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Antioxidative activity of chitosans of different viscosity in cooked comminuted flesh of herring (*Clupea harengus*)

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Abstract

Antioxidant efficacy of chitosans of different viscosity (14 cP, 57 cP and 360 cP) in cooked, comminuted flesh of herring (*Clupea harengus*), was investigated. The oxidative stability of treated fish flesh was determined and compared with those treated with conventional antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ) at a level of 200 ppm. The progress of oxidation was monitored by employing the peroxide value, 2-thiobarbituric acid-reactive substances (TBARS) and static headspace gas chromatographic analysis. In general, all chitosans exhibited varying antioxidant activities in a fish flesh model system. The formation of hydroperoxides and TBARS, in herring samples containing 200 ppm 14 cP chitosan, was reduced after 8 days of storage by 61 and 52%, respectively. Among the different viscosity chitosans, 14 cP chitosan was more effective than the higher viscosity chitosans in preventing lipid oxidation in the herring flesh model system.

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1. Introduction

The highly unsaturated fatty acids commonly found in seafoods are particularly sensitive to oxidative change during storage (Hsieh & Kinsella, 1989b; Shahidi, 1998). Tichivangana and Morrissey (1985) have shown that the oxidation of muscle foods occurs in the order of fish > poultry > pork > lamb. Although the process of lipid oxidation is thermodynamically favourable, the direct reaction between oxygen and highly unsaturated lipids is kinetically hindered (German & Kinsella, 1985; Hsieh & Kinsella, 1989b). Hence, an activating factor is necessary to initiate free radical chain reactions followed by their self-propagation (German & Kinsella, 1985; Shahidi, 1998). It has been proposed that lipid oxidation in fish may be initiated and promoted by a number of mechanisms involving autoxidation, photosensitized oxidation, lipoxygenase, peroxidase, and microsomal enzymes (Slabyj & Hultin, 1982; Frankel, 1985; Josephson, Lindsay, & Stuiber, 1987; Hsieh & Kinsella, 1989a).

Decker and Hultin (1992) identified several sources of protein-bound iron that exist in biological tissues, namely myoglobin, haemoglobin, ferritin, transferrin, and haemosiderin. St. Angelo (1996) reported that iron bound to these proteins may be released during post-harvest storage and cooking, activating oxygen and initiating lipid oxidation. There is a range of concentrations of haematin compounds in muscles from different species of fish and these are present in relatively large concentrations in the muscle of most fatty fish, especially their lateral band dark muscle (Castell & Bishop, 1969). Autoxidation of oxymyoglobin and oxyhaemoglobin (both in the Fe^{2+} oxidation state) may also result in the formation of superoxide anion, metmyoglobin and methaemoglobin (both in the Fe^{3+} oxidation state), respectively. The formation of superoxide anion from oxymyoglobin/oxyhaemoglobin may be catalyzed by anions such as SCN^-, OCN^-, F^- and Cl^- (Satoh & Shikama, 1981). Flick, Hong, and Knobl (1992) reported that increased oxidation of seafoods at lower humidities may be attributed to the concentration of prooxidants such as metal ions and haemoglobin. The main source of free iron or non-haem iron in cells is

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ferritin, which is a soluble iron storage protein found in liver, spleen and skeletal muscle and has a molecular mass of 450 kDa and contains 4500 iron atoms when fully loaded (Decker & Welch, 1990). Decker and Hultin (1990) observed that storage of unfrozen mackerel ordinary muscle at 4C for 7 days resulted in a 1.4-fold increase in low molecular weight iron-containing compounds from 0.16 to 0.23 μg Fe/g muscle. A small amount of iron is also bound to molecules such as ATP, ADP, organic acids and DNA. These compounds are capable of decomposing hydroperoxides (ROOH) in order to form free radicals (Kanner & Doll, 1991). Shahidi and Hong (1991) reported that metal ions such as those of copper and iron, can enhance lipid autoxidation to a greater extent in their lower valency states.

Tichivangana and Morrissey (1982, 1985) reported that ferrous ion at 1–10 ppm levels acts as a strong prooxidant in cooked fish muscles. Castell, Maclean, and Moore (1965) observed that the relative prooxidant activity of ions in fish muscle decreased in the order of $Cu^{2+} > Fe^{2+} > Co^{2+} > Cd^{2+} > Li^{+} > Ni^{2+} > Mg^{2+} > Zn^{2+} > Ca^{2+} > Ba^{2+}$. Superoxide anion may be dismutated to form hydrogen peroxide, resulting in the formation of hydroxyl radicals via the reaction of H_2O_2 with Fe^{2+} (Frankel, 1980; Yen, Chen, & Lee, 1999).

Chitosan, which is the deacetylated form of chitin, has been identified as a versatile biopolymer for a broad range of food applications (Shahidi, Kamil, & Jeon, 1999). Both chitin and chitosan have unusual multifunctional properties, including high tensile strength, bioactivity, and biodegradability which makes them an attractive speciality materials (Berkeley, 1979; Ikejma & Inoue, 2000). Furthermore, these polymers have been identified as being biocompatible, non-antigenic, nontoxic, and biofunctional (Hirano et al., 1990; Li, Dunn, Grandmaison, & Goosen, 1992). Recently, Rao and Sharma (1997) reported that acute systemic toxicity tests in mice did not show any toxic effect of chitosan; all mice injected with the test material lived during the entire period (72 h) of observation. These authors further observed that eye irritation tests in rabbits and skin irritation tests in guinea pigs did not produce any undesirable toxic effect due to chitosan.

Both chitin and chitosan are able to form complexes with many of the transition metals, as well as some of those from groups 3–7 of the periodic table (Muzzarelli, 1973). The heavy metal-polymer complexes are believed to form as a result of dative bonding with chitosan. This involves the donation of nonbonding pairs of electrons from the nitrogen, and/or the oxygen of the hydroxyl groups, to a heavy metal ion (Winterowd & Sandford, 1995). *N,O*-Carboxymethyl chitosan has been found to bond chemically with ions of numerous heavy metals, such as iron, copper, mercury and zinc, thus binding or sequestering them when present in even 10–1000 ppm (Hayes, 1986). The cupric ion seems to form one of the strongest complexes with chitosan in the solid state (Chuti, Mok, Nag, Luong, & Ma, 1996).

Synthetic antioxidants and chelating agents may be added to food products in order to prevent lipid oxidation. However, the growing consumer demand for food devoid of synthetic antioxidants has focused efforts on the discovery of new natural preservatives (Madsen & Bertelsen, 1995). Several sources of natural antioxidants are known (Shahidi, 1997), and some of them, such as those of rosemary and sage, are currently used in a variety of food products. However, fundamental studies on chitosan as a natural antioxidative agent in fish and seafood are lacking. Therefore, the objective of this study was to examine the effect of chitosans of different viscosity on lipid autoxidation in a fish model system.

2. Materials and methods

2.1. Materials

Fresh samples of crab processing discards, comprising intact cephalothorax and abdominal exoskeleton, were collected from local sources in Newfoundland. Samples were thoroughly washed with distilled water and vacuum-packed in Whirl pack plastic bags (Eastern Papaer, St. John's, NF) and subsequently stored at −60 °C (Ultra Low, Revco, West Columbia, SC) until used. Fresh samples of herring were acquired from a local source in Newfoundland and immediately cleaned, gutted, filleted, and skinned. Processed fillets were vacuum-packed in Whirl pack plastic bags (Eastern Paper, St. John's, NF) and frozen at −60 °C until used. Reagents, namely 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (TMP), tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), were obtained from Sigma Chemical Co. (St. Louis, MO). Aldehyde standards were purchased from Aldrich Chemical Co. (St. Louis, MO) and trichloroacetic acid was acquired from Fisher Scientific (Nepean, ON). Fatty acid methyl esters were purchased from either Supelco (Oakville, ON) or Nu-Check (Elysian, MN) companies. High performance liquid chromatographic (HPLC)-grade chemicals were used for analysis and preparation as required. All other chemicals used were of American Chemical Society (ACS) grade or better. Helium, hydrogen, nitrogen and compressed air were obtained from Canadian Liquid Air (St. John's, NF).

2.2. Methods

2.2.1. Preparation of chitosans and evaluation of their characteristics

Chitin was isolated from crab processing discards, using a modified version of methods of Mima, Miya,

Iwamoto, and Yoshikawa (1983) and Shahidi and Synowiecki (1991) (Fig. 1). Deproteinization and demineralization steps were carried out with 20 vol. of 4% (w/v) NaOH at 60 °C for 3 h and 10 vol. of 10% (w/v) HCl at 25 °C for 2 h, respectively. The alkali and acid treatments were repeated twice. The chitin residue, firmly complexed with the carotenoid pigments, was washed with 20 vol. of acetone and dried for 2 h at ambient temperature, followed by bleaching with a 0.32% (v/v) solution of sodium hypochlorite (containing 5.25% available chlorine) for 5 min with a solid to solvent ratio of 1:10 (w/v). Chitosan was prepared by alkali treatment of chitin using 10 vol. of 50% (w/v) NaOH in distilled water at 100C for 4, 10 and 20 h in a nickel crucible under a nitrogen atmosphere. The reactants were immediately filtered under vacuum after alkali treatment, washed with hot-deionized water to neutral pH and lyophilized for 72 h at −49 °C and 62 × 10^{-3} mbar (Freezone 6, Model 77530, Labconco Co., Kansas City, MO).

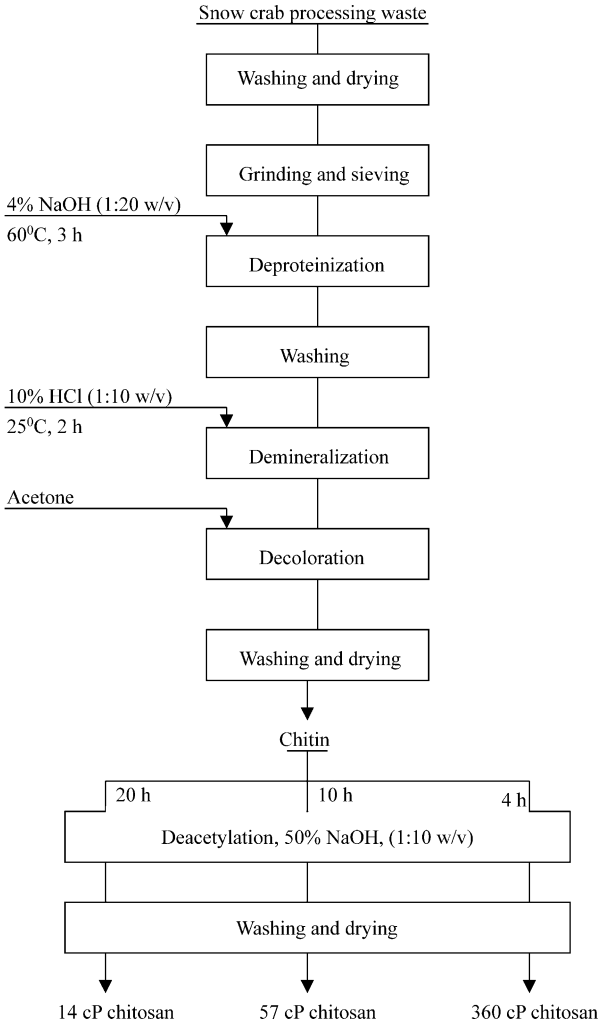

Fig. 1. Production of chitosans of different viscosity from crab processing waste.

Moisture, total nitrogen and ash contents of chitosan samples were then determined (Shahidi & Synowiecki, 1991). Apparent viscosity (at 2 Nm^{-2}) of a 1% (w/v) chitosan in a 1% (v/v) acetic acid solution at 25 °C was measured in triplicate using a rotational viscometer (Cole-Parmer Co. Vernon Hills, IL) equipped with a No. 5 spindle at 50 rpm and values reported in centipoise (cP) units. The degree of acetylation of chitosans was measured according to the picric acid method of Neugebauer, Neugebauer, and Brzezinski (1989).

The molecular weight of chitosan was expressed as the viscosity molecular weight (Mv) using a ViscoTek model Y-500 relative viscometer (Viscotek Co. Houston, TX). A chitosan solution in 0.1M acetic acid-0.2 M sodium chloride was diluted to give four concentrations ranging from 0.05 to 2 g/l which were used for determining of the specific viscosity (η_{sp}) at 25 °C. The specific viscosity was determined as follows: $\eta_{sp}=(\eta-\eta_s)/\eta_s$, where η and η_s are the solution and the solvent viscosities, respectively. The values of intrinsic viscosity (η) obtained ($\eta=\lim_{c\to 0}\ \eta_{sp}/C$) by extrapolating ($\eta_{sp}$)/$C$ versus C, where C is the concentration of the chitosan solutions, were fitted into the equation $Mv=[\eta/k]^{1/a}$, where constants of "k" and "a" are 1.81×10^{-5} and 0.93, respectively (Anthonsen, Varum, & Smidsrod, 1993; Kittur, Kumar, & Tharanathan, 1998; Roberts & Domaszy, 1982).

2.2.2. Preparation of cooked comminuted fish model system

A fish model system (herring) was prepared according to the modified method described by Shahidi and Pegg (1990). Skinless fillets were homogenized using a Waring Blender (Model 33BL73, Waring products, New Hartford, CT) and ground fish was mixed with 20% by weight of deionized water in Mason jars (height 10 cm, internal diameter 6 cm). Chitosans (50, 100, 150 and 200 ppm) and commercial antioxidants butylated hydroxyanisole + butylated hydroxytoluene (BHA + BHT 200 ppm), and tert-butylhydroquinone (TBHQ 200 ppm), were added separately to fish and thoroughly homogenized. A control sample containing no chitosan or commercial antioxidants was also prepared. Samples were cooked in a thermostatted water bath at 85±2 °C (internal temperature of 72±3 °C) for 40 min while stirring every 5 min with a glass rod. After cooling to room temperature, the fish system was homogenized in a Waring Blender (Model 33BL73, Waring products, New Hartford, CT) for 30 s, transferred into Whirl pack plastic bags (Eastern Paper, St. John's, NF) and then stored under refrigerated conditions at 4±1 °C for further analysis.

2.2.3. Extraction of lipids from cooked comminuted fish flesh

Total lipids were extracted into a mixture of chloroform and methanol as described by Bligh and Dyer (1959). The recovered lipids were stored at −60 °C

(Ultra Low, Revco, West Columbia, SC) for determination of fatty acid composition and peroxide value (PV).

2.2.4. Analysis of fatty acid composition of lipids

The fatty acid composition of fish lipids was determined after their conversion to fatty acid methyl esters (FAMEs) as detailed elsewhere (Wanasundara & Shahidi, 1997). The FAMEs were tentatively identified by comparison of their retention times with those of authentic standard mixtures (PUFA 1, Supelco Canada Ltd., Oakville, ON and GLC-416, Nu-Check, Elysian, MN). The area under each peak was calculated on a weight percentage basis using methyl tricosanoate (C23:0) as an internal standard.

2.2.5. Determination of peroxide value (PV)

The thiosulphate titration method described by the AOCS (1990) was used for determination of PV of oil samples.

2.2.6. Determination of 2-thiobarbituric acid-reactive substances (TBARS)

Fish samples were analyzed for their TBARS values over a 12-day period according to the method of Siu and Draper (1978), as described by Shahidi and Hong (1991). TBARS values were calculated using 1,1,3,3-tetramethoxypropane as a standard precursor of malonaldehyde. Inhibition of TBARS formation was determined using the equation: % inhibition = 100 (1−TBARS value for the treated sample/TBARS value for the control sample).

2.2.7. Static headspace gas chromatographic analysis

A Perkin-Elmer 8500 gas chromatograph and an HS-6 headspace sampler (Perkin-Elmer Corp., Montreal, PQ) were used for volatile analysis of fish samples. A high polarity Supelcowax 10 fused silica capillary column (30 m length × 0.32 m internal diameter, 0.10 mm film thickness, Supelco Canada Ltd., Oakville, ON) was used. Helium was the carrier gas, employed at an inlet column pressure of 17.5 psig with a split ratio of 7:1. The oven temperature was maintained at 40 °C for 5 min and then ramped to 200 °C at 20 °C/min and held there for 5 min. The injector and flame ionization detector (FID) temperatures were adjusted to 280 °C and held at this temperature throughout the analysis.

For headspace (HS) analysis, 2.0 g portions of homogenized fish samples were transferred to 5 ml glass vials. The vials were capped with teflon-lined septa, crimped and then frozen and kept at −60 °C (Ultra Low, Revco, West Columbia, SC) until used. To avoid heat shock after removal from storage, frozen vials were tempered at room temperature for 30 min and then preheated in the HS-6 magazine assembly at 90 °C for a 45 min equilibration period. The heating time of the vial was 6 s and the volume of the vapour phase drawn was approximately 1.5 ml. Chromatographic peak areas were expressed as integrator count units. Individual volatile compounds were tentatively identified by comparing their relative GC retention times with those of commercially available standards. Quantitative determination of dominant aldehydes was accomplished using 2-heptanone as an internal standard.

2.3. Statistical analysis

All experiments used completely randomized block designs (CRD) and analyses were carried out in triplicate. Mean values with standard deviations (SD) were reported when and where necessary. SigmaStat was used to normalize the data, analysis of variance (ANOVA) performed, and differences in mean values determined using Tukey's procedures of statistical analysis system (SAS, 1990).

3. Results and discussion

Characteristics of chitosans prepared usng different deacetylation times are listed in Table 1. Preparation of chitosan samples I, II, and III, shown in Table 1, involved deacetylation of chitin for 4, 10 and 20 h, respectively. The chitosans prepared from snow crab processing discards showed variations in their viscosity which were closely related to the duration of the deacetylation time. The highest viscosity was observed when deacetylation was carried out for 4 h, followed by those prepared over 10 and 20 h periods. As shown in Table 1, the nitrogen contents of chitosans were dependent on the deacetylation time and were 7.55, 7.63, and 7.70% for samples prepared over 4, 10 and 20 h, respectively, thus confirming a more effective deacetylation over longer periods. The corresponding apparent viscosity values were 360, 57, and 14 cP, for chitosan samples I, II and III, respectively. The observed viscosity values may indicate depolymerization of chitosans prepared over longer treatment times.

The extension of shelf-life of foods and specifically fish may be achieved using antioxidants (He & Shahidi, 1997; Tichivangana & Morrissey, 1984). Incorporation of an antioxidant into cooked fish meat or even glazing of fish fillets could slow down their deterioration (Khali & Mansour, 1998). In this study, a cooked comminuted herring model system was used and its oxidative stability monitored at 4±1 °C using peroxide value, TBARS and static headspace gas chromatographic analyses as oxidation indicators.

The peroxide values of cooked comminuted herring samples treated with chitosans of different viscosities at 50, 100 and 200 ppm are presented in Fig. 2. Control herring samples, devoid of chitosan or commercial

antioxidants, had a significantly ($P \leqslant 0.05$) higher peroxide value throughout the storage period and values in all samples increased up to day-8 of storage, but then gradually decreased. In general, herring samples treated with the 14 cP chitosan, regardless of the concentration used, had lower peroxide values than those of 57 and 360 cP chitosans. After day-8, peroxide values of herring samples treated with the 14 cP chitosan at the 200 ppm level were reduced by 61% as compared to that of the control. The corresponding values for 57 cP chitosans and 360 cP chitosans at the same concentration in herring samples were reduced by 53 and 40%, respectively. Among reference antioxidants, TBHQ was most effective in reducing the formation of hydroperoxides. Reduction in peroxide values of herring samples treated with 200 ppm BHA, BHT and TBHQ was in the range of 71–80% on day-8 of storage. Lower peroxide values were evident for herring samples containing 200 ppm chitosan (at all viscosities), possibly due to a concentration effect.

As shown in Fig. 3, TBARS values of cooked comminuted herring samples containing different viscosity chitosans and commercial antioxidants (BHA, BHT and TBHQ) increased over the entire storage period, but at slower rates than the control sample. The differences in TBARS values of herring samples containing different viscosity chitosans (14, 57 and 360 cP) were significant ($P \leqslant 0.05$) over the entire storage period. The 14 and 57 cP chitosans, at 50, 100 and 200 ppm levels, resulted in significantly ($P \leqslant 0.05$) lower TBARS values with marginal differences between lower and higher concentrations in the herring model system. After day-6 of storage, the TBARS values of herring samples containing 14 cP chitosan were approximately 49–56% lower than that of the control (Fig. 3). The 360 cP chitosan, at 50 ppm, reduced TBARS by 14–34% in treated herring samples whereas the effects for 14 and 57 cP chitosans at the same concentration exceeded that of the 360 cP chitosan and were significantly ($P \leqslant 0.05$) higher than that of the control.

The polyunsaturated fatty acid composition of total lipids of herring fillets is shown in Table 2. The results in this table indicate the highly unsaturated nature, and hence oxidative susceptibility, of lipids from herring fillets. The high PV of lipids from herring during the initial stages of storage of cooked comminuted fish, may be attributed to the high degree of unsaturation of their fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Moreover, characteristic features of lipid oxidation in fish flesh are influenced by factors such as lipid content (Ke, Linke, & Smith-Lall, 1982), the level of microsomal lipid present (Slabyj & Hultin, 1982), the level of haem compounds (Castell & Bishop, 1969) and the presence of metal ions (Tichivangana & Morrissey, 1985).

As expected, TBHQ exerted the strongest antioxidant effect at all concentrations in the herring fish model system. At 200 ppm, 14 cP chitosan exerted an effect similar to that of the commercial antioxidants (BHA, BHT and TBHQ) at the same level in a fish model system. Among different viscosity chitosans, 14 cP chitosan was more effective than higher viscosity chitosans in preventing lipid oxidation.

The content of total volatile aldehydes in the volatiles of cooked comminuted herring samples treated with different viscosity chitosans was also determined over a 12 day storage at $4 \pm 1^{\circ}C$. Formaldehyde and propanal were prominent aldehydes detected (Fig. 4). However, in fish muscle, formaldehyde may also accumulate as a result of decomposition of trimethylamine oxide by endogenous and microbial enzymes. Therefore, formaldehyde was not considered as a volatile aldehyde that could form only from autoxidation of fish muscle lipids. Thus, propanal was used as an indicator for assessment of the oxidative status of treated fish meat samples. Table 3 summarizes the effects of different viscosity chitosans and commercial antioxidants in lowering the formation of total volatile (TV) aldehydes and propanal (P) as compared with that of the control. The inhibitory effects of different viscosity chitosans were as follows: 14

Table 1
Characteristics of three different types of chitosans (I, II and III) prepared from crab shell waste[a]

Properties	I	II	III
Deacetylation time[b] (h)	4	10	20
Moisture (%)	4.50±0.30	3.95±0.34	3.75±0.21
Nitrogen (%)	7.55±0.10	7.63±0.08	7.70±0.19
Ash (%)	0.30±0.03	0.25±0.02	0.30±0.00
Colour	Cream white	Cream white	Cream white
Apparent viscosity (cP)	360±0.53	57±0.96	14±0.34
Degree of deacetylation (%)	86.4±2.1	89.3±1.2	91.3±1.3
Mv[c] (dalton)	1.8×10^6	9.6×10^5	6.6×10^5

[a] Results are mean values of three determinations±standard deviation.
[b] Deacetylation for preparation of chitosan I, II and III was achieved using 50% NaOH at 100 °C for 4, 10 and 20 h, respectively.
[c] Viscosity molecular weight

cP chitosan > 57 cP chitosan > 360 cP chitosan. As compared with the control sample, 200 ppm 14 cP chitosan-treated herring samples decreased the formation of total aldehydes in samples by over 68, 49, 48 and 57% on days 0, 4, 8 and 12, respectively.

Antioxidant activities of different viscosity chitosans in cooked comminuted herring may be attributed to their metal-binding capacities. Several sources of protein-bound iron exist in fish tissues, e.g. myoglobin, haemoglobin, ferritin and transferrin. The iron bound to these proteins may be released during storage and cooking, thus activating oxygen and initiating lipid oxidation (St. Angelo, 1996). Oxidative rancidity usually occurs more rapidly in cooked ground fish than in raw fish (Ramanathan & Das, 1992). Lee and Toledo (1977) also found that cooking significantly increased the TBA values of minced mullet (*Mugil* spp.) during refrigerated storage. The cooking process disrupts the muscle membrane system, thereby exposing the lipid components to oxygen and/or other reaction catalysts such as iron (Love & Pearson, 1976).

Chitosans may retard lipid oxidation by chelating ferrous ions present in the system, thus eliminating their prooxidant activity or their conversion to ferric ion. Furthermore, amino groups in chitosans may participate in the chelation of metal ions (Peng, Wang, &

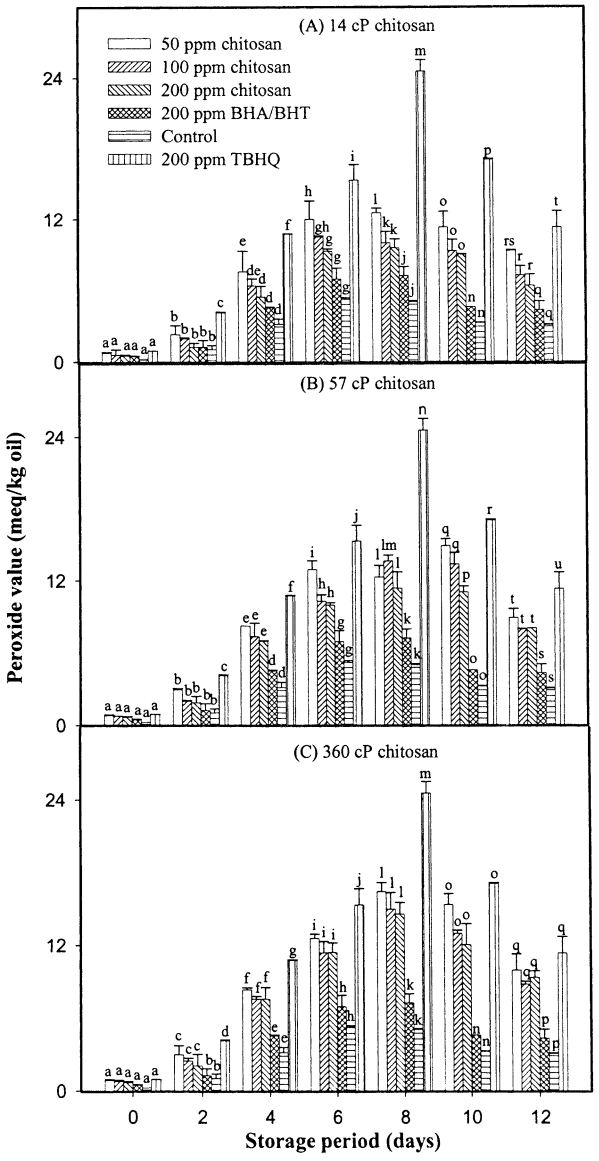

Fig. 2. Effect of chitosans of different viscosity and commercial antioxidants on peroxide value of a cooked comminuted herring model system. (A) 14 cP chitosan, (B) 57 cP chitosan, and (C) 360 cP chitosan. Each bar represents mean ± standard deviation of triplicate analyses. In each panel, bars sharing the same letter in a group of bars are not significantly different ($P > 0.05$) from one another.

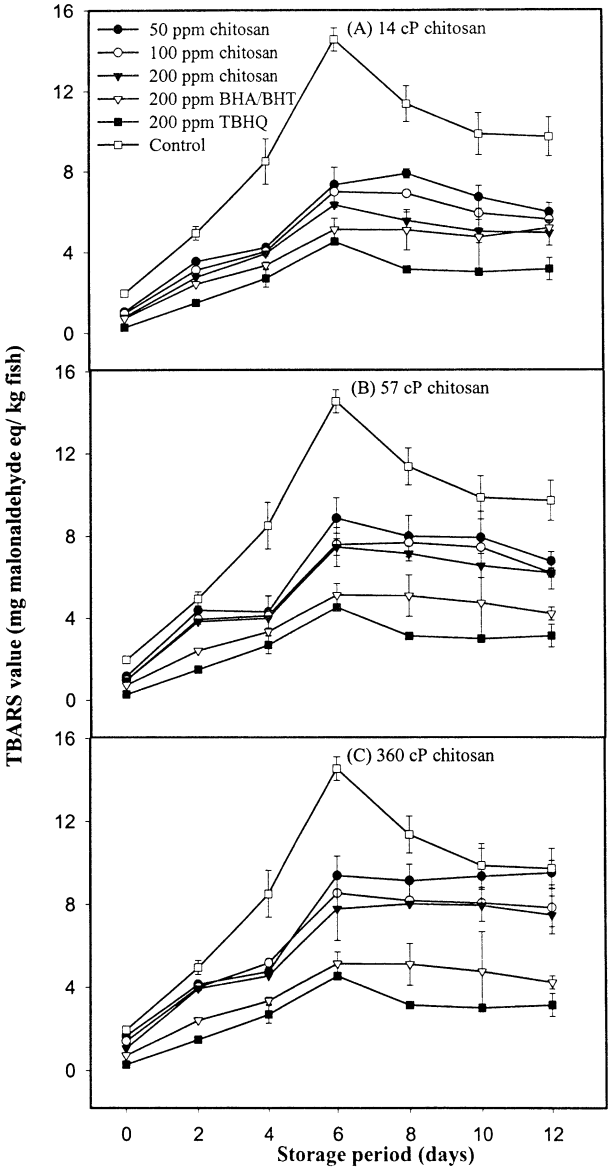

Fig. 3. Effect of chitosans of different viscosity and commercial antioxidants on 2-thiobarbituric acid reactive substances (TBARS) formation in a cooked comminuted herring model system. (A) 14 cP chitosan, (B) 57 cP chitosan, and (C) 360 cP chitosan.

Tang, 1998; Winterowd & Sandford, 1995). The varying antioxidant effect of chitosans of different viscosity in cooked comminuted fish model systems may be attributed to the molecular weight differences which determine the chelation of metal ions. In their charged state, the cationic amino groups of chitosans impart intramolecular electric repulsive forces, which increase the hydrodynamic volume by extended chain conformation (Anthonsen et al., 1993). Perhaps this phenomenon may be responsible for lesser chelation by high viscosity (high molecular weight) chitosans. Furda (1990) has reported that the degree of polymerization of the glucosamine unit is a major factor determining the viscosity of chitosans. Thus the degree of deacetylation is another factor that may be involved in chelaton ability of chitosans (see Table 2).

Xue, Yu, Hirata, Terao, and Lin (1998) reported that the liposoluble marine polysaccharides, hexanoyl chitin and *N*-benzoylhexanoyl chitosan solutions, retard the accumulation of hydroperoxides from methyl linoleate by effectively trapping peroxy radicals in organic solvents when the radical chain reaction had been initiated by 2,2′-azobis (2,4-dimethylvaleronitrile). Moreover, Xue et al. (1998) reported that water-soluble chitosans may chelate metals or combine with lipids to display a significant antioxidative effect. The effectiveness of chitosan treatment on the inhibition of oxidation of beef was studied by Darmadji and Izumimoto (1994) who observed that the addition of 1% chitosan resulted in a 70% reduction in TBARS values of meat after 3 days of storage at 4C. The effect of N-carboxymethylchitosan (NCMC) in preventing warmed-over flavour (WOF) in uncured meat was studied by St. Angelo and Vercellotti (1989) who reported 93 and 99% inhibition of TBARS

Table 2
Polyunsaturated fatty acids (weight%) of lipids of cooked comminuted herring[a]

Fatty acid	Weight%
18:3ω3	1.20±0.03
20:5ω3	11.3±0.16
22:4ω6	1.76±0.02
22:5ω3	3.14±0.03
22:6ω3	31.3±0.07

[a] All values are means of three determinations±standard deviation.

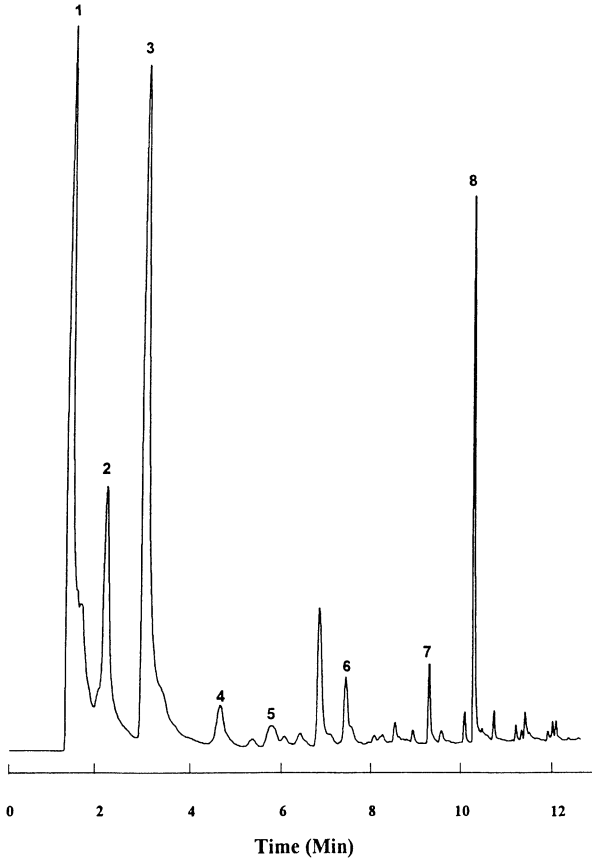

Fig. 4. Gas chromatogram of the headspace volatiles of an untreated cooked comminuted herring sample (control) after 6 days of storage at 4±1 °C. Peaks are: (1) formaldehyde; (2) acetaldehyde; (3) propanal; (4) iso-butanal; (5) butanal; (6) pentanal; (7) hexanal; and (8) heptanal.

Table 3
Content of propanal (P) and total volatiles (TV) (mg/kg fish) of cooked comminuted herring fish treated with different viscosity chitosans at 200 ppm level and stored at 4±1 °C[a]

Treatment	Storage days							
	Day 0		Day 4		Day 8		Day 12	
	P	TV	P	TV	P	TV	P	TV
Control	14.9±1.6e	62.9±3.4e	23.5±1.9e	78.9±0.6e	30.3±2.3e	91.6±1.2e	28.4±3.1d	84.3±2.4e
BHA/BHT	1.9±0.7b	19.7±2.3b	5.7±0.8b	32.4±1.3b	6.9±0.7b	39.3±2.4b	6.7±0.9b	37.4±1.2b
TBHQ	0.9±0.0a	12.5±1.0a	2.9±0.8a	19.4±0.7a	3.4±1.0a	19.2±0.9a	3.4±0.6a	20.3±0.2a
14 cP Chitosan	2.1±0.5b	20.3±1.4b	7.5±1.1c	40.6±0.9b	9.2±0.7c	47.8±1.6c	6.3±1.1b	36.2±0.9b
57 cP Chitosan	3.9±0.0c	27.2±0.6c	7.3±1.0c	40.9±1.3c	11.7±0.9d	55.0±2.4d	6.9±0.8b	43.4±0.2c
360 cP Chitosan	6.9±1.1d	34.3±0.6d	10.5±0.0d	52.4±0.7d	10.9±0.5cd	52.2±0.6d	13.8±0.5c	59.3±1.1d

[a] Results are expressed as mean±standard deviation of three determinations. Values with the same letters within each column are not significantly different ($P>0.05$). BHA/BHT were used at 100+100 ppm level.

and hexanal content in ground beef, respectively. Similarly, Shahidi (1995) reported that NCMC and its derivatives were effective in controlling the oxidation and flavour deterioration of pork during storage for nine days at refrigerated temperatures.

4. Conclusions

Cooked comminuted samples of herring flesh treated with chitosans of different viscosities showed lower peroxide vales, TBARS and total volatile aldehydes than control samples of three chitosans of different viscosity; low viscosity chitosan (14 cP) exhibited the strongest antioxidative effect. These findings have also demonstrated that chitosan extracted from crab processing waste may be considered as a potential natural antioxidant for stabilizing lipid containing foods.

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