TEXTURAL AND CHEMICAL CHANGES
IN THE MUSCLE OF CHUM SALMON (ONCORHYNCHUS KETA)
DURING SPAWNING MIGRATION

By
REBECCA ANNE REID
B.S.Ag., The University of Saskatchewan

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
in
THE FACULTY OF GRADUATE STUDIES
(Department of Food Science)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
May 1991
© Rebecca Anne Reid, 1991
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Food Science
The University of British Columbia
Vancouver, Canada

Date May 13, 1991
The spawning migration of chum salmon results in a deterioration of the eating quality of the flesh. There is a loss in the colour of the muscle from red-pink to white-grey, a distinct off-flavour develops and the firm texture becomes soft and watery. The reduced appeal of the canned and fresh product results in an economic loss of this important B.C. commodity.

Chum salmon were sampled at four stages during their spawning migration in order to describe the physical, chemical and structural changes which occurred as the fish matured. The fish were categorized into four grades based on their external characteristics. Grade one, silver-brights, were the least mature fish, grade two, intermediates, and grade three, darks, were progressively more mature. Grade four, spawning, were the most mature. Proximate analysis of the raw muscle revealed a significant decrease in protein and lipid and increase in moisture. The level of astaxanthin and the Hunter values a/L, a and a/b decreased significantly with increasing grade.

The texture of the canned fish was measured instrumentally with an Instron Universal Testing Machine using the texture profile analysis (TPA) method. A panel of semi-trained judges also evaluated the canned flesh based on its firmness, fibrousness, dryness, and chewiness. The
sensory panel determined that the silver-bright fish were significantly more firm, fibrous, dry, and chewy than the spawning fish. Of the ten instrumental TPA parameters, cohesiveness and slope 2 were able to distinguish between grade one and four. Poor correlation between the instrumental and sensory measure of texture suggested that the softening characterized by the sensory panel was not being measured by the Instron.

Thin and ultrathin sections of the muscle were photographed at the light and electron microscopic level. The light micrographs clearly showed an apparent loss in the myofibrillar diameter or number within muscle fibres as the fish became more mature. Focal degeneration of myofibrils was evident in the electron micrographs. Changes were described by four criteria, smearing, the loss of a distinct appearance of the actin and myosin, splitting, the tearing of myofibril bundles, thinning, a loss in myosin or actin filaments, and an increase in intermyofibril space.

Proteinase activity of the dorsal muscle was assayed at pH 3.5, 6.2 and 7.0. There was a significant increase in activity at pH 3.5 but no change at pH 6.2 or 7.0. Acid phosphatase, a lysosomal marker, also increased significantly with grade. These findings suggested that lysosomal enzymes largely contributed to the degradation in the muscle.
TABLE OF CONTENTS

ABSTRACT ......................................................... ii
LIST OF TABLES .................................................. vii
LIST OF FIGURES ................................................ ix
ACKNOWLEDGMENTS ............................................. xii

1. LITERATURE REVIEW .......................................... 1
   1.1. Introduction ............................................. 1
   1.2. Biochemical changes in the muscle of chum salmon .... 2
       1.2.1. Colour ............................................. 4
   1.3. Texture .................................................. 8
       1.3.1. Instrumental analysis ............................ 8
       1.3.2. Sensory analysis ................................ 18
   1.4. Ultrastructure of the muscle of chum salmon .. ....... 23
       1.4.1. Structure of muscle .............................. 23
       1.4.2. Muscle degeneration ............................. 29
       1.4.3. Starvation ....................................... 33
   1.5. Proteinase activity ................................... 36
       1.5.1. Lysosomal system ................................. 36
       1.5.2. Protein turnover ................................. 37
       1.5.3. Starvation ....................................... 39

2. CHEMICAL ANALYSIS .......................................... 42
   2.1. Introduction ............................................. 42
2.2. Materials and Methods ........................................ 43

2.2.1. Sampling ..................................................... 43
2.2.2. Grading ....................................................... 43
2.2.3. Proximate analysis .......................................... 44
  2.2.3.1. Total lipid analysis ................................. 44
  2.2.3.2. Moisture analysis ..................................... 46
  2.2.3.3. Protein determination ................................. 46
2.2.4. Colour ....................................................... 47
  2.2.4.1. Astaxanthin measurement ............................. 47
  2.2.4.2. Hunter Lab measurements .............................. 47

2.3. Results and Discussion ....................................... 48
  2.3.1. Astaxanthin content ..................................... 48
  2.3.2. Hunter Lab values ....................................... 50
  2.3.3. Proximate analysis ....................................... 54

3. TEXTURE ANALYSIS ............................................... 57
  3.1. Introduction ................................................ 57
  3.2. Materials and Methods ..................................... 60
    3.2.1. Instrumental analysis of texture .................... 61
    3.2.2. Sensory analysis of texture .......................... 62
  3.3. Results and Discussion .................................... 65
    3.3.1. Instrumental .......................................... 65
    3.3.2. Sensory analysis of texture .......................... 73
LIST OF TABLES

Table 1.1. Content and percentage composition of the major carotenoids in the chum salmon muscle during spawning migration (Ando and Hatano, 1987).

Table 2.1 Mean values for astaxanthin content in the dorsal muscle of chum salmon.

Table 2.2 Hunter L a b mean values for the dorsal muscle of chum salmon.

Table 2.3 Results from the correlation analysis between grade, Hunter L a b values and astaxanthin content.

Table 2.4 Mean values for percentage protein, moisture and lipid content of dorsal muscle of chum salmon on a wet basis.

Table 3.1 Mean instrumental TPA parameters of the four grades of chum salmon.

Table 3.2 Probability values produced from one-way ANOVAs on the square root transformed Instron TPA data.

Table 3.3 Mean untransformed results from the sensory panel’s evaluation of the textural differences between the four grades of chum salmon.

Table 3.4 Results from the analysis of variance of the sensory analysis to determine differences between grades and sequential rejective Bonferroni procedure for the multiple comparison of the data.

Table 3.5 Correlation values (r) extracted from a Pearson correlation matrix of the comparison between the instrumental parameters and the sensory attributes.

Table 4.1 Proteinase activity at pH 3.5, 6.2 and 7.0 during the different stages of spawning migration.
Table 4.2 Acid phosphatase activity (mM phosphate) in dorsal muscle of chum salmon during spawning migration.

Table 5.1 Percentage of silver-bright and spawning fish assigned to each score used to judge micrographs of chum dorsal muscle.

Table 8.1. Absorbance at U.V. 280 of soluble N versus grade as a measure of the proteinase activity of chum dorsal muscle at pH 3.5.

Table 8.2. Absorbance at U.V. 280 of soluble N versus grade as a measure of the proteinase activity of chum dorsal muscle at pH 6.2.

Table 8.3. Absorbance at U.V. 280 of soluble N versus grade as a measure of the proteinase activity of chum dorsal muscle at pH 7.0.

Table 8.4. Concentration of phosphate (mM) versus grade as a measure of the acid phosphatase activity of chum muscle during spawning migration.
LIST OF FIGURES

Figure 1.1 Generalized texture profile analysis .... 15 (TPA) curve (Bourne, 1982).

Figure 1.2 Texture profile score sheet (Bourne, .... 22 1982)

Figure 1.3 Appearance of musculature of a .... 25 typical fish. White spaces represent the parallel muscle cells, "dividing" lines represent the myocommata which separate the myomeres (Love, 1980).

Figure 1.4 Major subcellular components of .... 27 myotomal muscle (Peter, 1973).

Figure 2.1 Illustration of the four grades of .... 45 chum salmon.

Figure 3.1 Example of typical texture score .... 64 sheet used to analyze texture of the four grades of the canned chum samples.

Figure 3.2 Example of a typical Instron texture .... 66 profile analysis curve for a chum sample.

Figure 3.3 Standardized sensory scores for the .... 76 four grades of chum salmon for the attributes firmness, chewiness, dryness and fibrousness.

Figure 4.1 Flow diagram of the method used to .... 88 measure the proteinase activity in the dorsal muscle of four grades of chum muscle.

Figure 4.2 Standard curve of the absorbance at .... 94 405 nm versus the concentration of p-nitrophenol.

Figure 5.1 Light micrograph of a cross section .... 102 of silver-bright chum salmon stained with phosphotungstic acid haematoxylin. (Magnification 1500).

-ix-
Figure 5.2 Light micrograph of a cross section .... 103 of silver-bright chum salmon stained with phosphotungstic acid haematoxylin. (Magnification 600).

Figure 5.3 Light micrograph of a longitudinal .... 105 section of a silver-bright chum salmon stained with phosphotungstic acid haematoxylin. (Magnification 1500).

Figure 5.4 Light micrograph of a cross section .... 106 of a spawning chum salmon stained with phosphotungstic acid haematoxylin. (Magnification 1500).

Figure 5.5 Light micrograph of a cross section .... 107 of a spawning chum salmon stained with phosphotungstic acid haematoxylin. (Magnification 600).

Figure 5.6 Light micrograph of a longitudinal .... 108 section of a spawning chum salmon stained with phosphotungstic acid haematoxylin. (Magnification 1500).

Figure 5.7 Electron micrograph of the dorsal .... 109 muscle of a silver-bright chum salmon. (Magnification 27000).

Figure 5.8 Electron micrograph of the dorsal .... 110 muscle of a silver-bright chum salmon. (Magnification 27000).

Figure 5.9 Electron micrograph of the dorsal .... 111 muscle of a silver-bright chum salmon. (Magnification 27000).

Figure 5.10 Electron micrograph of the dorsal .... 112 muscle of a silver-bright chum salmon. (Magnification 27000).
Figure 5.11  Electron micrograph of the dorsal muscle of spawning chum salmon. Arrow indicates area of "smearing". (Magnification 26000).

Figure 5.12  Electron micrograph of the dorsal muscle of spawning chum salmon. Arrows indicate areas of "splitting" of the myofibrils. (Magnification 26000).

Figure 5.13  Electron micrograph of the dorsal muscle of a spawning chum salmon. Notice areas with "intermyofibrillar" spaces. (Magnification 26000).

Figure 5.14  Electron micrograph of the dorsal muscle of spawning chum salmon. Arrows point to areas which have myofibril "thinning". (Magnification 26000).
ACKNOWLEDGMENTS

No one could ever claim that a thesis was entirely an individual effort. Of the many people who's encouragement and advice helped me complete this project, I would like to extend my most sincere thanks towards my supervisor, Dr. T. Durance, for his patience, knowledge and constant encouragement. I would also like to thank Dr. D. Walker for sharing his invaluable knowledge of electron microscopy and for the Saturdays he spent helping me with my work. I thank Dr. D. Kitts and Dr. W. Powrie for fitting committee meetings into their busy schedules and for their helpful ideas and comments. I would also like to thank my father for his help in the lab and for his confidence in my work.

It is fitting to end this section with a quote from the author Douglas Adams, "So Long, and Thanks for all the Fish".

-xii-
1. LITERATURE REVIEW

1.1. Introduction

Chum salmon (*Oncorhynchus keta*) is an important commercial commodity in B.C., where it ranks as the #3 salmon fishery. In 1987, 11,000 tonnes were caught for a total landed value of 27 million dollars.

Chum are hatched in the gravel beds of fast running streams after incubating over the winter. Within a few days of emerging from the spawning beds, the fry drift downstream to the sea, where they may spend three to five years feeding on plankton and small crustacean. Spawning migration begins for 90 to 99% of the stock in their third or fourth year and may begin as early as July, or extend as late as January, depending on the stock (Beacham and Starr, 1982). Chum are often in an advanced stage of sexual maturity as they enter their natal streams, and often spawn not far from the sea (Neave, 1966). Even before the salmon enter fresh water, they stop feeding, and must survive on stored lipid and protein in their white muscle until they have spawned.

A result of these physiological changes in the salmon is a loss in the eating quality of the fish. The muscle loses its red-pink colour, and ultimately becomes a white-grey colour. The texture changes from firm and desirable to soft and watery. These changes were noted by Bilinski et al. (1984) who studied the effect of sexual
maturation on the quality of the flesh of coho salmon. These workers noticed changes in the canned product such as an increase in free liquid, a decrease in free oil, discolouration and softening of the flesh and a deterioration of the flavour and odour.

The quality changes which occur in the chum during maturation could be avoided by harvesting only the ocean-run fish. However, because fish of different stocks cannot be adequately distinguished until they begin their spawning run, mixed stock fisheries may result in the over harvesting of weaker stocks. Terminal fisheries management techniques reduce this problem and allow the fishing fleet to target specific stocks (Holmes, 1982).

1.2. Biochemical changes in the muscle of chum salmon

Ando and Hatano (1986a) measured changes in the colour, protein, fat and moisture content of the muscle of chum in order to assess the physiological state of the salmon throughout the spawning period. At the first stage tested (feeding migration), the muscle was high in total and sarcoplasmic protein, and low in ninhydrin positive, non-protein, nitrogenous compounds. As the spawning progressed, this relationship reversed so that the total and sarcoplasmic protein decreased, and the ninhydrin positive substances increased. The lipid content was markedly reduced during the early stages of migration, indicating it was used as a primary energy source for gonad development.
Idler and Bitners (1960) measured a 200% increase in the fat content of the ovaries, an 11% increase in ovary moisture, and no change in ovary protein, by the end of the migratory run. Analysis of the milt revealed a 33% increase in protein, a slow, steady increase in fat, and little change in moisture content.

Chang and Idler (1960) monitored the level of liver glycogen as an indicator of the physiological condition and nutritional state of the spawning sockeye salmon. Glycogen levels in the female were significantly lower than in the male, however for both sexes the level decreased during the early migratory phase, and increased in the later stage. Mommsen et al. (1980) found that the carbohydrate stores in the muscle were maintained throughout migration, and functioned as a source of short term emergency energy in the muscle.

Duncan and Tarr (1958) studied three protein fractions, actomyosin, myoalbumin and myogens, and collagen, and non-protein nitrogenous material in sockeye muscle. A decrease in the total nitrogen content was attributed primarily to the decrease in actomyosin and stroma fraction.

Ando and Hatano (1986b) followed the degree of myofibrillar protein degradation during spawning migration by measuring N-methylhistididine by ion exchange chromatography. This amino acid is useful as an index of myofibrillar protein degradation because it is contained only in actin.
and myosin, is not re-utilized for protein synthesis, and is excreted quantitatively into urine. In this manner, these workers were able to clearly show the degree of myofibrillar protein degradation during spawning migration of chum salmon.

1.2.1. Colour

Carotenoids are a group of pigments which, in animals such as Pacific salmon, contribute to the yellow, orange, and red colours found in the integument, muscle, and ovaries. Astaxanthin is an important carotenoid, and is the main pigment found in chum salmon (Simpson, 1982). Because salmon are unable to synthesize this carotenoid de novo, astaxanthin and other related carotenoids must be obtained from the diet. Thus the degree of pigmentation in the animal is a reflection of the ingestion and metabolism of specific dietary pigment sources (Ando et al., 1989).

The differences in the muscle and skin colour of chum salmon are closely associated with the physiological state of the fish (Ando et al. 1985). Ando et al. (1989) used HPLC techniques to follow the change in colour of chum salmon throughout the spawning migration. Astaxanthin was the primary carotenoid found in immature chum salmon. During migration there was an increase in serum carotenoid levels, and an increase in the carotenoid levels in the ovaries. With sexual maturity, a considerable proportion of 4-keto-zeaxanthin and zeaxanthin were detected in the muscle.
of the spent chum. The mechanism proposed for this change was via a reductive metabolic pathway from astaxanthin to 4-keto-zeaxanthin to zeaxanthin. Table 1.1 depicts this loss in astaxanthin, and the relative increase in zeaxanthin and 4-keto-zeaxanthin in the chum during four stages of its migration (Ando and Hatano, 1987).

The colour of the different carotenoids is a result of the presence of a system of conjugated double bonds. As the number of conjugated double bonds increase, the maximum absorbance of the compound occurs at the longer wavelengths, and the hue of the carotenoid becomes redder. In general, the carotenoids in food are found in the trans configuration. The shift to a cis isomer as a result of heat, light, or acidity, causes a gradual lightening of colour. Because astaxanthin is an unstable pigment, it is important during any quantification to protect it from excess heat, acid, exposure to light, oxygen, or lipoxygenases.

There is a need for instrumental techniques to provide consistent qualitative and quantitative information on colour (Skrede and Storebakken, 1986). The colour perceived when the eye views an illuminated object is related to three factors, the spectral composition of the light source, the chemical and physical characteristics of the object, and the spectral sensitivity properties of the eye. A system developed to quantitate the colour response mechanism of the human eye was proposed by the Commission International de
Table 1.1. Content and percentage composition of the major carotenoids in the chum salmon muscle during spawning migration (Ando and Hatano, 1987).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Sex</th>
<th>Astaxanthin</th>
<th>Zeaxanthin</th>
<th>4-keto-zeaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>14.5 (89)</td>
<td>0.19 (1.2)</td>
<td>0.34 (2.1)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>11.4 (91)</td>
<td>0.10 (0.8)</td>
<td>0.20 (1.2)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>10.7 (86)</td>
<td>0.32 (2.6)</td>
<td>0.45 (3.7)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9.4 (84)</td>
<td>0.31 (2.8)</td>
<td>0.48 (4.3)</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>0.4 (73)</td>
<td>0.07 (11.8)</td>
<td>0.03 (5.0)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.4 (78)</td>
<td>0.04 (8.4)</td>
<td>0.02 (4.3)</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>0.7 (64)</td>
<td>0.26 (23.7)</td>
<td>0.05 (4.9)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1.0 (70)</td>
<td>0.29 (20.0)</td>
<td>0.05 (3.4)</td>
</tr>
</tbody>
</table>

1 Brackets indicate percentage of total carotenoid.
2 Values are measured in mg/100 g whole muscle.
l’Eclairage (CIE) (de Man, 1980). However because the CIE scales were not able to provide a reasonably uniform estimate of colour as perceived by the human eye, other colour scales were developed based on the opponent colour theory of colour vision.

The opponent colour theory of colour vision was proposed by Ewald Hering in 1879, and was later refined by G.E. Mueller in 1930 (de Man, 1980). The theory stated that in the human eye there was an intermediate signal-switching stage between the light receptors in the retina and the optic nerve, which took colour signals to the brain. In the switching stage, the red colour responses were compared with green to generate a red-green colour dimension, denoted as "a". This red-green response was then compared with blue to generate a yellow to blue colour dimension, denoted as "b". A third dimension considered was lightness, \((L)\), a non-linear function which related to the percent reflectance of an object. Several systems of colour classification have been developed to provide quantitative information.

The Hunter \(L\ a\ b\) system has been used successfully to describe and measure colour. The modern model of this machine makes use of a reflectance spectrophotometer which is interfaced with an IBM personal computer. Visible light reflected from the sample is analyzed and the resultant spectrum is used to calculate the spectral values \(L\), \(a\), and \(b\).
1.3. Texture

1.3.1. Instrumental analysis

The texture of a food product is commonly considered the most difficult organoleptic property to describe accurately. Szczesniak, (1963) suggested this problem was caused first by the lack of an adequate bridge between theoretical rheology and practical applications, and second because most of the reported research had only considered specific textural characteristics of individual foods. A need was thus identified to develop a rational system, and nomenclature to describe and translate textural properties into precisely defined, measurable properties (Friedman et al., 1963).

Texture and consistency were defined by two elements, first the physical structure of the material (i.e. its geometry), and second the way the material felt in the mouth (i.e. its mechanical and surface properties). Szczesniak, (1963) grouped texture into three main classes, mechanical characteristics - those manifested by the reaction of food to stress, geometric characteristics - the arrangement of the constituents of food, and other characteristics - the mouth feel factors. This analysis of texture lead to the acceptance of five primary independent mechanical parameters, hardness, cohesiveness, adhesiveness, viscosity, and elasticity, and three secondary dependent parameters, brittleness, chewiness, and gumminess. Hardness was defined as
the force necessary to attain a given deformation, cohesiveness was the strength of internal bonds which made up the body of the product, viscosity was the rate of flow per unit force, elasticity (later changed to springiness) was the rate at which deformed material returned to an undeformed condition after the deforming force was removed, and adhesiveness was the work necessary to overcome the attractive forces between the surface of food and the surface of other materials with which the food came in contact. Brittleness, or fracturability, was the force with which the material fractured, and was related to the primary parameters of hardness and cohesiveness. Chewiness was the energy required to masticate a solid food to a state ready for swallowing, and was related to hardness, cohesiveness, and elasticity. Gumminess was the energy required to disintegrate a semisolid food product to a state ready for swallowing.

The geometric characteristics described the relative size and shape of the particles, and their relative orientation. Other characteristics described mouth feel quality and were related to the perception of moisture and fat in food.

Application of these parameters required the design of an instrumental unit capable of translating these definitions into an unbiased, recorded physical measurement. Friedman et al., (1963) recognized that in order to
characterize texture, a comprehensive objective test was needed. The objective measurement of texture was divided into three general categories, fundamental tests, empirical tests, and imitative tests. The first category measured the fundamental rheological properties of force, deformation, and time from which it was possible to calculate parameters such as ultimate strength, Poisson’s ratio, Young’s modulus and shear/bulk modulus (Bourne, 1978).

Texture measuring instruments cause the mechanical and structural failure of foods by imposing one or more forces. These have included tension, the application of uniaxial parallel forces to cause extension, compression, the uniaxial parallel force which causes flattening, and shear, a uniaxial tangential force which causes separation or cutting. Compression, an important measure of texture, can be tested in two ways. The first test is a bulk compression, in which the sample is compressed in three dimensions by means of hydraulic pressure. The second test is uniaxial compression whereby the sample is compressed in one direction, and unrestrained in the other two dimensions. The method of texture profile analysis utilizes the principle of uniaxial compression to crush food. The theoretical action of uniaxial compression was applied to Hookean solids of uniform cross-sectional area at a small strain (i.e. 1%) before rupture, is defined by Young’s modulus of elasticity (E) (Bourne, 1982).
\[ E = \text{Compressive stress} = \frac{F}{A} \]

\[ \text{Compressive strain} = \frac{\Delta L}{L} \]

Where:
- Stress = force per unit area
- Strain = deformation per unit dimension
- \( F \) = applied force
- \( A \) = cross sectional area
- \( L \) = unstressed height
- \( \Delta L \) = change in height due to \( F \)

Thus "\( E \)" is the slope of the stress/strain curve, and a measure of stiffness (Bourne, 1982). For most foods however, the pure definition of "\( E \)" rarely applies. This is because first, the sample is usually subjected to a large amount of strain, and second, because most foods are viscoelastic, not elastic. Viscoelasticity occurs when a weight placed on a food causes immediate compression ("instantaneous elastic deformation"), followed by a prolonged, continuous decelerating rate of deformation called "retarded deformation". When the weight is removed, the instantaneous partial elastic recovery is followed by a future recovery with respect to time, called "retarded recovery". Thus in a permanent deformation, the sample never returns to its original height. In order to maintain the "purity" of the definition of Young's Modulus, most foods are defined instead by a "modulus of deformability" (Johnson et al., 1980). The modulus of deformability is representative of a material's overall resistance to deformation. Actual measured values are dependent on test conditions, especially specific dimensions of the material, and deformation rates. It should be
noted however that if a high degree of uniaxial compression caused a product to rupture, spread, fracture, or break, then the modulus of deformability also would no longer apply (Bourne, 1982).

Fundamental tests such as Young’s modulus have tended to correlate poorly with sensory evaluation, and are often not meaningful in themselves because food does not have simple rheological properties that are independent of stress and strain conditions. Thus the primary function of fundamental tests has often been to provide a basis from which empirical tests may be developed (Brinton and Bourne, 1972).

Empirical tests measure parameters that have been found to be related to textural quality, but they do not include all the parameters necessary for a complete description of texture. Instruments used for these tests include penetrometers, compressors, consistometers, tenderometers, and shearing devices. Because these instruments have tended to concentrate only on a small phase of the mechanical properties involved in chewing, they have not provided a complete picture of how the mechanical parameters of texture were sensed in the mouth (Friedman et al., 1963).

Imitative tests were developed to imitate the actual conditions to which food was subjected in the mouth. Instruments that fall into this category include the Brabender Farinograph, Amylograph, and Alveograph, and the Instron Universal Testing Machine (Szczesniak, 1963). One of the
first instruments designed to simulate mastication was the denture tenderometer developed by the Food Technology Laboratory of the Massachusetts Institute of Technology (Friedman et al., 1963). This instrument was later used as a basic prototype by Friedman et al., (1963) for the construction of their "texturometer". Their texturometer consisted of four basic units, an articulator with a motor, variable-pulley drive, and strain-gauge sensing plate, a fast response pen recorder, a variable-voltage power supply, and a method to measure the strain-gauge circuit. The system produced a force-distance curve which represented an "integral picture of the textural characteristics of the product sample under test", and was thus called a "texture profile" (Friedman et al., 1963).

Bourne first used the Instron Universal Testing Machine to measure the deformation of foods with a high degree of precision (Brinton and Bourne, 1972). This machine is divided into two basic parts, a mechanism which drives a horizontal cross-head in a vertical direction at selected speeds within the range of 0.2 to 50 cm per minute, and a force sensing and recording system which consists of a load cell and a strip chart recorder. By use of a sensitivity selector switch and several different load cells, it is possible to obtain a full scale deflection of the pen over a force range of 0.2 to 500 kg force (2N to 5kN), and to produce a record of a sample's force-distance relationship. Since its
introduction, the Instron has gained widespread use in the evaluation of textural properties of solid food.

Texture profile analysis is a useful technique for the evaluation of textural parameters of a wide range of foods. Bourne (1982) used this technique to evaluate the texture profile parameters derived from force-deformation curves obtained from the Instron. Flat plates were attached to the Instron and used to compress bite-sized pieces of a sample twice in a reciprocating motion to imitate the action of the jaw. The cross-head, driven at a constant speed, approached the end of the compression stroke, then abruptly reversed, and continued with an upward stroke at the same speed. This action resulted in the production of a force-time curve from which a number of textural parameters could be measured which correlated well with sensory evaluations (Bourne, 1982). This technique was a reflection of the need for multidimensional analysis of texture. Figure 1.1 depicts a generalized texture profile curve.

Bourne et al. (1978) described Figure 1.1 as follows. A sample was placed between two flat plates on the Instron. The cross-head descended at constant speed and compressed the sample. The force at the first significant peak was known as fracturability. As the compression continued, a second peak, hardness 1, was formed at the point of maximum compression. The Instron cross-head then reversed
Figure 1.1. Generalized Texture Profile Analysis (TPA) curve (Bourne, 1982).
direction, and the force returned to zero. Area 1 was the area under the curve up to the maximum point of compression, and represented the work done to compress and crush food during the first bite. The positive area to the right of the dotted line (in Area 1) was theoretically the work returned by the food to the machine during compression. However, a significant part of the small area was possibly caused by pen response and was associated with substantial error. The negative portion of the curve under the zero force line represented the adhesion of the food to the compression plate as it moved upward during the decompression phase of the first bite. Area 3 was defined as the work done to pull the food apart, and was the area under the zero force line. The adhesive force was defined as the maximum negative force. Hardness 2 was the force at the point of the maximum second compression. Again, the positive area to the right of the dotted line was the work returned to the machine during decompression. Area 2 was the work done on the food by the machine during the second compression. The springiness was defined as "the distance the sample recovered in height between the end of the first rise in force greater than zero in the second bite, and its point of maximum compression". Bourne et al. (1978) also defined several parameters that could not be directly measured from the texture profile. Cohesiveness was derived by dividing area 2 by area 1, gumminess was the product of hardness and
cohesiveness, and chewiness was the product of gumminess and
springiness.

To obtain information from force-time graphs manually
was time consuming, difficult and of limited accuracy
(Abbott et al., 1982). As a solution to this problem, com-
puter attachments with automatic readouts of
force-deformation parameters have been developed. By inter-
facing the Instron with a microcomputer, a dual system was
formed, capable of measuring and recording load cell output
signals and force-deformation curves. Use of the computer
allowed the analysis of the data from a large number of sam-
ples in a relatively short time, and the analysis of a large
number of variables. However, Abbott et al., (1982) and
Bourne et al., (1978) cautioned that the original automatic
type systems were not appropriate for texture profile curves
because the variation common in these curves resulted in se-
provided a program which they claimed was able to record the
load cell output signal, control the Instron cross-head
speed and direction, and automatically extract data from
texture profile type compression tests. A solution to the
problems with computer analysis of the variable texture pro-
file curves was to allow the operator to select the peaks,
areas, and distances which the computer then calculated.

Despite the apparent simplicity of the Instron, Shama
and Sherman (1973) cautioned that instrumental evaluation
of textural properties as a method to predict sensory responses required that the instrumental test conditions be selected carefully. The magnitude and rate of the applied load influenced the instrumental evaluation of textural properties differently for each food depending on how it was normally masticated. In the Instron, as the rate of loading (the cross-head speed) increased, the force to achieve compression also increased and therefore influenced the force-deformation data. Thus it was suggested that when a comparison was made between foods with different textural properties, different instrumental test conditions may be necessary in order to simulate relevant masticatory patterns.

1.3.2. Sensory analysis

Organoleptic tests have frequently been used to study the impact of a process operation on quality attributes of a food. Aguilera and Stanley (1990) described three types of sensory panels; consumer panels, which measured the response of a "typical" consumer to the product; acceptance panels which asked hedonic questions, i.e. how much the sample was liked/disliked and why; and analytical panels which measured intensity differences in the textural attributes of the samples. The object of the analytical panels was to obtain numerical data which could be analyzed statistically. The problem with the first two techniques was that commonly "quality was in the mind of the observer", and a measured
response was a result of the judge's previous conditioning, and was subject to many variables outside the control of a food processor (Lund, 1982).

The development of a comprehensive and analytical sensory method to evaluate food texture was thus needed to produce a rational, well-defined system for classifying texture. Szczesniak et al. (1963) noted the common problem of poor correlation between instrumental and sensory evaluations and suggested that the machines were not evaluating the same property as the sensory tests. Szczesniak et al. (1963) developed a technique which was demonstrated to produce very good correlations with instrumental evaluations. With this method, texture of a food product was objectively analyzed by training an expert taste panel to create a "sensory texture profile" of any food product.

The term "texture profiling" was thus used to describe the organoleptic multidimensional analysis of a sample in terms of the degree and order of appearance of mechanical, geometric, fat and moisture characteristics, from the first bite through complete mastication. Requirements for this comprehensive sensory method included a panel of trained judges, a standard rating scale, and proper panel procedures (Brant et al., 1963).

Different textural characteristics were categorized by standard rating scales composed of foods with different textural intensities. These foods provided the basis for the
evaluation of the mechanical parameters of texture. Specific foods for each point on the scales were selected so that proper reference standards were always available. Rating scales were developed and included: hardness - the force required to penetrate a substance with molar teeth; brittleness - a secondary parameter which encompassed hardness and cohesiveness, defined as the ease or force with which a sample crumbled, cracked, or shattered; chewiness - the length of time in seconds required to masticate a sample at the rate of one chew per second in order to reduce it to a state satisfactory for swallowing; gumminess - a secondary parameter defined as density that persisted throughout mastication, adhesiveness - the force required to remove the material that adhered to the mouth during normal eating; and viscosity - the force required to draw a liquid from a spoon over the tongue. Use of these rating scales enabled the evaluation of a given textural parameter in an "unknown" product in terms of known products.

A comprehensive sensory method was then developed to evaluate the texture of a given food product. Brandt et al., (1963) used the three main classes of texture; mechanical, geometric and those properties relating to fat and moisture content as a systematic means of recording the textural characteristics of a given food by order of appearance. Texture was considered to have a definite pattern regarding the order in which characteristics were perceived.
First bite, masticatory, and residual phases were described. The first bite, or initial phase, included the mechanical characteristics of hardness, brittleness, viscosity, and other geometric characteristics initially observed. The second bite, or masticatory phase, included the mechanical characteristics of gumminess, chewiness, adhesiveness, and other geometric characteristics observed during chewing. The third bite, the residual phase, encompassed changes induced in the mechanical and geometric characteristics throughout mastication.

Panel selection was based on interest and availability of personnel. The sensory panel was trained to familiarize the judges with the standard rating scales, until unknown food samples could be accurately rated individually and through group discussion. The classification of the textural characteristics were studied, and the geometric characteristics of foods were evaluated. The panel then developed a complete texture profile for a sample using the predetermined standard rating scale. A typical texture profile score sheet is shown in Figure 1.2.

Properly trained panelists complied with the two criteria that defined an objective method; first the data obtained was independent of individual observation, and
Texture Profile Score Sheet

Product: ___________________________ Date: ______________
Time: ______________

1. Initial (perceived on first bite)
   a) Mechanical
      - Hardness
      - Fracturability
      - Viscosity
   b) Geometric
   c) Other

2. Masticatory (perceived during chewing)
   a) Mechanical
      - Gumminess
      - Chewiness
      - Adhesiveness
   b) Geometric
   c) Other characteristics

3) Residual
   - Rate of breakdown
   - Type of breakdown
   - Moisture absorption
   - Mouth coating

Figure 1.2. Texture profile score sheet (Bourne, 1982).
second, the results were repeatable, and verified by other panels held at different times and places.

This method of sensory texture profile analysis provided a means of helping food researchers obtain descriptive and quantitative sensory data on textural characteristics of food products. It had the advantage of flexibility, with application to any food product or textural characteristic, and objectivity through rigidly defined points of reference and nomenclature. One limitation of the method was related to the variable degree of the panelists' skill when applying the method.

1.4. Ultrastructure of the muscle of chum salmon

1.4.1. Structure of muscle

Although there are differences in the gross organization between fish muscle and terrestrial animals, Bremner and Hallett, (1985) found that there were enough similarities at the ultrastructural level that mammalian muscle could be used as a model to describe the muscle of many fish species. Shindo et al., (1986) compared the ultrastructure of skeletal muscle of eight species of fresh and salt water fish, and found them similar to each other and similar to that described for mammalian skeletal muscle. Differences which exist between fish and terrestrial animals included the arrangement of muscle fibrils, the amount of sarcoplasm, the relationship of nuclei and mitochondria to other muscle
components, and the arrangement of the sarcoplasmic reticulum (Bishop and Odense, 1967).

The gross structure of fish was described by Hallett and Bremner, (1988) as consisting of long sheets of muscle which extended from head to tail on both sides of the body. Adjacent muscle blocks called myotomes, were separated from each other by thin transverse collagenous sheets called myocommata (Hallett and Bremner, 1988). Figure 1.3 depicts the structure of this connective tissue. The myocommata interconnected with the myotomes, and were responsible for the transmission of the muscle's contractile forces. The myotomes on both sides of the axial skeleton take the shape of double cones which fit together along the long axis of the fish body, and give the appearance at the surface of a series of "W-shaped" sections resting on their sides. Within each myotome, the muscle fibres (myomeres) run approximately parallel to each other, but at varying angles to the myocommatal sheets. Each myomere is a single cell, and is composed of hundreds of myofibres which occupy most of the interior of muscle fibres. Myofibres are enveloped and separated from the extracellular environment by the sarcolemma, a double membrane composed of an inner semi-permeable membrane (plasmalemma) and an outer diffuse basement layer (Howgate, 1979). Myofibrils are the basic contractile units of the cells, and are close packed, non-branching, elongated, cylindrical structures of about
Figure 1.3. Appearance of musculature of a typical fish. White spaces represent the parallel muscle cells, "dividing" lines represent the myocommata which separate the myomeres. (Love, 1980).
one micron in diameter, which extend the length of the muscle fibre.

Surrounding the myofibrils is a lace-like network of tubules, the sarcoplasmic reticulum, and the T-system. Peter (1973) described the T-system as an invagination of the cell's plasma membrane which surrounded the myofibrils midway between the Z-lines and the centres of the A bands. Its function was to conduct electrical impulses from cell membranes into the sarcoplasmic reticulum of muscle during contraction and relaxation. The sarcoplasmic reticulum lies on either side of the T-tubules. The sarcoplasm was described by Dubowitz, (1985) as the cytoplasm of the fibre, and contains mitochondria, the tubule membrane system, microtubules, intermediate filaments, Golgi apparatus, glycogen, and free lipid droplets. Figure 1.4 describes the major subcellular components of a muscle cell.

Observation of a longitudinal section of a muscle fibre revealed a repetitious pattern of light and dark bands, which was the result of a regular overlapping arrangement of thick and thin filaments which made up the myofibril. These repetitious structures, sarcomeres, were a result of the regular alternation of different proteins, and were characteristic of the striated pattern of skeletal muscle (Dubowitz, 1985). Shindo, et al. (1986) found a marked difference in the relative width of I, and A bands of fish muscle as compared to mammalian muscle. In both white

-26-
Figure 1.4. Major subcellular components of myotomal muscle. (Peter, 1973)
and dark muscle, the relative width of the I bands was less than that usually illustrated for mammalian striated muscles. The workers were not convinced however, that this finding was a characteristic feature of all fish muscle, and suggested that it might be due to shrinkage which occurred during preparation of the specimens.

Bishop and Odense (1967) described two histologically distinct types of striated muscle in a marine teleost. The bulk of this tissue was a light brown, or pink coloured mass called white muscle. This tissue was thought to be similar to type II fibres in humans, and was characterized by large fibre size, less sarcoplasm, less glycogen, and fewer mitochondria than the second type of striated muscle. With few blood capillaries, it was thought to be constructed to facilitate the largest possible number of contractile units, and geared for anaerobic production of energy during swimming at high speeds (Dunn et al., 1981). At the surface of the white muscle, lying in a band along the fish’s mid-line, was a strip of brown coloured tissue called dark, or red muscle. These muscle fibres were similar to type I fibres in humans, and were smaller in diameter, with a longer sarcomere than the white fibres. They were richly vascularized, had a large number of mitochondria with well developed inner cristae, and tended to fatigue and contract more slowly than white muscle (Franzini-Armstrong and Porter, 1964; Peter, 1973). A cross section of the white
muscle fibres revealed that the myofibrils were elliptical, or nearly circular in the centre of muscle fibres, but rectangular, or more asymmetric at the periphery of muscle fibres. Dark muscle fibres were polygonal, and less asymmetric at any portion of its cross section (Shindo et al., 1986).

Proctor et al., (1980) noted the presence of a third fibre type called pink fibres in the musculature of Salmo trutta, brown trout. The pink fibres were situated between the red and white fibres, were intermediate in size and character between the large white, and small red fibre diameter, and were thought to have a role during intermediate swimming speeds.

1.4.2. Muscle degeneration

Kakwas and Adams, (1985) used a number of criteria to distinguish muscle diseases. Factors included the character and distribution of the changes of the muscle fibre and supporting tissue, the functional effect of the lesions, and the presence or absence of genetic, or other factors. Pathological changes found in muscle biopsies may affect the myofibrils, or their supporting membranes (Dubowitz, 1985). For example, interruptions or breaks in the continuity of the sarcolemma often occurred with necrotizing injuries, but were not necessarily lethal to the cell. The surface of the muscle cells may appear irregular, possibly a result of a decrease in fibre volume (Peter, 1973). Folding of the
sarcolemma is also common, and the basement membrane may split away from the plasma membrane, creating extensive folds in the extracellular space. Loss, or alteration of myofilaments was considered the most common abnormality in diseased muscle (Dubowitz, 1985). It was also one of the most difficult changes to assess, because even normal muscle often departs from the "classical appearance". Pathological changes observed in muscle biopsies included alterations of the fibre size and distribution, splitting, branching, and loss of myofibrils. In severe necrosis, characteristic myofilament structure may be lost and replaced by an amorphous granular material. Selective loss, or abnormalities of a particular region may include I or A band loss and Z-line alterations such as streaming, broadening (thickening), irregularities (zigzag appearance), or complete disintegration.

Kakwas and Adams, (1985) categorized changes in muscle fibres into three groups. The first group was called segmental necrosis in which there was a clustering of a few or many necrotic sarcomeres. The second group, myofibrillar disfigurement, was used to classify a series of subcellular lesions which were often difficult to distinguish from electron micrograph artifacts. The third group, morphological changes, included changes in fibre types and alterations of the organelles.
Based on mammalian studies, it is reasonable to believe that the physical stress that occurs during spawning migration contributes to muscle deterioration of chum. Factors which are likely responsible for changes in the ultrastructure of spawning salmon are related to first, the fatigue associated with both rapid bursts of energy and prolonged, repetitious, low force gathering types of contractions (Belcastro et al., 1985), second the effects of starvation during migration, and third, the hormonal changes occurring during development of the reproductive system.

Belcastro et al., (1985) observed changes in rat muscle which had undergone prolonged, repetitious contractions. The sarcomere appeared wavy and disoriented. Myofilament dissolution was observed at the last stage of the fatigue process, and was only associated with extreme conditions of muscle use. Friden et al., (1983) observed myofibrillar damage following intense eccentric exercise in man. Light micrographs of semi-thin sections revealed focal disturbances of the cross-striated band pattern of the myofilament. At the electron microscopic level, these focal disturbances were found to be a result of marked streaming, broadening, and total disruption of myofibril Z-bands. Gaps in the regular lattice pattern of the Z-bands, and an apparent loss of the thick myofilaments were also observed. It was found that exercise to the point of exhaustion resulted in fibre necrosis and an increase in the acid hydrolytic
activity of muscle enzymes. No mechanism for these changes was known, however it was suggested that lysosomal enzymes were liberated in the damaged fibres, and may have been responsible for the initiation of the degradation of myofibrillar material. George et al., (1987) studied ultrastructural changes in the breast muscle of the Canada goose after migration, a time of excess muscle use and fatigue. Excess Z-line streaming, and an increase in the number of lysosome-like bodies were apparent in the muscle. The histopathological changes in the muscle were correlated to lysosomal enzyme activity, and it was noted that flight-induced necrotic changes in the muscle mimicked the symptoms of human and animal myopathies.

Pale soft exudative (PSE) pork is a well known phenomenon whereby stressed animals exhibit an accelerated rate of post mortem glycolysis, which causes rigor mortis to occur at a low pH while the carcass temperature is still high. The ultrastructural differences between normal and PSE muscle were investigated by Cassens et al. (1963). Within 40 minutes post mortem, sarcoplasmic material was disrupted, I-bands were "clumped" together, and fibre bundles appeared disconnected. No marked alteration in myofibrils were observed in the first 24 hours of normal pork muscle. Cloke et al. (1981) also observed an unusual degree of disruption in the PSE muscle after death, including myofibrillar breakdown with loss of material at the Z-lines.
1.4.3. Starvation

The energy that non-feeding, maturing salmon require for survival, migration, and gonad development is obtained first by utilizing available lipid stores in the liver and muscles. When this supply becomes depleted, there is extensive use of the proteins from the white muscle for energy (Love, 1980).

Beardall and Johnston (1985) studied the ultrastructure of myotomal muscle of the low fat fish, Pollachius virens L. (saithe) during a period of starvation. A fifty percent loss in body weight, an 80% loss in tissue glycogen stores, and atrophy of the fast (white) and slow (red) twitch myotomal muscle fibres was observed. Atrophy of the muscle resulted in a decrease in the myofibril cross-sectional area. After 74 days of starvation, the volume of the myofibril reduced from 84 to 62% of the muscle cell. After 3 months, the mean cross-sectional area of the myotomal muscle had decreased by 50%, and the volume density of the myofibril had decreased by 25%. In addition, a preferential degeneration of M-line proteins, and of thick filaments at the periphery of the myofibril was observed. In severely atrophied muscle fibres, the myosin in myofibrils and whole fibres were degraded, however the sarcomeres still maintained their alignment. In 1981, Johnston studied the effects of starvation on the ultrastructure of skeletal muscle from a marine flatfish. Atrophy of white muscle fibres was
associated with a decrease in the diameter and fractional volume occupied by myofibrils. Also common was peripheral degeneration of Z-disks, which caused the unraveling of the thin filament lattice. Johnston suggested that this effect caused a partial decrease in myofibrillar diameter, and the maintenance of contractile function in starved fish. In severely degenerating white fibres, disorganized thick and thin filaments, and numerous multi-membrane lysosome-like vesicles were observed. It was suggested that this gradual reduction in myofibrillar diameter provided a mechanism whereby myofibrils could be used as an energy source while still maintaining some contractile functions. This hypothesis was supported by Love (1980) who studied histological changes in the muscle cells of the fish Cyprinus carpio. Even after 14 months of starvation, the parallel orientation of myofilaments in these fish, although largely destroyed, still had some recognizable myofibrils.

Beardall and Johnston (1985) listed four stages of starvation-induced degeneration that could be found in the white muscle of marine flatfish. At stage one, the myofibrils had a normal appearance. In the second stage, the diameter of the myofibrils were reduced, and the peripheral thick filaments (myosin) were absent. In the third stage, thick myofilaments were absent, leaving the thin filaments and Z-disc intact. In the fourth stage of degeneration, the myofibril had been completely, or largely
disrupted, and phagocytosed.

Haard (1987) observed that between 20 to 40% of the catch of the American plaice (Hippoglossoides platessoides) was unsuitable for market because of a jellied condition which was unacceptable to consumers. In contrast to the normal greyish colour and firm texture of the muscle, the fillets of these fish were jelly-like, glossy, and opalescent in appearance. The connective tissue septa was wide and translucent, and the connective tissue itself was loose, and indefinite in nature. The cut face of the fillets was smooth, and the myotomes were pulled away from each other (Templeman and Andrews, 1956). Proximate analysis of the normal and jellied fillets revealed that the water content in the jellied fillets increased from the normal 78%, to 89%, and the crude protein was reduced from 18% to 9%. Gel electrophoresis of the myofibrillar protein showed reduced levels of actin and myosin (Haard, 1987). Histological examination revealed that there were large spaces between muscle fibres, and less muscle material per unit area. Long section of the fibres exhibited waviness, possibly a result of fixation on the looser structure of the jellied tissue (Templeman and Andrews, 1956). This jellied condition was thought to be due partly to protein emaciation during sexual maturation, and also to starvation of the fish.
1.5. **Proteinase activity**

1.5.1. **Lysosomal system**

Dean and Barrett, (1976) described the lysosomal system as sedimentable intracellular membrane-limited vesicles of varying size and shape. Surrounded by a single thick membrane, they contained a diverse array of enzymes which generally had a pH optimum in the acid range, although some were active at neutral pH. These enzymes included proteinases, glycosidases, nucleases, phospholipases, phosphatases, and sulphatases. The function of these organelles was the digestion of complex macromolecules. For example, cell component proteins were degraded to small peptides and amino acids, complex lipids to free fatty acids, carbohydrates to monosaccharides, and nucleic acids to nucleosides and phosphates.

Although an overall increase in the number of lysosomes, and the amount of catheptic activity has been reported during periods of rapid protein degradation, the direct involvement of lysosomal enzymes in the initial degradation of myofibrillar proteins is unproven. There are many other proteolytic enzymes implicated in myofibrillar breakdown. Besides the enzymes in the lysosomal system, there are also alkaline proteinases, serine proteinases, and calcium activated neutral proteinases (CANP) (Segal and Doyle, 1978). However, circumstantial evidence which implicated lysosomes as an intracellular site for protein break-
down included a number of physiological and pathological studies in which the overall rates of proteolysis correlated with the activity of lysosomal proteinases, or with the size and number of lysosomes.

1.5.2. Protein turnover

Segal et al. (1978) described the process of protein turnover as the transformation of a protein to a susceptible form, its uptake to a lysosomal digestive system, and its breakdown by proteolysis. In a normal eukaryotic cell, it is accepted that there are two distinct features of the degradative process of protein turnover. First, there is a relationship between the molecular weight of the polypeptide and its half-life, for example large molecular weight proteins breakdown more rapidly than small molecular weight proteins. Second, the protein's isoelectric point and half-life are related, for example acidic proteins are degraded more rapidly than the neutral or basic ones. Proteolysis within the lysosomal system is initiated by endopeptidases which cleave bonds within a peptide, and is completed by exopeptidases which release newly exposed terminal residues (Dean and Barrett, 1976).

Control of protein turnover, and the degradation of both intra and extracellular protein was brought about through changes in the specific enzyme binding of the lysosomal membrane and other solid supports (Dean, 1978). The precise interaction between the membrane and the enzyme
was therefore important to its activity. For example, the conversion of fructose 1,6 diphosphatase from a form with a neutral pH optimum to a form with an alkaline pH optimum occurred most rapidly at pH 6.5 when bound to a membrane, but at pH 4.5 when solubilized.

To understand the role and mechanism of lysosomal enzyme action in catabolic processes, it is necessary to investigate their characteristics. A major endopeptidase found only in the lysosome is cathepsin D. Its points of cleavage were found to be strongly clustered in more hydrophobic parts of the polypeptide chains, preferably adjacent to aromatic side chains. This enzyme was unable to cleave low molecular weight substrates, and required peptides of five or more residues. Maximum activity against the substrate hemoglobin was between the pH 2.8-4.0, although precise optimum pH depended on the source of the enzyme and substrate (Barrett, 1978). This requirement for acidic conditions suggested that in vivo function could only occur within cells, or in very restricted extracellular locations. Incubation of cathepsin D with myofibrillar proteins caused extensive degradation of high molecular weight (> 200K) proteins such as connectin, nebulin, filamin, and myosin heavy chain. Calcium activated neutral proteinases (CANP) could not degrade the high molecular weight proteins or the myosin heavy chain to the same extent, however desmin, a protein which surrounds the myofibril at the Z-line was removed.
The incubation with CANP followed by cathepsin D produced an electrophoretic pattern similar to that of cathepsin D. Bird et al., (1978) suggested that cathepsin D caused substantial conformational changes in the molecule, which enabled CANP to then further degrade the molecule. However, it was not established conclusively that the two enzymes acted in a cooperative fashion to degrade myofibrillar proteins.

Another endopeptidase of importance to the formation of myofibrillar breakdown products was the thiol proteinase cathepsin B. Maximum activity with most substrates was at pH 6.0, although lower pH optimums were found with some substrates because of the denaturing effect of the acidity (Barrett, 1978). The incubation of cathepsin B at pH 5.2 with purified actin and myosin caused the degradation of denatured myosin to progressively smaller polypeptide fragments to an ultimate molecular size of less than 30,000. The degradation of native myosin was less extensive, and released a heterogeneous group of polypeptides with molecular weights from 10,000-50,000. In addition, a compound of molecular weight 150,000 was also produced which was resistant to further proteolysis.

1.5.3. Starvation

Millward et al., (1978) found that the muscle lysosomal systems during starvation were implicated in the catabolic increases in protein breakdown. For example, during
nutrient deprivation, protein catabolism was enhanced 30-150% in skeletal muscle, and morphological studies showed an increase in size and fragility of the lysosomes. The characteristics of intracellular protein degradation during starvation were described by Dice and Walker, (1978) who suggested that the increased protein catabolism was fundamentally different from normal protein degradation, and that the mechanism was not simply an acceleration of normal proteolysis. Several biochemical explanations have been put forward for this enhanced catabolism. The first suggestion was that the cell proteins became more susceptible to proteolytic attack. The normally stable proteins became preferentially proteinase-sensitive, and thus the accepted correlation between molecular size and half-life, and net charge and half-life was reduced. The second suggestion was that the susceptibility of cell proteins remained the same, but the activity of the degradative system was enhanced. Thus the system involved the normal protein degradative machinery, and the normal lysosomal uptake of cellular constituents. All types of proteins would be degraded at enhanced but proportional rates.

Other explanations for the enhanced degradation were hypothesized to include the involvement of non-lysosomal proteinases. Dice and Walker, 1978 observed the presence of non-lysosomal proteinases in muscle, and the increase in their activity during starvation. If these enzymes were to
use small and basic proteins as their substrate, then the normal correlation of protein breakdown with isoelectric point and molecular weight would be reduced.

Dice and Walker (1978) commented that whatever the mechanism occurring, during starvation conditions organisms benefit from the increased production of amino acids for use as a source of energy. Because the crucial regulatory enzymes tend not to be the stable proteins, it would be advantageous to degrade these stable proteins, because they could be sacrificed with the least disruption of the cell’s ability to adapt metabolically.

Other workers have also suggested the possibility of non-lysosomal proteinases in the initial stages of myofibrillar degradation (Asghar and Bhatti, 1987). For example, CANP have been shown to degrade intact myofibrils, remove Z-bands, and degrade troponin complex, tropomyosin, and C-protein. This enzyme was unable to degrade actin and myosin. However, it was thought to play an important role in the disassembly of thick and thin filaments prior to the specific cleavage of myosin light and heavy chains by myofibrillar proteinases. Final degradation was accomplished via the lysosomal pathway.
2. CHEMICAL ANALYSIS

2.1. Introduction

The spawning migration of chum salmon is a period in their life cycle in which the fish must endure severe environmental and physiological changes, and unaccustomed stresses. These include the movement from salt to fresh water, an often lengthy fresh water migration, bacterial and fungal disease, elevated water temperatures, starvation, and sexual maturation (McBride et al., 1965). The biochemical changes which occur in the fish impact upon their commercial quality. By quantitating these changes at different points of the migration, the state of the fish may be accurately described, and its economic value assessed.

The external appearance of the fish approximately follows the physiological state of the animal, and is commonly used to indicate the stage of maturity (Ando and Hatano, 1986a). Idler and Tsuyaki (1958) measured physical changes such as snout length, skin thickness, and weight, and related these to biochemical indices such as changes in fat, protein, and moisture, and visceral weight. Other changes included a near complete wasting of the alimentary tract, and other significant alterations in internal organs (Idler and Bitners, 1960). Both the male and female fish experienced loss in protein and lipid and an increase in moisture however, Idler and Bitners (1960) reported that the females
had 22% more fat loss and 25% more protein loss in the muscle.

Hatano et al. (1983) compared the chemical composition of fall (late) and summer (early) spawning chum salmon in Japan. The protein content decreased from 20 to 16%, the lipid dropped from 8.5 to 1.6%, and the moisture content increased from 72 to 80%.

The object of this study was to first assess the efficiency of a grading system which divided the fish into four grades based on their external appearance. The second object was to measure the changes in crude protein, fat, and moisture in the muscle of the migrating chum salmon.

2.2. Materials and Methods

2.2.1. Sampling

Fish were sampled over a 2 year period. In the first year, all fish were obtained from a local packing plant except for the spawning fish which were collected from the Chilliwack River Hatchery (Chilliwack, B.C.). All the fish were graded, sampled, washed, dressed, packaged in double heavy gauge polyethylene bags, and stored at -30°C.

2.2.2. Grading

The fish were graded by comparing their external appearance to a set of 9 pictures of chum composed by Anon. (1986). A tenth grade was added to include spawning fish. The 10 grades were then reduced to four grades by assigning grades A to D as grade 1, grades E to G as grade 2, grades H
to I as grade 3, and "grade J", any fish more mature than I, as grade 4. A brief description of the criteria used to grade the fish were as follows. Grade 1, silver-brights, were blue-black in colour with a silvery sheen, and no noticeable colour changes or water marks on the fish. Grade 2, intermediates, showed loss of the uniform silver skin colour, and distinct colour changes in the form of perpendicular colour bars which were light red to dark green or black in colour, from the ventral to dorsal surface. Grade 3, dark fish, had intensive, distinct, perpendicular colour bars of dark green or black, and the skin was thick and covered with a layer of slime. Grade 4, spawning fish, had very dark green, black or yellow skin colour, very thick skin and a heavy slime cover. Figure 2.1 illustrates the appearance of the four grades of salmon used in this study.

2.2.3. Proximate analysis

2.2.3.1. Total lipid analysis

The total lipid was determined by slightly modifying the method of Bligh and Dyer (1959). The lipid was extracted from the white muscle by homogenizing 25 g of the sample with 25 mL chloroform and 50 mL methanol for two minutes in a spark free blender. 25 mL of chloroform was then added twice to the mixture and homogenized each time for 30 seconds. The homogenate was filtered through Whatman No. 1 filter paper with a Buchner funnel. Additional lipid was
Figure 2.1. Illustration of the appearance of the four grades of chum salmon used in the study.
recovered by re-homogenizing and re-filtering the residual tissue. The filtrate was then allowed to settle in a graduated cylinder, the volume of the chloroform layer was measured, and the methanol layer was removed. The lipid content was found by evaporating to dryness 5 mL of the chloroform mixture in a tared round bottom flask. The chloroform was removed using a rotoevaporator in a water bath at 45°C, and drying the residue with a stream of nitrogen. The flasks were stored in the dark in a vacuum desiccator with granular silica gel until they were weighed. Total lipid was found using the following equation:

\[ \text{Total lipid} = W \times \frac{V_1}{V_2} \]

Where:  
- \( W \) = weight of lipid in aliquot  
- \( V_1 \) = volume of chloroform layer  
- \( V_2 \) = volume of aliquot

2.2.3.2. Moisture analysis

The moisture content of the fish was found by drying triplicate 5 g samples of the chum muscle in a vacuum oven at 70°C for 6 hours. The samples were weighed and re-dried for 1 hour to ensure constant weight.

2.2.3.3. Protein determination

The crude protein content of the chum muscle was found using a method for micro-Kjeldahl digestions (Anon., 1970). Approximately 9 g of dried chum muscle (0.5-2.5 mg N) was digested in 30 mL Kjeldahl digestion flasks with concentrated sulfuric acid and HgO catalyst. The mixture was
heated for 15 to 30 seconds and 1 mL of 30% hydrogen peroxide was added as an oxidant to speed up the decomposition of the organic material. Once the digestion was completed, the solution was diluted in 25 mL volumetric flasks, and the ammonia content of the samples was determined colourimetrically with a Techicon Autoanalyzer II. The percent protein was determined by multiplying the ppm N by the constant 6.25.

2.2.4. Colour

2.2.4.1. Astaxanthin measurement

The astaxanthin content was measured by resolubilizing the lipid extracted by the Bligh and Dyer method (1959) in reagent grade acetone, and filtering it through Whatman No. 2 filter paper. Absorbance readings were measured in 1 cm path length quartz cuvettes using a Shimadzu UV 160 U.V.-Visible Recording Spectrophotometer (Shimadzu Corp., Kyoto, Japan). The concentration of the carotenoid was calculated assuming an extinction coefficient (1%) of 1900 at 475 nm (Skrede and Storebakken, 1986).

2.2.4.2. Hunter Lab measurements

Colour parameters L, a, and b in the raw chum flesh were calculated from the reflectance spectra recorded by a HunterLab Labscan 6000 Spectrocolourimeter (Hunter Associates Lab. Inc., Virginia) in the region of 400-700 nm. A 1/4 inch aperture was used. Raw muscle segments of the approximate dimensions 2 cm X 1 cm X 0.5 cm were presented to
the instrument on glass cover slips. Triplicate samples of tissue from each fish were measured three times each at different locations, and the values were averaged to obtain L, a, and b readings for each sample.

2.3. Results and Discussion

2.3.1. Astaxanthin content

The grading of the fish was very important because it formed the basis of comparison from which all conclusions were made about differences between grades. For this reason it was important to confirm that the exterior appearance of the fish corresponded to other changes that occurred as the fish matured. In salmonids, carotenoids are of dietary origin and because they are mainly deposited in the muscle, they are responsible for its typical red-pink colour. With sexual maturation however, the carotenoids are mobilized from the muscle and transported to the integument and ovaries via the serum. Astaxanthin is the main carotenoid found in chum, and is the one often measured when following the loss in colour of the fish (Ando et al., 1985).

The mean values obtained from the analysis of the astaxanthin content of the fish are given in Table 2.1. Analysis of variance of the completely randomized design determined that there was a significant difference ($\alpha \leq 0.05$) between at least one of the grades. Multiple comparisons between the grades were made with single degree of freedom.
Table 2.1. Mean values for astaxanthin content in the dorsal muscle of chum salmon (mg/kg).  

<table>
<thead>
<tr>
<th>Grade</th>
<th>n $^2$</th>
<th>Astaxanthin (mg/kg muscle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>6.53 (2.6) $^a$</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>6.07 (3.3) ab</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>2.78 (1.8) bc</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>1.08 (0.7) c</td>
</tr>
</tbody>
</table>

1 Mean (standard deviation).  
2 "n" denotes sample size.  
3 Different letters signify a significant difference.
contrasts (Wilkinson, 1988) and corrected with the improved sequential rejective Bonferroni procedure (Holland and DiPonzio Copenhaver, 1987). Grade 1 was different from grades 3 and 4. Grade 2 was significantly different ($\alpha \leq 0.05$) from grade 4. Grades 3 was significantly different from grade 1 and 4.

2.3.2. Hunter Lab values

Because the astaxanthin was extracted without rigorous procedures to prevent oxidation, the Hunter colourimeter was also used to measure colour changes in the muscle itself. L, a and b values were measured, and are shown in Table 2.2. Analysis of variance of grades indicated that differences existed in the parameters L, a, and b. Multiple comparison by single degree of freedom contrasts and the rejective Bonferroni procedure detected the following differences between the grades. 'L' values were found to increase with increasing grade, and grade 1, 2, and 3 were different from grade 4. The 'a' values decreased with increasing grade, grades 1 and 2 were different from 3 and 4, and grade 3 was different from grade 4. The 'b' values decreased with increasing grade, and grades 1 and 2 were different from grade 3 and 4. The ratios a/L, and a/b proved to be significantly different between each grade except between 1 and 2.

Examination of Tables 2.1 and 2.2 revealed that mean astaxanthin content was roughly proportional to 'a' values.
Table 2.2. Hunter Lab mean values for the dorsal muscle of chum salmon.

<table>
<thead>
<tr>
<th>Grade</th>
<th>n</th>
<th>L value</th>
<th>a value</th>
<th>b value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>40.02 (0.51) a</td>
<td>10.05 (0.07) a</td>
<td>11.45 (0.16) a</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>35.82 (0.18) a</td>
<td>7.80 (0.13) a</td>
<td>10.52 (1.46) a</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>41.59 (0.41) a</td>
<td>4.40 (0.09) b</td>
<td>9.40 (0.08) b</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>47.08 (0.48) b</td>
<td>-1.30 (0.10) c</td>
<td>7.67 (0.13) b</td>
</tr>
</tbody>
</table>

1 "n" denotes sample size.
2 Mean (standard error of mean).
3 Different letters signify a significant (P ≤ 0.05) difference.
A more detailed look at the relationship was performed by correlation analysis which was carried out between the four grades of salmon, the concentration of astaxanthin, the Hunter L, a and b colour values and the ratios a/L and a/b for individual fish. The correlations were tested for their significance, and the results of this test are shown in Table 2.3. The colour values a/L, astaxanthin, a, and a/b were significantly negatively correlated to grade at the 1% level, so that as the grade increased, their values decreased. The parameters b and L were not significant.

The ratio a/L had the largest r value. Although this ratio is not commonly used in the literature as a measure of colour, it was a logical choice in this case. The decrease in 'a' reflected the loss in carotenoid, specifically astaxanthin, and the increase in 'L' value may have reflected the relative increase in connective tissue as the concentration of contractile protein in the muscle was diminished.

The colour of the muscle of salmon is an indicator commonly used in industry to assess its quality and maturity. In this study it was found that the loss in astaxanthin in the dorsal muscle did not clearly differentiate between each grade of chum. The 'a' Hunter value however, was able to distinguish between all of the grades except one and two. Aksnes et al. (1986) visually assessed the loss of colour in maturing Atlantic salmon, Salmo salar. Changes in colour
Table 2.3. Results from the correlation analysis between grade, Hunter Lab values, and astaxanthin content.

<table>
<thead>
<tr>
<th>Independent value</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a/L</td>
<td>-0.796 **</td>
</tr>
<tr>
<td>astaxanthin</td>
<td>-0.746 **</td>
</tr>
<tr>
<td>a</td>
<td>-0.736 **</td>
</tr>
<tr>
<td>a/b</td>
<td>-0.750 **</td>
</tr>
<tr>
<td>b</td>
<td>-0.468 NS</td>
</tr>
<tr>
<td>L</td>
<td>0.566 NS</td>
</tr>
</tbody>
</table>

1 'n', the number of samples, was 24.
2 Significant at the 1% level, NS = not significant at the 5% level.
were found to occur late in the maturation process, thus the colour of the maturing fish was significantly less vivid than the immature fish.

It is interesting to observe the lack of discrimination of the astaxanthin in distinguishing between the grades of the salmon, especially because the 'a' Hunter value was a measure of the redness of the fish, which is primarily due to astaxanthin content. An explanation of this inconsistency is that the isolation technique for astaxanthin resulted in loss of the carotenoid. This could have occurred from physical losses during the fat extraction process, or from chemical oxidation of the carotenoid at any time during the extraction, drying, or resolubilizing process. Because Hunter values are so easily obtained, and do not cause oxidation of the carotenoid, they were found to be a better method of determining the grade of the fish by its colour.

2.3.3. Proximate analysis

The results of the analysis of protein, moisture, and lipid are given in Table 2.4. A one-way analysis of variance was completed to determine whether there was a difference between the fish grades for the three parameters. The results for crude protein revealed significant ($P \leq 0.05$) differences between the grades. Grades 1, 2, and 3 were significantly different ($P \leq 0.05$) from grade 4. Moisture was also significantly different ($P \leq 0.05$) between grades. Grades 1, 2, and 3 were different ($P \leq 0.05$) from grade 4.
Table 2.4. Mean values (% on a wet basis) of protein, moisture and lipid content of dorsal muscle of chum salmon.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Protein</th>
<th>n</th>
<th>Moisture</th>
<th>n</th>
<th>Lipid</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.9 a(0.96)</td>
<td>6</td>
<td>74.17 a(0.29)</td>
<td>9</td>
<td>4.9 a(2.0)</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>19.0 a(0.04)</td>
<td>6</td>
<td>75.02 a(0.09)</td>
<td>7</td>
<td>3.8 ab(2.1)</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>18.3 a(0.13)</td>
<td>11</td>
<td>75.58 a(0.27)</td>
<td>11</td>
<td>4.3 ab(2.3)</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>15.8 b(0.45)</td>
<td>10</td>
<td>79.47 b(0.32)</td>
<td>11</td>
<td>1.0 b(0.92)</td>
<td>4</td>
</tr>
</tbody>
</table>

1. Mean values (standard error of mean).
2. "n" denotes sample size of mean.
3. Different letters signify significant difference.
The analysis of lipid content revealed a significant difference ($P \leq 0.05$) between mean scores for total lipids, however only grades 1 and 4 differed significantly from each other. These results suggest that the fish examined underwent a gradual loss of protein and lipid, however it was not until the fish was at an advanced stage of sexual maturity that the changes became dramatic. Aksnes et al. (1986) found that despite different energy needs, chemical changes in the proximate analysis of male and female farmed Atlantic salmon samples were the same. Bilinski et al. (1984) studied the effect of maturation on coho salmon and found a significant correlation between the moisture content of the raw flesh and the final quality of the canned product. It was concluded that moisture content of raw flesh was of potential value as a quality indicator to determine the acceptability of salmon for canning during progressive increase in sexual maturity.

Samples for the proximate analysis only from the dorsal muscle of the fish. This was done in order to reduce experimental errors by analyzing muscle from different parts of the fish. Karrick and Thurston (1964) took samples from the nape, centre, and tail portions of silver salmon to compare the values from the proximate analysis. The nape had the highest lipid and the lowest protein and moisture content. The tail contained the lowest lipid values, but the amount of protein was similar to the centre section.
3. TEXTURE ANALYSIS

3.1. Introduction

The textural characteristics of fish muscle are a reflection of the properties and concentrations of the structural elements of the tissue and of their complex arrangement in the muscle (Dunajski, 1979). The fibrous proteins constitute up to 70 to 80% of the total protein content in the muscle, and are responsible for the structure and rheological properties of the fish meat. Unlike mammalian tissue, collagenous tissues are present in relatively low concentrations in fish, are easily gelatinized by cooking, and do not contribute to the texture of the cooked product (Love et al., 1974). Hatae et al. (1990) found raw fish with a firm texture had a significantly higher collagen content than soft textured fish. Once the fish was cooked however, no relationship was found between collagen content and tenderness. Iso et al., (1984) stated that any changes in the texture of fish flesh during heat processing were primarily due to the heat denaturation of fibrous protein in the tissue.

Love et al. (1974) investigated differences in texture of steam-cooked cod muscle in relation to a number of parameters such as pH, body length, and physiological condition of the fish. A negative correlation was demonstrated between pH and texture. As the pH of the muscle increased,
the flesh became more tender. Body length was also found to correlate negatively with pH, however this relationship was not true for fish during their spawning cycle. The stress of spawning caused the depletion of protein, fat, and most important, glycogen in the cod muscle. Lower post-mortem glycogen levels resulted in less lactic acid being formed, thus the pH of the muscle did not drop as much as would be expected. Huynh (1989) measured the change in pH in spawning chum salmon and noted an increase in post mortem pH from 6.2 in the silver-brights to pH 6.5 for the dark fish and pH 7.0 for the spawned fish.

Hatae et al. (1990) used light microscopy and scanning electron microscopy to observe the role of muscle fibres in the texture of cooked fish. Cross sections of cooked muscle fibres were compared with firmness as measured by a Texturometer. A firm, dry texture was correlated with thinner, shorter fibres, and a soft, friable texture was correlated with thicker, longer muscle fibres.

Observation of the muscle samples after being tested on a Texturometer revealed that the fibres were not broken or deformed. Instead, the bundles appeared to have slid, or shifted over each other and separated into individual fibres. In firm textured fish, there was considerably less shifting of the bundles than in the soft textured fish. During mastication, discrimination of texture was thought to
be achieved by detecting differences in the sliding movement of muscle fibres.

Hatae et al. (1990) suggested that during the cooking process, sarcoplasmic proteins were squeezed into interstitial spaces, and once coagulated, acted to impede the sliding of muscle fibres over each other. Thus the presence or absence of heat coagulated protein in the inter- and intra-cellular spaces of the muscle fibres determined the firmness and friability of cooked muscle tissue.

The Instron Universal Testing Machine is an imitative type instrument that is able to measure the deformation characteristics of food with a high degree of precision. This machine is able to measure the mechanical failure of foods in many different ways depending on the test cell used. Texture profile analysis (TPA) is a technique that has been used successfully to measure a wide variety of textural attributes. This testing method simulates the mouth's chewing motion by compressing bite size samples with two flat plates, twice in a reciprocating motion. Based on the force-time curve obtained, a number of textural parameters are derived, or calculated to describe the texture of the food.

Aksnes et al. (1986) studied the changes in the sensory properties of maturing farmed Atlantic salmon. Significant changes in odour, flavour, and texture were observed between the immature, and mature fish. The texture of the immature
fish was described as filamentous, with a firm consistency, while the mature fish became watery, tough and "distinctly soft".

The biochemical changes, and physical stress of starvation during the spawning migration of chum salmon adversely affects the texture of the raw and cooked flesh. As the fish becomes more mature, the muscle loses its firm, desirable properties, and becomes soft and watery. This deterioration in texture has a significant effect on consumer acceptability of this important British Columbian commodity.

The object of this work was to develop an instrumental and sensory method to measure the textural changes in the canned fish. Measurements were made with the Instron Universal Testing Machine, and with a semi-trained taste panel.

3.2. Materials and Methods

The fish used for this work were obtained during the summer and fall of 1989 from B.C. Packers Ltd. (Richmond, B.C.), and from the Chilliwack hatchery (Chilliwack, B.C.). The fish were graded, washed, dressed, and frozen at -30°C for several months. After storage, the fish were thawed overnight at 4°C, washed in cold water, the skin and major bones were removed, and the fillets were butchered into 1.5 inch thick steaks. Three piece tinplate cans of the dimensions 307 X 200 (Wells Can Company Limited, Burnaby, B.C.) were packed with 213 g of the salmon, and 1% un-iodized salt
was added. The cans were retorted in pure steam at 248°F for an operator’s process time of 65 minutes in an FMC 500 W Laboratory retort (FMC Corporation, Santa Clara, CA), with water cooling. This process was based on known commercial practices (Collins, 1989). The cans were held for one month at room temperature before being tested further.

Cans from each of the four grades of salmon were examined by a sensory panel and also were tested objectively using an Instron Universal Testing Machine (Model 1122, Instron Corp., Canton, MA.). Three cans from each fish were opened, drained of free liquid, and combined to represent one experimental unit. The canned salmon was then flaked using two forks, into pieces of about 3-5 mm. Half of each sample was used for sensory evaluation and half for instrumental evaluation of texture. The numbers of experimental units and fish were as follows: grade 1 consisted of 11 experimental units, 33 cans, and 8 fish; grade 2, 4 experimental units, 12 cans, and 5 fish; grade 3, 16 experimental units, 48 cans, and 7 fish; and grade 4 was represented by 7 experimental units, 21 cans, and 8 fish.

3.2.1. Instrumental analysis of texture

Instrumental analysis was performed using the Instron Universal Testing Machine by the Texture Profile Analysis (TPA) method of Bourne (1978). Samples were prepared by severing the end of a 50 cc syringe at its zero line, placing a notched nylon disk in the bottom of the cylinder,
adding 10 g of the flaked fish and lightly compressing it with another nylon disk to a height of 2 cm and a diameter of 2.5 cm. This system ensured a strict, cylindrical sample geometry and allowed for correct uniaxial compression. A 5.5 cm flat-faced, teflon coated plate was used to compress the bite-size cylinders of fish to 25% of their original height, twice, in a reciprocating motion at a cross-head speed of 100 mm/min. Five replicate measures of each sample were collected. The Instron was interfaced to a computer to allow recording of force/deformation curves in digitized form at intervals of 0.02 seconds. Hardness, cohesiveness, chewiness, and gumminess were calculated as described by Bourne (1978). Incremental values of the slopes of the force/deformation curves were calculated from the digitized data over intervals of 0.06 seconds. The maximum slope associated with each compression was equated to instrumental firmness.

3.2.2. Sensory analysis of texture

Texture analysis of the salmon was modeled after the General Foods Texture Profile method (Brandt et al., 1963; Szczesniak et al. 1963). Nine panelists were selected based on prior panel experience and interest in the project. Three preliminary training sessions were conducted in order to familiarize the panelists with the range of textures to be expected, and to develop by consensus a list of texture attributes and definitions to describe the canned fish. The
definitions chosen included firmness, the force required to compress the material between the molars; dryness, the sensation of a progressive increase in free fluids in the oral cavity during mastication (i.e. the overall wetness of the sample); fibrousness, the perceived number of fibres evident during mastication; and chewiness, the total effort required to prepare the sample to a state ready for swallowing. The panelists assessed the four textural attributes by marking a vertical line for each sample on a 6 inch linear scale which was anchored on both ends with reference samples and descriptions. This mark represented the panelist’s judgement of the individual attribute for a sample, relative to other samples. Figure 3.1 is an example of the rating sheets used.

Ten half hour sessions were held to assess the texture of the fish and in each session each judge was given four unknown fish samples to analyze. Panelists were asked to compare the texture of the salmon samples to two reference samples, flaked ham and canned tuna. The flaked ham represented a product with a soft, juicy flesh, very few fibres present, and little effort needed to chew. The canned tuna represented a fish product which was firm, dry, fibrous and required considerable chewing effort. Because many cans of each reference product were needed over the ten sessions, an effort was made to purchase the same lot numbers for the tuna and ham. This helped reduce variation within the
EVALUATION OF TEXTURE ATTRIBUTES

Please evaluate the firmness, juiciness, chewiness and fibrousness of these samples by marking a vertical line for each sample on the horizontal lines to indicate your ratings. Label each vertical line with the code number of the sample it represents.

FIRMNESS
soft

firm

DRYNESS
wet

dry

FIBROUSNESS
few

many

CHEWINESS
little effort

much effort

COMMENTS:

Figure 3.1. Example of typical texture score sheet used to analyze texture of the 4 grades of the canned chum samples.
reference points which would affect the panelists judgement of the test samples. The judges were asked not to smoke or drink for one hour prior to the panel. Sensory analysis was performed in individual booths under red lights to mask any visual non-uniformity between the samples. Water, unsalted soda water, and unsalted crackers were available for cleaning the mouth after tasting each sample.

Four attributes of texture were considered by the judges and there was concern that bias would occur from the "halo effect" (Larmond, 1977) in which a general impression would be formed about the texture of the samples. To eliminate this effect, the judges were presented with differently coded samples for each of the four attributes and were not told when the samples were actually the same fish. The samples of fish were presented to the panelists in a random order.

3.3. Results and Discussion

3.3.1. Instrumental

Figure 3.2 shows a typical texture profile curve obtained from the Instron for the canned chum sample. Analysis of the curves produced by the data collection program are given in Table 3.1. This table represents the mean values of the parameters for each of the four grades, and their standard errors. Statistical analysis by a one-way analysis of variance (ANOVA) was performed to test the hypothesis that there was no difference between grades for the
Figure 3.2. Example of a typical Instron texture profile analysis curve for a chum sample.
Table 3.1. Mean\textsuperscript{1} instrumental TPA parameters of the four grades of chum salmon.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 1</td>
<td>202 (25.0)</td>
<td>152 (32.2)</td>
<td>170 (20.0)</td>
<td>139 (29.3)</td>
</tr>
<tr>
<td>Area 2</td>
<td>78.5 (9.58)</td>
<td>56.6 (11.5)</td>
<td>70.9 (15.7)</td>
<td>48.7 (11.0)</td>
</tr>
<tr>
<td>Hard 1</td>
<td>8.33 (1.02)</td>
<td>5.23 (1.20)</td>
<td>6.74 (0.85)</td>
<td>5.09 (1.13)</td>
</tr>
<tr>
<td>Hard 2</td>
<td>5.55 (0.72)</td>
<td>3.48 (0.85)</td>
<td>4.54 (0.59)</td>
<td>3.49 (0.57)</td>
</tr>
<tr>
<td>Cohes.</td>
<td>1.06 (0.04)</td>
<td>1.08 (0.03)</td>
<td>1.04 (0.02)</td>
<td>1.16 (0.03)\textsuperscript{2}</td>
</tr>
<tr>
<td>Spring.</td>
<td>1.83 (0.13)</td>
<td>1.99 (0.07)</td>
<td>2.00 (0.11)</td>
<td>1.64 (0.18)</td>
</tr>
<tr>
<td>Slope 1</td>
<td>36.3 (5.89)</td>
<td>19.2 (4.82)</td>
<td>28.8 (4.41)</td>
<td>20.4 (4.74)</td>
</tr>
<tr>
<td>Slope 2</td>
<td>38.2 (6.03)</td>
<td>20.6 (5.79)</td>
<td>30.2 (4.92)</td>
<td>20.5 (6.13)</td>
</tr>
<tr>
<td>Gummi</td>
<td>21.5 (2.74)</td>
<td>14.1 (3.39)</td>
<td>17.9 (2.38)</td>
<td>14.6 (3.13)</td>
</tr>
<tr>
<td>Chewi</td>
<td>96.4 (14.1)</td>
<td>70.9 (17.6)</td>
<td>84.1 (12.3)</td>
<td>60.6 (16.0)</td>
</tr>
<tr>
<td>n\textsuperscript{3}</td>
<td>12</td>
<td>06</td>
<td>14</td>
<td>05</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Standard errors are presented in brackets.
\textsuperscript{2}Two outliers were removed from the data set.
\textsuperscript{3}"n" = number of experimental units.
texture parameters. Upon testing assumptions of ANOVA, a systematic increase in standard errors with increasing mean was discovered. The lack of homogeneity of variances was a reflection of the larger biological variation inherent in the texture of less mature fish, than in spawning fish. A square root transformation was performed on the data in order to improve the homogeneity of the variances. Two fish were deleted from the data set as outliers because their standard deviations between replicate measures were much higher than the other fish. The results of the analysis of the transformed data are shown in Table 3.2. Slope 2 and cohesiveness were the only parameters which were significantly different between at least one of the grades ($\alpha \leq 0.05$), but hardness 1 and 2, and slope 1 were significant at $\alpha \leq 0.10$. Area 1 and 2, springiness, gumminess, and chewiness were not different between the four chum grades. Multiple comparisons between grades were performed with single degree of freedom contrasts, and corrected with the improved sequential rejective Bonferroni procedure. For the parameter cohesiveness, grade 4 was significantly different between grades 1, 2, and 3 ($\alpha \leq 0.05$). Grades 1 and 4 were significantly different for slope 2 ($\alpha \leq 0.05$), and for hardness 1 and 2, and slope 1 ($\alpha \leq 0.10$). The high degree of biological scatter in the instrumental measurements which lead to high standard deviations of the mean values contributed to the lack of significance for TPA variables.
Table 3.2  Probability values produced from one-way ANOVAs on the square root transformed Instron TPA data.

<table>
<thead>
<tr>
<th>TPA Parameter</th>
<th>Probability Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 1</td>
<td>0.28 (NS)</td>
</tr>
<tr>
<td>Area 2</td>
<td>0.10 (NS)</td>
</tr>
<tr>
<td>Hardness 1</td>
<td>0.06 (NS)</td>
</tr>
<tr>
<td>Hardness 2</td>
<td>0.08 (NS)</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.001 (NS)</td>
</tr>
<tr>
<td>Springiness</td>
<td>0.26 (NS)</td>
</tr>
<tr>
<td>Slope 1</td>
<td>0.06 (NS)</td>
</tr>
<tr>
<td>Slope 2</td>
<td>0.04 (NS)</td>
</tr>
<tr>
<td>Gumminess</td>
<td>0.15 (NS)</td>
</tr>
<tr>
<td>Chewiness</td>
<td>0.24 (NS)</td>
</tr>
</tbody>
</table>

1 Two outliers removed from the data set
2 Significant at the 10% level
3 Significant at the 5% level
Observation of general trends showed that grade 1 required the most force, or energy to compress the sample, both on the first, and second compression, while grade 4 required the least force. An exception to this observation was the TPA parameter cohesiveness, which was found to increase significantly from grade one to grade four. This finding can be explained by considering the physiological changes in the salmon during spawning, and the effects of heat processing. As the fish became more mature, there was a loss in myofibrillar proteins, a relative increase in connective tissue proteins, and an increase in the moisture content of the muscle. Aksnes et al. (1986) found an increase in the hydroxyproline content of maturing farmed Atlantic salmon. It was concluded that the metabolizing of protein from the muscle took place at the expense of non-collagenous, and cellular proteins. Upon retorting the fish, the connective tissue was gelatinized, and with the increased moisture content, it contributed to formation of a sticky, more cohesive final product.

Reproducibility of objective texture measurements was affected by the preparation of experimental units. Johnston, (1981) found that the objective evaluation of fish texture was difficult because of variations which existed in the flesh composition and structure along the length of the fish. Aksnes et al. (1986) reduced variation by using only the middle one third of the fillets for organoleptic
analysis. In the present study, three cans were sampled randomly from different locations along a fish, and were combined to obtain one experimental unit. This technique greatly reduced the possibility of introducing error based on differences in texture between the various locations of the fish that were canned.

In order to reduce error due to variation in muscle texture within the cans due to packing of the can, or location of connective tissue in the muscle, a flaked product was used for the instrumental and sensory evaluations. The decision to use flaked samples was based on two observations. First, canned salmon is not commonly served as a whole chunk, therefore flaking would more closely simulate the state in which canned salmon is commonly consumed. Second, in a study by Borderias et al. (1983), instrumental and sensory comparisons of cooked fillets and cooked mince of 5 fish species were observed. No correlation between sensory and instrumental data was found for the cooked fillets, but significant correlation was found for the minced products. In addition, the coefficient of variation in the raw and cooked fillets was higher than in the minced fish. Borderias et al. (1983) found that the application of a compression test onto the fillets caused the myomere layers to slide away from the force, thus making it impossible to reproduce the analysis. Szczesniak (1987) stated that mincing the fillets effectively eliminated the heterogeneity of the
test material which was the cause of the high degree of scatter in the instrumental test. A disadvantage of the mincing however was that some textural parameters could then not be detected.

Dunajski (1979) stated that a difficulty encountered in measuring the rheological properties of cooked fish, was that common instrumental methods were often ineffective. Because cooking of most fish resulted in total gelatinization of the collagen, the structural integrity of the myocommata was disrupted, and the muscle fibres became the only elements of resistance. It was recommended that instrumental tests should be designed to measure the resistance of the fibres to mechanical stress. However because of the organization of the fish's myomeres, positioning the sample to orient the fibres the same direction each time became a nearly impossible task.

The technique of forming the samples in a 50 cc syringe, was found to be very effective in preparing identical cylinders of fish. However despite having 5 replicate samples for each experimental unit, the standard deviations within experimental units were still quite high. In addition to inherent differences within and between fish of the same grade, incomplete draining of the can contents, or drying of the fish could also have caused variations in the measurement of texture. Because samples for both the instrumental and sensory analysis came from the same three
cans, instrumental measurements of texture were only possible after sensory analysis was completed. This delay resulted in some drying of the fish muscle, and may have contributed to the high standard deviations.

3.3.2. Sensory analysis of texture

Table 3.3 displays the mean results and standard deviations from the sensory panel's evaluation of the textural differences between the four grades of chum salmon. An analysis of variance was performed to test the hypothesis that there was no difference between the four grades for each attribute. This hypothesis was rejected however, because each attribute had at least one grade different from the other three.

Observation of the scoring system used by the individual judges lead to the conclusion that despite their training, different judges tended to use different parts of the linear scale to mark the samples. For example, judge "X" tended to use only the first half of the scale, judge "Y" grouped all the evaluations in the middle of the scale, and judge "Z" marked the results further to the end of the scale. The effect of this non-uniformity was that when calculating an average for each textural attribute, the extreme values cancelled each other out, and there appeared to be less difference between the grades than was expected. To eliminate the non-uniform use of the scale which resulted in non-homogeneous variance between judges, a Z-transformation
Table 3.3. Mean, untransformed results from the sensory panel’s evaluation of the textural differences between the four grades of chum salmon.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Firmness</th>
<th>Sensory Attributes</th>
<th>Sensory Attributes</th>
<th>Sensory Attributes</th>
<th>Sensory Attributes</th>
<th>n²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dryness</td>
<td>Fibrousness</td>
<td>Chewiness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.69 (2.6)¹</td>
<td>8.34 (2.3)</td>
<td>8.60 (2.3)</td>
<td>7.41 (2.8)</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.81 (3.5)</td>
<td>7.16 (3.2)</td>
<td>7.87 (3.0)</td>
<td>7.09 (3.0)</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6.54 (2.8)</td>
<td>5.22 (2.7)</td>
<td>7.00 (2.8)</td>
<td>7.42 (2.7)</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3.15 (2.3)</td>
<td>3.76 (2.5)</td>
<td>2.82 (1.6)</td>
<td>3.15 (2.2)</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

¹ Standard deviation are shown in brackets.
² n = total number of observations for a particular grade.
was performed on each of the textural attributes for each judge individually so that the mean of each of their scores was equal to zero and the variance was equal to one. This was justified because each judge tested the same samples. Figure 3.3 is a graphical representation of the standardized values for each grade of the 4 attributes. The bar graph showed a clear trend towards a decrease in firmness, dryness, chewiness, and fibrousness. Analysis of variance revealed a significant difference between at least one of the 4 grades. The statistical program Systat (Wilkinson, 1988) was used to perform single degree of freedom contrasts with the data. Multiple comparisons of the data were tested with the sequentially rejective Bonferroni procedure to control the overall comparison error rate for multiple hypotheses (Holland and DiPonzio Copenhaver, 1987). The results of this test are given in Table 3.4. The judges were able to distinguish between each of the 4 grades for the attribute dryness. Grade 1 was significantly dryer than grade 2, grade 2 was dryer than grade 3, and grade 4 was the least dry. For the attribute fibrousness, grades 1 and 2 could not be distinguished, and grade 3 and 4 were significantly less fibrous than any other grade. For the attribute firmness, no difference was found between grades 1 versus 2 which were the most firm. Grades 3 and 4 could also not be distinguished, and were less firm than grades 1 and 2. For the attribute chewiness, grades 1, 2, and 3 were not
Figure 3.3. Standardized sensory scores for the four grades of chum salmon for the attributes firmness, chewiness, dryness and fibrousness.
Table 3.4. Results from analysis of variance of the sensory scores to determine differences between grades, and sequential rejective Bonferroni procedure for the multiple comparison of the data.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Difference within the grades</th>
<th>1 vs 2</th>
<th>1 vs 3</th>
<th>1 vs 4</th>
<th>2 vs 3</th>
<th>2 vs 4</th>
<th>3 vs 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmness</td>
<td></td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Dryness</td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Fibrousness</td>
<td></td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Chewiness</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

* = significantly different at $\alpha = 0.05$.  
NS = not significantly different at $\alpha = 0.05$.  

-77-
significantly different, but they were chewier than grade 4.

3.3.3. Sensory and instrumental correlation

Correlation analysis was performed between the instrumental and sensory values using a Pearson correlation by Systat (Wilkinson, 1988). There was a significant correlation between the parameter cohesiveness and the attributes firmness, fibrousness, chewiness, and juiciness ($\alpha = 0.05$). Table 3.5 is part of the matrix created from comparing the instrumental and sensory attributes with a Pearson correlation matrix.

The use of sensory panels to evaluate the acceptability or quality of a product has the disadvantage that there may be a loss of accuracy due to fatigue if a large number of samples are examined in a short time, and unavoidable panel changes will mean the loss of members of the team. Lund (1982) commented that often sensory responses were non-linearly related to the physical and chemical changes that they reflect. Although many studies have used sensory analysis, few have generated data which could be quantified into a model useful to predict the effects of processing. The problems associated with sensory panels have prompted much work on developing effective instrumental techniques.

Levitt, (1974) described three steps used to determine whether an instrumental test could be used in place of sensory evaluations. First the sensory variables were selected to describe the organoleptic characteristic. Instrumental
Table 3.5. Correlation values (r) extracted from a Pearson correlation matrix of the comparison between the instrumental parameters, and the sensory attributes.

<table>
<thead>
<tr>
<th>Instrumental Parameters</th>
<th>Sensory Attributes(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Firmness</td>
</tr>
<tr>
<td>Area 1</td>
<td>0.347</td>
</tr>
<tr>
<td>Area 2</td>
<td>0.432</td>
</tr>
<tr>
<td>Hard 1</td>
<td>0.387</td>
</tr>
<tr>
<td>Hard 2</td>
<td>0.376</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.788*</td>
</tr>
<tr>
<td>Springiness</td>
<td>0.411</td>
</tr>
<tr>
<td>Slope 1</td>
<td>0.388</td>
</tr>
<tr>
<td>Slope 2</td>
<td>0.410</td>
</tr>
<tr>
<td>Gumminess</td>
<td>0.314</td>
</tr>
<tr>
<td>Chewiness</td>
<td>0.344</td>
</tr>
</tbody>
</table>

\(^1\) Sample size 'n' = 19.
* Significant at the 5% level.
variables were then selected so that they could describe the information contained in the sensory variables. The third step was to judge whether instrumental variables adequately described the underlying dimensions of the organoleptic characteristic. If it could be shown that variables measured by instrumental methods fulfilled the same function as the sensory panels, it would be possible to obtain a description of organoleptic characteristic of the product more quickly, accurately and economically.

Aguilera and Stanley, (1990) considered multivariate analysis to be the best approach for analysis of sensory and instrumental measurements of texture. The techniques of cluster analysis, principle component analysis, and stepwise discriminant analysis allowed for efficient simplification and interpretation of a large number of variables simultaneously. Other methods such as simple linear regression were limited because they only indicated how one set of numbers varied with another, and assumed parametric data i.e. normal distribution. Multiple regression and correlation was limited because results were affected by correlation of variables within each set (Lund, 1982).

The magnitude of an applied load and the rate that the load was applied influenced instrumental evaluation of textural properties. Shama and Sherman (1973) found that while chewing often occurred in a sinuosoidal pattern, the Instron’s force compression test was a linear application of
stress to the sample. Thus, it was speculated that correlation between instrumental and sensory data was often misleading. However, by evaluating textural properties during the down stroke of the chewing cycle (an approximately linear motion), the Instron was able to measure hardness, brittleness, and crispness.

Hatae et al. (1984) studied the texture of 5 species of fish by determining drip, residual weight, firmness, cohesiveness, penetration, fibre volume, fibre diameter, and fibre length. Statistical analysis was performed using analysis of variance and discriminant analysis. It was concluded that textural differences of various fish could be detected by discriminant analysis but not by evaluation of individual items of measurement. The correlations between sensory and instrumental texture measurements were usually of an associative, indirect nature, and reflected underlying effects which might have operated in the same or different directions of both sides of the correlation. Therefore correlation and prediction could not imply equality or an understanding of the mechanism behind the association.

Borderias et al., (1983) studied different instrumental methods to find which correlated best with sensory analysis. Raw and cooked skinned fillets and minced fish were tested by sensory TPA methods. These results were then compared to the data generated by shear, compression, and juiciness tests performed using an Instron Texturometer. The fish
minces correlated better with the sensory and instrumental techniques than did the fish fillets. Effective instrumental testing methods included the Kramer shear cell, the puncture test, and evaluation of juiciness.

Karl and Schreiber (1985) used canned blue whiting and mackerel to determine whether sensory evaluation of the texture could be correlated with the data obtained from an Instron with a Kramer shear cell, and a Brabender Farinograph. The value at the beginning of the Brabender curve correlated with the peak height of the Instron curve \( (r = 0.83) \). The Instron was more successful than the Brabender in predicting the sensory panel's "response to first bite", and "structure after prolonged chewing".

Dulce et al. (1987) compared the texture of de-boned, flaked, canned fish evaluated with a semi-trained sensory panel and with an Ottawa texturometer extrusion cell attached to an Instron Universal testing machine. Force measurements were highly correlated with both tenderness \( (r = 0.91) \) and juiciness \( (r = 0.87) \). It was concluded that the Ottawa texturometer was a good predictor of texture as perceived by a taste panel.

In this study, sensory analysis was clearly superior to instrumental TPA in discriminating power. None of the 10 TPA parameters were consistently different between grades, while sensory dryness was significantly different between all four grades. Correlation of the instrumental parameters
and sensory attributes showed that cohesiveness correlated with all four sensory parameters. However there were no other significant correlations.
4. PROTEINASE ACTIVITY

4.1. Introduction

The decrease in eating quality of chum salmon during the spawning migration is now well established. There is however, considerable interest in understanding the reasons for this deterioration. Konagaya (1983) found no evidence of the presence of sporozoan or other parasites in the fish, and concluded that biochemical changes were responsible for its poor texture. Hatano et al. (1983), and Konagaya (1983) used proximate analysis to characterize chum in Japanese waters at different maturity stages and showed a decrease in the protein content of the muscle from 20% to 16%, a decrease in lipid from 8.5% to 1.6%, and an increase in moisture from 72% to 80% for early and late-run chum respectively.

Mommsen et al. (1980) measured the activity of 13 metabolic enzymes, and the concentration of soluble and insoluble protein of sockeye during spawning migration. By the end of spawning migration, the activity of eleven of the thirteen enzymes was reduced to 30% of their original level. The soluble and insoluble protein was decreased by 70%, indicating its use in development of the gonads, and as an energy source. Two metabolic enzymes showed no change in level or activity, alanine aminotransferase (GTP), and malic enzyme. It was thought that these enzymes were selectively
maintained in order to sustain essential biochemical or physiological function throughout migration. Proteinase activity in muscle buffered to pH 4.05 increased linearly with the distance the salmon migrated so that end migration levels were seven times the pre-migratory levels. No change, or slight decreases in activity were detected at pH 6.8 and 8.5. Based on inhibition studies with pepstatin, these workers concluded that the proteinase activity at pH 4.0 was due to the presence of cathepsin D. The activity at the low pH indicated the involvement of muscle lysosomes. Two lysosomal enzymes, acidic carboxypeptidase A, and β-N-acetylglucosaminidase were also assayed. No change in activity during migration was detected for these two enzymes, thus it was concluded that the total number of lysosomes did not increase. Partial purification of cathepsin D lead to the conclusion that the observed increase in activity was due to an increase in the number of enzyme molecules, and not a result of enzyme activation.

Ando and Hatano (1986b) used the amino acid N-methylhistidine (MeHis) as an index of maturity of myofibrillar degradation in vivo. This amino acid is contained only in actin and myosin, is not utilized for either protein synthesis or energy, and is excreted quantitatively into the urine. It was suggested that the level of this amino acid in the muscle extracts of maturing chum salmon
reflected the degree of myofibrillar protein degradation. This approach clearly showed that the degree of myofibrillar degradation increased during spawning migration. It was also found that the level of MeHis was higher for females than for males.

Ando et al. (1986) found that serum sex steroids such as androgens and estrogens were closely related to protein degradation and to high levels of proteinase activity of chum salmon muscle during spawning migration. Enhanced protein catabolism was initiated by hormone levels which changed in response to nutrient depletion and increased activity levels, in order to provide the energy necessary for migration.

All the available evidence, such as the decrease in total protein, the increase in MeHis, and the apparent loss of myofilaments, suggested that myofibrillar proteins, including actin and myosin, may be degraded by muscle proteinases activated during spawning migration. In order to understand the changes in the chum, it was necessary to consider possible mechanisms of the physical degradation of the fish's body.

The object of this study was to determine the changes in the activity level of muscle proteinases buffered to pH 3.5, 6.2 and 7.0 during spawning migration. Because lysosomal enzymes have an important role in the degradation of the myofibrillar component of the total body’s protein,
the activity of acid phosphatase, a lysosomal marker, was also assayed.

4.2. Materials and Methods

The decision to collect chum samples in the year 1990 was made when it was discovered that some of the work planned was not possible on the samples collected in 1989 which were post-rigor fish which had undergone 3-5 days of post-mortem changes. In the second year, emphasis was placed on obtaining the fish in a pre-rigor state.

Silver-bright fish were collected by travelling to the N.E. side of Vancouver Island (Port McNeil), and taking the fish samples in Johnstone Strait aboard a Department of Fisheries and Oceans (DFO) test fishery vessel. Intermediate and dark fish were obtained on another DFO test fishery vessel, this time on the Fraser River. Spawning fish were obtained from the Chilliwack River Hatchery. Fish dorsal muscle was collected, sealed in ziplock freezer bags and stored at -30°C.

4.2.1. Measurement of proteinase activity

The proteinase activity of the muscle was measured by the method of Konagaya (1983), and is described in Figure 4.1. The dorsal muscle of the chum was homogenized in three parts distilled water/ice mixture at 0°C for 30 seconds in an Ultra-Turrax homogenizer. Two grams of the homogenate was added to each of 2 sets of 3 triplicate test tubes. The tubes were then diluted with 2 mL of 0.2 M citrate-phosphate
Muscle + ice, 1:3 w/w

homogenize at 0°C, 30s

2 g homogenate

2 mL citrate-phosphate buffer, pH 3.5, 6.2, or 7.0.
(6 aliquoats / pH)

Test

incubate 1 h at 37°C

Add cold TCA solution to 15%

Hold at 4°C, 1 h.

Clarify by filtration

Read absorbance at 280 nm

Blank

Add cold TCA stock to 15%

Hold at 4°C, 1 h.

Clarify by filtration

Read absorbance at 280 nm

Figure 4.1. Flow diagram of the method used to measure the proteinase activity in the dorsal muscle of four grades of chum muscle.
buffer adjusted to either pH 3.5, 6.2, or 7.0. Two sets of three tubes were diluted with pH 3.5 buffer, 2 sets of three tubes with pH 6.2 buffer, and 2 sets of 3 tubes with pH 7.0. One set of the tubes at each pH were incubated at 37°C in a water-bath shaker at approximately 100 RPM. After 1 hour, the incubated (test) samples were removed from the water bath and 8 mL of cold 15% trichloroacetic acid (TCA) was added to the incubated homogenate. The mixture was held for 1 hour in the cold before being filtered through Whatman #2 filter paper. The absorbance of the filtrate was measured at 280 nm on a Shimadzu U.V. 160 U.V - Visible Recording Spectrophotometer (Shimadzu Corp. Kyoto, Japan). The second set of tubes (blanks) at the three pH values were not incubated. Instead, 8 mL of cold 15% TCA was added immediately. The tubes were vortex mixed, held for one hour in an ice bath, and then filtered through Whatman #2 filter paper. The absorbance of the filtrate was measured at 280 nm. The mean values from the triplicate samples of the 'test' were subtracted from the mean 'blank' values to obtain a delta absorbance value for each of the four grades at the three pH values.

4.2.2. Measurement of acid phosphatase activity

The acid phosphatase activity of the muscle was measured using the method of Beeken and Roessner (1972). The dorsal muscle of the chum was homogenized (0.5 g tissue in 18 g distilled water/ice) for 30 seconds in an Ultra-Turrax
homogenizer, and then filtered through Whatman #2 filter paper. This enzyme solution (0.2 mL) was added to 1 mL of the substrate solution, p-nitrophenyl phosphate (PNPP). The substrate solution was prepared by mixing equal volumes of reagent 1, 0.4% disodium p-nitrophenyl phosphate in 0.001N HCl, and reagent 2, 0.1M Na citrate buffer pH 4.8. The substrate-enzyme mixture was incubated at 37°C for 30 minutes in a water-bath shaker at 100 RPM. Assays were performed in triplicate. The samples were then removed from the bath, 3 mL of 0.1 N NaOH was added, and the tubes were read at 405 nm. Standard solutions were prepared with p-nitrophenol (PNP) of concentrations 0.2, 0.4, 0.6, 0.8, and 1.0 mM. A standard curve was prepared by treating 0.15 mL of each solution with 3 mL of NaOH, and reading absorbance at 405 nm.

4.3. Results and Discussion

4.3.1. Proteinase activity

The activity of muscle proteinases was measured at three pH values. A pH of 3.5 represented the acidic conditions present in the lysosomal system of muscle, pH 6.2 was the typical muscle pH measured after resolution of rigor, and pH 7.0 was used to detect the activity of neutral, or slightly alkaline proteinases. The results of the experiment are given in Table 4.1. The bar graph of activity at pH 3.5 versus index of maturity is shown in the Appendix in Table 8.1. There was a significant correlation ($\alpha \leq 0.05$)
Table 4.1. Proteinase activity at pH 3.5, 6.2 and 7.0 during the different stages of spawning migration.

<table>
<thead>
<tr>
<th>Grade</th>
<th>pH 3.5</th>
<th>Proteinase Activity 1</th>
<th>pH 6.2</th>
<th>pH 7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0226 (0.018)</td>
<td>-0.0163 (0.017)</td>
<td>0.0219 (0.014)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0369 (0.024)</td>
<td>-0.0041 (0.006)</td>
<td>0.0344 (0.006)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-0.0129 (0.010)</td>
<td>-0.0310 (0.034)</td>
<td>0.0256 (0.005)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.1453 (0.025)</td>
<td>0.0799 (0.005)</td>
<td>0.0468 (0.080)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.0792 (0.049)</td>
<td>0.0347 (0.049)</td>
<td>0.0180 (0.011)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.2365 (0.028)</td>
<td>-0.0856 (0.002)</td>
<td>0.0976 (0.001)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.1295 (0.013)</td>
<td>-0.0015 (0.012)</td>
<td>0.0719 (0.013)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.1060 (0.008)</td>
<td>0.0144 (0.024)</td>
<td>0.0719 (0.013)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.2811 (0.008)</td>
<td>0.0714 (0.014)</td>
<td>0.0976 (0.001)</td>
<td></td>
</tr>
</tbody>
</table>

1 Means of triplicate determinations, standard deviations are presented in brackets.
between increased activity and increased maturity, with an r value of 0.701, and a sample size of 9. The bar graph of the proteinase activity at pH 6.2 versus index of maturity is shown in the Appendix, Table 8.2, however no significant correlation was found between these two factors. The proteinase activity at pH 7.0 is shown in the Appendix, Table 8.3, however no correlation between activity and maturity was found at this pH.

The findings of high proteinase activity in the acid range are consistent with the study by Konagaya (1982, 1983). This worker compared the optimum pH activity of chum dorsal muscle at three migratory stages. The autolytic activity was highest at pH 3.5 at each of the 3 stages. There was a four fold increase in activity from stage 1 to stage 2, and a six fold increase from stage 1 to stage 3. Because little proteinase activity was detected in the slightly alkaline region, even in the presence of 1 mM calcium, and 2 mM cysteine, calcium activated neutral proteinases (CANP) were not considered to contribute to the changes in the muscle.

In mammalian systems, skeletal muscle is very low in lysosomal content. However, Reddi et al. (1972) reported that the catheptic activity of fish muscle was ten times greater than that of mammalian tissue. To study the intracellular localization of catheptic activity in the skeletal muscle of flounder, Reddi et al. (1972) used
centrifugation to separate the muscle protein into five fractions. These fractions included (P1), unbroken cells and nuclei, (P2), mitochondria, (P3), lysosomes, (P4), microsomes, and (S4), final supernatant. The highest catheptic activity was found in the P3, lysosomal fraction, although the P2, and P4 fractions also had some activity. At pH 4.0 the P3 activity was optimum, at pH 6.5 there was less activity, and at pH 7.5, no activity could be detected.

4.3.2. Acid phosphatase activity

The enhanced activity of proteinases at pH 3.5 might be attributed to lysosomal cathepsins found in and around the muscle cells. Acid phosphatase has been commonly recognized as a lysosomal marker such that increasing levels of the enzyme indicated increasing concentration of lysosomes (Chayen et al. 1973). Therefore, its activity was monitored at the different maturity levels.

In this assay, incubation of the enzyme with PNPP resulted in formation of the breakdown products PNP and inorganic phosphate. Addition of NaOH converted the PNP into the yellow phenoxide ion, which was measured by colourimetric analysis. A standard curve of PNP was constructed to convert absorbance at 405 nm to phosphate concentration, and is shown in Figure 4.2. The activity of acid phosphatase was reported in the Appendix, Table 4.2 and as a graph in Appendix, Table 8.4.
Figure 4.2 Standard curve of the absorbance at 405 nm versus the concentration of p-nitrophenol.
Table 4.2. Acid phosphatase activity (mM phosphate) in dorsal muscle of chum salmon during spawning migration.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Phosphate Release (mM)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2541 (0.000)(^2)</td>
</tr>
<tr>
<td>1</td>
<td>0.2770 (0.011)</td>
</tr>
<tr>
<td>1</td>
<td>0.2826 (0.005)</td>
</tr>
<tr>
<td>2</td>
<td>0.2903 (0.003)</td>
</tr>
<tr>
<td>2</td>
<td>0.2943 (0.007)</td>
</tr>
<tr>
<td>3</td>
<td>0.3116 (0.010)</td>
</tr>
<tr>
<td>3</td>
<td>0.3071 (0.016)</td>
</tr>
<tr>
<td>4</td>
<td>0.3401 (0.022)</td>
</tr>
<tr>
<td>4</td>
<td>0.4060 (0.001)</td>
</tr>
<tr>
<td>4</td>
<td>0.4611 (0.003)</td>
</tr>
</tbody>
</table>

\(^1\) Means of triplicate determinations.  
\(^2\) Standard deviations are shown in brackets.
Correlation analysis showed there was a significant correlation ($\alpha \leq 0.05$) between the level of maturity and the increased activity of acid phosphatase, with an $r$ value of 0.829. The activity of the enzyme increased significantly between grades 1 and 4. The increased levels of proteinase activity at pH 3.5, together with the observed increase in acid phosphatase activity with increasing maturity, suggested that lysosomal cathepsins may be responsible for the softening of the chum salmon muscle tissue during spawning migration.

The effect of cold storage on the proteinase activity in the muscle is an important consideration. Warrier et al. (1988) studied the change in activity of aminopeptidase and cathepsin B in the muscle extract of 7 tropical fish species during storage at 0°C and frozen storage. Significant differences between the specific activities of the enzymes were found in the different species of fish. There was a general decrease in the activity of the aminopeptidase and cathepsin B during 4 months of cold storage, however the rate of decrease widely varied with each fish species.

It was expected that the activity of chum’s proteolytic enzymes would also decrease with time. However because of the relatively short time in frozen storage, less than 2 months, and the low storage temperature of $-30^\circ$C, the loss in activity was not expected to invalidate the results of the experiment.
There has been much disagreement among workers regarding the natural occurrence of lysosomes in muscle cells. Steiner et al., (1984) suggested that lysosomal enzymes in muscle tissue might be located either in macrophages of connective tissues, or within muscle cells. In order to obtain information about the occurrence and location of lysosomes in the cells of normal striated muscle, Steiner et al. (1984) used histochemical techniques for light and electron microscopy to detect acid phosphatase in the muscle of coho salmon. Light micrographs of 10 micron cryostat sections revealed a non-uniform distribution of lysosomes in the muscle. All of the acid phosphatase activity appeared to be limited to the connective tissue area of the endomysium and perimysium. No activity was found centrally located among the myofibrils. In order to determine whether the lysosomes were within the muscle cells at the peripheral cell membrane or in the connective tissue compartments, Epon embedded tissues were examined by electron microscopy. Three populations of lysosomes could be distinguished on the basis of their location within the tissue. The lysosomes were found in the connective tissue cells (identified as macrophages), at the periphery of, but within, muscle cells, and within the muscle cells distributed among the myofibrils. It was concluded that lysosomes occurred in salmon muscle tissue, but were not uniformly distributed. The conclusion that there was discrete localization of lysosomes in coho muscle
provided further evidence that lysosomal enzymes may be important in the deterioration of chum muscle during spawning. It would be interesting to determine the numbers and size of lysosomes in intact chum muscle during different stages of spawning migration. This would provide more evidence of the involvement of lysosomal proteinases in muscle degradation during spawning migration.
5. **STRUCTURAL CHANGES IN MUSCLE**

5.1. **Introduction**

Electron microscopy is a technology by which the structure of both normal and pathological striated muscle disorders can be studied. As a result, there is a well established body of knowledge available regarding the biological structure of muscle tissue and a wide range of useful techniques which have been established to study changes which occur in muscle at the ultrastructural level. In 1976, Stanley and Swatland commented that there was a certain reluctance on the part of food scientists investigating meat texture to borrow techniques from sources such as histochemistry and electron microscopy. Instead, much research time was directed to the complex study of the mechanical, or rheological properties of muscle.

The desire to improve the texture of the flesh of chum salmon has been addressed by empirical methods. Different processing techniques such as removing skin and bone, brine treatments, and heat processing in thin profile retortable pouches have been tested (Collins, 1989). Light, and electron microscopy may be employed to observe changes in muscle during spawning migration at the ultrastructural level. There are many factors which combine to affect the ultrastructural appearance of the fish’s skeletal muscle. The physical stresses of migration, together with the
process of sexual maturation and starvation contribute to myofibrillar degradation.

The object of this experiment was to observe the physical appearance of muscle from the spawning chum salmon at the ultrastructural level and compare this parameter to that of an ocean-run fish.

5.2. Materials and Methods

5.2.1. Light microscopy

Muscle from the four grades of chum salmon were sampled in a pre-rigor state. Tissue was excised from the dorsal muscle, trimmed into 5 mm blocks and allowed to rest for several minutes in a dry petri plate. The muscle was then fixed for one week in a 10% formalin solution. After fixation, the tissue was dehydrated with an ascending serial dilution of ethanol and xylene, and embedded in paraffin wax. Six micron sections were cut, the tissue was dewaxed with xylene, and rehydrated with a descending serial dilution of ethanol and water (Kiernan, 1981). The sections were then stained using phosphotungstic acid haematoxylin (Lillie, 1977). Photographs were taken using a Zeiss MC 100 light microscope.

5.2.2. Electron microscopy

Grades one and four of the chum salmon were sampled in a pre-rigor state. A 3 mm diameter core of tissue was taken from the dorsal muscle of the fish using a punch biopsy instrument. The tissue was allowed to rest for several
minutes, and rinsed with 0.1 M sodium cacodylate buffer (pH 7.3). The tissue was fixed with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.3, at 4°C overnight. The muscle was rinsed in buffer and post fixed in a 1% OsO₄/0.1 M sodium cacodylate buffer mixture for 1 hour, rinsed in distilled water three times, and serially dehydrated in ethanol and propylene oxide. The tissue was infiltrated and embedded with Spurr’s media. Ribbons of ultrathin "silver" sections (500-600 Angstroms) were cut from the blocks with an Om U3 ultramicrotome (Reichert, Austria), and placed on copper grids. The grids were stained for 20 minutes with 2% aqueous uranyl acetate and for 10 minutes with Sato’s lead citrate (Kiernan, 1981). The grids were viewed and photographed with a transmission electron microscope.

5.3. **Results and Discussion**

5.3.1. **Light microscopy**

The phosphotungstic acid haematoxylin histochemical stain was used to investigate the changes in the morphology of the muscle during spawning migration. Micrographs of sections from the dorsal muscle clearly showed a change between the silver-bright and spawning fish. Within the muscle cells of the grade 1 fish, the myofibrils were quite closely packed, as illustrated in the cross section, Figure 5.1, Figure 5.2 and the longitudinal section, Figure 5.3.
Figure 5.1. Light micrograph of a silver-bright chum salmon stained with phosphotungstic acid haematoxylin. Bar represents 10 microns. (Magnification 1500).
Figure 5.2. Light micrograph of a cross section of a silver-bright chum salmon stained with phosphotungstic acid haematoxylin. Bar represents 20 microns. (Magnification 600).
The grade 2 and 3 fish were similar, but appeared to have progressively more space between the myofibril bundles. In the spawning fish, dramatic changes were seen in the muscle cells. Figure 5.4 and Figure 5.5 show a cross section of muscle cells in which the large spaces within the cells indicate a loss of myofibrils. Figure 5.6 was a longitudinal section of the spawning muscle and demonstrated myofibrillar tearing, and loss in integrity of the muscle structure.

The changes observed by the light micrographs were also found by Greene (1926) who described the state of myofibrillar cheek muscle in the spawning chinook salmon. Muscle fibres were extremely disorganized, and had lost all structural characteristics. Striations had disappeared from the fibres, and the cells appeared unstructured and swollen. Similar changes were found in the white muscle, however they were less dramatic.

5.3.2. Electron microscopy

Thirty-six electron micrographic fields of different muscle cells in two grade 1 and two grade 4 chum fish were observed. Although in many micrographs there were distinct differences between the silver-bright and spawning chum, in other micrographs it was difficult to differentiate between the two grades. Figures 5.7 to 5.10 are electron micrographs of the muscle of silver-bright chum salmon. It was interesting to note that the micrographs of the silver-bright fish exhibited many of the same forms of degeneration
Figure 5.3. Light micrograph of a longitudinal section of a silver-bright chum salmon stained with phosphotungstic acid haematoxylin. Bar represents 10 microns. (Magnification 1500).
Figure 5.4. Light micrograph of a cross section of a spawning chum salmon stained with phosphotungstic acid haematoxylin. Bar represents 10 microns. (Magnification 1500).
Figure 5.5. Light micrograph of a cross section of a spawning chum salmon stained with phosphotungstic acid haematoxylin. Bar represents 20 microns. (Magnification 600).
Figure 5.6. Light micrograph of a longitudinal section of a spawning chum salmon stained with phosphotungstic acid haematoxylin. Bar represents 10 microns. (Magnification 1500).
Figure 5.7. Electron micrograph of the dorsal muscle of a silver-bright salmon. Bar on upper right corner of photograph represents 1 micron. (Magnification = 27,000).
Figure 5.8. Electron micrograph of the dorsal muscle of a silver-bright salmon. Bar on upper right corner of photograph represents 1 micron. (Magnification = 27,000).
Figure 5.9. Electron micrograph of the dorsal muscle of a silver-bright salmon. Bar on upper right corner of photograph represents 1 micron. (Magnification = 27,000).
Figure 5.10. Electron micrograph of the dorsal muscle of a silver-bright salmon. Bar on upper right corner of photograph represents 1 micron. (Magnification = 27,000).
as the spawning fish, but were present much less frequently. As a result, a list of criteria to distinguish between the micrographs of silver-bright and spawning fish was developed. For each micrograph, four criteria were scored by assigning a value of 0 (none observed), or 1 (observed somewhere on the photograph). The final score for each photograph was calculated as the sum of the 4 criteria and thus the scores ranged between 0 to 4. The criteria used were described as follows. Smearing, Figure 5.11, the partial or complete loss of a distinct pattern of actin and myosin filaments. If actin and myosin could be distinguished, a score of 0 was assigned. Splitting, Figure 5.12, were breaks or tears within the myofibril bundles. If any breaks or tears were observed, a score of 1 was assigned. Inter-myofibril spaces, Figure 5.13, was an increase in the space normally found between muscle fibrils. Swollen T-tubules were not sufficient for a score of 1. If the spaces between bundles were small and regular, a score of 0 was assigned even if the tubules appeared swollen. The entire photograph was considered to determine whether the spaces were unusual. Thinning, Figure 5.14, was the decreased density of the myofilaments within a fibril. Small spaces between the actin and myosin in the myofibrils were assigned a score of 1.

Five judges were asked to categorize the micrographs using the scoring system, and their results are given in
Figure 5.11. Electron micrograph of the dorsal muscle of spawning chum salmon. "Smearing" is present on the upper portion of the photograph. Bar on upper right corner of photograph represents 1 micron. (Magnification = 26,000).
Figure 5.12. Electron micrograph of the dorsal muscle of spawning chum salmon. "Splitting" is present to the right of the photograph. Bar on upper right corner of photograph represents 1 micron. (Magnification = 26,000).
Figure 5.13. Electron micrograph of the dorsal muscle of spawning chum salmon. Notice areas with "inter-myofibril spaces" Bar on upper right corner of photograph represents 1 micron. (Magnification = 26,000).
Figure 5.14. Electron micrograph of the dorsal muscle of spawning chum salmon. "Thinning" within the myofibril bundles is apparent. Bar on upper right corner of photograph represents 1 micron. (Magnification = 26,000).
Table 5.1. The judges had a 72 to 87% success rate in assigning the silver-bright samples a cumulative score of zero, or one. It was concluded that it was not possible to consistently distinguish between the silver-bright and spawning samples based on a single microscopic field. However, this inability of the grading system should not be considered as a failure of the method. Instead, it was a reflection of the focal nature of muscle degradation which occurred during sexual maturation. Although some areas in the muscle of spawning chum appeared severely degraded, other areas retained the structure and integrity of a silver bright sample. Conversely, it was not correct to assume that the silver-bright fish would not have any areas of muscle degradation. It was necessary to consider a number of micrographs in order to correctly assess the degree of maturity of the fish. It is expected therefore, that given a number of micrographs from the same fish, this scoring system could successfully distinguish between mature and immature fish.
Table 5.1. Percentage of silver-bright and spawning fish assigned to each score used to judge micrographs of chum dorsal muscle.

<table>
<thead>
<tr>
<th>Judge</th>
<th>Grade</th>
<th>Score 0</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Score 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Silver</td>
<td>28</td>
<td>13</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>87%</td>
</tr>
<tr>
<td></td>
<td>Spawn</td>
<td>1</td>
<td>3</td>
<td>13</td>
<td>19</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Silver</td>
<td>20</td>
<td>20</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>77%</td>
</tr>
<tr>
<td></td>
<td>Spawn</td>
<td>6</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Silver</td>
<td>10</td>
<td>19</td>
<td>19</td>
<td>7</td>
<td>0</td>
<td>72%</td>
</tr>
<tr>
<td></td>
<td>Spawn</td>
<td>0</td>
<td>3</td>
<td>13</td>
<td>19</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Silver</td>
<td>22</td>
<td>22</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>73%</td>
</tr>
<tr>
<td></td>
<td>Spawn</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>17</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Silver</td>
<td>7</td>
<td>44</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>75%</td>
</tr>
<tr>
<td></td>
<td>Spawn</td>
<td>3</td>
<td>17</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

1 Total correct classification. Silver-bright fish were classified as having scores less than 2.
6. CONCLUSIONS

The spawning migration of chum salmon (*Oncorhynchus keta*) adversely affected the appearance and eating quality of the canned product. Although harvesting the chum only as silver-brights in the ocean would improve the quality of the final product, other problems prevent this practice. In order to protect weaker stocks of fish, a large portion of the chum are harvested as they approach the mouth of the river.

The object of this work was to assess the physical, chemical and textural changes which occurred in the chum throughout their spawning migration. This was accomplished using methods such as proximate analysis, colour analysis, proteinase assays, light and transmission electron microscopy. In addition, sensory panels and an instrumental method were used to measure texture.

The fish were graded into four categories based on their external appearance. The skin colour, thickness and slime layer were important considerations in the determination of grade. Colour of the raw flesh was measured with a Hunter Lab colourimeter, and by calculating the concentration of astaxanthin in the fat-soluble portion of the muscle based on its absorbance at 475 nm.

The visual change in the muscle from a red-pink colour to a white-grey colour was a reflection of the loss of
astaxanthin in the muscle and the changes in the Hunter Lab values. The content of astaxanthin decreased significantly with increasing grade. The Hunter a and b values also decreased significantly, but the L value increased. Correlation analysis between grade and the colour values indicated that a/L, astaxanthin, 'a', and a/b were significantly negatively correlated with grade. The best indicator of grade was the ratio a/L.

Proximate analysis confirmed literature findings that there were significant decreases in protein, from 17.9 % to 15.8% and lipid from 4.9% to 1.0% and increases in moisture from 74% to 79% as the fish matured. These results demonstrated the severe depletion of the energy sources in the muscle as the fish progresses in its migration.

Instrumental texture profile analysis (TPA) was not effective in measuring the progressive softening of the salmon muscle. The only two parameters to detect a difference in grade (α = 0.05) were cohesiveness, the ratio of the work done during the first bite (Area 1) and the work done on the second bite (Area 2), and slope 2, the maximum slope of the first compression. Cohesiveness increased significantly with grade and slope 2 decreased with grade.

The sensory panelists were more successful in their ability to distinguish the four grades of salmon. All of the attributes decreased significantly with grade, however discrimination between each grade was most successful with
the sensory attribute dryness. By performing Z-transformations on the data from each judge, it was possible to eliminate the non-homogeneity of variances between judges thus the analysis of variance was more effective in distinguishing between the grades for each attribute. Correlation analysis between sensory and instrumental measures of texture indicated that cohesiveness was significantly negatively correlated with the four sensory attributes, and that fibrousness best correlated with cohesiveness. The negative correlation between sensory and instrumental measures indicated that although there was a definite softening of the texture of the canned product caused by the breakdown of myofibrillar proteins, the Instron was not measuring this change. Because the muscle fibres were not oriented in any particular direction in the sample presented to the Instron, it was not possible to measure the loss of myofibrillar strength. It is possible however that the Instron was measuring the force necessary for the muscle fibres to slide across each other. Starvation in the migrating salmon would result in a loss the myofibrillar proteins and an increase in the moisture content and the relative amount of connective tissue. As the content of gelatinized collagen, moisture and sarcoplastic proteins increased with increasing maturity of the fish, the force necessary to slide the muscle fibres across each other would also increase.
The proteinase activity of the muscle was measured at pH 3.5, 6.2 and 7.0. There was a significant increase in activity with grade at pH 3.5. The activity of acid phosphatase, a lysosomal marker, also increased significantly with grade. These results indicated the possible contribution of lysosomal enzymes to the degradation of muscle during migration. Further work would be useful to isolate and identify these enzymes.

Light and electron micrographs were used to observe visible changes in the structure of the muscle. Light micrographs revealed an apparent loss in the diameter, or number of myofibrils in the muscle cells. Longitudinal sections showed an increased occurrence of myofibrillar tearing, and loss of integrity of the muscle. Electron micrographs of the muscle revealed areas of focal degeneration. This degeneration was described by four criteria including smearing, a partial or complete loss the organization of the myofibrils, splitting, tears or breaks in myofibril bundles, intermyofibril spaces, excess cytoplasmic fluid in the cell, and thinning, a decease in the density of the myofilaments within a fibril. Use of these four criteria helped determine whether the micrograph was from a spawning or silver-bright fish. However, it was not possible to predict the maturity of a fish based on one photograph because of the focal nature of the degeneration. In addition, micrographs of silver-bright fish also demonstrated some of the
same changes, but to a lesser degree. It would be necessary to look at a number of photographs to accurately distinguish the fish.
7. REFERENCES


Holmes, A.W. 1982. Salmon Quality Considerations for Fisheries Management. Department of Fisheries and Oceans, Field Services Branch. Victoria, B.C.


Table 8.1. Absorbance at U.V. 280 of soluble N versus grade as a measure of the proteinase activity of chum dorsal muscle at pH 3.5.
Table 8.2. Absorbance at U.V. 280 of soluble N versus grade as a measure of the proteinase activity of chum dorsal muscle at pH 6.2.
Table 8.3. Absorbance at U.V. 280 of soluble N versus grade as a measure of the proteinase activity of chum dorsal muscle at pH 7.0.
Table 8.4. Concentration of phosphate (mM) versus grade as a measure of the acid phosphatase activity of chum muscle during spawning migration.