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Effects of Storage Atmosphere and Heme State on the Color and Visible Reflectance Spectra of Salmon (*Salmo salar*) Fillets

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ABSTRACT: It has previously been observed that the color of mackerel muscle is dependent on the status of heme as myoglobin and hemoglobin and hence the storage atmosphere. This study gives strong indications of this being the case also in salmon. Three different storage conditions were used to promote the oxidized, reduced, and carbon monoxide (CO) bound forms of heme in salmon and mackerel fillets. Color determination (instrumental color analysis, imaging, and sensory evaluation) and spectroscopic measurements were performed to study how spectral changes corresponded to color variations. Storage in CO significantly increased the redness in mackerel. This was also seen in salmon to such a degree that it was visible over normal levels of salmon carotenoids. Air storage increased the yellowness and reduced the redness in mackerel, but this effect was partly concealed in salmon by the astaxanthin absorption. The spectral differences due to storage condition could be ascribed to the spectral features characterizing heme of different oxidation states and bound to different ligands. The status of heme should therefore always be considered when experiments related to salmon color are performed. The findings could help in the understanding, control, and prediction of color loss in salmon during processing, storage, and transport.

KEYWORDS: color, salmon, storage, packaging, heme, carbon monoxide

INTRODUCTION

The red-orange color of salmon muscle is an important quality parameter largely affecting the market price.[1] The main pigment group responsible for this red color in aquatic animals is the carotenoids. Salmon cannot synthesize carotenoids itself and needs to obtain it through the diet. For fish farmers, this diet supplement is an item of expenditure and needs to be portioned carefully. The quick method for color assessment of salmon is by visual inspection and comparison with the Roche SalmoFan color wheel (F. Hoffmann-La Roche, Basel, Switzerland). Fish farmers employing this method have reported an observed change in color with time after filleting with a reduction of approximately one score on the SalmoFan. The cause of this effect is yet to be explained.

In fish species such as tuna, sardine, and mackerel a discoloration has been observed due to oxidation of myoglobin and formation of metmyoglobin.[2,3] This is especially important in fish species in which the concentration of heme (myoglobin and hemoglobin) in the muscle tissue is high. The oxidation leads to a darkening of the meat with a decrease in redness index (a^*/b^* ratio, where the a^* and b^* values are based on the $L^*a^*b^*$ lightness, redness, and yellowness 3D-color space defined by CIE). A reaction between carbon monoxide (CO) and myoglobin, on the other hand, leads to a brighter red color. When CO has been used to mask spoilage in, for instance, tuna, it can be revealed by spectroscopy.[4]

The concentration of myoglobin and hemoglobin in salmon muscle is expected to be low, and it is uncertain as to which degree any color effect of heme would be masked by the dominating absorption of astaxanthin. It has been found that the use of sodium nitrite in salt-curing of Atlantic salmon gives an increased reddish hue.[5] This could be explained by the formation of nitrosomyoglobin, which would mean that myoglobin is present in large enough concentrations in salmon to affect the color. Another explanation could, however, be that peroxynitrite reacts with astaxanthin.[6] An increased redness of fish has also been observed in experiments in which CO has been used to anesthetize tilapia and salmon.[7,8]

It is also possible that the change in salmon color during storage is related to astaxanthin. The shape of the astaxanthin spectra could vary due to solvent effects (astaxanthin is soluble in both water and lipid) and due to binding to other molecules. Astaxanthin in salmon is located on the surface of the proteins (actomyosin) with weak hydrophobic bonds.[9] Free astaxanthin has a single peak with λ_{max} at 468 nm, whereas the astaxanthin−actomyosin complex has a double peak with λ_{max} at 420 and 486 nm due to changes in the molecular plane.[10,11] When the proteins are decomposed during storage, it is possible that the astaxanthin molecules are released from the complex, which would result in a change in absorption.

In this study we have investigated the color change in salmon muscle under different storage conditions (CO, air, and vacuum). Visual reflectance spectroscopy and spectroscopy-based simulations were used to determine the chemical origin of the color variation. To separate between possible effects caused by astaxanthin and heme, we have in addition to salmon also studied mackerel muscle, which does not contain astaxanthin but does have a much higher concentration of heme. A sensory panel was used to determine whether heme-induced color changes were of a

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magnitude to be observable with the human eye and hence of relevance to the consumer.

MATERIALS AND METHODS

Fish Samples and Storage Conditions. Twelve whole Atlantic salmon (*Salmo salar*) supplied from a Norwegian fish farm (Bremnes Seashore AS, Bømlo) were studied in the experiment. The salmon were pre-rigor -filleted and kept without access to oxygen until they arrived at the laboratory 1 day post-mortem to keep control of the initial state of the raw material. The left fillet was used for sensory evaluation and the right fillet for instrumental analysis. Upon arrival at the laboratory, each fillet was cut into three equal samples of approximately 5 × 5 cm, giving a total of 72 samples (36 for instrumental analysis and 36 for sensory evaluation). The tail and belly parts were not used; neither was the part closest to the head. These regions were avoided to reduce the chemical variation between parallels. Nine fillets of mackerel (*Scomber scombrus*) were bought fresh from a fish market in Oslo (Naustvik Enghav). The exact time of storage was unknown. Each mackerel fillet was cut in two samples due to size limitations, giving a total of 18 samples. Instrumental analysis only was performed on the mackerel.

All samples were packed in bags of a polyamide/polyethylene laminate (Neemann PA 20/PE70, Neemann, Leer, Germany) with O_2 transmission rate per 24 h of 30 cm^3/m^2 at 23 °C and 85% relative humidity and vapor transmission per 24 h of 1.7 g/m^2 at 23 °C and 50% relative humidity. The samples were packed in either vacuum, air, or carbon monoxide. This was done in a systematic way to ensure that each fillet location was packed in all atmospheres the same number of times. One-third of the samples were vacuum packed with a vacuum chamber machine (Intevac IN30, Intevac Verpackungsmaschinen, Wallenhorst, Germany), applying a vacuum time of 7 s. The air-packed samples were placed in sealed polyamide/polyethylene bags to avoid unnecessary drying of the product. The bags were pierced and pulled apart to fill the bag with air and to avoid contact between the plastic material and the fillet surface. The samples to be stored in CO were placed in polyamide/polyethylene bags that were flushed for 1 min with a tube connected to a bottle of 100% CO (AGA, Oslo, Norway) to a concentration of 90−95% CO in the bags. The samples were kept for 6 days at 2 °C. They were measured by instrumental methods and evaluated by the sensory panel before they were packed and after 6 days immediately after the packaging had been removed.

Detection of Color Changes during Storage. *Colorimetric Measurements.* The color was measured before and after storage in the terms of $L^*a^*b^*$ values using a colorimeter (Chroma Meter CR300, Minolta, Osaka, Japan). Illuminant D65 (average daylight with a correlated temperature of 6504 K), a 2° standard observer angle, and an 8 mm viewer port were used. The instrument was calibrated against a white tile ($L^* = 97.16$, $a^* = 0.25$, $b^* = 2.09$). In the $L^*a^*b^*$ 3D-color space defined by CIE in 1976, the L^* value represents the lightness of the sample, the a^* value the red−green dimension, and the b^* value the yellow−blue dimension. The Minolta measurements were done directly on the sample surface and were repeated four times on the salmon (one measurement in each corner of the sample) and three times on the mackerel (along a line on the thickest part of the fillet).

Image Collection and Processing. RGB (red, green, blue) images were captured of each sample before and after storage with a digital camera (Canon ESO-1Ds, Tokyo, Japan). The images were collected in RAW format and then converted to DNG format with the Adobe digital negative converter (Adobe Systems Inc., San Jose, CA). In front of each photo series, a picture of a color standard (Colorchecker passport, X-Rite Inc., Grand Rapids, MI) was collected. The color standard was used to create a color profile in the Colorchecker passport software version 1.0.1 (X-Rite Inc.) that was then used for color adjustments on all the following images by uploading the profile in Adobe Photoshop CS5 (Adobe Systems Inc.). The pictures were used to calculate average RGB values from the whole sample surfaces using Matlab version 7.9 (The Mathworks Inc., Natick, MA). The average RGB values were then converted to $L^*a^*b^*$ values assuming daylight illumination (D65) and a 2° observer angle.[12]

Sensory Evaluation of Color. The salmon samples were evaluated by a trained sensory panel, using ISO 6564 (1985) sensory analysis methodology[13] and ISO 13299 (2003). The panel consisted of 12 subjects employed exclusively to work as sensory assessors 12 h per week at Nofima Mat. They have between 3 and 21 years of experience using descriptive analysis on various kinds of food, including fish. The panelists have been selected and trained according to the recommendations in ISO 8586-1 (1993). The sensory laboratory has been designed according to guidelines in ISO 8589 (1988) with separate booths and electronic data registration (EyeQuestion, v. 3.8.4 (Logic8 BV, Wageningen, The Netherlands)).

The visual appearance of the whole salmon samples was assessed on the sample surfaces according to the NCS system in terms of color hue, color intensity, and whiteness. Prior to the assessments, the panel went through a training session with three samples for the purpose of agreeing on the definitions of each attribute and variation in attribute intensity. Yellow/red (Y60R) was evaluated as no color hue, whereas red (R) was evaluated as maximum color hue. Black or maximum color intensity was evaluated as no whiteness.

A total of 36 samples in 4 sessions were assessed each day. The bags were opened about 10 min before each session, and evaluation lasted for approximately 20 min. The fish were put on gray board (20% black and 80% white), coded, and served in a randomized order by sample, session, and assessors. The samples were placed, one in each booth, and the assessors circulated from booth to booth to evaluate the samples. All samples were evaluated by each assessor, all of whom were allowed to work at their own speed. After all samples had been evaluated and the results recorded on paper forms, the panelists registered their own results on a 15 cm nonstructured continuous scale using EyeQuestion software, with the left side of the scale corresponding to the lowest intensity and the right side of the scale corresponding to the highest intensity. EyeQuestion transformed the responses into numbers from 1.0 (low intensity) to 9.0 (high intensity).

Spectroscopic Measurements. Visible and near-infrared spectra were collected in reflection mode (XDS Rapid Content Analyzer, Foss NIRSystems, Hillerød, Denmark) covering the region between 400 and 2500 nm with wavelength intervals of 0.5 nm. The measurement spot size was the standard 17.25 mm diameter. Previous tests on salmon have shown that a sample thickness of 1 cm is enough to avoid complete penetration of light. This criterion was fulfilled for all samples. The samples were taken out of the plastic bags and placed on the quartz plate of the instrument on top of a layer of cling film. Cling film was used to protect the instrument. The measurements were done on the center of the samples on the inside surface of the fillets and were repeated three times.

Spectra of pure myoglobin from equine skeletal muscle (Sigma-Aldrich, St. Louis, MO) in different oxidation states and bound to different ligands were measured with the same instrument using the XDS Rapid Liquid Analyzer module. These samples were measured in transmittance through a 2 mm quartz cuvette. The metmyoglobin (metMb) spectrum was collected directly after approximately 10 mg of myoglobin had been dissolved in 15 mL of 0.1 M phosphate buffer, pH 7.4. The deoxymyoglobin (DMb) spectrum was collected on the same sample after the addition of an excess of sodium dithionite (Sigma-Aldrich), and the carboxymyoglobin (COMb) spectrum was collected after the myoglobin and dithionite mixture had been bubbled with 100% CO gas.

Statistical Analysis. Possibly significant differences between the $L^*a^*b^*$ values before and after storage were identified by a paired t test with a 95% confidence level. Each fish species, packaging method, and

Table 1. Average Color Values (L^*, a^* and b^*) for Mackerel before and after Storage in CO, Vacuum, and Air As Determined by Imaging and Minolta

		before storage (T_1)			after storage[a] (T_2)		
color value	method	CO	vacuum	air	CO	vacuum	air
L^*	images	55.0	54.3	56.7	56.6#	56.3#	59.5#
	Minolta	47.2	45.8	47.7	49.3#	48.3#	49.4#
a^*	images	8.9	9.9	9.3	16.8#a	9.6b	5.7#c
	Minolta	4.0	3.1	2.9	8.6#a	3.6#b	2.6#b
b^*	images	3.0	2.7	4.1	4.2a	2.0#b	10.6#c
	Minolta	3.5	3.2	4.0	5.1#a	4.7#a	8.6#b

[a] #, $p < 0.05$ for T_2 compared to T_1; a−c, $p < 0.05$ between treatments

Table 2. Average Color Values for Salmon before and after Storage in CO, Vacuum, and Air As Determined by Imaging and Minolta (L^*, a^*, and b^*) and Sensory Evaluation (Whiteness, Color Intensity, and Color Hue)

		before storage (T_1)			after storage[a] (T_2)		
color value	method	CO	vacuum	air	CO	vacuum	air
L^*	images	47.0	46.8	47.2	48.7#a	46.9b	48.4#a
	Minolta	36.7	36.6	37.2	37.9#a	36.5b	38.7#a
a^*	images	41.6	41.4	41.2	42.9#a	41.8#b	40.9#c
	Minolta	14.9	14.8	14.8	16.5#a	15.9#b	14.9c
b^*	images	45.2	44.8	44.6	42.7#a	44.3b	43.9#b
	Minolta	12.5	12.6	12.3	12.9#	13.0a	12.1b
whiteness	sensory	3.4	3.3	3.2	3.3	3.3	3.2
color intensity	sensory	6.6	6.7	6.7	6.7	6.8	6.6
color hue	sensory	3.5	3.6	3.5	3.8#a	3.4	3.3

[a] #, $p < 0.05$ for T_2 compared to T_1; a−c, $p < 0.05$ between treatments

method for $L^*a^*b^*$ determination (Minolta and RGB imaging) was tested separately. Tests were also performed for significant differences between packaging methods after storage. For salmon this was done by a paired t test pairing samples from the same fish, whereas for mackerel this was done by an independent t test. Some variation within each fillet was expected. The treatment groups were therefore tested for significant differences before storage to rule out any false-positive results not related to storage condition. The results of the t tests for the two $L^*a^*b^*$ methods were then compared and discussed to draw a conclusion about actual color change. Significant differences between color hue, color intensity, and whiteness before and after storage and between packaging methods were tested in the same way. All statistical tests were done in Minitab Statistical Software 16 (Minitab Inc., State College, PA).

Two different multivariate methods were applied to study the variation in absorption spectra: principal component analysis (PCA) and partial least-squares regression (PLSR).[14] These methods help unravel the main underlying spectral features in overlapping spectra by compressing the data into a lower number of variables (components) describing the main variation in the data set. The data space is rotated so that the direction of the largest variance becomes the new coordinate axes decomposing the data into scores and loadings. The scores are the projection of the data to the new coordinate system, whereas the loadings define the size of the contribution of each original variable (wavelength in this case) to the component. In PLSR the components are found by maximizing the covariance between the spectra (x) and a measured quantity (y) (in this case, redness).

Only the wavelength region between 400 and 750 nm was used. Before multivariate analysis was performed, the spectra were standardized by standard normal variate (SNV) to remove some of the spectral variation connected to light scattering in the samples.[15] This preprocessing technique subtracts the mean of the spectrum from each wavelength of the spectrum and divides by the standard deviation of the spectrum. We are then left with mainly the chemical variation. PCA was performed on average spectra of salmon stored under the three different conditions together with spectra of myoglobin in the three different states. An average spectrum of salmon at day 1 was also included to determine the initial state of the salmon fillets. PLSR was performed between average absorbance spectra of salmon and mackerel and a^* values from the Minolta measurements. The main spectral features corresponding to the observed color variations could then be visualized in the regression coefficient vector. Spectral standardization, PCA, and PLSR were done with The Unscrambler 9.8 (Camo Software AS, Oslo, Norway).

Color Simulations. A measured diffuse reflectance spectrum of a salmon fillet of good coloration was modified by including effects of different absorption curves. Three different effects were studied: an increase in absorption of a single peak at 606 nm; an increase in COMb at the expense of DMb, representing packaging in CO; and an increase in metMb at the expense of DMb, representing packaging in air. Information about the sensitivity of the eye to different wavelengths of light (the three stimulus curve) was used to translate the created spectra into XYZ color values. These values were then converted into RGB and $L^*a^*b^*$ values and visually illustrated by a color card.[12]

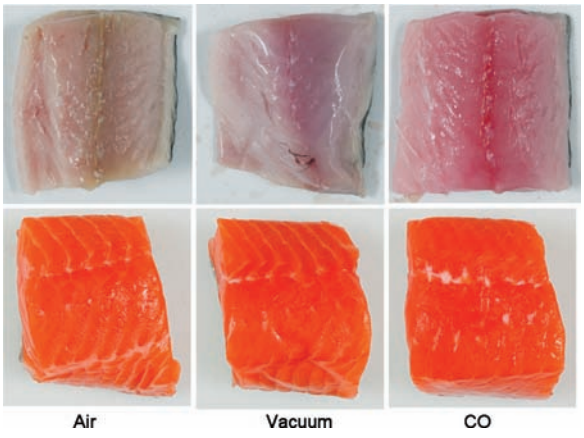

Figure 1. RGB images of mackerel (upper) and salmon (lower) after storage under different conditions as indicated below the images. All salmon samples are from the same fillet, whereas the mackerel samples are from two different fillets of similar initial color.

RESULTS AND DISCUSSION

Color Change during Storage. The color changes measured by Minolta and imaging corresponded well; although, the actual $L^*a^*b^*$ values were quite different (Tables 1 and 2). The values found were, however, in the same range as previously published results. Bjørlykke et al. reported a^* values in salmon of around 40 determined by digital imaging and approximately 12 determined by Minolta.[7] Misimi et al. reported a^* values in salmon between 17 and 38 and between 11 and 15 for the two methods, respectively.[16]

The focus in this paper has been on detecting significant differences before and after storage and between treatments. The absolute $L^*a^*b^*$ values were regarded as less important.

Storage in CO gave a more intense red color (Figure 1) and a higher mean a^* value (Table 1) of mackerel, as expected, with its relatively high heme content.[4] Bled mackerel muscle has been reported to contain 3.4 μmol hemoglobin/kg light muscle and 121.8 μmol hemoglobin/kg dark muscle.[17] For mackerel stored in air the a^* value decreased while the b^* value increased. A decrease in redness index (a^*/b^*) during ice storage has previously been observed in both sardine and mackerel and has been suggested to be caused by myoglobin oxidation.[2] A significant increase in redness (a^* value) was also detected (with both Minolta and RGB imaging) in salmon (Table 2), in which the heme content is normally much lower. Bled salmon muscle has been reported to contain between 0.93 and 2.08 μmol hemoglobin/kg light muscle depending on season.[18] The color change in CO was clearly visible to the naked eye (Figure 1). A similar increase in redness has previously been seen in salmon exposed to CO before slaughter.[7] A significant decrease in redness in salmon upon storage in air was detected only by the RGB images. Comparing the salmon treatments after storage revealed significant differences in redness between all sample treatments (CO > vacuum > air), although no difference was detected between the sample treatments before storage. The color stability in salmon therefore seemed to be largely dependent on the state of heme and packaging atmosphere. The results for the b^* value in salmon were inconclusive. Large differences were found between the two methods of color evaluation. As an example, the b^* value increased according to the Minolta measurements but strongly decreased according to the RGB images for salmon stored in CO.

A significant increase in lightness was detected with both Minolta and RGB imaging for salmon stored in air and CO, whereas the L^* value remained constant in vacuum. This could be related to mechanical pressure on the samples affecting the texture and light scattering. All samples were stored in vacuum for one day before the first measurement and then repacked in vacuum, CO, and air. The vacuum pressure would then be released on the CO- and air-stored samples while remaining constant on the vacuum-packed samples. The lightness increased in mackerel for all storage methods. The independency of storage method again indicated a stronger relationship to texture/light scattering than to chemical absorption.

Some variation was observed between the two color measurement methods as to which changes were detected as significant. One explanation to this could be the fact that there is a difference between how deep the two techniques measure. The images represent mainly the surface of the samples and are directly related to what the consumers see, whereas the Minolta measurements probed a small distance into the sample (approximately 7 mm). If we have color gradients as a function of depth, for instance, due to a lack of saturation of the gases or formation of oxymyoglobin (OMb) on the surface during analysis, the color values from the images would represent the observed color best. The Minolta color values would correspond better with the variation in the spectroscopic measurements. The spectroscopic method used probed approximately 10 mm into the sample and was the technique that probed deepest. The probing depth of the spectroscopic method in question and the Minolta measurements were tested by gradually reducing the thickness of a sample and investigating at what point the background started to influence the measured values.

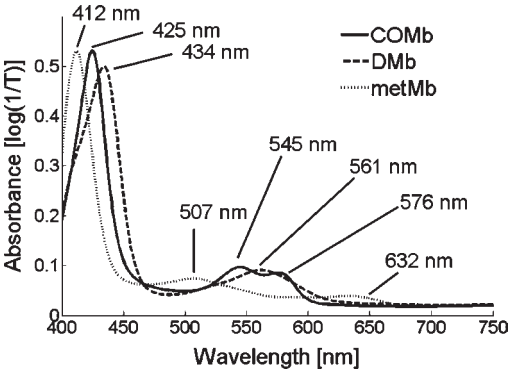

Figure 2. Spectra of myoglobin in different oxidation states and bound to different ligands.

Another fundamental difference between Minolta and digital imaging was the measurement area. On inhomogeneous materials such as fish, the Minolta results will be largely related to the measurement location due to the small measurement area (8 mm in diameter). Despite the efforts to measure on exactly the same spots at days 1 and 6, deviations may have occurred. The main focus was to detect changes, so the choice of measurement spots was not viewed as critical as long as they could be repeated. One could, however, argue that the chemical changes in the fish could be dependent on location. For instance, if the blood content varied greatly within the samples and the measurements were performed on a spot of low blood content, the detected change in color would be lower than the average value of the whole sample. This heterogeneity would be solved by using average RGB values from the images.

The sensory panel detected a significant increase in color hue in salmon samples stored in CO. The color hue remained constant in samples stored in air and vacuum. No change was detected in any of the three sample treatments with respect to color intensity and whiteness. The sensory panel was not able to detect any difference between storage in air and vacuum, although this was possible with instrumental methods. The time between removal of packaging and the assessment of samples was much longer for the sensory analysis as compared to the instrumental analysis. A small amount of OMb would therefore have been formed on the surface of the day 1 samples and the samples stored in vacuum. This represented a possible source of error. Formation of OMb would have resulted in larger differences between samples packed in vacuum and air. Because no significant difference was detected, the error was regarded as minor.

The salmon studied were of high astaxanthin concentration. Salmon containing a high content of blood and a low content of carotenoids could have a potentially higher color loss upon air storage. The degree of bleeding of the fish and filleting time could therefore be of importance. Further experiments are necessary to determine how color change during storage is affected by factors such as bleeding, carotenoid concentration, and distribution of heme pigments within the muscle. Experiments designed to study the change from OMb (as opposed to DMb as studied here) to metMB during storage in air could also potentially show a higher color loss.

Spectral Variation. The visible absorption spectra of pure myoglobin from equine skeletal muscle (Figure 2) showed a shift in position of the Soret band from 412 nm (metMb) via 425 nm

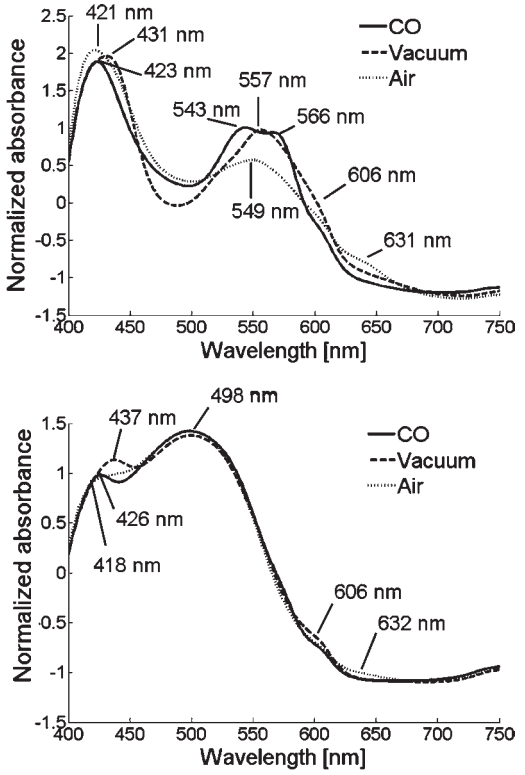

Figure 3. SNV corrected average spectra of mackerel (upper) and salmon (lower) after storage under different conditions.

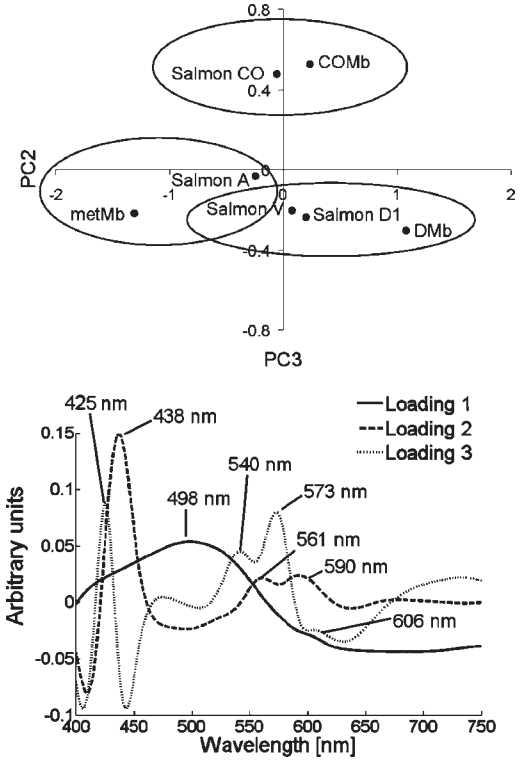

Figure 4. Results of PCA performed on the spectra of salmon stored under the three different conditions and the spectra of myoglobin in the three different states: scores (upper) and loadings (lower).

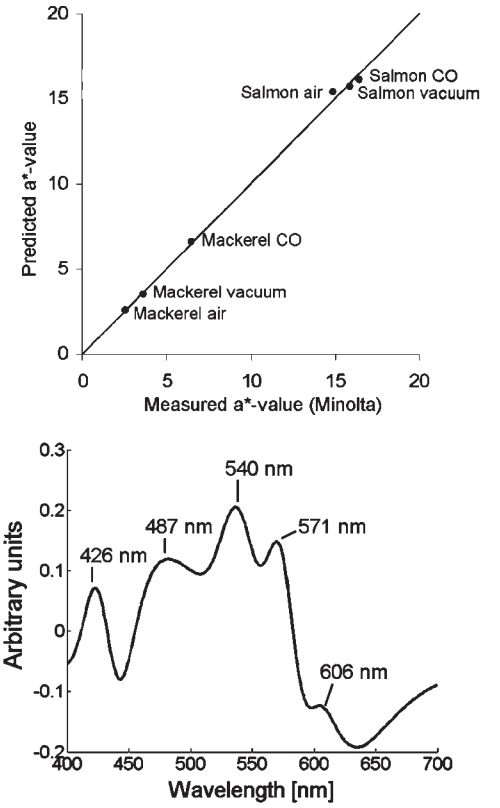

Figure 5. Regression of average salmon and mackerel spectra against a^* value as measured by Minolta: measured a^* value versus predicted (upper); regression coefficient vector (lower). The straight line in the upper plot represents the target values.

(COMb) to 434 nm (DMb) upon a change in oxidation state/ligand binding. There was also a variation in absorption minimum around 507 nm and a variation in the peak pattern between 545 and 576 nm. The peak at 632 nm was only present in metMb as expected.[19] The myoglobin molecule is highly conserved between species, and only minor differences can be found in its absorption pattern. Smulevich et al. reported similar absorption maxima of met- (406, 502, and 631 nm), carboxy- (420, 538, and 568 nm), and deoxy- (431 and 556 nm) myoglobin purified from tuna fish with the same red-shift in the Soret band in the order metMb−COMb−DMb.[4] The spectra of hemoglobin in its different forms are highly similar to the myoglobin spectra[20] and will therefore not be discussed here.

By studying the absorbance spectra from mackerel stored under different atmospheres (Figure 3, upper) it was clear that the spectral properties of myoglobin (and hemoglobin) at different oxidation states and bound to different ligands dominated the spectra and were hence responsible for a large part of the observed color variation. The Soret bands were, however, observed to be broad, and the absorption maxima did not correspond to the exact wavelengths of the pure forms of myoglobin, although still giving the expected red-shift. The Soret maximum for mackerel packed in air was 421 nm; in CO, 423 nm; and in vacuum, 431 nm. This suggested the presence of various myoglobin species as has been reported by other authors in complex organic material such as fish meat.[4,8] In salmon the myoglobin spectrum was much less pronounced (Figure 3, lower). The Soret band could be found at 418, 426,

and 437 nm depending on packaging atmosphere, whereas an increased absorption around 632 nm could be observed for samples stored in air. In the region of the astaxanthin peak (around 498 nm) the variation in myoglobin absorption was more difficult to observe directly in the salmon spectra. A small shift in peak position together with a small intensity increase could, however, be observed and was most likely due to the underlying spectral features of myoglobin. This could be better studied by PCA as described below. The SNV corrected spectra of mackerel and salmon collected at day 1 were nearly identical to the ones stored in vacuum (results not shown).

There was also another distinct curve-broadening feature at 606 nm in the spectra from both fish species. The presence in mackerel made it clear that it could not be related to a change in the chemistry of astaxanthin. The peak was more pronounced in the mackerel spectra, and the intensity was dependent on packaging method, suggesting that the compound could bind to both oxygen and CO. This could indicate a relationship to blood, perhaps as a breakdown product of heme with an intact porphyrin-like structure.

A PCA performed on the spectra of salmon stored under the three different conditions and the spectra of myoglobin in the three different states resulted in a clear grouping based on oxidation state/ligand binding and storage condition (Figure 4, upper). Salmon measured at day 1 had a score similar to that of salmon measured after 6 days in vacuum. The first loading (Figure 4, lower) represented the astaxanthin peak and explained nearly all of the variation (99.7%). This was a result of the salmon samples being fundamentally different from the myoglobin samples. The second and third loadings separated all samples based on myoglobin state/storage condition. The shape of the third loading could be recognized as the COMb spectrum seen in Figure 2 with peaks at 425, 540, and 573 nm. In addition, a small peak could be observed at 606 nm, which appears to be positively correlated to COMb. The results of the PCA can be seen as a strong indication of myoglobin being present in salmon in large enough amounts to be of importance to the visible spectrum of salmon.

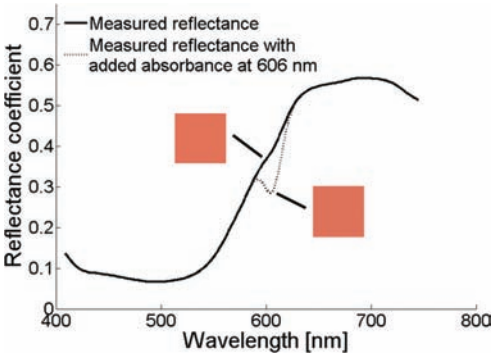

Figure 6. Reflectance spectra before (solid) and after (dotted) manipulation of the absorbance at 606 nm and their color cards before (left) and after (right).

To elucidate which spectral changes were responsible for the measured color variations, a PLS regression between absorbance spectra of salmon and mackerel and a^* values from Minolta was performed (Figure 5). A coefficient of determination of 0.999 and a root-mean-square error of cross-validation of 0.277 were achieved for a regression model of three factors. Both mackerel and salmon ended up higher on the regression curve after storage in CO and lower on the regression curve after storage in air (Figure 5, upper). The regression coefficient vector for predicting a^* values (Figure 5, lower) showed a clear resemblance to the COMb spectrum. Formation of COMb was positively correlated to the a^* value (positive peaks at 426, 540, and 571 nm) as so was also astaxanthin concentration (broad positive peak at 487 nm). The peak at 606 nm was also observed in the regression coefficient vector and was positively correlated to redness.

Color Simulations. The experimental results confirmed that the relatively small spectral changes due to heme that were detected in salmon muscle gave significant variations in observed color. By translation of absorption spectra to color, it is possible to determine more exactly how the different spectral changes affect color.

A simulated increase in absorbance at 606 nm (Figure 6) resulted in a significant decrease of both a^* and b^* values. This was in disagreement with the regression result that showed a positive correlation between redness and absorbance at 606 nm. An explanation to this could be that the regression is picking up an indirect correlation between the peak at 606 nm and carboxymyoglobin. Despite a significant decrease in both a^* and b^* values, no clear change could be visually detected on the color cards (Figure 6). This could be a result of the redness index (a^*/b^*) remaining approximately constant. Although its importance to observed color appears to be minimal, the peak at 606 nm could be an important factor to consider for the robustness of astaxanthin calibrations based on optical measurements[23] as it represents a large part of the spectral variation during storage. It could also prove to be of chemical importance, but so far the origin of the peak has yet to be identified.

Figure 7 shows how the color of salmon containing myoglobin gradually changes as DMb converts to COMb and metMb. Changing DMb into COMb significantly increased the redness. This was in agreement with the experimental observations of redder salmon during storage in CO. Changing DMb into metMb decreased the redness and increased the yellowness and lightness. This was in agreement with previous observations of lighter fillets during storage in air, which formerly has been explained as a light scattering effect.[21,22] The color changes could be observed visually, which illustrates how important the heme status is for the understanding of color development in salmon.

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Figure 7. Color change induced by a gradual conversion (from left to right) of DMb to COMb (upper bar) and to metMb (lower bar) as calculated by simulations.

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