JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chitosan as an Edible Invisible Film for Quality Preservation of Herring and Atlantic Cod

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The effect of chitosan with different molecular weights as coatings for shelf-life extension of fresh fillets of Atlantic cod (*Gadus morhua*) and herring (*Clupea harengus*) was evaluated over a 12-day storage at refrigerated temperature (4 ± 1 °C). Three chitosan preparations from snow crab (*Chinoecetes opilio*) processing wastes, differing in viscosities and molecular weights, were prepared; their apparent viscosities (360, 57, and 14 cP) depended on the deacetylation time (4, 10, and 20 h, respectively) of the chitin precursor. Upon coating with chitosans, a significant ($p \leq 0.05$) reduction in relative moisture losses of 37, 29, 29, 40, and 32% was observed for cod samples coated with 360 cP chitosan after 4, 6, 8, 10, and 12 days of storage, respectively. Chitosan coating significantly ($p \leq 0.05$) reduced lipid oxidation as displayed in peroxide value, conjugated dienes, 2-thiobarbituric acid reactive substances and headspace volatiles, chemical spoilage as reflected in total volatile basic nitrogen, trimethylamine, and hypoxanthine, and growth of microorganisms as reflected in total plate count in both fish model systems compared to uncoated samples. The preservative efficacy and the viscosity of chitosan were inter-related; the efficacy of chitosans with viscosities of 57 and 360 cP was superior to that of chitosan with a 14 cP viscosity. Thus, chitosan as edible coating would enhance the quality of seafoods during storage.

KEYWORDS: Chitosan; edible invisible film; lipid oxidation; herring; cod; moisture loss; coating; seafood; viscosity

INTRODUCTION

Health benefits associated with long-chain $n-3$ polyunsaturated fatty acids have stimulated interest for increased consumption of seafoods. However, seafood products are highly susceptible to quality deterioration caused by oxidation of their highly unsaturated fatty acids, catalyzed by the presence of high concentrations of hematin compounds and metal ions in the fish muscle (*1*). This problem may occur in both fatty and lean fish species. In some cases it has been observed that lipid oxidation may be even more severe in lean fish than fatty fish (*2*). A linkage between phospholipid hydrolysis and lipid peroxidation in low-fat fish muscle during low-temperature storage has been reported by Han and Liston (*3*). Thus, development of undesirable off-flavors and potentially toxic reaction products as well as loss of essential fatty acids and fat soluble vitamins in products is of concern (*4*, *5*). Furthermore, seafood quality is highly influenced by autolysis, contamination by and growth of microorganisms, and loss of protein functionality (*6*, *7*). Autolysis of fish results in the formation of peptides and free amino acids, all of which act as suitable nutrients for microbial growth and production of biogenic amines, which are known to affect the safety of fish meat (*8*, *9*). Cold storage and freezing do not always completely suppress quality deterioration of seafoods. Reactions leading to oxidative and enzymatic changes and protein degradation may still proceed under chilled storage conditions (*7*, *10*). Synthetic preservatives such as antioxidants, chelating agents, and antimicrobial compounds may be added to food products to improve their shelf life. However, growing consumer demand for foods devoid of synthetic antioxidants and preservatives has focused efforts in the discovery of new natural preservatives (*5*).

Chitosans have been identified as versatile biopolymers for a broad range of food applications (*11*). Chitosan may be produced from chitin, present in the exoskeleton of arthropods such as insects, crabs, shrimps, lobsters, and certain fungal cell walls. The use of chitosan in food applications is particularly promising because of its "biocompatibility" and nontoxicity. In 1992, Japan's Health Department approved chitin and its derivatives as functional food ingredients (*12*). The characteristics of chitosan, with respect to its nondigestibility, biodegradability, and bland taste, make it an excellent choice as a food additive component, particularly in the preparation of low-calorie foods (*13*). Due to its ability to bind lipids, chitosan may be considered to have a negative caloric value (*14*, *15*). Japan produces dietary cookies and noodles enriched with chitosan because of its hypolipidemic and hypocholesterolemic effects

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10.1021/jf011693l CCC: $22.00
Published on Web 07/25/2002

(*16*, *17*). The capacity of chitosan for binding metal ions such as those of copper, chromium, zinc, lead, vanadium, and iron has been demonstrated (*16*). Recently, it has been shown that chitosan and its derivatives exert antimicrobial effects against different groups of microorganisms such as bacteria, fungi, and yeast (*18*−*20*).

Chitosan-based materials may be used as edible films or coatings due to their unique property of increased viscosity upon hydration. Furthermore, chitosan films are tough, long-lasting, flexible, and very difficult to tear. Most mechanical properties of chitosan films are comparable to those of many medium-strength commercial polymers (*21*). Kittur et al. (*22*) have shown that chitosan films have moderate water permeability and could increase the storage life of fresh produce and foodstuff with high water activity. Studies on the extension of shelf life of foodstuff by chitosan coating have so far been limited to fruits and vegetables. Despite the many current and potential applications of chitosan, fundamental studies on chitosan coating of fish and seafood are lacking. On the other hand, even though chitosan has a wide range of viscosity when prepared, its efficacy as a coating material has not been investigated. Kaye (*23*) reported that the viscosity of chitosan was a major factor in determining its properties in different applications.

The objectives of this study were to prepare chitosans from snow crab (*Chinoecetes opilio*) processing discards using different deacetylation times in order to assess lipid oxidation, chemical spoilage, and microbial spoilage of chitosan-coated Atlantic cod (*Gadus morhua*), as a lean fish, and herring (*Clupea harengus*), as a fatty fish, during chilled storage. Evaluation of the preservative efficacy of different viscosity chitosans in the above model systems was also intended.

MATERIALS AND METHODS

Materials. Fresh samples of crab processing discards, composed of intact cephalothorax and abdominal exoskeleton, were collected from local sources in Newfoundland and subsequently washed with distilled water and vacuum packed in Whirl-pack plastic bags (Eastern Papaer, St. John's, NF) and kept at −60 °C (Ultralow, Revco, Inc., West Columbia, SC) until used. Fresh samples, namely, cod and herring, were acquired from a local source in Newfoundland and immediately cleaned, gutted, filleted, and deskinned. Processed fillets were vacuum packed in Whirl-pack plastic bags (Eastern Paper) and kept at −60 °C until used. Reagents, namely, 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (TMP), trimethylamine (TMA), hypoxanthine (Hx), xanthine oxidase (XO), picric acid (PA), and diisopropylethylamine (DIPEA), 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). Plate count agar and peptone were obtained from Difco Laboratories (Detroit, MI). 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 Ltd. (St. John's, NF).

Methods. *Preparation of Chitosans and Evaluation of Their Characteristics.* Chitin was prepared from crab processing discards using essentially the methods of Mima et al. (*24*) and Shahidi and Synoweiki (*25*). Chitosan was subsequently prepared by alkali treatment of chitin using 10 volumes of 50% (w/v) NaOH in distilled water at 100 °C for 4, 10, and 20 h.

Moisture, total nitrogen, and ash contents of chitosan preparations were determined (*25*). Apparent viscosity (at 2 Nm^{-2}) of a 1% (w/v) solution of chitosan in 1% (v/v) acetic acid at 25 °C was measured using a rotational viscometer (Cole-Parmer Co., Vernon Hills, IL). Measurements were made in triplicate using a No. 5 spindle at 50 rpm on solutions at 25 °C with values reported in centipoise (cP) units. The degree of acetylation of chitosans was measured according to the picric acid method of Neugebauer et al. (*26*). The fraction of amino groups acetylated (degree of *N*-acetylation, da) was calculated using the equation $da = m - 161n/m + 42n$, where m = weight of chitosan sample (mg), n = mmol of picric acid eluted from sample, 161 = molecular weight of d-glucosamine unit, and 42 = molecular weight of *N*-acetyl-D-glucosamine − molecular weight of d-glucosamine.

The molecular weight of chitosan was expressed as the viscosity molecular weight (M_v) using a ViscoTek model Y-500 relative viscometer (Viscotek Co., Houston, TX). Chitosan solution in 0.1 M 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 determination of the specific viscosity (η_{sp}) at 25 °C. Specific viscosity was determined as follows: $\eta_{sp} = (\eta - \eta_s)/\eta_s$, where η and η_s were the solution and 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 $M_v = [\eta/k]^{1/a}$, where constants of k and a are 1.81×10^{-5} and 0.93, respectively (*22*, *27*, *28*).

Preparation of Chitosan Coating Solution and Treatment of Fish. Each chitosan (10 g) was added to deionized water (1 L) containing 10 g of glacial acetic acid. The mixture was stirred at 40 °C for 1 h. The solutions in beakers were placed on a hotplate/magnetic stirrer, and glycerol was added at a level of 1.0 mL/g of chitosan as a plasticizer and stirred for 10 min. The resultant chitosan coating solution was filtered through a Whatman no. 3 filter paper to remove any undissolved particles. For coating of fish (herring and cod) with chitosan solutions, each fish fillet (5 × 15 cm) was immersed in the chitosan solution (5 °C) for 30 s and then allowed to stand for a 2-min period followed by a second immersion in the chitosan solution for 30 s. Fish fillets of the control group were left untreated. Coated fish samples were dried at 40 °C for 2 h in a forced air oven (Fisher Isotemp 300, Fair Lawn, NJ) in order to form the edible coatings and then stored at 4 ± 1 °C for subsequent quality assessment. All sample treatments were carried out in a cold room (4 ± 1 °C). Chemical and microbiological analyses were performed at 2-day intervals to determine the overall quality of fish.

Determination of Proximate Composition of Fish. Determinations of moisture, crude protein, and ash contents of the two types of fish were carried out according to the AOAC (*29*) procedures. Meanwhile, total lipids were determined by their extraction into a mixture of chloroform and methanol as described by Bligh and Dyer (*30*). The extracted lipids were quantified and then stored at −60°C (Ultralow, Revco, Inc.) for subsequent determination of peroxide value (PV), conjugated dienes (CD), and fatty acid composition.

Analysis of Lipid Fatty Acid Composition. Fatty acid composition of fish lipids was determined after their conversion to fatty acid methyl esters (FAMEs) as described by Wanasundara and Shahidi (*31*). 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.

Determination of Peroxide Value (PV), Conjugated Dienes (CD), and 2-Thiobarbituric Acid Reactive Substances (TBARS). The AOCS (*32*) method was used for determination of the PV of oil using 2 μg of samples. The PV was expressed as the uptake of milliequivalents of active oxygen (i.e., peroxide) per kilogram of oil.

The CD value of oil samples was measured according to the IUPAC (*33*) method using 0.02−0.04 g of material. Absorbance (A) readings were recorded at 234 nm and used for calculation of CD values from the equation $CD = (A/C)d$, where C is the concentration in grams of oil in 100 mL isooctane solvent and d is the length of the cell in centimeters.

Fish samples were analyzed for their TBARS values over a 12-day period according to the method of Siu and Draper (*34*) as described by Shahidi and Hong (*35*). The absorbance of the resulting pigment between the extract and the TBA reagent was read at 532 nm using a Hewlett-Packard diode array spectrophotometer (model 8452A, Hewlett Packard Co., Mississauga, ON). TBARS values were calculated from

absorbance values by using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde. Inhibition of TBARS formation was determined using the corresponding data for the control and treated samples, using the equation % inhibition = 100[1 − (TBARS of treated sample − TBARS of treated sample)/TBARS of untreated sample].

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.) 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.

The procedure for headspace (HS) analysis of samples has already been described elsewhere (*36*). 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.

Determination of Trimethylamine and Hypoxanthine. TMA determination was performed on trichloroacetic acid (TCA) extracts of fish using the modified picric acid methods described by Dyer (*37*) and Woyewoda et al. (*38*). The absorbance of the organic phase mixed with picric acid reagent was read at 410 nm using a Hewlett-Packard diode array spectrophotometer (model 8452A, Hewlett Packard Co.). TMA content (micrograms) was calculated by multiplying the absorbance reading by a factor of 73.36, determined from a standard line prepared using standard TMA. Final TMA content was expressed as milligrams of TMA-N per 100 g of fish using the equation $\{T[V_1 + (0.01MW)]\}/V_2W \times 10$, where T = equivalent TMA in μg determined from a standard curve, M = moisture of fish sample expressed in percent, V_1 = volume (mL) of TCA added for 1:2 extraction, V_2 = volume (mL) of extract added to test tube, and W = weight (g) of fish used in 1:2 extraction.

Monitoring of Hx levels in fish muscle during the storage was carried out according to the modified enzymatic method of Woyewoda et al. (*39*). The treated extracts in perchloric acids, following addition of xanthine oxidase, were incubated at 37 ± 1 °C for 30 min, and absorbance was read at 290 nm using a Hewlett-Packard diode array spectrophotometer (model 8452A, Hewlett Packard Co.). Hx content (micrograms) was calculated by multiplying the absorbance reading by a factor of 63.69, determined from a standard line prepared using Hx as a standard. Final Hx content was expressed as micromoles of Hx per gram of fish using the following equation: $\{H[V_1 + (0.01MW)]-(V_2 + V_3)\}/V_4WV_3G$, where $H = \mu$g of Hx determined from a standard curve, M = moisture content of fish expressed in percent, V_1 = volume (mL) of perchloric acid used in 1:4 extraction, V_2 = volume (mL) of KOH/phosphate buffer used for neutralization, V_3 = volume (mL) of extract neutralized by KOH/phosphate buffer, V_4 = volume (mL) of sample extract added to test tube, W = weight (g) of sample used in 1:4 extraction, and G = gram molecular weight of Hx, i.e., 136.1.

Determination of Total Volatile Basic-Nitrogen (TVB-N). TVB-N was determined according to a modified micro-Kjeldahl distillation technique described by Cobb et al. (*40*). The TVB-N was expressed as milligrams of nitrogen per 100 g of sample, determined by micro-Kjeldahl, from the following equation: TVB-N = $\{(V_1 - V_2)N \times 100 \times 14 \times 50\}/W \times 5$, where V_1 = volume (mL) of H_2SO_4 used for sample, V_2 = volume (mL) of H_2SO_4 used for blank, N = normality of H_2SO_4, and W = weight of sample in grams.

Quality Assessment Related to Microbial Spoilage. A sample of 11 g of fish with 99 mL of sterilized peptone water (10 g of peptone and 5 g of NaCl in 1 L of distilled water, pH 7.2−7.3) was put into sterilized plastic bags (Seward Medical Stomacher 400 bags) and homogenized in a laboratory stomacher (type BA 7021, Seward Medical, London, U.K.) for 30 s. From this mixture three, four, and five dilutions were obtained by mixing with peptone water. A 1 mL aliquot from each diluent was applied on a sterilized standard plate count agar (23.5 g of agar in 1 L of distilled water). The plates were triplicated and incubated at 20 °C for 72 h. Afterward, the colonies grown on the plates were counted using the colony counter, and total aerobic psychrotrophic plate count values were indicated as colony-forming units (CFU) per gram of fish.

Table 1. Characteristics of Three Different Types of Chitosans (I–III) Prepared from Crab Shell Waste[a]

property	I	II	III
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
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
M_v[b] (Da)	1.8×10^6	9.6×10^5	6.6×10^5

[a] Results are expressed as mean value of three determinations ± standard deviation. Chitosans I–III were prepared over 4, 10, and 20 h deacetylation times, respectively, using a 50% NaOH solution at 100 °C. The color of all chitosans so prepared was cream white. [b] Viscosity molecular weight.

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) was performed, and differences in mean values were determined using Tukey's procedures of statistical analysis system (*41*). Microbiological results were compared using the $\log_{10}$ transformation of counts; data were analyzed using the General Linear Models Procedure (PROC GLM) of SAS (*41*), and significant differences were determined using least-squares means ($p \leq 0.05$). Linear regressions were carried out to determine if there was a correlation between certain parameters.

RESULTS AND DISCUSSION

Characteristics of Chitosans Extracted from Crab Processing Discards. The characteristics of chitosans (samples I, II, and III) prepared from crab chitin using different deacetylation times of 4, 10, and 20 h are listed in **Table 1**. The chitosans so prepared exhibited variations in their viscosities, which were closely related to the duration of deacetylation time; the highest viscosity was observed for shorter deacetylation times (360, 57, and 14 cP for 4, 10, and 20 h of deacetylation time, respectively). The nitrogen contents of chitosans were also dependent on deacetylation time and were 7.55, 7.63, and 7.70%, respectively, for samples prepared over 4, 10, and 20 h (samples I, II, and III, respectively), thus confirming a more effective deacetylation over longer periods. As shown in **Table 1** there was a substantial difference in molecular weights measured by the viscometric method among products I−III, which reflects depolymerization of chitosans upon prolonged period of deacetylation. However, molecular weight differences were less than those observed for viscosity values, and molecular weight was not linearly related to viscosity.

The efficacy of demineralization is important for the quality of the isolated chitosan. Thus, a low ash content (0.25−0.30%) in all three chitosans indicates the effectiveness of the method used for demineralization. In this process, it is important that the amount of acid be stoichiometrically equal to or greater than all minerals presents in the shells to ensure complete reaction with them (*25*).

The degrees of deacetylation were 86.4, 89.3, and 91.3% for chitosans extracted from chitin due to changing of deacetylation time from 4 to 10 to 20 h, respectively. The large positive charge density due to the high degree of deacetylation (86.4−91.3%) makes crab chitosan unique for industrial applications. Li et al. (*42*) and Muzzarelli (*43*) reported that the term chitosan should

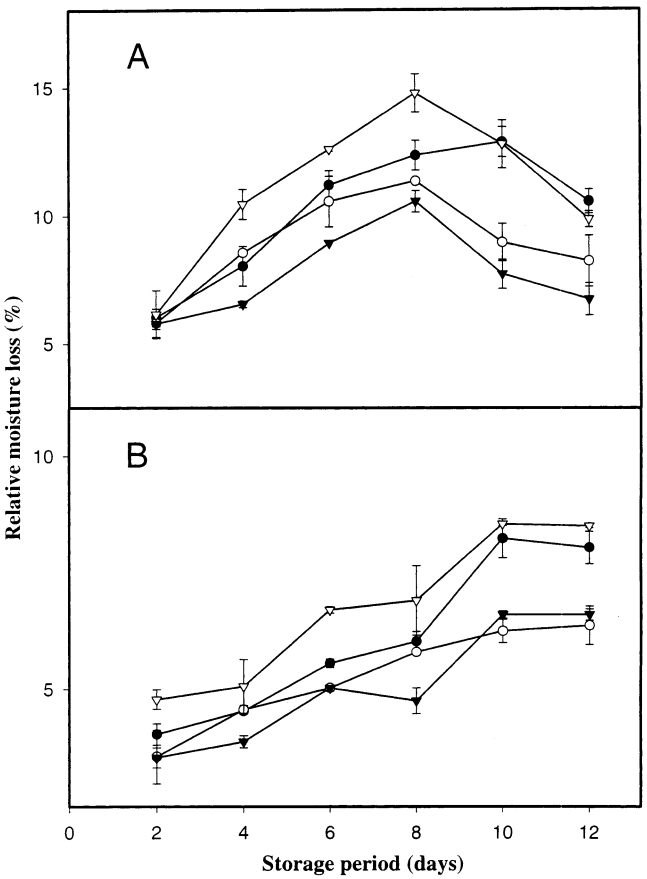

Figure 1. Effect of chitosan coating on relative moisture loss (percent) of cod (A) and herring (B) stored at 4 ± 1 °C: (●) 14 cP chitosan; (○) 57 cP chitosan; (▼) 360 cP chitosan; (▽) control uncoated.

be used when the degree of deacetylation is above >70% and the nitrogen content in the product is >7 wt %; both of these conditions were met for chitosans I−III. **Table 1** shows that the viscosity average molecular weight (M_v) of chitosan decreased with increasing deacetylation time. However, a very high degradation rate of chitin polymers was not observed in this study by increasing the deacetylation time from 10 to 20 h, perhaps due to intermittent washing of the intermediate product in distilled water two or more times during the alkali treatment. The procedure used for deacetylation in this investigation was similar to that of Mima et al. (*24*), who produced 96% deacetylation with three alkali washing treatments involving a shorter time (1 h) at a somewhat higher temperature of 110 °C.

Effect of Chitosan Coating on Relative Moisture Loss of Fish. The relative moisture loss of chitosan-coated cod and herring fillets stored at 4 ± 1 °C is summarized in **Figure 1**. The pattern of relative moisture loss in cod was different from that of herring, being lower in herring fillets than in cod fillets regardless of the type of chitosan employed (**Figure 1**). Moisture in cod fillets was evaporated much more rapidly during the first few days of storage, but decreased at day 6 of storage (except for the 14 cP chitosan-coated sample). According to Pham and Willix (*44*), the desiccated surface layer developed during cold storage produces a further resistance to mass transfer in the case of biological substances, thus perhaps bringing about a reduction in relative moisture loss in cod samples after a certain period of storage.

Cod samples coated with 57 and 360 cP chitosans exhibited a similar pattern of relative moisture loss as compared to that of the sample coated with 14 cP chitosan, but the latter exhibited a higher moisture loss after day 8 of storage, similar to that of the control. A significant ($p \leq 0.05$) reduction in relative moisture loss, by 37, 29, 29, 40, and 32%, over that of uncoated cod samples was observed for cod samples coated with 360 cP chitosan after 4, 6, 8, 10, and 12 days of storage, respectively. Corresponding values for 57 cP chitosan-coated cod samples were 18, 16, 23, 30, and and 17%. Water vapor permeability has been shown to be dependent on the relative polarity of the carbohydrate polymers. The more polar films tend to be more ordered and less porous, hence less water vapor permeable (*45*); this may explain comparatively lower relative moisture loss in 360 cP chitosan-coated cod samples as compared to that of 14 and 57 cP chitosan-coated cod samples. Water vapor transfer through hydrophilic coating/film by sorption and diffusion is affected by many factors (*46*). Therefore, the mechanism involved in the water vapor transfer through the chitosan coatings may require further studies.

Unlike cod, herring samples did not show a clear decrease in their relative moisture upon coating, perhaps due to their higher lipid content. There was no statistically significant ($p > 0.05$) difference in relative moisture loss of herring fillets subjected to different coatings until day 6 of storage, after which a significant ($p \leq 0.05$) effect was observed for samples coated with 57 and 360 cP chitosans compared to that of the uncoated herring. Both uncoated and 14 cP chitosan-coated herring samples exhibited the highest relative moisture loss and became dry on the surface (visual observation) after a 10-day storage period.

The water vapor barrier properties of edible films and coatings serve as important factors in their selection for use in several food systems. Stuchell and Krochta (*47*) reported efficient protection of salmon against water loss using a coating composed of a mixture of whey protein and acetylated monoacylglycerols. El Ghaouth et al. (*19*) used edible chitosan coatings to reduce water loss from cucumber and bell pepper. Coatings are generally composed of polysaccharides, proteins, lipids, or combinations thereof. At least one component should be a high molecular weight, long-chain polymer, to yield film or coating matrices with appropriate cohesive strength when deposited from a suitable solvent; chitosan possesses such a cohesive property due to its viscosity characteristics in aqueous solutions.

Quality Assessment Related to Lipid Oxidation. Assessment of lipid oxidation during refrigerated storage of fish fillets (moisture, protein, lipid, and ash contents were 73.53 ± 0.38, 13.21 ± 0.02, 12.43 ± 0.13, and 0.35 ± 0.01% for herring and 80.92 ± 0.93, 15.44 ± 0.07, 1.25 ± 0.04, and 0.52 ± 0.00% for cod, respectively) coated with chitosans was achieved by measuring PV and CD value for primary lipid oxidation products and TBARS and static HS gas chromatographic analysis for secondary lipid oxidation products. Due to the limitations of each of these assays, it is often recommended that more than one method be used to evaluate the oxidative status of foods and biological matters (*48*).

Effect of Chitosan Coating on Primary Lipid Oxidation Products of Fish. The effect of chitosan coating on changes of PV of fish lipids is depicted in **Figure 2**. Results indicate that the PV of the uncoated herring sample increases progressively up to day 10 of storage. On day 12 of storage, PVs of samples coated with chitosans were ∼48−63% lower than that of the control (**Figure 2**). Meanwhile, the PVs of all chitosan-treated herring samples were <10 mequiv/kg of oil up to day 8 of storage, but values for uncoated samples exceeded this level even after day 4 of storage. However, variable results were

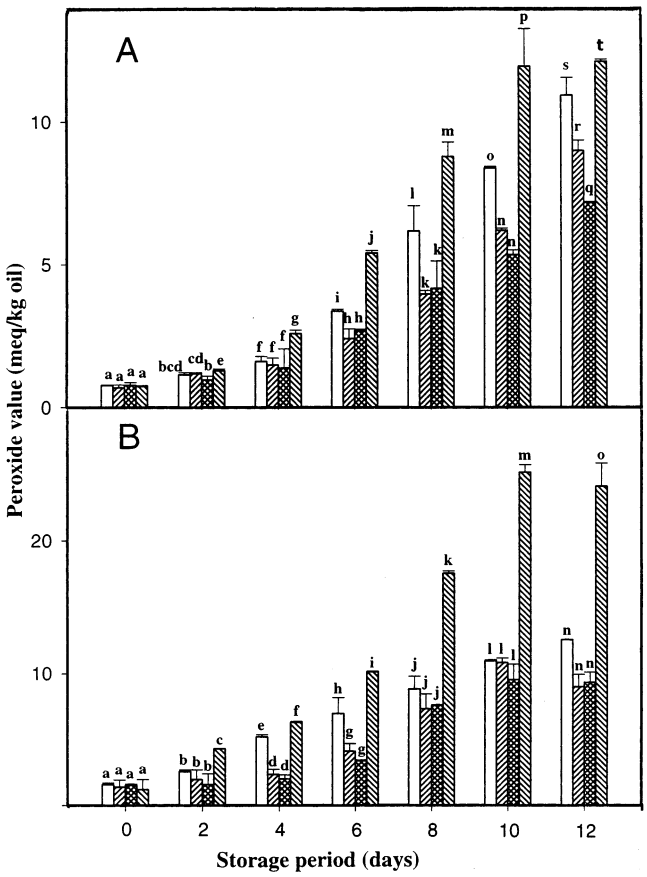

Figure 2. Effect of chitosan coating on peroxide value of cod (A) and herring (B) samples stored at 4 ± 1 °C. Within each grouping of bars, bars represent, from left to right, 14 cP chitosan, 57 cP chitosan, 360 cP chitosan, and control uncoated. Each bar represents the mean ± standard deviation of triplicate analyses. In each panel, bars sharing the same letter in a group of bars are not significantly different ($p > 0.05$) from one another.

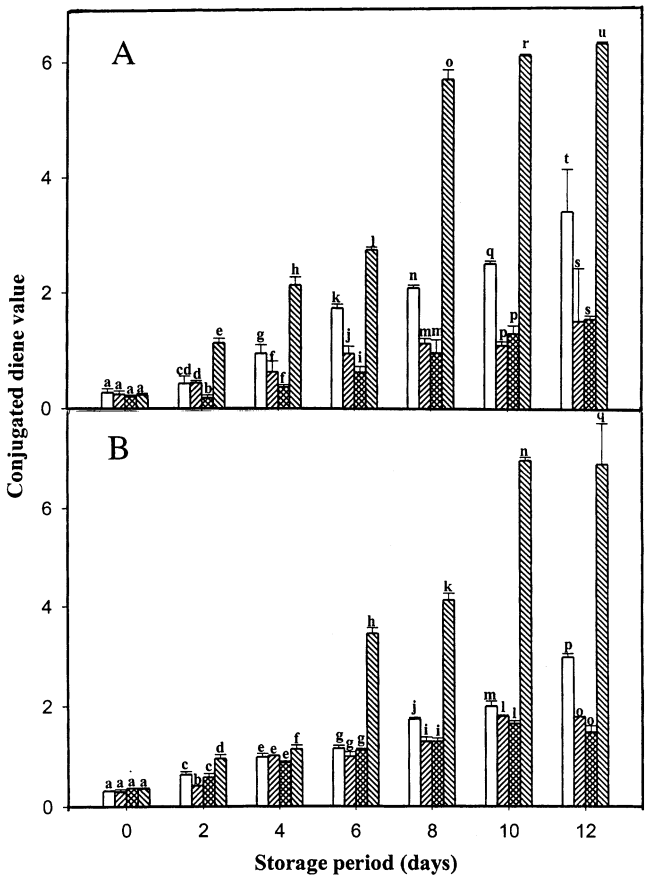

Figure 3. Effect of chitosan coating on conjugated diene values of cod (A) and herring (B) samples stored at 4 ± 1 °C. Within each grouping of bars, bars represent, from left to right, 14 cP chitosan, 57 cP chitosan, 360 cP chitosan, and control uncoated. Each bar represents the mean ± standard deviation 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.

observed for 14 cP chitosan-treated herring samples, where significantly ($p \leq 0.05$) different PVs were obtained compared to the other two types (57 and 360 cP) of chitosan-coated herring samples after 4, 6, and 12 days. The treatment of herring samples with 57 and 360 cP chitosans effectively decreased the production of peroxides over the entire storage period.

As shown in **Figure 2**, similar and lower PVs were observed for all chitosan-coated cod samples compared to that of the control. However, the inhibitory effect of chitosans against oxidation was viscosity-dependent as evidenced by lower PVs for samples coated with 57 and 360 cP chitosans compared to that coated with 14 cP chitosan. Furthermore, PVs of coated cod samples increased over time, but at a slower rate compared to the control. The observed differences in PVs of samples coated with 57 and 360 cP chitosan were either marginal or insignificant ($p > 0.05$) over the entire storage period, except after 12 days of storage. Meanwhile, the 360 cP chitosan-coated cod sample showed a PV ~ 40% lower than that of the uncoated samples after 12 days of storage. The PV (11.95 mequiv of peroxides/kg of oil) of uncoated cod samples exceeded 10 mequiv of peroxides/kg of oil, the maximum level generally regarded as acceptable, after 10 days of storage.

In concurrent studies CD values of chitosan-coated herring and cod samples were also monitored as shown in **Figure 3**. The pattern of CD values for herring and cod samples was similar to that of peroxide values of the corresponding samples with significantly ($p \leq 0.05$) lower CD values for coated samples. Furthermore, herring and cod samples treated with 14 cP chitosan showed higher CD values than those treated with 57 and 360 cP chitosans under similar experimental conditions. Sklan et al. (*49*) determined the content of conjugated dienes, trienes, and tetraenes, referred to as total conjugated products of oxidation, in total lipid extracts of turkey meat during a 60-day storage at 4 °C. Similar studies were carried out by Ahmad and Augustin (*50*) for fried fish during a 40-day storage at 60 °C. These authors indicated that the level of both dienes and trienes increased with increasing storage time. Medina et al. (*51*) reported that CD values in cooked tuna stored at both 40 and 60 °C were increased continuously during 4 days of storage.

CD values of chitosan-coated herring samples increased by ~4−9-fold at the end of a 12-day storage period, whereas the increase for the uncoated herring sample was 19-fold. In cod samples corresponding values were 6−12 and 27-fold, respectively. Storage of herring fillets beyond 10 days resulted in a reduction in both PV and CD of uncoated and 57 and 360 cP chitosan-treated samples, perhaps due to the breakdown of hydroperoxides or their interaction with muscle proteins.

The polyunsaturated fatty acid (PUFA) composition of total lipids of herring and cod fillets is shown in **Table 2**. The high PV and CD of lipids from herring and cod fillets during initial stages of storage of fillets may be attributed to their high content of PUFAs, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Moreover, characteristic features of lipid oxidation in fish flesh are influenced by factors such as lipid

Table 2. Polyunsaturated Fatty Acids (Weight Percent) of Herring and Cod Fillet Lipids[a]

fatty acid	cod	herring
18:3ω3	0.47 ± 0.11	1.20 ± 0.03
20:5ω3	9.93 ± 0.01	11.3 ± 0.16
22:4ω6	0.93 ± 0.05	1.76 ± 0.02
22:5ω3	1.60 ± 0.13	3.14 ± 0.03
22:6ω3	27.3 ± 0.18	31.3 ± 0.07

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

content, the level of microsome-associated lipid oxidation system present, the level of heme compounds, and the presence of metal ions.

As expected, both PVs and CD values were higher in herring samples as compared to those of cod throughout the storage period. The herring fillet has a large proportion of dark muscles, which are generally more prone to lipid oxidation than cod muscles, which are generally light. This is probably not only because dark herring muscle has a higher content of total lipids, including phospholipids per unit weight of tissue, but also because it contains higher amounts of pro-oxidant heme pigments and released metal ions (*1*, *52*). Herring and cod samples treated with the 14 cP chitosan showed higher PVs and CD values as compared to those treated with 57 and 360 cP chitosans. Furda (*53*) reported that the degree of polymerization of glucosamine units is directly proportional to the viscosity of chitosan solutions; hence, a better protection may be rendered to fish fillets by the higher molecular weight chitosans.

Chitosan films exhibited an extremely good barrier to oxygen permeation (*21*), and chitosan coating applied directly on the surface of fish meat might act as a barrier between fish meat and its surrounding, thus retarding diffusion of oxygen to the fish meat surface. Chen and Hwa (*54*) observed that tensile strength, tensile elongation, and enthalpy of the membrane prepared from high molecular weight chitosans were higher than those of low molecular weight chitosans. Furthermore, these authors reported that the permeability characteristics of high molecular weight chitosans (high viscosity) were lower than those of their low molecular weight counterparts (low viscosity). The results of the present study indicate that chitosan coating is effective in retarding the production of primary lipid oxidation products in herring fillets stored at 4 ± 1 °C. These results are in agreement with those of Stuchell and Krochta (*47*), who reported the effectiveness of whey protein isolate as an edible coating material for reduction of primary lipid oxidation products in salmon.

Effect of Chitosan Coating on Secondary Lipid Oxidation Products of Fish. The contents of TBARS in chitosan-coated herring and cod samples, depicted in **Figure 4**, were significantly ($p \leq 0.05$) lower than those of the uncoated samples throughout the storage period. However, TBARS values of herring samples were always higher than those of cod samples under similar storage conditions. This might originate from the higher content of total lipids and PUFA (12.4 and 48.77%, respectively) in herring as compared to cod (1.2 and 40.24%, respectively) (**Table 2**). Among the three different types of chitosan tested, 360 cP chitosan was most effective in retarding TBARS formation, irrespective of the type of fish tested. This is probably due to the presence of a large number of ionic functional groups, which create strong polymer interactions that restrict the chain motion in high-viscosity chitosans, which results in good oxygen barrier properties. The inhibitory effect of chitosan against

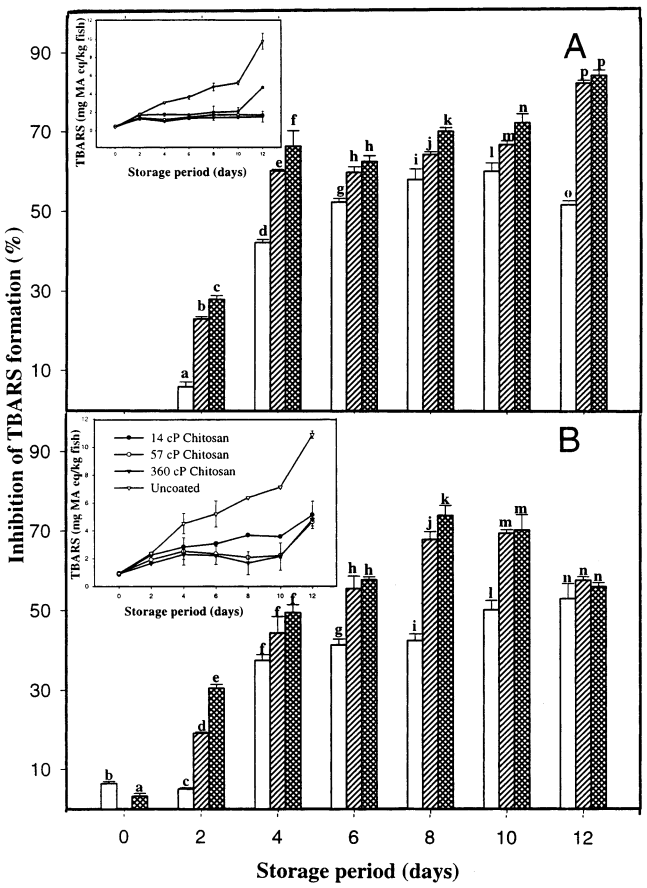

Figure 4. Inhibition of TBARS formation of chitosan-coated cod (A) and herring (B) samples stored at 4 ± 1 °C. Within each grouping of bars, bars represent, from left to right, 14 cP chitosan, 57 cP chitosan, 360 cP chitosan, and control uncoated. Each bar represents the mean ± standard deviation of 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.

herring oxidation increased with storage time, especially after day 4 for higher viscosity (57 and 360 cP) chitosans as compared 14 cP chitosan. However, percentage inhibition of TBARS formation for cod samples increased throughout the entire storage period. Thus, chitosan coating has a marked effect on the inhibition of TBARS in both herring and cod. Use of other edible coating materials for protection of muscle foods against oxidation has been reported in the literature. Wanstedt et al. (*55*) observed that calcium alginate coating served as an effective means for controlling lipid oxidation in ground pork patties as reflected in TBARS values. Corn zein coatings were also found to lower TBARS formation of precooked pork chops during a 9-day refrigerated storage (*56*). Furthermore, carrageenan films successfully inhibited the oxidation of fresh mackerel mince patties as measured by peroxide and TBA values (*57*).

Tables 3 and **4** show the changes of propanal and total volatile aldehydes (TVA) during storage of chitosan-coated herring samples, respectively. **Figure 5** shows the chromatograms of HS volatiles of uncoated and chitosan-coated herring samples after 12 days of storage at 4 ± 1 °C. Of the identified volatiles (formaldehyde, acetaldehyde, propanal, isobutanal, butanal, pentanal, hexanal, and heptanal), formaldehyde and propanal were most prominent. However, in fish muscle formaldehyde may also accumulate as a result of decomposition of trimethylamine oxide by endogenous and microbial enzymes and hence was not considered to arise only from fish lipid

Table 3. Content of Propanal (Milligrams per Kilogram of Fish) in Headspace Volatiles of Chitosan-Coated Herring Samples Stored at 4 ± 1 °C[a]

	storage period						
chitosan	0 days	2 days	4 days	6 days	8 days	10 days	12 days
uncoated	1.15 ± 0.85a	5.93 ± 1.05b	7.48 ± 1.05b	8.58 ± 0.48b	14.03 ± 1.00c	17.58 ± 0.60c	29.32 ± 0.78c
14 cP	1.45 ± 0.53a	4.58 ± 0.30ab	6.15 ± 0.30ab	7.73 ± 0.73b	8.25 ± 0.20b	9.93 ± 0.23b	11.15 ± 1.13b
57 cP	1.15 ± 0.75a	3.88 ± 0.53a	4.93 ± 0.65a	6.23 ± 0.40a	5.70 ± 0.48a	6.05 ± 0.48a	7.09 ± 0.09a
360 cP	1.55 ± 0.60a	3.93 ± 0.65a	4.40 ± 0.55a	5.05 ± 0.35a	4.58 ± 0.60a	5.68 ± 0.33a	7.15 ± 0.13a

[a] Results are expressed as mean ± standard deviation of three determinations. Values with the same letters within each column are not significantly different ($p > 0.05$).

Table 4. Content of Total Volatile Aldehydes (Milligrams per Kilogram of Fish) in Headspace Volatiles of Chitosan-Coated Herring Samples Stored at 4 ± 1 °C[a]

	storage period						
chitosan	0 days	2 days	4 days	6 days	8 days	10 days	12 days
uncoated	14.38 ± 3.50a	28.18 ± 3.35a	40.88 ± 4.83b	44.05 ± 5.03b	58.93 ± 5.88b	69.38 ± 1.73c	87.56 ± 3.11c
14 cP	15.53 ± 1.52a	30.33 ± 6.85a	33.35 ± 6.03ab	36.75 ± 3.98ab	39.75 ± 3.58a	42.05 ± 3.83b	58.17 ± 2.30b
57 cP	14.23 ± 2.47a	22.78 ± 3.15a	31.00 ± 2.93ab	32.08 ± 5.33a	34.40 ± 5.33a	35.98 ± 1.60a	44.03 ± 0.13a
360 cP	14.05 ± 2.65a	24.43 ± 1.03a	28.75 ± 2.50a	32.55 ± 1.58a	34.85 ± 1.85a	36.08 ± 0.88a	46.11 ± 1.33a

[a] Results are expressed as mean ± standard deviation of three determinations. Values with the same letters within each column are not significantly different ($p > 0.05$).

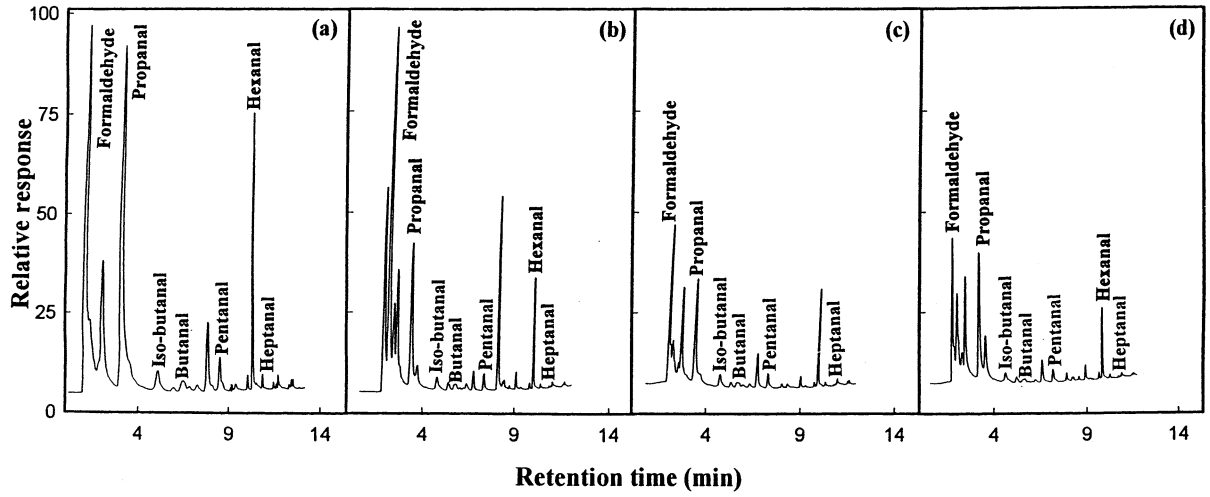

Figure 5. Day-12 gas chromatograms of chitosan-coated herring samples stored at 4 ± 1 °C: (a) uncoated; (b) 14 cP chitosan coated; (c) 57 cP chitosan coated; (d) 360 cP chitosan coated.

oxidation. Propanal is an oxidation product of $\omega-3$ fatty acids and was present at 45.81 and 38.84%, respectively, in both herring and cod. Frankel et al. (*58*) observed that propanal formation in fish oil highly and significantly correlated with the content of $\omega-3$ PUFA ($r = 0.950$, $p < 0.001$). Ota (*59*) showed that acetaldehyde and butanal were present in mackerel, sardine, and flat fish and that their content increased during room temperature storage of samples examined. The content of propanal in the control herring sample on day 0 was 1.2 mg/kg and increased to ~8.0 mg/kg on day 6 and 29 mg/kg on day 12 (**Table 3**). Propanal formation was reduced to different extents due to coating with chitosans. Both 57 and 360 cP chitosans exerted an inhibitory effect on the formation of propanal throughout the storage period. The 360 cP chitosan had the strongest inhibitory effect and reduced the formation of propanal in herring samples by 34, 70, and 76% on days 2, 6, and 12, respectively.

Boyd et al. (*60*) have shown that propanal is present in high amounts when samples of EPA and DHA are heated at 80 °C, and Girard and Durance (*61*) confirmed that propanal was a dominant aldehyde arising from the oxidation of canned pink and sockeye salmon samples. Medina et al. (*73*) observed no induction or lag period for propanal formation during oxidation of canned tuna that was stored at 40 °C for 4 days.

A linear relationship existed between TBARS and propanal content ($r = 0.9127$) and between TBARS and total volatile aldehyde content ($r = 0.9214$) for herring samples. The correlation coefficient ($r = 0.9127$) between propanal and TBARS indicates that propanal may serve as an alternative to TBARS and as a useful indicator for evaluation of oxidation state of seafoods.

Quality Assessment Related to Chemical Spoilage. Postmortem metabolism of nitrogenous compounds is mainly responsible for the gradual loss of fresh quality of fish (*6*). Chemical spoilage of fish samples coated with chitosans during storage was evaluated by measuring changes in the contents of TVB-N, TMA, and Hx.

Effect of Chitosan Coating on Total Volatile Basic Nitrogen Production in Fish. Table 5 shows the effect of chitosan coating on TVB-N production in cod and herring samples stored at 4 ± 1 °C. TVB-N content of treated cod samples increased by ~3−4-fold as compared to a 6-fold increase for the control sample at the end of a 12-day storage period, thus reflecting a 33−50% reduction in the formation of

Table 5. Content of Total Volatile Basic Nitrogen (Milligrams of N per 100 g of fish) of Chitosan-Coated Fish Samples Stored at 4 ± 1 °C[a]

	storage period						
chitosan	0 days	2 days	4 days	6 days	8 days	10 days	12 days
				Cod			
uncoated	9.83 ± 0.95a	11.98 ± 0.15a	18.18 ± 0.14c	29.83 ± 1.15c	37.10 ± 0.99c	41.25 ± 0.08c	53.39 ± 1.15c
14 cP	10.05 ± 0.7a	15.88 ± 0.95b	15.93 ± 1.13b	22.13 ± 1.37b	31.93 ± 1.18b	36.90 ± 1.95b	38.10 ± 0.95b
57 cP	8.13 ± 1.01a	11.55 ± 1.31a	14.45 ± 0.97b	16.75 ± 0.95a	20.88 ± 2.31a	23.18 ± 0.78a	25.33 ± 2.13a
360 cP	8.65 ± 0.95a	9.80 ± 0.94a	11.33 ± 0.86a	15.70 ± 0.19a	20.23 ± 0.09a	20.98 ± 0.93a	21.94 ± 0.68a
				Herring			
uncoated	8.65 ± 1.03a	10.70 ± 0.13a	17.00 ± 0.09c	21.88 ± 0.39c	27.50 ± 0.38d	37.33 ± 0.12d	48.91 ± 0.07c
14 cP	8.09 ± 1.74a	14.85 ± 0.66b	14.40 ± 0.18b	19.35 ± 0.18b	23.18 ± 1.16c	30.75 ± 0.09c	36.35 ± 0.18b
57 cP	9.60 ± 0.93a	9.83 ± 0.71a	13.23 ± 0.14a	16.50 ± 0.11a	20.75 ± 0.57b	22.15 ± 1.74b	24.19 ± 1.32a
360 cP	7.50 ± 0.99a	10.63 ± 0.11a	14.10 ± 0.54b	16.18 ± 0.14a	17.53 ± 0.12a	19.55 ± 0.54a	24.33 ± 0.89a

[a] Results are expressed as mean ± standard deviation of three determinations. Values with the same letters within each column are not significantly different ($p > 0.05$).

TVB-N in the coated samples. Reduction of TVB-N contents of herring samples treated with chitosans was in the range of 26−51% over the same period. Treatment with 57 and 360 cP chitosans of both cod and herring samples reduced ($p \leq 0.05$) TVB-N formation throughout the entire storage period. After day 6, the TVB-N levels in uncoated cod sample exceeded the acceptable level of 30 mg of N/100 g of flesh suggested for fish and shellfish (*40*, *62*). For herring samples, this level was reached after 8 days for the uncoated sample. Meanwhile, 14 cP chitosan-treated samples exceeded this acceptable level after 8 and 10 days for cod and herring, respectively. In general, the TVB-N levels were higher in the uncoated cod samples than in the uncoated herring samples, possibly due to the fact that cod has a relatively high content of TMAO as an osmoregulator, but in contrast to the trend observed for total microbial counts (**Figure 6**). Furthermore, factors such as age, locality, and culture method may influence the content of nonprotein nitrogenous compounds in fish muscle (*63*). Increase in TVB-N levels in fish during storage may be attributed to several enzymatic processes, namely, deamination of free amino acids, degradation of nucleotides, and oxidation of amines, among others (*8*).

Olafsdottir et al. (*64*) observed that the level of TVB-N in capelin stored at 0 °C reached ∼50 mg of N/100 g in 5 days, whereas that in capelin stored at 5 °C reached the same level in 4 days. According to Al-Kahtani et al. (*65*), TVB-N values for irradiated tilapia and Spanish mackerel remained within the permissible range (30 mg of TVB-N/100 g) for up to 20 days of storage at 2 °C.

Effect of Chitosan Coating on Trimethylamine Production in Fish. Chitosan coating resulted in a significant ($p \leq 0.05$) reduction in TMA production in both cod and herring samples (see **Table 6**). The low initial TMA-N contents (0.08−0.30 mg of TMA-N/100 g) indicated that the fillets (cod and herring) were procured in a very fresh condition. Furthermore, a slight increase ($p > 0.05$) in TMA over the initial storage period (4 days) reflects the low starting level of in the flesh of cod and herring fillets and precludes the usefulness of this compound as a freshness indicator.

On day-12 of storage, trimethylamine nitrogen content of cod samples coated with chitosans was 44−61% lower than that of the control (**Table 6**). The level of 5−10 mg TMA/100 g flesh, which is thought to indicate rejection limit for cod (*6*), was reached after 6 days in uncoated samples, whereas in all treated samples, the levels were well below the rejection limit throughout the storage period. However, significantly ($p \leq 0.05$) lower TMA levels were observed for 57 and 360 cP chitosan-coated cod samples compared to that of the sample treated with the 14 cP chitosan after 6 days. Meanwhile, herring samples did not reach the rejection limit of 5 mg/100 g, in agreement with values reported by Gill (*8*) and Sikorski et al. (*6*) for fatty fish species such as herring. Furthermore, comparatively higher amounts of TMA were produced in cod as compared to the herring samples, possibly due to the high content of the osmoregulator TMAO in cod (gadoid fish) tissues (*66*). Uncooked herring exhibited a significantly ($p \leq 0.05$) higher level of TMA as compared to its uncoated counterparts.

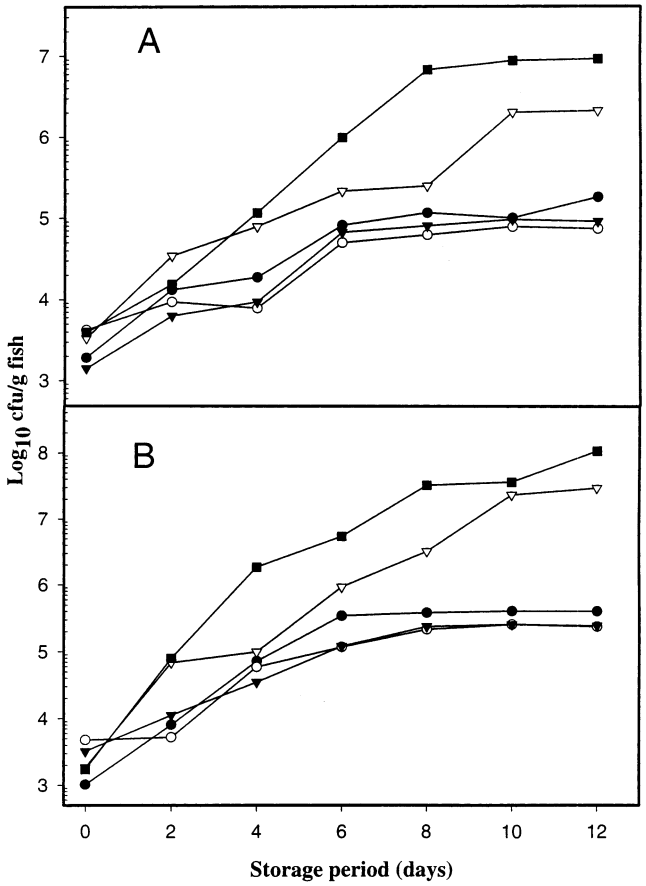

Figure 6. Effect of chitosan coating on total plate count of cod (A) and herring (B) samples stored at 4 ± 1 °C: (●) 14 cP chitosan; (○) 57 cP chitosan; (▼) 360 cP chitosan; (▽) 1% acetic acid; (■) control uncoated.

Effect of Chitosan Coating on Hypoxanthine Production in Fish. Considerable enzymatic dephosphorylation of inosine monophospahte (IMP) via inosine (Ino) to Hx occurs and results in the loss of fresh flavors and off-flavor development in several fish species (*66*). The Hx test is applicable to all species of fish during the early stages of refrigerated storage (*39*). However, a

Table 6. Content of Trimethylamine (Milligrams per 100 g of Fish) of Chitosan-Coated Cod Samples Stored at 4 ± 1 °C[a]

	storage period						
chitosan	0 days	2 days	4 days	6 days	8 days	10 days	12 days
				Cod			
uncoated	0.30 ± 0.02a	0.80 ± 0.09a	1.45 ± 0.24b	5.05 ± 0.28c	5.10 ± 0.36c	6.39 ± 0.09c	7.13 ± 0.05c
14 cP	0.30 ± 0.04a	0.97 ± 0.17a	1.00 ± 0.05a	2.01 ± 0.05b	2.65 ± 0.17b	2.73 ± 0.11b	3.98 ± 0.60b
57 cP	0.34 ± 0.04a	0.76 ± 0.07a	0.73 ± 0.08a	1.32 ± 0.10a	1.83 ± 0.07a	2.09 ± 0.10a	3.01 ± 0.01ab
360 cP	0.33 ± 0.03a	0.75 ± 0.14a	0.84 ± 0.06a	1.00 ± 0.12a	1.72 ± 0.20a	1.88 ± 0.13a	2.75 ± 0.63a
				Herring			
uncoated	0.09 ± 0.00a	0.73 ± 0.07b	1.20 ± 0.00c	2.86 ± 0.95b	3.07 ± 0.02c	3.58 ± 0.17d	3.98 ± 0.11c
14 cP	0.09 ± 0.01a	0.49 ± 0.03a	0.67 ± 0.13b	1.58 ± 0.04a	2.33 ± 0.00b	2.79 ± 0.06c	2.89 ± 0.04b
57 cP	0.09 ± 0.01a	0.34 ± 0.13a	0.21 ± 0.07a	1.01 ± 0.13a	1.18 ± 0.31a	1.38 ± 0.09b	2.05 ± 0.41a
360 cP	0.08 ± 0.00a	0.36 ± 0.05a	0.26 ± 0.07a	0.90 ± 0.03a	0.95 ± 0.04a	1.02 ± 0.10a	1.93 ± 0.13a

[a] Results are expressed as mean ± standard deviation of three determinations. Values with the same letters within each column are not significantly different ($p > 0.05$).

Table 7. Content of Hypoxanthine (Micromoles per Gram of Fish) of Chitosan-Coated Fish Samples Stored at 4 ± 1 °C[a]

	storage period						
chitosan	0 days	2 days	4 days	6 days	8 days	10 days	12 days
				Cod			
uncoated	1.32 ± 0.10a	2.17 ± 0.36b	2.33 ± 0.50ab	4.93 ± 0.13b	5.42 ± 0.90b	5.40 ± 0.61b	4.13 ± 0.13c
14 cP	1.64 ± 0.29a	1.64 ± 0.29ab	3.13 ± 0.43b	2.87 ± 0.10a	2.89 ± 0.37a	3.13 ± 0.97a	2.65 ± 0.34b
57 cP	1.34 ± 0.42a	1.39 ± 0.42ab	1.93 ± 0.08a	2.74 ± 0.76a	2.05 ± 0.06a	2.26 ± 0.33a	1.45 ± 0.06a
360 cP	1.26 ± 0.09a	1.27 ± 0.09a	2.07 ± 0.45a	2.39 ± 0.66a	2.10 ± 0.78a	1.96 ± 0.58a	1.72 ± 0.09a
				Herring			
uncoated	0.73 ± 0.11a	1.09 ± 0.66a	2.15 ± 0.90b	2.44 ± 0.42b	2.73 ± 0.76b	2.01 ± 0.39c	1.98 ± 0.09c
14 cP	0.94 ± 0.06a	1.45 ± 0.60a	1.69 ± 0.63a	1.77 ± 0.08a	1.79 ± 0.87ab	1.82 ± 0.09bc	1.80 ± 0.14bc
57 cP	0.44 ± 0.08a	0.96 ± 0.91a	1.74 ± 0.47a	1.73 ± 0.04a	1.13 ± 0.19a	1.10 ± 0.00a	0.95 ± 0.09a
360 cP	0.63 ± 0.39a	1.41 ± 0.01a	1.69 ± 0.19a	1.89 ± 0.17a	1.07 ± 0.09a	0.86 ± 0.08a	0.72 ± 0.10a

[a] Results are expressed as mean ± standard deviation of three determinations. Values with the same letters within each column are not significantly different ($p > 0.05$).

wide variation in the rate of nucleotide catabolism has been observed in different species of fish. Therefore, it is important to establish the rate of accumulation of Hx in a particular species before applying the level of Hx as an index of freshness or quality (*67*). **Table 7** shows that regardless of the viscosity of chitosan, significantly ($p \leq 0.05$) different Hx levels were reached for the treated and untreated samples of cod and herring after 4 days. After day 6, Hx formation in cod and herring samples treated with chitosan was reduced by 41−52 and 23−29% as compared to that of their uncoated counterparts, respectively. The critical level of Hx proposed for cod rejection is 2−3 μmol/g of fish (*68*), and this level was reached after 2, 4, and 6 days in uncoated and 14 and 57 cP chitosan-coated samples, respectively. However, the 360 cP chitosan-coated sample did not reach this level (2.5 μmol/g of fish) throughout the entire storage period. The content of Hx is first increased, followed by a decrease, with progression of spoilage (*39*, *69*) in seafoods. A similar pattern was observed in this study, perhaps due to the breakdown of Hx to xanthine and subsequently to uric acid.

Hx values of treated herring increased by ∼2−4-fold after day 6 of storage, whereas the increase for the control was ∼3-fold (**Table 7**). However, herring samples also exhibited the expected increase and decrease pattern similar to cod. This was in contrast to the findings of Kyrana et al. (*63*), who observed a linear increment of the Hx content in ice-stored gilthead sea bream over a 24-day storage period. The steady increase in the concentration of Hx has also been noted up to 19 days in seal meat stored at 0−4 °C (*70*). In general, samples coated with chitosans, regardless of their viscosity, had a lower Hx content throughout the entire storage period.

The development of Hx in many species parallels the production of TMA (*66*). Dalgaard et al. (*71*) showed a linear correlation ($r = 0.9000$) between the contents of TMA and Hx during storage of vacuum-packed cod fillets stored at 0 °C. However, in this study the observed correlation ($r = 0.6862$) between TMA and Hx was not strong, perhaps due to acceleration of breakdown of Hx during storage at 4 ± 1 °C; a reduction in microbial and autolytic activities under vacuum-pack conditions may also be operative.

Effect of Chitosan Coating on Microbial Spoilage of Fish. Total aerobic psychrotropic bacterial count in both cod and herring coated with chitosans was compared with uncoated samples and those treated with 1% acetic acid used for dissolving chitosans (**Figure 6**). Fresh cod and herring fillets had initial total aerobic psychrotropic counts of ∼3−3.7 log CFU/g. Samples of cod coated with chitosan contained $<10^6$ CFU/g of fish (psychrotropic bacterial count) during the entire storage period, whereas uncoated and 1% acetic acid-treated cod samples exceeded this level after 6 and 10 days, respectively. This acceptability limit of 10^6 CFU/g has been proposed for fresh fish (*72*). Stenstrom (*73*) observed that cod fillets stored at 2 °C surpassed this maximum acceptable level after 6 days of storage. A similar, but faster, pattern ($<10^6$ CFU/g) was observed with chitosan-coated herring samples. However, this acceptable limit was reached much more quickly in herring samples. Herring fillets treated with 57 and 360 cP chitosans had a lower ($p \leq 0.05$) aerobic psychrotropic count than 14 cP chitosan-treated fillets after day 6 of storage. Furthermore, a logarithmic increase of bacterial population was clearly observed only with uncoated and 1% acetic acid-treated samples. After 6 days, there were no further significant ($p \leq 0.05$) increases in total plate count (TPC), showing that stationary phase in bacterial growth had been attained in all chitosan-coated cod and herring samples. Moreover, data indicated that treatments with chitosan resulted in reductions of 10^3 and 10^2 TPC of

herring and cod samples, respectively, after 12 days of refrigerated storage.

Parkin et al. (*74*) reported the effectiveness of a modified atmosphere (80% CO_2 and 20% air) in extending the shelf life of fresh rockfish fillets stored in the dark at 2−4 °C. Gray et al. (*75*) studied refrigerated perch, seatrout, croaker, and bluefish packed with carbon dioxide and found 45−55% increase in stability, mainly due to an extension in the lag phase of psychrotropic organisms and their reduced growth rate in the logarithmic phase.

Treweek and Morgan (*76*) described the destabilization of negatively charged bacterial cells by a cationic polymer (polyethyleneimine, PEI). It was proposed that such a flocculation may be caused by one of three mechanisms, namely, double-layer coagulation, adsorption coagulation, or polymer bridging. It was further concluded that high molecular weight PEI produced rapid flocculation at low doses due to adsorption coagulation. Chitosan is believed to chelate certain ions from the lipopolysaccharide (LPS) layer of the outer membrane of bacteria. Thus, it has been suggested that alterations in the LPS layer may cause the outer cell surface to become more permeable, thereby releasing cellular components of bacteria (*18*, *20*). Furthermore, the chitosan coating acts as a barrier against oxygen transfer and leads to inhibition of growth of the aerobic bacteria. Tsai and Su (*77*) noted that chitosan caused leakage of glucose and lactate dehydrogenase from *Escherichia coli* cells. Furthermore, the antibacterial mechanism of chitosan involves a cross-linkage between the polycations of chitosan and the anions on the bacterial surface that changes the membrane permeability. Cuero (*78*) reported that the antimicrobial action of chitosan is influenced by intrinsic and extrinsic factors such as the type of chitosan (e.g., plain or derivative), degree of polymerization, host natural nutrient constituency, substrate chemical and/or nutrient composition, and environmental conditions.

Conclusions. Chitosans prepared from snow crab processing discards possessed varying viscosities (14, 57, and 360 cP chitosans), which were closely related to the duration of the deacetylation period. This study demonstrated the potential of chitosan as a preservative coating for herring and cod in reducing or preventing moisture loss, lipid oxidation, and microbial growth. Cod samples coated with 57 and 360 cP chitosans exhibited a significantly ($p \leq 0.05$) lower relative moisture loss as compared to the uncoated samples and fish coated with 14 cP chitosan throughout the storage period. However, a reduction in relative moisture loss due to chitosan coating was not clearly observed for herring samples. Furthermore, chitosan produced from crab processing discards exhibited a moderate to strong viscosity-dependent preservative effect in both fish model systems. In general, 360 cP chitosan exerted a better preservative effect (comparable to or better than 57 and 14 cP chitosans) in both fish model systems stored at 4 ± 1 °C.

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Received for review December 20, 2001. Revised manuscript received May 21, 2002. Accepted June 10, 2002. We are grateful to the Fisheries Diversification Program and Natural Sciences and Engineering Research Council (NSERC) of Canada for partial financial support and to the Canadian International Development Agency (CIDA) for the award of a fellowship to J.Y.V.A.K.

JF011693L