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Spectral Changes of Atlantic Salmon (*Salmo salar* L.) Muscle during Cold Storage As Affected by the Oxidation State of Heme

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ABSTRACT: The spectra of fresh salmon fillets change due to storage and packaging atmospheres. The aim of this study was to demonstrate the effects of heme oxidation states on spectral development in salmon fillets and to investigate the origin of a shoulder peak representing important spectral variations during storage. Hyperspectral images of fresh salmon fillets and mince with various water contents were collected during storage under different atmospheres. In addition, the absorption spectra of extracted salmon hemoglobin and its derivatives (methemoglobin and deoxyhemoglobin) were obtained. Air storage resulted in an increased similarity between spectra of methemoglobin and salmon fillets in principal component analysis. Results from the mince storage demonstrated that absorption features at the shoulder peak could be related to water content in the salmon muscle. This study established that the formation of oxidized heme is the primary source of spectral variations that occur during air storage of fresh salmon. Changes in the status of heme due to storage and packaging can influence the appearance of the underlying water absorption at the shoulder peak and create variations in the salmon spectra.

KEYWORDS: *salmon, storage, packaging, imaging, spectroscopy, heme, oxidation*

INTRODUCTION

Fresh fish and fishery products are highly perishable.[1] Quality changes occur as fish muscle deteriorates as a result of autolytic enzymatic processes in the first phase of storage. Subsequently, the growth and production of metabolites by microorganisms will cause spoilage.[2] Lipid oxidation may also contribute to deterioration of fish fillets during cold storage. This occurs particularly in dark muscle of unbled fish exposed to air.[3] It is, however, microbial growth that limits the storage time of fresh fish. Rapid determination of freshness and online monitoring of quality are essential to the modern fish industry. During recent decades, the potential for rapid and nondestructive spectroscopic methods has been investigated within the field of seafood quality.[4,5] Correlation between storage time and spectral parameters has been established,[6,7] and spectroscopic techniques have proven useful for the evaluation of quality attributes related to fish freshness.[8−12]

The visible region of the spectrum (400−700 nm) contains spectral features important for freshness prediction in cod fillets.[6,12,13] Sivertsen et al. proposed that the oxidation of heme proteins (hemoglobin, Hb; and myoglobin, Mb) may explain most variations in the visible region of the spectrum in cod fillet.[13] Color effects of heme are well recognized,[14−18] but for pigmented fish such as salmon, the concentration of heme in the muscle has been assumed to be small compared to that of astaxanthin. However, Bjørlykke et al. suggested that an increased redness observed in carbon monoxide treated salmon may have resulted from the cherry red color of carboxyHb.[19] In addition, the color of unbled salmon fillet may appear darker compared to that of well-bled fish,[20,21] probably because of a higher residual blood and Hb content in the fillet.[22] These results indicate that the color effects of heme could also be significant in salmon.

Recently, a possible contribution of the heme state to the spectral features of fresh salmon has been suggested. Spectra of fresh salmon fillets developed distinctive absorption characteristics during storage under different atmospheres.[23−25] The main spectral differences due to storage conditions occurred in the visible region at wavelengths centered at 600 and 630 nm. Air storage led to an increased intensity at a peak around 630 nm, whereas a shoulder peak surrounding 600 nm declined over time. Because similar spectral changes have been observed in cod and mackerel,[13,23] these spectral variations in salmon could not be ascribed to astaxanthin. An absorption peak around 630 nm has been associated with oxidized Hb (metHb) and Mb (metMb) present in mammalian and fish muscles.[23,26−28] The observed increase in the intensity of the peak was therefore tentatively related to the formation of oxidized heme during air storage of fresh salmon.[23−25] The origin of the shoulder peak at around 600 nm has not yet been identified. The intensity of the absorption shoulder appears to be dependent on both storage time and packaging similarly to that of the 630 nm peak.[23,24] However, absorption characteristics around 600 nm do not correspond with those of heme and its derivatives.[13] Sivertsen et al. suggested that the peak could arise from water in the muscle[13] as water exhibits an absorption shoulder at similar wavelengths.[29] The authors speculated that spectral variations at these wavelengths may occur due to changes in heme status during storage.

Previous studies provide indications of the significance of heme in determining spectral development of fresh salmon during storage. However, its spectral effects remain indicative

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and should be demonstrated. Further investigation must be conducted with heme proteins extracted from the species in question and under a pH comparable to that found in post-mortem fillet. The pH sensitivity and species specificity of fish heme may be significant and result in pH-induced variations in Hb spectra and cause analytical error.[26,30−32] The objective of this study was to demonstrate how different oxidation states of heme affected spectral properties of salmon fillets during storage. Furthermore, the study aimed to investigate whether the spectral shoulder around 600 nm may originate from absorption due to water.

MATERIALS AND METHODS

Storage Experiment of Salmon Fillets Under Different Atmospheres. *Fish Sample and Packaging.* A total of 144 prerigor fillets of Atlantic salmon (*Salmo salar* L.) were obtained from a commercial fish farm (Lerøy Aurora, Skjervøy, Norway) within 6 h of slaughter. The loin part of each fillet (mean weight = 1.60 ± 0.17 kg) was cut into a skin-on piece (254 ± 7.2 g). The 144 samples were randomly divided into three groups and individually packaged using one of three methods: (1) traditional overwrap packaging; (2) modified atmosphere (gas/product ratio 3:1) with a gas mixture of 60% carbon dioxide (CO_2) and 40% nitrogen (N_2) (Yara Praxair, Oslo, Norway); and (3) 90% vacuum. The oxygen (O_2) level inside the modified atmosphere packages was measured at each sampling by a CheckMate-3 headspace analyzer (PBI Dansensor, Ringsted, Denmark) and kept below 0.1% (0.05 ± 0.02%) during storage. The technical specifications of packaging materials used in this experiment are presented in Sone et al.[24] To prevent drying of the surface, each air-stored fillet was placed in a tray with high barrier properties as fillet under modified atmosphere, and the tray was wrapped with plastic film. A liquid absorbing pad (Dri-Loc absorbent pads, Sealed Air Corp., Epernon Cedex, France) was placed under each sample as described by Hansen et al.[33] Following packaging, the samples were kept at 4 ± 0.3 °C and evaluated on day 0 (i.e., 12 h after slaughter, 3−4 h after packaging) and on days 2, 4, 6, 8, 10, 12, and 14 after slaughter. At each sampling, six replicates of each packaging method were analyzed. Muscle pH was measured according to the method of Herland et al.[34]

Hyperspectral Imaging System. Hyperspectral interactance images of salmon fillets were collected using the imaging system described by Sivertsen et al.[13] The imaging system consisted of an imaging spectrometer (VNIR-640, Norsk Elektro Optikk, N-1497, Norway), two lines of custom-made fiber optical lights with focusing acrylic lenses, a white diffuse conveyer belt, and two black aluminum screens for light baffling. The conveyer belt ran at the industry standard speed of 400 mm/s. The spectrometer was placed 1030 mm above the conveyer belt and had a focal distance of 1000 mm and depth of field of 25 mm. Three 150 W (21 voltage direct current) halogen lamps each composed of a 200 mm long fiber optic light line were situated on both sides of the imaging area. The fiber lines were positioned 150 mm above the conveyer belt, and the width of each light line projected onto the belt was 10 mm. The spectrophotometer had a field of view of 1.0 × 320 mm, which was 20 mm apart from and ran parallel to the center of the projected light lines. The light in this region was recorded in one frame by a charge-couple device in the spectrometer and represented as 640 spectra. Individual spectra represented light intensity from a spatial area of 1.0 × 0.5 mm in the region of 400−1000 nm, with a spectral resolution of about 10 nm. Each sample was scanned line by line at 400 frames per second while moving through the spectrophotometer field of view. The data acquired were recorded as a hyperspectral image $R(\lambda,x,y)$ consisting of successive frames, $F(\lambda,x)$. At the start of each sampling day, a Teflon target (300 × 300 × 25 mm) was scanned and used for calibrating the imaging system. One hundred successive frames recorded from the Teflon target were used to obtain the average reference frame, $R_a(\lambda,x)$. The interactance image was calculated as $I(\lambda,x,y) = R_i(\lambda,x,y)/R_a(\lambda,x)$ and exported to Interactive Data Language (IDL) 8.1 (ITT Visual Information Solutions, Boulder, CO, USA) for analysis.

Spectral Acquisition of Salmon Hemoglobin. *Collection of Blood from Atlantic Salmon.* One Atlantic salmon (3.6 kg) was acquired from the Tromsø Aquaculture Research Station (Kårvika, Tromsø). The captured fish was immediately killed by a blow to the head, and fish blood was collected within 5 min. Blood was drawn from the caudal vein using lithium-heparinized vacutainers (Vacutainer, BD Diagnostics, Franklin Lakes, NJ, USA) and kept on ice during transport to Nofima (Tromsø, Norway).

Preparation of Hemolysate. Upon arrival, the hemolysate was prepared according to the method described by Richards and Hultin.[35] Four volumes of ice-cold 1.7% sodium chloride (NaCl) in 1 mM tris(hydroxymethyl)aminomethane/hydrochloride (Tris-HCl), pH 8.0, was added to the heparinized blood. Plasma was removed after centrifugation (70*g* for 10 min at 4 °C) (Heraeus Multifuge 1S-R, Thermo Electron Corp., USA), and red cells were washed three times with the above buffer. Washed red cells were lysed in 3 volumes of 1 mM Tris-HCl, pH 8.0, for 1 h. A $^1/_{10}$ volume of 1 M NaCl was added to the hemolyzed solution followed by centrifugation at 25000*g* for 15 min at 4 °C (centrifuge 5417R, Eppendorf, Germany). To remove NaCl from the hemolysate, the solution was dialyzed (4 °C) overnight against 1 mM Tris-HCl, pH 8.0, using a 12000−14000 molecular weight cutoff membrane. The dialysis buffer was changed twice. The hemolysate obtained was stored at −80 °C for later analysis.

Estimation of Hemoglobin Level in Hemolysate. The frozen hemolysate was thawed overnight at 4 °C and centrifuged at 15800*g* (centrifuge 5415 C, Eppendorf) for 1 min (4 °C) to remove denatured Hb. The supernatant of the hemolysate was diluted in 1 mM Tris-HCl, pH 8.0, to ensure that the Hb absorption in the Soret band had an optical density below 2.0. The sample was scanned from 450 to 400 nm against a blank (buffer), and the peak absorbance was recorded using a UV-1700 PharmaSpec UV−vis photospectrometer (Shimazu Corp., Kyoto, Japan). The standard curve was constructed using a bovine Hb standard (Sigma-Aldrich Norway AS, Oslo, Norway). An extinction coefficient of 4.42 × 10^5 L/cm mol was obtained.

Preparation of Oxidized and Deoxygenated Hemoglobin. The hemolysate was dialyzed against 0.1 M phosphate buffered saline (PBS) with 0.9% NaCl, pH 6.1, overnight to adjust the pH of the Hb solution to that of a post-rigor salmon fillet. The dialysis buffer was changed twice. The metHb was prepared by adding 100 μL of PBS containing 12.5 mg/mL solid potassium ferricyanide ($K_3Fe(CN)_6$) to 10 mL of the Hb solution. The amount of $K_3Fe(CN)_6$ added to the sample was equivalent to a 3-fold molar excess (heme basis) as described by Richards and Hultin.[35] The solution was stored on ice for 30 min to convert all of the Hb to metHb. The deoxyhemoglobin (deoxyHb) was prepared according to the method of Olsen et al.[26] Ten milliliters of the diluted hemolysate was bubbled for 1 min with a gas mixture containing 95% N_2 and 5% CO_2, and 1−2 mg of sodium dithionite ($Na_2S_2O_4$) was added to the solution immediately. For each Hb form, five duplicates were prepared.

Transmittance Measurements. Spectra of the diluted Hb solution and the metHb and deoxyHb solutions were recorded immediately after preparation using the XDS Rapid Liquid Analyzer module (FOSS NIRSystems, Inc., Laurel, MD, USA). The transmittance measurements were performed in a 10 mm quartz cuvette. The measured wavelength range was from 400 to 2500 nm with a spectral resolution of 0.5 nm. To make the transmittance spectra of Hb comparable to the interactance spectra of salmon fillets, Hb absorption spectra were interpolated at the wavelengths corresponding to the 64 wavelengths of the imaging data. The interpolated spectra were then exponentially transformed to the interactance: $I(\lambda,x,y) = 10^{-A(\lambda,x,y)}$.

Spectral Data Analysis of Salmon Fillet and Hb in Different State. A region of interest was defined as a rectangle in the center of each image to avoid possible interference from the edge of the sample. Nonoverlapping circular areas consisting of 81 pixels (40 mm^2) were randomly extracted from the defined region, and a mean spectrum was calculated from the spectra in each area. Spectral pretreatment by Standard Normal Variate (SNV)[36] was performed on the interactance spectra of salmon fillet and on the transformed spectra of salmon Hb.

A single spectrum of salmon fillet was randomly selected from each packaging type on the respective storage day. The 24 selected spectra were compiled together with the spectra of Hb, and principal component analysis (PCA) was developed with 17 wavelengths in the range of 600−765 nm. The selected range included five single wavelengths (606, 636, 665, 705, and 764 nm) previously reported to classify fresh salmon fillets by packaging type.[24] Following the construction of the PCA model, the spectra of the remaining salmon samples were projected onto the model. The spectral extraction and pretreatment routines as well as PCA were programmed in IDL.

Storage Experiment Using Minced Salmon with Different Water Contents. *Preparation of Minced Salmon and Packaging.* Six post-rigor fillets of Atlantic salmon (gutted weight = 4−5 kg) were obtained at a commercial fish processing company (Dragøy, Tromsø, Norway) after 3 days of storage on ice. The salmon fillets were cut into the belly and midline portions as described by Katikou et al.[37] After the caudal and ventral fat depots had been trimmed, part of the fillet posterior to the edge of the ventral fin was discarded. A cut was made along the lateral line to obtain the belly region of the fillet. The remainder of the fillet was divided along its length where the pattern in muscle fiber orientation changed. The dorsal portion was removed to obtain the midline region of the fillet. Following the portioning procedure, the belly and midline sections of the fillet were deep-skinned to remove all dark muscle. Bones and fat attached to the skin were also discarded. The belly and midline portions collected from the six salmon fillets were minced for 25 s to generate two batches of homogeneous muscle paste. From each homogenate, 20 samples of 70 g of mince were prepared and distributed evenly in a Petri dish with a plastic spatula. Thereafter, a hyperspectral interactance image of the mince sample was acquired using the imaging system described. Following acquisition of the hyperspectral image, the mince was individually placed in a MAPET tray with an oxygen transmission rate (OTR) of 100−160 cm^3/m^2 at 23 °C/0% relative humidity (RH). Half of the mince from each batch was packaged in 100% N_2 (AGA, Oslo, Norway) using a packaging machine (Webomatic Semiautomatic Tray Sealer TL 300, Bochum, Germany). Each tray was heat sealed with a lidding film (OTR = <3.0 cm^3/m^2 at 23 °C/0% RH) (R. Færch plast AS, Holstebro, Denmark). The O_2 level of modified atmosphere packages remained below 0.1% (0.09 ± 0.004%) during storage. The other half of the mince was wrapped with plastic film with low barrier properties (OTR = 7000 cm^3/m^2 at 23 °C/0% RH) (Maske group AS, Vinterbro, Norway). The samples were stored at 4 °C after packaging and analyzed on days 6 and 9 post-mortem by imaging spectroscopy. At each sampling, five replicates of the midline and belly mince were randomly selected from each packaging type for analysis. In addition, water content was measured according to the AOAC 950.46 method[38] using triplicates of the selected midline and belly samples in air and under modified atmosphere.

Spectral Data Analysis of Minced Salmon. Selection of a region of interest and spectral extraction were performed in IDL as described. The interactance spectra of mince samples were natural log transformed to absorbance $A(\lambda,x,y) = -\ln(I(\lambda,x,y))$ and pretreated by SNV. Absorption intensities at 932 and 972 nm were examined in the spectra of midline and belly mince to verify that the difference in water content between the two minces was large enough to give spectral variations.[39,40] To examine spectral features around 600 nm, cubic spline interpolation[41] was constructed at wavelengths of 586, 596, and 606 nm using two adjacent wavelengths on both sides (566, 576, 616, and 626 nm). The area surrounded by the three interpolated points $A_i(\lambda)$ and the true spectrum $A(\lambda)$ was estimated for each sample as

$$\text{area} = \sum_{606}^{\lambda=586} (A(\lambda) - A_i(\lambda)) \times \Delta\lambda$$

where $\Delta\lambda$ is the interval between two wavelengths. Significant difference between the estimated area of the belly and midline batch was tested by a t test with a 95% confidence level. Each sampling day and packaging method was tested separately.

RESULTS AND DISCUSSION

Heme Oxidation and Spectral Variations of Salmon During Storage. Mean spectra of extracted salmon Hb in the three different states are presented in Figure 1. The spectra of

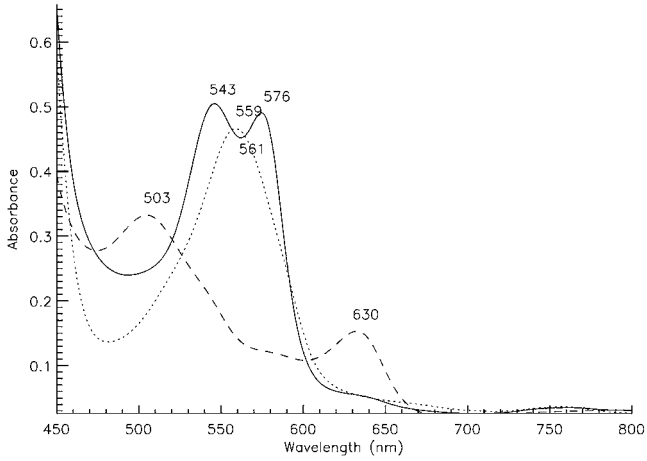

Figure 1. Mean absorbance spectra of extracted salmon Hb (solid), metHb (dashed), and deoxyHb (dotted) in the wavelength range of 450−800 nm.

the extracted Hb showed signs of oxygenation at pH 6.1. The two peaks at 543 and 576 nm correspond well with the absorption peaks of oxyHb in other fish species and mammals.[26,27,42−44] The mean spectrum of deoxyHb was characterized by a single peak at 559 nm, which is largely in accordance with spectra of deoxyHb acquired from Atlantic cod and mammals under different laboratory conditions.[26,45] The spectrum of salmon metHb showed the absorption maxima at 503 and 630 nm at pH 6.1 similarly to metHb spectra observed elsewhere.[27,46] The peak at 630 nm was present only in the metHb spectrum and not in any other forms of Hb prepared in this study.

A PCA was developed based on the spectra of extracted Hb in solution and its derivatives together with selected spectra of salmon fillets after pretreatment by SNV. The first principal component (PC1) accounted for 94.6% of the total variation. The spectra of extracted Hb in solution and its derivatives were completely segregated from the spectra of salmon fillets in PC1. The spectral variations contributing to this clear separation must have originated in components and properties that were exclusively or dominantly found in Hb in the different forms but not in salmon fillets, and vice versa. Astaxanthin is a component present only in the fillets. Absorption of astaxanthin was strongly represented in PC1 when PCA was run with the spectra of bovine myoglobin including the visible region (400−750 nm) by Ottestad et al.[23] The second (PC2) and third principal components (PC3) separated the spectra of salmon Hb in the three oxidation states. In the score plot with PC2 and PC3 (Figure 2), the spectra of metHb and deoxyHb appear at opposite ends along PC2. The PC2 loading (Figure 3) showed a leading contribution of spectral features occurring at 636 nm in the direction of PC2. The loading of PC3 was mainly characterized by spectral variations at 606 nm.

The spectra of salmon fillets were projected onto the PCA score plot according to the storage time (Figure 2). Little variation was obtained on day 0 between the different packaging types. During storage, the spectra of air-stored fillets

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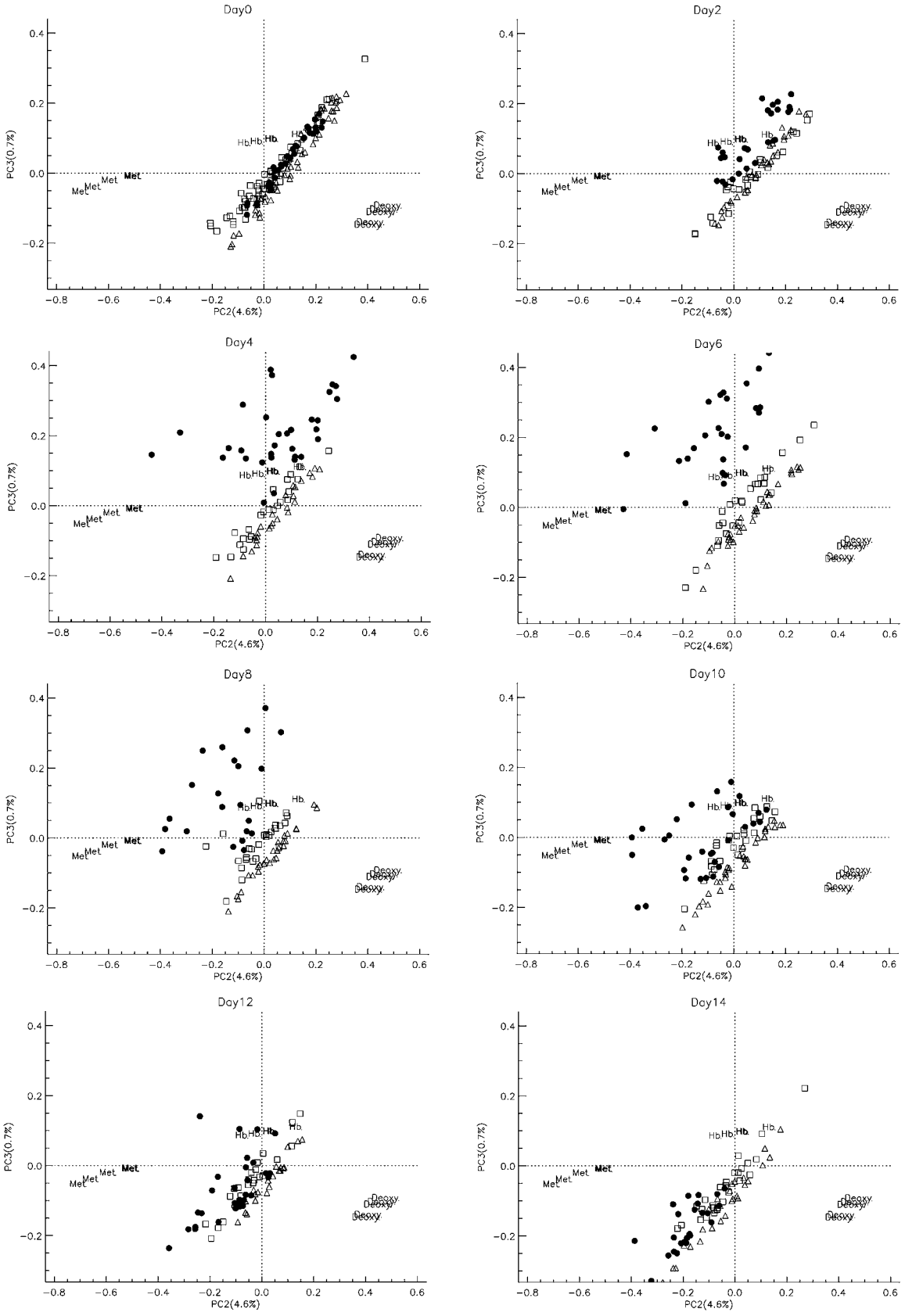

Figure 2. PCA projection of spectra of salmon fillets stored in air (solid circles), under modified atmosphere (squares), and vacuum (triangles) during storage from day 0 to day 14 post-mortem. The spectra of the extracted Hb and metHb and deoxyHb are labeled Hb, Met, and Deoxy, respectively. To demonstrate spectral development in salmon during storage, each plot shows projected spectra of fillets analyzed on the respective sampling day.

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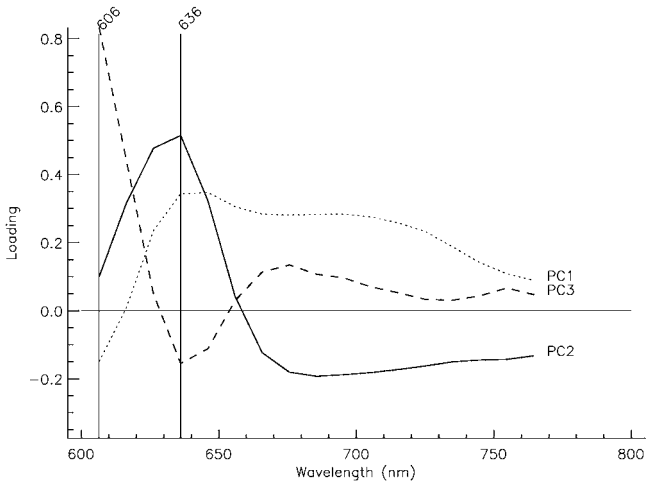

Figure 3. Loading plots of PC1 (dotted), PC2 (solid), and PC3 (dashed) obtained from PCA analysis of spectra of salmon Hb in the different oxidation states and selected spectra of salmon fillets.

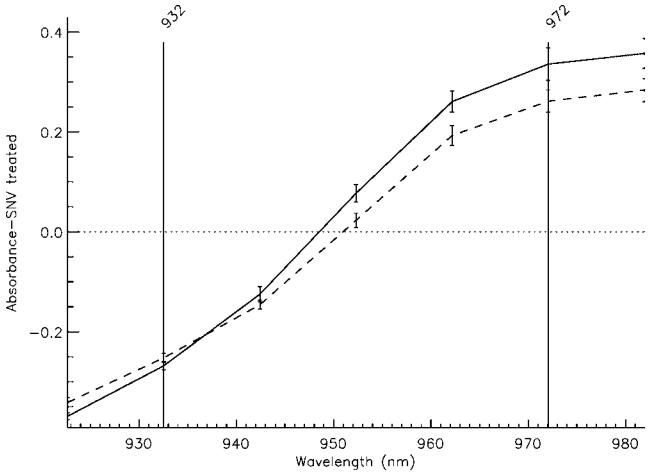

Figure 4. Mean absorption spectra (SNV-treated) of minced salmon collected from the midline (solid line) and belly (dashed) portion of the fillet on day 3 in the wavelength range of 920−980 nm.

showed gradual movement upward along PC3 (days 2−4) before they moved in the direction of metHb along PC2 (days 6−8). The loading and score plots illustrated an increased similarity between the spectra of salmon fillets and those of metHb during air storage. Fillets stored under modified atmosphere and vacuum showed little spectral shift in the direction of metHb. Following day 8, the spectra of air-stored salmon moved away from the spectra of metHb and toward the spectra of fillets under modified atmosphere and vacuum. This spectral development appears to occur along PC3. The loading of PC3 (Figure 3) suggests that changes occurring in spectral features at 606 nm may have a large influence on the spectra of air-stored fillets at the late stage of storage. The results demonstrated that an increase in oxidized heme is the primary source of spectral variations occurring in air-stored salmon in the first phase of air storage. In addition to spectral features at 606 nm, spectral changes brought about by heme oxidation contribute to separating spectra of air-stored salmon from those in the O_2-limited conditions.

Spectral Features with Various Water Contents, Storage Times, and Packagings. Storage- and packaging-dependent spectral changes were observed earlier at 606 nm (Figures 2 and 3). Similar spectral variations have been reported in cod, salmon, and mackerel at a shoulder peak of around 600 nm.[13,23−25] The chemical origin of the peak is yet to be determined. In this study, two batches of minced salmon were prepared from the midline and belly regions of post-rigor fillets and stored either in air or under modified atmosphere with 100% N_2. The difference in water content between the two batches was 7.5% (midline mince, 66.5 ± 0.4%; belly mince, 59.0 ± 0.1%) at the start of storage. The subsequent packaging and storage did not affect the water content of mince samples. The difference of 7.5% in the water content between the midline and belly mince was reflected in the mean spectra of the two batches (Figure 4). The inverse relationship between water and fat in the two types of mince could be verified by the difference in absorption intensity around 930 and 970 nm, which originate from the third overtone of carbon−hydrogen stretch in the methylene group of fat and the second overtone of the oxygen−hydrogen stretching band of water, respectively.[39,40]

The area defined by the interpolated points at 586, 596, and 606 nm and the true values at the respective wavelengths was significantly greater for the spectra of midline mince than for the spectra of belly mince ($p > 0.05$) (Figure 5). This was true for both number of storage days and packaging method. The area represented an estimated size of a curve-broadening feature at 586, 596, and 606 nm where it deviates from the line shape of the spectrum at the adjacent wavelengths. The shoulder peak surrounding 600 nm does not correspond with absorption by heme and is not related to astaxanthin.[13,23] At approximately 600 nm, water absorbs and exhibits a broad shoulder in the spectrum.[29] The difference in water content between the two batches was large enough to produce variations in absorption intensity at the water peak of 970 nm. It is possible that greater absorption intensity from water may have resulted in a more distinctive bump surrounding 600 nm in the spectra of midline mince than in the spectra of belly mince. The two batches of salmon mince also had different fat contents, but the spectral shape of fat does not exhibit a shoulder at these wavelengths. From the results it is probable that the origin of the peak around 600 nm can be connected with water in the muscle of fresh salmon and its absorption properties.

The estimated peak area of mince stored in air and under modified atmosphere varied distinctively during storage (Figure 5). The mean area decreased to a negative value from days 3 to 6 for air-stored samples. This illustrates that the intensity at 589, 596, and 606 nm may have declined during the 3 days of air storage. The result corresponds well with previously reported spectral changes occurring at these wavelengths.[24,25] An increase was observed during the same period for mince under modified atmosphere and remained until the end of storage. Samples stored in air taken on day 9 were characterized by an increase in the mean area to a value comparable to that of modified atmosphere (Figure 5). The PCA score plot showed a similar trend earlier, where the projected spectra of air-stored fillets more closely resembled the spectra of fillets under modified atmosphere after day 8 (Figure 2). The PCA loading showed that this change of course at late storage was largely influenced by spectral variations at 606 nm (Figure 3).

The area variations between the different packagings are not related to change in absorption intensity of water as neither

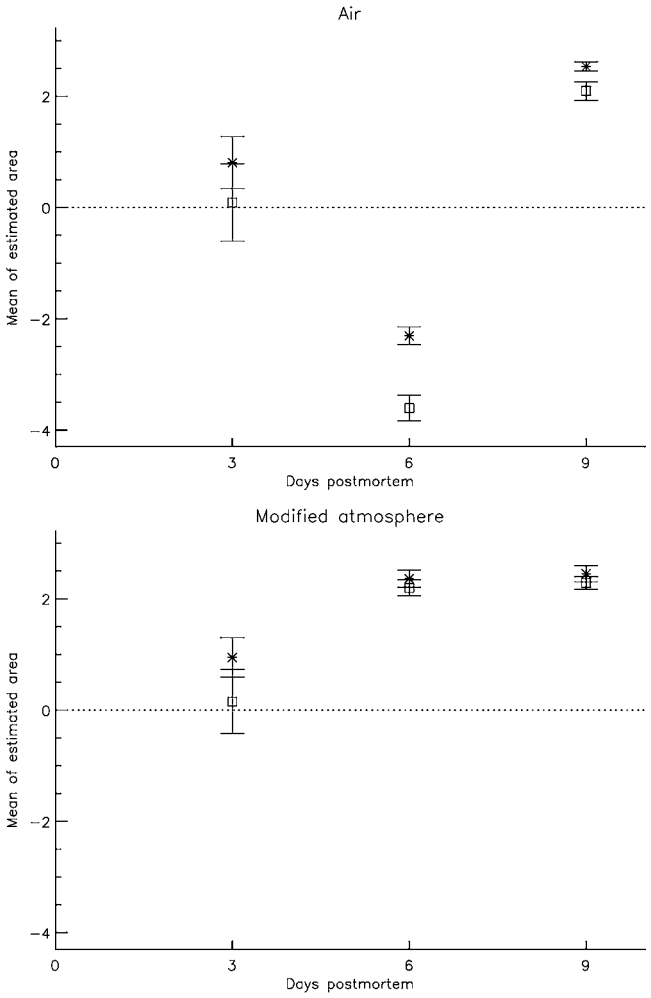

Figure 5. Means of estimated peak area for the midline (asterisk) and belly (square) mince packaged in air (top) and modified atmosphere (bottom) with standard deviations.

storage nor packaging affected the water content. On the basis of the inverse relationship between water and fat, the contribution of fat to the observed variations can also be assumed to be minimal. In addition to water and fat, astaxanthin and heme are the main constituents affecting the salmon spectrum in the visible region. The reduction of the 600 nm peak was reported during air storage of nonpigmented fish such as cod and mackerel.[13,23] These observations can therefore eliminate a possible contribution from astaxanthin to the packaging differences in the estimated area and to the previously observed spectral variations around 600 nm.

Extensive spectral effects of heme oxidation have been demonstrated earlier in this study. Air storage leads to heme oxidation, whereas most heme under O_2 limited conditions is converted to its deoxy form.[47,48] In addition, the number of spoilage bacteria such as *Pseudomonas* spp. and *Shewanella putrefaciens* increases rapidly in air-stored salmon.[49,50] The growing microorganisms may promote heme oxidation by reducing the oxygen partial pressure through oxygen consumption and the formation of a biofilm on the sample surface.[51,52] After prolonged air storage resulting in profound spoilage, several psychrophilic bacteria convert metMb into deoxyMb and red-colored heme derivatives. As a result, metMb content can decrease at late storage and the cessation of autoxidation may be observed spectroscopically.[15,52−54]

Air-stored salmon fillet suffered extensive microbial growth (>8 log cfu/g) after day 8 in the storage experiment.[24] At least a similar degree of spoilage can be expected in minced samples by day 9 of air storage. The observations in the estimated area (Figure 5) and the score plot (Figure 2) indicate that spectral features around 600 nm in air-stored samples resemble those under modified atmosphere at late storage. Absorption properties around 600 nm may have changed in salmon at this storage time because of decreased oxidized heme resulting from bacterial heme conversion. Our studies have previously demonstrated that the spectral features of the peak area at around 600 nm can arise from a water absorption shoulder at the respective wavelength. Change in heme state caused by microorganisms and the resulting spectral shift could have influenced the expression of the underlying water absorption at the shoulder peak in the spectra of samples. This could explain the increased mean area in air -stored mince on day 9 and the spectral development observed in salmon fillets after prolonged air storage. Overall, the results indicate that the shoulder at 600 nm and the surrounding bands decrease with the increased production of oxidized heme, whereas the predominance of deoxyheme can result in a more distinctive peak at the respective wavelengths.

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Notes

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REFERENCES

(1) Olafsdóttir, G.; Martinsdóttir, E.; Oehlenschläger, J.; Dalgaard, P.; Jensen, B.; Undeland, I.; Mackie, I. M.; Henehan, G.; Nielsen, J.; Nilsen, H. Methods to evaluate fish freshness in research and industry. *Trends Food Sci. Technol.* **1997**, *8*, 258−265.

(2) Huss, H. H. Fresh fish − quality and quality changes. *FAO Fisheries Technical Paper 348*; Food and Agriculture Organization of the United Nations: Rome, Italy, 1995.

(3) Richards, M. P.; Hultin, H. O. Contributions of blood and blood components to lipid oxidation in fish muscle. *J. Agric. Food Chem.* **2002**, *50*, 555−564.

(4) Nilsen, H.; Heia, K. VIS/NIR spectroscopy. In *Fishery Products: Quality, Safety and Authenticity*; Rehbein, H., Oehlenschlager, J., Eds.; Wiley-Blackwell Publishing: Chichester, UK, 2009; pp 89−104.

(5) Workman, J. J. Review of process and non-invasive near-infrared and infrared spectroscopy: 1993−1999. *Appl. Spectrosc. Rev.* **1999**, *34*, 1−89.

(6) Nilsen, H.; Esaiassen, M.; Heia, K.; Sigernes, F. Visible/near-infrared spectroscopy: a new tool for the evaluation of fish freshness? *J. Food Sci.* **2002**, *67*, 1821−1826.

(7) Sigernes, F.; Esaiassen, M.; Heia, K.; Eilertsen, G.; Wold, J. P.; Sørensen, N. K. Assessment of fish (cod) freshness by VIS/NIR spectroscopy. *Methods to Evaluate Fish Freshness in Research and Industry*; Olafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E., Heia, K., Eds.; International Institute of Refrigeration (IIR): Paris, France, 1998.
(8) Sone, I.; Olsen, R. L.; Dahl, R.; Heia, K. Visible/near-infrared spectroscopy detects autolytic changes during storage of Atlantic salmon (*Salmo salar* L.). *J. Food Sci.* **2011**, *76*, S203−S209.
(9) Lin, M.; Mousavi, M.; Al-Holy, M.; Cavinato, A. G.; Rasco, B. A. Rapid near infrared spectroscopic method for the detection of spoilage in rainbow trout (*Oncorhynchus mykiss*) fillet. *J. Food Sci.* **2006**, *71*, S18−S23.
(10) Bøknæs, N.; Jensen, K. N.; Andersen, C. M.; Martens, H. Freshness assessment of thawed and chilled cod fillets packed in modified atmosphere using near-infrared spectroscopy. *Food Sci. Technol.−Leb.* **2002**, *35*, 628−634.
(11) Warm, K.; Martens, H.; Nielsen, J.; Martens, M. Sensory quality criteria for five fish species predicted from near-infrared (NIR) reflectance measurement. *J. Food Qual.* **2001**, *24*, 389−403.
(12) Nilsen, H.; Esaiassen, M. Predicting sensory score of cod (*Gadus morhua*) from visible spectroscopy. *Food Sci. Technol.−Leb.* **2005**, *38*, 95−99.
(13) Sivertsen, A. H.; Kimiya, T.; Heia, K. Automatic freshness assessment of cod (*Gadus morhua*) fillets by Vis/Nir spectroscopy. *J. Food Eng.* **2011**, *103*, 317−323.
(14) Nakamura, Y.-N.; Ando, M.; Seoka, M.; Kawasaki, K.; Tsukamasa, Y. Changes of proximate compositions and myoglobin content in the dorsal ordinary muscles of the cultured Pacific bluefin tuna *Thunnus orientalis* with growth. *Fish. Sci.* **2007**, *73*, 1155−1159.
(15) Chaijan, M.; Benjakul, S.; Visessanguan, W.; Faustman, C. Changes of pigments and color in sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscle during iced storage. *Food Chem.* **2005**, *93*, 607−617.
(16) Lopez-Galvez, D.; de la Hoz, L.; Ordonez, J. A. Effect of carbon dioxide and oxygen enriched atmospheres on microbiological and chemical changes in refrigerated tuna (*Thunnus alalunga*) steaks. *J. Agric. Food Chem.* **1995**, *43*, 483−490.
(17) Richards, M. P.; Modra, A. M.; Li, R. Role of deoxyhemoglobin in lipid oxidation of washed cod muscle mediated by trout, poultry and beef hemoglobins. *Meat Sci.* **2002**, *62*, 157−163.
(18) Wetterskog, D.; Undeland, I. Loss of redness (a^*) as a tool to follow hemoglobin-mediated lipid oxidation in washed cod mince. *J. Agric. Food Chem.* **2004**, *52*, 7214−7221.
(19) Bjørlykke, G. A.; Roth, B.; Sørheim, O.; Kvamme, B. O.; Slinde, E. The effects of carbon monoxide on Atlantic salmon (*Salmo salar* L.). *Food Chem.* **2011**, *127*, 1706−1711.
(20) Heia, K.; Sivertsen, A. H.; Wold, J. P.; Ottestad, S.; Böcker, U.; Carlehög, M.; Altintzoglou, T.; Sone, I.; Gundersen, B. *Automatisk Kvalitetsdifferensiering av Laksefilet*; Report 7/12; Nofima: Tromsø, Norway, 2012 (in Norwegian).
(21) Roth, B.; Torrissen, O. J.; Slinde, E. The effect of slaughtering procedures on blood spotting in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture* **2005**, *250*, 796−803.
(22) Olsen, S. H.; Sorensen, N. K.; Stormo, S. K.; Elvevoll, E. O. Effect of slaughter methods on blood spotting and residual blood in fillets of Atlantic salmon (*Salmo salar*). *Aquaculture* **2006**, *258*, 462−469.
(23) Ottestad, S.; Sørheim, O.; Heia, K.; Wold, J. P. Effects of storage atmosphere and heme state on the color and visible reflectance spectra of salmon (*Salmo salar*) fillets. *J. Agric. Food Chem.* **2011**, *59*, 7825−7831.
(24) Sone, I.; Olsen, R. L.; Sivertsen, A. H.; Eilertsen, G.; Heia, K. Classification of fresh Atlantic salmon (*Salmo salar* L.) fillets stored under different atmospheres by hyperspectral imaging. *J. Food Eng.* **2012**, *109*, 482−489.
(25) Ottestad, S.; Isaksson, T.; Wold, J. P. Effect of varying optical properties on the modeling of astaxanthin concentration in salmon by visible spectroscopy. *Aquaculture* **2012**, *330*−*333*, 116−120.
(26) Olsen, S. H.; Elvevoll, E. O. pH-induced shift in hemoglobin spectra: a spectrophotometeric comparison of Atlantic cod (*Gadus morhua*) and mammalian hemoglobin. *J. Agric. Food Chem.* **2011**, *59*, 1415−1422.
(27) Zijlstra, W. G.; Buursma, A. Spectrophotometry of hemoglobin: absorption spectra of bovine oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, and methemoglobin. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* **1997**, *118*, 743−749.
(28) Millar, S. J.; Moss, B. W.; Stevenson, M. H. Some observations on the absorption spectra of various myoglobin derivatives found in meat. *Meat Sci.* **1996**, *42*, 277−288.
(29) Pope, R. M.; Fry, E. S. Absorption spectrum (380−700 nm) of pure water. II. Integrating cavity measurements. *Appl. Opt.* **1997**, *36*, 8710−8723.
(30) Richards, M. P.; Dettmann, M. A. Comparative analysis of different hemoglobins: autoxidation, reaction with peroxide, and lipid oxidation. *J. Agric. Food Chem.* **2003**, *51*, 3886−3891.
(31) Richards, M. P.; Nelson, N. M.; Kristinsson, H. G.; Mony, S. S. J.; Petty, H. T.; Oliveira, A. C. M. Effects of fish heme protein structure and lipid substrate composition on hemoglobin-mediated lipid oxidation. *J. Agric. Food Chem.* **2007**, *55*, 3643−3654.
(32) Maqsood, S.; Benjakul, S. Comparative studies on molecular changes and pro-oxidative activity of haemoglobin from different fish species as influenced by pH. *Food Chem.* **2011**, *124*, 875−883.
(33) Hansen, A. Å.; Mørkøre, T.; Rudi, K.; Rødbotten, M.; Bjerke, F.; Eie, T. Quality changes of prerigor filleted Atlantic salmon (*Salmo salar* L.) packaged in modified atmosphere using CO_2 emitter, traditional MAP, and vacuum. *J. Food Sci.* **2009**, *74*, M242−M249.
(34) Herland, H.; Esaiassen, M.; Cooper, M.; Olsen, R. L. Quality of farmed Atlantic cod: effects of season and storage. *Aquacult. Res.* **2010**, *41*, 1203−1210.
(35) Richards, M. P.; Hultin, H. O. Effect of pH on lipid oxidation using trout hemolysate as a catalyst: a possible role for deoxyhemoglobin. *J. Agric. Food Chem.* **2000**, *48*, 3141−3147.
(36) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc.* **1989**, *43*, 772−777.
(37) Katikou, P.; Hughes, S. I.; Robb, D. H. F. Lipid distribution within Atlantic salmon (*Salmo salar*) fillets. *Aquaculture* **2001**, *202*, 89−99.
(38) AOAC. *Official Methods of Analysis*, 15th ed.; AOAC International: Arlington, VA, 1990.
(39) Osborne, B. G.; Fearn, T. *Near Infrared Spectroscopy in Food Analysis*; Longman Scientific & Technical: Harlow, UK, 1986; p 200.
(40) van Veen, R. L. P.; Sterenborg, H. J. C. M.; Pifferi, A.; Torricelli, A.; Cubeddu, R. Determination of VIS-NIR absorption coefficients of mammalian fat, with time- and spatially resolved diffuse reflectance and transmission spectroscopy. *OSA Annual Biomedical Topical Meeting*; Optical Society of America: Washington, DC, 2004.
(41) Hastie, T. J.; Tibshirani, R. J.; Friedman, J. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*; Springer Science: New York, 2009; p 745.
(42) Zijlstra, W. G.; Buursma, A.; Meeuwsen-Van der Roest, W. P. Absorption spectra of human fetal and adult oxyhemoglobin, de-oxyhemoglobin, carboxyhemoglobin, and methemoglobin. *Clin. Chem.* **1991**, *37*, 1633−1638.
(43) Vitagliano, L.; Bonomi, G.; Riccio, A.; di Prisco, G.; Smulevich, G.; Mazzarella, L. The oxidation process of Antarctic fish hemoglobins. *Eur. J. Biochem.* **2004**, *271*, 1651−1659.
(44) Kristinsson, H. G.; Mony, S. S.; Petty, H. T. Properties of tilapia carboxy- and oxyhemoglobin at postmortem pH. *J. Agric. Food Chem.* **2005**, *53*, 3643−3649.
(45) Steinke, J. M.; Shepherd, A. P. Effects of temperature on optical absorbance spectra of oxy-, carboxy-, and deoxyhemoglobin. *Clin. Chem.* **1992**, *38*, 1360−1364.
(46) Naughton, J. J.; Zeitlin, H.; Frodyma, M. M. Tuna meat pigment studies, spectral reflectance studies of the heme pigments in tuna fish flesh. Some characteristics of the pigments and discoloration of tuna meat. *J. Agric. Food Chem.* **1958**, *6*, 933−938.

(47) McMillin, K. W. Where is MAP going? A review and future potential of modified atmosphere packaging for meat. *Meat Sci.* **2008**, *80*, 43−65.
(48) Shikama, K. The molecular mechanism of autoxidation for myoglobin and hemoglobin: a venerable puzzle. *Chem. Rev.* **1998**, *98*, 1357−1374.
(49) Gram, L.; Huss, H. H. Microbiological spoilage of fish and fish products. *Int. J. Food Microbiol.* **1996**, *33*, 121−137.
(50) Sivertsvik, M.; Rosnes, J. T.; Kleiberg, G. H. Effect of modified atmosphere packaging and superchilled storage on the microbial and sensory quality of Atlantic salmon (*Salmo salar*) fillets. *J. Food Sci.* **2003**, *68*, 1467−1472.
(51) Robach, D. L.; Costilow, R. Role of bacteria in the oxidation of myoglobin. *Appl. Microbiol.* **1961**, *9*, 529−533.
(52) Chan, W. K. M.; Joo, S.-T.; Faustman, C.; Sun, Q.; Vieth, R. Effect of *Pseudomonas fluorescens* on beef discoloration and oxymyoglobin oxidation in vitro. *J. Food Prot.* **1998**, *61*, 1341−1346.
(53) Faustman, C.; Johnson, J. L.; Cassens, R. G.; Doyle, M. P. Color reversion in beef. Influence of psychrotrophic bacteria. *Fleischwirtschaft* **1990**, *70*, 676−679.
(54) Motoyama, M.; Kobayashi, M.; Sasaki, K.; Nomura, M.; Mitsumoto, M. *Pseudomonas* spp. convert metmyoglobin into deoxymyoglobin. *Meat Sci.* **2010**, *84*, 202−207.