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Fluorescence of Muscle and Connective Tissue from Cod and Salmon

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Autofluorescence of salmon and cod muscle was measured and compared with autofluorescence of collagen type I and type V. Similarities between fluorescence of fish muscle and collagen were found in that the same peaks were obtained around 390, 430, and 480 nm. These similarities are supported by principal component analyses. Texture and gaping score were predicted from the fluorescence spectra by partial least-squares regression. However, the predictions did not perform well. Relating fluorescence to the gaping score gave a prediction error of 0.91 and a correlation of 0.43 when measuring gaping on a scale from 0 to 5. There was no relation between texture and fluorescence spectra. Fluorescence of fish muscle could be related to the storage time. However, this relation seemed not to be induced by changes in collagen.

KEYWORDS: Salmon; cod; collagen; connective tissue; fluorescence

INTRODUCTION

The collagen content of fish muscle varies considerably from species to species. Contents from approximately 3 to 20 g/kg of fish muscle have been found in a number of fish species (*1*). These values include the connective tissue of myocommata, perimysium, and endomysium. Collagen type I and type V have been identified, and it is stated that these two types of collagen make up the major part of the intramuscular connective tissue in fish muscle. Collagen type I is higher in content than collagen type V (*2*−*5*).

It has been shown that the texture of fish muscles and the degree of gaping depend on the content of connective tissue in that a higher collagen content was observed in situations with a firmer texture of the raw meat and a low collagen content was found in tender meat vulnerable to gaping (*1*, *6*). Since the connective tissue and thereby collagen are important for the overall quality of fish products, determination of the collagen content and its degradation by a fast on-line method, such as fluorescence spectroscopy, can be important for controlling and optimizing processing and marketing.

Fluorescence spectroscopy has been used to study connective tissue of ground bovine meat (*7*). The pure spectra of connective tissue was estimated, and it was shown that excitation at 332 nm gave emission with high intensities between 400 and 520 nm. Egelandsdal et al. (*8*) studied the fluorescence of connective tissue from bovine masseter muscle and found emission maxima at 380 and 465 nm after excitation at 335 nm. The sources of fluorescence from connective tissue are not fully understood at the molecular level yet, but it is supposed that different collagen cross-links and components such as pyridinoline are contributors (*9*, *10*).

It has been known for long time that fish muscle is able to exhibit autofluorescence (*11*, *12*), and it has been shown that autofluorescence changes during frozen storage (*13*). Previous experiments measuring fluorescence of fish products have primarily focused on the ability of fluorescence spectroscopy as a method to follow lipid oxidation. Increases in fluorescence of aqueous and organic extracts of cod, haddock, and sardine during freeze storage (*14*, *15*) and of blue whiting during chill storage (*16*) were correlated to some measures of lipid damage. Hasegawa et al. (*17*) used solid sample fluorescence spectrometry on a dried fish model system and found a correlation to the oxidative deterioration. Another application of fluorescence spectroscopy was the development of a method that can detect bones in fish fillets (*18*). Other sources contributing to the autofluorescence of fish muscle may be proteins, amino acids, NADH, FAD, pigments, and some nucleic acids. All these factors will make fluorescence of heterogeneous materials such as fish muscles very complex.

This paper presents an investigation of autofluorescence from fish muscle especially in relation to the occurrence of collagen type I and type V. By use of multivariate methods, the aim is to indicate the presence of these fluorophores within cod and salmon muscle. Changes in autofluorescence characteristics during storage are investigated as well as variations within the

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fillets. A second objective is to relate gaping and texture to the autofluorescence spectra of salmon. This relation is expected due to the influence of collagen on quality parameters such as texture and gaping (*1*, *6*) and the theory that collagen makes up an important part of the autofluorescence of fish muscle.

EXPERIMENTAL PROCEDURES

Materials and Sample Preparation. *Cod*. Four gutted and bled cod were obtained from Frederikstad, Norway, the day after catch. One fillet from each fish was measured the day after slaughtering. The other fillet was stored at 2 °C until the fifth day after slaughtering and then measured. Head and bones were kept on during storage. Another five fillets were bought from the fish auction hall in Oslo. The exact day of catch of these fish from the auction hall was not known. However, judged upon the odor, these fish were supposed to be older than the fish mentioned previously.

Fluorescence was measured on three samples from each cod fillet. Two samples were taken from the loin, one as close to the head as possible and the other from the middle of the fillet between the tail and the head. The third sample was taken as close to the tail as possible. This gave a total of 39 samples, 12 from storage day one, 12 from storage day five, and 15 obtained from the fish auction hall fillets. Skin was removed from the fillets before cutting out the samples. The samples were placed into specially designed sample cuvettes, which exposed a flat circular surface with a diameter of 5 cm for measurement. Samples were placed in the cuvettes such that the interior of the fillets was exposed for autofluorescence measurements. Thus, only fluorescence of the white muscle was measured. Before measurement, the samples were conditioned at 18 °C for at least 1 h.

Salmon. Salmon were raised, slaughtered, and filleted at the Institute of Aquaculture Research (Akvaforsk) in Norway. The fish were stored with ice at 2 °C for 1, 2, or 3 weeks before measuring. After 1 week of storage 60 salmon were analyzed, after 2 weeks 60 new ones were alalyzed, and after 3 weeks 30 additional salmon were analyzed. Texture and gaping were measured at Akvaforsk, after which the fillets were brought to the Norwegian Food Research Institute (Matforsk) for fluorescence measurements. These were measured 1 day after the texture and gaping score determinations. Samples of salmon were taken from the loin and were prepared as described for the cod. In addition, samples as close to the tail as possible were taken from the fish stored for 1 week.

Collagen. Fluorescence was measured on collagen type I purified from calf skin and collagen type V purified from bovine achilles tendon (Sigma Aldrich Sweden AB, Stockholm).

Autofluorescence Measurements. Fluorescence emission spectra were measured directly on the fish muscle. An optical bench system optimized for measuring rather large sample surfaces (up to diameter $\approx$ 6 cm) was used. The excitation light was generated by a 300 W xenon light source (Oriel 6258, Oriel Corp., Stratford, CT). The light was directed onto the samples at an angle of 45°. The spectra were collected by an imaging spectrograph (Acton SP-150, Acton Research Corp., Acton, MA) connected to a sensitive charge coupled device (CCD-camera) (Princeton TEA/CCD-512-TKBM1, Princeton Instruments Inc., Trenton, NJ). A cutoff filter was positioned in front of the spectrograph slit to suppress excitation light reflected from the samples. The samples were excited with a 332 nm (Oriel, bandwidth 10 nm) interference filter where after the emission was measured from 360 to 600 nm. The exposure time was 4 s, and the temperature of the samples ranged from 16 to 19 °C. For all samples, two spectra were collected and the average was used for further analysis. The sample illumination was not perfectly homogeneous, so samples were rotated 90−180° between the two exposures. Spectrograph and detector were controlled by the software WinSpec Ver. 1.4.3.4 (Princeton Instruments Inc., Trenton, NJ). Spectra were digitized to a resolution of 16 bit and the length reduced from 512 to 256 points by averaging two adjacent readings, resulting in one reading for approximately every 1 nm.

Images were collected with the same system. A Nikon 102 mm photographic lens was mounted on the imaging spectrograph, the spectrograph slit was removed, and the grating was exchanged with a mirror. Spectral images were created by placing a 40 nm bandwidth interference filter with center wavelength 450 nm in front of the lens. Samples were illuminated with 332 nm light, and fluorescence images were measured. The spatial resolution of the images were 512 pixels/35 mm. Exposure time for all images was 30 s. For both fish species, a few samples from each storage period were used for collecting the autofluorescence images.

Fluorescence emission from collagen type I and type V was measured with the same optical setup as for the fish muscles.

Gaping and Texture. Gaping was evaluated according to the procedure described by Andersen et al. (*19*). Each fillet was given a score from zero to five. Zero was given for a fillet without gaping, and five was given for a fillet with severe gaping.

Fillet texture was evaluated instrumentally by compression using a Texture Analyzer TA.XT2 (Stable Micro Systems, Surrey, England) equipped with a cylindrical plunger (12.5 mm diam). The plunger was pressed into the fillets at a constant speed of 1 mm s^{-1} until it reached 90% of the sample height. The resistance force was recorded at the maximum force obtained during compression. Two measurements were performed on each fillet, and the mean value was used for the analysis. The measurements were made in front of the dorsal fin, about 1.5 cm above the lateral line.

Data Analysis. The fluorescence spectra were analyzed using the multivariate methods principal component analysis (PCA) (*20*) and partial least-squares regression (PLSR) (*21*). By principal component analysis (PCA), the main systematic variation in data is extracted by resolving the information into principal components (PCs). The data (fluorescence spectra) are contained in a matrix **X** and decomposed into a score matrix **T** and a loading matrix **P**. The residuals, which are the variations that are not described by the scores and loadings, are collected in a residual matrix **E**.

The general purpose of PLSR is to find a mathematical relation between two data sets, **X** (fluorescence spectra) and **Y** (gaping score or texture). PLSR performs a simultaneous decomposition of the **X** and **Y** matrices in such a way that the information in the **Y** matrix is directly used as a guide for the decomposition of **X** after which regression of **Y** is done. The regression models were evaluated by the correlation between the measured and the predicted values and by the root-mean-squared error of prediction (RMSEP), which is defined by

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{N}}$$

where y_i is the measured value of sample i and $\hat{y}_i$ is the predicted value of the same sample. All models were validated with full cross validation. The data analysis was performed using The Unscrambler version 7.6, CAMO A/S, Trondheim, Norway.

RESULTS AND DISCUSSION

Spectral Characteristics of Fish Muscle and Collagen. Fluorescence emission spectra of purified collagen show that collagen type I has peaks around 430 and 480 nm irrespective of the excitation wavelength used (**Figure 1**). Collagen type V has a peak just below 400 nm and a shoulder around 480 nm.

The autofluorescence spectra of the two fish species (**Figure 2**) are somewhat different but have common features that are similar to those of collagen type I and type V. Especially for salmon, the peaks and shoulders obtained at 390, 430, and 480 nm agree with the three peaks of collagen. The figure also shows a difference in intensity between the salmon sample taken from the loin and the sample taken from the tail. A difference between the two cod samples is also seen, but it is not as clear as for the salmon.

The gray scale images visualize the difference in fluorescence measured around 450 nm between cod and salmon (**Figure 3**).

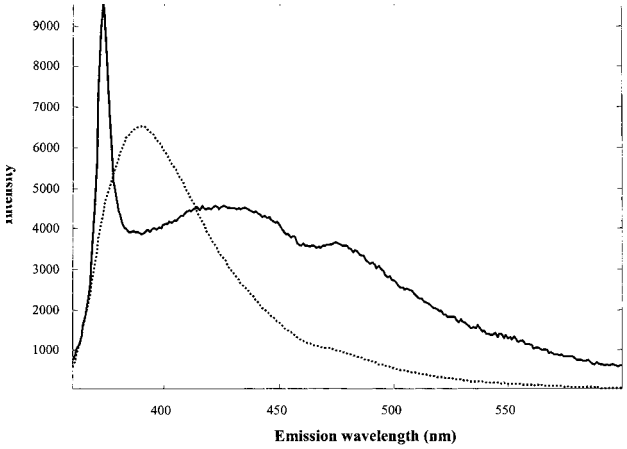

Figure 1. Autofluorescence spectra of calf skin collagen type I (solid) and bovine collagen type V (dotted) obtained after excitation at 330 nm.

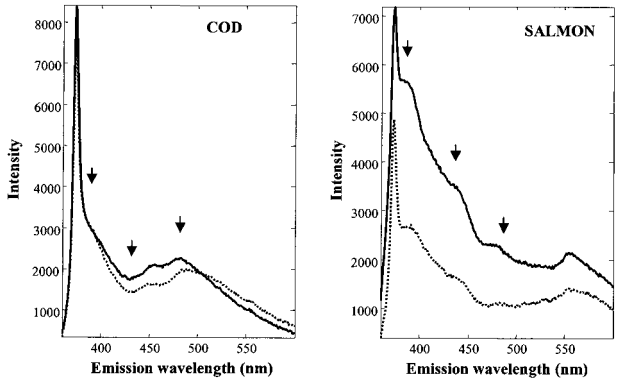

Figure 2. Autofluorescence of cod and salmon after excitation at 332 nm. Cod samples were stored for 5 days (left panel), from the middle section (solid) and froim the tail part (dotted) of the same fillet. Salmon samples (right panel) were from the middle section (solid) and the tail part (dotted) of the same fillet. The arrows indicate the peak of collagen as seen in **Figure 1**.

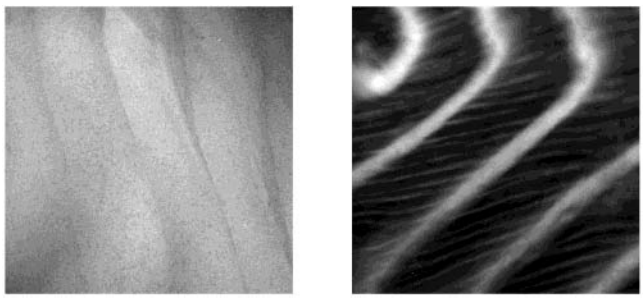

Figure 3. Autofluorescence images of cod stored for 1 day (left panel) and of salmon (right panel). The excitation wavelength was 332 nm, and the intensity of emitted light recorded at 450 nm.

The myocommata of salmon emits a bright white fluorescence, whereas the muscle fibrils are more reddish/pink. The thin fibrils of perimysial and endomysial connective tissue can be seen as white stripes connecting two thick stripes of myocommata. In contrast, the myocommata of cod emits slightly weaker fluorescence than the muscle fibrils. The whole fillet emits a blue/white fluorescence.

The actual fluorescence of the two species is probably quite similar. In salmon, however, fluorescence from collagen and other sources is most likely reabsorbed by the red pigments in the muscle fibrils. These pigments, astaxanthin and cantaxanthin, absorb strongly in the region 350−520 nm (*22*). This phenomenon creates the visible contrast between muscle fibers and connective tissue. Cod muscle is white, and no pigments will reabsorb the autofluorescence. Therefore, fluorescence of the muscle in cod is as pronounced as that of myocommata, and for the particular sample shown here, the fluorescence of the muscle fibers seems to be somewhat more intense. The peak at 430 nm of collagen type I can hardly be seen in the fluorescence spectra of cod, which might be due to the larger fluorescence of the muscle fibers, which do not allow this collagen peak to be detected. However, this phenomenon needs to be studied further.

Above, similarities between fluorescence of fish muscle and mammalian collagen type I and type V are illustrated. The collagen structure of fish muscle may not be exactly similar to the structure of collagen isolated from a mammalian source, and possibly the fluorescence of collagen from fish differs from that measured on mammalian collagen. However, it has been found that the structure and amino acid composition of collagen type I and type V from fish muscle is quite similar to that of mammalian and avian muscle (*2*, *3*). Hence, it is expected that the very small differences will not be detected by the method used here. Even though we have not found any publications stating that other types of collagen are present in fish muscles, it might be possible. However, collagen type I and V are the types found in largest concentration. Therefore, considering the low concentration of other collagen types and the complexity of the fish muscle, it is not expected that other types of collagen will be detected by the method used here. Elastin is another possible fluorophore but is only to be found in very low concentration and is shown to have an emission spectrum different from that of collagen (*23*).

Explorative Data Analysis. *Salmon.* Two PCAs based on emission spectra of salmon taken from the loin were made. One of the models included the samples of collagen, and the other was made only with the salmon samples. In this way 150 samples were used in the model without collagen and 152 samples were used in the model including collagen. Before modeling, the spectra of collagen were normalized to be of the same size as the salmon spectra. Three-component models explained 96% and 97% of the variation in data for the models with and without collagen, respectively. The observation that the addition of spectral features from collagen did not lead to an extra dimension in the models and that the two models explain approximately the same amount of variation suggests that collagen exhibits a major part of the autofluorescence of salmon.

The loadings of the three components obtained by the two models show similarities supporting the resemblance between the fluorescence of salmon and collagen (**Figure 4**). The PC1 loadings describe an average intensity in the emission spectra and illustrate peaks corresponding to those of collagen type I and type V. Thus, collagen may be a great contributor to the variation in intensity and, probably, the samples had different amounts of collagen exposed for illumination.

The PC2 loadings of the model including collagen have a clear peak around 390 nm corresponding to the peak of collagen type V. The significance of this peak is illustrated by the score values (**Figure 5**). All salmon samples have PC2 score values around zero whereas collagen type V has a high PC2 score value. This large difference between collagen type V and salmon indicates that phenomena other than collagen contribute to the autofluorescence of salmon. Both collagen type I and type V vary from the salmon in PC3. Thus, PC3 may explain some structure of collagen. Furthermore, in the model made without

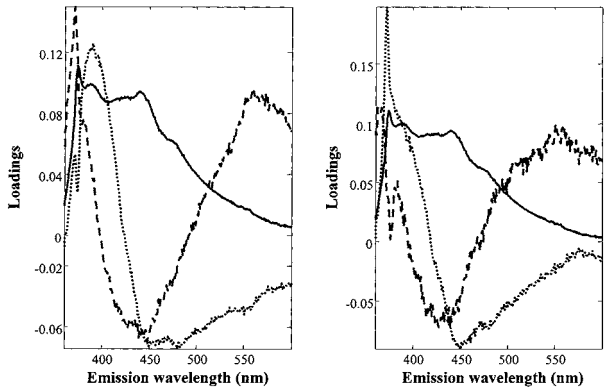

Figure 4. Loadings of PCAs based on emission spectra: (left) model including samples of salmon and collagen type I and type V, PC1 (86%) (solid), PC2 (8%) (dotted), and PC3 (3%) (dashed); (right) model including only salmon samples, PC1 (78%) (solid), PC2 (13%) (dotted), and PC3 (5%) (dashed).

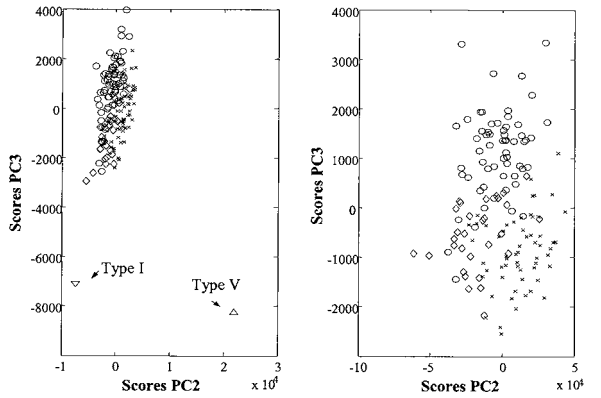

Figure 5. PC2 score versus PC3 score for PCAs based on emission spectra after excitation at 332 nm: (left) model including samples of salmon and collagen type I and V; (right) model including only salmon samples. Key: storage time 1 (○); storage time 2 (×); storage time 3 (◇).

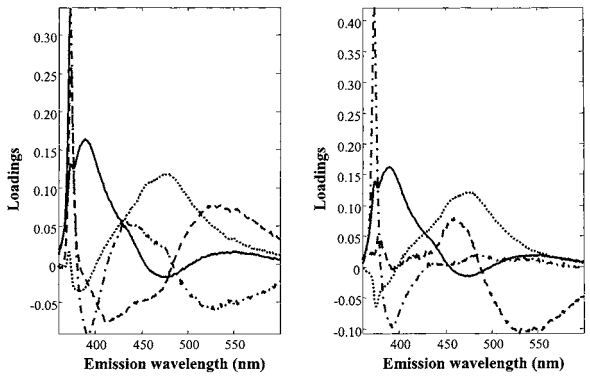

Figure 6. Loadings of PCAs based on emission spectra: (left) model including samples of cod and collagen type I and type V, PC1 (65%) (solid), PC2 (26%) (dotted), PC3 (6%) (dashed), and PC4 (2%) (dash–dotted); (right) model including only cod samples, PC1 (72%) (solid), PC2 (24%) (dotted), PC3 (2%) (dashed), and PC4 (2%) (dash–dotted).

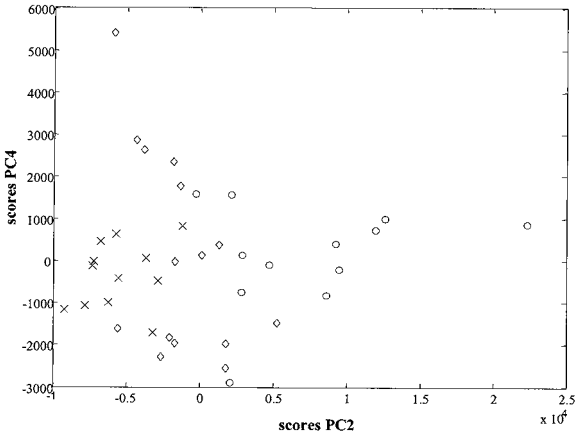

Figure 7. Scores of PC2 versus scores of PC4 from a PCA of the autofluorescence spectra after excitation at 332 nm. Storage time: 1 day (○); 5 days (×); old cod (◇). The PCA is made only on cod samples.

collagen there is a separation of the salmon samples as well. This may be due to a variation in the amount of pigment exposed for illumination. The large negative loadings of PC3 between 430 and 480 nm corresponds to the wavelengths with absorption of the pigments (*22*). Even though salmon may contain both tocopherol and vitamin A, these fluorophores do not seem to influence the salmon spectra due to low concentrations, low quantum yields, and excitation/emission maxima at other wavelengths (*24*).

A separation of data depending on the storage time is obtained both when modeling only salmon and when modeling collagen together with the salmon (**Figure 5**). The possibility of separating the samples according to the storage time illustrates the advantage of multivariate methods, since it was not possible to detect any variation of the storage time by studying the spectra visually. For the model made without collagen, PC2 separates samples stored for 2 or 3 weeks. PC3 separates samples stored 1 week from samples stored 2 or 3 weeks. There is no systematic separation of the sample groups in PC1 (not illustrated). As mentioned, this component mainly describes a variation in the overall fluorescence intensity.

Cod. Two PCAs based on autofluorescence spectra of cod were made. One included the two samples of collagen type I and type V, and the other was made only on cod samples. Thus, the two models were made using 41 and 39 samples, respectively. As for the models based on salmon, the collagen spectra were normalized to have approximately the same intensity as the cod samples. Four-component models explained 99% and 100% of the variation in data for the model with collagen and the model without collagen, respectively.

There are similarities in the PC1 and PC2 loadings of the two models (**Figure 6**). PC1 has a large peak below 400 nm corresponding to the peak of collagen type V shown in **Figure 1**. PC4 explains 2% of the variation when including collagen in the model and seems to describe the autofluorescence of both collagen types since the loadings have a negative peak below 400 nm and two positive peaks around 430 and 480 nm corresponding to the peaks obtained from pure collagen (**Figure 1**). When collagen is omitted from the model, there are still peaks in the loadings of PC4 corresponding to peaks in the spectra of the two types of collagen. Possibly, the fourth component describes the variation in collagen also in the model made on the basis of the data without collagen. However, there is no apparent systematic variation in the score values of PC4 (**Figure 7**) in any of the models.

A systematic variation of the storage time is seen in PC2 (**Figure 7**). Samples stored 1 day have higher score values corresponding to a larger peak between 450 and 480 nm. Samples from the fish auction hall have PC2 score values that are between the samples stored 1 day and the samples stored for 5 days. Conclusions based on samples with only three different storage times might be very weak. However, the separation in the score values may be due to an increase in the overall fluorescence intensity after longer storage. A broad peak

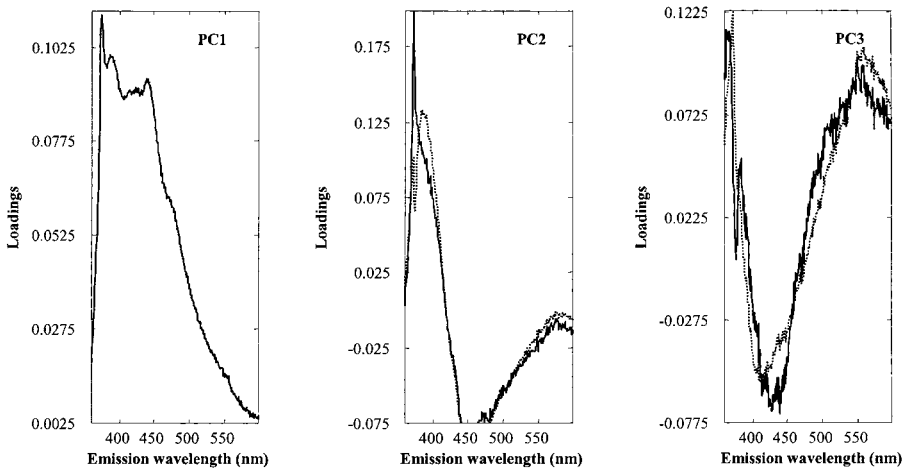

Figure 8. Loadings of a PCA based on emission spectra of salmon excluding samples of collagen (solid) and orthogonal rotated loadings of a PCA based on emission spectra of salmon and collagen (dotted).

above 500 nm developed after long storage may be so strong that it increases the intensity of the whole emission spectrum. This broad peak corresponds to the peak in the loadings of PC3 around 525 nm. As for the visual assessment (**Figure 2**) no clear variation according to the sampling site was seen in any of the components. Variation due to sampling site was expected since a variation in collagen content between head and tail has been shown for other fish species (*25*, *26*).

The development of the peak slightly above 500 nm during storage may be due to some oxidation phenomena (*16*, *17*, *27*). Adding a small amount of malondialdehyde directly to fresh cod muscle resulted in a strong increase in fluorescence intensity similar to the peak just above 500 nm (not illustrated). Wold et al. (*28*) used the same approach. They showed that fluorescence from poultry meat with added aldehydes exhibited fluorescence similar to that of freeze stored poultry. It was also shown that the emission from one sample can be caused by several oxidation products (*28*, *29*). Hasegawa et al. (*17*) described that emission developed just above 500 nm could originate from protein related oxidation. Due to the low fat content of cod of approximately 0.5%, it seems reasonable that the fluorescence developed by oxidation is related to the proteins.

The importance of the wavelength area around 450 nm in the PC2 loadings may be due to a decrease in the NADH content. NADH may be used as an indicator of freshness and makes up a large part of the fluorescence from fresh muscle. The content and thereby the intensity decreases during storage (*30*), which is in agreement with a decrease in the peak between 450 and 480 nm.

The fluorescence intensity obtained by the oxidation products and by NADH seems to be so strong that only weak indications of peak from collagen can be detected in the fluorescence spectra of cod. This may also explain why the peak at 430 nm of collagen type I is not seen in the fluorescence spectra. Probably, measuring only the connective tissue would reveal all the collagen peaks since NADH and oxidation products are found in the muscle fibers.

Collagen and Fish Muscle Fluorescence. The multivariate analysis indicated the presence of collagen type I and type V in the two fish species. Peaks possibly originating from fluorescence of collagen were clearly seen for salmon, but only collagen type V was easily detected in the spectra of cod. The identification of collagen can be further verified by rotating the loadings obtained from the PCAs. With application of orthogonal rotation, the loadings obtained from the two data sets (with and without collagen) should be approximately similar if the two sets span the same variation (*31*). By the rotation, the loadings obtained from PCAs on the basis of data containing collagen are regressed orthogonally on the loadings obtained from PCAs on the basis of data without collagen. The results thus obtained are illustrated in the **Figures 8** and **9**. For salmon, the rotated loadings corresponds well with the loadings of the model made on data without collagen. This similarity is not as clear for cod, where only the loadings of the two first components seem to be similar. These loadings may describe collagen type V, NADH, and lipid oxidation products. Thus, rotating the loadings by orthogonal rotation supports the results already indicated from the visual investigation. Another way to identify collagen could be to relate fluorescence spectra to a measured collagen content by partial least-squares regression.

The collagen content and its distribution depend on the swimming activity of the fish (*32*). Furthermore, higher collagen content in the tail part than near the head is found in flounder, hake, and trout (*25*, *26*). Therefore, it was expected that similar variations could be detected within the fillets. For salmon, there was an intensity difference between samples taken from the loin and samples taken from the tail. The myocommata is thicker in the middle section of the fillet compared to the tail part. Therefore, this variation in intensity may be explained by how much of the myocommata that was exposed for measurement. Furthermore, for ground beef it was shown that the connective and adipose tissue had almost the same emission spectra (*7*). Fluorescence microscopy showed that the major source of fluorescence from adipose tissue was from the connective tissue fibers located between the adipose cells (*33*). Therefore, the higher lipid content in the loin may add to the variation in fluorescence intensity between the two parts of the fillet. In addition, the larger amount of lipid and connective tissue in the loin reduces the relative amount of pigmented muscle and thus reduces the reabsorption by astaxanthin.

Cod did not show the same variations within one fillet. Probably this is due to the relatively low contribution of collagen to the fluorescence spectra, the low fat content of cod, and the fact that other fluorophores inside the muscle flesh are clearly more pronounced such as NADH and lipid oxidation products as mentioned above. Even though there is a variation in collagen content within the cod muscle, the content and the variation may be so low that it cannot be easily detected with the fluorescence techniques used here.

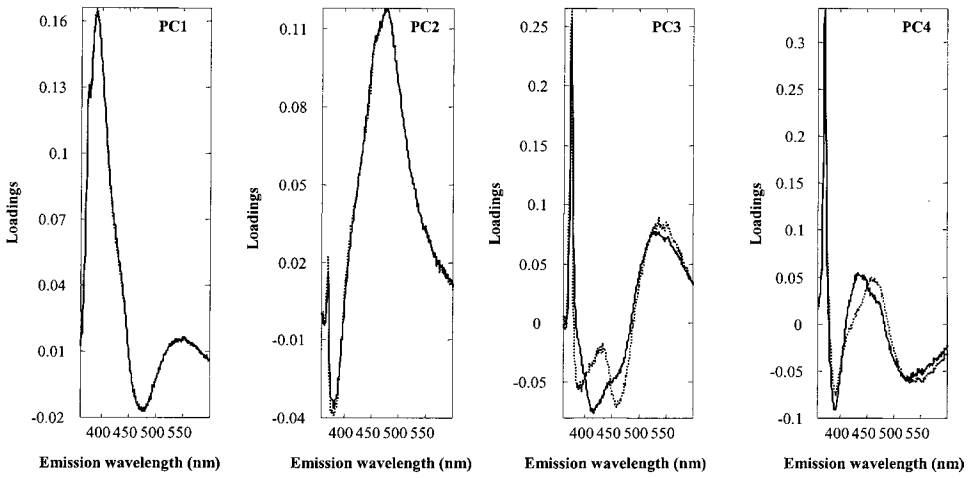

Figure 9. Loadings of a PCA based on emission spectra of cod excluding samples of collagen (solid) and orthogonal rotated loadings of a PCA based on emission spectra of cod and collagen (dotted).

Relating Fluorescence of Salmon to the Gaping Score and Texture. Autofluorescence spectra of salmon were correlated to the gaping score and to the texture by partial least-squares regression (PLSR). Above it was suggested that collagen type I and type V cause a major part of the structure in the autofluorescence spectra of salmon. Furthermore, texture and gaping are supposed to be influenced by the collagen content as well as its degradation during storage. Therefore, it was expected that the autofluorescence spectra could be correlated to either the texture, the degree of gaping, or both.

There seemed to be some relation between the autofluorescence spectra and the gaping score. A five-component PLS model gave a correlation of 0.43 between measured and predicted values and a RMSEP of 0.91 when the gaping scores varied between 0 and 3. The gaping score was evaluated subjectively and could be influenced by errors. Furthermore, the fluorescence intensity expressed how much collagen that was exposed at the surface of the sample and not the total amount of collagen within the fillet. Thus, the sample measured may not be representative for the whole fillet, which may induce errors in the predictions. It could be relevant to study the relation between gaping score and fluorescence in more detail using other instrumentation or sample preparation to develop on-line/at-line techniques for quality sorting. Since gaping occurs along myocommata, measurements can for instance be done in these areas.

The correlation between the measured texture, which varied between 5.5 and 14.1 N, and the texture predicted by the fluorescence measurements was close to zero. This low correlation may be explained by a variation due to the sampling technique, a very low variation in collagen content or texture, or that there is in fact no relation between texture and fluorescence.

As denoted by Sigurgisladottir et al. (*34*), optimizing the sampling technique is important for texture determinations. A relatively small area of the muscle is sampled by the cylindrical probe used here. Fluorescence is measured on another small part of the fillet. Thus, it may be that measurements performed on these small samples are not representative for the whole fillet and the two samples may not correspond exactly to each other. Furthermore, it has been shown that Kramer shear force measurements gave better texture determinations than the texture profile analysis used here (*35*) and should be used when more experiments are to be made.

Collagen contents of 2.9 g/kg (*5*) and 6.6 g/kg (*36*) have been found in salmon. Supposedly, the variation in collagen in the salmon muscle is so small that the correlation to texture and partly to the gaping score cannot be found. The relation between collagen content and texture or gaping found in other studies has been shown for different fish species with a much larger range in collagen content (*1*, *6*).

A relation to texture might be found if other instrumentation or a different sample preparation is used. One could either homogenize the samples or measure only myocommata.

Furthermore, concerning collagen degradation, we cannot exclude the possibility that even though collagen is degraded, the collagenous fluorophores may still remain active for which reason the degradation will not be measured with the technique used here.

In conclusion, collagen type I and type V seem to be major contributors to the structure of the fluorescence spectra. Although low correlations were obtained between fluorescence and texture and gaping on salmon, the method is still promising as a rapid tool for determining such quality parameters. Other sampling methods and instrumentation should be tested in this effort. The study shows that fluorescence from fish muscle is complex and depends on factors such as freshness, oxidation, and site of measurement.

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