A COMPARATIVE SURVEY OF CERTAIN FACTORS RESULTING FROM THE LYOPHILIZATION OF COOKED SHRIMP

A DISSERTATION

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CHAPTER I

I N T R O D U C T I O N

Freeze drying fulfills many of the long sought criteria for successful food preservation, particularly for relatively expensive raw material that is highly perishable in nature but at the same time is adversely affected in terms of quality by conventional drying methods. Shrimp is such a product, because of its characteristic flavor, taste and texture. Disadvantages in the shipping and storage of a frozen product make a method of preservation that does not necessitate special equipment highly desirable. Certain textural and reconstitutional properties resulting from lyophilization of shrimp remain a challenge to food technologists both as to cause and as to methods of prevention.

According to Bird (7) shrimp was one of the first foods to be freeze dried. In 1969 approximately 375,000 pounds were produced in the United States and the quantity is expected to increase in succeeding years (33). The Quartermaster Corps alone purchased 336,000 pounds in 1969 and plans to purchase 385,000 pounds in 1970. Freeze dried foods are extremely advantageous for service men in the field of action. The Quartermaster Corps of the Army is

currently purchasing the majority of freeze dried foods processed in the United States. The bulk of freeze dried foods is utilized in the form of pre-cooked food combinations that constitute a meal when rehydrated.

Moorjani and Dani (90) have emphasized that despite the many advantages of freeze drying as a method of preservation for shrimp, the fact is that an undesirable toughening occurs with rather severe histological changes, loss of flavor, loss of color and dryness of mouthfeel. In order to alleviate these problems more information as to the possible causes is needed.

STATEMENT OF PROBLEM

The prime objective of food product design is acceptability. This may be assessed through subjective analysis by means of a taste panel of trained judges and/or by objective analyses of chemical, physical and microbiological nature. This study investigated the changes in frozen cooked shrimp resulting from the process of lyophilization. The factors determining acceptability and the degree of acceptability were determined by organoleptic evaluation. Chemical and physical changes that could be related to the acceptance of the product were investigated. Emphasis was placed on factors relating to

tenderness. Data on changes resulting from the lyophilization of shrimp are limited.

According to Griswold (46), toughness in meats is generally attributed to connective tissue present and the changes therein resulting from the cooking process. The author of this study will investigate connective tissue changes and intramuscular protein changes resulting from freeze drying.

Goldblith, Karel and Lusk (40) have reviewed and delineated the problems associated with the freeze drying of foodstuffs. Major considerations include: the quality of the raw product, physical and chemical properties whose changes influence the quality of the freeze dried product, microbiological aspects, and food engineering parameters that affect quality. Few raw materials offer a greater quality control challenge than does shrimp.

Freeze drying, as a means of preservation, as reported by Harper and Tappell (51), offer advantages such as integrity of shape and appearance upon rehydration; extremely low microbiological activity as a result of low moisture level; low density for reduced shipping costs; long shelf life in fluctuating ambient atmospheres; and easy rehydration

necessitating no equipment or heat. The food products can be freeze dried in the cooked state as well as in the raw, thus eliminating preparation procedures before consumption.

Juiciness and favorable mouthfeel are associated with fat and moisture in meat. According to Chastain (13), little or no fat is present in shrimp. The author will investigate the "free" and "bound" water in an attempt to show cause for decreased acceptability of freeze dried shrimp.

OBJECTIVES OF STUDY

The objectives of this study are to determine the effects of lyophilization on cooked shrimp as to 1) microbiological quality, 2) changes in tenderness and 3) loss of juiciness.

CHAPTER II

REVIEW OF LITERATURE

The method of drying biologicals by sublimation of the ice under reduced pressure in vacuo has been known for over 50 years. Shackell, in 1909, applied vacuum pumps to his experiments to accelerate the process as cited by Harper and Tappell (51). It was not until shortly before World War II that primitive designs of laboratory freeze dryers were made commercially available. During this war much attention was given to the development of equipment and techniques for the purposes of supplying enormous quantities of dried plasma and penicillin to the armed forces. By the end of the war, the technique had become accepted as one of the best methods of preserving biological materials. Studies were undertaken during this period both in the United States and in Great Britain on freeze drying of meats, vegetables and fruit juices. A good review of early development of the process was prepared by Flosdorf (35).

The lyophilization systems in existence at the close of World War II were not of sufficient magnitude and the process was too costly to be feasable for food products. However, the renewed interest of the armed forces in

freeze dried foods at the onset of the Korean conflict provided the necessary stimulus for additional research. Harper and Tappell (51) reported that in 1952 research sponsored by the Quartermaster Food and Container Institute of the Armed Forces on freeze dried meat was begun.

By 1960 there were only two major processors marketing freeze dried foods in the United States. Interest in this method of food preservation grew so rapidly that by July, 1963, the United States Department of Agriculture published a list of 638 selected references including books and articles on freeze drying.

The most important application of freeze drying to food appears to be for meats, including beef, pork, chicken and fish. Freeze drying seems to overcome, in whole or in part, all the undesirable characteristics of ordinary high temperature or vacuum drying. Burke and Decareau (10) have listed changes wrought by drying as:

1) Pronounced shrinkage of solids
2) Migration of dissolved constituents to the surface of solid materials
3) Extensive denaturation of proteins
4) Case hardening - the formation of a relatively hard, impervious layer at the surface that slows the rate of rehydration and reconstitution
5) Formation of hard, impervious solids when drying liquid solution
6) Undesirable chemical reactions in heat sensitive materials

7) Excessive loss of desirable volatile constituents
8) Difficulty of rehydration as a result of one or more of the above changes.

The freeze drying technique is similar to ordinary vacuum distillation with one very essential difference. The material to be dried must first be solidly frozen and then subjected to a very low absolute pressure and a controlled heat input. Under these conditions the water content, in the form of an ice matrix, is subjectively removed via sublimation. Ice crystals form into vapor, completely by-passing the intermediary liquid phase.

Many of the various aspects of the freeze drying process have been investigated. Love (78) studied the effect of freezing rate on the denaturation of cod muscle protein. This investigator found that the rate of freezing does affect the rate of protein denaturation during cold storage of 30 and 90 days. From this study the postulation emerged that the locus of denaturation was at the end of the ice spears that formed in the center of each cell.

Hiner, Madsen and Hankins (60) worked with frozen beef to determine factors relating to drip loss. Histological studies indicated that drip losses decreased as the freezing temperatures were lowered from 18° F. to -114° F. Tenderness increased slightly with lowered freezing rates.

Deatherage and Hamm (26) showed that quick freezing (-55° C.) resulted in less protein denaturation than slow freezing (-15° C.) as shown by the amount of resulting drip. These workers hypothesized that the small crystals formed during fast freezing cause a loosening of the protein structure. This produced an increased electrical charge on the protein structure so that more water molecules could be bound.

The movement of water vapor from the ice interface to the exterior of the food was discussed by Tappell (121) and was further investigated by Robson and Rowe (105). Once the ice phase has disappeared, there is still bound water that must be removed for storage stability. Bound water is the moisture retained by capillary forces.

Heat transfer, or energy supply, is important to freeze drying since each pound of water sublimed absorbs approximately 1,200 BTU of energy as reported by Burke and Decareau (10). The typical freeze drying operation utilized radiant heat in the sublimation process; however, Hoover, Markantonatos, and Parker (62) successfully utilized ultra high frequency (uhf) power for accelerating the freeze drying of foods. Copson (18) successfully used microwaves in the sublimation process but the cost makes such use impractical industrially.

The optimum residual moisture content has been studied by Salwin (107). The accepted rate of 2.0 per cent or less is actually a holdover from the freeze drying of pharmaceuticals but Salwin and Slawson (108) indicated that this figure is too low for many food materials.

ORGANOLEPTIC EVALUATION

Selection Of A Taste Panel

Modern man's intellect is dominated by "higher" more highly developed senses of sight and hearing; however, tasting and smelling also play an important part in sensual and emotional life. Kalmus and Hubbard (70) described taste and smell as chemical senses since "it is believed that the presence of certain substances in appropriate concentrations provide the specific stimulus." Evidence suggests that the sense of smell is on an order of greater magnitude and is more acute than the sense of taste. Taste normally is active during drinking, biting, chewing, or swallowing. The complex sensations which make up one's impressions of specific kinds of foods are the ones people recognize. The Arthur D. Little Company (34) reports that it is possible to obtain quantitative and objective information concerning neural response of the sense receptors by using electrophysical techniques. The fact is, however, that only with the human subject can these different criteria

be related with any significantly reliable accuracy to human acceptance. As a consequence the use of taste panel judges has become an integral part of food acceptance research.

Pfaffman (98) states that taste sensations are directly related to "taste buds" containing gustatory cells with terminal microvilli found primarily in the fungiform, foliate and circumvallate papillae of the tongue and adjacent structures of the throat. The tongue surface is insensitive to taste according to Janousky (68) but there is sensitivity to salt around the edges, sensitivity to sweet primarily at the tip, sour at the sides and bitter at the back. Platt (99) indicates that the best measuring instrument for food acceptance is the human being but that humans vary widely in sensitivity, that is, in the strength of sensations which they can distinquish. Even with exactly the same degree of sensitivity they vary in their preferences. The interactions of every pair of human taste qualities - salty, sour, sweet and bitter - were studied by Kamen and others (71) and found to be positively related to each other but to varying degrees. Richter and MacLean (102), Henkin, Graziadei and Bradley (56), Caul (11), and Dawson et al (24, 25) have each found that threshold testing is of value in the selection of taste panel judges.

Baten (4) found that the young and the more mature individuals do not give the same evaluation to certain foods. Young people distinguish differences between varying strengths of materials while more mature individuals do not. Laird and Brun (74) found that, in individuals from 18 to 40 years of age, the taste preferences in degrees of sweetness and sourness are similar. Past age 40 there is a definite trend toward loss of taste sensitivity. Sex may also have some influence on ability to evaluate taste sensations. Wallen (126) found that strong flavored foods are more unpleasant to females than to males. Parr (96) showed the existance of trends in taste blindness among certain races.

Hardy (50) advanced the theory in 1908 that a genetic trend in ability to taste exists. This theory has been explored by many research workers including Blakeslie and Salmon (8) who tested threshold levels of sensitivity for phenylthiocarbamide; Fernberger (32), who tested para-ethoxy phenlthiocarbamide; Harris and Kalmus (53), who tested for sensitivity to phenylthiourea; and Hoover (61) and Peryam (97), who tested sodium benzoate. In 1960 taste test papers were developed by Hundley (63) for rapid and simple genetic taste testing to determine inability to taste.

Types Of Testing

Figure I represents an arbitary organizatien of sensory test methods by Ellis (30) into five groups - difference, analytical, sensitivity, ranking and rating. Other tables of sensory tests include those published by Caul (11), Juran (69) and the Committee on Sensory Evaluation of the Institute of Food Technologists (15).

Ellis (31) set forth criteria for the physical plant needed for reliable taste panel evaluation, for coding and order of sample presentation and for sample size. Amerine, Pangborn and Roessler (1) covered the entire scope of factors affecting sensory evaluation with multiple plans set forth for particular testing parameters. The negative effect of smoking immediately prior to taste testing was shown by Hall and Blakeslie (47). Mitchell (89) examined duration of tasting periods that result in reliable judgments.

MICROBIOLOGICAL ASPECTS

Since freeze drying does not depend upon sterilization but rather upon a low moisture content for the attainment of an appreciable shelf-life, the microbiological aspects of this process are of considerable importance. Freeze drying is used successfully for the preservation of bacterial strains. In the preservation of cultures, the

Method	Number of Samples Evaluated	Served	Number of panelsits	Purpose
I. Difference				
A. Triangle	1	3	6-25 Trained	Detecting difference when inter-sample effects are at a minimum.
B. Duo-Trio	1	3	6-25 Trained	Detecting difference when slight intersample effects may be present; training.
C. Paired Comparison	2	2	6-25 Trained	Comparing samples for some specific characteristic.
II. Sensitivity				
A. Threshold	5-10	5-10	12-24 Untrained for toler-ance	Selecting panel members evaluating ingredients, maintaining quality con-trol.
B. Dilution	5-10	5-10	12-24 Trained	As in threshold method.
III. Rating				
A. Hedonic	1-4	1-4	1-4 Trained	Determining of acceptability.

Figure 1

SENSORY TEST METHODS

organisms are not subjected to elevated temperatures during sublimation. This is in contrast to freeze dried foods in which the food material is, to hasten the drying process, subjected to elevated temperature programming during vacuum drying.

Food products contain mixed and charasteristic flora which appear in various physiological stages of growth. Each material will have undergone specific processing methods: freezing; drying under vacuum at elevated temperatures; packaging, usually in inert atmosphere; and storage at ambient temperatures until rehydrated for use. A report from an Institute of Food Technology symposium (66) indicates that the recovery of microorganisms will depend on many factors, for instance:

1) The initial physical state;
2) The locations of the organisms on or in the food;
3) The processing history of the food;
4) The composition of the food;
5) The conditions maintained during storage; and
6) The method of rehydration employed.

Each procedure of freeze drying exerts specific stresses on the product. Early research by Bird (7) indicated that freezing, per se, will normally result in the destruction of a portion of the microbiological flora. The rate of freezing affects survival of organisms as does the location of the organism. The surface organisms

are cooled at a much faster rate than those insulated in the interior of the material.

For sublimation to occur, water vapor must be removed from the solid state. Charm and Ronswalli (12), in analyzing the eutectic points in miscellaneous foods, found certain foods to have eutectic points as low as -40° C. and below. It is thus conceivable and probable that a portion of the water content associated with such a food material, and the bacteria therein, is not sublimed but is dried from the liquid state.

According to Luyet (84) bacteria can survive freezing due to their ability to undergo dehydration as the host material freezes and it is this tolerance to dehydration which actually accounts for the survival that occurs. The final temperature to which an organism is subjected can also influence the survival. Borgstrom (9) indicated the rate of survival is inverse to the decrease in temperatures.

The opinion that a number of cells may be injured after freezing and may be more susceptable to further injury imposed by drying resulting in a lower colony plate count for rehydrated freeze dried samples was suggested by Silverman and Goldblith (116). Sinskey and others (117) showed a lethal effect of high platen temperatures versus a minor effect of moderate platen temperatures.

The duration of drying and the final moisture content likely are important factors in microbial survival. Most investigators agree that excessive drying reduces viability. Investigations by Silverman and Goldblith (115) indicated that glucose may have effects other than moisture regulation on bacterial survival. Physiological saline also may have an effect though an injurious one to growth.

Sinskey and other (117) demonstrated that moisture level has a greater effect than storage in air or nitrogen on the survival of bacteria. Thus the packaging for proper closure in freeze dried foods is of utmost importance. Packaging in laminated foil and tin cans in nitrogen or inert gas is most effective.

Of the four bacterial strains freeze dried by Pablo, Sinskey and Silverman (95) at two platen temperatures, the Gram positive cocci were much more resistant than the Gram negative organisms. Charm and Ronswalli (12) also found a greater viability of Gram positive organisms. Spores are extremely resistant to drying and to storage according to Marshall, Murrell and Scott (85).

Silverman and Goldblith (116) conducted studies that indicated the bacteria flora of commercial freeze dried foods decreased during freeze drying. The principle microbiological problem in freeze dried foods lies in the preparation stages prior to freezing and in treatment following rehydration.

Foda and Waraki (36) reported fewer organisms surviving the freeze dehydration of green beans than survived the freezing step only. These workers found that the vitamin content had changed only slightly from the fresh state for thiamine, riboflavin, niacin and Vitamin A; however, the size of green beans was smaller and the shape was irregular in the freeze dried and rehydrated samples.

The most common analytical technique being employed for microbiological evaluation is that of the aerobic colony plate count which does not include obligate anaerobes. Though practical for in-plant testing, the method is criticized since the detection of a low number of organisms in a sample does not necessarily reflect the actual quality or the absence of pathogens.

Saleh, Silverman and Goldblith (106) analyzed eight commercial freeze dried meat products for total aerobic colony plate counts. enterococci, coliforms, salmonellae and coagulase-positive staphylococci. A comparison was made as to the effectiveness of two different media for recovery of these microorganisms. These investigators were unable to recover coliforms or Salmonella organisms but did detect Staphylococcus aureus and "fecal streptococci" in some samples. Although most total colony plate counts were low, these pathogens were present in small numbers. The media recommended for use in plating shrimp samples is tryptocase

soy agar with 0.5 per cent yeast. Clark, Reinbold and Rambo (14) recovered enterococci from 18 to 35 samples of freeze dried vegetables and recovered coliforms in 15 of the 18 samples tested.

Silverman and Goldblith (116) reported that according to Dack, all enterotoxigenic strains of Staphylococcus aureus are coagulase-positive but the converse is not true. Thus the presence of coagulase-positive strains has been accepted for the evaluation of the enterotoxin potential of a food. Insalada, Borker and Harrow (65) stressed the need for rapid in-plant methods of microbiological testing particularly for Staphylococcus aureus. These authors outlined perspectives for such tests but at the present time methods have not yet been developed. Immunological testing is not usually practiced because of the difficulty in securing sufficient quantitites of purified antisera.

Saleh, Silverman and Goldblith (106) evaluated two selective media for Staphylococcus aureus: the Baird Parker and the tellurite-polymyxin egg yolk agar of Cristley. Egg yolk reactions were most consistent after 48 hours at 37° C. The Baird Parker medium was also effective in recovery of Staphylococcus aureus from freshly hydrated freeze dried foods.

The fate of bacteria in chicken meat during freeze dehydration, rehydration and storage was studied by May and Kelly (86). Survival of the natural flora was determined after freeze dehydration and rehydration at varying temperatures including room temperature for 30 minutes. Meat samples were inoculated with Staphylococcus aureus, then dried, rehydrated, and stored at the same temperatures as those not inoculated. Numbers of surviving organisms in the inoculated samples were determined with the use of both selective and nonselective media. Approximately 32.0 per cent of the organisms survived the dehydration and rehydration at room temperature.

Rehydration studies by Pablo, Silverman Goldblith (93) showed Staphylococcus aureus and "fecal enterococci," as naturally occurring contaminants in freeze dried chicken, can grow in competition with the other natural flora at 20° C. and above. The competition may be due to the nature of the microbial distribution on the chicken surface.

Pablo, Silverman and Goldblith (94) studied the growth patterns of microorganisms in rehydrated freeze dried shrimp. Growth patterns indicated a change in the bacterial spectrum in response to temperature. This was pronounced when rehydrated shrimp was stored at 4° C.; the essentially mesophylic population were present in fewer numbers than

when stored at other temperatures. It was apparent that, in common with other types of perishable food products, rehydrated shrimp can have a significantly extended storage life by the use of low temperatures.

Silverman, Davis and Nickerson (115) compared several techniques for their ability to isolate coagulase-positive staphylococci. Selective enrichment involving inhibition of competing organisms was achieved by a 10.0 per cent sodium chloride solution followed by selection from mannitol salt agar or by the ability of this organism to produce halos on egg yolk agar and on Tween 80.

The fact that bruised tissues are subject to bacterial invasion of both aerobic and anaerobic organisms was shown by McCarthy, Brown and Hamdy (87). A study of poultry, bruised experimentally, revealed that 61.0 to 74.2 per cent of the tissue examined harbored aerobic and anaerobic bacteria. The number of bacteria increased in the early stages (one to two days) of healing and gradually decreased in four to six days. Isolated were 86 predominant bacterial strains of which 36.0 per cent of the Gram positive strains belonged to the genus Staphylococcus of which Staphylococcus aureus represented half the strains.

Silverman and Goldblith (116) reported that spore-formers such as Clostridium botulinum, Clostridium perfringens and

Bacillus cereus can be expected to be extremely resistant to normal freeze drying procedures in their spore state. The extent to which the presence of these organisms pose a threat to public health will depend on the extent of their contamination of the product and the treatment to which the product is subjected following rehydration.

Obrien (92), reported a rapid differentiation of bacteria by gas chromatography in foods of certain carbohydrate composition. Metabolic products produced by washed bacterial cells suspended in buffered glucose were analyzed by gas chromatography. Organisms tested included: Escherichia coli, Aerobacter aerogenes and species of Salmonella, Pseudomonas, Vibrio, Achromobacter, and Flavobacterium. Chromatograms of the metabolic products formed by each organism were different with the exception of Pseudomonas fluorescens and Vibrio percolans which did not produce any detectable products by the methods employed.

COLOR ANALYSES

Color, flavor and odor changes occuring in shrimp as a result of freezing are also evident in freeze dried shrimp. Lemon (75) noted these organoleptic changes as well as textural changes.

The red color of cooked shrimp is due to a carotenoid pigment, astaxanthin. The literature on the carotenoids,

including astaxanthin, is extensive and has been reviewed by Goodwin (42). Early literature refers to astaxanthin and astacin as the same compound. Goodwin and Srisukh (43) obtained two separate fractions on extracting the pigment from lobster. The reddish pink fraction was shown to be astaxanthin and the orange-red fraction, astacin. Sumner and Sumner (118) indicated that astacin is the oxidized form of astaxanthin.

The initial color change occurring in frozen cooked shrimp is the result of the conversion of the original pinkish red pigment to one which is more orange in color. The bleaching is apparently analogous to that which takes place when beta-carotene is oxidized and bleached. It is possible that the bleaching of astaxanthin is related to the oxidation of fat present; however, the amount of fat is so small in shrimp that Chastain (13) was not able to extract any. In freeze dried shrimp, this bleaching occurs markedly when packed in the presence of oxygen and to a limited extent when shrimp is packed in nitrogen. More research is needed to explain this oxidative color change.

Chastain (13) developed a colorimetric procedure for detection of color loss in order to obtain quantitative data on the degree of oxidation. The red pigments were

removed from ground fish tissue samples by acetone. The pigment extracts were analyzed spectrophotometrically. Peaks were observed for fresh and frozen shrimp at both 474 mμ and 450 mμ. The frozen shrimp extract produced a higher peak at 450 mμ. The curves for astaxanthin in carbon disulfide published by Goodwin (42) were smooth on either side of a peak at 475 mμ. Chastain attributes the second peak at 450 mμ to the presence of some beta-carotene and/or crytoxanthin in the extractant.

Lusk, Karel and Goldblith (82) studied astacene pigment loss (refering to pigment called astaxanthin by Chastain) in freeze dried shrimp and salmon as a function of freeze drying temperature, temperature of storage and the presence or absence of oxygen during storage. This study indicated that temperatures of storage and oxygen concentration were the most important factors determining the rate of loss of the pigment. The loss of color is greater in freeze dried shrimp than in frozen shrimp, thus the above study accounts for the greater degree of pigment degradation in freeze dried shrimp.

In another study, Lusk, Karel and Goldblith (83) investigated the effect of platen temperature, freezing temperature and chamber pressure on the rates of freeze drying for shrimp. No positive correlation was shown in relation to pigment change and the factors studied.

Moorjani and Dani (90) found the degree of color loss to be a more sensitive index of deterioration than organoleptic judgments. This color change was a more accurate measure of deterioration than the loss of texture due to protein denaturation.

Another method of reading color differences is by calibrated light reflection. The Agtron is a direct-reading reflectance spectrophotometer designed to measure the relative spectral qualties of product samples.

TENDERNESS

Toughness and lack of juiciness in fish have been noted by Connell (17) as the most common defect of freeze drying. Hamdy, Cahill, and Deatherage (48) refer to undesirable protein changes resulting from the freeze drying of flesh foods. An increase in cross linking of protein chains and a loss of gel forming ability may be responsible for this toughening. The cross linking could also cause dryness, which is due to poor water-binding capacity.

In a review of structures of various naturally occurring proteins by Seifter and Gallop (113), a microscopic examination revealed that fish muscle cells present all the usual features of skeletal muscle cells; namely, myofibrils, sarcoplasm, sarcoplasmic reticulum and nuclei. The muscle as a whole contains relatively small amounts of connective

tissue between cells of capillaries and of blood vessels. The cells and myofibrils contained with the muscle cells of fish are larger than those of beef, rabbit, and pork.

Measuring Tenderness

Methods other than chemical for determining tenderness of meats include: physical, enzymatic, histological and sensory. This study will relate physical measurement to sensory judgments.

Among the physical measurements of tenderness in meats that have been used, shearing devices are among the most commonly accepted criteria. The first shear device was assembled and described by Warner (127) in 1927. Later modifications were made by Bratzler and the instrument came to be known as the Warner-Bratzler shear. This device is the one most commonly used for measuring texture at the present time. Several modifications have been made but the basic mechanism remains the same.

Another device was developed by Kramer (73) and his associates at the University of Maryland. Highly significant correlations have been found in various laboratories between Kramer Shear press readings and sensory evaluation. This tool was employed in the present study to determine differences of tenderness in frozen cooked and freeze dried cooked shrimp.

Other devices for measuring tenderness in foods include: penetration methods such as the Christel Texturemeter, the Slice Tenderness Evaluator, the Lynn-Mitchell Maturometer and miscellaneous penetrometers. Biting devices such as the Lehmann Dexometer, the Volodkevick Bite Tenderometer, the Winkler Measurometer and the Massachusetts Institute of Technology Denture Tenderometer are used by some laboratories. Mincing and compression devices, consistometers and tensile strength apparati were reviewed by Szczesniak and Torgeson (120) in a report on methods of tenderness testing.

Connective Tissue

Seifter and Gallop (113) reported that little is known concerning fish muscle connective tissue, but much information that is known is related to texture. The amount of connective tissue is less in fish than in meat, varying from 3.0 per cent in gadoids to 10.0 per cent in elasmobranchs as indicated by Harrington and von Hippel (52). The gelatinization temperature is lower than that for meat as evidenced by the low temperature at which "flaking" or fragmentation becomes possible. The tenderness of fish can be attributed to two factors: its low content of connective tissue; and the definitely soft and easily degraded nature of the connective tissue. In practice, however, fish is more difficult to process texturally-speaking, than carcass meat. According to Bird (6) no

authenticated instances have been reported of the production of freeze dried fish which has not suffered some unacceptable degree of textural deterioration.

Connective tissue of meat consists of collagenous, elastic and reticular protein fibers embedded in an amorphous ground substance. The proportion of these fibers and the composition of the ground substance differ with the tissue. Little research has been directed toward the connective tissue of shrimp though an extensive amount of research has been reported on other flesh foods. Szczesniak and Torgeson (120) reported that as early as 1929, connective tissue was found to be the major factor contributing to toughness. Hiner, Anderson and Fellers (59) compared histological differences in the amount and character of connective tissue from a wide variety of beef samples of known history. The tender muscles of these samples contained both less elastic fibers and less collagenous fibers. Cover, Ritchey and Hostetler (19) found that muscles containing greater amounts of collagen became progressively more tender as internal temperature increased. In further studies, Cover, Ritchey and Hostetler (21) showed greater fragmentation at higher internal temperatures in muscle containing greater amounts of collagen but less fragmentation at higher internal temperatures in muscles containing less collagenous material.

Herring and Cassens (58) studied the factors affecting solubility of collagen in bovine muscle. Collagen solubility decreased with maturity though total collagen differed only slightly. The correlations for solubility of collagen and tenderness were low. Goll, Bray and Hoekstra (41) had previously reported lower nitrogen and higher moisture in young animals as compared to older groupings. Kim and Ho (72) correlated the tenderness of cooked beef with collagen content of the raw muscle and found a highly significant but somewhat low correlation. Loyd and Hiner (81) separated alkali-insoluble protein into three fractions and showed a significant correlation between the total hydroxyproline content of the fractions from beef muscles and their tenderness.

As a means of stydying collagen content, the determination of the amino acid hydroxyproline has been accepted as the most accurate index of connective tissue content of biological materials as indicated by Husaini and others (64). Neuman and Logan (91) employed a quantitative assay method that was well accepted though tedious and time consuming. Prockop and Undefriend (100) oxidized hydroxyproline in the presence of a known excess of alanine to reduce the interference of imino acids. In 1961, Woessner (130) published a method for the quantitative determination of hydroxyproline in biological materials containing as little

as one part hydroxyproline in 4,000 parts of amino acid. This method also calls for fewer steps in the procedure than the Prockop and Undefriend method (100), but was found to produce reliable results. Goll, Bray and Hoekstra (41) converted hydroxyproline values to collagen values with the factor 7.25.

Controversies are apparent in the literature as to causes of toughening. Griswold (44, 45) and Wilson, Bray and Phillips (129) concluded that the connective tissue content was not a critical measure of tenderness. Connell (16) reported that in one study undertaken in Japan in 1950 no relationship of toughness and connective tissue was found in fish. Literature is void of studies determining relative comparisons of collagen and tenderness in shrimp. This study will attempt to determine if the soluble collagen content of frozen cooked shrimp differs from that of freeze dried cooked shrimp.

Variation In Protein Fractions In Muscle

Loss of tenderness and flavor have been attributed to the denaturation of fish muscle proteins in frozen fish. Dyer (27) found that the extent to which this undesirable process had occurred in a muscle sample could be measured by the solubility of the proteins in sodium chloride solutions of varying strength following homogenization. For greater

accuracy of denaturation, Love and Mackay (79), developed a method of measuring the resistance of the individual cells to mechanical breakdown.

According to Love (77) the rate of denaturation in cod fish was governed by the sub-zero storage temperature, however, this process is accelerated as the temperature is reduced. It was concluded by Love (78) that the cell minerals and small organic molecules such as sugars become more concentrated at one end of each cell in freezing than in any other cell location. Seemingly the denaturation occurs in the presence of concentrated salts in liquid solution. The premise that denaturation would cease at low temperatures in which the last trace of liquid had disappeared was postulated by this research worker.

The effect of decreasing the amount of ice in the tissue by the use of glycerol and the effect of the abolition of ice altogether by ultra-rapid freeze method, ideally at -30° C. or below, was found to denature the proteins less than higher freezing rates. The complete prevention of surface desiccation also aided in the prevention of protein denaturation.

Sawant and Magar (112) showed that the solubility of proteins was less when the muscle was coated with a sealing

glaze before freezing. Of greatest importance in this study was the indication that the denaturation of proteins was restricted to the actomyosin fraction of proteins. The sarcoplasmic fraction remained unchanged.

A distinct alteration in the distribution of muscle protein fractions was noted by Love and Robertson (80) in cod fish suffering from starvation. The greatest change was in the actomyosin fraction, with little change occuring in the sarcoplasmic fraction.

Studies under varying conditions on the extractability of the protein fractions, and changes therein upon tenderness have recently been reported by Helander (55). Though a simple method for extracting actin was published by Szent-Gyorgyi (119) in 1951, food technologists did not assess this factor as of importance to tenderness for several years.

A procedure was developed to fractionate the major nitrogen containing components of muscle by Hegarty, Bratzler, and Pearson (54). The relationship of intracellular muscle proteins to tenderness was studied. Several factors were found to correlate with tenderness as measured by shear press and panel evaluation:

1) Sarcoplasmic protein nitrogen with total fibrillar protein nitrogen;
2) Soluble fibrillar protein nitrogen with total fibrillar protein nitrogen and;
3) Water released with total water content.

Fujimaki, Aralawa, Okitani and Takagi (38) analyzed certain fractions of beef sarcoplasmic proteins by chromatographic techniques on cellulose phosphate columns for enzyme activity. Activities were found for aldolase, lactic dehydrogenase and myokinase in three of 10 samples. It was concluded that the individual chromatograph peaks do not contain a particular homogenous protein nor is all of any one protein found in a single specific fraction.

The sarcoplasmic and myofibrillar proteins were extracted from the muscles of two different breeds of pigs by McLoughlin (88). The breed of pig did not affect the extractability of the proteins. It was concluded that the genetic background did not influence the inherent extractability of the muscle proteins.

Bovine sarcoplasmic proteins were studied by Thompson and others (122). Chromatograms showed that definite changes do occur in sarcoplasmic protein. Samples were aged for varying lengths of time and at different temperatures. The sarcoplasmic changes could be followed by the duration of the aging period but did not seem to be a direct measure of tenderness.

Davey and Gilbert (22) conducted a series of experiments on the relationship of the myofibrillar proteins to meat tenderness. Changes in the extractability of myofibrillar

proteins from beef and rabbit carcasses were examined, during aging, using a buffer which dissociates the actomyosin complex from the muscle cell. Approximately 52 per cent of the myofibrillar proteins of unaged meat were extracted in 40 minutes at 2° C.; whereas, from aged meat as much as 78 per cent was extracted. "The increase in the percentage of myofribrillar protein extracted during aging results either from a progressive weakening of the fibrous protein linkages with the insoluble stroma of the mat cell, or from a disintegration of the insoluble stroma itself." A complex mixture of other proteins were shown to make up the extractives of aged tissue in a subsequent study investigation by Davey and Gilbert (23). This indicates the difference to be due to the disintegration of the insoluble meat stroma.

Effect of Freeze Drying on Protein Denaturation

The effect of freeze drying on the denaturation of myosin A and myosin B was investigated by Yasui and Hashimoto (131). The measurement of adenosine triphosphatase activities and solubilities were used to show that the myosins undergo denaturation through the process of freeze drying.

The solubility of the nitrogenous compounds of freeze dried raw beef were observed (to change during storage) by El-Gharbawi and Dugan (29). Generally, total soluble nitrogen, soluble protein nitrogen and soluble non-protein

nitrogen decreased with increasing storage time. Free amino groups also decreased during storage.

Tuomy has published articles on factors relating to the acceptability of freeze dried pork. Tuomy and Feler (124) showed that the lower plate temperatures resulted in more tender freeze dried cooked pork. Tuomy and Helmer (125) investigated the extent of toughening produced by freeze drying on pork loin that could not be related to loin weight or conformation. These investigations showed that the frying method of cooking does not promote tenderness. The hypothesis that a revised cooking procedure could possibly offset the toughening effect of freeze drying was suggested for further study.

Bell and others (5) studied the texture of freeze dried chicken meat. The freeze dried meat was found to be readily distinguishable from the frozen. The freeze drying adversely affected the breast meat more than the thigh meat. Seltzer (114) recommended for economic feasability, that only the highest quality chickens could be freeze dried.

Histological Changes Resulting from Freeze Drying

Histological studies on freeze dried foods are much needed. Moorjani and Dani (90) studied the histological

differences in iced fresh shrimp, air dried shrimp and freeze dried shrimp. In freeze dried shrimp an extensive space system was formed and the muscle bundles shrank considerably. Fresh iced shrimp had a light microporous structure. The freeze dried shrimp were much superior to the air dried shrimp, however.

Water Holding Capacity

Press fluid is an index of "free" water and thus reflects the water binding capacity of meat. Because the juiciness and tenderness of meat may be dependent on the free water-bound water ratio, many workers have sought a valid means of determination.

The concept that fatness results in desirable qualities of juiciness and tenderness was postulated in 1908 by Armsby (2). Neither the role of fat or of water, nor the relationship between the two in meat, have been defined clearly. The quantity of fat in shrimp is too small to be extractable; therefore, juiciness must be relative to moisture within the sample.

Ramsbottom, Strandine and Koonz (101) reported tenderness to be directly related to the water holding capacity of various muscles. Satorius and Child (111) showed that press fluid and total moisture decreased with each increment of temperature increase in beef. The resulting products were

less juicy. The pH dependence of water holding capacity of beef was investigated by Hamm, Reiner and Deatherage (49) as well as the effect of temperature change. The greatest change in water holding capacity occurred between 40° and 50° C. at a nearly neutral pH. A rather complete review of methods of determining press fluids is included in the Hamm publication. One of the techniques recognized for accuracy was developed by this worker. A sample of 300 mg of meat was placed on filter paper between two plates and pressure was applied. Quantitatively the free water was determined by measuring the area wetted by the expressed water.

Wierbicki, Kunkle and Deatherage (128) developed a method using a specially constructed centrifuge tube, a constant temperature water bath, a centrifuge and a magnifying glass. The volume of extractant was read. This method is reproducible, rapid and accurate.

Sanderson and Vail (110) developed a modification of the Wierbicki method commonly called the one minute method. This method uses the difference in weight of the meat sample before and after pressing to determine the amount of press fluid released. A pressure of 2,000 psi was exerted on the sample for one minute. Using this method Sanderson and Vail (109) determined the fluid content and tenderness of three different muscles of beef cooked to three different

internal temperatures. Little relationship between fluid content and tenderness was shown.

In a series of tests on the tenderness of beef, Cover, Ritchey and Hostetler (20) considered juiciness and the softness components of tenderness. This work correlated scores for juiciness and two components of tenderness: softness to tongue and cheek and softness to tooth pressure. Steaks of the longissimus dorsi muscle were cooked to three different temperatures. Correlations calculated on a lot-muscle-temperature basis indicated that juiciness was not closely associated with any of the six components of tenderness.

Ritchey and Hostetler (104) determined free and bound water by a modified hydraulic press method. Losses of free and bound water were evident at each increase of temperature. A large percentage of loss of both occured between 74^{o} and 80^{o} C. Subjective scores indicated drier, harder meat with each increase in temperature.

Ritchey (103) later found that subjective scores for eating quality of two beef muscles, longissimus dorsi and bicepts femoris, cooked to two final internal temperatures, were related to fat content and to the amount of bound and free water. Softness scores were associated with

fat content. The amount of bound water remained relatively constant in raw and cooked steaks.

Literature is not available concerning the amounts or relationships of bound and free water to acceptability in shrimp. Indeed much of the literature cited for all aspects of this study pertain to tissue other than shrimp because of the lack of investigations reported concerning this crustacean.

CHAPTER III

PLAN OF PROCEDURE

SOURCE OF EXPERIMENTAL PRODUCT

Shrimp used in the experiments were of the Peneaus setiferus variety commonly known as white shrimp. The shrimp were obtained from a processing plant in McAllen, Texas. In size, the shrimp used were 40 to 50 count per pound.

In order to get high quality shrimp, company standards require consideration of the type of shrimp, cleanliness of the water in which the shrimp are grown, length of run from fishing ground to shore and the handling procedures employed on the "catch" craft. Even though the catch is iced in the holds, the normal voyage of two weeks permits too great an increase in microorganism count. To secure highest quality shrimp, company standards determine that only shrimp from boats that are out two or three days are purchased and quality control sampling on the dock determines acceptance or rejection. In addition, a trimethylamine test for free amines was used to check for degradation on purchased lots.

After deheading, the shrimp are quick frozen into 10 pound blocks for shipment to the processing plant where the blocks are held at -30° C. For processing the frozen blocks are defrosted in a bed of shaved ice to prevent temperature rise on the periphery causing deterioration. From a gravity flow bin, a conveyor belt carries the shrimp to a line of 10 automatic peelers and deveiners that operate at a capacity of 100 pounds per hour. An inspection at each deveining machine eliminates mechanical error and insures high quality control standards. By conveyor belt, the shrimp are spray washed and dropped onto perforated cooking trays with an automatically controlled capacity of 11 pounds per tray. The shrimp, placed on racks each holding 28 trays, are passed through a steam cooking and immediate spray cooling system. The cooking time, varying from three to seven minutes is dependent on the size of the shrimp and the size of the tray perforations. The cooling system reduces the shrimp temperature to 27° C. The weight of the shrimp following cooking and cooling is approximately nine pounds per tray. The racks are immediately placed in freezers operated at -10° C. A temperature-time limitation of four hours from thawing to freezing is imposed to further insure quality of the product.

The shrimp are retained in the freezer for a minimum of six hours to insure complete sublimation. At this stage

of processing frozen shrimp samples used for the experiment were removed and packaged in two pound units in heavy duty plastic bags. Samples of the same batch run of shrimp were lyophilized and packed under nitrogen in tin cans for experimental comparisons.

The lyophilization procedure includes controlled time-temperature gradations all under 1.5 mm mercury pressure. During freeze drying the temperature is held at 250° F. for three hours, at 200° F. for the next seven hours and then is maintained at 150° for eight hours. Heat is produced by passing oil through coils in the shelving of the lyophilization chamber. The freeze drying process spans a total of 18 hours and reduces the moisture level within the product to 0.5 per cent. Both Stokes Chambers and Del Vac Chambers are used nonpreferentially within the system.

A toluene distillation moisture test is used to determine moisture level at the time of packaging. Handling, even though the processing area is humidity controlled, accounts for about a 1.0 per cent moisture increase. The Quartermaster Corps standards require no more than 1.5 per cent moisture in the finished, packaged product of freeze dried shrimp.

ORGANOLEPTIC EVALUATION

Taste Panel Selection

The two basic types of taste panels are the difference panel and the preference panel. Since differences between the two products and changes resulting from processing methods were concerns of the study, for the most part, a difference panel was utilized.

The reliability of results from a difference panel depends primarily on the degree of training, the sensitivity of the individual panel members and the interest of the panel members in participation in this type of endeavor. The number of individuals used in a difference panel also affects the reliability of the results.

Since a difference panel provides no indication as to whether or not the difference is desirable, preference judging was included. An overall degree of preference was indicated between frozen cooked shrimp and rehydrated freeze dried cooked shrimp. A degree of tenderness was also preferentially indicated.

The individuals selected to serve on the taste panel had demonstrated the ability to detect relatively small differences in flavor and, perhaps more important, to detect these differences consistently. A group of undergraduate and graduate students, both male and female, were

tested for their ability to discern salty, sweet and sour tastes in varying low levels of concentrations. Sodium chloride levels varying incrementally from 0.0075 per cent to 0.10 per cent were presented to prospective taste panel members who had expressed an interest in, and a willingness to cooperate with a long term taste testing program. To test ability to taste sweet flavors, sucrose solutions varying from 0.175 per cent to 3.0 per cent were presented to the panelists. An ability to taste sour was tested using solutions of citric acid ranging from 0.01 per cent to 0.10 per cent.

Five levels of concentration for each of the three taste sensations were presented at three different testing periods. A total of eight threshold levels for each taste sensation was used. The same levels of flavor were not tested at each period. The position in the order of tasting the same concentration level varied from one series to the next.

Each panel member was requested to condition his mouth with distilled water, begin tasting until he could discern a particular taste sensation, then stop tasting. Each panelist was requested to record the taste detected and the number of the sample in which the taste was first identified and then continue to the next series of concentration

levels until the series of three taste sensations had been completed.

The panel members were requested not to smoke, drink beverages or eat for 30 minutes preceding the taste test periods. The taste testing was scheduled at either mid-morning or mid-afternoon sessions.

From this preliminary study of threshold level determination, a panel was chosen. The 15 members who had correctly identified the three tastes and who had shown consistency in identification of threshold levels were selected. These individuals ranged in age from 18 to 55 years; all were women with a home economics background. Each had had experience in evaluating food products.

The panel members chosen were tested for genetic taste inhibitions. At each of three different tasting periods, taste papers impregnated with chemicals for which known inhibitions occur, were presented for flavor evaluation. The three chemicals tested were: 1) phenylthiourea, 2) sodium benzoate, and 3) phenylthiocarbamide.

Taste Test Procedure

The taste panel evaluations of frozen cooked and freeze dried cooked shrimp were held at mid-afternoon sessions on Tuesdays each week. A foods laboratory, not

used by other classes, was set up for the panel's use, thus eliminating food odors and physical disturbances.

The panel group was trained in the use of the evaluation instrument, in descriptive word choices to relate meaning in a common vernacular, and in the testing procedure to be used. A group of from 10 to 15 trained panelists is recommended for difference testing by Hall (30). Though 15 panel members began the study two were unable to continue. The remaining group was determined to be adequate and to meet the requirements of training for a specific food problem. Two training periods for evaluating shrimp products preceded the actual tasting periods.

The same instrument was utilized throughout the taste testing procedure. A two-sample presentation was used. Two samples of two shrimp each were presented in petri plates to each judge. The order of presentation was rotated with half the judges testing the freeze dried sample and half testing the frozen sample first. The score sheets, with code numbers designating the appropriate sample, were prepared in advance of the testing period. The three digit code numbers used in coding the samples were changed at each testing period.

Panelists evaluated the shrimp samples for differences in 1) appearance, 2) flavor, 3) juiciness, 4) tenderness to tooth, 5) tenderness to tongue and cheek, and 6) fragmentation.

The degree of difference in the shrimp samples was indicated on a scale with a range of nine degrees of possible differences. Space was provided for an explanation of differences observed, following each category of evaluation. Panelists were requested to give some explanation of any degree of difference noted, omitting an explanation only if no difference was observed. A copy of the instrument used may be found in Appendix A.

In order to determine whether the differences indicated were desirable or undesirable, two rating scales were provided for each product. A nine point hedonic scale was utilized:

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

This hedonic scale was used to evaluate each of the two products as to overall acceptance and as to tenderness. This portion of the taste test was thus a preferential type of test.

Samples of thawed frozen shrimp and rehydrated freeze dried shrimp, both presented at room temperature, were compared in the first six judging periods. During the last

three tasting sessions "prepared" dishes were evaluated by the same method. The "prepared" dishes evaluated were shrimp creole, curried shrimp and shrimp gumbo. The "prepared" dishes were included after the results of the first evaluations indicated a definite trend in the preference for frozen shrimp over rehydrated freeze dried shrimp. An effort was made to determine if this preference would also be indicated in combination with other foods and other flavors.

MICROBIOLOGICAL ASPECTS

Procedure for Making Dilutions

Samples of five grams each of frozen cooked shrimp and rehydrated freeze dried cooked shrimp were aseptically weighed and blended with 25 ml of sterile water for two minutes in a microcup on a Waring blender at high speed. Dilutions of 1:10; 1:100; 1:1,000 were made for further plating and testing procedures.

Procedure for Total Plate Count

Total aerobic plate counts were made by overpouring a one ml sample of each dilution with trypticase soy agar supplemented with 0.5 per cent yeast. Plates were incubated at 37° C. and the total counts were made after 48 hours. Each sample was plated in triplicate for each of the dilutions.

Procedure for Coliform Tests

One ml samples of each dilution of freeze dried and frozen shrimp were pipetted into petri plates using sterile technique and were overpoured with sterile violet red bile agar at 45° C. The plates were incubated at 37° C. for 24 hours. The plating was repeated in triplicate.

The above procedure was repeated using eosin methylene blue agar medium. These plates were also incubated at 37° C. and were read following 24 and 48 hours of incubation.

Procedure for Staphylococci Tests

Five tubes of each of the three dilutions (1:10; 1:100; and 1:1,000) of the frozen and rehydrated freeze dried samples were prepared by the addition of 10 ml of sample to 10 ml of trypticase soy broth with 11 per cent sodium chloride. These tubes were incubated at 37° C. for 24 hours.

After this incubation period, a loop from each tube that appeared to be turbid or cloudy was streaked onto plates of egg yolk agar. The egg yolk agar base was prepared by aseptically adding the yolk of a fresh chicken egg to 100 ml of sterilized brain heart infusion broth. A volume of 25 ml of this mixture was added to 100 ml of sterile Bact-Colbeck egg yolk agar base. The egg yolk agar plates

were incubated at 37° C. for 48 hours. Colonies exhibiting a halo formation were tested for a coagulase-positive reaction.

To test for coagulase-positive colonies, trypticase soy broth was inoculated using a wire loop and sterile technique. These tubes were incubated at 37° C. for 24 hours. The tubes were read for settling out (little "buttons") and for cloudiness. One drop of turbid broth culture was added to 0.5 ml of reconstituted coagulase plasma. Control tubes of positive Staphylococcus aureus and a negative Sarcina lutea were compared with the suspect cultures. Tubes containing known and unknown solutions were incubated in a 37° C. water bath and were read at 30 minute intervals for a period of three hours.

COLOR ANALYSES

Pigment Extraction

The extraction procedure for astaxanthin pigment adopted for this study was as follows: 100 gm each of frozen and rehydrated freeze dried shrimp were put through a food grinder and thoroughly mixed; a 7 gm aliquot of each shrimp mixture was blended with 10 gm sodium sulfate and 30 ml acetone in a microcup of a Waring Blender at high speed for two minutes. The mixture was filtered through filter paper into a 50 ml flask and made to volume with washings. Triplicate extractions were made of both frozen

shrimp and freeze dried rehydrated shrimp. Samples of 3 ml were pipetted into quartz cuvettes and the absorbance was read from 350 mμ to 550 mμ to establish that wave length at which greatest absorbancy could be read. A comparison between the two products could then be made on a quantitative basis.

Measurement of Light Reflection

Measurements of light reflection were read on an Agtron Solid State Model M. 400-A reflectance spectrophotometer. The instrument embodies gaseous discharge tubes for illuminating the sample and interference filters for isolating one of three monochromatic lines from the light sources. Relative spectral reflectance is measured by a phototube and a solid state amplifier and meter. Alternating pulses of light generated by the light sources, are reflected from the bottom of the sample well onto the sensitive area of the phototube, after passing through one of the monochromatic filters. The filters include blue (436 mμ), green (546 mμ) and red (640 mμ) (67).

Samples of shrimp of 25 gm each were finely chopped and placed in the sample cup of the instrument. Samples were read in the blue mode, in the green mode and in the red mode. The same samples were then blended with 100 ml of

distilled water in an Osterizer for two minutes at high speed. This suspension was also read in the blue, the green and the red modes for degree of light reflectance.

FACTORS RELATING TO TENDERNESS

Procedure for Total Protein Determination

The Biuret Method for quantitative protein determination as described by Henry (57) was used for this study. Ten each of frozen and rehydrated freeze dried shrimp were dessicated for total moisture determinations, then were ground in a mortar with a pestal. The ground dessicated material was well mixed and samples of 70 mg each were weighed on a Mettler analytical balance. These samples were placed in 10 ml volumetric flasks to which 5 ml of 6.0 per cent sodium hydroxide were added. The samples were allowed to stand overnight in a darkened area for solubilization of the protein. To completely solubilize the protein, a hot water bath at 60° C. followed by a rotation period was used alternately. When the solution was clear, water was added to make a total volume of 10 ml. A 1.0 ml sample of soluble protein was diluted to 25 ml with physiological saline and was well mixed. Of this mixture, 2.5 ml was mixed with 2.5 ml of 6.0 per cent sodium hydroxide and 1 ml of Biuret Reagent was added to the combined mixture. The tubes were shaken and allowed to stand 15 minutes. Absorbancy was read at 545 mμ on a Hitashi-Perkin-Elmer Spectrophotometer.

The unknowns were read against a blank of 5 ml of 3.0 per cent sodium hydroxide and 1 ml of Biuret Reagent and compared with a protein standard. The standard consisted of 7 gm albumin dissolved in 100 ml distilled water. A 2. 5 ml portion was reacted with 2.5 ml 6.0 per cent sodium hydroxide and 1 ml Biuret Reagent.

Procedure for Collagen Determination

The distribution of hydroxyproline is confined almost exclusively to the connective tissue; thus hydroxyproline has been widely used as an indicator of both the presence and the metabolism of collagen. The procedure developed by Woessner (130) was utilized in this investigation since accuracy of the procedure has been shown in biological materials containing as little as one part hydroxyproline in 4,000 part of amino acids.

The samples of frozen and rehydrated freeze dried shrimp that had been oven dried for moisture determinations were finely ground and utilized for securing aliquots. Samples of 0.1 gm of dessicated tissue were weighed and placed in test tubes. To each sample tube 3.0 ml of concentrated hydrochloric acid and 3.0 ml distilled water were added. The tubes were then sealed and autoclaved at 15 pounds pressure for three hours. After the tubes were opened 2 ml of the acid solutions was pipetted into clean

test tubes. Two drops of 0.02 per cent methyl red indicator were added to each tube and the acidity was adjusted to a pH of 6-7 with 2.5 N sodium hydroxide. Of the neutralized solution, 2 ml were pipetted into a 25 ml volumetric flask and brought to a volume of 25 ml with distilled water.

Hydroxyproline oxidation was initiated by adding 1 ml of Chloramine T solution to 2 ml of unknown samples in a predetermined sequence. The tube contents were well mixed and allowed to stand 20 minutes. The Chloramine T was destroyed by adding 1 ml of perchloric acid. The contents were mixed and allowed to stand five minutes. Finally, 1 ml para-dimethylaminobenzaldehyde was added. The mixture was shaken until no schlieren could be seen. The tubes were placed in a 60° C. water bath for 20 minutes; then cooled in tap water for five minutes. The absorbancy of the solutions was determined spectophotometrically at 557 mμ. The hydroxyproline concentrations were determined directly from the standard curve.

The standard curve was determined by following the above procedure using two tubes each of 0 and 5 μg of hydroxyproline. A stock solution was prepared by dissolving 25 mg of vacuum dried L-hydroxyproline in 250 ml of 0.001 N hydrochloric acid.

Procedure for Protein Fractionation

Protein fractionation and a differential study of the proteins within the fractions were investigated and related to tenderness. The procedure for fractionation was adapted from one developed by Hegarty, Bratzler and Pearson (54). The major changes were in sampling method, centrifugation and buffer systems.

A quantity of approximately 100 gm each of frozen and rehydrated freeze dried shrimp were blotted twice between paper toweling, then ground with a food grinder. The ground material was thoroughly mixed prior to the removal of five gm aliquots.

Each sample was placed in a microblender jar with 70 ml of a phosphate buffer (0.156 M di-potassium phosphate and 0.0035 M potassium biphosphate) at a pH of 7.6. An attempt was made to achieve smallest particle size possible with a minimum amount of foaming. This was achieved by blending for one minute at a blender speed of 8,000 rpm (adjusted with a Powerstat transformer setting of 40). After blending, the material was divided into three 50 ml certrifuge tubes. The blender jar was rinsed with 30 ml of extracting solution that was also divided into the same three centrifuge tubes. After one hour the material was centrifuged for 15 minutes, the supernatant was decanted

and the volume was recorded. A volume of 100 ml of extracting solution was divided between the three tubes. The tubes were stoppered and shaken thoroughly. After one hour the material was centrifuged for 15 minutes, the supernatant decanted and its volume recorded. The residue remaining in the three tubes from the extraction was reacted with 200 ml of 0.1 M sodium hydroxide overnight at room temperature. The mixture was filtered through eight layers of gauze to remove alkali-insoluble material, i. e., connective tissue.

A similar procedure also using five gm aliquots of sample was performed using a high ionic strength extracting buffer of 0.5 M potassium chloride and 0.03 M sodium bicarbonate at pH of 8.75. The residue from this extraction was discarded and only the supernatants retained for analyses.

A model PR-4 International Electronics Corporation Refrigerated Centrifuge was used throughout the experiment. Centrifuging was done at 10,000 rpm at a temperature of 4° C. All fractionation procedures were carried on in duplicate at 4° C. pH measurements were made on a Sargent pH meter, model DR.

The extracted fractions were analyzed quantitatively by the Folin-Ciocalteu Method. Reagents employed in this

procedure were as follows:

1) Reagent A was a solution of 2.0 per cent sodium carbonate in 0.1 N sodium hydroxide.
2) Reagent B was a solution of 0.5 per cent cuprous sulfate in 1.0 per cent sodium acetate.
3) Reagent C was made by combining 50 ml Reagent A with 1 ml Reagent B.

Upon the addition of 1 ml of reagent C to 0.1 and 0.2 ml samples, the solutions in the tube were thoroughly mixed and allowed to stand at room temperature for 10 minutes. Then, immediately after the addition of 0.1 ml of the Folin-Ciocalteu Reagent, each tube was thoroughly mixed. After one hour the total volume of each sample was adjusted to 3.0 ml. Each sample was transferred to a quartz cuvette and mixed thoroughly prior to the determination of the absorbance at 500 mμ by means of a Hitashi-Perkin-Elmer Spectrophotometer, Model 139. A comparison of these values of the protein fraction being analyzed with those of the standard permitted the determination of the protein content therein.

The standard used was an albumin solution containing 40 mg per 100 ml. Samples of 0.1 and 0.2 were combined with reagent C and Folin-Ciocalteu Reagent and the procedure was completed as for the unknown samples. A blank of the working alkaline copper reagent and water was used for adjusting the instrument.

Three of the fractions resulting from the protein fractionation procedure were analyzed quantitatively for

hydroxyproline. The supernatant extracted by the first extraction procedure with a low ionic strength buffer, the supernatant extracted by the second extraction procedure with a high ionic strength buffer and the supernatant resulting from the reaction of residual material with sodium hydroxide solution. The Woessner (130) method of procedure was again utilized for hydroxyproline determinations.

Aliquots of 3 ml of each of the supernatants from the extraction procedure of both frozen and freeze dried shrimp samples were sealed with 3 ml of 6 N hydrochloric acid. The tubes were autoclaved for three hours at 15 pounds pressure. The tube contents of 6 ml were neutralized with 6 N sodium hydroxide solution and were brought to a total volume of 10 ml with distilled water. Aliquots of 2 ml were pipetted into test tubes for completion of the hydroxyproline procedure as previously discussed in this chapter.

TOTAL MOISTURE AND WATER HOLDING CAPACITY

Total Water Determinations

Total moistures were determined by two methods: evaporation and toluene extraction. For all moisture tests shrimp samples were blotted lightly twice between paper towels before moisture determination procedure was begun.

Evaporative moisture determinations were made by drying samples in an oven at 160° F. under a vacuum equivalent to 29 inches of Mercury. Weight losses during drying were used as total moisture content. For this determination 10 each of frozen and rehydrated freeze dried shrimp were used.

The procedure used for moisture determination by toluene extraction was the same as that used at the processing plant from which the sample material was obtained. The procedure is routinely used in quality control to check moisture levels in freeze dried products prior to packaging.

The moisture determination procedure was as follows: a 50 gm sample of blotted shrimp was quickly transferred to a 500 ml Erlenmeyer flask and 75 ml of toluene were poured into the flask covering the sample. The flask and a trap were connected to a condenser through which cold water circulated. The amount of heat applied to the flask containing the sample was regulated so that the toluene condensed in the trap at the rate of about four drops per second.

After 60 minutes of distillation the moisture level in the trap was read at 15 minute intervals until two readings were in agreement. The milliliters of water in the trap multiplied by two is equal to the percentage of moisture in the sample.

Press Fluid Determinations

Press fluid determinations were made by weighing 0.5 gm samples of both frozen and rehydrated freeze dried shrimp between discs of aluminum foil. The discs were placed between double filter papers and were pressed at 5,000 pounds with a Carver laboratory press. The foil discs with the pressed sample were weighed on a Mettler analytical balance. The press fluid was the difference between the sample weight before and after pressing.

CHAPTER IV

PRESENTATION AND ANALYSIS OF DATA

The data for this study were obtained by means of both subjective and objective techniques. Certain factors usually related to total product acceptance were inveti-gated in determining the effect of lyophilization on cooked frozen shrimp. The factors included were: appearance, flavor, juiciness, tenderness, and micro-biological population.

ORGANOLEPTIC EVALUATION

Subjective data were secured through a series of organoleptic evaluations by a panel of 13 trained judges. The panel members were selected on the basis of accuracy and consistency in identifying three basic tastes. A pilot study in taste testing was conducted for the identi-fication of threshold levels of solutions of varying strengths representing three of the four basic tastes. Solutions of sucrose, citric acid and sodium chloride were each tasted on three separate tasting periods. The judges participating in the organoleptic evaluation of shrimp were those who had correctly identified each of three basic tastes at the threshold levels presented during each

pilot testing session. Furthermore, the identification had been made within a close range of threshold levels.

Each panel member was tested for certain genetic taste inhibitions. Commercial taste papers impregnated with thiourea, sodium benzoate and phenylthiocarbamide (PTC) were used for testing the taste sensitivity.

The judges were requested to check one and only one of the following categories on the "Taste Sensitivity Test" questionnaire; no taste, salty, sweet, sour or bitter. The panelists were classified according to responses as to taste ability for sodium benzoate and PTC. The taste classifications developed by Fox (37) and adapted by Townsend (123) were used for this study as follows:

Tasters	Nontasters
(PTC - Sodium Benzoate)	(PTC Sodium Benzoate)
Bitter - bitter	No taste - sweet
Bitter - salty	No taste - salty
Bitter - sour	No taste - sour
Bitter - sweet	No taste - no taste

For this study both tasters and nontasters were chosen. Fox (37) reported a 76 per cent probable distribution of tasters in the human race; therefore, to include acceptance of any given product by the masses, both tasters and non tasters should be included. Based on tests conducted

with 1,500 individuals, Fox found that individuals who were classified as "no taste - no taste" in respect to PTC-sodium benzoate tend to have the lowest number of food dislikes; whereas, those classified as "bitter-salty" have the highest number of food dislikes. These results indicate that those categorized as "bitter-salty" are more discriminant tasters and could bias a panel if their numbers weighted the total panel population. Table I shows the responses of tasters participating in the present study for the three chemical compounds according to the basic taste reported. Both tasters and nontasters were chosen as panel members in order that taste evaluation would be more nearly equated with the dispersion of tasters and nontasters in the general population.

During the shrimp tasting periods two samples, one frozen and one rehydrated-freeze dried, were presented to the testing panel at each evaluation session. In the first six periods of testing, the shrimp was presented in a thawed and rehydrated form with no further preparation. The last three test sessions concluding the organoleptic evaluation included "prepared" dishes of shrimp - creole, curry and gumbo.

Two types of evaluation used in the study were difference testing and preferential ratings. In the

TABLE I

RESPONSES OF PANEL MEMBERS TO THREE CHEMICAL COMPOUNDS ACCORDING TO THE BASIC TASTE REPORTED

Judge Number	Taste		
	Thiourea	Sodium Benzoate	PTC
1	Bitter	Salty	Bitter
2	Bitter	Salty	No taste
3	Bitter	Sour	Salty
4	Bitter	Sweet	Salty
5	Bitter	Sweet	Bitter
6	Bitter	Salty	No taste
7	Bitter	Salty	No taste
8	Bitter	Sweet	Bitter
9	Bitter	Sweet	No taste
10	Bitter	Salty	Bitter
11	Bitter	Salty	Bitter
12	No taste	Sweet	No taste
13	Bitter	Salty	Bitter

difference testing, the judges evaluated the degree of difference observed between the two samples by checking a point along a scale of 10 gradations ranging from no difference to a pronounced difference. This type of evaluation was used for each of the following areas of comparison: appearance, flavor, juiciness, softness to tooth, softness to tongue and cheek and fragmentation. Six sessions were used for evaluating appearance and flavor and five sessions for evaluating juiciness, softness to tooth, softness to tongue and cheek and fragmentation. The observed differences were verbally described if any degree of dissimilarity was indicated on the scale.

For the evaluation of preference, each sample was scored on a nine point hedonic scale for tenderness and overall acceptance. The panelists were requested to circle the number that best described each product. The hedonic scale was as follows:

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

The form "Evaluation of Shrimp" may be found in Appendix A. A copy of the "Criteria for Judging," displayed

as a reference for the panel members at each evaluation session, may be found in Appendix B.

Difference scores for each of the organoleptic factors investigated were subjected to an overall analysis of variance. The differences between replications within each factor were compared by use of the t-test. Only the test results showing a significance in observed differences will be discussed in this dissertation.

Preferential rating scores were also subjected to an overall analysis of variance. The ratings evaluating both overall acceptance and tenderness for frozen and freeze dried samples were analyzed. Differences between replications were analyzed by use of the t-test. The differences between tasting sessions were compared with the differences within a particular tasting session.

Analysis of Appearance Difference Testing

The differences between frozen and freeze dried shrimp samples were not significant when either "plain" shrimp or when "prepared" dishes were served to the panel members. However, a significant difference was found between testing sessions in which shrimp was served "plain" and those testing sessions when "prepared" dishes were served. The t-values shown in Table II indicate the significance of the observable differences in appearance between the stated

TABLE II

ANALYSES OF APPEARANCE DIFFERENCES

BETWEEN TESTING SESSIONS

Comparisons	Mean	Standard Deviation	t-value**
Rep 1	7.00	1.24	9.499
Rep 7	0.77	1.05	
Rep 1	7.00	1.24	7.154
Rep 8	2.31	2.33	
Rep 1	7.00	1.24	7.858
Rep 9	1.85	1.87	
Rep 2	6.54	1.50	8.796
Rep 7	0.77	1.05	
Rep 2	6.54	1.50	6.450
Rep 8	2.31	2.33	
Rep 2	6.54	1.50	7.154
Rep 9	1.85	1.87	
Rep 3	6.92	1.27	9.382
Rep 7	0.77	1.05	
Rep 3	6.92	1.27	7.037
Rep 8	2.31	2.33	
Rep 3	6.92	1.27	7.740
Rep 9	1.85	1.87	

**$p < 0.01$

TABLE II (Continued)

ANALYSES OF APPEARANCE DIFFERENCES

BETWEEN TESTING SESSIONS

Comparisons	Mean	Standard Deviation	t-value**
Rep 4	7.00	1.30	7.154
Rep 8	2.31	2.33	
Rep 4	7.00	1.30	7.858
Rep 9	1.85	1.88	
Rep 5	7.23	1.67	9.851
Rep 7	0.77	1.05	
Rep 5	7.23	1.67	7.506
Rep 8	2.31	2.33	
Rep 5	7.23	1.67	8.209
Rep 9	1.85	1.87	
Rep 6	6.31	1.81	8.444
Rep 7	0.77	1.05	
Rep 6	6.31	1.81	6.099
Rep 8	2.31	2.33	
Rep 6	6.31	1.81	6.802
Rep 9	1.85	1.87	

**$p < 0.01$

replications. In replications 1 through 6, shrimp was served "plain" while replications 7 through 9 represent "prepared" dishes; namely, creole, curry and gumbo.

Analysis of data concerning observed appearance difference revealed that the F-value was highly significant as shown below:

Appearance	Sum of squares	Degrees freedom	Mean square	F-value
Between	724.77	8	90.60	32.40*
Within	302.00	108	2.80	
Total	1026.77	116		

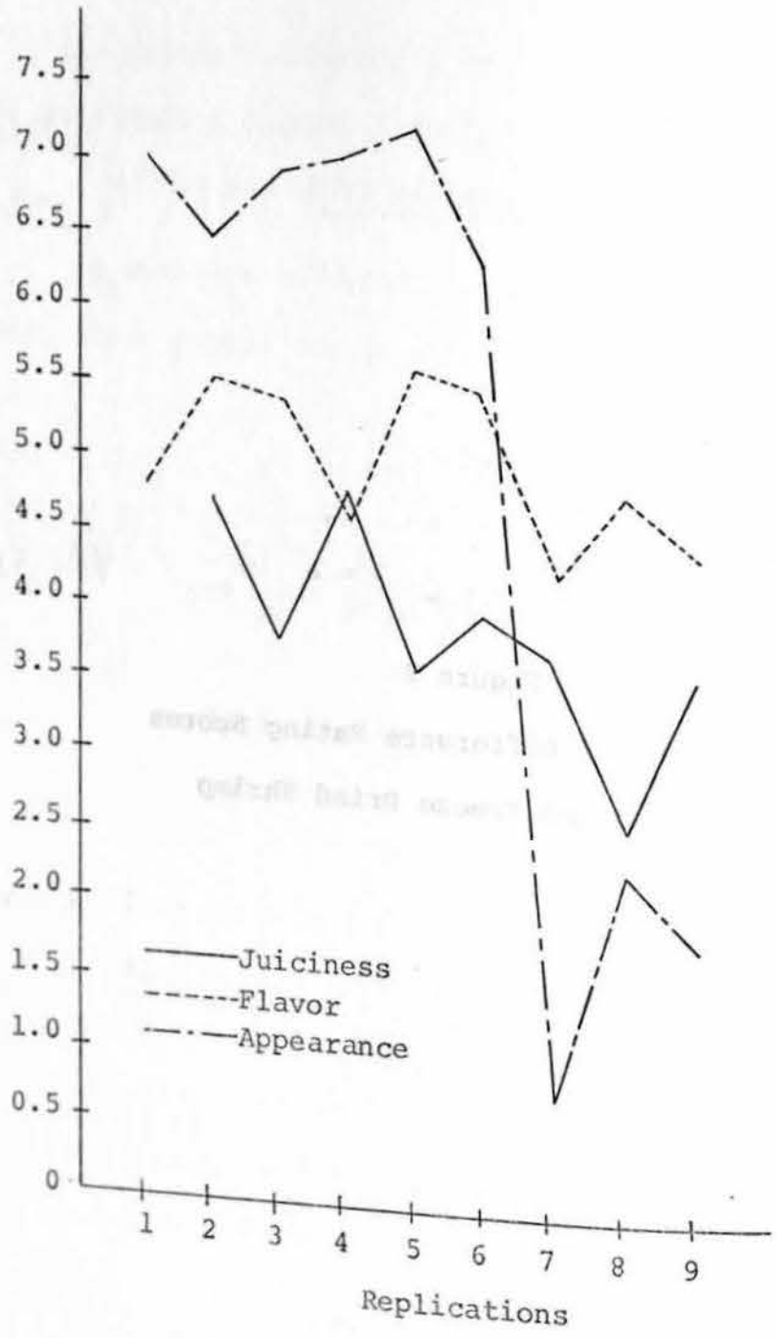
*$P < 0.01$

Figure 2 compares the means of the difference scores for each testing period in three areas of comparison: appearance, flavor and juiciness. The high difference scores found in evaluating "plain" samples (replications 7 through 9) for "prepared" dishes, indicate that in so far as appearance is concerned freeze dried shrimp could successfully be substituted for frozen shrimp in "prepared" dishes. The sauces in the "prepared" dishes tended to disquise differences in the pigment and flesh tones of the frozen and freeze dried shrimp. This substitution could, however, be easily detected if shrimp were served "plain."

Figure 2

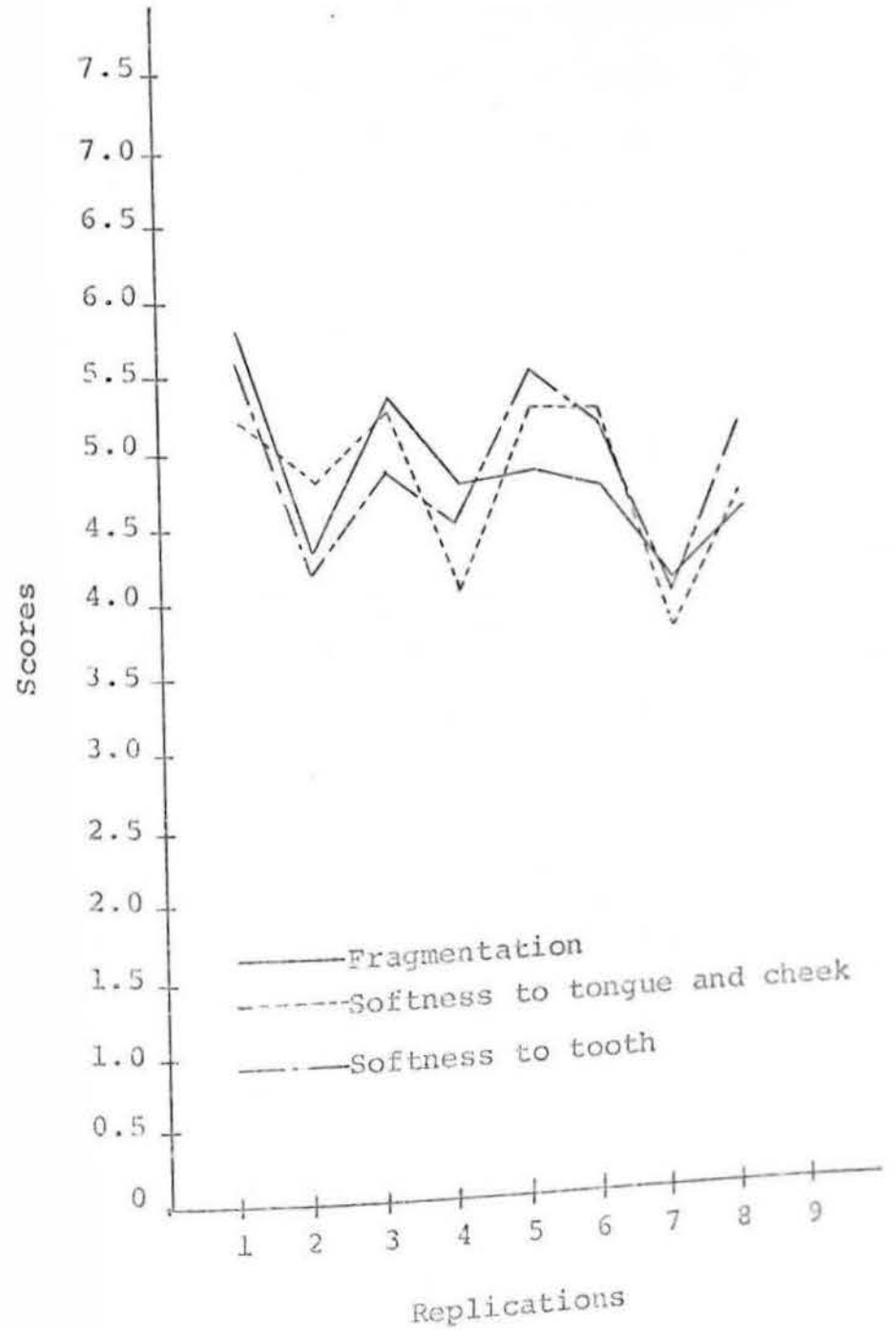
A Comparison of Difference Rating Scores

for Frozen and Freeze Dried Shrimp



Part I

A Comparison of Difference Rating Scores for Appearance, Flavor and Juiciness



Part II

A Comparison of Difference Rating Scores for Softness to Tooth, Softness to Tongue and Cheek and Fragmentation

The most frequently observed difference between frozen and freeze dried shrimp was that of color. Both the highly pigmented color of the shrimp and the color of the flesh were observed to be different. Exemplary comments in order of decreasing occurrence describing frozen shrimp were: pigment pink, flesh pale; brighter appearance; brighter color, more red; more natural appearance; and dry appearance. Counterpart comments pertaining to reconstituted freeze dried shrimp were: pigment orange, flesh creamy; yellowish appearance; off color; too pale; color faded; coral rather than pink; shinier looking; wet appearance; and plumper appearance.

Differences observed in moistness and plumpness were reported infrequently but many have been prompted by the freeze dried product having more exterior water adhering to the flesh. The samples were removed from the water bath of rehydration and were blotted twice lightly between paper towels. However, a more moist appearance was observed in some samples. The notation of a plumper appearance may have been the result of this more moist surface. The weight and size classifications of the two sample types were not different.

Analysis of Flavor Difference Testing

Differences in flavor as indicated by the analysis of variance of responses of the judges were insignificant as shown below:

Flavor	Sum of squares	Degrees freedom	Mean square	F-value
Between	28.07	8	3.51	0.673*
Within	562.92	108	5.21	
Total	590.99	116		

*P $<$ 0.01

Some judges consistently detected flavor differences at each session, other judges could not. Of the 13 judges, five were inconsistent in their responses to flavor differences within the six tasting periods in which shrimp samples were served "plain." Three of these five judges were categorized as nontasters on the basis of PTC-sodium benzoate classifications. Those judges who were consistent in responding to flavor differences, indicated high level flavor differences. Those judges who were the more consistant in their evaluations indicated a flavor preference for the frozen samples during each tasting session. This inconsistency by some panel members could have biased the results.

Comments concerning flavor differences favored the frozen product. In order of frequency, flavor difference comments for the frozen product were: mild flavor; fresher tasting; bland; sweet, nice flavor; and no aftertaste. Though the flavor differences were not statistically significant, the derogatory comments indicated not only an observed difference but a preference. Comments pertaining to the flavor evaluation of freeze dried shrimp were: strong fish taste; fishy flavor; old taste; off flavor; and "cooked twice" flavor.

Table III shows the taste sensitivity classifications as related to flavor difference scores. The two judges (11 and 12) who observed the lowest degree of flavor differences were inconsistent in difference observations in five of the six factors evaluated. Judge Number 7 also rated observable differences inconsistently in regard to flavor, juiciness, softness to tooth, softness to tongue and cheek, and fragmentation. Consistency in rating for all judges was found only for the appearance factor. Inconsistency in one factor only, and that for flavor was found in the difference ratings for Judges Number 2 and 6.

The 13 panel members were chosen by the accuracy and consistency registered in a pilot taste test study. Various concentrations of sucrose, sodium chloride and citric acid solutions were presented to a large panel of judges.

TABLE III

TASTE SENSITIVITY CLASSIFICATION OF JUDGES AS RELATED TO MEANS OF FLAVOR DIFFERENCE SCORES

Judge Number	Taste Test Classification		Means of differences in flavor scores
	PTC	Sodium Benzoate	
	Tasters of PTC		
1	Bitter	Salty	7.3
3	Salty	Sour	7.3
4	Salty	Sweet	5.7
5	Bitter	Sweet	5.5
8	Bitter	Sweet	5.0
10	Bitter	Salty	4.3
11	Bitter	Salty	2.8
13	Bitter	Salty	6.3
	Nontasters of PTC		
2	No taste	Salty	6.3
6	No taste	Salty	5.5
7	No taste	Salty	5.5
9	No taste	Sweet	4.3
12	No taste	Sweet	2.7

Only those individuals who correctly identified the basic tastes at each of the three tasting sessions and who identified the flavor at approximately the same concentration at each taste session were chosen for participation in the shrimp evaluation study. However, inconsistency of the scoring of observable differences in the shrimp study indicated that consistency and accuracy in one area of flavor evaluation does not necessarily qualify an individual for evaluating another product. A pilot study for the selection of taste panel members should test the individual's discriminatory powers relating to the particular food or beverage being evaluated.

Analysis of Differences in Juiciness

Analysis of difference scores evaluating juiciness resulted in a non-significant F-value for the overall group as shown below:

Juiciness	Sum of squares	Degrees freedom	Mean square	F-value
Between	32.58	7	4.65	0.780*
Within	572.77	96	5.97	
Total	605.35	103		

*P < 0.01

A significant t-value ($P<0.05$) was found between only two replications, replication 1, "plain" shrimp, and replication 7, shrimp creole, as shown:

Comparison	Mean	Standard deviation	t-value
Rep 1	4.69	2.33	2.168*
Rep 7	2.62	2.31	

*$P< 0.05$

In evaluating juiciness, five judges were inconsistent in their responses. Four of the five were the same panelists who had shown inconsistency in evaluating flavor. Four of the five inconsistent panel members were classified as nontasters according to the PTC-sodium benzoate criteria. This number of inconsistent judges could have biased the overall analysis.

Figure 2 illustrating the means of difference scores pertaining to juiciness, shows that less difference was observed in the juiciness of shrimp in "prepared" dishes than was observed in shrimp served "plain.: In the "Criteria for Judging", Appendix B, two factors for juiciness evaluation were to be considered. One factor was the amount of "juice" and the other was the relative degree of flavor in the juice. The same judges who were unable to detect flavor differences were also unable to detect differences in juiciness. Few

comments were made relating to the frozen samples but description of the freeze dried samples indicated the difference observed in the flavor of the juice was a major factor in the difference value assigned. Statements frequently made were that the amount of moisture appeared to be similar but the flavor of the juice was superior for the frozen product.

Concerning the freeze dried samples, comments in order of frequence were: the taste was "watery"; the mouthfeel became dry before swallowing; juicy at first, then dry. This dryness of mouthfeel was observed by several panelists in both the "prepared" dish samples as well as in the samples served plain.

Analysis of Tenderness Data

Analysis of data for the factors of tenderness difference: softness to tooth, softness to tongue and cheek, and fragmentation indicated that the F-values for each factor were not significant as indicated by the data in Table IV.

Three of the five judges who had shown incensistency in rating differences in flavor and juiciness continued this pattern in the tenderness evaluations. Of these three, two were classified as nontasters of PTC-sodium benzoate.

TABLE IV

ANALYSES OF VARIANCE FOR SOFTNESS TO TOOTH, SOFTNESS TO TONGUE AND CHEEK AND FRAGMENTATION FOR FROZEN AND FREEZE DRIED SHRIMP SAMPLES

Subject of Evaluation	Sum of squares	Degrees freedom	Mean square	F-value*
Softness to tooth				
Between	34.45	7	4.92	0.95
Within	496.92	96	5.18	
Total	531.37	103		
Softness to tongue and cheek				
Betweem	29.55	7	4.22	0.71
Within	580.51	97	5.98	
Total	610.06	104		
Fragmentation				
Between	26.66	7	3.81	0.68
Within	535.74	95	5.64	
Total	562.40	102		

* Not significant

Although the 13 panel members were individuals with training in food tasting and had participated in two preliminary tasting sessions prior to the study, the factors of tenderness appeared to be confusing. The factors of softness to tooth, softness to tongue and cheek, and fragmentation have been used successfully by research workers investigating beef tenderness. These three areas of tenderness are possibly not as discriminatory for shrimp as for beef. Figure 3 shows the mean difference scores for each tasting session for each of the three tenderness factors as compared to differences of appearance, flavor and juiciness.

Not only was inconsistency apparent among some panelists in scoring of like samples, but difference rating scores were inconsistent with the comments made by these same judges. Comments concerning tenderness differences for frozen shrimp samples, in the order of frequency expressed, were as follows: more tender, easier to cut with the teeth, easier to chew, little tooth pressure needed, and fragments more readily. Comments in the order of frequency reported for the rehydrated freeze dried samples were: more resistant to tooth pressure; chewy and tough; takes more chewing; more chewing pressure needed; more resilient; spongy; does not fragment.

Tenderness was also scored on a nine point hedonic scale in the preferential rating segment of the organoleptic

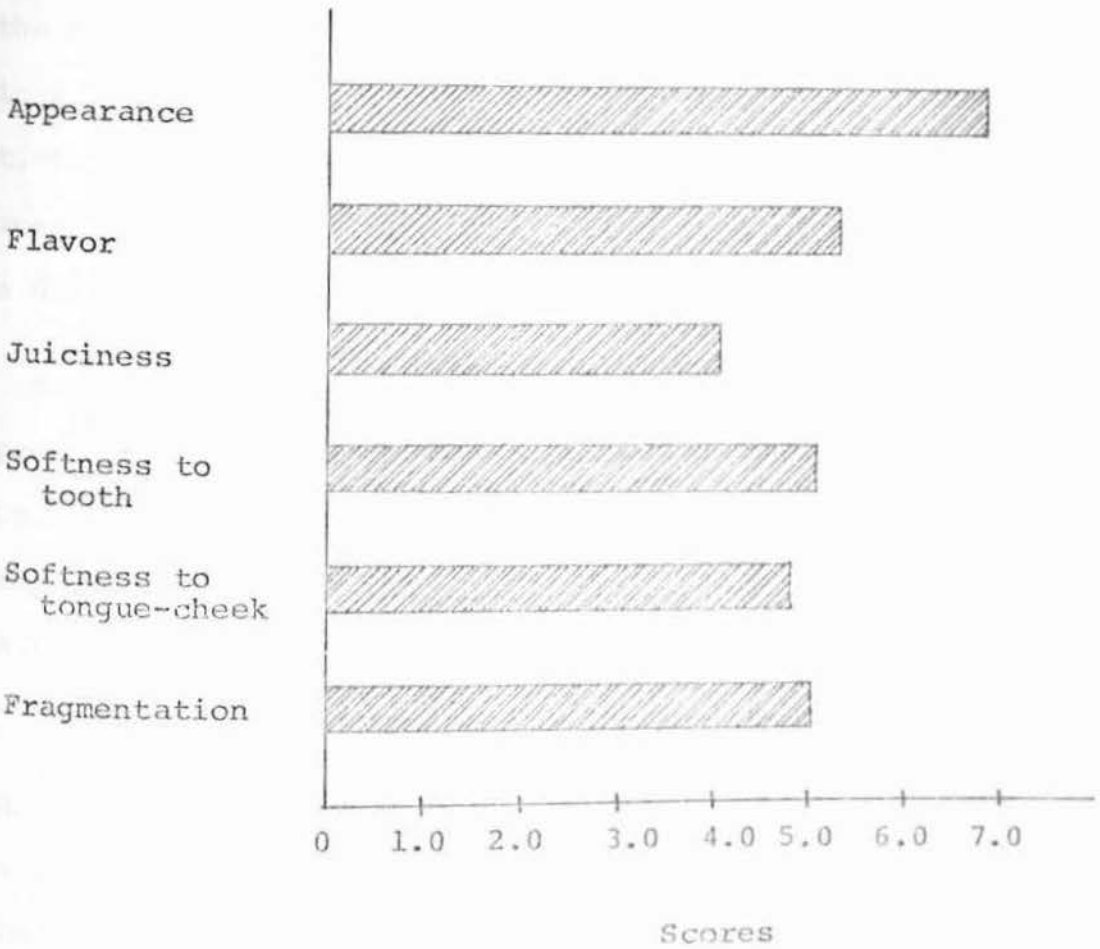


Figure 3

Means of the Difference Scores for Organoleptic Evaluation of Frozen and Freeze Dried Shrimp

evaluations. The inconsistencies observed in individual ratings and the inconsistency between comments and difference ratings by a given panel member were not as apparent in the preferential rating system as in the three difference ratings for tenderness. The data for this study indicated that, in the area of tenderness, there were a higher agreement in responses on a preferential rating type of scoring than on a differential scale.

Analysis of the data from the preferential ratings of product tenderness indicated a significant F-value ($P < 0.01$). Differences between frozen and freeze dried shrimp samples were greater than differences within replications as shown in Table V.

The significantly lower ratings for the freeze dried product indicated that a textural change had taken place as a result of lyophilization. Figure 4 shows the mean ratings for each testing period for both the frozen and freeze dried products.

An overall acceptance score based on a nine point hedonic scale was recorded for the frozen and the freeze dried samples at each of nine tasting sessions. The F-values determined by analysis of variance showed a preference for the frozen sample, significant at the 1.0 per cent level, at each tasting session as shown by Table VI.

TABLE V

ANALYSES OF VARIANCE FOR TENDERNESS RATINGS FOR FROZEN AND FREEZE DRIED SHRIMP SAMPLES FOR EIGHT TESTING PERIODS

Testing Periods	Sum of squares	Degees freedom	Mean square	F-value*
1				
Between	67.85	1	67.85	30.41
Within	53.54	24	2.23	
Total	121.39	25		
2				
Between	81.39	1	81.38	67.18
Within	29.08	24	1.21	
Total	110.46	25		
3				
Between	47.12	1	47.12	28.82
Within	39.23	24	1.63	
Total	86.35	25		
4				
Between	30.15	1	30.15	17.95
Within	40.31	24	1.68	
Total	70.46	25		

*P < 0.01

TABLE V (Continued)

ANALYSES OF VARIANCE FOR TENDERNESS RATINGS FOR FROZEN AND FREEZE DRIED SHRIMP SAMPLES FOR EIGHT TESTING PERIODS

Testing Periods	Sum of squares	Degrees freedom	Mean square	F-value*
5				
Between	74.46	1	74.46	47.41
Within	37.69	24	1.57	
Total	112.15	25		
6				
Between	71.12	1	71.12	31.88
Within	53.54	24	2.23	
Total	124.66	25		
7				
Between	15.38	1	15.38	9.23
Within	40.00	24	1.67	
Total	55.38	25		
8				
Between	30.15	1	30.15	13.96
Within	51.85	24	2.16	
Total	82.00	25		

*P < 0.01

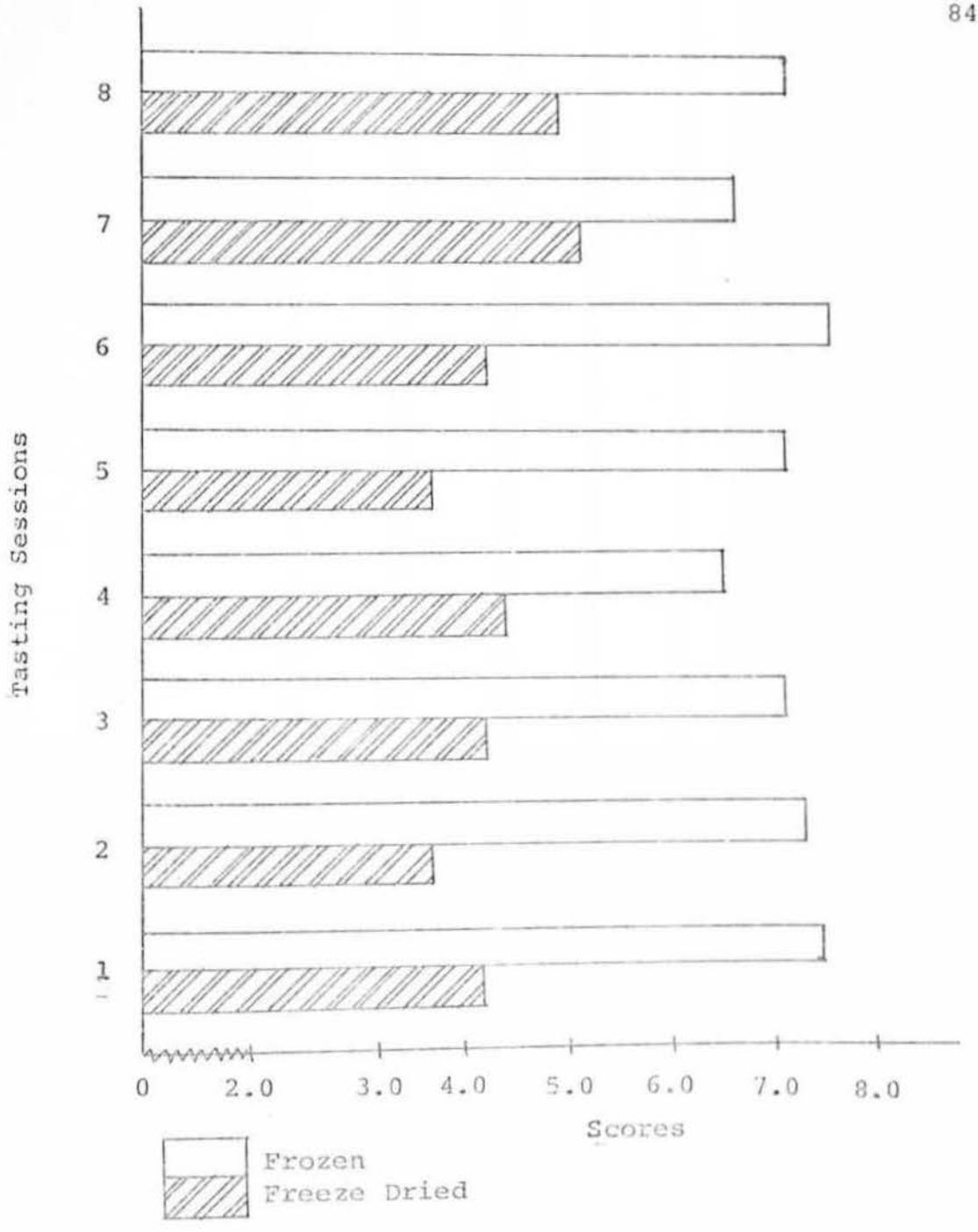


Figure 4

Means of Preferential Rating Scores for Tenderness for Frozen and Freeze Dried Shrimp

TABLE VI

ANALYSES OF VARIANCE FOR OVERALL ACCEPTANCE RATINGS FOR FROZEN AND FREEZE DRIED SHRIMP SAMPLES FOR NINE TESTING PERIODS

Testing Periods	Sum of squares	Degrees freedom	Mean square	F-value*
1				
Between	30.15	1	30.15	13.99
Within	51.69	24	2.15	
Total	81.84	25		
2				
Between	44.46	1	44.46	23.75
Within	44.92	24	1.87	
Total	89.38	25		
3				
Between	49.85	1	49.85	27.19
Within	44.00	24	1.83	
Total	93.85	25		
4				
Between	58.50	1	58.50	23.34
Within	60.15	24	2.51	
Total	118.65	25		
5				
Between	55.54	1	55.54	27.77
Within	48.00	24	2.00	
Total	103.54	25		

*$P < 0.01$

TABLE VI (Continued)

ANALYSES OF VARIANCE FOR OVERALL ACCEPTANCE RATINGS FOR FROZEN AND FREEZE DRIED SHRIMP SAMPLES FOR NINE TESTING PERIODS

Testing Periods	Sum of squares	Degrees freedom	Mean square	F-value*
6				
Between	58.50	1	58.50	44.09
Within	31.85	24	1.33	
Total	90.35	25		
7				
Between	52.65	1	52.65	20.90
Within	60.46	24	2.52	
Total	103.11	25		
8				
Between	41.88	1	41.88	17.02
Within	59.08	24	2.46	
Total	100.96	25		
9				
Between	52.65	1	52.65	23.40
Within	54.00	24	2.25	
Total	106.65	25		

*$P < 0.01$

The significantly lower scores for the freeze dried product indicated further that degradation does result from the lyophilization of shrimp. Figure 5 indicates the comparison by mean scores for each product at each session as to overall acceptance.

ANALYSES OF OBJECTIVE TESTING

Various objective evaluations were made in an effort to determine the factors contributing to the undesirable changes resulting from the lyophilization of cooked shrimp. A microbiological survey of the growth potential of certain pathogenic microorganisms was included. A pigment loss evaluation was made both as to the amount of extractable pigment and the degree of change in light reflectance.

Tenderness was studied in relation to protein and hydroxproline within samples of tissue of the frozen and freeze dried shrimp and also within samples of tissue following fractionation. Sarcoplasmic protein was extracted with a low ionic strength buffer and myofibrillar proteins were extracted by a residual treatment with sodium hydroxide. Proteins extractable with a high ionic strength buffer (pH 8.75) were extracted to compare the effect of lyophilization on protein solubility. Protein and hydroxyproline values were determined for each fraction.

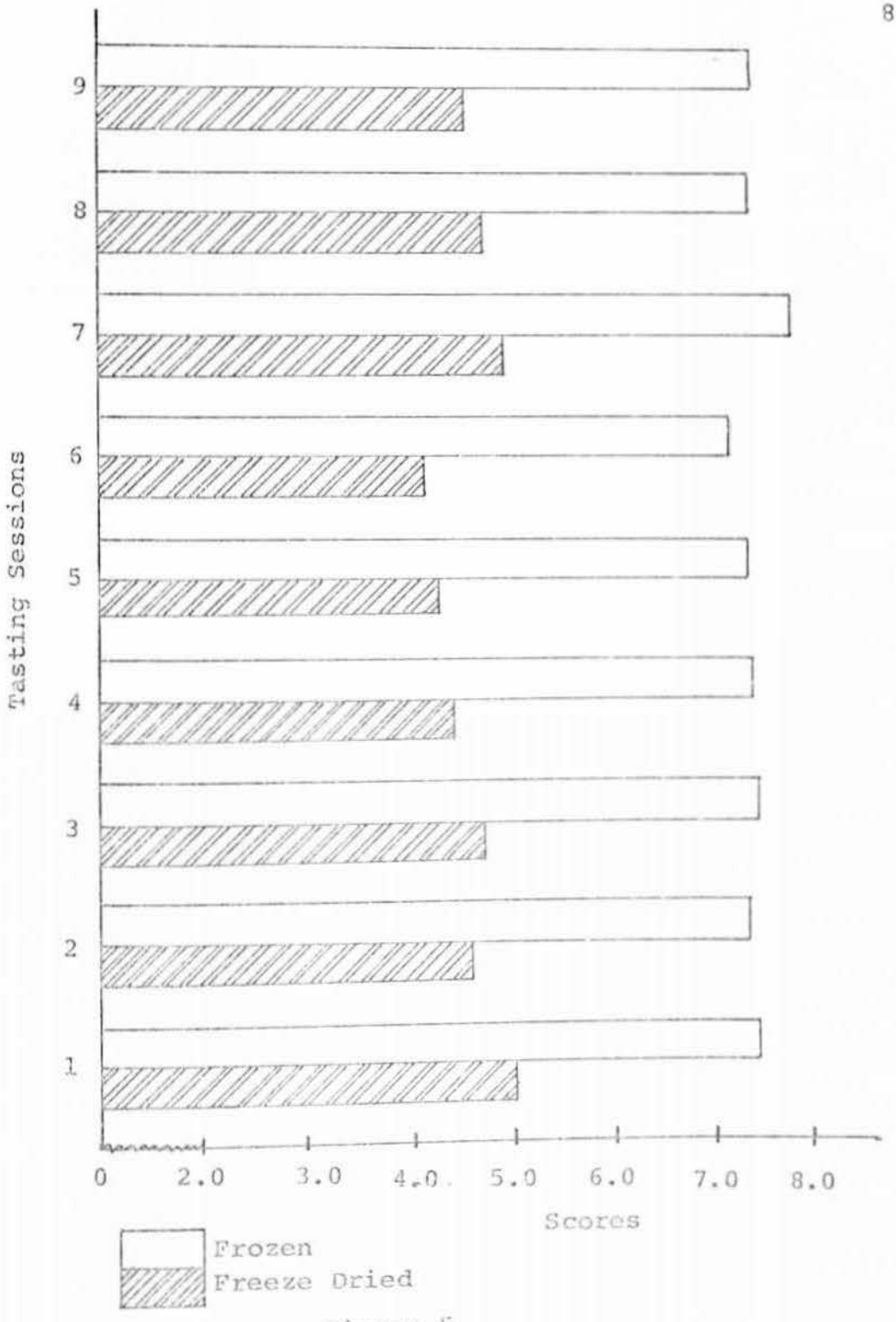


Figure 5

Means of Preferential Rating Scores for Overall Acceptance for Frozen and Freeze Dried Shrimp

The total moisture content and percentage of press fluid lost under given conditions were investigated. The relationship of "bound" and "free" water was considered relative to the organoleptic factor of juiciness.

MICROBIOLOGICAL RESULTS

Since freeze drying does not depend on sterilization but rather upon a low moisture content for the attainment of an appreciable shelf life, the microbiological aspects of this process are of considerable importance. This dissertation reports an investigation into the recovery of certain pathogenic microorganisms from samples of frozen cooked shrimp and freeze dried cooked shrimp obtained from the same processing "batch". Staphylococcus aureus and "fecal enterococci" have been shown to be natural contaminants in some freeze dried foods; therefore, recovery counts of these organisms were investigated in this study. Positive identification of a pathogenic strain of staphylococci was accomplished by means of immunological tests for coagulase-positive organisms. Total aerobic plate counts were also made on frozen and rehydrated freeze dried shrimp samples.

Samples of 5 gm of each type of shrimp were blended with 25 ml of sterile water. For plating purposes, dilutions of 1:10; 1:100; and 1:1,000 were prepared under sterile conditions with both frozen and rehydrated freeze

dried shrimp mixtures. The procedures for microbiological analysis were carried out in triplicate. Freeze dried shrimp samples were rehydrated in sterile distilled water.

Total aerobic plate counts were made in trypticase s oy agar supplemented with 0.5 per cent yeast extract. Plates were incubated at 37° C. and were counted after 48 hours. Plate counts were based on the original 5 gm shrimp in 25 ml sterile water. Serial dilutions did not produce growth of countable colonies in the statistically valid range. Means of the total plate counts based on calculations from the dilutions for the frozen and freeze dried samples were as follows:

Sample	Cells/gram of shrimp
Frozen	4×10^3
Freeze dried	9×10^2

The total plate count for the frozen samples was greater than the total plate count for the freeze dried samples. These results conform with comparisons of freeze dried and non-freeze dried shrimp by other research workers (95).

"Fecal enterococci" were enumerated after growth on violet red bile agar. Aliquots of 1.0 ml of each of the three dilutions of both frozen and freeze dried samples

were pipetted into sterile petri plates and the violet red bile agar medium was added. After incubation at 37° C. for 24 hours on this medium the coliform colonies, if present, are purplish-red surrounded by a reddish zone of precipitated bile. No such colonies appeared on plates of either frozen or freeze dried shrimp at any of the three dilution levels tested. In order to cross check for the presence of coliforms, the same procedure was repeated using eosin methylene blue (EMB) agar. On EMB agar, coliform colonies appear as fluorescent green colonies. There were no positive plates for either type of shrimp at any of the three dilution levels after 48 hours incubation at 37° C. The samples of shrimp tested were thereby shown to be free of any coliform contamination.

Coagulase-positive staphylococci were detected in the frozen shrimp samples at the 1:10 dilution in two of the three samples but no coagulase-positive staphylococci were recovered from the freeze dried samples. Trypticase soy broth with 11.0 per cent sodium chloride was the growth medium used for Staphylococcus aureus determinations. Five tubes of each dilution was inoculated. After 24 hours, egg yolk agar plates were streaked with turbid broth from the trypticase soy broth medium. Colonies

of Staphylococcus aureus, if present, appear in a halo formation that is either pigmented or unpigmented.

Organisms producing halo formations on egg yolk agar were subjected to the coagulase test. Trypticase soy broth in test tubes was inoculated with presumed staphylococcus like organisms as based on colony morphology and allowed to incubate at 37° C. for 24 hours. Reconstituted coagulase plasma from rabbits was dispensed into small test tubes in the amount of 0.5 ml. To the reconstituted plasma a drop of a very cloudy broth culture was added and tubes were kept at room temperature. The tubes were compared with control tubes containing known coagulase-positive Staphylococcus aureus and known coagulase-negative Sarcina lutea at 30 minute intervals for a total of three hours. The presence of and the extent of clotting constituted the basis for the scoring of the degree of the coagulase-positive reaction. Tables VII and VIII show the progression of staphylococci presumptive and coagulase-positive identification tests.

The freeze dried samples did not indicate the presence of a pathogenic coagulase-positive staphylococcus in any of the three samples tested. Of the three samples of frozen shrimp, two reacted positively to the antisera for coagulase-positive pathogens. The frozen samples, however, were not obtained from a normal processing flow pattern. Shrimp

TABLE VII

STAPHYLOCOCCUS PRESUMPTIVE AND COAGULASE-POSITIVE

IDENTIFICATION TESTS ON FROZEN SHRIMP SAMPLES

Experimental step	Description of procedure	Replications			Description of reaction
		I	II	III	
1	Trypticase soy broth inoculated with shrimp dilutions 1:10*	1/5**	2/5	1/5	Turbidity and/or scum formation
2	Inoculated from (1) to egg yolk agar plates	No growth	Halos	Halos	Halo formations, not pigmented
3	Trypticase soy broth inoculated from (2)	Slight clouding	Cloudy buttons	Cloudy buttons	Clouding and formation of buttons
4	Reconstituted plasma inoculated from (3)	No growth	Full clot	Loose, gel-like clot	Clot formations

* Dilutions of 1:100 and 1:1,000 showed no growth.
** Only 1 tube out of 5 showed turbidity indicating bacterial growth.

TABLE VIII

STAPHYLOCOCCUS PRESUMPTIVE AND COAGULASE-POSITIVE

IDENTIFICATION TESTS ON FREEZE DRIED SHRIMP SAMPLES

Experimental step	Description of procedure	Replications			Description of reaction
		I	II	III	
1	Trypticase soy broth inoculated with shrimp dilutions 1:10*	1/5**	2/5	1/5	Turbidity
2	Inoculated from (1) to egg yolk agar plates	No growth	No growth	---	No halo formations present
3	Trypticase soy broth inoculated from (2)	No growth	No growth	---	No clouding, no button-like formations
4	Reconstituted plasma inoculated from (3)	No growth	No growth	---	No clot formations

* Dilutions of 1:100 and 1:1,000 showed no growth.
** Only 1 tube out of 5 showed turbidity indicating bacterial growth.

samples used in the study were removed from freeze trays by hand and were placed into plastic bags which were twisted and tied. The processing plant from which samples were obtained was not equipped for the sterile packaging of frozen foods. The shrimp source was a plant whose operation was devoted exclusively to the freeze drying of food material.

When conditions for rehydration were controlled so that bacterial contamination was at a minimum, the data from shrimp samples indicated that fewer microorganisms were recoverable from the freeze dried than from the frozen shrimp samples. The low level of moisture of freeze dried shrimp, essential for prolonged storage, seems to provide too great a stress for bacterial survival.

COLOR ANALYSES

The red pigment (astoxanthin) was extracted from shrimp with acetone and was quantitated spectrophotometrically as described in Chapter III. Both frozen and rehydrated freeze dried shrimp samples were extracted in triplicate. The samples were the same weight to the fourth decimal place, thus averages of the light absorbancies were calculated so that one characteristic absorbancy curve for each type sample could be determined. Figure 6 shows the representative absorbancy spectra for extracts of frozen and freeze dried shrimp.

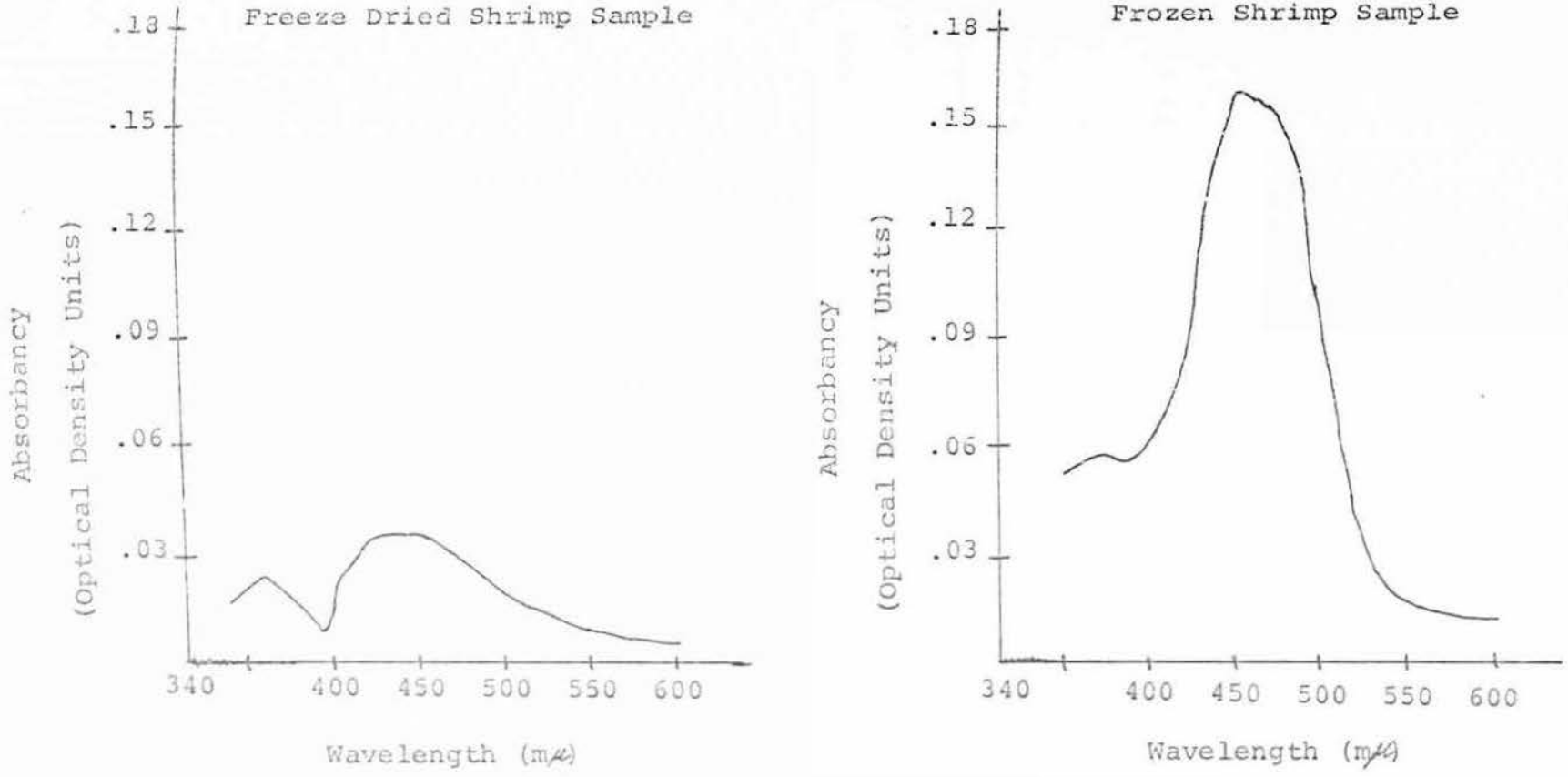


Figure 6

Absorption Spectra of Acetone Extract of Shrimp

The absorption maxima for each of the two types of shrimp occurred between 450 mμ and 460 mμ. A second small peak at 350 mμ was also presented for both frozen and freeze dried samples. No information as to the identity of the component causing this peak could be found in the literature.

The purpose of extracting the pigment was to utilize a quantitative test for the color loss that takes place during storage. If color loss is an oxidative change, measurements of color loss should provide an index of the amount of oxidation having taken place. Oxidative degradation is thought to be responsible for lowered product acceptability.

Color reflectance of chopped and pulverized shrimp samples were read on an Agtron reflectance spectrophotometer. The light source in this instrument can be passed through one of three filters. The filters provided include blue (436 mμ), green (546 mμ), and red (640 mμ). Twenty-five gram samples of chopped and blended shrimp were read in each of the color modes in triplicate.

Means of the three readings in each color mode are shown below:

Sample	Filter	Freeze dried shrimp	Frozen shrimp
Chopped	Red mode	57	58
	Green mode	40	39
	Blue mode	21	25
Blended	Red mode	55	66
	Green mode	46	50
	Blue mode	29	38

The data were analyzed on the basis of the triplicate readings for the two types of shrimp in each of the three color modes. It is realized that this number of replications is inadequate for any definite conclusions; however, data analyses based on the three replications for each of the three modes for both blended and chopped shrimp are shown in Tables IX and X.

Data analyses indicated that there appeared to be a real difference in light reflectance for both the chopped and blended samples in the blue mode. There was a difference in the light reflectance between the frozen and freeze dried shrimp in the blended sample scanned in the red mode but no difference of reflectance was indicated for the chopped sample. The blue mode would be preferred for quality control evaluation of cooked shrimp. The wave length for the blue mode (436 mμ) is within the spectra of greatest

TABLE IX

ANALYSES OF VARIANCE IN LIGHT REFLECTANCE OF CHOPPED SAMPLES OF FROZEN AND FREEZE DRIED SHRIMP AS INDICATED BY AGTRON COLOR MODES

Color Mode	Sum of squares	Degrees freedom	Mean square	F-value
Red Mode				
Between	20.17	1	20.17	3.03*
Within	26.67	4	6.67	
Total	46.84	5		
Green Mode				
Between	2.67	1	2.67	2.29*
Within	4.67	4	1.17	
Total	7.34	5		
Blue Mode				
Between	20.17	1	20.17	120.99**
Within	0.67	4	0.17	
Total	20.84	5		

* Not significant
** $P < 0.01$

TABLE X

ANALYSES OF VARIANCE IN LIGHT REFLECTANCE OF BLENDED SAMPLES OF FROZEN AND FREEZE DRIED SHRIMP AS INDICATED BY AGTRON COLOR MODES

Color Mode	Sum of squares	Degrees Freedom	Mean square	F-value
Red Mode				
Between	181.50	1	181.50	12.66**
Within	57.33	4	14.33	
Total	283.83	5		
Green Mode				
Between	24.00	1	24.00	2.53*
Within	38.00	4	9.50	
Total	62.00	5		
Blue Mode				
Between	112.67	1	112.67	15.72**
Within	28.67	4	7.17	
Total	141.34	5		

* Not significant
** $P < 0.01$

absorbancy (450 mμ to 460 mμ) of the astaxanthin extracted as previously shown in Figure 5. The use of the Agtron as a less costly instrument than the spectrophotometer and a less time consuming procedure, is highly indicated for quality control use, if further testing with a larger number of samples indicates that the results will remain consistent.

Data analysis of the organoleptic evaluation of differences in appearance, flavor, juiciness, softness to tooth, softness to tongue and cheek and fragmentation indicated that the only difference that was significant was for one factor, and that factor was appearance. The difference in color was the contributing factor mentioned most frequently by the panel members. Loss of color resulted in a low overall acceptancy rating. The objective data for pigment analysis and the subjective evaluation of color were in agreement in that loss of color results in a less desirable product. Both the objective and subjective evaluations indicated this loss of color occurs in the freeze dried shrimp samples.

ANALYSIS OF DATA PERTAINING TO TENDERNESS

The denaturation of proteins is responsible for changes in tenderness in specified cooked foods as compared to the same food in an uncooked form. This study investigated only changes resulting from this lyophilization of cooked shrimp. A change in tenderness as a result of lyophilization

was shown by the highly significant t-values in the preferential rating evalution tests. An accepted method used by food technologists for determining relative tenderness is a type of shearing device. The Kramer Shear Press was employed in this study to determine the pounds of pressure needed to shear the fibers of the shrimp. Shrimp samples of 10 gm each were sheared using a ring of 200/3,000 pounds tolerance. Analysis showed that the t-value for differences in shear press readings was highly significant ($P < 0.01$) as shown below:

Shrimp sample	Mean	Standard deviation	t-value
Freeze dried	87.57	7.57	4.773**
Frozen	69.57	5.29	

**$P < 0.01$

The average pounds of force needed to shear a 10 gm sample of freeze dried shrimp was 87.57 while a mean force of 69.57 pounds could shear the frozen samples as shown in Figure 7. The significantly greater force needed to shear the freeze dried samples was in agreement with the significantly lower preferential rating scores in regard to organoleptic evaluation of tenderness for freeze dried shrimp. If the loss of tenderness could be alleviated, the

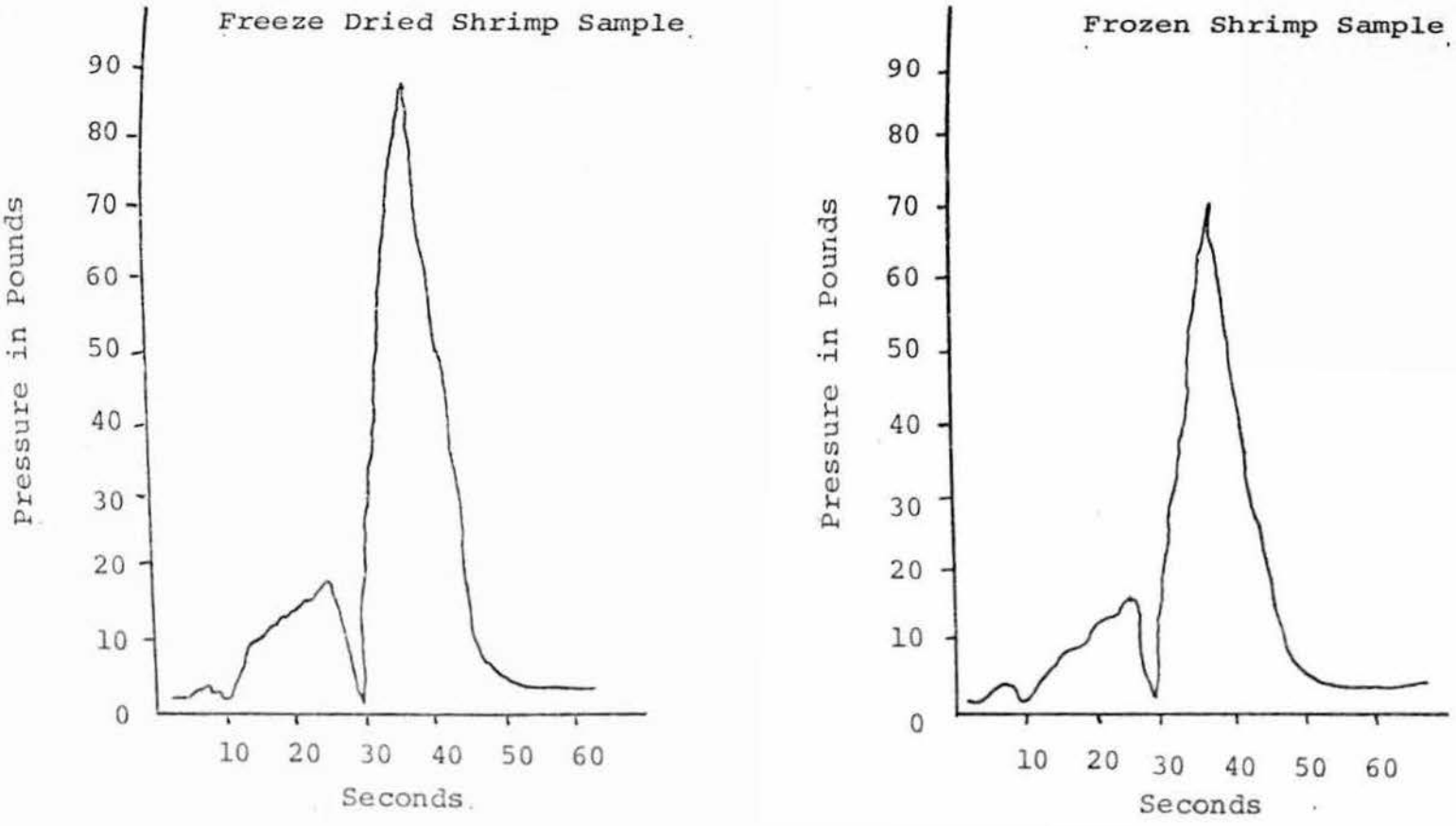


Figure 7

Means of Kramer Shear Press Readings for Freeze Dried and Frozen Shrimp

freeze dried product would likely be a more acceptable substitute for frozen shrimp.

Protein Comparison

The effect of lyophilization on the proteins of shrimp causing a less tender product was a factor of interest in this study. The quantitative presence of peptide linkages was determined by the Biuret Test as outlined in Chapter III.

The means of the optical density readings for the Biuret Tests were 0.061 for frozen and 0.060 for freeze dried shrimp as compared to a standard of 70 mg/ml which gave an optical density reading of 0.059. These results indicate the amount of protein or polypeptides in the frozen and freeze dried samples of shrimp were similar. In view of the fact that the dessicated samples were reconstituted to a concentration of 70 mg/ml, it appears that samples were essentially protein.

Hydroxyproline Comparison

Connective tissue within the muscle plays an important role in determining tenderness. Collagen is the constituent of connective tissue that is most frequently used as an index of tenderness. The quantitative determination of hydroxyproline is a standard technique used to quantitate

collagen; therefore, hydroxyproline analyses were conducted on dessicated samples of freeze dried and frozen shrimp.

No confirmed evidence was found in the literature as to the factor for converting hydroxyproline to collagen in fish or shrimp. A factor of 7.25 was used by Coll, Bray and Hoekstra (41) in the conversion of hydroxyproline to collagen in beef; however, sufficient differences occur between beef and fish to make this conversion factor questionable for shrimp. Therefore, hydroxyproline extraction values will be compared in this study of frozen and freeze dried shrimp rather than converting such values to collagen for comparisons.

The Woessner technique (130) was carried out for the determination of hydroxyproline using 0.10 gm of dessicated tissue sample (weighed to the nearest thousandth) for digestion in 3.0 ml of concentrated hydrochloric acid, then made up to 6.0 ml with distilled water. The procedure was carried out in triplicate for both the frozen and freeze dried samples. Tubes were prepared according to the method described in Chapter III.

The freeze dried samples were found to contain an average of 6.3 μg of hydroxyproline as calculated from optical density readings. The frozen samples contained

an average of 5.3 μg of hydroxyproline. The means for the optical densities read at 557 mμ were as follows: standard (40 mg/ml) - 0.285: frozen samples - 0.373; and freeze dried samples - 0.448.

Two possible factors could account for the greater amount of hydroxyproline recovery in freeze dried samples. The higher concentrations of "hydroxyproline" in the freeze dried samples may be the result of the production of a p-aminobenzaldehyde reactive breakdown product during the lyophilization process. Additional analyses are needed to determine the chemical nature of this product.

Intracellular Protein Comparison

The relationship of certain intracellular protein characteristics to the tenderness factor in frozen and freeze dried shrimp was investigated. Sarcoplasmic proteins were extracted with a low ionic strength buffer (two extractions, for one hour each). Myofribrillar protein was extracted from the residue with sodium hydroxide. Proteins extractable with a high ionic strength buffer were also determined. Figure 8 shows the scheme for the quantitative determination of sarcoplasmic proteins and total fibrillar proteins as discussed in Chapter III. Figure 9 schematically illustrates the extraction procedure for proteins extractable with a high ionic strength buffer.

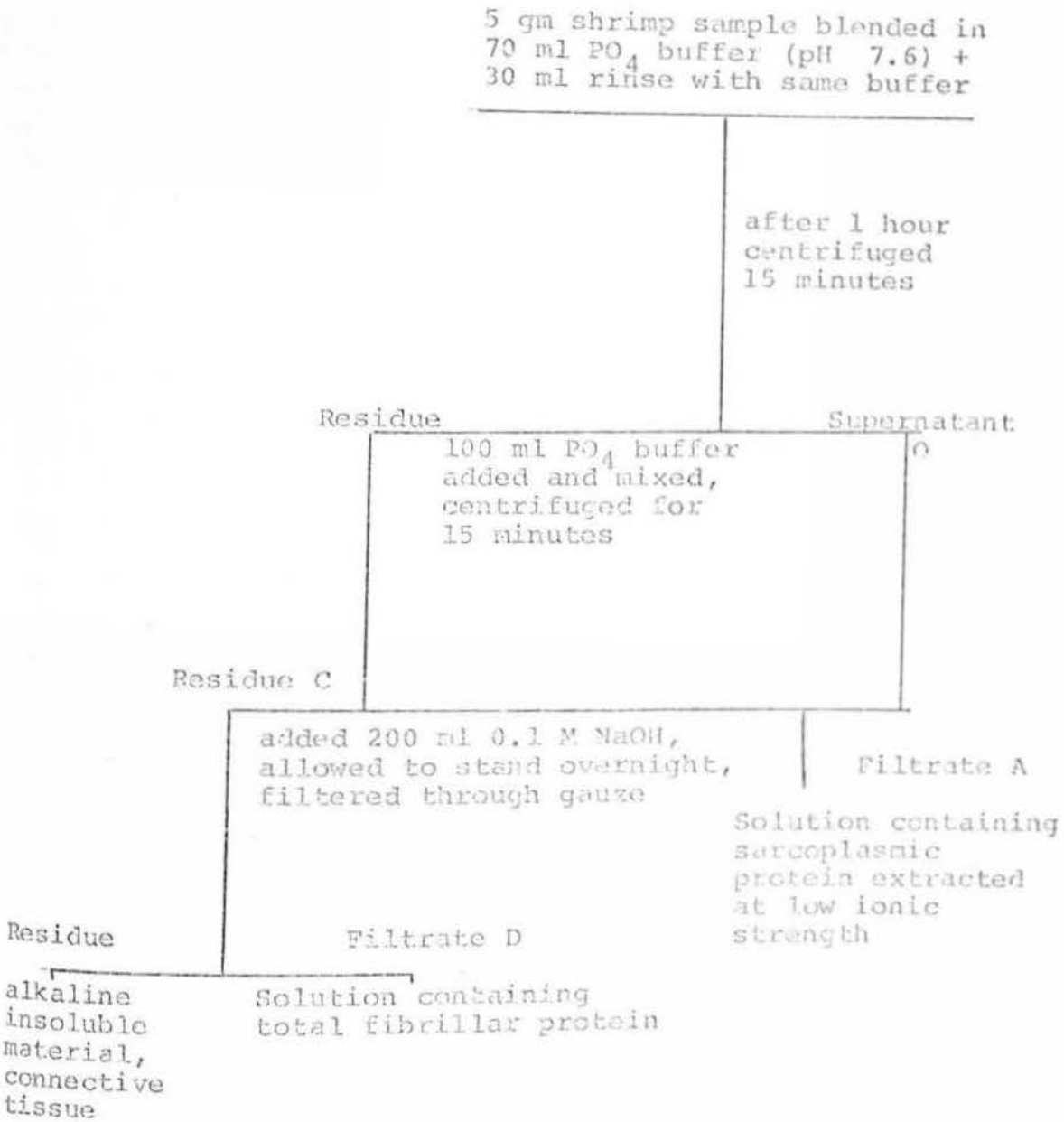


Figure 8

SCHEME FOR THE QUANTITATIVE DETERMINATION

OF SARCOPLASMIC AND FIBRILLAR PROTEINS

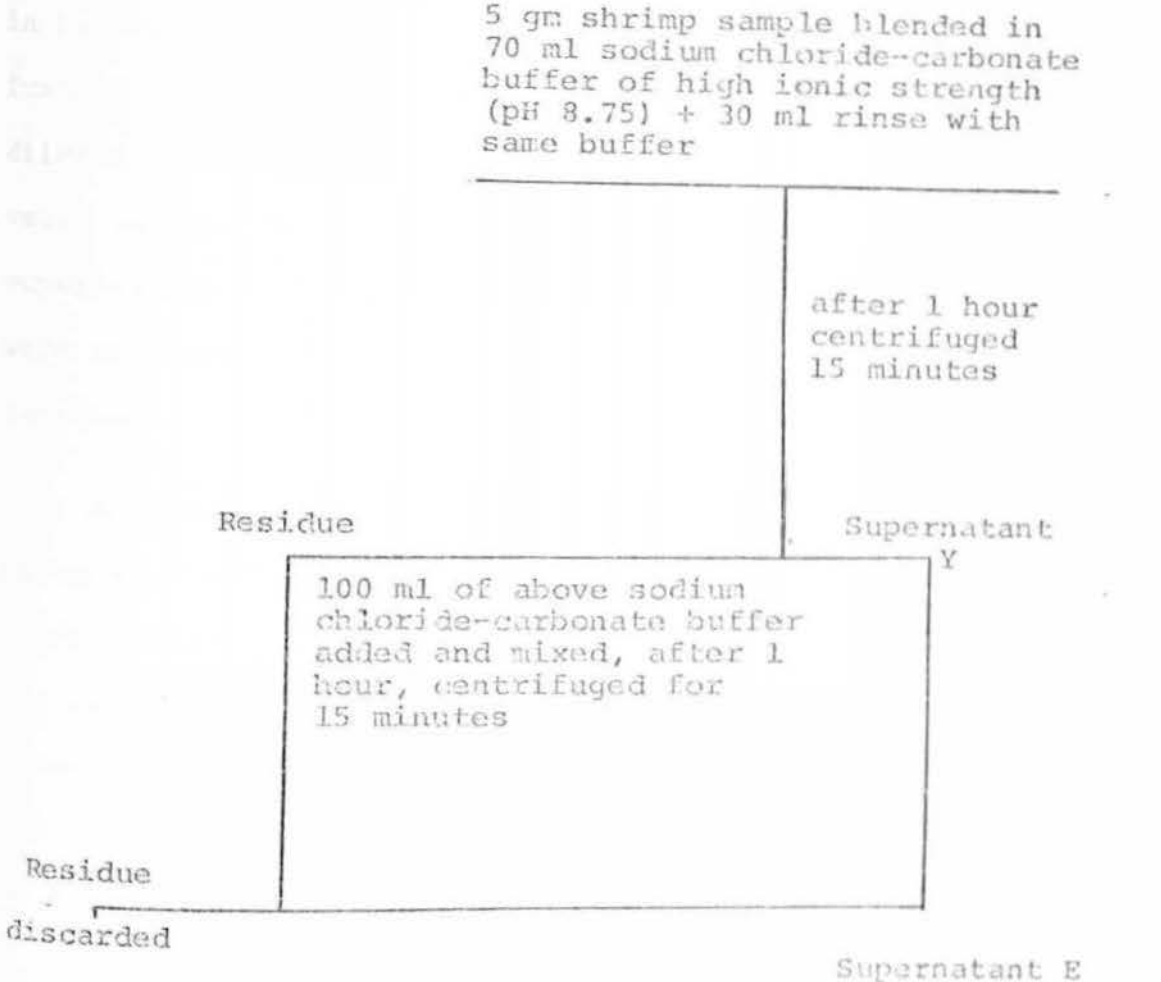


Figure 9

SCHEME FOR THE QUANTITATIVE DETERMINATION OF PROTEINS SOLUBLE IN A BUFFER OF HIGH IONIC STRENGTH

Only the supernatant extractions indicated as "0" in Figure 8 and as "Y" in Figure 9 were utilized for further reporting because total extraction volumes diluted protein and hydroxyproline to such an extent that valid optical density readings were not possible using supernatants "A" and "E". The same extraction levels were compared for both frozen and freeze dried shrimp samples.

Quantitative protein determinations were carried out on each of the three protein fractions: sarcoplasmic, myofribrillar, and proteins extracted at high ionic strength. The extraction method used was that described by Bailey (3) using the Folin Ciocalteu reagent as described in Chapter III. The milligrams of protein for each 5 mg of shrimp tissue in each of the fractions are shown below:

Shrimp samples	Proteins in mg		
	Sarcoplasmic	Fibrillar	Fraction soluble at 8.75 pH
Frozen	0.10	0.86	0.12
Freeze dried	0.08	0.67	0.18

The lyophilization process changed the solubility of proteins in the extractant buffers used in this study. The frozen shrimp sample indicated a 21 per cent greater

solubility (pH 7.60) of intracellular proteins, both sarcoplasmic and fibrillar, than did the freeze dried sample. A slightly greater amount of sarcoplasmic protein was extracted with the phosphate buffer of low ionic strength from the frozen sample than from the freeze dried. The residue from the extraction from freeze dried samples, when treated with sodium hydroxide solution, yielded less extractable fibrillar protein than did the same reaction when carried out with frozen shrimp samples. With the high ionic strength sodium carbonate buffer (pH 8.75) the protein solubility was greater in the freeze dried samples. The freeze dehydration process may in some way affect the bonds that are due to electrostatic interaction between polar groups and to van der Waals forces between non-polar groups of the proteinaceous material.

The association of sarcoplasm to fibrils in bovine muscle was found by Hegarty, Bratzler and Pearson (54) to be proportionant to sarcoplasmic protein and fibrillar protein. The proportion of these two fractions was also related to the kind of work that the muscle performs. Investigations indicated that there is a definite relationship of sarcoplasmic protein to fibrillar protein and muscle activity. These authors expressed the opinion that the ratio of these two proteins could be related to tenderness. The higher proportion of sarcoplasmic protein to fibrillar protein results in greater tenderness in beef.

In the present study organoleptic evaluation of tenderness indicated that the frozen shrimp samples were significantly more tender. Analyses of the protein fractions in this study indicated a one to eight ratio of sarcoplasmic protein to fibrillar protein in each type sample. The actual values were 0.10 mg sarcoplasmic to 0.86 mg fibrillar for the frozen samples and for freeze dried samples, 0.08 mg/ 0.67 mg. Despite the similar relationship between the two protein fractions, a distinct tenderness difference was noted by panel members.

Hydroxyproline values were determined for each protein fraction that had been analyzed quantitatively for protein. The procedure used was the same Woessner method employed in total hydroxyproline analyses and is described in Chapter III. The micrograms of hydroxyproline for each 5 mg of shrimp tissue in each fraction are compared below:

Shrimp sample	Proteins in µg		
	Sarcoplasmic	Fibrillar	Fraction soluble at pH 8.75
Frozen	1.57	2.16	1.19
Freeze dried	1.84	2.21	1.46

The freeze dried samples had greater light absorbancy for each of the three above fractions than did the frozen samples; although the extractable protein was less in the

fibrillar sample. The sarcoplasmic fraction contained a higher ratio of hydroxyproline to protein than did the fibrillar extract. The freeze dried samples indicated a 38 per cent greater light absorbancy in hydroxyproline determinations for intracellular protein than the frozen.

Reconstituted freeze dried shrimp was used for fractionation rather than dessicated shrimp which was utilized for total hydroxproline determinations. The fact that hydroxyproline values were higher in each fraction of freeze dried shrimp than in the corresponding fractions of frozen shrimp seems to indicate that lyophilization does result in a breakdown of some protein fraction that alters the light absorbancy from that of tissue that had not been freeze dried.

Hydroxyproline was quantitated for the intracellular fractions of sarcoplasmic and fibrillar proteins and for total shrimp samples in this study. The difference in intracellular hydroxyproline and total hydroxyproline content calculated on the basis of 5 gm of sample would represent that content of hydroxyproline in connective tissue in shrimp.

There is an inverse relationship between tenderness and the amount of connective tissue present in beef muscle. This study indicated that the frozen cooked shrimp, which contained the lesser amount of extractable hydroxyproline,

was more tender than the freeze dried samples, containing the greater amounts of extractable hydroxyproline. This inverse relationship appears to be present in shrimp and in freeze dried shrimp to a greater extent than in frozen shrimp.

Statistical comparisons on the chemical analyses were not calculated because of the low number of replications. Analyses of the quantitative determinations for protein and hydroxyproline were performed in duplicate only.

TOTAL MOISTURE AND WATER HOLDING CAPACITY

The relationships of the moisture within the tissue of meat and fish and the sensations of juiciness associated with eating are not clearly understood. The water component of flesh foods would seem to influence flavor, juiciness and tenderness. The possible effect of change in water holding capacity, produced by lyophilization was investigated. The author was of the opinion that decreased flavor and change in mouthfeel might be associated with moisture changes.

As a check for validity, total moisture in both frozen and rehydrated freeze dried shrimp samples was obtained by two different procedures. The samples were weighed on a Mettler analytical balance and were placed in a drying oven at 160° F. for 15 hours, after which each sample was again weighed on the same balance. Analysis of the data by the

t-test showed that the percentage moisture within the samples as determined by use of a drying oven did not differ significantly for frozen and freeze dried samples as shown below:

Shrimp sample	Mean	Standard deviation	t-value
Freeze dried	73.8	0.979	0.574*
Frozen	72.9	0.900	

*Not significant

Total moisture determinations for frozen rehydrated freeze dried shrimp samples were also made by a combined toluene extraction distillation procedure. A method developed by the lyophilization plant furnishing the shrimp was employed using a 50 gm sample of product in 100 ml toluene and distilling off the moisture within the product. The toluene extraction method appears to be the more precise method of determining moisture content as evidenced by the comparison of the means of the two methods of moisture extraction as follows:

Shrimp sample	Moisture Extracted		Difference
	Toluene method	Drying oven	
	Per cent	Per cent	Per cent
Freeze dried	74.5	73.8	0.7
Frozen	73.4	72.9	0.5

Toluene extractions are employed to determine exact moisture levels of freeze dried products accurately to less than one-half of one per cent. Drying oven methods are accurate only to 2.0 per cent as a result of a small amount of residual bound water that is difficult to evaporate from the product (10).

The insignificant difference in total moisture content as determined by the objective tests was in agreement with comments made by those panel members who were consistent in their observations. Comments concerning juiciness indicated that little differences in amount of juice between the frozen and freeze dried products were observed but the juice in the frozen shrimp was "flavorful" while the juice in the freeze dried sample was "watery."

Several methods were undertaken to determine either the water within the flesh or the water holding capacity of the tissue. Ten gram samples were subjected to moisture extraction on an Ohaus Moisture Balance with readings taken at 10 minute intervals, in an effort to differentiate bound and free water. The author anticipated the percentage moisture extracted would form a curve with two plateaus; one plateau at the point at which the free water was removed and another at the point at which all moisture was removed.

The curves for both the frozen and freeze dried samples showed a regular pattern of moisture level to the point of total dessication.

A Carver laboratory press was employed for a determination of extractable press fluid that is considered by some research workers (104) to be the free water within a product. The customary use of a dye for sample extraction of free moisture was unsuccessful. The shrimp fibers were sufficiently tender that portions of the product were extruded around the dye thus making the reweighing of the residual material inaccurate.

A method of placing 0.5 gm of sample between aluminum foil discs was devised. After determining the size discs needed, samples were weighed between two aluminum foil discs on a Mettler analytical balance. The sample and discs were placed between double filter papers and were pressed in the Carver Hydraulic Press with 5,000 pounds pressure for one minute duration. The aluminum foil discs together with the residual material from the shrimp sample were reweighed in order to determine the percentage of water lost.

Analysis of data indicated that a significantly greater amount of fluid could be extracted from the rehydrated

freeze dried than from the frozen shrimp as indicated below:

Shrimp sample	Mean	Standard deviation	t-value
Freeze dried	42.40	0.800	12.79**
Frozen	36.40	0.490	

**$p < 0.01$

The fact that a greater amount of press fluid could be extracted from rehydrated freeze dried shrimp was likely the cause for comments in the organoleptic evaluation pertaining to a dry mouthfeel after chewing. Some research workers call this extractable water the free water in a meat product. The residual moisture is then called bound water. The author feels that a more extensive study would be necessary to determine the exact amount of pressure, temperature, humidity conditions, and time needed to clearly differentiate between bound and free water in the investigation of shrimp. The data clearly indicate, however, that under the given conditions of this investigation a greater amount of press fluid is extractable from the rehydrated freeze dried product.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Some of the factorial differences resulting from the lyophilization of cooked shrimp were investigated and were related to organoleptic acceptance of the freeze dried product. The factors studied were the effect of lyophilization on 1) color; 2) microbiological flora; 3) tenderness and 4) juiciness.

Organoleptic evaluations of frozen cooked shrimp and rehydrated freeze dried cooked shrimp were obtained by means of difference and preference testing employing a panel of 13 trained judges. The judges were selected on the basis of accuracy and consistency in identifying threshold levels of sucrose, citric acid and sodium chloride solutions. These judges, following a training period in shrimp evaluation and experience in using the test form designed for use in this study, participated in nine sessions of taste testing. At each session the observed differences in appearance; flavor; juiciness; and tenderness to tooth, to tongue and to cheek and fragmentation were scored on a 10 point scale ranging from no difference to a pronounced difference. Comments describing the difference were required of the panel members if any degree of observable difference was indicated. Preference between the two products, as related

to overall acceptance and to tenderness, was indicated by assigning a value to each sample of frozen and freeze dried shrimp. A nine point hedonic scale was used.

Organoleptic evaluations indicated the difference in appearance was highly significant, the greater preference being for the frozen product. The preference for frozen shrimp was significantly higher both as to overall acceptance and as to tenderness. Color was ranked as the foremost difference factor in the area of appearance. Frozen and freeze dried shrimp did not differ significantly as to the amount of juice but comments indicated a real difference in the mouthfeel and flavor of the juice present. Again the preference was for the frozen samples.

Of the nine taste testing sessions, six compared "plain" shrimp. Three of the evaluation periods evaluated differences in frozen and freeze dried shrimp in "prepared" dishes although the observable differences were less apparent.

For further studies, this author feels that choice of panel members on the basis of pre-testing should be accomplished by means of evaluation of the particular product to be tested and not on the basis of threshold levels of substances unrelated to that food. Furthermore, the panel members in a taste test study should be trained for a period of time sufficient to establish complete clarity

in vernacular used in evaluations. A more nearly synchronized use and choice of words would help to eliminate inconsistency between judges.

To determine if differences in the number of organisms or the presence of pathogens occur in samples of frozen and freeze dried shrimp, a microbiological survey was conducted. Total aerobic plate counts at three dilutions; 1:10, 1:100, and 1:1,000, indicated that fewer microorganisms could be recovered from the freeze dried samples than from the frozen samples. Of the two pathogenic types of bacteria for which determinations were made, "fecal enterococci" were not present in either frozen or freeze dried samples. Coagulase-positive staphylococci were not present in the freeze dried sample. Based on this data, freeze drying as a means of preservation appears to be bacteriologically safe.

Color change represented a major factor of criticism in freeze dried shrimp. Shrimp samples were subjected to two different quantitative colorimetric analyses procedures. Astaxanthin pigment, responsible for the red color, was extracted chemically and was quantitated spectrophotometrically. The amount of light reflectance of each sample, frozen and freeze dried, was determined by a color reflectance spectrophotometer. Objective tests indicated a significant pigment loss in the freeze dried product and this color

loss was associated with a lower overall product acceptance as indicated by organoleptic evaluation. A significant difference in light reflectance at approximately the same wave length at which the peak of light absorbancy was observed in pigment extraction seems to indicate that light reflectance could be used successfully as a quality control tool for the determination of product quality.

The taste panel rating scores for tenderness indicated a highly significant preference for the frozen product. As an objective measure of tenderness, Kramer Shear Press readings of pounds per square inch necessary for shearing fibers of shrimp were obtained. The frozen samples showed a significantly lower shear value.

In order to account for the lower degree of tenderness indicated for the freeze dried product an investigation was made to determine the relationship of protein to hydroxyproline both in the total shrimp protein content and that of three intracellular fractions, namely, sarcoplasmic, fibrillar and protein extractable at high ionic strength. The quantitative determinations of hydroxyproline has been accepted as a valid method for quantitating collagen in material. Collagen, as an important constituent of connective tissue, is associated with tenderness.

Tissue samples of freeze dried shrimp and also aliquots of extractions of sarcoplasmic protein, fibrillar protein and the protein fractions extractable at high ionic strength indicated greater optical density readings than did like samples of frozen shrimp in hydroxyproline determinations. Dessicated tissue samples were used for the hydroxyproline extraction of the total protein, whereas, fresh frozen and freshly rehydrated samples were employed for fractionation. In the opinion of the author, the increased light absorbancy of the freeze dried material was a result of some change in protein as a result of lyophilization. Data from a greater number of samples are necessary, however, before this assumption can be validated.

Fractional solubility differences in frozen and freeze dried shrimp samples were indicated by the data in this study. Fibrillar protein extractability was markedly diminished by lyophilization. The relationship of intracellular proteins was approximately one part sarcoplasmic protein to eight parts of fibrillar protein. In beef studies, tenderness has been shown to be related to higher proportions of sarcoplasmic proteins to that of fibrillar proteins. In the shrimp samples investigated in this study the ratio of the two fractions were the same. Nevertheless, tenderness differed greatly both by organoleptic evaluation and by shear press values.

A greater number of replications of data of the type obtained in this study are needed in order to determine statistical reliability. In addition to the study of hydroxyproline content of each intracellular fraction, hydroxyproline determinations should be made on the residue containing alkali insoluble matter. Identification of the factor or factors responsible for the greater light absorbancy in the freeze dried samples is needed. This determination would represent the hydroxyproline content of the connective tissue of shrimp. A conversion factor for hydroxyproline to collagen for shrimp should be determined and set forth in the literature.

The factor of juiciness and acceptability of mouthfeel was investigated in frozen and freeze dried shrimp. Although fat is associated by some research workers as the most significant influence on the juiciness of beef and other meats, the fat content of shrimp is negligible and therefore could not be a contributory factor to juiciness. The amount of moisture within a shrimp sample and the rate at which that moisture is released was assumed by the author to be responsible for either a juicy product or one producing a dry mouthfeel. The percentage of press fluid extractable from the freeze dried samples was significantly greater than that from frozen samples under similar conditions. The difference in the greater moisture release

from the freeze dried sample under like chewing conditions could account for the comments by taste panel members pertaining to a dryness of product after the first few chews.

The author accounted only for press fluid under given conditions since no method for an absolute determination of "bound" and "free" water was ascertained. If such a technique could be devised, a valid differential factor could be investigated for shrimp as well as for many other food products.

The need for further investigation into the causes of the toughening and loss of color in shrimp as a result or lyophilization with recommendations for the alleviation of these undesirable characteristics is needed for this product to become more acceptable to consumers. The high cost of freeze dried food necessitates a product of optimum quality in order to appeal to buyers. Little toughness occurs in fresh and frozen shrimp; as a consequence, little research has been directed to the problem of shrimp toughening. A vast number of investigations have been carried out on bovine and porcine muscle samples but these tissues differ greatly from shrimp muscle tissue. Research must determine if the reactions of shrimp proteins are sufficiently similar to the same reactions of beef and pork proteins to allow research workers to make direct comparisons.

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A P P E N D I C E S

APPENDIX A

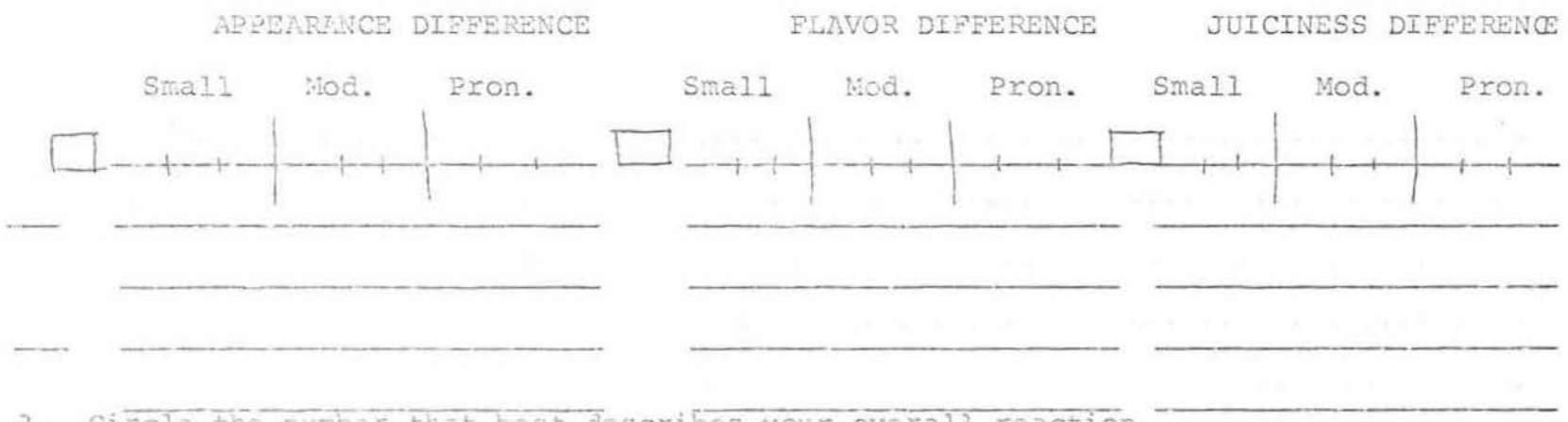
EVALUATION OF SHRIMP

DIFFERENCE EVALUATION DATE_____

Judge_____

The purpose of this test is to determine if there is any difference in flavor, juiciness and appearance between these samples.

1. Order of testing - _____, _____
2. Indicate whether or not these samples differ by marking the proper amount of difference on the scale. Check between the lines. If no detectable difference or not certain, check in the box. Describe the differences.



3. Circle the number that best describes your overall reaction.

	Code	Code
LIKE		
9 -- Extremely	9	9
8 -- Very much	8	8
7 -- Moderately	7	7
6 -- Slightly	6	6
NEUTRAL		
5 -- Neither like nor dislike	5	5
DISLIKE		
4 -- Slightly	4	4
3 -- Moderately	3	3
2 -- Very much	2	2
1 -- Extremely	1	1

JUDGE_____ TENDERNESS EVALUATION DATE_____

5. Circle the number that best describes your overall tenderness evaluation.

	Code___	Code___
TENDER		
9 -- Extremely	9	9
8 -- Very tender	8	8
7 -- Moderately	7	7
6 -- Slightly	6	6
NEUTRAL		
5 -- Neither like nor dislike	5	5
NOT TENDER		
4 -- Slightly	4	4
3 -- Moderately	3	3
2 -- Very tough	2	2
1 -- Extremely	1	1

6. Indicate whether these samples differ in tenderness aspects and fragmentation by marking the proper amount of difference on the scale. Check between the lines. If no detectable difference exists, check in the box. Describe the differences.

SOFTNESS TO TOOTH PRESSURE	SOFTNESS TO TONGUE AND CHEEK	FRAGMENTATION ACROSS THE GRAIN
Small Mod. Pron.	Small Mod. Pron.	Small Pron. Pron.
☐ ——+——+——+——	☐ ——+——+——+——	☐ ——+——+——+——
___ _____	_____	_____
_____	_____	_____
___ _____	_____	_____
_____	_____	_____

APPENDIX B

CRITERIA FOR JUDGING

CRITERIA FOR JUDGING

I. Appearance factors to be judged:

1. Color of pigment as to color differences (pink tones or orange tones)
2. Color of flesh (white to creamy color)
3. Moistness or dryness
4. Shriveled or plump

II. Flavor factors to be judged:
1. Fresh, mild flavor compared to fishy strong flavor
2. Off flavors

III. Juiciness to be tested by squeezing the meat between the teeth in the first few gentle chews. Indicate amount of juice and flavor of juice.

IV. Softness to tooth pressure to be judged by muscular force exerted to sink teeth into the meat during first few gentle chews. Cutting with the teeth is not necessary.

V. Softness to tongue and cheek to be judged by feel.

VI. Ease of fragmentation judged by amount of cutting or breaking required across the grain. Toughening results from less ease in fragmentation as fibers tend to clump and adhere together.