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Effects of Edible Coatings on the Moisture Content and Lipid Oxidation of Pink Salmon (*Oncorhynchus gorbuscha*) Fillets during Three Months of Frozen Storage

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Abstract

The effects of a variety of edible coatings, cooked arrowtooth flounder coating (AFC), hydrolysed arrowtooth flounder protein (AFH), acid solubilized arrowtooth flounder protein (AFP), soy protein (SP), whey protein (WP) and non-coated fillets as control (NC) on the biochemical and physical changes in pink salmon fillets stored at - 30°C were evaluated in this study. The AFC and AFH coatings significantly reduced the relative moisture loss (RML) of the fillets, while the thiobarbituric reactive substance (TBARS) assays for AFH and SP were significantly lower than in treatments. The AFP significantly increased coating yield as well as the moisture content of cooked fillets. This study demonstrated the efficacy of arrowtooth flounder protein coatings as a means of maintaining fish fillet quality.

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Introduction

Freezing is an effective fish preservation method that minimizes undesirable chemical changes and maintains the characteristics of fresh fish. However, fish do deteriorate, though at a much slower rate, during long-term frozen storage. Fish and fish products lose their quality due to loss of moisture to the environment and diffusion of oxygen into the flesh of the fish which enhances lipid oxidation. Oxidized, unsaturated lipids bind to proteins to form complexes that produce toughened texture, poor flavors and rancid odors (Khayat and Schwall 1983).

Due to increased consumer demand for high quality foods with long shelf-life, edible coatings may become a viable alternative to traditional packaging. During the past few decades, considerable work has been done on use of edible coatings to extend the shelf-life and improve the quality of fresh, frozen and fabricated foods. A variety of polysaccharides, proteins and lipids have been used alone or in combinations to produce edible coatings (McHugh and Krochta 1994a). Edible coatings can regulate water vapor, oxygen, carbon dioxide and lipid transmission in foods; they can be used on foods of different sizes (Gennadios and Weller 1990), such as fish fillets, fish bites and fish sticks.

Many types of coatings have been tested in attempts to minimize chemical changes in fish during frozen storage. Ice glazing is often used to retard moisture loss and lipid oxidation (Wheaton and Lawson 1985). Whey proteins have been shown to provide an excellent oxygen barrier which slows the lipid oxidation (McHugh and Krochta 1994b; Khwaldia et al. 2004). Soy proteins also provide resistance to loss of water vapor and drastically reduce the aldehyde products of lipid oxidation (Rhim et al. 2000).

Arrowtooth flounder (*Atheresthes stomias*) is an underutilized flatfish found in abundance in seas surrounding Alaska, USA. Human consumption of arrowtooth flounder is limited due to the presence of endogenous proteolytic enzymes that break down the flesh, rendering it undesirable for food (Wasson et al. 1992; Babbitt et al. 1993). Sathivel et al. (2004) successfully produced purified powders from arrowtooth flounder fillets and showed that proteins from arrowtooth flounder exhibit useful functional properties such as water retention, gelation, foam stability and emulsion capacity, which make them suitable for use as emulsifiers in food supplements (Sathivel 2005). The goals of this project were to 1) prepare edible coatings of proteins from arrowtooth flounder fillets and soy and whey protein concentrates; 2) apply these coatings to pink salmon fillets to determine their effects on chemical changes (i.e. relative moisture loss, lipid oxidation, color change and pH in the fillets during three months of chilled storage (- 30° C); and, 3) determine raw yield, cooked yield, cooked protein moisture content, and drip loss.

Materials and Methods

Preparation of arrowtooth flounder protein powders

Arrowtooth flounders were procured from a commercial fish processing plant in Kodiak, Alaska. The fish were immediately filleted and stored at -30° C until processed into protein powders. The frozen arrowtooth flounder fillets were thawed at 4° C. The thawed samples were minced in a Hobart grinder (K5SS Hobart Corp., Troy, Ohio, USA) through a 6-mm-dia-pore size plate.

Cooking method: Soluble protein powders from arrowtooth flounder fillets were produced according to the methods reported by Sathivel et al. (2004). The resulting arrowtooth flounder crude protein powder AFCP (Table 1) was packaged and stored at 4° C in a refrigerator.

Table 1. Proximate composition of protein powders used in edible coatings for pink salmon fillets during the 3 mo frozen storage. Mean \pm percent relative standard deviation (RSD)

Ingredient ²	Protein % ¹	Lipid % ¹	Moisture % ¹	Ash % ¹
AFC	76.9 ± 1.7	4.4 ± 0.5	4.6 ± 1.1	8.6 ± 0.3
AFH	82.1 ± 0.9	12.4 ± 1.9	7.8 ± 0.1	7.9 ± 0.0
AFP	85.9 ± 0.2	2.4 ± 0.2	2.0 ± 0.1	10.9 ± 1.3
SPC^3	90.0 ± 0.4	4.0 ± 0.3	6.0 ± 0.1	5.0 ± 0.1
WPC ³	97.8 ± 0.2	0.4 ± 0.2	4.9 ± 0.2	1.9 ± 0.2

¹Values are mean \pm SD of 3 determinations.

 2 AFC = arrowtooth flounder protein from cooked method; AFH = arrowtooth flounder protein from enzyme hydrolysis method; AFP = arrowtooth flounder protein from pH extraction method; SP = soy protein concentrate; WP = whey protein concentrate.

³Proximates of SPC and WPC are referred from the laboratory testing from the manufacturing companies.

Enzyme hydrolysis method: The soluble protein powders from arrowtooth flounder fillets were prepared by the enzyme hydrolysis method

cited by Sathivel et al. (2005). The hydrolysis conditions were those described by Liceaga-Gesualdo and Li-Chan (1999). This arrowtooth flounder hydrolyzed protein powder AFHP (Table 1) was packaged and stored at 4°C in a refrigerator.

Protein powders by pH method: Acid solubilized protein from arrowtooth flounder fillets was extracted by altering pH as described by Kristinsson et al. (2005). The proximate composition pH solubilized arrowtooth flounder powder AFPP was recorded in table 1.

Soy and whey protein powders

Soy protein concentrates (Archer Daniels Midland Co., Decatur, IL 62526, USA) and whey protein concentrates ('Bipro', Davisco Foods International Inc., Le Sueur, MN 56058, USA) were used to prepare the soy protein (SP) and whey protein (WP) coating solutions, respectively. The proximate composition of soy protein concentrate (SPC) and whey protein concentrate (WPC) is shown in table 1.

Preparation of coating solutions

Protein coatings solutions: Three arrowtooth flounder protein coating solutions, AFC (cooked method), AFH (enzyme hydrolysis method), AFP (pH extraction method), SP (soy protein concentrate) and WP (whey protein concentrate) were used to coat pink salmon fillets. All the coatings were prepared by the method of Rhim et al. (2000). Protein powders of AFC, AFH, AFP, SP and WP (23.5 g at 4.7 % w/v) were stirred continuously as 9 g of glycerol was added to the solution as a plasticizer. The pH of the solution was adjusted to 10 ± 0.1 with 1 M sodium hydroxide and the mixture was heated for 20 min in a temperature-controlled bath. Alkaline conditions assisted protein dispersal during the formation of coating solutions (Gennadios et al. 1993) and aided the dissolution of AFP and WP in water. The coating solutions were filtered through eight layered cheesecloth to remove particulate materials and stored in a refrigerator at 4°C until used.

Application of coatings on salmon fillets

Fresh skinless, boneless fillets of pink salmon were procured from Western Alaska Co., Kodiak, Alaska. The fillets were cut into approximately 100 g portions of uniform size and shape. The portions were weighed and dipped into each of the freshly prepared coating solutions for 60 sec, drained for 30 sec on racks, weighed and packed into ziplock polyethylene freezer bags. The coating solutions were kept cold by placing them on an ice bath during this process. The bagged fillets were also placed in an ice bath until stored in a freezer at -30° C. Fillets were subjected to measurements of weights, color, moisture, lipid oxidation, cooked moisture, texture at the start of the study and after three months of storage.

Determination of proximate composition of pink salmon fillets

Determination of moisture and ash were done following standard AOAC procedures 930.15 and 942.05 (AOAC 1995). Crude protein content was measured using the Leco FP-2000 Nitrogen Analyzer (Leco Corp., St. Joseph, MI USA) and total lipid content was measured using the ASE 200 solvent extractor (Dionyx Corp., Sunnyvale, CA USA). Protein content was calculated as percent nitrogen x 6.25 (Table 2).

Analyses	Values ¹
Protein (%)	18.6 ± 4.6
Lipid (%)	2.2 ± 0.4
Moisture (%)	75.8 ± 0.1
Ash (%)	4.6 ± 0.4
TBA (µmoles MDA 100g ⁻¹ sample)	0.2 ± 0.1
Color	
L*	48.1 ± 4.0
a*	10.5 ± 4.8
B*	13.6 ± 4.5
whiteness	44.9 ± 2.2
Hardness (N)	28.6 ± 12.8

Table 2. Proximate composition, TBA, color and texture of fresh non-coated pink salmon fillets. Mean \pm % RSD

¹Values are means \pm SD of 3 determinations.

Color measurements

Color of the coated pink salmon fillets was measured in nine replicates using a Minolta CIELAB Chromameter (Model CR-300, Minolta Co. Ltd, Osaka, Japan); the values were reported as L*, a* and b*. The coated fillets were cooked at 95°C in a hot water bath for 3 min before the color was measured. Whiteness index was calculated by the formula, (WI) = $100-[(100-L)^2 + a^2 + b^2]^{1/2}$ (Bolin and Huxsoll 1991).

Determination of yield and cooking yield

The weight of the fillets was recorded before and after coating. The yield (weight gain %), cooked yield (%) and drip loss (%) of the coated fillets were calculated using the formula described by Sathivel (2005).

Determination of pH

A modified method of Ingolfsdottir et al. (1998) was used to measure the pH of the coated salmon fillets after 3 months. Twenty grams of minced fillets were placed in a 400 mL beaker and homogenized with 80 mL of distilled water for 1 min with a motorized homogenizer (Model 6-105- AF, Virtis Co., Gardner, NY USA). The pH (Table 6) of homogenized sample was measured using a pH meter (model 300 Beckman, USA)

2-Thiobarbituric acid reactive substances (TBARS) assay

The thiobarbituric acid reactive substances (TBARS) test was conducted on fresh (Table 2) and coated pink salmon fillets using the method of Lemon (1975). Malondialdehyde (MDA) content in the samples was measured and expressed as values of TBARS in units of μ moles of MDA 100g⁻¹ of tissue.

Relative moisture loss (RML)

The relative moisture loss (RML, %) of pink salmon fillets was calculated using the method described by Sathivel (2005).

Statistical Analysis

Mean values f rom replicate analyses were compared following the analysis of variance (ANOVA) procedure (SAS version 9.0, SAS Inst., Cary, NC, USA). When significant differences were indicated, Tukey's test was used to identify the differences among the multiple comparisons ($P \le 0.05$).

Results

Color, TBARS and pH

The thawed fillets (Table 3) showed that the L* and whiteness of the non-coated fillets was significantly higher than the coated fillets. Within coated fillets, AFC and SP treatments showed significant L* and whiteness. There were no differences in a* and b* values. Yellowness (a*) in cooked fillets (Table 4) was higher in AFH and AFP treatments among all coatings. Whiteness in cooked fillets was lower in AFH and AFP treatments.

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Samples	Coatings ¹	L*	a*	b*	whiteness
Thawed	AFC	$48.7 \pm 1.1^{\rm c}$	6.9 ± 1.6^{a}	15.7 ± 1.4^{a}	$45.8\pm1.2^{\rm c}$
	AFH	53.9 ± 5.1^{ab}	6.1 ± 1.7^{ab}	16.1 ± 1.8^{a}	50.7 ± 4.5^{abc}
	AFP	54.6 ± 5.0^{ab}	6.5 ± 1.7^{ab}	16.5 ± 1.1^{a}	51.1 ± 4.5^{ab}
	SP	$48.2\pm3.4^{\rm c}$	5.5 ± 2.5^{ab}	13.7 ± 2.2^{a}	$46.1 \pm 3.9^{\circ}$
	WP	$51.0\pm1.9^{\rm bc}$	5.7 ± 2.3^{ab}	$15.2\pm1.0^{\rm a}$	$48.3 \pm 1.3^{\text{bc}}$
	NC	$58.4\pm2.8^{\rm a}$	6.1 ± 3.2^{ab}	15.3 ± 3.6^{a}	$55.0\pm4.0^{\rm a}$

Table 3. Color L* a* b* and whiteness values (means \pm SD, n=9) of thawed pink salmon fillets coated with different coating solutions after 3 mo frozen storage. Mean \pm % RSD

^{abcd} Within treatments, means with same letter in each column are not significantly different. ${}^{1}AFC =$ arrowtooth flounder protein coating from cooked method; AFH = arrowtooth flounder protein coating from pH extraction method; SP = soy protein coating; WP = whey protein coating; NC = control. P > 0.05

Table 4. Color L* a* b* and whiteness values (means \pm SD n=9) of cooked pink salmon fillets coated with different coating solutions after 3 mo frozen storage. Mean \pm % RSD

Samples	Coatings ¹	L*	a*	b*	whiteness
Cooked	AFC	$74.4\pm1.0^{\rm a}$	5.0 ± 1.1^{a}	$15.7\pm0.9^{\rm b}$	69.5 ± 1.4^{ab}
	AFH	$75.6\pm1.4^{\rm a}$	4.7 ± 2.8^{a}	$21.8\pm2.1^{\rm a}$	$66.8\pm2.1^{\text{b}}$
	AFP	$74.4\pm1.8^{\rm a}$	3.9 ± 1.4^{a}	$20.7\pm3.5^{\rm a}$	66.7 ± 3.4^{b}
	SP	$76.2\pm2.0^{\rm a}$	$4.2\pm2.7^{\rm a}$	18.2 ± 4.2^{ab}	69.4 ± 3.7^{ab}
	WP	$76.8\pm2.5^{\rm a}$	$5.9\pm2.3^{\rm a}$	15.2 ± 2.2^{b}	71.6 ± 3.6^{a}
	NC	$75.9 \pm \! 1.9^a$	2.8 ± 2.3^{a}	$15.9\pm1.8^{\rm b}$	$70.9\pm2.4^{\rm a}$

^{abcd} Within treatments, means with same letter in each column are not significantly different. ¹AFC = arrowtooth flounder protein coating from cooked method; AFH = arrowtooth flounder protein coating from enzyme hydrolysis method; AFP = arrowtooth flounder protein coating from pH extraction method; SP = soy protein coating; WP = whey protein coating; NC = control. P \geq 0.05

The lipid oxidation values expressed as μ moles of malondialdehyde (MDA) end product per 100 g of sample is depicted in figure 1. The oxidation in pink salmon fillets coated with AFH (0.75 μ moles MDA 100g⁻¹ sample), and AFP (0.95), was significantly lower than the NC (1.32). There were no significant differences in the rest of the treatments.

Small differences were observed in the pH (Table 6) of the thawed fillets after 3 month frozen storage. The non-coated fillets showed higher pH than all the other treatments while AFH treatments showed the lowest values.

Relative moisture loss (RML) and moisture content

The relative moisture loss (%) in pink salmon fillets coated with different coatings is shown in figure 2. The non-coated fillet exhibited significantly high percent moisture loss (3.76), while AFH treatment (0.19) and AFC (0.37) successfully minimized the moisture loss. There were no

differences in AFP, SP and WP treatments. Similarly, the moisture contents after thawing the fillets and after cooking the thawed fillets are shown in table 5. There were no differences in the rest of the treatments except the presence of lower moisture in non coated fillets than the other treatments in the thawed fillets. The cooked fillets showed no significant differences.



Fig. 1. Thiobarbituric acid (TBA) of pink salmon fillets during a 3 month frozen storage. ^{abc} Means with same letters are not significantly different (p > 0.05). AFC = arrowtooth flounder protein coating from cooked method; AFH = arrowtooth flounder protein coating from enzyme hydrolysis method; AFP = arrowtooth flounder protein coating from pH extraction method; SP = soy proteincoating; WP = whey protein coating; NC = control. (P >0.05)

Fig. 2. Relative moisture loss in pink salmon fillets, coated with different edible coatings during a 3 month frozen storage. ^{ab}Means with same letters are not significantly different. P > 0.05AFC = arrowtooth flounder protein coating from cooked method; AFH = arrowtooth flounder protein coating from enzyme hydrolysis method; AFP = arrowtooth flounder protein coating from pH extraction method; SP = soy protein coating; WP = wheyprotein coating; NC = control.



Yields and drip loss

There was an increase in yield of AFP over all the treatments. There were no differences in the rest of the treatments for drip loss and cooked yield (Table 6).

Samples ²	Moisture content after thawing $(\%)^1$	Moisture content after cooking $(\%)^1$
AFC	$75.5 \pm 0.4^{\mathrm{a}}$	$71.8\pm0.9^{\mathrm{ab}}$
AFH	$75.6\pm0.4^{\rm a}$	72.7 ± 0.5^{ab}
AFP	73.7 ± 1.4^{ab}	$73.1\pm0.4^{\rm a}$
SP	74.6 ± 0.7^{ab}	71.6 ± 0.7^{ab}
WP	74.8 ± 0.9^{ab}	$73.0\pm0.4^{\rm a}$
NC	72.9 ± 0.6^{b}	71.9 ± 0.2^{ab}

Table 5. Moisture content (means \pm SD n=3) of thawed and cooked pink salmon fillets coated with different coating solutions after 3 month frozen storage. Mean \pm % RSD

 ab Within treatments, means with same letter in each column are not significantly different. $P \geq 0.05$

 1 AFC = arrowtooth flounder protein coating from cooked method; AFH = arrowtooth flounder protein coating from enzyme hydrolysis method; AFP = arrowtooth flounder protein coating from pH extraction method; SP = soy protein coating; WP = whey protein coating; NC = control.

Table 6. Yield, drip loss, thaw yield, cook yield and pH of pink salmon fillets with different coatings after 3 month frozen storage

Samples ³	Yield $(\%)^1$	$\frac{\text{Drip loss}}{(\%)^1}$	Cook Yield $(\%)^2$	pH^2
AFC	101.1 ± 0.3^{b}	$1.0\pm0.5^{\mathrm{b}}$	$92.3\pm1.8^{\rm a}$	6.4 ± 0.1^{bcd}
AFH	101.2 ± 0.3^{ab}	$1.9\pm1.0^{\mathrm{ab}}$	$90.8 \pm 1.4^{\rm a}$	6.3 ± 0.0^{d}
AFP	$102.3\pm1.5^{\rm a}$	$1.1\pm0.5^{\mathrm{b}}$	$92.4\pm2.7^{\rm a}$	6.4 ± 0.1^{bcd}
SP	101.7 ± 0.3^{ab}	2.0 ± 0.9^{ab}	$92.5\pm0.8^{\rm a}$	6.5 ± 0.0^{abc}
WP	101.5 ± 0.3^{ab}	1.9 ± 0.9^{ab}	92.6 ± 0.9^{a}	6.6 ± 0.0^{ab}
NC	100.7 ± 0.3^{b}	2.0 ± 1.2^{ab}	$87.8\pm0.5^{\rm a}$	$6.7\pm0.0^{\rm a}$

¹Values are means \pm SD of 6 determinations.

²Values are means \pm SD of 3 determinations.

 abcd Within, treatments, means with same letter in each column are not significantly different. $P \geq 0.05$

 ${}^{3}\text{AFC}$ = arrowtooth flounder protein coating from cooked method; AFH = arrowtooth flounder protein coating from enzyme hydrolysis method; AFP = arrowtooth flounder protein coating from pH extraction method; SP = soy protein coating; WP = whey protein coating; NC = control. P \geq 0.05

Discussions

The results of this study show that the coatings on the salmon fillets are effective in minimizing the relative moisture loss and lipid oxidation. Antioxidant effects are highly dependent on molecular size of peptides and amino acid composition. It is possible that the peptides from the AFH could be aiding the retardation of lipid oxidation compared to AFC and AFP. The peptide formation may vary according to the degree of hydrolysis in cooking, enzymatic hydrolysis and in pH solubilization process. The enzymatic hydrolysis produces a mixture of peptides of different lengths having polar amino acids at each end which aid in retarding the oxidation (Kristinsson and Rasco 2000) better than hydrolyzed peptides from AFC and AFP. Although the relation of degree of hydrolysis among different extraction methods have not been understood, studies have found powerful anti-oxidant properties in intact proteins and hydrolysates (Shahidi et al. 1995). Sathivel et al. (2003) reported the anti-oxidant activity in fish protein hydrolysates. Arrowtooth flounder proteins have smaller molecular weight due to partial hydrolysis (Sathivel et al. 2004). Peptides of many proteins like soy protein (Pratt 1972), whey proteins (Cervato et al. 1999) and fish proteins (Kim et al. 2001; Rajapakse et al. 2004) have been reported to possess anti-oxidation properties which might attribute to lower TBARS values of soy and whey proteins to the non coated fillets

The acceptability and price of commercial fish such as salmon depends on quality parameters which includes flesh coloration (Skrede and Storebakkan 1986). The color of fillets was significantly lighter (L*), yellow (b*) and whiter in thawed fillets from the fresh fillets (Tables 2 and 3) recorded 3 months prior, could suggest the onset of oxidation. This reduction of pigments could be due to degradation of carotenoids (Shaheen et al. 1998; Christinansen et al 1995) during the frozen storage.

The AFH and AFC treatments have observed to allow less that 10% moisture loss compared to that of the non-coated fillets. The water-protein interaction plays an important role in food systems since water holding protein increases properties like texture. Proteins and peptides from the muscles of fish and beef have shown their ability to bind water and avoid the loss of moisture (Damodaran 1996). It seems that peptides from AFH and AFC are more effective than peptides from AFP to avoid the moisture loss in salmon fillets. This could be due to less hydrolyzed fractions, bigger peptide chain lengths with non polar amino acids or intactness of proteins in fish protein (Kristinsson 1998). Although the soy and whey proteins have shown to improve water binding (Kristinsson and Rasco 2000; Khwaldia et al. 2004), the application in this study was found to be less effective.

The yield data suggests the formation of thicker coat by AFP treatment that can also explain the higher moisture content in cooked fillets. Shahidi et al. (1995) have reported that the addition of protein hydrolysates to the comminuted meats increase their cooking yield and observed large reduction in drip loss. A report by Chapman et al. (1997), on the contrary, suggests that coated fillets showed increased drip loss than the non-coated fish fillets.

Conclusions

The results presented in this study conclude that edible coatings prepared from proteins extracted from underutilized arrowtooth flounder have a definite potential to enhance the shelf life of commercially valuable fishes like salmon, along with other protein sources like soy and whey, during the frozen storage. Arrowtooth flounder protein coatings have shown to retard the unfavorable chemical changes in fish fillets like lipid oxidation and moisture loss, during frozen storage. The color and texture properties of the fish also seem to be better protected by the edible coatings than that of the non-coated fillets. An increased coating yield, cooked yield and reduced drip losses in the fish fillets will certainly help the seafood industry in the U.S to consider the potential of arrowtooth flounder proteins as an invisible protective edible coating on seafood.

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