AN ABSTRACT OF THE THESIS OF

Phillip John Akel for the degree of Master of Science in Food Science and Techology presented on AUG. 24, 1981

Title: Characterization of the Collagen Protein in Smooth Pink Shrimp *(Pandalus jordani)*

Abstract approved: David L. Crawford

The collagen content and composition of collagens in different age classes of shrimp were determined. Their physical and chemical characteristics were investigated. The interrelationship of shrimp size and muscle collagen content to raw and cooked meat yield was established.

Total collagen content for three lots of round shrimp with weights averaging 2.58±.39, 5.27±.55 and 7.72±.96 g was determined to be 2.36, 3.35 and 3.47 mg collagen/g total musculature N, respectively. Unformed collagen comprised 53.85, 35.52 and 0.86% of the total collagen content, respectively. Maturation, as reflected by shrimp size, was accompanied by a near linear increase in formed collagen.

A molecular weight of 310,000 for shrimp collagen was determined using SDS gel electrophoresis. The accuracy of this determination was compromised by limited mobility and lack of standard reference proteins of appropriate molecular weight, but did establish a molecular weight in a range common to other collagens.

Variations in the amino acid composition of formed and unformed collagen reflected the function of the tissues in the musculature from which they were derived. Formed collagen contained higher amounts of glycine, proline and hydroxylysine than unformed collagen, providing a chemical basis for its structural function in formed connective tissues. Remaining amino acids, except histidine, glutamate and arginine were contained in higher amounts in unformed collagen. Unformed collagen also contained a substantial amount of unidentified components which were suspected to be amino sugar derivatives. Only trace amounts of these components were found in formed collagen.

Shrimp collagen contained unusually low levels of glycine, only trace amounts of hydroxyproline and substantial quantities of tryptophan. Glycine and hydroxyproline are important amino acids in mammalian collagens, but tryptophan is usually not present. Shrimp collagen also contained higher levels of threonine, tyrosine, hydroxylysine, valine, methionine, leucine, isoleucine and phenylalanine than most other reported collagens. These variations in amino acid composition seem to reflect a requirement for a structural protein possessing unique characteristics commensurate with the anatomical structure of the species.

The yield (% dry wt.) of raw and cooked (100 sec; 101^{o}C in steam) derived through hand peeling round shrimp, was correlated ($P \geq .001$) in a positive manner by well defined

power functions. Raw meat yield (% dry wt.) declined during ice storage in a linear ($P \geq .001$) manner at a rate dependent upon shrimp size. The more rapid loss of solids from large shrimp reduced yield differences as storage was extended. Raw meat losses during ice storage ranged from 0.298 to 0.318 g raw meat dry matter/100 g round shrimp/day for 2.5 and 7.5 g shrimp respectively. Dry matter weight loss from raw meat through the washing action of melting ice, was replaced in a linear ($P \geq .05$-$P \geq .005$) manner with water to maintain yield (% wet wt.) during storage. Ice storage expanded cooked yield (% dry wt.) differences between shrimp sizes. Meat losses through cooking mediated by ice storage, ranged from 0.421 to 0.303 g cooked meat dry matter/100 g round shrimp/day for 2.5 and 7.5 g shrimp, respectively.

The age class dependent content and composition of collagens in the musculature of shrimp was reflected in the recovery of raw and cooked meat. Meat from small shrimp contained higher levels of unformed collagen which possessed less dry matter and degraded more rapidly in ice storage. Proteolytic action on elevated levels of unformed collagen was not reflected in the rate of ice storage losses. But, it markedly increased heat induced solubilization of solids and enhanced moisture retention through steam precooking over larger shrimp. Maturation of shrimp associated with more formed and less unformed collagen reduced solids solubilization and moisture retention through steam precooking.

Characterization of the Collagen Protein in Smooth Pink Shrimp *(Pandalus jordani)*

by

Phillip John Akel

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed August 1981

Commencement 1982

APPROVED:

Professor of Food Science and Technology
in charge of major

Head of Department of Food Science and Technology

Dean of Graduate School

Date thesis is presented August 24, 1981

Typed by Nancy Caton for Phillip John Akel

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. David L. Crawford for his guidance throughout the course of my research.

Special thanks is extended to Dr. Robert Becker for his assistance in conducting the amino acid analyses, and the staff of Oregon State University Seafoods Laboratory for their help and encouragement.

Appreciation is extended to the National Oceanic and Atmospheric Administration (maintained by the U. S. Department of Commerce) Institutional Sea Grant NA 79 AA-D-00106 and the Otter Trawl Commission of Oregon for their partial support of this work, and to Marine Construction and Design Company of Seattle, Washington, for the loan of their laboratory scale mechanical shrimp peeler.

Finally, I would like to express my thanks and deep appreciation to my family and friends for their patience and support.

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INTRODUCTION

Collagen is the most abundant animal protein on Earth. It is an important constituent of the supporting structure of both vertebrates and invertebrates. Although it is found to a large extent in fibrous connective tissues, collagen appears in some form in virtually all tissues. Examples include skin, tendon, bone, cornea, basement membrane and muscle. Its principal role lies in its mechanical properties and its ability to support and interconnect body tissues, making possible organization of the whole body or organism.

Being a family of proteins with unique structural features, collagen has been the subject of structure-function correlation studies and research involved with its structural organization, from amino acid sequence to the nature of the fibrils. Its role in tissue development and differentiation, wound healing and pathological fibrosis has recently been studied, as well. Thus, the recent number of advances in collagen biochemistry have, in turn, led to improvements in product technologies where collagen is concerned, such as the food industry. Even so, most collagen studies to date have focused on vertebrates. Consequently, little is known on the characteristics of invertebrate col-

lagen. What little work that has been done, has revealed close structural similarities between vertebrate and invertebrate collagen. Now, comparitive studies should give us an idea of structural modifications in various collagen molecules, and may even reveal new functions.

Collagen may be subdivided into formed and unformed collagen. Formed collagen represents fully developed collagen, making up the fibrous network in connective tissue proteins. The term unformed collagen, or protocollagen as written in current literature, is used to describe the individual polypeptide chain or group of chains making up the precursor to the fibrous connective tissue collagen.

The existence of protocollagen as a collagen precursor has been questioned for several years. However, some researchers have provided indirect evidence for its existence (Peterkofsky and Udenfriend, 1963; Lukens, 1965). Still others have been able to isolate and characterize protocollagen (Thompson and Thompson, 1969). Further research involved in the characterization of collagen precursors may lead to a better understanding of the biochemical basis for structural breakdown and yield losses in meat resources.

Cooked shrimp meat yields, for example, are of economic importance to both processors and consumers. Reduced meat yields mean higher costs for the processor, which in turn, are passed on to the consumer. Soluble solid losses occurring during processing, can result in a drier and

tougher cooked product. Also, the nutritive value of the cooked meat may decrease substantially because of protein and mineral leaching.

This investigation was designed to characterize the collagen protein of smooth pink shrimp *(Pandalus jordani)*, one of the Pacific Northwest's biggest seafood resources. Analyses of the amino acid content, total nitrogen, and molecular weight determinations were done for the collagen on both immature and mature shrimp. A quantitative determination of variations existing in collagen content between different age classes was also examined, along with relationships existing between age classes and meat yields through steam precooking.

REVIEW OF LITERATURE

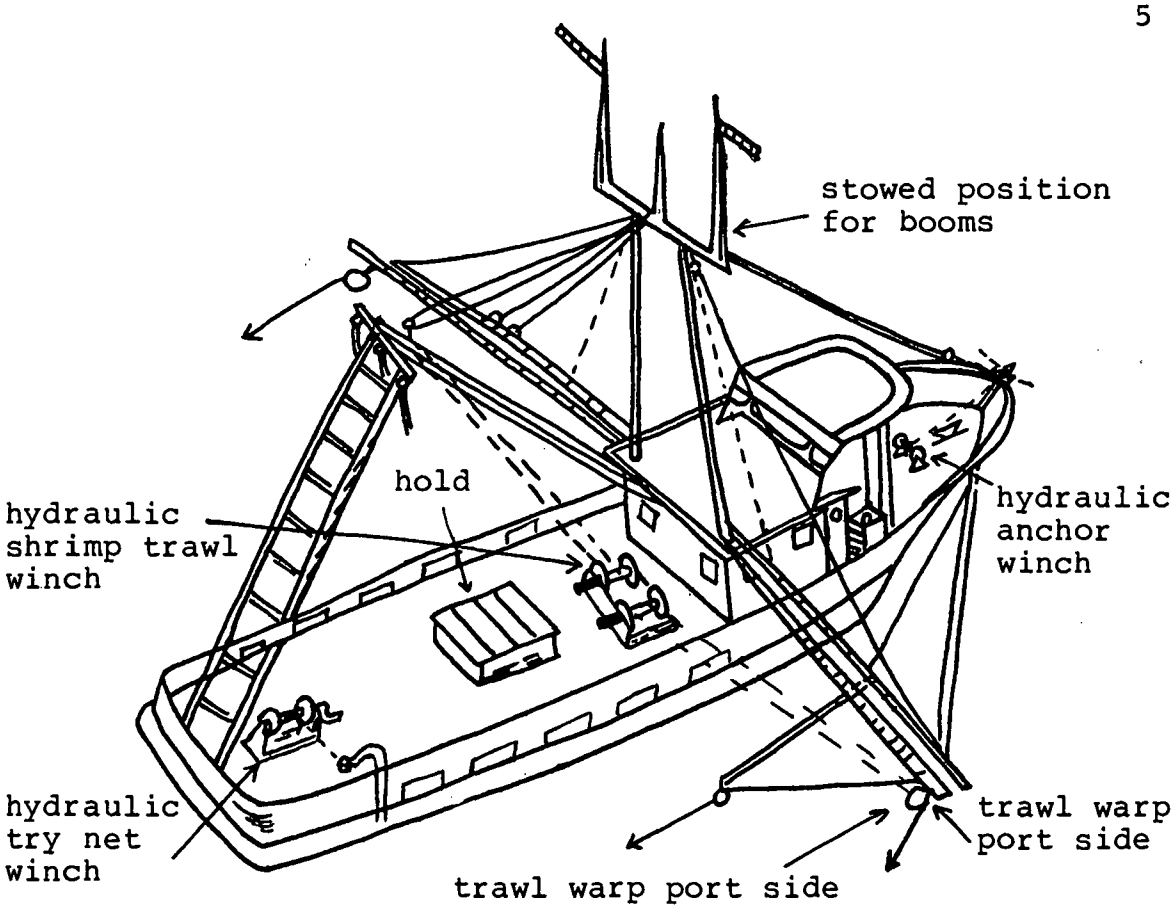
Shrimp as a Resource

More than eighty-five different species of shrimp can be found off the Pacific coast from Northern California to Alaska (Butler, 1980). They are an important part of the marine ecosystem and are a major part of the diet of fishes, marine mammals, and large invertebrates. But, from an economic standpoint, shrimp's greatest importance is as a food resource for man. Since 1952, it has been the most valuable marine resource in the United States (Idyll, 1976). In 1980, total shrimp catches in the United States amounted to 208 million pounds.

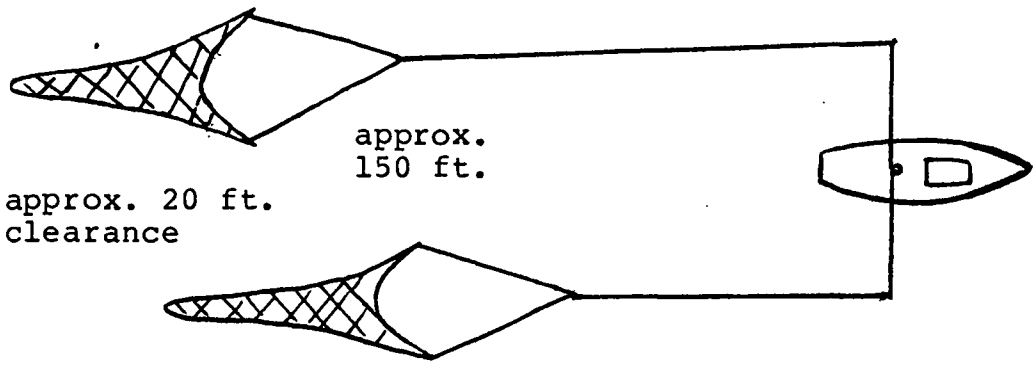
Shrimp Harvesting

The species is primarily found on a green mud or mixed sand and mud bottom (Dahlstrom, 1970). They are usually caught at depths of 120 to 240 feet. In the fall, they move into deeper waters ranging from 240 to 1500 feet deep, where females carry eggs from November to April. After the eggs hatch, shrimp move inshore again. This movement to deeper waters makes shrimping difficult and a major reason shrimp fishing is seasonal. The shrimp reach a marketable size at about 50 mm.

Commercial shrimp boats catch shrimp by towing a trawl net from the stern or sides of the trawler (Figure 1). As the net scrapes over the mud bottom, shrimp are funneled



LAYOUT OF A DOUBLE RIG SHRIMP TRAWLER



TWIN RIG TRAWLING

Each net is towed by a single warp and the starboard net is usually towed ahead.

Figure 1

Sainsbury (1971)

into the bag or tail of the net. After about three hours of trawling, the usual time necessary to fill the bag, the net is hauled to the surface and swung aboard the boat. The contents are emptied into a holding bin. The shrimp are separated from the trash fish, washed with sea water and stored in alternate layers of ice in bins within the hold of the vessel. The catch date is marked on each bin in order to insure the earliest catches get processed first. Landed shrimp are washed immediately after unloading and transported to the processing plant.

Shrimp Processing

Because most shrimp harvested in northern Pacific waters are sold in a canned or frozen state, some type of processing is required. This frequently involves precooking and peeling before packaging. Since labor costs for hand peeling are high, large scale production in the Northwest is carried out with the aid of a mechanical peeler. Two kinds of machine peelers are used. One peels shrimp in the raw state, and one conditions the carcasses with a short steam precook prior to peeling (Lapeyre, 1968). These peelers utilize counter rotating rollers to peel and separate the shell from the musculature of the shrimp (Lapeyre and Coret, 1972). Collins (1960), demonstrated the difficulties of machine peeling freshly caught raw shrimp. He proposed the common practice of holding shrimp on ice or

in refrigerated sea water for at least two days to facilitate the machine peeling operation. But holding shrimp increases the cost and reduces the yield, due to biochemical changes and subsequent physical damage of the shrimp during storage.

Precooking of shrimp has also been shown to improve peelability (Lapeyre, 1966). But, precooking encourages moisture and drip losses. This is accompanied by a loss of heat soluble protein constituents resulting in a decreased yield of the shrimp meat (Nouchpramool and Crawford, 1980).

Recently, the use of condensed phosphates, followed by a short precook, has produced optimum yield and quality attributes of cooked shrimp meat while at the same time improving peelability (Nouchpramool and Crawford, 1980). But, a basic understanding of the biochemistry of protein solubilization and the mechanisms responsible for the reduction of meat yields through steam precooking is needed. Proteolysis and heat solubilization of the connective tissue proteins is believed partly responsible for this occurance (San Ramon and Crawford, 1980).

Life Cycle and Morphology

The life cycle of *Pandalus jordani* begins with the larvae hatching from late March to early April. Following an unknown larval life, metamorphosis takes place to the adult form in August (Butler, 1980). The species is a protrandous hermaphrodite. Individual shrimp mature and function first

as males. They then undergo a sex change during the second and third year of life and function as a female. Though in some years, up to 40% of an age group may mature first as females. Generally, gonads of the shrimp begin developing during the summer months. The gonads become visible as bluish-green ovaries within the carapace in autumn, and by late November, females are carrying eggs. Following hatching in the spring, females usually die. There are instances, however, where they may live into their fourth year. The size of the mature shrimp ranges from 125 mm to 175 mm.

The color of *Pandalus jordani* ranges from light to dark pink, with the shell surface being covered with fine red dots. The shrimp body is slender and compressed. The shell is thin and its surface is smooth (Rathburn, 1902). The shell is composed primarily of chitin, a structural polysaccharide containing units of polyacetylglucosamine. Noncollagenous proteins comprise the remaining portion of the chitin structure (Rudall, 1955; Dennell, 1960; Lockwood, 1967). The chitinous integument protects the body against mechanical injuries and also serves as an exoskeleton. The chitinous shield prevents free growth of the body, however. Therefore, shrimp grow only when they shed their shell, a process known as molting. Molting plays a role in other physiological functions as well; e.g., fertilization of the female (Burukovskii and Bulanevkov, 1969).

The shrimp body is divided into two separate regions.

The anterior end is the cephalothorax, and the posterior end, the abdomen (Figure 2). The cephalothorax consists of the head and the thorax, which are covered by a shield known as the carapace. The sides of the carapace are called the pleura. The carapace conceals the majority of the internal organs and offers protection to the animal. The eyes extend from stalks and are faceted. This species has two sets of antennae which serve mainly as tactile organs. The mouth appendages are situated below the antennae in the antero-ventral section of the cephalothorax (Burukovskii and Bulanekov, 1969). Three kinds of appendages serve for locomotion: five pairs of walking legs on the cephalothorax, five pairs of pleopods and one pair of uropods on the abdomen. This organization allows for movement in three different directions. The ventral side of the abdomen is divided into seven segments. The first five bear lateral appendages known as the pleura. Each segment contains a pair of pleopods or swimmerets. In all shrimp, the inner branches of the first two sets of pleopods are modified into copulatory appendages for reproduction.

In the internal anatomy, the mouth opens directly into the stomach leading to the midgut region where food is digested and absorbed. The midgut forms a complex tubular gland commonly known as the liver. The hindgut passes through the whole body and opens in the anus on the ventral side of the telson. The internal sex organs of shrimp

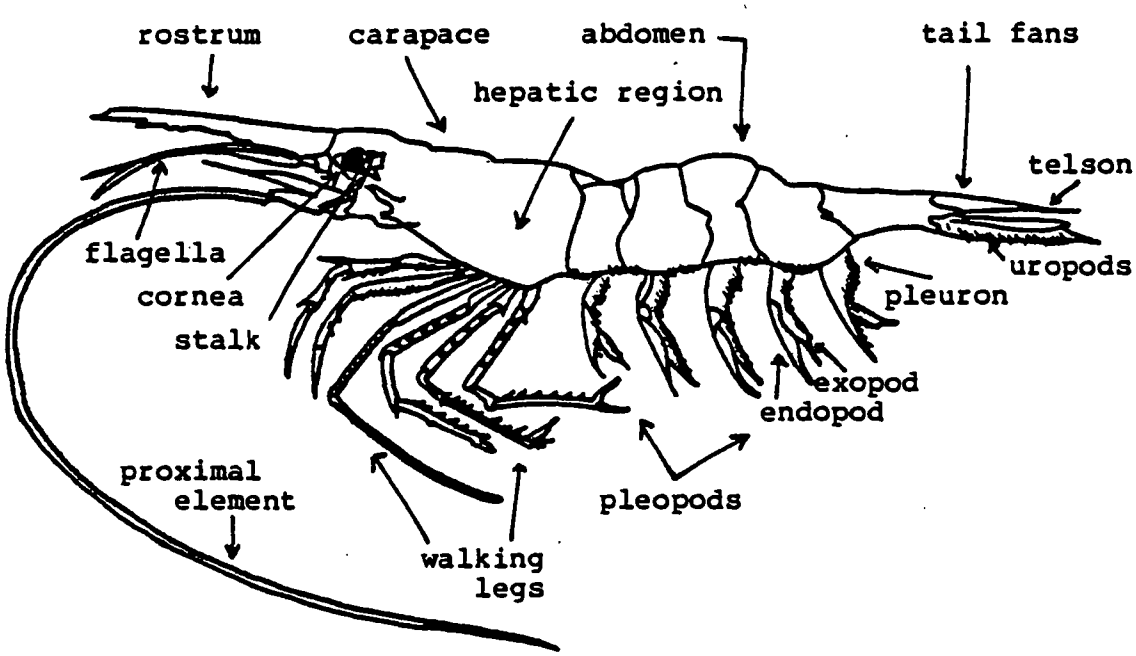


Figure 2

The Pandalid Shrimp

Butler (1980)

are located above the liver and intestine. In females, the reproductive system consists of paired ovaries, oviducts, outer genital openings and the telicum (Burukovskii and Bulanekov, 1969). In male shrimp, paired testes are situated in the cephalothorax and have lateral lobes in proximity to the sides of the liver. The vasa deferentia form ampullae where sperm is stored. The ampullae open in the genital openings, which are situated at the juncture between the fifth set of walking legs and the body. The heart is located in the posterior section of the cephalothorax above the sex organs. Shrimp have an open circulatory system. Gills are located under the pleura of the carapace. The nervous system is comprised of two circumesophagal ganglia and a ventral nerve chain consisting of two adjacent nerve stems with paired ganglia in each body segment. Forty to 45 percent of the shrimp's total weight though, consists of musculature located in the abdominal region. This is the edible portion of shrimp. Interdispersed between this muscle and comprising the structural framework of the muscle is the collagen.

Collagen

Collagen is ubiquitous in nature and functions as the major connective tissue protein in animals. Collagen's biochemical structure has been elucidated in most vertebrates, however, its nature and organization is relatively unknown

in most invertebrates.

Structural Features

Much information has been known about the basic structure of collagen for many years (Traub and Piez, et al., 1971), but new information is being continually added. The definitive property of all collagen molecules is the triple helix, a unique protein conformation that is a coiled coil of three polypeptide chains. These polypeptide chains are commonly known as alpha chains and each alpha chain has a unique amino acid sequence. A nomenclature for the alpha subunits has been developed where $[\alpha 1(I)]_2\alpha 2$ is type 1, $[\alpha 1(II)]_3$ is type 2, $[\alpha 1(III)]_3$ is type 3 and $[\alpha 1(IV)]_3$ is type 4. The functional form of each of these types is the fibril, an ordered molecular polymer (Figure 3).

In vertebrate collagen, every third amino acid residue is typically glycine with the repeating tripeptide glycine-x-y, where x and y frequently represent proline and hydroxyproline, respectively. Whether this pattern holds true for invertebrate collagen, has yet to be determined. A probable reason for glycine's abundance in vertebrate collagen is it is the only amino acid small enough to cluster down the central core of the molecule and allow the association of the alpha chains in a superhelical structure. The glycine residues can also form hydrogen bonds to the amino group of the peptide bond on adjacent chains. This contri-

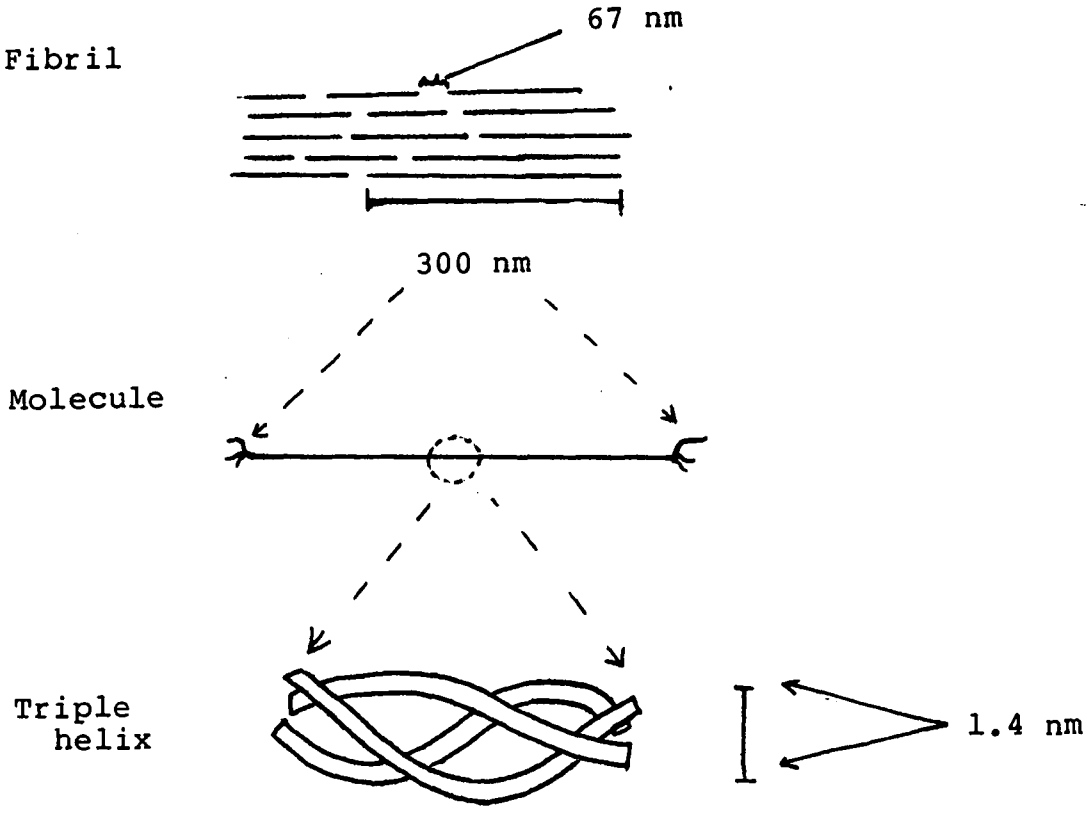


Figure 3. Molecular features of vertebrate collagen structure from primary sequence up to the fibril (Eyre, 1980).

butes to the stability of the molecule. Among invertebrate collagens, differences in the content of hydroxylated amino acids have been reported, especially in the ratios of proline to hydroxyproline and lysine to hydroxylysine (Kimura, 1972; Thompson and Thompson, 1970).

Most of the well characterized vertebrate collagens have molecular weights in the range of 300,000, and assemblies of three peptide chains of approximately equal lengths. But again, there is little information on the molecular weight and subunit composition of invertebrate collagen. In the few instances where molecular weights of invertebrate collagen have been determined, they appear to resemble the vertebrate norm (Nordwig and Hayduk, 1973).

Water is also an important constituent of collagen with about 0.5 grams of water needed per gram of procollagen to maintain its native structure (Dahl, 1970). Water is involved in interchain hydrogen bonding (Ramanchandron and Chandrasekharan, 1968), and upon complete dehydration, collagen becomes insoluble. Freeze drying of collagen is believed to decrease the solubility as well.

Interstitial collagens of vertebrate origin are glycoproteins containing a small number of hydroxylysyl residues substituted with galactose or glucosylgalactose moieties (Fessler and Fessler, 1978). In contrast to the low numbers of carbohydrates found in vertebrate collagen though, some invertebrate collagens studied are rich in carbohydrates

(Eyre, 1978). In addition to modified hydroxylysyl residues, serine and threonine can take part in glycosidic linkages in invertebrate collagen (Adams, 1978).

Biosynthesis and Molecular Packing

The basic sequence of events in collagen biosynthesis are the production of individual polypeptide chains by the processes of transcription and translation, followed by alterations or modifications in the polypeptide chains, such as glycosylation and hydroxylation, and alignment of these chains to precursor molecules. These precursor molecules are commonly referred to as protocollagen or unformed collagen. Polymerization to formed collagen occurs with the last step being the aggregation of the collagen molecules into fibers.

Laying down of collagen fibers cannot occur until extension peptides at the amino terminus and carboxyterminus of unformed collagen chains are removed. A number of proteases have been implicated in their ability to remove these non-helical peptides (Dutson, et al., 1976).

As stated previously, the structural stability of the collagen molecule allows it to act as the major supporting framework of animal tissues. Collagen can satisfy this function because of its unique molecular configuration, the specific alignment during aggregation and most importantly, by the formation of covalent crosslinks which give the

fibers high tensile strength and resistance to chemical attack. Modified amino acids, hydroxylysine, lysine, proline and hydroxyproline have been shown to add stability to collagen structure. Therefore, the relative amounts of these amino acids in collagen are an important feature in distinguishing it from other proteins.

Crosslinking

Crosslinking amino acids are necessary for the strength and normal function of collagen fibrils. Crosslinking of collagen occurs by three major mechanisms: intramolecular, intermolecular and disulfide bonds. The initial stage in intramolecular crosslinking involves an oxidative deamination of certain lysine and hydroxylysine amino acids catalyzed by the enzyme lysyl oxidase (Figure 4). The actual crosslinks are formed from the aldol condensation of these lysine or hydroxylysine derived aldehydes (Tanzer, 1973) (Figure 5). The aldol bond serves only to crosslink chains within a triple helix collagen molecule and does not account for the increasing stability of the fibrils. Intermolecular crosslinks are believed to be partly responsible for collagen tensile strength and stability. The crosslinks are formed from the condensation of reactive groups such as the epsilon-amino group of hydroxylysine to form intermolecular bonds of the aldimine type. During maturation, these reducible bonds are converted to a non-reducible, more

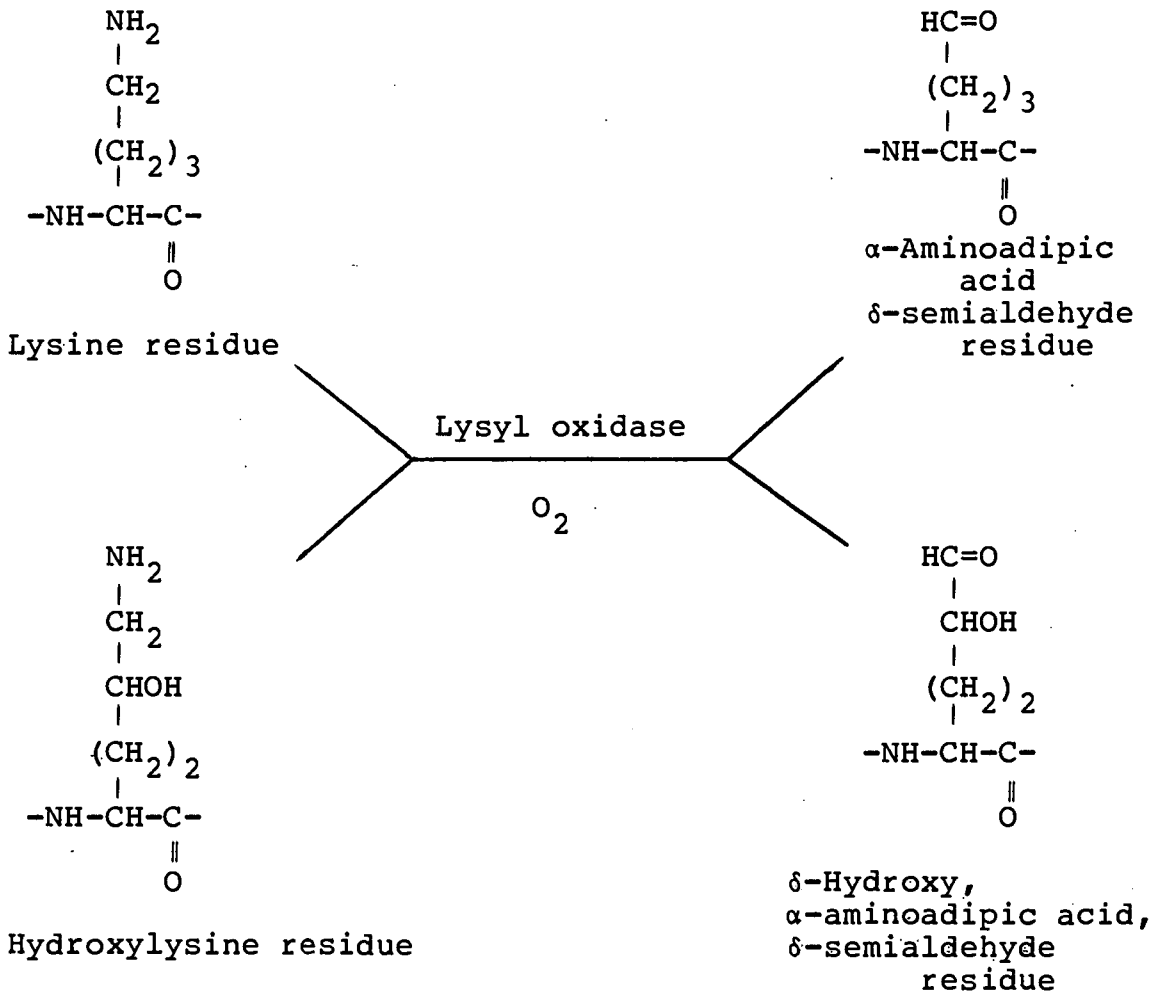
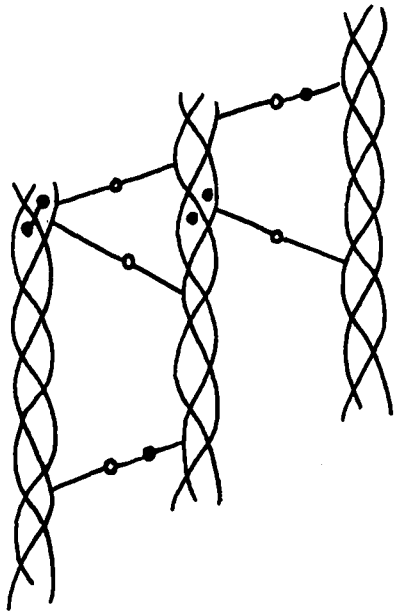


Figure 4 Formation of collagen aldehydes by enzymatic action of lysyl oxidase on collagen molecules. (Tanzer, 1973).

A

Lateral
view



B

Cross
sectional
view

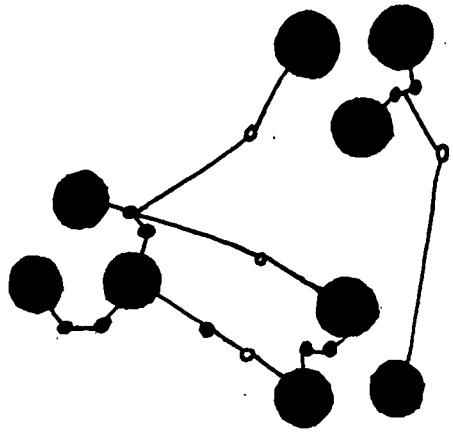


Figure 5

General scheme of intermolecular cross-link formation, by reaction of collagen aldehydes with other collagen amino acids.

● = Aldehydic groups
O = Other reactive groups

(Tanzer, 1973)

stable form. The mechanism is unknown. Disulfide bonds are another type of crosslink occuring in collagen. Due to their stability, they may prove to be quite important in maintaining collagen structure. These bonds involve cystine and other sulfhydryl containing amino acids. These crosslinks occur within the helical portion of the collagen molecule and are very resistant to chemical or mechanical disruption.

The degree and types of crosslinking occuring in intramuscular collagen varies with animal age, post mortem aging, nutritional status, and from muscle to muscle within the same animal (McClain, 1976). Present data (Eyre, 1980; McClain, 1976) indicates that not only the amount of collagen, but also the degree and types of crosslinking may be important in the quality attributes of meat.

EXPERIMENTAL

Characterization of Shrimp Collagen

Sample lots of freshly caught shrimp were obtained from a local commercial source. All buffers were prepared using reagent grade chemicals and deionized water. The methods that were used for the isolation of formed and unformed shrimp collagen followed the procedures of Thompson and Thompson, et al. (1968 and 1969). All work was carried out in a cold room maintained a 1.5°C and in a refrigerated centrifuge also maintained at 1.5°C.

Formed Collagen Isolation

Shrimp were brought to the laboratory, rinsed and allowed to drain for a few minutes. Each shrimp was weighed and placed in one of three size lots: small, medium or large, based upon mean whole weight. They were headed, peeled by hand, and a 75 gram portion was taken for collagen analysis from each age class. The remaining portions were used for moisture and total nitrogen determinations to be done later. These portions were packed in styrofoam cups with plastic lids (approximately 200 grams per unit), and frozen at -34°C. After freezing, the styrofoam cups were vacuum sealed in moisture proof pouches and stored at -18°C.

The 75 gram portions taken for formed collagen analysis were homogenized in a Waring blender with 750 ml of cold 10%

NaCl. They were allowed to stand for 24 hours. The suspensions were centrifuged at 9220 x g for 20 minutes, and the supernatants discarded. The residues were washed free of chlorides with cold deionized water, and the NaCl extractions were then repeated. The resulting residues were dehydrated with 150 ml of cold acetone. The acetone was decanted, and the residues were extracted four times with 200 ml of ether. The ether was decanted, and the residual ether was removed with cold distilled water. The residues were washed again four more times to ensure total removal of the acetone and ether. The residues were then stored in cold distilled water overnight. The suspensions were centrifuged at 9220 x g for 20 minutes, and the supernatants decanted and discarded. The residues were extracted overnight with 250 ml of pH 8.0 (0.1M) phosphate buffer. The suspensions were centrifuged at 9220 x g for 20 minutes, and the supernatants were decanted and discarded. Cold distilled water was used to wash the residues free of phosphate buffer. The residues were then extracted overnight with 250 ml of pH 3.5 (0.65M) citrate phosphate buffer. The suspensions were again centrifuged with the supernatants discarded. Cold distilled water was used to wash the residues free of buffer. Both the phosphate and citrate phosphate buffer extractions were repeated a second time with a thorough water washing after each extraction. The suspensions were centrifuged at 9220 x g for 20 minutes, with the water

being decanted and discarded. The purified collagen residues were frozen, lypholized, and stored at 15°C until further analysis was required.

Unformed Collagen Isolation

The dialysis tubing was obtained from VWR Scientific Company. The tubing is made of cellulose and has a width of 41 mm.

The same weighing, peeling and sorting procedure was used for the unformed collagen isolation as was done for the formed collagen isolation procedure. Again, a 75 gram portion was taken for collagen analysis, while the remainder of the peeled shrimp was stored for moisture and total nitrogen determinations.

The 75 gram portions taken from each age class for unformed collagen analysis were teased and placed in flasks, each containing 750 ml of cold pH 3.5 (0.65M) citrate-phosphate buffer. The contents were mechanically stirred for 72 hours. The resulting suspensions were centrifuged at 20,800 x g for 20 minutes. Supernatants were decanted and dialyzed against deionized water. Solutions were changed every 12 hours for a period of 7 days. A cloudy white precipitate was obtained in each of the dialysis bags. The bags were shaken to give a suspension. They were then slit and the suspensions collected. Each suspension was centrifuged at 20,800 x g for 20 minutes. The supernatants were de-

canted and discarded. The precipitates were collected and washed free of buffer with cold distilled water. They were then lypholized and stored at -15°C until further analysis was required.

Chemical Analysis

Amino Acid Analysis

Preparation of hydrolysates

Lypholized formed and unformed collagen preparations were analyzed for their amino acid content. The acid hydrolysates were prepared by placing approximately 1.0 mg of the lypholized shrimp collagen in 1 ml of 6 N HCl. These were then evacuated to 30 μ Hg pressure and were digested at 110°C for 22 hours. The HCl was removed from the hydrolysates by flash evaporation, and the amino acids were redissolved in sodium citrate buffer at pH 2.2 and stored at 0°C until required for analysis.

An alternate hydrolysate procedure was necessary for the determination of tryptophan, because tryptophan is readily destroyed in HCl by oxidation. This method utilized methanesulfonic acid (CH_3SO_3H), rather than HCl as a catalyst for hydrolysis (Liu, 1976). It is well known that sulfonic acids are non oxidizing strong acids, therefore, tryptophan can be retained under these conditions. 1 mg taken from each of these lypholized collagen preparations was hydrolyzed in vacuo (20μ) at 110°C for 24 hours with 1.0 ml

of 4 N methanesulfonic acid containing 0.2 percent 3-(2-aminoethyl) indole. Following hydrolysis, the hydrolysates were partially neutralized with 1.0 ml of 3.5 N NaOH, and centrifuged for amino acid analysis.

Chromatographic separation and colorimetric estimation of amino acids

Amino acid analysis was performed on a Beckman 120 B Amino Acid Analyzer, according to the protocol set down in Beckman's Operating Parameters for Beckman Amino Acid Analyzers, 1976. .5 ml hydrolyzed samples were run and the system used employed three sodium citrate buffers at pH 3.25, 4.12, and 6.4 in sequence for elution. A D 6A anion resin was used in a .6 cm x 32 cm column. Ninhydrin was detected and measured by a Gilson Fluorometer. A Spectrophysics Autolab System IVB integration system was used for the recording of the data. Flow rates for both the buffers and ninhydrin solution were 12 ml/hr. and the total run time was about 180 minutes.

Amino acid analysis was also performed on a Durrum Microbore system (J. R. Benson, 1975). This system used the same buffers as employed with the Beckman analyzer, plus one additional sodium citrate buffer employed at pH 10.1. Orthopthalaldehyde (OPA), was used as the fluoremetric reagent instead of ninhydrin. OPA is more sensitive in detecting tryptophan levels than ninhydrin (Roth & Hampai, 1973). The flow rates and integration system employed with the Durrum

analyzer remained the same as the ones used in the Beckman analyzer. However, the total run time was reduced to 96 minutes.

Molecular Weight Determination

The technique of acrylamide gel electrophoresis was used to determine approximate molecular weight ranges for both formed and unformed shrimp collagen. The specific procedure used was developed by Dean Malencik in the Department of Biochemistry at Oregon State University.

A running gel containing 7.5 percent acrylamide was prepared from a 30 ml gel solution containing percent by volume amounts as follows: 10.1 parts acrylamide + bis (22.2 g acrylamide, 0.22 g methylenebisacrylamide, water to 100 ml), 10.9 parts of distilled water, 7.5 parts gel buffer B (96.88 g Tris, 30 ml H_3PO_4, 8 g SDS, 400 ml Glycerol, 10^{-4} EDTA, water to 2 liters, pH will be ca. 6.8), 1.5 parts of ammonium persulfate and 0.04 parts of Temed (tetramethylethylenediamine). Total parts: 30.

The acrylamide gel was polymerized chemically by the addition of the temed and ammonium persulfate. The acrylamide was polymerized into a thin rectangular slab between two glass plates having dimensions of 127 mm x 127 mm x 3.2 mm. Sample wells were made at one end of the gel by placing a comb-shaped jig into the reaction mixture before polymerization occured. After polymerization, the jig was re-

moved leaving the sample wells molded into the polymerized acrylamide.

The electrode buffer consisted of a solution containing 96.88 g Tris, 30 ml H_3PO_4 and 8 g SDS dissolved in 2 liters of distilled water.

Lypholized formed and unformed shrimp collagen samples and high molecular weight standards were dissolved 1:1 in a sample buffer consisting of 0.01 M Tris-PO_4, pH 6.8, 1 percent SDS, 1 percent 2-mercaptoethanol, 0.04 percent bromophenol blue as the tracking dye, 20 percent glycerol and 10^{-4} EDTA. The proteins were dissociated by immersing the samples for 1 minute in boiling water. Samples containing 20 µg of formed collagen, unformed collagen or one of the standard proteins of known molecular weight were then injected into individual sample wells.

Electrophoresis was run at 150 volts for approximately 4.5 hours. The gel was then separated from the slab plates and stained overnight in a 0.15 percent Coomassie brilliant blue solution made up freshly in 50 percent methanol and 10 percent glacial acetic acid. The gel was destained by repeated washings in a 5 percent methanol, 7.5 percent glacial acetic acid solution. Standard protein migrations were compared to sample migrations and molecular weight ranges determined utilizing a standard curve.

Moisture Content Determination

Frozen peeled shrimp samples from each age class were thawed for 24 hours at 10°C prior to analysis. Samples in styrofoam containers were prepared by blending the entire contents (about 200 grams) in a Waring blender at room temperature.

Moisture was determined as outlined in the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC). 24.002, 1980.

Total Nitrogen Determination

Frozen peeled shrimp samples from each age class were thawed for 24 hours at 10°C prior to analysis.

In order to determine total nitrogen content in the shrimp collagen, collagen extracts were first prepared as outlined in the isolation procedures for formed and unformed collagen. Total nitrogen was then measured by the microkjeldahl procedure as outlined in the Official Methods of the Association of Official Analytical Chemists (AOAC), 7.032 and 24.027, 1980.

To determine total raw nitrogen content in shrimp meat, thawed samples from each age class were minced, and total raw nitrogen was measured by the microkjeldahl procedure in AOAC. To determine non-protein nitrogen, thawed samples were prepared by homogenizing one part shrimp mince with two parts 5% trichloroacetic acid solution prior to Kjeldahl

analysis.

Interaction of Shrimp Size to Meat Yield

Lots of freshly caught shrimp (2000 g/treatment lot) were obtained from a local commercial source. Lots were placed in pans equipped with a drainer, packed in a deep bed of ice and stored at 2-4°C for varying periods of time. Single shrimp meat yield evaluations were carried out according to prescribed conditions at the following time periods post-catch: <1, 3, 5 or 7 days. The prescribed conditions were as follows: raw and cooked (100 sec, 101°C surface temperature).

Fifty intact shrimp, chosen to represent the size variations existing in a lot, were used for each of the conditions outlined above. The weight of the round shrimp and the weight of the derived meat (wet and dry) was determined. The weight of the meat (wet and dry) for various example round shrimp sizes was then computed from the linear regression of meat weight (wet and dry) on round shrimp weight. These weights (wet and dry) were, in turn, utilized to compute meat yields (wet and dry) and moisture contents.

RESULTS AND DISCUSSION

Evaluation of the Collagen Isolation Procedure

The shrimp obtained for this study were freshly caught and less than 1 day old when headed and peeled. Iced shrimp from commercial sources have been shown to have connective tissue damage due to bacterial-enzymatic degradation within a few days after being caught by trawling (Love & Thompson, 1966). So, it was important to use freshly caught shrimp to reduce the possibility of obtaining degraded component contaminents in the preparation.

Individual shrimp were weighed and placed in one of three size lots: small, medium or large, based upon mean whole weight. Whole mean weights of 2.58 ± .3906 for small, 5.27 ± .5500 for medium and 7.72 ± .9600 for large were used, in turn, for the age class distributions.

Most of the reported methods for the isolation and purification of insoluble collagens are essentially the same (Veis, et al, 1960). The method used for the isolation of purified formed shrimp collagen followed the procedure of Thompson and Thompson, (1968). Homogenizing the shrimp with a cold 10% NaCl solution prior to the collagen extraction, better facilitated the extraction of the salt soluble impurities.

As mentioned previously, the existence of a precursor to formed collagen has been demonstrated by Lukens, (1965);

Peterkofsky and Udenfriend, (1963); and Thompson and Thompson (1969). In an effort to isolate and characterize the connective tissue proteins of Pacific Smooth Pink Shrimp, the procedure of Thompson and Thompson was employed to extract the acid-soluble collagen from shrimp meat.

Attempts to isolate and characterize both the formed and unformed shrimp collagen in this study were successful. However, there are some limitations with these methods. Both extraction procedures have been demonstrated to be good qualitative measures of collagen purity and composition. Whether this holds true from a quantitative standpoint, has yet to be determined. It is possible there was not 100% recovery of the formed and unformed collagen. But, because the methodology employed was the same from one sample lot to the next, experimental yields were very consistent.

The major drawbacks of these isolation procedures were that they were much too long. In the isolation of unformed collagen, two weeks of extractions and dialysis were required to obtain amorphous precipitates of citrate soluble collagen. The minimum amount of time for isolating a sample of formed collagen was a week, with the time frame being strictly dependent on the original shrimp sample size.

Evaluation of Amino Acid Analyses

Chemical analyses were carried out to determine the amino acid composition of pink shrimp's formed and unformed

collagen. No measurable differences exist in the amino acid contents detected by the Beckman 120 B analyzer versus the Durrum Microbore System. Results of the amino acid analyses are presented in Table 1. Amino acid concentrations are given in residues/1000 total residues.

In a comparison between the formed collagen and unformed collagen, a number of differences in amino acid contents are reported. Of considerable interest, are those differences occuring in glycine, proline and hydroxylysine, amino acids which are partly responsible for the conformation and stability of the collagen molecule. The formed collagen contained 147.7, 67.9 and 16.6 residues/1000 total residues of glycine, proline and hydroxylysine, respectively. These levels are significantly higher than for unformed collagen which contained 78.1 and 39.6 residues/1000 total residues of glycine and proline respectively. No hydroxylysine was detected in unformed collagen. From a compositional standpoint, this data alone suggests formed collagen may be structurally more stable and therefore, less subject to enzymatic degradation and less subject to heat solubilization than unformed collagen.

In the chromatographic separation of collagen, unknown peaks appeared between leucine and tyrosine, and between lysine and ammonia. They are labelled U1 and U2 in Table 1. The unknowns are present in substantial amounts in the unformed collagen, but only in trace amounts in the formed

Table 1 Amino acid composition of *Pandalus jordani* (residues/1000 total residues)[1]

Amino acid	Formed Collagen	Unformed Collagen
Aspartic acid	90.8	104.6
Threonine	39.3	56.7
Serine	62.2	67.3
Glutamate	119.7	117.1
Glycine	147.7	78.1
Alanine	65.1	87.2
Valine	46.7	63.8
Methionine	25.5	29.9
Isoleucine	45.5	47.9
Leucine	67.9	78.4
Tyrosine	23.5	32.8
Phenylalanine	33.4	44.5
Lysine	59.1	65.8
Histidine	20.4	18.2
Arginine	52.1	45.3
Proline	67.9	39.6
Hydroxyproline	trace[3]	--
Hydroxylysine	16.6	--
Cystine/2	8.2	9.4
Tryptophan	8.5	14.5
U1[2]	trace	9.6
U2[2]	trace	44.1

[1]The values presented are averages of single amino acid analyses performed using the Beckman analyzer and Durrum Microbore System.

[2]Represent unknown peaks found in the collagen isolates.

[3]Hydroxyproline's presence was noted, but amounts were too small to measure.

collagen. There is a good possibility these residues may be amino sugar derivatives in the developing stages of collagen. However, no conclusive evidence exists that will support this theory.

Of the remaining amino acids, all but histidine, glutamate and arginine are contained in higher amounts in the unformed collagen than in the formed collagen. The magnitude of these differences suggests a considerable change in amino acid composition and structure is taking place in shrimp collagen as it matures.

The amino acid compositions of formed and unformed shrimp collagen were compared to amino acid compositions of some structural proteins reported from other sources (Table 2). Shrimp collagen is unusual among connective tissue proteins. It contains a lower than usual glycine content when compared to other collagen. Also, hydroxyproline, generally considered a unique constituent of collagen, is absent in shrimp unformed collagen, and only in trace amounts in formed collagen. This is quite unusual, in that hydroxyproline is normally a principal binding amino acid in most collagens. The data indicates the usual repeating amino acid sequence of glycine-X-Y typifying most collagens (where X and Y frequently represent proline and hydroxyproline respectively) is not the pattern of shrimp collagen. Glycine does not represent one third of the total amino acid residues in the collagen, nor does hydroxyproline appear to play

Table 2 Comparison of the Amino acid composition of *Pandalus jordani* with the amino acid composition of various structural proteins (residues/1000 total residues).

Amino acid	Shrimp collagen (formed)	Shrimp collagen (unformed)	Calfskin collagen (unformed)[1]	Cod collagen[2]	Tendon (acid extract) Human[3]	Human bone collagen[4]
aspartic acid	90.8	104.6	52.2	52.0	50.5	50.6
threonine	39.3	56.7	19.0	25.0	19.3	19.8
serine	62.2	67.3	40.7	69.0	38.5	38.7
glutamate	119.7	117.1	75.9	75.0	75.5	77.5
glycine	147.7	78.1	354.3	345.0	338.0	344.0
alanine	65.1	87.2	115.9	107.0	115.6	119.5
valine	46.7	63.8	21.0	19.0	26.5	25.4
methionine	25.5	29.9	6.5	13.0	6.0	5.7
isoleucine	45.5	47.9	14.3	11.0	9.5	14.3
leucine	67.9	78.4	28.5	23.0	27.2	27.5
tyrosine	23.5	32.8	5.5	3.5	3.8	4.8
phenylalanine	33.4	44.5	14.2	13.0	14.8	15.0
lysine	59.1	65.8	27.1	29.0	22.5	30.2
histidine	20.4	18.2	4.5	7.5	5.6	6.2
arginine	52.1	45.3	47.2	51.0	51.2	50.8
proline	67.9	39.6	125.4	102.0	127.2	133.0
hydroxyproline	trace	0.0	97.9	53.0	96.2	108.0
hydroxylysine	16.6	0.0	7.1	6.0	9.3	3.8
cystine/2	8.2	9.4	0.0	NA	0.0	0.0
tryptophan	8.5	14.5	0.0	NA	0.0	0.0

[1]Data from Traub & Piez (1971)

[2]Data from Piez & Gross (1960)

[3]Data from Eastoe, J.E. (1955)

[4]Data from Eastoe, J.E. (1955)

a role in the amino acid sequential pattern. There may be some question then whether the connective tissue protein extracted from Smooth Pink Shrimp can be classified as a true collagen. Matsumura (1972) has suggested a purified collagen preparation must contain greater than 300 glycine residues in a total amino acid content of 1000 residues. Exceptions have been made for certain invertebrate collagens, containing less glycine than 300 residues/1000 total residues in purified preparations (McBride & Harrington, 1967; Pikkarainen, Rantanen, et al., 1968). Although shrimp collagen contains more glycine than any other amino acid in a total amino acid content of 1000 residues, it clearly contains much less than the 300 residues/1000 total residues which Matsumura has defined as a collagen criterion. It also contains a higher level of tyrosine (23.5-32.8 residues/1000 total residues) than what Matsumura has suggested to be the upper limit (10 residues/1000 total residues). In fact, shrimp collagen exhibits higher levels of the hydroxylic amino acids threonine, tyrosine, and hydroxylysine; and higher levels of the hydrophobic amino acids valine, methionine, leucine, isoleucine and phenylalanine, than most other reported collagens. It also contains a significant amount of tryptophan, whereas most other collagens contain only trace amounts, or none at all. Because of the limited amount of work done in the characterization of invertebrate collagens, there is not enough verification for the assump-

tion that a true collagen must contain a glycine content of at least 300 residues/1000 total residues. However, evidence presented in this study does indicate the amino acid composition in shrimp connective tissue protein to deviate from the norm of most collagens.

Evaluation of Molecular Weight Determination by SDS Gel Electrophoresis

The molecular weights of formed and unformed collagen were determined by comparing their mobilities in SDS gels with those of marker proteins with known molecular weights. Whenever this method is used for molecular weight determination, it is advisable to include at least three to four standard proteins with known molecular weights to plot a standard curve. The following standard proteins were used: myosin (210,000), beta-galactosidase (150,000), phosphorylase b (100,000), bovine serum albumin (75,000), ovalalbumin (45,000) and trypsin inhibitor (21,500). Their mobilities yielded a straight line plotted versus the logarithm of their molecular weights (Figure 6).

The results of electrophoretic analysis of the shrimp collagen extracts and standard proteins dissolved in the SDS Tris-PO_4 buffer are presented in Table 3. Molecular weights of the extracts were estimated to be in the range of 310,000 for both the formed and unformed collagens. This figure was based on relative mobilities of the extracts and appropriate positioning on the standard curve. This is designated on

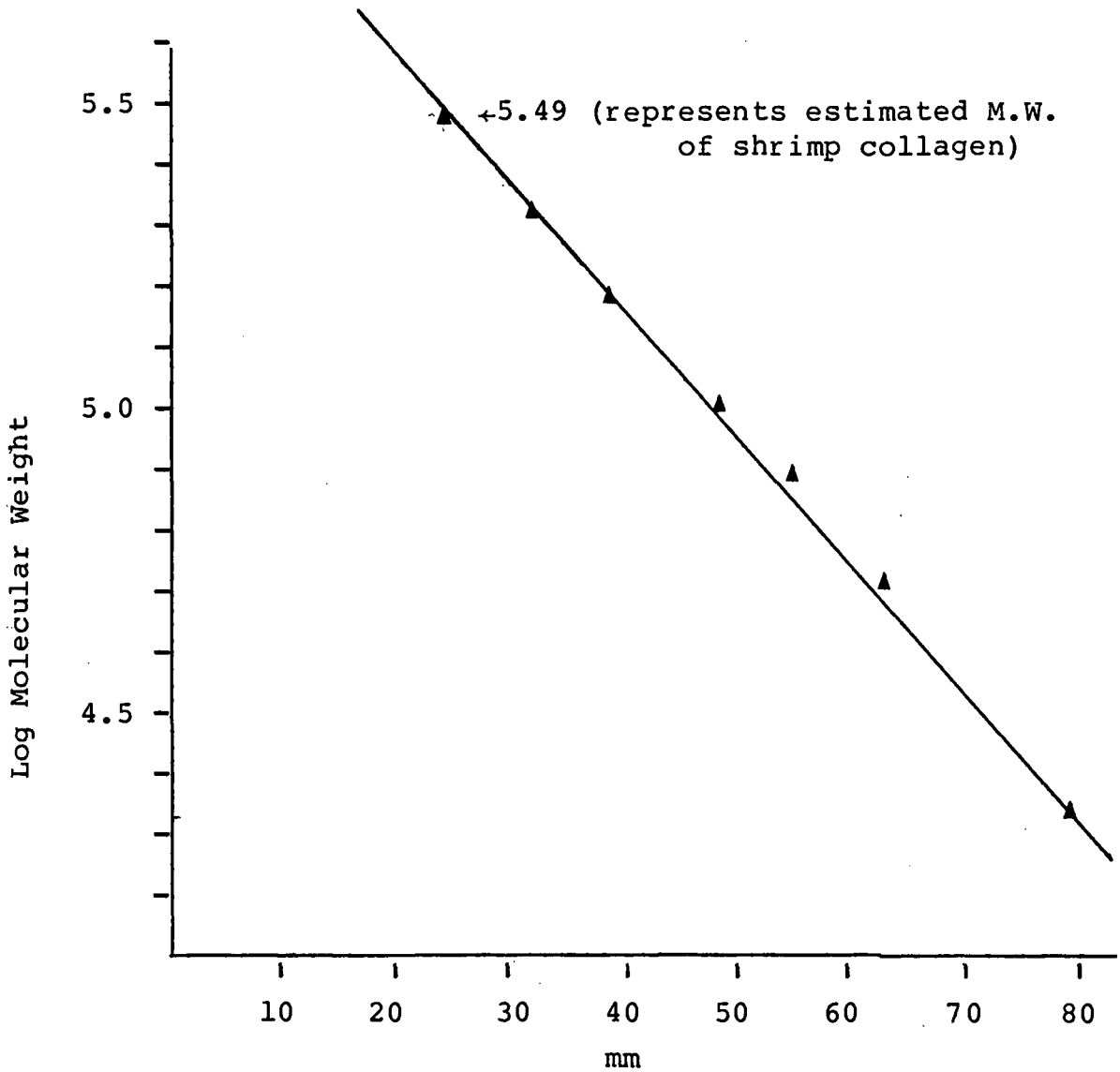


Figure 6. Plot of logarithm of molecular weights of proteins vs distance from start in a 7.5% polyacrylamide gel. The figures correspond to those of Table 1.

Table 3 Electrophoretic mobility of native proteins as a function of molecular weight

	Proteins	Molecular weight	Mobility (mm)
1.	Formed shrimp collagen	310,000	24
2.	Unformed shrimp collagen	310,000	24
3.	Myosin	210,000	33
4.	B-galactosidase	150,000	39
5.	Phosphorylase B	100,000	48
6.	Bovine serum albumin (monomer)	75,000	54
7.	Ovalalbumin	45,000	63
8.	Trypsin inhibitor	21,500	80

Figure 6. The values obtained here correspond well to collagen molecular weights reported in literature. As expected, the collagen preparations emerged very slowly. The complete absence of other bands in the gel pattern indicates the high degree of purity in the sample preparations.

An exact determination of the molecular weight of shrimp collagen has not been determined in this study. Only an approximate range has been estimated. To get an exact molecular weight and total characterization of shrimp collagen, depolymerization of the collagen molecules is necessary to achieve individual polypeptide migrations. Depolymerization of certain multimers to monomeric proteins has been successfully demonstrated using guanidinium hydrochloride treatment of the protein multimers (Lorentz, 1976). Whether this procedure will work for the relatively insoluble collagens, has yet to be determined. It would be of considerable interest to see if the monomeric chains of shrimp collagen resemble those of the alpha subunits of mammalian collagens. These analyses, however, go beyond the scope of this study. Within the limitations of this experiment, the electrophoretic method used was still valuable to estimate an approximate molecular weight range of shrimp collagen, where none had previously been estimated.

Evaluation of Nitrogen and Protein Determination

Table 4 shows the protein, moisture and nitrogen con-

Table 4 Protein, moisture and nitrogen content in *Pandalus jordani* as related to raw mean meat yield and shrimp size

	Shrimp size		
	Small	Medium	Large
Round shrimp wt.[1] (g)			
mean	2.58	5.27	7.72
S.D.	.39	.55	.96
% Moisture	79.70	78.70	79.33
Raw meat Avg.[2] meat yield (wet wt.%)	41.30	40.81	40.92
mg N/g raw meat wet wt.	31.36	33.35	30.14
% TN[3]	3.14	3.34	3.01
% NPN[4]	0.64	0.65	0.62
% Protein[5]	15.62	16.85	15.00

[1]Individual whole shrimp weighed and placed in age class

[2]$\frac{\text{total lot peeled wt.}}{\text{total lot whole weight}}$

[3]total nitrogen

[4]non-protein nitrogen

[5]protein = (TN-NPN) x 6.25

tent of freshly caught smooth pink shrimp as related to shrimp size and raw meat yield. The three size lots of small, medium and large were based on whole mean weights (grams) of 2.58±.39, 5.27±.55 and 7.72±.96 respectively.

Protein content [(TN-NPN)x6.25] in the three age classes varied only from 15.00 to 16.85%. Moisture contents varied from 78.7 to 79.70%. Non-protein nitrogen (NPN) content was approximately the same in all three lots, ranging from .62 to .65%. Total nitrogen (TN) content varied from 3.01 to 3.34%. Raw meat yields in all three age classes were also consistent with one another, ranging only from 40.81 to 41.30%

These slight differences may be attributed to a number of factors. Size may be important as smaller shrimp grow more rapidly and they molt more frequently than larger size shrimp (Wilaichon and Cobb, 1976). A soft shell is usually indicative of a recent molt or beginning molt. Some soft shells were evidenced in a small percentage of the shrimp used in this study. Lockwood (1968) indicated an increase in moisture content occurs in raw shrimp meat after molting. Results of this study support the results of Lockwood, in that, a slightly higher moisture content occurred in the small shrimp lot than in the medium or large sized shrimp lots.

Differences in the salinity of the water from which shrimp are taken have been demonstrated to affect NPN levels

(Cobb et al., 1975). However, since the shrimp used in this study were taken from the same lot off the boat, salinity changes can be ruled out as a factor here.

The type and levels of microorganisms occurring on shrimp, and the autolytic enzymes or enzymatic contamination from microorganisms during ice storage, can influence post-morten biochemical changes in shrimp musculature (Cobb and Vanderzant, 1971). But, because shrimp used in this analyses were freshly caught, storage on ice was minimal, and protein degradation and other biochemical changes were probably still negligible.

Effect of Shrimp Size on the Collagen content as Related to Total Nitrogen

Commercial samples of freshly caught shrimp were used to evaluate the relationship between shrimp size and collagen content. Ratios of total muscle nitrogen to total collagen in each age class were determined, as well as the relative percentages of formed collagen and unformed collagen in shrimp musculature. Also, the ratio of formed collagen to unformed collagen in each age class was determined in order to evaluate the structural changes occurring in shrimp collagen during maturation.

Results of the effect of shrimp size on the collagen content as related to total nitrogen are presented in Table 5. Again, the three size lots of small, medium and large were based on whole mean weights (grams) of 2.58±.39,

Table 5 Effect of shrimp size on the collagen content in *Pandalus jordani*

	Shrimp Size		
	Small 2.58 ± 39 g	Medium 5.27 ± .55 g	Large 7.72 ± .96 g
Total musculature N (mg/g)	31.36	33.35	30.14
Mg collagen N/g total N			
Total collagen	2.36	3.35	3.47
Unformed collagen[1]	1.26	1.19	0.03
Formed collagen[2]	1.08	2.16	3.44
Collagen relationships:			
Unformed collagen (%)	53.85	35.52	0.86
Formed collagen (%)	46.15	64.48	99.14
Formed/unformed	0.86	1.82	114.67

[1]Soluble in citrate phosphate buffer

[2]Insoluble in NaCl (10%)

5.27±.55 and 7.72±.96, respectively.

The collagen content varied as follows: total collagen content ranged from 2.34 in the small size lot to 3.47 mg N/g total N in the large size lot; unformed collagen content ranged from 0.03 in the large size lot to 1.26 mg N/g total N in the small size lot; and formed collagen content ranged from 1.08 in the small size lot to 3.44 mg N/g total N in the large size lot. Percent formed collagen was calculated to be 99.14 in the large size lot of shrimp, 64.48 in the medium size lot and 46.15 in the small size lot. Similarly, percent unformed collagen was calculated to be 0.86 in the large size lot of shrimp, 35.52 in the medium size lot and 53.85 in the small size lot. The ratio of total muscle nitrogen to total collagen nitrogen in each age class ranged from 13.4 to 8.7, while the ratio of formed collagen to unformed collagen nitrogen in each age class ranged from 114.67 to 0.86.

Analysis of the results show an increase in total muscle collagen content occurs as shrimp mature. Another measure of this is given by the ratio of total muscle nitrogen to total collagen nitrogen within each age class. This ratio decreases with increasing shrimp size. Marked differences occur in the relative percentages of formed collagen to unformed collagen in each age class. The percentage of formed collagen increases sharply as shrimp size increases, while the percentage of unformed collagen sharply decreased.

Another measure of this is given by the ratio of formed collagen to unformed collagen in each age class. This ratio increases dramatically with increasing shrimp size. Similarly, Dutson (1974) indicated that in beef, there is no significant change in the amount of collagen per gram of muscle tissue due to aging, but that soluble unformed collagen content decreases with increasing animal age.

In the young immature shrimp, new collagen is continually synthesized to accomodate increases in the size of muscles. New collagen, or unformed collagen as it is highly undeveloped, undergoes structural and compositional changes as aging proceeds. Cross-linking of modified amino acids within and between collagen molecules is taking place giving collagen its fibrous character, hence the term formed collagen. The differences in the collagen type and composition seen in the results of this study lend support to the accepted theories of collagen development with age. The amino acid variations existing between formed and unformed collagen, as presented earlier in this study, give a structural basis for these differences. Because large shrimp exhibit a higher content of formed or insoluble muscle collagen than do small shrimp, better structural stability can be expected in the shrimp meat from large shrimp than from small shrimp.

Evaluation of the Interrelationship of Shrimp Size and Muscle Collagen to Raw and Cooked Meat Yield Characteristics

The relationship of shrimp size to raw meat yield characteristics during ice storage was investigated. Table 6 lists the changes occurring in the yield and moisture content of raw meat derived from round shrimp stored in ice at 2-4°C after periods of 1, 3, 5, and 7 days post-catch. The total meat yield derived from each treatment lot followed a linear regression of meat weight (wet and dry) on round weight, with r significant to $p \geq .001$, x=round wt., y=meat wt. (wet or dry) and n=50. The recovery of raw meat from round shrimp was directly related to ice storage time. Total yield of raw meat from round shrimp stored in ice <1 day was 9.044% (dry wt.). Increasing ice storage time to 3, 5 and 7 days reduced the raw meat yield to 8.485%, 7.697% and 7.301% (dry wt.), respectively (Table 6). The moisture content in round shrimp was also directly related to the ice storage time. The moisture content of raw meat from round shrimp stored in ice <1 day was 79.09%. Increasing ice storage time to 3, 5 and 7 days increased moisture content to 80.82%, 81.34% and 83.68% respectively. Replacement of dry matter lost through the washing action of melting ice with water maintained yield (wet weight) during storage.

To determine the relationship between shrimp size and raw meat yield, the linear regression of meat weight (wet and dry) on round weight was used to calculate similar data for individual shrimp representing a wide range of age

Table 6 Yield and moisture content of raw meat derived from round shrimp stored in ice at 2 - 4°C.

Storage time (days in ice)	Parameter	Weight (g) Mean	S.D.	Total	Linear regression of meat wt. (wet and dry wt.) on round wt.[1] r	m	b
	Round	3.50	1.16	175.04			
<1	Meat wet	1.51	0.52	75.71	.974016[2]	.432548	-.000067
	Meat dry	0.32	0.11	15.83	.968086[2]	.093518	-.010790
	Round	3.87	1.38	193.52			
3	Meat wet	1.71	0.59	85.62	.986621[2]	.419004	.090685
	Meat dry	0.33	0.12	16.42	.985246[2]	.086973	-.008221
	Round	3.99	1.26	199.42			
5	Meat wet	1.76	0.52	87.99	.989741[2]	.411515	.118272
	Meat dry	0.31	0.10	15.35	.957897[2]	.078268	-.005164
	Round	3.91	1.21	195.58			
7	Meat wet	1.75	0.54	87.49	.987955[2]	.439350	.031236
	Meat dry	0.29	0.09	14.28	.969577[2]	.074309	-.005068

Round wt. (g)	Percent	Storage time (days in ice) <1	3	5	7
	Yield wet	43.25	44.24	44.12	44.73
Total[1]	Yield dry	9.044	8.485	7.697	7.301
	Moisture	79.09	80.82	81.34	83.68
	Yield wet	43.25	45.53	45.88	45.18
2.5	Yield dry	8.920	8.367	7.620	7.228
	Moisture	79.38	81.62	83.39	84.00
	Yield wet	43.25	43.71	43.52	44.56
5.0	Yield dry	9.136	8.533	7.723	7.329
	Moisture	78.88	80.48	82.25	83.55
	Yield wet	43.25	43.11	42.73	44.35
7.5	Yield dry	9.208	8.588	7.758	7.363
	Moisture	78.71	80.08	81.84	83.40

[1] n = 50

[2] Sig. P≥.001

classes (Table 6). The recovery of meat from round shrimp was directly related to shrimp size at individual time periods of ice storage. The yield of raw meat from a 2.5 g round shrimp stored in ice <1 day on a dry weight basis was 8.920%. The yield of raw meat derived from a 5.0g and 7.5g round shrimp stored in ice <1 day increased to 9.136% and 9.208% (dry wt) respectively. Similarly, at individual ice storage times of 3, 5 and 7 days, the raw meat yields (% dry wt) increased as size of round shrimp increased. This change in dry weight yield with respect to shrimp size followed well defined power functions: <1 day: $y=8.658x^{.0320}$ ($r=.9839$); 3 day: $y=8.167x^{.0261}$ ($r=.9842$); 5 day: $y=8.494x^{.0179}$ ($r=.9845$); 7 day: $y=7.104x^{.0186}$ ($r=.9841$) ($p \geq .001$. $n=8$). An expanded group of calculations was done to include eight variations in sizes ranging from 2.0 to 7.5 grams. Results of the data indicate yield (% dry wt) decreases with respect to storage time, but increases with respect to shrimp size. The highest yield was obtained with the 7.5 g round shrimp stored <1 day in ice (9.208% dry wt). In contrast, the lowest yield was obtained with the 2.5 g round shrimp stored for 7 days in ice (7.228% dry wt.).

The moisture content of round shrimp was also directly related to shrimp size at individual times of ice storage. The moisture content of raw meat derived from a 2.5 g round shrimp stored in ice <1 day was 79.38%. The moisture content of raw meat derived from a 5.0 g and 7.5g round shrimp

stored in ice <1 day decreased to 78.88% and 78.71% respectively. Similarly, at individual ice storage times of 3, 5 and 7 days, the moisture content decreased as round shrimp size increased. Results of the data indicate % moisture increases with respect to storage time increases, but decreases overall with increasing shrimp size. The highest moisture content was obtained with the 2.5 g shrimp stored for 7 days in ice (84.00%). In contrast, the lowest moisture content was obtained with the 7.5 g shrimp stored <1 day in ice (78.71%).

From the presented data in Table 6, it was also determined that as shrimp size increases, the rate at which solids are lost increases. This is reflected in the linear regression of meat yield (% dry weight) on storage time days shown in Table 7. Similarly, from the presented data, larger shrimp are gaining more moisture in ice storage than are the smaller shrimp. This is reflected in the linear regression of meat moisture (%) on storage time (days) shown in Table 8.

The relationship of shrimp size to raw meat yield characteristics during ice storage can be summarized as follows: Shrimp are losing solids but gaining moisture with respect to time. This is reflected in % dry weight yield and % moisture presented in Table 6. At individual times of ice storage, the dry weight yield increases as shrimp size increases. It is also apparent that smaller sized round

Table 7 Linear regression of raw meat yield (% dry wt) on storage time, days[1] (% dry wt. = m (storage time) + b)

Round wt. (g)	r	m	b
mean	-.9929[2]	-.2980	9.3190
2.5	-.9937[2]	-.2916	9.1984
5.0	-.9923[2]	-.3116	9.4265
7.5	-.9918[2]	-.3183	9.5023

[1]n=4 [2]sig P≥.001

Table 8 Linear regression of raw meat moisture (%) on storage time days[1] (moisture = m (storage time) + b)

Round wt. (g)	r	m	b
mean	.9740[2]	.7145	78.3745
2.5	.9729[2]	.7815	78.9715
5.0	.9982[3]	.7890	78.1340
7.5	.9989[3]	.7915	77.8415

[1]n=4 [2]sig P≥.05 [3]sig P≥.005

shrimp have higher initial moisture levels and lower initial solids than larger sized shrimp. However, larger shrimp are gaining more moisture and losing more solids in ice storage than are smaller sized shrimp (Tables 7 and 8). These results suggest that as shrimp size increases, the rate at which solids are lost also increases. But large shrimp still retain a greater overall solids content than small shrimp, due to the differences in initial levels of solids and percent water.

The relationship of shrimp size to cooked meat yield characteristics during ice storage was also investigated. Table 9 lists the changes occurring in the yield and moisture content of cooked meat (100 seconds, 101°C) derived from round shrimp stored in ice at 2-4°C for periods of <1, 3, 5 and 7 days post-catch. A 100 second precooking time in steam at 101°C approximates the usual heat processing time for shrimp in the Pacific Northwest.

The total meat yield derived from each treatment lot followed a linear regression of meat weight, with r significant to $p \geq .001$, and n=50. As was true with raw meat recovery, the recovery of cooked meat from round shrimp stored in ice was directly related to ice storage time. Yield of cooked meat from round shrimp stored in ice <1 day on a dry weight basis, was 7.844%. Increasing ice storage time to 3, 5, and 7 days reduced the cooked meat yield to 7.159%, 6.365% and 5.706% (dry wt) respectively. The moisture

content in cooked meat from round shrimp stored in ice <1 day was 75.99%. Increasing ice storage time to 3, 5 and 7 days increased moisture content to 78.31%, 79.74% and 80.85% respectively.

To determine the relationship between shrimp size and cooked meat yield, the linear regression of cooked meat weight (wet and dry) on round weight was used to calculate similar data for individual shrimp representing a wide range of age classes (Table 9). The recovery of meat from round shrimp was directly related to shrimp size at individual times of ice storage. The dry weight yield of cooked meat from a 2.5g round shrimp stored in ice <1 day was 7.841%. The yield of cooked meat derived from a 5.0g and 7.5g round shrimp stored in ice <1 day increased to 7.845% and 7.846%, respectively. Similarly, at individual ice storage times of 3, 5, and 7 days, the cooked meat yields (% dry wt) increased as shrimp size increased. The change in dry weight cooked yield with respect to shrimp size also followed a well defined power function; <1 day: $y=7.835x^{.0007}$ ($r=.9855$); 3 day: $y=6.434x^{.0715}$ ($r=.9826$); 5 day: $y=5.570x^{.0872}$ ($r=.9821$); 7 day: $y=4.661x^{.1394}$ ($r=.9802$) ($p \geq .001$, $n=8$). Again, an expanded group of calculations was done to include eight variations in sizes ranging from 2.0 to 7.5g. Results of the data indicate yield (% dry wt) decreases with respect to storage time, but increases with respect to shrimp size (Table 9).

Table 9 Yield and moisture content of cooked (100 sec.) meat derived from round shrimp stored in ice at 2 - 4°C.

Storage time (days in ice)	Parameter	Weight (gm) Mean	S.D.	Total	Linear regression of meat wt. (wet and dry) on round wt. r	m	b
	Round	3.74	1.56	187.16			
<1	Meat wet	1.22	0.55	61.13	0.987077[2]	0.351175	-0.091919
	Meat dry	0.29	0.13	14.68	0.927879[2]	0.078491	-0.000209
	Round	3.97	1.50	198.90			
3	Meat wet	1.31	0.50	65.65	0.972097[2]	0.326362	+0.014732
	Meat dry	0.28	0.12	14.24	0.978747[2]	0.076348	-0.018911
	Round	4.13	1.30	206.45			
5	Meat wet	1.30	0.45	64.86	0.930617[2]	0.321881	-0.031848
	Meat dry	0.26	0.09	13.14	0.980202[2]	0.068583	-0.020381
	Round	3.78	1.17	188.75			
7	Meat wet	1.12	0.38	56.23	0.977553[2]	0.320568	-0.085544
	Meat dry	0.22	0.08	10.77	0.971830[2]	0.064790	-0.029181

Round wt. (gm)	Percent	Storage time (days in ice) <1	3	5	7
	Yield wet	32.66	33.01	31.42	29.79
Total[1]	Yield dry	7.844	7.159	6.365	5.706
	Moisture	75.99	78.31	79.74	80.85
	Yield wet	31.44	33.23	30.91	28.64
2.5	Yield dry	7.841	6.878	6.043	5.312
	Moisture	75.06	79.30	80.45	81.45
	Yield wet	33.23	32.93	31.55	30.35
5.0	Yield dry	7.845	7.257	6.451	5.895
	Moisture	76.43	77.96	79.55	80.57
	Yield wet	33.89	32.83	31.76	30.92
7.0	Yield dry	7.846	7.383	6.587	6.090
	Moisture	76.85	77.51	79.26	80.30

[1]n = 50 [2]Sig. P≥.001

With the exception of shrimp lots stored <1 day in ice, moisture contents decreased as round shrimp size increased with respect to individual ice storage times (Table 9). The lower moisture content of cooked meat derived from small shrimp at <1 day of ice storage over larger shrimp is directly related to relative differences in raw meat moisture content. At <1 day of storage, the moisture content of raw meat from a 2.5g shrimp was only 0.67 percentage points higher than a 7.5g shrimp; at 3 days storage the difference was 1.54 percentage points. Differences in the moisture content of raw meat from small and large shrimp were not sufficient to overcome the more extensive loss of moisture from small shrimp exposed to more heating through steam precooking.

Some general comparisons can be made between cooked and raw meat yields in *Pandalus jordani* during ice storage. Both raw and cooked shrimp lose solids, but gain moisture with time. This is reflected in % dry weight yield and % moisture presented in Tables 6 and 9. Also, at individual times of ice storage, dry weight yield increases as shrimp size increases.

However, it is evident from the results of this investigation that overall % meat yields during ice storage are significantly higher in raw shrimp than in cooked shrimp. A summary of the yield and moisture content of both raw and cooked meat derived from round shrimp stored

in ice at 2-4°C is presented in Table 10.

Further evidence of the differences exhibited in raw versus cooked meat yield characteristics is demonstrated by comparing the linear regressions of raw and cooked meat yield (% dry wt) on storage time (Tables 7 and 11) and the linear regressions of raw and cooked meat moisture (%) on storage time (Tables 8 and 12).

From the presented data, it was determined that as shrimp size increases, the rate in moisture gain decreases (Table 12). These effects contrast sharply with those exhibited in raw meat yield characteristics, where the rate at which solids are lost and moisture is gained, increases with increasing shrimp size.

From a processor's point of view, the relative effects of shrimp size on cooked meat yield characteristics may be better represented by estimating the total meat loss of small, medium and large shrimp stored 3 days in ice and cooked at 101°C for 100 seconds. Shrimp stored 3 days in ice prior to processing is a typical holdover period for processors. As an example, 2.5, 5.0 and 7.5g shrimp stored and cooked under these conditions would have lost approximately 98.65, 85.25 and 81.15 lb./1000 lb. round shrimp, respectively. Selective processing of larger more mature shrimp could increase wet meat yields as much as 17.5 lb/1000 lb round shrimp. This, in turn, would result in a marked increase in the economic return from processing.

Table 10 Summary of the yield and moisture content of raw and cooked (100 sec.) meat derived from round shrimp stored in ice at 2 - 4°C.

Round wt. (gm)	Percent	Storage time (days in ice) <1 Raw	<1 Cooked	3 Raw	3 Cooked	5 Raw	5 Cooked	7 Raw	7 Cooked
Total[1]	Yield wet	43.25	32.66	44.24	33.01	44.12	31.42	44.73	29.79
	Yield dry	9.044	7.844	8.485	7.159	7.697	6.365	7.301	5.706
	Moisture	79.09	75.99	80.82	78.31	81.34	79.74	83.68	80.85
2.5	Yield wet	43.25	31.44	45.53	33.23	45.88	30.91	45.18	28.64
	Yield dry	8.920	7.841	8.367	6.878	7.620	6.043	7.228	5.312
	Moisture	79.38	75.06	81.62	79.30	83.39	80.45	84.00	81.45
5.0	Yield wet	43.25	33.23	43.71	32.93	43.52	31.55	44.56	30.35
	Yield dry	9.136	7.845	8.533	7.257	7.723	6.451	7.329	5.895
	Moisture	78.88	76.43	80.48	77.96	82.25	79.55	83.55	80.57
7.5	Yield wet	43.25	33.89	43.11	32.83	42.73	31.76	44.35	30.92
	Yield dry	9.208	7.846	8.588	7.383	7.758	6.587	7.363	6.090
	Moisture	78.71	76.85	80.08	77.51	81.84	79.26	83.40	80.30

[1]n = 50.

Table 11 Linear regression of cooked (100 sec, 101°C) meat yield (% dry wt) on storage time[1] days

Round wt. (g)	r	m	b
mean	-.9994[2]	-.3604	8.2101
2.5	-.9981[3]	-.4211	8.2101
5.0	-.9975[3]	-.3328	8.1932
7.5	-.9945[4]	-.3032	8.1893

[1]n=4 [2]sig P≥.001 [3]sig P≥.005 [4]sig ≥.01

Table 12 Linear regression of cooked (100 sec, 101°C) meat moisture (%) on storage time[1] days

Round wt. (g)	r	m	b
mean	.9854[3]	.8005	75.5205
2.5	.9333[2]	1.0160	75.0010
5.0	.9957[4]	.7005	75.8255
7.5	.9867[3]	.6050	76.0600

[1]n=4 [2]sig P≥.10 [3]sig P≥.025 [4]sig P≥.005

The differences in the rate of solids lost and moisture gained by age classes of shrimp during ice storage and subsequent steam cooking may be directly attributable to collagen content differences. Greater heat solubilization and moisture gain will occur in unformed collagen than formed collagen due to their differences in structural stability. It can be assumed unformed collagen in situ has the ability to hydrate more water than formed collagen because it has more functional groups free. As evidence suggests, smaller immature shrimp containing higher percentages of unformed collagen also contain more moisture than the larger shrimp. In addition, unformed collagen will exhibit greater enzymatic degradation than the more fully developed formed collagen. Smaller shrimp containing higher percentages of unformed collagen than formed collagen, exhibit greater enzymatic degradation and heat solubilization with time than larger shrimp containing higher percentages of formed collagen. These facts help explain the variations which occurred in solid losses and % moisture gains between the different age classes leading to differences in cooked meat yields. The amount and type of collagen present in shrimp musculature then can be used as an index of physical stability. The formed and unformed collagen variations existing between different age classes are a major function of yield variation in raw and cooked shrimp meat.

SUMMARY AND CONCLUSIONS

A quantitative determination of variations existing in the collagen content between different age classes of *Pandalus jordani* was examined. Total collagen quantity was comprised of both formed and unformed collagen. The two fractions were separated on the basis of formed being insoluble in 10% NaCl, and unformed being soluble in a pH 3.5 (0.65M) citrate phosphate buffer. The collagen fractions were physically and chemically characterized and the relationship of collagen content to meat yield characteristics was determined.

The quantity of collagen present in shrimp was determined to be 2.36, 3.35 and 3.47 mg total collagen N/g total N for small (2.58±.39g), medium (5.27±.55g) and large (7.72±.96g) mean size age classes, respectively. Percent unformed collagen was calculated to be 0.86% in the large, 35.55% in the medium and 53.85% in the small size lots. Total collagen content increased as shrimp size increased. A decrease in unformed and an increase in formed collagen was observed with maturation.

Formed collagen contained much higher glycine, proline and hydroxylysine levels than unformed collagen; 1.89 and 1.76 times as much for glycine and proline respectively. No hydroxylysine was detected in the unformed collagen. Significant peaks appearing in the chromatographic separation of

unformed collagen were suspected to be amino sugar derivatives. Formed collagen contained only traces of these components.

Of the remaining amino acids, all but histidine, glutamate and arginine were contained in higher amounts in the unformed than in formed collagen. The magnitude of these differences give evidence for structural differences between formed and unformed shrimp collagen.

The technique of SDS gel electrophoresis was used in estimating the molecular weight of shrimp collagen. The derived figure of 310,000 daltons compares favorably with molecular weights of those collagens reported in literature.

Shrimp collagen is quite unusual among connective tissue proteins. It possesses similar structural functions and molecular weight characteristics, yet its amino acid composition differs greatly from the mammalian norm. It contains a lower than usual glycine content and very limited amounts of hydroxyproline. It also contains a substantial amount of tryptophan, unlike most other reported collagens. Questions may then be raised as to its classification as a true collagen. But, it does function as a connective tissue and possesses an approximate molecular weight of collagen. This amino acid alteration from mammalian collagens may be related to some unique requirements of shrimp's anatomical structure.

The yield (% dry wt) of raw and cooked meat (100 sec

in steam) derived through hand peeling round shrimp was correlated ($P \geq .001$) in a positive manner by well defined power functions. Raw meat yield (% dry wt) declined during ice storage in a linear ($P \geq .001$) manner at a rate dependent upon shrimp size. The more rapid loss of solids from large shrimp reduced yield differences as storage was extended. Raw meat losses during ice storage ranged from (g/100g/day) 0.298 to 0.318 g raw meat dry matter/100 g round shrimp/day for 2.5 and 7.5 g shrimp respectively. Dry matter weight loss from raw meat through the washing action of melting ice, was replaced in a linear ($P \geq .05 - P \geq .005$) manner with water to maintain yield (% wet wt) during storage. Ice storage expanded cooked yield (% dry wt) differences between shrimp sizes. Meat losses through cooking mediated by ice storage, ranged from 0.421 to 0.303 g cooked meat dry matter/100 g round shrimp/day for 2.5 and 7.5 g shrimp, respectively. A linear ($P \geq .10 - P \geq .005$) increase in moisture retention through cooking modified differences in yield (% wet wt). The rate of moisture retention was dependent on shrimp size; 1.016 and 0.605 in water/100 g cooked meat/day for 2.5 and 7.5 g round shrimp, respectively.

The differences in yield reductions and moisture gains achieved between the different age classes may be attributed to the relative content and composition of collagens. Greater heat solubilization and moisture gain occurred in unformed collagen than formed collagen due to their res-

pective differences in structural stability and moisture holding capacities. Heat induced solubilization was further enhanced by proteolytic attack during ice storage. Enzymatic action on proteins not already solubilized and lost through ice storage, increased the water holding capacity of shrimp meat. Smaller immature shrimp containing higher percentages of unformed collagen also contained more moisture than the larger shrimp. Smaller shrimp exhibited greater enzymatic degradation and subsequent meat solubilization with time than the larger shrimp which contained higher percentages of formed collagen. This resulted in a greater reduction of meat yield from small shrimp than from large shrimp. The amount and type of collagen present in shrimp musculature can be then used as an index of physical stability. The variations in the collagen content existing between different age classes are a major factor in yield variation in raw and cooked shrimp meat.

Meat yield losses associated with ice storage and precooking could be reduced if processors handled shrimp more quickly. Young shrimp are more labile; separation by size with more rapid processing of small shrimp could increase cooked meat yields over-all.

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