Classification of fresh Atlantic salmon fillets by packaging type using hyperspectral imaging

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Introduction

Fresh fish are highly perishable. Freshness is therefore an important attribute of quality.[1,2] The type of atmosphere packaging can influence the rate and nature of freshness loss and subsequent spoilage during storage. Visible/near infrared (NIR) hyperspectral imaging was used to investigate how different packaging affected spectral properties of fresh salmon fillets during storage at 4^{o}C. The potential of hyperspectral imaging to classify salmon fillets by packaging type was also evaluated.

Materials and Methods

Samples

Farmed Atlantic salmon (*Salmo salar* L.) were pre-rigor filleted and arrived in our laboratory within 6 hours after slaughtering. Upon arrival, the loin of each fillet was cut into skin-on pieces (254 ± 7.2 g) and individually packed using one of three methods; 1) traditional overwrap packaging ("AIR"), 2) modified atmosphere packaging with a 60% CO_2 and 40% N_2 gas mixture and gas/product ratio 3:1 ("MAP"), and 3) 90% soft vacuum ("VAC"). Following the packaging, samples were kept at 4 ± 0.3^{o}C and evaluated at day 0, 2, 4, 6, 8, 10, 12, 14 and 16 post mortem. At each time point, 6 replicates of each packaging method were analysed.

Hyperspectral interactance imaging

Hyperspectral images of salmon fillets were collected using the imaging system described by Sivertsen et al.[3] On the interactance image, a region of interest was defined in IDL 7.1 (ITT Visual Information Solutions, Boulder, USA), including as much area as possible of the fillet but avoiding possible interference from the thickest dorsal part of the loin and small bones. From the defined region, non-overlapping circular areas consisting of 81 pixels (40 mm^2) were randomly selected and a mean spectrum was calculated from the spectra in each area. Spectral pretreatment in the form of standard normal variate (SNV) was performed in The Unscrambler version 9.8 (CAMO, Oslo, Norway).[3] Principal component analysis (PCA) was run in The Unscrambler to see whether samples could be separated by packaging type based on the pretreated spectra.

Classification

Classification was performed in IDL using the K-nearest neighbour classifier (Knn)[4] with Euclidian distance and three neighbours. Following pretreatment with SNV, 90% of fillets in each packaging group were randomly chosen and used as a prototype to classify the remaining 10% of each group by packaging type. The Knn classification was performed excluding day 0 and 2 and run 500 times for each iteration, which provided the average and standard deviation of the correct classification rate. In order to reduce the number of features (wavelengths), partial least squares regression (PLS) was run without day 0 and 2 in The Unscrambler on two of the three groups each time, where the two groups were assigned reference values of either +1 or -1. Weighed beta coefficients were obtained by dividing each variable (wavelength) by its standard deviation and used to identify relevant wavelengths for packaging classification.

Results and Discussion

Little variation was found between the packaging groups AIR, MAP and VAC at day 0 (not shown) but the mean spectra of the three groups clearly showed deviation at day 4 (Figure 1). The major spectral differences between the packaging appeared at 606 and 636 nm. In PCA (Figure 2a), the spectra of AIR could be separated from those of MAP and VAC at day 4. The distinction between the MAP and VAC samples also became more apparent at day 6 and 8. The groups were separated along the second PC at days 4, 6 and 8. The largest spectral variations along PC2 occurred at 606 and 636 nm where the peak at 606 nm was negatively correlated with that at 636 nm (Figure 2b).

The Knn classifier was applied to SNV pretreated spectra. Based on the loading plots (Figure 3a-c) showing weighed beta coefficients, the best classification result (88.3 ± 4.2%) was obtained using five

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wavelengths (606, 636, 665, 705 and 764 nm) and excluding days 0 and 2. The two wavelengths at 606 and 636 nm could alone classify 69.4 ± 5.9 % of the test sample. Four of the five selected wavelengths fell in the visible region. Sivertsen et al.[3] suggested that oxidation of heme proteins (myoglobin and hemoglobin) may be detected in this region of the spectrum during storage of fresh and frozen-thawed cod (*Gadus morhua* L.). For astaxanthin pigmented fish like salmon, it had previously been assumed that absorptions by heme proteins were small compared to astaxanthin. However, more recent studies have suggested that heme proteins do contribute to the colour change of fresh salmon during storage.[5,6]

Also in this study it is possible that storage and packaging resulted in a shift in the oxidation state of heme in the fish muscle and influenced spectral development of salmon fillets during storage. Consequently, different spectral features may have appeared in AIR, MAP and VAC and this may have permitted the successful classification of fillets by packaging type. The line plot (Figure 1), the PCA loading (Figure 2b) as well as the results in the classification give a strong indication that such storage- and packaging-dependent spectral variations appeared at 606 and 636 nm.

The line plot (Figure 1) showed that the absorption at 636 nm was greater for the AIR samples than for those under the limited O_2 conditions (MAP and VAC) at day 4. Increased absorption around at 630 nm has been observed during air storage of cod[3] and tuna (*Thunnus thynnus* L. and *T. obesus* L.).[7] Oxidized heme proteins, methemoglobin and metmyoglobin are known to absorb around at 630 nm[8,9] In addition, bacterial growth and lipid oxidation have been reported to promote heme oxidation and subsequent formation of methemoglobin and metmyoglobin during air storage[10-12]. The results therefore indicated that the increase in absorption intensity at 636 nm could originate from the formation of oxidized heme in the air stored salmon fillets.

Unlike 636 nm, the intensity around 606 nm decreased for the AIR samples (Figure 1). The opposite correlations between 606 and 636 nm in the PCA and PLS loadings indicated possible relation of spectral changes at 606 nm to heme oxidation during air storage (Figure 2a and 3a-b). A distinct shoulder peak has been described at 606 nm in the spectra of cod, mackerel and salmon[3,6,13]. This illustrates that the shoulder peak is not related to astaxanthin in the salmon muscle. The origin of the peak is not of heme as it does not correspond with the absorption characteristics of heme in any oxidation states. Sivertsen et al.[3] suggested that the peak could arise from water in the muscle as water exhibits a shoulder at a similar wavelength.[14] Ottestad et al.[6] speculated that spectral changes occurring at this wavelength could be linked to a breakdown product of heme with an intact porphyrin-like structure. The results of their works well as ours suggest that spectral variations observed at 606 nm reflect structural and physicochemical changes occurring in the heme proteins as a result of oxidation during storage.

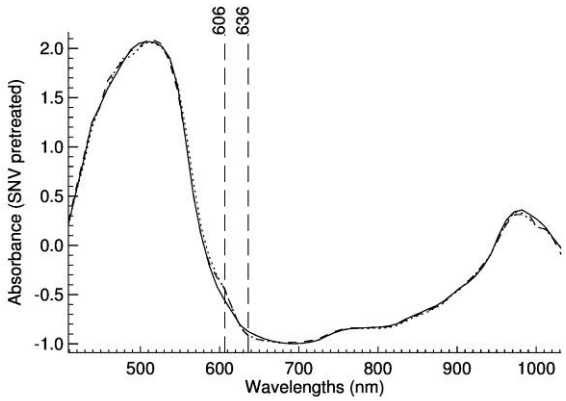

Figure 1. Mean spectra of AIR (solid), MAP (dotted) and VAC (dashed) samples at day 4. The interactance spectra were natural log transformed to the absorbance $A(\lambda,x,y) = -\ln I(\lambda,x,y)$ and pretreated by SNV.

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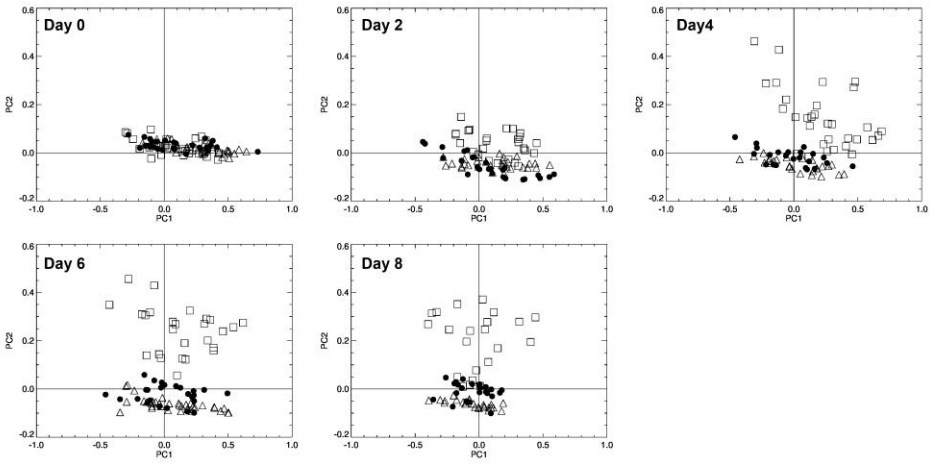

Figure 2a. PCA score plots at day 0, 2, 4, 6 and 8. The AIR, MAP and VAC samples are shown as square, filled circle and triangle, respectively.

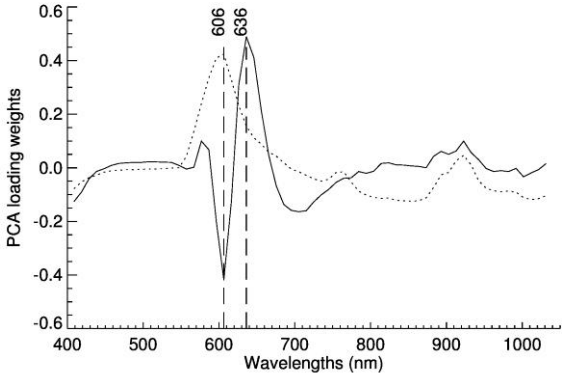

Figure 2b. X loading weights at day 8. The explained variance was 51% (the first PC in dotted line) and 31% (the second PC in solid line).

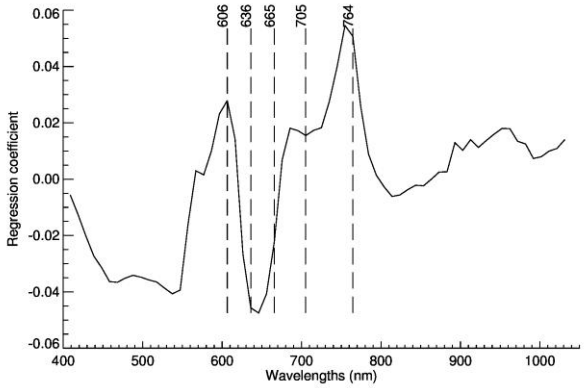

Figure 3a. Weighed beta coefficients in the first PC in PLS with the assigned reference value +1 for AIR and -1 for MAP.

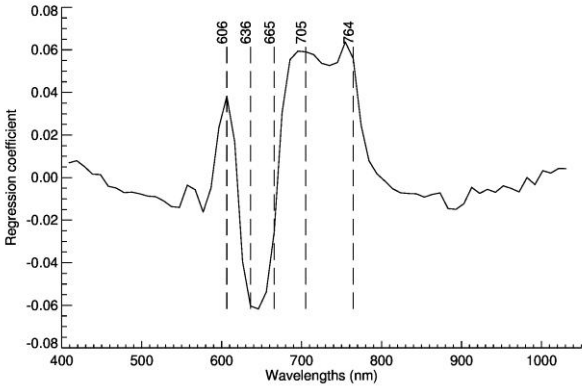

Figure 3b. Weighed beta coefficients in the first PC in PLS with the assigned reference value +1 for AIR and -1 for VAC

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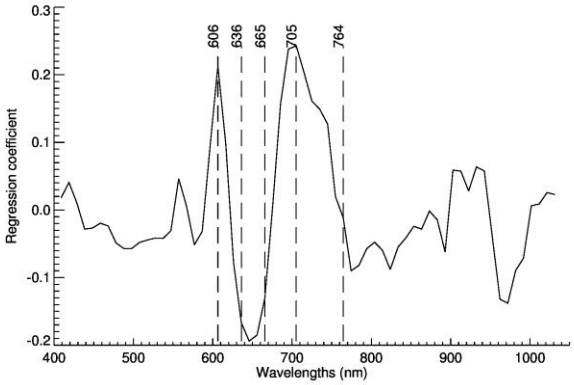

Figure 3c. Weighed beta coefficients in the third PC in PLS with the assigned reference value +1 for MAP and -1 for VAC.

Conclusion

Fresh salmon fillets were correctly classified by packaging type based on the spectra obtained by visible/near infrared hyperspectral imaging. The successful classification was largely dependent on the spectral variations occurring at 606 and 636 nm, probably due to storage- and packaging-dependent changes in the oxidation state of heme proteins in the muscle.

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