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# Multi-spectral Image Analysis for Astaxanthin Coating Classification

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**Abstract.** Industrial quality inspection using image analysis on astaxanthin coating in aquaculture feed pellets is of great importance for automatic production control. In this study multi-spectral image analysis of pellets was performed using LDA, QDA, SNV and PCA on pixel level and mean value of pixels for each pellet. Classification using LDA or QDA on pellet mean or median values showed better results than using the pixel values or PCA.

**Keywords:** astaxanthin, multi-spectral, image analysis

## 1 Introduction

Industrial quality inspection using image analysis is an area of extensive development. Pigment inclusion in aquaculture feed pellets is of great interest for automatic visual analysis for statistical production control and optimisation.

Astaxanthin is a naturally occurring carotenoid with a high antioxidant activity essential for reproduction, growth and survival, and important for the development of colour in salmonide fishes [1]. The primary use of astaxanthin within aquaculture is as a feed additive to ensure that farmed salmon and trout have similar appearance as their wild counterparts [2]; it is the pigment that makes salmonide fishes red. The colour appearance of fish products is important for the customers. Astaxanthin is highly expensive [3] and therefore optimisation of its use in fish feed production is of importance.

An automatic vision system for on-line quality control of pigment inclusion will be of great benefit to the industry both in relation to process control and process optimisation.

This paper is based in part on an earlier study by Ljungqvist et al. (2010) [4]. Besides this no further work has to the authors' knowledge previously been done on analysing the coating of fish feed using image analysis. Multi-spectral image analysis has shown good results in previous biological applications [5, 6, 7, 8] where it is of interest to detect subtle differences in colour and surface chemistry.

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## 2 Multi-spectral Image Analysis for Astaxanthin Coating Classification

The aim of this project is to investigate the possibility of distinguishing between feed pellets coated with fish oil with and without added astaxanthin using multi-spectral image analysis and in this way investigate what spectral features are of interest for further analysis of astaxanthin coating.

## 2 Material and Methods

### 2.1 Material

The feed type used is EcoLife20 and AquaLife R90, both with the radius of 4.5 mm. The fish feed pellets are divided into two groups. One class constitutes pellets coated with fish oil with 50 ppm added of a synthetic version of astaxanthin; class A (astaxanthin). The other class is the same pellet types with fish oil coated without additional astaxanthin included; class B (base). (The fish oil typically contains a small amount of natural astaxanthin, but this is assumed to be less than 1 ppm and should therefore not affect the results.) The distribution of the surface coating is unknown and some amount of variation is likely to occur.

A total of 2223 EcoLife20 pellets were used, and a total of 2158 AquaLife R90 pellets were used, see Table 1.

### 2.2 Imaging Equipment

The equipment used was a camera and lighting system called VideometerLab which supports a multi-spectral resolution of up to 20 wavelengths. These are distributed over the ultra-violet A (UVA), visible (VIS) and first near infrared (NIR) region. The range is from 385 to 1050 nm.

This system uses a Point Grey Scorpion SCOR-20SOM grey-scale camera and the objects of interest are placed inside an integrating sphere (Ulbricht sphere) with uniform diffuse lighting from light emitting diodes (LED) placed around the rim of the sphere. The curvature of the sphere and its white matte coating ensures a uniform diffuse light so that specular effects are avoided and likewise minimising the amount of shadows. The device is calibrated radiometrically with a following light and exposure calibration. The system is geometrically calibrated to ensure pixel correspondence for all spectral bands [9].

The image resolution is  $1280 \times 960$  pixels. Each file contains 20 images, one for each spectral band. This results in a multi-spectral image cube with dimensions of  $1280 \times 960 \times 20$ .

**Table 1.** Number of analysed pellets in each group.

	Size (mm)	Class A samples	Class B samples	Total samples
EcoLife	4.5	1165	1058	2223
R90	4.5	1207	951	2158

### 2.3 Spectral Equipment

In order to further explore the spectral properties of astaxanthin a spectrometer was used. Absorption spectra of synthetic astaxanthin in a solution of fish oil along with plain fish oil were recorded in the VIS and NIR range using a NIRSystems 6500 absorption spectrometer. The absorption spectra was transformed to reflection values using the standard relation  $A = -\log(R)$ , where  $A$  is absorption values and  $R$  is the reflection values.

### 2.4 Image Analysis

The pellets were segmented from the background using a grey-scale threshold.

The basic pellet compound gives a spectral response which will be present in both class A and B. Each pixel is thus a combination of the reflectance of a set of constituents. This mix is assumed to be of equal amount for each pellet type except for the difference of the astaxanthin coating that we want to isolate in our classification.

The ground truth is that we know that certain pellets are coated with synthetic astaxanthin, but since the surface distribution is unknown it is unclear how much synthetic astaxanthin each of those pixels contains. This gives us an uncertain one-to-many relationship situation.

A way to solve this uncertainty is to represent each pellet using the mean or median of all pixels in a pellet as sample values. In this manner we even out the variance of all pixels in a pellet and each pellet becomes a distinct observation.

In addition to the pellet pixel mean and median values further summary statistics features to describe the coating distribution were extracted based on pellet pixel values: Skewness, kurtosis, variance and maximum value.

**PCA** Our multivariate data from the images was analysed using principal component analysis (PCA) for exploratory purpose. PCA is the most optimal method with respect to maximising the variance [10] and has been commonly used for dimension reduction for dealing with ill-posed problems. If the relation of interest contains large variation then PCA is a good method for analysing the data.

The pre-processing method standard normal variate (SNV) [11] was used to reduce any variation in concentration level of the overall coating concentration between pellets.

**Discriminant Analysis** To discriminate between the two classes we want the within group deviation to be small compared to that between groups. Wilk's  $\Lambda$  consists in principle of the ratio of the within group variation ( $\mathbf{W}$ ) and the total variation ( $\mathbf{T}$ ), i.e. the within group plus the between group variation.  $\Lambda = \frac{\det(\mathbf{W})}{\det(\mathbf{T})}$ . A value of Wilk's  $\Lambda$  which is close to zero indicates that the two groups are well separated.

For statistical discriminant analysis methods we use linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA) [10]. They are both based

on the Mahalanobis distance, and assumes that the variables in each class are normally distributed. LDA and QDA are based on a distance to the class mean weighted by the variance. A training set of 70% of the samples were used here, along with a test set of 30% of the samples.

### 3 Results and Discussion

It turned out that spectral band number 20 showed some artefacts for about half of the EcoLife20 class A due to temperature variations so therefore statistical tests were also performed without this band. Results show that this problem did not affect the classification in a negative manner (results not shown).

Comparing the SNV-normalised mean spectra of the two classes of EcoLife20 elucidates the largest difference being at 970, 950 and 565 nm (in order of magnitude). Both 970 and 950 nm are in the NIR range, while 565 nm represents the green colour which is next to yellow. For AquaLife R90 the largest difference between the class spectra are in the visual range around 400 nm and also slightly above 600 nm.

The spectrometer results show a large deviation between synthetic astaxanthin in fish oil and plain fish oil to be in the range of 500 – 600 nm, see Figure 1. This corresponds well with the results from the VideometerLab images and partly corresponds with previous studies of astaxanthin [12, 13].

The mean spectra of the two groups of both EcoLife20 and AquaLife R90 are significantly different at a 0.1% level. This is promising for classification between the two coating groups. On the other hand, Wilk's lambda of the class means of EcoLife20 pellet mean values equals 0.987, and for AquaLife R90 it is 0.826. The high values here are reflecting the situation of high variation within the groups and a low variation between the groups. So even though the class means are well separated, there is a vast overlap of the two groups.

Classification tests of EcoLife20 show that LDA on the pellet means or pellet medians gave the best result with a classification correctness of about 93%. See Table 2 for test results.

Classification tests of AquaLife R90 show that QDA on the pellet medians gave the best result with a classification correctness of 100%.

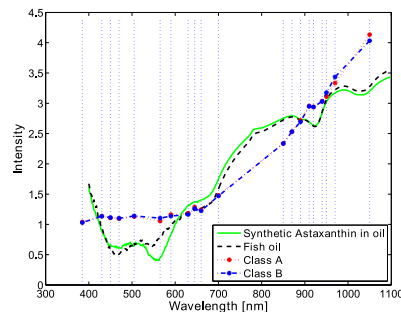
Using LDA and QDA on the other summary statistics features (skewness, kurtosis, variance and maximum value) gave results of lower correctness for both pellet types (results not shown).

Using PCA before doing LDA or QDA on the pellet mean values did not improve the results, see Table 2. This may be an indication that maximising the variance is not a well-suited method for this particular problem, which also was indicated by the high variation within groups in comparison to the variation between groups. PC2 shows the largest difference between the two classes, see Figure 2. The first five principal components explain 98% of the total variance of the pellet mean values, and still the result of the discriminant analysis on these five components rendered worse classification in comparison to using the plain data itself.

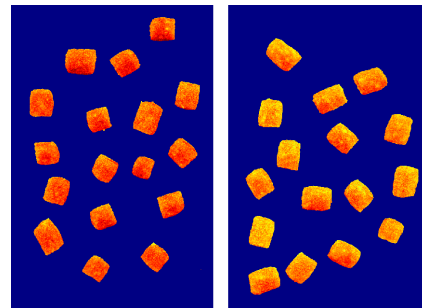
**Table 2.** The misclassification of pellet coating type for different kinds of features. Displayed values are total test error for classification of the two groups A (astaxanthin) and B (base).

EcoLife20	LDA	QDA
Mean	0.0646	0.0901
Median	0.0736	0.0931
Mean, SNV, PC1-5	0,1396	0,2162
AquaLife R90		
Mean	0.0046	0.0031
Median	0.0015	0.0000
Mean, SNV, PC1-5	0.0185	0.0201

To sum up, the results show that it is possible to distinguish between feed pellets with and without inclusion of synthetic astaxanthin in the coating using multi-spectral image analysis. However, more work is needed in order to make the method robust for various pellet types and also for various amount of astaxanthin. Since astaxanthin is expensive it is desired to have a good accuracy in the method. This will further on be of importance for developing on-line quality food and feed products with optimal use of pigment and minimum amount of waste.



**Fig. 1.** Spectrometer reflectance of synthetic astaxanthin in oil (*green*) and plain fish oil (*black*). Multi-spectral images (reflectance) mean of class A (synthetic astaxanthin in fish oil) (*red*) and class B (fish oil) (*blue*) of the EcoLife20 type.



**Fig. 2.** The 2nd principal component of the multi-spectral image (reflectance) of EcoLife20 pellet pixels. Pellets coated with synthetic astaxanthin in fish oil, class A (*left*). Pellets coated with fish oil, class B (*right*). Red colour indicates high values.

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