# Classification of prior temperature history of chilled chicken breasts by near infrared spectroscopy

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## Introduction

Labeling of "fresh" or "frozen" in reference to muscle foods is controversial because the measurable basis for defining these terms has been the temperature, either of the products themselves and/or of the environmental chamber containing the product. The labelling of raw poultry products as "fresh" has been used by the Food Safety Inspection Service (FSIS) to describe products that have not been previously frozen to -18°C. In 1993, California enacted a state law restricting the use of the term "fresh" to poultry products that had never been kept at or below -4°C. As a result, several industrial poultry groups brought suit against California, charging that the State law preempted Federal law. Recently, the FSIS reexamined its policy for use of the term "fresh" on products whose temperature has ever been below -3°C. The objective of this work was to investigate the feasibility of using NIR to classify the temperature to which poultry has been chilled to ensure that products distributed to consumers are accurately labeled.

## Materials and methods

### Samples

Mixed sex, 42 day old, commerically processed broiler carcasses were obtained from a local plant and transported to the laboratory. All carcasses were held on ice at the processing plant at least 8 h prior to pick up. Skinless breast fillets were removed from each carcass. Left breast sides were placed on ice in trays, scanned within 20 minutes of deboning for baseline NIR data and then packaged immediately in polyethylene bags.

## Experimental design

Four complete replicates were conducted, involving 150 birds at each replication. Packaged breast fillets were placed in holding chambers set at temperatures of  $-18^{\circ}$ C,  $-12^{\circ}$ C,  $-3^{\circ}$ C,  $0^{\circ}$ C and  $4^{\circ}$ C. Temperatures of the chambers were monitored 24 hours a day and controlled to  $\pm 3^{\circ}$ C. Samples were stored in the holding chambers for 48 hours (treatment A), 7 days (treatment B) and at  $-18^{\circ}$ C for 7 additional days (treatment C) after initial seven day temperature storage (treatment B).

#### Near infrared reflectance spectroscopy

Samples were scanned on a NIRSystems 6500 monochromator (NIRSystems, Silver Spring, MD) equipped with a sample transport module and a half coarse sample cell. Reflectance measurements were recorded at 2 nm intervals from 400 to 2500 nm and averaged over 32 scans. The data were collected and analyzed using ISI software (Infrasoft International Inc., State College, PA) and Unscrambler (CAMO, Trondheim, Norway), respectively.

Samples designated as "fresh" were scanned within 20 minutes of deboning (n = 600). After treatment, A and B samples from  $-18^{\circ}$ C,  $-12^{\circ}$ C,  $-3^{\circ}$ C and  $0^{\circ}$ C were tempered back to  $4^{\circ}$ C and scanned (n = 320). Samples from  $4^{\circ}$ C were scanned immediately after removal from storage (n = 80). Samples from treatment C were tempered back to  $4^{\circ}$ C and scanned (n = 200).

#### Classification models

Classification models were developed for each of the five storage temperatures. Samples from treatment A and B were combined within storage temperature (N = 80). Using an algorithm called SELECT,<sup>1</sup> samples were divided into calibration and validation sets. By trial and error, a cut-off value of H = 0.25 was chosen to obtain 50 calibration samples for each storage temperature. From the remaining 30 samples, 10 were chosen at random and combined across storage temperature for a total of 50 validation samples.

The method of principal component analysis (PCA) was used for all chemometric models. A first derivative difference with a gap = 6 nm and 3 nm average was performed on the spectra to reduce sample-to-sample baseline variation. The wavelength region was then reduced to 700–1850 nm because preliminary results (not shown), which utilized 700–2498 nm, indicated that the models were not as accurate as those based on the reduced region, due to a non-linear response at longer wavelengths. Cross-validation was performed during model development with 20 cross-validation segments, whereby three calibration samples at a time were temporarily removed from the calibration set. Performance statistics were accumulated for each group of removed samples. The first local minimum in the X-residual cross-validation variance was used to select the optimal number of PCA factors. Model performance was reported as the number of samples correctly classified in the validation set.

## Results and discussion

The spectrum of a typical "fresh" chicken breast fillet is shown in Figure 1. The effects of O–H absorbance due to water<sup>2</sup> are present throughout the spectrum. In general, the shape and appearance of this spectrum is typical for any high moisture sample scanned in reflectance. Reflectance spectra of high moisture materials exhibit a loss of measured reflectance at the higher wavelengths. In our case, this was caused by specular reflectance, which was a function of light scattering from the mirror like surface of the skinless breast fillets<sup>3</sup> as well as the high absorbance due to water.<sup>4</sup> These phenomenon caused a non-linear log (1/R) signal at the longer wavelengths.

Discriminant analysis and classification techniques are concerned with separating distinct sets of observations (or samples) and with allocating new observations (or samples) to previously defined groups. The goals of discrimination and classification are: (i) to describe either graphically or algebraically, the differential features of observations from several known sample sets (populations). The goal is to find "discriminants" whose numerical values are such that the populations are separated as much as possible; and (ii) to sort observations into two or more labeled classes. The emphasis is on deriving a rule that can be used to optimally assign a "new" sample to the labeled classes. In our case, we tried to find disciminants in the spectra of breast fillets tempered to 4°C after storage in the five temperature holding chambers. The philosophy behind this is that the fillets from a given storage temperature show similar rather than identical behavior. With this



Figure 1. Log (1/reflectance) spectra of a typical unfrozen chicken breast fillet.

approach, you allow for the samples to have individualities and model only the common properties of the temperature storage class. Consequently, the complete classification model consisted of several PC models, one for each temperature class.

Classification of the validation samples using five PCA discriminant models are shown in Table 1. The optimal number of PCs varied from seven to nine and explained on average 91% of the spectral variation. Within a storage class, no samples were uniquely classified as belonging to a class model. There was more ambiguity in the classification of "unfrozen" samples (samples stored at 4, 0 and  $-3^{\circ}$ C) than frozen samples. Fifty and seventy percent of the frozen samples could be classified as belonging to the  $-12^{\circ}$ C or the  $-18^{\circ}$ C class, respectively. The reason why unfrozen samples cannot be separated into their storage classes may be because they are very similar. The model-to-model distance between the five class models was used to determine how different the models were. Model distances compared to class model 4°C were 1.9, 8.5, 2.6 and 2.5 for 0, -3,

Table 1. Classification of unfrozen and frozen chicken breast fillets using object-to-	
model distances with five PCA discriminant models on 10 validation samples per stor	-
age class.	

	Class models					
Storage classes	4/0	4/-12	4/-18	0/-18	-12/-18	NO
4	1	2	4	3		1
0	6	1	2	1		
-3	4	1	1	4		
-12			1	2	5	2
-18				3	7	

	Class models					
Storage classes	4/0	-3	-12/-18	NO		
4/0	8 (12) <sup>a</sup>		(12)			
-3	1 (19)		(19)			
-12	(13)		6 (13)	1		

Table 2. Classification as unfrozen or frozen chicken breast fillets using object-to-model distances with three PCA discriminant models.

<sup>a</sup>Value in parentheses indicate the number of samples cross-classified.

-12 and -18°C class models, respectively. A larger number than three indicates good class separation, (i.e. the models are different). It is clear that the 3°C model is very different from the other models that are quite similar. Thus, some misclassification can be expected.

Due to the similarities of the unfrozen models (i.e. 4 and 0°C) and frozen models (i.e. -12 and  $-18^{\circ}$ C) a three class model approach was investigated. The three class models were samples from treatment A and B stored at 4/0°C,  $-3^{\circ}$ C and  $-12/-18^{\circ}$ C. Using SELECT and a cut-off value of H = 0.25, 113 and 78 calibration samples were chosen for the 4/0°C and  $-12/-18^{\circ}$ C class models, respectively. The  $-3^{\circ}$ C class model reported previously was used without recalibration. The validation set (N = 50) consisted of 20 samples from 4/0°C and  $-12/18^{\circ}$ C storage temperatures and 10 from  $-3^{\circ}$ C.

Classification results using three PCA discrimimant models are shown in Table 2. The optimal number of PCs varied from seven to nine and explained on average 91% of the spectral variation. Within a storage class, a few samples were uniquely classified as belonging to a class model. Combining the  $4/0^{\circ}$ C and  $-12/-18^{\circ}$ C storage classes did not improve the discriminating power of these models. We see that 60 and 65% of the 4/0 and  $-12/-18^{\circ}$ C storage classes were cross-classified as unfrozen and frozen. The model-to-model distance for these two classes was 1.9, thus some misclassification can be expected. The greatest ambiguity occurred for the  $-3^{\circ}$ C storage class where 90% of the samples were cross-classified.

The classification data presented in Tables 1 and 2 were based on the object-to-model distance for the two closest models. When samples are doubly classified, one can study both the object-tomodel distance and the leverage (i.e. object-to-model center distance) to find the best fit. At a similar object-to-model distance, the sample is probably closest to the model to which it has the smallest leverage. Utilizing this approach, the models correctly classified 85% and 75% of the

 Table 3. Classification as unfrozen or frozen chicken breast fillets using object-to-model

 and leverage distances with three PCA discriminant models.

	Class models				
Storage classes	4/0	-3	-12/-18	NO	
4/0	20				
-3	6		4		
-12/-18	4		15	1	

unfrozen and frozen samples, respectively (Table 3). However, there were still problems with the classification of samples stored at  $-3^{\circ}$ C. No samples stored at  $-3^{\circ}$ C were classed with the  $-3^{\circ}$ C model. These samples were classed either as unfrozen, frozen or cross-classified. Poultry meat is not frozen at  $-3^{\circ}$ C. Freezing of poultry is initiated over a range of temperatures between  $-6^{\circ}$ C and  $-3^{\circ}$ C. If one designates the  $-3^{\circ}$ C storage class as unfrozen, 87% of the samples were classified correctly (Table 3).

## Conclusions

In this study, the combined three temperature storage class models performed better than the five class models. In addition, the use of sample leverage in conjunction with sample-to-model distance improved the classification. The ambiguity of the  $-3^{\circ}$ C class could be due to these samples having properties of both unfrozen and frozen classes. The FSIS in the US needs a method for determining whether poultry meat has previously been frozen. However, the classification models in their current state are not accurate enough for regulatory purposes. The classification accuracy of the NIR method might be further improved by weighting the classes and the use of drip loss data. This approach is currently being studied.

# References

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