellfish during storage which can lead to darkening of the product. By dipping the shellfish into an acid solution, the problem may be alleviated. He also says that acidulants in seafoods help to preserve the quality by lowering the pH which can prevent the growth of bacteria. Acidulants also act as synergistic and chelating agents.

Landgraf (47) stated that citric acid brine used in canned wet-pack shrimp will help to stop graying and will give a better over-all product by reducing pH.

Baily and Fieger (5) found that Black Spot (melanogenesis) could be inhibited for a limited amount of time by use of an ascorbic acid-citric acid solution.
Fieger et al. (23) said that a mixture of CTC and iso-ascorbic acid could be effective in preventing melanosis if the shrimp were processed soon after catching. It was also effective in keeping bacterial population down.

Ascorbic acid helps to prevent discoloration and unwanted reactions in food substances. It has the property of acting both as an antioxidant and as an acidulant (28) which helps to maintain the good properties of the product. For instance, ascorbic acid has been found to be helpful in preventing the discoloration caused by enzymatic browning (27). According to Schultz (63), the ascorbic acid will reduce the products oxidized by the enzyme involved, in this case polyphenol oxidase, and this will eventually lead to inactivation of the enzyme.

Timberlake (73) studied the oxidation of ascorbic acid in black currant juice in the presence of copper and iron. The reaction rate with copper was approximately proportional to the square root of the copper concentration, while the reaction with iron alone was very small. However, when traces of iron are in the presence of 0.85 ppm of copper the oxidation of ascorbic acid increased greatly. He also tested the effects of metal chelating agents for inhibiting ascorbic acid oxidation by copper. Ethylenediamine-NNN'N'-tetraacetic acid (EDTA), diethylenetriamine-NNN'N'-pentaacetic acid (DTPA), 1,2-diaminocyclohexane-NN'N'-tetraacetic acid (CDTA), 1,3-diaminopropan-2-ol-
NNN'N'-tetraacetic acid (DPTA) and 2,9-dimethyl-o-phenanthroline (neocuproine) were the most effective chelating agents used.

Hawkins and others (32) found that ascorbic acid, in relatively large amounts, was effective in preventing a darkening discoloration in french-frying potatoes when they were cooked by boiling or put though the oil-blanced french-fried process.

Greig and Fliehman (7) showed that the shelf life of fresh-water, lake herring fillets increased from about 3 months to 7 months with a 2% ascorbic acid treatment.

Tsychiya and coworkers (7) showed that a yellow discoloration present in frozen scallops could be prevented by use of an ascorbic acid mixture.

Bauernfeind and Pinkert (7) report that for some types of seafood discolorations, it is better to use a combination dip of ascorbic acid and a copper chelator. Kakimoto and Kanazawa (7) showed that ascorbic acid was beneficial in delaying the black discoloration of lobsters. Waters (78) showed that EDTA could not prevent iron discoloration in crab meat.

Smith (67) says that EDTA and the polyphosphates make good sequestering agents in the removal of calcium from cheese in order to make the product softer. He also said that citric acid has been very valuable as a chelating agent in frozen fruits because of its ability to reduce
the action of iron and copper.

The beverage industry uses a great deal of citric acid, along with other organic acids as sequestering agents to remove metallic ions (67).

**Polyphosphates**

According to *The Handbook of Food Additives* (19), the polyphosphates form soluble complexes with nearly all of the metallic ions. This includes those ranging from two to many phosphate units per molecules. Van Wazer (76) said that the phosphorus atom of all phosphates is surrounded by four oxygen atoms. This gives a tetrahedral type structure. Many properties of the polyphosphates are explained by the presence of this tetrahedral, such as the ability they have to sequester metallic ions (19) in food systems, especially the long chain phosphates. When the metal ions have been sequestered, they are not able to carry out their usual function (76). Mahon and others (52) point out the fact that tripolyphosphate helps to control thawing drip in shrimp. They point out that much of the organoleptic qualities of shrimp are prevented by use of a tripolyphosphate dip, in this case especially tenderness and juiciness. They also point out that there is a reduction in oxidative changes which could also improve the quality of the product.

The structure of tripolyphosphate is shown below:
Kibbel (41) said that the polyphosphates are valuable in eliminating metal ions from the environment by acting as sequestering agents and therefore alleviating their chemical reactions.

Matsuhashi (53) demonstrated the ability of polyphosphates to chelate iron ions in an agar solution.
EXPERIMENTAL PROCEDURES

The purposes of these experiments were to study and evaluate the causes and to develop methods for preventing undesirable and detrimental discoloration reactions in breaded shrimp.

The pH changes of the shrimp were determined over a definite storage period, under conditions of constant temperature, and temperature fluctuations. During these experiments, the degree of decomposition was also evaluated. The pH was measured at both the anterior and posterior regions of the shrimp tail.

Sodium tripolyphosphate, and an ascorbic acid-citric acid mixture were tested as possible preventive agents for inhibiting discoloration. Iron ions, constant temperature versus temperature fluctuations, stage of decomposition, and storage time were factors tested in these experiments.

Other experiments were conducted to measure volatile bases changes in peeled deveined shrimp stored under frozen conditions, freeze-thaw conditions, and in crushed ice.
I. Materials

All materials and equipment used in the experimental procedures are generally accepted for the designated purpose or have been designated in such a way as to give the same conditions necessary for true experimental results. All materials used, with the intended use and methods of preparation, are given below.

Copper Solution

The copper solution was made by dissolving 1 g of pure copper powder in a small amount of HNO₃. After adding 5 ml of HCl, the solution was evaporated almost to dryness and diluted to 1 liter with 0.1N HCl. The solution was stored in a glass flask and all dilutions requiring a copper ion solution were made from this stock (4).

Iron Solution

The iron solution was made by dissolving 1 g of pure iron powder in 30 ml of 6N HCl and boiling. The solution was diluted to 1 liter. The solution was stored in a glass flask and all dilutions requiring an iron ion solution were made from this stock (4).

Sodium Tripolyphosphate Solution

The sodium tripolyphosphate solution was prepared by dissolving 4 g of sodium tripolyphosphate powder in 100 ml of distilled water at room temperature. In order
to insure thorough dissolving, it was necessary to mix the solution on an automatic stirrer.

**Ascorbic Acid-Citric Acid Solution**

An ascorbic acid-citric acid mixture was incorporated into the batter mix by dissolving 0.20 g ascorbic acid powder and 1 g of solid citric acid in approximately 1 ml of water. This solution was then incorporated into a 100 ml liquid batter.

**Shrimp**

Shrimp used in the experiments evaluating factors causing and influencing discoloration, and in the experiment which evaluated the role of pH, were received from a commercial processor in the fresh frozen state soon after capture. The shrimp were thawed in ice and remained on crushed ice throughout the pre-breading experimental procedures.

Shrimp used in tests dealing with volatile bases and microbiological examinations were received from a local dealer without ever having been frozen. They had been caught the day before laboratory processing. The shrimp were deheaded, peeled, and deveined and then were quickly placed under the desired conditions shortly after this preparation.

It should be pointed out that all peeled deveined shrimp retained the shell on the posterior end of the
shrimp tail. This is a practice commonly used by commercial shrimp batters.

Breaded shrimp used in preliminary studies were discolored and control samples received from a commercial processor. These samples were maintained under frozen condition (-20°C) until needed. When the connotation ±0°C is used in this dissertation, this means that the sample was stored under freeze-thaw conditions.

**Tashiro's Reagent**

Tashiro's reagent was prepared by adding 200 ml of a 0.1% alcoholic solution of methyl red to 50 ml of a 0.1% methylene blue alcoholic solution. This stock solution was stored in a brown reagent bottle. In order to use it as an indicator, 1 part of the stock solution was added to 1 part of alcohol and 2 parts of distilled water (15).

**Batter Solution**

The batter solution was prepared by mixing 1 part of batter powder mix with 1 part of distilled water. The solution was mixed well to insure even dispersion. The batter was chilled throughout the dipping procedures by the addition of ice.

**Ba(OH)\(_2\)** Solution

It was necessary to make up the Ba(OH)\(_2\) solution just prior to titration to insure proper normality because of the tendency of Ba(OH)\(_2\) to react with CO\(_2\) which in turn
forms an insoluble precipitate. The powdered Ba(OH)$_2$ should be as fresh as possible for the same reason.

**Phosphate Buffer**

Butterfield's buffered stock solution was prepared by dissolving 34 g of KH$_2$PO$_4$ in 500 ml of H$_2$O and adjusting the pH to 7.2 with approximately 175 ml of 1N NaOH. After diluting to 1 liter with H$_2$O, the solution was stored at 40°F. To use the buffer in water blanks, 1.25 ml of the stock solution was diluted in 1 liter of distilled water (13).

**Glassware**

All glass containers and pipettes used in microbial work were sterilized by autoclaving at 121°C for 30 minutes and dried. Pipettes used in all work were selected to deliver only the designated volume of the highest calibration on the pipet. It was essential that all glassware be clean for all experiments.

**Eugon Agar**

Eugon Agar (Difco) is recommended for culturing a large variety of microorganisms (16). It was prepared according to manufacturer's instructions. The agar was sterilized at 121°C for 15 minutes. It was used in pour plate procedures.
II. Methods

Measurements of Metallic Content of Breaded Shrimp

Discolored breaded shrimp samples were categorized according to the appearance of the sample. Of the different types, iron and copper analyses were run on four types: (1) breaded shrimp with a yellowish discoloration at the posterior end of the tail, (2) breaded shrimp with a distinct green color at the posterior end of the tail and other parts of the breading, (3) breaded shrimp with graying throughout the breading, and (4) breaded shrimp that had a dark-greenish yellow discoloration throughout the entire shrimp.

Various parts of these breaded shrimp samples were examined for copper and iron in a preliminary study to determine the highest areas of metallic contamination. The whole shrimp debreaded shrimp and breading material was analyzed. A control sample, of non-discolored shrimp, was also evaluated. A Jarrell-Ash Atomic Absorption was used to make all metallic ion analyses.

The samples were prepared by using a dry ashing method. This was done by weighing out approximately 25 g of the sample into a platinum ashing dish and ashing for about 24 hours at 500°C. The ashes were carefully washed into beakers with deionized water, being careful that all particles were collected. To this, 50 ml of a perchloric-
nitric acid solution (3:1) was added. The beakers were covered with a watch glass and digested on a hot plate until the solution became clear. The solution was poured into a 50 ml volumetric flask. The beaker was washed well with deionized water to collect all traces of the solution. The washings were also poured into the 50 ml volumetric flask containing the solution, after which the flask was filled to the 50 ml mark with deionized water. The solutions were stored in bottles until readings were made on the atomic absorption. Standard solutions of both copper and iron were prepared by making the proper dilutions from a stock solution containing 1000 ppm of the respective ion. Calculations were made directly from the recorder scale.

**Breading Procedures**

Visits were made to the W. R. Grace seafood processing plant (Sea-Pak) in order to observe methods of handling and breading the shrimp. Procedures utilized by them to bread shrimp were used in a similar manner throughout these experiments in our laboratories in Baton Rouge, Louisiana. Advice and counsel of Sea-Pak personnel were used in formulation of techniques of shrimp preparation.

Shrimp were dipped so as to completely submerge and cover the entire shrimp with batter. The shrimp were removed and excess batter was allowed to drip off. Immediately, the shrimp were covered with dry breader mix to assure complete covering. The shrimp were then frozen at -20°C.
During periods of storage, the shrimp used in the pH study were packaged in boxes exactly like those used commercially; however, the breaded shrimp used in evaluating the factors responsible for creation and prevention of discoloration were packaged in Whirl-Paks during storage periods for ease of separation.

Measurements of pH in Discoloration Breaded Shrimp

The purpose of these pH experiments was to examine the role that pH plays in discoloration. Again, in this preliminary study, pH determinations were made on categorized discolorations in breaded shrimp samples received from a commercial processor. They were as follows:
(1) breaded shrimp which had a yellow coloration at the posterior end of the tail, but with a non-discolored anterior end; (2) samples which had a gray discoloration throughout the breading material; (3) breading material that had become green; and (4) newly prepared breading material which had no discoloration.

The A.O.A.C. electrometric method for determining pH was used to calculate the pH of shrimp breading samples (4). This was done by weighing 10.0 g of breading material into a clean, dry Erlenmeyer flask and adding 100 ml of recently boiled H$_2$O. The water was allowed to cool to 25°C before being used. It should be noted that pH measurements were made only from breading material. The flasks were
shaken for a digestion period of 30 minutes by using a Lab-Line Junior Orbit Shaker, rotating between 150-200 rpm. After the shaking cycle was complete, the samples were allowed to settle for 10 minutes, after which the supernatant was decanted into a 50 ml beaker. The pH of the solution was determined immediately by using a Leeds & Northrup pH meter. The pH meter was standardized with buffer solutions at pH 7 and pH 8. Measurements were accurate to the nearest tenth pH unit.

In order to evaluate the influence of pH on discoloration in breaded shrimp, the experimental design shown in Diagram 1 was used. The shrimp were removed from the ice at 0 days (fresh), 8 days, and at 16 days. They were then breaded. Samples were kept at -20°C and under freezing and thawing conditions in order to determine the effects of temperature. Freeze-thaw cycles occurred at 4-week intervals. Measurements of pH were made at 0 weeks, 8 weeks and at 16 weeks of storage. The anterior and posterior portions of the shrimp tail were evaluated. All samples were run in replicas of 3. A statistical analysis of these results was performed to calculate the significance of the factors involved.

As a point of clarification, when the term shrimp "tail" is used in this dissertation, it refers to all the abdominal segments plus the telson. The shrimp were headed at the anterior end of the "tail." The shell
Diagram 1. Schematic of Experimental Procedures Used to Evaluate pH Changes in Breaded Shrimp
covering was peeled from the shrimp except for the shell on the last abdominal segment and telson at the posterior end of the tail.

Measurement of Discoloration in Breaded Shrimp

The purpose of this experiment was to evaluate factors which cause and influence discoloration, as well as to determine the effectiveness of an ascorbic acid-citric acid mix and sodium tripolyphosphate as possible agents for inhibiting or retarding discoloration in breaded shrimp. Diagram 2 shows the experimental procedures that were employed to test the effects of the above mentioned factors.

The effects of temperature were evaluated by exposing some samples to a freeze-thaw cycle at regular intervals of 4 weeks. Other samples were maintained at -20°C throughout the duration of the study. The shrimp undergoing freeze-thaw cycles were allowed to thaw by exposure to room temperature for five hours.

The effects of iron on breaded shrimp discoloration were evaluated by incorporating iron ions into the batter mix before the shrimp were dipped. A 100 ppm iron solution was prepared by pipetting 10 ml of a 1000 ppm iron solution into 90 ml of batter. All shrimp to be contaminated with iron ions were dipped into this batter solution. In order to test the effectiveness of citric acid and ascorbic acid, an ascorbic acid-citric acid power mixture was dissolved in
Diagram 2. Schematic of Experimental Procedures Used to Evaluate Factors in Discolored Breaded Shrimp
a small quantity of distilled water and then added to batter to give a 0.2 and 1.0% mixture, respectively. The shrimp were dipped in this batter, breaded and stored under the specified conditions.

Sodium tripolyphosphate treated shrimp were prepared by submerging the shrimp in a sodium tripolyphosphate solution (4%) for 60 seconds, then dipped in batter, and breaded. These samples were then stored under the specified conditions. In order to prepare a control, shrimp were dipped in batter and breaded with no treatment and stored under the proper conditions as outlined in the schematic.

The quality of the shrimp prior to breading was evaluated as a factor in discoloration of breaded shrimp by keeping the shrimp on ice over-regulated periods of time. This allowed for the buildup of bacterial populations, breakdown of organic components and thus decomposition. Inoculations were made after shrimp had been on ice for 0 (fresh), 8 and 16 days.

Discoloration of the breaded shrimp was measured by means of a sight panel. The panel consisted of 8 judges using a hedonic scale numbered from 1 to 7. Figure 1 shows the questionnaire used by panelists to judge the breaded shrimp. The lowest number (1) indicates no discoloration, while the highest number (7) indicates very heavy discoloration. The samples were judged after 9 weeks of storage and
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**Discoloration** A B C D E F G H I J K L

- **NONE**
  - 1
  - 2
  - 3

- **MODERATE**
  - 4
  - 5
  - 6

- **HEAVY**
  - 7

*Figure 1. Questionnaire Used by Panelist to Evaluate Discoloration in Breaded Shrimp*
then again after 18 weeks of storage. The value given for the initial judging was 1 because discoloration does not take place in the breading immediately. At all judging thereafter, a freshly prepared control was used for comparison with the other samples. The control always had a discoloration value of 1.

A statistical analysis using a randomized complete block design with a factorial arrangement of treatments was performed on the above procedures.

Measurement of Volatile Bases

The purpose of this experiment was to measure the changes in volatile bases. It is conceivable that a buildup of the volatile bases could change the pH in the breading material, which in turn could initiate discoloration. Diagram 3 shows the schematic used to evaluate changes in volatile bases. Fresh, peeled, deveined shrimp were placed in plastic bags and stored in ice at -20°C and under freeze-thaw conditions. Volatile bases were measured at 0 day (fresh state) and then at 5-day intervals thereafter up to 20 days. In all samples, measurements were made of the anterior and posterior ends of the shrimp tail. Three replicas were run on each sample.

The method employed by Lagarde (46) was used to measure volatile bases. The sample was prepared by weighing out 25 g of shrimp, adding 225 ml of 4% trichloroacetic acid to give a 1:10 dilution factor, and blending for 2
Diagram 3. Schematic of Experimental Procedures Used to Measure Volatile Base Changes in Shrimp under Varying Storage Conditions
minutes in a Waring Blender. This solution was centrifuged for 15 minutes at 2500 rpm. The supernatant was poured off and filtered to remove particles of precipitate.

The diffusion apparatus was composed of 150 ml wide mouth bottles containing a 10 ml beaker. The beaker held 2 ml of 0.005N HCl and Tashiro's indicator. To the bottom of the bottle was added 0.5 ml of the supernatant from the sample and 1 ml of 40% KOH. Care was exercised not to contaminate the solution in the 10 ml beaker. After tightly sealing the bottle, the system was distilled at room temperature for 6 hours. Work done by Lagarde (46) shown in Figure 2 below indicates that 5-6 hours is enough time to allow for complete distillation:

![Figure 2. Isothermal Distillation Standardization](image-url)
After the proper elapse of time, the excess HCl in the 10 ml beakers was titrated with 0.01N Ba(OH)$_2$ using a microburet, accurate to 2/100 ml. The formula below, as used by Lagarde (46), was used in calculating the milligrams of volatile base per 100 grams of sample.

\[
\text{Milligrams of V.B. per 100 gram of sample} = \frac{(\text{control-sample})(14)(N)(D)}{(\text{sample weight}) \times 1000} \times 1000 \times 100
\]

where
- \(14 = \) molecular weight of nitrogen
- \(N = \) normality of Ba(OH)$_2$
- \(D = \) dilution factor

A statistical analysis was performed on these experimental procedures involving volatile bases.

**Total Plate Count**

The purpose of this experiment was to determine changes in microorganisms population in shrimp during storage conditions. The quality of shrimp before they are breaded is a factor in maintaining good color properties in the breading material, and this is related to bacterial populations.

A schematic similar to Diagram 3 used to measure volatile bases was also used to evaluate microorganisms. All storage conditions were exactly the same. The only difference was that the section of shrimp was not involved.

The pour plate technique was used to determine
microorganism numbers by direct counting of the colonies. The sample was prepared by aseptically weighing 25 g of shrimp and blending for 2 minutes in a Waring Blender after the addition of 225 ml of sterile Butterfield's phosphate buffer. The proper dilutions were made from this 1:10 solution for each particular sample. All dilutions were made into sterile 90 ml phosphate buffer blanks, using 10 ml aliquots from the previous dilution. After the proper dilutions of sample had been placed into sterile petri dishes, Eugon agar was used as a growth medium. The plates were incubated 48 hrs at 38°C, and the colonies counted. By multiplying the inverse of the dilution times the number of colonies, the number of bacteria per gram were calculated.
RESULTS AND DISCUSSION

1. Preliminary Studies

In order to develop a scientific understanding of the factors which cause or influence discoloration in breaded shrimp, it was necessary to investigate the basic nature of discolored breaded shrimp samples. Upon initial examination, it was found that the discolored areas were restricted almost exclusively to the breading material. Only in old samples did any shrimp flesh contain darkening. Since the discoloration reactions are restricted to the breading material in these experiments, the factors responsible were evaluated from the breading.

It was noted, upon initial examination of shrimp samples received from a processor, that several types of discoloration were present in the breading. This indicated that there are various factors or conditions which are responsible for causing or influencing discolorations and that perhaps different conditions are needed for different types of discolorations. One of the commonest types of discolorations was a "yellowing." This type of discoloration seems to occur mostly in the posterior end of samples, but could be found anywhere on the sample. If the "yellowing" was dark enough, it appeared to be brown,
and if it was light enough it appeared to be greenish-yellow. A "graying" discoloration was also common. It occurred not only at the posterior end of the shrimp sample, but throughout the breading material. These samples varied from light gray to dark gray-black. Other types of discolorations also occur in breaded shrimp, but they are not as common. A distinct green to blue-green pigmentation can sometimes be found. It usually occurs in spots near the tail, but can be found in other areas, and is usually found in combination with other types of discolorations.

Preliminary studies reaffirm the findings of Thomp­son and Farragut (72) that the pigment from discolored portions of breaded shrimp (breading) is soluble in water; however, it was necessary to make the water slightly alkaline to maintain the highest degree of color. The extracted material could be made colorless by the addition of acid, but the color returned by making the solution slightly alkaline again.

The ability of volatile bases to cause discoloration in breading material was demonstrated in a preliminary study by exposing the breading material to an ammonia atmosphere using a Conway Diffusion Dish. Almost immediately the breading became greenish or yellowish. This experiment showed that not only could discoloration of this type result from processing plant environmental conditions,
for example, escaping ammonia gas used in the cooling system, but it could also be a result of volatile bases, such as ammonia, TMA, and DMA, being produced from the shrimp flesh as a result of protein degradation.

Many times metallic ions are responsible for discoloration reactions in foods. Based on findings in the literature and in laboratory research, iron and copper were examined. A preliminary study of iron and copper was performed to evaluate the extent of involvement in the discoloration reactions. Table 1 shows the ppm of iron in various parts of the shrimp, while Table 2 shows the ppm of copper. From Table 1, it can be seen that the highest concentration of iron in breading material is found in the black breading, a form of "graying" discoloration. Whole shrimp with black and green discoloration also seem to have a relatively high iron content. This indicates that iron functions in discoloration reactions. The sample that appeared gray with greening also had a high iron content in the debreaded shrimp. The shrimp components with "yellowing" discoloration, as well as the control samples, did not exhibit as high an iron content. From this preliminary study, it was shown that perhaps iron is involved more in the "graying" discoloration than in the "yellowing" discoloration.

From Table 2, it can be seen that the concentration of copper is nearly the same in all samples, except for
TABLE 1
Iron Content of Various Types of Discolored Breaded Shrimp and Components

<table>
<thead>
<tr>
<th>Type of Discoloration</th>
<th>ppm Iron</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>av</td>
</tr>
<tr>
<td>Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) Yellow breading</td>
<td>8.80</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>8.80</td>
</tr>
<tr>
<td>(2) debreaded shrimp</td>
<td>10.06</td>
<td>4.90</td>
<td>4.10</td>
<td>6.35</td>
<td></td>
</tr>
<tr>
<td>(3) Shrimp and breading</td>
<td>5.89</td>
<td>7.87</td>
<td>6.90</td>
<td>6.89</td>
<td></td>
</tr>
<tr>
<td>Black with Green</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) green breading</td>
<td>8.17</td>
<td>4.68</td>
<td>5.36</td>
<td>6.07</td>
<td></td>
</tr>
<tr>
<td>(2) black breading</td>
<td>10.65</td>
<td>9.30</td>
<td>10.12</td>
<td>10.02</td>
<td></td>
</tr>
<tr>
<td>(3) debreaded shrimp</td>
<td>7.20</td>
<td>4.20</td>
<td>5.82</td>
<td>5.74</td>
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</tr>
<tr>
<td>(4) shrimp and breading</td>
<td>11.01</td>
<td>15.50</td>
<td>8.90</td>
<td>11.80</td>
<td></td>
</tr>
<tr>
<td>Gray with Green</td>
<td></td>
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</tr>
<tr>
<td>(1) gray-green breading</td>
<td>11.66</td>
<td>6.81</td>
<td>7.11</td>
<td>8.53</td>
<td></td>
</tr>
<tr>
<td>(2) debreaded shrimp</td>
<td>7.94</td>
<td>17.05</td>
<td>11.73</td>
<td>12.24</td>
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<tr>
<td>(3) shrimp and breading</td>
<td>7.99</td>
<td>5.88</td>
<td>6.91</td>
<td>6.93</td>
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<tr>
<td>Non-discolored (control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) breading</td>
<td>6.28</td>
<td>6.12</td>
<td>5.94</td>
<td>6.11</td>
<td></td>
</tr>
<tr>
<td>(2) debreaded shrimp</td>
<td>2.73</td>
<td>4.08</td>
<td>3.00</td>
<td>3.27</td>
<td></td>
</tr>
<tr>
<td>(3) shrimp and breading</td>
<td>7.41</td>
<td>--</td>
<td>--</td>
<td>7.41</td>
<td></td>
</tr>
<tr>
<td>Type of Discoloration</td>
<td>ppm Copper</td>
<td></td>
<td></td>
<td>av</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------</td>
<td>---</td>
<td>---</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) yellow breading</td>
<td>1.18</td>
<td></td>
<td></td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>(2) debreaded shrimp</td>
<td>1.61</td>
<td>0.98</td>
<td>1.28</td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td>(3) shrimp and breading</td>
<td>1.13</td>
<td>1.16</td>
<td>1.70</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td>Black with Green</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) green breading</td>
<td>1.07</td>
<td>1.29</td>
<td>1.55</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>(2) black breading</td>
<td>2.66</td>
<td>1.70</td>
<td>1.20</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td>(3) debreaded shrimp</td>
<td>2.43</td>
<td>1.50</td>
<td>2.39</td>
<td>2.11</td>
<td></td>
</tr>
<tr>
<td>(4) shrimp and breading</td>
<td>1.02</td>
<td>1.49</td>
<td>1.16</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td>Gray with Green</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) gray-green breading</td>
<td>0.77</td>
<td>0.79</td>
<td>0.74</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>(2) debreaded shrimp</td>
<td>2.04</td>
<td>2.14</td>
<td>1.50</td>
<td>1.89</td>
<td></td>
</tr>
<tr>
<td>(3) shrimp and breading</td>
<td>0.88</td>
<td>0.96</td>
<td>1.33</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Non-discolored (control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) breading</td>
<td>0.75</td>
<td>0.78</td>
<td>0.76</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>(2) debreaded shrimp</td>
<td>1.66</td>
<td>1.02</td>
<td>1.50</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td>(3) shrimp and breading</td>
<td>1.11</td>
<td></td>
<td></td>
<td>1.11</td>
<td></td>
</tr>
</tbody>
</table>
the relatively high level in the black and green debreaded shrimp samples and the low level in the gray-green breading and in the control breading. Based on the results of this experiment, iron and copper ions were incorporated separately into batter mixes, making a 100 ppm solution, and applied to shrimp. After 3 months of frozen storage, with freeze-thaw cycles, the shrimp with iron ions showed considerable "graying" discoloration, while the shrimp with the copper ions demonstrated somewhat less discoloration. The preliminary studies of iron and copper were used to design later experiments in measuring and evaluating the amount of discoloration.

The pH of the breading material was examined, in a preliminary study, to determine possible pH involvement as a factor in discoloration. Thompson and Farragut (72) found that normal shrimp had a pH of 7.4 but discolored shrimp had a pH of 7.8. The examination of the discolored breading, as shown in Table 3, demonstrated that it did indeed have a higher pH than non-discolored breading. The control sample (freshly breaded shrimp) had a pH of 7.00, and green breading had a pH of 7.78.

It was shown that the same shrimp sample may have different pH readings. Yellow breading taken from the posterior end of shrimp samples had an average pH of 8.00, whereas non-discolored breading from the same shrimp displayed an average pH of 7.33. This indicates that a high
**TABLE 3**

**pH Measurements of Shrimp Breading**

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>av</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow breading*</td>
<td>8.05</td>
<td>7.90</td>
<td>8.05</td>
<td>8.00</td>
</tr>
<tr>
<td>White breading*</td>
<td>7.20</td>
<td>7.40</td>
<td>7.40</td>
<td>7.33</td>
</tr>
<tr>
<td>Green breading</td>
<td>7.85</td>
<td>7.70</td>
<td>--</td>
<td>7.78</td>
</tr>
<tr>
<td>Gray breading</td>
<td>7.60</td>
<td>7.55</td>
<td>--</td>
<td>7.58</td>
</tr>
<tr>
<td>Control</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
</tr>
</tbody>
</table>

*The yellow and the white breading were taken from the same shrimp samples, the yellow being the discolored portion.*
pH is necessary for discoloration in the breading. The addition of NaOH to breading material quickly turned it yellowish-green, manifesting this point. The average pH of the gray breading was only 7.58, which indicates that other factors are necessary to produce "graying." The main point to be made from this experiment is that pH variances can occur within the breading of the same shrimp sample and that a high pH can cause discoloration in breaded shrimp.

Results of the examination of microorganisms are shown in Figure 3. It was the purpose of this test to show the storage conditions necessary to retard or enhance microorganism populations. The graph shows that very little increase in growth occurred in samples maintained at -20°C but that high increases were observed in samples held under freeze-thaw conditions and on ice after 20 days. It should be mentioned that the populations in the freeze-thaw samples remained relatively low up to 10 days and then began to climb rapidly, but the iced samples began a rapid climb after 5 days. Because of the inconsistent results of both this test and other preliminary tests, it is difficult to evaluate the role microorganisms play in discoloration. However, large numbers of them can speed up the production of decomposition compounds and raise the pH. A more detailed and comprehensive study would be necessary to ascertain the types and relationships of microorganisms on discoloration in breaded shrimp.
Figure 3. The Influence of Storage Conditions on Bacterial Populations
II. pH Results and Discussion

Using raw data, Figures 4, 5, and 6 show changes in pH in breading from shrimp stored on ice at various time intervals. The shrimp which were breaded and placed in frozen storage at 0 day showed the smallest amount of pH rise. Large increase intervals were noted when the shrimp were held at 8 and 16 days on ice. It should also be noted that the pH in the breading increased the longer it was held in storage and that the increase was greater as the quality of the product decreased. These graphs also show that differences in pH do occur within the same samples. The posterior end of the breaded shrimp tail had a higher pH in most cases than did the anterior end.

Preliminary studies showed that discolored breading, especially "yellowing," always had a higher pH than normal breading. This indicates that pH is a primary factor governing this type of discoloration.

A statistical analysis of pH involvement in breaded shrimp was performed to evaluate the significance of pH rises in the breading. The schematic in Diagram 1 was used for the experimental design.

Results from the statistical analysis run on data from pH determinations of breading show that all the main effects, (1) temperature of storage, (2) days on ice prior to breading, (3) weeks of storage, and (4) section of the shrimp, i.e., the anterior end of the shrimp tail as
Figure 4. Changes in pH in Shrimp During Iced-Storage at 0 Day

Δ = anterior end, at -20°C
□ = posterior end, at -20°C
○ = anterior end, at ±0°C
● = posterior end, at ±0°C
Figure 5. Changes in pH in Shrimp During Iced-Storage at 8 Days
Figure 6. Changes in pH in Shrimp During Iced-Storage at 16 Days
opposed to the posterior end of the tail, were found to be highly significant ($P < 0.01$). Thus all the main effects have a role in influencing pH which in turn controls discoloration. Table 4 shows the analysis of variance for this test.

A highly significant interaction between temperature of storage and weeks of storage was observed. It is apparent from the data shown in Figure 7 that temperature fluctuations above $0^\circ$C cause an acceleration of pH shift towards the alkaline range. Consequently, storage temperature for frozen breaded shrimp should be maintained as low as economically possible and fluctuations above $0^\circ$C should always be avoided. This interaction also shows that pH increases as time in weeks increases regardless of the temperature of storage, but the increase is greater when fluctuations above $0^\circ$C occur. This manifests the importance of eliminating unnecessary delays in moving the product to the consumer.

The interaction between days on ice prior to breading and weeks of cold storage was highly significant. The data is plotted in Figure 8. Clearly, it can be seen that as the number of days on ice prior to breading increases, a large increase in pH occurs. This indicates that compounds produced as a result of decomposition in shrimp are more rapidly built up during this phase of the breading operation than in the storage phase. Results from this
%TABLE 4
Analysis of Variance for Variable pH in Breaded Shrimp

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.0890</td>
<td>14.785**</td>
</tr>
<tr>
<td>Days on Ice</td>
<td>2</td>
<td>2.9407</td>
<td>488.600**</td>
</tr>
<tr>
<td>Weeks of Storage</td>
<td>2</td>
<td>3.9195</td>
<td>651.246**</td>
</tr>
<tr>
<td>Section of Shrimp</td>
<td>1</td>
<td>0.4408</td>
<td>73.246**</td>
</tr>
<tr>
<td>T X D</td>
<td>2</td>
<td>0.0001</td>
<td>0.015</td>
</tr>
<tr>
<td>T X W</td>
<td>2</td>
<td>0.0590</td>
<td>9.800**</td>
</tr>
<tr>
<td>D X W</td>
<td>4</td>
<td>0.7548</td>
<td>125.415**</td>
</tr>
<tr>
<td>T X D X W</td>
<td>4</td>
<td>0.0026</td>
<td>0.431</td>
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<tr>
<td>T X S</td>
<td>1</td>
<td>0.0157</td>
<td>2.600</td>
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<tr>
<td>D X S</td>
<td>2</td>
<td>0.0258</td>
<td>4.292*</td>
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<tr>
<td>T X D X S</td>
<td>2</td>
<td>0.0168</td>
<td>2.785</td>
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<tr>
<td>W X S</td>
<td>2</td>
<td>0.1158</td>
<td>19.246**</td>
</tr>
<tr>
<td>T X W X S</td>
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<td>0.0045</td>
<td>0.754</td>
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<tr>
<td>D X W X S</td>
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<td>0.0092</td>
<td>1.523</td>
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<tr>
<td>T X D X W X S</td>
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<td>0.0107</td>
<td>1.769</td>
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<tr>
<td>Error</td>
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<td>0.0060</td>
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<tr>
<td>Corrected Total</td>
<td>107</td>
<td></td>
<td></td>
</tr>
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</table>

All main effects and interactions were tested using the error value.

*Significant at the 5% level
**Significant at the 1% level
Figure 7. Effects of Various Frozen Temperature and Storage Time as Related to pH of the Breading
Figure 8. Effects of Days on Ice and Weeks of Storage as Related to pH of the Breading
interaction demonstrate the importance of using fresh shrimp and show again that pH increases as the length of time of cold storage increases.

Figure 9 illustrates the interaction between the number of days the shrimp were retained on ice prior to breading and the pH of the section of shrimp. The interaction shows that the pH in the posterior end of the shrimp was higher than the pH in the anterior end of the shrimp and that pH increased in each section significantly as the length of time on ice prior to breading increased. It should be mentioned here that the shell remaining on the posterior end of the shrimp is apparently responsible for the increase of pH. This shell lends itself to harboring bacteria, which are not easily washed off because of the porous nature of the shell, which in turn produce decomposition products capable of causing pH increases. The shell may also work to funnel and concentrate compounds, such as volatile bases, causing a higher pH in that area. Many times discoloration of the breaded shrimp begins and is more intense at the lip of the shell. Notice in Figure 23 how distinct the dividing line is between discolored and non-discolored breading. This line marks the spot where the tail shell ends and illustrates how volatile bases and other complexing materials from under the shell are concentrated at this particular area.

A highly significant interaction occurs between the
Figure 9. Effects of Days on Ice and Section of Shrimp as Related to pH of the Breading
weeks of cold storage and the section of the shrimp. This relationship is shown in Figure 10. The interaction shows that the posterior section of the breaded shrimp sample has a higher pH than the anterior section, as noted in the above interaction for the same reasons, and that the pH increases in both sections as the weeks of storage increase.

Summarizing, it was shown that the main effects of temperature, days on ice prior to breading, and weeks of storage have a relationship to pH increases in breaded shrimp. It was also shown that there is a significant difference in pH within the same shrimp sample.

III. Sight Panel Results and Discussion

Results from the statistical analysis run on data from the sight panel test show that all the main effects, (1) presence of iron ions, (2) temperature of storage, (3) treatments, (4) number of days on ice prior to breading, and (5) weeks of storage, were found to be highly significant (P < 0.01). This indicates that all of the above effects have a definite relationship in discoloration reactions. Table 5 shows the analysis of variance for this test.

A significant interaction between the presence of iron ions and the temperature of storage was observed. This relationship is shown in Figure 11. The graph shows
Figure 10. Effects of Weeks of Storage and Section of Shrimp as Related to pH of the Breading
### TABLE 5
Analysis of Variance for Discoloration in Breaded Shrimp

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>F Value</th>
</tr>
</thead>
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<tr>
<td>Judge</td>
<td>7</td>
<td>4.9603</td>
<td>8.931**</td>
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<tr>
<td>Presence of Iron</td>
<td>1</td>
<td>13.5000</td>
<td>24.305**</td>
</tr>
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<td>Storage Temperature</td>
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<td>151.6713</td>
<td>273.063**</td>
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<tr>
<td>Treatment</td>
<td>2</td>
<td>52.9618</td>
<td>95.350**</td>
</tr>
<tr>
<td>Days on Ice</td>
<td>2</td>
<td>8.1979</td>
<td>14.759**</td>
</tr>
<tr>
<td>Weeks of Storage</td>
<td>2</td>
<td>281.9201</td>
<td>507.558**</td>
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<tr>
<td>I X S</td>
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<td>2.4491</td>
<td>4.409*</td>
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<td>I X T</td>
<td>2</td>
<td>9.2118</td>
<td>16.585**</td>
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<tr>
<td>S X T</td>
<td>2</td>
<td>16.7789</td>
<td>30.208**</td>
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<tr>
<td>I X S X T</td>
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<td>4.3623</td>
<td>7.854**</td>
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<tr>
<td>I X D</td>
<td>2</td>
<td>0.2188</td>
<td>0.394</td>
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<tr>
<td>S X D</td>
<td>2</td>
<td>8.3831</td>
<td>15.093**</td>
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<td>I X S X D</td>
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<td>0.4039</td>
<td>0.727</td>
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<td>T X D</td>
<td>4</td>
<td>5.9722</td>
<td>10.752**</td>
</tr>
<tr>
<td>I X T X D</td>
<td>4</td>
<td>1.5556</td>
<td>2.801*</td>
</tr>
<tr>
<td>S X T X D</td>
<td>4</td>
<td>4.4491</td>
<td>8.010**</td>
</tr>
<tr>
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<td>0.1296</td>
<td>0.023</td>
</tr>
<tr>
<td>I X W</td>
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<td>8.333**</td>
</tr>
<tr>
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<td>2</td>
<td>51.2651</td>
<td>92.296**</td>
</tr>
<tr>
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<td>9.204**</td>
</tr>
<tr>
<td>T X W</td>
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<td>17.2569</td>
<td>31.069**</td>
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### TABLE 5 (continued)

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<td>5.789**</td>
</tr>
<tr>
<td>S X T X W</td>
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<td>11.361**</td>
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</tr>
<tr>
<td>D X W</td>
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<td>14.0966</td>
<td>25.379**</td>
</tr>
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<td>8</td>
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<td>8.197**</td>
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<td>3.762**</td>
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<tr>
<td>Total</td>
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</tbody>
</table>

All main effects and interactions were tested using the error value.

*Significant at the 5% level  
**Significant at the 1% level
Figure 11. The Effects of Iron Ions and the Temperature of Storage as Evaluated by a Sight Panel.
that as the temperature increases so does the discoloration. Samples containing iron ions increased in discoloration faster than samples which did not contain iron ions. Accordingly, samples should always be maintained at as low a temperature as possible to eliminate the possibility of accelerated discoloration in samples that may have become contaminated with iron. Figures 21, 22, 23, and 24 show some of the shrimp samples used in the sight panel test. Figure 22 shows the "graying" which occurs when shrimp have been contaminated with iron ions and have undergone temperature fluctuations. Breaded shrimp without iron ions and stored under freeze-thaw conditions are in Figure 21. Notice the "yellowing" that occurred. Observations made in processing plants showed that iron contamination can occur in several areas. The walls and floors of the ice supply bin can become rusty as can instruments used in transporting the ice in the plant (such as shovels, carts, trays, augars, etc.) if they are made of iron and/or galvanized iron. All implements that come in contact with the shrimp or substances used in the breading, either directly or indirectly, should be made of corrosion resistant materials such as stainless steel or plastic. It should also be mentioned that water used in processing must not be overlooked as a possible source of iron contamination.

A highly significant interaction between the
presence of iron ions and treatment of the shrimp is shown in Figure 12. Treatments consisted of adding ascorbic acid-citric acid mix to samples and sodium tripolyphosphate to samples to test for inhibition of discoloration. The analysis demonstrated that an ascorbic acid-citric acid mixture could prevent discoloration in breading when compared to a control. No increase in discoloration was noted in samples containing an ascorbic acid-citric acid mixture with added iron ions. The control breaded shrimp samples increased rapidly with the addition of iron ions. The graph shows that sodium tripolyphosphate has no effect in preventing discoloration in the presence of iron ions. In fact, these shrimp samples showed a larger increase in discoloration than the control. From the findings, it is apparent that ascorbic acid-citric acid mixture should be incorporated into the batter in order to prevent discoloration reactions even when iron ions are involved. The level used in these experiments was 0.2% ascorbic acid mixed with 1.0% citric acid in the batter. Sodium tripolyphosphate served no useful purpose, according to these experiments, and should not be used to inhibit discoloration in breaded shrimp when iron ions are responsible for causing it. It should be mentioned that an ascorbic acid-citric acid blend and sodium tripolyphosphate were tested separately for inhibiting discoloration. It may be that a synergistic effect could be possible using them together, but this was
Figure 12. The Effects of Iron Ions and Treatment of Breaded Shrimp as Evaluated by a Sight Panel.
not tested. Figure 24 shows samples treated with ascorbic acid-citric acid mix and Figure 23 shows samples treated with sodium tripolyphosphate. These samples were stored under freeze-thaw conditions. Notice the lack of discoloration in ascorbic acid-citric acid treated samples, even though subjected to stress conditions. The samples treated with sodium tripolyphosphate and containing iron ions obviously demonstrate no discoloration inhibition properties.

A highly significant interaction was observed between the temperature of storage and the treatments tested to inhibit discoloration. Figure 13 illustrates that as temperature increases an ascorbic acid-citric acid mixture is significantly effective in preventing discoloration in breaded shrimp. The graph shows that sodium tripolyphosphate treated samples increase in discoloration more rapidly than the control or ascorbic acid-citric acid treated shrimp as temperature increases. This interaction manifests the point that sodium tripolyphosphate does not act to prevent discoloration due to temperature variations and may even cause discoloration when utilized as in these experiments. A rise in discoloration was evident in all the samples as the temperature rose. This, again, emphasizes the importance of maintaining as low a temperature as economically feasible.

The interaction between the storage temperature of
Figure 13. The Effects of Storage Temperature and Treatment of the Breaded Shrimp as Evaluated by a Sight Panel
the product and the days on ice prior to breading is highly significant. According to Figure 14, shrimp breaded at 0 days on ice had the lowest index of discoloration at -20°C followed by breading at 8 and 16 days. However, with an increase in temperature discoloration occurs faster in samples breaded at 8 days than samples breaded at 16 days. This inconsistent trend was probably caused by the greater degree of decomposition in the shrimp after 16 days on ice. The discoloration at this stage creates a uniform discoloration throughout the sample, possibly causing the panelist to give it a lower discoloration rating. This interaction illustrates that shrimp should be breaded in the freshest state possible and then maintained at a constant low temperature while in storage.

The highly significant interaction between the type of treatment given to shrimp and the number of days the shrimp are on ice prior to breading is shown in Figure 15. The response to discoloration in ascorbic acid-citric acid treated breaded shrimp was low and consistent when compared to the control and sodium tripolyphosphate treated samples. It is apparent from this relationship that the ascorbic acid-citric acid treatment is efficient in retarding discoloration reactions in breaded shrimp that have been on ice prior to breading as long as 16 days. It is recommended that breaded shrimp be treated with an ascorbic acid-citric acid mixture. Sodium tripolyphosphate is
Figure 14. The Effects of Storage Temperature and Days on Ice before Breading as Evaluated by a Sight Panel
Figure 15. The Effects of Treated Breaded Shrimp and the Days on Ice before Breading as Evaluated by a Sight Panel
inconsistent and does not show a trend of discoloration prevention in breaded shrimp.

The presence of iron ions in breaded shrimp interacting with the length of time the shrimp were kept in cold storage is highly significant. It can be seen from Figure 16 that the presence of iron ions increases discoloration at a faster rate at the end of 18 weeks of storage than if no ions are present. This interaction demonstrates the importance of moving the breaded shrimp as quickly as possible through the distribution chain without unnecessary delays.

Figure 17 plots the highly significant interaction between the temperature of storage and the length of time the samples are maintained at their respective temperature in cold storage. Discolorations are more prevalent and occur at a faster rate in samples stored at ±0°C than in samples stored at -20°C. This again illustrates the important role of low temperature in storage, especially when the storage period is over several months. Many times temperature fluctuations occur while the frozen products are in transit to distribution points.

The highly significant interaction between mode of treatment and weeks of storage, illustrated in Figure 18, points out that ascorbic acid-citric acid treated breaded shrimp are less prone to discoloration than either the control or sodium tripolyphosphate treated shrimp. The
Figure 16. The Effects of Iron Ions and the Length of Storage Time as Evaluated by a Sight Panel
Figure 17. The Effects of Storage Temperature and the Length of Storage Time as Evaluated by a Sight Panel
Figure 18. The Effects of Treated Breaded Shrimp and the Length of Storage Time as Evaluated by a Sight Panel.
data from the sodium tripolyphosphate treated shrimp is inconsistent and appears to have little effect in preventing discoloration.

A highly significant interaction between weeks of cold storage and number of days the shrimp are maintained on ice was observed. Clearly, as shown in Figure 19, the earlier the shrimp are breaded, the longer they can be kept in cold storage. Again, the shrimp held 16 days on ice gave inconsistent plots.

**IV. Volatile Bases Analysis**

An examination of volatile bases was conducted to determine the volume of volatile nitrogen produced under different conditions of storage. Since volatile bases produced from the shrimp are responsible for pH increases in breading material and since a high pH in breading will cause discoloration reactions to take place on breaded shrimp, this experiment was designed to show the amount of volatile bases given off under various conditions of storage that may be encountered by the shrimp from the time it is caught until it is consumed. Milligrams of volatile nitrogen per 100 grams of sample were calculated over a 20-day period of time (1) on ice, (2) under freeze-thaw conditions, and (3) under constant frozen temperature of -20°C. Reference to Figure 25 shows the relationship of the volatile nitrogen in each of these categories. Notice
Figure 19. The Effects of Days on Ice before Breading and Length of Storage Time as Evaluated by a Sight Panel
Figure 20. Discolored Samples Produced Commercially by a Breaded Shrimp Processor
Figure 21. Control Breaded Shrimp Stored at ±0°C for Approximately 3 Months (dark areas denote "yellowing").

Figure 22. Control Breaded Shrimp, Containing Iron Ions, and Stored at ±0°C for Approximately 3 Months (dark areas denote "graying").
Figure 23. Sodium Tripolyphosphate Treated Breaded Shrimp Containing Iron Ions and Stored at ±0°C, for Approximately 3 Months

Figure 24. Ascorbic Acid-Citric Acid Treated Breaded Shrimp Stored at ±0°C for Approximately 3 Months
Figure 25. The Influence of Storage Conditions on the Production of Volatile Bases in Shrimp
the increase in volume of volatile base production when the samples were maintained on ice. A smaller increase is evident in samples undergoing freezing and thawing, while samples under a constant temperature of -20°C showed little increase. Differences are also noted in different sections of the breaded shrimp samples.

In order to determine the significance of the differences in volatile base production, a statistical analysis of the data was performed. Table 6 shows the main effects and interactions found in this experiment along with the significance of each.

Findings shows that the main effects of the temperature of storage and the days in storage were highly significant. This indicates that all these effects have a relationship in influencing the amount of volatile bases produced. The main effect of shrimp tail section was found to be non-significant, showing that volatile bases are given off equally in any one area of the shrimp flesh. However, it should be kept in mind that the shell at the posterior end may funnel and concentrate the volatile bases, thus creating concentration areas and giving rise to pH increases, which in turn cause discoloration.

A significant interaction was observed between the temperature of storage and the amount of volatile base production in each section of the shrimp. According to Figure 26, the shrimp stored under freeze-thaw conditions
TABLE 6
Analysis of Variances for Volatile Base Production in Shrimp

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage Temperature</td>
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<td>35558.5000</td>
<td>1195.6620**</td>
</tr>
<tr>
<td>Days of Storage</td>
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<td>14167.6875</td>
<td>476.3907**</td>
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<tr>
<td>Section of Shrimp</td>
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<td>192.5607**</td>
</tr>
<tr>
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<td>131.4757</td>
<td>4.4209*</td>
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</tr>
<tr>
<td>Total</td>
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</tbody>
</table>

All main effects and interactions were tested using the error value.

*S*Significant at the 5% level

**S**ignificant at the 1% level
Figure 26. The Effects of Storage Temperature versus the Section of Shrimp in Volatile Base Production
and at \(-20^\circ\text{C}\) showed very little difference in volume of volatile bases in the anterior end as opposed to the posterior end. In fact, smaller amounts of volatile bases were present in the posterior area than in the anterior area. However, when the shrimp were maintained on ice, more volatile bases were produced in the posterior region as compared to the anterior area. This could be due to the increased bacterial growth in that area because of the protective characteristics of the shell at that end.

An observation made between storage temperature and days of storage shows a highly significant interaction. From Figure 27, it can be seen that as the days on ice increase so does the amount of volatile bases. Samples held at \(-20^\circ\text{C}\) show the least amount of increase, while samples undergoing freezing and thawing show a higher increase. Shrimp samples held on ice show a very large increase of volatile bases after 20 days. This graph manifests again the importance of processing fresh shrimp to avoid discoloration.
Figure 27. The Effects of Storage Temperature versus the Days of Storage in Volatile Base Production
SUMMARY

The factors responsible for causing or influencing discoloration in breaded shrimp were studied. Preliminary studies were conducted to ascertain the basic factors responsible for discoloration.

By means of a sight panel, the factors of (1) iron ions, (2) temperature, (3) treatments, (4) days on ice, and (5) weeks in storage were evaluated. The treatments consisted of (1) breading with an ascorbic acid-citric acid mixture, and (2) sodium tripolyphosphate treated shrimp to test the effectiveness of each to prevent discoloration.

When the pH of the breading was alkaline, it was shown to cause discoloration. Based on this, an experiment was designed to test (1) temperature of storage, (2) days in ice prior to breading, (3) weeks in storage, and (4) section of shrimp, as factors capable of causing or influencing pH. The section of the shrimp refers to the shrimp tails divided into the anterior end and the posterior end.

Due to their ability to cause changes in alkalinity, volatile base production was also evaluated as a factor influencing discoloration.

Statistical analyses were performed on the data to
determine the significance of the factors involved in discoloration.

From the results of these experiments, a summary of conclusions is listed below.

1. It was shown that the condition of the shrimp at the time of breading can influence discoloration. The presence of iron ions can also affect discoloration. Breaded shrimp should always be maintained at as low a temperature as economically feasible, while avoiding freezing and thawing. Excessive storage times should be avoided by moving the product to the consumer as rapidly as possible.

2. Findings showed that temperature fluctuation, quality of shrimp at the time of breading and length of time of storage, all contribute to an increase in pH in breaded shrimp. Significant differences of pH in definite parts of the same shrimp samples were observed.

3. Volatile base production is high if shrimp are iced during storage, and therefore shrimp should be processed as rapidly as possible. Apparently, discoloration is enhanced when the shell is not removed from the shrimp tail.

4. An ascorbic acid-citric acid mixture was very effective in preventing discoloration in breaded shrimp, even in stressed samples. Sodium tripolyphosphate, in concentrations used in the existing experimental conditions, did not prevent discoloration.
SELECTED BIBLIOGRAPHY


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VITA

The author was born on September 19, 1946, in Alexandria, Louisiana. He attended Nederland High School located in Nederland, Texas, and was graduated in May, 1965. He entered Louisiana State University and received his Bachelor of Science degree in January, 1970. He was commissioned a Second Lieutenant in the United States Army Reserves in May, 1970.

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Major Field: Food Science

Title of Thesis: "Causes and Prevention of Discoloration Reactions in Breaded Shrimp"

Approved:

[Signatures of Major Professor and Chairman, Dean of the Graduate School, and Examining Committee members]

EXAMINATION AND THESIS REPORT

Date of Examination: November 14, 1973