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Detection and Quantification of Bacterial Spoilage in Milk and Pork Meat Using MALDI-TOF-MS and Multivariate Analysis

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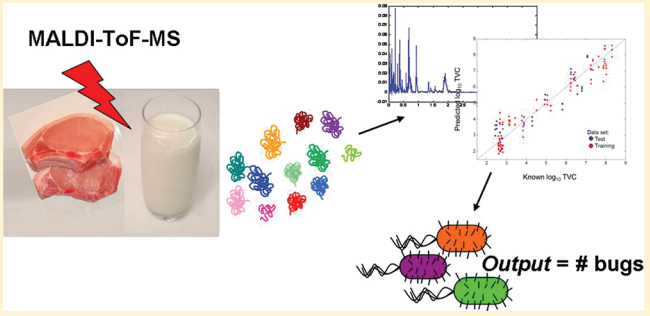
Supporting Information

ABSTRACT: Microbiological safety is one of the cornerstones of quality control in the food industry. Identification and quantification of spoilage bacteria in pasteurized milk and meat in the food industry currently relies on accurate and sensitive yet time-consuming techniques which give retrospective values for microbial contamination. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), a proven technique in the field of protein and peptide identification and quantification, may be a valuable alternative approach for the rapid assessment of microbial spoilage. In this work we therefore developed MALDI-TOF-MS as a novel analytical approach for the assessment of food that when combined with chemometrics allows for the detection and quantification of milk and pork meat spoilage bacteria. To develop this approach, natural spoilage of pasteurized milk and raw pork meat samples incubated at 15 °C and at room temperature, respectively, was conducted. Samples were collected for MALDI-TOF-MS analysis (which took 4 min per sample) at regular time intervals throughout the spoilage process, with concurrent calculation and documentation of reference total viable counts using traditional microbiological methods (these took 2 days). Multivariate statistical techniques such as principal component discriminant function analysis, canonical correlation analysis, partial least-squares (PLS) regression, and kernel PLS (KPLS) were used to analyze the data. The results from MALDI-TOF-MS combined with PLS or KPLS gave excellent bacterial quantification results for both milk and meat spoilage, and typical root mean squared errors for prediction in test spectra were between 0.53 and 0.79 log unit. Overall these novel findings strongly indicate that MALDI-TOF-MS when combined with chemometric approaches would be a useful adjunct for routine use in the milk and meat industry as a fast and accurate viable bacterial detection and quantification method.



Animal milk commonly derived from cows, sheep, and goat, and meat such as pork, have been part of the human diet for thousands of years. The reason behind their widespread use is their highly nutritious nature, being good dietary sources for proteins and certain vitamins and minerals.[1,2] Microbial spoilage of these types of food has been a constant nuisance and an unavoidable problem throughout history, as an immense number of fungi and bacteria use the same food (meant for human consumption!) as nutrient sources, affecting them in a variety of ways.[3−7]

Some of the microorganisms that grow in food can cause unacceptable sensory alterations, through the production of metabolites, such as off-flavors or odors or changes in texture or appearance, termed collectively as sensory spoilage.[8−10] These are the microorganisms that should be termed as specific spoilage organisms for food, as other microorganisms may also grow in food but without causing any sensory changes. The type of sensory changes causing food spoilage appear to depend on the type of microorganism species present in food which are sequentially influenced by the chemical composition of food, and in turn affected by the processing and preservation processes, and finally the storage conditions of foods.[5,9] In addition, the good bacterial growth promoting properties of milk and meat, especially when meat is undercooked or milk is inadequately processed, can favor the growth of some species that are considered to be pathogenic for humans, such as *Listeria* spp., *Salmonella* spp., *Campylobacter jejunum*, *Staphylococcus aureus*, and *Yersina enterocolytica*,[11] which sometimes lead to outbreaks of bacterial food poisoning,[12] which in the immune-compromised, very young or elderly may lead to death.

The role of milk and meat product analysis, involving the detection of spoilage and pathogenic organisms through quantitative and qualitative processes, respectively, is of paramount importance for food product assessment and in the promotion of public health. In an attempt to preserve high-quality manufacturing and food production levels and at the same time safeguarding the public, European Union legislation (Directive 92/46 EEC) has been introduced while the Hazard

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Analysis and Critical Control Point (HACCP) system has been implemented worldwide.

A number of different techniques have been explored and utilized over the years for the detection and enumeration of microbiological spoilage in food products. These include ATP bioluminescence, electrical and microscopy methods, immunoassays, polymerase chain reaction, nucleic acid probing, and electronic nose techniques,[1,13−26] each with positive and negative aspects in regard to their application and final outcome. Significant drawbacks of these techniques include the high demand on operator skills and relatively slow sample turnaround times, which limit them for everyday use in the food industry.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been shown to possess very positive characteristics in its ability to identify and more recently quantify proteins,[27−33] potentially making it a very useful tool in the analysis of food products such as milk and meat. As an ionization technique, MALDI works on the principle of laser irradiation of a sample incorporated into a UV-absorbing matrix, leading to desorption and ionization subsequently detected using TOF-MS.[34] The use of small sample quantities, the simple preparation and analysis techniques, and the successful application on heterogeneous food samples are some of the potential advantages of the technique.[35,36] The aim of our study was therefore to examine the ability of MALDI-TOF-MS for quantifying bacterial contamination in whole pasteurized cows' milk and pork meat. To achieve this, we employed a variety of multivariate analysis techniques and used the entire spectral peak range acquired during analysis.

MATERIALS AND METHODS

Bacterial Spoilage. Milk and pork meat were allowed to naturally spoil for 168 and 78 h, respectively, and sampled regularly for MALDI-TOF-MS. The full details of this and the microbiological methods for calculating the bacterial numbers (total viable counts (TCVs)) can be found in the Supporting Information.

MALDI-TOF-MS Sample Preparation. *Milk.* Milk sample preparation was undertaken as previously described by Cozzolino and colleagues[29] and further optimized in ref 37. Dilution of 100 μL of each homogenized sample was performed in 1 mL of water containing 0.1% trifluoroacetic acid. Further dilution with the same solvent then ensued to a ratio of 1:10 before 5 μL of each sample was mixed with the same quantity of the matrix solution. The latter was composed of sinapinic acid saturated in a matrix solution composed of 50% acetonitrile and 50% water. One microliter volumes of the final mixtures were then used for MALDI-TOF-MS analysis, after being placed on a stainless steel target plate and allowed to dry for 1 h at room temperature.[37]

Meat. The 1 g pork samples were diluted and mixed in 10 mL of water containing 0.1% trifluoroacetic acid; for most of the meat to be suspended, this was vortexed for 15 min. These samples were then further diluted and mixed in a 1:10 ratio with the same solvent. The next steps for mixing the sample with the matrix preparation were undertaken as described above, except before the samples were spotted onto the target plates, the meat was allowed to settle so that only the liquid phase was sampled.

MALDI-TOF-MS. A MALDI-TOF mass spectrometer (AXIMA-CRF*plus*, Shimadzu Biotech, Manchester, U.K.), equipped with a nitrogen pulsed UV laser (337 nm) was employed in the analysis as developed previously.[37] Ionization and separation was undertaken using a positive ion source in linear mode and 90 mW of laser power. A random raster of 500 profiles, individually containing data from five laser shots, was used to analyze each spot. Typical collection times were 4 min per sample, and each sample was analyzed three times.

Data Analysis. *Preprocessing.* The mass spectral data were processed and analyzed once they were imported into the Matlab 2010a (The Math Works, Natick, MA) program. Data were baseline corrected using asymmetric least squares and normalized by dividing each individual baseline-corrected spectrum with the square root of the sum of squares of the spectrum[38] (the effect of this process is shown in Figure S1 in the Supporting Information). Associations between the MALDI-TOF-MS spectra and the total viable count (TVC) numbers in milk and meat were then investigated employing multivariate statistical methods, including cluster analysis, correlation analysis, and the supervised regression-based techniques of partial least squares (PLS) and kernel PLS (KPLS).

Trajectory Analysis. A full description of the two-step trajectory analysis undertaken in this study has been provided elsewhere.[39] Principal component analysis (PCA) was used to transform a large number of correlated variables in the MALDI-TOF-MS spectra to a smaller number of uncorrelated variables, named principal components (PCs).[40] Subsequently, with the use of prior knowledge of which spectra are biological replicates, discriminant function analysis (DFA) is able to discriminate data on the basis of the first 20 retained PCs. The use of biological replicates in the cluster analysis negates the introduction of bias as the algorithm is not told the level of bacterial spoilage.

Quantitative Analysis. In contrast to the trajectory analysis during supervised learning, this statistical process constructs models to associate inputs and outputs; this necessitates that the knowledge of both input (*viz.*, MALDI-MS spectra) and the target/output values (TVCs) is used in model calibration. In this study, canonical correlation analysis was performed to assess whether there is any significant correlation between the MALDI-MS spectra and the TVCs, and then such correlation was tested by constructing linear and nonlinear models using PLS and KPLS, respectively. The validation of the PLS and KPLS methods for both milk and meat samples was performed by randomly selecting 70% of the total spectra to use as a training set while using the remaining 30% as a test set. This process was repeated four times, and the average prediction accuracies of the models were reported.[41]

CCA,[42] PLS,[43] and KPLS[44] statistical analyses were undertaken as previously reported by us elsewhere[37,45] and described in the Supporting Information. All the calculations were performed using Matlab 2010a, and the calculations of model validation, latent variable selection, and prediction errors were executed as described elsewhere.[37,41,45]

RESULTS AND DISCUSSION

Total Viable Count. Table S1 (Supporting Information) illustrates the results obtained from the spoilage experiments for milk and meat. With regard to the milk experiments, these are also illustrated as growth curves (Figure 1A). The initial mean log(TVC) was 2.67, and after 7 days (78 h) of incubation at 15 °C the final mean log(TVC) was 6.95. The bacterial lag phase during these experiments was consistently between 0 and

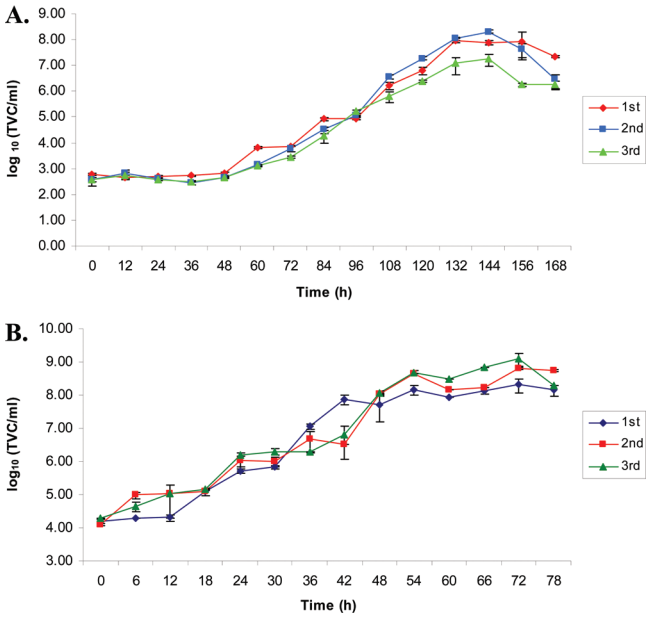

Figure 1. log(TVC/mL) against time for (A) full fat milk samples spoiled at 15 °C for 168 h and (B) pork meat samples spoiled at room temperature for 78 h. Each point plotted represents the average value derived from three replicate measurements, and the error bars indicate the standard deviation.

48 h, with a mean log(TVC) of 2.67−2.71. The 48 h time point marked the start of the exponential bacterial growth phase which ended at ∼132 h (mean log(TVC) = 2.71−7.86).

The bacterial growth dynamics in the spoilage experiments for pork (Figure 1B), as expected, show a pattern similar to those described above for milk, except that spoilage was quicker. The initial mean log(TVC) of 4.20 was followed by a ∼12 h lag phase and an exponential phase initiating between 12 and 18 h and ending at ∼60 h with a mean log(TVC) of 5.11−8.25; finally from then on the stationary phase ensued (mean log(TVC) of ∼8.51).

Mass Spectra. It is possible that specific peaks in the MALDI spectra may be exploited to identify and potentially quantify protein components in spoiled milk and meat samples, and this may be aided by reference to previously published protein mass spectra. However, this is not straightforward as consideration has to be given to the potential variability of the molecular mass of proteins and thus the position of specific peaks of some of these proteins. Genetic and nongenetic polymorphism, food processing (which may lead to protein denaturation), differences between the raw and processed food product, and small variations in molecular mass between different animal breeds or even among the same animal species can influence the molecular mass.[46−50]

Due to the lack of any obvious single protein that could be used to quantify the bacterial spoilage level, we decided to use whole spectral analysis methods. Baseline correction and normalization of data were two processes required prior to chemometric analysis, and we have previously optimized them for MALDI-TOF-MS from milk.[37] A typical MALDI mass spectrum of fresh full fat pasteurized cows' milk illustrating the effect of the above processes on a baseline spectrum is shown in Figure S1 (Supporting Information). Characterization of proteins through spectral peaks (Figure 2A,B) mostly appears to be similar between fresh and spoiled milk with some variations in the exact position of the molecular mass signal of a few of the proteins, probably due to the factors explained above, and the presence of some additional proteins. A small number of distinctions in regard to the existing numbers of proteins in each type of milk can also be visualized. Utilizing information derived from previously published molecular mass data for proteins in milk,[27,29,30,33,51] a number of protein peaks can be identified (Figure 2A,B). For example, proteoso peptone can be identified at the $m/z \approx 9000$ peak, and peaks relating to α-lactalbumin and β-lactoglobulin are seen at $m/z \approx 16000$ and m/z 18500, respectively. The α-lactalbumin peak appears to almost disappear from 0 to 168 h, while the proteoso protein and β-lactoglobulin peaks appear to decrease in intensity during the same time period. Dimeric and trimeric species also make their appearance as broad peaks over m/z 32000 and 43000,[51] but again disappear from 0 to 168 h.

Typical MALDI spectra for fresh and spoiled pork meat are also illustrated in Figure 2C,D. Similar to the milk spectra prior to chemometric analyses, the original data were baseline corrected. Apart from the absence of some proteins between the fresh and spoiled pork meat spectra, qualitatively both spectra displayed very similar protein patterns. Furthermore, some quantitative differences were visible in regard to some types of protein. The lack of published information on molecular mass data of pork meat in relation to the protein peaks derived directly from MALDI makes specific identification of proteins based on the obtained spectral peaks (Figure 2C,D) difficult. Indirect information though through the initial isolation of proteins through polyacrylamide gel electrophoresis (PAGE) and subsequent characterization using MALDI-MS on pork meat can potentially reveal some useful information.[52] On the basis of this information, a number of protein peaks can be identified by closely inspecting the MALDI mass spectrum derived from pork meat (Figure 2). This reveals two peaks between $m/z \approx 32500$ and m/z 33000 representing tropomyosin α- and β-chains for skeletal muscle, respectively; the myosin regulatory light chain 2 for skeletal muscle is represented at $m/z \approx 18800$ m/z, and potentially the actin α-1 skeletal muscle peak is represented at $m/z \approx 39000$, showing a lower intensity at 78 h compared to 0 h, while a peak at $m/z \approx 36000$ almost disappears from 0 to 78 h.

Trajectory Analysis. The first stage of the chemometric analysis was to observe the relationship between the MALDI-TOF-MS spectra without using any prior information on the bacterial spoilage levels, and this employed the cluster analysis using PC-DFA. Figure 3A shows the relevant results from the analysis of the milk samples. Trajectory analysis of these data shows that during the first 108 h there is a downward trend from the right upper part of the PC-DFA space downward in DF1 which relates with the sampling time (although there is overlap between neighboring time points). After 108 h a clearer distinction between time points is revealed with samples collected between 120 and 168 h which now follows a different direction, a right to left upward trajectory in DF1 and DF2. During this period the mean log(TVC) values change from 6.96 to 8.00 to 6.95. This suggests that, despite little bacterial growth during this time period, significant changes are occurring in relation to the milk proteins as DF1 is extracted to explain the most variance in the plot.

By contrast, a different type of trajectory trend is seen in the PC-DFA space constructed from the MALDI-MS data collected from the pork meat (Figure 3B) with a good distinction between all time-points from 0 to 78 h. The spread of time points is greater and more distinct compared with those from

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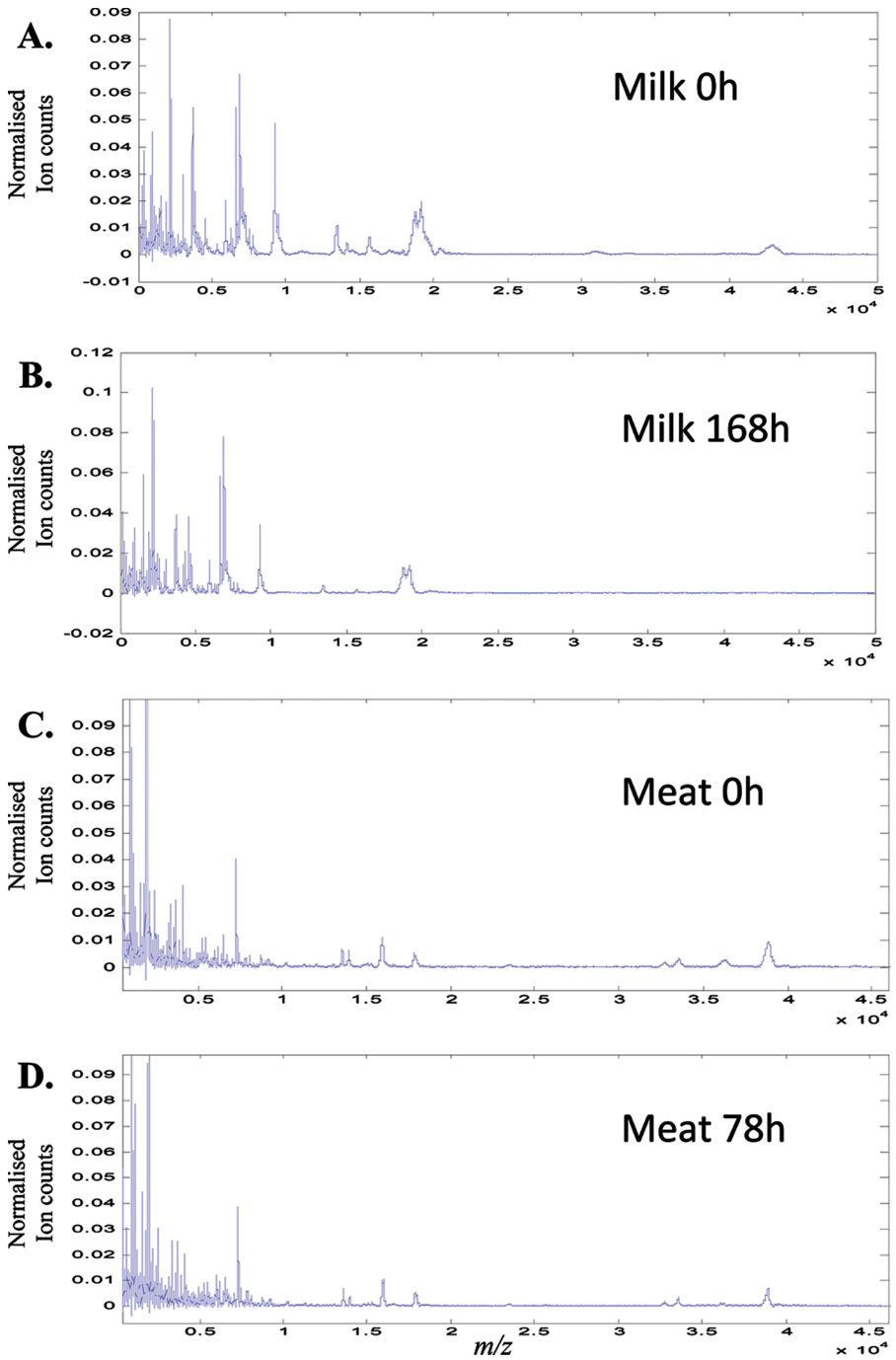

Figure 2. MALDI-TOF-MS mass spectra for whole milk at (A) 0 h and (B) 168 h and pork meat samples at (C) 0 h and (D) 78 h. Spectra plotted are those after data preprocessing.

the milk samples. Overall trajectory analysis shows a double dip trend from 0 to 54 h from a left to right direction, spreading across the DF1 with relatively clear distinction between time points representing the initial lag and the exponential bacterial growth phases, with a typical bacterial growth increase of log(TVC) from ~4.20 to ~8.56. Unlike the milk trajectory, which had a clear gradient effect, according to the progression of the spoilage, during the spoilage of the pork samples, several distinctive trajectory patterns can be observed. The early spoilage (<48 h) samples are located in the negative part of DF1 and late spoilage (>54 h) samples on the positive side of DF1. The gradient in between follows a "W"-shaped pattern, with a middle peak at spoilage times between 48 and 54 h. This suggests that the changes in the proteomic profiles in pork spoilage might not be a continuous process but one with multiple phases. This may be due to the sequential colonization of different bacteria on the surface as the succession proceeds due to nutrient limitation; although there is no direct evidence of this for pork in this experiment, similar nutrient catabolism has been reported elsewhere.[8] Similar to the milk PC-DFA, a clearer time point distinction and trajectory spread across DF1 (from left to right this time) is seen after the initiation of the stationary phase (after 60 h of growth) when the mean log(TVC) changes minimally from 8.25 to 8.48.

Examination of the PC-DFA loadings (Figure S2, Supporting Information) allowed for the identification of some important

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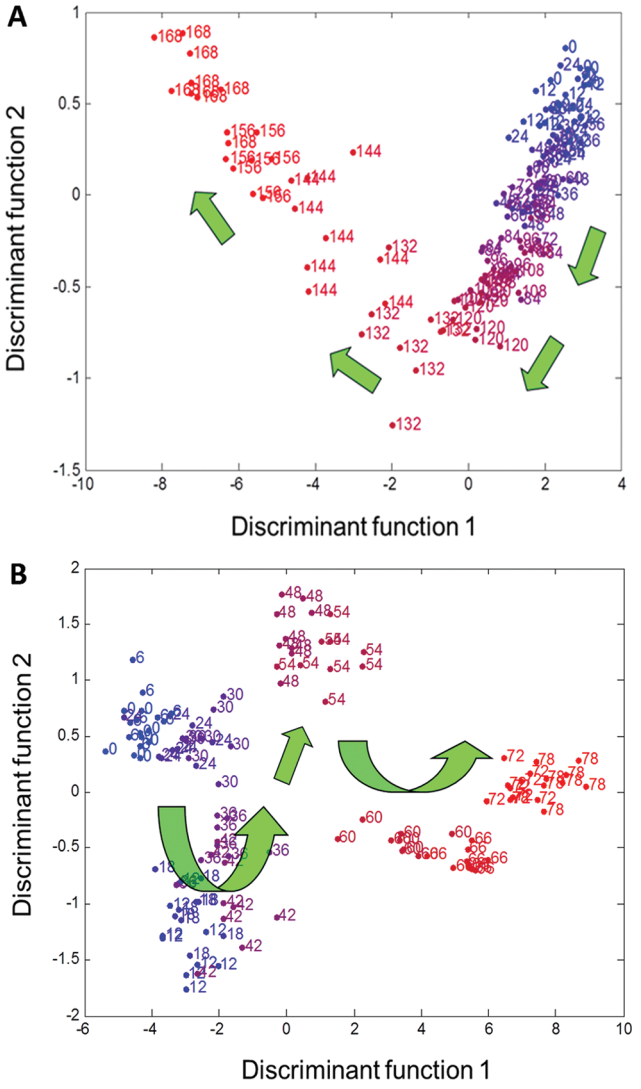

Figure 3. PC-DFA plot of MALDI-TOF-MS spectra for (A) milk and (B) pork meat samples. The DFA algorithm used PCs 1−20 (accounting for (A) 99.76% and (B) 99.93% of the total variance) with *a priori* knowledge of biological replicates. Distinct time points are differentiated in terms of color difference, while data trends are illustrated using block arrows.

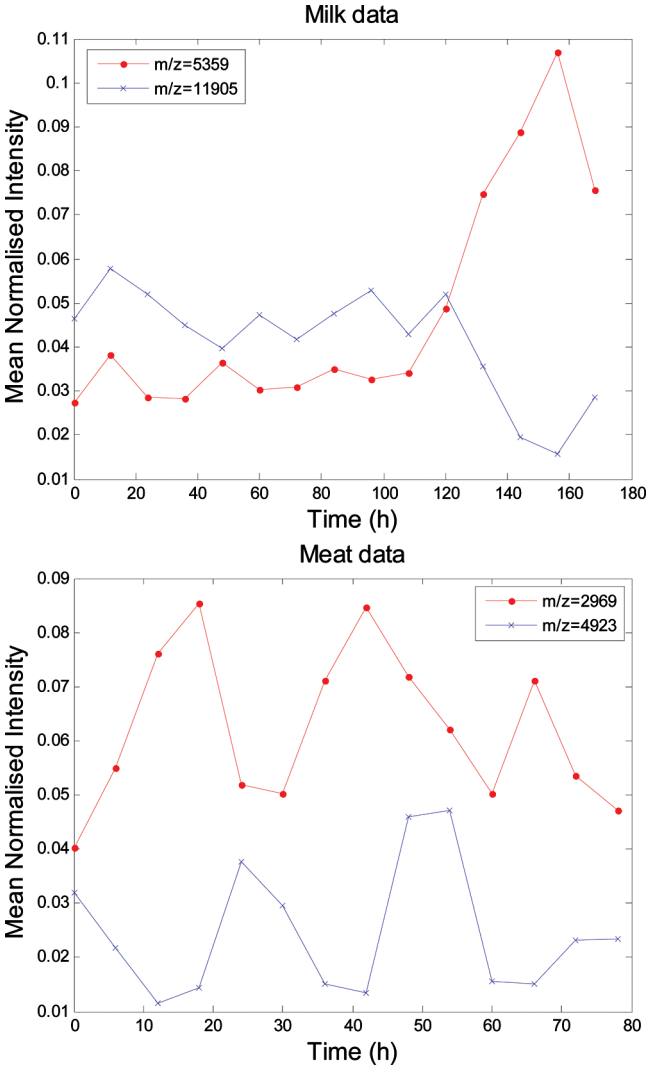

Figure 4. Changes in normalized peak intensity against time of two randomly selected representative proteins in milk and meat samples. Full statistics on these peaks are shown in Figure S3, Supporting Information.

peaks used to create the PC-DFA model (Figure 3). Two significant peaks from milk and two significant peaks from meat PC-DFA loadings were selected to illustrate how the peak areas change with respect to time (Figure 4; Figure S3, Supporting Information). These peaks show some multiple-stage trends, and the two pairs of milk and pork protein peaks appear to be almost perfectly anticorrelated. This may indicate internal biological processes taking place within the samples across the time points, such as the "consumption" of proteins/peptides by the bacteria and the generation of new peptides/proteins (which could be protein degradation products or new bacterial proteins). In milk where proteins at m/z 11905 and 5359 have been identified as γ2-casein and fragments of β-casein,[53] respectively, the process potentially illustrated in Figure 4 shows the effect of spoilage and in particular the catabolism of higher molecular weight proteins with respect to time and accumulation of lower molecular weight fragment proteins.

Canonical Correlation Analysis. To investigate the correlation of MS with bacterial spoilage levels, further data analysis using CCA was undertaken separately on the MALDI-TOF-MS data obtained from the milk and pork meat samples. Canonical correlation coefficients (R) of canonical variables against log(TVC) were 0.94 for milk and 0.95 for meat samples. The CCA plots from milk and pork meat are shown in Figure S4 (Supporting Information), and in general, an approximately linear correlation between the bacterial concentration levels and the MALDI mass spectra is observed.

Bacterial Quantification Using PLS and KPLS. As there appears to be a clear correlation of MS with TVC, linear and nonlinear regression analyses were employed in an attempt to perform bacterial quantification. As described above, all PLS and KPLS models were constructed four times, where 70% of the data were used to calibrate the model and the remaining 30% for model validation (and in this process replicate samples were kept together). The average prediction values were calculated, and these are reported in Table 1 and include error calculations for the training (root-mean-square error for the calibration, RMSEC), cross-validation (root-mean-square error for the cross-validation, RMSECV), and test set prediction (root-mean-square error for the prediction, RMSEP) along

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Table 1. Determination of the log(TVC) from Milk and Pork from MALDI-TOF-MS Spectra Using PLS Regression and the Nonlinear KPLS[a]

	PLS		kernel PLS	
	mean	std dev	mean	std dev
Milk				
RMSECV (log)	0.89	0.06	0.89	0.06
RMSEC (log)	0.59	0.04	0.55	0.20
RMSEP (log)	0.79	0.10	0.64	0.20
correlation coeff in the training set (R^2)	0.92	0.01	0.95	0.03
correlation coeff in the test set (Q^2)	0.82	0.03	0.82	0.03
Pork				
RMSECV (log)	0.75	0.06	0.77	0.01
RMSEC (log)	0.32	0.03	0.40	0.07
RMSEP (log)	0.72	0.09	0.53	0.11
correlation coeff in the training set (R^2)	0.96	0.01	0.96	0.01
correlation coeff in the test set (Q^2)	0.80	0.04	0.81	0.04

[a]RMSECV = root-mean-square error for the cross-validation, RMSEC = root-mean-square error for the calibration, and RMSEP = root-mean-square error for the prediction, all produced from the training set.

with the correlation coefficients for the training (R^2) and test (Q^2) sets. The representative results for PLS from milk and meat from one of the four models (the model closest to the average) are plotted in Figure 5, where it is clear that the expected $\log(y) = \log(x)$ line is seen for both the training data and most important for the test data.

Inspection of the highest variable importance for prediction (VIP) scores can reveal the most significant features used by the PLS models to detect milk and meat spoilage. The VIP scores for both milk and meat are shown in Figure S5 (Supporting Information) and Table 2. For milk the most significant features at m/z 11623 and 11891 represent γ3-casein and γ2-casein, respectively.[29,51] Even though these proteins did not appear on the fresh and spoiled milk spectra, PLS suggests that despite their apparent small "spectral" magnitude these proteins are highly important. Additional peaks at m/z 5057, 6405, and 7227 also had significant VIP scores, representing fragments of a_{s1}-casein, β-casein, and β-lactoglobulin, respectively.[54,53,55] The peaks at m/z 6405 and 7097 although significant are not yet identified. Low molecular weight species below m/z 9000 are perhaps not surprisingly overrepresented in these spectra as they are likely to arise from the bacterial proteolysis of higher molecular weight proteins; however, due to the very complex microbial community present on milk and meat during spoilage,[2,5,8,24,56] these proteins are not readily identifiable.

With respect to spoilage of pork, although many peaks are significant according to VIP (Figure S5, Supporting Information), we have not been able to identify them because of the lack of published information on molecular mass data of pork meat in relation to the proteins in this foodstuff, let alone the microbial community that may be involved in the spoilage process. These peaks could potentially be identified using other MS methods such as LC–MS–MS; however, this requires complex sample preparation techniques and interpretation (the known amino acid sequence of these proteins from these bacteria would need to be available,[57–59] and many food spoilage organisms are yet to be brought into pure culture) and is outside of the scope of the present study.

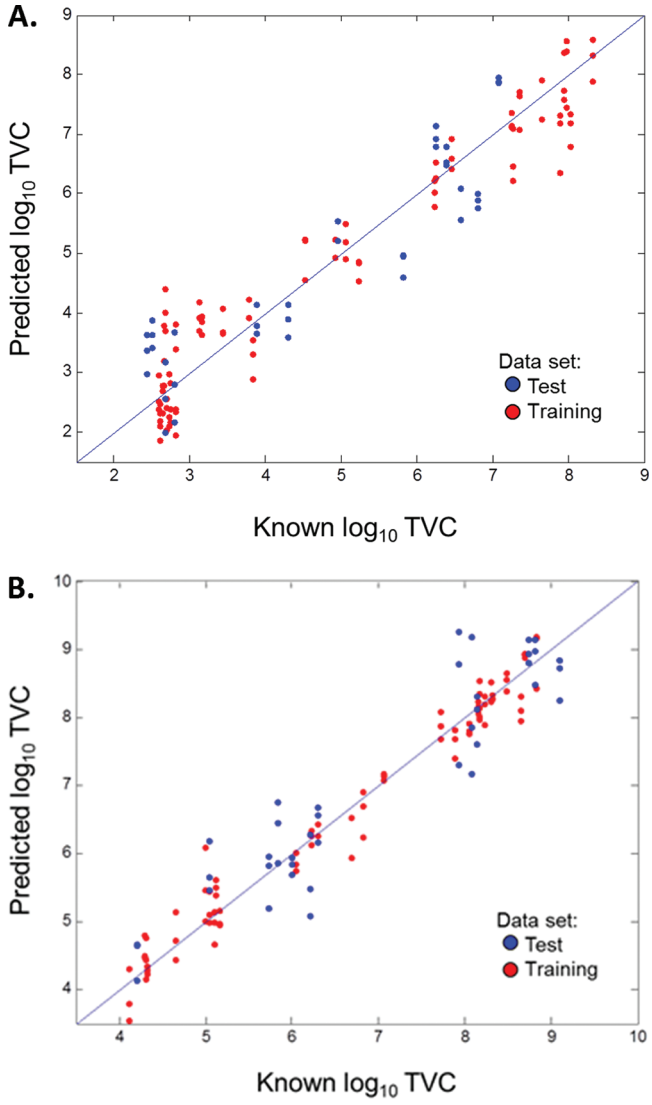

Figure 5. Illustration of the average predicted log(TVC) values derived from PLS regression on MALDI-TOF-MS *versus* the average measured log(TVC) for (A) milk samples and (B) pork meat samples. Representative plots are shown.

Table 2. Spectral Peaks with the Highest Average Variable Importance for the Prediction (VIP) Scores Used in the PLS Modeling for Milk and Meat Samples

peak m/z	av VIP score	std dev	peak m/z	av VIP score	std dev
		Milk			
5057	692.3	112	7227	324.4	11.47
5359	286.9	26.7	11623	193.3	22.1
6405	184.7	27.9	11891	465.7	41.1
7097	621.5	24.6			
		Meat			
3382	107.2	9.9	4816	117.3	3.4
3911	1418.3	152.3	4918	171.4	16.7
4098	156.0	17.2			

Good predictive values were obtained from both PLS and KPLS analyses using MALDI-TOF-MS data derived from both the milk and pork meat samples (Table 1). In the milk data the RMSEC derived from PLS analysis was 0.59 (log) and from the KPLS 0.55 (log), both providing similar prediction values,

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while the RMSEP was slightly better in the PLS analysis compared to KPLS, with values of 0.79 (log) and 0.64 (log), respectively. The correlation coefficients in the test set and training set were again similar for both types of analyses, with values for Q^2 of 0.82 and 0.82 and R^2 of 0.92 and 0.95 for PLS and KPLS, respectively. RMSEC and RMSEP values for pork meat data derived from PLS and KPLS were similar, with Q^2 and R^2 values ranging between 0.80 and 0.81 for Q^2 and 0.96 for R^2 for the two types of analyses. PLS appeared to yield slightly better results than KPLS; KPLS was implemented using an Radial Basis Function (RBF) kernel with a broad Gaussian width. This suggests that the multivariate modeling of the spoilage process is predominantly a linear regression problem as also indicated in the CCA plots (Figure S2, Supporting Information). Overall, predictions of bacterial enumeration in both milk and pork meat samples appeared to be highly encouraging using either of the PLS and KPLS strategies.

CONCLUDING REMARKS

Although selective peaks derived from MALDI-TOF-MS spectra have been utilized in the past, in both milk[27−33] and meat,[52] this has been limited to assessing good protein qualitative qualities rather than microbial spoilage. In this study no single peaks could be used to estimate the bacterial spoilage load accurately, so multivariate analyses were investigated. Previously we have used the whole MALDI-TOF-MS spectra in combination with multivariate analysis for quantifying milk adulteration with milk from different animal species;[37] thus, we decided to adopt a similar approach here for the estimations of bacterial load in milk or on pork. In this study we used the entire MALDI-TOF mass spectrum in conjunction with CCA, linear PLS, and nonlinear kernel PLS. Good predictions of total bacterial counts causing natural spoilage in food samples have successfully been demonstrated; for the test set samples RMSEP was between 0.64 and 0.79 and 0.53 and 0.72 log unit for milk and pork meat, respectively.

In our opinion the use of MALDI-TOF-MS appears to be advantageous compared to other analytical techniques. We have shown in this study that MALDI-TOF-MS is a sensitive technique in regard to detecting microbiological spoilage in milk and meat, and it is also very fast: a spectrum can be generated within minutes following sample preparation. In addition, the soft-ionization process utilized causes minimal or no fragmentation of the analytes (peptides and proteins), allowing for the detection of their molecular ions even in mixtures. Fourier transform infrared (FT-IR) spectroscopy has similar properties with regard to the quantification of bacterial spoilage,[4,60] in that it is a rapid technique also requiring minimal sample preparation, with low running costs. However, the molecular specificity of MS potentially enables the identification of important proteins, which is a major advantage compared to FT-IR spectroscopy.

In conclusion, the typical sample times for MALDI-TOF-MS were only 4 min per sample, and this is certainly much faster than classical microbiological plating approaches which take up to 2 days. These rapid and accurate virtues of MALDI-TOF-MS in relation to bacterial detection and enumeration make this approach a very good candidate for potential use in the dairy and meat industries. Such methods are urgently needed within HACCP, and such a general spoilage detection technique will be important for improving consumer safety and product quality.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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