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Detection of adulteration in fresh and frozen beefburger products by beef offal using mid-infrared ATR spectroscopy and multivariate data analysis

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Abstract

A series of authentic and offal-adulterated beefburger samples was produced. Authentic product (36 samples) comprised either only lean meat and fat (higher quality beefburgers) or lean meat, fat, rusk and water (lower quality product). Offal adulterants comprised heart, liver, kidney and lung. Adulterated formulations (46 samples) were produced using a D-optimal experimental design. Fresh and frozen-then-thawed samples were modelled, separately and in combination, by a classification (partial least squares discriminant analysis) and class-modelling (soft independent modelling of class analogy) approach. With the former, 100% correct classification accuracies were obtained separately for fresh and frozen-then-thawed material. Separate class-models for fresh and frozen-then-thawed samples exhibited high sensitivities (0.94 to 1.0) but lower specificities (0.33 – 0.80 for fresh samples and 0.41 – 0.87 for frozen-then-thawed samples). When fresh and frozen-then-thawed samples were modelled together, sensitivity remained 1.0 but specificity ranged from 0.29 to 0.91. Results indicate a role for this technique in monitoring beefburger compliance to label.

Keywords: beefburger; authenticity; adulteration; offal; class-modelling; discrimination.

1. Introduction

Intentional or economically-motivated adulteration of food has recently been defined as “the fraudulent addition of non-authentic substances or removal or replacement of authentic substances without the purchaser’s knowledge for economic gain of the seller” (Moore, Spink and Lipp, 2012). Recent scandals involving adulteration of milk powder with melamine (BBC, 2010), spices with Sudan Red dye (EC, 2010), milk with glucose and skim milk powder (Times of India, 2012) and processed beef products with horsemeat (Reuters, 2013) have reverberated around the world and impinged on the consciousness of informed consumers. Not only have these scandals involved economic fraud but the Sudan Red and melamine adulterations in particular raised significant issues of food safety which had not always been associated with food adulteration incidents. A recent trend in the USA has been to consider all food adulterations as potential food safety issues on the basis that most people adulterating food are assumed to have limited or no knowledge of any potential food safety implications of their acts. To offer greater protection to society, therefore, a regulatory strategy based on the assumption that food safety may be compromised by adulteration is merited (Moore, Spink and Lipp, 2012).

Meat is an important constituent of diets of the developed world; consumption in the developing world is also increasing rapidly. In the USA and UK, the most important meat sources are pigs, sheep and cattle while in other regions such as India, the Middle East and Africa, goat and camel are the main meats consumed. Meat products such as sausages, burgers, pork pies, etc. account for almost half of all meat consumed in developed countries. In many of these countries, beef consumption has been in slow decline and has lost market share at the expense of pork and poultry (chicken) in

particular (Kearney, 2010). Meat offal consumption in Ireland is intermittent and generally in decline (McCarthy and Barton, 1998), thereby creating a challenge for the beef industry to find a market outlet for offal materials.

Once comminuted, beef is no longer identifiable as such to the naked eye (McElhinney, Downey and O'Donnell, 1999). When they buy such products, consumers rely on a producer's reputation for reassurance that products are actually what they claim to be. Beefburgers represent a significant class of comminuted beef product which is regularly consumed by many people in the developed world. In 2012, 1.1 billion hamburgers were produced annually to supply McDonald's outlets in Ireland and Britain alone (Irish Farmers Journal, 2012). Consumer trust is of critical importance to the modern food processing industry (Gellynck, Verbeke and Vermeire, 2006) and the meat sector has been subjected to a number of food scares in the recent past e.g. the Belgian and Irish dioxin crises and outbreaks of foot-and-mouth disease in several countries. These events each produced a sudden reduction in demand for meat products followed by a slow and often incomplete recovery (Verbeke, 2001). The immediate and dramatic impact of the BSE outbreak in 2000 on home meat consumption in Spain (down 12%), France (decrease of 40%), Germany (reduction of 60%), Italy (42% drop) and Portugal (30% reduction) has been documented (Angulo and Gil, 2007). Of additional concern is the extent to which consumer responses to a specific food scare may impact on other, even unrelated, foods and the purchasing decisions consumers subsequently make. Food safety is increasingly recognised as a credence attribute and consumers depend on trust in the processing industry, regulators and retailers to avoid the purchase of food which may be deleterious to health (Lobb, Mazzocchi and Traill, 2007). Analysis of information processing by

112 consumers during a food crisis is not readily available (Grunert, 2002) but it seems
113 reasonable to anticipate a general increase in distrust in the food processing industry
114 following food crisis events. Development of control mechanisms capable of
115 detecting potential safety problems as early as possible in the food chain are therefore
116 in the interest of producers, processors, retailers and consumers alike.
117 Detection of adulteration in meat and meat products using vibrational spectroscopic
118 techniques has been the subject of some previous reports (Karoui, Downey & Blecker,
119 2010; Rodriguez-Saona and Allendorf, 2011; Rohman et. al., 2011; Rohman and Che
120 Man, 2011; Weeranantanaphan et al., 2011); research efforts have also been
121 expended on various data analysis approaches (Panagou et al., 2011; Argyri et al.,
122 2013) and emerging techniques such as image analysis (Dissing et al., 2013).
123 Admixture of offal with beef to produce beefburgers is a potential adulteration issue
124 and no established methods are currently available to detect such adulteration. A
125 number of publications have demonstrated experimental models based on near
126 infrared or mid-infrared spectroscopy for the detection of offal or meat from other
127 species in mixtures with minced beef (McElhinney, Downey and O'Donnell, 1999;
128 Meza-Márquez, Gallardo-Velázquez, Osorio-Revilla, 2010; Al-Jowder, Defernez,
129 Kemsley and Wilson, 1999, 2002; Prieto, Roehe, Lavín, Batten and Andrés, 2009;
130 Norsy and Sun, 2013; McElhinney, Downey and Fearn, 1999). However, with
131 particular regard to offal, these studies were often based on simple admixtures and the
132 aim of some reports was to quantify offal content in minced meat products which the
133 authors knew to contain offal. The actual type of offal involved was also often not
134 clearly described. In this work we undertook experiments (1) which included
135 authentic and adulterated beefburgers of two quality categories to correspond to
136 commercial practice, (2) made use of an experimental design approach to facilitate the

involvement of four types of offal (heart, lungs, kidney and liver) and other normal components (water and rusk) either individually or in combination so as to cover the widest possible range of inclusion values and (3) studied the products developed in both the fresh and frozen-then-thawed states to be consistent with normal commercial practice for the lower quality beefburgers in particular. The goal was to develop a capability to discriminate between authentic beefburgers and beefburgers adulterated with offal irrespective of the quality class (high or low) of the meat product.

2. Material and methods

2.1 Beefburger formulation and manufacture

Authentic beefburgers were produced in two groups – called lean and fat– which correspond to higher (lean) and lower (fat) quality levels. Higher quality beefburgers contained only lean beef and beef fat. Lean meat content was varied between 80 and 100% $^{w}/_{w}$ of burger in 2.5% $^{w}/_{w}$ increments with fat accounting for the remainder. Lower quality beefburgers contained rusk (5% $^{w}/_{w}$) and water (20% $^{w}/_{w}$) in addition to lean beef (45 - 62.5% $^{w}/_{w}$ in 2.5% $^{w}/_{w}$ increments) and beef fat (22.5 – 10% $^{w}/_{w}$ in 2.5% $^{w}/_{w}$ increments). Beefburgers 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 sometimes required 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 was prepared.

Adulterated beefburgers were formulated with lean beef, beef fat, water, rusk and offal (liver, lung, kidney and heart). Formulations were produced according to 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% $^{w}/_{w}$) and rusk (0-5% $^{w}/_{w}$) set by the operator; each formulation also contained liver, lung, kidney and heart, each at variable amounts in the range 0-20% $^{w}/_{w}$. A total of 46 different beef burger formulations was generated by the design software to efficiently represent the design space for the multitude of possible combinations of these ingredients. These beefburgers were produced over a period of several days; each formulation was produced once. The recipe formulations followed in this work are shown in Table 1.

Table 1 near here

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 manually excised 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 tap water were blended into the minced meat by hand for 2-3 mins. 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 ºC. Ten burgers were made for each formulation; four out of the ten were randomly-selected for analysis as fresh samples on the following day; two out of the ten were stored at -20ºC for 14 days prior to thawing 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. One of the formulations dictated by the experimental design software (run #5) contained only beef and beef fat and therefore properly belonged to the authentic class of product. To avoid confusion, both of these samples were eliminated, one each from fresh and frozen-then-thawed adulterated sample sets.

2.2. 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 container. The Robot Coupe mincing bowl was washed with detergent, rinsed with tap water and wiped dry after each sample.

For spectroscopic analysis, homogenised samples were placed onto the in-compartment benchmark horizontal attenuated total reflectance (ATR) ZnSe crystal (11 internal reflections; Specac Ltd., Kent, UK) of a Bio-Rad Excalibur FTS3100 mid-infrared spectrometer (Bio-Rad, Philadelphia, USA). Samples were applied so as to cover the ATR crystal surface entirely and were pressed to the crystal surface using

a spatula to ensure efficient contact between sample and crystal along length of the latter. ATR spectra [log(1/R)] were recorded over the wavelength range 800-4000 cm^{-1} at a nominal resolution of 4 cm^{-1}; 64 sample scans were averaged and corrected using an air blank reference scan which was recorded before each sample. Samples were scanned in random order at ambient temperature (~20ºC). Spectral acquisition and file conversion were performed using manufacturer's software (Resolutions Pro v 4.0) Each sample was scanned in duplicate (with sub-sampling) and the mean of these duplicates was used in later chemometric operations.

2.3. Data analysis

Spectra were exported from Resolutions Pro software in GRAMS format and imported directly into The Unscrambler (v 9.7; CAMO A/S, Trondheim, Norway) for all multivariate operations. Spectra in the attenuated fingerprint range (900-1800 cm^{-1}; 467 variables) were used in all chemometric operations. Preliminary data analysis was performed by principal component analysis (PCA) of mean-centred, unmodified spectral data. Models were constructed using raw spectral data and after pre-processing by multiplicative scatter correction (MSC), standard normal variate (SNV) transformation and Savitzky-Golay derivatisation (1^{st} derivative with 9 points, 2^{nd} derivative with 11 points). 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. Calibration models were developed and evaluated on separate calibration and validation sample sets. Each set represented approximately 50% of the total sample numbers which were selected on the basis of their position in the spectral file. Samples 1, 3, 5 etc until the end 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 and best models were selected based on a number of criteria including the position of the first local minimum in the leverage and residual X-variance plots, the correct classification rate, the number of loadings or components involved and whether data pre-treatment was required. Models were developed separately for fresh and frozen-then-thawed samples and both in combination.

For PLS1-DA models, a dummy Y-value was given to each sample – a value of 0 was given to all adulterated samples and a value of 1 to all the authentic samples. After prediction on the validation sample set, a sample with a predicted Y-value ≥ 0.5 was identified as authentic while a Y-value <0.5 was identified as adulterated. In subsequent steps, models involving a reduced number of spectral variables were developed following application of the Martens Uncertainty Test (Martens and Martens, 2000), as reduction in variable numbers may lead to increased model stability and also enable transfer of models to cheaper instrumentation. Performance of these models was then compared to those using the full fingerprint spectral region.

SIMCA models of authentic beefburgers were developed using 50% of the authentic samples; model performance was estimated using all remaining samples. A 5% significance value was utilised for membership determination. A limitation of SIMCA involves the fact that the principal components are calculated on the basis of spectroscopic measurements and these need not be clearly related to chemical data of relevance in any particular class-model. In contrast, PLS regression involves Y-data in the calculation of PLS factors and scores. In an attempt to improve SIMCA-modelling,

261 therefore, PLS scores were input to the modelling process in a separate exercise.
262 Model performances were evaluated on the basis of sensitivity, specificity and
263 efficiency. Sensitivity is defined as the fraction of samples belonging to the modelled
264 class which is correctly accepted by the respective model; specificity is that fraction
265 of samples not belonging to the modelled class that is correctly rejected by the model
266 (Oliveri and Downey, 2012). Efficiency summarises the sensitivity and specificity of
267 model performance by calculating the geometric mean of their values (Oliveri and
268 Downey, 2012). Index values of efficiency vary between 0 and 1. In our case,
269 sensitivity was described as the fraction of authentic samples correctly identified by
270 the model of authentic beefburgers. Specificity was the fraction of adulterated
271 samples correctly rejected by the same model. Efficiency was calculated as:

272 $$\text{Efficiency} = (\text{Sensitivity}*\text{Specificity})^{0.5}$$

273 Conventional PLS regression was applied to try to quantify content of total offal and
274 individual offal types in adulterated beefburgers but no model of acceptable accuracy
275 was obtained. This is most likely due to the absence of any specific, detectable FT-IR
276 signal specifically associated with any or all of the offal types tested and at the levels
277 encountered in this study. Given that the offal was obtained from the same animal
278 species, this may be expected but it also raises the possibility that offal from different
279 species may be detected.
280

281 # 3. Results and discussion

282

283 **3.1 Spectral data**

284 A plot of the untransformed spectral data of all samples in the 900-1800 wavenumber
285 range is shown in Figure 1a. Plots of mean spectra for authentic and adulterated

material are shown in Figures 1b and 1c respectively. These spectra show typical noise levels for this type of sample and sample presentation but ,on either side of

Figure 1 near here

the 900-1800 fingerprint wavenumber segment , spectral noise was judged by visual examination to be excessive and, unless otherwise stated, chemometric operations were performed using this attenuated range only. In general, the beefburger spectra appear very similar with an obvious baseline offset between individual samples; the main features are a large absorbance peak centred around 1640 cm^{-1}, ascribed to O-H bending vibration of water (Al-Jowder, Defernez, Kemsley and Wilson, 1999) and amide I (C=O) stretching vibrations (Meza-Márquez, Gallardo-Velázquez and Osorio-Revilla, 2010) together with and the, with smaller but significant peaks around 1543 and 1556 cm^{-1} probably arising from amide II absorptions (Al-Jowder, Defernez, Kemsley and Wilson, 1999). Apparent spectral detail around the 1640 wavenumber peak is more likely to may reflect differences in water structure rather than secondary structure of the different muscle proteins because of the relative absorbance of these two moieties. Spectral differences may be related to different degrees of hydration of e.g protein. Smaller, broader peaks are evident centred around 1026 and 1082 cm^{-1} with a double peak feature at 1157 and 1176 wavenumbers. Absorptions in the 950-1200 cm^{-1} region have been reported to reflect the content of carbohydrate moieties, specifically muscle glycogen (Al-Jowder, Defernez, Kemsley and Wilson, 1999) while the weakly absorbing amide III band is found around 1300 cm^{-1} (Bandekar, 1992) , absorptions of amino acid side-chains occur between 1480 – 1800 wavenumbers (Goormaghtigh, Cabiaux and Ruysschaert, 1994) and peptide bonds absorb between 1500-1700 cm^{-1}. A peak around 1744 cm^{-1} is generally taken as arising from C=O absorptions in lipids (Al-Jowder, Defernez, Kemsley and Wilson,

1999) and this did exhibit considerable from sample to sample depending on formulation. Relatively strong absorptions arising from acyl chain C-H linkages were also found between 2800 and 3000 cm^{-1} (not shown) while absorption from triacylglycerol ester linkage C-O has been reported around 1175 cm^{-1} (Dufour, 2009). Some small sharp details are also evident at 1734, 1748, 1773 and 1792 cm^{-1} while two peaks centred around 1458 and 1472 cm^{-1} were also a feature of all spectra. In summary, interpretation of spectra is complex but absorptions found in this work closely resemble those previously published (Meza-Márquez, Gallardo-Velázquez and Osorio-Revilla, 2010; Al-Jowder, Defernez, Kemsley and Wilson, 1999; 2002) and fit in with expectations based on muscle food composition. There is no obvious, visible segregation into fresh and frozen-then-thawed samples or authentic or adulterated material detectable in these spectra.

Principal component analysis (PCA) was used to determine the major sources of variance in the total dataset and to detect any unusual or outlying samples. PCs 1 to 3 accounted for 72.9, 11.3 and 8.4% respectively of the variability in the complete (i.e. fresh and frozen-then-thawed authentic and adulterated) spectral data set. The first 6 PCs accounted for 98.8% of total variability. No clear separation between fresh and frozen-then-thawed samples was discernible in any of the scores plots (not shown) although PC1 did suggest some limited separation. This indicates that the processing history of these samples did not impact significantly on their FT-IR spectra. When comparing authentic vs adulterated samples, the only discernible pattern was a more diffuse clustering of the adulterated beefburger scores than those of authentic material. When fresh samples only were studied, a limited separation of the majority of authentic and adulterated samples was evident in the scores plot of PC 1 and 6 (Figure 2a).

Figure 2 near here

Interpretation of the PC6 loading plots (Figure 2b) is not straightforward but the major features are peaks centred at 1540 and 1650 cm^{-1} with smaller, broader maxima around 960 and 1136-1176 wavenumbers. A broad interpretation of this loading pattern would be that protein (content and/or structure) and lipid components account for the separation obtained between authentic and adulterated beefburger samples. In the case of frozen-then-thawed samples only, tentative separations were visible in certain plots (e.g. PC 3 versus PC2; Figure 2c) but these were generally not as definitive as was the case for fresh samples. The loading plot for PC3 (Figure 2d) revealed a profile with major maxima around 1175, 1510 and 1749 wavenumbers; there were also minima centred at 1026 and 1640 cm^{-1}. Features at 1749 and 1175 wavenumbers are indicative of lipid involvement, which may be expected given the formulation of adulterated beefburgers and the lipid content of certain offal types e.g. liver. Interestingly, these features are in opposition to protein-related absorbances i.e. around 1640 cm^{-1}.

3.2 PLS modelling

Models were developed separately and together for fresh and frozen-then-thawed samples. Accuracy of each PLS discriminant model was assessed on the basis of the percentage correct classification of validation sample sets. Performance of combined models (fresh plus frozen-then-thawed samples) was generally much poorer than their separate counterparts and will not be discussed further. From a practical point of view this is disappointing since it would be preferable to have a single model for authentication of beefburgers irrespective of their thermal history. It does, however,

indicate that in future expanded studies of this problem, separate calibration models are required for fresh and frozen-then-thawed material.

A summary of the PLS models obtained for fresh samples is shown in Table 2. As is

Table 2 near here

clear from this table, each of the models produced 100% accurate classification results in both calibration and validation. Selection of a single model to use is difficult but, as a general rule, models with fewer loadings are preferred because they are likely to be more robust i.e. stable. There are no hard and fast rules governing the maximum number of loadings to use because each model is specific to its own dataset. Models containing up to 13 loadings as is reported here are not unusual in discriminant applications and simply reflect the complexity of the analytical task being addressed. Most models reported here contain between 3 and 9 loadings, numbers which give no cause for concern. Regarding pre-treatments, one which is is internally generated i.e. does not require the use of any specific individual or mean spectrum, is preferred. The model based on SNV-treatment of the spectral data therefore represents a good choice. PLS scores for this model are shown in Figure 3a. While detailed interrogation of regression vectors is complex and

Table 3 near here

needs care, examination of the vector associated with this model does reveal structural detail (Figure 3b). A single major maximum is evident at 1653 cm^{-1} (possible amide I or water absorptions) while significant negative features are present at 1055, 1080 and 1560 (perhaps amide II absorption) wavenumbers. Minima at 1055 and 1080 cm^{-1} may be related to carbohydrate (eg glycogen; Al-Jowder, Defernez, Kemsley and Wilson, 1999) which would explain the position of the adulterated samples in the scores plot. A similar summary of results obtained for frozen-then-thawed samples is

385 also shown in Table 2. In this case, model performances varied considerably but the
386 most accurate was that produced using MSC pre-treated data even though it required
387 13 loadings, much more than any other model. Derivative spectra produced models
388 which were almost as accurate but SNV pre-treatment resulted in poor results, worse
389 even than raw spectra. High classification accuracies were obtained for all of the
390 combined (fresh plus frozen-then-thawed) models developed (Table 2) although no
391 model produced completely correct classifications in both calibration and validation
392 sample sets. Best overall results for the combined sample collections were obtained by
393 MSC pre-treated data, once again involving 13 PLS loadings. Only two mis-
394 classifications occurred in the calibration sample set while all of the validation
395 samples were correctly identified. While a joint modelling approach may have some
396 merit, higher correct classification accuracies were obtained using separate
397 calibrations for fresh and for frozen-then-thawed samples. This suggests that when the
398 temperature history of a beefburger is known, use of the relevant model is
399 recommended. When this history is uncertain, the deployment of the model based on
400 the combined sample set will allow accurate classification.
401 If models of acceptable accuracy can be obtained using fewer variables than are
402 involved in those described above, then the possibility of their deployment in simpler
403 instrumentation becomes a possibility. To explore this, PLS regression models were
404 developed after deployment of the Martens Uncertainty Test; summary results of the
405 models' performances are shown in Table 3. As a general observation, it may be
406 stated that these reduced-variable models maintained the accuracy levels of the
407 fingerprint models with the possible exception of frozen-then-thawed validation
408 adulterated samples. The number of loadings required in the attenuated models was
409 generally less than was the case for their greater wavenumber range counterparts; in

some cases, a very significant reduction in the number of variables involved was also apparent. Examination of the regression vectors for the most accurate fresh, frozen-then-thawed and combined models is complex but line plots of both these and the full fingerprint models are shown in Figure 1. The complexity is further enhanced by the fact that spectral pre-treatments are applied for the frozen-then-thawed and combined models. In both these cases, retained variables are spread more or less evenly across the spectral range. For fresh samples, the negative peaks around 1200 cm^{-1} may reflect absorbances located at 1157 and 1176 cm^{-1} in the original spectra which may be attributed to glycogen; a group of small negative peaks around 1700 cm^{-1} may arise from lipid absorptions. Further interpretation is difficult.

3.3 SIMCA modelling

As above, separate models for fresh and frozen-then-thawed samples were developed; model performances are summarised in Table 4. For both fresh and frozen-then-

Table 4 near here

thawed samples, sensitivities were high, ranging from 0.94 to 1.0, but specificity values varied considerably over the range 0.33 – 0.80 (fresh samples) and 0.41 – 0.87 (frozen-then-thawed samples). Efficiency values varied from 0.57 – 0.87 for fresh and 0.62 to 0.91 for frozen-then-thawed beefburgers. These results indicate that the SIMCA method was able to accurately and efficiently model authentic beefburgers (sensitivity values of up to 1) but that some adulterated material overlapped with this model (specificity values less than 1) leading to false positive identifications. Overall, the most efficient models were produced using either raw spectral data (fresh samples) or after 2^{nd} derivative pre-treatment (frozen-then-thawed beefburgers). In general, only low numbers of principal components (≤ 5) were required for SIMCA

models which suggests that they are likely to be robust. When fresh and frozen-then-thawed samples were modelled together (Table 4), sensitivity remained perfect at 1.0 but specificity produced a range of values from 0.29 to 0.91 although most were around 0.65. Efficiency values varied from 0.54 to 0.95 with most equal to 0.81. The best overall model was produced by this composite sample collection; this involved MSC pre-treatment of spectral data and required 5 principal components.

On the basis of these results, SIMCA models may have a useful role to play in industrial quality control by e.g. confirming the authenticity of beefburgers produced according to the recipe used in this work. Such models could be deployed in a production facility where they would indicate any significant change in beefburger formulation arising e.g. from the use of different or contaminated ingredients. Deployment in a regulatory test environment may not yet be recommended given the failure to achieve specificity values of 1. However, the value of 0.91 in the overall best model suggest that alternative data treatments or variable selection steps may achieve perfect sensitivity and specificity values, paving the way for use by control agencies.

4. Conclusions

Offal-adulteration of beefburger products has been investigated using FTIR ATR spectroscopy and chemometric data analysis. Both discriminant (PLS-DA) and class-modelling (SIMCA) methods have been investigated. Models have been developed separately for fresh and frozen-then-thawed; models for both sample types together have also been generated. With regard to the classification approach, highest accuracies were achieved using individual (i.e. fresh or frozen-then-thawed) models

while a SIMCA model involving both sample types proved optimum. On this basis, class-modelling results allow highly accurate identification of beefburger samples of the types studied whether their thermal history is known or not. A limitation of this approach however lies in the fact that we have only demonstrated a capability which is exclusive for these formulations. In the case of analysis of a beefburger made to a different formulation, there is no guarantee that this model will work. Regarding SIMCA, a model developed using the combined fresh and frozen-then-thawed sample set provides 100% accuracy in identification of authentic samples. This enables its use in a quality control setting. Its advantage lies in the fact that the model is only concerned with authentic beefburger formulations. Therefore, beefburgers which have been adulterated by means other than beef offal admixture should still be identified as non-authentic. The results reported in this manuscript represent a feasibility study only but demonstrate the potential of this method for regulatory or quality control purposes; a larger study is merited to confirm the findings reported.

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

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

576
577 **Table 2.** Summary of PLS classification model results (fingerprint wavenumber range) for beefburger samples

			Calibration Samples		**Validation Samples**	
			# Correctly Classified[b]		**# Correctly Classified**	
Sample Type	**Data pre-treatment**	**Loadings[a]**	**Authentic**	**Adulterated**	**Authentic**	**Adulterated**
Fresh	none	10	18/18	22/22	18/18	23/23
	SNV	6	18/18	22/22	18/18	23/23
	MSC	5	18/18	22/22	18/18	23/23
	1 DER 7	6	18/18	22/22	18/18	23/23
	2 DER 11	6	18/18	22/22	18/18	23/23
Frozen-then-thawed	None	3	15/18	20/23	18/18	20/22
	SNV	5	16/18	15/21[a]	17/18	16/22
	MSC	13	18/18	23/23	18/18	22/22
	1 DER 7	9	17/18	22/23	18/18	22/22
	2 DER 11	8	18/18	22/23	18/18	22/22
Fresh plus frozen-then-thawed	None	8	36/36	41/45	35/36	43/45
	SNV	10	35/36	44/45	35/36	44/45
	MSC	13	34/36	45/45	36/36	45/45
	1 DER 7	6	34/36	44/45	34/36	44/45
	2 DER 11	6	35/36	44/45	36/36	44/45

578 [a] number of PLS loadings in each model; [b]no. of correctly classified samples/total number of samples in relevant set

579 **Table 3.** Summary of PLS classification model results (reduced number of variables[a]) for beefburger samples

				Calibration Samples		Validation Samples	
				# Correctly Classified		# Correctly Classified	
Sample Type	**Data pre-treatment**	**Loadings[b]**	**Variables retained**	**Authentic**	**Adulterated**	**Authentic**	**Adulterated**
Fresh	none	7	29	18/18	22/22	18/18	23/23
	SNV	3	22	18/18	20/21[d]	16/18	22/23
	MSC	3	30	18/18	21/22	18/18	23/23
	1 DER 7	5	61	18/18	22/22	18/18	23/23
	2 DER 11	4	70	18/18	22/22	18/18	23/23
Frozen-then-thawed	None	9	29	17/18	22/23	17/18	19/22
	SNV	4	25	18/18	16/22[a]	18/18	17/22
	MSC	8	27	18/18	22/23	16/18	20/22
	1 DER 7	6	66	18/18	23/23	18/18	20/22
	2 DER 11	6	119	18/18	22/23	18/18	20/22
Fresh plus frozen-then-thawed	None	7	49	34/36	42/45	36/36	41/45
	SNV	8	153	35/36	43/45	35/36	43/45
	MSC	10	47	35/36	44/45	35/36	44/45
	1 DER 7	6	171	34/36	45/45	36/36	43/45
	2 DER 11	6	219	35/36	44/45	36/36	44/45

580 [a] after Martens Uncertainty Test; [b]number of PLS loadings in each model; [c]no. of correctly classified samples/total number of samples in relevant
581 set; [d]1 sample removed
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586 **Table 4.** Summary of SIMCA class-modelling results for beefburger samples

			Validation Samples		
Sample Type	**Data pre-treatment**	**PCs[a]**	**Sensitivity**	**Specificity**	**Efficiency**
Fresh	none	4	0.94	0.80	0.87
	SNV	3	1.0	0.65	0.81
	MSC	3	1.0	0.70	0.84
	1 DER 7	3	1.0	0.33	0.57
	2 DER 11	3	1.0	0.46	0.68
Frozen-then-thawed	none	4	1.0	0.83	0.91
	SNV	2	0.94	0.41	0.62
	MSC	5	0.94	0.87	0.90
	1 DER 7	4	1.0	0.78	0.88
	2 DER 11	4	1.0	0.83	0.91
Fresh plus frozen-then-thawed	None	4	1.0	0.65	0.81
	SNV	5	1.0	0.66	0.81
	MSC	5	1.0	0.91	0.95
	1 DER 7	3	1.0	0.29	0.54
	2 DER 11	5	1.0	0.66	0.81

587 [a]number of PCs used in each model

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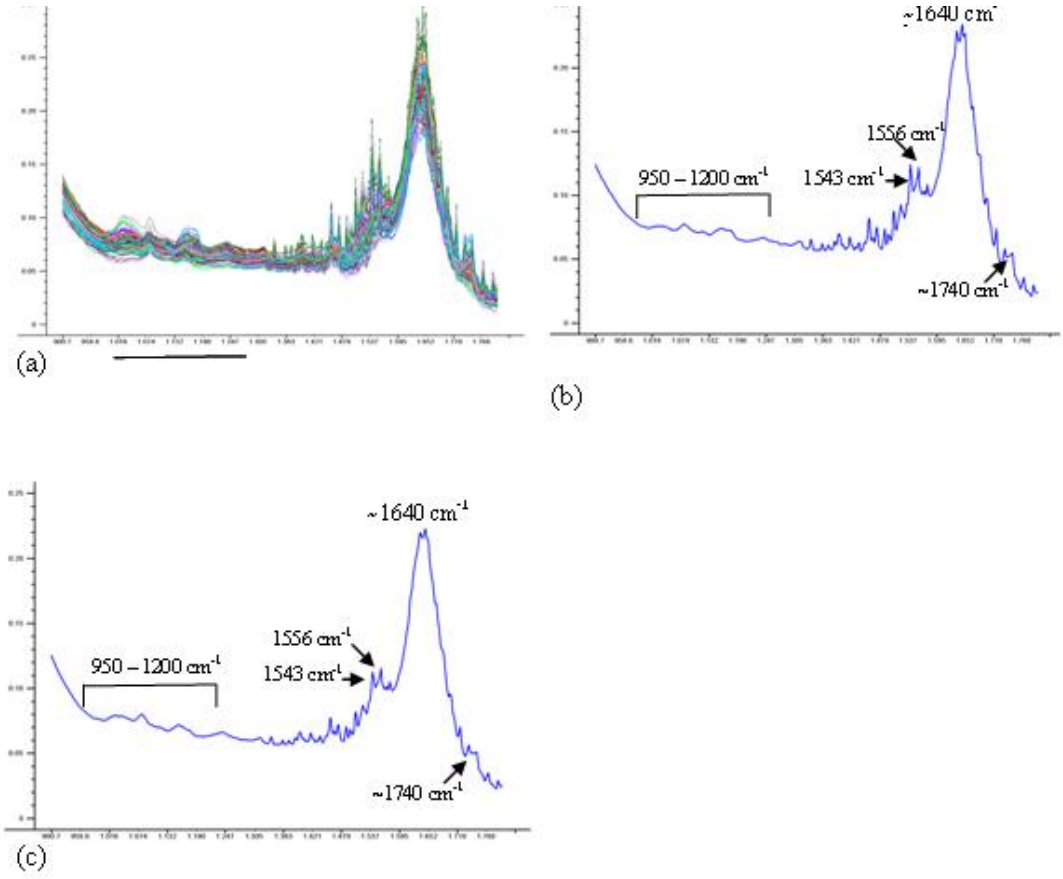

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591 **Figure 1.** Plots of (a) all untransformed beefburger spectra and mean spectra of
592 untransformed (b) authentic and (c) adulterated beefburgers.
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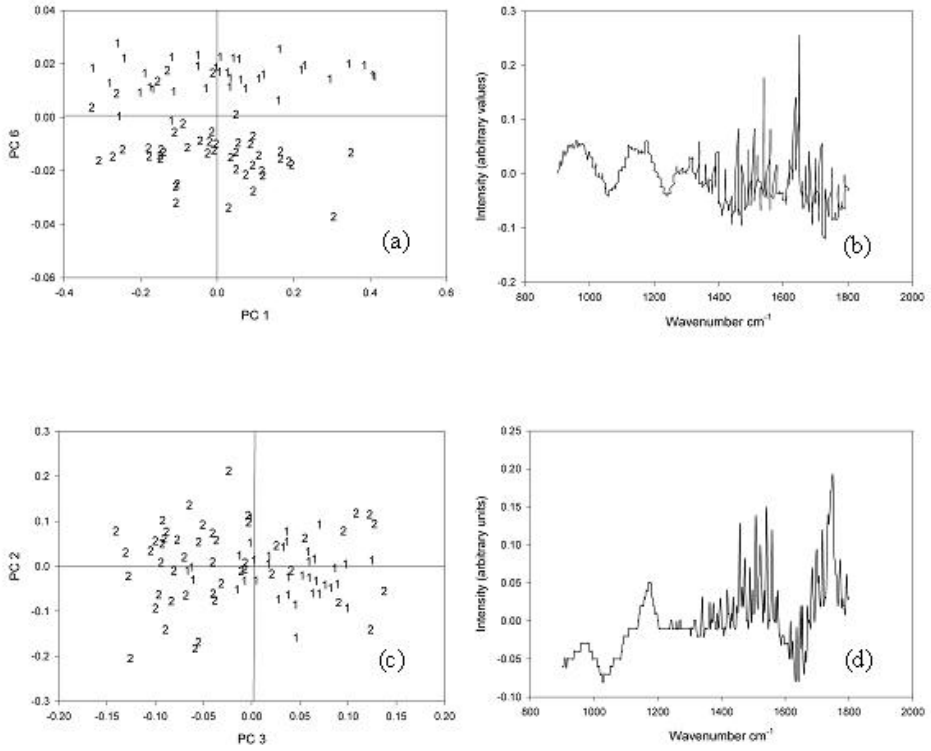

Figure 2. (a) PC scores plot (PCs 1 & 6) of fresh beefburger samples; (b) loading of PC6 for fresh beefburger samples; (c) PC scores plot (PCs 2 & 3) for frozen-then-thawed beefburger samples; (d) loading plot of PC 3 for frozen-then-thawed beefburgers.