TITLE: Detection of offal adulteration in beefburgers using near infrared reflectance spectroscopy and multivariate modelling

AUTHORS: Ming Zhao, Colm P. O'Donnell, and Gerry Downey

This article is provided by the author(s) and Teagasc T-Stór in accordance with publisher policies.

Please cite the published version.

The correct citation is available in the T-Stór record for this article.

NOTICE: This is the author's version of a work that was accepted for publication in *Journal of Near Infrared Spectroscopy*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. A definitive version was subsequently published in *J. Near Infrared Spectrosc.*, 21(4), 237–248 (2013). DOI: 10.1255/jnirs.1057

This item is made available to you under the Creative Commons Attribution-Non commercial-No Derivatives 3.0 License.

Detection of offal adulteration in beefburgers using near
infrared (NIR) reflectance spectroscopy and multivariate
modelling

Zhao, M.¹, O'Donnell, C.P.² and Downey, G.¹

¹ Teagasc Food Research Centre Ashtown, Ashtown, Dublin 15, Ireland

² School of Biosystems Engineering, University College Dublin, Belfield, Dublin 4

*corresponding author: ming.zhao@teagasc.ie

Abstract

The main aim of this study was to develop a rapid and reliable tool using near infrared (NIR) reflectance spectroscopy to confirm beefburger authenticity in the context of offal (kidney, liver, heart and lung) adulteration. Experimental design was used to develop beefburger formulations to simultaneously maximise the variable space describing offal-adulterated samples and minimise the number of experiments required. Authentic (n=36) and adulterated (n=46) beefburger samples were produced using these formulations. Classification models (partial least squares discriminant analysis, PLS1-DA) and class-modelling tools (soft independent modelling of class analogy, SIMCA) were developed using raw and pre-treated NIR reflectance spectra (850-1098nm wavelength range) to detect authentic and adulterated beefburgers in (1) fresh, (2) frozen-then-thawed and (3) fresh or frozen-then-thawed states. In the case of authentic samples, the best PLS1-DA models achieved 100% correct classification for fresh, frozen-then-thawed and fresh or frozen-then-thawed samples. SIMCA models correctly identified all the fresh samples but not all the frozen-then-thawed and fresh or frozen-then-thawed samples. For the adulterated samples, PLS1-DA models correctly classified 95.45% of fresh, 91.30% of frozen-then-thawed and 88.89% of fresh or frozen-then-thawed beefburgers. SIMCA models exhibited specificity values of 1 for both fresh and frozen-then-thawed samples, 0.99 for fresh or frozen-then-thawed samples; sensitivity values of 1, 0.88 and 0.97 were obtained for fresh, frozen-then-thawed and fresh or frozen-then-thawed products respectively. Quantitative models (PLS1 regression) using both 850-1098nm and 1100-2498nm wavelength ranges were developed to quantify (1) offal adulteration and (2) added fat in adulterated beefburgers, both fresh and frozen-then-thawed. Models predicted

added fat in fresh samples with acceptable accuracy (RMSECV= 2.0; RPD=5.92); usefully-accurate predictions of added fat in frozen-then-thawed samples were not obtained nor was prediction of total offal possible in either sample form.

Keywords: near infrared reflectance spectroscopy, partial least squares (PLS) regression, principal component analysis (PCA), soft independent modelling of class analogy (SIMCA), offal-adulterated beefburgers, authenticity

Introduction

Beef is an important component of the typical European diet. In 2012, beef consumption within the European Union reached 6.94 million tonnes, a 1% drop from the 2011 figure; however, overall beef consumption values for the last 50 years have been roughly constant.¹ In recent years, the cost of beef to the consumer has increased significantly.² Taken in combination with the recent economic downturn in Europe, consumers have turned to the purchase of cheaper beef cuts and beef products.² Commercial minced beef is produced from trimmings including forequarter cuts; compared to the relatively more expensive steak and beef joints (hindquarter cuts of beef), low-cost and convenient minced beef is popular as the main ingredient of commercially - manufactured meat products (e.g. beefburgers, meatballs, sausages etc.). In parallel with this shift of consumption to cheaper beef products, the issue of meat authenticity has become of increasing concern to consumers, regulatory bodies, trade groups and retailers especially after the recent horsemeat scandal in Europe.³ An

authentic product is one that is what it claims to be. Deliberate meat adulteration or fraud arises when an unscrupulous trader substitutes some or all raw meat with ingredients which are inferior and therefore of lower price.⁴ Adulterated meat products become much more difficult to uncover when the meat product is comminuted since all gross physical characteristics of the meat are lost. For this reason, a beefburger is a popular minced beef product that may offer opportunities for economic fraud.^{5,6} Common frauds may involve adulteration of beefburgers with other non-beef meats, mechanically - recovered meat, offal, egg or other cheaper proteins of unspecified animal species or vegetable origin.^{7,8}

In addition to economic impacts, meat adulteration can have food safety and religious repercussions. Since, by definition, adulterated meat products do not comply with quality considerations implied by name or label, consumers could be cheated or misled to purchase and consume products which are not desired. Food safety hazards may arise in the case of consumers who are intolerant of certain adulterants (e.g. egg or gluten intolerance); while mis-labelling can be critical for consumers with particular lifestyles (e.g. specialised diets) or religious (e.g. pork consumption is banned for Hindus and Muslims) concerns.⁸ Moreover, selling adulterated foods has economic repercussions for honest traders and consumers due to unfair competition.^{6,9} Therefore, in order to protect authentic foods and fight against food fraud, appropriate rapid analytical methods and procedures are required to confirm authenticity or detect adulteration.¹⁰

In the past three decades, a variety of screening methods such as chromatography,^{11,12} mass spectrometry,^{13,14,15} microscopy,¹⁶ enzymatic assays,¹⁷ electrophoresis¹⁸ and

DNA-based techniques (e.g. polymerase chain reaction (PCR) amplification; DNA hybridisation etc.)^{19,20} have been reported to detect adulteration of meat products . However, these methods may be expensive, time-consuming and require sophisticated laboratory facilities and procedures. More recently, spectroscopic methods, including near infrared (NIR) spectroscopy, have been shown to be effective in the detection of adulteration in the case of minced and related meat products. NIR with multivariate data analysis has been used to distinguish kangaroo meat from beef,⁶ lamb from beef,²¹ to discriminate between turkey, chicken, lamb, pork and beef²² and to detect and quantify pork, fat and offal in both fresh and frozen-thawed minced beef.⁹ Furthermore, NIR reflectance spectroscopy has been reported to detect adulteration of beefburgers by pork, mutton, skim milk powder and wheat flour.⁶ The two latter studies have been focused on the authenticity of minced beef; the general approach has been to detect adulteration based on a comparison of spectra of the matrices of the selected meat types plus adulterants with a standard model developed with a defined matrix of pure beef. However, most minced beef is consumed in the form of commercial products such as beefburgers and these are considerably more complex than simple minced beef since they can include other components such as water, extra fat and certain cereal products (rusk) used as binders and flavouring agents; these ingredients can account for a significant portion of beefburger weight. Some vegetables (spring onion, onion, etc.) may also be present in greater or lesser amounts. This picture is complicated even further by the availability of beefburgers which represent both higher and lower quality products; the former are generally purchased fresh from a butcher or retailer while the later are normally purchased in the frozen state from retailers.

In the present study, NIR spectroscopy has been investigated as a tool to detect the adulteration of higher and lower quality beefburgers by beef offal (heart, lungs, kidney and liver). Given that offal is considerably cheaper than beef, its incorporation into beefburger formulations would represent a significant cost saving. The authors are unaware of any other published method for detection of this particular adulteration. A key feature of this work was the use of an experimental design to maximise the variable space describing the adulterated beefburger formulations while also minimising the number of required experiments. This study therefore aimed to (1) develop classification and class-modelling models to distinguish between authentic and offal-adulterated beefburger formulations in both the fresh and thawed states and (2) quantify total offal adulteration and added fat content.

Materials and Methods

Beefburger formulation and manufacture

Beefburger formulation

Authentic beefburgers comprised two groups – so-called lean burgers and fat burgers – which correspond to higher and lower quality levels. Higher quality burgers contained only lean beef and beef fat; lean meat content was varied between 80 and 100% w/w of burger in 2.5% increments with fat accounting for the remainder. Lower quality burgers contained rusk (5% w/w) and water (20% w/w) in addition to lean beef (45 - 62.5% w/w in 2.5% increments) and beef fat (22.5 – 10% w/w in 2.5% increments). Burgers in each of the two groups were made on separate occasions

beginning with the highest lean meat content and moving to the lowest. Depending particularly on the quality of the lean meat purchased, production of either group could require more than 1 day. Each group of beefburgers was produced on two separate occasions; therefore, a total of 36 (18 higher quality and 18 lower quality) authentic beefburgers were prepared.

Adulterated beefburgers were formulated with lean beef, beef fat, water, rusk and offal (liver, lung, kidney and heart). Formulations were produced using a D-optimal experimental design (Design Expert v. 7.6.1, Stat-Ease Inc., Minneapolis, MN, USA) with minimum and maximum incorporation levels of meat (0-75% w/w), fat (0-25% w/w), water (0-15% w/w), rusk (0-5% w/w) set by the operator; each formulation also contained liver, lung, kidney and heart, each at 0-20% w/w. A total of 46 different beefburger formulations were generated by the design software to efficiently represent the design space for the multitude of possible combinations of the ingredients. These beefburgers were produced in random order over a period of several days, because of time constraints; each formulation was produced once. Adulterated beefburger formulations are shown in Table 1.

Variability in data sets of both authentic and adulterated beefburgers was maximised by sourcing meat and offal from 4 suppliers and on different occasions.

Beefburger manufacture

Fresh beef (brisket), beef offal (kidney, liver, lungs and heart) and beef fat were purchased from local stores and stored overnight at 4 °C at Teagasc Food Research

Centre Ashtown. Skin, fatty tissue, connective tissue, visible blood vessels and cartilage were removed to ensure the highest possible quality of the raw materials. Raw materials were cut into cubes, weighed and mixed according to each formulation before mincing; mixed meat samples were minced twice (Mainca meat mincer, Cheshire, England) through a mesh plate (5 mm diameter holes). In between the two mincing occasions and when required by the formulation, sifted pinhead rusk (Redbrook, Damastown, Dublin) and iced water were blended into the minced meat by hand for 2-3 minutes. After the second mincing step, the mixture was pressed into a standard beefburger mould. Between each formulation, the meat mincer was washed with warm water and detergent, rinsed with warm water and then wiped dry with tissue paper. Once produced, samples were placed in a tray sealed with cling film and stored overnight at 4 °C. Ten burgers were made for each formulation; four out of the ten were randomly picked for analysis as fresh samples. At the same time, two out of the ten were placed in storage at -20°C for 14 days prior to overnight thawing at room temperature and analysis.

In total, 82 fresh beefburger samples (36 authentic, 46 adulterated) and 82 frozen-then-thawed beefburger samples (36 authentic, 46 adulterated) were prepared. Two samples with one particular formulation were eliminated, one each from fresh and frozen-then-thawed adulterated sample sets. Their formulations were generated by the experimental design software but included no offal; therefore they were removed to avoid mis-calculations in the correct classification determinations.

Spectroscopic measurements

Before spectroscopic analysis, frozen beefburgers were removed from -20°C storage and allowed to thaw at room temperature for ~16 hours; fresh beefburgers were removed from chill storage at 4°C. Each fresh or frozen-then-thawed sample was homogenised using a Robot Coupe R301 ultra (Vincennes, France) for 30s and transferred into a sterile plastic container. The Robot Coupe mincing bowl was washed with detergent, rinsed with tap water and wiped dry after each sample.

For analysis, homogenised sample duplicates with re-packing were placed into a small ring cup (3.8 cm inner diameter) and a disposable backing disc applied. Packing pressure was standardised by feel and adjusted by varying sample thickness. Reflectance spectra [$\log (1/R)$] were recorded using a NIRSystems 6500 instrument (NIRSystems. Inc., Maryland, USA) over the wavelength range 400-2500 nm (2 nm intervals) and using a 16-32 reference-sample scan sequence. Samples were scanned in random order at ambient temperature (~20°C). Spectral acquisition and file conversion were performed using WINISI software (version 1.04; Infrasoft International, Port Matilda, USA). Each sample was scanned twice with rotation of the sample cup through ~ 120° between the sequential scans; the mean of these repetitions was used in later chemometric operations.

Chemometric operations

Spectra were exported from WINISI software as JCAMP.DX files and imported directly into The Unscrambler software (v9.7; Camo, Trondheim, Norway).

Raw spectral data in the range 850 to 1098nm were used in all chemometric operations. Qualitative models were also investigated using the 1100-2498nm wavelength range but results were inferior to the 850-1098nm range; therefore, results of this wavelength range will not be discussed in this paper. Quantitative models for offal and added fat prediction were developed using both spectral ranges. Spectra were used without modification (raw) and after pre-processing by multiplicative scatter correction (MSC), standard normal variate (SNV) transformation, Savitzky-Golay (1st derivative with 9 points and 2nd derivative with 17 points). Additionally, models were produced using the raw spectra and all of the pre-treatments in the order shown. These pre-treatments were able to remove baseline shifts, slope changes, scatter and other effects from spectral data; moreover, Savitzky-Golay derivatives reveal greater structure in the spectral data which should make it easier to interpret the chemical basis of the observed signals. Principal component analysis (PCA), partial least squares - discriminant analysis (PLS1-DA) and soft independent modelling of class analogy (SIMCA) were performed using The Unscrambler software (v9.7).

To discriminate between authentic and offal-adulterated beefburgers, PLS1-DA calibration models were developed and evaluated on separate calibration and validation sample sets. Each set represented approximately 50% of the total sample numbers and were selected on the basis of their position (spectral data of authentic and adulterated samples were separately sorted together) in the spectral file containing all sample spectra. Odd-numbered samples were selected as calibration samples while the remainder were used for validation. Calibration models were developed using full, i.e. leave-one-out, cross-validation; optimum model complexity was determined using the first local minimum in PLS loading plots. For PLS1-DA models, a dummy Y-

value was given to each sample – 0 for adulterated samples and 1 for authentic samples. During validation, samples with a predicted value ≥ 0.5 were identified as authentic while those with a value < 0.5 were identified as adulterated. Model performance was evaluated on the basis of percentage of correct classification.

In a class-modelling approach, SIMCA models of authentic beefburgers were developed using 50% of the authentic samples; model performance was estimated using the other 50% of the authentic samples and all the adulterated samples. A 5% significance value was utilised for membership determination. The characteristic limitation of SIMCA relates to the fact that principal components are calculated on the basis of variability in spectroscopic measurements alone and individual PCs may not be clearly related to chemical data of specific relevance in any class-model. Principal components describe decreasing amounts of variability in spectral data and the calculated components may not be directly linked to membership of any given class. In contrast, PLS regression involves Y-values in the calculation of PLS factors and therefore includes a targeted contribution to PLS scores from Y. In an attempt to improve SIMCA modelling, therefore, PLS scores were input to the SIMCA modelling process in a separate exercise. Model performances were evaluated on the basis of sensitivity, specificity and efficiency. Sensitivity is defined as the fraction of samples belonging to the modelled class which is correctly accepted by the respective model; specificity is that fraction of samples not belonging to the modelled class that is correctly rejected by the model.²² Efficiency summarises the sensitivity and specificity of model performance by calculating the geometric mean of their values.²² Index values of efficiency vary between 0 and 1. In our case, sensitivity was described as the fraction of authentic samples correctly identified by the model of

authentic beefburgers; specificity was the fraction of adulterated samples correctly rejected by the same model; efficiency was calculated as:

$$\text{Efficiency} = (\text{Sensitivity} * \text{Specificity})^{0.5}$$

PLS1-DA classification models and SIMCA models were developed separately using the spectral data of fresh and frozen-then-thawed. Models using spectral data of both fresh and frozen-then-thawed samples were also produced to detect samples which are in an unknown fresh or frozen-then-thawed state in future applications. In this case, 50% of total authentic and adulterated samples in both fresh and frozen-then-thawed states were utilized as calibration samples; the rest was for validation use.

To quantify offal adulteration and added fat in beefburgers, PLS1 linear regression models were developed; fresh and frozen-then-thawed beefburgers were modelled separately. Y- values were ascribed to each offal-adulterated sample according to total quantity (% w/w) of (a) offal (i.e. heart, liver, kidney and lung) and (b) added fat in each formulation. Models were developed using leave-one-out full cross-validation. The correlation coefficient of determination (r^2), root mean square error of cross-validation (RMSECV) and the residual predictive deviation (RPD) were used to evaluate these models. RPD values ≥ 3 are stated to be satisfactory for screening purposes, 5-10 are good for quality control and >10 are excellent.²³

Results and discussion

NIR spectral data

NIR spectra of fresh and frozen-then-thawed authentic and adulterated samples over the 850 to 1098nm wavelength range are shown in Figure 1(a-d). All spectra are very similar and show no potential to segregate them visually. There is one unusual spectrum off-scale which was clearly shown as an outlier by a PCA of the relevant spectral set. Therefore, it is not shown in the frozen-then-thawed authentic group (Figure 1b); this was most likely due to a sample presentation issue and it was removed before any data analysis was performed. These spectra reveal two major features; the first is a vertical offset likely due to particle size and packing density differences; while the second involves a single broad peak centred around 964 nm. This is likely due to absorbance by water.⁴ There is the suggestion of an increased range in $\log(1/R)$ values after freezing-and-thawing for both authentic and adulterated samples; this likely reflects physical changes in sample structure following freeze-thaw processes, involving the symmetric and asymmetric stretching vibration modes of the water molecules.^{24,25} A comparison of spectra from authentic and adulterated material suggests a greater range in $\log(1/R)$ values for the latter and slight differences in spectral shape.

Principal component analysis (PCA) was used to determine the major sources of variance in the overall dataset and to detect any unusual or outlying samples. Firstly, PCA was used to detect outlying samples within each group separately. In the cases of both fresh and frozen-then-thawed authentic samples, two components (PC1 and PC2) explained about 100% of the spectral variance; for frozen-then-thawed samples, three components accounted for 100% of the spectral variance.

PCA score plots for the fresh and frozen-then-thawed beefburger spectra are shown in Figure 2. For fresh beefburgers, PC1 and PC2 accounted for 94% and 5% of variance respectively. It is clear from Figure 2(a) that some separation between authentic (label-1) and adulterated (label-0) samples has occurred. The authentic samples have formed a relatively tight cluster while the adulterated sample cluster is more dispersed as might be expected given the different formulations involved. It is notable that the samples adulterated with similar formulations are located in the same region of the PCA plot; the samples adulterated at low level are located close to the authentic samples. (To avoid complexity and consequent difficulty in reading sample labels, this extra information was not added to Figure 2 a, c.) While the authentic sample cluster does not lie parallel to PC1, PC2 contributes significantly to the separation of the two groups. A plot of PC2 (Figure 2c) is shown as a relatively featureless line sloping from the highest intensity at 850nm (involving C-H stretch vibration from aliphatic compounds)²⁶ down to an apparent minimum at 1078nm (probably involving O-H stretch of water around 1065nm).²⁶ This loading is responsible for the relative location of authentic and adulterated samples in Figure 2a. The relevant PC1 loading plot shows a small broad peak at 960-980nm in an otherwise flat line which resembles the raw spectra (Figure 2c). Frozen-then-thawed beefburgers exhibited very similar behaviour, except that the spread of sample clusters larger than was the case for fresh beefburgers.

PLS1 – Discriminant Analysis

Accuracy of each individual PLS discriminant model was assessed on the basis of the percentage correct classification of validation sample sets. Summary results of PLS1-DA models are shown in Table 2.

In the case of fresh beefburger identification, the model developed using 2nd derivative (17-point Savitzky-Golay filter) pre-treated data and 8 PLS loadings gave the most accurate results. In this case, 100% (18 of 18 samples) of authentic samples and 95.45% (21 of 22 samples) of adulterated samples were predicted correctly. Models involving MSC, SNV, 1st derivative (9-point Savitzky-Golay filter) pre-treated data and 3 PLS loadings achieved 100% (18 of 18 samples) correct classification of authentic samples and 90.91% (20 of 22 samples) classification of adulterated samples. For frozen-then-thawed beefburgers, MSC pre-treated data produced accuracy figures for authentic and adulterated samples of 100% (18 of 18 samples) and 91.3% (21 of 23 samples) respectively. Moreover, in the case of identification of fresh or frozen-then-thawed samples, the model developed using MSC pre-treated data obtained correct classification rates of 100% (35 of 35 samples) of authentic samples and 88.89% (40 of 45 samples) of adulterated samples. It is noteworthy that all models identified authentic samples with 100% accuracy irrespective of spectral pre-treatment; the modelling challenge was therefore to correctly classify the adulterated material.

Examination of results for fresh beefburgers showed the same two adulterated samples (sample numbers 38 and 42) were mis-classified by three of the models (models using MSC, SNV and 1st derivative pre-treated data); sample no. 42 was also the single adulterated sample mis-classified by the model developed using 2nd

derivative pre-treated data. It is noteworthy that sample no. 38 contained 45% lean beef, 25% beef fat, 15% water, 5% rusk and 10% liver – only one offal adulterant at a low level. Sample 42 contained 65% lean beef, 15% water and 20% lung. However, these two samples were not the only adulterated samples which contain a single offal type; therefore, the failure to identify them correctly seems likely to arise from properties which are specific to these offal types.

The 2D scatter plots of the most accurate PLS model for fresh and frozen-then-thawed beefburgers are shown in Figure 3(a, c). In selecting the optimum number of PLS loadings for each PLS-DA model, the number of loadings producing the first local minimum was selected when the plots of residual Y-variance vs. no. of PLS loadings were examined. Figure 3 (b, d) are the regression coefficient plots corresponding with model (a) and (c) shown as Figure 3 (a, c). Spectral data pre-treatment methods of model (a) and model (c) are Savitzky-Golay filter (2nd derivative with 17 points) and MSC separately. Examination of regression coefficient plots showed multiple peaks mainly accumulated over the 912-980nm and 1004-1088nm wavelength ranges (fresh beefburgers Figure 3b) or 976nm (frozen-then-thawed beefburgers; Figure 3d). These peaks are likely to relate to absorbance of water (970nm),⁴ aliphatic acids and hydrocarbons (915-930nm ,1021nm ,1041nm)²⁷ and aromatic amine around 1015-1019nm, 1021-1026nm.²⁷ Differences in the regression coefficient plots shown in Figure 3 (b, d) arose chiefly from differences in spectral data pre-treatments. As an evidence, MSC-pre-treatment of spectral data for generation of model (a) produced a regression coefficient plot (not shown in this paper) similar to that in Figure 3d.

SIMCA modelling

420

421 For fresh samples, results of SIMCA modelling (Table 3) show that models produced
422 using MSC, SNV and Savitzky-Golay filter (2nd derivative with 17 points) pre-treated
423 data sets exhibited high sensitivity (1) and high specificity (0.84-1). In validation, all
424 authentic samples (18/18) and high numbers of adulterated samples (44/45 or 45/45)
425 were correctly identified. Efficiencies of model performance were in the range of
426 0.92-1. The model produced using raw spectral data performed worst of all on the
427 fresh adulterated samples. For the frozen-then-thawed samples, an efficiency value of
428 0.94 was obtained for the model using MSC pre-treated data; 15 out of 17 authentic
429 samples and all adulterated samples (45/45) were correctly identified. Models of fresh
430 or frozen-then-thawed samples exhibited efficiency values varying from 0.88 to 0.95;
431 the model (developed using MSC pre-treated data) with highest efficiency value
432 exhibited sensitivity (0.91) and specificity (0.99); 32 out of 35 authentic samples and
433 89 out of 90 adulterated samples were correctly identified.

434

435 SIMCA models were additionally developed using PLS scores extracted from the first
436 10 PLS loadings of the relevant PLS1-DA models, for the PLS1-DA models with
437 good performance can be developed within 10 PLS loadings (Table 2). Compared to
438 models based on PCA scores, models using PLS scores may be expected to contain
439 not only information relating to spectral variance but to corresponding Y-data i.e.
440 classification information. Results (Table 4) reveal improved classification accuracy
441 in the case of raw spectral data, especially for the fresh adulterated samples;
442 specificity value increased from 0.84 to 0.98 although this was accompanied by a
443 marginal reduction (from 1 to 0.94) in correct identification of authentic samples.
444 Efficiency value increased from 0.92 to 0.96. Moreover, results from raw, MSC and

SNV- treated spectra were quite similar; they all mis-classified the same adulterated sample – sample no. 9. For frozen-then-thawed beefburgers, results for the model produced using MSC-treated spectra (Table 4) gave the highest efficiency value, although raw and SNV- treated spectra produced similar outcomes. Only in the case of 2nd derivative spectra did model performance improve over the PCA-based equivalent (Table 3) with the specificity value increasing from 0.80 to 0.91. Classification accuracy of models for fresh or frozen-then-thawed samples also revealed improvements with the range of efficiency values increasing from 0.88-0.95 to 0.91-0.96. In Tables 3 and 4, slightly fewer principal components were used to form SIMCA models based on PCA scores. All things being equal, models formed with fewer principal components are preferred as they may be expected to be more robust.

PLS quantitation

Performance of PLS quantitative models for prediction of total offal adulteration in fresh and frozen-then-thawed samples using spectra in the 850-1098nm wavelength range (Table 5) was better than models developed using the 1100-2498nm spectral range; the latter will not be discussed here. Generally, models for prediction of total offal adulteration were not satisfactory, producing RPD values <3; RMSECV values were relatively high. For frozen-then-thawed samples, RPD values were lower (1.31-1.49) than those for fresh samples (1.69-2.31). Possible reasons for this difference must involve both the complex chemical composition of offal and effects, including light scatter, related to changes in sample physical structures during freezing and thawing steps. None of these models are suitable for practical usage.

Table 6 shows the performance of PLS quantitative models for predicting added fat in fresh and frozen-then-thawed samples over both the 850-1098nm and 1100-2498nm wavelength ranges. For fresh samples, RPD values of models are all >4. In particular, the model using 2nd derivative spectra (850-1098nm) and that using 1st derivative spectra (1100-2498nm) produced RPD values >5, which are claimed to be high enough for use in quality control. However, RPD values of models for frozen-then-thawed samples over both spectral ranges were <3, likely due again to the freezing and thawing procedures. Results of 1100-2498nm wavelength range were generally better than results using the other wavelength range, probably because of the greater fat informational content regarding in this range i.e. C-H combination regions of fatty acids around 1300-1550nm and above 2040nm; only one C-H combination region attributed to fatty acids can be detected in the 850-1098nm spectral range (around 960-1070nm).²⁸

Conclusions

This study has explored the potential of NIR reflectance spectroscopy to (1) discriminate between authentic and offal-adulterated beefburgers, and (2) quantify offal and added fat content in adulterated material. Use of an experimental design in formulation of offal-adulterated beefburgers facilitated efficient capture of the potentially large variation in this sample type. Using a multivariate discriminant approach, high levels of correct classification ($\geq 89\%$) were achieved for the adulterated validation samples; authentic material was identified with 100% accuracy. In the case of a SIMCA class-modelling approach, sensitivity values of models were

slightly higher for fresh than frozen-then-thawed and fresh or frozen-then-thawed authentic samples. Adulterated samples were predicted with similar specificity in the case of both fresh and frozen-then-thawed samples, slightly higher than specificity of models for fresh or frozen-then-thawed samples. The innovative use of PLS scores in the SIMCA modelling process resulted in only slight improvement in efficiency. Prediction of offal content proved not to be possible as might be expected, given the unlikely possession of a unique spectral signal by offal. Quantification of added fat content was possible with useful accuracy for fresh beefburger samples but not for frozen-then-thawed material.

It is anticipated that these methods may form the basis of a rapid and reliable tool to confirm general authenticity of beefburgers and especially to detect offal-adulterated products. More generally, the meat and other food industries require methods which will enable them to confirm with a high degree of confidence that their products are not adulterated. NIR spectroscopy combined with multivariate data treatment has shown the potential to provide a solution to meet this need. Such a wide-ranging solution will require the testing of large numbers of authentic commercial samples to establish classification boundaries and challenging of such boundaries with known adulterated material. This paradigm for using NIR spectroscopy will aid the development and uptake of Process Analytical Technology (PAT) in the fields of meat safety and quality control.

These results were obtained on a limited number of samples but they indicate the merit of a larger study, which would include vegetable and other flavouring

ingredients in formulations, to confirm the commercial regulatory utility of this approach.

Acknowledgements

This project was financed by the Food Safety Authority of Ireland; opinions expressed in this paper are those of the authors solely. We thank Dr. Derek Keenan, Mr. Eugene Vesey and Ms. Astrid Malechaux for technical support. Ming Zhao is a Teagasc Walsh Fellow.

References

1. BordBia, "Meat and livestock review & outlook 2011/12," <http://www.bordbia.ie/industryservices/information/publications/MeatLivestockReview/Meat%20and%20Livestock%20-%20Review%202011-12.pdf> (2012).
2. J. Burke, "Lower priced cuts absorb cattle price increase," <http://www.farmersjournal.ie/site/farming-Lower-priced-cuts-absorb-cattle-price-increase-16459.html> (2013).
3. http://en.wikipedia.org/wiki/2013_meat_adulteration_scandal#cite_note-51
4. N. Z. Ballin, "Authentication of meat and meat products," *Meat Sci.* **86** (3), 577-587 (2010).
5. G. Downey and D. Beauchêne, "Authentication of fresh vs. frozen-then-thawed beef by near infrared reflectance spectroscopy of dried drip juice," *LWT - Food Sci. Technol.* **30**(7), 721-726 (1997).
6. H. B. Ding and R. J. Xu, "Near-Infrared spectroscopic technique for detection of beef hamburger adulteration," *J. Agr. Food Chem.* **48**(6), 2193-2198 (2000).
7. O. Al-Jowder, E. K. Kemsley, and R. H. Wilson, "Mid-infrared spectroscopy and authenticity problems in selected meats: a feasibility study," *Food Chem.* **59**(2), 195-201 (1997).

8. O. Al-Jowder, E. K. Kemsley, and R. H. Wilson, "Mid-infrared spectroscopy and chemometrics for the authentication of meat products," *J. Agr. Food Chem.* **47**, 3210-3218 (2002).
9. N. Morsy and D.-W. Sun, "Robust linear and non-linear models of NIR spectroscopy for detection and quantification of adulterants in fresh and frozen-thawed minced beef," *Meat Sci.* **64**(4), 587-591 (2013).
10. G. Downey, "Food and food ingredient authentication by mid-infrared spectroscopy and chemometrics," *TrAC-Trend Anal. Chem.* **17** (7), 418-424 (1998).
11. W. N. Sawaya, T. Saeed, M. Mameesh, E. El-Rayes, A. Husain, S. Ali, and H. Abdul Rahman, "Detection of pork in processed meat: Experimental comparison of methodology," *Food Chem.* **37**(3), 201-219 (1990).
12. S. H. Ashoor, W.C. Monte and P.G. Stiles, "Liquid chromatographic identification of meats," *J. AOAC Int.* **71**(2), 397-403 (1988).
13. J. Lembcke, U. Ceglarek, G.M. Fiedler, S. Baumann, A. Leichtle and J. Thiery, "Rapid quantification of free and esterified phytosterols in human serum using APPI-LC-MS/MS," *J. Lipid Res.* **46**(1), 21-26 (2005).
14. P. Varelis and R. Jeskelis, "Preparation of [13C3]-melamine and [13C3]-cyanuric acid and their application to the analysis of melamine and cyanuric acid in meat and pet food using liquid chromatography-tandem mass spectroscopy," *Food Addit. Contam.* **25**(10), 1208-1215 (2008).
15. G. Frick, S. Dubois, C. Chaubert and S. Ampuero, "Identification by microscopy and MS-based electronic nose of a fraudulent addition to maize gluten," *Biotechnol. Agron. Soc. Environ.* **13**(S), 45-50 (2009).
16. K. M. Angood, J. D. Wood, G. R. Nute, F. M. Whittington, S. I. Hughes and P. R. Sheard, "A comparison of organic and conventionally-produced lamb purchased from three major UK supermarkets: Price, eating quality and fatty acid composition," *Meat Sci.* **78**(3), 176-184 (2008).
17. N. K. Sharma, A. K. Srivastava, J. P. S. Gill, and D. V. Joshi, "Differentiation of meat from food animals by enzyme assay," *Food Control* **5**(4), 219-221 (1994).
18. A. Skrökki and O. Hormi, "Composition of minced meat part A: Methods," *Meat Sci.* **38**(3), 497-501 (1994).

19. K. F. Ebbehøj and P. D. Thomsen, "Species differentiation of heated meat products by DNA hybridization," *Meat Sci.* **30(3)**, 221-234 (1991).
20. K. F. Ebbehøj and P. D. Thomsen, "Differentiation of closely related species by DNA hybridization," *Meat Sci.* **30(4)**, 359-366 (1991).
21. J. McElhinney, G. Downey, and C. O'Donnell, "Quantitation of Lamb Content in Mixtures with Raw Minced Beef Using Visible, Near and Mid-Infrared Spectroscopy," *J. Food Sci.* **64(4)**, 587-591 (1999).
22. J. McElhinney, G. Downey and T. Fearn, "Chemometric processing of visible and near infrared reflectance spectra for species identification in selected raw homogenised meats," *J. Near Infrared Spec.* **7**, 145-154 (1999).
23. P. Oliveri and G. Downey, "Multivariate class modelling for the verification of food-authenticity claims," *TrAC-Trend Anal. Chem.* **35**, 74-86 (2012).
24. P. C. Williams and D. C. Sobering, "Comparison of commercial near infrared transmittance and reflectance instruments for analysis of whole grains and seeds^a," *J. Near Infrared Spec.* **1**, 25-32 (1993).
25. J. Workman and J.L. Weyer, Practical Guide to Interpretive Near-Infrared Spectroscopy. CRC Press, Oxon, UK, p.63-65 (2008).
26. D. Bertrand and É. Dufour, La spectroscopie infrarouge et ses applications analytiques (2 e édition). TEC & DOC, Paris, France, p.100-101 (2000).
27. D. Burns and E. Ciurczak, Handbook of New Infrared Analysis (2nd ed.), Marcel Dekker, p.431-433 (2001).
28. O.H. Wheeler, "Near-Infrared spectra of organic compounds," *Chem. Rev.* **59**, 629-666 (1959).

Table 1. D-optimal experimental design of adulterated beefburger formulations.

Table 2. Summary of PLS discriminant models performance (850-1098nm) on (1) fresh, (2) frozen-then-thawed and (3) fresh or frozen-then-thawed validation samples. (Most accurate models in bold)

Table 3. Summary of SIMCA model performances (850-1098nm) on (1) fresh, (2) frozen-then-thawed and (3) fresh or frozen-then-thawed validation samples. (Most accurate models in bold)

Table 4. Summary of SIMCA model performances (850-1098nm) based on PLS scores of (1) fresh, (2) frozen-then-thawed and (3) fresh or frozen-then-thawed validation samples. (Most accurate models in bold)

Table 5. Summary of PLS quantitative models (850-1098nm) for prediction of total offal adulteration of fresh and frozen-then-thawed samples. (Most accurate models in bold)

Table 6. Summary of PLS quantitative models (850-1098nm, 1100-2498nm) for prediction of added fat content of fresh and frozen-then-thawed samples. (Most accurate models in bold)

Figure 1. NIR reflectance [$\log(1/R)$] spectra of fresh and frozen-then-thawed beefburgers (850-1098nm). (a) fresh authentic; (b) frozen-then-thawed authentic; (c) fresh adulterated; (d) frozen-then-thawed adulterated.

Figure 2. Score plots of all beefburger samples (a) fresh, (b) frozen-then-thawed; PC1 and PC2 loading plots of all beefburger samples (c) fresh, (d) frozen-then-thawed.

Figure 3. (a) Prediction of fresh beefburger validation samples by PLS1 using Savitzky Golay filter (2nd derivative with 17 points) pre-treated data; (b) regression coefficient plots of the model of fresh samples. (c) Prediction of frozen-then-thawed beefburger validation samples by PLS1 using MSC pre-treated data; (d) regression coefficient plots of the model of frozen-then-thawed samples. [Numbers in figures (a) and (c) are sample ID numbers]

Table 1

Run	% Meat	%Fat	%Water	%Rusk	%Heart	%Liver	%Kidney	%Lung
1	0	25	10	5	20	20	20	0
2	32.5	12.5	15	0	20	0	0	20
3	30	0	15	5	0	20	20	10
4	75	0	0	5	0	0	20	0
5	75	25	0	0	0	0	0	0
6	20	25	0	5	0	10	20	20
7	47.5	12.5	0	0	0	0	20	20
8	60	0	0	0	0	20	0	20
9	42.5	25	7.5	5	0	0	0	20
10	20	25	15	0	0	20	0	20
11	0	5	15	0	20	20	20	20
12	22.5	12.5	0	5	0	20	20	20
13	55	0	15	0	10	0	20	0
14	65	0	0	5	0	0	10	20
15	17.5	25	15	2.5	20	0	20	0
16	60	0	0	0	20	20	0	0
17	30	25	15	0	20	10	0	0
18	60	0	0	0	20	0	0	20
19	10	10	15	5	20	0	20	20
20	45.5	10.4	6.4	0	9.4	9.4	9.4	9.5
21	45.5	10.4	6.4	0	9.4	9.4	9.4	9.5
22	52.5	25	0	2.5	0	20	0	0
23	0	25	15	0	0	20	20	20
24	25	0	0	5	20	20	20	10
25	37.5	0	0	2.5	20	0	20	20
26	30	0	15	5	20	20	10	0
27	60	0	0	0	0	20	20	0
28	55	0	15	0	10	0	20	0
29	50	0	15	5	20	0	0	10
30	75	0	2.5	2.5	20	0	0	0
31	30	0	15	5	0	20	20	10
32	60	0	0	0	20	0	20	0
33	40	25	0	5	20	0	10	0
34	20	25	15	0	0	20	20	0
35	30	0	15	5	10	20	0	20
36	30	0	15	5	10	20	0	20
37	0	25	15	5	20	20	0	15
38	45	25	15	5	0	10	0	0
39	37.5	25	15	2.5	0	0	20	0
40	65	0	15	0	0	20	0	0
41	0	25	15	0	20	0	20	20
42	65	0	15	0	0	0	0	20
43	20	25	0	5	20	10	0	20
44	40	25	0	5	20	0	10	0
45	0	20	0	0	20	20	20	20
46	52.5	0	7.5	0	0	0	20	20

Table 2

Sample type	Data Type	No. of PLS loadings	% Correct classification of validation sample set	
			Authentic	Adulterated
Fresh	Raw	2	100 (18/18)	86.36 (19/22)
	MSC - treated	3	100 (18/18)	90.91 (20/22)
	SNV - treated	3	100 (18/18)	90.91 (20/22)
	1stDer4+4 - treated	3	100 (18/18)	90.91 (20/22)
	2ndDer8+8 - treated	8	100 (18/18)	95.45 (21/22)
Frozen-then-thawed	Raw	5	100 (17/17)	82.61 (19/23)
	MSC - treated	3	100 (17/17)	91.30 (21/23)
	SNV - treated	3	100 (17/17)	86.96 (20/23)
	1stDer4+4 - treated	11	100 (17/17)	73.91 (17/23)
	2ndDer8+8 - treated	8	100 (17/17)	86.96 (20/23)
Fresh or Frozen-then-thawed	Raw	2	100 (35/35)	80.00 (36/45)
	MSC - treated	3	100 (35/35)	88.89 (40/45)
	SNV - treated	2	100 (35/35)	68.89 (31/45)
	1stDer4+4 - treated	10	100 (35/35)	84.44 (38/45)
	2ndDer8+8 - treated	10	100 (35/35)	88.89 (40/45)

Table 3

Sample type			Model performance		
Fresh	Data Type	Principal component used	Sensitivity	Specificity	Efficiency
	Raw	1	1	0.84	0.92
	MSC - treated	1, 2	1	1	1
	SNV - treated	1, 2, 3,	1	0.98	0.99
	2ndDer8+8 - treated	1, 2, 3, 4	1	0.98	0.99
Frozen-then-thawed	Raw	1	0.82	0.96	0.90
	MSC - treated	1, 2	0.88	1	0.94
	SNV - treated	1, 2, 3, 4	0.82	1	0.91
	2ndDer8+8 - treated	1, 2, 3	0.82	0.80	0.81
Fresh or Frozen-then-thawed	Raw	1	0.91	0.94	0.92
	MSC - treated	1,2	0.91	0.99	0.95
	SNV - treated	1,2,3	0.91	0.97	0.94
	2ndDer8+8 - treated	1,2,3	0.94	0.82	0.88

Table 4

Sample type			Model performance		
Fresh	Data Type	Principal component used	Sensitivity	Specificity	Efficiency
	Raw	1, 2, 3	0.94	0.98	0.96
	MSC - treated	1, 2, 3	1	0.98	0.99
	SNV - treated	1, 2, 3	1	0.98	0.99
	2ndDer8+8 - treated	1, 2, 3, 4, 5	1	1	1
Frozen-then-thawed	Raw	1	0.82	0.96	0.89
	MSC - treated	1, 2, 3	0.88	1	0.94
	SNV - treated	1, 2, 3	0.82	0.98	0.90
	2ndDer8+8 - treated	1, 2, 3, 4	0.82	0.91	0.87
Fresh or Frozen-then-thawed	Raw	1,2,3	0.89	0.99	0.94
	MSC - treated	1,2,3	0.89	0.98	0.93
	SNV - treated	1,2,3	0.97	0.94	0.96
	2ndDer8+8 - treated	1,2,3	0.97	0.96	0.96

Table 5

Sample type	Data type	No. of loadings	r^2	RMSECV	RPD
Fresh	raw	11	0.82	6.7	2.31
	MSC-treated	11	0.81	6.8	2.28
	SNV-treated	3	0.66	9.2	1.70
	1stDer4+4-treated	5	0.66	9.2	1.69
	2ndDer8+8-treated	6	0.73	8.2	1.89
Frozen-then-thawed	raw	6	0.56	10.4	1.49
	MSC-treated	3	0.52	11.0	1.41
	SNV-treated	3	0.55	10.5	1.47
	1stDer4+4-treated	6	0.43	11.9	1.31
	2ndDer8+8-treated	6	0.45	11.6	1.34

Table 6

Wavelength range 850-1098nm	Data type	No. of loadings	r^2	RMSECV	RPD
Fresh	raw	6	0.96	2.4	4.93
	MSC-treated	6	0.95	2.7	4.29
	SNV-treated	6	0.95	2.7	4.32
	1stDer4+4- treated	4	0.95	2.5	4.56
	2ndDer8+8- treated	3	0.96	2.2	5.17
Frozen-then- thawed	raw	7	0.81	5.1	2.29
	MSC-treated	6	0.81	5.0	2.30
	SNV-treated	6	0.82	5.0	2.32
	1stDer4+4- treated	4	0.82	5.0	2.33
	2ndDer8+8- treated	3	0.84	4.6	2.50
1100-2498nm					
Fresh	raw	3	0.96	2.4	4.81
	MSC-treated	4	0.96	2.4	4.85
	SNV-treated	5	0.97	2.1	5.55
	1stDer4+4- treated	3	0.97	2.0	5.92
	2ndDer8+8- treated	1	0.96	2.3	4.98
Frozen-then- thawed	raw	3	0.86	4.3	2.67
	MSC-treated	4	0.86	4.4	2.62
	SNV-treated	4	0.86	4.4	2.61
	1stDer4+4- treated	2	0.87	4.3	2.69
	2ndDer8+8- treated	1	0.86	4.3	2.69

Figure 1

Figure 2

Figure 3