Rapid and Quantitative Detection of the Microbial Spoilage of Meat by Fourier Transform Infrared Spectroscopy and Machine Learning

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Fourier transform infrared (FT-IR) spectroscopy is a rapid, noninvasive technique with considerable potential for application in the food and related industries. We show here that this technique can be used directly on the surface of food to produce biochemically interpretable “fingerprints.” Spoilage in meat is the result of decomposition and the formation of metabolites caused by the growth and enzymatic activity of microorganisms. FT-IR was exploited to measure biochemical changes within the meat substrate, enhancing and accelerating the detection of microbial spoilage. Chicken breasts were purchased from a national retailer, comminuted for 10 s, and left to spoil at room temperature for 24 h. Every hour, FT-IR measurements were taken directly from the meat surface using attenuated total reflectance, and the total viable counts were obtained by classical plating methods. Quantitative interpretation of FT-IR spectra was possible using partial least-squares regression and allowed accurate estimates of bacterial loads to be calculated directly from the meat surface in 60 s. Genetic programming was used to derive rules showing that at levels of 107 bacteria g−1 the main biochemical indicator of spoilage was the onset of proteolysis. Thus, using FT-IR we were able to acquire a metabolic snapshot and quantify, noninvasively, the microbial loads of food samples accurately and rapidly in 60 s, directly from the sample surface. We believe this approach will aid in the Hazard Analysis Critical Control Point process for the assessment of the microbiological safety of food at the production, processing, manufacturing, packaging, and storage levels.

The last decade has seen an exponential increase in the consumer demand for poultry and poultry products, fueled in part by dietary health considerations. Fears over microbiological food safety issues, especially the incidence of Salmonella spp. (23, 49) and Campylobacter spp. (11, 39), in conjunction with consumer demand for a product of consistently high quality, have focused attention on a particular area of the food production industry, namely, the requirement for a rapid (less than a few minutes) and accurate detection system for microbiologically spoiled or contaminated meat (3).

At present, no such technology exists in the food industry within the Hazard Analysis Critical Control Point system for the microbiological safety and quality of meat and poultry products (20, 26, 48).

Muscle foods, such as meat and poultry, are described as spoiled if organoleptic changes make them unacceptable to the consumer. These organoleptic characteristics may include changes in appearance (discoloration), the development of off odors, slime formation, changes in taste, or any other characteristic which makes the food undesirable for consumption (25, 26). While endogenous enzymatic activity within muscle tissue postmortem can contribute to changes during storage (1, 25, 32, 44), it is generally accepted that detectable organoleptic spoilage is a result of decomposition and the formation of metabolites caused by the growth of microorganisms (28, 40). The organoleptic changes which take place will also vary according to the species of microflora present, the characteristics of the meat, processing methods, product composition, and the environment in which the food is stored (25).

Provided that the atmosphere is moist, a consortium of bacteria is responsible for spoilage of meat stored at between −1 and 25°C. It is agreed that spoilage organisms belong primarily to the genus Pseudomonas, and these have been observed to attach more rapidly to meat surfaces than other spoilage bacteria (25). The other major members of the spoilage flora of meat stored aerobically include Moraxella spp., Psychrobacter spp., and Acinetobacter spp. While the gram-negative motile and nonmotile aerobic rods and coccobacilli generally dominate the spoilage microflora of meat, the initial population may also contain various levels of gram-positive genera, usually represented by micrococci and then lactic acid bacteria and Bronchothrix thermosphacta (13).

To date, in excess of 40 methods have been proposed to measure and to detect bacterial spoilage in meats (13, 26). These include enumeration methods based on microscopy, ATP bioluminescence, and the measurement of electrical phenomena (10, 45), as well as detection methods based on either immunological or nucleic acid-based procedures (42). The major drawback with the range of protocols available is that they are time-consuming and labor-intensive and give retrospective information. However, in a modern food-processing environment, monitoring procedures need to give results in real time so that corrective action can be taken as soon as possible.

The ideal method for the on-line microbiological analysis of meat would be rapid, noninvasive, reagentless, and relatively inexpensive, and these requirements can be met via the application of a spectroscopic approach, in combination with any appropriate data deconvolution strategy based on statistics or machine learning. Such statistical methods include partial
least-squares (PLS) regression (36), while a popular and powerful series of machine learning strategies (37) are based on methods of evolutionary computing (4), such as genetic algorithms (GAs) (4, 19, 24) and genetic programming (GP) (6, 33). Fourier transform infrared (FT-IR) spectroscopy involves the observation of vibrations of molecules that are excited by an infrared beam, and an infrared absorbance spectrum represents a “fingerprint” which is characteristic of any chemical or biochemical substance (18, 43). This technique is also very rapid (taking seconds) and has been shown to be a valuable tool for the characterization of axenically cultured bacteria (22, 34, 38, 47), including single-gene knockout strains (41).

With the prior knowledge that organoleptic spoilage is the result of decomposition and the formation of metabolites caused by the growth of microorganisms, this information can be exploited through spectroscopic analysis. Therefore, rather than measuring exclusively the presence of bacteria per se on the meat surface, vibrational spectroscopy can also be used to measure biochemical changes within the meat substrate, enhancing and accelerating the detection of microbial spoilage. The objective of the present study was to establish this technology through a series of experiments undertaken on chicken breast muscle at room temperature and analyzed by horizontal attenuated total reflectance (HATR) FT-IR spectroscopy with PLS analysis. Our research demonstrates the utility of a novel analytical approach based on FT-IR that can enhance and accelerate the detection of microbial spoilage, providing rapid, accurate, and quantitative results. PLS methods are not always transparent as to which variables are used (2), whereas certain other methods use input variables explicitly and directly in the construction of the relationship between the spectra observed and the property of interest. GA and GP analyses fall into this category and are used here to suggest that proteinolysis is the key indicator for the onset of spoilage.

### MATERIALS AND METHODS

**Sample preparation.** Fillets of prepacked fresh chicken breast meat were purchased from national retail outlets on the morning of each experiment. No preparation of the meat, such as removal of fat or connective tissue, was undertaken. In order to accelerate the spoilage preparation of the meat, such as removal of fat or connective tissue, washing, or inoculation with bacteria, was undertaken. The population of P. aeruginosa in a particular meat sample was determined by plating meat homogenate and incubating for 48 h at 25°C, and the total viable counts (TVC) were recorded as CFU.

**Supervised analysis.** ASCII data were exported from the Opus software used to control the FT-IR instrument and imported into Matlab version 5.3 (The MathWorks, Inc., Natick, Mass.), which runs under Microsoft Windows NT on an IBM-compatible PC. To minimize problems arising from unavoidable baseline shifts, the spectra were scaled so that the smallest absorbance was set to 0 and the highest was set to +1 (47).

When the desired responses (targets) associated with each of the inputs (spectra) are known, then the system may be supervised. The goal of supervised learning is to find a model that correctly associates the inputs with the targets; this is usually achieved by minimizing the error between the target and the model’s response (output) (16, 36). For quantititative interpretation of the FT-IR spectra, the multivariate linear regression method of PLS was applied as detailed previously (27) following computations given elsewhere (36). The input (x) data sets for the supervised-learning method contained the full HATR FT-IR spectra (441 absorbances representing a band of 16 wavenumbers) and known log_{10}(TVC) values (y data) from the first two spoilage experiments, and these were partitioned into training and cross validation sets according to our established modeling practices (31, 46). During calibration of the model, the root mean squared (RMS) error between the true and desired levels for the cross validation data was calculated, and the lowest RMS error from this was used to find the optimal calibration which would give the best general predictive model. Following calibration, the PLS model was challenged with the independent test set of data from the entirely separate third and final experiment.

**Evolutionary computation.** Although PLS is an excellent tool for the quantitative analysis of biological systems (36), the information as to which wave numbers in the infrared spectrum are important is not readily available. The use of PLS, therefore, is often perceived as a “black box” approach to modeling spectra and so has limited use for the deconvolution of hyperspectral data in chemical or biochemical terms. Therefore, in this study, evolutionary computation methods based on GAs and GP were employed to aid in the deconvolution of these hyperspectra.

**GA.** A GA is an optimization method based on the principles of Darwinian selection (24), where, over a series of generations, a population of parameter sets evolves until an optimal, or near-optimal, solution to a given problem is found. The chromosomes (or chromosomes) in a GA are represented as a string of numbers or binary digits representing the parameters of the problem to be optimized. The population is randomized so that n sets of “unique” parameter values can be evaluated and assigned a fitness value (usually a single numerical value). Once all n fitness values have been assigned, the next generation of chromosomes is created. In order for this new generation to be fitter than the last, principles analogous to those of sexual and asexual reproduction are applied. Using a stochastic selection method, based on parent fitness, the chromosomes are chosen to reproduce, swapping sections of their respective sequences (the probability of a particular parent chromosome being selected for sexual reproduction is proportional to its fitness) and chromosomes with a respectively high fitness value will have a greater chance of selection). This process creates two new child chromosomes inheriting characteristics of their parents. The child chromosomes are then subjected to mutation, where the value of each parameter may be randomly changed. The probability of this change is normally very small. The process of selection followed by reproduction followed by mutation is then repeated until n new chromosomes are created (i.e., a new population to replace the old). The fitness value is then evaluated for each of the new chromosomes, and the whole process repeats itself. The algorithm continues until a stopping criterion is reached. For example, the criterion may be that a given optimal fitness value has been reached or the number of generations has passed, or the chromosomes have converged to similar parameter values.

The GA-multiple linear regression (MLR) wavelength selection methodology (9) uses a GA to determine the subset of n wavenumbers, taken from the total spectral data set, which, when applied to an MLR model, will optimally discrim-
TABLE 1. Data matrix of results from three spoilage experiments

<table>
<thead>
<tr>
<th>Exp</th>
<th>Initial pH</th>
<th>Final pH</th>
<th>Initial log_{10}(TVC)</th>
<th>Final log_{10}(TVC)</th>
<th>Room temp (°C)</th>
<th>Spoilage (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.02</td>
<td>6.79</td>
<td>6.86</td>
<td>9.20</td>
<td>21.5</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>5.52</td>
<td>6.05</td>
<td>6.62</td>
<td>8.64</td>
<td>23.1</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>5.94</td>
<td>6.83</td>
<td>6.77</td>
<td>9.04</td>
<td>23.3</td>
<td>10</td>
</tr>
</tbody>
</table>

Mean (SD) 5.87, 6.67, 6.76, 9.02, 22.6 (0.99), 13.6 (3.5)

"The onset of spoilage is taken as the point when total bacterial numbers reach 10^5 CFU g^-1."

RESULTS AND DISCUSSION

The comminution of samples in order to accelerate the spoilage process was successful, as the final mean log_{10}(TVC) of 9.02 (Table 1) was an order of magnitude above the 10^5 CFU g^-1 generally accepted as the point at which organoleptic spoilage becomes readily detectable (13, 15). Using 10^5 CFU g^-1 as the indicator for postspoilage, the average spoilage time over the series of experiments was 13.6 h (Table 1). The spoilage of the samples within 24 h at room temperature was anticipated, as comminution ruptures cell walls, releasing a source of nutrients; increases the surface-area-to-volume ratio; and distributes bacteria that would normally be restricted to the surface throughout the meat substrate. The initial mean pH range of fresh samples during the three experiments (5.7 to 5.9) was within those described previously in the literature (13, 15). The use of pH as an indicator of spoilage or remaining shelf life in meats would be insufficient, as the pH fluctuates prior to spoilage, only rising significantly when levels of bacte-
so show that this approach can be used to accurately assess the spoilage status of meat. As can be seen in Fig. 3 and Table 2, the lowest level of spoilage encountered was \(2 \times 10^6\) CFU g\(^{-1}\), and this necessarily restricts the detection limit. The TVC for chicken immediately postslaughter is \(10^3\) cm\(^{-2}\), rising to \(10^4\) to \(10^5\) cm\(^{-2}\) after packaging (26). That PLS gave accurate results at \(2 \times 10^6\) CFU g\(^{-1}\) suggests that it will be possible to reach lower levels, and this will be the subject of further study with freshly killed chickens. From Fig. 3, it is evident that the spectra obtained by direct FT-IR analysis of meat do contain biochemical information that allows correlation with the spoilage status of the chicken, for data used to produce the PLS model and, more importantly, for data from a completely new experiment. The obvious question that needs to be addressed is that of which biochemical species the FT-IR is measuring that are related to the spoilage status of the chicken.

The Pearson correlation coefficients between the absorbances at each wave number in the FT-IR spectra from experiments 1 and 2 and the log10(TVC) were calculated and are also plotted in Fig. 2. It can be seen that most peaks from 1,500 to 700 cm\(^{-1}\) are positively correlated with spoilage, but no single peak appears uniquely dominant; this necessarily means that it is difficult to pinpoint the cause of microbial spoilage to a single (or a small group of) biochemical species using this correlation approach. Therefore, GAs and GPs were evolved to discriminate qualitatively between meat carrying \(<10^7\) and \(\geq10^7\) bacteria (as TVC) per cm\(^2\).

GA-MLR was applied so as to extract subsets of two, three, and five wave numbers that could discriminate between fresh (<\(10^6\) bacteria/cm\(^2\)) and spoiled (\(\geq10^7\) bacteria/cm\(^2\)) chicken. Because the starting population for each GA run was random, 60 GA-MLR runs were performed, and the following subsets were found to be optimal for selecting just two or three wave numbers, respectively: (1,096, 1,227 cm\(^{-1}\)) and (1,312, 1,235, 1,088 cm\(^{-1}\)). When the algorithm was used to look for five wave numbers, it was found that the degree of discrimination did not improve compared with selecting subsets of three, and no consistent areas of the FT-IR spectra were found to be dominant in the GA expressions; however, vibrations at 1,096 and 1,305 cm\(^{-1}\) were found within the best subsets.

### TABLE 2. Log10(TVC) of bacteria acquired from comminuted meat samples from three experiments

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Log10(TVC) for expt: Arithmetic mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.87(^e) 6.62(^c) 6.77(^t) 6.77</td>
</tr>
<tr>
<td>1</td>
<td>6.87(^e) 6.35(^v) 6.54(^t) 6.45</td>
</tr>
<tr>
<td>2</td>
<td>6.93(^e) 6.38(^e) 6.60(^t) 6.70</td>
</tr>
<tr>
<td>3</td>
<td>6.48(^e) 6.61(^t) 6.75(^t) 6.63</td>
</tr>
<tr>
<td>4</td>
<td>6.71(^e) 6.64(^t) 6.76(^t) 6.71</td>
</tr>
<tr>
<td>5</td>
<td>6.82(^e) 6.50(^t) 7.06(^t) 6.85</td>
</tr>
<tr>
<td>6</td>
<td>7.07(^e) 6.75(^t) 6.90(^t) 6.93</td>
</tr>
<tr>
<td>7</td>
<td>7.31(^v) 6.53(^v) 7.25(^t) 7.14</td>
</tr>
<tr>
<td>8</td>
<td>7.10(^v) 6.67(^v) 7.32(^t) 7.11</td>
</tr>
<tr>
<td>9</td>
<td>7.12(^v) 6.76(^v) 7.91(^t) 7.52</td>
</tr>
<tr>
<td>10</td>
<td>7.41(^v) 6.75(^v) 8.00(^t) 7.64</td>
</tr>
<tr>
<td>11</td>
<td>7.66(^v) 6.65(^v) 8.04(^t) 7.73</td>
</tr>
<tr>
<td>12</td>
<td>7.55(^v) 6.92(^v) 8.31(^t) 7.92</td>
</tr>
<tr>
<td>13</td>
<td>7.79(^v) 7.07(^v) 8.47(^t) 8.09</td>
</tr>
<tr>
<td>14</td>
<td>8.07(^v) 6.89(^v) 8.56(^t) 8.21</td>
</tr>
<tr>
<td>15</td>
<td>8.54(^v) 7.42(^v) 8.85(^t) 8.56</td>
</tr>
<tr>
<td>16</td>
<td>8.34(^v) 7.84(^v) 8.89(^t) 8.55</td>
</tr>
<tr>
<td>17</td>
<td>8.44(^v) 8.36(^v) 8.93(^t) 8.66</td>
</tr>
<tr>
<td>18</td>
<td>8.48(^e) 8.28(^e) 8.88(^e) 8.62</td>
</tr>
<tr>
<td>19</td>
<td>8.70(^e) 8.49(^e) 8.89(^t) 8.72</td>
</tr>
<tr>
<td>20</td>
<td>8.68(^e) 8.55(^e) 9.00(^t) 8.78</td>
</tr>
<tr>
<td>21</td>
<td>8.96(^e) 8.49(^e) 9.12(^t) 8.93</td>
</tr>
<tr>
<td>22</td>
<td>8.88(^e) 8.62(^e) 9.00(^t) 8.86</td>
</tr>
<tr>
<td>23</td>
<td>9.10(^e) 8.56(^e) 9.07(^t) 8.97</td>
</tr>
<tr>
<td>24</td>
<td>9.21(^e) 8.65(^e) 9.04(^t) 9.02</td>
</tr>
</tbody>
</table>

\(^a\) All measurements were taken in triplicate after incubation at 25°C for 48 h and were used, in conjunction with FT-IR spectra, to calibrate (\(^e\)), cross validate (\(^v\)), and test (\(^t\)) the PLS model.
GP analyses (i) using the same \(10^7\)-bacteria/cm\(^2\) threshold as above and (ii) evolved to predict the log \(10\) (TVC) levels produced trees which could easily discriminate between fresh and spoiled chicken and quantify the level of spoilage, respectively; a typical GP parse tree is shown in Fig. 4. As with the GAs, the initial populations were produced randomly; therefore, 10 separate GPs were evolved. For the threshold GP analysis, the number of times each input (wave number) was used for the 10 evolved populations was calculated and plotted against the wave number of the infrared light (Fig. 5). Figure 5 clearly shows that the dominant area of the spectra for discriminating between fresh (<\(10^7\) bacteria/cm\(^2\)) and spoiled (\(\geq 10^7\) bacteria/cm\(^2\)) chicken was 1,088 to 1,096 cm\(^{-1}\); moreover, these wave numbers were also selected by the GA-MLR method. The functional group vibration in the region 1,088 to 1,096 cm\(^{-1}\) is ascribable to C-N stretching, most plausibly from amines (14, 35).

The most intense peaks that appear in fresh meat are the amide I (C=O vibration at 1,640 cm\(^{-1}\)) and amide II (N-H deformation at 1,550 cm\(^{-1}\)) bands from proteins and peptides, and from the Pearson correlation coefficients, the amide II band is the only vibration that is negatively correlated with spoilage (Fig. 2). This strongly suggests that the protein content of the meat was decreasing during spoilage. By contrast, the peaks at 1,240 and 1,088 cm\(^{-1}\), which are both ascribable to C-N stretching from amines from free amino acids, are positively correlated. Indeed, the rule in Fig. 4 shows that spoilage can be ascribed simply to the ratio of 1,096 to 1,683 cm\(^{-1}\) from these vibrations from amines and amides, respectively.

Plots of the absorbances of these vibrations versus the time for the second experiment are shown in Fig. 6. It is clear that the amide I and II bands are constant, although the amide II band does decrease very slightly after 16 h while the peaks at 1,240 and 1,088 cm\(^{-1}\) start to increase significantly after 16 h. It is noteworthy that the onset of spoilage, as characterized by a TVC of >\(10^8\) g\(^{-1}\), was at 17 h, and this was the point at which

\[
[0 < 10^7 \geq 1] = \frac{1}{1 + \exp\left[-0.17912X - 34.91251\right]}
\]

FIG. 4. Typical GP tree evolved to discriminate between chicken carrying <\(10^7\) and \(\geq 10^7\) bacterial counts. The use of the logistic function \(Pr(Y) = 1/(1 + \exp^{-\text{Model Expression}})\), defines a maximum-likelihood \(Pr(Y)\) decision boundary for an output being either false (0) or true (1).
the absorbance due to free amines started to increase. This was also found to be the case for experiments 1 and 3 (data not shown). These correlations, and the fact that the GAs and GPs both pick the region 1,088 to 1,096 cm\(^{-1}\) as the most significant area of the FT-IR spectra for the prediction of spoilage of chicken which is attributable to free amino acids, makes it clear that the most significant metabolic process that occurs at spoilage is the start of proteolysis. This is indeed highly likely, since it is known that spoilage in meat is most frequently associated with the postglucose utilization of amino acids by aerobic microorganisms, such as pseudomonads, and the onset of the enzymatic degradation of proteins and peptides, leading to the production of free amino acids (8, 12, 40).

In conclusion, FT-IR spectroscopy, in combination with appropriate machine learning methods, presents itself as a novel method for the quantitative detection of food spoilage. Using FT-IR, we were able to acquire a metabolic snapshot (30) and quantify, noninvasively, the microbial loads of food samples accurately and rapidly (within 60 s) directly from the sample surface. We believe that this approach has considerable potential for further development and will aid both the food safety regulatory bodies and the Hazard Analysis Critical Control Point system. In particular, we will conduct future studies testing our method for quantifying the numbers of spoilage organisms on muscle foods at the production, processing, packaging, and storage levels.

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