J Food Sci Technol (June 2015) 52(6):3859–3869
DOI 10.1007/s13197-014-1457-9

ORIGINAL ARTICLE

Detection of poultry meat specific bacteria using FTIR spectroscopy and chemometrics

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Revised: 16 June 2014 / Accepted: 18 June 2014 / Published online: 1 July 2014

Abstract FTIR spectra of poultry meat specific bacteria viz. Salmonella enteritidis, Pseudomonas ludensis, Listeria monocytogenes and Escherichia coli were collected and investigated for identification of spectral windows capable of bacterial classification and quantification. Two separate datasets obtained at different times were used in the study to check reproducibility of results. Multivariate data analysis techniques viz. principal component analysis (PCA), partial least-squares discriminant analysis (PLSDA) and soft independent modelling of class analogy (SIMCA) were used in the analysis. Using full cross-validation and separate calibration and prediction datasets, the highest correct classification results for SIMCA and PLSDA were achieved in spectral window (1800-1200 cm-1) for both datasets. The window was also tested then for quantification of different bacteria and it had been observed that PLS models had better R values for classification (R=0.984) than predicting various concentration levels (R=0.939) of all four poultry specific bacteria inoculated in distilled water. The identified spectral window 1800–1200 cm-1 also demonstrated potential for 100% correct classification of chicken salami samples contaminated with S. enteritidis and P. ludensis from control using SIMCA. However, this wavenumber range yielded few misclassifications using PLS-DA approach. Thus FTIR spectroscopy in combination with chemometrics is a powerful technique that can be developed further to differentiate bacteria directly on poultry meat surface.

Keywords FTIR · Poultry meat · SIMCA · PLSDA · Bacteria detection

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Introduction

The increase in the consumer demand for poultry and poultry products has also increased concerns and fears for microbiological safety and quality issues. A large outbreak of *Salmonella* infections were reported in European Union (EU) in 2001 with 1, 57, 822 illnesses and in USA in 2010 causing nearly 2000 illnesses (CDC 2013; Cavitte 2003; Mbata 2005). Poultry was widely recognized as a major reservoir in each case. This has focused attention on the requirement for rapid and accurate detection methods/systems for microbiologically contaminated/spoiled meat. Existing methods for detection, identification and classification of bacteria are based on microscopy, ATP bioluminescence, biochemical or serological tests, measurement of electrical phenomena and the molecular methods. The major drawback with the existing protocols is that they are time-consuming, labour-intensive and give retrospective information. However, in a modern food-processing scenario, real time monitoring procedures are needed so that timely corrective action can be taken. The spectroscopy in combination with chemometrics has the potential to meet the requirements of a rapid, non-destructive and relatively inexpensive method for testing microbiological safety of meat.

Infrared spectroscopy became useful in the analysis of bacteria only with the advent of Fourier transform infrared (FTIR) instruments, improvements in laser design, higher signal to noise ratio coupled with significant advances in computing power and the utilization of chemometric analysis (Naumann et al. 1991). Chemometrics techniques enabled replacement of complex multidimensional spectral dataset by a simplified version with fewer dimensions which facilitates easy analysis of variance. Moreover recent advances in detector technology and attenuated total reflectance (ATR) accessory have made the analysis of biological samples simpler and faster (Movasaghi et al. 2008). The major advantage of infrared spectroscopy is reagent-less analysis. Fourier transform infrared (FT-IR)

spectroscopy combined with multivariate statistical tools has been used to identify and classify microorganisms based on their specific biochemical fingerprint in various fields such as clinical applications (Horbach et al. 1988; Helm et al. 1991a, 1991b; Maquelin et al. 2002; Miguel Gómez et al. 2003); food industry (Amiel et al. 2000; Lefier et al. 2000; Lucia et al. 2001; Guibet et al. 2003) and to monitor microbial spoilage in food products (Ellis and Goodacre 2001; Kummerle et al. 1998; Ellis et al. 2002; Nicolaou and Goodacre 2008; Lu and Rasco 2010). FTIR spectroscopy has been successfully compared with the classical methods of identification and classification (Van der Mei et al. 1993). Al-Qadiri et al. (2006) had successfully employed FTIR spectroscopy and multivariate analysis to detect *Pseudomonas aeruginosa* and *Escherichia coli* in bottled drinking water.

A contaminated food may present a distinct infrared spectrum if the bacteria metabolism products are specific. Based on this, FTIR methods had been developed for detection of different pathogens in different food matrices viz. apple juice (Yu et al. 2004); apple surface (Yang and Irudayaraj 2003); fruit juices (Burgula et al. 2007). But the detection of bacteria in meat matrix is challenging due to the presence of high protein and lipid content. Although efforts has been made to develop FTIR methods for rapid and quantitative detection of microbial spoilage of meat products based on the changes in physicochemical quality (Argyri et al. 2010; Ellis et al. 2002), few studies have been reported about detection of microbe/strain specific contamination in meat and its products (Amamcharla et al. 2010; Davis et al. 2010). To develop a rapid, non-destructive FTIR based method for detection and identification for poultry meat specific pathogenic/spoilage bacteria, first step would be identification of different spectral windows able to classify them into different groups. Three pathogenic bacteria specific to poultry viz. *Salmonella enteritidis*, *Listeria monocytogenes* and *Escherichia coli*and one spoilage bacterium viz. *Pseudomonas ludensis* were identified. Spectral signatures of four microbes were acquired using FTIR and analysed using chemometrics. Thus, the aim of this study was to identify spectral windows able to classify above four bacterial species specific to poultry meat suspended individually in sterile water. The study also investigated the potential of above spectral windows identified for bacterium in isolated system in detection of two major microbial threats (pathogenic microbe *S. enteritidis* and spoilage microbe *P. ludensis*) in presence of meat matrix.

Material and methods

Material

Four pure bacterial cultures (*Salmonella enteritidis*, *Pseudomonas ludensis*, *Listeria monocytogenes*, *Escherichia coli*) isolated from poultry were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. The lyophilized cultures of *S. enteritidis*, *P. ludensis*, *L. Monocytogenes* and *E. coli* were revived and cultivated on Brain Heart Infusion Agar, AMP, Trypticase Soy agar and Nutrient agar media respectively obtained from HIMEDIA and also maintained on culture slants.

Sample preparation

Isolated single colony of each bacterium (*Salmonella enteritidis*, *Pseudomonas ludensis*, *Listeria monocytogenes and Escherichia coli*) was collected from plate with a sterile loop, suspended in 10 ml of respective media broth and incubated at 37 °C for 48 h. The bacterial cultures were centrifuged (5,000 rpm for 10 min at 4 °C), washed twice and resuspended in sterile distilled water yielding a concentrated working solutions ofapproximately 10^8 cfu/ml. The working solution of each bacterial culture was further serially diluted (dilution factor 10^1, 10^2, 10^3, 10^4,10^5, 10^6 and 10^7) in sterile distilled water just before spectra acquisition.

For detection of microbes in an isolated system, two separate sets of experiments were conducted at two different times to check reproducibility of results. First set of experiment had been referred as dataset1 and it had a total of 172 samples comprising of 43 samples each of four selected bacteria with eight different bacterial concentrations. Dataset 2 had 297 samples comprising of 74 samples each of four selected bacteria with eight different bacterial concentrations. The concentration of bacteria in different samples (varying dilutions) was further validated by the standard plate count (SPC) method. Aliquots (0.1 ml) of the 10^1–10^8 dilutions were plated (in triplicate) on agar plates of respective media and colonies were counted after plates had been incubated at 37ºC for 24 h.

For detection experiments in meat matrix, frozen chicken salami belonging to a single lot were purchased from a local processing plant and transported under refrigeration to the laboratory. Salami packs were stored in freezer till further analysis. The salami samples were thawed and total viable number of bacteria was estimated by standard plate count (10^2 cfu/g). Thawed salami samples (10±2 g) were weighed, transferred to sterile petri plates and inoculated with different dilutions (10^4, 10^5, 10^6, 10^7,10^8 cfu/ml) of 48 h grown cultures of *P. ludensis and S. enteritidis*. Control samples were dipped in sterile distilled water. Both control and spiked samples were kept in respective solutions for three hours. Thereafter, the FTIR spectra of drained salami samples were immediately acquired. This had been referred as dataset 3 in the manuscript.

Spectra acquisition

Spectral signatures of four microbes suspended in distilled water (dataset 1 and 2) were acquired using FTIR

spectrometer in the range of 4,000–375 cm^{-1} with ZnSe ATR crystal cell (Spectrum 100, Bruker). The data were recorded at room temperature (25±2 °C) at 1.42 cm^{-1} interval. The scan speed per sample was 2 cms^{-1} and instrument control and spectral collection were performed using OPUS software. The reference (background) spectra of distilled water using a blank ATR crystal were recorded at an interval of five samples. After scanning each sample, the ATR crystal was washed twice with alcohol and wiped dry using a soft tissue paper. Twenty four independent scans were performed for each sample and the average spectrum was saved for analysis.

FTIR spectra of chicken salami samples (spiked and control) was acquired using same instrument in the range of 4,000–575 cm^{-1}. A spectrum of blank ATR crystal was recorded as background in this case. The background was recorded after every sample. The other instrument parameters and ATR crystal cleaning procedure was kept same. Spectra were exported from Opus 6.5 as excel files and imported directly into the Unscrambler for data analysis.

Chemometrics

Pre-processing

Spectral data of dataset 1 was standard normal variate transformed while no transformation was applied on dataset 2. The spectrum was split into three different segments to reduce the dimensionality of dataset. Three spectral regions (windows) were selected based on the appearance of higher order peaks. These regions are 700–600 cm^{-1}, 1,800–1,200 cm^{-1} and 3,998–3,500 cm^{-1}. Fourth region was the combination of the above three selected spectral subsets (windows). For comparison, the full spectrum (3,998–375 cm^{-1}) was taken as fifth region to develop the classification models.

Spectra obtained were processed using the Unscrambler software (version 9.8; CAMO AS, Trondheim, Norway). For all three datasets, samples were randomized and divided into two groups viz. calibration set and testing set. Every third sample was assigned membership of testing set. Due care was taken that all dilutions of each bacterial species were well represented in calibration and testing set of dataset 1 and 2. On the same note, dataset 3 was also divided into calibration and testing groups and care was taken that all the chicken salami samples of dataset 3 (control and spiked) were well represented in calibration and testing set.

Principal component analysis (PCA)

PCA was used as an unsupervised classification method to reduce the dimensionality as well as to visualize the resemblance and the differences among different samples for all spectral regions of three datasets. Principal component analysis of spectra of calibration samples of all three datasets was performed to investigate grouping of different samples into different clusters and to identify any outliers in the spectral datasets. Effect of various mathematical pre-treatments was also examined before PCA. PCA analyses of FTIR data was performed using all the five different wavenumber segments.

SIMCA classification

Soft Independent Modelling Class Analogy (SIMCA) classification was used to predict class memberships. In dataset 1 and 2 each bacterial species was described by a principal component model which was developed using calibration samples. These models were developed for all the five selected spectral windows. The developed models were used to classify the members of testing set. Same classification technique was also applied for classification of different types (spiked and control) of chicken salami samples in testing set of dataset 3.

Classification using PLS

Partial Least Square Discriminant analysis (PLSDA) was also used to develop models to discriminate between different bacteria in dataset 1 and 2. As per this approach, a sample has to be a member of one of the classes included in the analysis. Each class was represented by an indicator variable (Y_1) i.e. for four different organisms. Y_1 variable had been given value 0, 1, 2 and 3. Class membership from the X-variables describing any given sample was predicted by building a PLS1 model with indicator variable Y_1. Model output was a predicted value for an unknown sample. Correct predictions for bacteria would have ideally same assigned Y_1 value. In this study, $Y_1<0.5$ was interpreted as indicating membership for bacteria class assigned Y_1 value 0; $0.5<Y_1<1.5$ as indicating membership for bacteria class assigned Y_1 value 1; $1.5<Y<2.5$ as indicating membership for bacteria class assigned Y_1 value 2; $Y_1>2.5$ as indicating membership

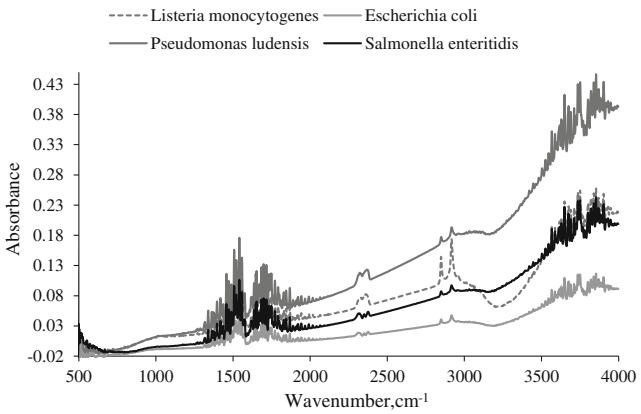

Fig. 1 Original spectra of different bacterial suspensions in the range of 4,000–500 cm^{-1} (Dataset 2)

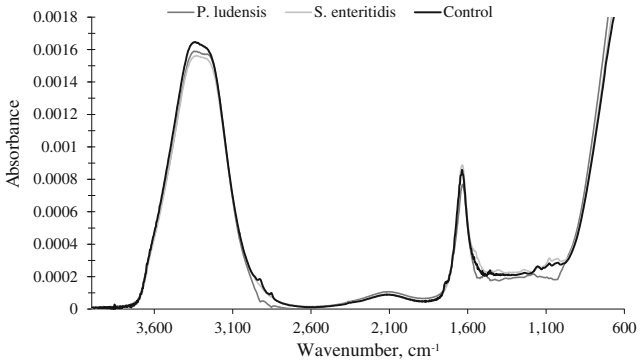

Fig. 2 FT-IR spectra (4,000–600 cm^{-1}) of chicken salami samples spiked with *P. ludensis* and *S. enteritidis*

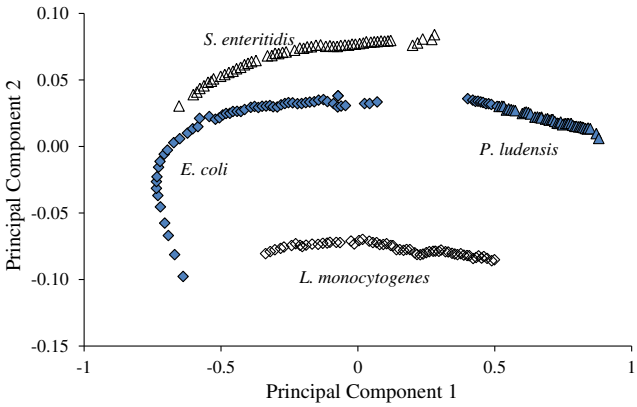

Fig. 4 PCA of original spectral dataset 2 over the wavenumber range 1,200–1,800 cm^{-1}

for bacteria class assigned Y_1 value 3. Bacterial samples *L. monocytogenes*, *E. coli. P. ludensis* and *S. enteritidis* were assigned Y_1 value 0, 1, 2 and 3 respectively in dataset 1 and 2. Similarly in dataset 3 for discrimination between control and spiked chicken salami samples, indicator variable Y had been given values −1, 0 and 1 for chicken salami samples spiked with *P. ludensis*, *S. enteritidis* and control respectively. Partial least squares (PLS) method was also used for quantification of different bacteria in region 1,800–1,200 cm^{-1} for dataset 1 and 2. For quantification, Y_2 variable was given values 4, 5, 6, 7 and 8 for concentrations 10^4, 10^5, 10^6,10^7 and 10^8 respectively. All predicted values were accompanied by a deviation which was an estimate of reliability of the prediction. Full cross validation method with all samples was used to evaluate predictability of the model i.e. by removingone sample from the data set at a time and applying acalibration to the remaining samples (Davis et al. 2010). The suitability of the developed models for predicting concentration, classification of different types of bacteria and discrimination between control and spiked chicken salami samples was assessed by correlation coefficient *R*, number of PLS factors (rank), root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP).

Results and discussion

FTIR spectra

The different functional groups present in the biochemical structure contribute to a distinct absorbance in the mid infrared region. The differences in amount and type of cell wall constituents of microorganisms viz. peptidoglycan layer, lipoproteins, phospholipids, proteins, and lipopolysaccharides could be responsible for the unique and reproducible mid infraredspectral patterns. The spectra of different bacterial suspensions in dataset 2 showed almost identical patterns for all bacterial samples except in the 3500to 3,000 cm^{-1} region due to different water content in sample and the background

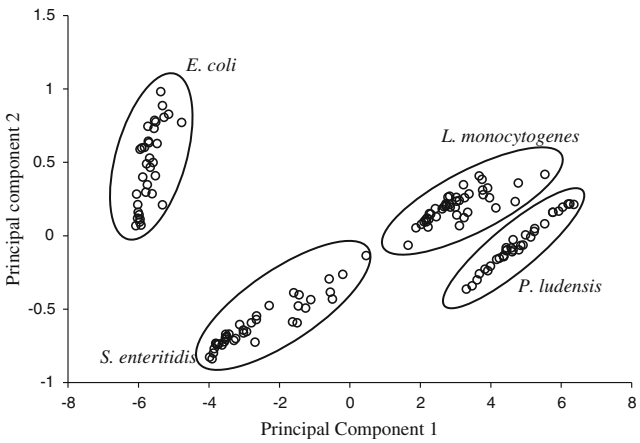

Fig. 3 PCA of SNV transformed spectral data dataset 1 over the wavenumber range 1,200–1,800 cm^{-1}

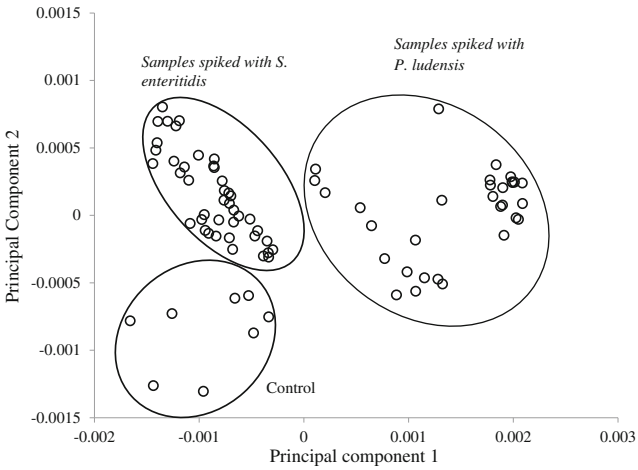

Fig. 5 PCA of normalized spectral dataset 3 over the wavenumber range 4,000–600 cm^{-1}

Table 1 SIMCA classification results for different spectral regions for different bacterial cultures suspended in water and for chicken salami samples spiked with *P. ludensis* and *S. enteritidis*

Dataset	Spectral region (cm^{-1})	Sample	Samples classified	Classification accuracy (%)	False negatives	False positives
1	600–700	*Escherichia coli*	11	90.90	1	0
		Listeria monocytogenes	14	92.86	1	1
		Pseudomonas ludensis	10	90	1	10
		Salmonella enteritidis	13	100	0	0
	1,200–1,800	*Escherichia coli*	11	100	0	0
		Listeria monocytogenes	14	90.90	1	0
		Pseudomonas ludensis	10	92.86	1	0
		Salmonella enteritidis	13	100.0	0	0
	3,500–3,998	*Escherichia coli*	11	90.90	0	0
		Listeria monocytogenes	14	92.86	0	0
		Pseudomonas ludensis	10	100	0	0
		Salmonella enteritidis	13	100	0	0
	600–700, 1,200–1,800, 3,500–3,998	*Escherichia coli*	11	90.90	0	0
		Listeria monocytogenes	14	92.86	0	0
		Pseudomonas ludensis	10	100	0	0
		Salmonella enteritidis	13	100	0	0
	375–3,998	*Escherichia coli*	11	100	0	0
		Listeria monocytogenes	14	100	0	17
		Pseudomonas ludensis	10	100	0	0
		Salmonella enteritidis	13	92.30	1	0
2	600–700	*Escherichia coli*	23	95.65	1	39
		Listeria monocytogenes	20	95	1	22
		Pseudomonas ludensis	18	94.44	1	8
		Salmonella enteritidis	22	81.82	4	0
	1,200–1,800	*Escherichia coli*	23	91.30	2	7
		Listeria monocytogenes	20	100.0	0	0
		Pseudomonas ludensis	18	100.0	0	0
		Salmonella enteritidis	22	95.45	1	6
	3,500–3,998	*Escherichia coli*	23	95.65	1	12
		Listeria monocytogenes	20	95	1	3
		Pseudomonas ludensis	18	100	0	0
		Salmonella enteritidis	22	100	0	0
	600–700, 1,200–1,800, 3,500–3,998	*Escherichia coli*	23	91.30	2	14
		Listeria monocytogenes	20	100	0	0
		Pseudomonas ludensis	18	94.44	1	0
		Salmonella enteritidis	22	86.36	3	0
	375–3,998	*Escherichia coli*	23	100	0	37
		Listeria monocytogenes	20	100	0	42
		Pseudomonas ludensis	18	94.44	1	5
		Salmonella enteritidis	22	100	0	29
3	3,000-2,500	Chicken salami spiked with *Pseudomonas ludensis*	16	100	0	0
		Chicken salami spiked with *Salmonella enteritidis*	20	100	0	0
		Control	6	100	0	7
	4,000–600	Chicken salami spiked with *Pseudomonas ludensis*	16	100	0	0
		Chicken salami spiked with *Salmonella enteritidis*	20	100	0	0

Table 1 (continued)

Dataset	Spectral region (cm^{-1})	Sample	Samples classified	Classification accuracy (%)	False negatives	False positives
		Control	6	100	0	7
	1,800–1,200	Chicken salami spiked with *Pseudomonas ludensis*	16	100	0	0
		Chicken salami spiked with *Salmonella enteritidis*	20	100	0	0
		Control	6	100	0	0
	1,200–800	Chicken salami spiked with *Pseudomonas ludensis*	16	100	0	0
		Chicken salami spiked with *Salmonella enteritidis*	20	100	0	0
		Control	6	100	0	8
	3,000–2,500, 1,800–800	Chicken salami spiked with *Pseudomonas ludensis*	16	100	0	0
		Chicken salami spiked with *Salmonella enteritidis*	20	100	0	0
		Control	6	100	0	0

(Fig. 1). The spectra showed strong absorption bands in region 900–600 cm^{-1}, 1,800–1,200 cm^{-1}, and 4,000–3,500 cm^{-1}. The 900–600 cm^{-1} is referred to as the 'Fingerprint region' which contains weak but specific absorbance characteristic of bacteria. The 1,800–1,200 cm^{-1} region is commonly assumed to be dominated by chemical groups related to protein, carboxylic groups of proteins, free amino acids, polysaccharides, RNA/DNA and phospholipid constituents (Naumann 2001; Naumann et al. 1991). The spectral regions 900–600 cm^{-1}(I), 1,800–1,200 cm^{-1}(II), 4,000–3,500 cm^{-1}(III), combination of 700–600, 1,800–1,200, 3,998–3,500 cm^{-1} (IV) and 4,000–375 cm^{-1}(V) were selected for classification and discriminant analysis for dataset 1 and 2.

Raw spectra of dataset 3 comprising of chicken salami samples spiked with *Salmonella enteritidis* and *Pseudomonas ludensis* and control were normalized to enhance the differences in the IR spectra between contaminated and control salami samples (Fig. 2). Normalization eliminates the variation in path length and also reduces the differences between each single measurement of the same sample (Yu and Irudayaraj 2005). The spectra showed strong and different absorption bands and patterns in region 1,200–800 cm^{-1}, 1,800–1,200 cm^{-1}, and 3,000–2,500 cm^{-1} (Fig. 2). The spectral region 1,800–1,200 cm^{-1} has also been identified as predominant region in spectra of these bacteria in isolated system. In presence of meat matrix, strong absorbance peaks had been also been observed in 1200–800 cm^{-1} and 3,000–2,500 cm^{-1}. The peaks in region 3,000–2,500 cm^{-1} had been referred as corresponding to C-H asymmetric and symmetric stretching of $-CH_3$ and $-CH_2$ in fatty acids, C-H stretching of ≥ C-H of amino acids. The peaks in region 1,200–900 cm^{-1} correspond to C-O-C, C-O dominated by ring vibrations in various polysaccharides, P = O symmetric stretching in DNA, RNA and phospholipids (Davis and Mauer 2010). As mentioned in earlier paragraph region < 900 cm^{-1}corresponds to "Fingerprint region" in bacterial identification. Brandily et al. (2011) had also identified regions 3,000–2,800 cm^{-1} and 1,800–1,000 cm^{-1} as providing the greatest contribution to the total variance in the FT-IR spectral data acquired for detection of *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enteritidis* in minced meat, sausage meat and raw cheese matrix. Thus the spectral regions 3,000–2,500 cm^{-1} (I), 1,800–1,200 cm^{-1} (II), 1,200–800 cm^{-1} (III), 4,000–575 cm^{-1} (IV) and combination of 3,000–2,500 cm^{-1}, 1,800–1,200 cm^{-1} and 1,200–800 cm^{-1} (V) were selected for classification and discriminant analysis in dataset 3.

PCA

PCA of the four selected spectral regions of datasets 1 and 2 revealed that the best separation of the bacterial species into four different clusters was found using raw data in dataset 2 and with standard normal variate transformation in dataset 1 in the wavelength range 1,800–1,200 cm^{-1} (Figs. 3 and 4). It had been observed that bacterial concentrations less than 10^3 cfu/ml for all four bacteria were resulting in merging of different clusters. So these concentrations were not included in further analysis. Also two sample outliers were detected in both datasets, and were therefore not used in subsequent chemometric analysis. Figs. 3 and 4 revealed that each bacterial species in concentration range 10^4–10^8 cfu/ml formed well-defined clusters in both datasets. This suggested that

Table 2 PLS-DA prediction results for different spectral regions for different bacteria and chicken salami samples spiked with *P. ludensis* and *S. enteritidis*

Dataset	Spectral region (cm^{-1})	Sample	Samples classified	Classification accuracy (%)	False negatives	False positives
1	600–700	*Escherichia coli*	11	80	4	0
		Listeria monocytogenes	14	84.62	3	4
		Pseudomonas ludensis	10	80	5	3
		Salmonella enteritidis	13	83.33	8	3
	1,200–1,800	*Escherichia coli*	11	81.81	2	0
		Listeria monocytogenes	14	100.0	0	2
		Pseudomonas ludensis	10	100	0	0
		Salmonella enteritidis	13	100.0	0	0
	3,500–3,998	*Escherichia coli*	11	100	0	0
		Listeria monocytogenes	14	100	0	0
		Pseudomonas ludensis	10	100	0	0
		Salmonella enteritidis	13	100	0	0
	600–700, 1,200–1,800, 3,500–3,998	*Escherichia coli*	11	100	0	0
		Listeria monocytogenes	14	100	0	0
		Pseudomonas ludensis	10	100	0	0
		Salmonella enteritidis	13	100	0	0
	375–3,998	*Escherichia coli*	11	100	0	0
		Listeria monocytogenes	14	92.86	1	1
		Pseudomonas ludensis	10	80	2	1
		Salmonella enteritidis	13	100	0	1
2	600–700	*Escherichia coli*	23	70	6	8
		Listeria monocytogenes	20	41.12	7	0
		Pseudomonas ludensis	18	93.33	1	10
		Salmonella enteritidis	22	78.95	4	0
	1,200–1,800	*Escherichia coli*	23	100.0	0	0
		Listeria monocytogenes	20	100.0	0	0
		Pseudomonas ludensis	18	100.0	0	0
		Salmonella enteritidis	22	100.0	0	0
	3,500–3,998	*Escherichia coli*	23	100	0	0
		Listeria monocytogenes	20	100	0	1
		Pseudomonas ludensis	18	93.33	1	0
		Salmonella enteritidis	22	100	0	0
	600–700, 1,200–1,800, 3,500–3,998	*Escherichia coli*	23	80	4	0
		Listeria monocytogenes	20	100	0	1
		Pseudomonas ludensis	18	100	0	4
		Salmonella enteritidis	22	94.74	1	0
	375–3,998	*Escherichia coli*	23	95	1	5
		Listeria monocytogenes	20	82.35	3	1
		Pseudomonas ludensis	18	80	3	3
		Salmonella enteritidis	22	89.47	2	1
3	3,000–2,500	Samples spiked with *Pseudomonas ludensis*	16	100	0	0
		Samples spiked with *Salmonella enteritidis*	20	100	0	0
		Control	6	100	0	0
	4,000–600	Samples spiked with *Pseudomonas ludensis*	16	100	0	0
		Samples spiked with *Salmonella enteritidis*	20	100	0	0
		Control	6	100	0	0
	1,800–1,200	Samples spiked with *Pseudomonas ludensis*	16	100	0	0

Table 2 (continued)

Dataset	Spectral region (cm^{-1})	Sample	Samples classified	Classification accuracy (%)	False negatives	False positives
		Samples spiked with *Salmonella enteritidis*	20	100	0	1
		Control	6	83.33	1	0
	1,200–800	Samples spiked with *Pseudomonas ludensis*	16	100	0	0
		Samples spiked with *Salmonella enteritidis*	20	100	0	2
		Control	6	66.64	2	0
	3,000–2,500, 1,800–800	Samples spiked with *Pseudomonas ludensis*	16	100	0	0
		Samples spiked with *Salmonella enteritidis*	20	100	0	1
		Control	6	83.33	1	0

differentiation between the different bacterial species in an isolated system i.e. in absence of food matrix in concentration range 10^4–10^8 cfu/ml was possible on the basis of their mid infrared spectra. As the scanning order of the bacterial suspension samples was fully randomized and taken at two different times, the observed differentiation is not an artefact. The clusters were slightly dispersed and this might be due to presence of a large concentration range within each bacterial data set (Alexandrakis et al. 2008). PCA of normalized spectral dataset 3 comprising of chicken salami samples spiked with *S.enteritidis*and *P. ludensis* in wavenumber range of 4,000–600 cm^{-1} resulted in best separation of samples into different clusters (Fig. 5).

SIMCA classification of different bacteria

The highest correct classification results for SIMCA were achieved in spectral window II (1,800–1,200 cm^{-1}) for datasets 1 and 2. Evaluation of the accuracy of these models was made on the basis of errors in identification and fall into one of two classes. False-negative identifications refer to samples actually belonging to a class that are not classified as such by the class model. False-positive identifications relate to samples that do not belong to a given class but are incorrectly identified as so by the class model.

In dataset 1, models developed in spectral windows 1,800–1,200 cm^{-1} (II), 4,000–3,500 cm^{-1} (III), combination of 700–600, 1,800–1,200, 3,998–3,500 cm^{-1} (IV) produced 100 % correct classification rates for *Salmonella enteritidis* without any misclassifications. Classification rates in spectral windows II, III and IV for *E. coli* was 100, 90.90 and 90.90 % without any false positives; for *Listeria monocytogenes* was 90.90, 92.86 and 92.86%and for *Pseudomonas ludensis* was 92.86, 100 and 100 % respectively (Table 1). Models for all four pathogens did not produce any false-positive errors. In dataset 2, only spectral window II (1,800–1,200 cm^{-1}) gave good classification rates with minimum number of false positives. The model developed using window II gave 100 %

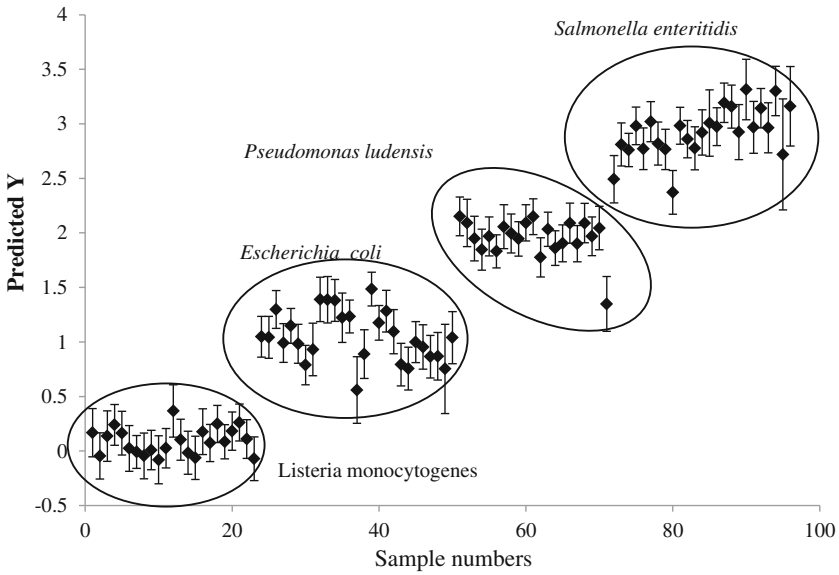

Fig. 6 Prediction results for different bacteria (PLS-DA, dataset 2, 1,200–1,800 cm^{-1})

classification for *L. monocytogenes* and *P. ludensis* without any misclassification. However classification rates for *E. coli* and *S. enteritidis* was 91.30 % with 2 false positives and 7 false negatives and 95.45 % with 1 false negative and 6 false positive respectively (Table 1). Spectral window II (1,800–1,200 cm^{-1}) consistently gave the best classification results for all four microorganisms in both datasets. This window includes the amide II region (1,550 to 1,520 cm−1), mixedregion of proteins, lipids, and carbohydrates (1,500 to 1,300 cm−1) and nucleic acid region (1,240 to 1,220 cm−1) (Davis et al. 2010). Use of all the bacterial samples or different randomly selected samples in the calibration and validation sample sets did not change the overall pattern of SIMCA results.

In dataset 3, models developed in spectral window 1,800–1,200 cm^{-1}(II) produced 100 % correct classification rates for samples spiked with *Salmonella enteritidis* and *Pseudomonas ludensis* and control without any misclassifications. Though classification rates in spectral windows 3,000–2,500 cm^{-1} (I), 1,200–800 cm^{-1} (III), 4,000–575 cm^{-1} (IV) and combination of 3,000–2,500 cm^{-1}, 1,800–1,200 cm^{-1} and 1,200–800 cm^{-1} (V) was 100 % without any false negatives; but had seven false positives in model for control samples (Table 1). These samples belonged to samples spiked with *S. enteritidis*. Thus, the spectral window 1,800–1,200 cm^{-1} identified for best classification of same bacterial species suspended in distilled water had shown best classification results even in the presence of meat matrix. A summary of model performance is shown in Table 1 for different spectral regions and three datasets.

Classification and quantification of different poultry specific bacteria using PLS

The highest correct classification results for Partial Least Square Discriminant Analysis (PLSDA) were achieved in spectral window II (1,800–1,200 cm^{-1}) for datasets 1 and 2 (Table 2). The output of the PLS-DA model is a series of regressions of each bacterial organism against all of the others.

Table 3 Partial least square (PLS) models from the 1,800–1,200 cm^{-1} region of IR spectra for classification of poultry specific microbes[a] with dilution ranging from 10^4–10^8 inoculated into distilled water

Dataset	Rank	R	RMSEC[b]	RMSEP[c]
1	7	0.960	0.306	0.352
2	11	0.984	0.240	0.201

PLS models were developed for all four microbes (Salmonella enteritidis, Pseudomonas ludensis, Listeria monocytogene, and Escherichia coli); Rank = number of PLS factors; R = correlation coefficient

[b] RMSEC Root-mean square error of calibration

[c] RMSEP Root-mean square error of prediction

Table 4 Partial least square (PLS) models from the 1,800–1,200 cm^{-1} region of IR spectra for quantification of poultry specific microbes[a] with dilution ranging from 10^4–10^8 inoculated into distilled water

Dataset	Rank	R	RMSEC[b]	RMSEP[c]
1	7	0.923	0.810	0.98
2	11	0.939	0.639	0.810

PLS models were developed for all four microbes (Salmonella enteritidis, Pseudomonas ludensis, Listeria monocytogene, *and Escherichia coli*); Rank = number of PLS factors; R = correlation coefficient

[b] *RMSEC* Root-mean square error of calibration

[c] *RMSEP* Root-mean square error of prediction

A graphical display of regression which is a plot of predicted *Y* value for all prediction samples is shown in Fig. 6. In this figure, the prediction value is shown as a rhombus shaped point and the vertical lines around this point indicate deviation that is reliability of prediction. Evaluation of the accuracy of PLS models wasalso made on the basis of number of false-negative or false positive identificationsas in case of SIMCA. The ability of PLS model to classify different bacteria inoculated in distilled water issummarized in Table 3. In dataset 2, PLS model for *Salmonella enteritidis*, *Listeria monocytogenes*, *Escherichia coli* and *Pseudomonas ludensis* showed 100 % correct classification of class members without any false negatives and false positive classifications (Table 2). In case of dataset 1, PLS model developed for four bacterial classes showed 100 % correct classification for *Salmonella enteritidis*, *Listeria monocytogenes* and *Escherichia coli* but the correct classification rate for*Pseudomonas ludensis* with two false negatives indicated ad false positives of *Listeria monocytogenes* and *Salmonella enteritidis* class (Table 2). Deviations for the prediction samples are all very similar, as shown by the magnitude of the error bars associated with each sample.

Quantification of pathogenic/spoilage bacteria in poultry is one of the important criteria for its safety and quality

Table 5 Partial Least Square (PLS) models for classification of chicken salami samples spiked with *P. ludensis* and *S. enteritidis*

Wavenumber range (cm^{-1})	Rank	Rcal	Rval	RMSEC[b]	RMSEP[c]
3,000–2,500	5	0.988	0.978	0.420	0.223
4,000–600	9	0.989	0.976	0.136	0.202
1,800–1,200	7	0.978	0.970	0.197	0.228
1,200–800	9	0.984	0.970	0.168	0.227
3,000–2,500,1,800–800	9	0.972	0.962	0.221	0.255

Rank = number of PLS factors; R = correlation coefficient

[b] *RMSEC* Root-mean square error of calibration

[c] *RMSEP* Root-mean square error of prediction

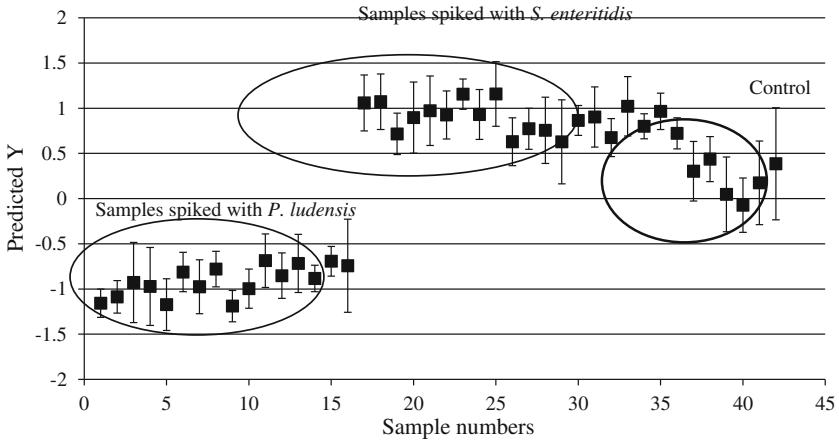

Fig. 7 Prediction results for different test samples belonging to dataset 3 (PLS-DA, 4,000–600 cm^{-1})

assessment. Results of PLS analysis of the FT-IR spectrato quantify different poultry specific bacteria inoculated in distilled water are summarized in Table 4. For developing the PLS models, wavenumber regions 1,800–1,200 $cm^{-1}$was selected based on the best performance for both datasets 1 and 2. PLS models had better R values for classification (R=0.984 for dataset 2) than predicting various concentration levels (R= 0.939 for dataset 2) of all four poultry specific bacteria inoculated in distilled water (Tables 3 and 4).

PLS model developed in spectral window 1,800–1,200 cm^{-1}(II) for three classes of samples showed 100 % correct classification for samples contaminated with *Salmonella enteritidis* and *Pseudomonas ludensis* but the correct classification rate for control samples was 83.33 % with one false negatives indicated as false positives of *Salmonella enteritidis* class (Table 2). The highest correct classification results for Partial Least Square Discriminant Analysis (PLSDA) of dataset 3 were achieved in spectral window I (3,000–2,500 cm^{-1}) and 4,000–575 cm^{-1} (IV) (Table 2). PLS model for samples contaminated with *Salmonella enteritidis*, *Pseudomonas ludensis* and control showed 100 % correct classification of class members without any false negatives and false positive classifications (Table 2). PLS models in wavenumber range 4,000–575 cm^{-1} had better R values for classification (R=0.989) of different samples than all other spectral windows (Table 5). Plot of predicted Y value for all prediction samples of dataset 3 in wavenumber range 4,000–575 cm^{-1} is shown in Fig. 7.

Conclusion

FTIR spectral profile of bacteria specific to poultry meat in an isolated system were analysed with chemometric tools which identified spectral window1800–1,200 cm^{-1}as region having potential of classification into different groups. Further experimental work carried on chicken salami food samples demonstrated that although the wavenumber range of 1,800–1,200 cm^{-1} had potential to classify contaminated samples from control using SIMCA, this range yielded few misclassification using PLS-DA approach. However 100 % correct classification was achieved using PLS-DA in the wavenumber range of 3,000–2,500 cm^{-1} and 4,000–575 cm^{-1}. Therefore, FTIR spectroscopy in combination with chemometrics as a successful technique that can be developed further to differentiate bacteria directly on poultry meat surface.

Acknowledgments Financial support received from Indian Council of Agricultural Research (ICAR) and facilities provided by Central Institute of Post-Harvest Engineering and Technology (CIPHET) under institute project number IX10155 are gratefully acknowledged.

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