AN ABSTRACT FOR THE DISSERTATION OF

Jasvinder Kaur for the degree of Doctor of Philosophy in Food Science and Technology presented on July 7th, 1999. Title: Concentration Of Anserine And Carnosine In Surimi Wash Water And Their Antioxidant Activity.

Abstract approved: ______________________________

Michael T. Morrissey

Anserine and carnosine are water-soluble dipeptides that have antioxidant properties and are found in the skeletal muscle of mammals and fishes. These dipeptides are removed through the washing process in surimi production. The objective of this research was to determine the concentration of anserine and carnosine in surimi wash water (SWW) at all stages of surimi processing, and undertake preliminary methods to remove and concentrate the two dipeptides and study the effect of surimi wash water extract on color. Wash water samples were collected from a local surimi plant. The samples were treated by the following methods: centrifugaion, heat-treatment at 60, 80 and 100°C and filtration using different ultrafiltration (UF) membranes. The concentrations of the protein and the two dipeptides were analyzed using Lowry and high performance liquid chromatography with a fluorescent detector, respectively. Iron content was determined in SWW samples using atomic absorption spectrometry and colorimetry. Effect of SWW extract and other antioxidants on the color of

fresh-farmed salmon were studied using color parameters-hue angle, chroma and lightness. Results showed that there was a trend: content of protein and dipeptides (anserine and carnosine) in SWW (raw) was higher in the first two stages of surimi processing. In the second set of experiment, where different heat treatments were used, it was found that the proteins and dipeptides showed similar trends. Additionally, 80°C followed by 100°C treatment were effective in removal of proteins and recovery of dipeptides. Among UF treatments, 1K molecular weight cut-off membrane was the most effective in recovery of dipeptides. Iron was less than 1 ppm in all SWW samples. Color measurement of fresh farmed salmon patties revealed that treatments of SWW extract (1%) in addition to other food antioxidants such as butylated hydroxy toluene and carnosine (1%), maintained salmon color until day 5. Therefore, SWW extract at lower concentrations may have an economical and potential use as a food antioxidant.

©Copyright by Jasvinder Kaur
July 7th, 1999
All Rights Reserved

# CONCENTRATION OF ANSERINE AND CARNOSINE IN SURIMI WASH WATER AND THEIR ANTIOXIDANT ACTIVITY

by

Jasvinder Kaur

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirement for the
degree of

Doctor of Philosophy

Presented July 7th, 1999
Commencement June 2000

Doctor of Philosophy dissertation of Jasvinder Kaur presented on July 7th, 1999

APPROVED:

Major Professor, representing Food Science and Technology

Chair of Department of Food Science and Technology

Dean of Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Jasvinder Kaur, Author

# Acknowledgements

I wish to take this opportunity to thank everyone involved directly or indirectly with the completion of this dissertation. First of all my thanks are to my adviser and dissertation committee chairman, Dr. Michael T. Morrissey, for his consistent support, valuable guidance, suggestions and conversations throughout the course of this project. I am thankful to Dr. Wilbert Gamble, Dr. Shawn Mehlenbacher, Dr. Daniel Selivonchik, Dr. Jim Leklem for being committee members, for advising me and updating and reviewing this work on ongoing basis. My sincere thanks are to Dr. Haejung An, Dr. Jae Park, Dr. Daniel Farkas, Dr. Mina McDaniel, Dr. Ron Wrolstad, Dr. Donald Reed, Dr. E.A. Decker, Dr. Tori Hagen, Dr. Ajoy Velayudhan and Dr. Jack Higginbotham for their valuable advise and support. Special thanks to Retd. Lewis Richardson, Marda Brown, and Roger, who were always ready to help me; Nancy Chamberlain and Joey Heimberg for their unending support and understanding; I am grateful to Dr. Barry H. Gump, who always supported and helped me as and when I needed help. I am also grateful to all my colleagues and others whose names are not mentioned, for their support and help. I am thankful to National Oceanic and Atmospheric Administration (Saltonstall Kennedy Project #: NA76FD0212) and the Mamie Markham (Research Award, 1997) for providing funds for this research work.

Last but not the least, my thanks go to my loving parents, late mother-in-law, brothers and sisters who endlessly supported my education and always encouraged me. In addition, to my beloved husband, who cheered me up and supported me, and to my

precious little son who amazingly adjusted well with my parents during the course of my study.

## TABLE OF CONTENTS

Page

INTRODUCTION....1

Seafood Waste And Utilization....1

Lipid Oxidation And Antioxidants....4

Ultrafiltration....8

Iron And Colorimetry....9

Color And Pigment Effect In Salmon....10

CONCENTRATION OF ANSERINE AND CARNOSINE IN SURIMI WASH WATER....13

Abstract....13

Introduction....14

Materials And Methods....16

Results And Discussion....21

Summary....32

References....32

EFFECT OF HEAT TREATMENT ON THE CONCENTRATION OF DIPEPTIDES OBTAINED FROM SURIMI WASH WATER....37

Abstract....37

Introduction....38

Materials And Methods....39

TABLE OF CONTENTS (CONTINUED)

Page

Results And Discussion........................................................41

Summary........................................................................52

References......................................................................53

EFFECT OF ULTRAFILTRATION AND IRON CONTENT ON THE CONCENTRATION OF DIPEPTIDES OBTAINED FROM SURIMI WASH WATER........................................................56

Abstract........................................................................56

Introduction....................................................................57

Materials And Methods...........................................................59

Results And Discussion..........................................................61

Summary.........................................................................71

References......................................................................72

ANTIOXIDANTS AND THEIR EFFECT ON THE COLOR OF FARMED SALMON..........................................................................76

Abstract........................................................................76

Introduction....................................................................76

Materials And Methods...........................................................80

Results And Discussion..........................................................83

Summary.........................................................................95

**TABLE OF CONTENTS (CONTINUED)**

Page

References....96

SUMMARY....102

BIBLIOGRAPHY....106

# LIST OF FIGURES


| Figure | | Page |
|---|---|---|
| 1.1 | Chemical structures of histidine-related dipeptides (anserine and carnosine)... | 3 |
| 1.2 | Simplified scheme showing various steps in auto-oxidation... | 6 |
| 2.1 | Surimi processing flow and sample collection... | 17 |
| 2.2 | Proteins in surimi wash water samples (raw treatment)... | 23 |
| 2.3 | Total dipeptides in surimi wash water samples (raw treatment)... | 25 |
| 2.4 | Total dipeptides in surimi wash water samples (freeze dried)... | 26 |
| 2.5 | Total dipeptides in surimi wash water samples obtained from Alaska Pollock... | 28 |
| 3.1 | Comparison of proteins (ppm) of all surimi wash water samples treated at different temperatures... | 43 |
| 3.2 | Relative amounts (%) of total dipeptides in heat-treated surimi wash water samples (Sample Set A)... | 46 |
| 3.3 | Comparison of relative amounts (%) of dipeptides over heat treatments of Dehydrator 1 and Dehydrator 2... | 48 |
| 3.4 | Relative amounts (%) of dipeptides from surimi wash water sample (Sample Set B) treated at different temperatures... | 50 |
| 3.5 | Comparison of relative amounts (%) of dipeptides in surimi wash water samples collected at the first 2 stages... | 51 |
| 4.1 | Comparison of dipeptide (anserine and carnosine) content of ultrafiltered samples using Dehydrator 1 (DH1) sample... | 64 |
| 5.1 | Hue angle of individual antioxidants with acceptable range for salmon (*Salmo salar*) treated with antioxidants (5°C)... | 88 |
| 5.2 | Hue angle of combined antioxidants with acceptable range for salmon (*Salmo salar*) treated with antioxidants (5°C)... | 94 |

# LIST OF TABLES


| Table | | Page |
|---|---|---|
| 2.1 | Protein content (ppm) of surimi wash water samples determined by the Lowry method | 22 |
| 2.2 | Concentration (ppm) of anserine and carnosine in randomly collected surimi wash water samples | 24 |
| 2.3 | Concentration (ppm) of anserine and carnosine in a surimi wash water sample | 29 |
| 3.1 | Protein content (ppm) of surimi wash water samples determined by the Lowry method | 42 |
| 3.2 | Concentration (ppm) of anserine and carnosine in heat-treated surimi wash water samples | 45 |
| 3.3 | Concentration (ppm) of anserine and carnosine in surimi wash water samples collected from six stages: percent recovery of spiked samples | 47 |
| 4.1 | Concentration (ppm) of anserine and carnosine in surimi wash water samples filtered through different ultrafiltration membranes | 62 |
| 4.2 | Relative standard deviations of the replicated samples for high performance liquid chromatography with a fluorescent detector | 66 |
| 4.3 | Iron levels (ppm) determined by AAS and colorimetric methods in surimi wash water samples | 67 |
| 4.4 | Iron levels (ppm) determined by AAS and colorimetric methods in heat-treated surimi wash water samples | 69 |
| 4.5 | Ranges of iron levels (ppm) determined by AAS and colorimetric methods in heat-treated surimi wash water samples | 69 |
| 4.6 | Concentration (ppm) of iron in surimi wash water samples filtered through different ultrafiltration membranes | 70 |

## LIST OF TABLES (CONTINUED)

Table | Page

5.1 Antioxidant treatments added to farmed salmon (w/w)...................... 82

5.2 Color parameters of individual antioxidants applied to farmed salmon (*Salmo salar*) over 7 days (5°C)--hue angle.................................. 85

5.3 Color parameters of individual antioxidants applied to farmed salmon (*Salmo salar*) over 7 days (5°C)--chroma.................................... 86

5.4 Color parameters of individual antioxidants applied to farmed salmon (*Salmo salar*) over 7 days (5°C)--lightness.................................. 87

5.5 Color parameters of combined antioxidants applied to farmed salmon (*Salmo salar*) over 7 days, hue angle........................................ 91

5.6 Color parameters of combined antioxidants applied to farmed salmon (*Salmo salar*) over 7 days, chroma.......................................... 92

5.7 Color parameters of combined antioxidants applied to farmed salmon (*Salmo salar*) over 7 days, lightness........................................ 93

# CONCENTRATION OF ANSERINE AND CARNOSINE IN SURIMI WASH WATER AND THEIR ANTIOXIDANT ACTIVITY

## Chapter 1

## Introduction

### Seafood Waste and Utilization

In the current seafood industry, only 20-40% of raw material from fish is used for primary products. The remaining 60-80% of raw material is considered processing waste, and is used for low value products such as fish meal, pet foods and fish hydrolysate or disposed of at sea (Keller, 1990). A small percentage of fish byproducts is used for colorants, seafood flavorings, chitosan and enzymes, etc. However, the recovery of bioactive components from seafood waste is an area of expanded research as well as high economic potential that has expanded the utilization of the seafood byproducts. There are a number of useful compounds that have already been isolated and identified in seafood waste. These include seafood proteins having biotechnological properties (Haard et al., 1994), chitin and chitosan from shrimp waste (Skaugrud and Sargent, 1990), seafood flavorings from crab waste and fish hydrosylate (In, 1990; Chung and Cadwallader, 1993), and carotenoid pigments from shrimp waste (Simpson and Haard, 1985). The potential for recovery of natural antioxidants from shrimp waste has also been demonstrated (Li et al., 1994).

Anserine (B-alanyl-1-methylhistidine) and carnosine (B-alanylhistidine) are imidizole dipeptides that have been shown to have antioxidant properties (**Fig. 1.1**) (Abe, 1995). The molecular weight of these 2 naturally occurring dipeptides, carnosine and anserine, is 226.2 and 242.3, respectively, and the difference in their molecular weight is due to presence of an extra methyl ($CH_3$) group in anserine (Dawson et al., 1969).

The dipeptides anserine and carnosine are found in skeletal muscle of different animals, including fish (Crush, 1970; Boldyrev and Severin, 1990; Suzuki et al., 1990; Johnson and Hammer, 1992; Aursand et al., 1995; Choi et al., 1996). According to Boldyrev and Severin (1990), the biological significance of histidine-containing dipeptides can be presented as consisting of buffering (pH stabilizing), antioxidative properties and membrane stabilizing activities. Carnosine is a highly valuable pharmacological agent due to its high biological activity. The concentrations of anserine and carnosine range from 0 to 5590 ppm and 0 to 5090 ppm, respectively, in different species of fish (Suzuki et al., 1990; Choi et al., 1996). The dipeptides concentration varies from 1 species to another (Suzuki et al.; 1990, 1991). However, their antioxidant activity is similar (Kohen et al., 1988). According to Suzuki et al. (1991), anserine is present in high concentrations in the muscle of migrating fish and contributes to the buffering capacity of fish muscle. White muscle contains higher concentrations of dipeptides to protect against ischemic reperfusion injury that occurs in anaerobic conditions when oxygenated blood enters muscle tissue (Crush, 1970).

Synthetic production of carnosine can cost 3 times more than other food grade antioxidants. This indicates that the possibility of extracting carnosine and anserine from

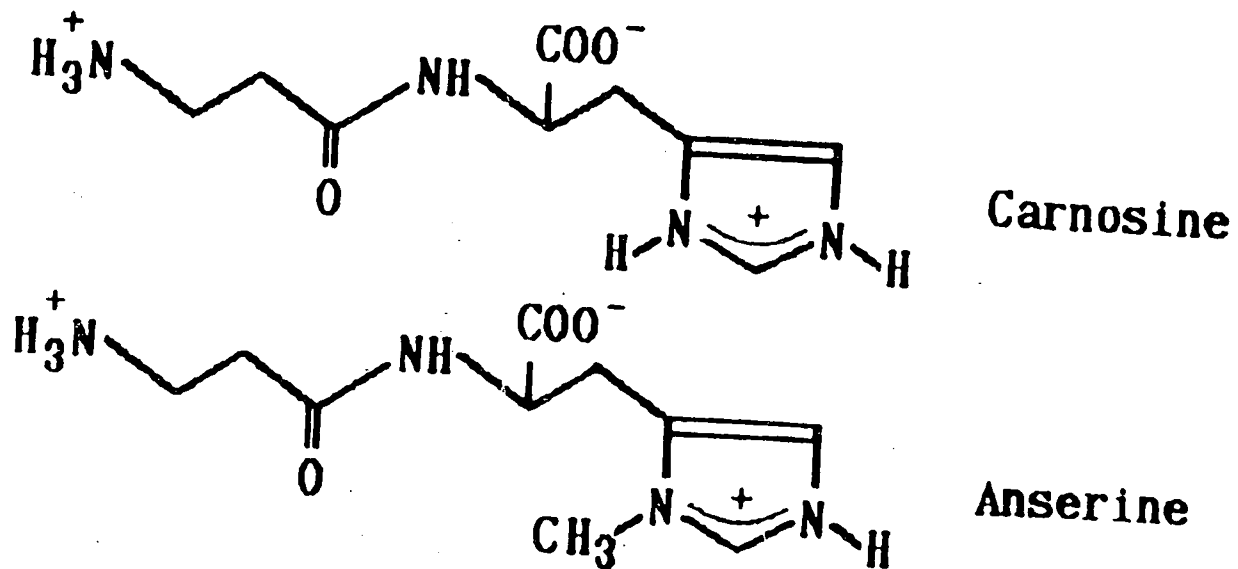



**Fig 1.1-Chemical structures of histidine-related dipeptides (anserine and carnosine)**

Adapted from: Abe, H. 1995.

surimi wash water (SWW) could be an alternative method of producing these compounds cost effectively. This would increase the waste utilization of a major byproduct of the surimi industry as well as help alleviate environmental problems associated with SWW disposal.

Surimi production from Pacific Whiting (*Merluccius productus*) is a recent development in the Pacific Northwest, which has resulted in considerable capital investment (Morrissey et al., 1992). Surimi is produced as a result of mincing fish muscle, washing the mince 2-3 times with water, and freezing it with cryo-protectants (Lee, 1984). The amount of water required to wash the mince is at least 2-3 times its weight. The washing steps concentrate the myofibrillar proteins that are used for surimi production. The remaining components such as pigments, enzymes, low molecular weight compounds, lipids and sarcoplasmic proteins are soluble in water and hence are expected to be found in SWW.

Both dipeptides are water-soluble compounds and are therefore removed through the washing process in surimi production. Wash water can possibly contain other soluble components such as pro-oxidative metals and iron-containing proteins that decrease antioxidant activity (Chan and Decker, 1994).

**Lipid Oxidation and Antioxidants**

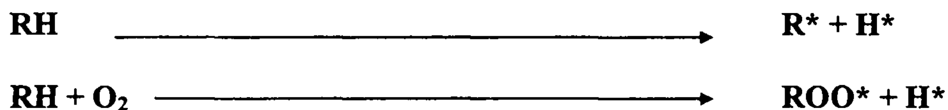
Anserine and carnosine are capable of inhibiting lipid oxidation and are natural antioxidants. The 2 dipeptides have peroxyl radical-trapping ability, are reducing agents,

chelate metal ions, quench singlet oxygen and bind hydroperoxides (Kohen et al., 1988). Lipid oxidation in food is a major cause of chemical spoilage in foods. Foods high in unsaturated fatty acids are extremely susceptible to lipid oxidation (Decker and Faraji, 1990). Polyunsaturated fatty acids (PUFA) such as linoleic and arachidonic acids undergo the greatest oxidative deterioration in fish, red meats and poultry during frozen storage (Igene et al., 1979; Lai et al., 1995). The major pathway of auto-oxidation of unsaturated fatty acids involves a self catalytic, free radical mechanism that produces lipid peroxides and is summarized in **Fig. 1.2** (Khayat and Schwall, 1983; Decker and Faraji, 1990).

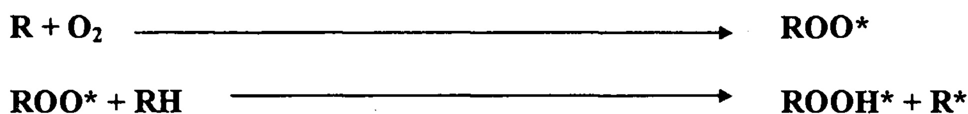
Lipid oxidation is also a major cause of food deterioration affecting color, flavor, texture and nutritional value (Chan et al., 1993). Atherosclerosis is partially caused by toxic substances formed during lipid oxidation (Addis and Park, 1989). There are numerous antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) that are used in the food industry to control lipid oxidation. There is an increased demand for natural products by consumers. The natural antioxidants are available in the form of herbs and spices (Ramanathan and Das, 1993) and tocopherol, which have a limited use due to high cost, color and flavor attributes (Chan et al., 1993). The formation of non-enzymatic browning products as a result of drying extracts lead to increased antioxidant activity (Bailey, 1988; Ramanathan and Das, 1993).

Carnosine has antioxidant properties and has been found to inhibit lipid oxidation caused by iron, copper, hemoglobin, singlet oxygen and lipoxygenase (Boldyrev et al., 1988; Kohen et al., 1988; Decker and Faraji, 1990; Decker et al., 1992). Carnosine was

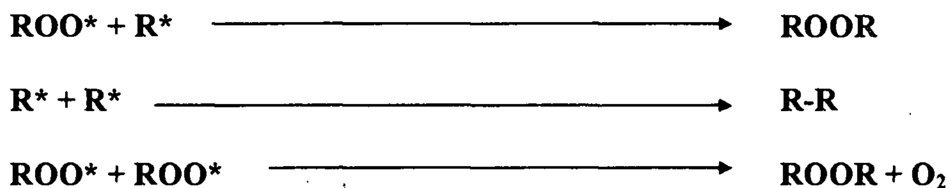
**Initiation:**




**Propagation:**




**Termination:**




**Where ROO* = lipid peroxy radical**
**RH = unsaturated lipid**

**R* = lipid radical**
**ROOH = hydroperoxides**

**Fig. 1.2-Simplified scheme showing various steps in auto-oxidation**

Adapted from: Khayat, A., and Schwall, D. 1983.

also found to be effective at protecting oxidative rancidity and color changes in salted ground pork after 1 month of frozen storage (-15°C) (Decker and Crum, 1991). Also, carnosine has been found to reduce lipid oxidation in cooked ground pork during refrigerated storage (Decker and Crum, 1993). There are numerous water-soluble components that lead to lipid oxidation: active oxygen species, transition metals, pork heme-containing proteins, myoglobin, and hemoglobin in skeletal muscles (Ashghar et al., 1988; Kanner et al., 1988; Decker and Hultin, 1992).

According to Boldyrev et al. (1988) "the inhibiting effect of the dipeptides is enhanced either by the rise in their concentration or by the reduction of the other components in the membranes." The addition of the dipeptides leads to a marked decrease in the level of primary molecular products of lipid oxidation (Boldyrev et al., 1988). The naturally occurring dipeptides regenerate $\alpha$-tocopherol, suggesting their synergistic effect to inhibit lipid oxidation (Boldeyrev et al., 1988; Decker and Faraji, 1990). However, carnosine can form a complex with copper that leads to a reduction in its catalytic activity (Decker et al., 1992). Carnosine in conjunction with ascorbic acid may be useful as a meat additive for increasing shelf life and stabilizing color of meat products (Lee et al., 1999).

The SWW samples also contain pro-oxidative metals and heme-containing proteins that decrease antioxidant activity (Chan and Decker, 1994). According to Chan et al., (1993), "In order to produce an effective antioxidant extract from skeletal muscle, techniques are needed to reduce the level of pro-oxidants while maintaining high levels of carnosine." The antioxidant extract having high concentration of the 2 dipeptides can

only be produced when heme-containing proteins are removed either through heat precipitation at selective temperature or ultrafiltration..

**Ultrafiltration**

Anserine and carnosine and low molecular weight, water-soluble compounds are possibly present in SWW. Ultrafiltration is a technique that can be used to remove pro-oxidants and other compounds from SWW. Ultrafiltration is a unit operation in which water and some solutes in a solution are selectively removed through a semi-permeable membrane (Fellows, 1988; Toyomoto and Higyuchi, 1992; Schagger, 1994; Potter and Hotchkiss, 1995). Ultrafiltration membranes have high porosity and retain only large molecules that have a low osmotic pressure. Smaller solutes are transported across the membrane with the water and can therefore can operate at lower pressures (50-2000kPa) (Fellows, 1988). Ultrafiltration membranes are made of different materials such as cellulose acetate and polyamide, with considerable control of their physical and chemical properties (Singh and Heldman, 1993; Potter and Hotchkiss, 1995). Ultrafiltration can help separate low molecular weight compounds and is presently the more commercially proven and cost-effective recovery technique (Singh and Heldman, 1993; Haard et al., 1994; Lanier, 1994; Scott, 1995). Ultrafiltration applications in recycling of food processing waste water and in recovery of valuable components of food processing wastes are currently being developed (Toledo, 1991).

**Iron and Colorimetry**

The concentration and the antioxidant activity of anserine and carnosine may be affected by iron. Heme iron in animal flesh is highly assimilated compared to non-heme iron in plants. Absorption of non-heme iron is improved by ascorbic acid and animal protein. Substituting fish for red meat in modern diets would reduce the intake of both heme and total iron. However, this decreased intake of iron may be offset by enhancement of absorption of non-heme iron from the diet by fish protein (Chao and Gordon, 1983). One mg of hemoglobin contains 3.4 μg of iron (Weissman and Pileggi, 1974). The iron levels found in white flesh finfish were 0.31 mg/100 g (Gordon and Roberts, 1977).

Colorimetric techniques are commonly used in the food industry for mineral analysis and usually the colored complex is formed which is read at a specific wavelength, depending on the mineral. Ferrous (iron) forms stable, intensely colored species with compounds such as 3-(2-pyridyl)-5,6-bis (4-phenylsulfonic acid)-1,2,4-triazine (ferrozine) and O-phenanthroline (Caldwell and Adams, 1946), which can be used for the quantitative determination of iron (Stookey, 1970). Ferrozine reacts with ferrous to form a stable magenta-colored complex in water and can be used for the direct determination of iron in water (Stookey, 1970; Gordon, 1978). The colored complex is more stable between pH 4 and 9 (Persijn et al., 1971). This technique avoids protein precipitation (Goodwin et al., 1966; Persijn et al., 1971) and minimizes interference from other trace metals (Stookey, 1970). The absorption wavelength used for the technique is 560 nm (Persijn et al., 1971).

**Color and Pigment Effect in Salmon**

Redness is one of the important quality criteria of salmonoids (Francis and Clydesdale, 1975; Haard, 1988; Hong and Storebakken, 1991). Color is helpful in providing sorting (separating) information in fish in addition to size and shape (Strachan, 1993). Carotenoids are the pigments responsible for the yellow or red color of salmon flesh. Canthaxanthin and astaxanthin are fed to salmonoids, rainbow trout and rockfish for pigmentation (Skrede and Storebakken, 1986a; Skrede et al., 1990; Choubert et al., 1992; Ingemansson et al., 1993; Li et al., 1998). Astaxanthin is the dominant carotenoid pigment in the flesh of wild salmon (Khare et al., 1973). The pigments are absorbed from the diet and distributed to the fish muscle where they bind to actomyosin (Skrede et al., 1989; Swatland et al., 1997).

Oxidation is the major cause of carotenoid degradation in foods. The rate of oxidation depends on contact with oxygen, light, heat, and presence of pro-oxidants and antioxidants (Francis and Clydesdale, 1975; Haard, 1988; Hong and Storebakken, 1991; Li et al., 1998). The influence of light can induce unwanted changes such as oxidation of pigments associated with loss of natural color, photosynthesized oxidation of lipids and loss of vitamin activity (Bjerkeng and Johnsen, 1995). Carotenoid degradation proceeds in an analogous fashion to lipid degradation, i.e., auto-oxidation and/or photosynthetic oxidation (reaction with singlet oxygen) (Wasson et al., 1991).

The color of salmon may be assessed by various analytical methods including but not limited to sensory analysis using trained panelists (Ostrander et al., 1976; Skrede and Storebakken, 1986a) or standardized colors (McCallum et al., 1987; Skrede et al., 1990).

Assessment can also be done by instrumental analysis based on reflectance spectra (Skrede and Storebakken; 1986a,b). According to Hunter (1975), the reflectance spectra and further transformation into CIE XYZ tri-stimulus values and other uniform color systems are used for color studies (Skrede and Storebakken, 1986a). Salmon color in these systems is described by the parameters L*, a*, and b*, where L represents lightness, a redness and b yellowness. In the L* C* h* color system, L* represents lightness similar to L*, a*, b* system, C*, Chroma, which is the saturation of the color numerically defined as the square root of $a^2 + b^2$, and h*, hue angle, which is numerically equal to $\tan^{-1}$ (b* / a*) (Hunter 1991; Minolta®, 1994).

It is difficult to compare results from different analytical methods because of the lack of intensity references in sensory analysis or lack of standardized colors. Instrumental design and sample presentation influence the values of instrumental color analysis (Skrede and Storebakken, 1986b). The results can also vary when considering the pigment of the salmonoid. For example, with astaxanthin the color is more red than canthaxanthin (Skrede et al., 1989). For all practical purposes, it is important to understand the color and its relationship to the chemical and instrumental methods. Lipid oxidation and processing causes changes in the perceived salmonoid color (Chen et al., 1984; Skrede et al., 1989). The hue shifts from red to orange-red in salmonoid flesh (Skrede et al., 1989).

The right coloration for cultivated fish is required to be provided by the farmers to satisfy consumer expectations (Choubert et al., 1992; Williams, 1992; Metusalach et al., 1997). Salmon is often priced according to the intensity of its hue and is an important feature of salmon quality (Sigurgisladottir et al., 1994; Metusalach et al., 1997).

Therefore, hue angle, chroma and lightness were used as 3 parameters of color measurement. In the case of farmed salmon, hue angle indicates redness at $0^{\circ}$ and yellowness at $90^{\circ}$ and is a good indicator of how the sample is perceived visually. Chroma is indicative of color saturation. Lightness varies from 1 to 100 as in the L*, a*, b* system. There is a radial increase in the pigmentation towards the backbone (McCallum et al., 1987). Considerable variation in lipid oxidation may occur within salmon species and there is significant color variance of farmed salmon reared in different farms (Ostrander et al., 1976).

In the chapters following this introduction, first the concentration of anserine and carnosine in SWW samples will be determined and trends discussed. Next, the effect of heat treatment and ultrafiltration techniques on removal of proteins and iron and maximum recovery of dipeptides will be evaluated. The content of iron will also be determined in SWW samples, due to its effect on the concentration as well as antioxidant activity of dipeptides. Thereafter, the antioxidant activity or inhibition of oxidative rancidity by the SWW extract and other antioxidants would be investigated via measuring color parameters viz hue angle, chroma and lightness. Finally, the results of this research have been summarized.

# Chapter 2

# DETERMINATION OF ANSERINE AND CARNOSINE CONCENTRATIONS IN SURIMI WASH WATER

## Abstract

In surimi processing, 60-80 % of the raw material is considered processing waste and is used for low value products including fish meal, pet foods, fish hydrolysate or disposed of at the sea. The dipeptides anserine and carnosine have been shown to be present in the skeletal muscles of fish. These naturally occurring dipeptides are water-soluble, have a very low molecular weight and are known to exhibit antioxidant properties. Therefore, it was hypothesized that surimi wash water (SWW) might be a good source for recovering these dipeptides. Thus, the objective of this experiment was to determine the concentration of dipeptides in SWW samples. SWW samples were collected from a local surimi processing plant that has 6 processing stages: Dehydrator 1 (DH1), Dehydrator 2 (DH2), Dehydrator 3 (DH3), Screw Press (SP), Rotary Screen 1 (RS1) and Rotary Screen 2 (RS2). The protein and dipeptides concentrations were determined by the Lowry method and high performance liquid chromatography with the fluorescence detection method, respectively. Results showed that the SWW samples that had a higher amount of total protein also had a higher amount of dipeptides. In this study, the highest amounts of dipeptides were present in the first 2 stages, DH1 and DH2. This experiment was the first step in establishing that SWW extract can be obtained from surimi waste.

**Introduction**

In seafood processing only 20-40% of the raw material from the fish is used for primary products. The remaining 60-80% of the raw material is considered processing waste and is used for low value products including fish meal, pet foods, fish hydrolysate or is disposed of at the sea (Keller, 1990). A small percentage of this fish byproduct is used for colorants, seafood flavorings, chitosan and enzymes. However, the recovery of bio-active components from seafood waste is an area of expanded research and high economic potential that has expanded the utilization of seafood byproducts. A number of useful compounds have been isolated and identified in seafood waste. These include seafood proteins with biotechnological properties (Haard et al., 1994); chitin and chitosan from shrimp waste (Skaugrud and Sargent, 1990); seafood flavorings from crab waste and fish hydrosylate (In, 1990; Chung and Cadwallader, 1993) and carotenoid pigments from shrimp waste (Simpson and Haard, 1985). The potential recovery of natural antioxidants from shrimp waste has also been demonstrated (Li et al., 1994).

Consumers are increasingly looking for natural foods. The dipeptides anserine and carnosine are found in skeletal muscle of different animals including fish (Crush, 1970; Boldyrev and Severin, 1990; Suzuki et al., 1990; Johnson and Hammer, 1992; Aursand et al., 1995; Choi et al., 1996). According to Boldyrev and Severin (1990), the biological significance of histidine-containing dipeptides includes buffering (pH stabilizing), antioxidative properties and membrane stabilizing. The high biological activity of carnosine makes it a highly valuable pharmacological agent. The concentrations of anserine and carnosine range from 0 to 5590 and 0 to 5090 ppm,

respectively, in different species of fish (Suzuki et al., 1990; Choi et al., 1996). The dipeptide concentration varies from 1 species to another (Suzuki et al.; 1990, 1991), however, their antioxidant activity is similar across species (Kohen et al., 1988). According to Suzuki et al. (1991), anserine is present in high concentrations in the muscle of migrating fish and contributes to the buffering capacity of fish muscle. White muscle contains higher concentrations of dipeptides to protect against ischemic reperfusion injury which occurs in anaerobic conditions when oxygenated blood enters muscle tissue (Crush, 1970).

Synthetically produced carnosine can cost 3 times more than other food grade antioxidants. Extracting carnosine and anserine from surimi wash water (SWW) is an alternative way of cost effectively producing these compounds. This would increase the waste utilization of a major byproduct in the surimi industry as well as help alleviate environmental problems associated with SWW disposal. Both dipeptides are water-soluble compounds and are removed by the washing process in surimi production. Wash water can possibly contain other soluble components such as pro-oxidative metals and iron-containing proteins which decrease antioxidant activity (Chan and Decker, 1994). Surimi production from Pacific Whiting (*Merluccius productus*) is a recent development in the Pacific Northwest, which has resulted in considerable capital investment (Morrissey et al., 1992). Surimi is produced as a result of mincing fish muscle, washing the mince 2-3 times with water and freezing it with cyro-protectants (Lee, 1984). The amount of water required to wash the mince is at least 2-3 times its weight. The washing steps concentrate the myofibrillar proteins that are used for surimi production; the

remaining components such as pigments, enzymes, low molecular weight lipids and sarcoplasmic proteins are soluble in water and hence are expected to be found in SWW.

Anserine (B-alanyl-1-methylhistidine) and carnosine (B-alanylhistidine) are imidizole dipeptides that have been shown to have antioxidant properties (Abe, 1995). The difference in the molecular weight of these 2 naturally occurring dipeptides—226.2 for carnosine and 243.2 for anserine—is due to the presence of an extra methyl ($CH_3$) group in anserine (Dawson et al., 1969). These 2 dipeptides are water soluble and are found in skeletal muscle of the fish and exhibit antioxidant activity. The objective of this research was to determine the concentration of anserine and carnosine in SWW at all stages of surimi processing.

## Materials and Methods

The SWW samples were collected from Point Adams Co., Warrenton, Oregon. The samples were collected from each surimi processing stage in clean 1-liter bottles and brought to the Oregon State University (OSU) Laboratory, Astoria, Oregon on ice. The samples were identified based on the surimi processing stage collected: DH1 (Dehydrator 1); DH2 (Dehydrator 2); DH3 (Dehydrator 3); SP (Screw Press); RS1 (Rotary Screen 1); and RS2 (Rotary Screen 2) (**Fig. 2.1**). The surimi made in this plant was from Pacific Whiting (*Merluccius productus*). The samples were prepared for analysis according to the following procedures. The concentration (ppm) of the 2 dipeptides anserine and carnosine in all the stages of SWW was determined using high performance liquid

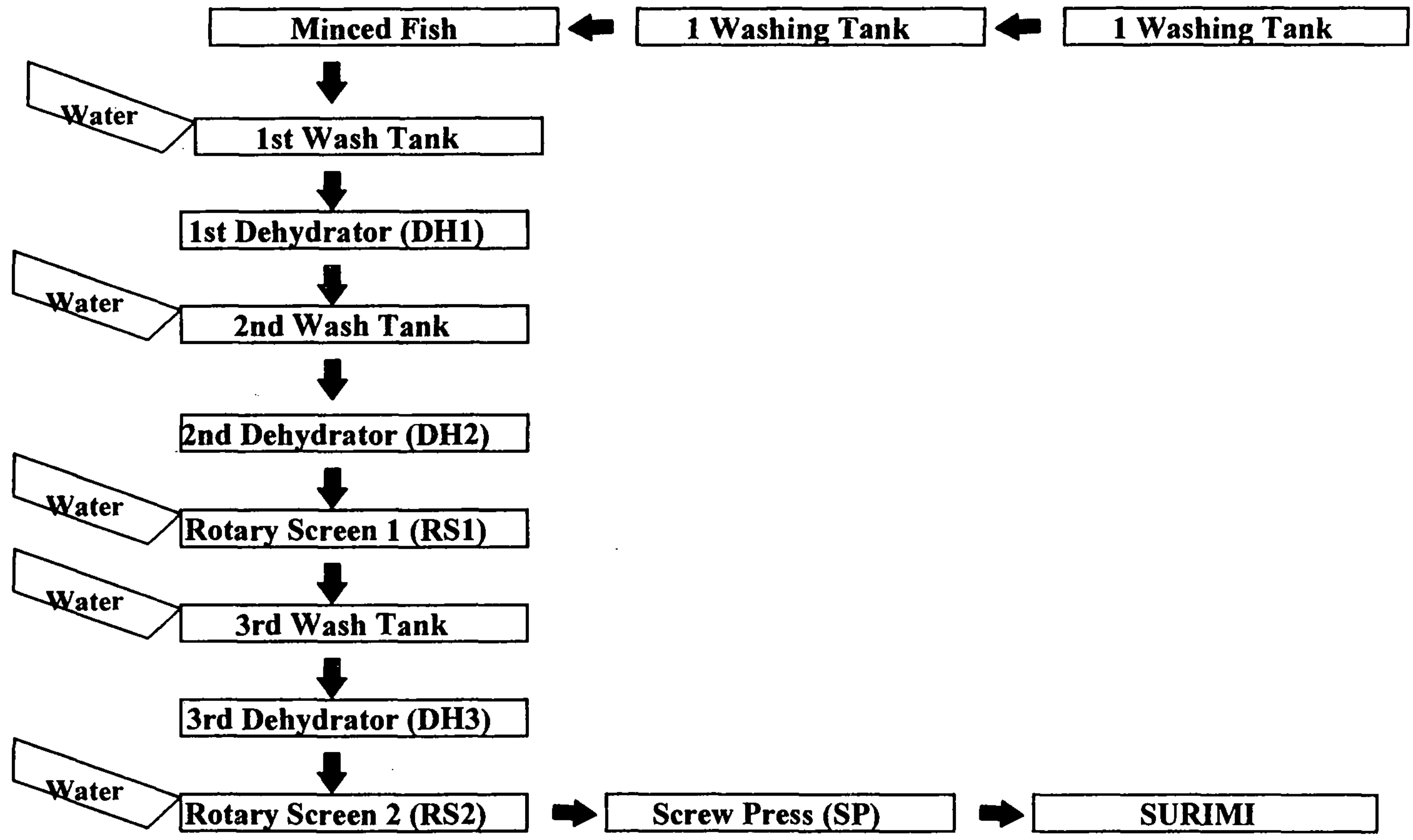



**Fig. 2.1-Surimi processing flow and sample collection.**

chromatography (HPLC) with a fluorescent detector. Water was used as a blank in the experiment. The concentration of total protein was determined using the standard Lowry method (Lowry et al., 1951). The chemicals for this method were purchased from Sigma Chemical Company, St. Louis, MO.

The first set of samples (Sample Set A) was taken directly from the surimi processing plant in 1-liter bottles and transported to the OSU Seafood Lab on ice; samples were centrifuged at 7000 x g for 30 minutes at 4°C (Sorvall RC-5B refrigerated super speed centrifuge, Newton, CT). The supernatant was filtered through filter paper (Whatman 41). The filtrates were subjected to blast freezing for rapid freezing, vacuum packed and stored at -80°C for further analysis (Chan et al., 1993). All the samples (2 replicates) were collected on 3 different days from the same plant. The filtered SWW samples (25-50 ml) were freeze dried (2 replicates) for approximately 24 hours (Labconco, Kansas, MO), vacuum packed and frozen at -80°C until further analysis. Freeze dried samples (1 gram) were reconstituted with HPLC grade water (40 ml) and analyzed for dipeptides similar to other SWW samples.

SWW samples received from Alaska (1 each) were analyzed for comparison purposes. The surimi at the Alaska plant is made from Pollock (*Theragra chalcogramma*). The samples were shipped on dry ice and were immediately stored at -80°C on arrival at the OSU Seafood Lab. The layout plan of the Alaska surimi plant is different than that at Point Adams. The Alaska plant has 4 processing stages: First Wash, Second Wash, Screw Press and Decanter.

A second set of samples were collected from Point Adams Co., in a single day and treated on the same day in a similar fashion as described above. This set of samples is referred to as Sample Set B.

Before analysis with HPLC, all the samples were uniformly treated according to the procedure described by Teahon and Rideout (1992) and Decker (1998), and Ortho-phthaldehyde (OPA) was used as a derivatizing agent. Standard solutions of concentrations ranging from 0 to 1000 ppm of carnosine and anserine (Sigma Chemical Co., St. Louis, MO) were prepared.

For the first set of samples (Sample Set A), the concentration of the 2 dipeptides was analyzed using HPLC (Perkin Elmer [PE] 200 LC pump/PE series 200 LC autosampler) with a fluorescent detector (PE LS 40)(Perkin Elmer, Norwalk, CT). The column used for the experiment was Hypersil ODS (C18), 5 µM, 250X4.6 mm (Alltech No. 98507), Guard Cartridge 7.5 x 4.6mm (Alltech No. 96013) and Guard Column Holder (Alltech No. 80101) (Alltech, Deerfield, IL). Data collection was done using a PE 970 interface box connected to a PC running Turbochrom® software (Perkin Elmer, Norwalk, CT).

The HPLC conditions used were a) flow rate: 1.8 mL/min; b) mobile phase: 0.3M sodium acetate (75%):methanol (25%), pH-5.5; c) injection conditions (auto sampler): 150 µL injection volume for standards and 21.5-min cycle time for standards, 25 or 50 µL injection volume for samples and 45-min cycle time for samples; d) the derivatization method used with auto sampler: sample volume: 150 µL/reagent volume: 150 µL/mix cycles: 2/ reaction time: 0.1 min; e) detector conditions: excitation wavelength 310 nm and emission wavelength-375 nm/response-2.

Sample Set B was analyzed similarly except that the HPLC system was different. In this case, the concentration of the 2 dipeptides was analyzed using HPLC (Spectraphysics P 200 pump) /Valco injector valve (manual injection) with a fluorescent detector (Spectraphysics FL 2000) (Spectraphysics, Mountain View, CA). The column used for this sample set was the same as described previously. Data were collected by using a Shimadzu C-R5A chromatopac integrator (Milton Keynes, UK).

The HPLC conditions used were a) flow rate: 1.5 mL/min; b) mobile phase was same as described before; c) injection conditions (manual): 100 μL injection volume for standards and 30-min cycle time for standards, 200 μL injection volume for samples and 60-min cycle time for samples; d) derivatization: sample volume: 200 μL/reagent volume: 200 μL; e) detector conditions: excitation wavelength 310 nm / emission wavelength 376 nm/volt 600. The analysis for dipeptides was initially run as a single set analysis due to the time involved for each sample run (1 hour), number of samples and manual injection. In addition, 2 samples, DH1 and DH3, were run in duplicate and triplicate respectively for the purposes of repeatability and statistical analysis. This will be clearer from the discussion of Table 4.2 in which the standard deviation and relative standard deviation have been included. The samples were also spiked to confirm the retention times and the peaks of the 2 dipeptides. This was done by adding 5-20 μL of standard solution (100-400 ppm) in the SWW samples and derivatizing the sample as described in the preceding paragraph.

Statistical analysis was performed with the Statistical Analysis System (SAS Institute, Inc., 1988) using ANOVA and Duncan's Multiple Range Test and Student-Neuman-Kuel's (SNK) multiple range test.

**Results and Discussion**

The mean concentration of protein (ppm) is shown in **Table 2.1 (Fig. 2.2)**. The mean concentration of protein was highest in processing stages DH1 (30%) and DH2 (18%) followed by RS1, SP, RS2 and DH 3 (**Table 2.1, Fig. 2.2**). The trends and the amounts of the protein in SWW samples correspond with those already reported by other researchers (Lin et al., 1995; Huang and Morrissey, 1996). According to Lin et al. (1995), the levels of protein were found to be highest in the first dehydrator and successive stages revealed decreased levels of protein.

The concentration of anserine and carnosine in SWW at all stages of surimi processing (raw samples) was determined based on the standard curves obtained after HPLC analysis for both the dipeptides. The first 2 stages of surimi processing, DH1 (36%) and DH2 (22%) had the highest content of the 2 dipeptides as compared to the other stages (**Table 2.2, Fig. 2.3**) (Kaur et al., 1998).

Samples were freeze dried to determine the effects of this treatment on the concentration of the dipeptides. The content of the dipeptides decreased as compared to raw samples but the over all trend was almost similar, i.e, stages DH1 and DH2 had the highest amounts of dipeptides compared to the other samples. However, RS1 also showed higher amounts of dipeptides in this case (**Table 2.2, Fig. 2.4**). Freeze-drying reduced the content of dipeptides as compared the content of dipeptides in raw samples and therefore may not be a suitable method for their recovery. The particular reason for this decrease is unidentified at this time. However, possible reasons are high solubility of dipeptides in water and their low molecular weight, reaction of dipeptides with other

**Table 2.1-Protein content (ppm) of surimi wash water samples determined by the Lowry method**

| Sample name | Protein |
|---|---|
| Dehydrator 1[a] | 5266 ± 765 a[b,c] |
| Dehydrator 2 | 3206 ± 615 b |
| Dehydrator 3 | 1921 ± 192 d |
| Screw Press | 2320 ± 342 cd |
| Rotary Screen 1 | 2929 ± 389 bc |
| Rotary Screen 2 | 2022 ± 92 d |

[a]Samples were collected on 2 different days.
[b]Overall means are shown with standard deviation.
[c]a-d: treatment means in the same column for a given surimi wash water sample marked with different letter differs ($p \leq 0.05$).

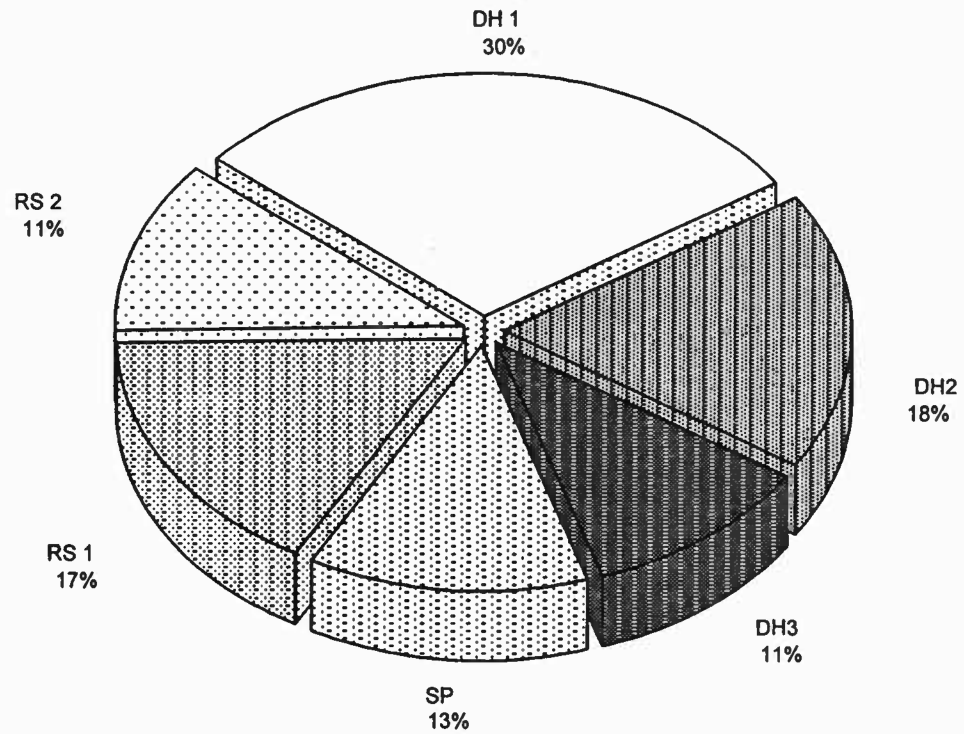



**Fig. 2.2-Proteins in surimi wash water samples (raw treatment)**

**Table 2.2-Concentration (ppm) of anserine and carnosine in randomly collected surimi wash water samples**

| Raw Samples | Anserine | Carnosine |
|---|---|---|
| DH1 | 424 ± 22 a[a,b] | 620 ± 25 a |
| DH2 | 154 ± 8 b | 468 ± 2 b |
| DH3 | 19 ± 3 e | 291 ± 18 d |
| SP | 61 d[d] | 239 e[d] |
| RS1 | nd f[c] | 371± 0.13 c |
| RS2 | 95 c[d] | 92 f[d] |
| **Freeze Dried Samples** | | |
| DH1 | 61 ± 16 abc | 162 ± 52 ab |
| DH2 | 86 ± 19a | 174 ± 46 a |
| DH3 | 52 ± 1 abc | 92 ± 5 bc |
| SP | 32 ± 9 c | 74 ± 9 c |
| RS1 | 69 ± 19 ab | 217 ± 14 a |
| RS2 | 38 ± 8 bc | 80 ± 10 c |
| **Raw Samples (Alaska)** | | |
| 1ST Wash | 101[d] | 1091[d] |
| 2ND Wash | nd[d] | 543[d] |
| Screw Press | 33[d] | 462[d] |
| Decanter | nd[d] | 1130[d] |

[a]Overall means are shown with standard deviation.
[b]a-f:Treatment means in the same column for a given SWW sample marked with different letter differs ($p \leq 0.05$).
[c]nd:not detectable.
[d]1measurement.

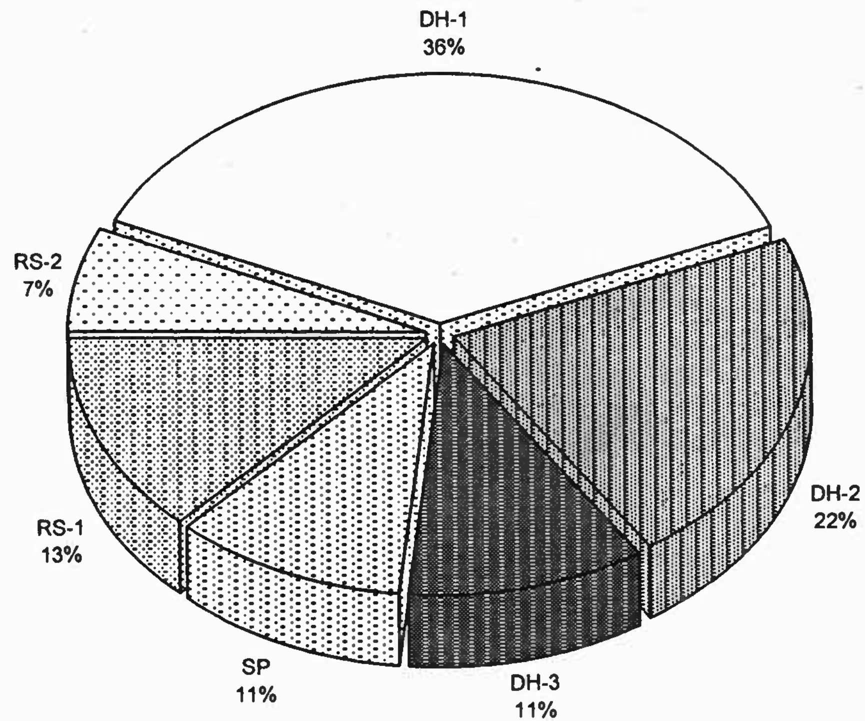



**Fig. 2.3-Total dipeptides in surimi wash water samples (raw treatment)**

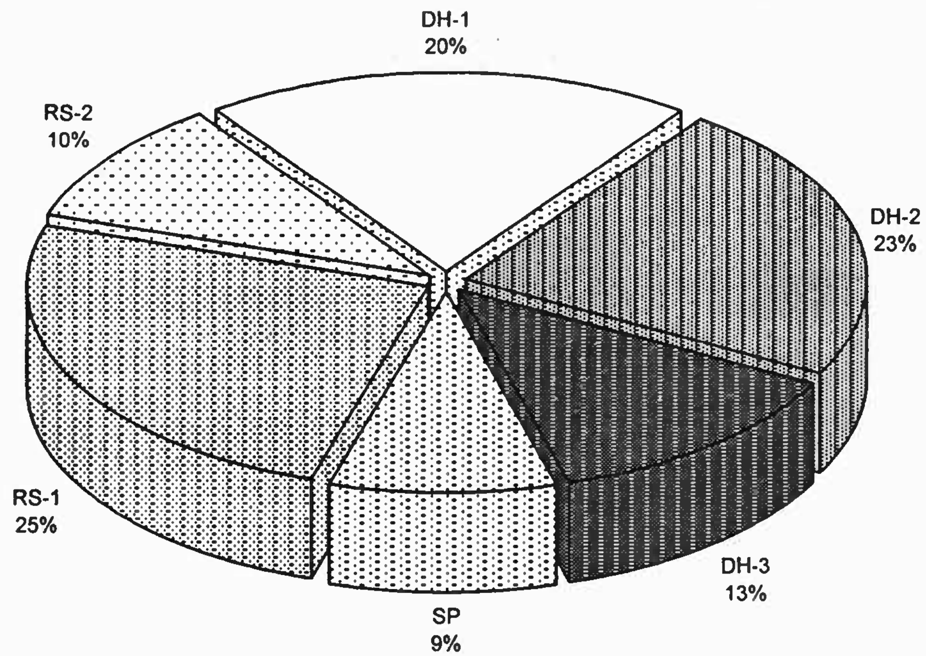



**Fig. 2.4-Total dipeptides in surimi wash water samples (freeze dried)**

chemicals and further breakdown to amino acids, presence of other interfering components such as pro-oxidants, enzymes, pigments and metals, etc.

The first and last stage in the processing of Alaskan Pollock samples showed higher concentrations of dipeptides than the other 2 stages (**Fig. 2.5**). The total dipeptide content of SWW samples obtained from Alaskan Pollock was similar to the Pacific Whiting (**Table 2.2**). This similarity may be because Alaskan Pollock and Pacific Whiting belong to the same family (Gadidae). However, Alaska SWW samples had a higher content of carnosine and a lower content of anserine whereas the SWW samples collected from Point Adams had both anserine and carnosine concentrations distributed almost evenly. This difference in SWW samples may be due to species specificity of anserine and carnosine as discussed previously. The concentration of dipeptides differs from various processing stages in different surimi processing plants. This difference occurs because of the variation in the plant layouts as well as processing parameters.

The concentration of dipeptides differs from various processing stages in a SWW sample collected from Point Adams on the same day. Sample set B also showed that stages DH1 and DH2 had higher amounts of dipeptides than the other samples (**Table 2.3**) ($P \leq 0.05$). The percent recovery of the spiked samples (**Table 2.3**) varied between 99.5 and 101.7% and was within the limits of analytical variability even though the samples had other compounds present in them. This confirms that HPLC with a fluorescent detector method is a viable method of determining the concentrations of carnosine and anserine.

The trend seen in the 2 data sets of raw samples obtained from Point Adams (**Table 2.2, 2.3**) was similar, even though the absolute numbers were different, i.e.,

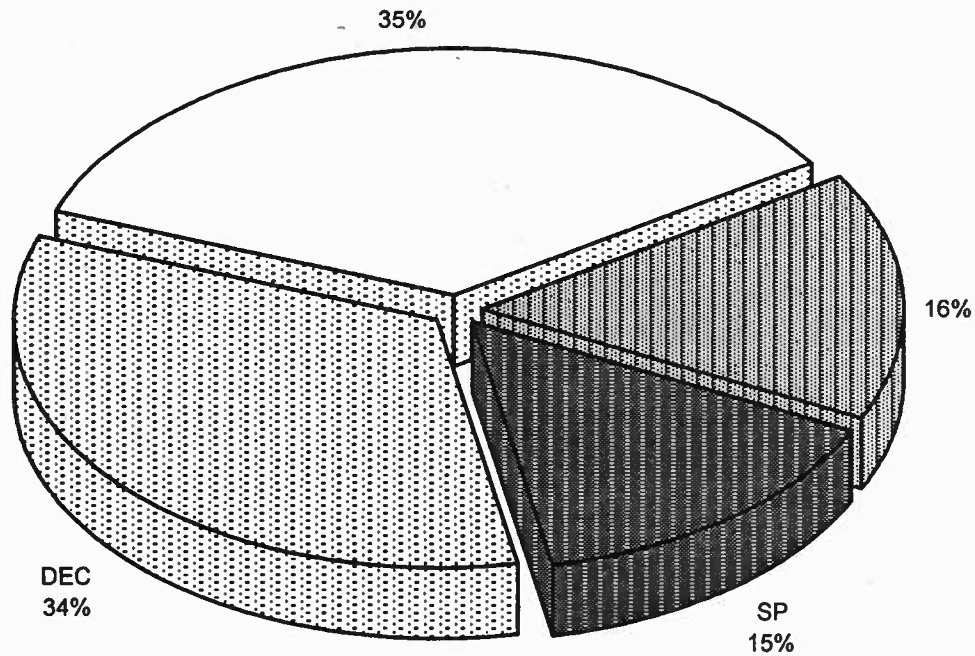



**Fig. 2.5-Total dipeptides in surimi wash water samples obtained from Alaska Pollock**

**Table 2.3-Concentration (ppm) of anserine and carnosine in a surimi wash water sample**

| Raw Samples | Number of Samples | Anserine | Carnosine | Percent recovery of spiked samples | |
|---|---|---|---|---|---|
| | | | | Anserine | Carnosine |
| DH1 [a] | 2 | 243 a[b] | 339 a | 101.7 | 96.9 |
| DH2 | 1 | 99 b | 123 b | | |
| DH3 [a] | 3 | 81 c | 110 c | 99.5 | 100.6 |
| SP | 1 | 58 d | 42 d | | |
| RS1 | 1 | 21 e | 38 d | | |
| RS2 | 1 | nd[c] | nd | | |

[a] spiked samples.
[b] a-e: If any two enteries in the same column are followed by different letters, this indicates significant difference by SNK test ($p \leq 0.05$).
[c] not detectable.

the total concentration of the dipeptides in the first 2 stages of surimi processing was higher than that of the other stages. This may be because SWW samples are biological samples and the differences may be caused by various reasons, such as the samples were collected and processed on different days. The variability and conflicting data on an individual run may indicate seasonal differences and/or variability in sampling or methodology, i.e., the samples in the first data set were collected on 3 different days whereas the samples of the second data set were collected and processed in a single day. The dipeptides are antioxidants and can possibly be affected by microbial contamination, chemical reaction with other compounds in wash water, enzymatic changes, physical damage, possible breakdown to other components and reaction with proteins. For example, the surimi processing plant was cleaner on some days than others. Fewer contaminants were present in the SWW samples collected after the weekly cleaning versus a day before. There were differences in the 2 instruments used (HPLC and detector); age of the columns used; temperature maintenance of the column; injecting solutions; and derivatization technique during the experiment. In the first case, the samples were derivatized by the auto sampler itself whereas in second case, the sample and reagent were mixed manually and injected. The injector, manual or auto, sampler may have had possible contamination from the surfaces, pipettes, etc.

Not only the trend of the protein and dipeptides in raw samples was similar but also the trends of the 2 dipeptides in both data sets (Sample Set A and B) were similar. In other words, stages DH1 and DH2 contained the highest amounts of proteins as well as dipeptides, compared to the other stages (**Table 2.1, 2.2, 2.3**). Therefore, it is proposed that before the dipeptides are analyzed, the protein content should be determined. This

will help to evaluate which particular stage of surimi processing plant will have a higher content of anserine and carnosine or both (Kaur et al., 1998). This can save time and cost in the long run in running dipeptide analysis.

The content of dipeptides has not been determined in SWW so far. However, the logical comparison can be made for confirmation purposes on the basis of hierarchy of categories of fishes. The SWW is made from Pacific Whiting (Gadidae), which belongs to Order 16, whereas eels (Anguilidae) belong to Order 10 and the tuna (Scrombidae) belong to Order 20 (Bond, 1965). The amounts of dipeptides detected in SWW are within range of the 2 extremes of the amounts found in the fish species of Order 10 and Order 20 as described earlier (Bond, 1965; Suzuki et al., 1990). The content of anserine and carnosine is within the range of the contents of the 2 dipeptides found in fish species as discussed before. Additionally, the range of anserine and carnosine in Scrombidae species varies between 0 and 3550 ppm. In other fish species, the dipeptides concentration ranging between 0 and trace has also been reported (Suzuki et al., 1990). When compared with other sea animals in general, the numbers of dipeptides are within the limits of the amounts indicated in literature (Crush, 1970; Choi et al., 1996). The advantage of collecting dipeptides from SWW is that the surimi process itself readily removes these water-soluble compounds. Consequently, the economical feasibilty of dipeptide recovery in SWW should be greater than that of other processing systems.

**Summary**

Anserine and carnosine are natural antioxidants found in the skeletal muscles of fish. These dipeptides are water-soluble and help in reducing lipid oxidation that affects flavor, aroma, texture, color and nutritional composition. Determining the presence of these dipeptides in SWW is the first step in demonstrating their potential use as antioxidants. Protein, anserine and carnosine were analyzed using the Lowry method and HPLC with a fluorescent detector. From this experiment, it is determined that SWW may be an economical source for these dipeptides. Results showed that the SWW samples with a higher amount of total protein also had higher amounts of dipeptides. However, due to SWW being a biological sample and dipeptides being antioxidants, the concentration of dipeptides can vary from 1 day to another and 1 surimi processing plant to another. This experiment was the first step in establishing that SWW extract can be obtained from surimi waste.

**References**

Abe, H. 1995. Histidine-related dipeptides: distribution, metabolism, and physiological function. Ch. 14 in *Biochemistry and Molecular Biology of Fishes*, Vol. 4, P.W. Hochachka and T.P. Mommsen (Eds.), p. 309-333.

Aursand, M., Jorgensen, L., and Grasdalen, H. 1995. Quantitative high-resolution C nuclear magnetic resonance of anserine and lactate in white muscle of Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. 112B(2): 315-321.

Boldyrev, A.A. and Severin, S.E. 1990. The histidine-containing dipeptides, carnosine and anserine: distribution, properties and biological significance. Adv. Enzyme Regul. 30: 195.

Bond, C.E. 1965. Outline of classification of fishes. Unpubl. Rep., Dept. of Fisheries and Wildlife, Oregon State Univ., Corvallis.

Chan, K.M. and Decker, E.A. 1994. Endogenous skeletal muscle antioxidants. Crit. Rev. Food Sci. and Nutr. 34: 403-426.

Chan, K.M., Decker, E.A., and Means, W. 1993. Extraction and activity of carnosine, a naturally occurring antioxidant in beef muscle. J. Food Sci. 58: 1.

Choi, J.Y., Kim, I., Lee, K., Kim, G., Lee, N., and Cho, Y. 1996. Available components of cooking drips, dark muscle, head and raw viscera from skipjack. J. Korean Fish. Soc. 29: 701-708.

Chung, H.Y. and Cadwallader, K.R. 1993. Volatile components in blue crab (*Callinectes sapidus*) meat and processing by-product. J. Food Sci. 58: 1203-1207, 1211.

Crush, K.G. 1970. Carnosine and related substances in animal tissues. Comp. Biochem. Physiol. 34: 3.

Dawson, R.M.C., Elliot, D.C., Elliot, W.H., and Jones, K.M. (Eds.) 1969. *Data for Biochemical Research.* Oxford Univ. Press, Oxford, U.K.

Decker, E.A. 1998. Personal communication. Univ. of Massachusetts, Amherst.

Haard, N.F., Simpson, B.K., and Sikorski, Z.E. 1994. Biotechnological applications of seafood proteins and other nitrogenous compounds. In *Seafood Proteins*, Z.E. Sikorski, B.S. Pan, and F. Shahidi (Ed.), p. 194-216. Chapman & Hall Publ., New York.

Huang, L. and Morrissey, M.T. 1996. Heat-coagulation of proteins from washed mince of Pacific whiting. Presented at the 48th Ann. Mtg., Pacific Fisheries Technologists, San Diego, CA., Feb. 18-21.

In, T. 1990. Seafood flavorants produced by enzymatic hydrolysis. In *Making Profits Out of Seafood Wastes*, S. Keller (Ed.), p. 197. Alaska Sea Grant Publ., Fairbanks.

Johnson, P. and Hammer, J. 1992. Histidine containing dipeptide levels in aging and hypertensive rat skeletal and cardiac muscles. Comp. Biochem. Physiol. 103B(4): 981-984.

Kaur, J., Morrissey, M.T., Decker, E.A., and Yang, L. 1998. Concentration of anserine and carnosine in surimi wash water. Presented at Ann. Mtg., Inst. of Food Technologists, Atlanta, GA, June 20-24.

Keller, S. (Ed.). 1990. Making profits out of seafood wastes. Proc. of the Int. Conf. on Fish By-products, Alaska Sea Grant College Program, Fairbanks.

Kohen, R., Yamamoto, Y., Cundy, K.C., and Ames, B. 1988. Antioxidant activity of carnosine, homocarnosine, and anserine present in muscle and brain. Proc. Natl. Acad. Sci. USA 85: 3175.

Lee, C. 1984. Surimi process technology. Food Technol. 38(11): 69-80.

Li, S.J., Morrissey, M.T., and Seymour, T. 1994. Isolation of a natural antioxidant from shrimp waste. Presented at Ann. Mtg., Oil Chemists Soc., Atlanta, GA, May 9-12.

Lin, T.M., Park, J., and Morrissey, M.T. 1995. Recovered protein and reconditioned water from surimi processing waste. J. Food Sci. 60(1): 4-9.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Morrissey, M.T., Peters, G., and Sylvia, G. 1992. Quality issues in the Pacific whiting fisheries. In *Pacific Whiting: Processing, Marketing and Quality Assurance*, G. Sylvia and M.T. Morrissey (Ed.), p. 9-16. Oregon Sea Grant, Corvallis, OR.

Simpson, B. D. and Haard, N. F. 1985. The use of proteolytic enzymes to extract carotenoproteins from shrimp wastes. J. Appl. Biochem. 7: 212.

Skaugrud, O. and Sargent, G. 1990. Chitin and chitosan: crustacean biopolymers with potential. In *Making Profits Out of Seafood Wastes*, S. Keller (Ed.), p. 61-70. Alaska Sea Grant Publ., Fairbanks.

Statistical Analysis System. 1988. SAS Institute Inc., Cary, N.C.

Suzuki, T., Shirai, T., and Hirano, T. 1990. Distribution of extractive nitrogenous constituents in white and dark muscles of fresh-water fish. Comp. Biochem. Physiol. 96B(1): 107-111.

Suzuki, T., Shirai, T., and Hirano, T. 1991. Anserine and free amino acid contents in the egg and fry stages of developing rainbow trout (*Onchorhynchus mykiss*). Comp. Biochem. Physiol. 100A(1): 105-108.

Teahon, K., and Rideout, J.M. 1992. A sensitive and specific high performance liquid chromatographic assay for imidizole dipeptides and 3-methylhistidine in human muscle biopsies, serum and urine. Biomed. Chromatogr. 6: 16.

# Chapter 3

# EFFECT OF HEAT TREATMENT ON THE CONCENTRATION OF DIPEPTIDES OBTAINED FROM SURIMI WASH WATER


## Abstract

Anserine and camosine are water-soluble dipeptides found in significant amounts in the skeletal muscle of fish, and their presence in surimi wash water (SWW) has been confirmed. Surimi wash water samples contain pro-oxidative metals and heme-containing proteins that decrease antioxidant activity. In order to produce an effective antioxidant extract from skeletal muscle and to reduce the level of pro-oxidants, extraction techniques need to be investigated. Therefore in this experiment, the effects of 3 different heat treatments (60, 80 and 100°C) were evaluated. The Lowry method and high performance liquid chromatography with a fluorescent detector were used to determine the levels of protein and dipeptides in heat-treated SWW. The highest content of protein and dipeptides was found in Dehydrator 1 (DH1) and Dehydrator 2 (DH2) processing stages. Based on dipeptides obtained from the DH1 and DH2 samples and the removal of protein, 80°C was found to be the best treatment for the recovery of dipeptides, followed by 100°C. This experiment also confirms findings of the previous study that protein and dipeptide follow similar trends, i.e., the first 2 stages, DH1 and DH2, of SWW samples have the highest concentrations of anserine and camosine. Therefore, it is recommended that it is better to establish the content of proteins in SWW before analyzing dipeptides.

**Introduction**

Anserine and carnosine are water-soluble dipeptides found in surimi wash water (SWW). The SWW samples also contain pro-oxidative metals and heme-containing proteins that decrease antioxidant activity (Chan and Decker, 1994). According to Chan et al. (1993), "techniques are needed to reduce the level of pro-oxidants while maintaining high levels of carnosine to produce an effective antioxidant extract from skeletal muscle." An antioxidant extract having a high concentration of the 2 dipeptides can be produced when heme-containing proteins are removed through heat precipitation at selective temperatures.

Lipid oxidation is a major cause of food deterioration affecting color, flavor, texture and nutritional value (Chan et al., 1993). Atherosclerosis may be caused in part by toxic substances formed during lipid oxidation (Addis and Park, 1989). Numerous antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are used in the food industry to control lipid oxidation. There is an increased demand by consumers for natural products. Natural antioxidants are available in the form of herbs and spices (Ramanathan and Das, 1993) and tocopherol, which have a limited use due to high cost, color and flavor attributes (Chan et al., 1993). The formation of non-enzymatic browning products as a result of drying extracts lead to increased antioxidant activity (Bailey, 1988; Ramanathan and Das, 1993).

Carnosine and anserine are present in large amounts in skeletal muscle of different animals including fish. These dipeptides are capable of inhibiting lipid oxidation catalyzed by iron, hemoglobin, lipoxidase and singlet oxygen in vitro (Decker

and Faraji, 1990). According to Decker and Crum (1991), carnosine effectively inhibits the oxidative rancidity in uncooked salted ground pork during frozen storage and reduces red color loss. Carnosine has been found to reduce lipid oxidation in cooked ground pork during refrigerated storage (Decker and Crum, 1993). Numerous water-soluble components lead to lipid oxidation: active oxygen species, transition metals, heme-containing proteins, and myoglobin and hemoglobin in skeletal muscles (Ashghar et al., 1988; Kanner et al., 1988; Decker and Hultin, 1992). Antioxidants are helpful in chelating iron and inactivating heme proteins and scavenging free radicals in seafood (Flick et al., 1992; Kelleher et al., 1994). As mentioned earlier, before the SWW extract that has antioxidant activity is produced, it is important to evaluate different techniques used to remove heme-containing proteins and iron. This is because the heme-containing proteins and iron affect the concentration and antioxidant activity of the dipeptides. Therefore, the objective of this experiment was to study the effect of heat treatments on SWW samples at different temperatures and to evaluate which of these treatments is most effective.

**Materials and Methods**

Surimi wash water (SWW) samples were collected from Point Adams Co., Warrenton, Oregon. The SWW samples were collected in clean 1-liter bottles and transported on ice to Oregon State University, Seafood Laboratory, Astoria, Oregon. The samples were identified based on the surimi processing stage at which they were

collected: DH1 (Dehydrator 1), DH2 (Dehydrator 2), DH3 (Dehydrator 3), SP (Screw Press), RS1 (Rotary Screen 1) and RS2 (Rotary Screen 2). The concentration of total protein was analyzed in all samples using the Lowry method (Lowry et al., 1951). The reagents were obtained from Sigma Chemical Co., St. Louis, MO. The concentration (ppm) of the 2 dipeptides, anserine and carnosine, in all the stages of SWW was determined using high performance liquid chromatography (HPLC) with a fluorescent detector. Water was used as the control in this experiment.

The SWW samples (Sample Set A) (50 mls) were heat treated at 60, 80 or 100°C for 10 minutes in a water bath (1120 Lindberg Bluem, Rochester, NY), centrifuged at 7000 x g for 30 minutes at 4°C (Sorvall RC-5B refrigerated superspeed centrifuge, Newton, CT). The supernatant was filtered through filter paper (Whatman 41). The filtrates were put in vials (Wheaton disposable scintillation vials), vacuum packed and frozen at -80°C until further analysis (Chan et al., 1993). All SWW samples were collected and processed from Point Adams over a period of 3 different days. Each SWW sample had 2 replicates.

Before analysis with HPLC, all the samples were uniformly treated according to the procedure described by Teahon and Rideout (1992) (Decker, 1998), and Ortho-phthaldehyde (OPA) was used as a derivatizing agent (Sigma Chemical Co., St. Louis, MO). Standard solutions ranging from 0 to 1000 ppm of carnosine and anserine were prepared (Sigma Chemical Co., St. Louis, MO). The concentration of the 2 dipeptides was analyzed using HPLC with a fluorescent detector and auto sampler (Perkin Elmer, Norwalk, CT). The system and the conditions used were the same as described in the Materials and Methods section of previous study.

In order to study the effect of each heat treatment specifically, the second set (Sample Set B) of SWW samples (1 each) was collected from 6 different stages of SWW processing from Point Adams in a single day and processed at the 3 heat treatments (60, 80 and 100°C) on the same day. The samples were frozen at -80°C until further analysis. This set of samples was analyzed as described above, except that the HPLC system used was Spectraphysics (Spectraphysics, Mountain View, CA) and the samples were injected manually as described in the Materials and Methods section of previous study. For this set, the analysis for dipeptides was initially run as a single set analysis due to the time involved for each sample run (1 hour), number of samples and manual injection. In addition, Raw: dehydrator 1 (2 reps) and dehydrator 3 (3 reps); 60°C: rotary Screen 1 (3 reps); 80°C: rotary Screen 2 (3 reps); and 100°C: dehydrator 1(3 reps) and Dehydrator 2 (2 reps) were run for the purposes of evaluating repeatability and statistical analysis. This will be clearer from the discussion of Table 4.2. The samples were also spiked to confirm the retention times and the peaks of the 2 dipeptides as described in the preceding chapter. Statistical analysis was performed with Statistical Analysis System (SAS Institute, Inc., 1988) using ANOVA, Duncan's Multiple Range Test and Student-Neuman-Keul's (SNK) multiple range test.

**Results and Discussion**

The first 2 stages of SWW processing, DH1 and DH2, contained higher amounts of proteins than the other 4 stages (**Table 3.1, Fig 3.1**). The trends are similar to those of

**Table 3.1-Protein content (ppm) of surimi wash water samples determined by the Lowry method**

| Sample Name | Raw | 60°C | 80°C | 100°C |
|---|---|---|---|---|
| Dehydrator 1 | 5266 ± 765a[a,b] | 3393 ± 530 a | 3771 ± 420 a | 2801 ± 86 a |
| Dehydrator 2 | 3206 ± 615 b | 2634 ± 634 b | 1152 ± 17 b | 1250 ± 53 b |
| Dehydrator 3 | 1921 ± 192 d | 907 ± 146 d | 400 ± 11 c | 457 + 21 c |
| Screw Press | 2320 ± 342 cd | 1861 ± 151 c | 1166 ± 140 b | 535 ± 254 c |
| Rotary Screen 1 | 2929 ± 389 bc | 1322 ± 192 cd | 613 ± 88 c | 942 ± 18 b |
| Rotary Screen 2 | 2022 ± 92 d | 1090 ± 145 d | 212 ± 170 c | 1152 ± 205 b |

[a]Overall means are shown with standard deviation.
[b]a-d: treatment means in the same column for a given surimi wash water samples marked with different letter differs ($p \leq 0.05$).

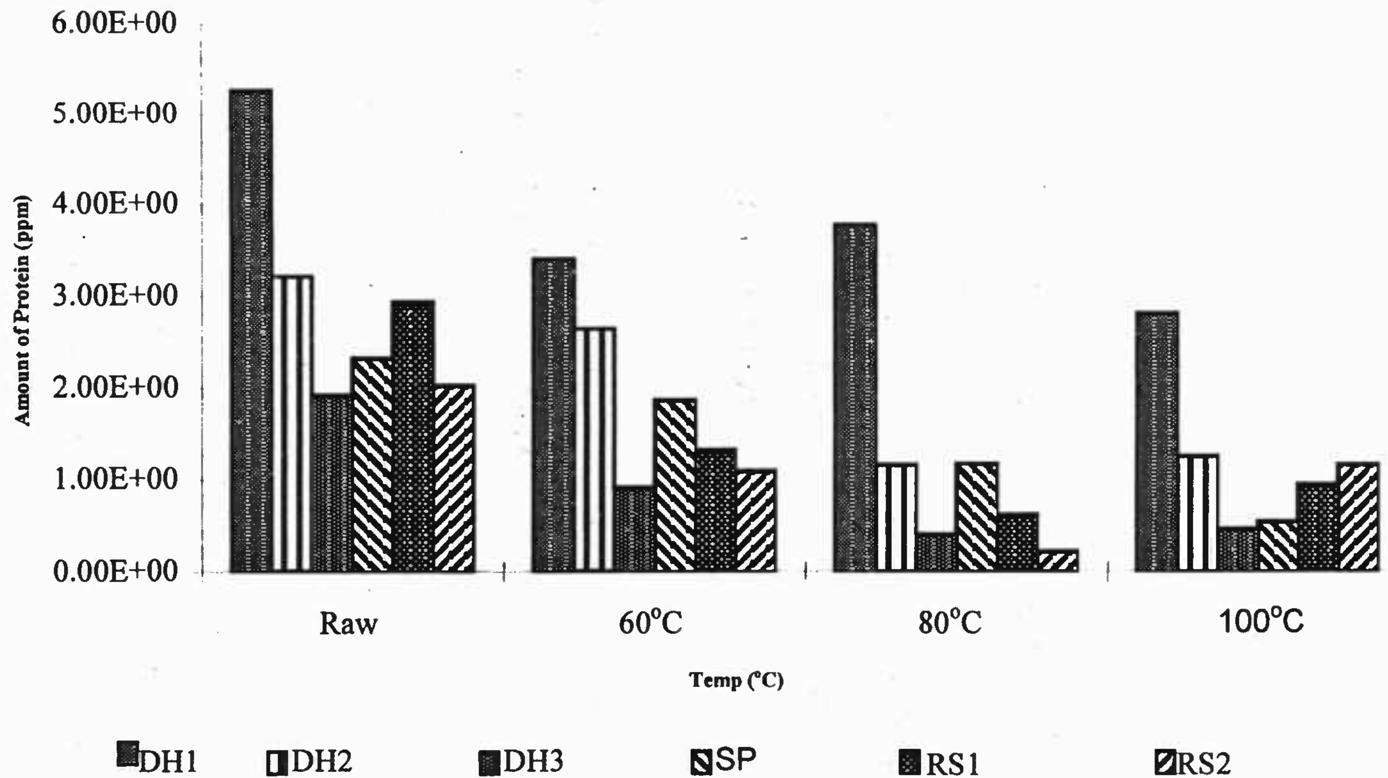



**Fig. 3.1-Comparison of proteins (ppm) of all surimi wash water samples treated at different temperatures**

the previous experiment, i.e., the first 2 stages of surimi processing had higher amounts of proteins at all 3 heat treatment levels (60°, 80° and 100°C). According to Huang (1997), the majority of the heat sensitive proteins are removed at temperatures above 70°C. In this experiment, the protein content decreased with different heat treatments as compared to control SWW samples, with the higher heat treatments (80 and 100°C) showing the greatest decrease **(Table 3.1, Fig 3.1).**

In the first data set (Sample Set A) anserine and carnosine concentrations were determined for all heat-treated SWW samples. DH1 and DH2 samples contained significantly more of these 2 dipeptides compared to the other SWW samples **(Table 3.2, Fig. 3.2).** Dipeptide contents of raw SWW samples from a previous study have been included for comparison purposes **(Table 3.3)** (Kaur et al., 1998). The results of the heat-treated SWW samples showed the same trends as found in the raw samples. Therefore, the comparison of relative amounts of dipeptides was only done for DH1 and DH2 for the raw and all 3 heat treatments **(Fig. 3.3).** The trends for dipeptides content were also similar to protein **(Fig. 3.1, 3.3).** The relative amounts (%) of total dipeptides (anserine and carnosine) in SWW after heat treatments remained comparable to the raw samples. The relative amounts were calculated by adding the absolute amounts of the 2 dipeptides together and changing them to percentage in relation to the total amount of dipeptides for that particular treatment. This helps to evaluate the trends concisely **(Fig. 3.3).** This indicates that heat treatment may be used to precipitate proteins which can then be removed by either centrifuging or filtering out from the wash water and help recover the dipeptides. To study the effect of each heat treatment on individual SWW wash water samples, the samples were collected from different stages in a single day and

**Table 3.2-Concentration (ppm) of anserine and carnosine in heat-treated surimi wash water samples**

| Samples | Anserine | Carnosine |
|---|---|---|
| **Raw** | | |
| DH1 | 424 ± 22a[a,b] | 620 ± 25 a |
| DH2 | 154 ± 8b | 468 ± 2 b |
| DH3 | 19 ± 3e | 291 ± 18 d |
| SP | 61d[d] | 239 e[d] |
| RS1 | nd f[c] | 371 ± 0.13 c |
| RS2 | 95 c[d] | 92 f[d] |
| **60°C** | | |
| DH1 | 229 ± 5 a | 688 ± 11 a |
| DH2 | nd c[c] | 259 ± 24 c |
| DH3 | nd c | 327 ± 10 b |
| SP | nd c | 275 ± 9 c |
| RS1 | nd c | 153 ± 33 d |
| RS2 | 26 ± 6 b | 41 ± 5 e |
| **80°C** | | |
| DH1 | 542 ± 65 a | 548 ± 88 a |
| DH2 | 373 b[d] | 516 a[d] |
| DH3 | 166 ± 4 c | 209 ± 13 b |
| SP | 189 ± 5 c | 279 ± 27 b |
| RS1 | 141 ± 2 cd | 187 ± 60 b |
| RS2 | 69± 0.19 d | 25 ± 1 c |
| **100°C** | | |
| DH1 | 719 ± 26 a | 526 ± 60 a |
| DH2 | 363 ± 21 b | 515 ± 28 a |
| DH3 | 259 ± 16 d | 327 ± 62 bc |
| SP | 12 ± 2 f | 317 ± 22 bc |
| RS1 | 187 ± 0.22 e | 247 ± 17 c |
| RS2 | 314 c[d] | 387 b[d] |

[a] Overall means are shown with standard deviation.
[b]a-f: treatment means in the same column for a given sww sample marked with different letter differs ($p \leq 0.05$).
[c] nd: not detectable.
[d] 1 Measurement.

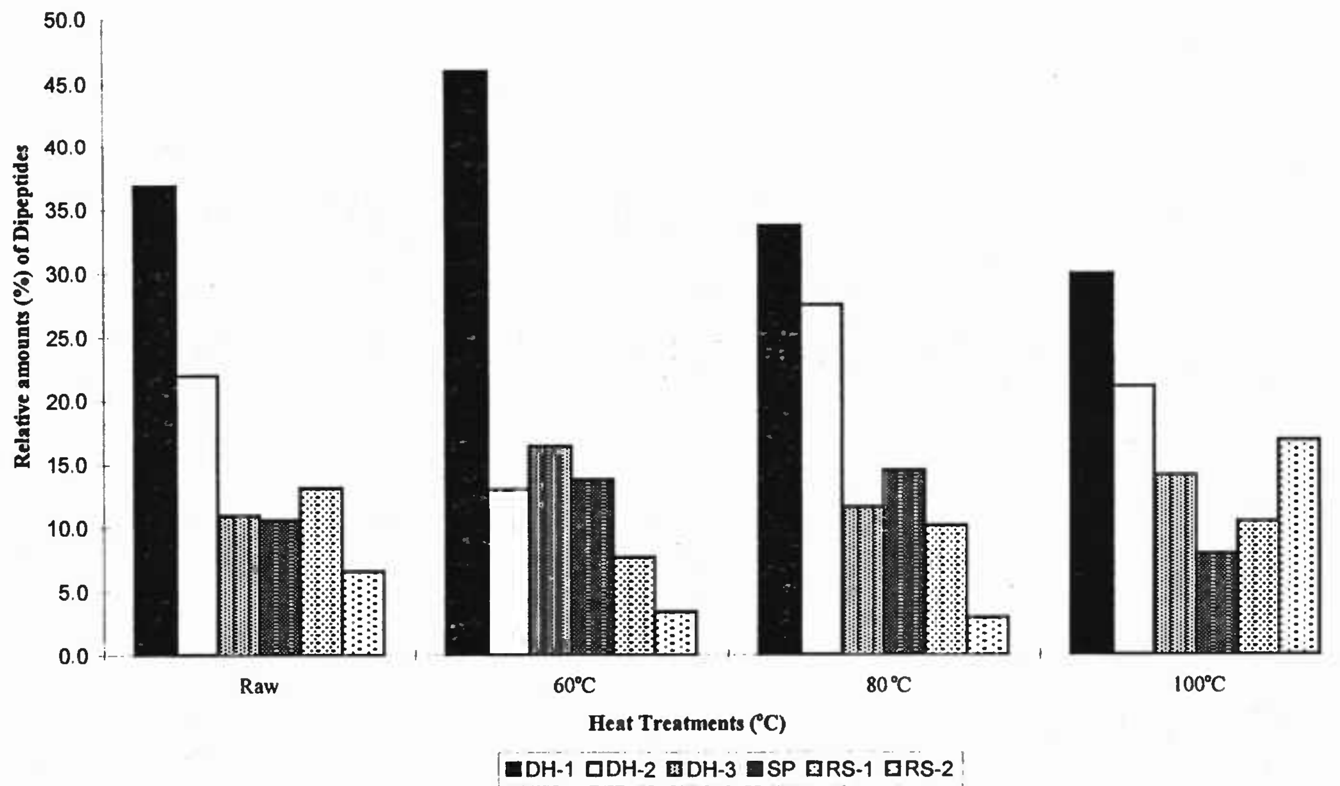



Fig 3.2-Relative amounts (%) of total dipeptides in heat-treated surimi wash water samples (Sample Set A)

**Table 3.3-Concentration (ppm) of anserine and carnosine in surimi wash water samples collected from six stages: percent recovery of spiked samples**

| Samples | Number of samples | Anserine | Carnosine | Anserine (% recovery) | Carnosine (% recovery) |
|---|---|---|---|---|---|
| **Raw** | | | | | |
| Dehydrator 1 *[a] | 2 | 243 a[b] | 339 a | 101.7 | 96.9 |
| Dehydrator 2 | 1 | 99 b | 123 b | | |
| Dehydrator 3 * | 3 | 81 c | 110 c | 99.5 | 100.6 |
| Screwpress 1 | 1 | 58 d | 42 d | | |
| Rotary Screen 1 | 1 | 21 e | 38 d | | |
| Rotary Screen 2 | 1 | nd e[c] | nd e | | |
| **60°C** | | | | | |
| Dehydrator 1 | 1 | 199 a | 246 a | | |
| Dehydrator 2 | 1 | 88 b | 116 b | | |
| Dehydrator 3 | 1 | 66 d | 53 d | | |
| Screwpress 1 | 1 | 74 c | 89 c | | |
| Rotary Screen 1 | 3 | 14 e | 25 e | 108.0 | 104.1 |
| Rotary Screen 2 | 1 | nd f | 2 f | | |
| **80°C** | | | | | |
| Dehydrator 1 | 1 | 187 a | 240 a | | |
| Dehydrator 2 | 1 | 121 b | 162 b | | |
| Dehydrator 3 | 1 | 37 d | 43 d | | |
| Screwpress 1 | 1 | 63 c | 81 c | | |
| Rotary Screen 1 | 1 | 14 e | 25 e | | |
| Rotary Screen 2 | 3 | 22 f | 31 e | | |
| **100°C** | | | | | |
| Dehydrator 1 | 3 | 111 a | 124 a | 101.0 | 106.0 |
| Dehydrator 2 | 2 | 54 c | 18.8 c | 99.6 | 99.5 |
| Dehydrator 3 | 1 | 1 e | 3 d | | |
| Screwpress 1 | 1 | 75 b | 101 b | | |
| Rotary Screen 1 | 1 | 14 d | 27 c | | |
| Rotary Screen 2 | 1 | nd | nd | | |

[a] spiked samples.
[b]a-f: If the enteries in the same column are followed by different letter, this indicates significant difference by SNK test ($p \leq 0.05$).
[c]nd: not detectable.

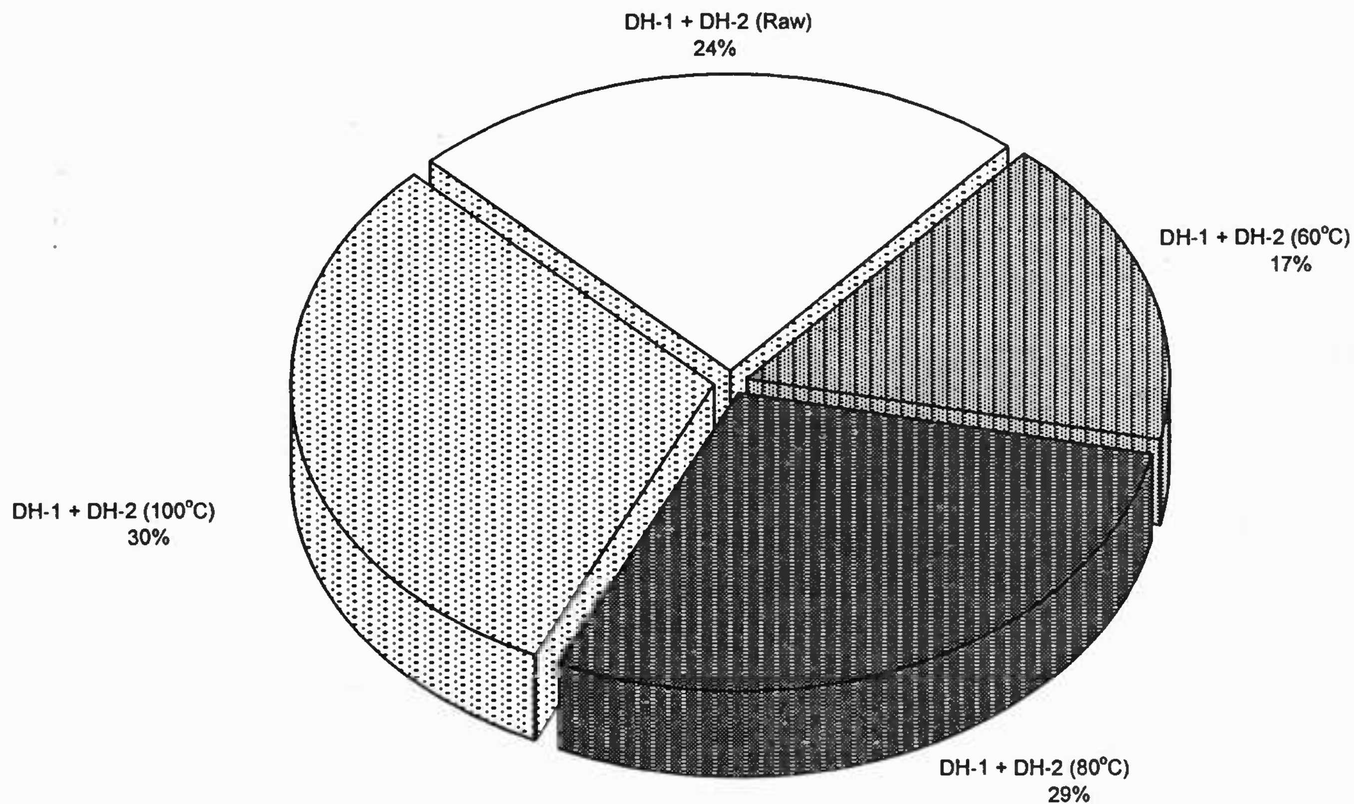



**Fig. 3.3-Comparison of relative amounts (%) of dipeptides over heat treatments of Dehydrator 1 and Dehydrator 2**

each of the samples was exposed to different temperatures (60, 80, 100°C) on the same day (Sample Set B). The total dipeptides content was higher in DH1 and DH2 than all the other stages of SWW samples except the samples tested at 100°C. In the 100°C treatment, the content of dipeptides in the SP stage was also high.

Percent recovery of spiked samples shows the accuracy of sample testing and dipeptide recovery. For all the spiked samples in the concentration range of 0-243 ppm of anserine and 0-339 ppm of carnosine, the range of recovery was 99.5-108% and 96.9-106% respectively. The percent recovery for spiked samples was within the limits of analytical variability of both high and low dipeptide concentrations **(Table 3.3)** (Kaur et al., 1999).

The overall trend of Sample Set B showed that the concentration of dipeptides was higher in first 2 stages of surimi processing than that of other stages. This trend was similar to Sample Set A even though the absolute numbers were different. Statistical analysis also confirmed this trend **(Table 3.3, Fig. 3.4)**. DH1 and DH2 were again combined for comparison of relative amounts of total dipeptides to evaluate the effect of different heat treatments **(Fig. 3.5)**. The 80°C treatment had a higher dipeptide concentration than those of the other heat treatments. When the first data set was compared to the second **(Fig. 3.3)**, the relative amount of dipeptides was 29% at 80°C for both the sample sets.

There are various reasons for similar trends and different concentrations of total dipeptides in the first and second data sets: samples in the first set were collected and processed randomly and, in the second case, 1 SWW sample was collected and exposed to 3 heat treatments on a single day. Dipeptides are antioxidants and can possibly be

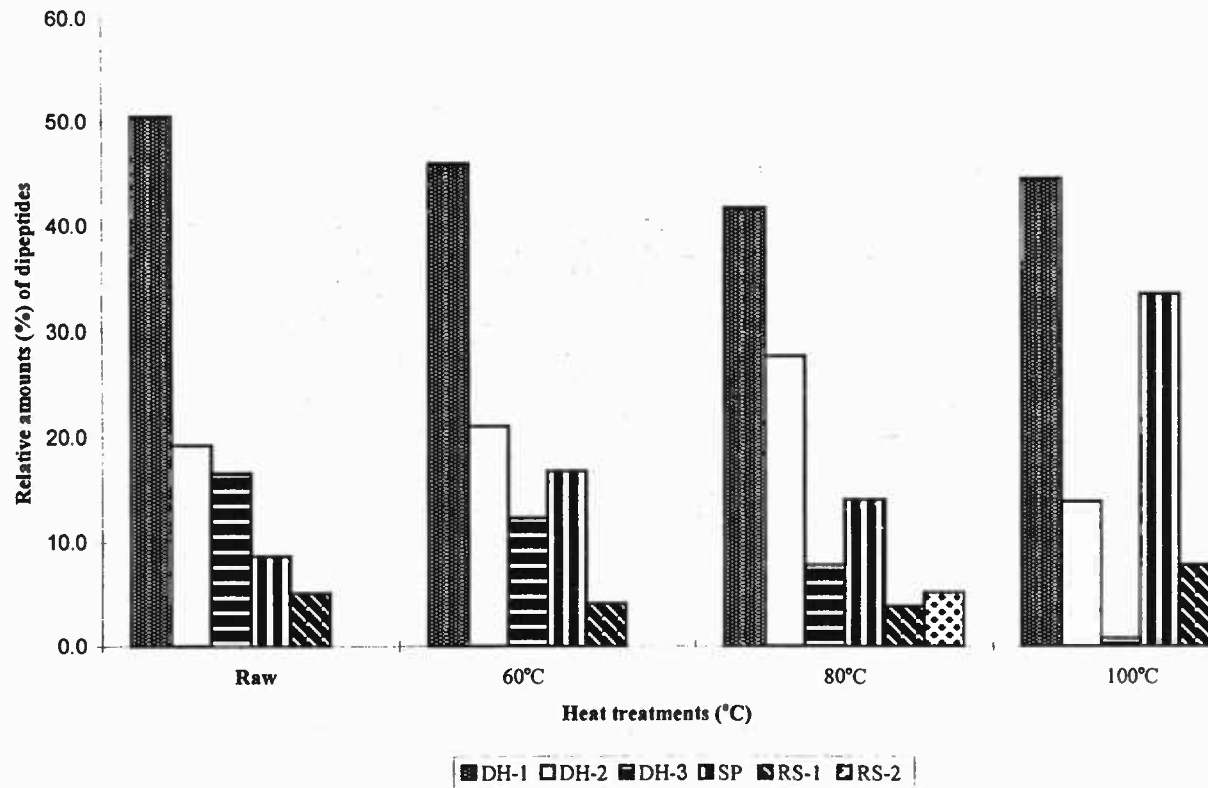



**Fig 3.4-Relative amounts (%) of dipeptides from surimi wash water sample (Sample Set B) treated at different temperatures**

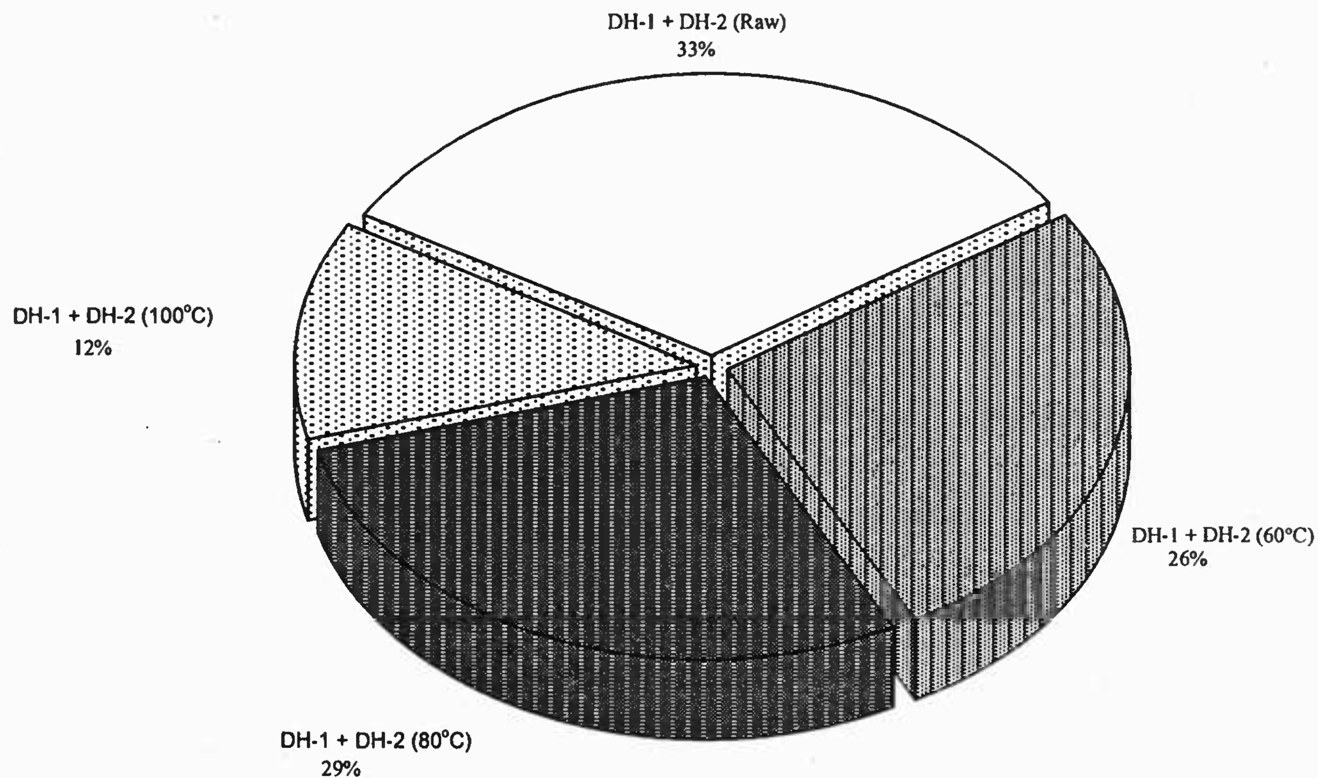



**Fig. 3.5-Comparison of relative amounts (%) of dipeptides in surimi wash water samples collected at the first 2 stages.**

affected by microbial contamination, chemical reaction with other compounds in wash water, enzymatic changes, physical damage, possible breakdown to other components and reaction with proteins. For example, the surimi processing plant was cleaner on some days than others. There were differences in 2 instruments used (HPLC and detector), age of the columns used, temperature maintenance of the column, injecting solutions, derivatization technique during the experiment, whether the injector is manual or auto sampler, and there may have been contamination from the surfaces and pipettes, etc.

Of all the heat treatments tested, the 80°C treatment was found to be the best followed by 100°C. These results coincide with those obtained by Chan et al. (1993); they found that the 100°C treatment was more effective than 60°C with beef extracts for the recovery of dipeptides.

**Summary**

Surimi wash water samples were obtained from Point Adams and were exposed in 2 different trials to 3 different heat treatments: 60, 80 and 100°C respectively. In the first data set (Sample Set A), the samples were collected over several days; in the second set the SWW samples were collected during a single day's surimi run. Protein content was determined for all SWW samples. Results showed that the trends of the relative amounts of proteins and total dipeptides were similar, i.e., the first 2 stages of SWW (DH1 and DH2) had higher amounts of proteins and dipeptides compared to the other SWW

samples. Also, the 2 data sets showed similar trends for dipeptides as described above; however, absolute numbers (concentrations) determined were different. The 80°C heat treatment was the most effective.

**References**

Addis, P.B. and Park, S.W. 1989. Role of lipid oxidation products in atherosclerosis. In *Food Toxicology: A perspective on the Relative Risks*. S.L. Taylor and R.A. Scanlan (Ed.), p. 297. Marcel Dekker, Inc., New York.

Ashghar, A., Gray, J.I., Buckley, A.M., Pearson, A.M., and Booren, A.M. 1988. Perspectives on warmed-over-flavor. Food Technol. 42(6): 102.

Bailey, M.E. 1988. Inhibition of warmed-over-flavor, with emphasis on Milliard reaction products. Food Technol. 42(6): 123.

Chan, K.M. and Decker, E.A. 1994. Endogenous skeletal muscle antioxidants. Crit. Rev. Food Sci. and Nutr. 34: 403-426.

Chan, W.K.M., Decker, E.A., and Means, W. 1993. Extraction and activity of carnosine, a naturally occurring antioxidant in beef muscle. J. Food Sci. 58(1): 1.

Decker, E.A. 1998. Personal communication. Univ. of Massachusetts, Amherst.

Decker E.A. and Crum, A.D. 1991. Inhibition of oxidative rancidity in salted ground pork by carnosine. J. Food Sci. 56: 1179.

Decker E.A. and Crum, A.D. 1993. Antioxidant activity of carnosine in cooked ground pork. Meat Sci. 34: 245.

Decker, E.A. and Faraji, H. 1990. Inhibition of lipid oxidation by carnosine. JAOCS 67(1): 650.

Decker, E.A. and Hultin, H.O. 1992. Lipid oxidation in muscle foods via redox iron. In *Lipid Oxidation in Foods*, A.J. St. Angelo (Ed.), p. 33-54. American Chemical Society Symposium Series Vol. 500, American Chemical Society Books, Washington, D.C.

Flick, G.J., Hong, G.P., and Knoll, G.M. 1992. Lipid oxidation in seafood during storage. In *Lipid Oxidation in Foods*, A.J. St. Angelo (Ed.), p. 183-207. American Chemical Society Symposium Series Vol. 500, American Chemical Society Books, Washington, D.C.

Huang, L. 1997. Application of membrane filtration to recover solids from protein solutions. Ph.D. dissertation, Oregon State Univ., Corvallis.

Kanner, J., Shegalovich, I., Harel, S., and Hazan, B. 1988. Muscle lipid peroxidation dependent on oxygen and free metal ions. J. Agric. Food Chem. 36: 409.

Kaur, J., Morrissey, M.T., Decker, E.A., and Yang, L. 1998. Concentration of anserine and carnosine in surimi wash water. Presented at Ann. Mtg., Inst. of Food Technologists, Atlanta, GA, June 20-24.

Kaur, J., Morrissey, M.T., Decker, E.A., and Yang, L. 1999a. Concentration of dipeptides--anserine and carnosine in surimi wash water. Presented at 51st Ann. Mtg., Pacific Fisheries Technologists, Parksville, B.C., Feb. 7-10.

Kelleher, S.D., Hultin, H.O., and Wilhelm, K.A. 1994. Stability of mackerel surimi prepared under lipid-stabilizing processing conditions. J. Food Sci. 59: 269-271.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Ramanathan, L., and Das, N.P. 1993. Natural products inhibit oxidative rancidity in salted cooked ground fish. J. Food Sci. 58: 318-320, 360.

Statistical Analysis System, 1988. SAS Institute Inc., Cary, N.C.

Teahon, K., and Rideout, J.M. 1992. A sensitive and specific high performance liquid chromatographic assay for imidizole dipeptides and 3-methylhistidine in human muscle biopsies, serum and urine. Biomed. Chromatogr. 6: 16.

# Chapter 4

# EFFECT OF ULTRAFILTRATION AND IRON CONTENT ON THE CONCENTRATION OF DIPEPTIDES OBTAINED FROM SURIMI WASH WATER

## Abstract

Ultrafiltration is a unit operation in which water and some solutes in a solution are selectively removed through a semi-permeable membrane. It was hypothesized that the recovery of dipeptides and removal of pro-oxidants present in surimi wash water (SWW) could be done using an ultrafiltration technique. SWW samples that had a higher concentration of dipeptides were first passed through a 50K molecular weight cut-off (MWCO) membrane and then individually through 30K, 10K and 1K MWCO membranes. The concentration of dipeptides was determined using high performance liquid chromatography (HPLC) with a fluorescent detector. The dipeptide content of the samples passed through all the membranes were similar. Therefore, it is recommend that after passing the water through a 50K cut-off membrane, a 1K MWCO membrane should be the membrane of choice.

Because iron affects the concentration and the antioxidant activity of the dipeptides anserine and carnosine, it is necessary to remove it from wash water. Iron concentration was determined in all untreated and treated SWW samples using atomic absorption spectrometry (AAS) and colorimetry. The results showed that almost all of

the samples had an iron content of ≤1 ppm, indicating that the removal of iron out of SWW was unnecessary.

**Introduction**

The recovery of bioactive components from seafood waste is an area of expanded research and high economic potential that has expanded the use of seafood by-products. The dipeptide concentration varies from 1 species to another (Suzuki et al., 1990, 1991). The molecular weight of 2 naturally occurring dipeptides, carnosine and anserine, is 226.2 and 242.3, respectively and the difference in their molecular weight is due to presence of an extra methyl ($CH_3$) group in anserine (Dawson et al., 1969). Surimi wash water (SWW) may contain other soluble components such as pro-oxidative metals and iron-containing proteins that decrease antioxidant activity of dipeptides (Chan and Decker, 1994).

Ultrafiltration (UF) is a unit operation in which water and some solutes in solution are selectively removed through a semi-permeable membrane (Fellows, 1988; Toyomoto and Higuchi, 1992; Schagger, 1994; Potter and Hotchkiss, 1995). UF membranes have higher porosity and retain only large molecules that have a low osmotic pressure. Smaller solutes are transported across the membrane with the water and can therefore operate at lower pressures (50-2000kPa) (Fellows, 1988). Ultrafiltration membranes are made of different materials such as cellulose acetate and polyamide, and their physical and chemical properties can be considerably controlled (Singh and Heldman, 1993; Potter

and Hotchkiss, 1995). UF can help separate low molecular-weight compounds (Singh and Heldman, 1993; Haard et al., 1994; Scott, 1995). UF is a commercially proven and cost-effective recovery technique and its application in recycling food processing waste water and in recovery of valuable components of food processing wastes are being developed (Lanier, 1994; Toledo, 1991).

Heme iron in animal flesh is highly assimilated, compared to non-heme iron in plants. Absorption of non-heme iron is improved by ascorbic acid and animal protein. Substituting fish for red meat in the modern diet would reduce the intake of both heme and total iron. However, this decreased intake of iron may be offset by enhancement of absorption of non-heme iron by fish protein in the diet (Chao and Gordon, 1983).

Colorimetric techniques are commonly used in food industry for mineral analysis and usually the colored complex is formed which is read at a specific wavelength depending on the mineral. For quantitative determination of ferrous (iron) content, compounds such as (o-phenanthroline) and 3-(2-pyridyl)-5,6 -bis (4-phenylsulfonic acid)-1,2,4-triazine (ferrozine) are used to form stable, intensely colored species with ferrous and iron (Caldwell and Adams, 1946; Stookey, 1970). One mg of hemoglobin contains 3.4 µg of iron (Weissman, 1974). The iron levels found in white flesh finfish are 0.31 mg/100 g (Gordon and Roberts, 1977). The mineral analysis in fish was determined by atomic absorption spectrometry (AAS) (Gordon and Roberts, 1977; Gordon, 1978). Ferrozine reacts with iron to form a stable magenta-colored complex in water and can be used for the direct determination of iron in water (Stookey, 1970; Gordon, 1978). The colored complex is stable between pH 4 and 9 (Persijn et al.,1971). This technique does not cause protein precipitation (Goodwin et al., 1966; Persijn et al., 1971) and minimizes

interference from other trace metals (Stookey, 1970). The absorption wavelength used for the technique is 560 nm (Persijn et al., 1971).

The SWW contains iron and heme-containing proteins that may affect the concentration and the antioxidant activity of carnosine and anserine. UF may be helpful in removing these compounds. Additionally, determining the iron content in SWW would help establish the potential need to remove iron from the SWW. Therefore, the objective of this experiment is to study the effect of UF on SWW samples through different cut-off membranes and to evaluate which cut-off membrane is most effective. The second objective of this study is to determine the concentration of iron in SWW samples in order to evaluate if the iron needs to be removed from SWW before the SWW extract is made.

## Materials and Methods

Surimi wash water samples were obtained from Point Adams Co., Warrenton, Oregon. Based on the data and results obtained from previous experiments (Chapter 3), only SWW samples from processing stages Dehydrator 1 (DH1), Dehydrator 2 (DH2) and Rotary Screen 1 (RS1) were ultrafiltered. Prior to UF, the samples were collected from the above mentioned surimi processing stages in clean 1-liter bottles and brought to the Oregon State University (OSU) Seafood Laboratory on ice. The samples were collected and processed on different days.

Ultrafiltration was conducted using stirred cell (Amicon, Bedford, MA) bench top equipment. Amicon UF membranes (Amicon, Bedford, MA) of different molecular weight cut-off (MWCO), 50K, 30K, 10K and 1K were used to fractionate the SWW samples. Seventy-five percent of the SWW samples were passed through these membranes under pressure (55 psi) with nitrogen gas and collected as permeate. The retentate could not be used because of the solubility and the smaller molecular weight of the dipeptides and hence was discarded.

The samples were first passed through the 50K MWCO membrane, and then individually through 30K,10K and 1K MWCO membranes. The permeates from each MWCO membrane were collected and stored at -80°C until further analysis. The samples were then shipped on dry ice to and from Colorado State University, Fort Collins, for UF processing as described above. The samples (1 each) were analyzed using high performance liquid chromatography (HPLC) with a fluorescent detector and a manual injector (Spectraphysics, Mountain View, CA) (Teahon and Rideout, 1992; Decker, 1998). Ortho-phthaldehyde (OPA) was used as a derivatizing agent (Sigma Chemical Co., St. Louis, MO). Standard solutions ranging from 0 to 1000 ppm of carnosine and anserine were prepared (Sigma Chemical Co., St. Louis, MO). In this experiment, the analysis for dipeptides was initially run as a single set analysis due to the time involved for each sample run (1 hour), number of samples and manual injection. In addition, DH1 sample for all UF treatments (3 reps) were run for the purposes of evaluating repeatability and statistical analysis. The samples were also spiked to confirm the retention times and the peaks of the 2 dipeptides. This was done by adding 5-20 μL of standard solution

(100-400 ppm) in the SWW samples and derivatizing the sample as described in the preceding study.

The iron concentration was determined by using AAS and colorimetry. The 2 methods were used based on the availability of the equipment and timing. For AAS, the standard method to determine the concentration of iron was used (Gordon, 1978; Horneck et al.,1989). The SWW samples (3 replicates) from all stages of SWW processing were used. The AAS method used for the samples involved measurement of iron in different ways: direct (measurement of iron in the SWW sample); filtered (SWW sample was filtered through Whatman paper); and the wet ash method. The wet ash method helped to determine the content of iron in SWW samples that had solid particulates present in them. For colorimetry, the standard procedure number (565) for colorimetric determination of total iron was used (Sigma Chemical Co., St. Louis, MO). In this procedure, iron color reagent (0.85% ferrozine (w/v) in hydroxylamine hydrochloride solution) was used as an iron color reagent. The absorption was read at 560 nm (Presijn et al., 1971; Sigma Chemical Co., 1998).

Statistical analysis was performed with Statistical Analysis System (SAS Institute, Inc., 1988) using ANOVA and Duncan's Multiple Range Test and Student-Neuman-Keul's (SNK) multiple range test.

**Results and Discussion**

The concentration of SWW (raw) samples is included from a previous experiment for comparison purposes **(Table 4.1).** High concentration of the dipeptides (carnosine

**Table 4.1-Concentration (ppm) of anserine and carnosine in surimi wash water samples filtered through different ultrafiltration membranes**

| Samples | Number of Samples | | | Percent Recovery of Spiked Samples | |
|---|---|---|---|---|---|
| **Raw**[a] | | **Anserine** | **Carnosine** | **Anserine** | **Carnosine** |
| Dehydrator 1*[b,c] | 2 | 243 a[d] | 339 a | 101.7 | 96.9 |
| Dehydrator 2 [b] | 1 | 99 b | 123 b | | |
| Dehydrator 3* | 3 | 81 c | 110 c | 99.5 | 100.6 |
| Screwpress 1 | 1 | 58 d | 42 d | | |
| Rotary Screen 1 | 1 | 21 e | 38 d | | |
| Rotary Screen 2 | 1 | nd[e] | nd | | |
| **UF (50K)** | | | | | |
| Dehydrator 1* | 3 | 188 a | 359 a | 102.7 | 99.4 |
| Dehydrator 2 | 1 | 144 b | 193 b | | |
| Rotary Screen 1 | 1 | 6 c | 12 c | | |
| **UF (30K)** | | | | | |
| Dehydrator 1 | 3 | 158 a | 304 a | | |
| Dehydrator 2 | 1 | nd b | 3 b | | |
| Rotary Screen 1 | 1 | nd b | nd c | | |
| **UF (10K)** | | | | | |
| Dehydrator 1* | 3 | 165 a | 297 a | 93.7 | 102.5 |
| Dehydrator 2 | 1 | nd b | 3 b | | |
| Rotary Screen 1 | 1 | nd b | nd c | | |
| **UF (1K)** | | | | | |
| Dehydrator 1* | 3 | 147 a | 287 a | 100.9 | 101.2 |
| Rotary Screen 1 | 1 | 1 b | nd b | | |

[a] Name of the treatment
[b] Name of the sample
[c] Spiked samples.
[d] a-e: If the enteries in the same column are followed by different letter, this indicates significant difference by SNK test ($p \leq 0.05$).
[e] Not detectable.

and anserine) in processing stage DH1 was retained when SWW sample was passed through the 50K, 30K, 10K and 1K MWCO membranes **(Table 4.1)**. The concentration of dipeptides in DH2 was significant only when passed through the 50K MWCO membrane and insignificant when passed through the other membranes. The decrease in concentration of dipeptides in the DH2 SWW sample is notable and its cause is difficult to discern. The concentration of dipeptides in the RS1 sample was negligible when passed through all MWCO membranes.

There are several possible reasons for the reduced amount of dipeptides in the DH2 SWW sample passed through 30K and 10K MWCO membranes. These SWW samples were shipped from Colorado to the OSU Seafood Laboratory, Astoria, Oregon, and the samples may have been contaminated during transport. There may have been operational, experimental and human errors involved. The UF equipment used may have been contaminated. Other possible reasons were described in previous studies. Some of the SWW samples were spiked to evaluate accuracy and reliability of the HPLC method. For all spiked samples in the range of 0-243 ppm for anserine and 0-339 ppm for carnosine, recovery was 93.7-102.7 % and 96.9-102.5%, respectively. This percent recovery is within limits of analytical variability given that some samples had a very low concentration of anserine and carnosine, and that other compounds were present.

Comparison of the effectiveness of different MWCO membranes was conducted to determine which particular MWCO membrane would be best to use in recovering dipeptides. Processing stage DH1 was used because it was the only sample that underwent UF treatment through all 4 membranes **(Fig. 4.1)** and had appreciable amounts of dipeptides in all of its permeates. The dipeptide content of the DH1 SWW samples

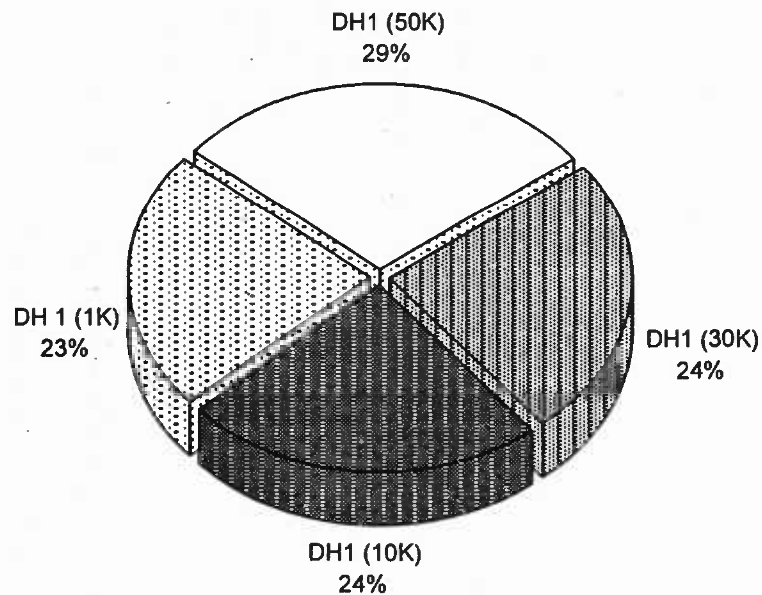



**Fig 4.1-Comparison of dipeptide (anserine and carnosine) content of ultrafiltered samples using Dehydrator 1 (DH1) sample**

passed through all 4 membranes similarly **(Fig. 4.1)** (Kaur et al.; 1998, 1999a). This indicates that the 1K MWCO membrane is the recommended choice because the permeate obtained will have fewer contaminants than permeates from10K and 30K MWCO membranes.

Relative standard deviation (RSD) or coefficient of variance (CV) of the replicated samples (3) for all treatments including raw samples, heat treated (see Chapter 3) and ultrafiltered samples were calculated to evaluate precision **(Table 4.2)**. In the experiments where the manual injector was used, analysis for each SWW sample took about 1 hour and therefore all the samples could not be run in triplicate. Therefore, for all replicated samples (12), in the range of 14.2-188.4 ppm of anserine and 25.4-358.7 ppm of carnosine, the CV was 0.33-8.65% and 1.17-4.96% respectively **(Table 4.2)**. This indicates that the processing methods (heat treatment and ultra filtration) used in this research were reliable and reproducible (Decker, 1998; Kaur et al., 1998).

Iron affects the concentration of the dipeptides and their antioxidant activity. Therefore, the iron concentration was determined in SWW samples to evaluate whether the iron needed to be removed from SWW before the SWW extract is made. The concentration of iron in raw SWW samples was determined by AAS (direct), AAS (filtered) and the colorimetry method. In all cases, the amount of iron was ≤1 ppm. However, because the samples did not include the solid particulates, it prompted use of the wet ash method to evaluate whether the total iron in the SWW samples, including the solid particulate, changed. Only the DH1 sample had iron levels of 2.3 ppm when wet ash method was used **(Table 4.3)**. Because dipeptides are water-soluble and the amount

**Table 4.2-Relative standard deviations of the replicated samples for high performance liquid chromatography with a fluorescent detector.**

| **Treatment** | **Avg Conc (ppm) ± Std dev** | | **Relative Standard Deviation Or CV[a]** | |
|---|---|---|---|---|
| **Raw[b]** | **Anserine** | **Carnosine** | **Anserine** | **Carnosine** |
| Dehydrator 3[c] | 81 ± 1.18[d] | 110 ± 1.28 | 1.46 | 1.17 |
| **60°C** | | | | |
| Rotary Screen 1 | 14 ± 0.78 | 25 ± 1.26 | 5.49 | 4.96 |
| **80°C** | | | | |
| Rotary Screen 2 | 22 ± 1.92 | 31 ± 1.20 | 8.65 | 3.84 |
| **100°C** | | | | |
| Dehydrator 2 | 110 ± 0.37 | 124 ± 4.27 | 0.33 | 3.45 |
| **UF (50K)** | | | | |
| Dehydrator 1 | 188 ± 5.24 | 359 ± 11.91 | 2.78 | 3.32 |
| **UF (30K)** | | | | |
| Dehydrator 1 | 158 ± 0.63 | 304 ± 9.32 | 0.40 | 3.06 |
| **UF (10K)** | | | | |
| Dehydrator 1 | 165 ± 6.94 | 297 ± 6.02 | 4.20 | 2.03 |
| **UF (1K)** | | | | |
| Dehydrator 1 | 147 ± 2.71 | 287 ± 4.98 | 1.84 | 1.74 |

[a] CV Co-efficient of variance.
[b]Name of different treatments used.
[c]Name of the specific samples (3) used for analysis.
[d]Means are shown with standard deviation

**Table 4.3-Iron levels (ppm) determined by AAS and colorimetric methods in surimi wash water**

| Sample name | Direct | Filtered | |
|---|---|---|---|
| | AAS 1[a] | AAS 2 | Colorimetry |
| Dehydrator 1 | 0.27 ± 0.01[b] | 0.42 ± 0.01 | 1.16[c] |
| Dehydrator 2 | <0.05 ± 0 | 0.14 ± 0.02 | 0.08 |
| Dehydrator 3 | <0.05 ± 0 | 0.10 ± 0.01 | 0.22 |
| Screwpress 1 | 0.1 ± 0 | 0.19 ± 0.04 | 0.19 |
| Rotary Screen 1 | <0.05 ± 0 | 0.10 ± 0.01 | 0.4 |
| Rotary Screen 2 | <0.05 ± 0 | 0.10 ± 0.01 | 0.38 |
| | Wet Ash[d] | | |
| | AAS | | |
| Dehydrator 1 | 2.3 | | |
| Dehydrator 2 | 0.8 | | |
| Dehydrator 3 | 0.5 | | |
| Screwpress 1 | 0.9 | | |
| Rotary Screen 1 | 0.4 | | |
| Rotary Screen 2 | 0.2 | | |

[a]AAS1 and AAS2-Atomic Absorption Spectrometry (1-First analysis)(2-Second analysis).
[b]Means are shown with standard deviation (3-reps).
[c] SWW samples (1 each).
[d]These samples included the solid phase or particulates.

of iron in liquid phase was ≤1 ppm, the removal of iron out of raw SWW samples was unnecessary. The treated (heat and UF) SWW samples were collected and treated on different days as described earlier and therefore may have contained varied amounts of pro-oxidants including iron. Therefore, to evaluate the effect of treatment (heat and UF) on the content of iron in SWW samples, the iron concentration was determined in treated SWW samples.

For all heat-treated samples, the concentration of iron in almost all cases was ≤1 ppm except DH1 at 60°C. However, in the heat-treated DH1 sample, the iron content determined by AAS ranged between 0.12 and 1.33 ppm; the iron content from colorimetric analysis was found to range between 0.07 and 3.02 ppm. The higher value of 3.02 ppm may due to experimental error or solid particulates present in the sample **(Table 4.4)**. For heat-treated SWW samples, the iron content in 80°C treated SWW samples was usually lowest, followed by 100°C and then 60°C **(Table 4.5)**. These results confirm that among heat treatments, 80°C is the treatment of choice for SWW samples, followed by 100°C.

Among all ultrafiltered samples, analyzed with either AAS or colorimetry, iron content was <1 ppm **(Table 4.6)**. The concentration of iron was not affected by any UF membrane used in the experiment. Therefore, the SWW can be passed though a 50K MWCO membrane and then through a1K as indicated earlier. This confirms that if the1K MWCO membrane is used, iron will not interfere either with the recovery or the antioxidant activity of the dipeptides as the concentration ratio of dipeptides:iron is large. The concentration of iron was ≤1 ppm in almost all the samples, regardless of treatment

**Table 4.4-Iron levels (ppm) determined by AAS and colorimetric methods in heat-treated surimi wash water samples**

| | 60°C | | | 80°C | | | 100°C | | |
|---|---|---|---|---|---|---|---|---|---|
| **Sample name** | **AAS 1[a]** | **AAS 2** | **Colorimet** | **AAS 1** | **AAS 2** | **Colorimet** | **AAS 1** | **AAS 2** | **Colorimet** |
| Dehydrator 1 | 0.12 ± 0.01[b] | 1.13 ± 0.05 | 3.02[c] | 0.13 ± 0.02 | 1.1 ± 0 | 0.07 | 0.19 ± 0.02 | 1.33 ± 0.05 | 0.36 |
| Dehydrator 2 | 0.05 ± 0.02 | 1 ± 0.10 | 0.07 | 0.07 ± 0.01 | 1.1 ± 0.2 | 0.10 | 0.12 ± 0.02 | 1.27 ± 0.11 | 0.24 |
| Dehydrator 3 | 0.07 ± 0.01 | 0.8 ± 0 | 0.09 | 0.10 ± 0.01 | 0.93 ± 0.1 | 0.16 | 0.08 ± 0.02 | 1.1 ± 0.17 | 0.47 |
| Screwpress 1 | 0.1 ± 0 | 0.9 ± 0.10 | 0.63 | 0.12 ± 0.01 | 1.13 ± 0.1 | 0.12 | 0.07 ± 0.01 | 1.17 ± 0.11 | 0.23 |
| Rotary Screen 1 | 0.09 ± 0.01 | 0.83 ± 0.15 | 0.33 | 0.06 ± 0.01 | na[d] | 0.26 | 0.17 ± 0.01 | 1 ± 0 | 0.31 |
| Rotary Screen 2 | 0.05 ± 0.01 | 0.93 ± 0.06 | 0.24 | 0.11 ± 0.01 | 1.07 ± 0.1 | 0.39 | 0.09 ± 0.01 | 1.17 ± 0.21 | 0.26 |

[a] AAS1 and AAS2-Atomic Absorption Spectrometry (1-First analysis)(2-Second analysis).
[b] Means are shown with standard deviations, (3-reps).
[c] SWW samples (1 each).
[d] Not available.

**Table 4.5-Ranges of iron levels (ppm) determined by AAS and colorimetric methods in heat-treated surimi wash water samples**

| Heat Treatment | AAS1 | AAS2 | Colorimet |
|---|---|---|---|
| 60°C | 0.05-0.12 | 0.8-1.13 | 0.07-3.02 |
| 80°C | 0.06-0.13 | 0.93-1.1 | 0.07-0.39 |
| 100°C | 0.07-0.19 | 1.0-1.33 | 0.24-0.36 |

**Table 4.6-Concentration (ppm) of iron in surimi wash water samples filtered through different ultrafiltration membranes**

| Sample Name | AAS | Colorimetry |
|---|---|---|
| **UF (50K)**[a] | | |
| Dehydrator 1[b] | 0.93 ± 0.11[c] | na[d] |
| Dehydrator 2 | 0.9 ± 0.1 | 0.28[e] |
| Rotary Screen 1 | 0.73 ± 0.25 | 0.34 |
| **UF (30K)** | | |
| Dehydrator 1 | 0.63 ± 0.40 | na |
| Dehydrator 2 | 0.5 ± 0.17 | na |
| Rotary Screen 1 | 0.27 ± 0.20 | na |
| **UF (10K)** | | |
| Dehydrator 1 | 0.4 ± 0.2 | 0.34 |
| Dehydrator 2 | 0.7 ± 0.1 | na |
| Rotary Screen 1 | 0.57 ± 0.15 | 0.35 |
| **UF (1K)** | | |
| Dehydrator 1 | 0.55 ± 0.07 | na |
| Dehydrator 2 | 0.55 ± 0.07 | na |
| Rotary Screen 1 | 0.63 ± 0.15 | 0.15 |

[a]Name of the treatment.
[b]Name of the samples used.
[c]Means are shown with standard deviation (3 reps).
[d]Not available.
[e]SWW samples (1 each).

(heat and UF) and analysis method (AAS and colorimetry), indicating it is unnecessary to remove the iron from the surimi wash water.

Pacific Whiting (*Merluccius productus*) SWW samples in general do not have a high iron content as is clear from the present study. However, it is recommended that the concentration of iron should be determined in all SWW samples, regardless of the fish species from which the surimi is obtained, for 2 reasons. First, anserine and carnosine are water-soluble dipeptides whose concentrations are species specific, and second, the iron content in SWW samples can vary across samples. The determination of the iron content in all SWW samples before the extract is made may help to determine the need to remove the iron. This study reveals that either of the 2 methods (AAS and/or colorimetry) can be used for the determination of iron.

**Summary**

The concentration of anserine and carnosine varies among species. Surimi wash water samples may contain other soluble components, such as pro-oxidative metals and iron-containing proteins which decrease the antioxidant activity of dipeptides. For the removal of these components, SWW samples were previously heat treated and it was determined that DH1 and DH2 had higher amounts of dipeptides than other stages of surimi processing. Therefore, UF was performed on the selected SWW processing stage samples DH1, DH2 and RS1. First, the samples were passed through a 50K MWCO membrane and then individually through 30K, 10K and 1K MWCO membranes. After

the SWW is passed through a 50K MWCO membrane, a 1K membrane can be used for the recovery of dipeptides. Dipeptide recovery was most successful from processing stage Dehydrator 1 (DH1).

The iron content in all SWW samples (raw, heat treated and ultrafiltered) was determined using AAS and colorimetry; the concentration of iron in almost all the samples was negligible (≤1 ppm). Therefore, the removal of iron from SWW for our purpose was nonessential.

**References**

Caldwell, D.H. and Adams, R.B. 1946. Colorimetric determination of iron in water with o-Phenanthroline. J. Am. Water Works Assoc. 38: 727-730.

Chan, K.M. and Decker, E.A. 1994. Endogenous skeletal muscle antioxidants. Crit. Rev. Food Sci. & Nutr. 34: 403-426.

Chao, L.S. and Gordon, D.T. 1983. Influence of fish on bioavailability of plant iron in the anemic rat. Mtg. of Am. Inst. of Nutr. Fed. Proc. 39: 1044.

Dawson, R.M.C., Elliot, D.C., Elliot, W.H., and Jones, K.M. (Ed.). 1969. *Data for Biochemical Research.* Oxford University Press, U.K.

Decker, E.A. 1998. Personal communication. Univ. of Massachusetts, Amherst.

Fellows, P.J. (Ed.) 1988. *Membrane Concentration. Food Processing Technology, Principles andPractice.* Ellis Horwood Limited, Hertfordshire, U.K.

Goodwin, J.F., Murphy, S., Bramlett, K., and Guillemette, M. 1966. Direct measurement of serum iron and binding capacity. Clinical Chem. 12(2): 47-57.

Gordon, D.T. 1978. Atomic absorption spectrometric and colorimetric determination of iron in seafoods. J. Assoc. Off. Anal. Chem. 61: 715-719.

Gordon, D.T. and Roberts, G.L. 1977. Mineral and proximate composition of Pacific coast fish. J. Agric. Food Chem. 25: 1262-1268.

Haard, N.F., Simpson, B.K., and Sikorski, Z.E. 1994. Biotechnological applications of seafood proteins and other nitrogenous compounds. In *Seafood Proteins*, Z.E. Sikorski, B.S. Pan, and F. Shahidi (Ed.), p. 194-216. Chapman & Hall Publ., New York.

Horneck, D.A., Hart, J.M., Topper, K., and Koepsell, B. (Ed.). 1989. *Methods of Soil Analysis Use in Soil Testing Laboratory.* Oregon State Univ., Corvallis.

Kaur, J., Morrissey, M.T., Decker, E.A., and Yang, L. 1998. Concentration of anserine and carnosine in surimi wash water. Presented at Ann. Mtg., Inst. of Food Technologists, Atlanta, GA, June 20-24.

Kaur, J., Morrissey, M.T., Decker, E.A., and Yang, L. 1999. Concentration of dipeptides--anserine and carnosine in surimi wash water. Presented at 51st Ann. Mtg., Pacific Fisheries Technologists, Parksville, B.C., Feb 7-10.

Lanier, T.C. 1994. Functional food protein ingredients from fish. In *Seafood Proteins*, Z.E. Sikorski, B.S. Pan, and F. Shahidi (Ed.), p. 127-159. Chapman & Hall Publ., New York.

Persijn, J.P., Slik, W.V., and Riethorst, A. 1971. Determination of serum iron and latent iron-binding. Clin. Chem. Acta 35: 91-98.

Potter, N.N. and Hotchkiss, J.H. 1995. *Food Science*. 5th ed. Chapman & Hall Publ., New York.

Schagger, H. 1994. Chromatographic techniques and basic operations in membrane protein purification. In *A Practical Guide to Membrane Protein Verification*, G.V. Jagow and H. Schagger (Ed.), p. 23. Academic Press, San Diego, CA.

Scott, K. 1995. Introduction to membrane separations. In *Handbook of Industrial Membranes,* 1st ed., p. 27. Elsevier Advanced Technology, Oxford, U.K.

Sigma Chemical Company. 1998. Personal communication. St. Louis, MO.

Singh, R.P. and Heldman, D.R. 1993. *Introduction to Food Engineering*, 2nd ed. Academic Press, New York

Statistical Analysis System. 1988. SAS Institute Inc., Cary, N.C.

Stookey, L.L. 1970. Ferrozine--a new spectrophotometric reagent for iron. Anal. Chem. 42: 779-781.

Suzuki, T., Shirai, T., and Hirano, T. 1990. Distribution of extractive nitrogenous constituents in white and dark muscles of fresh-water fish. Comp. Biochem. Physiol. 96B: 107-111.

Suzuki, T., Shirai, T., and Hirano, T. 1991. Anserine and free amino acid contents in the egg and fry stages of developing rainbow trout (*Onchorhynchus mykiss*). Comp. Biochem. Physiol. 100A: 105-108.

Teahon, K. and Rideout, J.M. 1992. A sensitive and specific high performance liquid chromatographic assay for imidizole dipeptides and 3-methylhistidine in human muscle biopsies, serum and urine. Biomed. Chromatogr. 6: 16.

Toledo, R.T. 1991. *Fundamentals of Food Process Engineering*, 2nd ed. Van Nostrand Reinhold, New York.

Toyomoto, K. and Higuchi, A. 1992. Microfiltration and ultrafiltration. In *Membrane Science and Technology*. Y. Osada and T. Nakagawa (Ed.), p. 289-331. Marcel Dekker Inc., New York.

Weissman, N. and Pileggi, V.J. 1974. Inorganic ions. In *Clinical Chemistry Principles and Techniques*, 2nd ed., R.J. Henry, D.C. Cannon and J.W. Winkelman (Ed.), p. 684, 685, 695. Harper & Row, New York.

# Chapter 5

# ANTIOXIDANTS AND THEIR EFFECT ON THE COLOR OF FARMED SALMON


## Abstract

Oxidation is the major cause of carotenoid degradation in foods. The rate of oxidation depends on contact with oxygen, light, heat, and presence of pro-oxidants and antioxidants. Salmon color is described by the parameters L*, a*, b*, where L represents lightness, a redness and b yellowness. The objective of this study was to evaluate the effect of antioxidants, particularly surimi wash water (SWW), on the color of fresh salmon over a period of 1 week. For this study the fresh-farmed salmon fillets were minced and formed into patties. The color was measured by using a Minolta Chroma Meter CR300; parameters used were hue angle, chroma and lightness. Results showed that the SWW extract at the lower concentration (1%) maintained color parameters until day 5. SWW extract may have a potential to be used as a natural food antioxidant in future.


## Introduction

Lipid oxidation decreases the shelf life of seafood by causing deterioration of flavor, color and texture. Oxidative reactions lead to the formation of lipid oxidation

products such as aldehydes, epoxides, and oxidized sterols which are potentially toxic and lead to diseases like atherosclerosis and cancer (Addis and Park, 1989). In order to produce high quality meat products, lipid oxidation should be reduced. In skeletal muscles, heme-containing proteins, iron, copper and other transition metals, as well as pigments such as hemoglobin and myoglobin lead to lipid oxidation (Ashghar et al., 1988; Kanner et al., 1988; Decker and Hultin, 1992 ).

Antioxidants, which have the ability to chelate iron, deactivate heme-containing proteins and scavenge free radicals, are capable of inhibiting oxidative rancidity in seafoods (Flick et al., 1992; Kelleher et al., 1994). Carnosine and anserine exhibit similar antioxidative activity. According to Boldyrev et al. (1988) "the inhibiting effect of the dipeptides is enhanced either by the rise in their concentration or by the reduction of the other components in the membranes." The addition of the dipeptides leads to a marked decrease in the level of primary molecular products of lipid oxidation (Boldyrev et al., 1988). The 2 dipeptides have peroxyl radical-trapping ability; they chelate metal ions, quench singlet oxygen, bind hydroperoxides, and are reducing agents, (Kohen et al., 1988). The naturally occurring dipeptides regenerate tocopherol, suggesting their synergistic effect to inhibit lipid oxidation (Boldeyrev et al., 1988; Decker and Faraji, 1990).

Carnosine has antioxidant properties and has been found to inhibit lipid oxidation caused by iron, copper, hemoglobin, singlet oxygen and lipoxygenase (Boldyrev et al., 1988; Kohen et al., 1988; Decker and Faraji, 1990; Decker et al., 1992). Carnosine was also found to be effective at preventing oxidative rancidity and color changes in salted ground pork after 1 month of frozen storage (-15°C) (Decker and Crum, 1991). In farmed

salmon, the color of salmon flesh depends on multiple factors and can vary based on pigment, the farm where they were raised, their feed and the age of the salmon.

Redness is one of the important quality criteria of salmonoids (Francis and Clydesdale, 1975; Haard, 1988; Hong and Storebakken, 1991). Color is helpful in providing sorting (separating) information in fishes in addition to shape (Strachan, 1993). Carotenoids are the pigments responsible for giving the yellow or red color to the salmon. According to Hari et al. (1994), pigment is applied to a material of known or unknown physical state or to an unanalyzed and unknown colored material. Canthaxanthin and astaxanthin are fed to salmonoids, rainbow trout and rockfish for pigmentation (Skrede and Storebakken,1986a; Skrede et al.,1990; Choubert et al.,1992; Ingemansson et al., 1993; Li et al., 1998). Astaxanthin is the dominant carotenoid pigment in the flesh of wild salmon (Khare et al., 1973). The pigments are absorbed from the diet and distributed to the fish muscle where they bind to actomyosin (Skrede et al., 1989; Swatland et al., 1997).

Oxidation is the major cause of carotenoid degradation in foods. The rate of oxidation depends on contact with oxygen, light, heat, and presence of pro-oxidants and antioxidants (Francis and Clydesdale, 1975; Haard, 1988; Hong and Storebakken, 1991; Li et al., 1998). The influence of light can induce unwanted changes such as oxidation of pigments associated with loss of natural color, photosynthesized oxidation of lipids and loss of vitamin activity (Bjerkeng and Johnsen, 1995). Carotenoid degradation proceeds in an analogous fashion to lipid degradation, i.e., auto-oxidation and/or photosynthetic oxidation (reaction with singlet oxygen) (Wasson et al., 1991).

The color of salmon may be assessed by various analytical methods including but not limited to sensory analysis using trained panelists (Ostrander et al., 1976; Skrede and Storebakken, 1986a) or standardized colors (McCallum et al., 1987; Skrede et al., 1990). Assessment can also be done by instrumental analysis based on reflectance spectra (Skrede and Storebakken; 1986a,b). According to Hunter (1975), the reflectance spectra and further transformation into CIE XYZ tri-stimulus values and other uniform color systems are used for color studies (Skrede and Storebakken,1986a). Salmon color in these systems is described by the parameters L*, a*, and b*, where L represents lightness, a redness and b yellowness. In the L* C* h* color system, L*, lightness, is same as in the L*, a*, b* system, C, Chroma, is the saturation of the color numerically defined as the square root of $a^2 + b^2$, and h, hue angle, is numerically equal to $\tan^{-1}$ (b* / a*) (Minolta®, 1994; Hunter, 1991).

It is difficult to compare results from different analytical methods because of the lack of intensity references in sensory analysis or lack of standardized colors. Instrumental design and sample presentation influence the values of instrumental color analysis (Skrede and Storebakken, 1986b). The results can also vary when considering the pigment of the salmonoid. For example, with astaxanthin the color is more red than canthaxanthin (Skrede et al., 1989). For all practical purposes, it is important to understand the color and its relationship to the chemical and instrumental methods. Lipid oxidation and processing causes changes in the perceived salmonoid color (Chen et al., 1984; Skrede et al., 1989). The hue shifts from red to orange-red in salmonoid flesh (Skrede et al., 1989).

The right coloration for cultivated fish is required to be provided by the farmers to satisfy consumer expectations (Choubert et al., 1992; Williams, 1992; Metusalach et al., 1997). Salmon is often priced according to the intensity of its hue and is an important feature of salmon quality (Sigurgisladottir et al., 1994; Metusalach et al., 1997). Therefore, hue angle, chroma and lightness were used as 3 parameters of color measurement. In the case of farmed salmon, hue angle indicates redness at $0^{\circ}$ and yellowness at $90^{\circ}$ and is a good indicator of how the sample is perceived visually. Chroma is indicative of color saturation . Lightness varies from 1 to 100 as in the L*, a*, b* system. There is a radial increase in the pigmentation towards the backbone (McCallum et al., 1987). Considerable variation in lipid oxidation may occur within salmon species and there is significant color variance of farmed salmon reared in different farms (Ostrander, 1976). The main objective of this study was to evaluate the effect of different antioxidants, particularly SWW extracts, on the oxidative rancidity of farmed salmon mince using different color parameters over a period of time. The second objective was to study the synergistic effect of antioxidants on the color parameters of farmed salmon.

**Materials and Methods**

Fresh-farmed salmon (*Salmo salar*) was obtained from a local retailer. The fillets from 5 different fishes were cut and brought to the Oregon State University Seafood Laboratory on ice within minutes. The fillets were de-skinned and homogenized in a

food processor in order to avoid the problems associated with varied thickness, pigmentation in the backbone, different fish samples, variable color and the amount of lipids in the fish (Skrede, 1997; Wrolstad,1998). The acid hydrolysis method was used to determine fat content of the mince (AOAC, 1995). The fat content was used to determine the amount of butylated hydroxy toluene (BHT) and tocopherol (0.02% of the fat content) to be added to the mince.

All the antioxidants (carnosine, BHT and tocopherol) were obtained from Sigma Chemical Company (St. Louis, MO). However, the SWW extract was obtained from Decker's Laboratory at the University of Massachusetts, where it was made from processing stage Dehydrator 1 (DH1) using ion exchange chromatography. Prior to making an extract, the SWW sample (DH1) was sent from the OSU Seafood Laboratory to Decker's Laboratory on dry ice.

Antioxidant solutions were made and appropriate amounts of these solutions were added to the mince to obtain the desired final concentration of antioxidants (**Table 5.1**). The samples were brought to the same weight by the addition of either water or ethanol, depending on the solubility of the antioxidant. The samples obtained were placed in small dishes in the form of patties and immediately covered and placed in the dark to avoid artifactual oxidation. Because, it is difficult to compare the color of salmon with a standardized system, fresh salmon (mince) was used for comparison.

Instrumental color analysis was performed using Minolta Chroma Meter CR 300 (Minolta Camera Co., Osaka, Japan). The instrument was standardized using a Minolta calibration plate [Y(CIE) = 94.5, x (CIE) = .3160 and y (CIE) = .330] and a Hunter Lab

**Table 5.1-Antioxidant treatments added to farmed salmon (*w/w*)**

| Treatment No. | Treatment name |
|---|---|
| 1 | Control |
| 2 | Carnosine 1% |
| 3 | Carnosine 5% |
| 4 | BHT (0.02% of fat) |
| 5 | Tocopherol (0.02% of fat) |
| 6 | Extract 1% |
| 7 | Extract 5% |
| 8 | Carnosine 1%+BHT (0.02% of fat) |
| 9 | Carnosine 1%+Tocopherol (0.02% of fat) |
| 10 | Extract 1%+BHT (0.02% of fat) |
| 11 | Extract 5%+BHT (0.02% of fat) |
| 12 | Extract 1%+ Tocopherol (0.02% of fat) |
| 13 | Extract 5%+ Tocopherol (0.02% of fat) |

standard hitching tile ($L^*$ = 82.13, $a^*$ = -5.24 and $b^*$ = -.55) with D-65, illuminant and 2° observer. Color of the farmed salmon samples with antioxidants was read over a period of 7 days at refrigerated temperature. Conditions were standardized beforehand for color analysis. Exposure of samples to light, adverse temperatures and surfaces was avoided to prevent artifactual oxidation. All samples were treated in the same manner.

Statistical analysis was performed with Statistical Analysis System (SAS Institute, Inc., 1988) using ANOVA and Duncan's Multiple Range Test.

**Results and Discussion**:

The fat content of the fish sample was approximately 14%. The antioxidant treatments were used as described in **Table 5.1**.

Considering the 3 parameters selected for studying color, the fresh salmon with a hue angle of 79.3°, chroma -26.89, and lightness -62.87 ($\pm$ 3%) were used as an acceptable standard measure in terms of quality (Skrede, 1997). No and Storebakken (1991) reported a hue angle of 73.7° in tissue homogenates of trout fillets. Skrede and Storebakken (1986b), the hue angle for raw salmon flesh was 53.1°. The hue angle value for homogenate of the fresh-farmed salmon in our study was within the analytical limits of the values reported in the literature (Skrede, 1997). The difference in the values was expected due to different species, feed, pigmentation, and lipid oxidation.

Considering the criteria selected, i.e., the values of color parameters ($\pm$ 3%) as mentioned above, the color parameters for the control (no antioxidant) were out of the

designated color range on day 3, indicating deterioration in quality due to oxidation (**Table 5.2, 5.3, 5.4;Fig. 5.1**).

The value of hue angle for the control decreased during storage toward a redder hue angle. The particular reason for this change is unknown at this time. The possible reasons that may contribute to this change include: the reduction in the values of chroma (saturation) and lightness; exposure to refrigerated temperature, darkness, as well as the presence of carotenoid pigments (astaxanthin or canthaxanthin) present in the flesh of the farmed salmon. Other contributing factors can be endogenous antioxidants, other transition metals, proteins, lipids present in the flesh of the salmon and other possible chemical reactions (Hagen, 1998; Wrolstad 1998). All antioxidants were expected to cause differences in the values of color parameters due to differences in chemical composition, and in their ability to react with pigments and other compounds and inhibit lipid oxidation or oxidative rancidity. Among antioxidant treatments, BHT (0.02% of fat) had hue angle, chroma and lightness within the range until day 7 followed by carnosine which had hue angle in range but reduced chroma and lightness. However, SWW extract (extract 1%) had hue angle values within the range until day 5 but the chroma and lightness were also reduced (**Table 5.2, 5.3, 5.4;Fig. 5.1**). Carnosine (5%) showed the hue angle, chroma and lightness reduced on all days except on day 7 when the chroma was similar to day 1. Tocopherol exhibited an erratic pattern, indicating that it is not a good antioxidant for the study because it had some adverse reaction with carotene (Skrede, 1997), which made the salmon look darker (**Table 5.2, 5.3, 5.4; Fig. 5.1**). Surimi wash water extract (5%) showed a constant increase in hue angle ranging

**Table 5.2-Color parameters of individual antioxidants applied to farmed salmon (*Salmo salar*) over 7 days (5°C)--hue angle(°)**

| Treatment name | Day 1 | Day 3 | Day 5 | Day 7 | |
|---|---|---|---|---|---|
| Control | 79.3 ± 0.5d[a,b] | 73.9 ± 0.15d | 75.2 ± 0.25c | 76.2 ± 1.88b | ns[c] |
| Carnosine 1% | 84.1 ± 0.26b | 79.5 ± 0.28b | 79.0 ± 0.16b | 78.1 ± 0.5b | ns |
| Carnosine 5% | 78.2 ± 0.21d | 74.2 ± 0.22d | 73.9 ± 0.13d | 71.2 ± 1.42c | **[d] |
| BHT (0.02% of fat) | 78.0 ± 0.30d | 76.6 ± 0.27c | 78.7 ± 1.02b | 81.7 ± 0.77b | ns |
| Tocopherol (0.02% of fat) | 82.8 ± 0.46c | 72.0 ± 0.26e | 77.4 ± 0.18b | 82.8 ± 2.27b | ns |
| Extract 1% | 81.8 ± 0.91c | 77.4 ± 0.14b | 78 ± 0.67b | 82.7 ± 0.48b | ns |
| Extract 5% | 87.3 ± 0.56a | 86.3 ± 1.47a | 100.9 ± 0.86a | 104.3 ± 0.97a | ***[e] |

[a] Overall means are shown with standard deviation (reps-4).
[b] a-e: treatment means in the same column for a given antioxidant marked with different letter differs ($p \leq 0.05$).
[c] Not significant.
[d] These treatments are significantly different over a period of 7 days at ($\alpha$ = 0.05, 0.01).
[e] These treatments are significantly different over a period of 7 days at ($\alpha$ =0.05, 0.01, 0.001).

**Table 5.3-Color parameters of individual antioxidants applied to farmed salmon (*Salmo salar*) over 7 days (5°C)--chroma**

| Treatment Name | Day 1 | Day 3 | Day 5 | Day 7 | |
|---|---|---|---|---|---|
| Control | 26.9 ± 0.28b[a,b] | 24.8 ± 0.00c | 24.4 ± 0.05c | 24.2 ± 0.5c | *[c] |
| Carnosine 1% | 27.1 ± 0.08b | 25.4 ± 0.04b | 25.1 ± 0.02b | 25.1 ± 0.13b | ns[d] |
| Carnosine 5% | 26.7 ± 0.03b | 23.7 ± 0.01d | 23.2 ± 0.13d | 22.4 ± 0.60d | **[e] |
| BHT (0.02% of fat) | 26.8 ± 0.11b | 26.4 ± 0.03a | 26.1 ± 0.24a | 26.6 ± 0.23a | ns |
| Tocopherol (0.02% of fat) | 28.2 ± 0.05a | 24.9 ± 0.12c | 25.6 ± 0.04b | 26.3 ± 0.83a | ns |
| Extract 1% | 27.0 ± 0.02b | 25.6 ± 0.12a | 22.1 ± 0.04e | 23.9 ± 0.33c | ***[f] |
| Extract 5% | 27.3 ± 0.31b | 21.8 ± 0.99e | 18.2 ± 0.07f | 17.9 ± 0.67e | ** |

[a] Overall means are shown with standard deviation (reps-4).
[b] a-f: treatment means in the same column for a given antioxidant marked with different alphabet.
[c] These treatments are significantly different over a period of 7 days at ($\alpha$ = 0.05).
[d] Not significant.
[e] These treatments are significantly different over a period of 7 days at ($\alpha$ = 0.05, 0.01).
[f] These treatments are significantly different over a period of 7 days at ($\alpha$ =0.05, 0.01, 0.001).

**Table 5.4-Color parameters of individual antioxidants applied to farmed salmon (*Salmo salar*) over 7 days (5°C)--lightness**

| Treatment Name | Day 1 | Day 3 | Day 5 | Day 7 | |
|---|---|---|---|---|---|
| Control | 62.9 ± 0.15a[a,b] | 59.9 ± 0.04b | 59.0 ± 0.01a | 56.2 ± 0.5c | ***[c] |
| Carnosine 1% | 58.2 ± 0.09c | 57 ± 0.26d | 56 ± 0.02c | 54.5 ± 0.28d | *** |
| Carnosine 5% | 56.6 ± 0.01d | 54 ± 0.03e | 53.1 ± 0.11d | 51.9 ± 0.40e | *** |
| BHT (0.02% of fat) | 62.1 ± 0.02b | 60.4 ± 0.04a | 58.3 ± 0.39b | 57.3 ± 0.09b | *** |
| Tocopherol (0.02% of fat) | 61.5 ± 0.42b | 56.6 ± 0.33d | 55.4 ± 0.01c | 54.2 ± 0.23d | *** |
| Extract 1% | 61.7 ± 0.07b | 56.2 ± 0.16d | 53.8 ± 0.39d | 52.4 ± 0.47e | *** |
| Extract 5% | 62.5 ± 0.35a | 58.2 ± 0.09c | 59.5 ± 0.44a | 59.3 ± 0.23a | *** |

[a] Overall means are shown with standard deviation (reps-4).
[b] a-e: treatment means in the same column for a given antioxidant marked with different alphabet.
[c] treatments are significantly different over a period of 7 days at ($\alpha$ =0.05, 0.01, 0.001).

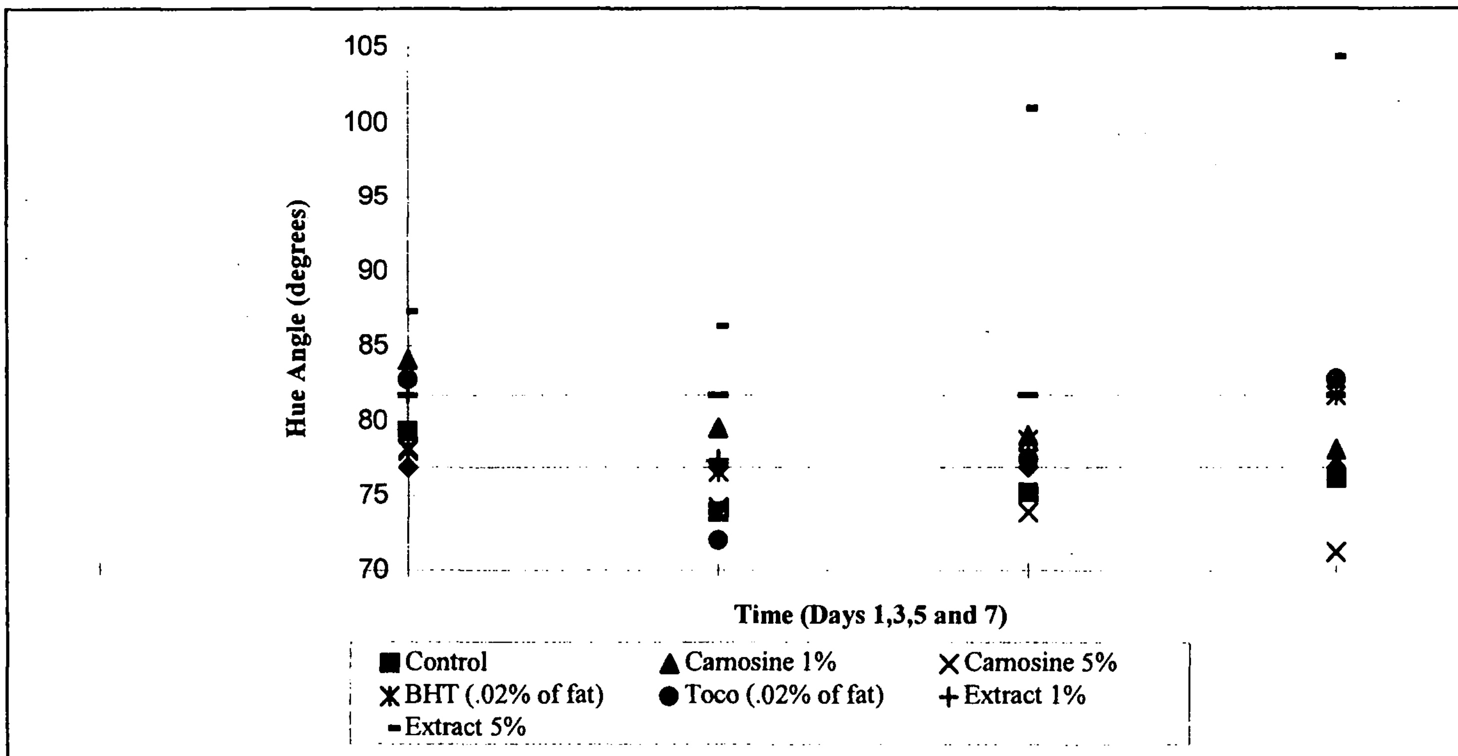



**Fig. 5.1-Hue angle of individual antioxidants with acceptable range for salmon (*Salmo salar)* treated with antioxidants (5°C)**

from 87.3 on day 1 to 104.3 on day 7, but with a reduction in chroma and a slight consistency in lightness (**Table 5.2, 5.3, 5.4; Fig. 5.1**).

Analysis of Variance (ANOVA) and Dunnett's test were run to confirm the results. A statistically significant effect of individual antioxidants on all 3 parameters of color was defined at $\alpha = 0.05$ (P<0.0001). For Dunnett's test for hue, there were statistically significant differences between fresh salmon and carnosine 5% and extract 5% at $\alpha = 0.05$ (**Table 5.2**). For chroma, antioxidant treatments carnosine 5%, extract 5% and 1% had statistically significant differences (**Table 5.3**). For lightness, all the antioxidants were found to have statistically significant effects (**Table 5.4**). However, the treatment with extract (1%) showed better results compared to extract (5%); therefore, the extract may be more effective at a lower concentration (1%) than at higher concentration (5%). This may be due to an increase in the concentration of pro-oxidants, such as metals, that reduce antioxidant activity of the extract at a higher concentration. However, further research is required to confirm this hypothesis. According to Decker and Crum (1991) "carnosine (0.5-1.5%) effectively inhibited formation of lipid peroxides and thiobarbituric acid reactive substances in frozen (-15°C) salted ground pork during six months of frozen storage." This indicates that future experiments using a lower concentration of SWW extract may be beneficial.

For combined antioxidants, synergistic effects were evaluated. Synergistic effects occur when antioxidants work in combination without any interference. In the measurement of color, if the value of hue angle, chroma, or lightness of the combined antioxidant is lower than that of the individual antioxidant, the antioxidants have a synergistic effect. However, if the value is similar to that of an individual antioxidant it

has additive effect and if the value happens to be more than the individual antioxidant value, it may be due to unknown reaction (Reed, 1998).

In combination, almost all antioxidants exhibited a synergistic effect but some values were greater than the individual antioxidants. Among the combined treatments (**Table 5.4, 5.5, 5.6; Fig. 5.2**), carnosine 1% + BHT (0.02% of fat) was found to be most effective followed by extract 1% + tocopherol (0.02% of fat). The effectiveness of the treatment combinations were based on hue angle and chroma (**Table 5.5, 5.6; Fig. 5.2**). For all 3 parameters of color there was a statistically significant effect of antioxidants on color (**Table 5.5, 5.6, 5.7**). There was a statistically significant difference between extract 1% + BHT, extract 5% + BHT and extract 5% + tocopherol as compared to the fresh salmon control. This confirms results that carnosine 1% + BHT (0.02% of fat) and extract 1% + tocopherol (0.02% of fat) are effective combined antioxidant treatments and treated salmon did not significantly differ from fresh salmon in terms of appearance. Lightness was less than that of standard range in all the antioxidant combinations (**Table 5.7**); the antioxidants showed a statistically significant effect on lightness (Kaur et al.; 1999a,b).

For all individual antioxidants, BHT was found to be most effective in inhibiting oxidative rancidity followed by carnosine 1%, and extract 1%. Carnosine 5%, tocopherol, and extract (5%) were not as effective in inhibiting lipid oxidation, as indicated by changes in color parameters over 7 days (Kaur et al.; 1999a,b).

Among the combined antioxidants, carnosine 1% + BHT was found to be most effective, followed by extract 1% + tocopherol, in inhibiting oxidative rancidity as indicated by color parameters. Other combined antioxidants were not as effective in

**Table 5.5-Color parameters of combined antioxidants applied to farmed salmon (*Salmo salar*) over 7 days, hue angle**

| Treatment name | Day 1 | Day 3 | Day 5 | Day 7 | |
|---|---|---|---|---|---|
| Control | 79.3 ± 0.5d[a,b] | 73.9 ± 0.15e | 75.2 ± 0.25d | 76.2 ± 1.88de | ***[c] |
| Carnosine 1%+BHT (0.02% of fat) | 82.6 ± 1.64c | 76.1 ± 0.43d | 75 ± 0.63d | 77.7 ± 0.21d | *** |
| Carnosine 1%+Toco[d] (0.02% of fat) | 79.6 ± 0.23d | 75.5 ± 0.31d | 74.3 ± 0.41d | 75.6 ± 0.24de | *** |
| Extract 1%+BHT (0.02% of fat) | 77.9 ± 0.85e | 72.9 ± 0.27e | 74.7 ± 0.54d | 75.2 ± 0.93e | *** |
| Extract 5%+BHT (0.02% of fat) | 87.6 ± 0.64a | 88.9 ± 0.32a | 82.4 ± 0.83b | 83.6 ± 2.73b | *** |
| Extract 1%+Toco (0.02% of fat) | 86.8 ± 0.41b | 78.0 ± 2.14c | 78.7 ± 0.40c | 80.9 ± 0.17c | ns[e] |
| Extract 5%+Toco (0.02% of fat) | 85.8 ± 1.59b | 86.8 ± 0.76b | 98.2 ± 1.37a | 108.6 ± 1.39a | *** |

[a] Overall means are shown with standard deviation (reps 4).

[b] a-e: treatment means in the same column for a given antioxidant marked with different alphabet differs ($\alpha = 0.05$).

[c] These treatments are significantly different over a period of 7 days at ($\alpha$ =0.05, 0.01, 0.001).

[d] Toco = tocopherol.

[e] Not significant.

**Table 5.6-Color parameters of combined antioxidants applied to farmed salmon *(Salmo salar)* over 7 days, chroma**

| Treatment Name | Day 1 | Day 3 | Day 5 | Day 7 | |
|---|---|---|---|---|---|
| Control | 26.9 ± 0.28d[a,b] | 24.8 ± 0.00b | 24.4 ± 0.05b | 24.2 ± 0.5c | ***[c] |
| Carnosine 1%+BHT (0.02% of fat) | 28.6 ± 0.19a | 26.1 ± 0.19a | 25.4 ± 0.33a | 26 ± 0.5a | *** |
| Carnosine 1%+Toco[d] (0.02% of fat) | 26.8 ± 0.07d | 25.0 ± 0.04b | 24.2 ± 0.08b | 24.6 ± 0.03b | *** |
| Extract 1%+BHT (0.02% of fat) | 25.7 ± 0.06e | 23.5 ± 0.06d | 23.4 ± 0.19c | 24.1 ± 0.24c | *** |
| Extract 5%+BHT (0.02% of fat) | 28.1 ± 0.12b | 24.1 ± 0.12c | 19.6 ± 0.18d | 17.6 ± 0.34e | *** |
| Extract 1%+Toco (0.02% of fat) | 28.0 ± 0.05b | 24.7 ± 0.81b | 23.5 ± 0.09c | 22.8 ± 0.01d | *** |
| Extract 5%+Toco (0.02% of fat) | 27.3 ± 0.67c | 23.0 ± 0.01e | 19.4 ± 0.35d | 17.8 ± 0.02e | *** |

[a] Overall means are shown with standard deviation (reps 4).
[b] a-e: treatment means in the same column for a given antioxidant marked with different alphabet differs ($\alpha = 0.05$).
[c] These treatments are significantly different over a period of 7 days at ($\alpha$ =0.05, 0.01, 0.001).
[d] Toco = tocopherol.

**Table 5.7-Color parameters of combined antioxidants applied to farmed salmon (*Salmo salar*) over 7 days, lightness**

| Treatment Name | Day 1 | Day 3 | Day 5 | Day 7 | |
|---|---|---|---|---|---|
| Control | 62.9 ± 0.15b[a,b] | 59.9 ± 0.04a | 59.0 ± 0.01b | 56.2 ± 0.5c | ***[c] |
| Carnosine 1%+BHT (0.02% of fat) | 60.0 ± 0.26d | 57.8 ± 0.32c | 56.0 ± 0.38e | 55.1 ± 0.11d | *** |
| Carnosine 1%+Toco[d] (0.02% of fat) | 59.6 ± 0.35e | 57 ± 0.18d | 55.2 ± 0.02f | 54.4 ± 0.05e | *** |
| Extract 1%+BHT (0.02% of fat) | 57.5 ± 0.40f | 55.3 ± 0.26f | 54.2 ± 0.22g | 53.3 ± 0.38f | *** |
| Extract 5%+BHT (0.02% of fat) | 63.3 ± 0.13a | 56.7 ± 0.12e | 58.4 ± 0.02c | 59 ± 0.06b | *** |
| Extract 1%+Toco (0.02% of fat) | 62.0 ± 0.16c | 58.5 ± 0.15b | 57.0 ± 0.15d | 56.2 ± 0.01c | *** |
| Extract 5%+Toco (0.02% of fat) | 63.6 ± 0.29a | 60.0 ± 0.05a | 60.4 ± 0.48a | 60.7 ± 0.03a | *** |

[a] Overall means are shown with standard deviation (reps–4).
[b] a-g: treatment means in the same column for a given antioxidant marked with different alphabet differs ($\alpha = 0.05$).
[c] Treatments are significantly different over a period of 7 days at ($\alpha$ =0.05, 0.01, 0.001).
[d] Toco = tocopherol.

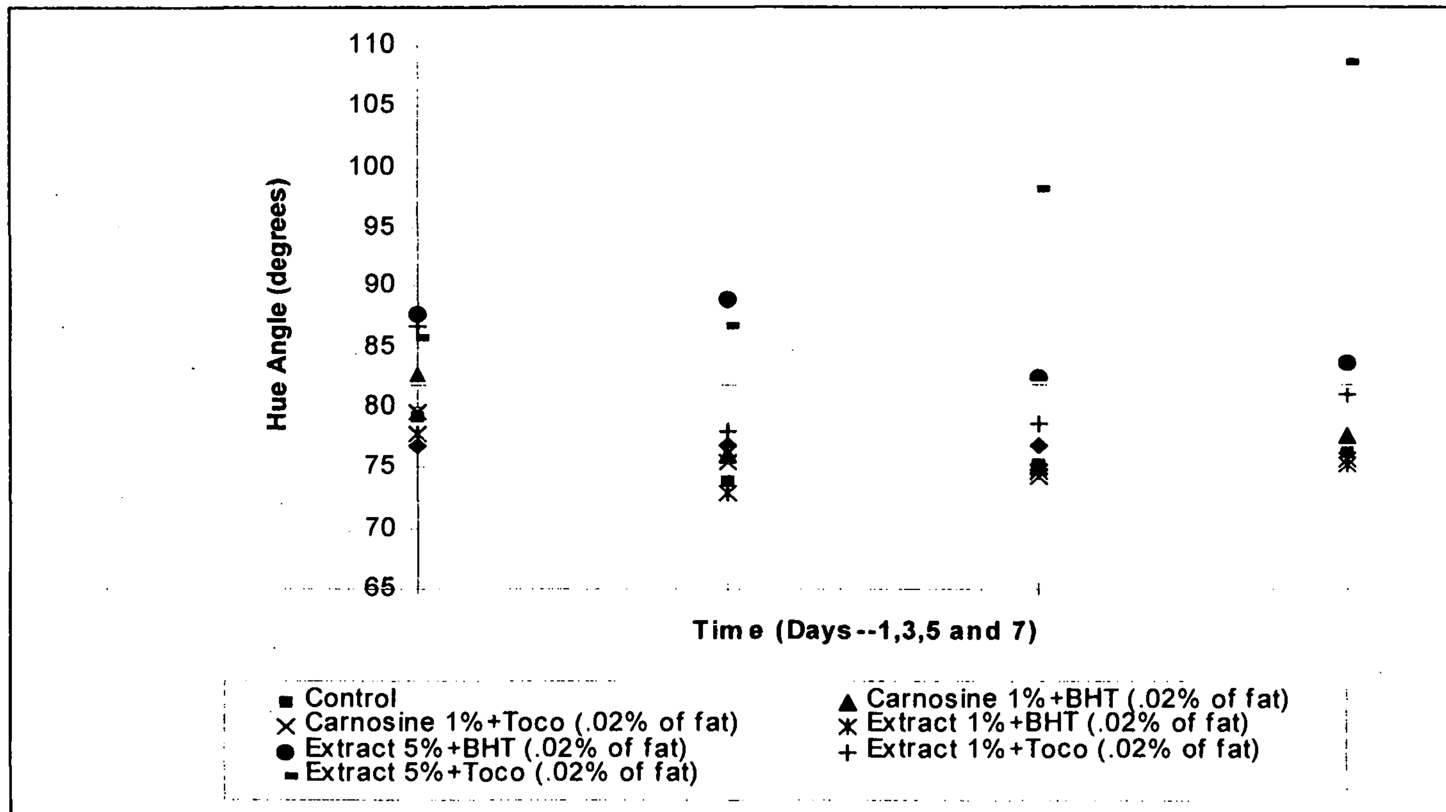



**Fig 5.2-Hue angle of combined antioxidants with acceptable range for salmon (*Salmo salar*) treated with antioxidants (5°C)**

inhibiting lipid oxidation. The color differences were also quite apparent visually (Kaur et al.; 1999a,b).

The use of antioxidants can help to improve fish quality, shelf life and consumer satisfaction. The use of SWW extract containing dipeptides is economical for 2 reasons. First, salmon is an expensive seafood product and increasing the shelf life is beneficial. Secondly, SWW extract is effective at lower concentrations and can be obtained more economically from surimi wash water. However, further research is required to test the effectiveness of SWW extract at lower concentrations. It is suggested by this study that different concentrations (0.5-2.5%) can be used for evaluation purposes. This will enable researchers to evaluate the maximum concentration level at which SWW extract can inhibit oxidative rancidity effectively. More research will be needed to investigate the potential use of SWW extract as a food grade antioxidant.

**Summary**

Lipid oxidation decreases the shelf life of seafood by causing the deterioration of flavor, color and texture due to the formation of lipid oxidation products such as peroxides and aldehydes. Carotenoids are the pigments responsible for giving the yellow or red color to salmon. Oxidation is the major cause of carotenoid degradation in foods. Carotenoid degradation proceeds in an analogous fashion to lipid degradation. In this study, lipid oxidation was studied by analyzing color parameters: hue angle, chroma and lightness. The results showed that amongst individual antioxidants, after BHT and

carnosine (1%), SWW extract (1%) is an effective antioxidant in inhibiting oxidative rancidity and hence color maintenance over a 7-day period at refrigerated temperature. In combined antioxidants, carnosine 1% + BHT was most effective in inhibiting oxidative rancidity and hence color. However, extract 1% was more effective in inhibiting oxidative rancidity in combination with tocopherol than BHT.

It is noteworthy that the SWW extract at a lower concentration (1%) was more effective than at a higher concentration (5%). This may be due to increased presence of interfering compounds or pro-oxidants in the extract at higher concentrations as mentioned earlier. Results show that the SWW extract can be used as a potential food antioxidant (natural food additive) that is economical and can help to increase the shelf life of fish (salmonoids). Also, SWW extract will help to improve fish quality and consumer satisfaction. However, more research is required for evaluating its use as a food antioxidant.

**References**

Addis, P.B. and Park, S.W. 1989. Role of lipid oxidation products in atherosclerosis. In *Food Toxicology: A perspective on the relative risks,* S.L. Taylor and R.A. Scanlan (Ed.), p. 297. Marcel Dekker, Inc., New York.

AOAC. 1995. Official Methods of Analysis, 16$^{th}$ ed. Association of Official Analytical Chemists, Arlington, VA.

Ashghar, A., Gray, J.I., Buckley, A.M., Pearson, A.M., and Booren, A.M. 1988. Perspectives on warmed-over-flavor. Food Technol. 42(6): 102.

Bjerkeng, B. and Johnsen, G. 1995. Frozen storage quality of rainbow trout (*Oncorhynchus mykiss*) as affected by oxygen, illumination and fillet pigment. J. Food Sci. 60: 284-288.

Boldyrev, A.A., Dupin, A.M., Pindel, E.V., and Severin, S.E. 1988. Antioxidative properties of histidine-containing dipeptides from skeletal muscles of vertebrates. Comp. Biochem. Physiol. 89B: 245.

Chen, H., Meyers, S.P., Hardy, R.W., and Biede, S.L. 1984. Color stability of astaxanthin pigmented rainbow trout under various packaging conditions. J. Food Sci. 49: 1337-1340.

Choubert, G., Blanc, J., and Courvalin, C. 1992. Muscle carotenoid content and color of farmed rainbow trout fed astaxanthin or canthaxanthin as affected by cooking and smoke-curing procedures. Int. J. Food Sci. & Tech. 27: 277-284.

Decker E.A. and Crum, A.D. 1991. Inhibition of oxidative rancidity in salted ground pork by carnosine. J. Food Sci. 56: 1179.

Decker, E.A. and Faraji, H. 1990. Inhibition of lipid oxidation by carnosine. JAOCS 67: 650.

Decker, E.A. and Hultin, H.O. 1992. Lipid oxidation in muscle foods via redox iron. In *Lipid Oxidation in Foods*, A.J. St. Angelo (Ed.), p. 33-54. American Chemical Society Symposium Series Vol. 500, American Chemical Society Books, Washington, D.C.

Decker, E.A., Crum, A.D., and Calvert, J.T. 1992. Differences in the antioxidant mechanism of carnosine in the presence of copper and iron. J. Agric. Food Chem. 40: 756.

Flick, G.J., Hong, G.P., and Knoll, G.M. 1992. Lipid oxidation in seafood during storage. In *Lipid Oxidation in Foods*, A.J. St. Angelo (Ed.), p.183-207. American Chemical Society Symposium Series Vol. 500, American Chemical Society Books, Washington, D.C.

Francis, F.J. and Clydesdale, F.M. 1975. Color measurement of foods. Food Product Dev. 5(7): 33.

Haard, N.F. 1988. Biochemistry and chemistry of color and color change in seafoods. In *Advances in Seafood Biochemistry*, G.J. Flick and R.E. Martin (Ed.), p. 305. Technomic Publ. Co., Inc., Lancaster, PA.

Hagen, T. 1998. Personal communication. Linus Pauling Institute, Oregon State Univ., Corvallis.

Hari, R.K., Patel, T.R., and Martin, A.M. 1994. An overview of pigment production in biological systems: functions, biosynthesis, and applications in food industry. Food Rev. Int. 10(1): 49-70.

Hong, K.N. and Storebakken, T. 1991. Color stability of rainbow trout fillets during frozen storage. J. Food Sci. 56(4): 969-972, 984.

Hunter, R.S. 1975. *The Measurement of Appearance*. John Wiley & Sons, New York.

Hunter 1991. Hunter lab, ISO 9001 certified. Hunter Assoc. Lab., Inc., Reston, VA.

Ingemansson, T., Pettersson, A., and Kaufmann, P. 1993. Lipid hydrolysis and oxidation related to astaxanthin content in light and dark muscle of frozen stored rainbow trout (*Oncorhynchus mykiss*). J. Food Sci. 58(3): 513-518.

Kanner, J., Shegalovich, I., Harel, S., and Hazan, B. 1988. Muscle lipid peroxidation dependent on oxygen and free metal ions. J. Agric. Food Chem. 36: 409.

Kaur, J., Morrissey, M.T., Decker, E.A., and Yang, L. 1999a. Concentration of dipeptides--anserine and carnosine in surimi wash water. Presented at 51st Ann. Mtg., Pacific Fisheries Technologists, Parksville, B.C., Feb 7-10.

Kaur, J., Morrissey, M.T., Decker, E.A., and Yang, L. 1999b. Concentration of anserine and carnosine in surimi wash water and its antioxidant activity. Presented at Biological Colloquium, Corvallis, OR. Feb 24-25.

Kelleher, S.D., Hultin, H.O., and Wilhelm, K.A. 1994. Stability of mackerel surimi prepared under lipid-stabilizing processing conditions. J. Food Sci. 59: 269-271.

Khare, A., Moss, G. P., Weedon, B.C.L., and Mattews, A.D. 1973. Identification of astaxanthin in Scottish salmon. Comp. Biochem. Physiol. 45B: 971.

Kohen, R., Yamamoto, Y., Cundy, K.C., and Ames, B. 1988. Antioxidant activity of carnosine, homocarnosine, and anserine present in muscle and brain. Proc. Natl. Acad. Sci. USA 85: 3175.

Li, S.J., Seymour, T.A., King, A.J., and Morrissey, M.T. 1998. Color stability and lipid oxidation of rockfish as affected by antioxidant from shrimp shell waste. J. Food Sci. 63: 438-441.

McCallum, I.M., Cheng, K.M., and March, B.E. 1987. Carotenoid pigmentation of two strains of chinook salmon (*Oncorhynchus tshawytscha*) and their crosses. Aquaculture 67: 291.

Metusalach, S., Brown, J.A., and Shahidi, F. 1997. Effects of stocking density on color characteristics and deposition of carotenoids in cultured Arctic charr (*Salvelinus alpinus*). Food Chem. 59(1): 107-114.

Minolta®. 1994. *Precise Color Communication, Color Control from Feeling to Instrumentation.* Minolta Corp., New Jersey.

No, H.K. and Storebakken, T. 1991. Color stability of rainbow trout fillets during frozen storage. J. Food Sci. 56 (4): 969-984.

Ostrander, J., Martinsen, C., Liston, J., and McCollough, J. 1976. Sensory testing of pen-reared salmon and trout. J. Food Sci. 41: 386.

Reed, R. 1998. Personal communication. Oregon State Univ., Corvallis.

Sigurgisladottir, S., Parrish, C.C., Lall, S. P., and Ackman, R.G. 1994. Effects of feeding natural tocopherols and astaxanthin on Atlantic salmon (*Salmo salar*) fillet quality. Food Res. Int. 27: 23-32.

Skrede, G. and Storebakken, T. 1986a. Characteristics of color in raw, baked and smoked wild and pen-reared Atlantic salmon. J. Food Sci. 51(3): 804-808.

Skrede, G. and Storebakken, T. 1986b. Instrumental colour analysis of farmed and wild Atlantic salmon when raw, baked and smoked. Aquaculture 53: 279-286.

Skrede, G., Storebakken, T., and Naes, T. 1989. Color evaluation in raw, baked and smoked flesh of rainbow trout (*Onchorhynchus mykiss*) fed astaxanthin or canthaxanthin). J. Food Sci. 55: 1574-1578.

Skrede, G., Risvik, E., Huber, M., Enersen, G., and Blumlein, L. 1990. Developing a color card for raw flesh of astaxanthin-fed salmon. J. Food Sci. 55: 361-363.

Skrede, G. 1997. Personal communication. Oregon State Univ., Corvallis.

Statistical Analysis System. 1988. SAS Institute Inc., Cary, N.C.

Strachan, N.J.C. 1993. Recognition of fish species by color and shape. Image & Vision Computing 11(1): 2-8.

Swatland, H.J., Haworth, C.R., Darkin, F., and Moccia, R.D. 1997. Fiber-optic spectrophotometry of raw, smoked and baked Arctic charr (*Salvelinus alpinus*). Food Res. Int. 30(2): 141-146.

Wasson, D.H., Reppond, K.D., and Kandianis, T.M. 1991. Antioxidants to preserve rockfish color. J. Food Sci. 56(6): 1564-1566.

Williams, W.D. 1992. Origin and impact of color on consumer preference of food. Poultry Sci. 71:744-746.

Wrolstad, R. 1998. Personal communication. Oregon State Univ., Corvallis.

# Chapter 6

# SUMMARY

Anserine and carnosine are natural antioxidants found in skeletal muscles of fish. These water-soluble dipeptides help reduce lipid oxidation which affects flavor, aroma, texture, color and nutritional composition. Determining the presence of these dipeptides in SWW was the first step in demonstrating their potential use as antioxidants. High performance liquid chromatography with a fluorescence detector was used to determine the concentrations of dipeptides. The results showed that the concentration of proteins and dipeptides is higher in the first 2 stages of surimi processing. SWW samples at different surimi processing stages that had higher amount of proteins also had high amount of dipeptides. Before analyzing dipeptides from SWW, it is helpful to determine protein content at all SWW stages. Additionally, results showed that SWW is a possible source for these dipeptides.

Heat treatment was used to evaluate different techniques to remove proteins and have maximum recovery of dipeptides out of SWW. Surimi wash water samples were obtained from Pt. Adams Co., Warrenton, Oregon, and were exposed to 3 different treatments: 60, 80 and 100°C, respectively, at 2 different times. Sample Set A was collected and treated on 3 different days, and in a second case (Sample Set B) a SWW sample was collected and exposed to all 3 heat treatments in a single day. Results showed that the trends of the relative amounts of proteins and total dipeptides were

similar. Also the 2 data sets of dipeptides showed similar trends; however, absolute numbers (concentration) determined were different.

There are various reasons for similar trends and different concentrations of total dipeptides in the first and second data sets: samples in the first set were collected and processed randomly and in the second case, 1 SWW sample was collected and exposed to 3 heat treatments on a single day. Dipeptides are antioxidants and may possibly be affected by microbial contamination, chemical reaction with other compounds in wash water, enzymatic changes, physical damage, possible breakdown to other components and reaction with proteins. For example, the surimi processing plant was cleaner on some days than others. Microbial contaminants and chemicals also affect the levels of antioxidants. There were differences in the 2 instruments used (HPLC and detector), age of the columns used, temperature maintenance of the column, injecting solutions, derivatization technique during the experiment, whether the injector is a manual or auto sampler, and there may have been contamination from the surfaces and pipettes, etc. Among heat treatments, 80°C, followed by 100°C, can be used for removal of proteins and the recovery of dipeptides.

The concentration of anserine and carnosine varies from 1 species to another. Dehydrator 1 (DH1) and Dehydrator 2 (DH2) had higher amounts of dipeptides than those of other stages of surimi processing. Ultrafiltration was done for selected SWW samples: DH1, DH2 and Rotary Screen 1 (RS1). The samples were first put through a 50K MWCO membrane and then through 30K, 10K and 1K MWCO membranes individually. The results showed that the 1K MWCO membrane can be used for the recovery of dipeptides. Also, the recovery of dipeptides was best from DH1.

The iron content in all SWW samples (raw, heat treated and ultrafiltered) was determined using AAS and colorimetry. The results showed that the concentration of iron in almost all the samples was negligible (≤1 ppm) in liquid portion or water. Therefore, the removal of iron out of SWW for our purpose was not necessary. SWW extract was obtained using ion exchange chromatography from Decker's laboratory at the University of Massachusetts, Amherst, MA and was used for antioxidation studies.

Lipid oxidation decreases the shelf life of seafood by causing deterioration of flavor, color and texture due to the formation of lipid oxidation products such as peroxides and aldehydes. Carotenoids are the pigments responsible for giving the yellow or red color to salmon. Oxidation is the major cause of carotenoid degradation in foods. Carotenoid degradation proceeds in an analogous fashion to lipid degradation. In this study, lipid oxidation was studied via analyzing color parameters: hue angle, chroma and lightness. BHT, carnosine 1%, and SWW extract 1% were effective antioxidants in inhibiting oxidative color changes over a 7-day period at refrigerated temperature. In combination, carnosine 1% + BHT was effective in preventing color deterioration However, SWW extract 1% was more effective in inhibiting oxidative color changes in combination with tocopherol than BHT. It is note worthy that the extract at a lower concentration (1%) was more effective than at a higher concentration (5%). This may be due to increased presence of impurities or pro-oxidants in the extract at higher concentrations. Results show that the SWW extract can be used as a potential food antioxidant (natural food additive) that is economical and can help to increase the shelf life of fishes (salmonoids). Also, SWW extract may help to improve fish quality and consumer satisfaction.

To conclude, the first step was to determine the concentration of proteins and dipeptides in SWW samples. Next, heat treatment and ultra filtration techniques were used to remove iron and proteins from SWW samples and recover dipeptides. Iron content was determined and finally the SWW extract was obtained using ion exchange chromatography. The antioxidant activity of SWW, along with other antioxidants, was determined. The extraction of dipeptides out of SWW and their ability to inhibit oxidative color changes s in fresh-farmed salmon indicates that SWW extract may have potential use as a natural food antioxidant in the future.

# BIBLIOGRAPHY


Abe, H. 1995. Histidine-related dipeptides: distribution, metabolism, and physiological function. In *Biochemistry and Molecular Biology of Fishes*, Vol. 4, P.W. Hochachka and T.P. Mommsen (Eds.), p. 309-333.

Addis, P.B. and Park, S.W. 1989. Role of lipid oxidation products in atherosclerosis. In *Food Toxicology: A perspective on the Relative Risks*. S.L. Taylor and R.A. Scanlan (Ed.), p. 297. Marcel Dekker, Inc., New York.

AOAC. 1995. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Arlington, VA.

Ashghar, A., Gray, J.I., Buckley, A.M., Pearson, A.M., and Booren, A.M. 1988. Perspectives on warmed-over-flavor. Food Technol. 42(6): 102.

Aursand, M., Jorgensen, L., and Grasdalen, H. 1995. Quantitative high-resolution C nuclear magnetic resonance of anserine and lactate in white muscle of Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. 112B(2): 315-321.

Bailey, M.E. 1988. Inhibition of warmed-over-flavor, with emphasis on Milliard reaction products. Food Technol. 42(6): 123.

Bjerkeng, B. and Johnsen, G. 1995. Frozen storage quality of rainbow trout (*Oncorhynchus mykiss*) as affected by oxygen, illumination and fillet pigment. J. Food Sci. 60: 284-288.

Boldyrev, A.A. and Severin, S.E. 1990. The histidine-containing dipeptides, carnosine and anserine: distribution, properties and biological significance. Adv. Enzyme Regul. 30: 195.

Boldyrev, A.A., Dupin, A.M., Pindel, E.V., and Severin, S.E. 1988. Antioxidative properties of histidine-containing dipeptides from skeletal muscles of vertebrates. Comp. Biochem. Physiol. 89B: 245.

Bond, C.E. 1965. Outline of classification of fishes. Unpubl. Rep., Dept. of Fisheries and Wildlife, Oregon State Univ., Corvallis.

Caldwell, D.H. and Adams, R.B. 1946. Colorimetric determination of iron in water with o-Phenanthroline. J. Am. Water Works Assoc. 38: 727-730.

Chan, K.M. and Decker, E.A. 1994. Endogenous skeletal muscle antioxidants. Crit. Rev. Food Sci. & Nutr. 34: 403-426.

Chan, K.M., Decker, E.A., and Means, W. 1993. Extraction and activity of carnosine, a naturally occurring antioxidant in beef muscle. J. Food Sci. 58: 1.

Chao, L.S. and Gordon, D.T. 1983. Influence of fish on bioavailability of plant iron in the anemic rat. Mtg. of Am. Inst. of Nutr. Fed. Proc. 39: 1044.

Chen, H., Meyers, S.P., Hardy, R.W., and Biede, S.L. 1984. Color stability of astaxanthin pigmented rainbow trout under various packaging conditions. J. Food Sci. 49: 1337-1340.

Choi, J.Y., Kim, I., Lee, K., Kim, G., Lee, N., and Cho, Y. 1996. Available components of cooking drips, dark muscle, head and raw viscera from skipjack. J. Korean Fish. Soc. 29: 701-708.

Choubert, G., Blanc, J., and Courvalin, C. 1992. Muscle carotenoid content and color of farmed rainbow trout fed astaxanthin or canthaxanthin as affected by cooking and smoke-curing procedures. Int. J. Food Sci. & Tech. 27: 277-284.

Chung, H.Y. and Cadwallader, K.R. 1993. Volatile components in blue crab (*Callinectes sapidus*) meat and processing by-product. J. Food Sci. 58: 1203-1207, 1211.

Crush, K.G. 1970. Carnosine and related substances in animal tissues. Comp. Biochem. Physiol. 34: 3.

Dawson, R.M.C., Elliot, D.C., Elliot, W.H., and Jones, K.M. (Ed.). 1969. *Data for Biochemical Research.* Oxford Univ. Press, Oxford, U.K.

Decker, E.A. 1998. Personal communication. Univ. of Massachusetts, Amherst.

Decker, E.A. and Crum, A.D. 1991. Inhibition of oxidative rancidity in salted ground pork by carnosine. J. Food Sci. 56: 1179.

Decker, E.A. and Crum, A.D. 1993. Antioxidant activity of carnosine in cooked ground pork. Meat Sci. 34: 245.

Decker, E.A. and Faraji, H. 1990. Inhibition of lipid oxidation by carnosine. JAOCS 67: 650.

Decker, E.A. and Hultin, H.O. 1992. Lipid oxidation in muscle foods via redox iron. In *Lipid Oxidation in Foods*, A.J. St. Angelo (Ed.), p. 33-54. American Chemical Society Symposium Series Vol. 500, American Chemical Society Books, Washington, D.C.

Decker, E.A., Crum, A.D., and Calvert, J.T. 1992. Differences in the antioxidant mechanism of carnosine in the presence of copper and iron. J. Agric. Food Chem. 40: 756.

Fellows, P.J. (Ed.) 1988. *Membrane Concentration. Food Processing Technology, Principles andPractice.* Ellis Horwood Limited, Hertfordshire, U.K.

Flick, G.J., Hong, G.P., and Knoll, G.M. 1992. Lipid oxidation in seafood during storage. In *Lipid Oxidation in Foods*, A.J. St. Angelo (Ed.), p.183-207. American Chemical Society Symposium Series Vol. 500, American Chemical Society Books, Washington, D.C.

Francis, F.J., and Clydesdale, F.M. 1975. Color measurement of foods. Food Product Dev. 5(7): 33.

Goodwin, J.F., Murphy, S., Bramlett, K., and Guillemette, M. 1966. Direct measurement of serum iron and binding capacity. Clinical Chem. 12(2): 47-57.

Gordon, D.T. 1978. Atomic absorption spectrometric and colorimetric determination of iron in seafoods. J. Assoc. Off. Anal. Chem. 61: 715-719.

Gordon, D.T. and Roberts, G.L. 1977. Mineral and proximate composition of Pacific coast fish. J. Agric. Food Chem. 25: 1262-1268.

Haard, N.F. 1988. Biochemistry and chemistry of color and color change in seafoods. In *Advances in Seafood Biochemistry*, G.J. Flick and R.E. Martin (Ed.), p. 305. Technomic Publ. Co., Inc., Lancaster, PA.

Haard, N.F., Simpson, B.K., and Sikorski, Z.E. 1994. Biotechnological applications of seafood proteins and other nitrogenous compounds. In *Seafood Proteins*, Z.E. Sikorski, B.S. Pan, and F. Shahidi (Ed.), p. 194-216. Chapman & Hall Publ., New York.

Hagen, T. 1998. Personal communication. Linus Pauling Institute, Oregon State Univ., Corvallis.

Hari, R.K., Patel, T.R., and Martin, A.M. 1994. An overview of pigment production in biological systems: functions, biosynthesis, and applications in food industry. Food Rev. Int. 10: 49-70.

Hong, K.N. and Storebakken, T. 1991. Color stability of rainbow trout fillets during frozen storage. J. Food Sci. 56: 969-972, 984.

Horneck, D.A., Hart, J.M., Topper, K., and Koepsell, B. (Ed.). 1989. *Methods of Soil Analysis Use in Soil Testing Laboratory.* Oregon State Univ., Corvallis.

Huang, L. 1997. Application of membrane filtration to recover solids from protein solutions. Ph.D. dissertation, Oregon State Univ., Corvallis.

Huang, L. and Morrissey, M.T. 1996. Heat-coagulation of proteins from washed mince of Pacific whiting. Presented at the 48th Ann. Mtg., Pacific Fisheries Technologists, San Diego, CA, Feb. 18-21.

Hunter. 1991. Hunter lab, ISO 9001 certified. Hunter Assoc. Lab., Inc., Reston, VA.

Hunter, R.S. 1975. *The Measurement of Appearance*. John Wiley & Sons, New York.

Igene, J.O., King, J.A., Pearson, A.M., and Gray, J.I. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem. 27(4): 838-841.

In, T. 1990. Seafood flavorants produced by enzymatic hydrolysis. In *Making Profits Out of Seafood Wastes*, S. Keller (Ed.), p. 197. Alaska Sea Grant Publ., Fairbanks.

Ingemansson, T., Pettersson, A., and Kaufmann, P. 1993. Lipid hydrolysis and oxidation related to astaxanthin content in light and dark muscle of frozen stored rainbow trout (*Onchorhynchus mykiss*). J. Food Sci. 58: 513-518.

Johnson, P. and Hammer, J. 1992. Histidine containing dipeptide levels in aging and hypertensive rat skeletal and cardiac muscles. Comp. Biochem. Physiol. 103B: 981-984.

Kanner, J., Shegalovich, I., Harel, S., and Hazan, B. 1988. Muscle lipid peroxidation dependent on oxygen and free metal ions. J. Agric. Food Chem. 36: 409.

Kaur, J., Morrissey, M.T., Decker, E.A., and Yang, L. 1998. Concentration of anserine and carnosine in surimi wash water. Presented at Ann. Mtg., Inst. of Food Technologists, Atlanta, GA, June 20-24.

Kaur, J., Morrissey, M.T., Decker, E.A., and Yang, L. 1999a. Concentration of dipeptides--anserine and carnosine in surimi wash water. Presented at 51st Ann. Mtg., Pacific Fisheries Technologists, Parksville, B.C., Feb 7-10.

Kaur, J., Morrissey, M.T., Decker, E.A., and Yang, L. 1999b. Concentration of anserine and carnosine in surimi wash water and its antioxidant activity. Presented at Biological Colloquium, Corvallis, OR. Feb. 24-25.

Kelleher, S.D., Hultin, H.O., and Wilhelm, K.A. 1994. Stability of mackerel surimi prepared under lipid-stabilizing processing conditions. J. Food Sci. 59: 269-271.

Keller, S. (Ed.). 1990. Making profits out of seafood wastes. Proc. of the Int. Conf. on Fish By-products, Alaska Sea Grant College Program, Fairbanks.

Khare, A., Moss, G. P., Weedon, B.C.L., and Mattews, A.D. 1973. Identification of astaxanthin in Scottish salmon. Comp. Biochem. Physiol. 45B: 971.

Khayat, A., and Schwall, D. 1983. Lipid oxidation in seafood. Food Technol. 63(6): 130.

Kohen, R., Yamamoto, Y., Cundy, K.C., and Ames, B. 1988. Antioxidant activity of carnosine, homocarnosine, and anserine present in muscle and brain. Proc. Natl. Acad. Sci. USA 85: 3175.

Lai, S., Gray, J.I., Booren, A.M., Crackel, R.L., and Gill, J.L. 1995. Assessment of off-flavor development in restructured chicken nuggets using hexanal and TBARS measurements and sensory evaluation. J. Sci. Food Agric. 67: 447-452.

Lanier, T.C. 1994. Functional food protein ingredients from fish. In *Seafood Proteins*, Z.E. Sikorski, B.S. Pan, and F. Shahidi (Ed.), p. 127-159. Chapman & Hall Publ., New York.

Lee, C. 1984. Surimi process technology. Food Technol. 38(11): 69-80.

Lee, B.J., Hendricks, D.G., and Cornforth, D.P. 1999. A comparison of carnosine and ascorbic acid on color and lipid stability in a ground beef pattie model system. Meat Sci. 51:245-253.

Li, S.J., Morrissey, M.T., and Seymour, T. 1994. Isolation of a natural antioxidant from shrimp waste. Presented at Ann. Mtg., Oil Chemists Soc., Atlanta, GA, May 9-12.

Li, S.J., Seymour, T.A., King, A.J., and Morrissey, M.T. 1998. Color stability and lipid oxidation of rockfish as affected by antioxidant from shrimp shell waste. J. Food Sci. 63: 438-441.

Lin, T.M., Park, J., and Morrissey, M.T. 1995. Recovered protein and reconditioned water from surimi processing waste. J. Food Sci. 60: 4-9.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

McCallum, I.M., Cheng, K.M., and March, B.E. 1987. Carotenoid pigmentation of two strains of chinook salmon (*Oncorhynchus tshawytscha*) and their crosses. Aquaculture 67: 291.

Metusalach, S., Brown, J.A., and Shahidi, F. 1997. Effects of stocking density on color characteristics and deposition of carotenoids in cultured Arctic charr (*Salvelinus alpinus*). Food Chem. 59(1): 107-114.

Minolta™. 1994. *Precise Color Communication, Color Control from Feeling to Instrumentation.* Minolta Corp., New Jersey.

Morrissey, M.T., Peters, G., and Sylvia, G. 1992. Quality issues in the Pacific whiting fisheries. In *Pacific Whiting: Processing, Marketing and Quality Assurance*, G. Sylvia and M.T. Morrissey (Ed.), p. 9-16. Oregon Sea Grant, Corvallis.

No, H.K. and Storebakken, T. 1991. Color stability of rainbow trout fillets during frozen storage. J. Food Sci. 56: 969-984.

Ostrander, J., Martinsen, C., Liston, J., and McCollough, J. 1976. Sensory testing of pen-reared salmon and trout. J. Food Sci. 41: 386.

Persijn, J.P., Slik, W.V., and Riethorst, A. 1971. Determination of serum iron and latent iron-binding. Clin. Chem. Acta 35: 91-98.

Potter, N.N. and Hotchkiss, J.H. 1995. *Food Science*. 5th ed. Chapman & Hall Publ., New York.

Ramanathan, L. and Das, N.P. 1993. Natural products inhibit oxidative rancidity in salted cooked ground fish. J. Food Sci. 58: 318-320, 360.

Reed, R. 1998. Personal communication. Oregon State Univ., Corvallis.

Schagger, H. 1994. Chromatographic techniques and basic operations in membrane protein purification. In *A Practical Guide to Membrane Protein Verification*, G.V. Jagow and H. Schagger (Ed.), p. 23. Academic Press, San Diego, CA.

Scott, K. 1995. Introduction to membrane separations. In *Handbook of Industrial Membranes,* 1st ed., p. 27. Elsevier Advanced Technology, Oxford, U.K.

Sigma Chemical Company. 1998. Personal communication. St. Louis, MO.

Sigurgisladottir, S., Parrish, C.C., Lall, S. P., and Ackman, R.G. 1994. Effects of feeding natural tocopherols and astaxanthin on Atlantic salmon (*Salmo salar*) fillet quality. Food Res. Int. 27: 23-32.

Simpson, B. D. and Haard, N. F. 1985. The use of proteolytic enzymes to extract carotenoproteins from shrimp wastes. J. Appl. Biochem. 7: 212.

Singh, R.P. and Heldman, D.R. 1993. *Introduction to Food Engineering*, 2nd ed. Academic Press, New York.

Skaugrud, O. and Sargent, G. 1990. Chitin and chitosan: crustacean biopolymers with potential. In *Making Profits Out of Seafood Wastes*, S. Keller (Ed.), p. 61-70. Alaska Sea Grant Publ., Fairbanks.

Skrede, G. 1997. Personal communication. Oregon State Univ., Corvallis.

Skrede, G. and Storebakken, T. 1986a. Characteristics of color in raw, baked and smoked wild and pen-reared Atlantic salmon. J. Food Sci. 51(3): 804-808.

Skrede, G. and Storebakken, T. 1986b. Instrumental colour analysis of farmed and wild Atlantic salmon when raw, baked and smoked. Aquaculture 53: 279-286.

Skrede, G., Storebakken, T., and Naes, T. 1989. Color evaluation in raw, baked and smoked flesh of rainbow trout (*Onchorhynchus mykiss*) fed astaxanthin or canthaxanthin). J. Food Sci. 55: 1574-1578.

Skrede, G., Risvik, E., Huber, M., Enersen, G., and Blumlein, L. 1990. Developing a color card for raw flesh of astaxanthin-fed salmon. J. Food Sci. 55: 361-363.

Statistical Analysis System. 1988. SAS Institute Inc., Cary, N.C.

Stookey, L.L. 1970. Ferrozine--a new spectrophotometric reagent for iron. Anal. Chem. 42: 779-781.

Strachan, N.J.C. 1993. Recognition of fish species by color and shape. Image & Vision Computing 11:2-8.

Suzuki, T., Shirai, T., and Hirano, T. 1990. Distribution of extractive nitrogenous constituents in white and dark muscles of fresh-water fish. Comp. Biochem. Physiol. 96B: 107-111.

Suzuki, T., Shirai, T., and Hirano, T. 1991. Anserine and free amino acid contents in the egg and fry stages of developing rainbow trout (*Onchorhynchus mykiss*). Comp. Biochem. Physiol. 100A: 105-108.

Swatland, H.J., Haworth, C.R., Darkin, F., and Moccia, R.D. 1997. Fiber-optic spectrophotometry of raw, smoked and baked Arctic charr (*Salvelinus alpinus*). Food Res. Int. 30: 141-146.

Teahon, K. and Rideout, J.M. 1992. A sensitive and specific high performance liquid chromatographic assay for imidizole dipeptides and 3-methylhistidine in human muscle biopsies, serum and urine. Biomed. Chromatogr. 6: 16.

Toledo, R.T. 1991. *Fundamentals of Food Process Engineering*, 2nd ed. Van Nostrand Reinhold, New York.

Toyomoto, K. and Higuchi, A. 1992. Microfiltration and ultrafiltration. In *Membrane Science and Technology*. Y. Osada, and T. Nakagawa (Ed.), p. 289-331. Marcel Dekker Inc., New York.

Wasson, D.H., Reppond, K.D., and Kandianis, T.M. 1991. Antioxidants to preserve rockfish color. J. Food Sci. 56: 1564-1566.

Weissman, N. and Pileggi, V.J. 1974. Inorganic ions. In *Clinical Chemistry Principles and Techniques*, 2nd ed., R.J. Henry, D.C. Cannon, and J.W. Winkelman (Ed.), p. 684, 685, 695. Harper & Row, New York.

Williams, W.D. 1992. Origin and impact of color on consumer preference of food. Poultry Sci. 71: 744-746.

Wrolstad, R. 1998. Personal Communication. Oregon State Univ., Corvallis.