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THE EFFECT OF CHITOSAN-BASED COATING ON THE QUALITY OF FRESH REDFISH (*Sebastes marinus*) FILLET DURING COLD STORAGE

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ABSTRACT

The main aim of this study was to evaluate the improvement of quality obtained through use of chitosan-based coating on redfish fillets under cold storage. For this purpose, four different skinned fillet groups treated with different solutions (water, ascorbic acid 1%, chitosan 1.5 %, chitosan 1.5% combined with gelatine 2%) was stored at 2 -3^{o}C for 12 days to determine the changes (physical, chemical, microbiological, sensory) during preservation. The results revealed that chitosan-based coating significantly increased the whiteness as well as significantly limited the lipid oxidation of redfish fillets during cold storage ($p<0.05$). It slowed down the increase in TVC in the first 2 days of cold storage. Chitosan combined with gelatine also showed a delay on TVC and *Pseudomonas* spp. during cold storage. Chitosan-based coating could significantly prolong the freshness of redfish fillets, as well as increase the shelf life and quality of redfish fillet during cold storage ($p<0.05$).

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1 INTRODUCTION

Fish products play an important role in the Vietnamese economy and are one of the top ten main exported commodities (Vietnam Customs, 2019). Vietnam exports fish products to over 70 countries in the world with a value of over 4 billion US dollars per year (Vasepa, 2019). The main export markets are the US, the EU and Japan (Vasepb, 2019). In Vietnam, frozen fish is an export orientated product (Vasepb, 2019). Frozen products are relatively easy to export to foreign countries due to their long shelf life. By contrast, fresh or chilled fish is more preferred and highly priced, when compare to frozen fish. Fresh fish also represents the largest share of fish for direct human consumption in the world (FAO, 2018). Vietnamese people have a long tradition of consumption of fresh fish. Fresh fish has high levels of moisture and is rich in digestible proteins and polyunsaturated fatty acids. As a result, it is highly perishable and has a short shelf life even under chilling. Hence, to enhance the economic value of fish products, Vietnamese fish producers nowadays are concerned with prolonging the storage time and quality of fresh fish during cold storage.

Food packaging can help to prevent oxidative and microbial spoilage and extend the shelf-life of fish products (Tharanathan, 2003). In recent years, the use of biodegradable and edible materials for fish packaging, especially from agro-industrial by-products as well as marine food processing industry wastes, has increased (Baldwin, Hagenmaier, & Bai, 2012). The edible coatings have so far improved gas and moisture barriers, sensory attributes, microbial protection, and prolonged the storage time of various fish products (Krochta, 2002).

Chitosan is a partially deacetylated derivative of chitin, which is produced from the exoskeletons of crustacean shellfish, e.g., crabs and shrimps (Muzzarelli, 1996; Zhang, Xue, Li, Zhang, & Fu, 2006). Chitosan has attracted increased attention since derived coatings to improve the fish quality and the shelf life of the protected fish due to its adequate mechanical properties, excellent gas barrier properties as well as antimicrobial properties (No, Xu, & Meyers, 2007; López-Caballero, Gómez-Guillén, Pérez-Mateos, & Montero, 2005; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003; López-Caballero, Gómez-Guillén, Pérez-Mateos, & Montero, 2005; Coma, Martial-Gros, Garreau, Copinet, & Deschamps, 2002). Although chitosan is not currently allowed in the EU, it is accepted as a food additive in the US, Japan and other countries (Tanaka, Kaneda, Suguro, & Bara, 2004; EPA, 2019; Primex, 2018).

Vietnam is a major exporter of shrimp with the amount of 202 thousand metric tons to the world market (FAO, 2019). Shrimp is usually processed into shrimp meat for export. Approximately 35 - 45% of leftovers are shells and heads considered by-products. In recent years, Vietnamese scientists have researched successfully on shrimp shells to produce chitosan (Trang & Pham, 2012). Economically, chitosan is cheap due to being produced from the fishery processing industry wastes. Though chitosan has been used as an edible coating on fruit products, it is not currently used in application as a method for preservation of fresh fish products in Vietnamese fisheries industry.

Based on the above facts, utilization of chitosan for fresh fish preservation has a significant meaning for the fishery industry in Vietnam. A better understanding of the influence of the chitosan-based coating on fresh fish would offer prospects to increase the shelf life and the quality of cold-stored fresh seafood products in Vietnam.

The main goal of this project is to provide an innovative solution for fresh fish preservation in Vietnam by using chitosan-based coating. Redfish is chosen in this project due to the similar properties found with species present in Vietnam (pangasius, cobia, etc.).

1.1 Project objectives

- Determine the changes (physical, chemical, microbiological, sensory) of chitosan-coated fresh Redfish fillet occurring during cold storage;
- Evaluate any improvement of quality obtained through use of the chitosan-based coating.

2 LITERATURE REVIEW

2.1 Edible coating in fish preservation

An edible coating or film is primary packaging made from edible components from biopolymers (Marsuelli, 2018). A thin layer of edible/biopolymeric material can be directly coated on food or be used as a food wrap without changing the original ingredients or the processing method (Galus & Kadzinska, 2015). Whereas edible films are defined as a thin layer or solid sheets of material placed on or between food components, edible coatings can be applied in liquid form (Falgueraa, Pablo, Jimenez, & Munoz, 2011; Galus & Kadzinska, 2015). Biopolymers can be consumed by humans or other animals in whole or part via the oral cavity and are harmless in terms of effects on health (Shit & Shah, 2014). The various naturally occurring biopolymeric materials of use in composite film making and coating formulations are shown in Table 1 (Tharanathan, 2003). Chitosan, starch, cellulose, alginate, carrageenan, gelatine, zein, gluten, whey, carnauba, beeswax and fatty acids are the most commonly used compounds to form edible coatings (Baldwin, Hagenmaier, & Bai, 2012; Shit & Shah, 2014).

Table 1. The various naturally biopolymeric materials use in composite film making and coating formulations.

Source	Edible/biopolymeric material
Animal origin	Collagen/gelatine
Marine by-product	Chitin/Chitosan; Collagen/gelatine; Free fatty acids
Agriculture	Lipids/Fats - Beeswax - Carnauba wax - Free fatty acids Hydrocolloids - Protein: Zein, Soy, Whey, Wheat gluten - Polysaccharides: Cellulose, Fibre (lignocellulosic complex), Starch, Pectin/gums
Microbial	Pullulan Polylactic acid Polyhydroxyalkanoates

Biopolymers can be applied as additional protection to preserve food quality and stability (Shit & Shah, 2014). Edible coatings can be applied by dipping or spraying products in coating

materials and then allowing excess coating to drain as it dries and solidifies (Baldwin, Hagenmaier, & Bai, 2012). When food products are coated by dipping or spraying, a thin film forms on the food surface that acts as a semipermeable membrane, which in turn controls the moisture loss and/or suppresses gas transfer (Lin & Zhao, 2007). The dip method of coating is commonly used method for fruits, vegetables, and meat or fish products.

In recent years, there has been an increasing interest to develop edible materials with film-forming capacity and having antimicrobial properties that help to improve safety and shelf life of products (Fakhouria, Martelli, Caon, Velasco, & Mei, 2015). The edible coatings have so far improved gas and moisture barriers, mechanical properties, sensory attributes, convenience, microbial protection, and prolonged the shelf life of various fish products (Krochta, 2002).

2.2 Chitosan

Chitosan is a polycation consisting of randomly distributed β-(1-4)-linked N-acetyl-d-glucosamine and d-glucosamine with specific structure and properties (Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003) (Figure 1). It is a partially deacetylated derivative of chitin, from the exoskeletons of crustacean shellfish, e.g., crabs and shrimps (Muzzarelli, 1996; Zhang, Xue, Li, Zhang, & Fu, 2006; Khan, Peh, & Ch'ng, 2000; No, Xu, & Meyers, 2007).

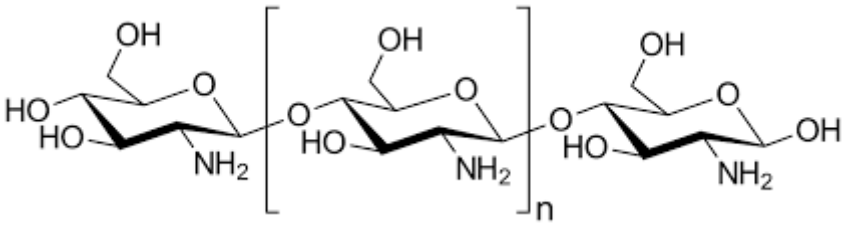


Figure 1. Chitosan formula

The degree of deacetylation (DDA) and the molecular weight (M_w) are two important parameters used to characterize chitosan (Tharanathan, 2003). The DDA of chitosan usually ranges from 75–80 % in foods and food products, and from 90–95 % in pharmaceuticals (Tsai, Su, Chen, & Pan, 2002; Kumar, Muzzarelli, Muzzarelli, & Sashiwa, 2004). The M_w of chitosan is a key parameter in the preparation of chitosan complexes. The commercial molecular weights of chitosan are in the range of 2000 to 2050 kDa (Ilium, 1998). Chitosan with high M_w is a very stable complex, but due to its bulky molecules, the interaction efficiency is very low. By contrast, chitosan with low M_w presents a higher activation energy and can effectively interact with other negatively charged compounds (Zhang, Oh, Allen, & Kumacheva, 2004). On the other hand, chitosan is insoluble in water, alkali, and organic solvents but soluble in most organic acids with a pH below 6, because of the positive charge amino group on the C2 of the glucosamine monomer. Chitosan can be formed into viscous solutions and they may function as thickeners, stabilizers, suspending agents. Chitosan has the ability to form protective films (Coma, Martial-Gros, Garreau, Copinet, & Deschamps, 2002; Fernandez-Saiz, Lagaron, & Ocio, 2009; Cho, Jang, Park, & Ko, 2000). In addition, the viscosity of chitosan is affected by DDA, M_w, concentration, types of solvents, pH value of the prevailing solution and ionic strength, as well as temperature (Kumar M. N., 2000).

Chitosan has several favourable biological properties. It is natural, biodegradable, biocompatible, bland in taste, non-toxic, analgesic, antitumorigenic, haemostatic, hypocholesterolaemia, and has antioxidant properties (Muzzarelli, 1996; Kumar M. N., 2000; Chitin-the undisputed biomolecule of great potential, 2003). The oral LD50 (median lethal dose) of chitosan in mice was found to be in excess of 16 g/kg of body weight per day, which

is higher than that of sucrose (Singla & Chawla, 2001; Bowman & Leong, 2006). Additionally, chitosan shows antimicrobial properties which can inhibit the growth of a wide range of microorganisms such as bacteria, fungi, and yeast (Sagoo, Board, & Roller, 2002; Kumar, Varadaraj, Gowda, & Tharanathan, 2005). Chitosan is reportedly more effective in inhibiting growth of gram-positive bacteria than that of gram-negative bacteria (No, Xu, & Meyers, 2007) (No et al., 2002). On the other hand, chitosan is inexpensive because it is a natural compound obtained by deacetylation of chitin, which is produced from shrimp, crab, and crawfish shells waste (Knorr, 1994). As a result, chitosan is an excellent choice for a natural food additive component and can be used as a prospectively edible coating for food preservation.

2.3 Gelatine

Gelatine, a polyanion, is a water-soluble animal protein resulting from partial hydrolysis of collagen, a fibrous protein mainly found in certain parts of vertebrate and invertebrate animals as bones, skins, connective tissues and tendons, as well as of by-products obtained from the fishing industry, such as heads, skin, bones, fins, muscle pieces, scales, viscera (Shankar, Jaiswal, & Rhim, 2016; Alfaro, Balbinot, Weber, & Tonial, 2014).

Gelatine can be divided into two groups based on distinctive functional properties. The first group has properties related to surface behaviour such as protective colloid function, emulsion and foam formation and stabilization, adhesion and cohesion and film-forming capacity. The second group is associated with gelling behaviour, such as gel formation, thickening, texturizing and water binding capacity. So, gelatine can be widely used in the food, packaging, pharmaceutical, cosmetic and photographic industries. Additionally, gelatine shows good ability to form coatings (Amiri et al., 2018; Samsi et al., 2019). It has been used in packaging of highly perishable food products such as meat and fish. This is based on some particular properties such as cost, availability, functional attributes, mechanical (flexibility, tension) and optical (brightness and opacity) properties, barrier effect against gas flow, structural resistance to water and microorganisms and sensory acceptability (Shankar, Jaiswal, & Rhim, 2016). In recent research, chitosan has been combined with gelatine to enhance the properties of film forming and protect products (Yung-Shin, Guan-Wen, & Shao-Ching, 2019; Nowzari, Shábanpour, & Ojagh, 2013; Feng, Bansal, & Yang, 2016).

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Redfish fillet

Fresh redfish fillets were provided by the Brim company. A total of 144 skinned redfish fillets were used for pre-trials and training the sensory panellist as well as 324 fillets for the main trials. Redfish with average weight was 70.6 ± 8.5 g. The fishing day was 4th of January, the processing day was 8th of January

3.1.2 Chitosan

Chitosan with 90% degree of deacetylation (DDA) was used, supplied by the Primex Company.

3.1.3 Gelatine

Tilapia skin gelatine with 200 bloom was used, supplied by the Louis Francois Company.

3.1.4 Ascorbic acid

L-ascorbic acid with 97,5-100% purity, from Sigma Aldrich was used.

3.2 Methods

3.2.1 Pre study

Before doing the main experiments, pre-trials were carried out, including:

- Pre-trial 1: To determine the concentration of chitosan as well as type and concentration of acid solution to be used in the main experiments. Chitosan (0.5 %; 1%; 1.5%; 2 %) was dissolved in solution of acetic acid (0.5 %; 1 %) and ascorbic acid solution (0.5 %, 1 %) respectively. The mixtures were evaluated the soluble ability, the coating and dipping capacity (visual), pH value and general taste evaluation on cooked coated fish. The chitosan solution was realised at room temperature.

- Pre-trial 2: To determine the concentration of gelatine solution to use in the main experiment. Gelatine solution with concentration 1%, 2%, 3%, 4% were evaluated by their coating and dipping capacity (visual) respectively as well as the weight gain after coating. This trial was also conducted to determine if when the gelatine was added, the chitosan coating was replaced by it, or not. To show that the chitosan layer stayed on the fish, the chitosan layer was coloured with blue and after 10 second of drying it was dipped into the gelatine. It was shown that no colour went in the gelatine solution and stayed on the fillet, which means that the chitosan layer remained intact.

Making gelatine solution: Fish gelatine powder was dissolved in distilled water and stirred at 45^{o}C for 30 minutes. Then gelatine solution was kept at 25^{o}C during coating progress.

- Pre-trial 3: To evaluate the drying time after chitosan coating and chitosan combined gelatine coating. After coating, the coated fillet was dried at 2 – 3^{o}C for 1; 1.5; 2; 2.5 hours respectively. Then, different methods were used such as visual (dry/wet), touch (stick/non-stick) to determine the optimum drying time.

3.2.2 Experimental design

Flowchart of the experimental design is presented in Figure 2. Fresh redfish fillets were divided into four different treatment groups following the dipping method, including:

- Group 1 – W, water coating – the control sample.
- Group 2 – A, acid solution coating (control of chitosan groups coating)
- Group 3 - Ch, chitosan solution coating.
- Group 4 – Ch-Ge, double layers coating, chitosan and gelatine.

Fillets were immersed for 5 s in the water/solution. Samples in the group 4 – Ch-Ge, after dipping in chitosan solution and then allowed to stand for a 10 s period followed by immersion in gelatine solution for 5 s.

Then all coated fish fillets were allowed to dry at 2 - 3°C in order to form an edible coating. All samples were placed on Styrofoam trays with wrapping by linear low-density polyethylene material (LLDPE), stored at 2 - 3°C for 12 days to be evaluated for quality changes.

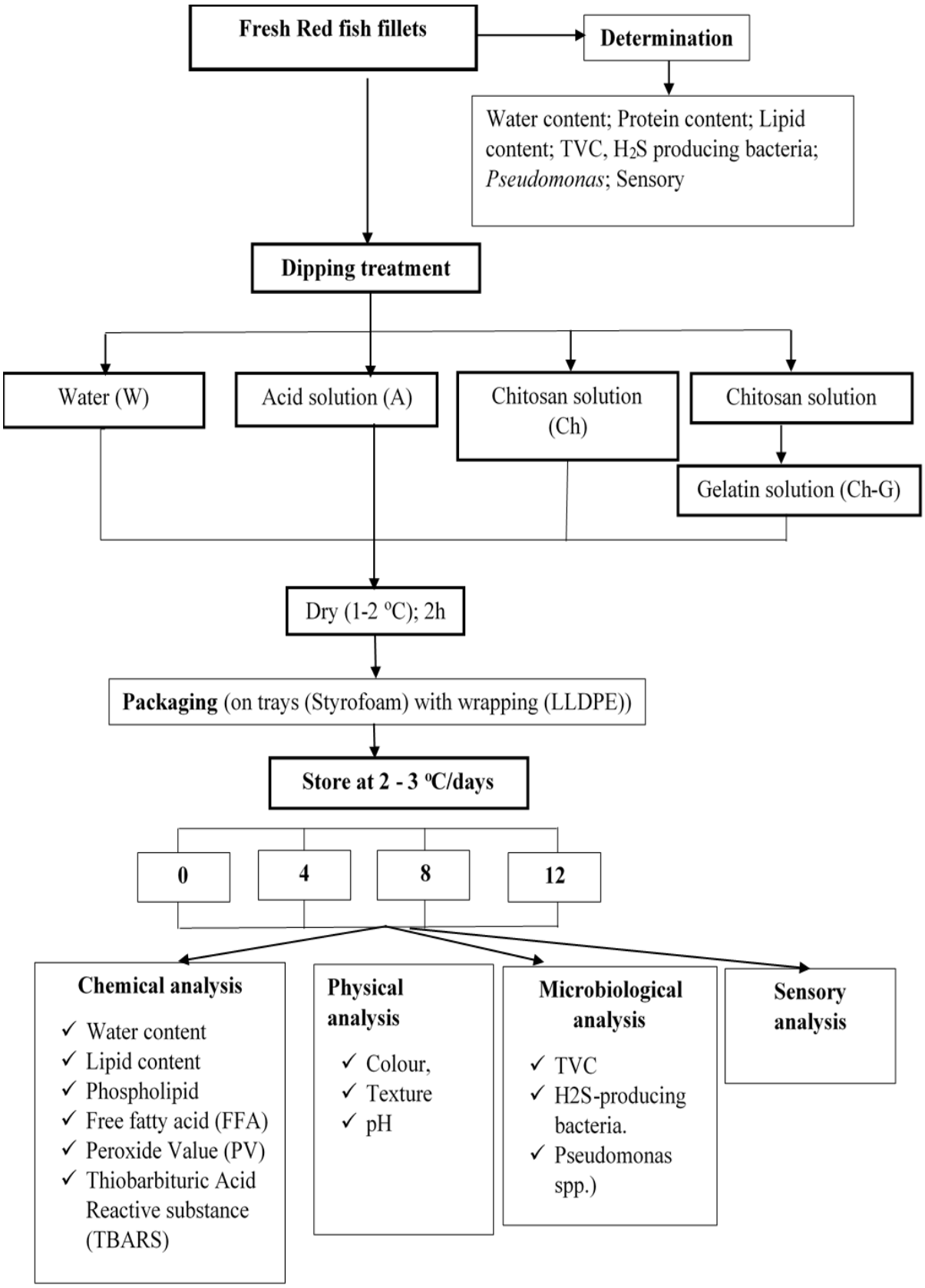


Figure 2. The flowchart of the main experiments for Redfish fillet preservation.

3.2.3 Methods of analysis

Microbial analysis

Total Viable Counts (TVC), H_2S-producing bacteria

A sample of 20 g were mixed with 180 g of dilution buffer (0.85 % NaCl + 0.1 % peptone) and the mixture was then blended in the Stomacher for one minute. The extracted solution from the mixture was diluted to the desired decimal dilutions. 1 mL of the decimal dilution was pipetted and poured into a Petri plate. Then, approximate 15 mL of melted iron agar (IA) medium was poured and mixed with the dilution in culture plates. When the medium turned to solid, the plates were covered by a thin layer of melted iron agar medium. The plates were then incubated at 22^{o}C for 48 hours.

Total viable bacteria (total white and black colonies) were counted on the plates using the Colony Counter (CFU/g).

Pseudomonas spp.

Enumeration of presumptive pseudomonas was performed using modified Cephaloridine Fucidin Cetrimide (mCFC) agar as described by Stanbridge and Board (1994). Plates were spread-plated and incubated incubated at 22°C for 3 days.

Pseudomonas Agar Base (Oxoid, UK) with CFC selective Agar Supplement (Oxoid) was used.

Chemical analysis

Water content

Water content was measured according to ISO 6496:1999. Approximately 5.0 g of homogenized sample was weighed and placed in a small porcelain bowl. The porcelain bowl of sample was left to dry for 4 hours in the oven at 103±2°C. The bowl was removed from the oven and allowed to cool to ambient temperature in a desiccator for about 30 minutes. The water content was calculated by the formula as follows:

$$\mathbf{W} = \frac{m2 - m3}{m2 - m1} * 100\ (\%)$$

$W = m2{-}m3\ m2{-}m1 * 100\ (\%)$

Where: m1 was the mass of the bowl (g)

m2 was the mass of the bowl, test portion (g)

m3 was the mass of the dish, dried test portion (g).

Protein content

Protein content was determined by the Kjeldahl method (ISO 5983-1:2005). 5 g homogenized sample was digested in sulphuric acid in the presence of copper as a catalyst. Thereafter, the sample was placed in a distillation unit, 2400 Kjeltec Auto Sample System. The digested sample was made alkaline by a sodium hydroxide solution, the nitrogen was distilled as ammonia. The ammonia was absorbed by boric acid solution and then the amount of ammonia nitrogen was quantified by titration with standardized H_2SO_4 solution. The nitrogen content was multiplied by the factor 6.25 to get the ratio of crude protein.

Lipid content

A homogenized sample of 25 g was extracted with 50 mL of methanol, 50 mL of chloroform and 25 ml KCl 0.88% (Bligh & Dyer, 1959). 3 mL of the chloroform phase of the Bligh and Dyer extraction was evaporated at 55°C under a nitrogen jet. The weight of the tube was recorded before adding the sample (m1) and after the evaporation (m2). The weight of the sample used to conduct the Bligh and Dyer extraction was recorded and named w.

$$Fat(\%) = \frac{\frac{(m2 - m1) * 50}{3}}{w} * 100$$

Phospholipid

The total lipid extracts were used to measure phospholipid content (PL) by the colorimetric method (Stewart, 1980). This method was based on the formation of a complex between phospholipids and ammonium ferrothiocyanate. A standard curve was prepared with phosphatidylcholine in chloroform (5 - 50 µg/ml) by evaluation of absorbance at 488 nm (UV1800 spectrophotometer, Shimadzu, Kyoto, Japan). The results were expressed as a percentage of the total lipid content.

Free fatty acids

Free fatty acids (FFA) were determined according to method from (Lowry & Tinsley, 1976) with a modification made by (Bernárdez, Pastoriza, Sampedro, Herrera, & Cabo, 2005). 3 mL of the lower phase resulting from lipid extraction (Bligh & Dyer, 1959) was added in a screw cap culture tube. Any solvent present was removed at 55^{o}C using nitrogen jet. After cooling, 3 mL of cyclohexane were accurately added by 1 mL of cupric acetate – pyridine reagent and vortex for approximate 40 seconds. After centrifugation at 2000 rounds per minute (rpm) for 10 min at 4^{o}C, the upper layer was read at 710 nm in spectrophotometer. The FFA concentration in the sample was calculated as µmol oleic acid based on a standard curve spanning a 0-20 µmol range. The results were indicated as µgram oleic acid per 100g lipid of sample.

Peroxide value (primary oxidation product)

Lipid hydroperoxides (PV) were determined with a modified version of the ferric thiocyanate method. Total lipids were extracted from 5 g of samples with 10 mL ice-cold solvent, including chloroform: methanol (1:1) solution with 500 ppm BHT to prevent further peroxidation during the extraction process. 5mL of sodium chloride (0.5 M) was added into the mixture and homogenized for 30 seconds before centrifuging at 5100 rpm for 5 minutes (TJ-25 Centrifuge, Beckmann Coulter, USA). The chloroform layer was collected (100 µL) and completed with 900 µL chloroform: methanol solution. A total amount of 5 µL of ammonium thiocyanate (4 M) and ferrous chloride (80 mM) mixture (1:1) was finally added. The samples were incubated at room temperature for 10 minutes and read at 500 nm (Tecan Sunrise, Austria). A standard curve was prepared using cumene hydroperoxides. The peroxide value was amount of µmol lipid hydroperoxides per kg of sample.

Thiobarbituric acid reactive substance (secondary oxidation product)

A modified method of Lemon (1975) was used for measuring thiobarbituric acid reactive substance (TBARS). 5 g of sample was homogenized with 10 mL of trichloroacetic acid (TCA) extraction solution (7.5% TCA, 0.1% propyl gallate and 0.1% EDTA mixture prepared in ultrapure water) using a homogenizer at maximum speed for 10 seconds (Ultra-Turrax T-25 basic, IKA, Germany). The homogenized samples then centrifuged at 5100 rpm for 20 minutes (TJ-25 Centrifuge, Beckmann Coulter, USA). 0.1mL supernatant was collected and mixed with the 0.9 mL thiobarbituric acid (0.02 M) and heated in a water bath at 95 °C for 40 min. The samples were cooled down on ice and immediately loaded into 96-wells microplates (NUNC A/S Thermo Fisher Scientific, Roskilde, Denmark) for reading at 530 nm (Tecan Sunrise, Austria). A standard curve was prepared using tetraethoxypropane. The results were indicated as µmol of malonaldehyde diethylacetal per kg of sample.

Physical analysis

Colour

The intensity of the flesh colour was measured with a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) using the CIE Lab system. The instrument recorded the L value, lightness on the scale of 0 to 100 from black to white; a value, (+) red or (-) green; b value, (+) yellow or (-) blue. The colour was measured above the lateral line at 3 point of fillets. The whiteness was calculated the formula as follows: Whiteness = L – 3*b (Hunter, 1960).

Texture

Firmness was evaluated by a compressing test using a TA. XT2i Texturer. To ensure the flesh temperature was kept constant during testing, each fish was kept on ice until the flesh samples were excised. The probe with 2.5 cm in diameter was applied on 3 points of each fillet (top-middle-tail). The distance of penetration was 5mm, the time was 5 seconds and the speed test was set at 1 mm s− 1. The maximum peak force in Newton required to compress on the sample was recorded as a compressing force.

pH

Orion Star A111 pH meter was used to measure the pH value. The electrode was submerged into the homogenised muscle fish.

Sensory evaluation

Generic Descriptive Analysis (GDA) and Torry freshness scale for medium fat fish were used to evaluate all samples in this study (Lawless H.T., 2010; Shewan JM, 1953). GDA expressed odour, appearance, flavour, and texture of cooked redfish with 26 different attributes listed in Appendix 1, table 1. GDA scale was described by using a 15 cm unstructured scale which in analysis was transformed to numbers from 0-100 from evaluation. The Torry scale ranged from 10 (good quality) to 3 (bad quality) and is shown in Appendix 1, table 2. Shelf life was defined as the time until the redfish reaches an average value of 5,5 on the Torry scale.

Twelve panellists all trained and experienced in evaluation of redfish participated in the evaluation (EN-ISO-8586, 2014).

Two panel training sessions were carried out prior to the evaluation to review both the GDA scale and Torry scale, as well as synchronising the panellists use of the scales. The GDA scale had been developed in earlier experiments on redfish. Samples of redfish of different treatments were used as references in the training.

Each sample was ~50 g of crosscut redfish loin. Ten fillets were used in each sensory session and two samples were taken from each fillet. The samples were cooked for 6 minutes in a steam oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) and presented warm to the panellists in a 140 ml aluminium box with a transparent plastic lid. Four samples were evaluated in each session. All samples were coded with three-digit numbers and a duplicate was evaluated for each sample group. The sensory evaluation program FIZZ (2.50B, Biosystémes) was used to collect sensory data.

The program Panelcheck (V1.4.0, Nofima, Tromsø, Norway) was used to evaluate the performance of the panel and individual panellists. The statistical program NCSS 2000 (NCSS, Utah, USA) was used to calculate Analysis of Variance (ANOVA, general linear model) to compare results from the four sample groups, per sampling day. A correction was made for different use of scale by the panellists. Duncan´s test was used to calculate multiple comparisons between sample groups. The significance level was set at 5%. Principal Component Analysis (PCA) was calculated using the program Unscrambler ® (Version 9.7, CAMO, Trondheim, Norway).

Data analysis

Microsoft Excel for office 365 was used to calculate means and standard deviations (STDV) and to build graphs. One-way ANOVA (analysis of variance) and post hoc Tukey's test were carried out with the software SPSS version 26.0 to compare the mean values for a statistic significantly level of 0.05.

4 RESULTS AND DISCUSSION

4.1 Pre-trials

4.1.1 Solubility of chitosan

The solubility of chitosan in different solvents is indicated in Table 2. The results showed that chitosan did not solubilise in water but dissolved in the acetic acid as well as the ascorbic acid solution. However, the effect of chitosan solubility in acetic acid solution was higher than in the ascorbic acid solutions in the room temperature. Chitosan could solute well in acetic acid solution with 0,5%, 1% and ascorbic acid solution 1%, meanwhile, it could not fully dissolve in ascorbic acid solution 0.5%.

Table 2. Solubility of chitosan with 90% DDA in different solutions.

Solvents		Chitosan (%)			
		0.5	1	1.5	2
Acetic acid (%)	0.5	*	*	*	*
	1	*	*	*	*
Ascorbic acid (%)	0.5	o	o	o	o
	1	*	*	*	*
Water		u	u	u	u

Note: *: Dissolved [a]; o: Partially dissolved [a]; u: Undissolved [a]
a: Evaluation of chitosan dissolution was based on visual assessment of the solution

4.1.2 The dipping and coating capacity of chitosan solution

A description of chitosan solutions in different concentrations is shown in Table 3. The results showed that chitosan solution 1% and 1.5% had viscous and sticky attributes. It was without problems to dip fish fillet inside these solutions and form a coating membrane on the surfaces of fillet.

Table 3. A visual evaluation of chitosan solution in different concentrations

Chitosan solution (%)	Description
0.5	Too liquid, almost non-sticky liquid. Easy for dipping fish fillets, the liquid almost disappears out of the fillet surface after coating.
1	Viscous and sticky liquid. Easy for dipping fish fillets, a layer of sticky liquid maintains on the fillet surface after coating.
1.5	Viscous and sticky liquid. Easy for dipping fish fillets, a layer of sticky liquid maintains on the fillet surface after coating.
2	Too dense and thick. Difficult to dip fish fillets in.

4.1.3 The pH value of solutions and the taste evaluation on cooked coated fillets

The results showed that chitosan influenced the pH value of the acid solution (Table 4). The pH value of acid solutions increased with an increase in concentration of chitosan. For example, the pH value of ascorbic acid 1% was 2.62. This value went up to 4.26 and 5.31 when adding chitosan powder 1 and 1.5 % respectively. The results also demonstrated that chitosan had a different effect on the pH value of different types of acid. For instance, the ascorbic and acetic solution with 1% had the same pH value (6.62). However, the pH value of these acid solutions was different when adding the same 1.5% chitosan concentration, As1Ch1.5 (Chitosan 1.5% in ascorbic acid solution 1%) was 5.31 and Ac1Ch1.5 (Chitosan 1.5% in acetic acid solution 1%) was 4.1.

The results also indicated that all coated samples did not have any acidity odour except for the fillet coated by acetic acid solution 1% (Table 4).

Table 4. The description of taste on cooked fillet coated by different solutions as well as pH

Coating solutions (%)	pH	Description of taste on cooked fish fillets
Ac0.5	2.9	No comment
Ac0.5Ch1	4.4	No comment
Ac0.5Ch1.5	4.77	No comment
As1	2.62	No comment
As1Ch1	4.26	No comment
As1Ch1.5	5.31	No comment
Ac1	2.62	Acidity, TMA odour
Ac1Ch1	3.84	No comment
Ac1Ch1.5	4.1	No comment

Note: Ac-Acetic acid; As-Ascorbic acid; Ch-Chitosan
AcCh: Chitosan dissolved in the acetic acid solution; AsCh: Chitosan dissolved in the ascorbic acid.

4.1.4 The concentration of gelatine solution for coating

A description of gelatine solutions, coated fillets, and the percentage of weight gain of samples after coating in different concentrations is presented in Table 5. The results showed that the gelatine solution with a concentration of 2% could be used effectively to form a thin membrane on fish fillets.

Table 5. A visual description of gelatine solutions, coated fillets and the weight gain of samples after coating in different concentrations.

Gelatine solution (%)	Description	Weight gain (%)
1	Too liquid. Easy to dip fish fillets, the liquid almost disappears out of the fillet surface after coating.	0.05
2	Viscous liquid. Easy to dip fish fillets, a thin layer of gelatine maintains on the fillet surface after coating and drying.	0.97
3	Viscous and sticky liquid. Easy to dip fish fillets, a rather thick film of gelatine maintains on the fillet surface after coating and drying.	2.37
4	Viscous and sticky liquid. Easy to dip fish fillets, a thick film of gelatine remains on the fillet surface after coating and drying.	3.67

4.1.5 The drying time after coating chitosan and chitosan combined gelatine

The results indicated that the optimum time for drying is 2 hours at 2-3^{o}C (Table 6). At this time, surface of coated fish fillet was dried and a bit sticky.

Table 6. The visual description of fish fillet's surface in different drying time.

Time (hours)	Chitosan 1.5%	Chitosan 1.5%- Gelatine 2%
1	Wet and sticky surface	Wet and sticky surface
1.5	Dried and sticky surface	A bit wet and sticky surface
2	Dried and a bit sticky surface	Dried and a bit sticky surface
2.5	Totally dried surface	Totally dried surface

Generally, both ascorbic and acetic acid solution dissolved chitosan. The acetic acid solution more effectively dissolved chitosan than the ascorbic acid solution at room temperature. According to study of Romanazzi et al. (2009) on the effect of chitosan dissolved in different acids, ascorbic acid solutions should be heated to 60^{o}C to dissolve chitosan (Romanazzi, Smilanick, Gabler, Margosan, & Mackey, 2009). On the other hand, the pH value of the acid solutions increased with an increase in the concentration of chitosan. Chitosan was a polycationic, it might play a role as a basic reagent and neutralize the proton released by the acid. This neutralization also caused the chitosan to dissolve in the aqueous phase and the pH value of solutions went up (Chen, Hwang, Kuo, & Liu, 2007).

Acetic acid, which had a strong and unpleasant smell, gave negative attributes to the coated fillets. Ascorbic acid solution with 1% of concentration dissolved chitosan at room temperature and did not give any negative tastes. Ascorbic acid was also known as an antioxidant using widely in food preservation. In this research, ascorbic acid solution of 1% was used to dissolve chitosan. This may enhance the antioxidant ability of chitosan coating.

4.2 Main trials

4.2.1 Chemical compositions and number of microorganisms of initial redfish fillet

The chemical compositions and the total viable count (TVC) as well as the number of Specific Spoilage Organisms (SSOs) including H_2S-producing bacteria and *Pseudomonas* spp. of raw redfish fillets are indicated in Table 7. The result showed that redfish was a semi-fatty fish species with a low percentage of lipid content (2.35%) and a high-water content (81.45%). Lipid content was variable depending on the individual or catching season, ranging from 2.5% to 4.46% (Philp, Sveinþórsdóttir, & Hjaltadóttir, 2014; Lauzon, et al., 2011).

Table 7. Chemical compositions and number of microorganisms of initial Redfish fillet.

			Microbiology		
Water content (%)	Lipid content (%)	Protein content (%)	TVC (cfu/g)	H_2S-producing bacteria	*Pseudomonas* spp.
81.45 ± 0.22	2.35 ± 0.31	14.95 ± 0.49	(4.65 ± 4.88)*10^3	(9.1 ± 1.9)*10^2	(6.05 ± 1.91)*10^3

Besides, the quality of raw material was quite good, under the minimum acceptable limit of the total viable count for fresh fish (< 10^5 cfu/g) (Huss, 1993). However, the standard deviation of TVC was rather high (4.88*10^3). This may be explained by the non-uniform quality of raw redfish fillets.

In the present study, the raw material was almost fresh with SSOs counts around 10^3 cfu/g. SSOs were found in different seafood products which can give rise undesirable flavours and related to seafood spoilage. While cold marine fish was indicated by *Shewanella putrefaciens*

(H_2S-producing bacteria), SSOs of cold freshwater fish was known as *Pseudomonas* spp. (Lone Gram, 2002). Scientists had combined analysing microbial ecology including SSOs growth, analytical chemistry as well as sensory analysis in order to build mathematical modelling for shelf life predictions of products (Mai Nga, 2017). The fresh fish with SSOs counts should less than 10^2 cfu/g for shelf life prediction and gets spoilage when SSOs counts reach to 10^6- 10^8 cfu/g depending on preservation methods (Lone Gram, 2002; Huss., 1995).

4.2.2 The effect of coating on chemical properties of fillets during cold storage time

Water content

The changes in water content in redfish fillets during cold storage influenced by different treatments are demonstrated in Figure 2. As can be seen from the graph, the water content was different among groups, and had a decreasing trend during cold storage. Chitosan-based groups had a higher water content than other groups from day 4 to day 12 of storage. Meanwhile, the water content of the Ch-Ge group was the highest at day 0 and day 4 (approximately 82%). This amount always maintained above 80% during the period of storage. The Ch group was stable in water content during the first 8 days of storage.

By contrast, the water and acid groups presented the lower water content from day 4 to day 12. For example, the water content of control sample (W) was always under 80% during the period 12 days. These results showed that the chitosan-based could keep moisture for fish fillet due to the ability of formatting a thin film and wrapped the fillets.

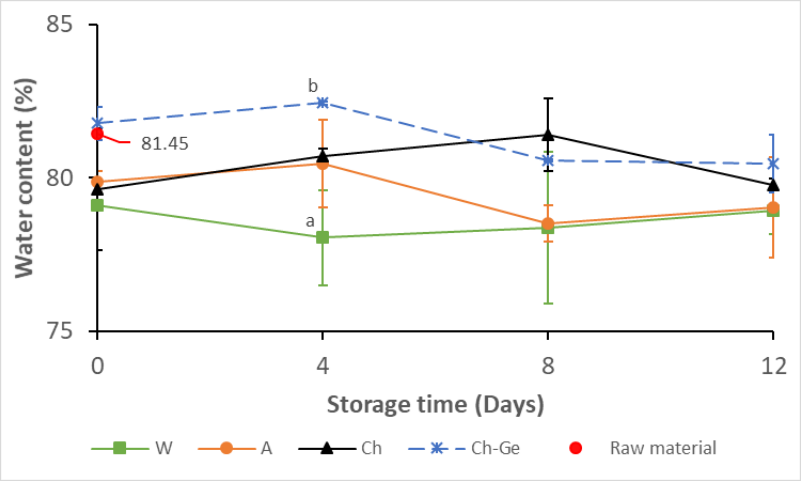


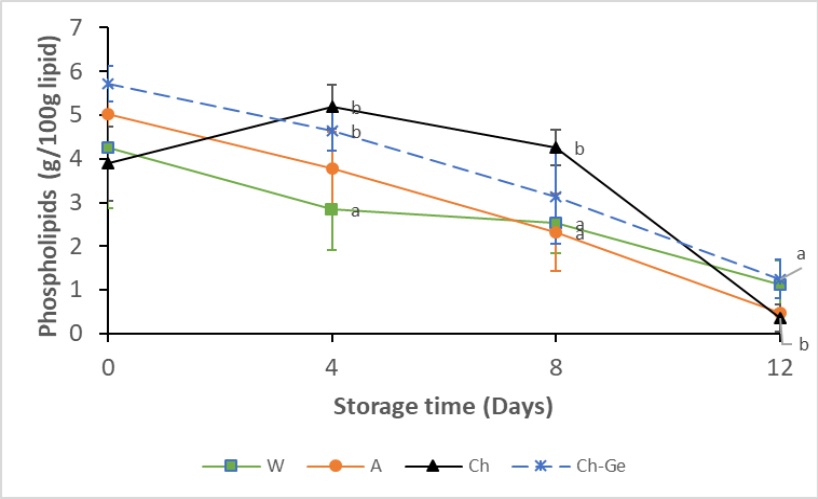
Figure 3. Water content of Redfish fillets during cold storage time.
W: Control sample, A: Ascorbic acid coated fillets, Ch: Chitosan coated fillets, Ch-Ge: Chitosan combined Gelatine coated fillets. Different letters (a, b, c) indicate significantly different whiteness, b-value and a-value ($p<0.05$) between groups at the same day of storage.

Phospholipids (PL) and Free fatty acid (FFA)

The changes of PL and FFA in redfish fillets during cold storage of the different treatments showed that PL gradually decrease over the last 8 days of storage in all groups (Figure 4). For example, the amount of PL in 100g lipid in the coated acid fillets dropped from 3.8 g/100g lipid (day 4) to 2.3 g/100g lipid (day 8) and 0.5 g/100g lipid (day 12). On the other hand, there was a significant difference in the PL values between groups on the same day of storage, except

for day 0. The groups treated with chitosan-based coating revealed a significantly higher PL value compared to other groups in day 4 and day 8 of storage ($p<0.05$).

The drop in phospholipid content could be explained by hydrolysis of phospholipids through storage time. The enzymes responsible are believed to be cellular phospholipases (Huss., 1995). The fact was that PL content in groups treated with chitosan-based was significantly higher than in other groups at day 4 and 8 ($p<0.05$) means that the chitosan-based coating could slow down the degradation of phospholipids in fish muscle.



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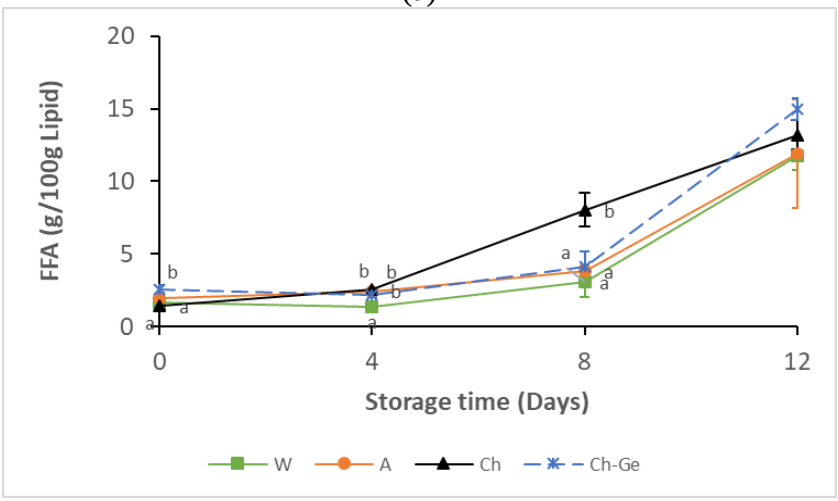


(b)

Figure 4. PL (a) and FFA (b) of Redfish fillets during cold storage for up to 12 days.
W: Control sample, A: Ascorbic acid coated fillets, Ch: Chitosan coated fillets, Ch-Ge: Chitosan combined Gelatine coated fillets. Different letters (a, b, c) indicate significantly different whiteness, b-value and a-value ($p<0.05$) between groups at the same day of storage.

By contrast, the amount of FFA was stable during the first 4 days of storage (1.4 – 2.5 g/100g lipid) in all 4 groups. These values slightly increased from day 4 to day 8 and significantly over the rest 4 days of storage ($p<0.05$) in the groups treated by water, acid, and chitosan combined with gelatine.

Meanwhile, the group treated with chitosan showed a dramatic increase in the FFA value after 4 days of storage, from 2.5 g/ 100g lipid (day 4) to 13.5 g/100 lipid (day12). There was no

significant difference in the FFA value between groups at the same day of storage except for day 8. On this day, the FFA content was approximately 8.0 g/100 lipid. This number was significantly higher compared to rest groups (3 – 4.1 g/100 g lipid) ($p<0.05$). These differences could come from the difference found in each individual fillet.

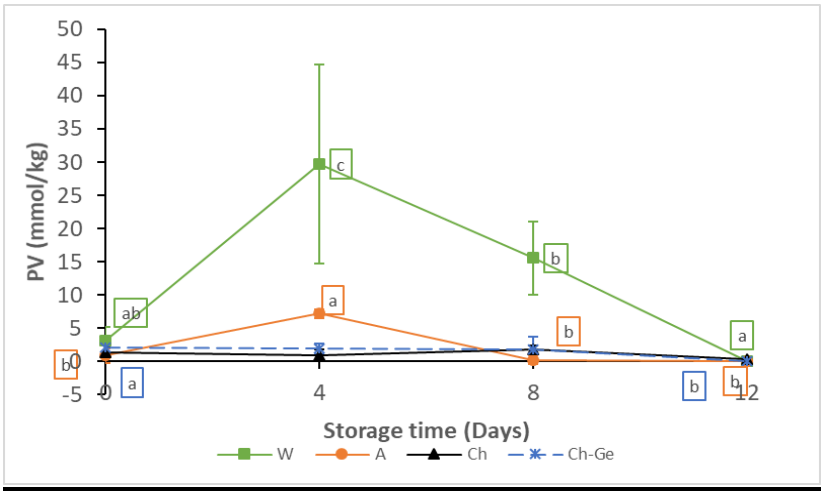
Production of free fatty acids tended to increase due to hydrolysis of phospholipids and triglycerides happening (Huss., 1995). Triglyceride lipases originating from the digestive tract or excreted by certain microorganisms promoted hydrolysis of triglycerides. This led to an increase of FFA on redfish fillet during cold storage. The formation of FFA could cause texture deterioration by interacting with proteins, had associated with lipid oxidation development and caused undesirable taste (Lauzon, et al., 2011). The current study revealed that chitosan-based coating did not affect on FFA formation on redfish fillets during cold storage.

Peroxide value (PV) and Thiobarbituric Acid Reactive Substances (TBARS)

According to the present study, PV and TBARS had a slight and insignificant increase in fillets treated with chitosan-based coating through 12 days of cold storage ($p<0.05$) (Figure 5). For instance, the PV was stable at the level of 1 to 2.1 mmol/kg in chitosan-based coated samples over the first 8 days of storage. The TBARS had a slight increase from 3.8 µmol/kg to 11 µmol/kg (treated chitosan fillets).

On the contrary, PV and TBARs increased significantly ($p<0.05$) and reached a peak at day 4 of storage in the control group (PV at the level of 7.2 mmol lipid hydroperoxide/kg and TBARS at 47.7 µmol malonaldehyde diethyl acetal (MDA)/kg) and in the acid ascorbic group (PV at the level of 29.7 mmol lipid hydroperoxide/kg and TBARS at 21.1 µmol MDA/kg). The results indicated that ascorbic acid also has a positive influence on PV and TBARS of fish muscle. PV and TBARS in acid ascorbic group was significantly lower than in the control group over the period of storage time ($p<0.05$).

The level of lipid oxidation in fish fillets was evaluated by measuring PV (the primary oxidation) which was expressed as lipid hydroperoxide content and by measuring TBARS (the secondary oxidation) which were expressed as MDA content. The lipid oxidation could be promoted with oxygen, transition metal, haem ion, singlet oxygen, enzymes – lipoxygenase and cyclooxygenase (Shahidi., 1994). Chitosan could form a coating film which could be a good oxygen barrier and not allow oxygen contact to the fish. So, it could limit the lipid oxidation of redfish fillets during storage. Chitosan also had antioxidant properties (Muzzarelli, 1996; Kumar M. N., 2000; Chitin-the undisputed biomolecule of great potential, 2003). The antioxidant mechanism of chitosan could be explained as the primary amino groups of chitosan, which form a stable fluorosphere with volatile aldehydes which was derived from the breakdown of fats during the oxidation (the secondary oxidation) (Falgueraa, Pablo, Jimenez, & Munoz, 2011).



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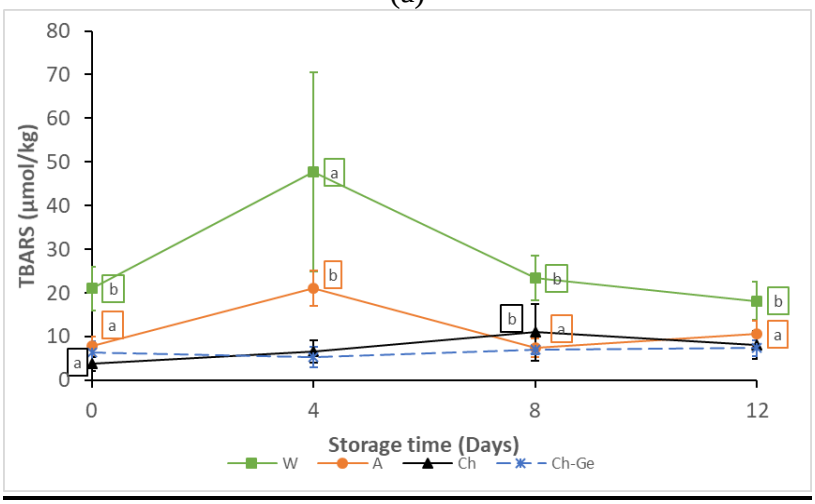


(b)

Figure 5. PV (a) and TBARS (b) of Redfish fillets during cold storage time.
W: Control sample, A: Ascorbic acid coated fillets, Ch: Chitosan coated fillets, Ch-Ge: Chitosan combined Gelatine coated fillets. Different letters (a, b) indicate significantly different PV and TBARs values ($p<0.05$) between storage days of the same group.

4.2.3 The effect of the coating on microbial properties of fillets during cold storage times

The changes of Total Viable Count and Specific Spoilage Organisms (SSOs) on fillets with different treatments during cold storage are shown in Figure 6 and 7.

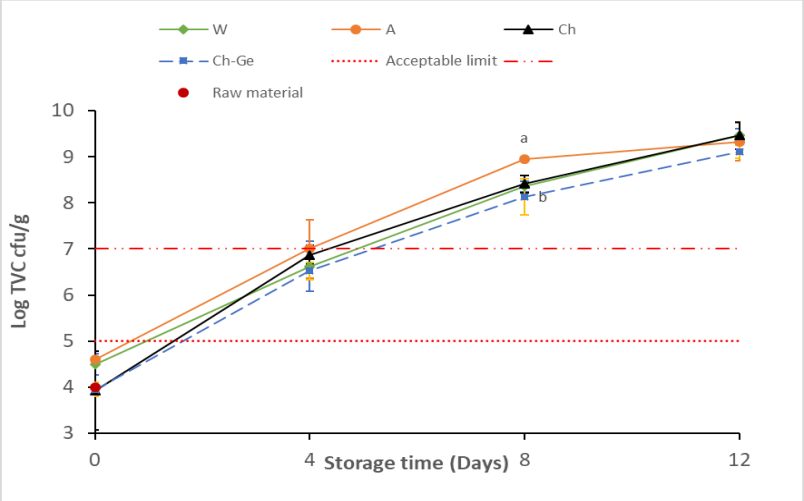
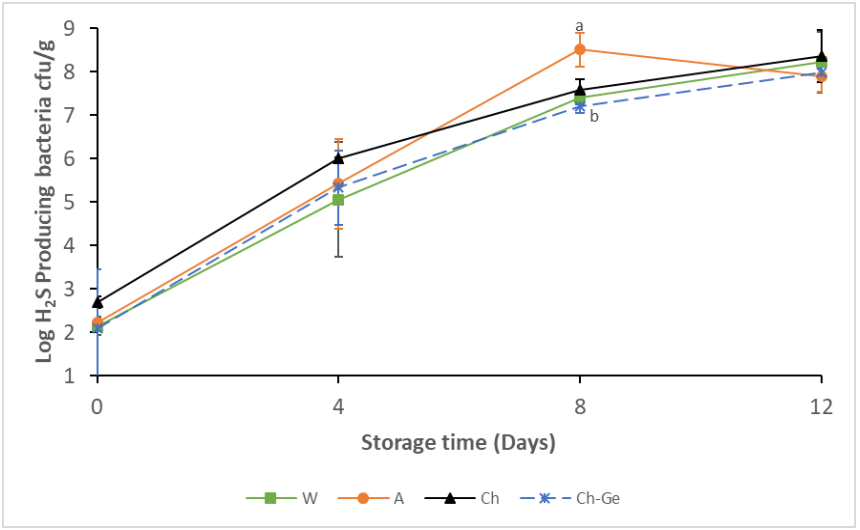


Figure 6. Total Viable Counts (log cfu/g) of Redfish fillets during cold storage time.
W: Control sample, A: Ascorbic acid coated fillets, Ch: Chitosan coated fillets, Ch-Ge: Chitosan combined Gelatine coated fillets. Different letters (a, b, c) indicate significantly different whiteness, b-value and a-value ($p<0.05$) between groups at the same day of storage.



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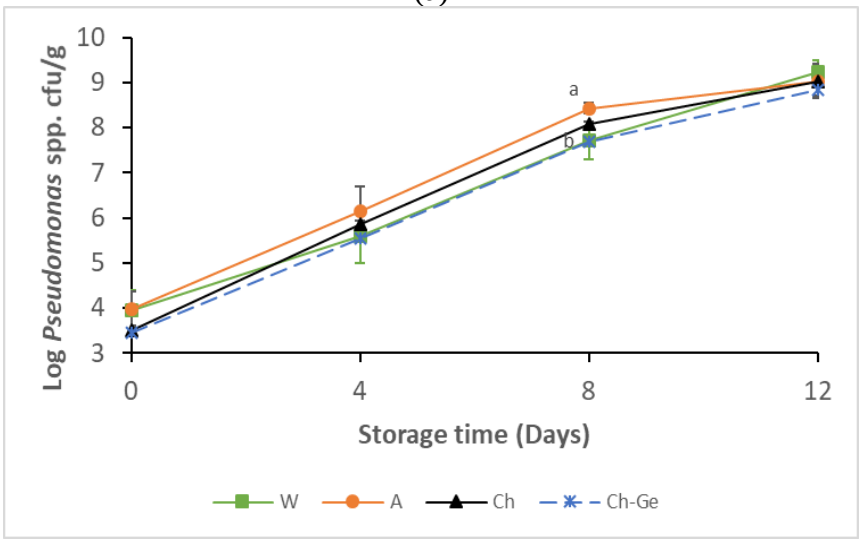


(b)

Figure 7. The total of H_2S producing bacteria (log cfu/g) (a) and *Pseudomonas* spp. (log cfu/g) (b) of Redfish fillets during cold storage time.
W: Control sample, A: Ascorbic acid coated fillets, Ch: Chitosan coated fillets, Ch-Ge: Chitosan combined Gelatine coated fillets. Different letters (a, b, c) indicate significantly different whiteness, b-value and a-value ($p<0.05$) between groups at the same day of storage.

The results indicated that TVC and SSOs increased dramatically during cold storage. Meanwhile, chitosan-based showed that it could slow down the TVC and *Pseudomonas* spp. growth compared to the other groups in the first 2 days of preservation.

On the order hand, Ch-Ge group revealed a delaying in TVC growth during cold storage. Chitosan combined gelatine had a significant TVC, SSOs lower than in acid treated samples at day 8 of storage ($p<0.05$).

Chitosan showed antimicrobial properties which could inhibit the growth of a wide range of microorganisms such as bacteria, fungi, and yeast (Sagoo, Board, & Roller, 2002; Kumar, Varadaraj, Gowda, & Tharanathan, 2005). The antimicrobial activity of chitosan could be explained by the presence of the positively charged amino groups which interacted with negatively charged macromolecules on the microbial cell surface, leading to the leakage of intracellular constituents of the microorganisms. The mechanism of action of chitosan appeared to be related to the disruption of the lipopolysaccharide layer of the outer membrane of gram-negative bacteria. Moreover, chitosan could form a film as a barrier against oxygen transfer. This could limit the growth of aerobic bacteria.

The gelatine solution also had an antimicrobial ability due to the presence of oligopeptide chains from the hydrolysis of gelatine. These chains were suspected of having antimicrobial activity because of the presence of side-chain amino groups (M. Pereda, 2011; C.O. Jeon, 2002). In the current research, it was also seen that group treated by chitosan and gelatine could retard the growth of TVC and *Pseudomonas* spp. better than other groups tested ($p>0.05$).

4.2.4 The effect of the coating on physical properties of fillets during cold storage time

Colour

Figure 8 and 9 illustrate the results obtained by colour measurement, with a-value (index of redness) and b-value (index of yellowness). The whiteness was calculated by the formula, Whiteness = L – 3*b, with L was lightness value (Hunter, 1960).

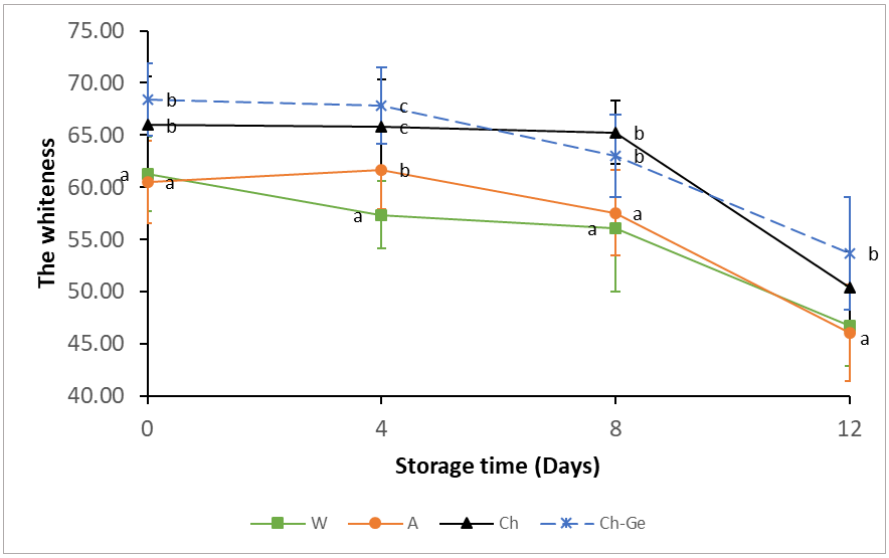
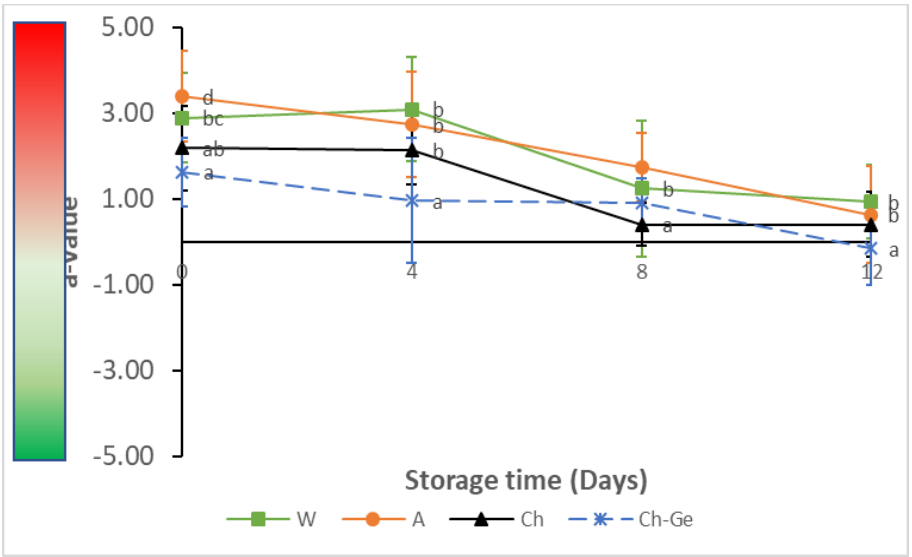


Figure 8. The whiteness of Redfish fillets during cold storage.
W: Control sample, A: Ascorbic acid coated fillets, Ch: Chitosan coated fillets, Ch-Ge: Chitosan combined Gelatine coated fillets. Different letters (a, b, c) indicate significantly different whiteness, b-value and a-value ($p<0.05$) between groups at the same day of storage.

Generally, the whiteness and redness of fillets gradually decreased during storage, except for the fillets treated with chitosan. The whiteness in chitosan treated fillets was almost steady over the first 8 days of cold storage and sharply decreased in the last 4 days of preservation. Chitosan-based coating had a positive influence on the whiteness of redfish fillets. Chitosan-based coated fillets had a significantly higher whiteness ($p<0.05$) and less redness compared to control groups. By contrast, the yellowness formation tended to go up during storage in all groups. However, the chitosan coated fillets had less yellowness formation than on fillets in remaining groups ($p>0.05$).



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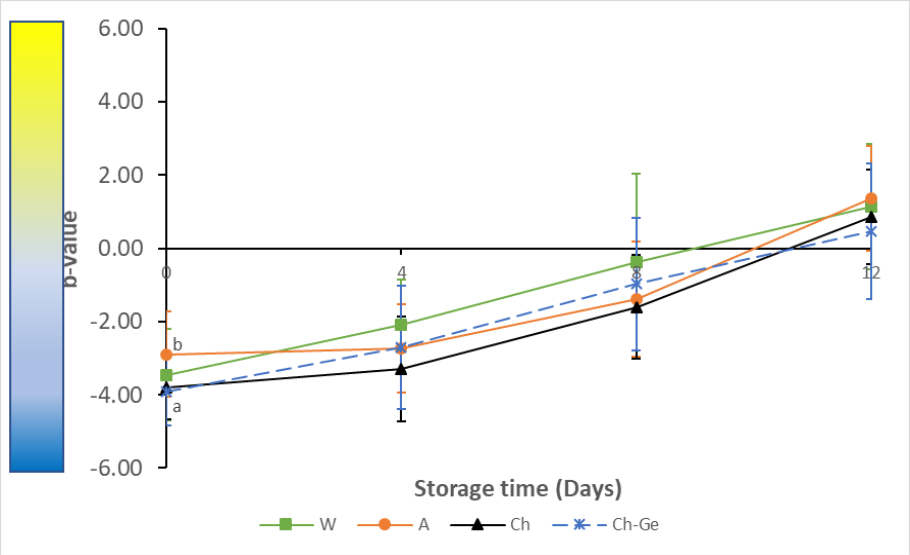


(b)

Figure 9. a-value (a) and b-value (b) of Redfish fillets during cold storage time.
W: Control sample, A: Ascorbic acid coated fillets, Ch: Chitosan coated fillets, Ch-Ge: Chitosan combined Gelatine coated fillets. Different letters (a, b, c) indicate significantly different whiteness, b-value and a-value ($p<0.05$) between groups at the same day of storage.

The changes in colour, especially increases in yellowness, can be explained by promotion of lipid oxidation on fish fillets during storage. According to previous results in this study, the groups treated chitosan-base were shown to significantly delay the lipid oxidation due to its ability in forming a barrier against oxygen and known antioxidant properties. This evidenced again chitosan-based coating could slow down lipid oxidation and retard off-colour formation on fresh fish fillets.

Texture

The texture was measured by the compressing force (Newton) on the fillets and shown in Figure 10. Based on the results, there was a significant difference in texture of fillets between treated chitosan and control sample fillets on day 8 and day 12 of storage ($p<0.05$). The fillets treated with chitosan had a significantly higher firmness compared to the control sample on day 4 of storage ($p<0.05$). This reflected that the texture of control samples became softer than the samples treated with chitosan at day 8 and day 12 of storage.

Previous research has shown that fish muscle becomes softer during cold storage after catching (Nielsen & Hyldig, 2001). This might be due to the degradation of proteins by the action of endogenous enzymes and microbial enzymes.

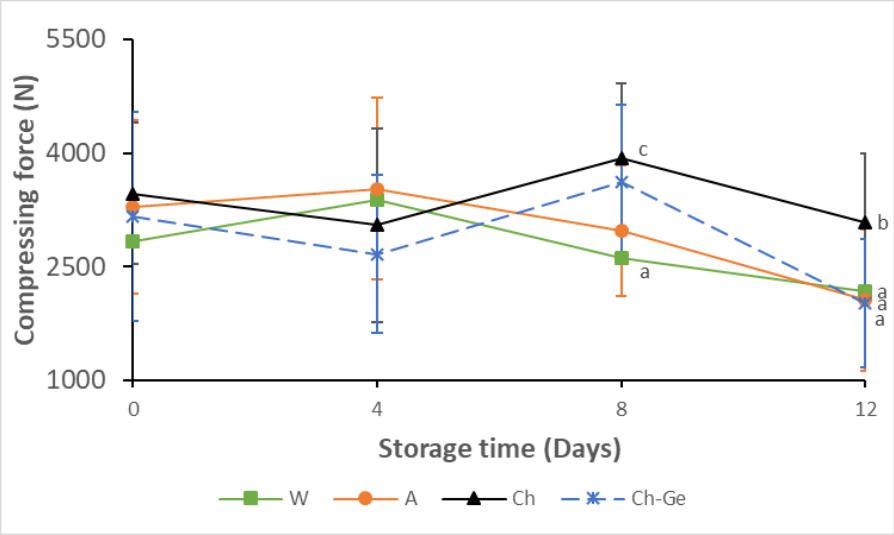


Figure 10. The compressing force (N) on Redfish fillets during cold storage time.
W: Control sample, A: Ascorbic acid coated fillets, Ch: Chitosan coated fillets, Ch-Ge: Chitosan combined Gelatine coated fillets. Different letters (a, b, c) indicate significantly different whiteness, b-value and a-value ($p<0.05$) between groups at the same day of storage.

pH

The pH value of redfish fillet muscle was approximate 6.55 – 6.7 at day 0 of storage and tended to increase during cold storage (Figure 11). However, the pH value of fillets treated with chitosan was significantly lower than the control group (W) during the period of preservation, except for day 0 ($p<0.05$). Chitosan combined with gelatine could retard the increase in the pH value in fish muscle compared to the control samples at day 8 and 12 of storage.

The increase in pH value could be due to an increase in volatile bases produced, e.g., ammonia and trimethylamine, by endogenous enzymes as well as microbial enzymes (Briones-Labarca, Perez-Won, Zamarca, Aguilera-Radic, & Tabilo-Munizaga, 2012). This is true in the current study. This result was similar to the previous TVC results. The group treated with chitosan combined gelatine could retard the growth of TVC.

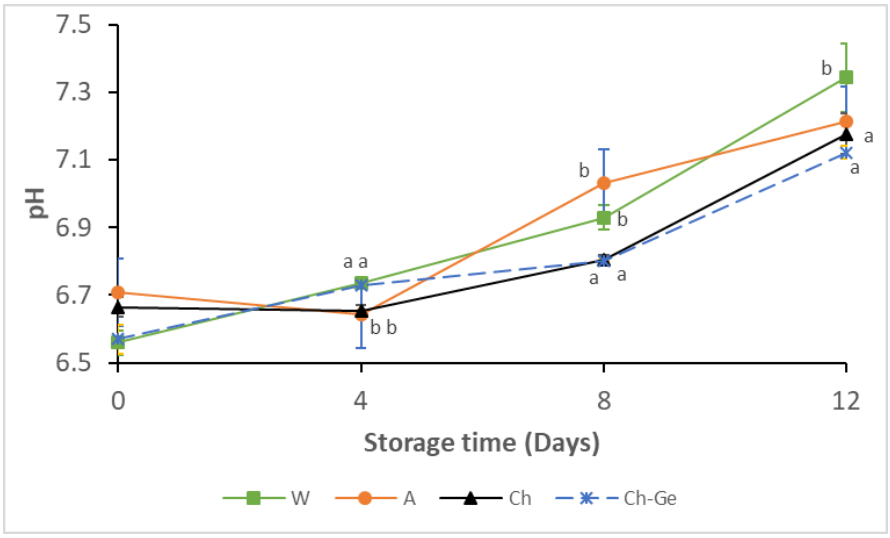


Figure 11. The pH value of Redfish fillets during cold storage.

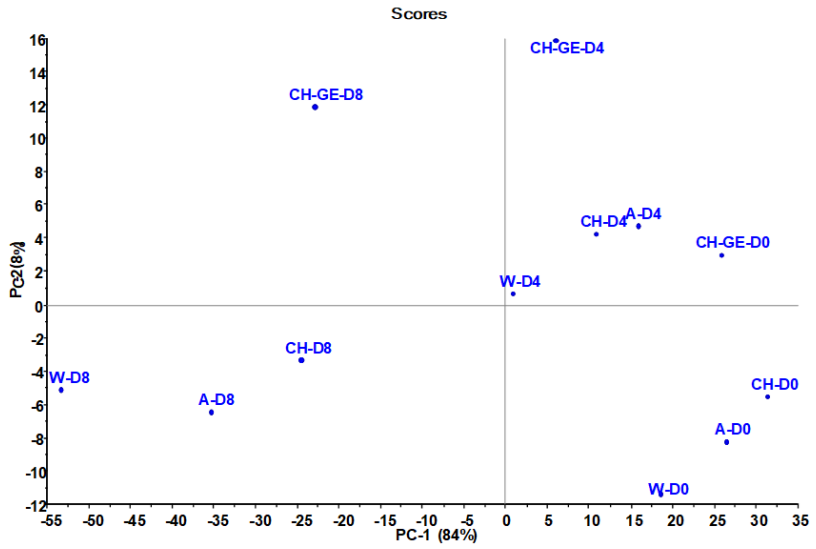
W: Control sample, A: Ascorbic acid coated fillets, Ch: Chitosan coated fillets, Ch-Ge: Chitosan combined Gelatine coated fillets. Different letters (a, b) indicate significantly different whiteness, b-value and a-value ($p<0.05$) between groups at the same day of storage.

4.2.5 The effect of the coating on sensory properties of fillets during cold storage time

Average values and results from statistical analysis of GDA scores are in Appendix 3. The results indicated that there was no significant difference in sensory score among groups, except for colour and precipitation characteristics on day 0. Meanwhile, the colour of groups treated by chitosan-base were significantly whiter than another groups. Hence, chitosan-based coating could be applied directly on fish fillets for consumption without any negative odours, flavours and textures.

Changes were also seen in odour and flavour so that the fish became more undesirable. On day 4 of storage, TMA odour (a spoilage characteristic) in the control sample was significantly higher than chitosan and acid group. Flavour of spoilage on day 8 showed a similar situation. These revealed again that chitosan can slow down spoilage in redfish fillets.

Principal Component Analysis (PCA) of GDA averages showed difference between treatments and changes over storage time (Figure 12). Principal Component 1 (PC1) explains 84% of the data variance and was mostly described with differences in spoilage-, and freshness characteristics. Principal Component 2 (PC2) explains 8% of the data variance and was mostly due to differences in amount of precipitation. With longer storage time, spoilage characteristics increased for all sample groups and freshness characteristics decreased. Group W was, on all sampling days, described with fewer freshness characteristics and more with spoilage characteristics than other sample groups. Redfish treated with gelatine scored higher for precipitation than other groups on all sampling days. This could be explained by remaining trace of chitosan-gelatine-based film on fish.



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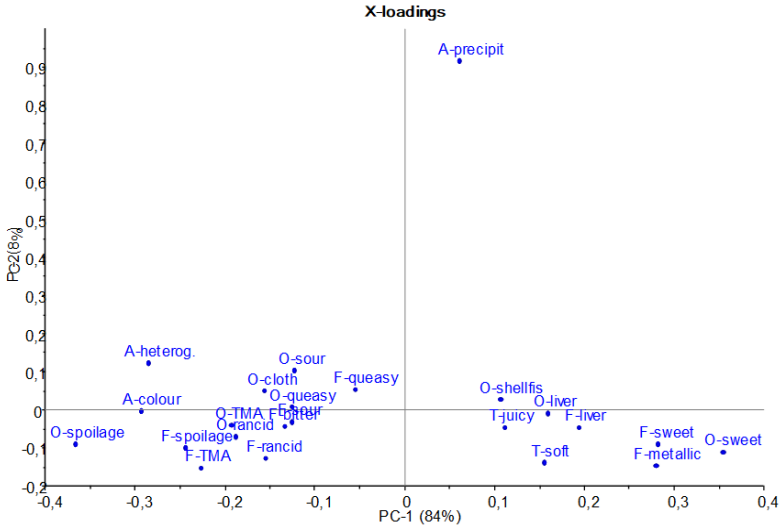

(b)

Figure 12. Principal Component Analysis (PCA) for average Generic Descriptive Analysis (GDA) scores. Upper graph - PCA scores.
W: control sample, CH: treated with chitosan solution, A: treated with acidic solution, CH-GE: treated with chitosan solution in gelatine.

Figure 13 shows changes of Torry score of redfish fillets during storage. Freshness period was often defined as the time from catching until the fillet reached average value of 7 on Torry freshness scale. At that point, the fish had lost most of its freshness characteristics and a neutral odour and flavour had developed. Shelf life was defined as the time until the redfish reached average value of 5.5 on the Torry scale, since a score of 5 indicated that slight spoilage characteristics had developed. In this research, the Torry results supported results from the GDA.

Differences were seen in the freshness period and shelf life between the sample groups. The control group (W) was on all sampling days described with less freshness characteristics and more with spoilage characteristics than other sample groups. The results indicated that group water had a freshness period of two to three days but other groups from three five days. Shelf life was estimated slightly above six days for group W and slightly less than eight days for group acid. The shelf life of groups chitosan-based was estimated longer than eight days.

The shelf life of redfish fillets needed to be changed according to the day of catch. Based on current data, day 0 of preservation was later by 1 day compared to the day of processing, and 5 days compared to the day of catching. Hence, the shelf life of redfish fillets coated by chitosan-base should be longer than 13 days when coated fish fillets preserved at 2- 3^{o}C.

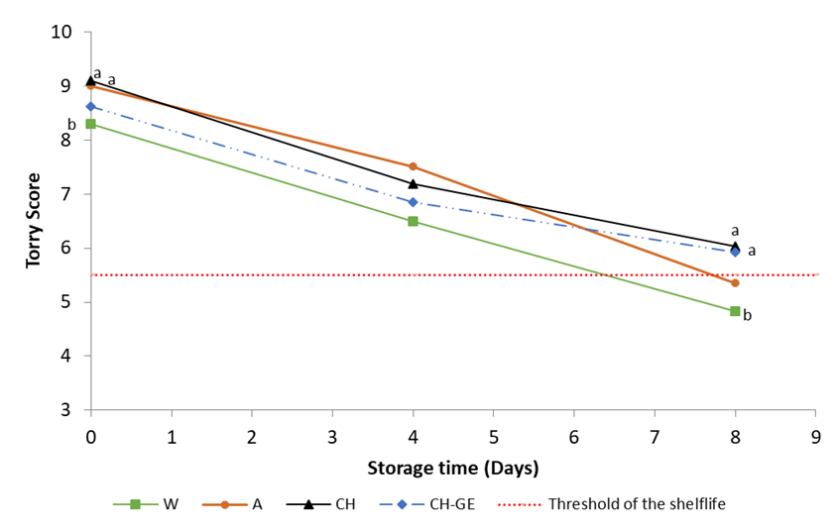

Figure 13. The change of Torry score of Red fish fillets during storage
W: Control sample, A: Ascorbic acid coated fillets, Ch: Chitosan coated fillets, Ch-Ge: Chitosan combined Gelatine coated fillets. Different letters (a, b) indicate significantly different Torry score ($p<0.05$) between groups at the same day of storage.

5 CONCLUSION AND RECOMMENDATION

Chitosan-based treatment significantly limited the lipid oxidation of redfish fillets during cold storage. There was no significant difference in limiting the lipid oxidation between groups of chitosan coating and chitosan and gelatine combined coating. Meanwhile, chitosan combined gelatine coating significantly increased the whiteness of redfish fillets. Chitosan coating retarded the yellowness formation on redfish fillets during storage.

The chitosan-based treatment slowed down the increase in TVC in the first two days of cold storage. Chitosan had no significant influence on the growth of SSOs on redfish fillet. Chitosan combined with gelatine showed a delay of TVC and *Pseudomonas.* spp during cold storage.

Chitosan-based coating significantly prolonged the freshness of redfish fillets and increased the shelf life and the quality of redfish fillet during cold storage.

In the current research, there are some recommendations for further study. First, quality of raw material might influence results of the study as well as the shelf life of fish. It should be repeated with the fresher fillet samples to know how exactly chitosan coating can effect quality of fillets, especially effects on microbiology. Secondly, it is necessary to have more information about using chitosan for preserving other fish species (fatty/lean fish) as well as by other storage methods. Finally, research on groups treated exclusively with gelatine is needed to understand how far gelatine can have potential use in fish preservation.

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APPENDICES

Appendix 1. Sensory methodology.

Table 1. Sensory attributes, short names, scale anchors and attribute definition for GDA of cooked redfish.

Sensory attribute	short name	scale	definition
ODOUR			
sweet	O-sweet	none || much	Sweet odour of fresh redfish
cod liver	O-liver	none || much	Odour of boiled cod liver
shellfish, algae	O-shellfish	none || much	Characteristic, fresh odour
dish cloth	O-cloth	none || much	Reminds of a dish cloth (damp cloth to clean kitchen table, left for 36 hrs.)
TMA	O-TMA	none || much	TMA odour, reminds of dried salted fish, amine
rancid	O-rancid	none || much	Rancid odour
queasy sweet	O-queasy	none || much	Queasy sweet odour, spoilage characteristic, overripe fruit
sour	O-sour	none || much	spoilage characteristic, odour of sour milk, vinegar, butyric acid
spoilage	O-spoilage	none || much	Strength of spoilage odour, (all spoilage odours)
APPEARANCE			
colour	A-colour	light || dark	Surface of sample. Light; white colour, dark: yellow, brown, grey
heterogenous color	A-heterog.	none || much	Surface of the sample, heterogenous colour, e.g. stains, discoloured edge
precipitations	A-precipit.	none || much	Amount of precipitation on the surface and in liquid
FLAVOUR			
cod liver	F-liver	none || much	Flavour of boiled cod liver
metallic	F-metallic	none || much	Characteristic metallic flavour of fresh redfish
sweet	F-sweet	none || much	Characteristic sweet flavour of fresh redfish
bitter	F-bitter	none || much	Bitter flavour
queasy sweet	F-queasy	none	Queasy sweet flavour, spoilage characteristic, overripe fruit
sour	F-sour	none	spoilage characteristic, flavour of sour milk, vinegar, butyric acid
TMA	F-TMA	none || much	TMA flavour, reminds of dried salted fish, amine
rancid	F-rancid	none || much	Rancid flavour
spoilage	F-spoilage	none || much	Strength of spoilage flavour (all spoilage flavours)
TEXTURE			
soft	T-soft	firm || soft	Softness in first bite
juicy	T-juicy	dry || juicy	Dry: draws liquid from mouth. Juicy: releases liquid when chewed
tender	T-tender	tough || tender	Tenderness when chewed a few times
mushy	T-mushy	little || much	Mushy, porridge like texture
fatty mouthfeel	T-fatty	little || much	Fatty mouthfeel after chewing

Table 2. Torry score sheet for freshness evaluation of cooked medium.

Odour	Flavour	score
Initially weak odour of boiled cod liver, fresh oil, starchy	Boiled cod liver, watery, metallic	10
Shellfish, seaweed, boiled meat, oil, cod liver	Oily, boiled cod liver, sweet, meaty, characteristic	9
Loss of odour, neutral odour	Sweet and characteristic flavours but reduced in intensity.	8
Woodshavings, woodsap, vanillin	Neutral	7
Condensed milk, boiled potato	Insipid	6
Milk jug odours, boiled clothes- like	Slight sourness, trace of "off"-flavours, rancid	5
Lactic acid, sour milk, TMA	Slight bitterness, sour, "off"-flavours, TMA, rancid	4
Lower fatty acids (e.g. acetic or butyric acids) composed grass, soapy, turnippy)	Strong bitter, rubber, slight sulphide, rancid	3

Appendix 2. Dipping treatment method.

1. Dipping in the solution for 5s

2. Standing for 10s

3. Dipping in gelatine solution 5s

4. Drying

5. Packing

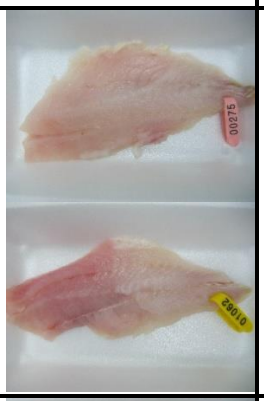
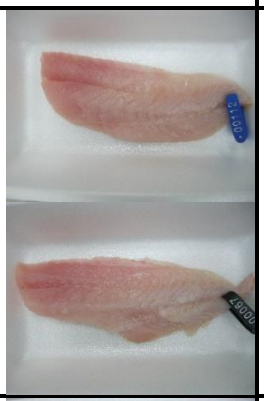
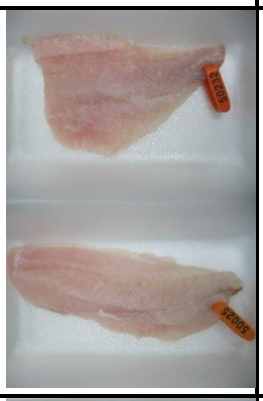
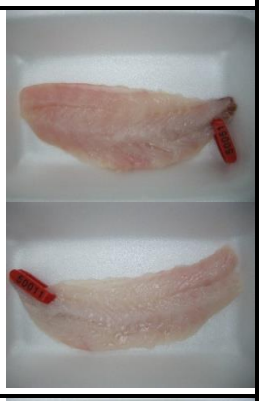
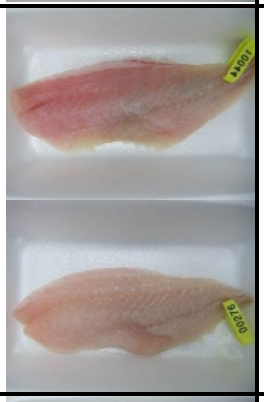
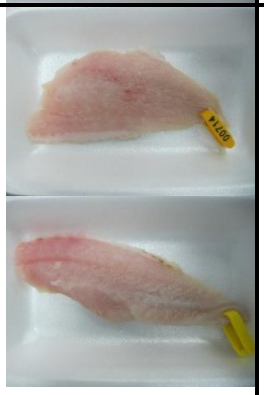
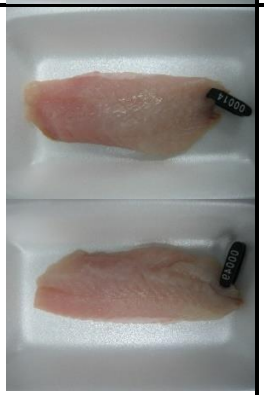
Appendix 3. Sensory results.

Table 3. Generic Descriptive Analysis (GDA) mean values of sensory attributes (scale 0-100) for the four sample groups evaluated. Different letters within a column per day, indicate a significant difference between the relevant groups.

	ODOUR									APPEARANCE		
Group	**O-sweet**	**O-liver**	**O-shellfish**	**O-cloth**	**O-TMA**	**O-rancid**	**O-queasy**	**O-sour**	**O-spoilage**	**A-colour**	**A-heterog.**	**A-precipit.**
Day 0												
W	34	22	16	0	1	1	0	0	0	19 **a**	18	13 **c**
A	38	25	15	0	1	0	0	0	0	15	13	17 **bc**
CH	41	26	17	0	0	0	0	0	0	11 **b**	11	21 **b**
CH-GE	37	22	14	0	1	1	0	0	1	13 **b**	13	29 **a**
p-value	0.215	0.444	0.704	0.332	0.280	0.733	0.465	0.594	0.282	0.008	0.075	0.000
Day 4												
W	24	18	14	6	9 **a**	2	1	2	6	22	17	21 **b**
A	33	20	18	1	1 **b**	0	1	2	1	16	15	26 **b**
CH	31	23	15	3	3 **b**	1	1	5	6	17	19	26 **b**
CH-GE	29	21	13	4	3	2	3	3	4	22	20	38 **a**
p-value	0.242	0.509	0.371	0.151	0.035	0.493	0.097	0.374	0.146	0.113	0.435	0.000
Day 8												
W	11	14	6	11	16	19	9	7	30	35	34	17 **b**
A	14	11	9	10	12	7	9	9	22	34	29	13 **b**
CH	23	15	12	10	10	9	6	8	19	28	29	19 **b**
CH-GE	17	16	11	9	10	8	7	11	17	30	34	32 **a**
p-value	0.077	0.596	0.064	0.861	0.603	0.139	0.661	0.631	0.058	0.307	0.325	0.001

	FLAVOUR									TEXTURE					
Group	**F-liver**	**F-metallic**	**F-sweet**	**F-bitter**	**F-queasy**	**F-sour**	**F-TMA**	**F-rancid**	**F-spoilage**	**T-soft**	**T-juicy**	**T-tender**	**T-mushy**	**T-fatty**	**Torry**
Day 0															
W	38	32	24 **b**	3	0	0	1	3	2	69	64	66	31	9	8.3 **b**
A	38	33	31 **a**	2	0	0	0	0	0	67	62	67	36	6	9.0 **a**
CH	40	33	32	2	0	0	0	0	0	68	67	67	35	6	9.1 **a**
CH-GE	37	30	33	2	3	1	0	1	1	66	62	63	31	7	8.6
p-value	0.917	0.852	0.049	0.753	0.451	0.463	0.100	0.264	0.241	0.827	0.126	0.365	0.525	0.286	0.015
Day 4															
W	33	25	19	4	2	0	3	2	3	55	57	66	44	7	6.5
A	34	24	25	2	4	0	1	0	0	60	60	65 **a**	38	7	7.5
CH	33	23	26	1	1	1 **b**	1	0	2	61	58	66	47	7	7.2
CH-GE	30	23	18	2	2	3 **a**	2	1	5	62	63	59 **b**	39	10	6.8
p-value	0.955	0.953	0.279	0.116	0.549	0.054	0.465	0.098	0.115	0.505	0.617	0.068	0.292	0.636	0.145
Day 8															
W	22	10	8	12	6	9	20	14	24 **a**	54	50	63	36	7	4.8 **b**
A	24	11	12	10	3	8	12	8	12 **b**	57	59	68	35	7	5.4
CH	26	18	16	8	5	10	12	10	12 **b**	57	63	68	33	7	6.0 **a**
CH-GE	32	18	16	10	4	5	7	6	9 **b**	59	59	67	32	5	5.9 **a**
p-value	0.301	0.126	0.108	0.783	0.744	0.628	0.150	0.202	0.018	0.596	0.083	0.237	0.675	0.527	0.019

Appendix 4.Red fish fillets with different treatments during storage.

Treatments / Storage time	Water	Ascorbic Acid	Chitosan	Chitosan_Gelatine
Day 0				
Day4				
Day 8				
Day 12