Attempted authentication of cut pieces of chicken meat from certified production using near infrared spectroscopy

Olivier Fumière, Georges Sinnaeve and Pierre Dardenne

Département Qualité des Productions Agricoles (CRAGx), Chaussée de Namur 24, B-5030 Gembloux, Belgium.

The control of the origin of meat is of major interest not only to consumers but also to producers and retailers. The number of poultry meat products with quality marks continues to increase. Near infrared (NIR) reflectance spectroscopy was investigated as a mean of discriminating between "slow-growing" and "industrial" chicken strains. Seventy-two chickens (39 "slow-growing" chickens and 33 "industrial" ones) were analysed using two instruments (NIRSystems 6500 and Perten DA 7000). The NIRSystems 6500 is characterised by a wide wavelength range and a fine resolution of the spectra; the Perten DA 7000 is able to acquire the spectra of whole chicken carcasses within a second. Various cut pieces (leg with skin, breast without skin, carcass with skin and skin) corresponding to commercial products were considered. The performance of the discriminant models developed range between 80% and 100% of correct classification in either the calibration or the validation step. These positive results show that the technique could be integrated in an analytical system of surveillance of certified meat products.

Keywords: meat, chicken, NIR, near infrared spectroscopy, authentication.

Introduction

In the late 1980s, discriminant analysis was already being applied to distinguish wheat species.[1] Near infrared (NIR) reflectance spectroscopy and principal component analysis (PCA) were used to authenticate Basmati rice which is much more expensive than other rice types.[2,3] The same procedure was used to authenticate wheat flours,[4] orange juices[5,6] and coffee blends.[7] Fourier transform infrared spectroscopy (FT-IR) is also often used for liquid products such as fruit juices.[8,9] The authentication of food and food ingredients by NIR spectroscopy has been reviewed by Downey.[10]

As far as meat and meat products are concerned, spectroscopic techniques are used for the quantitative determination of major constituents, such as moisture, fat and protein,[11,12] for estimating organoleptic quality,[13] for classifying pig carcasses objectively,[14] for detecting fraud[15] and for meat speciation.[16] Discrimination between fresh and frozen-then-thawed samples of meat from beef was also successfully attempted.[17,18] At least, discrimination of raw pork, chicken and turkey meat by spectroscopy in the visible, near- and mid-infrared ranges was studied.[19]

The chicken production sector is characterised by growing economic importance and the occurrence of products with special characteristics. A large number of products with legally certified brands or quality marks (e.g. "Label de Qualité Wallon" in Belgium, "Label Rouge" in France, special labelling according to European regulation 91/1538/CEE, Denominations of Origin, ...) exist in Europe. If these products are to fetch higher prices than their more ordinary counterparts, it is essential that adulteration is prevented and that efficient analytical methods for checking their conformity to specific norms are developed. Therefore

NIR spectroscopy—a sensitive, fast and affordable technique—was studied to determine its potential for the authenticating cut pieces of chicken meat of certified production.

Material and methods

Poultry samples

Seventy-two chickens were analysed and two groups were defined. The "Slow Growing Chickens" group (SGC) contained 39 chickens belonging to strains with a low growth rate. They are slaughtered between 81 and 91 days old. All samples came from animals bearing a quality mark ascribed by an official certification organism (e.g. "Label de Qualité wallon", "Label Rouge" or organic farming). "Poulets Villages" (poultry of higher quality carrying the "Label de Qualité wallon" quality mark) were obtained through Professor André Théwis (Faculté Universitaire des Sciences Agronomiques de Gembloux, Belgium), a member of the Professional Union of Certified Poultry Producers.

The "Industrial Chickens" group (IC) contained 33 chickens belonging to strains selected for their high feed efficiency and fast growth rate. The age of slaughtering is rarely mentioned but is much lower than for the "slow growing chicken" group (42–50 days old).

Seven types of samples were analysed: legs with skin, legs without skin, breasts with skin, breasts without skin, carcasses with skin, carcasses without skin and skins. Three of these (legs with skin, breasts without skin and carcasses with skin) correspond to commercial products. A fourth type (skins), prepared in our laboratory from other samples, is also very interesting because of its fat composition.

The sample analysis procedure is described in Figure 1: the chicken is divided in two half-carcasses and analysed using the Perten instrument. The first half-carcass is then skinned and analysed again using the Perten. After that, leg and breast are separated and analysed individually using the Perten. The second half-carcass is not skinned but is analysed using the same procedure. When the spectra of all the samples are acquired using the Perten, they are homogenised using a mixer and their spectra are collected using the NIRSystems 6500. A sample of homogenised carcass with skin was made by pooling homogenised leg with skin and

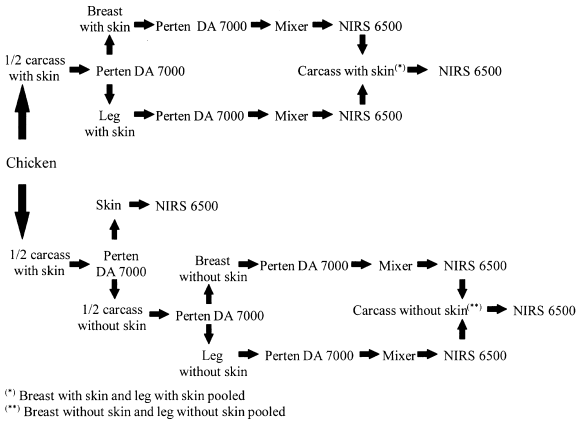

Figure 1. Sample analysis procedure using a NIRSystems 6500 and a Perten DA 7000.

homogenised breast with skin; a similar procedure was used for the skinned samples.

All the spectra were collected in triplicate. The spectral data treatment was performed on the average spectra.

NIR spectral acquisition

Chicken meat spectra were obtained using two spectrometers. A NIRSystems Model 6500 dispersive scanning visible/NIR spectrometer (400 to 2500 nm, spectral resolution of 2 nm) (Foss-NIRSystems, Silver Spring, MD, USA) was used to acquire the spectral data in reflectance mode using the DCA (Direct Contact Analysis) module. The samples of homogenised chicken meat were analysed in small glass ring cups. The area of sample scanned was 1 cm^2.

A Perten DA 7000 (Perten Instruments Inc., Chatham, IL, USA) was also used to acquire the spectral data of whole cut pieces and carcasses of chicken. This spectrometer is a post-dispersive polychromator which generates spectra from 400 to 1700 nm with a spectral resolution of 5 nm. The instrument was inverted and the area scanned was a circle with a diameter of 13.5 cm. Apart from the half-carcass samples, the triplicate scans used the same section of the samples.

Spectral data management

All manipulations and processing of the spectra were carried out using the software ISI-NIRS 3 ver. 4.0

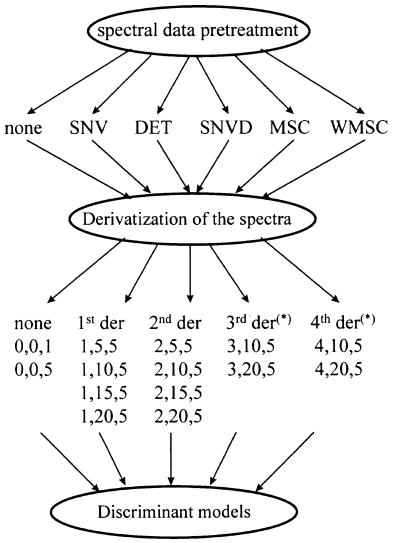

Figure 2. Flow chart for the global calibration trials used using a NIRSystems 6500. Scatter correction [Standard Normal Variate (SNV), Detrend (DET), Standard Normal Variate and Detrend (SNVD), Multiplicative Scatter Correction (MSC), Weighted Multiplicative Scatter Correction (WMSC)]; Mathematical treatment (derivative, gap in data points, segment in data points).

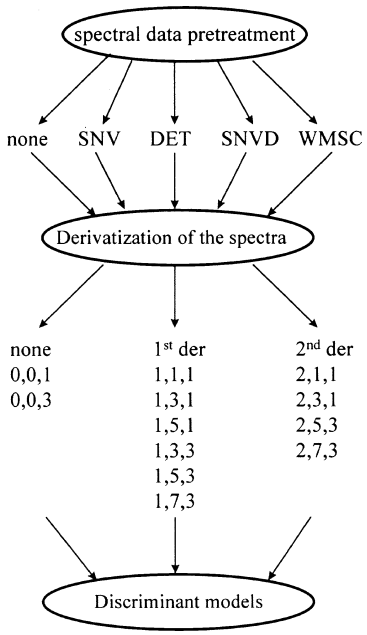

Figure 3. Flow chart for the global calibration trials using a Perten DA 7000. Scatter correction [Standard Normal Variate (SNV), Detrend (DET), Standard Normal Variate and Detrend (SNVD), Multiplicative Scatter Correction (MSC), Weighted Multiplicative Scatter Correction (WMSC)]; Mathematical treatment (derivative, gap in data points, segment in data points).

(Infrasoft International, Port Matilda, PA, USA). In the first stage, global calibrations were obtained by testing 64 and 60 spectral data treatments with the spectra acquired using the NIRSystems 6500 and the Perten DA 7000, respectively (Figures 2 and 3). For global calibrations, a class variable was defined based on the following formula, as described by Dagnelie:[20]

$$X_{SGC} = \frac{-n_{IC}}{n_{SGC} + n_{IC}} \qquad X_{IC} = \frac{n_{SGC}}{n_{SGC} + n_{IC}}$$

where n_{SGC} and n_{IC} are the number of "Slow Growing Chicken" and the number of "Industrial Chicken", respectively.

In a second stage, the performances of the best calibrations were tested using the "Discriminate groups" option of the software. This part of the ISI-NIRS 3 ver. 4.0 software is particularly useful for mathematically separating sample groups using partial least squares regression PLS2.[21,22] In our case, where the aim was to discriminate between two sample groups, PLS2 could be assimilated to PLS. The PLS2 model was calculated using one dummy-variable for each group. Each of the two variables was set to "+2" for samples of the actual group and "+1" otherwise. Each sample was classified into the group of highest predicted dummy values. A similar procedure was used by Thyholt *et al.*[16]

The number of cross-validation groups (CV) is equal to the number of samples used to build the models (full cross-validation).

Global calibrations and statistical discriminant models were made with two-thirds of the samples of the two groups (48 chickens divided in 26 "Slow Growing" ones and 22 "Industrial" ones). The samples were randomly selected according to an algorithm defined by the software;[21] the final third (24 chickens divided in 13 "Slow Growing" ones and 11 "Industrial" ones) was used to validate the models. All the models were made with the samples corresponding to the same chickens. In this way, it was easier to compare the performances of the models obtained using the two spectrometers and each type of samples.

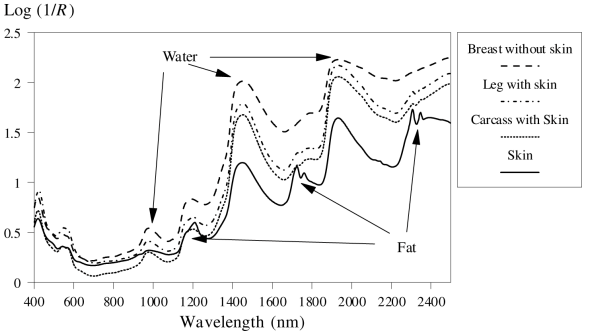

Figure 4. Mean visible/NIR reflectance spectra of samples of legs with skin, breasts without skin, carcasses with skin and skins obtained using a NIRSystems 6500.

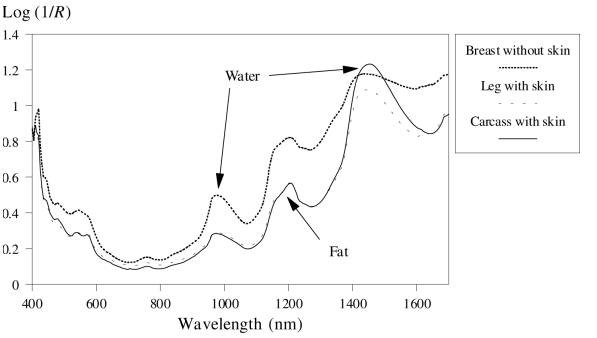

Figure 5. Mean visible/NIR reflectance spectra of samples of legs with skin, breasts without skin and carcasses with skin obtained using a Perten DA 7000.

The final stage of the process involved testing the discriminant models with a set containing the 72 samples. Here again, the number of cross-validation groups (CV) was equal to the number of samples.

Results and discussion

Spectral characterisation

Figure 4 shows the mean spectra of samples of legs with skin, breasts without skin, carcasses with skin and skins obtained using the NIRSystems 6500. The spectra show bands at 1208 nm (CH stretch second overtone), 1724 and 1762 nm (CH stretch first overtone), 2310 and 2348 nm (CH combination bands) due to fat. Characteristic bands of water are also observable at 980 nm (OH second overtone), 1450 nm (OH first overtone) and 1934 nm (OH combination tone). The spectrum of skin is particularly illustrative of the fatty composition of this sample.

Figure 5 shows the mean spectra of samples of legs with skin, breasts without skin, and carcasses with skin obtained using the Perten DA 7000. The spectra show bands at 1205 nm (CH stretch second overtone) due to fat. The bands of water are observable at 975 nm (OH second overtone), 1440 nm (OH first overtone). The spectra are also characterised by a high noise level under 460 nm.

Figure 6 shows the mean spectra of "Slow Growing Chickens" (SGC) and "Industrial Chickens" (IC) samples obtained using the NIRSystems 6500. Although the two profiles are essentially similar, a sharper lipid absorption bands can be observed with the "Industrial Chickens" in the CH first overtone and in the CH combination regions. Similar observations in chicken meat spectra were attributed by Cozzolino *et al.*[12] to a higher fat content. This explanation is also in accords with fat analysis by gas chromatography on chicken meat belonging to "Label" and "standard" populations.[23]

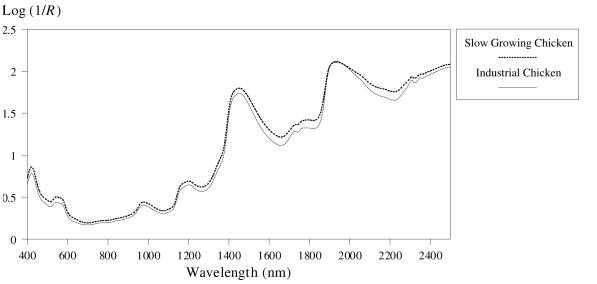

Figure 6. Mean visible/NIR reflectance spectra of all samples of "Slow Growing Chickens" (SGC) and "Industrial Chickens" (IC) obtained using a NIRSystems 6500.

Discriminant analysis

The best discriminant models we obtained and their performances are given in Tables 1–4 and in Figure 7. From these results, we can make the following comments.

The analytical performances of the discriminant models obtained with the NIRSystems 6500 were always better than or equal to those obtained with the Perten DA 7000 (Figure 7). This is due mainly to the

Table 1. Results of the discriminant analysis of legs with skin: (a) percentages of correct assignment in calibration and in validation with 48 samples; (b) percentages of correct assignment in calibration with 72 samples.

Instruments	Treat.[a]	CV[c]	T[b]	Calibration set	Validation set	CV[c]	T[b]	Calibration set
NIRSystems 6500	SNV 1,5,5,1	48	4	SGC 96% IC 95% Total 96%	SGC 92% IC 92% Total 91%	72	4	SGC 97% IC 97% Total 97%
Perten DA 7000	SNV 1,5,3,1	48	5	SGC 92% IC 77% Total 85%	SGC 100% IC 90% Total 95%	69	4	SGC 97% IC 88% Total 93%

[a] treatment of the spectra (scatter correction, derivative, gap in data points, segment in data points, smooth)
[b] number of terms in the models
[c] number of cross-validation groups

Table 2. Results of the discriminant analysis of breasts without skin: (a) percentages of correct assignment in calibration and in validation with 48 samples; (b) percentages of correct assignment in calibration with 72 samples.

Instruments	Treat.[a]	CV[c]	T[b]	Calibration set	Validation set	C.V.[c]	T[b]	Calibration set
NIRSystems 6500	MSC 2,5,5,1	48	5	SGC 92% IC 77% Total 85%	SGC 85% IC 82% Total 83%	72	3	SGC 90% IC 88% Total 89%
Perten DA 7000	SNVD 2,1,1,1	48	7	SGC 77% IC 82% Total 79%	SGC 75% IC 60% Total 68%	70	8	SGC 82% IC 81% Total 81%

[a] treatment of the spectra (scatter correction, derivative, gap in data points, segment in data points, smooth)
[b] number of terms in the models
[c] number of cross-validation groups

Table 3. Results of the discriminant analysis of carcasses with skin: (a) percentages of correct assignment in calibration and in validation with 48 samples; (b) percentages of correct assignment in calibration with 71 samples.

Instruments	Treat.[a]	CV[c]	T[b]	Calibration set	Validation set	CV[c]	T[b]	Calibration set
NIRSystems 6500	SNVD 2,10,5,1	48	3	SGC 92% IC 100% Total 96%	SGC 100% IC 100% Total 100%	71	5	SGC 97% IC 97% Total 97%
Perten DA 7000	SNV 1,5,3,1	48	3	SGC 92% IC 95% Total 94%	SGC 100% IC 90% Total 95%	70	7	SGC 95% IC 97% Total 96%

[a] treatment of the spectra (scatter correction, derivative, gap in data points, segment in data points, smooth)
[b] number of terms in the models
[c] number of cross-validation groups

Table 4. Results of the discriminant analysis of skins: (a) percentages of correct assignment in calibration and in validation with 38 samples; (b) percentages of correct assignment in calibration with 58 samples.

Instruments	Treat.[a]	CV[c]	T[b]	Calibration set	Validation set	CV[c]	T[b]	Calibration set
NIRSystems 6500	SNV 0,0,5,1	38	8	SGC 94% IC 95% Total 95%	SGC 89% IC 91% Total 90%	58	7	SGC 85% IC 97% Total 91%

[a] treatment of the spectra (scatter correction, derivative, gap in data points, segment in data points, smooth)
[b] number of terms in the models
[c] number of cross-validation groups

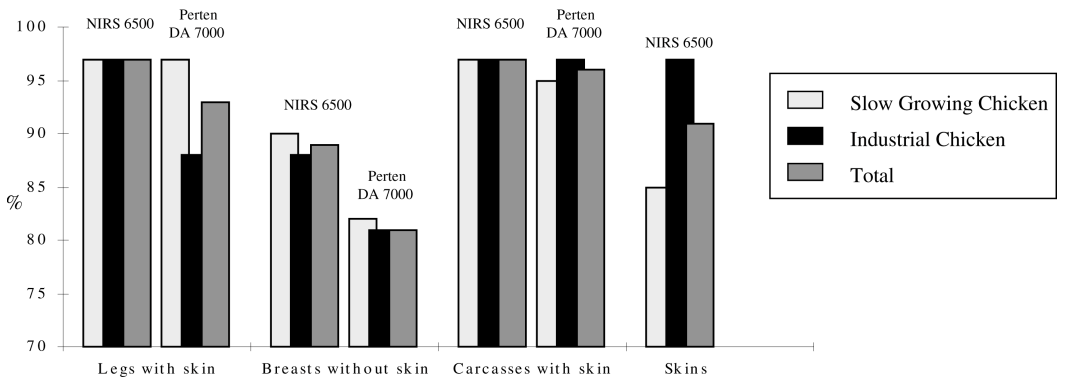

Figure 7. Best percentages of correct classification obtained with the final discriminant analysis (with 72 chickens) for each tissue (legs with skin, breasts without skin, carcasses with skin and skins), chicken strains (Slow Growing Chicken, Industrial Chicken and Total) and instruments (NIRSystems 6500 and Perten DA 7000).

characteristics of the instruments (wavelength ranges and spectra resolutions) and to the sample presentation (whole cut pieces with the Perten DA 7000, mixed homogenised meats with the NIRSystems 6500).

The analytical performance of the best models obtained with 48 chickens generally ranged between 80% and 100% of correct classification in either the prediction or the validation step. In many cases, more than 90% of the samples were assigned to the correct group.

The analytical performance of the discriminant models obtained for breasts without skin (48 samples) are slightly lower than for the other sample types (Table 2). The mean correct classification rate was around 80–85 % with the NIRSystems 6500 and around 70–80% with the Perten DA 7000. The predominant localisation of fat in the skin or directly under the skin could explain this result. The qualitative and quantitative fat composition would be a discriminant criterion between the slow-growing chicken strains and the industrial ones. Girard *et al.*[23] had already applied a discriminant analysis to two species of poultry (chickens and ducks) based on lipids and fatty acids composition determined by gas chromatography. Using this approach, they were able to assign 100% of the chickens to their corresponding group (“label” or “standard”).

The performances of the discriminant models were mostly identical with the calibration and the validation set [e.g. legs with skin—NIRSystems 6500—SNV 1,5,5,1 (Table 1)]. However, with a few discriminant models, the performances differed in calibration vs in validation (e.g. legs with skin—Perten DA 7000—NONE 0,0,3,1; breasts without skin—Perten DA 7000—SNV 2,1,1,1; skins—NIRSystems 6500—SNVD 1,10,5,1: data not shown). For this reason, new discriminant models were built with a set containing all samples. The same mathematical treatments as in the previous stage were tested. Globally, the discriminant models built with the larger set showed better per-

formances than the corresponding ones built with the set of 48 samples. It is very important that we see whether we can improve the performances of the discriminant models using new samples.

The discriminant analysis of carcasses with skin (Table 3) and skins (Table 4) produced results leading to comments similar to those made above.

The two spectrometers used in this study have very different characteristics. Their use in an industrial context will depend of the control strategy adopted: Perten DA 7000 is well adapted to on-line screening and other similar applications with this instrument are developed by our Department (e.g. the on-line determination of apple internal quality[24] or the analysis of fresh forages on harvest machine[25]). The NIRSystems 6500 is more useful for single control procedures.

Conclusions

The performance of the discriminant models developed on 48 chickens show the ability of NIR spectroscopy to distinguish slow-growing chicken strains from industrial ones in more than 80% of cases. The discrimination was further improved with a larger set of 72 chickens. If these results are confirmed using new samples, the technique could be integrated in an analytical system of surveillance of certified meat products and used as a screening technique prior to the use of more expensive and time-consuming analyses such as lipid composition, protein composition or DNA testing.

The choice of the spectrometer depends on the number of samples to control. An on-line control procedure requires the Perten DA 7000 (no removal of the samples and fast data acquisition); a single control procedure could be completed successfully with a NIRSystems 6500 (higher classification rate).

The fatty composition of chicken meat seems to be a discriminant criterion between slow growing chicken strains and industrial ones:

- the analytical performance of models built with lean samples (i.e. meat without skin) are lower than when skin is included;
- the differences between the analytical performance of the models built with the NIRSystems 6500 and the Perten DA 7000 could be explained by the fact that the wavelength range of the Perten DA 7000 is limited to 400–1700 nm. The region above 1700 nm, which contains important absorption bands corresponding to fat and seems to be very informative, cannot be explored with this instrument.

According to Dennis,[26] spectroscopic approaches now occupy a pivotal position in authenticity research. Two quite different tactics could be used: either the spectrum is the basis for a statistical pattern recognition or it is used to measure a particular chemical entity. In the first case, it is difficult to falsify a food to defeat the class separation approach because usually the basis of the classification is unknown. We are aware that no biochemical analysis can explain the discrimination observed but care was taken to ensure that simple parameters did not influence our measurements; hence we included as large a variety as possible in our data bases.

We also observed the development of intermediate quality products. These productions are often characterised by feed restrictions (e.g. no meat and bone meal, no antibiotics, percentage of cereals etc.) even if they are very similar to intensive systems of production. The performances of our discriminant models may be improved by distinguishing a third group containing those products. However, the heterogeneity of this class of intermediate products could work against a good discrimination.

We are continuing to add samples to our databases and to check the performances of the models.

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