AN ABSTRACT OF THE THESIS OF

Shiao Jing Li for the degree of Doctor of Philosophy in Food Science and Technology presented on August 2, 1994.

Title: Isolation of a Natural Antioxidant from Shrimp Waste.

Abstract approved: _____

Michael T. Morrissey

Shrimp waste samples were extracted with a variety of organic solvents. Each fraction was measured for antioxidant activity by determining the rate of oxidation of β-carotene-linoleic acid in an emulsion system. An ethanol extract exhibited the highest antioxidant activity. Purification of the most active fraction was accomplished by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Antioxidant activity was not significantly effected by heating at 100°C for 4 hr or 30 days storage at 4°C.

Purified antioxidant samples were further analyzed by several spectroscopy methods such as Fourier transformed-infrared spectroscopy (FT-IR), mass spectrometry and nuclear magnetic resonance (NMR). The antioxidant was characterized as an ortho-disubstituted benzene. The content of antioxidant in shrimp waste was estimated to be 1.80 ppm.

Antioxidant from shrimp waste was extracted and partially purified by silica gel glass column chromatography. Two species of rockfish (*Sebastolobus alascanus, Sebastes ruberriumus*) were treated with crude antioxidant solution respectively, while rockfish fillets (*Sebastes alutus*) were treated with different concentrations of antioxidant solutions from the column chromatography. Higher a* values were found in rockfish samples treated with antioxidants compared to the control without antioxidant during iced storage. Furthermore, rockfish fillets treated with 0.20%, and 0.50% (w/v) antioxidant had lower 2-thiobarbituric acid (TBA) values compared to the control group of rockfish fillets (*Sebastes alutus*).

Crude extract (0.50% w/w), and purified antioxidant (0.10%, 0.20%, and 0.50% w/w) from shrimp waste were applied to sablefish mince and evaluated for their effectiveness to inhibit oxidative and hydrolytic rancidity of mince samples. Treatments with crude extract (0.5%), partially purified antioxidant (0.2%, 0.5%) had a significantly lower TBA, and peroxide value (PV) compared to the control group during refrigerated ($4^0$C) and frozen storage ($-20^0$C). The results from free fatty acid values suggested that antioxidant from shrimp waste had no effect on hydrolytic rancidity in sablefish mince.

# Isolation of a Natural Antioxidant from Shrimp Waste

by

Shiao Jing Li

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed August 2, 1994
Commencement June 1995

Doctor of Philosophy thesis of Shiao Jing Li presented on August 2, 1994

APPROVED:

Professor in Food Science and Technology in charge of major

Head of Department of Food Science and Technology

Dean of the Graduate School

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

Shiao Jing Li, Author

**ACKNOWLEDGMENTS**

I would like to thank my major professor, Dr. Michael Morrissey, for his encouragement, guidance, and support during the entire time of my Ph.D study. I would also like to express my sincere gratitude to my thesis committee members, Dr. Michael Penner, Dr. Henry Schaup, Dr. Tom Seymour and Dr. Lloyd Swanson for their time and advise. Thanks also to Dr. Ronald Wrolstad and other faculty in the Department of Food Science, at OSU for their help.

Special thanks to Dr. An, Dr. Park, Lewis Richardson, Nancy Chamberlain, Toni, Tami, Jovi, Lynne, etc. for their assistance. I also would like to take this opportunity to thank my other friends at the Seafoods Lab for their help.

Finally, I would like to thank my parents, brother, and sisters for their spiritual support. This thesis is dedicated to my daughter, Anqi Li. She is my inspiration.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

LIST OF APPENDIX TABLES

# ISOLATION OF A NATURAL ANTIOXIDANT FROM SHRIMP WASTE

## I. INTRODUCTION

The West Coast shrimp fishery has maintained an annual harvest of more than 50 million lb of shrimp since 1987 (Talley, 1993). More than 50% of the harvest was landed in Oregon during this time period, however, the states of Washington and California also contributed significantly to the capture. In most cases, shrimp is landed at processing plants where it is cooked, peeled, packaged, and frozen for market. Cooked, peeled shrimp has an average recovery of 28-30%, and the resulting shrimp discard represents more than 30 million lb of waste material on the Pacific Coast. However, the shellfish industry as a whole is faced with an increasing dilemma due to recent enforcement of pollution laws which now prohibit the disposal of shellfish waste into the ocean or in landfill dumping sites (Mandeville et al., 1992). It is critical that avenues be found for various aspects of waste utilization.

### Utilization of Shrimp Waste

It is well documented that shrimp waste has been used for animal feed. Kirk et al. (1967) reported that shrimp waste can have value as a feed supplement, primarily for its protein content, if offered at low levels in combination with traditional feedstuffs. Watkins et al. (1982) also

indicated that shrimp waste could be a satisfactory protein supplement for mink. Feeding experiments with rainbow trout and salmon showed that the protein, in the acid preserved shrimp waste had a good nutritional value (Tidemann et al., 1982). Shrimp waste contains a variety of feeding stimulants, such as, amino acids which increase the value of the diet considerably in terms of its attractiveness for various aquatic species (Meyer, 1986).

Shrimp waste, either in a fresh, frozen, or dried form, was used as a natural source of carotenoids for pigmentation purposes (Meyer, 1986). Earlier reports have shown that feeding dried red shrimp waste was used to yield desirable color in a cultured sea bream and colored carp (Saito and Kondo, 1967). Shrimp waste as a pigment source for trout has been extensively studied. Tunison et al. (1947) first reported the coloration in trout was increased by feeding carotenoid-rich diets, including shrimp waste. A U.S. Dept. of Interior report (1968) indicated that trout color can be improved by feeding a high quality vacuum-dried shrimp meal, while high-temperature processed shrimp meal did not affect flesh color. The shrimp waste, as a source of carotenoid pigments, was also used as aquaculture diet for brook trout (*Salvelinus fontinalis*) (Saito and Regier, 1971). They found that the carotenoid compounds in dried shrimp waste can be transferred to the flesh and skin of brook trout. Moreover, Steel (1971) reported that shrimp waste meal

significantly increased the pigmentation in the skin and muscular tissue of rainbow trout (*Salmo gairdneri*). By-products of shrimp waste were proposed as sources of astaxanthin for pigmentation of Atlantic salmon (Tidemann et al., 1982).

Tidemann et al. (1982) showed a good recovery of astaxanthin from shrimp waste achieved by using hydrochloric acid. Extraction of crustacean wastes with soy oil can effectively reduce both the ash and the chitin levels and facilitate a good recovery of pigments (Spinelli et al., 1974; Chen and Meyer, 1982). Raa and Hansen (1982) indicated results on the extraction of astaxanthin from shrimp waste using hydrochloric acid, ethyl acetate and soybean oil. Shrimp waste was also used to extract carotenoprotein, which is a promising source of feed supplement for farmed fish or as a colorant and flavorant for application in food products (Simpson and Haard, 1985; Cano-Lopez et al., 1987).

Another way of utilizing shrimp waste is to extract flavor-active compounds. Kubota et al. (1986) illustrated that pyridine was a flavor constituent of roasted and boiled shrimp. Mandeville et al. (1991) reported that flavor-active components were extracted from raw commercial shrimp waste. These compounds were also isolated from cooked commercial shrimp waste (Mandeville et al., 1992). The main

use of such flavor extracts can be found in the manufacture of surimi-type products.

Attention has also focused on the manufacture and application for chitin and chitosan from shrimp shell. Chitin consists of N-acetyl glucosamine linked by β-1,4, while chitosan is made either by chemical or biochemical deacetylation of chitin. It has been shown that shell wastes were a potential source of raw material for the production of many value-added products including chitin/chitosan (Ashford et al., 1977; Johnson and Peniston, 1982). The chitin content in the shrimp shells was estimated to be 40.4% on dry basis (Naczk and Shahidi, 1990). The production of chitin and chitosan from shrimp waste is currently being commercially manufactured. The preparation of chitin from shrimp shell consists of two steps (Tsugita, 1990). The first step in the production of chitin is the removal of protein with a dilute aqueous sodium hydroxide solution, and the second step involves the calcium carbonate separation with a dilute aqueous hydrochloric acid solution in which the mineral matter is reacted out. Once the chitin stage is achieved, rinsing and drying follow. The dried chitin can be stored indefinitely until conversion to chitosan, or some other use (Ornum, 1992). The first step in producing chitosan is the selection of a chitin lot. Deacetylation reaction is an essential step to manufacture chitosan.

Sugano et al. (1980) reported that rats fed cholesterol-free diets with 0.5% chitosan contained more high-density lipoproteins (HDL)-cholesterol and less very low density lipoproteins (VLDL)-cholesterol compared to the control without chitosan treatment. Furthermore, there are numerous potential applications for chitin and chitosan; including food processing, agriculture, bio-medical, biotechnology, water treatment, cosmetics, etc. (Ornum, 1992).

**Isolation of Natural Antioxidants from Various Sources**

Recently, much attention has focused on natural antioxidants because there are some concerns about the possible health risks from the use of synthetic antioxidants (Inatani et al., 1983; Ito et al., 1985; Chen et al., 1986). Consumer preference might lead to increased interest in natural antioxidants by the food industry. In addition, research has shown that natural antioxidants such as β-carotene and tocopherols have positive health effects against cancer and other diseases (Bjelke, 1975; Hirayama, 1979; Floyd, 1982; Knekt et al., 1988).

Tocopherols, which are a group of closely related phenolic benzochroman derivatives having extensive ring alkylation, are the most widely used natural antioxidant compounds. Although tocopherols have been widely used in various foods; such as, fish oil, potato chips, lard, cake,

etc. (Dugan and Kraybill, 1956; Niki, 1988), one disadvantage of tocopherols are their high manufacturing cost. Tocopherols may act as pro-oxidants at a very high concentration (Nawar, 1985). Thus, increased efforts have been made to search for other natural antioxidants from various sources.

Spices and herbs have been used throughout history, not only for flavoring but also for their antioxidant effects. Much investigation has been focused on the isolation of active antioxidant compounds from some spices and herbs; especially rosemary and sage. Chang et al. (1977) first described the extraction and fractionation of natural antioxidants from sage and rosemary. They found that the antioxidant activity increased with increasing polarity of extraction solvents. Later, chemical structures of the antioxidant compounds from rosemary; rosmariphenol, rosmariquinone, rosmanol, epirosmanol, and isorosmanol etc. were determined (Inatani et al., 1982; Wu et al., 1982; Nakatani and Inatani, 1984; Nozaki, 1989). However, Chang et al. (1977) also indicated that rosemary extract imparted a strong and undesirable taste to products when it was used in high concentration.

Natural antioxidants derived from other spices and herbs have also been studied. Lee and Ahn (1985) reported that an antioxidant component named gingerol was isolated from ginger by thin layer chromatography. Furthermore,

active antioxidants were isolated from rhizome of *Curcuma longa L* (Toda et al., 1985). They found that curcumin was the most active antioxidant component, while other compounds 4-hydroxycinnamoyl methane and bis(4-hydroxy-cinnamoyl) methane were also shown to possess antioxidant activity. All of these components have higher antioxidant activity than α-tocopherol. Kikuzaki and Nakatani (1993) illustrated that crude extract of ginger rhizomes was purified by preparative HPLC. They reported that five gingerol related compounds, including curcumin, and eight diarylheptanoids, were responsible for antioxidant activity. The overall common characteristic of these compound structures is phenolic and an ortho methoxyl as the side chain.

Another herbal spice, oregano (*Origanum valgare L.*), was also found to possess antioxidant activity (Kikuzaki and Nakatani, 1993). Several phenolic acids extracted from this plant including glucoside, protocatechnic acid, caffeic acid, and rosmarinic acid were responsible for antioxidant activity. It was also reported that the antioxidant compounds of flavonoid and flavonoid glucosides were isolated from leaves of *Polygonum hydropiper* (Haraguchi et al., 1992).

Antioxidants extracted from other plant sources have also been reported. Osawa et al. (1992) reported that a novel antioxidant was isolated from young green barley leaves and the active antioxidant was identified, which

belongs to the flavonoid glucoside. Recently, Kanner et al. (1994) reported that naturally occurring phenolic antioxidants were also present in grapes and wines.

Although numerous antioxidants have been reported from various sources, very little is known about natural antioxidants from crustacean origin. Rosenzweig-Pasquel and Babbitt (1991) reported that an ethanol extract from shrimp meat (*Pandalus jordani*), the main species of shrimp harvested from the West Coast, had considerable antioxidant activity. The isolation and characterization of a natural antioxidant from shrimp waste could have important quality implications for the seafood industry, as well as to present a viable alternative to shrimp waste utilization on the West Coast. Therefore, the objective of the first study was to isolate and identify the natural antioxidant from shrimp waste.

**Lipid Oxidation in Fish**

One potential use for a natural antioxidant from shrimp waste is to prolong the shelflife of various seafood products by inhibiting lipid oxidation. Lipid oxidation, which is one of the major causes of food spoilage, generally causes deleterious changes in flavor, texture, nutrition, and color. It was recognized that seafood is considered a nutritious source of protein, omega-3 fatty acids, and low levels of cholesterol. Dietary polyunsaturated fatty acids

(PUFA) decrease and saturated fatty acids increase blood cholesterol level (Grundy, 1984). The omega-3 fatty acids are a group of polyunsaturated fatty acids that reduce the risk of cardiovascular disease (Stansby, 1982; Kinsella, 1986; Kinsella, 1987). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the predominant polyunsaturated fatty acids (PUFA) in fish (Kinsella, 1987; Wang et al., 1990). However, these fatty acids are also very susceptible to oxidation which is a major cause of deterioration in fatty fish (Hultin et al., 1982; Hultin, 1988; Nawar et al., 1990).

It is well known that lipid oxidation is affected by several factors such as fatty acid composition, storage temperature, light, oxygen, pro-oxidant, antioxidant, etc. Enser (1974) illustrated that the rate of lipid oxidation increased by increasing the number of double bonds of fatty acids. Ke et al. (1977) indicated that lipid oxidation occurred much faster in the skin of Atlantic mackerel than it did in either the white or dark muscles due to high fat content in the skin. Khayat and Schwall (1983) also indicated that lipid oxidation in fish was affected by the fatty acid composition.

The most common method to retard lipid oxidation is to lower the storage temperature as in chilled and frozen storage. As with most chemical reactions, lipid oxidation rates increase with increasing temperature. Ke et al.

(1977) reported that the storage temperature significantly influenced the lipid oxidation in mackerel during frozen storage. The oxidation rate in mackerel at -40°C was significantly lower compared to storage temperature at -15°C. Hsieh and Kinsella (1989) reported that a decrease in storage temperature lowered the rate of oxidation of PUFA and decomposition of hydroperoxides.

A critical component of the oxidation of fish muscle lipids is molecular oxygen (Hultin, 1992). Eliminating the oxygen by vacuum packaging or using inert gases have also been demonstrated as effective means to prevent lipid oxidation (Bilinski et al., 1979; Josephson et al., 1985).

Other factors; such as, metal ions, catalyze lipid oxidation by increasing the rate of free radical formation (Ladikos and Lougovois, 1990). It has been known that $Fe^{2+}$ and $Cu^{2+}$ were found to accelerate lipid oxidation in fish tissue (Castell and Bishop, 1969; Ke and Ackman, 1976; Mizushima et al., 1977; Khayat and Schwall, 1983; Kanner et al., 1987; Decker and Hultin, 1990a). In addition to those ions occurring naturally in muscle tissues, contaminants from processing equipment or wash water may result in increased content of metal ions (Hultin, 1992). Thus, metal chelating agents such as EDTA can effectively reduce lipid oxidation (Igene et al., 1979; Harel and Kanner, 1985; Decker and Hultin, 1990b).

Alternately adding synthetic and natural antioxidants was another effective way to retard lipid oxidation. Although synthetic, commercial antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tertiary-butylhydroquinone (TBHQ), etc. have been widely used to inhibit lipid oxidation in food products, there has been some concern over the safety of synthetic antioxidants (Branen, 1975; Waldrop, 1980), as well as a ban on selected antioxidants in some international circles (Boyd et al., 1993). In addition, there are some other disadvantages such as cost and/or the fear of an adverse reaction by the consumer in utilizing synthetic additives. Overall, the advantage of natural antioxidants over the synthetic antioxidants might include: lowered assumed toxicity, and greater consumer acceptability as natural additives and enhanced masking of off-flavors (Chang et al., 1977; Boyd et al., 1993).

**Effects of Natural Antioxidants on Lipid Oxidation**

Korczack et al. (1988) reported that the oxidative stability of pork meat balls was maintained with rosemary and sage extracts during refrigerated storage. Furthermore, it was reported that increasing the concentration of rosemary from 125 to 500 ppm decreased the hexanal and TBA values in both fresh and stored beef patties (Angelo et al., 1990). Lee et al. (1986) also indicated that ginger extract

from ginger rhizome can decrease the rate of lipid oxidation in fresh, frozen and precooked pork patties. Dawson and Gartner (1983) illustrated that the storage stability of chicken was greater than that of turkey due to higher levels of tocopherol in chicken. Recently, it was reported that carnosine, a dipeptide natural antioxidant, was found to reduce the oxidative rancidity of uncooked and cooked ground pork, respectively (Decker and Crum, 1991; 1993).

Research has also been done to investigate the prevention of lipid oxidation in fish with various natural antioxidants. Zama et al. (1979) reported that α-tocopherol effectively decreased the rate of oxygen consumption in fish, thus preventing its lipid oxidation. In addition, Ke et al. (1981) noted that the oxidation in mackerel skin can be effectively decreased by α-tocopherol. Ramanathan and Das (1992) reported that flavonoids such as quercetin, morin, myricetin, etc. were effective in inhibiting lipid oxidation in raw, ground fish (*Scomberomorus commersoni*). Moreover, oxidative rancidity in salted, cooked, ground fish (*Scomberomorus commersoni*) could be controlled by dried spices such as clove, ginger, tumeric, and garlic, etc. (Ramanathan and Das, 1993). Ascorbic acid has been shown to be very effective in inhibiting lipid oxidation of menhaden mince, while tocopherols and rosemary extracts showed limited effectiveness (Hwang and Regenstein, 1988). However, it was noted that ascorbic acid could act as a pro-

oxidant at low concentrations (Benedict et al., 1975; Deng, 1978). A water-soluble extract of rosemary, Herbolox-w (HERB) commercial antioxidant, was found to be effective in preventing lipid oxidation of grey trout (*Cynoscion regalis*) during frozen storage (Boyd et al., 1993).

**Effects of Antioxidant on Color**

Various attempts have been made to maintain the color of poultry products with antioxidant treatments. It was reported that antioxidants, propyl gallate (PG), BHA, and ascorbic acid can retard the degradation of the red color of ground, raw beef during refrigerator storage (Greene, 1969; Greene et al., 1971). Furthermore, it was found that ascorbic acid treatment retarded pigment oxidation and color loss of beef steak (Harbers et al., 1981; Okayama et al., 1987; Mitsumoto et al., 1991). Chastain et al. (1982) indicated that BHA was very effective in preserving color of beef and pork steaks.

Recently, increased efforts have been made in expanding markets from the West Coast fisheries to Japan where there exists a large demand for whole rockfish, including several species that are captured off the West Coast of the United States. Over the past year, there has been a trial marketing of fresh West Coast rockfish in the main Tokyo fish markets. These initial attempts were only moderately successful as the fish were marked down for appearance due

to a rapid discoloration of the red pigments common to the species involved (Read, 1991).

Carotenoid, mainly astaxanthin and tunaxanthin, are the main pigments responsible for the red color of rockfish. It is believed that the fading of the red skin color is due to the oxidation of these pigments (Tsukuda and Amano, 1967; Frankel, 1985). It has also been suggested that lipid oxidation can cause a yellow-to-brown discoloration in fish (Dyer et al., 1956; Goldman et al., 1983). However, very few papers reports that antioxidants preserve the red color of rockfish. Wasson et al. (1991) reported that tocopherols, sodium erythorbate, and BHT significantly improved the red skin color of rockfish (*Sebastolobus alascanus*) compared to a control containing no antioxidant. Overall, the objectives of these study were as follows:

1. To isolate, purify, and characterize the natural antioxidant from shrimp waste and also study stability in various testing conditions.
2. To evaluate the effect of the natural antioxidant extracted from shrimp waste on color stability of several species of red rockfish.
3. To test whether the antioxidant from shrimp waste could prevent the oxidative and hydrolytic rancidity in the sablefish mince.

**References**

Angelo, A.J., Crippen, K.L., Dupuy, H.P., and James, C.J. 1990. Chemical and sensory studies of antioxidant treated beef. J. Food Sci. 55(6):1501.

Ashford, N.A., Hattis, D., and Murray, A.E. 1977. Industrial prospects for chitin and protein from shellfish wastes, MIT Sea Grant Report MISG 77-3:MIT; Cambridge, MA.

Benedict, R.C., Strange, E.D., and Swift, C.E. 1975. Effect of lipid antioxidants on the stability of meat during storage. J. Agric. Food Chem. 23:167.

Bilinski, E., Jonas, E.E., and Lau, Y.C. 1979. Control of rancidity in frozen Pacific herring, *(Clupea harengus pallasi)*: Use of sodium erythorbate. J. Fish. Res Board. Can. 36:219.

Bjelke, E. 1975. Dietary vitamin A and human lung cancer. Int. J. Cancer. 15:561.

Boyd, L.C., Green, D.P., Giesbrecht, F.B., and King, M.F. 1993. Inhibition of oxidative rancidity in frozen cooked fish flakes by tert-butylhydroquinone and rosemary extract. J. Sci. Food Agric. 61:87.

Branen, A.L. 1975. Toxicology and biochemistry of BHA and BHT. J. Am. Oil Chem. Soc. 52:59.

Cano-Lopez, A., Simpson, B.K., and Haard, N.F. 1987. Extraction of carotenoprotein from shrimp process wastes with the aid of trypsin from Atlantic cod. J. Food Sci. 52(2):503.

Castell, C.H. and Bishop, D.M. 1969. Effect of hematin compounds on the development of rancidity in muscle of cod, flounder, scallops, and lobster. J. Fish. Res Board. Can. 26:2299.

Chang, S.S., Ostric-Matijasevic, B., Hsieh, O.L., and Huang, C. 1977. Natural antioxidants from rosemary and sage. J. Food Sci. 42(4):1102.

Chastain, M.F., Huffman, D.L., Hsieh, W.H., and Cordray, J.C. 1982. Antioxidants in restructed beef/pork steaks. J. Food Sci. 47:1779.

Chen, H.M. and Meyer, S.P. 1982. Extraction of astaxanthin pigment from crawfish waste using a soy oil process. J. Food Sci. 47:892.

Chen, C., Pearson, A.M., Gray, J.I., and Bennink, M.R. 1986. Antioxidant inhibition of mutagenicity in fried meat. Proc. European Meeting. Meat Res Work. I(32):211.

Dawson, L.E. and Gartner, R. 1983. Lipid oxidation in mechanically deboned poultry. Food Technol. 37(7):112.

Decker, E.A. and Crum, A.D. 1991. Inhibition of oxidative rancidity in salted ground pork by carnosine. J. Food Sci. 56(5):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 Hultin, H.O. 1990a. Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. J. Food Sci. 55(4):947.

Decker, E.A. and Hultin, H.O. 1990b. Nonenzymic catalysts of lipid oxidation in mackerel ordinary muscle. J. Food Sci. 55(4):951.

Deng, J.C. 1978. Effect of iced storage on free fatty acid production and lipid oxidation in mullet muscle. J. Food Sci. 43:337.

Dugan, L.R. and Kraybill, H.R. 1956. Tocopherol as carry-through antioxidant. J. Am. Oil Chem. 33:527

Dyer, W.J., Morton, M.L., Fraser, D.I., and Bligh, E.G. 1956. Storage of frozen rockfish fillets. J. Fish. Res. Board Can. 13:569.

Enser, M. 1974. Factors affecting the development of oxidative rancidity in frozen meat. Meat Res. Inst. (MRI) Symp. Proc. 3:11.

Floyd, R.A. 1982. Free radicals and cancer. p. 201, Marcel Dekker, Inc, New York.

Frankel, E.N. 1985. Chemistry of autoxidation: Mechanism, products and flavor significance. Ch. 1. In *Flavor Chemistry* of *Fats* and *Oils*, D.B. Min and T.H. Smouse(Ed.), p 1. American Oil Chemists' Society, Champaign, IL.

Goldman, M., Horev, B. and Saguy, I. 1983. Decolorization of β-carotene in model systems simulating dehydrated food. Mechanism and kinetic principle. J. Food Sci. 48:751.

Greene, B.E. 1969. Lipid oxidation and pigment changes in raw beef. J. Food Sci. 34:110.

Greene, B.E., Hsin, I. and Zipser, M.W. 1971. Retardation of oxidation color changes in raw ground beef. J. Food Sci. 36:940.

Grundy, S.M. 1984. AHA special report: Recommendations for the treatment of hyperlipidemia in adults: A joint statement of the nutrition committee and the council on atherosclerosis of the American Heart Association. Atherosclerosis. 4:445A.

Haraguchi, H., Hashimoto, K., and Yagi, A. 1992. Antioxidative substances in leaves of *(polygonum hydropiper* ). J. Agric. Food Chem. 40:1349.

Harbers, C.A.Z., Harrison, D.L. and Kropf, D.H. 1981. Ascorbic acid effects on bovine muscle pigments in the presence of radiant energy. J. Food Sci. 46:7.

Harel, S. and Kanner, J. 1985. Muscle membranal lipid peroxidation initiated by $H_2O_2$-activated metmyoglobin. J. Agric. Food Chem. 33:1188.

Hirayama, T. 1979. Diet and cancer. Nutr. Cancer. 1:67.

Hsieh, R.J. and Kinsella, J.E. 1989. Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. In *Advances* in *Food* and *Nutrition Research*. 33:233.

Hultin, H.O. 1988. Potential lipid oxidation problems in fatty fish processing. In *Fatty Fish Utilization*: *Upgrading From Feed to Food*, N. Davis (Ed.). p. 185. UNC Sea Grant College Program, Raleigh, NC.

Hultin, H.O. 1992. Lipid Oxidation in Fish muscle. In *Advances in Seafood Biochemistry*. G.J. Flick. and R.E. Martin (Ed.), p.99. Technomic Publishing Co., Lancaster, PA.

Hultin, H.O., McDonald, R.E., and Kelleher, S.D. 1982. Lipid oxidation in fish muscle microsomes. In *Chemistry* and *Biochemistry* of *Marine Food Products*, R.E. Martin., G. J. Flick, C.E. Hebard, and D.R. Ward. (Ed.), p. 1. AVI Publishing Co., Westport, CT.

Hwang, K.T. and Regenstein, J.M. 1988. Protection of menhaden mince lipids from rancidity during frozen storage. J. Food Sci. 54(5):1120.

Igene, J.O., King, J.A., and Pearson, A.M. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warm-over flavor in cooked meat. J. Agric. Food Chem. 27:838.

Inatani, R., Nakatani, N., and Fuwa, H. 1983. Antioxidative effect of the constituents of rosemary (*Rosmarinus officinalis L.*) and their derivatives. Agric. Biol. Chem. 47:521.

Inatani, R., Nakatani, N., Fuwa, H., and Seto, H. 1982. Structure of a new antioxidative phenolic diterpene isolated from rosemary (*Rosmarinus officinalis L.*). Agric. Biol. Chem. 46(6):1661.

Ito, N., Fukushima, S., and Fsuda, H. 1985. Carcinogenicity and modification of the carcinogenicity response by BHA, BHT, and other antioxidants. CRS Ctit. Rev. 15(2):109.

Johnson, E.L. and Peniston, Q.P. 1982. Utilization of shellfish waste for chitin and chitosan production. Ch. 9. In *Chemistry* and *Biochemistry* of *Marine Food Products*. R.E. Martin., G.J. Flick., C.E. Hebard., and D.R. Ward (Ed.). AVI Publishing Co., Westport, CT.

Josephson, D.B., Lindsay, R.C., and Stuiber, D.A. 1985. Effect of handling and packing on the quality of frozen whitefish. J. Food Sci. 50:5.

Kanner, J., Frankel, E., Granit, R., German, B., and Kinsella, J.E. 1994. Natural antioxidants in grapes and wines. J. Agric. Food Chem. 42:64.

Kanner, J., German, J.B., and Kinsella, J.E. 1987. Initiation of lipid peroxidation in biological systems. CRC Crit. Rev. 25:317

Ke, P.J. and Ackman, R.G. 1976. Metal-catalyzed oxidation in mackerel skin meat lipids. J. Am. Oil Chem. Soc. 53:636.

Ke, P.J., Ackman, R.G., Linke, B.A., and Nash, D.M. 1977. Differential lipid oxidation in various parts of frozen mackerel. J. Food Technol. 12:37.

Ke, P.J., Nash, D.N., and Ackman, R.G. 1981. Mackerel skin lipids as an unsaturated fat model system for the determination of antioxidative potency of TBHQ and other antioxidant compounds. J. Am. Oil Chem. Soc. 54:417.

Khayat, A. and Schwall, D. 1983. Lipid oxidation in seafood. Food Technol. 7:130.

Kikuzaki, H. and Nakatani, N. 1993. Antioxidant effects of some ginger constituents. J. Food Sci. 58(6):1407.

Kinsella, J.E. 1986. Food components with potential therapeutic benefits: the n-3 polyunsaturated fatty acids of fish oils. Food Technol. 40(2):89.

Kinsella, J.E. 1987. Seafoods and Fish Oils in Human Health and Disease. Dekker, New York.

Kirk, W.G., Shirley, R.L., Easley, J.F., and Peacock, F.M. 1967. Composition and feed value of shrimp meal. Quart. J. Florida Acad. Sci. 30:51.

Knekt, P., Aromaa, A., Maatela, J., Aaran, R., Nikkari, T., Hakama, M., Hakulinen, T., Peto, R., Saxen, E., and Teppo, L. 1988. Serum vitamin E and risk of cancer among Finnish men during a ten-year follow-up. Am. J. Epidemiol. 127:28.

Korczack, J., Flaczyk, E., and Pazola, Z. 1988. Effects of spices on stability of meat products kept in cold storage. Fleischwirtschaft. 68:64.

Kubota, K., Shijimara, H., and Kobayashi, A. 1986. Volatile components of roasted shrimp. Agric. Biol. Chem. 50:2867.

Ladikos, D. and Lougovois, V. 1990. Lipid oxidation in muscle foods: A review. Food Chem. 35:295.

Lee, I.K. and Ahn, S.Y. 1985. The antioxidant activity of gingerol. Korean J. Food Sci. Tech. 17(2):65.

Lee, Y.B., Kim, Y.S., and Ashmore, C.R. 1986. Antioxidant property in ginger rhizome and its application to meat products. J. Food Sci. 51:20.

Mandeville, S., Yaylayan, V., and Simpson, B. 1992. GC-MS analysis of flavor-active compounds in cooked commercial shrimp waste. J. Agric. Food Chem. 40:1275.

Mandeville, S., Yaylayan, V., Simpson, B., and Pare, J. 1991. GC-MS analysis of flavor active compounds from raw commercial shrimp waste. Spectrosc: Int J. 9:61.

Meyer, S.P. 1986. Utilization of shrimp processing wastes. Infofish. 4:18.

Mitsumoto, M., Cassens, R.G., Schaefer, D.M., Arnold, R.N., and Scheller, K.K. 1991. Improvement of color and lipid stability in beef longissimus with dietary vitamin E and vitamin C dip treatment. J. Food Sci. 56(6): 1489.

Mizushima, Y., Takama, K., and Zama, K. 1977. Effect of copper, iron and hemin on lipid oxidation in fish flesh homogenate. Bull. Faculty Fish. Hokkaido U. 18(4):207.

Naczk, M. and Shahidi, F. 1990. Chemical composition and chitin content of crustacean offal. Ch. VI. In *Advances in Fisheries Technology* and *Biotechnology* for *Increased Profitability*. M.N. Voigt and J. Botta (Ed.), p. 299. Technomic Publishing Co., Lancaster, PA.

Nakani, N. and Inatani, R. 1984. Two antioxidative diterpenes from rosemary (*Rosmarinus officinalis L.*) and a revised structure for rosmanol. Agric. Biol. Chem. 48(8):2081.

Nawar, W.W. 1985. Lipids. In *Food Chemistry*. O.R. Fennema, (Ed.), p. 139. Marcel Dekker, Inc, NY.

Nawar, W., Hultin, H., Li, Y., Xing, Y., Kelleher, S., and Wilhelm, C. 1990. Lipid oxidation in seafoods under conventional conditions. Food Rev. Internat. 6:647.

Niki, E. 1988. Action of vitamin E as an antioxidant. Vitamins. 62(11):601.

Nozaki, K. 1989. Antioxidant activity of rosemary. New Food Ind. 31:27.

Okayama, T., Imai, T., and Yamanoue, M. 1987. Effect of ascorbic acid and alpha-tocopherol on storage stability of beef steaks. Meat Sci. 21:267.

Ornum, J.V. 1992. Shrimp waste-must it be wasted? Infofish Inter. 6:48.

Osawa, T., Katsuzaki, H., Hagiwara, Y., Hagiwara. H., and Shibamoto, T. 1992. A novel antioxidant isolated from young green barley leaves. J. Agric. Food Chem. 40:1135.

Raa, J. and Hansen, F. 1982. Norwegian Patent No. 147365B.

Ramanathan, L. and Das, N.P. 1992. Studies on the control of lipid oxidation in ground fish by some polyphenolic natural products. J. Agric. Food Chem. 40:17.

Ramanathan, L. and Das, N.P. 1993. Natural products inhibit oxidative rancidity in salted cooked ground fish. 58(2):318.

Read, R. 1991. Oregon seeks to crack Japanese fish market. The Oregonian. Jan, 27.

Rosenzweig-Pasquel, L.J.D. and Babbitt, J.K. 1991. Isolation and partial characterization of a natural antioxidant from shrimp (*Pandalus jordani*). J. Food Sci. 56(1):143.

Saito, K., and Kondo, I. 1967. Symposium paper, Annual. Meeting Fish. Soc. Jap.

Saito, A. and Regier, L.W. 1971. Pigmentation of brook trout (*Salvelinus fontinalis*) by feeding dried crustacean waste. J. Fish. Res. Board. Can. 28:509.

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

Spinelli, J., Lehman, L., and Weig, D. 1974. Composition, processing, and utilization of red crab (*Pleuronocodes planipes*) as an aquacultural feed ingredient. J. Fish. Res. Board. Can. 31:1025

Stansby, M.E. 1982. A clinical study on the role of fish oil in alleviating human heart disease. In *Nutritional Evaluation* of *Long-chain Fatty acids* in *Fish Oil*, S.M. Barlow and M.E. Stansby (Ed.), p.263. Academic Press, New York.

Steel, R.E. 1971. Shrimp processing waste as a pigment source for rainbow trout (*Salmo gairdneri*). MS Thesis. Oregon State University, Corvallis.

Sugano, M., Fujikawa, T., Hiratsuji, Y., Nakashima, K., Fukuda, N., and Hasegawa, Y. 1980. A novel use of chitosan as a hypocholesterolemic agent in rats. Am. J. Clin. Nutr. 33(4):787.

Talley, K. 1993. Stats pack. Pacific Fish. 15(3):73.

Tidemann, E., Raa, J., Stormo, B., and Torrisen, O. 1982. Processing and utilization of shrimp waste. Tromiso, Norway: Ins. Fish. Tech. Res. p. 583.

Toda, S., Miyase, T., Arichi, H., Tanizawa, H., and Takino, Y. 1985. Natural antioxidants. III. Antioxidative components isolated from rhizome of *Curcuma longa L.* Chem. Pharm. Bull. 33(4):1725.

Tsugita, T. 1990. Chitin/chitosan and their applications. Ch. 6. In *Advances* in *Fisheries Technology* and *Biotechnology* for *increased Profitability.* M.N. Voigt. and J. Botta (Ed.), p. 287. Technomic Publishing Co, Lancaster, PA.

Tsukuda, N. and Amano, K. 1967. Studies on the discoloration of red fishes. IV. Discoloration of astaxanthin, tunaxanthin, and β-carotene by tissue homogenates of fishes. Bull. Japan Soc Sci. Fish. 33(10):962.

Tunison, A.V., Brockway, D.R., Maxwell, J.M., Dorr, A.L., and McCay, C.M. 1947. Coloration of fish. Prog Fish Cult. 9:53.

United States Department of the Interior. 1968. Danes use vacuum-dried shrimp meal in trout feed. Fishery Market News Report. 5(11):2.

Waldrop, M. 1980. Firm takes new approach to food additives. Chem. Eng. News. 58(18):22.

Wang, Y.J., Miller, L.A., Perren, M., and Addis, P.B. 1990. Omega-3 fatty acids in lake superior fish. J. Food Sci. 55(1):71.

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

Watkins, B.E., Adair, J., and Oldfield, J.E. 1982. Evaluation of shrimp and king crab processing byproducts as feed supplement for mink. J. Ani Sci. 55(3):578.

Wu, J., Lee, M.H., Ho, C.T., and Chang, S. 1982. Eluciation of the chemical structures of natural antioxidants isolated from rosemary. J. Am. Oil Chem. Soc. 59:339.

Zama, K., Takama, K., and Mizushima, Y. 1979. Effect of metal, salt and antioxidants on the oxidation of fish lipids during storage under the condition of low and intermediate moisture. J. Food Proc. Preser. 3:249.

# II. ISOLATION OF A NATURAL ANTIOXIDANT FROM SHRIMP WASTE

## ABSTRACT

Shrimp waste samples were extracted with a variety of organic solvents. Each fraction was measured for antioxidant activity by determining the rate of oxidation of β-carotene-linoleic acid in an emulsion system. An ethanol extract exhibited the highest antioxidant activity. Purification of the most active fraction was accomplished by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). This antioxidant was relatively polar and had a UV absorption at 268 nm which suggests an aromatic moiety. Antioxidant stability was measured in samples heated at 100°C for 4 hr and stored for 1 mo at 4°C. Antioxidant activity was not significantly effected by heating.

## INTRODUCTION

The West Coast shrimp fishery has maintained an annual harvest of more than 50 million lb of shrimp since 1987 (Talley, 1993). More than 50% of the harvest was landed in Oregon during this time period, although the states of Washington and California contributed significantly to the capture. In most cases, shrimp are landed at processing plants where it is cooked, peeled, packaged and frozen for market. Cooked, peeled shrimp have an average recovery of 28-30%. The resulting shrimp discard represents more than 30 million lb of waste material per year on the Pacific Coast. A number of attempts have been made to utilize shrimp waste (Meyer, 1988). These have included: extraction of pigments and flavorants (Saito and Regier, 1971), utilization of shell material (Muzarelli and Parisher, 1978), and production of shrimp meal (Simpson and Haard, 1985). Some of these tests have led to utilization of shrimp waste materials, especially in the area of aquaculture diets. Another area of shrimp shell utilization is to produce chitosan which has numerous applications including food processing, agriculture, bio-medical, biotechnology, water treatment, cosmetics, etc. (Ornum, 1992). The bulk of the waste materials, however, are still underutilized and represent a valuable resource in terms of potential isolated compounds. With increasingly restrictive environmental legislation and solid waste management

regulations, it is critical that avenues be found for various aspects of waste utilization.

Antioxidants can effectively prevent lipid oxidation which is a major cause of chemical spoilage in foods. Much attention has recently been focused on natural antioxidants because of their positive health effects against cancer and heart disease (Bjelke, 1975; Floyd, 1982; Knekt et al., 1988).

Although various natural antioxidants have been extracted from plant sources (Chang et al., 1977; Pratt and Birac, 1979; Dugan, 1980; Pratt and Miller, 1984; Gopalakrishna and Prabhakar, 1985; Papadopoulos and Boskou, 1991; Chevolleau et al., 1992; Onyeneho and Hettiarachchy, 1992; Osawa et al., 1992), little is known about antioxidants of crustacean origin. Previous studies indicated that shelf-life of minced fish incorporated with shrimp meat was improved due to antioxidant activity from shrimp (*Pandalus jordani*) (Babbitt et al., 1976). Furthermore, Rosenzweig-Pasquel and Babbitt (1991) also reported that an ethanol extract from shrimp meat had considerable antioxidant activity.

The objective of this study was to isolate and purify a natural antioxidant from shrimp waste and determine its stability under various test conditions.

# MATERIALS & METHODS

## Shrimp Waste Samples

Shrimp waste, from the species *Pandalus jordani*, was obtained from an Oregon seafood processor during the shrimp fishing season (May through October). Samples were kept on ice and transported to the Oregon State University Seafood Laboratory in Astoria. Two hundred gram samples (200 g) were vacuum packed, blast frozen, and stored at -20°C. Frozen samples, to be analyzed, were thawed at refrigerated temperatures.

## Solvent Extraction

Extract from shrimp waste was prepared in the following manner. Fifty gram (50 g) samples were extracted with 100 ml of organic solvents which include ethyl ether, chloroform, acetone, isopropanol, and ethanol respectively, mixtures were blended for 2 min using a Waring blender. Each slurry was filtered through a Buchner funnel using Whatman No. 1 filter paper. Each fraction was evaporated to dryness and redissolved in the same volume of 95% ethanol and measured for antioxidant activity. Negative controls consisted of a 95% ethanol solution. Since ethanol extracts showed the highest antioxidant activity, these fractions were further purified.

A sample of 100 g shrimp waste was also extracted with chloroform, methanol and water mixtures according to the method of Bligh and Dyer (1959). The ability to partition in the chloroform or aqueous layer would serve to characterize the antioxidant as polar or non-polar.

**Antioxidant Activity Testing**

The antioxidant activity was evaluated in a β-carotene-linoleic acid emulsion as described by Marco (1968). The ability of antioxidant to prevent the oxidative destruction of β-carotene and linoleic acid emulsion was expressed as the decrease in absorbance at 470 nm. A 2 mg sample of crystalline β-carotene was dissolved in 10 ml of chloroform. One ml of this solution was then pipetted into a round-bottomed flask which contained 20 mg of purified linoleic acid and 200 mg of Tween 40 emulsifier. After removal of chloroform on a rotary evaporator, 50 ml of deionized water was added to the flask with vigorous stirring. A 5 ml aliquot of the aqueous emulsion which formed was then pipetted into each of a series of spectrophotometer tubes which contained 0.2 ml portions of extract solution. A zero reading was taken at 470 nm on the reaction mixture in each tube immediately after addition of the emulsion to the extract solution. The tubes were then stoppered and placed

in a water bath at 50°C. Subsequent readings were taken at regular intervals.

**Thin-Layer Chromatography (TLC)**

Crude ethanol extract was applied to analytical silica gel thin-layer plates (Kiesel $60F_{254}$, 20x20cm, 0.25 mm thickness, EM Science, Gibbstown, NJ) in a straight line 2.0 cm above the lower edge. Each plate was developed with benzene-methanol-acetic acid (45:8:4 v/v).

Phenolic compounds were visualized by spraying plates with one of the following reagents:

1. Folin-Ciocalteu's phenol reagent followed by an overspray of 14% sodium carbonate.
2. Ferric chloride-potassium ferricyanide reagent(1:1 v/v).

Plates were also sprayed with a β-carotene-linoleic acid solution. After spraying, the plates were exposed to normal room light for about 4 hr. The yellow colored band indicated the presence of antioxidant activity.

Plates were sprayed with ferric chloride solution to detect whether the active antioxidant compound had dihydroxy or tri-hydroxy phenolic groups.

Crude ethanol extract was purified by preparative TLC (1.0 mm thickness) using benzene-methanol-acetic acid (45:8:4). Each fraction was scraped off the TLC plates with a razor blade and measured for antioxidant activity. In

order to check the purity of the isolated compound, analytical TLC plates were run with a more polar phase, butanol:water:acetic acid (12:5:3).

Samples prepared by the Bligh and Dyer method consisting of the methanol-water layer were also applied to analytical TLC plates developed by benzene-methanol-acetic acid (45:8:4 v/v). Plates were then rotated $90^0$ and run with solvents butanol-water-acetic acid (12:5:3 v/v). Plates were sprayed with one of the following reagents to detect the presence of the phenolic and amine group, respectively:

1. Folin-Ciocalteu's phenol reagent followed by an overspray of 14% sodium carbonate.
2. 0.1% ninhydrin in isopropanol.

**High-Performance Liquid Chromatography (HPLC)**

HPLC was performed on a Bio-Rad 2700 gradient pump system equipped with a Bio-Rad model 1706 UV detector (BioRad, Hercules, CA). A preparative column packed with R Sil C18 HL column (250 x 10mm i.d., 5 $\mu$m particle size) was used. A 500 ul sample with TLC fraction $R_f$ 0.05 from TLC was injected onto HPLC. The eluents used were water and acetonitrile with a gradient of 0-20% acetonitrile for 15 min and a flow rate of 2.50 ml/min. Absorbance at 280 nm was monitored. Peak fractions were collected and measured for antioxidative activity. Active fractions (retention

time 8.4 min) were concentrated by a rotary evaporator and rechromatographed onto an analytical HPLC equipped with a 250 mm x 4.6 mm i.d. R Sil C18 HL column to evaluate purity. Two different kinds of conditions were used. The first analytical condition was carried out with the mobile phase of 2.5% acetic acid in water (pump A) and 2.5% acetic acid in methanol (pump B) with a gradient of 0-50% (pump B) for 30 min. A flow rate of 1.0 ml/min was used. The second condition was carried out with the mobile phase of 5.0% acetic acid in water (pump A) and acetonitrile (pump B) with a gradient of 0-20% pump B in 15 min and a flow rate of 1.0 ml/min.

**UV Spectrum**

An active fraction (pH at 7.0), collected from preparative HPLC at retention time 8.45 min was run within UV range (200-380 nm) using a Backman Spectrophotometer DU 640 (Beckman, Fullerton, CA). The scan speed was 1200 nm/min.

**Heat Treatment**

Samples of ethanol extract and purified solutions from HPLC were placed in 10 ml test tubes and heated at 100°C for 0, 15, 30, 60, 120, and 240 min. Heated crude extracts were centrifuged at 10,000 xg for 15 min to remove precipitated

substances. The tubes were then cooled to room temperature and antioxidant activity of each sample was measured with methods previously described.

**Storage Experiment**

Samples of ethanol extract and purified solutions were also stored at refrigerated temperature (4°C) for 0, 5, 15, 20, and 30 days. Samples were then assayed for antioxidant activity.

## RESULTS AND DISCUSSION

Extracts of all solvents except diethyl ether improved the β-carotene-linoleic acid emulsion stability over the negative control containing only 95% ethanol solution (Fig. 1). It was found that crude ethanol extract resulted in the highest antioxidant activity compared with other crude extracts. Therefore, crude ethanol extract was further analyzed by TLC and HPLC. Ethanol extracts, when analyzed by TLC in benzene:methanol:acetic acid solvents, exhibited four spots when visualized by Folin-Ciocalteu's with $R_f$ values of 0.05, 0.40, 0.48, and 0.64. Faint blue spots were visualized by spraying with Folin-Ciocalteu's phenolic reagent; and blue spots were immediately observed after spraying with ferric chloride-potassium ferricyanide reagent. Positive reactions with both indicators shows the presence of phenolic compounds. When antioxidant activity of each band was determined by measuring the decrease of the absorbance at 470 nm of β-carotene-linoleic acid emulsion, the $R_f$ 0.05 band showed the strongest activity, while $R_f$ values of 0.40, 0.48 and 0.64 gave almost no antioxidant activity (Fig. 2).

The separated compounds in TLC developed by benzene-methanol-acetic acid solvents were also sprayed with a solution of β-carotene-linoleic acid. It was found that only the band ($R_f$ 0.05) maintaining the yellow color on TLC

plates, after exposure to daylight for about 4 hr, possessed antioxidant activity. The results from TLC suggests that a polar phenolic compound from shrimp waste was responsible for antioxidant activity.

It was also observed that the methanol-water layer prepared by the Bligh and Dyer method gave one spot with $R_f$ 0.05 using benzene:methanol:water (45:8:4 v/v) in the first dimension of two-dimensional TLC after spraying either Folin-Ciocalteu's phenol reagent or ninhydrin. When this spot was developed by the second dimension using butanol:water:acetic acid (12:5:3) solvents, two spots with $R_f$ 0.20 and 0.32 were separated. The results indicated that an antioxidant compound was present in the polar fraction. Increased solvent strength in the second dimension separated the $R_f$ 0.05 band into two spots. Therefore, the $R_f$ 0.05 band was subjected to further purification by HPLC.

There were two major peaks from preparative HPLC at retention times of 8.45 and 12.53 min (Fig. 3) when water and acetonitrile with a gradient of 0-20% acetonitrile in 15 min was used. The fraction collected from HPLC at 8.45 min showed strong antioxidant activity (Fig. 4). A combination of water and acetonitrile was used as a polar mobile phase to elute the polar fraction. Fractions were concentrated by rotary evaporator and rechromatographed on R-Sil $C_{18}$ HPLC under two different conditions. The combination of acetic acid, water and methanol was used as the first elution

condition while the solvents of acetic acid, water, and acetonitrile were used as the second elution condition. Both conditions resulted in a single peak (Fig. 5). Moreover, when the active fraction was separately applied to analytical TLC plates developed with either the mobile phase of butanol: water: acetic acid (12:5:3, v/v) or benzene:methanol:acetic acid (45:8:4), single spots appeared at either $R_f$ 0.21 or 0.05, respectively. These observations confirm the purity of the active fraction collected from HPLC.

Purification of natural antioxidants by HPLC was shown by several authors (Osawa and Namiki, 1985; Ramarathnan et al., 1988; Osawa et al., 1992). Reverse phase HPLC was successfully used to purify very polar antioxidants from green barley leaves and rice hull (Ramarathnan et al., 1988; Osawa et al., 1992). The combination of preparative TLC and normal phase HPLC was adopted to purify a novel antioxidant from *eucalyptus* leaf waxes (Osawa and Namiki, 1985). Several natural antioxidants extracted from different sources such as rosemary, rice hull, young barley leaves, *Polygonum hydropiper* leaves, and tea have shown to have polar properties (Chang et al., 1977; Ramarathnan et al., 1988; Haraguchi et al., 1992; Ho et al., 1992).

Ferric chloride spray was used to determine whether this phenolic compound contained a dihydroxy and/or trihydroxy group. The negative reaction excluded the

possibility of the existence of such a group. The UV spectrum indicated a major absorption at 268 nm and a minor peak at 323 nm (Fig. 6) further confirming the presence of aromatic moiety. Based on the absorbance and concentration of purified sample, extinction coefficient($\epsilon^{1cm}_{1\%}$) at 268 nm and 323 nm was calculated to be 64,000 and 7,600, respectively.

Babbitt et al. (1976) reported that ethanol extracts from shrimp meat exhibited antioxidant properties. Furthermore, various solvents were used to extract antioxidant from shrimp meat. Ethanol extract was the most effective solvent (Rosenzweig-Pasquel and Babbitt, 1991). It was also reported that extracts of sage and rosemary made with non-polar solvents are less effective as an antioxidant (Chang et al., 1977). Our results showed that crude ethanol extract had strong antioxidant activity. However, all of those extracts showed lower antioxidant activity compared to Tenox 6 which consisted of a mixture of BHA, BHT, propyl gallate, and citric acid (Fig. 1).

A polar phenolic compound isolated from shrimp meat with the same TLC solvent system also showed antioxidant activity; and had a strong absorption at 204 nm (Rosenzweig-Pasquel and Babbitt, 1991), which differed from the result presented here. Lack of major absorption at 205 or 210 nm rules out antioxidant as a peptide. Positive reaction with

ninhydrin indicated that the compound contains an amine group.

Polar phenolic compounds acting as natural antioxidants have been suggested by several authors (Pratt and Miller, 1984; Ramarathnan et al., 1988; Papadopoulos and Boskou, 1991; Haraguchi et al., 1992; Ho et al., 1992). Hydroxytyrosol and caffeic acid from olive oil showed greater antioxidant activity than synthetic antioxidant BHT, while protocatechnic acid and syringic acid also from olive oil were found to have lesser antioxidant activity (Papadopoulos and Boskou, 1991). Antioxidant activities varied with different polyphenolic compositions of different tea processing methods (Ho et al., 1992). It was well documented that water soluble compounds, flavonoids and flavonid glucoside possessed strong antioxidative activity (Pratt and Birac, 1979; Pratt and Miller, 1984; Haraguchi et al., 1992; Osawa et al., 1992). Flavonoids possess a phenolic structure, which is shared by α-tocopherol, BHA and BHT. Recently, it was reported that the presence of phenolic compounds in grapes were shown to have antioxidative effects (Kanner et al., 1994). Lipid soluble natural phenolic antioxidants, tocopherols, are the most widely distributed antioxidants in nature (Nawar, 1985). Another phenolic antioxidant from a tropical tree, gum quaiac, showed an ability to inhibit lipid oxidation in cooked pork during refrigerated storage (Shahidi et al.,

1987).

Although most antioxidants are phenolic compounds, another water soluble natural antioxidant, carnosine is a β-ala, his dipeptide found in the skeletal muscle and nervous tissue (Crush, 1970). The antioxidant mechanism of carnosine was proposed as a combination of its ability to act as a chelator, free radical scavenger and hydrogen donor (Decker and Crum, 1993). In addition, natural antioxidants, rosmariquinone isolated from rosemary (*Rosmarinus officinalis L*) were superior to BHT in heated lard (Houlihan et al., 1985).

Antioxidant activity of both crude and purified samples was unchanged after heating for up to 4 hr (Fig. 7). It was reported that antioxidant activity of 60°C heated extract from beef muscle was not significantly different from untreated extract. However, antioxidant activity was increased 8.6-fold at 100°C due to removal of prooxidants such as iron- and heme-containing compounds by heat treatment (Chan et al., 1993). In addition, antioxidant(s) from acid whey proteins was found to be stable when heated at 90°C for 30 min (Colbert and Decker, 1991). On the other hand, phenolic antioxidants such as BHA, BHT lose effectiveness after deep-fat frying due to decomposition and evaporation (Lin et al., 1981; Hamama and Nawar, 1991).

When crude ethanol extract and purified samples were stored at 4°C for 1 mo, antioxidant activity changed very

little (Fig. 8). The naturally occurring α-tocopherol content in seaweed meal decreased with increased storage temperature (Jensen, 1969). Furthermore, stability of α-tocopherol in a dehydrated model food system containing methyl linoleate with varying storage temperatures (20°C, 30°C, and 37°C) was also studied (Widicus and Kirk, 1981), the degradation rate of α-tocopherol increased as a function of storage temperature.

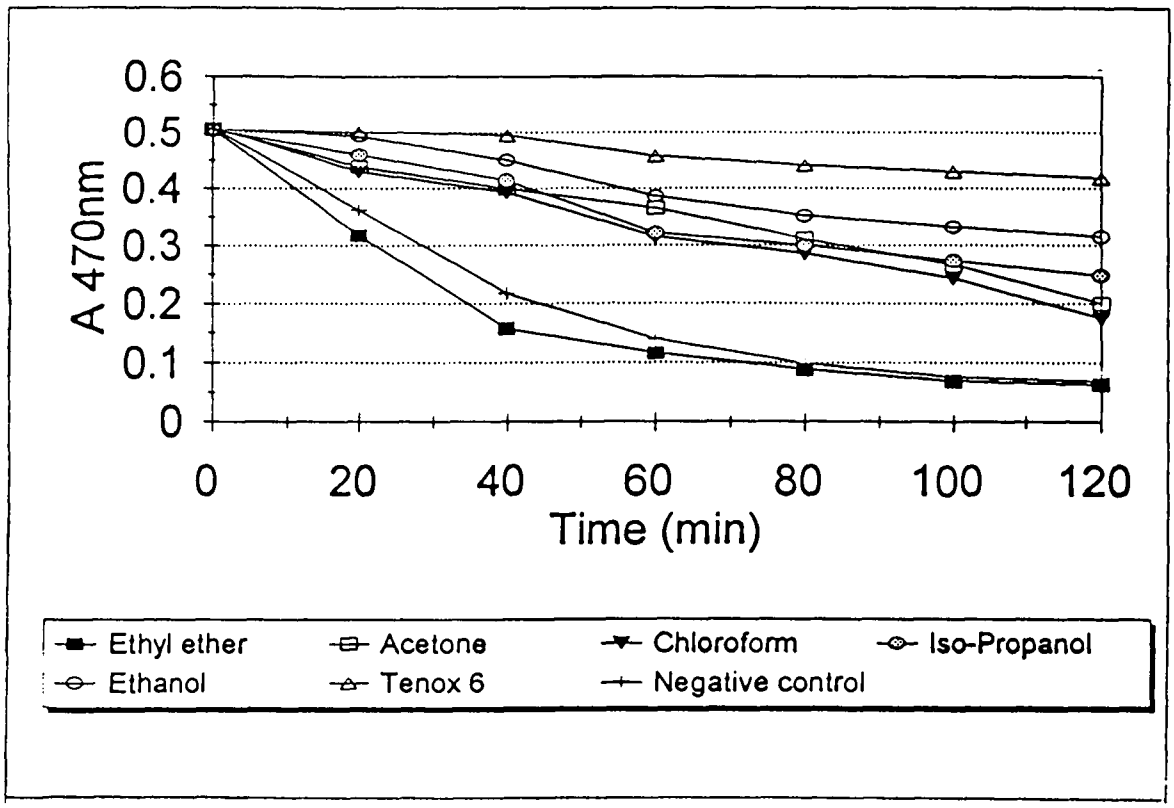


Figure II. 1. Antioxidant activity of shrimp waste extracted with different kinds of solvents. Decrease in absorbance at 470nm of β-carotene and linoleic acid emulsion was monitored for 120 min.

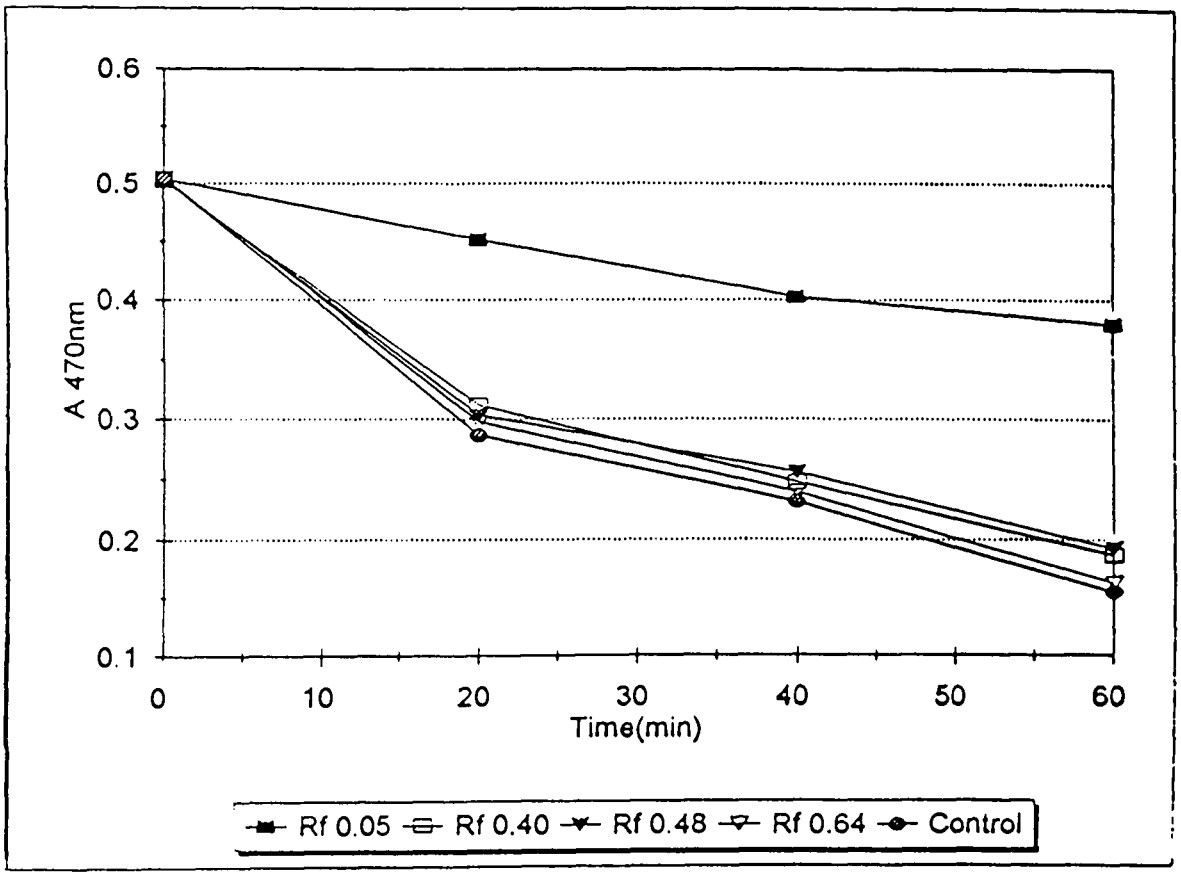


Figure II. 2. Antioxidant activity of different $R_f$ value of phenolic compounds separated by TLC from shrimp waste. TLC plate developed with benzene-methanol-acetic acid(45:8:4 v/v). Antioxidant activity was represented by change in A470nm for 60min.

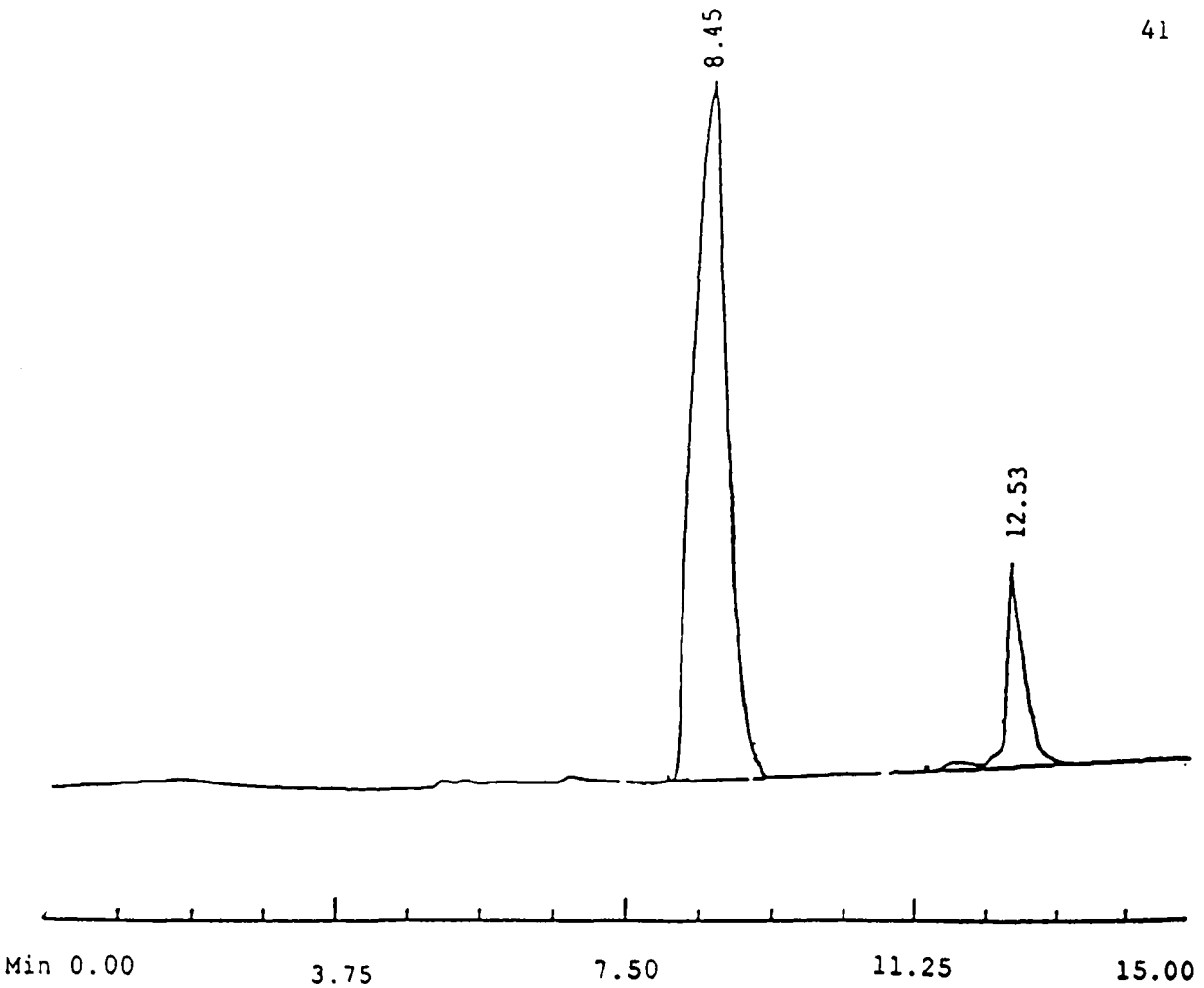


Figure II. 3. HPLC chromatogram of the TLC fraction of Rf 0.05. The mobile solvents used were water (pump A) and acetonitrile (pump B) with a gradient of 0-20% acetonitrile in 15 min.

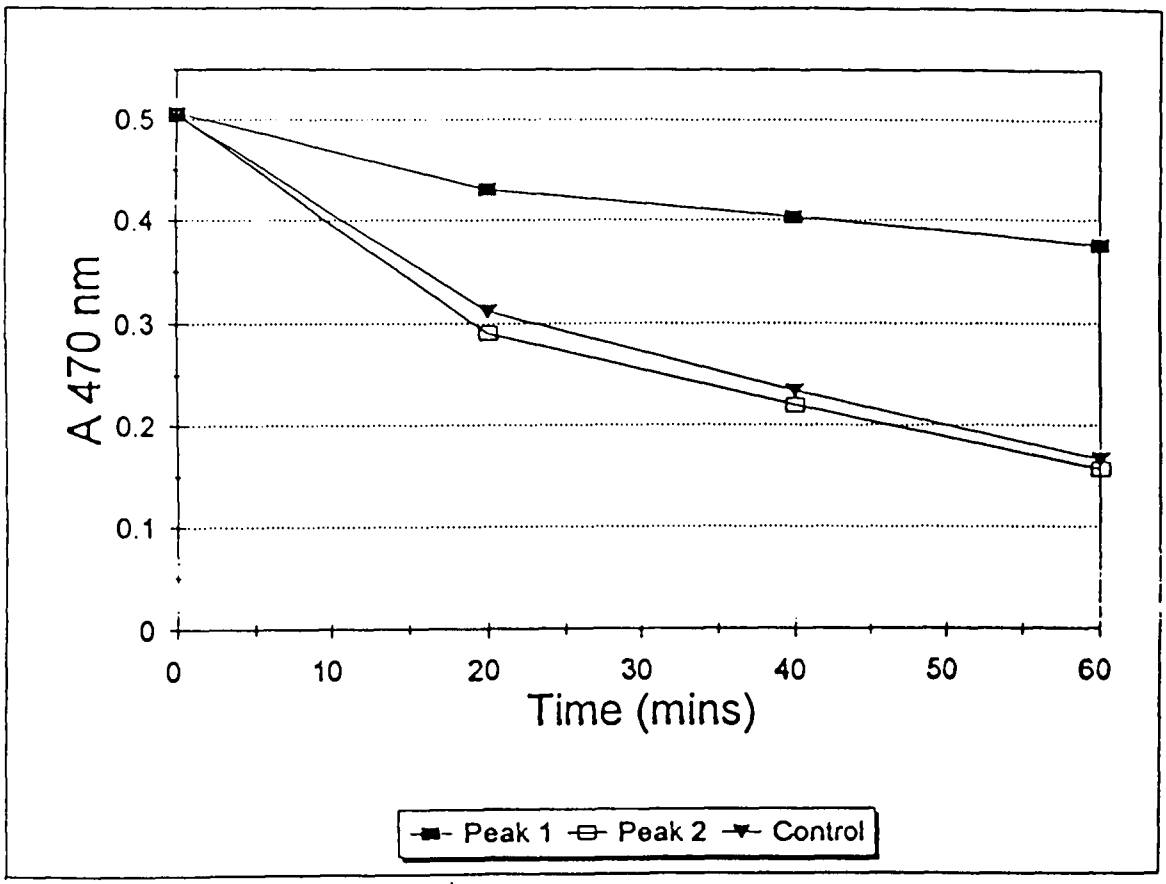


Figure II. 4. Antioxidant activity of the collected fraction of peaks from R-Sil $C_{18}$ HPLC. Peak 1 is referred to the peak at retention time 8.45 min and peak 2 is referred to the peak at retention time 12.53 min.

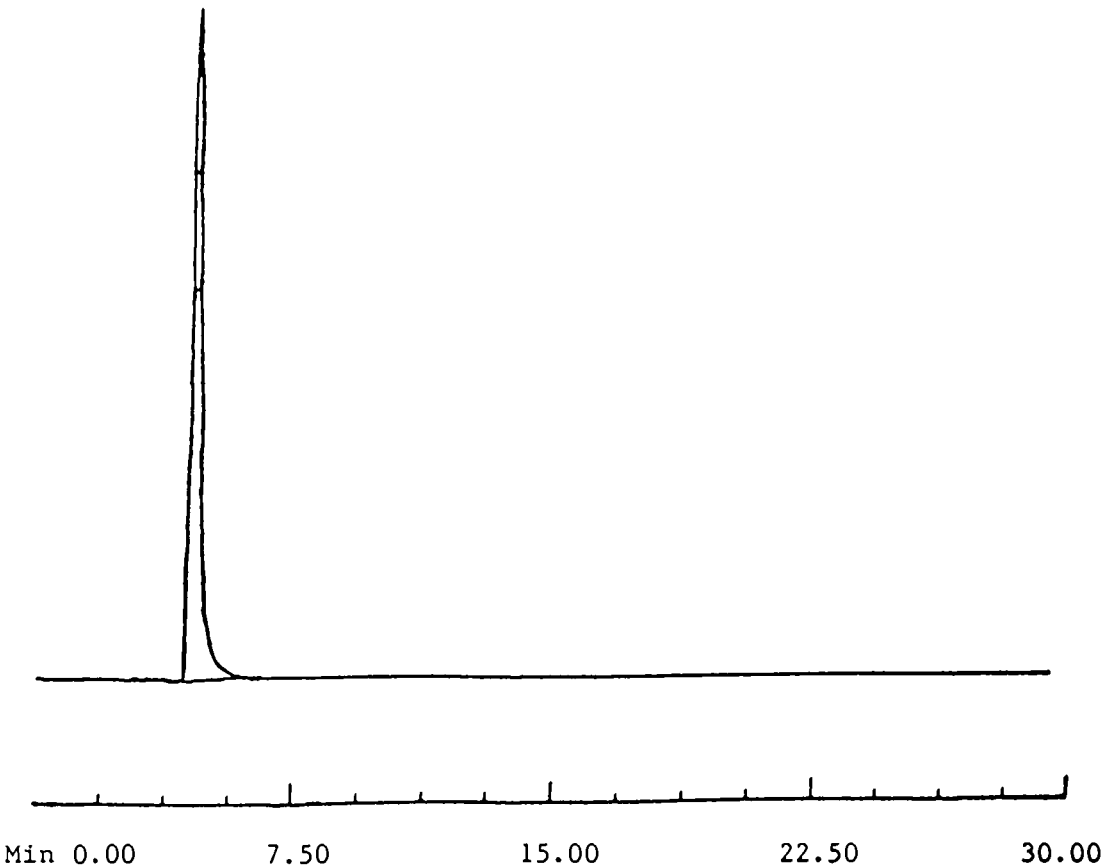


Figure. II. 5(A). Rechromatogram of a fraction collected from preparative HPLC with retention time at 8.45 min. The mobile solvents used were 2.5% acetic acid in water(pump A) and 2.5% acetic acid in methanol (pump B) with a gradient of 0-50% (B) in 30 min.

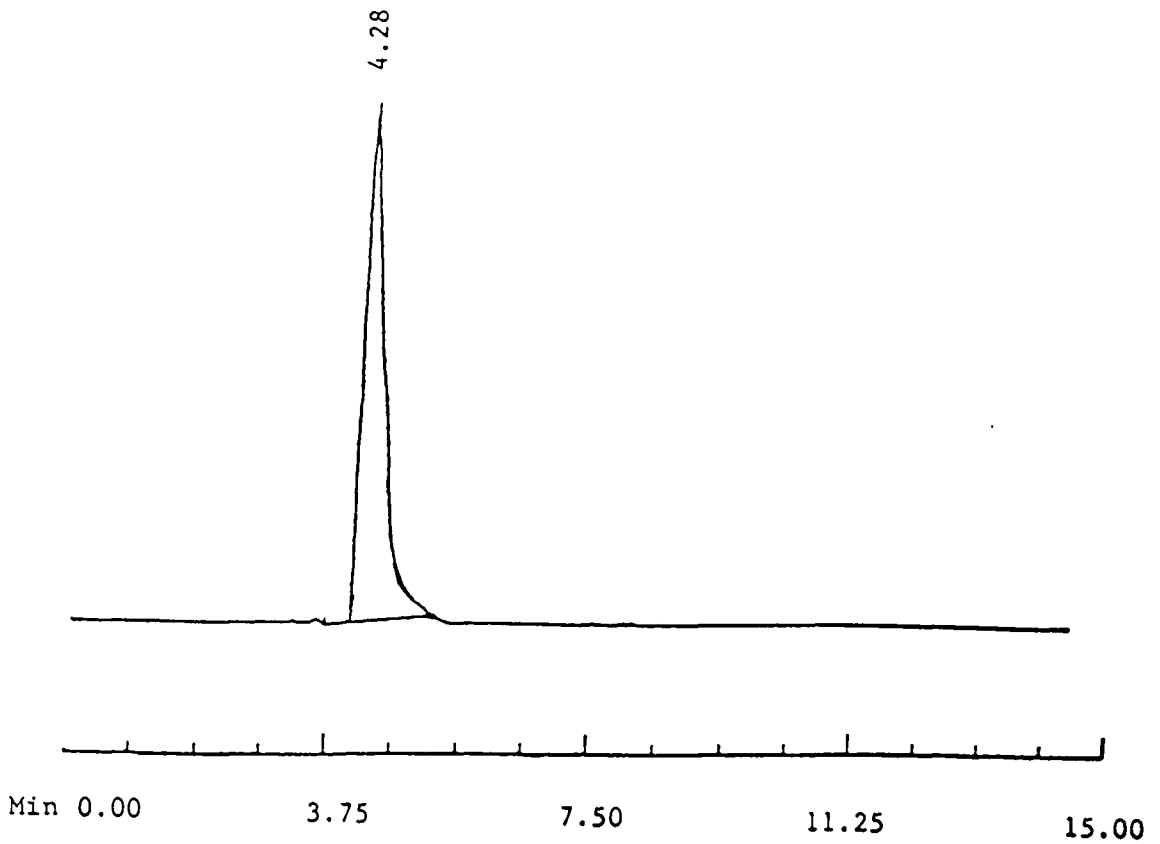


Figure. II. 5(B). Rechromatogram of a fraction collected from preparative HPLC with retention time at 8.45 min. The mobile solvents used were 5.0% acetic acid in water(pump A) and acetonitrile (pump B) with a gradient of 0-20% (B) in 15 min.

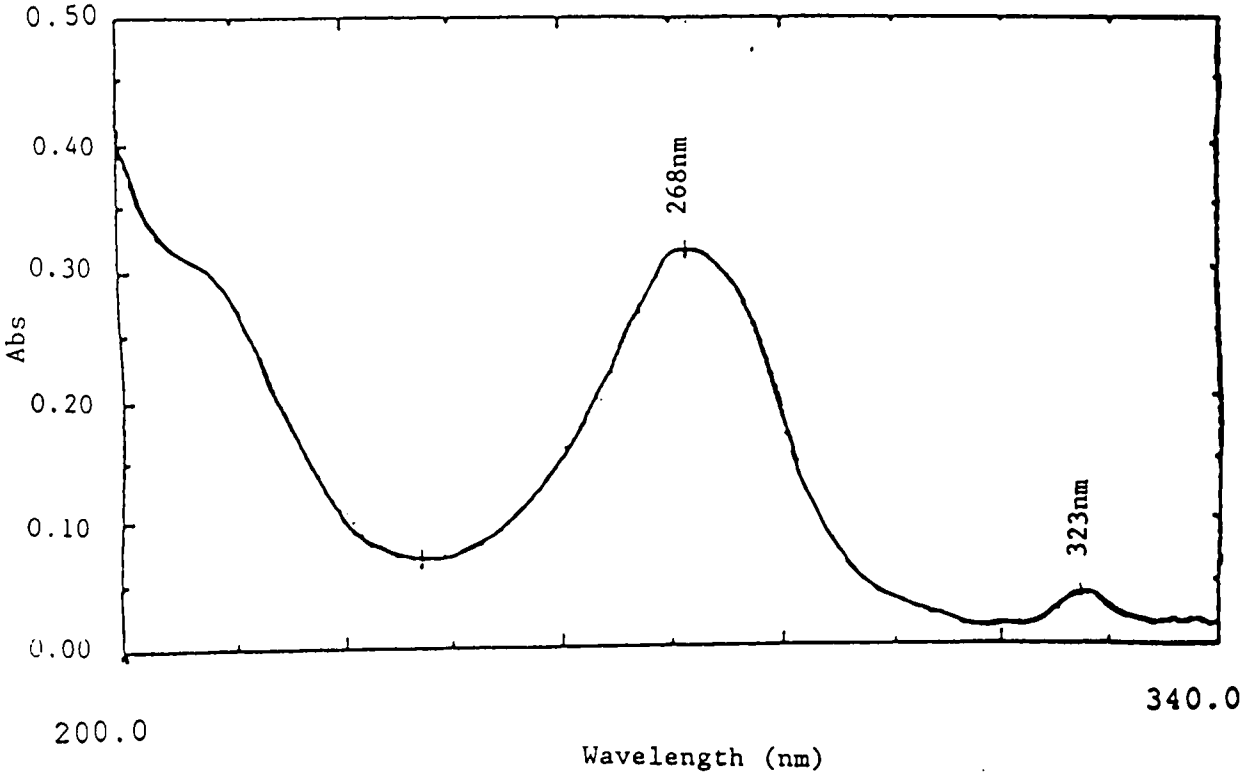


Figure. II.6. UV spectrum of a fraction collected from preparative HPLC with retention time at 8.45 min.

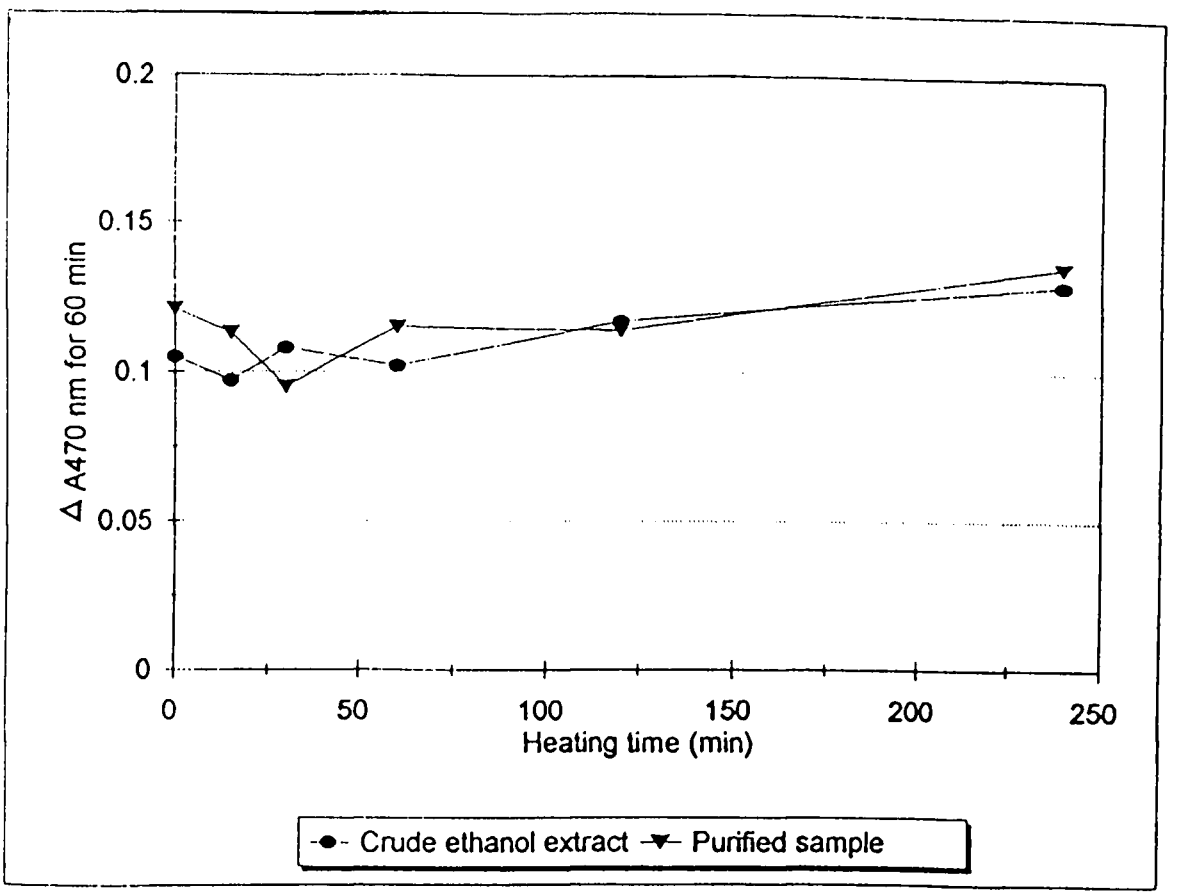


Figure. II.7. Different heating time effects on antioxidant activity of both crude ethanol extract and purified samples. Antioxidant activity was represented by the decrease in A470nm over 60 min. Samples collected from HPLC are referred to as purified samples.

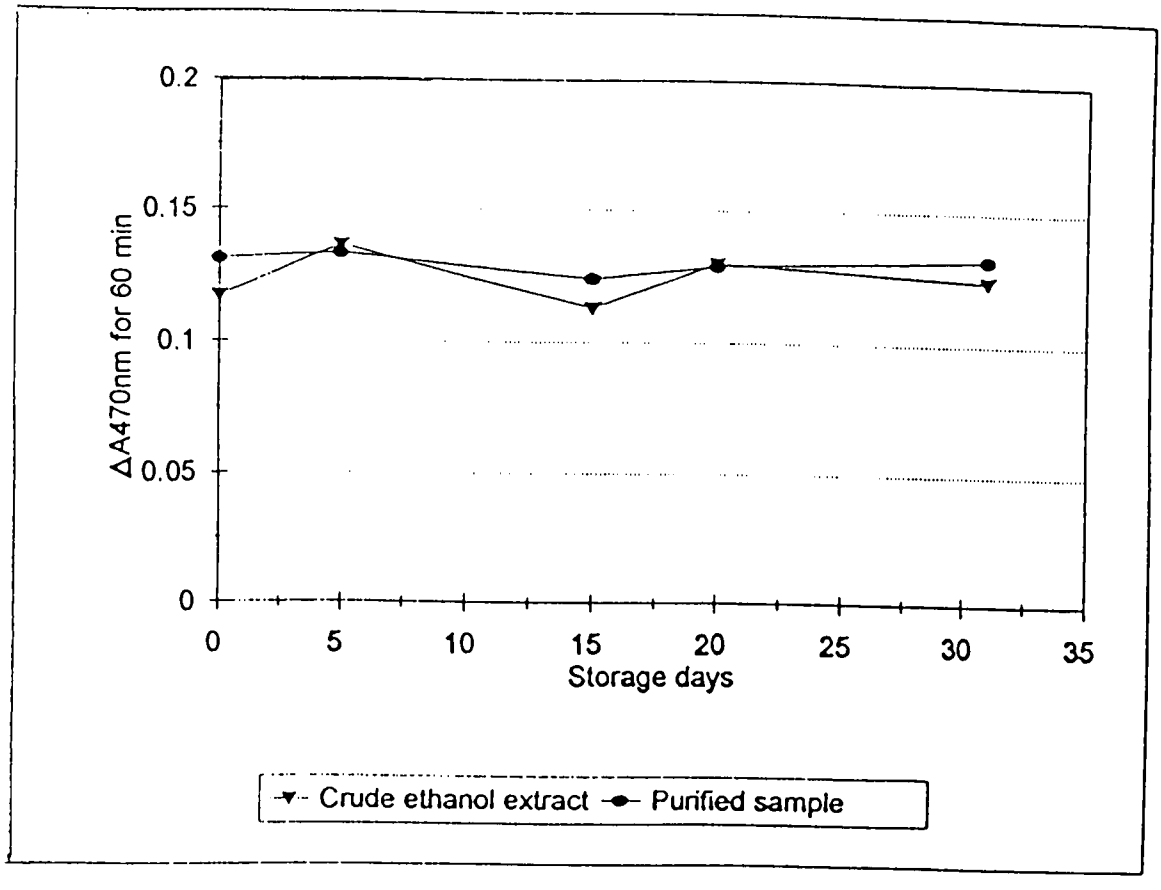


Figure. II.8. Different storage days at the 4°C effect on antioxidant activity of both crude ethanol extract and purified samples. Antioxidant activity was represented by ΔA470 nm in 60 min.

**REFERENCES**

Babbitt, J.K., Law, D.K., and Crawford, D.L. 1976. Improved acceptance and shelf life of frozen minced fish with shrimp. J. Food Sci. 41:35.

Bjelke, E. 1975. Dietary vitamin A and human lung cancer. Int. J. Cancer. 15:561.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37(8):911.

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

Chang, S.S., Ostric-Matijasevic, B., Hsieh, O.L., and Huang, C. 1977. Natural antioxidants from rosemary and sage. J. Food Sci. 42(4):1102.

Chevolleau, S., Mallet, J.F., Ucciani, E., Gamisans, J., and Gruber, M. 1992. Antioxidant activity in leaves of some mediterranean plants. J. Am. Oil Chem. Soc. 69(12):1269.

Colbert, L.B. and Decker, E.A. 1991. Antioxidant activity of an ultrafiltration permeate from acid whey. J. Food Sci. 56(5):1248.

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

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

Dugan, L.R. 1980. Autoxidation in foods and biological systems; M.G. Simic, and M. Karel (Ed.), p. 261. Plenum Press: New York.

Floyd, R.A. 1982. Free radicals and cancer. p. 201, Marcel Dekker, Inc, New York. 201.

Gopalakrishna, A.G. and Prabhakar, J.V. 1985. Antioxidant efficacy of water washed gums as a function of relative humidity in peanut oil. J. Am. Oil Chem. Soc. 62(11):1581.

Hamama, A.A. and Nawar, W.W. 1991. Thermal decomposition of some phenolic antioxidants. J. Agr. Food Chem. 39:1063.

Haraguchi, H., Hashimoto, K., and Yagi, A. 1992. Antioxidative substances in leaves of *(polygonum hydropiper)*. J. Agric. Food Chem. 40:1349.

Ho, C.T., Chen, Q., Shi, H., Zhang. K.Q., and Rosen, R.T. 1992. Antioxidative effect of polyphenol extract prepared from various Chinese teas. Prev Med. 21:520.

Houlihan, C.M., Ho, C.T., and Chang, S.S. 1985. The structure of rosmariquinone-A new antioxidant isolated from *Rosmarinus officinalis L.* J. Am. Oil Chem. Soc. 62(1):96.

Jensen, A. 1969. Tocopherol content of seaweed and seaweed meal. III. Influence of processing and storage on the content of tocopherols, carotenoids and ascorbic acid in seaweed meal. J. Sci. Food. Agr. 20:622.

Kanner, J., Frankel, E., Granit, R., German, B., and Kinsella, J.E. 1994. Natural antioxidants in grapes and wines. J. Agr. Food Chem. 42:64.

Knekt, P., Aromaa, A., Maatela, J., Aaran, R., Nikkari, T., Hakama, M., Hakulinen, T., Peto, R., Saxen, E., and Teppo, L. 1988. Serum vitamin E and risk of cancer among Finnish men during a ten-year follow-up. Am. J. Epidemiol. 127:28.

Lin, F.S., Warner, C.R., and Fazio, T. 1981. Alteration of phenolic antioxidants in heated vegetable oil. J. Am Oil. Chem Soc. 58:789.

Marco, G.J. 1968. A simplified method for the evaluation of antioxidants. J. Am. Oil Chem. Soc. 45:594.

Meyer, S.P. 1986. Utilization of shrimp processing waste. Infofish. 4:18.

Muzarelli, R.A. and Parisher, E.R. 1978. Proceedings from the First International Conference on Chitin/Chitosan. MIT Sea Grant Program, Cambridge, MA.

Nawar, W.W. 1985. Lipids. In *Food Chemistry*. O.R. Fennema, (Ed.), p. 139, Marcel Dekker, Inc, New York, NY.

Onyeneho, S.N. and Hettiarachchy, N.S. 1992. Antioxidant activity of durum wheat bran. J. Agric. Food Chem. 40:1496.

Ornum, J.V. 1992. Shrimp waste-must it be wasted? Infofish Inter. 6:48.

Osawa, T., Katsuzaki, H., Hagiwara, Y., Hagiwara. H., and Shibamoto, T. 1992. A novel antioxidant isolated from young green barley leaves. J. Agric. Food Chem. 40:1135.

Osawa, T. and Namiki, M. 1985. Natural antioxidants isolated from *eucalyptus* leaf waxes. J. Agr. Food Chem. 33(5):777.

Papadopoulos, G. and Boskou, D. 1991. Antioxidant effect of natural phenols on olive oil. J. Am. Oil Chem. Soc. 68(9):669.

Pratt, D.E. and Birac, P.M. 1979. Source of antioxidant activity of soybean and soy products. J. Food Sci. 44:1720.

Pratt, D.E. and Miller, E.E. 1984. A flavonoid antioxidant in spanish peanuts (*Arachia hypogoea*). J. Am. Oil Chem. Soc. 61(6):1064.

Ramarathnam, N., Osawa, T., Namiki, M., and Kawakishi, S. 1988. Chemical studies on novel rice hull antioxidants. 1. Isolation, fractionation, and partial characterization. J. Agric. Food Chem. 36:732.

Rosenzweig-Pasquel, L.J.D. and Babbitt, J.K. 1991. Isolation and partial characterization of a natural antioxidant from shrimp (*Pandalus jordani*). J. Food Sci. 56(1):143.

Saito, A. and Regier, L.W. 1971. Pigmentation of brook trout (*Salvelinus fontinalis*) by feeding dried crustacean waste. J. Fish. Res. Bd. Can. 28:509.

Shahidi, F., Rubin, L.J. and Wood, D.F. 1987. Control of lipid oxidation in cooked ground pork with antioxidants and dinitrosyl ferrohemochrome. J. Food Sci. 52(3):564.

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

Talley, K. 1993. Statspack. Pacific Fish. 15(3):73.

Widicus, W.A. and Kirk, J.R. 1981. Storage stability of α-tocopherol in a dehydrated model food system containing methyl linoleate. J. Food Sci. 46:813.

# III. CHARACTERIZATION OF A NATURAL ANTIOXIDANT FROM SHRIMP WASTE

## ABSTRACT

Shrimp waste samples were extracted with ethanol and purified by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Purified antioxidant samples were further analyzed by several spectroscopy methods such as Fourier transformed-infrared spectrometry (FT-IR), mass spectrometry (EI and CI), and proton nuclear magnetic resonance (NMR). FT-IR spectrum shows the presence of phenolic hydroxyl and amide groups. Results from EI and CI show that this compound has a molecular weight of 164. Proton NMR indicates that it is an ortho-disubstituted benzene. The content of antioxidant in shrimp waste was also estimated.

## INTRODUCTION

Recently, natural antioxidants have received considerable interest from the food industry because there has been some concern over the safety of synthetic antioxidants (Branen, 1975; Waldrop, 1980; Ito et al., 1985; Chen et al., 1986). Consumers prefer natural products instead of synthetic additives (Colbert and Decker, 1991). Tocopherols are the most widely used antioxidants found in plant tissue as a blend of alpha, beta, gamma and delta homologs (Becker, 1993). Although tocopherols have been widely used in food as safe antioxidants, the disadvantages of tocopherols are high manufacturing costs and low effectiveness in some food products such as cereals, and citrus oils (Osawa and Namiki, 1981; 1985; Becker, 1993). Thus, these situations led to the search for new natural antioxidants from various sources (Haumann, 1990).

Many spices have been extensively studied for their natural antioxidant characteristics, especially rosemary. Chang et al. (1977) described a patented procedure to extract antioxidants from rosemary and sage. Later, a number of antioxidant components from *Rosemarinus officianalis L.* were isolated, such as carnosol, rosmanol, rosmariquinone and rosmaridiphenol, etc. (Inatani et al., 1982; Wu et al., 1982; Houlihan et al., 1985). Natural rosemary antioxidant is currently being marketed (Kanner et al., 1994). Recently, several antioxidants from sage

(*Salvia officinalis*) have also been identified (Cuvelier et al., 1994).

Furthermore, various antioxidants have also been found in other sources such as young green barley leaves, tanshen (*Salvia miltiorrhiza bung*), *Polygonum hydropiper* leaves, oregano (*Origanum vulgare L.*), *Eucalyptus* leaf waxes and red turnip (*Brassica campestris L.*). The majority of these natural antioxidants possessed phenolic rings (Osawa and Namiki, 1985; Kikuzaki and Nakatani, 1993; Zhang et al., 1990; Ishikawa et al., 1991; Haraguchi et al., 1992; Osawa et al., 1992).

We have previously reported that a polar compound was responsible for antioxidant activity in shrimp waste. There is an increasing need in the seafood industry for the production and utilization of natural antioxidants. The disposal and/or utilization of seafood processing wastes is a critical issue for the next decade. The isolation and characterization of a natural antioxidant from shrimp waste could have important quality implications for the seafood industry, as well as present a viable alternative to shrimp waste utilization on the West Coast.

The objective of this study was to characterize the natural antioxidant in shrimp waste and quantify its content.

# MATERIALS & METHODS

## Materials

Waste, from the species *Pandalus jordani*, was obtained from an Oregon seafood processor during the shrimp fishing season (April through October). Samples were kept on ice and transported to Oregon State University Seafood Laboratory in Astoria. Two hundred gram samples (200 g) were vacuum packed, blast frozen, and stored at -20°C. Frozen samples to be analyzed were thawed at refrigerated temperatures.

## Purification of the Antioxidant

One kg samples of shrimp waste were extracted with 2 L of 95% ethanol. Mixtures were blended until homogeneous using a Waring blender. The slurry was filtrated through a Buchner funnel using Whatman No. 1 filter paper. The filtrate was evaporated by a rotary evaporator and redissolved into 50 ml ethanol solution. The crude extract was applied to preparative TLC plates developed by solvents benzene-methanol-acetic acid (45:8:4). The fractions at $R_f$ 0.05 were scraped off the TLC plates and further purified by high performance liquid chromatography (HPLC).

HPLC was performed on a Bio-Rad 2700 gradient pump system equipped with a Bio-Rad model 1706 UV detector (BioRad, Hercules, CA). A preparative column packed with R

Sil C18 HL column (250 x 10mm i.d., 5$\mu$m particle size) was used. 500 $u$l of sample with TLC fraction $R_f$ 0.05 from TLC was injected onto HPLC. The eluents used were water and acetonitrile with a gradient of 0-20% acetonitrile for 15 min at a flow rate of 2.50 ml/min. Absorbance at 280 nm was monitored. Active fractions (retention time 8.4 min) were collected. Samples were run several times, pooled together and freeze-dried for further analysis.

**Mass Spectrometry**

Electron impact (EI) and positive chemical ionization (CI) were operated with HP 5985 mass spectrometer. In the EI, the samples were introduced into the sources of the instrument via direct insertion. The source was maintained at 200$^0$C and the electron energy was 70eV. Positive CI spectra was taken with methane as the reagent gas. The source temperature was 150$^0$C, source pressure was $2x10^{-4}$ Torr, and the electron energy was 235 eV.

**Nuclear Magnetic Resonance Spectroscopy (NMR)**

$^1$H NMR spectra was recorded with a Bruker AC 400-MHz spectrometer. Samples were dissolved in $D_2O$ into a semimicrotube with a cylindrical cavity.

**Infrared Spectroscopy (IR)**

IR spectrum was performed in an IR Nicolet 510P apparatus with Fourier transformed (FT-IR). Dried sample was run after putting into NaCl disk.

**Quantitative Measurement of the Antioxidant**

Purified antioxidant samples from HPLC were quantified using salicylamide as a standard, which is a monohydroxylphenolic compound substituted with amide group in ortho position and possesses a similar structure to the phenolic antioxidant from shrimp waste. The phenolic content was determined spectrophotometrically by the Folin-Ciocalteau reagent. A 1.0 ml sample was mixed with 1 ml of phenol reagent (1 part phenol reagent:2 parts water). Then 3 ml of 14% $Na_2CO_3$ solution was added and absorbance determined at 660 nm after 15 min with a Beckman spectrophotometer (Beckman DU-600, Fullerton, CA), the concentration of phenolic antioxidant eluted from HPLC was determined by comparing with absorbances of different concentrations of standard salicylamide. The content of antioxidant from shrimp waste was then calculated by a conversion factor of sample volume.

## RESULTS & DISCUSSION

Purification of the active antioxidant fraction was accomplished by both TLC and HPLC. Molecular weight of the antioxidant samples was determined by electron impact (EI) and chemical ionization (CI) methods, respectively. The EI mass spectra of purified antioxidant is shown in Fig. 1, which indicated that the molecular ion ($M^+$) could be detected at m/z 164 with intensity of 20.9% of the base peak. The EI mass spectrum showed the following peaks (relative intensity, %): 164($M^+$,20.9) 123(8.8), 115(9.8), 107(17.7), 97(11.4), 96(14.8), 95(54.5), 94(100), 83(14.2), 81(11.2), 80(26.8), 79(18.5), 71(15.8), 69(30.9), 67(12.2), 58(9.6), 57(49.8), 56(22.2), 55(48.4), and 53(9.8).

Positive CI spectra gave $[M+H]^+$ at 165 (Fig. 2), confirmed that the molecular weight of the compound is 164. CI spectra also showed that the molecular ion of CI was also base peak. The CI mass spectrum showed the following peaks (relative intensity, %): 165($M^+$+H, 100%), 130(13.3), 123(14.1), 108(17.4), 104(17.3), 94(21.0), 84(39.2), 80(18.6), 69(22.0), 61(69.8), 58(88.3), and 55(35.9). It was found that the major fragments of CI matched with those of EI very well. Furthermore, CI mass spectra gave fewer fragments compared to EI.

EI is the most common method used, and CI is the second choice in determining molecular weights of compounds, which are complementary techniques. Several researchers have

shown that EI-MS was successfully used to detect the molecular weights of natural antioxidants (Wu et al., 1982; Houlihan et al., 1985; Ishikawa et al., 1991; Cuvelier et al., 1994). Extensive fragmentation of EI often occurs. One disadvantage of the EI method is that the molecular ion may not be present or apparent. Therefore, CI-MS was used to locate or confirm the molecular ion (Phillips et al., 1990). The empirical molecular formula ($C_8H_8N_2O_2$) of the antioxidant was obtained by high resolution mass spectrometer which gave a molecular ion peak at m/z 164.0611.

Strong and moderate infrared absorptions were observed at 3415, 3078, 1650, 1640, 1420, 1368, 1300, 1150, 960, and 800 $cm^{-1}$ as shown in Fig. 3. Major absorption at 3400 $cm^{-1}$ and 3078 $cm^{-1}$ indicates the presence of phenolic hydroxyl group. The results from IR corresponded to UV absorption at 268 nm, which indicated the presence of aromatic moiety. A strong amide I band and amide II band were found at 1650 $cm^{-1}$ and 1640 $cm^{-1}$, respectively, these bands results from C=O stretching and $NH_2$ bending, respectively. Moreover, The C-N stretching band of primary amide occurs near 1400$cm^{-1}$ which further supported the presence of amide band.

The result from $^1H$ NMR showed that 4 free protons are in the aromatic range, $\delta$ 8.75(1H, d), $\delta$ 8.58 (1H, t), $\delta$ 8.10(1H, t), $\delta$ 7.95(1H, d) (Fig. 4), suggesting a

disubstituted benzene. Furthermore, these substitutions are found only in the ortho position.

Mass spectrometer is the most facile and initial approach to the identification of microquantities of compounds eluted from an HPLC column, while infrared and nuclear magnetic resonance spectrometries require relatively larger quantities to be analyzed compared to the mass spectrometer (Phillips and Hedin, 1990). UV absorption and infrared spectroscopy identified the phenolic moiety and amide group. The double bonds in side chain of the compound also shifted the UV absorption to 323 nm. The proton NMR detected the presence of an ortho di-substituted benzene compound.

Most synthetic and natural antioxidants have phenolic hydroxyl groups in the structures although some exceptions have been shown (Osawa and Namiki, 1981; Houlihan et al., 1985; Zhang et al., 1990). Generally, the mechanism of primary antioxidant is to act as a free radical scavenger by donating hydrogen or electrons while synergists, such as EDTA, as metal chelators. Thus, the phenolic antioxidant in shrimp waste obviously could play a role in providing hydrogen or electrons to a free radical.

It was reported that antioxidant activity of monophenols was increased substantially by alkyl or methoxy substitution at ortho position relative to the hydroxyl because the ortho-substitution with electron donor of alkyl

or methoxy group increases the stability of aryloxyl radical (Cuvelier, 1992; Pokorny, 1987). These theories may explain the increased antioxidant efficient of the active compound.

The phenolic antioxidant content in shrimp waste was estimated to be 0.18 mg per 100 g shrimp waste, by measuring the phenolic content of the collected samples from HPLC according to the standard curve of salicylamide. Very few papers have reported on the quantitative analysis of antioxidant content from natural sources. The antioxidant content was quantified in sage and rosemary extracts (Cuvelier et al., 1994). The content in carnosol, rosmadial, carnosic acid, and methyl carnosine was quantified by analytical HPLC.

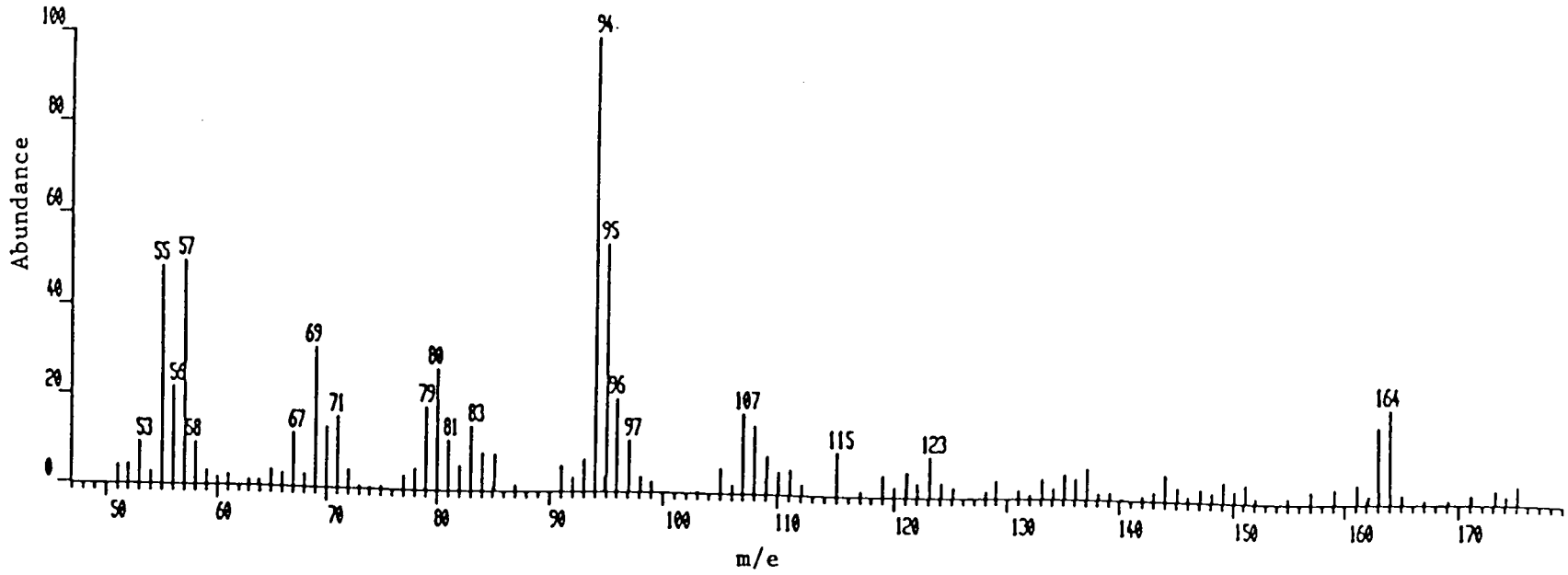


Figure III.1. Electron impact(EI) mass spectra of the antioxidant compound isolated from shrimp waste.

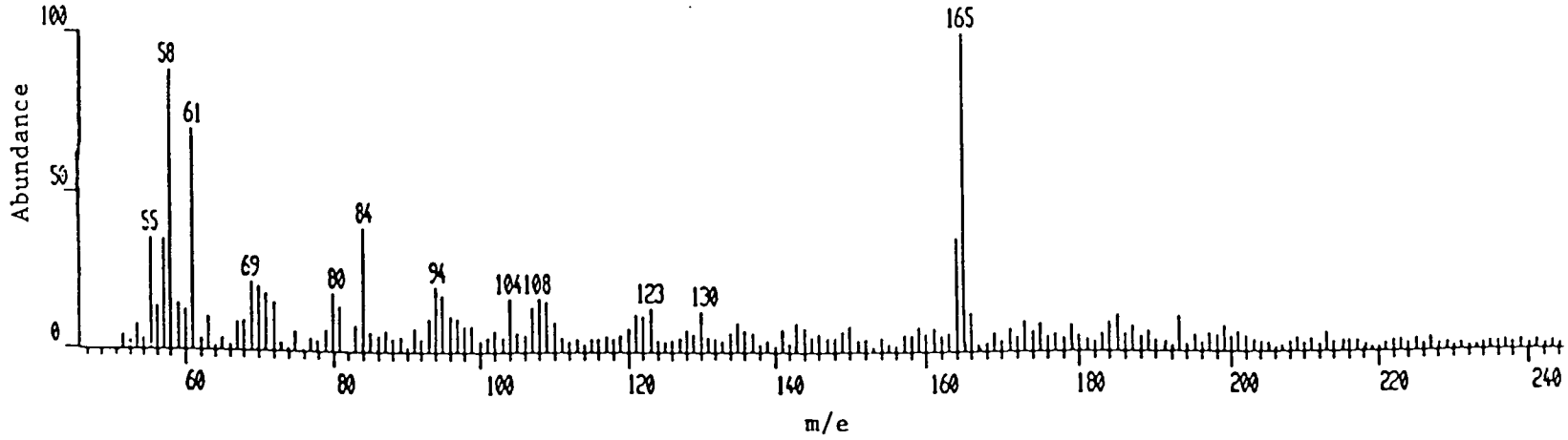


Figure III.2. Positive chemical ionization (CI) mass spectra of the antioxidant compound isolated from shrimp waste.

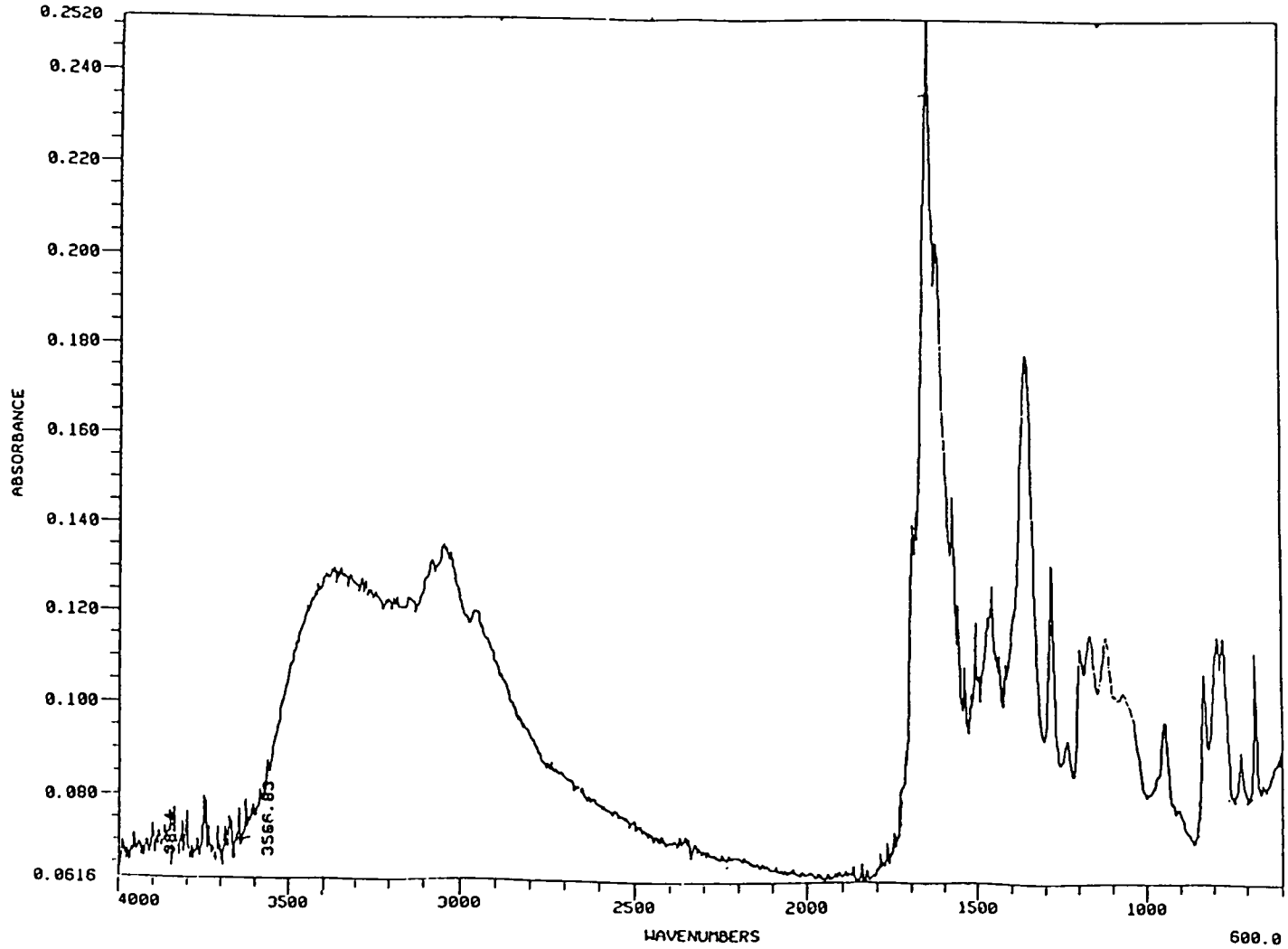


Figure III.3. Fourier transformed-infrared spectrometry (FT-IR) spectra of the antioxidant compound isolated from shrimp waste.

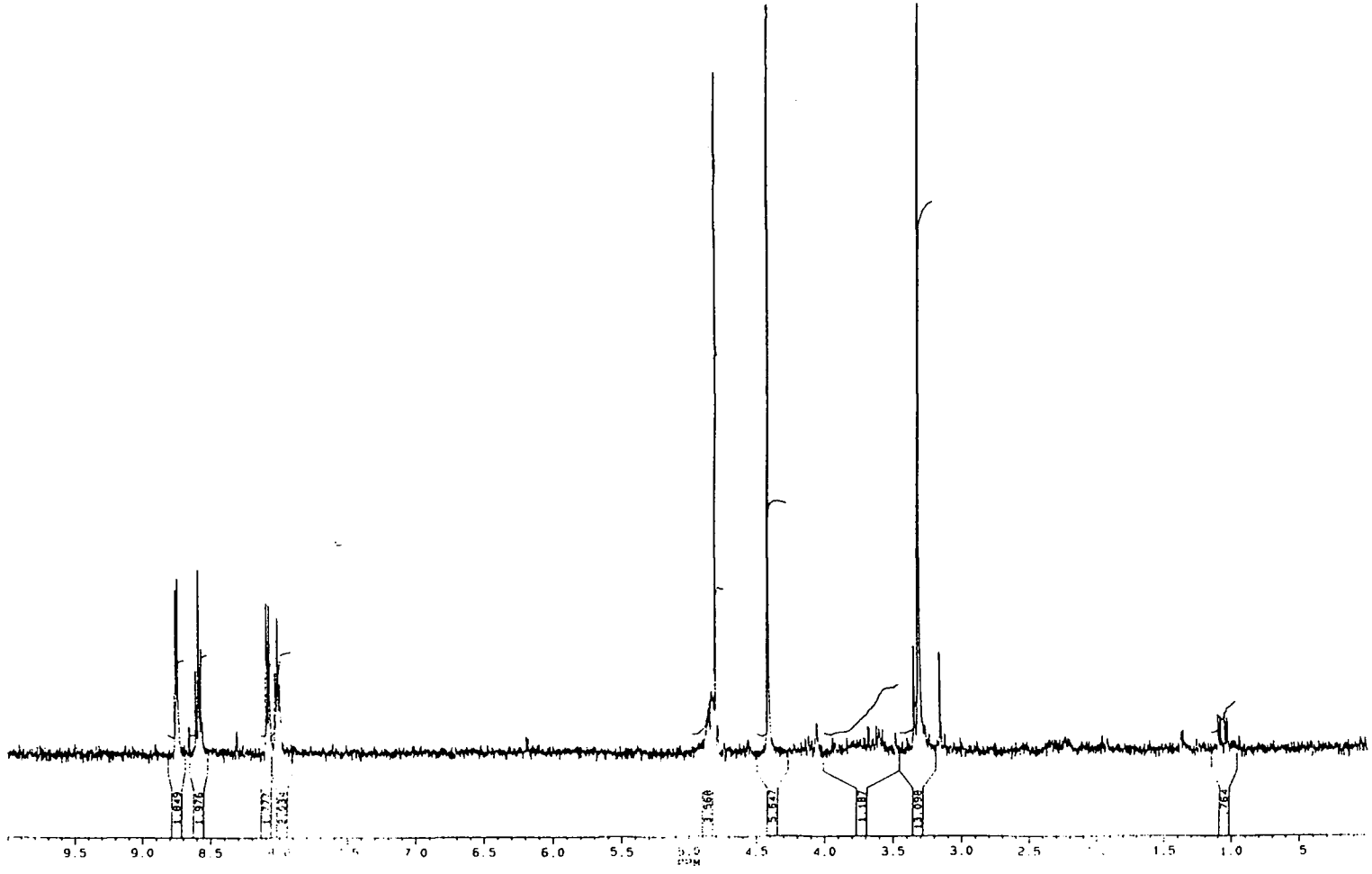


Figure III.4. Proton nuclear magnetic resonance spectroscopy (NMR) spectra of the antioxidant from shrimp waste.

**REFERENCES**

Becker, G.L. 1993. Preserving food-and health: Antioxidants make functional, nutritious preservatives. Food Proc. 12:54.

Branen, A.L. 1975. Toxicology and biochemistry of BHA and BHT. J. Am. Oil Chem. Soc. 52:59.

Chang, S.S., Ostric-Matijasevic, B., Hsieh, O.L., and Huang, C. 1977. Natural antioxidants from rosemary and sage. J. Food Sci. 42(4):1102.

Chen, C., Pearson, A.M., Gray, J.I., and Bennink, M.R. 1986. Antioxidant inhibition of mutagenicity in fried meat. Proc. European Meeting. Meat Res Work. 1(32):211.

Colbert, L.B. and Decker, E.A. 1991. Antioxidant activity of an ultrafiltration permeate from acid whey. J. Food Sci. 56(5):1248.

Cuvelier, M.E., Richard, H., and Berset, C. 1992. Comparison of the antioxidative activity of some acid-phenolis: Structure-activity relationship. Biosci. Biotech. Biochem. 56(2):324.

Cuvelier, M.E., Berset, C., and Richard, H. 1994. Antioxidant constituents in sage (*Salvia officinalis*). J. Agric. Food Chem. 42:665.

Haraguchi, H., Hashimoto, K., and Yagi, A. 1992. Antioxidative substances in leaves of *( polygonum hydropiper )*. J. Agric. Food Chem. 40:1349.

Haumann, B.F. 1990. Antioxidants: Firms seeking products they can label as natural. Inform. 1(12):1002.

Houlihan, C.M., Ho, C.T., and Chang, S. 1985. The structure of rosmariquinone-A new antioxidant isolated from *Rosmarinus oficinalis L.* J. Am. Oil Chem. Soc. 62(1):96.

Inatani, R., Nakatani, N., Fuwa, H., and Seto, H. 1982. Stucture of a new antioxidative phenolic diterpene isolated from rosemary *( Rosmarinus oficinalis L )*. Agric. Biol. Chem. 46:1661.

Ishikawa, Y., Morimoto, K., and Iseki, S. 1991. Atrovenetin as a potent antioxidant compound from penicillium species. J. Am. Oil Chem. Soc. 68:9.

Ito, N., Fukushima, S., and Fsuda, H. 1985. Carcinogenicity and modification of the carcinogenicity response by BHA, BHT, and other antioxidants. CRS Crit. Rev. 15(2):109.

Kanner, J., Frankel, E., Granit, R., German, B., and Kinsella, J.E. 1994. Natural antioxidants in grapes and wines. J. Agric. Food Chem. 42:64.

Kikuzaki, H. and Nakatani, N. 1993. Antioxidant effects of some ginger constituents. J. Food Sci. 58(6):1407.

Osawa, T. and Namiki, M. 1981. A novel type of antioxidant isolated from leaf wax of *Eucalyptus* leaves. Agric. Biol. Chem. 45(3):735.

Osawa, T. and Namiki, M. 1985. Natural antioxidants isolated from *eucalyptus* leaf waxes. J. Agric. Food Chem. 33(5):777.

Osawa, T., Katsuzaki, H., Hagiwara, Y., Hagiwara. H., and Shibamoto, T. 1992. A novel antioxidant isolated from young green barley leaves. J. Agric. Food Chem. 40:1135.

Phillips, V.A. and Hedin, P.A. 1990. Spectral technique for structural analysis of the coton terpenoid aldehydes gossypol and gossypolone. J. Agric. Food Chem. 38:525.

Phillips, V.A., Hedin, P.A., Alley, E.G., and Oldham, P. 1990. Mass spectral techniques for the identification of cotton terpenoid aldehydes. J. Agric. Food Chem. 38:521.

Pokorny, J. 1987. Autoxidation of unsaturated lipid. H, Chan (Ed), p. 141, Academic Press, London.

Waldrop, M. 1980. Firm takes new approach to food additives. Chem. Eng. News. 58(18):22.

Wu, J., Lee, M.H., Ho, C.T., and Chang, S. 1982. Eluciation of the chemical structures of natural antioxidants isolated from rosemary. J. Am. Oil Chem. Soc. 59:339.

Zhang, K. Q., Bao, Y., Wu, P., Rosen, R.T., and Ho, C.T. 1990. Antioxidative components of tanshen (*Salvia miltiorrhiza Bung*). J. Agric. Food Chem. 38:1194.

# IV. THE COLOR STABILITY OF ROCKFISH AFFECTED BY ANTIOXIDANT EXTRACTED FROM SHRIMP WASTE

## ABSTRACT

Antioxidant from shrimp waste was extracted and partially purified by silica gel glass column chromatography. Two species of rockfish (*Sebastolobus alascanus, Sebastes ruberriumus*) were treated with crude antioxidant solutions, respectively, while another species of rockfish (*Sebastes alutus*) was treated with different concentrations of antioxidant solutions from column chromatography. Higher a* values found in rockfish samples treated with antioxidants were compared to the control without antioxidant treatment. Furthermore, the dipping of 0.20%, and 0.50% antioxidant lowered TBA values compared to the control group of rockfish fillets (*Sebastes alutus*).

## INTRODUCTION

The color of rockfish is a primary factor in determining whether consumers will purchase the product. Several species of rockfish; such as, *Sebastolobus alascanus, Sebastes ruberriumus,* and *Sebastes alutus* have a red skin color. These red species of rockfish usually are more attractive and command a higher price than the yellow or brown species although they have a similar taste (Sackton, 1986). Pacific ocean perch (*Sebastes alutus*) have a bright red skin color which is one of the most prized of the *Sebastes* complex (Haard, 1988).

Recently, increased efforts have been made in expanding markets for the West Coast fisheries in Japan where there is a large demand for whole rockfish, including several other species captured off the West Coast of the United States. Over the past years, there have been a number of marketing trials of fresh West Coast rockfish in the main Tokyo fish markets. These initial attempts were only moderately successful as the fish were marked down in price due to a rapid discoloration of the red pigments (Read, 1991).

Carotenoids, mainly astaxanthin and tunaxanthin, are pigments responsible for the red color of rockfish (Tsukuda and Amano, 1967). In addition, Haard (1988) indicated that a wide range of skin color of California rockfish (*Sebastes sp.*) appears to be related to the total amount of carotenoids as well as the percentage of astaxanthin. The

major cause of carotenoid degradation in foods is oxidation with rates dependent on contact with oxygen, light, heat, and presence of pro- and antioxidants (Francis, 1985; Haard, 1988).

Various attempts have been made to use antioxidants to stabilize the color and pigment of poultry products during storage (Greene et al., 1971; Harbers et al., 1981; Chastain et al., 1982; Mitsumoto et al., 1991); however, there are different mechanisms involved in color or pigment degradation between poultry/meat muscle and rockfish skin. The degradation of meat color is due to the oxidation of myoglobin to metmyoglobin, while carotenoid oxidation results in color degradation in rockfish skin.

The mechanism of carotenoid degradation is thought to be similar to lipid oxidation (Frankel, 1985). It has been reported that antioxidants which inhibit lipid oxidation also decrease the degradation of color and carotenoid. Research has been conducted by Wasson et al. (1991) to determine the effect of several antioxidants on rockfish color degradation. The results showed that all antioxidant-treated rockfish had significantly higher red color scores than the control group. They also found that tocopherol is the most effective antioxidant to maintain the red color of rockfish. Butylated hydroxytoluene (BHT) was not as

effective as tocopherol. A previous study showed that an antioxidant compound was isolated from shrimp waste (Chapter. II).

The objective of this study is to evaluate the effect of a natural antioxidant extracted from shrimp waste on color stability of several species of rockfish.

# MATERIALS & METHODS

## Materials

Samples of shrimp (*Pandalus jordani*) waste and rockfish (*Sebastolobus alascanus, Sebastes ruberriumus,* and *Sebastes alutus*) were obtained from a local seafood company. Approximately 200 g of shrimp waste samples were vacuum packed, blast frozen, and stored at -20°C. Frozen samples to be analyzed were thawed at room temperature. Rockfish samples were stored in an ice box and transferred to the Oregon State University Seafood Laboratory in Astoria, Oregon. The samples were subjected to treatments both with and without antioxidants.

## Preparation of Crude Extract of Antioxidant

One kg samples of shrimp waste were extracted with 2 L of 95% ethanol solution. The mixture was blended until homogeneous using a Waring blender. The slurry was filtered through a funnel using Whatman No. 1 filter paper. The filtrate was evaporated to dryness and a solution of 0.5% in water was prepared.

## Silica Gel Glass Column Chromatography

A 2000 ml crude ethanol extract was concentrated to about 20 ml by a rotary evaporator and subjected to column

chromatography. A glass column with a diameter of 2.5 cm and length of 58.4 cm was packed with silica gel 60-200 mesh in hexane slurries. The fractions were first eluted with 150 ml 100% hexane, and then 20% acetone in hexane A/H, 40% A/H, 60% A/H, 80% A/H, 100% A, 20% methanol in acetone (M/A), 40% M/A, 60% M/A, 80% M/A, and, finally 100% methanol. The flow rate of eluents was kept at 2.5-3.0 ml/min. Each fraction was concentrated to a 5.0 ml solution and its antioxidant activity was measured. Active fractions from multiple runs were collected and evaporated to dryness, and solutions in 0.1%, 0.2%, 0.5% water were prepared.

**Antioxidant Activity Testing**

Antioxidant activity was evaluated in a β-carotene-linoleic acid emulsion as described by Marco (1968). The ability of antioxidant to prevent the oxidative destruction of the β-carotene and linoleic acid emulsion was expressed as the decrease in absorbance at 470 nm. A 2.0 mg sample of crystalline β-carotene was dissolved in 10 ml of chloroform. One ml of this solution was then pipetted into a round-bottomed flask which contained 20 mg of purified linoleic acid and 200 mg of Tween 40 emulsifier. After removal of chloroform on a rotary evaporator, 50 ml deionized water was added to the flask with vigorous stirring. A 5.0 ml aliquot of the aqueous emulsion was formed and then pipetted into

each of a series of spectrometer tubes which contained 0.2 ml portions of extract solution. A zero reading was taken at 470 nm on the reaction mixture in each tube immediately after addition of the emulsion to the extract solution. The tubes were then stoppered and placed in a water bath (at 50°C. Subsequent readings were taken at regular intervals.

**Antioxidant Treatments**

Samples of rockfish (*Sebastolobus alascanus, Sebastes ruberriumus*) were individually dipped into various solutions of different treatments for 5 min: negative control containing only water solution; 0.5% (w/v) crude antioxidant solution; and 0.05% (w/v) sodium erythorbate solution. For each treatment, 3 whole fish were used as replicates. After treatment, fish samples were put into individual plastic bags and stored in the ice box at 4°C. In another set of experiments, fillet samples (*Sebastes alutus*) with skin were dipped into the following solutions for 5 min: 0.1%; 0.2%; and 0.5% antioxidant solution purified from silica gel glass column chromatography; 0.05% sodium erythorbate; and negative control. These samples were put into individual plastic bags and stored in the ice box at 4°C, and color and TBA values were measured.

**Color Measurement**

The skin color of these species of rockfish was measured with a Minolta Chroma Meter CR 300 (Osaka, Japan) before and during ice storage. Tristimulus L* a* b* values were obtained (CIE 1976) using a white color standard. The measuring head of chroma meter CR 300 has an 8 mm diameter measuring area and uses diffuse illumination. Each fish was measured in 10 locations in the head, middle, and tail regions. And the average of L*, a*, b* values was calculated based on these observations.

**TBA Analysis**

2-Thiobarbituric acid value (TBA) was determined on rockfish (*Sebastes alutus*) samples as described by Yu and Sinnhuber (1975). The fillet meat was ground, mixed, and the samples subjected to analysis. The TBA number is expressed as mg malonaldehyde/kg sample.

**Statistics Analysis**

All treatments were conducted in triplicate. Data was subjected to one factor analysis of variance (ANOVA) to compare treatments at each specified time of color and TBA measurements. The Least Significant Difference (LSD) test determined the differences between treatments. Pearson's

correlation coefficient was calculated for TBA and a* values of rockfish (*Sebastes alutus*).

## RESULTS & DISCUSSION

The antioxidant activity of crude extract from shrimp waste is shown in Fig. 1. The loss of antioxidant activity was negligible due to evaporation and resolubilization in water. However, the antioxidant activity of the extracts was lower than Tenox 6.

The crude ethanol extract was further subjected to purification by silica gel glass column chromatography. An active fraction (10) eluted with methanol in acetone (80%) showed a strong antioxidant activity while fractions 3 and 9, eluted with 40% acetone in hexane and 60% methanol in acetone, respectively, also had some antioxidant activity (Fig. 2). Basically, polar compounds would have longer retention times than nonpolar compounds under these conditions. It was previously reported that antioxidants from ginger and *Eucalyptus* leaves were fractionated by silica gel column chromatography (Osawa and Namiki, 1981; Kikuzaki and Nakatani, 1993). These antioxidants were eluted by nonpolar solvents such as hexane; hexane and ethyl acetate (9:1 v/v), etc. The data shown here from silica gel column chromatography was consistent with our previous results in Chapter 2, which indicated that the antioxidant possesses polar characteristics. Due to its water solubility, the property of the antioxidant compound described here explains the effect on stabilizing surface colors in the skin of a number of rockfish species.

**Rockfish (*Sebastolobus alascanus*)**

Results clearly indicated that a* value (redness) with no antioxidant treatment decreased more rapidly than other treatments during the ice storage (Fig. 3A). Results showed that a* values of controls significantly decreased during storage ($P<0.05$). After 4 days of ice storage, it could be visually observed that the red color of rockfish skin samples, without antioxidant, had faded, while the fish treated with antioxidants maintained the red skin color. At the end of storage, the control of a* value was significantly lower compared to antioxidant treatments ($P<0.05$), while no significant difference was found between crude extract and sodium erythorbate treatment.

Samples were also analyzed for L* value, which represents the lightness characteristic in sample appearance. All of the treatments showed a decreasing trend and then increased after 2 days (Fig. 3B). A slight loss of b* values, as indicated by the fading of yellowness, was shown in Fig. 3C. With respect to L* and b* values respectively, no significant difference was noticed among treatments and storage times.

**Rockfish (*Sebastes ruberriumus*)**

After 2 days of iced storage samples treated with either crude extract or sodium erythorbate apparently

maintained more skin redness than controls in which a* values decreased rapidly from initial values of 19.48±1.83 to 10.92±1.17 (Fig. 4A). Statistical results showed that decrease in a* value of the control groups were highly significant throughout the storage period ($P<0.001$). Moreover, after 7 days, a significant difference among treatments in a* value could be detected ($P<0.05$).

No significant differences in L* values were detected among treatments and storage times (Fig. 4B). Higher b* value indicates that the rockfish species contains higher initial yellowness than another species, *Sebastolobus alascanus*. Fig. 4C exhibited that all of the treatments resulted in a slight loss in b* values, but there were no significant differences among storage times and treatments, respectively.

**Rockfish (*Sebastes alutus*)**

Antioxidant solutions from shrimp waste were applied at 3 levels to this species to evaluate the effect of color. Results indicated that all of the treatments showed a significantly higher a* value than the control sample on days 6 and 9 respectively ($P<0.05$)(Fig. 5A). Furthermore, no significant difference was found among antioxidant treatments. The L* value of a negative control group was significantly greater than other treatments on days 0, 3, and 6, respectively, (Fig. 5B). Antioxidant treatments

appear to not affect the b* value from this species in which no statistical difference was observed among treatments (Fig. 5C).

Overall, antioxidant extracts from shrimp waste had an effect on red color stability for these species of rockfish. The effectiveness was lower compared to sodium erythorbate, a water soluble antioxidant, which has been commercially used to stabilize the red skin color of rockfish. Furthermore, it appears that both L* and b* values were not affected by antioxidant treatments. It was reported that pigment oxidation did not affect L* value (lightness) (Ledward and MacFarlane, 1971; Brewer and Harbers, 1991). Carotenoid degradation probably involves both non-enzymatic and enzymatic oxidation. It has been suggested that enzymatic oxidation can be catalyzed by lipoxygenase in fish while non-enzymatic oxidation included autoxidation and photosensitized oxidation (Tsukuda and Amano, 1967; Tsukuda, 1970; Frankel, 1985; German and Kinsella, 1985). Fish lipoxygenase tends to be particularly active in red skinned fish (Tsukuda and Amano, 1967; Tsukuda, 1970). Hsieh and Kinsella (1989) illustrated that lipid hydroperoxides interact with pigments and other macromolecular in fish causing discoloration and off-flavor. Wasson et al. (1991) showed that tocopherols stabilized the skin color of rockfish.

TBA increased most rapidly for both the control group and 0.1% antioxidant solution compared with other antioxidant treatments between days 0 to 6 (Fig. 6). The results also indicated that 0.2%, and 0.5% antioxidant can effectively inhibit lipid oxidation, but not as well as sodium erythorbate which gave the lowest TBA value during storage. After 6 days of iced storage, TBA values with the control and 0.1% extract decreased. Statistical results show there is a highly significant difference among treatments ($p<0.01$) on 0, 3, 6 and 9 days, respectively.

The relationship between TBA and a* values in rockfish (*Sebastes alutus*) is shown in Table 1. The correlation for all treatments was not significant except for the control, in which the correlation coefficient was -0.72 ($P<0.01$). The results suggest that both color degradation and TBA value were related in the control sample, and both of them significantly correlated in the early stage of iced storage. It was previously shown that the correlation between lipid peroxidation and the discoloration of raw beef product was highly significant (Rhee et al., 1985). Torres et al. (1988) reported that pigment oxidation occurred earlier than lipid oxidation in beef. It was also suggested that the delay in lipid oxidation was due to greater proportions of saturated to unsaturated fatty acids in ground beef (Akamittath et al., 1990); On the other hand, lipid and pigment oxidation occurred simultaneously because of greater

unsaturated fatty acids in poultry product (Akamittath et al., 1990). Goldman et al. (1983) suggested that free radicals produced by lipid oxidation may interact with carotenoid to intensify carotenoid oxidation. Since rockfish contains polyunsaturated fatty acids, it would explain our results that lipid oxidation and color degradation both occurred in the early stages of storage.

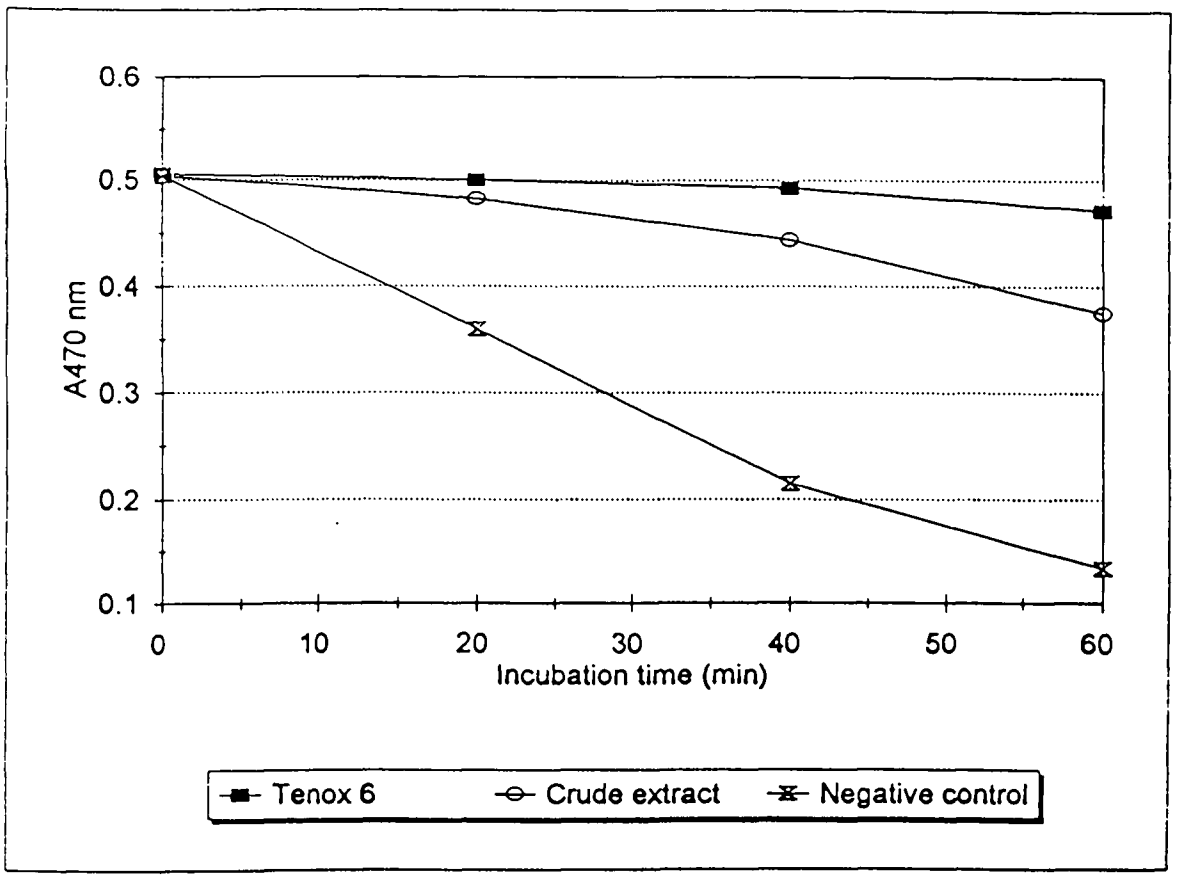


Figure IV.1. Antioxidant activity of crude extract from shrimp waste compared to Tenox 6 and control. Crude extract: samples of shrimp waste extracted with ethanol and redissolved in water. Tenox 6: 0.02% Tenox 6 which is a mixture of BHA, BHT, and citric acid. Control: only 95% ethanol.

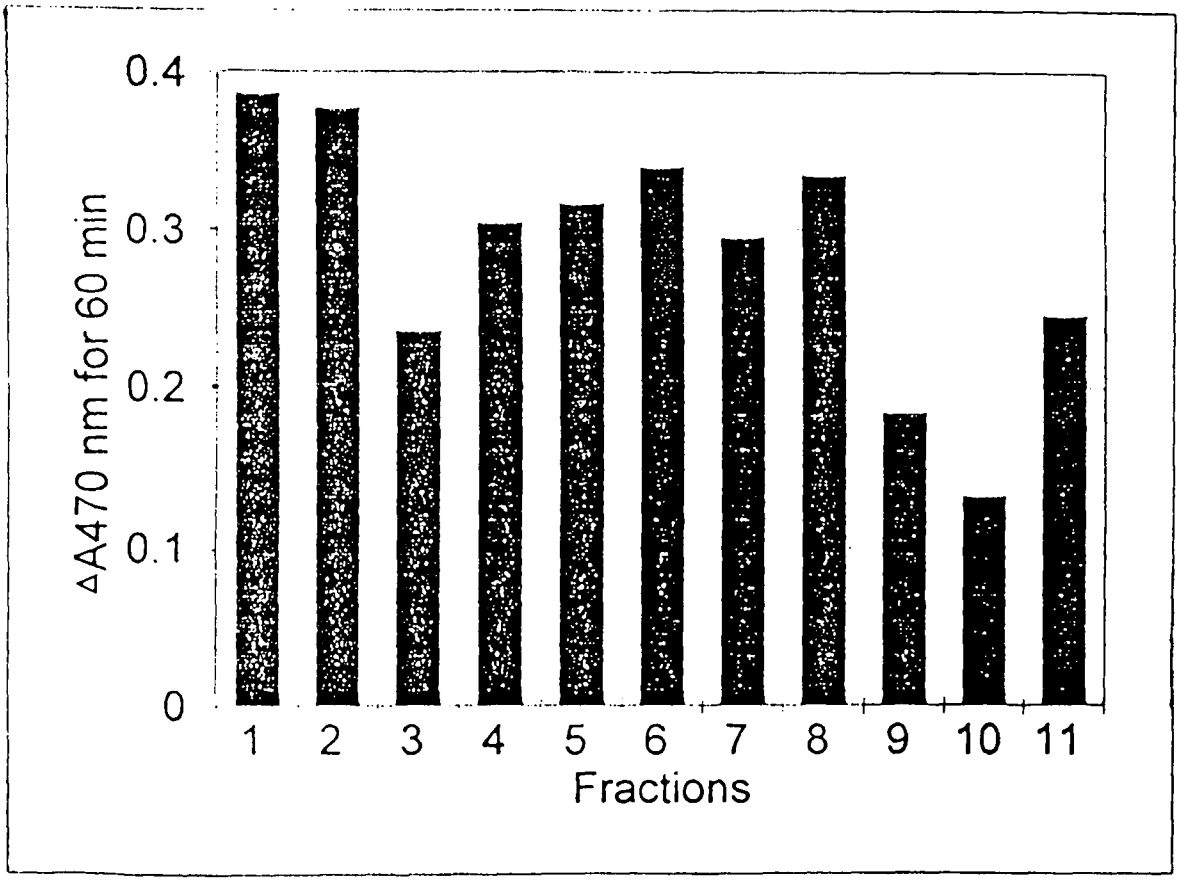


Figure IV.2. Antioxidant activity of different fractions eluted from silica gel glass column chromatography.

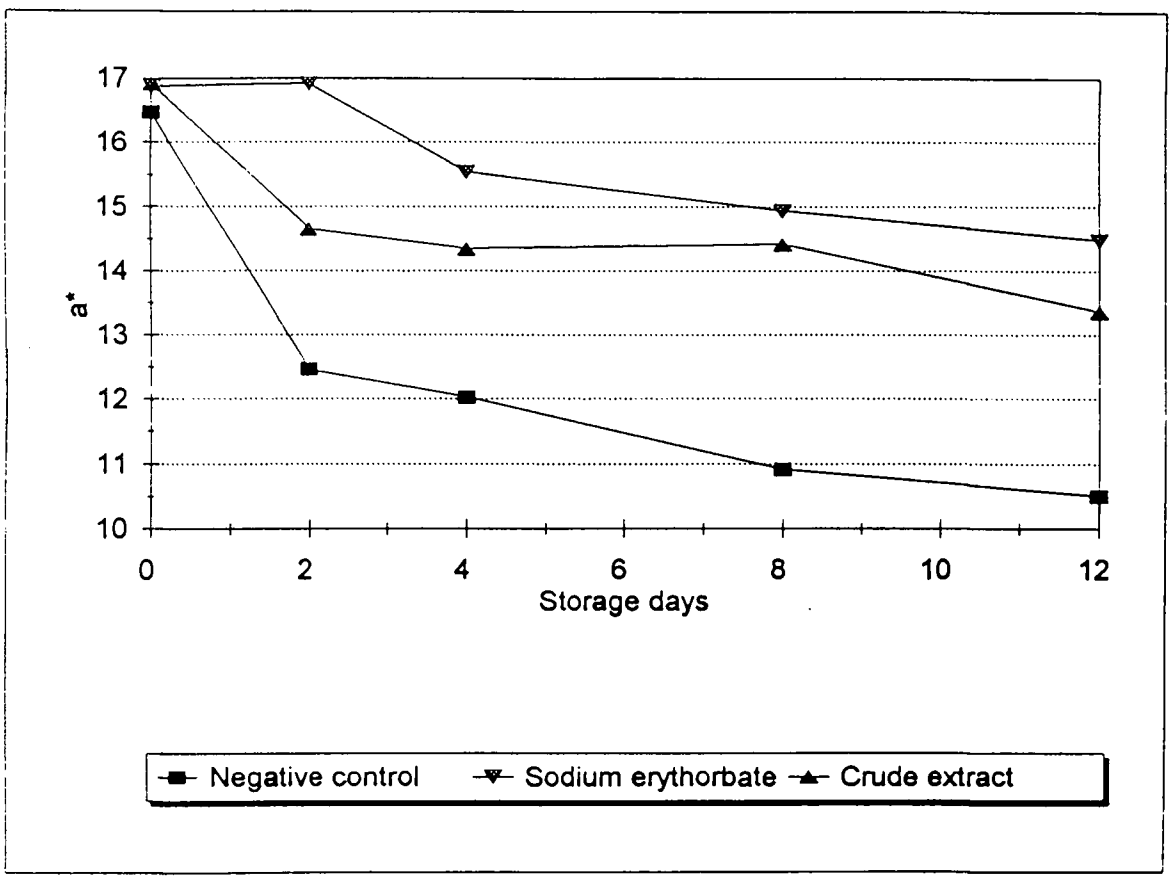


Figure IV. 3(A). Change of a* values of the skin of rockfish(*Sebastolobus alascanus*) treated with crude extract, sodium erythorbate (0.05%W/V), and control containing no antioxidant solutions during ice storage.

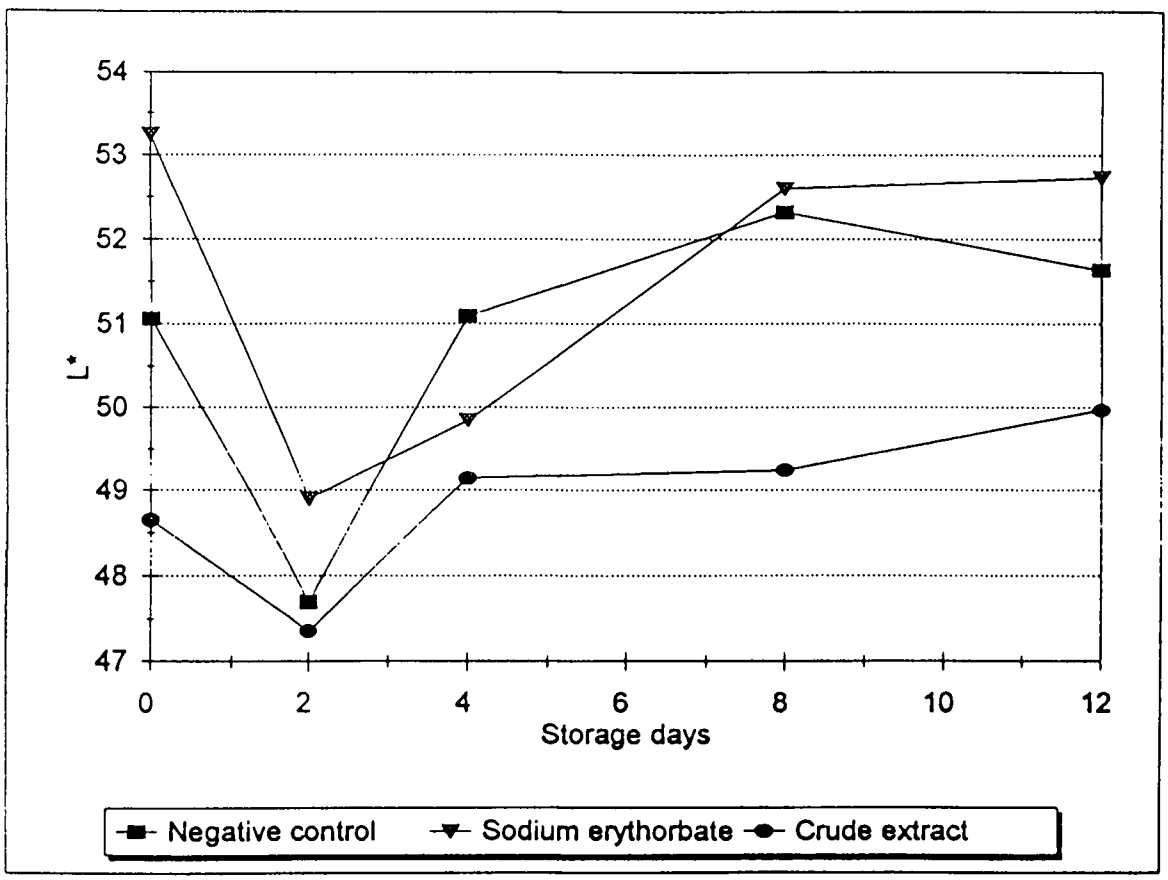


Figure IV.3(B). Change of L* values of the skin of rockfish (*Sebastolobus alascanus*) treated with crude extract, sodium erythorbate (0.05% W/V), and control containing no antioxidant solutions during ice storage.

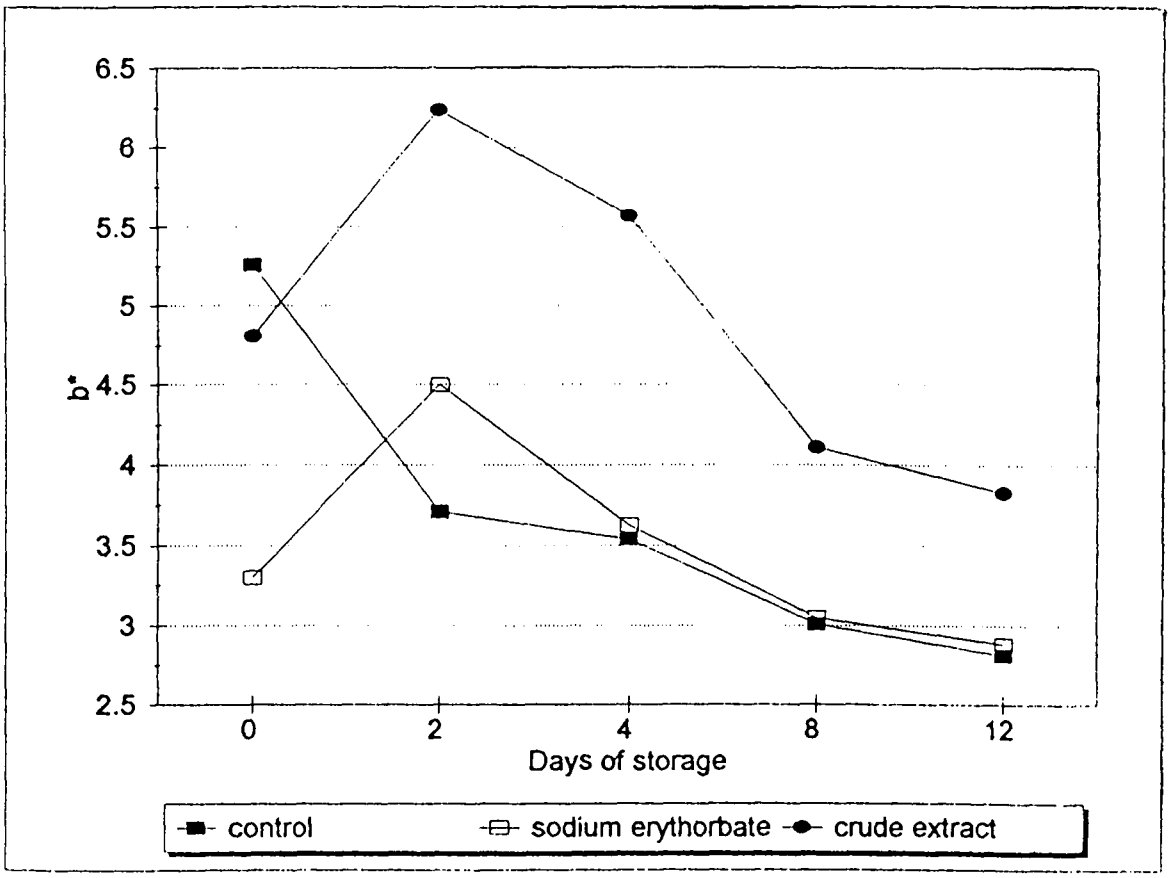


Figure IV.3(C). Change of b* values of the skin of rockfish (*Sebastolobus alascanus*) treated with crude extract, sodium erythorbate (0.05% W/V), and control containing no antioxidant solutions during ice storage.

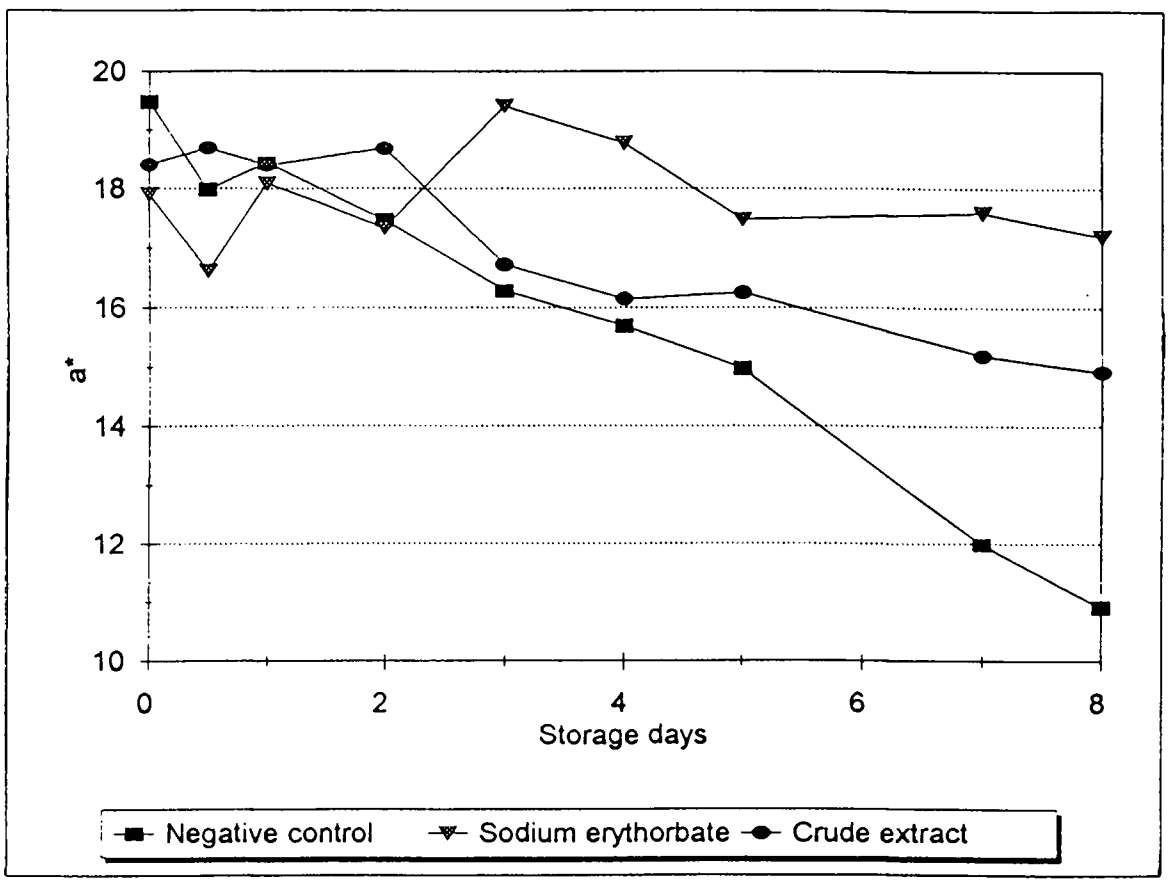


Figure IV.4(A). Change of a* values of the skin of rockfish (*Sebastes ruberriumus*) treated with crude extract, sodium erythorbate (0.05% W/V), and control containing no antioxidant solutions during ice storage.

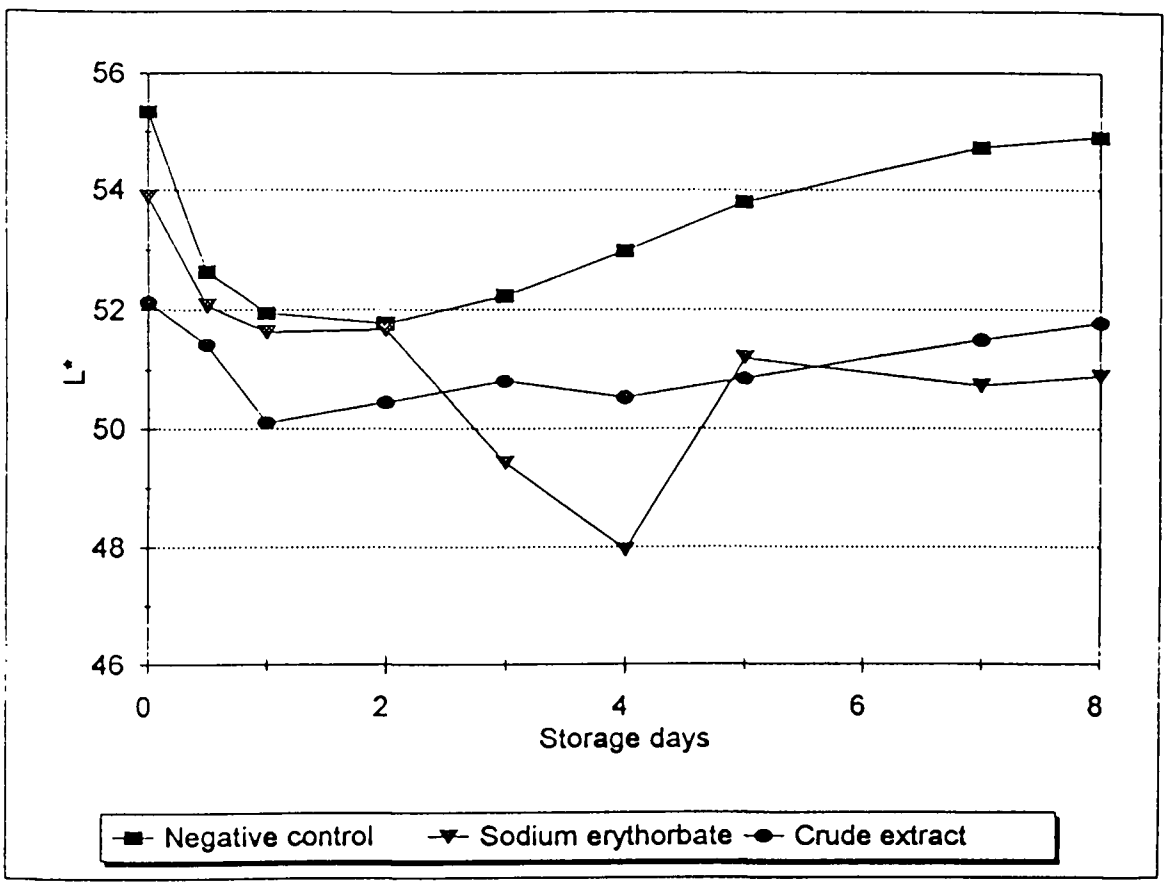


Figure IV.4(B). Change of L* values of the skin of rockfish (*Sebastes ruberriumus*) treated with crude extract, sodium erythorbate (0.05% W/V), and control containing no antioxidant soultions during ice storage.

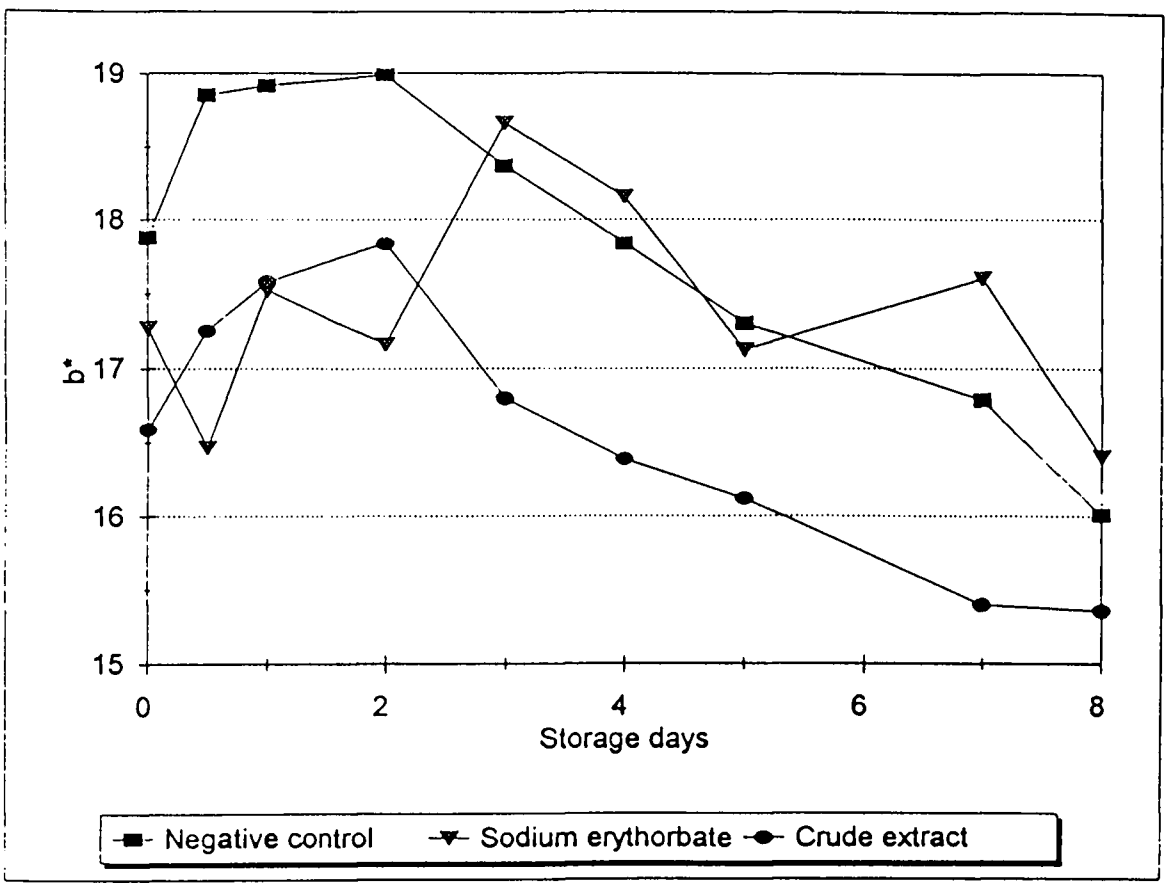


Figure IV.4(C). Change of b* values of the skin of rockfish (*Sebastes ruberriumus*) treated with crude extract, sodium erythorbate (0.05% W/V), and control containing no antioxidant solutions during ice storage.

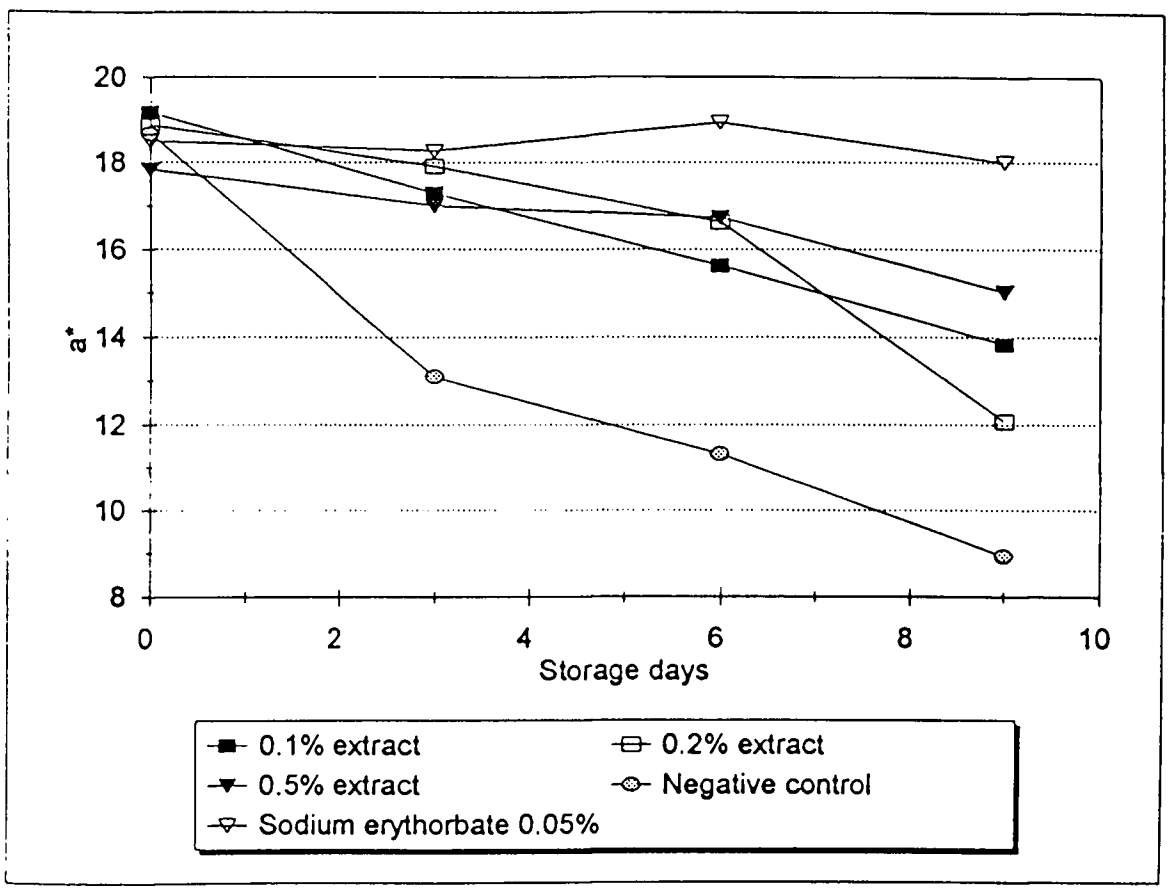


Figure IV.5(A). Change of a* values of the skin of rockfish(*Sebastes alutus*) treated with 0.10%; 0.20%; and 0.50% (W/V) partially purified antioxidant, sodium erythorbate (0.05% W/V) and control containing no antioxidant solutions during ice storage.

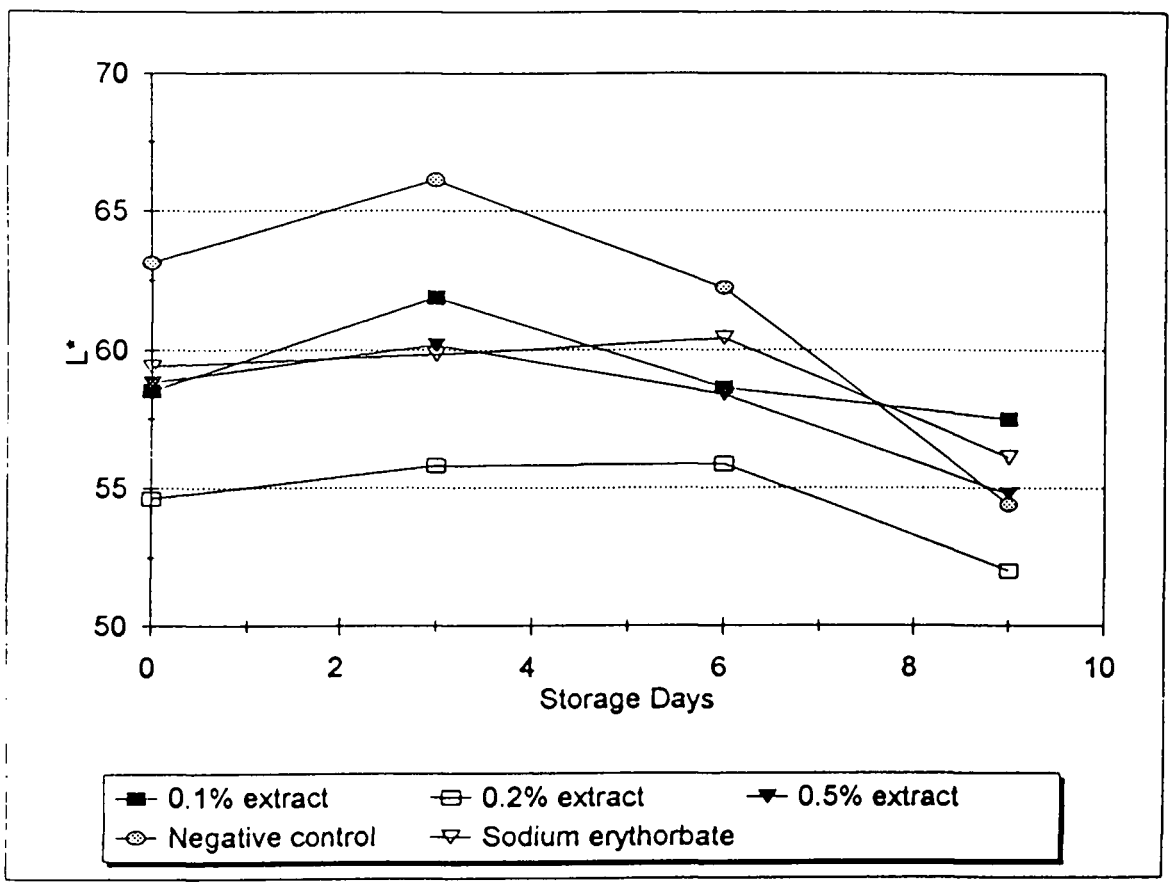


Figure IV.5(B). Change of L* values of skin of rockfish(*Sebastes alutus*) treated with 0.10%; 0.20%; and 0.50% (W/V) partially purified antioxidant, sodium erythorbate(0.05% W/V), and control containing no antioxidant solutions during ice storage.

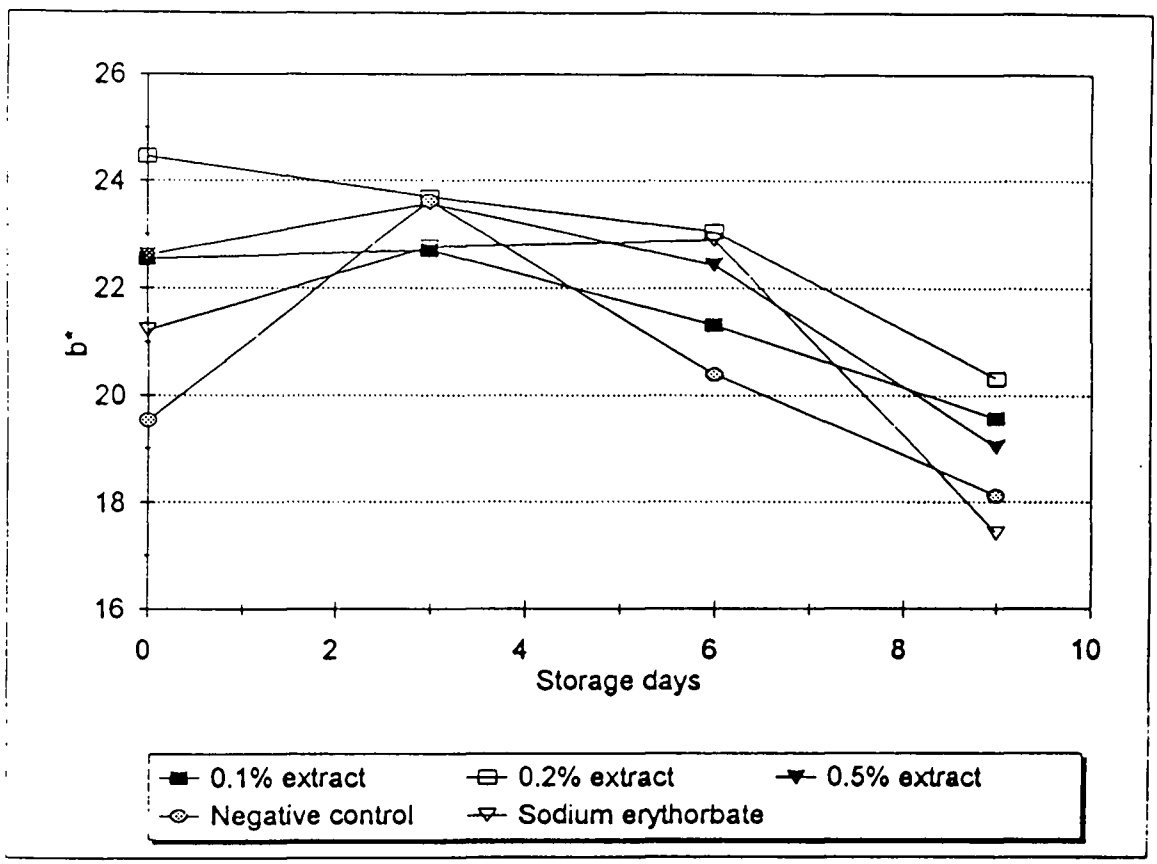


Figure IV.5(C). Change of b* values of skin of rockfish (*Sebastes alutus*) treated with 0.10%, 0.20%, 0.50% partially purified antioxidant, sodium erythorbate (0.05% W/V), and control containing no antioxidant solutions during ice storage.

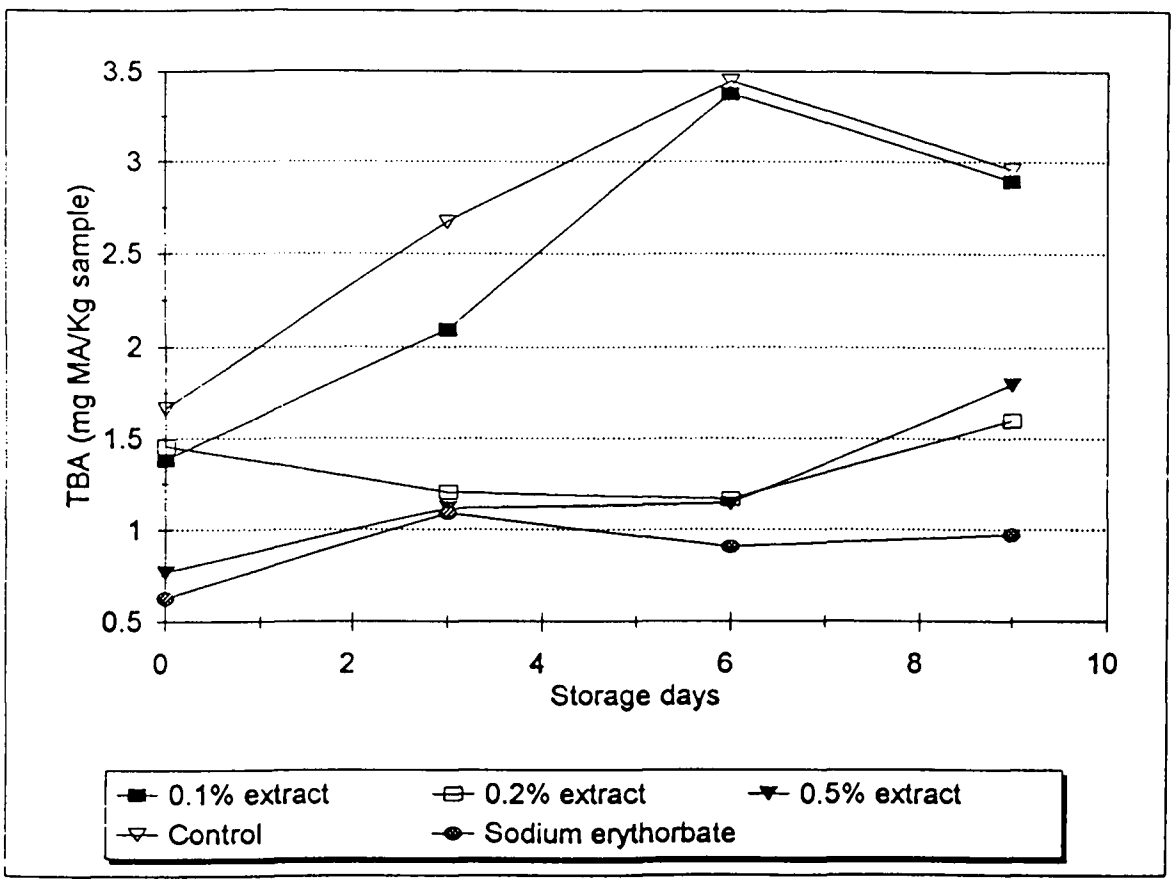


Figure IV.6. Change in Thiobarbituric Acid (TBA) of rockfish (*Sebastes alutus*) treated with 0.10%, 0.20%, 0.50% (W/V) partially purified antioxidant, sodium erythorbate (0.05% W/V), and control solutions during ice storage.

Table 1. Correlation coefficients and p-value between TBA and a* values of rockfish (*Sebastes alutus*).

| | Treatments | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| r | -0.56 | -0.34 | -0.38 | -0.72 | 0.13 |
| p-value | 0.059 | 0.273 | 0.212 | 0.009 | 0.673 |

Treatment 1, 2, and 3 are referred to 0.1%, 0.2%, and 0.5%(W/V) antioxidant purified from silica gel glass column chromatography respectively.
Treatment 4: Control.
Treatments 5: 0.05% (W/V) sodium erythorbate.

## CONCLUSIONS

Crude extract and a fraction eluted from silica gel glass column chromatography at 80% methanol in acetone showed a strong antioxidant activity. The crude extract of antioxidant in water-dispersible form contributed to high a* values of rockfish (*Sebastolobus alascanus and Sebastes ruberriumus*) compared to the control group during iced storage, but not as well as sodium erythorbate treatment. Both L* and b* values are not affected by antioxidant treatments. Treatment at 0.1%, 0.2%, and 0.5% concentrations of partially purified antioxidant resulted in higher a* values of the skin of rockfish (*Sebastes alutus*) than the control sample during storage. 0.2%, and 0.5% purified samples were effective in decreasing TBA values in rockfish fillets (*Sebastes alutus*). The correlation between TBA and a* values in rockfish (*Sebastes alutus*) for all treatments were not significant except the control.

**REFERENCES**

Akamittath, J.G., Brekke, C.J., and Schanus, E.G. 1990. Lipid oxidation and color stability in restructured meat systems during frozen storage. J. Food Sci. 55(6):1513.

Brewer, M.S. and Harbers, C.A.Z. 1991. Effect of packing on color and physical characteristics of ground pork in long-term frozen storage. J. Food Sci. 56(2):363.

Chastain, M.F., Huffman, D.L., Hsieh, W.H., and Cordray, J.C. 1982. Antioxidants in restructed beef/pork steaks. J. Food Sci. 47:1779.

Francis, F.J. 1985. Pigments and Other Colorants. In Food Chemistry. O.R. Fennema (Ed.), p. 545. Marcel Dekker, New York, NY.

Frankel, E.N. 1985. Chemistry of autoxidation: Mechanism, products and flavor significance. Ch. 1. In *Flavor Chemistry* of *Fats* and *Oils*, D.B. Min and T.H. Smouse (Ed), p. 1. American Oil Chemists' Society, Champaign, IL.

German,J.B., and Kinsella, J.E. 1985. Lipid oxidation in fish tissue. Enzymatic initiation by lipoxygenase. J. Agric. Food Chem. 23:680.

Goldman, M., Horev, B. and Saguy, I. 1983. Decolorization of β-carotene in model systems simulating dehydrated food. Mechanism and kinetic principle. J. Food Sci. 48:751.

Greene, B.E., Hsin, I. and Zipser, M.W. 1971. Retardation of oxidation color changes in raw ground beef. J. Food Sci. 36:940.

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 Publishing Co., Inc. Lancaster, PA.

Harbers, C.A.Z., Harrison, D.L. and Kropf, D.H. 1981. Ascorbic acid effects on bovine muscle pigments in the presence of radiant energy. J. Food Sci. 46:7.

Hsieh, R.J. and Kinsella, J.E. 1989. Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish: In *Advances* in *Food* and *Nutrition Research*. 33: 233.

Kikuzaki, H. and Nakatani, N. 1993. Antioxidant effects of some ginger constituents. J. Food Sci. 58(6):1407.

Ledward, D.A. and MacFarlane, J.J. 1971. Some observations on myoglobin and lipid oxidation in frozen beef. J. Food Sci. 36:987.

Marco, G.J. 1968. A rapid method for evaluation of antioxidants. J. Am. Oil Chem. Soc. 45:594.

Mitsumoto, M., Cassens, R.G., Schaefer, D.M., Arnold, R.N., and Scheller, K.K. 1991. Improvement of color and lipid stability in beef longissimus with dietary vitamin E and vitamin C dip treatment. J. Food Sci. 56(6): 1489.

Osawa, T. and Namiki, M. 1981. A novel type of antioxidant isolated from leaf wax of *Eucalyptus* leaves. Agric. Biol. Chem. 45(3):735.

Read, R. 1991. Oregon seeks to crack Japanese fish market. The Oregonian. Jan, 27.

Rhee, K.S., Vanderzant, C., Keeton, J.T., Ethlers, J.G., and Leu, R. 1985. Microbiological and shelf-life properties of ground beef containing glandless cottonseed flour. J. Food Sci:1388.

Sackton, J. 1986. The Seafood Handbook. Seattle Washington: Seafood Business, p. 70.

Torres, E., Pearson, A.M., Gray, J.I., Booren, A.M., and Shimakomaki, M. 1988. Effect of salt on oxidative changes in pre-and post-rigor ground beef. Meat Sci. 23:151.

Tsukuda, N. 1970. Studies on the discoloration of red fishes. VI. Partial purification and specificity of the lipoxygenase-like enzyme responsible for carotenoid discoloration in fish skin during refrigerated storage. Bull. Japan Soc Sci. Fish. 36(7):725.

Tsukuda, N. and Amano, K. 1967. Studies on the discoloration of red fishes. IV. Discoloration of astaxanthin, tunaxanthin, and β-carotene by tissue homogenates of Fishes. Bull. Japan Soc Sci. Fish. 33(10):962.

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

Yu, T.C. and Sinnhuber, R.O. 1975. An improved 2-thiobarbituric acid (TBA) procedure for the measurement of autoxidation in fish oil. J. Am Oil. Chem Soc. 44:256.

# V. THE EFFECT OF NATURAL ANTIOXIDANT FROM SHRIMP WASTE ON LIPID OXIDATION OF SABLEFISH MINCE

## ABSTRACT

Crude extract (0.50% w/w), and purified antioxidant (0.10%, 0.20%, and 0.50% w/w) from shrimp waste were applied to sablefish mince on a total weight basis and evaluated for their effectiveness to inhibit oxidative and hydrolytic rancidity of mince samples stored at refrigerated temperature (4°C) for 8 days and in frozen storage (-20°C) for 4 mo, respectively. Crude extract, 0.20%, and 0.50% antioxidant from silica gel glass column chromatography had a significantly lower 2-thiobarbituric acid value (TBA), peroxide value (PV) compared to the control group during refrigerated and frozen storage. Results from free fatty acid values suggested that antioxidant from shrimp waste had no effect on hydrolytic rancidity in sablefish mince.

## INTRODUCTION

Lipid oxidation causes various off-flavor, off-odor, texture, and nutrient damage in muscle food (Simic and Karel, 1980; Chan, 1987). Seafood is especially susceptible to the deterioration process which makes it less palatable and of causes quality defects due to highly unsaturated fatty acids. Fatty fish, such as, sablefish, and mackerel, make them extremely susceptible to autoxidation and rapid deterioration resulting in unstable food products.

Sablefish, or black cod (*Anoplopoma fimbria*) is distributed in the North Pacific Ocean from northern Mexico northward to the Bering Sea, and along the Asian Coast from Kamchatka southward to the northeastern Coast of Japan. As sablefish are particularly well suited for salting and smoke curing, they are actively fished for local markets by both Canada and the United States.

Oxidative rancidity in seafood can be decreased by several methods (Allen and Hamilton, 1983). The most common method is to lower the temperature as in chilled and frozen storage. Another method is to eliminate oxygen by vacuum packing and/or modified storage atmosphere. A third method uses the addition of synthetic and natural antioxidants to retard lipid oxidation. Although synthetic commercial antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tertiary-butylhydroquinone (TBHQ), etc. have been widely

used to inhibit lipid oxidation of food products, there has been some concern over the safety of synthetic antioxidants (Branen, 1975; Waldrop, 1980; Inatani et al., 1983; Ito et al., 1985). There are some other disadvantages; such as, cost and/or the fear of an adverse reaction by the consumer in utilizing synthetic additives. Several authors have reported that various antioxidants were used to inhibit lipid oxidation in fish systems (Benedict et al., 1975; Zama et al., 1979; Ke et al., 1981; Hwang and Regenstein, 1988). Moreover, some natural products such as polyphenols, dried spices, and fresh spices were used to inhibit oxidative rancidity in ground fish (*Scomberomorus commersoni*) (Ramanathan and Das, 1992; 1993).

Previous results have shown that the natural antioxidant extract from shrimp waste could decrease the fading of the skin color of rockfish and also produce lower TBA values compared to the control group of rockfish fillets (*Sebastes alutus*). The objective was to test whether the antioxidant from shrimp waste could inhibit the oxidative and hydrolytic rancidity in sablefish mince.

# MATERIALS & METHODS

## Materials

Samples of shrimp waste including peel and head parts (*Pandalus jordani*), and fresh sablefish (*Anoplopoma fimbria*) were obtained from a local seafood company and transported in ice to the Oregon State University Seafood Laboratory in Astoria, Oregon. Shrimp waste samples were vacuum packed, blast frozen, and stored at -20°C. Samples of sablefish mince were prepared and subjected to treatments both with and without antioxidants.

## Preparation of Antioxidant Extract

A 1 kg sample of shrimp waste was extracted with 2000 ml 95% ethanol solution. The mixture was blended until homogeneous, using a Waring blender. The slurry was filtered through a funnel using Whatman No. 1 filter paper. The solvent was evaporated to dryness by rotary evaporation.

## Silica Gel Glass Column Chromatography

A 2000 ml crude ethanol extract was concentrated to about 20 ml by rotary evaporator (Buchi, Switzerland) and subjected to column chromatography. A glass column of a diameter of 2.5 cm and length of 58.4 cm was packed with silica gel 60-200 mesh in hexane slurries. The fractions

were first eluted with 150 ml 100% hexane, and then 20% acetone in hexane (A/H), 40% (A/H), 60% (A/H), 80% (A/H), 100% acetone, 20% methanol in acetone (M/A), 40% (M/A), 60% (M/A), 80% (M/A), and finally 100% methanol. The flow rate of the eluents was kept at 2.5-3.0 ml/min. The most active fraction eluted with methanol in acetone (80%) was repeatedly collected and evaporated to dryness.

**Preparation of Sablefish Mince**

Fresh sablefish were cleaned, and gutted. After filleting, the fillets were ground using a Hobart Model N50 grinder. Approximately 26% mince was obtained from raw fish. The fat content of the fish was analyzed according to modified Folch extract method (Bligh and Dyer, 1959). Samples of 0.50% (w/w) crude extract and 0.10%, 0.20%, and 0.50% (w/w) purified antioxidant from shrimp waste were added to the mince on a total weight basis, respectively. A commercial antioxidant, 0.02% Tenox 6 (the mixture of BHA, BHT, propyl gallate, and citric acid), was added as a percentage of fat content of the sablefish mince. The control group contained no antioxidant. Each mixture was homogenized with a Kitchen Aid mixer. Samples of each treatment, 60 g of mince, were put into individual plastic bags (Ziploc brand). The samples were stored in the blast freezer at -20$^{0}$C for 4 mo. 2-Thiobarbituric acid value (TBA), peroxide value (PV), and free fatty acid (FFA) were

analyzed at 0, 1, 2, 3, and 4 mo. Another set of samples was stored at refrigerated temperature (4°C) for 8 days and sampled at 0, 4, and 8 days for TBA, PV and FFA analyses.

**TBA Analysis**

2-Thiobarbituric acid values (TBA) were determined as described by Yu and Sinnhuber (1975) with some modification. About 200 mg of minced samples were mixed with 3 drops of antioxidant (TBHQ in propylene glycol and Tenox 6) mixture, 3 ml of TBA solution, and 17 ml of trichloroacetic acid (TCA)-HCL reagent. Samples were flushed with nitrogen and tightly closed with a screw cap, placed in a boiling water bath for 30 min and cooled in tap water. 5 ml of chloroform was added and about 15 ml of the colored solution was transferred into a 50 ml conical centrifuge tube. The tubes were then centrifuged for 10 minutes at 3000 rpm. A portion of the clear, colored, aqueous solution was transferred to a cuvette for absorbance measurement at 535 nm.

**Determination of Peroxide Value**

The fat from fish mince was extracted utilizing the procedure of the Bligh and Dyer (1959), and peroxide values (PV) of extracted fat were measured as described in AOAC (28.022, 28.023, 50.037, and 50.038), 1975. Peroxide value (PV) was calculated as PV (milliequiv. Peroxide/kg sample)=

S x N x 1000/g sample where S=ml $Na_2S_2O_3$ and N=normality $Na_2S_2O_3$ solution.

**Determination of Free Fatty Acid**

Free fatty acid (FFA) was determined according to the procedure of Ke and Woyewada (1978). Aqueous sodium hydroxide was added until an end-point was reached as indicated by a color change of meta-cresol purple. The FFA was represented as μmol $g^{-1}$ of tissue sample.

**Statistical Analysis**

Analysis was performed, in duplicate, with two replications. Data was subjected to one factor analysis of variance (ANOVA) to compare treatments. Least Significant Difference (LSD) was further applied to determine differences between treatments if F-test was significant.

## RESULTS & DISCUSSION

### Effect of Antioxidants at 4°C Storage.

The results from fat analysis showed that the sablefish mince contained about 7.60% total fat content according to the Bligh and Dyer method (1959).

The TBA values for sablefish mince samples with treated and untreated antioxidants for 8 days of storage are shown in Fig. 1. After 4 days of storage, the samples treated with crude extract, 0.20%, and 0.50% antioxidant from shrimp waste had significantly lower TBA than the control sample containing no antioxidant($P<0.01$); while 0.10% antioxidant had no effect on decreasing TBA values. Furthermore, differences in TBA values between crude extract, 0.20%, and 0.50% antioxidant were not significant during storage tests. However, all levels of antioxidant treatments from shrimp waste had a lower effectiveness compared to that of Tenox 6 (0.02%).

The change in peroxide value of the mince samples with and without antioxidant treatments stored at 4°C is shown in Fig. 2. Crude extract, 0.20%, and 0.50% antioxidant, had significantly lower PV than the control throughout the refrigerated storage ($P<0.01$), while 0.10% treatment was not effective in decreasing PV for the entire 8 days of storage. Further statistical results indicated that there was no significant difference between crude extract, 0.20%, and

0.50% purified antioxidant on days 4 and 8. It was found that the results from PV were consistent with those of TBA values, which indicated that antioxidant at 0.10% concentration was not sufficient to inhibit lipid oxidation; while crude extract, 0.20%, 0.50% antioxidant supressed lipid oxidation in the sablefish mince to some degree.

Free fatty acid values of samples with treated and untreated antioxidants increased rapidly during 8 days of storage at $4^{0}$C (Fig. 3). After 4 days, the samples treated with 0.10% and 0.50% antioxidant had significantly higher free fatty acid production than other samples ($P<0.05$), but 0.20% purified antioxidant and crude extract did not affect the free fatty acid. The inconsistent results suggested that antioxidant from shrimp waste had no effect on free fatty acid production during refrigerated storage.

Research has been conducted to compare the effectiveness of varying levels of natural antioxidants. It was reported that α-tocopherol at a level of 200 ppm was effective in preventing lipid oxidation of the ground fish, while a lower concentration (30 ppm) had no effect during storage at $4^{0}$C (Ramanathan and Das, 1992). Furthermore, it was reported that polyphenols had a concentration-dependent effect on inhibition of lipid oxidation in which the concentration at 0.005% level was more effective than at 0.001% in fresh cooked ground fish (Ramanathan and Das, 1993). Decker and Crum (1993) reported that all 3 carnosine

concentrations (0.50%, 1.0%,and 1.5%) significantly reduced the formation of TBA values in cooked, unsalted ground pork compared to the control sample after 3 days of storage at 4°C. No significant differences were detected between the 3 carnosine concentrations; however, only 1.5% carnosine inhibited TBA formation in cooked ground pork during refrigerated storage.

**Effect of Antioxidants during Frozen Storage**

TBA values of all samples increased after 1 mo of storage as shown in Fig. 4. Samples treated with crude extract, 0.20%, 0.50% antioxidant, and Tenox 6 had significantly lower TBA values than the control group after 2 mo of frozen storage ($P<0.05$). TBA values showed that 0.10% level of antioxidant had no effect on controlling oxidative rancidity during frozen storage. Furthermore, there were no differences ($P>0.05$) in TBA values between samples with 0.20%, 0.50% levels, and crude extract treatments. However, all antioxidant treatments from shrimp waste had significantly higher TBA values than Tenox 6. The results indicated that the effectiveness of crude extract and purified antioxidants was less than that of Tenox 6.

Peroxide values of sablefish mince samples with or without antioxidant treatments increased after 2 mo of frozen storage at -20°C (Fig. 5). The control consistently had the highest PV among all treatments for 0, 1, 2, 3, and

4 mo. Crude extract, 0.20%, and 0.50% antioxidant had significantly lower PV compared to the control group.

The effectiveness of several antioxidants on protecting oxidative rancidity in menhaden mince has been reported by Hwang and Regenstein (1988). They indicated that ascorbic acid and erythorbic acid showed very strong antioxidants with only a very little change in PV during frozen storage. It was found that Tenox 20 (0.08% TBHQ) had an effect in decreasing PV compared to the control group, but did not completely control lipid oxidation; while commercial rosemary extracts alone or in combination with tocopherols, had little effect on PV. However, Boyd et al (1993) reported that rosemary extract was effective in decreasing TBA values in cooked sea trout. Our results showed that Tenox 6, and the natural antioxidant from shrimp waste did not completely retard the lipid oxidation in sablefish mince.

Several natural antioxidants have been used to inhibit lipid oxidation in food. For example, spices and herbs have been chosen as sources for natural antioxidants to prevent the oxidative rancidity in various food systems. Rosemary spice extract at 0.05% has been shown to exhibit substantially a lower TBA value than the untreated sample of deboned turkey meat during refrigerated storage (MacNeil et al., 1973). Antioxidant activity of rosemary oleoresin was comparable to a commercial blend of BHA/BHT/citric acid in

suppressing lipid autoxidation in turkey sausage (Barbut et al., 1985). Other spices such as cloves, cinnamon, black pepper, thyme, etc. also exhibited antioxidative activity to inhibit lipid oxidation in a variety of food systems (Chipault et al., 1952; Al-Jalay et al., 1987).

Tsukuda (1976) observed an increase from 79 to 338 mg/100 g free fatty acid in skipjack tuna muscle during frozen storage (10$^0$C) after 80 days. Nair et al. (1976) reported that free fatty acid significantly increased as a function of frozen storage periods in mackerel. Our results showed that free fatty acid values of all samples increased with increased storage times up to 4 mo (Fig. 6). After 4 mo of storage, 0.20% antioxidant had a significantly higher free fatty acid compared to other treatments, which differed from the results in refrigerated storage.

It has been suggested that TBHQ possibly stimulated the activity of lipase which released more free fatty acid in mullet muscle during frozen storage (Deng, 1978). Boyd et al. (1993) also reported that cooked fish flakes treated with antioxidants such as TBHQ and rosemary extract produced higher FFA values compared with the untreated control. Ke et al. (1981) indicated that TBHQ retarded the formation of carbonyl from lipid hydrolysis and secondary oxidation reaction. On the other hand, Hwang and Regenstein (1988) reported that Tenox 20 (0.08% TBHQ) treatment at -7$^0$C did

not inhibit lipid hydrolysis on menhaden mince. They further indicated that the release of free fatty acid from the menhaden mince could be minimized by frozen storage at -20°C.

## CONCLUSIONS

Crude extract, 0.20%, and 0.50% (w/w) antioxidant from shrimp waste significantly lowered TBA and peroxide values of sablefish minces stored at 4°C for 8 days and frozen condition for 4 mo, respectively, while 0.10% purified antioxidant had no effect in decreasing TBA and peroxide values. Results indicated that crude extract, 0.20%, and 0.50% purified antioxidant can effectively inhibit lipid oxidation. Inconsistent results from free fatty acid suggested that antioxidant from shrimp waste had no effect on the hydrolytic rancidity of sablefish mince stored at 4°C and frozen storage, respectively.

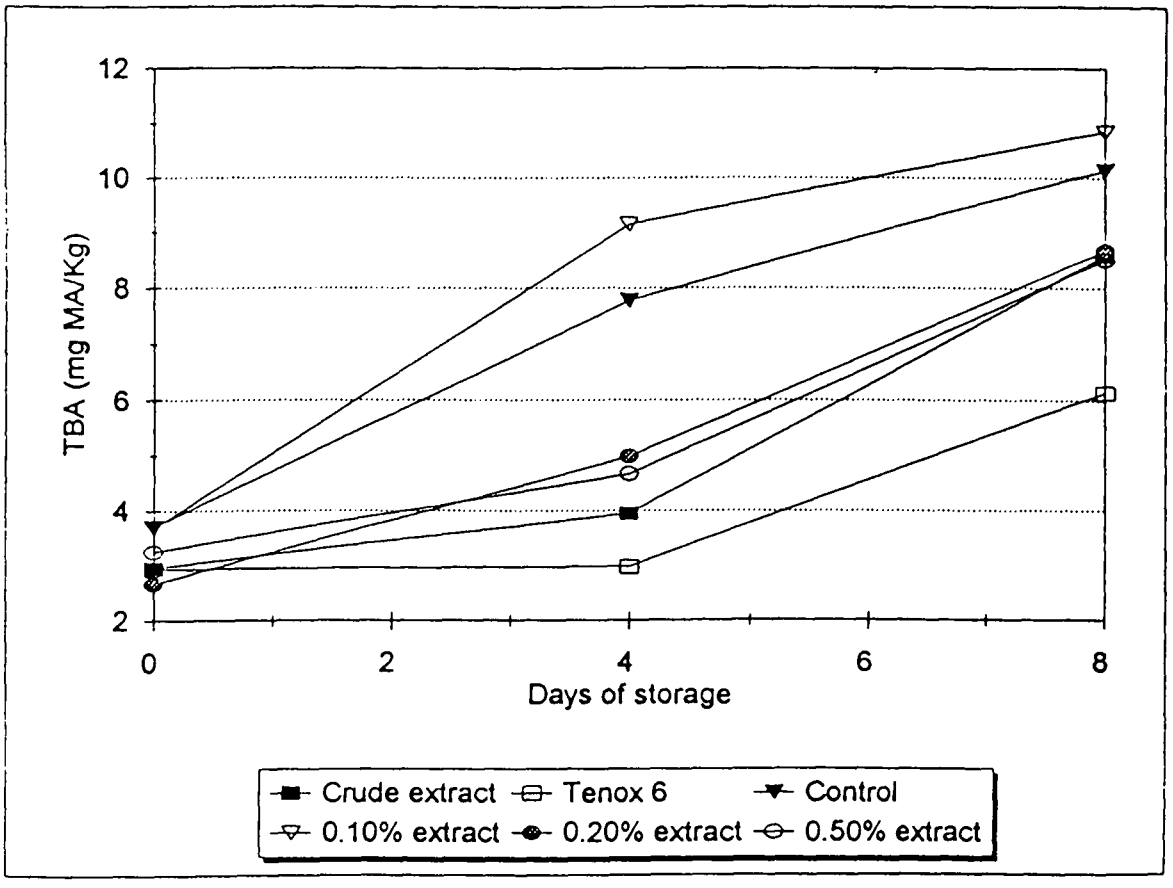


Figure V.1. The effect of antioxidant treatments on thiobarbituric acid (TBA) values of sablefish mince samples stored at refrigerator temperature (4°C) for 8 days. Crude extract: 0.50% crude extract added to the mince samples on a total weight basis; 0.10% extract, 0.20% extract and 0.50% extract are referred to 0.10%, 0.20%, and 0.50% purified antioxidant from silica gel glass column chromatography.

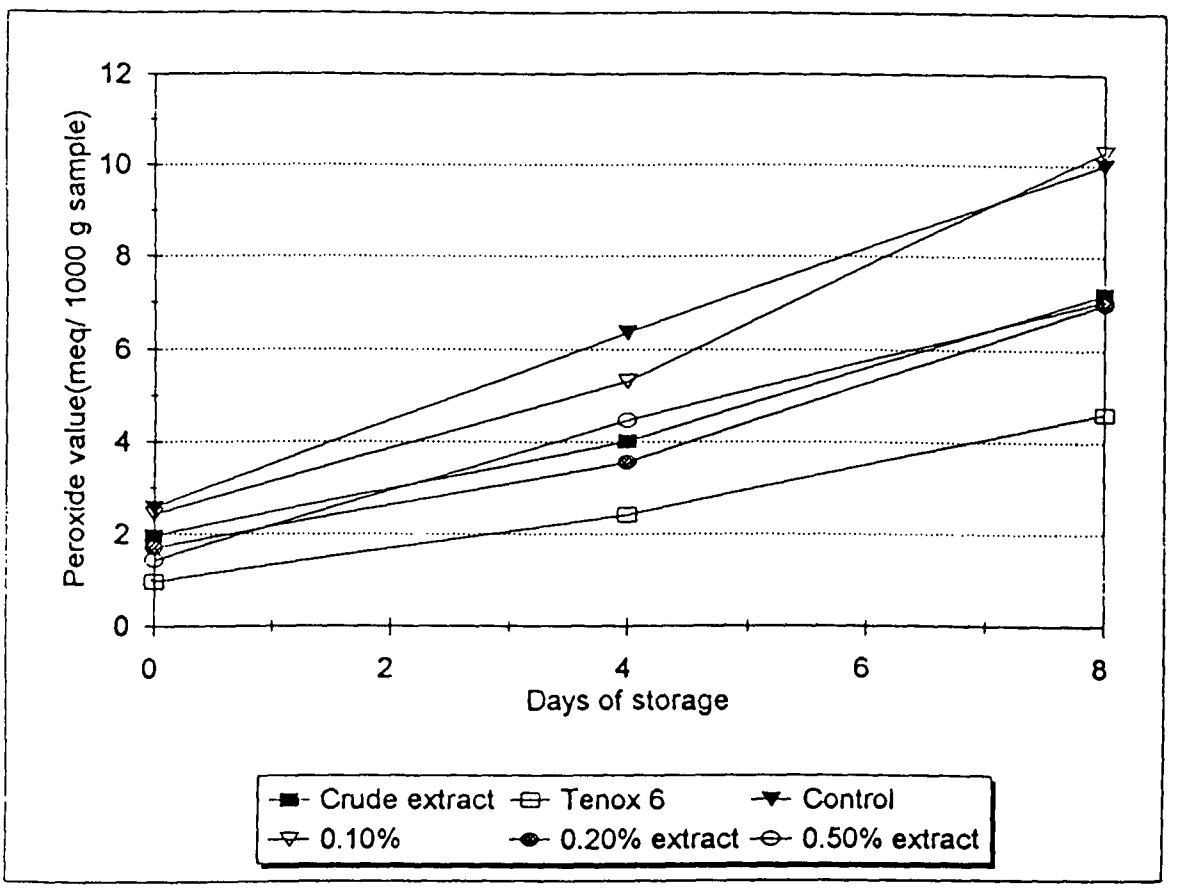


Figure V.2. Effect of antioxidant treatments on peroxide values (PV) of sablefish mince samples stored at refrigerator temperature (4°C) for 8 days.

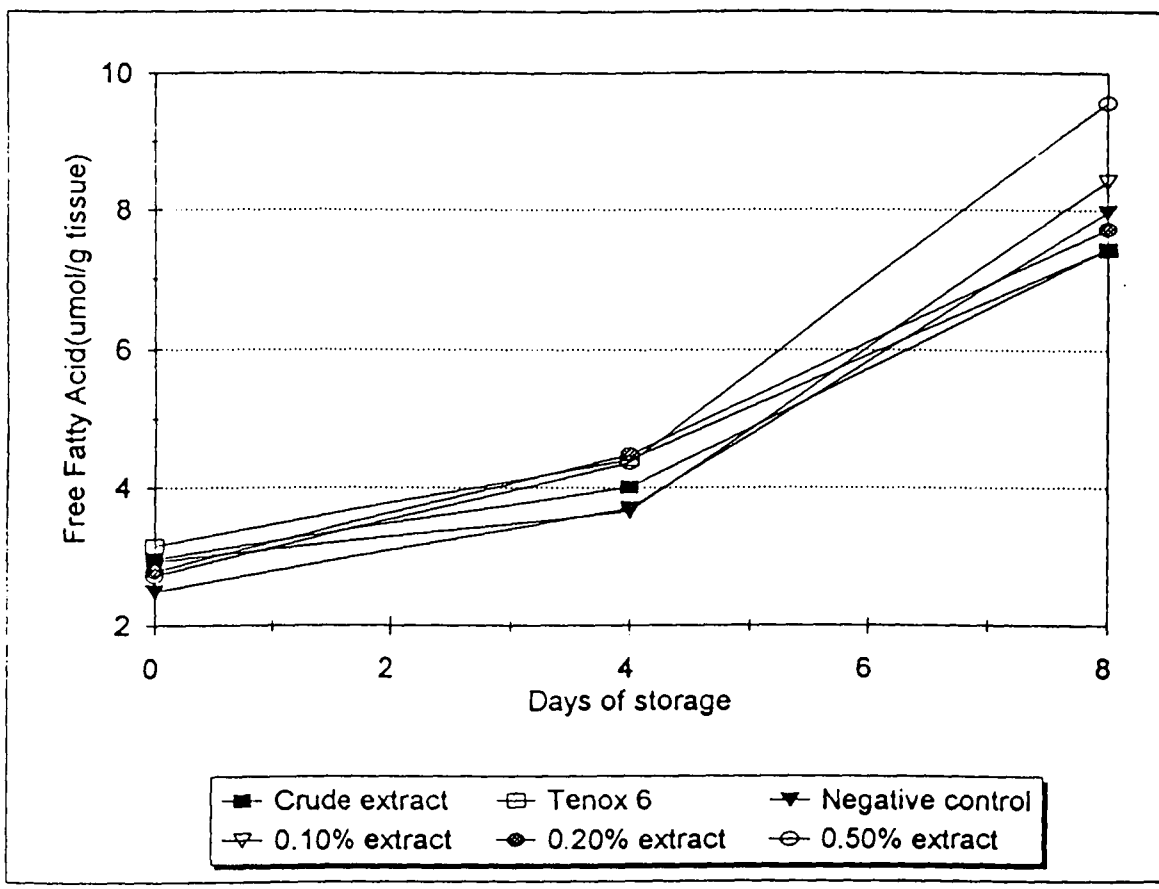


Figure V.3. Effect of antioxidant treatments on free fatty acid(FFA) of sablefish mince samples at refrigerator temperature (4°C) for 8 days.

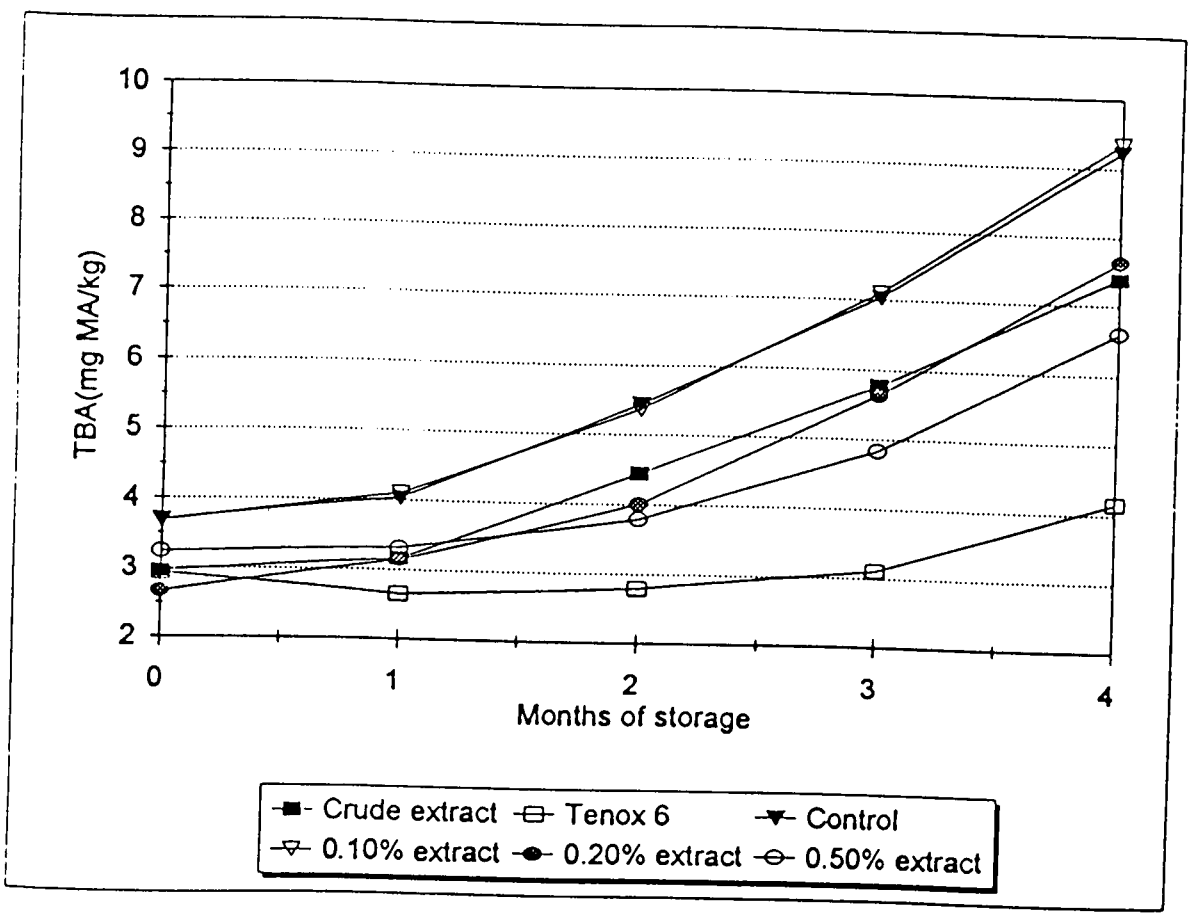


Figure V.4. Effect of antioxidant treatments on 2-thiobarbituric acid (TBA) values of sablefish mince samples in frozen storage (-20°C) for 4 months.

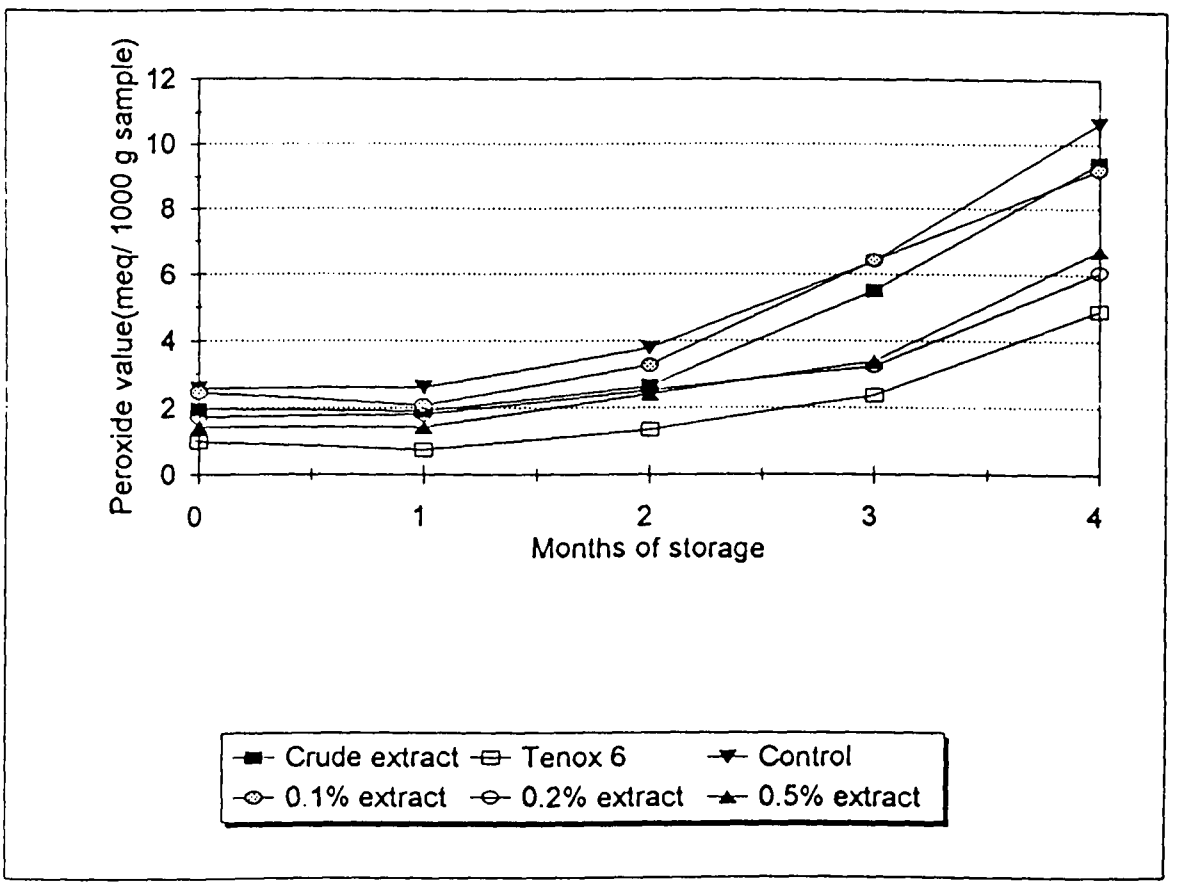


Figure V.5. Effect of antioxidant treatments on peroxide values (PV) of sablefish mince samples in frozen storage(-20°C) for 4 months.

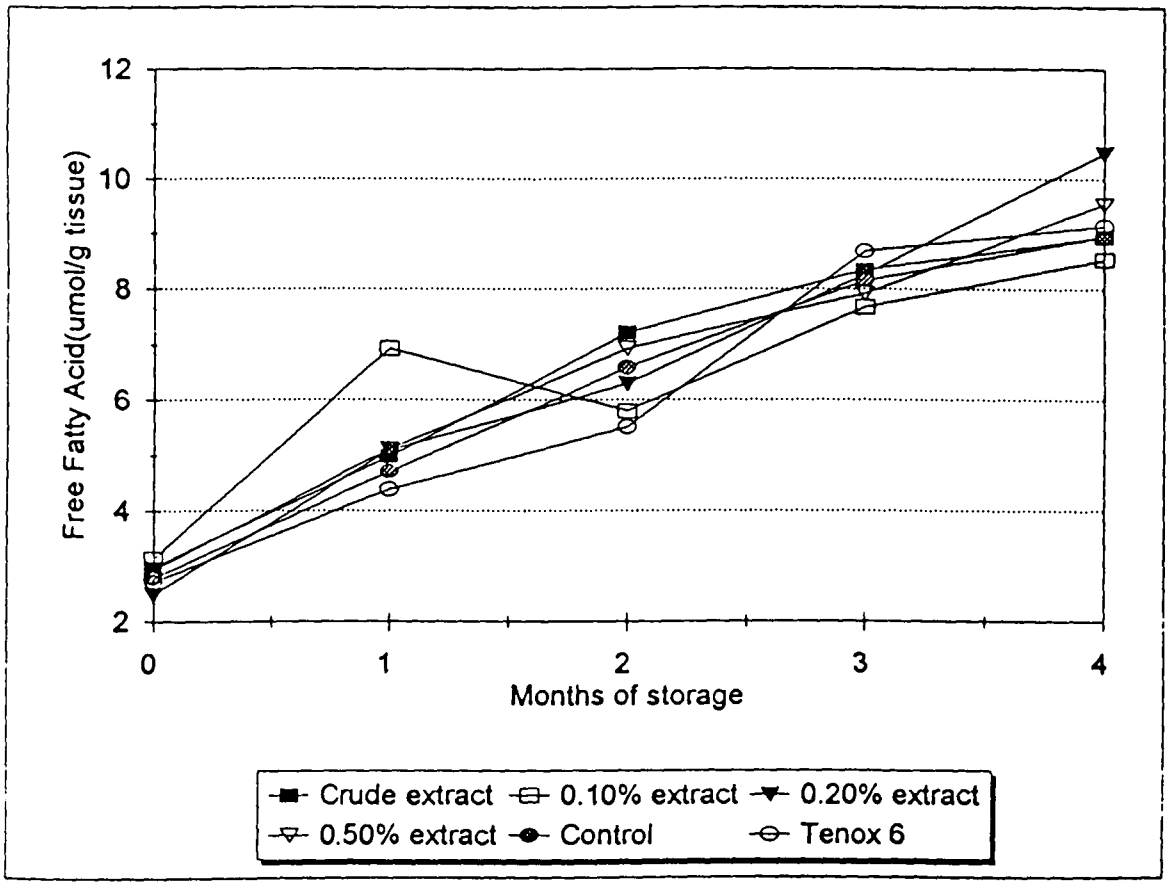


Figure V.6. Effect of antioxidant treatments on free fatty acid (FFA) of sablefish mince samples in frozen storage (-20°C) for 4 months.

## REFERENCES

AOAC. 1975. Official Method of Analysis, 12th. ed. Association of Official Analytical Chemists, Washington, D.C.

Al-Jalay, B., Blank, G., McConnell, B., and Al-Khayat, M. 1987. Antioxidant activity of selected spices used in fermented meat sausage. J. Food Protect. 50:25.

Allen, J.C. and Hamilton, R.J.(Ed.). 1983. Rancidity in Foods. Applied Science Publishers, New York, NY.

Barbut, S., Josephson, D.B., and Maurer, A.J. 1985. Antioxidant properties of rosemary oleoresin in turkey sausage. J. Food Sci. 50:1356.

Benedict, R.C., Strange, E.D., and Swift, C.E. 1975. Effect of lipid antioxidants on the stability of meat during storage. J. Agric. Food Chem. 18:167.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911.

Boyd, L.C., Green, D.P., Giesbrecht, F.B., and King, M.F. 1993. Inhibition of oxidative rancidity in frozen cooked fish flakes by tert-butylhydroquinone and rosemary. J. Sci. Food Agric. 61:87.

Branen, A.L. 1975. Toxicology and biochemistry of BHA and BHT. J. Am. Oil Chem. Soc. 52:59.

Chan, H.W.S. 1987. The mechanism of autoxidation. In Autoxidation of unsaturated lipids. H.W.S. Chang (Ed.), p.1. Academic Press, New York.

Chipault, J.R., Mizuno, G.R., Hawkins, J.M., and Lundberg, W.O. 1952. The antioxidant properties of natural spices. Food Res. 17:46.

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

Deng, J.C. 1978. Effect of iced storage on free fatty acid production and lipid oxidation in mullet muscle. J. Food Sci. 43:337.

Deng, J.C., Matthews, R.F., and Watson, C.M. 1977. Effect of chemical and physical treatments on rancidity development of frozen mullet (*Magil cephalus*) fillets. J. Food Sci. 42:344.

Hwang, K.T. and Regenstein, J.M. 1988. Protection of menhaden mince lipids from rancidity during frozen storage. J. Food Sci. 54(5):1120.

Inatani, R., Nakatani, N., and Fuwa, H. 1983. Antioxidative effect of the constituents of rosemary (*Rosmarinus officinalis L.*) and their derivatives. Agric. Biol. Chem. 47:521.

Ito, N., Fukushima, S., and Fsuda, H. 1985. Carcinogenicity and modification of the carcinogenicity response by BHA, BHT, and other antioxidants. CRC Ctri. Rev. 15(2):109.

Ke, J., Nash, D.N., and Ackman, R.G. 1981. Mackerel skin lipids as an unsaturated fat model system for the determination of antioxidative potency of TBHQ and other antioxidant compounds. J. Am. Oil Chem. Soc. 54:417.

Ke, P.J. and Woyewoda, A.D. 1978. A titrimetric method for determination of free fatty acids in tissues and lipids with ternary solvents and m-cresol purple indicator. Anal Chem Acta. 99:387.

MacNeil, J.H., Dimick, P.S., and Mast, M.G. 1973. Use of chemical compounds and a rosemary spice extract in quality maintenance of deboned poultry meat. J. Food Sci. 38:1080.

Nair, P.G.V., Gopakumar, K., and Nair, M.R. 1976. Lipid hydrolysis in mackerel (*Rastrellinger kanagurta*) during frozen storage. Fish Tech. 13(2):111.

Ramanathan, L. and Das, N.P. 1992. Studies on the control of lipid oxidation in ground fish by some polyphenolic natural products. J. Agric. Food Chem. 40:17.

Ramanathan, L. and Das, N.P. 1993. Natural products inhibit oxidative rancidity in salted cooked ground fish. J. Food Sci. 58(2):318.

Simic, M.G. and Karel, M. 1980. (Ed.). Autoxidation in food and biological systems. Plenum, New York.

Tsukuda, N. 1976. Change in the lipids of frozen fish. Bull. Tokai Reg. Fish. Lab. 84:31.

Waldrop, M. 1980. Firm takes new approach to food additives. Chem. Eng. News. 58(18):22.

Yu, T.C. and Sinnhuber, R.O. 1975. An improved 2-thiobarbituric acid(TBA) procedure for the measurement of autoxidation in fish oil. J. Am Oil. Chem Soc. 44:256.

Zama, K., Takama, K., and Mizushima, Y. 1979. Effect of metal, salt and antioxidants on the oxidation of fish lipids during storage under the condition of low and intermeditae moisture. J. Food Process. Preser. 3:249.

# VI. CONCLUSIONS

Ethanol extract from shrimp waste exhibited the highest activity compared to ethyl ether, chloroform, acetone, and isopropanol extracts. The $R_f$ 0.05 band showed strong antioxidant activity when ethanol extract was separated by thin layer chromatigraphy (TLC) in benzene:methanol:acetic acid (45:8:4). Further purification of this fraction was accomplished by high performance liquid chromatography (HPLC) using a $C_{18}$ preparative column. A fraction collected from HPLC at 8.45 min was shown to have strong antioxidant activity. It was concluded that the antioxidant is a polar compound.

Antioxidant activity of crude and purified samples was not significantly affected by heating at 100°C for 4 hr. Furthermore, the activity was also not changed significantly when samples were stored at 4°C for 1 mo.

The results of the electron impact (EI) and chemical ionization (CI) mass spectra indicated that the molecular weight of the antioxidant was 164. Fourier transformed-infrared spectrometry (FT-IR) spectrum showed the presence of phenolic hydroxyl and amide groups. The results of the proton nuclear magnetic resonance (NMR) showed that the antioxidant was an ortho-disubstituted benzene. Content of the antioxidant in shrimp waste was also estimated to be 0.18 mg/100 g sample.

The crude extract and a fraction eluted from silica gel glass column chromatography at 80% methanol in acetone showed strong antioxidant activity. The crude extract of antioxidant in water-dispersible form contributed to higher a* values of rockfish (*Sebastolobus alascanus and Sebastes ruberriumus*) compared to the control group during iced storage, but lower a* value compared to sodium erythorbate treatment. Both L* and b* values are not affected by antioxidant treatments. Treatment by 0.1%, 0.2%, and 0.5% concentrations of partially purified antioxidant resulted in higher a* values of the skin of rockfish (*Sebastes alutus*) than the control sample during storage. Samples treated with 0.2%, and 0.5% antioxidant were effective in decreasing TBA values in rockfish fillet (*Sebastes alutus*). The correlation between TBA and a* values in rockfish (*Sebastes alutus*) for all treatments were not significant except the control.

Crude extract, 0.20%, and 0.50% (w/w) antioxidant from shrimp waste had significantly lower TBA and peroxide values of sablefish minces stored at $4^0$C for 8 days and frozen condition for 4 mo, respectively, while 0.10% purified antioxidant had no effect on decreasing TBA and peroxide values. The results indicated that crude extract, 0.20%, and 0.50% purified antioxidant can effectively inhibit lipid oxidation. Inconsistent results from free fatty acid suggested that antioxidant from shrimp waste had no effect

on the hydrolytic rancidity of sablefish mince stored at $4^{0}C$ and frozen storage, respectively. Further work needs to be done to focus on the development of a commercial scale to extract the antioxidant from shrimp waste and fully identification of chemical structure of the antioxidant.

## BIBLIOGRAPHY

AOAC.1975. Official Method of Analysis, 12th. ed. Association of Official Analytical Chemists, Washington, D.C.

Akamittath, J.G., Brekke, C.J., and Schanus, E.G. 1990. Lipid oxidation and color stability in restructured meat systems during frozen storage. J. Food Sci. 55(6):1513.

Al-Jalay, B., Blank, G., McConnell, B., and Al-Khayat, M. 1987. Antioxidant activity of selected spices used in fermented meat sausage. J. Food Protect. 50:25.

Allen, J.C. and Hamilton, R.J.(Ed.). 1983. Rancidity in Foods. Applied Science Publishers. New York, NY.

Angelo, A.J., Crippen, K.L., Dupuy, H.P., and James, C.J. 1990. Chemical and sensory studies of antioxidant treated beef. J. Food Sci. 55(6):1501.

Ashford, N.A., Hattis, D., and Murray, A.E. 1977. Industrial prospects for chitin and protein from shellfish wastes, MIT Sea Grant Report MISG 77-3:MIT; Cambrige, MA.

Babbitt, J.K., Law, D.K., and Crawford, D.L. 1976. Improved acceptance and shelf life of frozen minced fish with shrimp. J. Food Sci. 41:35.

Barbut, S., Josephson, D.B., and Maurer, A.J. 1985. Antioxidant properties of rosemary oleoresin in turkey sausage. J. Food Sci. 50:1356.

Becker, G.L. 1993. Preserving food and health-antioxidants make functional, nutritious preservatives. Food Proc. 12:54.

Benedict, R.C., Strange, E.D., and Swift, C.E. 1975. Effect of lipid antioxidants on the stability of meat during storage. J. Agric. Food Chem. 23:167.

Bilinski, E., Jonas, E.E., and Lau, Y.C. 1979. Control of rancidity in frozen Pacific herring, *(Clupea harengus pallasi)*: Use of sodium erythorbate. J. Fish. Res Board. Can. 36:219.

Bjelke, E. 1975. Dietary vitamin A and human lung cancer. Int. J. Cancer. 15:561.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911.

Boyd, L.C., Green, D.P., Giesbrecht, F.B., and King, M.F. 1993. Inhibition of oxidative rancidity in frozen cooked fish flakes by tert-butylhydroquinone and rosemary extract. J. Sci. Food Agric. 61:87.

Branen, A.L. 1975. Toxicology and biochemistry of BHA and BHT. J. Am. Oil Chem. Soc. 52:59.

Brewer, M.S. and Harbers, C.A.Z. 1991. Effect of packing on color and physical characteristics of ground pork in long-term frozen storage. J. Food Sci. 56(2):363.

Cano-Lopez, A., Simpson, B.K., and Haard, N.F. 1987. Extraction of carotenoprotein from shrimp process wastes with the aid of trypsin from Atlantic cod. J. Food Sci. 52(2):503.

Castell, C.H. and Bishop, D.M. 1969. Effect of hematin compounds on the development of rancidity in muscle of cod, flounder, scallops, and lobster. J. Fish. Res Board. Can. 26:2299.

Chan, H.W.S. 1987. The mechanism of autoxidation. In Autoxidation of unsaturated lipids. H.W.S. Chang (Ed.), p.1. Academic Press, New York.

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

Chang, S.S., Ostric-Matijasevic, B., Hsieh, O.L., and Huang, C. 1977. Natural antioxidants from rosemary and sage. J. Food Sci. 42(4):1102.

Chastain, M.F., Huffman, D.L., Hsieh, W.H., and Cordray, J.C. 1982. Antioxidants in restructed beef/pork steaks. J. Food Sci. 47:1779.

Chen, C., Pearson, A.M., Gray, J.I., and Bennink, M.R. 1986. Antioxidant inhibition of mutagenicity in fried meat. Proc. European Meeting. Meat Res Work. 1(32):211.

Chen, H.M. and Meyer, S.P. 1982. Extraction of astaxanthin pigment from crawfish waste using a soy oil process. J. Food Sci. 47:892.

Chevolleau, S., Mallet, J.F., Ucciani, E., Gamisans, J., and Gruber, M. 1992. Antioxidant activity in leaves of some mediterranean plants. J. Am. Oil Chem. Soc. 69(12):1269.

Chipault, J.R., Mizuno, G.R., Hawkins, J.M., and Lundberg, W.O. 1952. The antioxidant properties of natural spices. Food Res.17:46.

Colbert, L.B. and Decker, E.A. 1991. Antioxidant activity of an ultrafiltration permeate from acid whey. J. Food Sci. 56(5):1248.

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

Cuvelier, M.E., Berset, C., and Richard, H. 1994. Antioxidant constituents in sage (*Salvia officinalis*). J. Agric. Food Chem. 42:665.

Cuvelier, M.E., Richard, H., and Berset, C. 1992. Comparison of the antioxidative activity of some acid-phenolis: Structure-activity relationship. Biosci. Biotech. Biochem. 56(2):324.

Dawson, L.E. and Gartner, R. 1983. Lipid oxidation in mechanically deboned poultry. Food Technol. 37(7):112.

Decker, E.A. and Crum, A.D. 1991. Inhibition of oxidative rancidity in salted ground pork by carnosine. J. Food Sci. 56(5):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 Hultin, H.O. 1990a. Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. J. Food Sci. 55(4):947.

Decker, E.A. and Hultin, H.O. 1990b. Nonenzymic catalysts of lipid oxidation in mackerel ordinary muscle. J. Food Sci. 55(4):951.

Deng, J.C. 1978. Effect of iced storage on free fatty acid production and lipid oxidation in mullet muscle. J. Food Sci. 43:337.

Dugan, L.R. 1980. Autoxidation in foods and biological systems; M.G. Simic. and M. Karel. (Ed.), p. 261, Plenum Press: New York.

Dugan, L.R. and Kraybill, H.R. 1956. Tocopherol as carry-through antioxidant. J. Am. Oil Chem. 33:527

Duxbury,D.D. 1989. Decolorized, deflavorized rosemary extracts antioxidant properties retard oxidative rancidity. Food Proc. 50(5):62.

Dyer, W.J., Morton, M.L., Fraser, D.I., and Bligh, E.G. 1956. Storage of frozen rockfish fillets. J. Fish. Res. Board Can. 13:569.

Enser, M. 1974. Factors affecting the development of oxidative rancidity in frozen meat. Meat Res. Inst. (MRI) Symp. Proc. 3:11.

Floyd, R.A. 1982. Free radicals and cancer. p. 201, Marcel Dekker, Inc, New York.

Francis, F.J. 1985. Pigments and other Colorants. In *Food Chemistry*. O.R. Fennema, (Ed.). p. 545. Marcel Dekker, New York.

Frankel, E.N. 1985. Chemistry of autoxidation: Mechanism, products and flavor significance. Ch 1. In *Flavor Chemistry* of *Fats* and *Oils*, D.B. Min and T.H. Smouse (Ed), p 1. American Oil Chemists' Society, Champaign, IL.

Fukuda, N., and Hasegawa, Y. 1980. A novel use of chitosan as a hypocholesterolemic agent in rats. Am. J. Clin. Nutr. 33(4):787.

German, J.B., and Kinsella, J.E. 1985. Lipid oxidation in fish tissue. enzymatic initiation by lipoxygenase. J. Agric. Food Chem. 23:680.

Goldman, M., Horev, B. and Saguy, I. 1983. Decolorization of β-carotene in model systems simulating dehydrated food. Mechanism and kinetic principle. J. Food Sci. 48:751.

Gopalakrishna, A.G. and Prabhakar, J.V. 1985. Antioxidant efficacy of water washed gums as a function of relative humidity in peanut oil. J. Am. Oil Chem. Soc. 62(11):1581.

Greene, B.E. 1969. Lipid oxidation and pigment changes in raw beef. J. Food Sci. 34:110.

Greene, B.E., Hsin, I. and Zipser, M.W. 1971. Retardation of oxidation color changes in raw ground beef. J. Food Sci. 36:940.

Grundy, S.M. 1984. AHA special report: Recommendations for the treatment of hyperlipidemia in adults: A joint statement of the nutrition committee and the council on atherosclerosis of the American Heart Association. Atherosclerosis. 4:445A.

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

Hamama, A.A. and Nawar, W.W. 1991. Thermal decomposition of some phenolic antioxidants. J. Agric. Food Chem. 39:1063.

Haraguchi, H., Hashimoto, K., and Yagi, A. 1992. Antioxidative substances in leaves of *(Polygonum hydropiper*). J. Agric. Food Chem. 40:1349.

Harbers, C.A.Z., Harrison, D.L. and Kropf, D.H. 1981. Ascorbic acid effects on bovine muscle pigments in the presence of radiant energy. J. Food Sci. 46:7.

Harel, S. and Kanner, J. 1985. Muscle membranal lipid peroxidation initiated by $H_2O_2$-activated metmyoglobin. J. Agric. Food Chem. 33:1188.

Haumann, B.F. 1990. Antioxidants: Firms seeking products they can label as natural. Inform. 1(12):1002.

Hirayama, T. 1979. Diet and cancer. Nutr. Cancer. 1:67.

Ho, C.T., Chen, Q., Shi, H., Zhang. K.Q., and Rosen, R.T. 1992. Antioxidative effect of polyphenol extract prepared from various Chinese teas. Prev Med. 21:520.

Houlihan, C.M., Ho, C.T., and Chang, S. 1985. The structure of rosmariquinone-A new antioxidant isolated from *Rosmarinus oficinalis L.* J. Am. Oil Chem. Soc. 62(1):96.

Hsieh, R.J. and Kinsella, J.E. 1989. Oxidation of polyunsaturated fatty acids: Mechanisms, products, and inhibition with emphasis on fish. In *Advances* in *Food* and *Nutrition Research*, 33:233.

Hultin, H.O. 1988. Potential lipid oxidation problems in fatty fish processing. In *Fatty Fish Utilization*: *Upgrading* From *Feed* to *Food*, N. Davis (Ed.), p. 185. UNC Sea Grant College Program, Raleigh, NC.

Hultin, H.O. 1992. Lipid oxidation in Fish muscle. In *Advances* in *Seafood Biochemistry*. G.J. Flick. and R.E. Martin (Ed.), p.99. Technomic Publishing Co., Lancaster, PA.

Hultin, H.O., McDonald, R.E., and Kelleher, S.D. 1982. Lipid oxidation in fish muscle microsomes. In *Chemistry* and *Biochemistry of Marine Food Products*, R.E. Martin, G.J. Flick, C.E. Hebard, and D.R. Ward, (Ed.), p. 1. AVI Publishing Co., Westport, CT.

Hwang, K.T. and Regenstein, J.M. 1988. Protection of menhaden mince lipids from rancidity during frozen storage. J. Food Sci. 54(5):1120.

Igene, J.O., King, J.A., and Pearson, A.M. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warm-over flavor in cooked meat. J. Agric. Food Chem. 27:838.

Inatani, R., Nakatani, N., and Fuwa, H. 1983. Antioxidative effect of the constituents of rosemary (*Rosmarinus officinalis L.*) and their derivatives. Agric. Biol. Chem. 47:521.

Inatani, R., Nakatani, N., Fuwa, H., and Seto, H. 1982. Structure of a new antioxidative phenolic diterpene isolated from rosemary (*Rosmarinus officinalis L.*). Agric. Biol. Chem. 46(6):1661.

Ishikawa, Y., Morimoto, K., and Iseki, S. 1991. Atrovenetin as a potent antioxidant compound from penicillium species. J. Am Oil. Chem Soc. 68:9.

Ito, N., Fukushima, S., and Fsuda, H. 1985. Carcinogenicity and modification of the carcinogenicity response by BHA, BHT, and other antioxidants. CRS Crit. Rev. 15(2):109.

Jensen, A. 1969. Tocopherol content of seaweed and seaweed meal. III. Influence of processing and storage on the content of tocopherols, carotenoids and ascorbic acid in seaweed meal. J. Sci. Food. Agric. 20:622.

Johnson, E.L. and Peniston, Q.P. 1982. Utilization of shellfish waste for chitin and chitosan production. Ch. 9. In *Chemistry* and *Biochemistry* of *Marine Food Products*. R.E. Martin, G.J, Flick, C.E. Hebard, and D.R. Ward (Ed.). AVI Publishing Co., Westport, CT.

Josephson, D.B., Lindsay, R.C., and Stuiber, D.A. 1985. Effect of handling and packing on the quality of frozen whitefish. J. Food Sci. 50:5.

Kanner, J., German, J.B., and Kinsella, J.E. 1987. Initiation of lipid peroxidation in biological systems. CRC Crit Rev. 25:317.

Kanner, J., Frankel, E., Granit,R., German, B., and Kinsella, J.E. 1994. Natural antioxidants in grapes and wines. J. Agric. Food Chem. 42:64.

Ke, P.J. and Ackman, R.G. 1976. Metal-catalyzed oxidation in mackerel skin meat lipids. J. Am. Oil Chem. Soc. 53:636.

Ke, P.J., Ackman, R.G., Linke, B.A., and Nash, D.M. 1977. Differential lipid oxidation in various parts of frozen mackerel. J. Food Tech. 12:37.

Ke, P.J., Nash, D.N., and Ackman, R.G. 1981. Mackerel skin lipids as an unsaturated fat model system for the determination of antioxidative potency of TBHQ and other antioxidant compounds. J. Am. Oil Chem. Soc. 54:417.

Ke, P.J. and Woyewoda, A.D. 1978. A titrimetric method for determination of free fatty acids in tissues and lipids with ternary solvents and m-cresol purple indicator. Anal Chem Acta. 99:387.

Khayat, A. and Schwall, D. 1983. Lipid oxidation in seafood. Food Technol. 7:130.

Kikuzaki, H. and Nakatani, N. 1993. Antioxidant effects of some ginger constituents. J. Food Sci. 58(6):1407.

Kinsella, J.E. 1986. Food components with potential therapeutic benefits: the n-3 polyunsaturated fatty acids of fish oils. Food Technol. 40(2):89.

Kinsella, J.E. 1987. Seafoods and Fish Oils in Human Health and Disease. Dekker, New York.

Kirk, W.G., Shirley, R.L., Easley, J.F., and Peacock, F.M. 1967. Composition and feed value of shrimp meal. Quart. J. Florida Acad. Sci. 30:51

Knekt, P., Aromaa, A., Maatela, J., Aaran, R., Nikkari, T., Hakama, M., Hakulinen, T., Peto, R., Saxen, E., and Teppo, L. 1988. Serum vitamin E and risk of cancer among Finnish men during a ten-year follow-up. Am. J. Epidemiol. 127:28.

Korczack, J., Flaczyk, E., and Pazola, Z. 1988. Effects of spices on stability of meat products kept in cold storage. Fleischwirtschaft. 68:64.

Kubota, K., Shijimara, H., and Kobayashi, A. 1986. Volatile components of roasted shrimp. Agric. Biol. Chem. 50:2867.

Ladikos, D. and Lougovois, V. 1990. Lipid oxidation in muscle foods: A review. Food Chem. 35:295.

Ledward, D.A. and MacFarlane, J.J. 1971. Some observations on myoglobin and lipid oxidation in frozen beef. J. Food Sci. 36:987.

Lee, I.K. and Ahn, S.Y. 1985. The antioxidant activity of gingerol. Korean J. Food Sci. Tech. 17(2):65.

Lee, Y.B., Kim, Y.S., and Ashmore, C.R. 1986. Antioxidant property in ginger rhizome and its application to meat products. J. Food Sci. 51:20.

Lin, F.S., Warner, C.R., and Fazio, T. 1981. Alteration of phenolic antioxidants in heated vegetable oil. J. Am Oil. Chem Soc. 58:789.

Longard, A.A. and Regier, L.W. 1974. Cold and some composition changes in ocean perch (*Sebastes marinus*) held in refrigerated Sea water with and without carbon dioxide. J. Fish. Res. Board Can. 31:456.

MacNeil, J.H., Dimick, P.S., and Mast, M.G. 1973. Use of chemical compounds and a rosemary spice extract in quality maintenance of deboned poultry meat. J. Food Sci. 38:1080.

Mandeville, S., Yaylayan, V., and Simpson, B. 1992. GC-MS anlysis of flavor-active compounds in cooked commercial shrimp waste. J. Agric. Food Chem. 40:1275.

Mandeville, S., Yaylayan, V., Simpson, B., and Pare, J. 1991. GC-MS analysis of flavor active compounds from raw commercial shrimp waste. Spectrosc: Int J. 9:61.

Marco, G.J. 1968. A rapid method for evaluation of antioxidants. J. Am. Oil Chem. Soc. 45:594.

Meyer, S.P. 1986. Utilization of shrimp processing waste. Infofish. 4:18.

Mitsumoto, M., Cassens, R.G., Schaefer, D.M., Arnold, R.N., and Scheller, K.K. 1991. Improvement of color and lipid stability in beef longissimus with dietary vitamin E and vitamin C dip treatment. J. Food Sci. 56(6):1489.

Mizushima, Y., Takama, K., and Zama, K. 1977. Effect of copper, iron and hemin on lipid oxidation in fish flesh homogenate. Bull. Faculty Fish. Hokkaido U. 18(4):207.

Muzarelli, R.A. and Parisher, E.R. 1978. Proceedings from the First International Conference on Chitin/Chitosan. MIT Sea Grant Program, Cambridge, MA.

Naczk, M. and Shahidi, F. 1990. Chemical composition and chitin content of crustacean offal. Ch. VI. In *Advances in Fisheries Technology* and *Biotechnology for Increased Profitability*. M.N. Voigt. and J. Botta (Ed.), p. 299. Technomic Publishing Co., Lancaster, PA.

Nair, P.G.V., Gopakumar, K., and Nair, M.R. 1976. Lipid hydrolysis in mackerel (*Rastrellinger kanagurta*) during frozen storage. Fish Tech. 13(2):111.

Nakani, N. and Inatani, R. 1984. Two antioxidative diterpenes from rosemary (*Rosmarinus officinalis L.*) and a revised structure for rosmanol. Agric. Biol. Chem. 48(8):2081.

Nawar, W.W. 1985. Lipids. In *Food Chemistry*. O.R. Fennema, (Ed.), p.139. Marcel Dekker, Inc, New York, NY.

Nawar, W., Hultin, H., Li, Y., Xing, Y., Kelleher, S., and Wilhelm, C. 1990. Lipid oxidation in seafoods under conventional conditions. Food Rev. Internat. 6:647.

Niki, E. 1988. Action of vitamin E as an antioxidant. Vitamins. 62(11):601.

Nozaki, K. 1989. Antioxidant activity of rosemary. New Food Ind. 31:27.

Okayama, T., Imai, T., and Yamanoue, M. 1987. Effect of ascorbic acid and alpha-tocopherol on storage stability of beef steaks. Meat Sci. 21:267.

Onyeneho, S.N. and Hettiarachchy, N.S. 1992. Antioxidant activity of durum wheat bran. J. Agric. Food Chem. 40:1496.

Ornum, J. V. 1992. Shrimp waste-must it be wasted? Infofish Inter. 6:48.

Osawa, T., Katsuzaki, H., Hagiwara, Y., Hagiwara. H., and Shibamoto, T. 1992. A novel antioxidant isolated from young green barley leaves. J. Agric. Food Chem. 40:1135.

Osawa, T. and Namiki, M. 1981. A novel type of antioxidant isolated from leaf wax of *Eucalyptus* leaves. Agric. Biol. Chem. 45(3):735.

Osawa, T. and Namiki, M. 1985. Natural antioxidants isolated from *eucalyptus* leaf waxes. J. Agric. Food Chem. 33(5):777.

Papadopoulos, G. and Boskou, D. 1991. Antioxidant effect of natural phenols on olive oil. J. Am. Oil Chem. Soc. 68(9):669.

Phillips, V.A. and Hedin, P.A. 1990. Spectral technique for structural analysis of the coton terpenoid aldehydes gossypol and gossypolone. J. Agric. Food Chem. 38:525.

Phillips, V.A., Hedin, P.A., Alley, E.G., and Oldham, P. 1990. Mass spectral techniques for the identification of cotton terpenoid aldehydes. J. Agric. Food Chem. 38:521.

Pokorny, J. 1987. Autoxidation of unsaturated lipid. H. Chan (Ed.), p. 141. Academic press, London.

Pratt, D.E. and Birac, P.M. 1979. Source of antioxidant activity of soybean and soy products. J. Food Sci. 44:1720.

Pratt, D.E. and Miller, E.E. 1984. A flavonoid antioxidant in spanish peanuts (*Arachia hypogoea*). J. Am. Oil Chem. Soc. 61(6):1064.

Raa, J. and Hansen, F. 1982. Norwegian Patent No. 147365B.

Ramanathan, L. and Das, N.P. 1992. Studies on the control of lipid oxidation in ground fish by some polyphenolic natural products. J. Agric. Food Chem. 40:17.

Ramanathan, L. and Das, N.P. 1993. Natural products inhibit oxidative rancidity in salted cooked ground fish. J. Food Sci. 58(2):318.

Ramarathnam, N., Osawa, T., Namiki, M., and Kawakishi, S. 1988. Chemical studies on novel rice hull antioxidants. 1. Isolation, fractionation, and partial characterization. J. Agric. Food Chem. 36:732.

Read, R. 1991. Oregon seeks to crack Japanese fish market. The Oregonian. Jan, 27.

Rhee, K.S., Vanderzant, C., Keeton, J.T., Ethlers, J.G., and Leu, R. 1985. Microbiological and shelf-life properties of ground beef containing glandless cottonseed flour. J. Food Sci:1388.

Rosenzweig-Pasquel, L.J.D. and Babbitt, J.K. 1991. Isolation and partial characterization of a natural antioxidant from shrimp (*Pandalus jordani*). J. Food Sci. 56(1):143.

Sackton, J. 1986. The Seafood Handbook. Seattle Washington: Seafood Business, p. 70.

Saito, A. and Regier, L.W. 1971. Pigmentation of brook trout (*Salvelinus fontinalis*) by feeding dried crustacean waste. J. Fish. Res. Board. Can. 28:509.

Saito, K., and Kondo, I. 1967. Symposium paper, Annual. Meeting Fish. Soc. Jap.

Scharow, S. 1969. Fish packing-neglected but needed. Food Prod. Dev. 11: 22.

Shahidi, F., Rubin, L.J. and Wood, D.F. 1987. Control of lipid oxidation in cooked ground pork with antioxidants and dinitrosyl ferrohemochrome. J. Food Sci. 52(3):564.

Simic, M.G. and Karel, M. 1980. Autoxidation in food and biological systems. Plenum, New York.

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

Spinelli, J., Lehman, L., and Weig, D. 1974. Composition, processing, and utilization of red crab (*pleuronocodes planipes*) as an aquacultural feed ingredient. J. Fish. Res. Board. Can. 31:1025

Stansby, M.E. 1982. A clinical study on the role of fish oil in alleviating human heart disease. In *Nutritional Evaluation* of *Long-chain Fatty acids* in *Fish Oil*, Edited by S.M. Barlow and M.E. Stansby, (Ed.) p. 263. Academic Press, New York.

Steel, R.E. 1971. Shrimp processing waste as a pigment source for rainbow trout (*Salmo gairdneri*). MS Thesis. Oregon State University, Corvallis.

Sugano, M., Fujikawa, T., Hiratsuji, Y., Nakashima, K., Fukuda, N., and Hasegawa, Y. 1980. A novel use of chitosan as a hypocholesterolemic agent in rats. Am. J. Clin. Nutr. 33(4):787.

Talley, K. 1993. Stats pack. Pacific Fish. 3:73.

Tidemann, E., Raa, J., Stormo, B., and Torrisen, O. 1982. Processing and utilization of shrimp waste. Tromiso, Norway: Ins. Fish. Tech. Res, p. 583.

Toda, S., Miyase, T., Arichi, H., Tanizawa, H., and Takino, Y. 1985. Natural antioxidants. III. Antioxidative components isolated from rhizome of *Curcuma longa L.* Chem. Pharm. Bull. 33(4):1725.

Torres, E., Pearson, A.M., Gray, J.I., Booren, A.M., and Shimakomaki, M. 1988. Effect of salt on oxidative changes in pre-and post-rigor ground beef. Meat Sci. 23:151.

Tsugita, T. 1990. Chitin/chitosan and their applications. Ch. 6. In *Advances* in *Fisheries Technology* and *Biotechnology* for *increased profitability*. M.N. Voigt. and J. Botta (Ed.), p. 287. Technomic Publishing Co., Lancaster, PA.

Tsukuda, N. 1970. Studies on the discoloration of red fishes. VI. Partial purification and specificity of the lipoxygenase-like enzyme responsible for carotenoid discoloration in fish skin during refrigerated storage. Bull. Japan Soc Sci. Fish. 36(7):725.

Tsukuda, N. 1976. Change in the lipids of frozen fish. Bull. Tokai Reg. Fish. Lab. 84:31.

Tsukuda, N. and Amano, K. 1967. Studies on the discoloration of red fishes. IV. Discoloration of astaxanthin, tunaxanthin, and β-carotene by tissue homogenates of fishes. Bull. Japan Soc Sci. Fish. 33(10):962.

Tunison, A.V., Brockway, D.R., Maxwell, J.M., Dorr, A.L., and McCay, C.M. 1947. Coloration of fish. Prog Fish Cult. 9:53.

United States Department of the Interior. 1968. Danes use vacuum-dried shrimp meal in trout feed. Fishery Market News report. 5(11):2.

Waldrop, M. 1980. Firm takes new approach to food additives. Chem. Eng. News. 58(18):22.

Wang, Y.J., Miller, L.A., Perren, M., and Addis, P.B. 1990. Omega-3 fatty acids in Lake Superior fish. J. Food Sci. 55(1):71.

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

Watkins, B.E., Adair, J., and Oldfield, J.E. 1982. Evaluation of shrimp and king crab processing byproducts as feed supplement for mink. J. Ani Sci. 55(3):578.

Wheeler, T.L., Seideman, S.C., Davis, G.W., and Rolan, T.L. 1990. Effect of chloride salts and antioxidants on sensory and storage traits of restructured beef steaks. J. Food Sci. 55(5): 1274.

Widicus, W.A. and Kirk, J.R. 1981. Storage stability of α - tocopherol in a dehydrated model food system containing methyl linoleate. J. Food Sci. 46:813.

Wu, J., Lee, M.H., Ho, C.T., and Chang, S. 1982. Eluciation of the chemical structures of natural antioxidants isolated from rosemary. J. Am. Oil Chem. Soc. 59:339.

Yu, T.C. and Sinnhuber, R.O. 1975. An improved 2-thiobarbituric acid (TBA) procedure for the measurement of autoxidation in fish oil. J. Am. Oil Chem. Soc. 44:256.

Zama, K., Takama, K., and Mizushima, Y. 1979. Effect of metal, salt and antioxidants on the oxidation of fish lipids during storage under the condition of low and intermeditae moisture. J. Food Proc. Preser. 3:249.

Zhang, K. Q., Bao, Y., Wu, P., Rosen, R.T., and Ho, C.T. 1990. Antioxidative components of tanshen (*Salvia miltiorrhiza Bung*). J. Agric. Food Chem. 38:1194.

# APPENDIX

Table A.1. Data of TBA values of sablefish with and without antioxidant treatments in refrigerated storage.

| Treatments | Days of storage | | |
|---|---|---|---|
| | 0 | 4 | 8 |
| Crude extract | 2.96±0.19*[a] | 3.95±0.47[ab] | 8.59±0.44[a] |
| Tenox 6 | 2.94±0.22[a] | 2.98±0.30[a] | 6.14±0.20[b] |
| Negative control | 3.71±0.57[a] | 7.78±0.46[c] | 10.16±0.59[ac] |
| 0.10% extract | 3.69±0.59[a] | 9.15±1.24[c] | 10.85±1.01[c] |
| 0.20% extract | 2.67±0.45[a] | 4.99±0.64[b] | 8.67±0.42[a] |
| 0.50% extract | 3.25±0.21[a] | 4.67±0.28[ab] | 8.51±0.21[a] |

*:average±S.E($p<0.01$)

Table A.2. Data of peroxide values of sablefish with and without antioxidant treatments in refrigerated storage.

| Treatments | Days of storage | | |
|---|---|---|---|
| | 0 | 4 | 8 |
| Crude extract | 1.96±0.07*[a] | 4.02±0.45[a] | 7.19±0.80[a] |
| Tenox 6 | 0.97±0.12[b] | 2.42±0.05[b] | 4.62±0.18[b] |
| Negative control | 2.58±0.15[c] | 6.36±0.17[d] | 10.01±0.21[c] |
| 0.10% extract | 2.44±0.04[c] | 5.31±0.82[cd] | 10.30±0.40[c] |
| 0.20% extract | 1.71±0.07[a] | 3.56±0.31[ab] | 6.98±0.34[a] |
| 0.50% extract | 1.44±0.03[d] | 4.46±0.25[ac] | 7.05±0.36[a] |

*:average±S.E($p<0.01$)

Table A.3. Data of peroxide values of sablefish with and without antioxidant treatments in frozen storage.

| Treatments | Months of storage | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| Crude extract | 1.96±0.07*[a] | 1.90±0.14[a] | 2.66±0.29[bc] | 5.51±0.36[c] | 9.43±0.82[cd] |
| Tenox 6 | 0.97±0.12[c] | 0.75±0.15[b] | 1.34±0.15[a] | 2.37±0.13[a] | 4.91±0.24[a] |
| Negative control | 2.58±0.15[b] | 2.62±0.14[d] | 3.77±0.23[d] | 6.38±0.53[c] | 10.66±0.05[d] |
| 0.10% extract | 2.44±0.04[b] | 2.07±0.25[a] | 3.26±0.40[cd] | 6.41±0.29[c] | 9.20±0.58[c] |
| 0.20% extract | 1.71±0.07[a] | 1.81±0.02[ac] | 2.53±0.10[bc] | 3.23±0.08[ab] | 6.05±0.36[ab] |
| 0.50% extract | 1.44±0.03[d] | 1.43±0.11[c] | 2.40±0.39[b] | 3.39±0.28[b] | 6.72±0.32[b] |

*: average±S.E($p<0.01$)

Table A.4. Data of free fatty acid of sablefish with and without antioxidant treatments in refrigerated storage.

| Treatments | Days of storage | | |
|---|---|---|---|
| | 0 | 4 | 8 |
| Crude extract | 2.97±.15*[a] | 4.01±.44[a] | 7.44±.32[a] |
| Tenox 6 | 3.14±.17[a] | 4.42±.21[a] | 7.45±.55[a] |
| Negative control | 2.49±.24[a] | 3.71±.43[a] | 7.96±.25[a] |
| 0.10% extract | 2.93±.09[a] | 3.66±.51[a] | 8.42±.51[ab] |
| 0.20% extract | 2.79±.18[a] | 4.49±.19[a] | 7.73±.33[a] |
| 0.50% extract | 2.72±.12[a] | 4.37±.41[a] | 9.57±.62[b] |

*: average±S.E($p<0.05$)

Table A.5. Data of free fatty acid of sablefish with and without antioxidant treatments in frozen storage.

| Treatments | Months of storage | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| Crude extract | $2.97\pm.15^{*a}$ | $4.97\pm.05^{a}$ | $7.21\pm.17^{bc}$ | $8.36\pm.10^{c}$ | $8.95\pm.12^{cd}$ |
| Tenox 6 | $3.14\pm.17^{b}$ | $4.39\pm.30^{c}$ | $5.52\pm.25^{a}$ | $8.69\pm.18^{a}$ | $9.15\pm.05^{a}$ |
| Negative control | $2.49\pm.24^{c}$ | $4.71\pm.28^{d}$ | $6.59\pm.12^{d}$ | $8.16\pm.06^{c}$ | $8.96\pm.09^{d}$ |
| 0.10% extract | $2.93\pm.09^{c}$ | $6.93\pm.33^{a}$ | $5.80\pm.03^{cd}$ | $7.68\pm.15^{c}$ | $8.55\pm.04^{c}$ |
| 0.20% extract | $2.79\pm.18^{c}$ | $5.09\pm.60^{ab}$ | $6.30\pm.11^{bc}$ | $8.27\pm.13^{ab}$ | $10.47\pm.54^{ab}$ |
| 0.50% extract | $2.72\pm.12^{d}$ | $5.11\pm.14^{b}$ | $6.93\pm.5^{b}$ | $7.92\pm.07^{b}$ | $9.54\pm.27^{b}$ |

*: average ± S.E($p<0.01$)

Table A.6. Data of TBA values of sablefish with and without antioxidant treatments in frozen storage.

| Treatments | Months of storage | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| Crude extract | 2.96±.19*[a] | 3.18±.55[a] | 4.45±.35[a] | 5.77±.13[a] | 7.39±.53[a] |
| Tenox 6 | 2.94±.22[a] | 2.66±.07[a] | 2.80±.31[c] | 3.12±.35[c] | 4.17±.23[c] |
| Negative control | 3.71±.57[a] | 4.03±.44[a] | 5.44±.28[b] | 7.04±.06[d] | 9.23±.17[b] |
| 0.10% extract | 3.69±.58[a] | 4.11±.29[a] | 5.37±.25[b] | 7.12±.07[d] | 9.36±.18[b] |
| 0.20% extract | 2.67±.45[a] | 3.16±.47[ba] | 4.02±.11[a] | 5.64±.45[a] | 7.63±.58[a] |
| 0.50% extract | 3.25±.21[a] | 3.33±.16[a] | 3.80±.15[a] | 4.84±.09[b] | 6.60±.26[a] |

*: average ± S.E($p<0.05$)

Table A.7. Data of L*, a*, and b* values of rockfish(*Sebastolobus alascanus*) with and without antioxidant treatments in refrigerated storage.

| Treatments | Days of storage | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 8 | 12 |
| Control (L*) | 51.00±2.14*[a] | 47.70±2.11[a] | 51.10±1.03[a] | 52.32±1.05[a] | 51.64±0.73[a] |
| Sodium erythorbate | 53.24±2.46[a] | 48.90±2.26[a] | 49.85±1.40[a] | 52.60±1.03[a] | 52.73±0.81[a] |
| Crude extract | 48.65±2.32[a] | 47.36±0.78[a] | 49.14±1.50[a] | 49.24±1.83[a] | 49.97±1.87[a] |
| Control (a*) | 16.48±1.16[a] | 12.48±1.59[a] | 12.04±0.87[a] | 10.91±0.88[a] | 10.51±0.44[a] |
| Sodium erythorbate | 16.89±0.99[a] | 16.93±1.79[a] | 15.55±1.68[a] | 14.94±1.14[a] | 14.48±1.29[b] |
| Crude extract | 16.93±1.08[a] | 14.66±1.73[a] | 14.42±1.29[a] | 14.42±1.03[a] | 13.37±0.22[b] |
| Control (b*) | 5.26±2.79[a] | 3.71±1.89[a] | 3.54±1.80[a] | 3.01±1.93[a] | 2.81±1.84[a] |
| Sodium erythorbate | 3.30±0.80[a] | 4.50±1.70[a] | 3.63±0.28[a] | 3.05±0.42[a] | 2.88±0.60[a] |
| Crude extract | 4.81±1.76[a] | 6.24±1.12[a] | 5.57±0.75[a] | 4.11±2.05[a] | 3.83±1.55[a] |

*: average ± S.E($p<0.05$)