AN ABSTRACT OF THE THESIS OF

LORENZO JOSE DE ROSENZWEIG PASQUEL for the degree of MASTER OF SCIENCE in FOOD SCIENCE AND TECHNOLOGY presented on December 2, 1977.

Title: ISOLATION AND PARTIAL CHARACTERIZATION OF A NATURAL ANTIOXIDANT FROM SHRIMP (PANDALUS JORDANI)

Abstract approved: Jerry K. Babbitt

The nature of a natural antioxidant present in shrimp was investigated.

Shrimp extracts were prepared using different solvents to ascertain the best extraction method. The antioxidant activity was determined using the β-carotene-linoleate model system and the oxygen weighing method. Ethanol proved to be the best solvent for extracting the natural antioxidant.

Different shrimp materials extracted with ethanol proved to be equally effective as antioxidant sources.

The non-lipid nature of the antioxidant was established by separating the lipid from the non-lipid material and measuring its antioxidant activity.

The phenolic nature of the antioxidant was further established

and its isolation achieved by means of preparative thin layer chromatography. The Rf values with different solvents and its wavelength of absorption maxima were determined. Its chemical nature was further investigated using different visualizing reagents.

The colorimetric quantitation of the antioxidant factor revealed that it is present in very low concentrations in its natural source. Its effectiveness as an antioxidant at low concentrations together with the fact that it is a natural product makes it a valuable compound of potential use in the food industry.

Isolation and Partial Characterization of a Natural Antioxidant from Shrimp (*Pandalus jordani*)

by

Lorenzo Jose de Rosenzweig Pasquel

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1978

APPROVED:

Associate Professor of Food Science and Technology
in charge of major

Head of Department of Food Science and Technology

Dean of Graduate School

Date thesis is presented December 2, 1977.

Typed by Opal Grossnicklaus for Lorenzo Jose de Rosenzweig Pasquel

ACKNOWLEDGEMENTS

I want to express my gratitude to Dr. Jerry K. Babbitt for his valuable guidance throughout this research.

Sincere thankfulness is extended to Dr. Ron Wrolstad, Dr. Dennis Gordon and Mr. Lou Roberts for their generous help.

I want to thank also Dr. David L. Crawford and the staff of the Oregon State University Seafoods Laboratory for their constant cooperation.

My deepest gratitude is directed towards my parents Jenaro and Graciela and my wife Linda for their continuous encouragement and help during my graduate study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ISOLATION AND PARTIAL CHARACTERIZATION OF A NATURAL ANTIOXIDANT FROM SHRIMP (PANDALUS JORDANI)

INTRODUCTION

The use of antioxidants in marine products is under extensive investigation to determine better ways of preventing oxidative deteriorations. Among the large variety of seafoods stored, frozen fish is particularly susceptible to oxidative rancidity.

Mechanically deboned minced fish muscle is a potential protein source for the formulation of frozen seafood products. However, oxidative rancidity in the frozen minced fish muscle is greatly accelerated.

A recent study (Babbitt et al., 1976) showed that the incorporation of shrimp muscle (Pandalus jordani) into a fish-shrimp portion greatly increased the acceptance and storage stability of this product. It was suggested that the enhanced shelf-life of the portion may be due to a natural antioxidant present in shrimp.

The purpose of this research was to confirm the presence of this antioxidant factor in shrimp (Pandalus jordani) and to isolate and characterize it.

LITERATURE REVIEW

An antioxidant can be defined as a compound which, when present in an oxidizable substrate in relatively low concentration, markedly inhibits the reaction of this substrate with oxygen (Olcott, 1967). Antioxidants can be divided into natural and synthetic food-grade antioxidants. The second category comprises BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) which are the most commonly used antioxidants at the present time (Chang et al., 1977). There is a growing concern about the use of synthetic food additives and several studies have been directed towards the enzyme-inducing effect of BHT on the microsomal enzyme systems of the body (Food Chemical News, 1976). The possibility of having a restriction in the use of these synthetic antioxidants in the forthcoming years creates the necessity of searching for possible substitutes. The use of natural antioxidants obtained from common foodstuffs would be a logical alternative and would be more acceptable by consumers who are questioning the use of synthetic chemicals in foods. Research in this area has been particularly extensive in the last ten years and a large number of natural antioxidants of potential use in the food industry have been reported (Masuyama, 1976).

The phospholipids present in a chloroform-methanol extract of a marine algae (Porphyra tenera) known as purple laver were

found to have antioxidant activity when tested on safflower oil (Kaneda and Ando, 1972). The phospholipid fraction also showed synergistic activity when used in combination with BHT.

Tocopherols are the most known and widely distributed natural antioxidants and their presence has been determined in a large number of foodstuffs and plant materials. Their presence accounts to a certain degree for the stability of the products in which they occur. Dolev and Olcott (1965) reported that sable fish (Anoplopoma fimbria) oil has a singularly high amount of tocopherol and also shows a relatively great oxidative stability.

A method of protecting oils against oxidation has been found by Vaskovskii and Syskin (1973) in which fats are stabilized with antioxidants of natural origin by adding an extract containing the non-polar fraction of marine invertebrate fat.

Amino acids and esterified amino acids have been reported to have synergistic activity when present in lard containing natural tocopherols. The benzyl esters of methionine, histidine, lysine and tryptophan were specially effective (Yuki et al., 1974). Jurewicz and Salmonowicz (1973) studied the pro- and antioxidant effects of some amino acids upon fish oil and found that valine, methionine, cysteine and proline had a high antioxidant activity even at a concentration of 0.1%. The antioxidant activity of proline in fish oil was also observed by Revankar (1974). At 0.02% in fresh sardine oil, proline

was as effective as BHA and at 0.1% it was much more effective than BHA.

The antioxidant properties of a large number of phenolic compounds of plant origin have been established in the search of natural antioxidants.

Cantarelly and Montedoro (1974) report the presence of phenolic acids and polyphenols in the non-saponifiable fraction of crude olive oil and after evaluating its antioxidant activity in other oils concluded that the compounds may be useful as food additives.

The presence of natural antioxidant factors was also observed by Qureshi et al. (1974) when working with tomato skin and pulp. These antioxidant substances were tentatively identified as quercetin and caffeic acid.

The occurrence of antioxidant materials in bacteria was determined by Smith and Alford (1970) who found active antioxidants in the washed cells of 14 different species. The methanol soluble-benzene insoluble fraction obtained from extracts of Pseudomonas ovalis proved to be the most effective antioxidant.

A method of stabilizing edible oils against oxidation with dihydroxyphenylalanine (DOPA) or DOPA derivatives was patented in the United States (1975) and Great Britain (1976) by Ninagawa and Takeshita (1975). They found the compound to be effective at concentrations of 0.001-1% by weight in a large range of foods that includes

vegetable and fish oils.

Babbitt et al. (1976) suggested that the antioxidant activity observed in shrimp extracts might be due to the presence of carotenoid pigments, tocopherols or phenolic compounds. The relatively large amounts of proline present in shrimp (Cobb et al., 1974) and the role of DOPA in the cuticle sclerotization in crustaceans (Bailey et al., 1959; Summers, 1967) could be also related to this antioxidant activity.

EXPERIMENTAL

Materials and Handling Procedures

Analyses were carried out on samples of Pacific shrimp, Pandalus jordani, caught off the North Oregon coast and supplied by Pacific Shrimp Inc. of Warrenton, Oregon.

The starting material used for the antioxidant extractions included:

1) Mechanically peeled raw shrimp collected at the outlet of the peeling machine. Prior to the peeling operation the shrimp had been on ice for three days.
2) Mechanically peeled shrimp cooked for 60 seconds at 99°C.
3) Shrimp waste composed of shrimp heads and shells obtained from the waste water screen during the peeling process.
4) Shrimp cannery effluent collected from the discharge of the peeling machines and screened to remove shrimp fragments.

The first three items listed above were packed in 100 g portions in polyethylene bags and stored at -28°C until analyzed. The frozen samples were thawed at room temperature prior to extraction. The shrimp cannery effluent was refrigerated overnight at 2° C and analyzed the next day.

Preparation of Extracts

Peeled Raw Shrimp

Peeled raw shrimp portions (50 g) were extracted with the following solvents:

Chloroform:2-propanol (1:1). A sample was extracted according to the procedure of Kelly and Harmon (1972). The chloroform:2-propanol filtrate was evaporated to dryness in a rotary vacuum evaporator and the residues redissolved in 100 ml of absolute ethanol.

Acetone. The acetone fraction prepared by the same extraction procedure as above was evaporated to dryness and redissolved in the same volume of absolute ethanol.

Ethanol. The 50 g shrimp sample, together with 100 ml of ethanol and 10 g of silica gel were blended for 30 seconds at high speed using an Osterizer blender. The slurry was filtered through a Büchner funnel using Whatman No. 1 filter paper.

Diethyl ether, chloroform and water. The same procedure used in the ethanol extraction was repeated using diethyl ether, chloroform and water as the extracting solvents. The diethyl ether and chloroform extracts were evaporated to dryness and the residues redissolved in 100 ml of absolute ethanol.

Chloroform:water-methanol. The lipid material present in a 100 g peeled raw shrimp sample was extracted according to the

method of Bligh and Dyer (1959). The chloroform layer was evaporated in the rotary evaporator. Nitrogen was used to break the vacuum and the bright orange oil residue was redissolved in 200 ml of absolute ethanol. The water-methanol layer was separated from the protein band and filtered through Whatman No. 1 filter paper.

Peeled Cooked Shrimp and Shrimp Shell Waste

A sample of cooked shrimp, together with 100 ml of etnanol and 10 g of silica gel were blended for 30 seconds at high speed in an Osterizer blender. The homogenate was filtered through a Büchner funnel using Whatman No. 1 filter paper and the filtrate saved. The same procedure was repeated using a 100 g sample of shrimp shell waste composed of shrimp shells and heads.

Shrimp Cannery Effluent Precipitate

A 500 ml sample of shrimp cannery effluent (pH = 8.35) was filtered through cheesecloth to remove large particles. The pH of the filtrate was lowered with 50% sulfuric acid to a value of 4.5. After letting it stand for 15 minutes the precipitated material was removed by centrifuging. The precipitate was extracted with ethanol following the same procedure as above.

All the prepared extracts were stored in amber bottles at 2^{o}C.

Measurement of Antioxidant Activity

The antioxidant properties of the different extracts were determined with the antioxidant evaluation method of Miller (1971) and Marco (1968) which is based on the ability of the different extracts to decrease the oxidative destruction of β-carotene in a β-carotene-linoleic acid emulsion. This antioxidant activity was expressed as the decrease in absorbance at 470 nm during 1 and 2 hour reaction periods.

In order to compare this method to other methods used to evaluate antioxidants, the oxygen weighing method of Olcott and Einset (1958) was used to determine the effect of a raw shrimp ethanol extract on the induction period of salmon oil. The oil samples (1 g) were incubated in 50 ml beakers at 60°C in a draft oven. The antioxidants were added before weighing the oil and the ethanol evaporated under a stream of nitrogen.

Determination and Quantitation of Phenolics

Phenols were determined colorimetrically with the Folin-Ciocalteau phenol reagent according to the method used by Babbitt et al. (1973). The shrimp antioxidant was quantitated using a propyl gallate standard curve.

Thin Layer Chromatography

Phenolic compounds present in the chloroform and water-methanol layers of the Bligh and Dyer extraction as well as those present in the ethanol extracts were separated by TLC using .25 mm silica gel G plates. Freshly prepared butanol:acetic acid:water (12:3:5, v/v) BAW and benzene:methanol:acetic acid (45:8:4, v/v) BeAM were used as solvent systems. The plates were visualized with Folin-Ciocalteau phenol reagent followed by an overspray of 14% sodium carbonate, Bray and Thorpe (1961). Preparative TLC was used for the quantitative fractionation of the different phenolic compounds.

The following reagents were found useful in the chemical characterization of the shrimp antioxidant:

Detection of α-amino groups: 0.1% ninhydrin in isopropanol.

Detection of ortho substituted dihydroxyphenols: 1:1 1% ferric chloride:1% potassium ferricyanide solution and the chromogenic reagent of Bhatia (1973).

Reported in studies dealing with cuticular phenolics present in arthropods: a solution of phloroglucinol in 10% sodium hydroxide and the sucrose reagent of Roux (1951).

The presence of organic compounds other than phenolics was determined with a 50% sulfuric acid spray followed by a high temperature treatment.

The chromatographic homogeneity of each of the bands was confirmed by two dimensional chromatography using benzene:methanol: acetic acid (45:8:4, v/v) and chloroform:ethyl acetate:formic acid (5:4:1 v/v) ChEF as second dimension solvents.

RESULTS AND DISCUSSION

The antioxidant activity of raw shrimp meat extracts prepared using different solvents is shown in Figure 1 and Table 1. This antioxidant activity, expressed as accumulated decrease in absorbance over a period of 120 minutes, is proportional to the oxidative destruction of β-carotene.

Listing the solvents in increasing order of polarity it appears that the higher the polarity of the solvent (water excluded) the higher the antioxidant activity of the extract. It should be noted that the chloroform extract had very little antioxidant activity and that the diethyl ether extract showed no activity at all. Also of interest is the fact that while extraction with water resulted in a poor antioxidative solution, the water-methanol layer of the Bligh and Dyer lipid extraction showed strong antioxidant properties.

There is no information reported about natural antioxidants of crustacean origin besides the work of Babbitt et al. (1976). However, Chang et al. (1977) report that the yield of antioxidant extracted from Rosemary and sage is increased by using more polar solvents. Also, Sugimoto et al. (1972) reported the presence of two water and ethanol soluble fractions in a fermented soybean preparation which show an antioxidant behavior against linoleic acid.

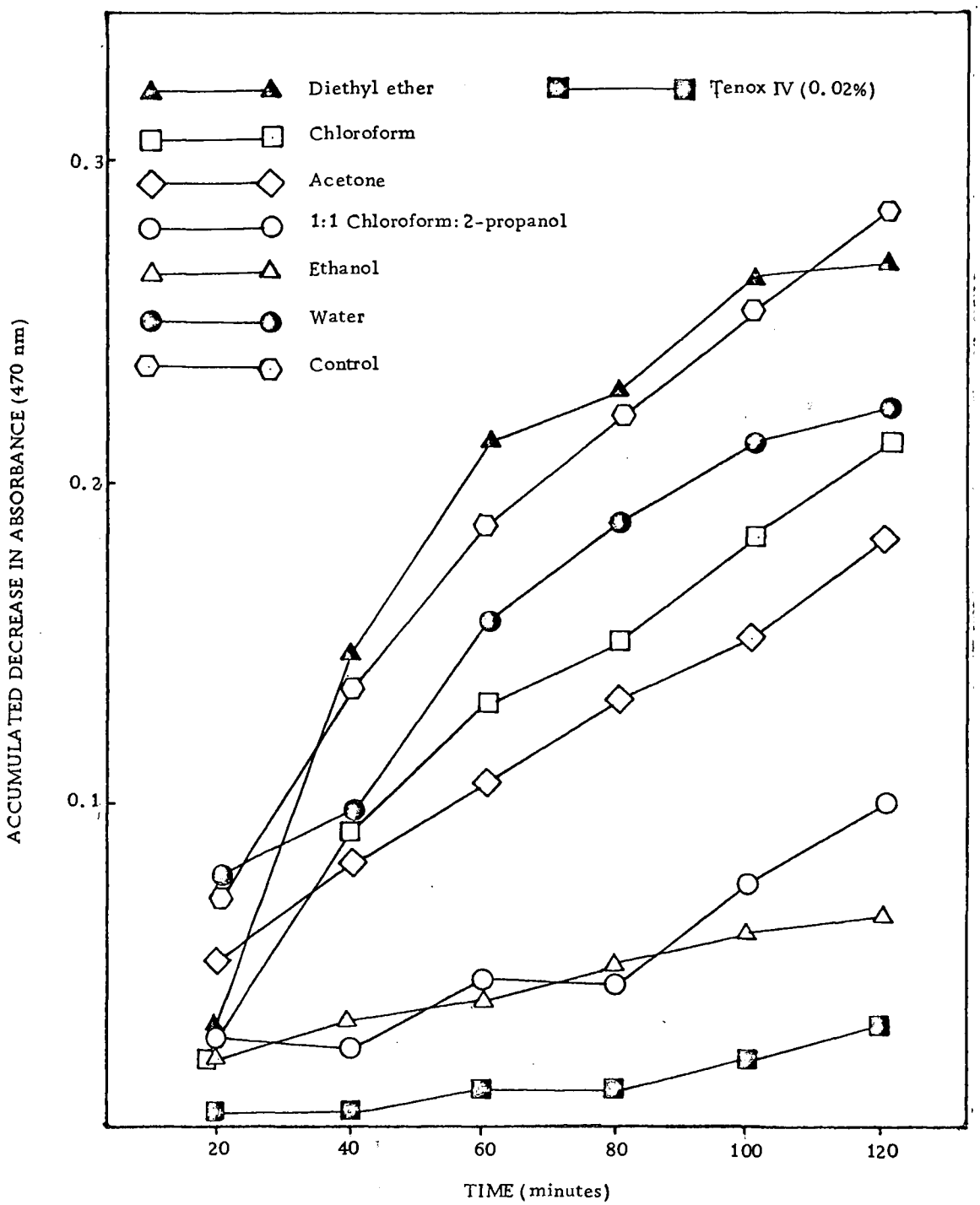

Figure 1. Antioxidant activity of raw shrimp extracts using different solvents.

Table 1. Effect of raw shrimp meat extracted with different solvents on the rate of carotene decolorization.

Solvent	Decrease in carotene absorbance[a] ($\Delta A_{470\ nm}$ x100 for 120 min)
Diethyl ether	26.5
Chloroform	21
Acetone	18
(1:1) Chloroform:2-propanol	10
Ethanol	6.5
Water	22
Control	28
Tenox IV (0.02%)	3

[a]Mean of duplicate samples

The antioxidant was also found in the ethanolic extracts of raw and cooked shrimp meat, shrimp cannery effluent precipitate and shell waste. The antioxidant activity of these four extracts are shown in Figure 2 and Table 2. These results indicate that the antioxidant factor present in shrim was not affected at all by the heat treatment received by the cooked shrimp sample. The presence of this antioxidant in both the shrimp cannery effluent precipitate and the shrimp shell waste suggests that it might be particularly abundant in the cuticular layer of the shrimp, i. e., the union between the shell and the muscle.

Additional observations in this part of the experiment indicated

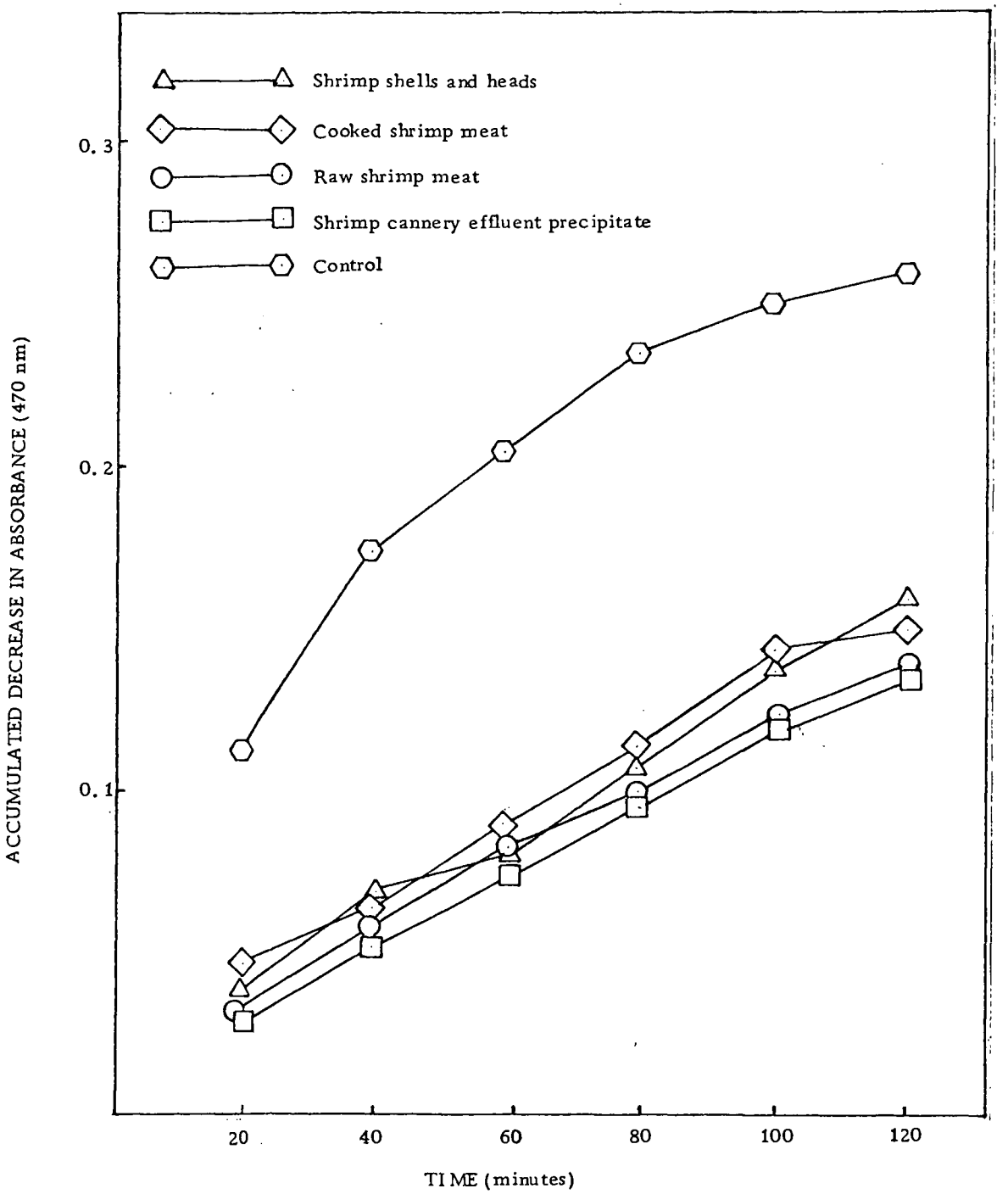

Figure 2. Antioxidant activity of different shrimp materials extracted with ethanol.

that only three test tubes held in a 50°C water bath had a yellow color due to the presence of unoxidized β-carotene after 12 hours. One of the test tubes contained 0.02% of Tenox IV; the other two had 0.2 ml aliquots of cooked shrimp and precipitated shrimp cannery effluent ethanol extracts.

Table 2. Effect of ethanol extracts from different shrimp materials on the rate of carotene decolorization.

Source	Decrease in carotene absorbance[a] ($\Delta A_{470\,nm}$ x100 for 120 min)
Raw shrimp muscle	14
Cooked shrimp muscle	15
Shrimp shells and heads	16
Shrimp cannery effluent precipitate	14
Control	26
Tenox IV (0.02%)	15.5

[a]Mean of duplicate samples

Figure 3 shows the effect of BHT and a shrimp ethanol extract on the induction period of salmon oil at 60°C in a draft oven. The shrimp antioxidant had a considerable protecting effect extending the induction period to 60 hours. It was also of interest to observe that the salmon oil samples containing the shrimp antioxidant retained its orange color while the other samples turned to a pale yellow after a few hours.

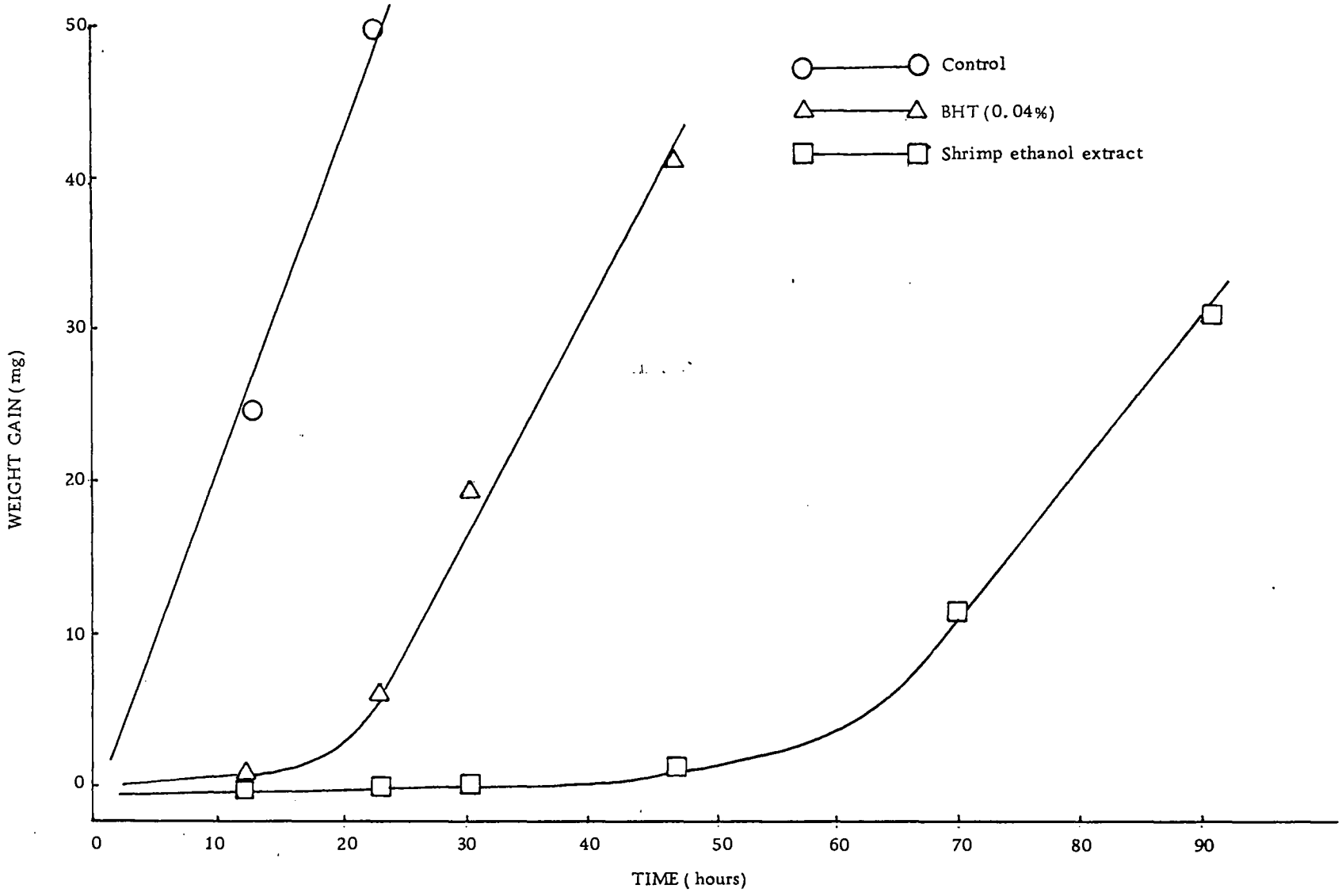

Figure 3. Effect of BHT and a shrimp ethanol extract on the induction period of salmon oil at 60°C. (1 g samples)

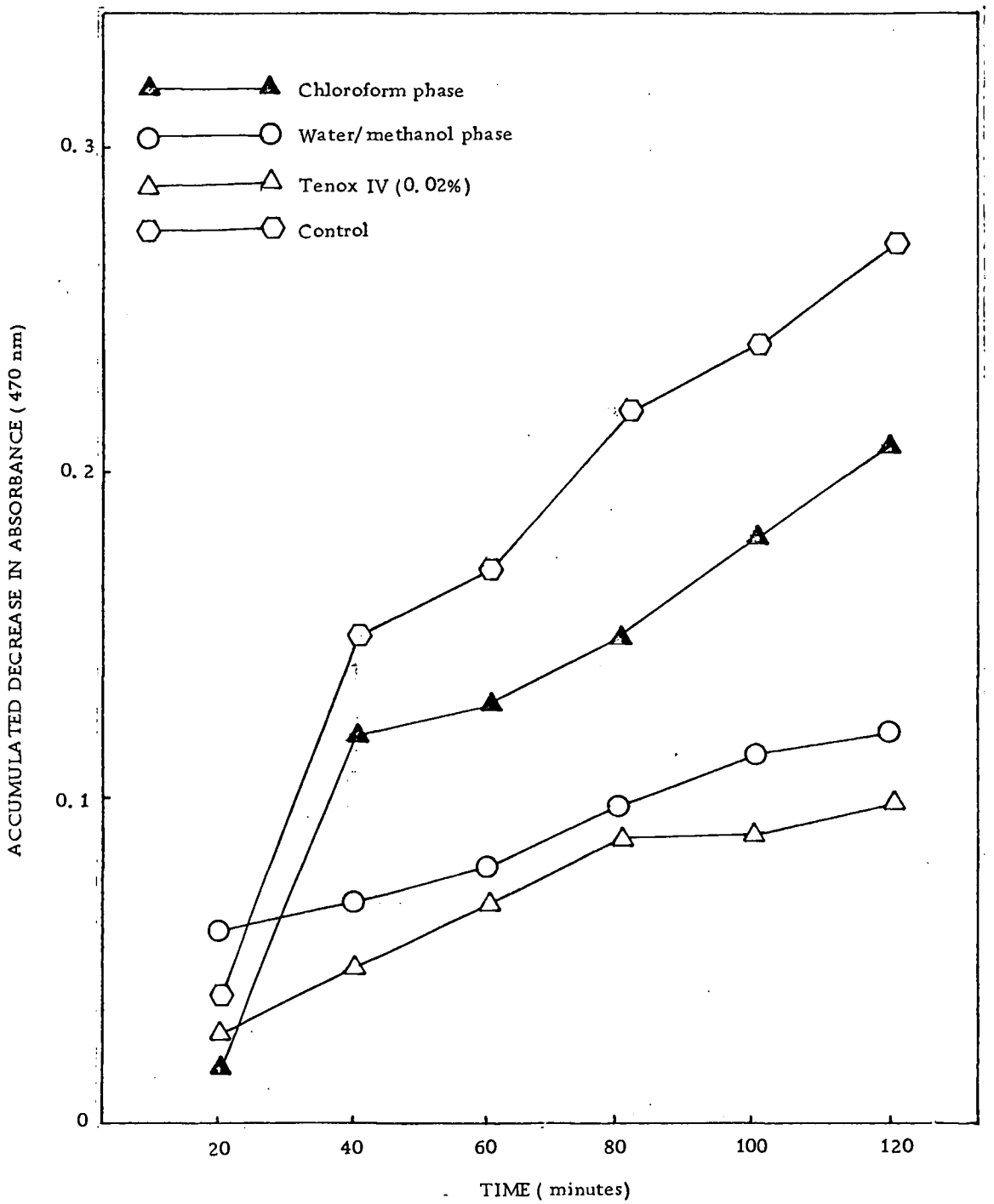

Figure 4. Antioxidant activity of lipid and non-lipid fractions from raw shrimp meat.

The non-lipid nature of the antioxidant factor was further established by the antioxidant activity of the chloroform and water-methanol fractions. The results shown in Figure 4 and Table 3 indicate that the antioxidant factor is located in the water-methanol phase.

Table 3. Effect of lipid and non-lipid fractions of raw shrimp meat on the rate of carotene decolorization.

Treatment	Decrease in carotene absorbance[a] ($\Delta A_{470\ nm}$ x100 for 120 min)
Water-methanol fraction	12
Chloroform fraction	21
Control	27
Tenox IV (0.02%)	10

[a]Mean of duplicate samples

All fractions showing antioxidant properties gave a positive reaction when tested with the Folin-Ciocalteau phenol reagent. The solubility behavior of the antioxidant further suggested that the antioxidant may be a phenolic compound. Figure 5 shows the separation of six different phenolic compounds by TLC using a solvent system of benzene:methanol:acetic acid (45:8:4 v/v). Parallel runs of the water-methanol and chloroform extracts revealed that four of them were lipid soluble while only one was exclusively present in the water-methanol fraction. The sixth spot was present in both extracts

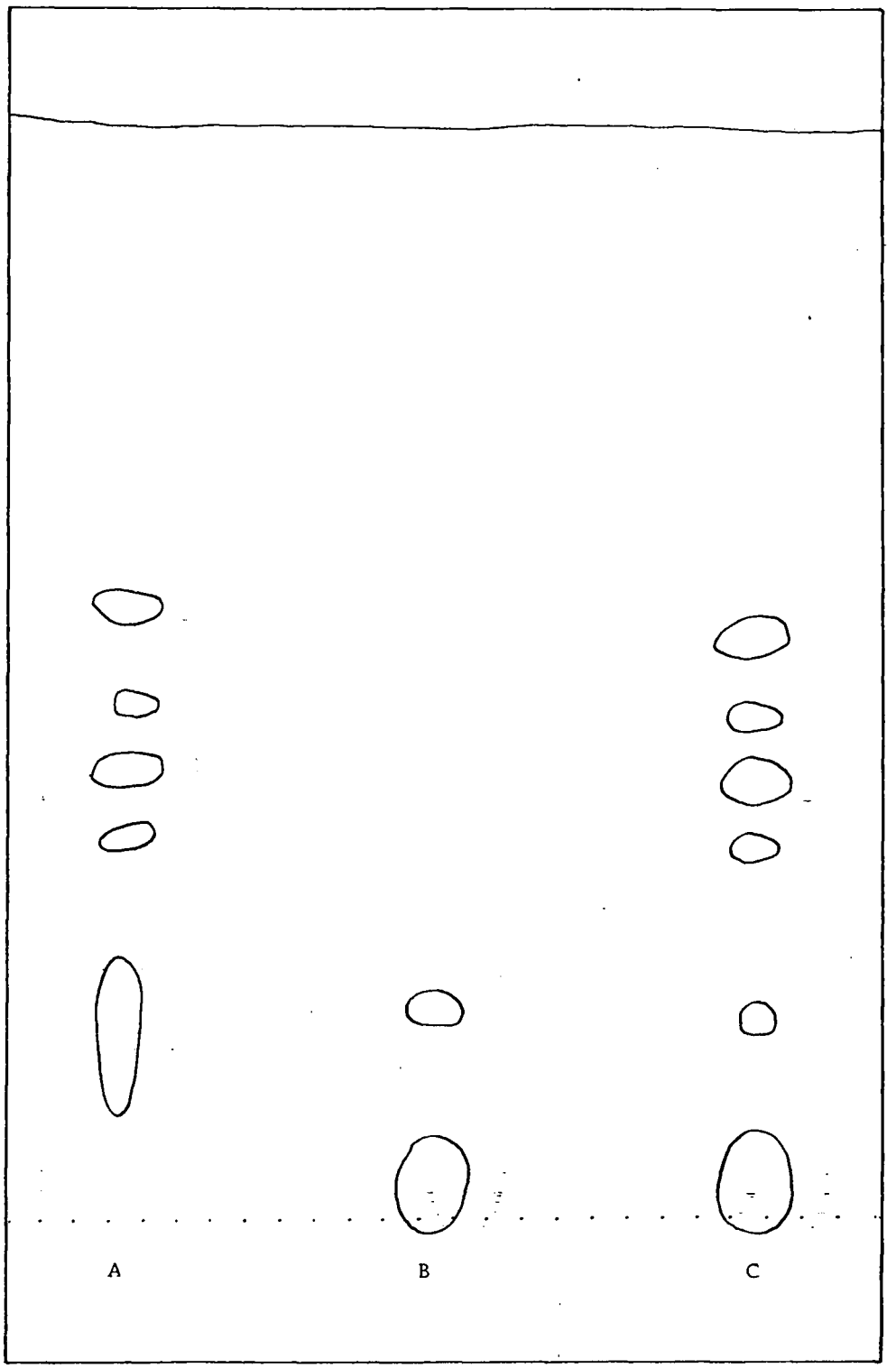

Figure 5. Thin layer chromatography separation[a] of phenolic compounds present in a shrimp ethanol extract (A = chloroform fraction B = water/methanol fraction C = shrimp ethanol extract).

[a] Benzene:methanol:acetic acid (45:8:4 v/v) as solvent system.

(Figure 5).

The six compounds were quantitatively separated by means of a preparative plate and their antioxidant activity measured by the β-carotene-linoleate method. The results are shown in Table 4. The band with an Rf value of 0.02 had the highest antioxidant activity and acted in approximately the same manner as the original shrimp ethanol extract. When this band was chromatographed using a more polar solvent system of butanol:acetic acid:water (12:3:5 v/v) two spots resulted with Rf values of 0.17 and 0.37. These two spots proved to be chromatographically homogeneous when run in a second dimension with chloroform:ethyl acetate:formic acid (5:4:1 v/v) and sprayed with the Folin-Ciocalteau reagent.

Table 4. Antioxidant activity of phenolic compounds present in raw shrimp meat.

Rf[a]	Present in chloroform fraction (lipid)	Present in MeOH/H_2O fraction (non-lipid)	Decrease in carotene absorbance[b] ($\Delta A_{470\,nm}$ x 100 for 60 min)
.02	-	+	2.5
.18	+	+	7.5
.32	+	-	9
.38	+	-	9
.45	+	-	10
Control			12
Raw shrimp meat ethanol extract			2

[a] Rf using benzene:methanol:acetic acid (45:8:4 v/v)

[b] Mean of duplicate samples

Another preparative plate was run using butanol:acetic acid: water (12:3:5 v/v) to ascertain which of the two fractions had antioxidant activity. The results, presented in Table 5, indicate that the substance with an Rf value of 0. 17 with the above solvent system is the one responsible for the antioxidant activity in a shrimp ethanol extract.

Table 5. Antioxidant activity of phenolic compounds present in raw shrimp meat.

Rf[a]	Present in chloroform fraction (lipid)	Present in MeOH/H_2O fraction (non-lipid)	Decrease in carotene absorbance[b] ($\Delta A_{470\,nm}$ x100 for 60 min)
. 17	-	+	5. 7
. 37	-	+	13
Control			14
Propyl gallate (0. 02%)			1. 5
Raw shrimp meat ethanol extract			4. 5

[a]Rf using butanol:water:acetic acid (12:5:3) v/v)
[b]Mean of duplicate samples

This compound is present in very low amounts as revealed by colorimetric quantitation measured against propyl gallate. The calculated concentration of the natural antioxidant in the β-carotene assay was 1 μg/ml when using 0. 2 ml of a water-methanol aliquot and of 0. 5 μg/ml when using 0. 2 ml of an ethanol aliquot. According to these figures the total content of the antioxidant in shrimp muscle was calculated to be approximately 2. 4 mg per 100 g of shrimp.

In order to learn more about the chemical structure of the active compound, different reagents were used to visualize it. It formed a pronounced blue with the Folin-Ciocalteau reagent only

after being made alkaline, characteristic of para substituted dihydroxy phenols. However no reaction occurred when tested with the ferric chloride:potassium ferricyanide solution and the chromogenic reagent of Bhatia indicating the absence of ortho substituted hydroxyl groups. The sucrose reagent of Roux gave a negative test and an intense brown color resulted when sprayed with alkaline phloroglucinol. There was a positive reaction to ninhydrin producing a purple spot with a yellow tint in the center. These results are summarized in Table 6. The above information suggests that the phenolic compound acting as an antioxidant has one or more hydroxyl groups but these are not ortho substituted. The yellowish purple spot observed with ninhydrin is characteristic of substituted α-amino groups.

Table 6. Reactions of the antioxidant fraction with different color reagents.

Reagent	Observed reaction
Ninhydrin (0.1% in isopropanol)	Purple with yellow tint
Sucrose reagent of Roux[a]	Negative
Folin-Ciocalteau phenol reagent	Blue
Alkaline phloroglucinol[b]	Brown
Ferric chloride:potassium ferricyanide	Negative
Bhatia chromogenic reagent[c]	Negative

[a]Roux (1951)

[b]Malek (1961)

[c]Bhatia (1973)

Also the compound had absorption maxima at 204 and 278 nm (Figure 6) and fluoresced strongly with a light blue color when observed under a long wave ultraviolet lamp.

The origin, chromatographical behavior and chemical characteristics of this unknown compound suggests the possibility of dealing with an intermediate of the cuticle sclerotization process in crustacea. Further evidence supporting this would be the findings of Krishnan (1951) and Dennell (1947) that showed that the newly formed cuticle of moulting crustacea has a polyphenol oxidase system capable of hydroxylating aromatic amino acids. Also of interest are the findings reported by Lissitzky and Rolland (1962) that describe an intermediary compound of the melanogenesis process using mushroom polyphenol oxidase. This intermediate showed a very small Rf value using polar solvent systems. A trihydroxylated derivative of phenylalanine was proposed as the unknown intermediate.

Another alternative to be considered is the possibility that the unknown antioxidant factor present in shrimp is of bacterial origin. Smith and Alford (1970) report the presence of antioxidant compounds in bacteria of the genus Pseudomonas that were soluble in methanol and insoluble in benzene. In another study Larway and Evans (1962) showed the existence of a polyphenol oxidase system in a small vibrio of the genus Microspira capable of forming 2, 4, 5-trihydroxyphenylalanine.

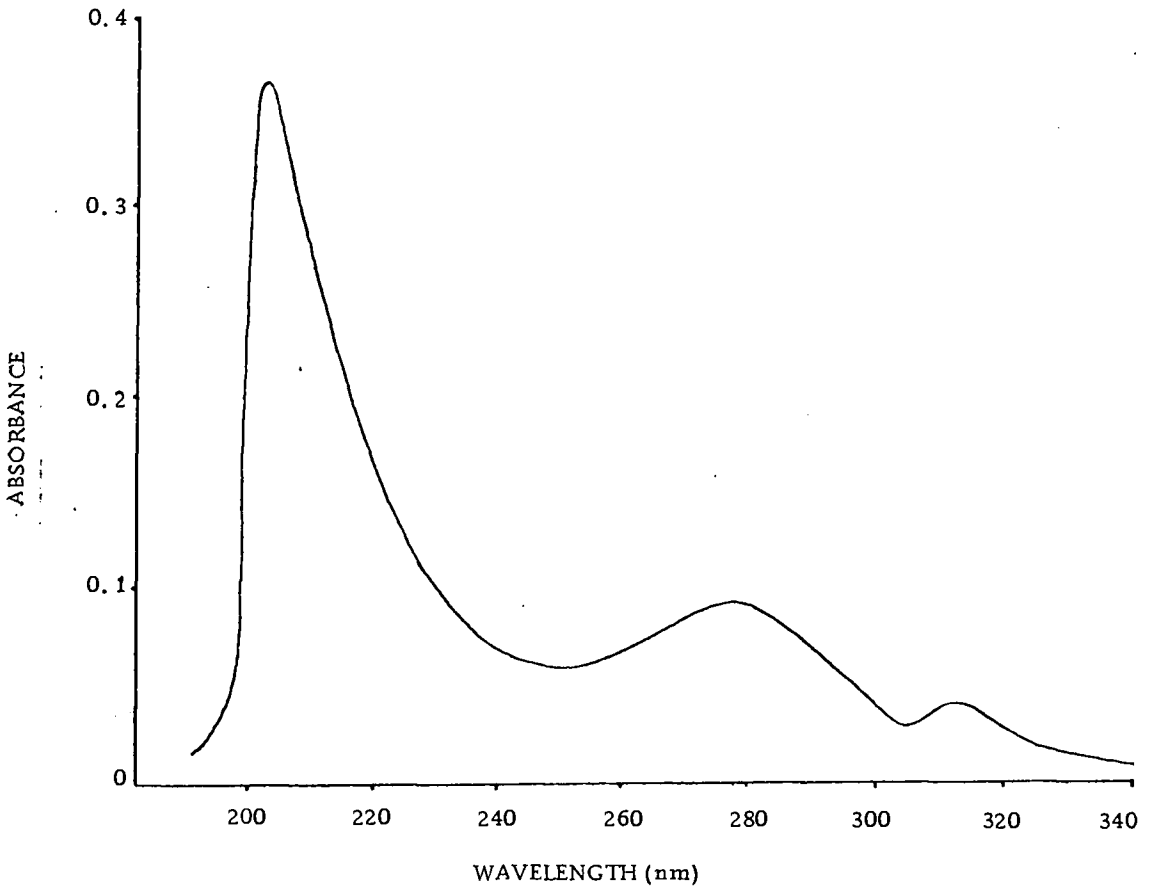

Figure 6. UV spectrum of the active fraction of a shrimp ethanol extract.

SUMMARY AND CONCLUSIONS

The strong antioxidant activity of a shrimp ethanol extract was detected using the β-carotene-linoleate model system and the weighing oxygen method.

From the different solvents used in the extraction process, ethanol proved to be the most effective. A diethyl ether extract exhibited no antioxidant activity.

The compound responsible for the antioxidant effect was found to be heat stable. Shrimp ethanol extracts were still effective after three months when kept at $2^{\circ}C$ in amber bottles.

The antioxidant was present in raw and cooked shrimp muscle, in shrimp shell waste and in precipitated shrimp cannery effluent.

The chemical characteristics and chromatographic properties of this compound suggests that it is a polyhydroxylated derivative of an aromatic amino acid.

The precise chemical structure of this naturally occurring antioxidant could not be determined at this stage. Further work is required to find a fractionation method capable of yielding larger amounts of the compound in a pure form so that its exact nature can be established.

BIBLIOGRAPHY

Babbitt, J. K., Law, D. K. and Crawford, D. L. 1973. Blueing discoloration in canned crab meat (Cancer magister). J. Food Sci. 38:1101.

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.

Bailey, M. E., Fieger, E. A. and Novak, A. F. 1960. Phenol oxidase in shrimp and crab. Food Res. 25:565.

Bhatia, I. S., Joginder, S. and Bajaj, K. L. 1973. A new chromogenic reagent for the detection of phenolic compounds on thin-layer plates. J. Chromatogr., 79:350.

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

Bray, H. G. and Thorpe, W. V. 1961. Analysis of phenolic compounds of interest in metabolism. In Methods of Biochemical Analysis (Edited by Glick, D.) Vol. 1, pp. 27-52. Interscience, New York.

Cantarelli, C. and Montedoro, G. 1974. Extraction of natural olive antioxidants: Experimental study. Nat. Synth. Zusatzstoffe Nahr. Menschen, Int. Symp., 14th 84:93.

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

Cobb, B. F., Vanderzant, C. and Hyder, K. 1974. Effect of ice storage upon the free amino acid contents of tails of white shrimp (Penaeus setiferus). J. Agr. Food Chem. 22(6):1052.

Dennell, R. 1947. The occurrence and significance of phenolic hardening in the newly formed cuticle of Crustacea Decapoda. Proc. Roy. Soc. B134:485.

Dolev, A., and Olcott, H. S. 1965. The triglycerides of sablefish (Anaplopoma fimbria). I Quantitative fractionation by column chromatography on silica gel impregnated with silver nitrate. J. Am. Oil Chemists' Soc. 42:624.

Food Chemical News 1976. 17(50):March 1.

Jurewicz, I. and Salmonowicz, J. 1973. Pro- and antioxidant effects of some amino acids upon fish oil. Zesz. Probl Postepow Nauk Roln. 136:119.

Kaneda, T. and Ando, H. 1972. Component lipids of purple laver and their antioxygenic activity. Proc. 7th Int. Seaweed Symp., pp. 553-557.

Kelley, C. E. and Harmon, A. W. 1972. Method of determining carotenoid contents of Alaska pink shrimp and representative values for several shrimp products. Fish. Bull. 70 (1):111.

Krishnan, G. 1951. Phenolic tanning and pigmentation of the cuticle in Carcinus maenas. Quart. J. Microscop. Sci. 92:333.

Larway, P. and Evans, W. C. 1962. The oxidation of tyrosine by Microspira tyrosinatica. Biochem. J. 85:22P.

Lissitzky, S. and Rolland, M. 1962. New intermediate of melanogenesis in vitro. Nature 193:88.

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

Masuyama, S. 1976. Natural antioxidants. Kagaku To Kogyo. 50 (5):157.

Miller, H. E. 1971. A simplified method for the evaluation of antioxidants. J. Am. Oil Chemists' Soc. 48:91.

Ninagawa, S. and Takeshita, S. 1975. Method of stabilizing organic compounds against oxidation with dopa or dopa derivatives. United States Patent 3883674.

Olcott, H. S. and Einset, E. 1958. A weighing method for measuring the induction period of marine and other oils. J. Am. Oil Chemists' Soc. 35:161.

Olcott, H. S. 1967. Antioxidants. In Fish Oils: their Chemistry, Technology, Stability, Nutritional Properties and Uses (Edited by Stansby, M. E.) pp. 164-170. The Avi Publishing Company, Inc.

Qureshi, M., Jamil, M. and Blain, J. A. 1974. Isolation and identification of antioxidant factors in tomatoes. Nucleus (Karachi). 11(1-2):25.

Revankar, G. D. 1974. Proline as an antioxidant in fish oil. J. Food Sci. Technol. (India). 11(1):10.

Roux, D. G. 1951. Color reagents for the paper chromatography of di- and tri-hydroxy phenols. Nature. 168:1041.

Smith, J. L. and Alford, J. A. 1970. Presence of antioxidant materials in bacteria. Lipids 5(10):795.

Sugimoto, Y. and Murata, K. 1972. Antioxidant factors in tempeh, a fermented soybean preparation. Osaka Shiritsu Daigaku Kaseigakubu Kiyo 20:13.

Summers, N. M. 1967. Cuticle sclerotization and blood phenol oxidase in the fiddler crab, Uca pugnax. Comp. Biochem. Physiol. 23:129.

Vaskovskii, V. E. and Syskin, G. A. 1973. Method of stabilizing fats against oxidation. USSR Patent 380694.

Yuki, E., Ishikawa, Y. and Yoshiwa, T. 1974. Antioxidant activities of amino acids and their esters under the condition of active oxygen method. Yukagaku 23(8):497.