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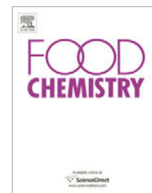

Quality enhancement in fresh and frozen lingcod (*Ophiodon elongates*) fillets by employment of fish oil incorporated chitosan coatings

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ABSTRACT

The 3% chitosan solutions incorporating 10% fish oil (w/w chitosan, containing 91.2% EPA and DHA) with or without the addition of 0.8% vitamin E were prepared. Fresh lingcod (*Ophiodon elongates*) fillets were vacuum-impregnated in coating solution at 100 mm Hg for 10 min followed by atmospheric restoration for 15 min, dried, and then stored at 2 °C or −20 °C for 3-weeks and 3-months, respectively, for physicochemical and microbial quality evaluation. Chitosan–fish oil coating increased total lipid and omega-3 fatty acid contents of fish by about 3-fold, reduced TBARS values in both fresh and frozen samples, and also decreased drip loss of frozen samples by 14.1–27.6%. Chitosan coatings resulted in 0.37–1.19 and 0.27–1.55 log CFU/g reductions in total plate and psychrotrophic counts in cold stored and frozen stored samples, respectively. Chitosan–fish oil coatings may be used to extend shelf-life and fortify omega-3 fatty acid in lean fish.

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1. Introduction

Fish is an important source of high-quality proteins for humans (Tidwell & Allan, 2001). However, it is highly susceptible to both microbiological and chemical deterioration, due to its high water activity, neutral pH, relatively large quantities of free amino acids, and presence of autolytic enzymes (Jeyasekaran, Ganesan, Anandaraj, Shakila, & Sukumar, 2006). Cold storage and freezing are the normally employed methods for fish preservation, but they do not completely inhibit the quality deterioration of fish (Jeon, Kamil, & Shahidi, 2002).

Oily fish, such as salmon, mackerel, and sardine, are rich in omega-3 fatty acids. As the significant structural components of the phospholipids of cellular membranes, omega-3 fatty acids, particularly α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are very important for human health (Connor, 2000). EPA and DHA play vital roles in prevention of cardiovascular diseases and some types of cancers, including colon, breast, and prostate (Das, 2008; Matsumoto, Nakayama, Ishida, Kobayashi, & Kamata, 2009). DHA is essential for brain functioning in both infants and adults. The inclusion of plentiful DHA in the diet not only prevents brain ageing and Alzheimer's disease, but also improves the recovery from certain visual dysfunctions (Horrocks & Yeo, 1999). American Heart Association (AHA) recommends the intake of 1 g omega-3 fatty acids per day as a safe and effective way to obtain the heart health benefits (Kris-Etherton, Harris, & Appel, 2002). Omega-3 fatty acids are found mainly in fat-rich fishes, but many lean fishes, such as catfish, cod, flounder, grouper, haddock, hake, perch, and swordfish, contain very low content (<0.5 g/100 g portion) of this nutraceutical compound (Exler & Wehrauch, 1988).

Chitosan, a cationic polysaccharide obtained from crustacean shells, is a well-known film-forming biopolymer with a broad antimicrobial activity against bacteria and fungi (Cagri, Ustunol, & Ryser, 2004; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). Chitosan based films and coatings have been applied as a microbial hurdle in a variety of food, including fruits and vegetables (Devlieghere, Vermeulen, & Debevere, 2004), eggs (Kim, Daeschel, & Zhao, 2008), cheeses (Duan, Park, Daeschel, & Zhao, 2007), and meat (Ouattara, Simard, Piette, Bégin, & Holley, 2000), for improving overall food quality and prolonging storage life. Chitosan has also been applied as an edible invisible film for preserving fresh fillets of Atlantic cod and herring, and its preservative efficacy has been exhibited by the reduced moisture loss, lipid oxidation, and growth of microorganisms in the tested fishes (Jeon et al., 2002). When forming into films or coatings, chitosan can effectively carry other functional substances, such as nutraceuticals, antioxidants, and antimicrobial agents, due to the presence of a high density of amino groups and hydroxyl groups in the chitosan polymer structure (Park, Daeschel, & Zhao, 2004).

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Previously, we reported that chitosan was a good film-forming material for developing mineral and vitamin E fortified edible films and coatings (Park & Zhao, 2004). However, the application of omega-3 fatty acids fortified chitosan films and coatings to improve seafood quality have not been reported. Lingcod, a popular eating fish, is unique to the west coast of North America and economically important in coastal and estuarine environments from Alaska to California. Although it is a good source of protein and is high in vitamin B12 and selenium, its fat content is very low, with 1.06% total lipid and 0.3% total polyunsaturated fatty acids being reported in USDA nutrient database (USDA, 2008). This study aimed to evaluate the application of fish oil incorporated chitosan coatings for improving physicochemical and microbial qualities and enhancing the omega-3-fatty acids content of lingcod fish during cold and frozen storages.

2. Materials and methods

2.1. Materials

Lingcod fish (*Ophiodon elongates*) was obtained from a local source in Clackamas, OR, USA. It was harvested, stored at 2 °C for 4 d, and then cleaned, gutted, filleted and skinned at the plant. Processed fillets were immediately shipped to our lab in a cooler with ice and kept at 2 °C overnight before use.

Shrimp derived chitosan from Primex ehf (1.49×10^5 Da molecular weight, 9 cPs viscosity of a 1% (w/w) aqueous acetic acid solution at 25 °C, and 97% deacetylation; Siglufjordur, Iceland) was used without further purification. Fish oil (fatty acid ethyl ester – ED85 EE, containing 91.2% EPA and DHA) were obtained from Proparts S.A. (Lugano, Switzerland).

Reagent-grade acetic acid was purchased from Baker and Adamson (Morristown, NJ, USA), glycerol, chloroform, and methanol HCl from EMD Biosciences (Darmstadt, Germany), Tween 80, hexane, thiobarbituric acid, trichloroacetic acid, and 1,1,3,3-tetraethoxypropane (TEP) from Sigma–Aldrich (St. Louis, MO, USA), and methanolic HCl from Supelco (Bellefonte, PA, USA). Vitamin E500 (tocopheryl acetate spray dried powder) was obtained from D-BASF Co. (Florham Park, NJ, USA), commercial bleach solution (Clorox Regular Bleach, ~6% [w/w] NaOCl) from Clorox Co. (Oakland, CA, USA), and plate count agar (PCA) from Becton Dickinson and Co. (Sparks, MD, USA).

2.2. Preparation of fish oil incorporated chitosan coating solution

Chitosan solution was prepared by dissolving 3% chitosan in a 1% acetic acid solution with an addition of 25% glycerol (w/w chitosan) in the mixture. Ten percent fish oil (w/w chitosan) was mixed into chitosan solution with addition of 4× Tween 80 (w/w fish oil). Vitamin E500 powder was then mixed into fish oil incorporated chitosan solution to a concentration of 0 or 0.8% (w/v chitosan solution). The solution mixtures were homogenised using a homogeniser (Polytron PT 10–35, Kinematica, Luzernerstrasse, Switzerland) at 3000 rpm for 1 min and used for coating fish fillets right after the preparation. The pH of fish oil incorporated chitosan solution with and without addition of vitamin E was 4.58 ± 0.3 and 4.63 ± 0.16, respectively.

2.3. Treatment and storage of fish samples

The lingcod fillets were cut into ~50 g pieces and washed for 1 min in NaOCl solution containing 250 ppm total chlorine prepared by diluting commercial bleach solution with distilled water. The fish samples were then rinsed with distilled water for 1 min twice to remove the residual chlorine and hanged in the open air under room temperature until the surface was dried. Chlorine was not detected in water of the 2nd rinsing when measured with the chlorine test kit (limit of detection was 0.02 ppm, Hach Co., Loveland, CO, USA). The fish samples were coated using vacuum impregnation procedures by immersing the samples in the container with chitosan coating solution at a fish:coating solution ratio of 1:2. Vacuum impregnation was applied to achieve more complete and uniform coatings and introducing solvents and solutes of choice into the porous space on the surface of fillets. The container was then placed inside a chamber connected to a vacuum pump (Model 0211-P204, Gast Mfg. 149 Corp., Benton Harbor, MI, USA). A vacuum pressure of 100 mm Hg was applied at room temperature for 10 min, and the system was then restored to atmospheric pressure for 15 min. Fish samples were removed from solutions, air dried at room temperature for approximately 30 min, packed in storage bags (Ziploc, Johnson and Son, Inc., Racine, WI, USA), and stored at 2 ± 1 °C for up to 3 weeks, or packed in freezer bags (Ziploc, Johnson and Son, Inc.) and stored a −20 ± 1 °C for up to 3 months. Physicochemical, microbial, and lipid qualities of the fish samples were measured on 0–3 weeks of cold storage or on 0–3 months of frozen storage. Uncoated samples were used as controls.

2.4. Measurements of physicochemical qualities

For pH measurement, approximately 20 g of minced fish samples were placed in a 400-mL beaker and homogenised with 80 mL distilled water with a homogeniser (Polytron PT 10-35, Kinematica) at 3000 rpm for 30 s. The pH of homogenised sample was measured using a pH meter (Model 125, Corning Inc., Corning, NY, USA). Colour of fish samples was determined using a colorimeter (LabScan, Hunter Associates Laboratory, Inc., Reston, VA, USA) and reported as L^*, a^*, and b^* values, where L^* denotes the lightness of colour (0–100, black to white), a^* corresponds to red to green, and b^* presents yellow to blue. Moisture content was measured by drying about 10 g of minced fish samples in a conventional oven (Model 17, Precision Scientific Co., Chicago, IL, USA) at 102 °C for 16–18 h. The weight of the initial and dried samples was recorded. The moisture content (%) was calculated as weight change after drying divided by the initial weight of samples × 100%. Drip loss (%) was determined by thawing frozen samples at refrigerated temperature (2 °C) for 22 h, and calculated as weight change after thawing divided by the initial weight of frozen sample × 100%.

2.5. Analysis of total lipid, fatty acid composition, and lipid oxidation

Lipid extraction, total lipid, and fatty acid analyses were conducted by the method of Cherian, Traber, Goeger, and Leonard (2007) with slight modification. Approximately 2.0 g minced fish sample were homogenised with 18 mL of chloroform/methanol (2:1, v/v) (Folch, Lees, & Sloane-Stanley, 1957) at 3000 rpm for 30 s. After an overnight incubation at 4 °C, the homogenate was filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England) into a 50-mL graduated cylinder, and 4 mL of 0.88% sodium chloride solution was added and mixed. After phase separation, the volume of the lipid layer was recorded, and the top layer was completely siphoned off. Total lipids were determined gravimetrically.

For fatty acid methyl ester (FAME) preparation, 1 mL of the lipid extract was dried in a block heater (VWR International, LLC, West Chester, PA, USA) under nitrogen atmosphere. The dried lipid was redissolved in 2 mL 3 M methanolic HCl and heated in a water bath (Precision, Winchester, VA, USA) for 1 h at 60 °C. After cooling to room temperature, the FAME was separated by hexane and distilled water. Analysis of fatty acid composition was performed with a Agilent 6890 gas chromatograph (Agilent Technologies Inc., Palo

Alto, CA, USA) equipped with an autosampler, flame ionisation detector, and SP-2560 fused silica capillary column, 100 m × 0.25 mm × 0.2 μm film thickness (SP-2560, Supelco). Sample (2 μL) was injected with He as a carrier gas (1.0 mL/min) onto the column programed for ramped oven temperatures (initial temperature was 110 °C, held for 1 min, then ramped at 15 °C/min to 190 °C and held for 55 min, then ramped at 5 °C/min to 230 °C and held for 5 min). Inlet and detector temperatures were both 220 °C. Peak areas and percentages were calculated using Agilent ChemStation software (Hewlett Packard Co., Wilmington, DE, USA). FAME was identified by comparison with retention times of authentic internal standards including a comprehensive set of fatty acids (Matreya Inc., Pleasant Gap, PA, USA). Fatty acid values and total lipids were expressed as weight percentages.

The lipid oxidation in fish samples were measured by thiobarbituric acid-reactive substances (TBARS) assay as described by Buege and Aust (1978) with slight modification. Approximately 3.0 g of minced fish samples was homogenised with 25 mL of solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 M HCl, with a homogenisation at 3000 rpm for 30 s. The mixture was heated in a boiling water bath (Precision) (100 °C) for 10 min for developing a pink colour, then cooled and centrifuged (RC2-B, Ivan Sorvall, Inc., Norwalk, CT, USA) at 3600g at 25 °C for 20 min. The absorbance of the supernatant was measured by a spectrophotometer (UV160U, Shimadzu Scientific Instruments, Kyoto, Japan) at 532 nm, and 1,1,3,3-tetraethoxypropane (TEP) (Sigma–Aldrich) was used as the standards. TBARS was expressed as mg malonaldehyde equivalents/kg (mg MA eq/kg) muscle.

2.6. Microbiological analysis

Approximately 20 g of minced fish samples was homogenised with 9× sterile phosphate buffered saline (PBS) using a stomacher (Sward Stomacher 400, Brinkmann, Westbury, NY, USA) at 230 rpm for 1 min to prepare 1:10 sample suspension. Additional 10-fold dilutions were prepared with sterile PBS. Pour-plate method using plate count agar was used to determine the total plate and psychrotrophic counts in the fish samples. The inoculated agar plates were incubated at 35 °C for 48 h for determining total plate counts, and at 4 °C for 7 d for psychrotrophic counts.

2.7. Statistical analysis

Two independent replications with two samples each replication were performed for all treatments. Four individual samples were used for each measurement at each sampling point. Data analyses were performed by ANOVA (analysis of variance) using SAS statistical software 9.01 (SAS Institute, Cary, NC, USA). Multiple comparisons among the treatments with significant differences tested in ANOVA were conducted by using LSD (least significant difference) at $P < 0.05$.

3. Results and discussion

3.1. Effect of chitosan coatings on physicochemical qualities of lingcod fish fillets

During 3 weeks cold storage, the pH of uncoated fish sample slightly but not significantly increased from 7.00 to 7.19 (Fig. 1a) ($P > 0.05$). The coating treatments reduced the pH of fish samples to 6.86–6.89 and the pH of coated samples were lower than uncoated samples during the subsequent cold storage ($P < 0.05$). No significant difference was found between the pH of the samples coated with chitosan only and chitosan incorporated with vitamin E ($P > 0.05$). The reduction in pH was probably caused by the acidic coatings (pH 4.58–4.63) formed on the surface of fish fillets. The pH of coated fish samples was also increased with storage time (except the 2nd week), reaching a value of 7.00–7.25 at the end of 3 weeks storage. The increase in pH indicated the growth of bacteria in the fish (Jay, Loessner, & Golden, 2005). Spoilage bacteria utilise low molecular weight compounds such as amino acids present in fish muscle and induce the accumulation of alkaline ammonia components, resulting in the rise of pH (Campos, Rodríguez, Losada, Aubourg, & Barros-Velázquez, 2005). Similar increases in pH have also been reported on other fish species stored in flake ice, such as sardine and hake (Ababouch et al., 1996; Nunes, Batista, & Morão de Campos, 1992; Ruiz-Capillas & Moral, 2001).

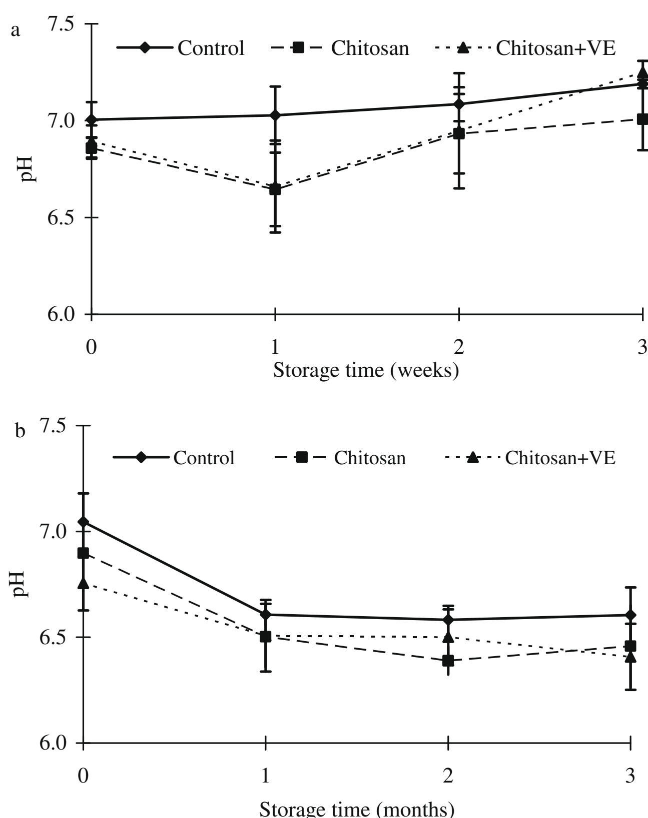

Fig. 1. pH of fish fillets during: (a) cold storage at 2 °C and (b) frozen storage at −20 °C. The results are the mean of four replications and the bars indicate the standard deviation. V_E: vitamin E500.

The pH of coated fish samples was also lower than uncoated ones during frozen storage ($P < 0.05$) and vitamin E incorporation in the coating solutions did not affect the pH of coated samples ($P > 0.05$) (Fig. 1b). However, a decrease in pH was observed in both coated and uncoated samples during frozen storage, in which the pH of fish samples dropped from 6.76–7.05 on day 0 to 6.50–6.61 after 1-month storage and stayed at 6.40–6.61 in the remaining storage time. During frozen storage, the growth of microorganism was inhibited by the low temperature. The decrease in pH of the fish samples was probably resulted from the protein breakdown and the release of phosphoric and lactic acids occurred during freezing and thawing processes (Singh & Balange, 2005).

Considerable variations in colour were observed among individual lingcod fillets, but in general they were in white to yellowish colour, which was illustrated by L^* value >55, a^* value ranging from 1 to −5, and b^* value ranging from 8 to 16 (Fig. 2). The coating treatments, either with or without vitamin E incorporation, did not affect the L^*, a^*, and b^* values of fish samples in both cold and frozen storages ($P > 0.05$). The L^*, a^*, and b^* values of cold

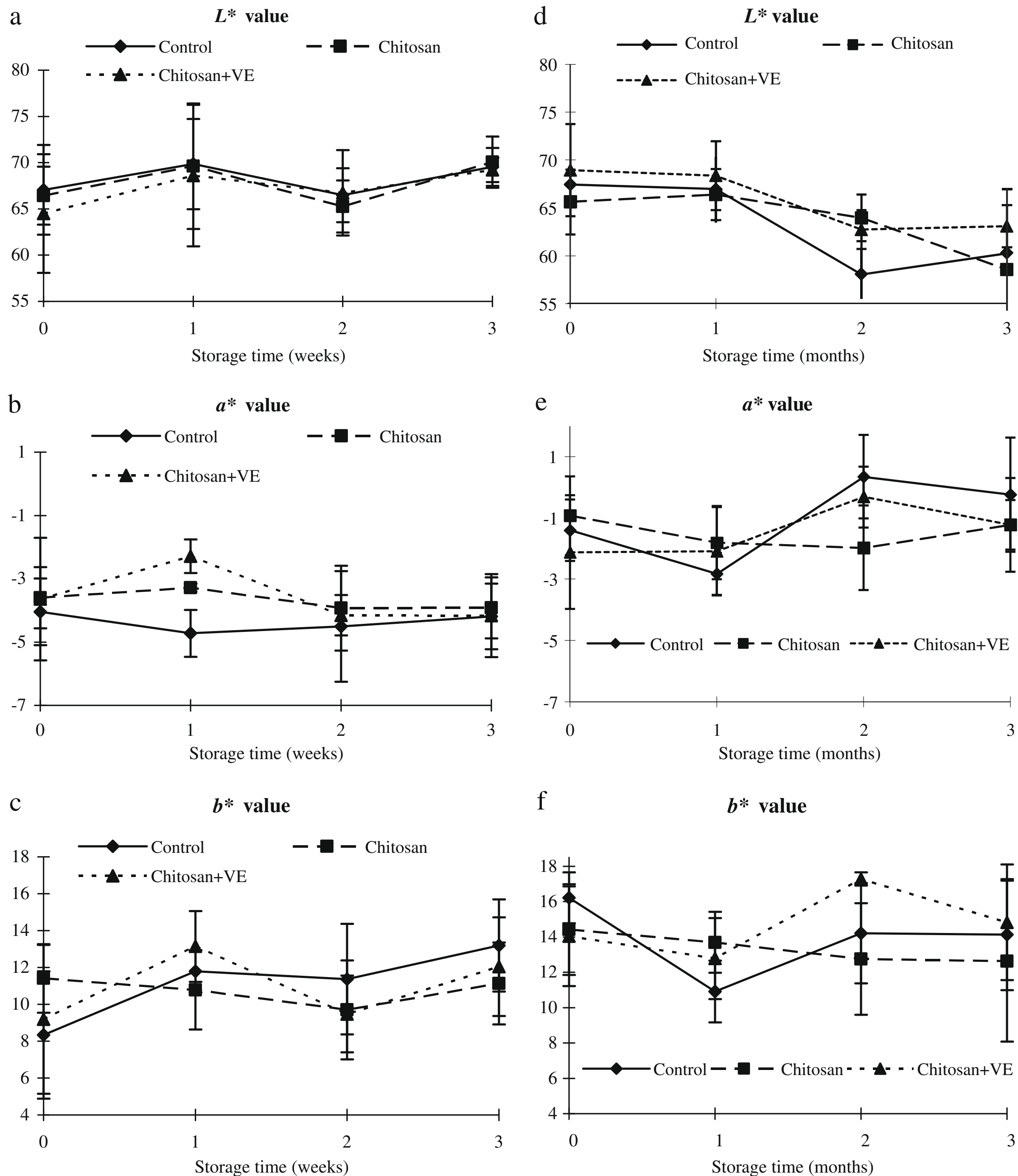

Fig. 2. Colour of fish fillets during (a–c) cold storage at 2 °C and (d–f) frozen storage at −20 °C. The results are the mean of four replications and the bars indicate the standard deviation. V_E: vitamin E500.

stored fish samples and a^* and b^* values of frozen stored samples did not change along with the storage time ($P > 0.05$). However, the L^* value of frozen stored samples decreased from 65.6–68.9 on day 0 and 1 month to 58.1–63.9 on 2 and 3 months ($P < 0.05$), indicating a darkening of the fish colour with the extension of frozen storage. This colour change may be caused by the ice crystals formed during freezing, which lead to extensive mechanical damage on cell membranes and deterioration of cellular components, such as protein denaturation (Tironi, LeBail, & de Lamballerie, 2007).

Moisture contents of fresh cod fillets ranged from 79.98% to 80.88% (Table 1). The coating treatments lowered the moisture content of fresh fish samples to 78.70–79.65%, and the moisture content of coated fish samples were lower than those of uncoated samples during the subsequent cold and frozen storages, but the difference was not significant on the 2nd and 3rd weeks and months ($P > 0.05$). During coating process, the fish fillets were air dried under room temperature for about 30 min after being vacuum-impregnated in coating solution. The water molecules evaporation from the fish surface along with the drying of coating solution may be the cause of the decreased moisture content in coated fish samples.

The moisture content did not change with the storage time for both cold and frozen storages ($P > 0.05$). However, other researcher

Table 1
Moisture content of cold (2 °C) and frozen (−20 °C) stored fish fillets, and drip loss of frozen stored fish fillets.[a]

Cold storage				Frozen storage						
Time (week)	Moisture content (%)			Time (mo)	Moisture content (%)			Drip loss (%)		
	Control	Chitosan	Chitosan + V_E[b]		Control	Chitosan	Chitosan + V_E	Control	Chitosan	Chitosan + V_E
0	A80.89 ± 1.26a	A79.65 ± 1.16ab	A79.00 ± 0.66b	0	A79.99 ± 1.23a	A78.03 ± 1.20ab	A78.27 ± 1.21b	–	–	–
1	A80.91 ± 0.86a	A78.72 ± 1.12b	A78.26 ± 1.21b	1	A79.6 ± 0.60a	A78.32 ± 1.29ab	A78.29 ± 0.37b	A4.64 ± 1.39a	A3.14 ± 2.01ab	A3.58 ± 2.31b
2	A80.17 ± 1.02a	A78.75 ± 1.08a	A79.03 ± 1.15a	2	A80.00 ± 0.55a	A79.11 ± 0.77a	A79.05 ± 1.12a	AB6.09 ± 0.50a	B5.65 ± 0.50a	AB4.13 ± 1.26b
3	A80.23 ± 1.15a	A79.89 ± 0.67a	A79.31 ± 0.36a	3	A80.24 ± 1.09a	A79.54 ± 0.89a	A79.26 ± 1.08a	B6.76 ± 0.67a	B5.93 ± 0.32b	B5.68 ± 0.83b

Means preceded by the same capital letters in the same column are not significantly different ($P > 0.05$).
Means followed by the lowercase letters in the same row within each storage method and each measurement are not significantly different ($P > 0.05$).
[a] The results are the mean of four replications ± standard deviation.
[b] V_E: vitamin E500.

reported a 4.1% moisture loss in pink salmon fillets after 3 months of frozen storage (Sathivel, 2005). The difference could be caused by the different fish species and package materials used. The freezer bag used in this study might have a high water vapour barrier property, which prevented the escape of the water molecules from the fish surface.

Drip loss of frozen fish fillets increased with the storage time for both coated and uncoated samples (Table 1). Drip loss in frozen fish fillets is a complicated process, which may be caused by myosin aggregation during frozen storage, thus leading to muscle toughening and a loss in water-holding capacity (Mackie, 1993). Bahuaud et al. (2008) observed a high amount of myofibre

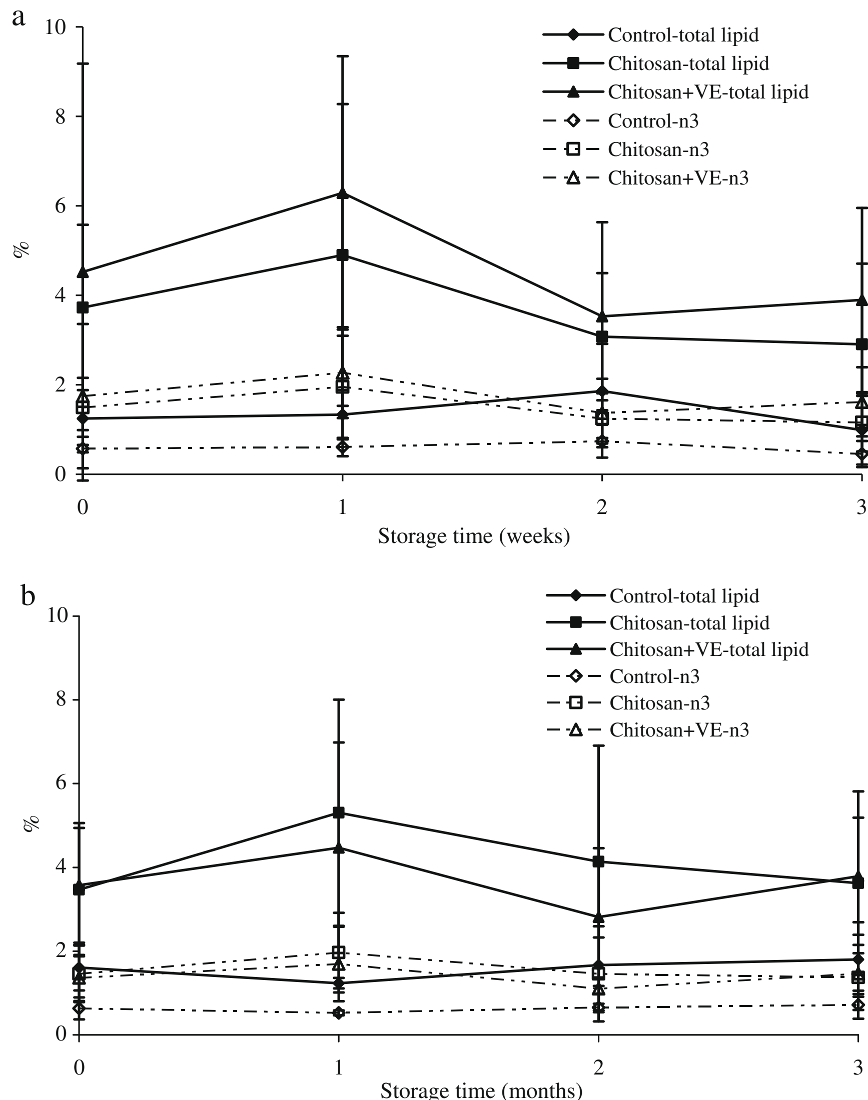

Fig. 3. Total lipid and omega-3 fatty acid contents of fish fillets during: (a) cold storage at 2 °C and (b) frozen storage at −20 °C. Omega-3 fatty acids include ALA, EPA, DHA, DPA, and ETA. The results are the mean of four replications and the bars indicate the standard deviation. V_E: vitamin E500.

breakages in super-chilled Atlantic salmon fillets (45 min in a super-chilling tunnel at −25 °C to reach a core temperature of −1.5 °C) due to the formation of ice crystals, which might also cause a decrease in water-holding capacity. Compared to uncoated samples, the coating treatments reduced the drip loss ($P < 0.05$) by 27.6, 19.7, and 14.1% on 1–3 months of storage, respectively. Since the chitosan coatings only covered the surface of fish, they might not prevent the loss in water-holding capacity of fish muscle during frozen storage, but they might reabsorb and hold the water expelled from the muscle during thawing and cause the reduction in drip loss. Addition of vitamin E in the coating solution did not affect the drip loss between coated samples ($P > 0.05$).

3.2. Effect of chitosan coatings on total lipid, omega-3 fatty acid contents, and TBARS values of lingcod fillets

The total lipid content of uncoated fish fillets ranged from 0.98% to 1.86% (Fig. 3), indicating the low fat content in lingcod fish. Fish oil incorporated chitosan coatings significantly increased the total lipid content of the fish samples ranging from 2.81% to 6.29% ($P < 0.05$), which was an approximately 3-fold increase when compared to uncoated samples. The incorporated fish oil in chitosan coatings was stable, with no significant change being observed in total lipid content of coated samples during both cold and frozen storages ($P > 0.05$).

Chitosan has been well-known for its good film-forming property and excellent compatibility with other substances. The use of chitosan films or coatings to incorporate antimicrobials, antioxidants, and nutrients in food systems has been reported in several previous studies (Han, Zhao, Leonard, & Traber, 2004; Park & Zhao, 2004; Park et al., 2004; Sebti, Chollet, Degraeve, Noel, & Peyrol, 2007). The presence of the high density of amino groups and hydroxyl groups in its polymer structure makes chitosan a cationic polyelectrolyte, one of the few found in nature. Chitosan possesses high positive charge on $-NH_3^+$ groups when dissolved in aqueous acidic solution, and therefore it is able to adhere to or aggregate with negatively charged lipids and fats (Wydro, Krajewska, & Hac-Wydro, 2007).

The fatty acid composition showed that omega-3 fatty acids, including ALA, EPA, DHA, docosapentaenoic acid (DPA), and eicosatetraenoic acid (ETA), accounted for 33.3–55.8% of total lipid of the fish samples (data not shown). Uncoated fish samples contained only 0.45–0.74% of omega-3 fatty acids, while coated samples showed significantly ($P < 0.05$) enhanced omega-3 fatty acid con-

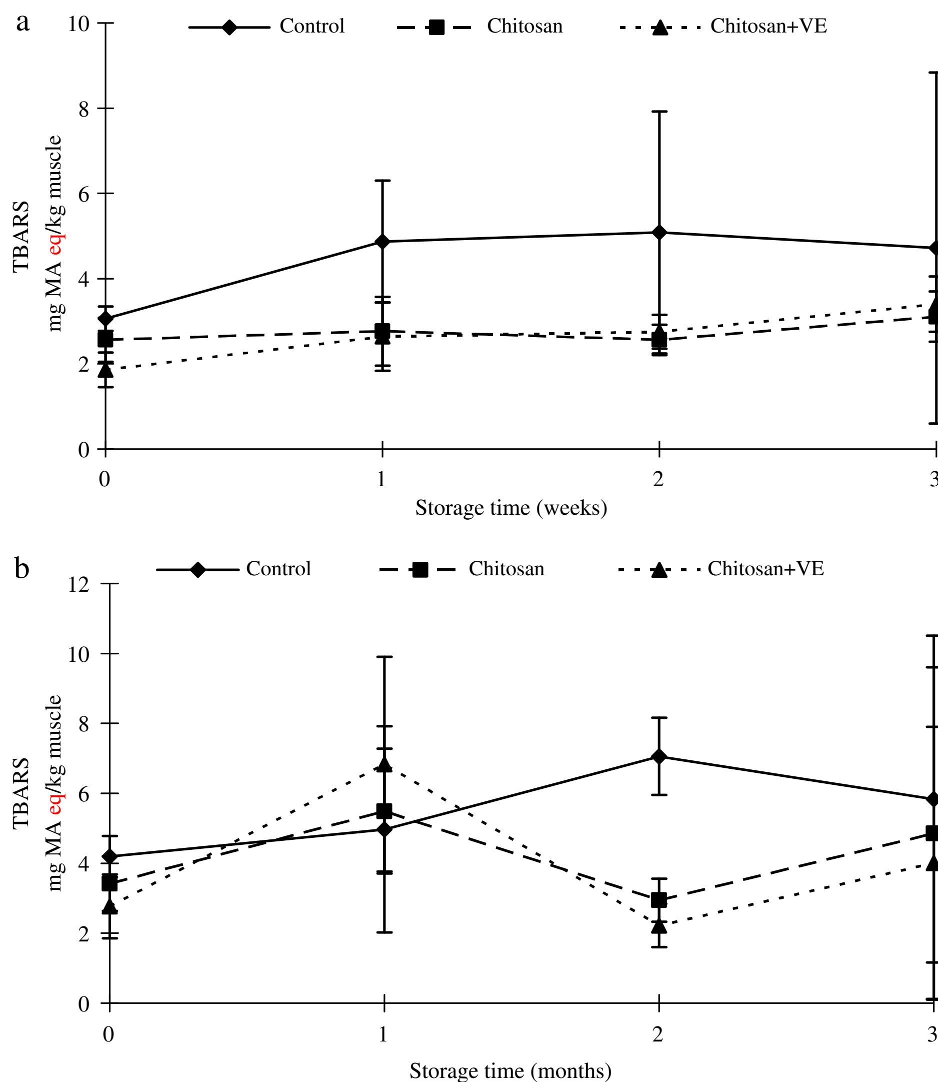

Fig. 4. Thiobarbituric acid-reactive substances (TBARS) of fish fillets during (a) cold storage at 2 °C and (b) frozen storage at −20 °C. The results are the mean of four replications and the bars indicate the standard deviation. V_E: vitamin E500.

tent ranging from 1.11% to 2.27%, which was comparable to the omega-3 fatty acid content of ~1.0–2.2% in salmon (Kris-Etherton et al., 2002) (Fig. 3). The omega-3 fatty acids were also stable in cold and frozen stored fish, with no significant change being found during both cold and frozen storages ($P > 0.05$). The addition of vitamin E in coating solution did not affect the total lipid and omega-3 fatty acid contents in coated samples ($P > 0.05$).

For cold storage, the initial TBARS value of fresh fish fillet was 3.06 mg MA eq/kg muscle (Fig. 4a). The TBARS value of uncoated samples increased to 4.87 mg MA eq/kg muscle after 1 week cold storage and ranged from 4.72 to 5.08 mg MA eq/kg muscle over the 3 weeks storage. The chitosan-coated samples showed lower TBARS values (ranging from 1.86 to 3.40 mg MA eq/kg muscle) throughout the cold storage ($P < 0.05$), indicating that the coatings might be effective in reducing lipid oxidation. However, the addition of vitamin E, a natural antioxidant, in chitosan coatings did not enhance the antioxidant effect of the coatings ($P > 0.05$). The lipid oxidation was not severe in lingcod fish, since it is a lean fish species. The chitosan coatings might be sufficient to protect the fish from lipid oxidation. Therefore, the antioxidant activity of vitamin E was masked by that of chitosan in this study.

The initial TBARS value of fresh fish for frozen storage was 4.19 mg MA eq/kg muscle. The TBARS value of uncoated samples increased from 4.19 to 7.06 mg MA eq/kg muscle after 2 months frozen storage, and decreased to 5.83 mg MA eq/kg muscle at the end of 3 months frozen storage (Fig. 4b), indicating that the freezing temperature did not prevent the lipid oxidation in fish flesh. Chitosan coating did not show antioxidant effect during the 1st month storage ($P > 0.05$), but it significantly lowered the TBARS values of coated samples in the subsequent storage time ($P < 0.05$). Similar to the cold storage, the addition of vitamin E in the coating solution did not cause greater reduction in lipid oxidation in coated samples ($P > 0.05$).

Similar results were found by other researchers. Jeon et al. (2002) found lower TBARS values in chitosan-coated herring and Atlantic cod samples throughout a 12 d cold storage. Sathivel (2005) reported that chitosan coatings reduced the lipid oxidation in pink salmon fillets during the frozen storage. Lipid oxidation causes undesirable rancid off-flavours and potentially toxic products, which lead to the qualitative deterioration of fish (Eymard et al., 2005). The free radical chain reactions in lipid oxidation are initiated by the attacks of oxygen to the double bond in fatty acids. With the good oxygen barrier properties, chitosan coatings applied on the surface of fish may act as a barrier between the fillet and its surroundings, thus slowing down the diffusion of oxygen from the surrounding to the surface of fillet and retarding the lipid oxidation (Sathivel, 2005). In addition, chitosan may reduce lipid oxidation by chelating ferrous ions present in fish proteins, thus eliminating their prooxidant activity or their conversion to ferric ion (Kamil, Jeon, & Shahidi, 2002). The ability of chitosan to combine with lipid also plays a role in its antioxidative activity (Xue, Yu, Hirata, Terao, & Lin, 1998).

3.3. Effect of chitosan coatings on microbiological qualities of fish

Microorganism grew fast in cold stored fish fillets. The initial total plate count of fresh fish fillets was 2.18 log CFU/g, but the population increased to 6.21 log CFU/g by the end of the 1st week, and

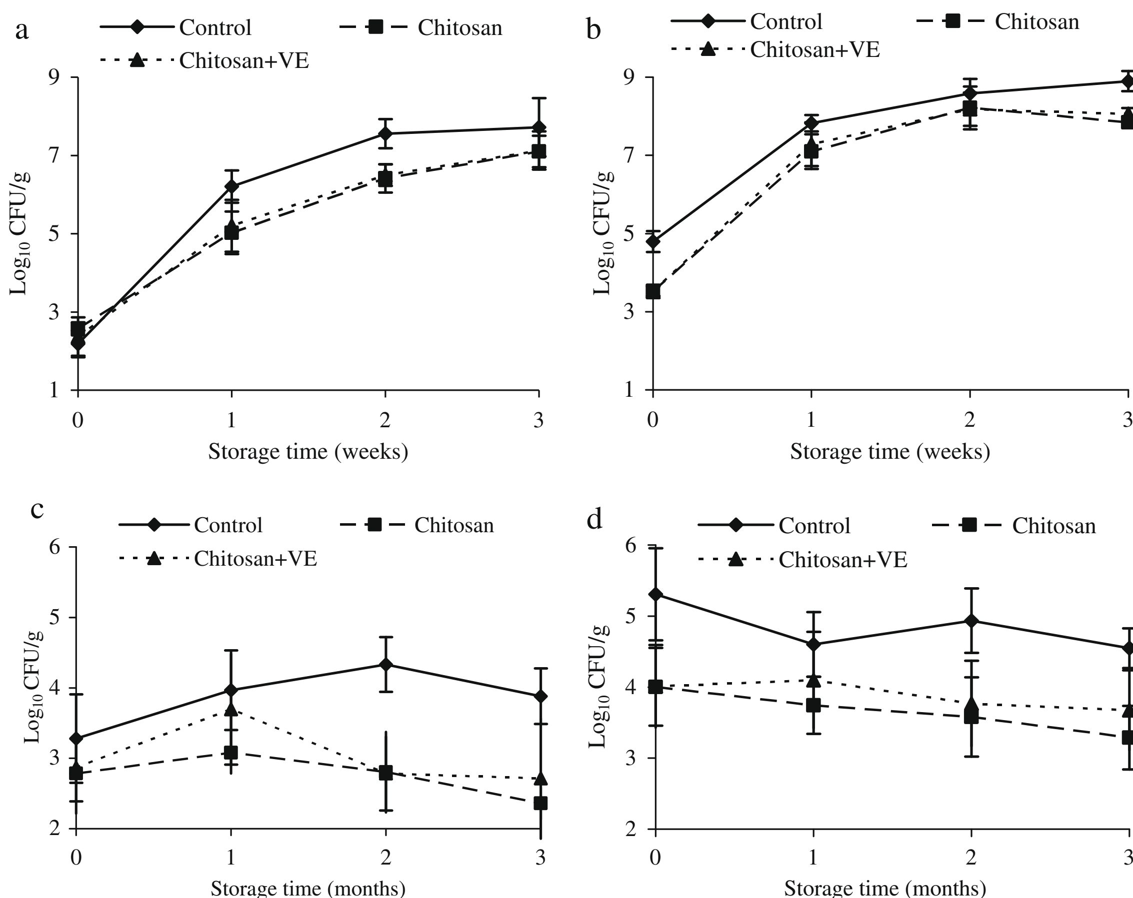

Fig. 5. Microbiological quality of fish fillets. (a) Total plate count during cold storage at 2 °C; (b) psychrotrophic count during cold storage at 2 °C; (c) total plate count during frozen storage at −20 °C; (d) psychrotrophic count during frozen storage at −20 °C. The results are the mean of four replications and the bars indicate the standard deviation. V_E: vitamin E500.

reached 7.55 log CFU/g by the end of the 2nd week storage in uncoated samples (Fig. 5a). Chitosan coatings significantly lowered the total plate count in fish samples ($P < 0.05$), with 0.60–1.19 log CFU/g reductions being obtained in the coated samples, and the total plate counts of coated samples were below 10^7 CFU/g during the first 2 weeks of cold storage. Jeon et al. (2002) reported that chitosan coatings resulted in 2–3 log reductions in total plate counts of herring and cod samples after 12 d of refrigerated storage. The greater inhibitory effect might be related with the higher molecular weight (6.6×10^5–1.8×10^6 Da) of chitosan used in their study.

Psychrotrophic counts of cold stored fish fillets was about 1 log CFU/g higher than total plate counts, indicating that fish bacterial flora is composed mainly of psychrotrophic bacteria. Psychrotrophic counts of uncoated fish rapidly increased to 7.82 log CFU/g by the end of the 1st week storage and exceeded 8.58 log CFU/g after 2 weeks of cold storage (Fig. 5b). Fish is highly perishable under chilled conditions. The shelf-life of cold stored fish in this study was estimated as only about 5 d, if the acceptability limit of 10^7 CFU/g was applied. Chitosan coatings resulted in 0.37–1.05 log CFU/g reductions in psychrotrophic counts of cold stored fish ($P < 0.05$), which extended the shelf-life of fresh fish fillets to 7 d.

The growth of microorganism in frozen fish fillets was inhibited by the freezing temperature. The total plate count of uncoated fish increased slowly from 3.28 log CFU/g on day 0 to 4.33 log CFU/g after 2 months of frozen storage, but dropped down to 3.88 log CFU/g at the end of 3 months (Fig. 5c). The chitosan coatings resulted in 0.27–1.55 log CFU/g reductions in total plate count, and the total plate counts of coated samples were lower than 10^3 CFU/g by the end of 2 and 3 months storage.

Similar to cold storage, the psychrotrophic counts of frozen fish fillets were higher than total plate counts. The frozen fish samples showed a decreasing trend in psychrotrophic counts, which was probably caused by the intra- and extra-cellular ice crystals formed during the freezing process that induce the irreversible damage to both the outer and the cytoplasmic membranes of bacteria (Uljas & Ingham, 1999). However, the inactivating activity from freezing process was limited. The population of psychrotrophic bacteria in uncoated fish fillets slightly decreased from 5.31 log CFU/g on day 0 to 4.55 log CFU/g at the end of 3 months frozen storage (Fig. 5d). The chitosan coatings significantly lowered the psychrotrophic counts by 0.50–1.31 log CFU/g ($P < 0.05$) and kept the populations of psychrotrophic bacteria lower than 10^4 CFU/g by the end of 2 and 3 months frozen storage.

Chitosan is well-known for its excellent film-forming property and broad antimicrobial activity against bacteria and fungi (Rabea et al., 2003). The antimicrobial action of chitosan appears to be mediated by the interactions between the positively charged chitosan and negatively charged microbial cell membranes, which induces the leakage of cellular proteins and other intracellular constituents. Chitosan also inhibits the microbial growth by the chelation of essential metals and nutrients, spore components, as well as the penetration of the nuclei of the microorganisms, which leads to the interference with protein synthesis by binding with DNA. Furthermore, chitosan coatings act as an oxygen barrier and thus inhibit the growth of aerobic bacteria (Devlieghere et al., 2004; Shahidi, Arachchi, & Jeon, 1999).

4. Conclusions

Chitosan coatings have broad antimicrobial activity and excellent compatibility with other functional substances. This study demonstrated that chitosan coatings incorporating fish oil significantly reduced TBARS values, inhibited growth of total and psychrotrophic bacteria, and enhanced the total lipid and omega-3 fatty acid contents in lingcod fish fillets during the cold and frozen storages. The drip loss of coated frozen samples was also reduced when compared to uncoated samples. Chitosan coatings did not affect the colour of fish fillets, but lowered the pH and moisture content of the coated samples, which was caused by the acidity of coating solution and the air dry process during coating application, respectively. The addition of vitamin E in the coating solutions did not further enhance the antioxidant effect of chitosan coatings. This study suggested that the fish oil incorporated chitosan coatings could be applied in fish packaging to extend shelf-life and enhance omega-3 fatty acid content of lean fish. A separate sensory study will be conducted to evaluate the sensory characteristics and consumer acceptance of treated fish fillets.

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References

Ababouch, L. H., Souibri, L., Rhaliby, K., Ouahdi, O., Battal, M., & Busta, F. F. (1996). Quality changes in sardines (*Sardina pilchardus*) stored in ice and at ambient temperature. *Food Microbiology, 13*(2), 123–132.

Bahuaud, D., Mørkøre, T., Langsrud, Ø., Sinnes, K., Veiseth, E., Ofstad, R., et al. (2008). Effects of −1.5 °C super-chilling on quality of Atlantic salmon (*Salmo salar*) pre-rigor fillets: Cathepsin activity, muscle histology, texture and liquid leakage. *Food Chemistry, 111*(32), 9–339.

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology, 52*, 302–310.

Cagri, A., Ustunol, Z., & Ryser, E. T. (2004). Antimicrobial edible films and coatings. *Journal of Food Protection, 67*(4), 833–848.

Campos, C. A., Rodríguez, Ó., Losada, V., Aubourg, S. P., & Barros-Velázquez, J. (2005). Effects of storage in ozonised slurry ice on the sensory and microbial quality of sardine (*Sardina pilchardus*). *International Journal of Food Microbiology, 103*(2), 121–130.

Cherian, G., Traber, M. G., Goeger, M. P., & Leonard, S. W. (2007). Conjugated linoleic acid and fish oil in laying hen diets: Effects on egg fatty acids, thiobarbituric acid reactive substances, and tocopherols during storage. *Poultry Science, 86*(5), 953–958.

Connor, W. E. (2000). Importance of $n-3$ fatty acids in health and disease. *American Journal of Clinical Nutrition, 71*(1), 171S–175S.

Das, U. N. (2008). Can essential fatty acids reduce the burden of disease(s)? *Lipids in Health and Disease, 7*, 9.

Devlieghere, F., Vermeulen, A., & Debevere, J. (2004). Chitosan: Antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology, 21*(6), 703–714.

Duan, J., Park, S.-I., Daeschel, M. A., & Zhao, Y. (2007). Antimicrobial chitosan–lysozyme (CL) films and coatings for enhancing microbial safety of Mozzarella cheese. *Journal of Food Science, 72*(9), M355–M362.

Exler, J., Wehrauch, J. L., & USDA. (1988). Provisional table on the content of omega-3 fatty acids and other fat components in selected foods. *Human Nutrition Information Service*, HNS/PT-103.

Eymard, S., Carcouët, E., Rochet, M.-J., Dumay, J., Chopin, C., & Genot, C. (2005). Development of lipid oxidation during manufacturing of horse mackerel surimi. *Journal of the Science of Food and Agriculture, 85*(10), 1750–1756.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–507.

Han, C., Zhao, Y., Leonard, S. W., & Traber, M. G. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria × ananassa*) and raspberries (*Rubus ideaus*). *Postharvest Biology and Technology, 33*(1), 67–78.

Horrocks, L. A., & Yeo, Y. K. (1999). Health benefits of docosahexaenoic acid (DHA). *Pharmacological Research, 40*(3), 211–225.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). *Modern food microbiology* (7th ed.). New York: Springer.

Jeon, Y.-J., Kamil, J. Y. V. A., & Shahidi, F. (2002). Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. *Journal of Agricultural and Food Chemistry, 50*(18), 5167–5178.

Jeyasekaran, G., Ganesan, P., Anandaraj, R., Shakila, R. J., & Sukumar, D. (2006). Quantitative and qualitative studies on the bacteriological quality of Indian white shrimp (*Penaeus indicus*) stored in dry ice. *Food Microbiology, 23*(6), 526–533.

Kamil, J. Y. V. A., Jeon, Y.-J., & Shahidi, F. (2002). Antioxidative activity of chitosans of different viscosity in cooked comminuted flesh of herring (*Clupea harengus*). *Food Chemistry, 79*(1), 69–77.

Kim, W. B., Daeschel, M., & Zhao, Y. (2008). Edible coatings for enhancing microbial safety and extending shelf life of hard-boiled eggs. *Journal of Food Science, 73*(5), M227–235.

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation, 106*, 2747–2757.

Mackie, I. M. (1993). The effects of freezing on flesh proteins. *Food Reviews International, 9*(4), 575–610.

Matsumoto, T., Nakayama, N., Ishida, K., Kobayashi, T., & Kamata, K. (2009). Eicosapentaenoic acid improves imbalance between vasodilator and vasoconstrictor actions of endothelium-derived factors in mesenteric arteries from rats at chronic stage of type 2 diabetes. *The Journal of Pharmacology and Experimental Therapeutics, 329*(1), 324–334.

Nunes, M., Batista, I., & Morão de Campos, R. (1992). Physical, chemical and sensory analysis of sardine (*Sardina pilchardus*) stored in ice. *Journal of the Science of Food and Agriculture, 59*(1), 37–43.

Ouattara, B., Simard, R. E., Piette, G., Bégin, A., & Holley, R. A. (2000). Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *International Journal of Food Microbiology, 62*(1–2), 139–148.

Park, S.-I., Daeschel, M. A., & Zhao, Y. (2004). Functional properties of antimicrobial lysozyme–chitosan composite films. *Journal of Food Science, 69*(8), M215–221.

Park, S.-I., & Zhao, Y. (2004). Incorporation of a high concentration of mineral or vitamin into chitosan-based films. *Journal of Agricultural and Food Chemistry, 52*(7), 1933–1939.

Rabea, E. I., Badawy, M. E., Stevens, C. V., Smagghe, G., & Steurbaut, W. (2003). Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules, 4*(6), 1457–1465.

Ruiz-Capillas, C., & Moral, A. (2001). Correlation between biochemical and sensory quality indices in hake stored in ice. *Food Research International, 34*(5), 441–447.

Sathivel, S. (2005). Chitosan and protein coatings affect yield, moisture loss, and lipid oxidation of pink salmon (*Oncorhynchus gorbuscha*) fillets during frozen storage. *Journal of Food Science, 70*(8), E455–459.

Sebti, I., Chollet, E., Degraeve, P., Noel, C., & Peyrol, E. (2007). Water sensitivity, antimicrobial, and physicochemical analyses of edible films based on HPMC and/or chitosan. *Journal of Agricultural and Food Chemistry, 55*(3), 693–699.

Shahidi, F., Arachchi, J. K. V., & Jeon, Y.-J. (1999). Food applications of chitin and chitosans. *Trends in Food Science and Technology, 10*(2), 37–51.

Singh, R. K., & Balange, A. K. (2005). Characteristics of pink perch (*Nemipterus japonicus*) surimi at frozen temperature. *Journal of Food Processing and Preservation, 29*(1), 75–83.

Tidwell, J. H., & Allan, G. L. (2001). Fish as food: Aquaculture's contribution. *EMBO Reports, 2*(11), 958–963.

Tironi, V., LeBail, A., & de Lamballerie, M. (2007). Effects of pressure-shift freezing and pressure-assisted thawing on sea bass (*Dicentrarchus labrax*) quality. *Journal of Food Science, 72*(7), C381–387.

Uljas, H. E., & Ingham, S. C. (1999). Combinations of intervention treatments resulting in 5-Log_{10}-unit reductions in numbers of *Escherichia coli* O157:H7 and *Salmonella typhimurium* DT104 organisms in apple cider. *Applied and Environmental Microbiology, 65*(5), 1924–1929.

USDA. (2008). USDA national nutrient database for standard reference, release 21. Available from: <http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl> Accessed 16.06.09.

Wydro, P., Krajewska, B., & Hac-Wydro, K. (2007). Chitosan as a lipid binder: A langmuir monolayer study of chitosan–lipid interactions. *Biomacromolecules, 8*(8), 2611–2617.

Xue, C., Yu, G., Hirata, T., Terao, J., & Lin, H. (1998). Antioxidative activities of several marine polysaccharides evaluated in a phosphatidylcholine–liposomal suspension and organic solvents. *Bioscience, Biotechnology, and Biochemistry, 62*(2), 206–209.