Metabolic profiling of meat: assessment of pork hygiene and contamination with *Salmonella typhimurium*†

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Spoilage in meat is the result of the action of microorganisms and results in changes of meat and microbial metabolism. This process may include pathogenic food poisoning bacteria such as *Salmonella typhimurium*, and it is important that these are differentiated from the natural spoilage process caused by non-pathogenic microorganisms. In this study we investigated the application of metabolic profiling using gas chromatography-mass spectrometry, to assess the microbial contamination of pork. Metabolite profiles were generated from microorganisms, originating from the natural spoilage process and from the artificial contamination with *S. typhimurium*. In an initial experiment, we investigated changes in the metabolic profiles over a 72 hour time course at 25 °C and established time points indicative of the spoilage process. A further experiment was performed to provide in-depth analysis of the metabolites characteristic of contamination by *S. typhimurium*. We applied a three-way PARAllel FACtor analysis 2 (PARAFAC2) multivariate algorithm to model the metabolic profiles. In addition, two univariate statistical tests, two-sample Wilcoxon signed rank test and Friedman test, were employed to identify metabolites which showed significant difference between natural spoiled and *S. typhimurium* contaminated samples. Consistent results from the two independent experiments were obtained showing the discrimination of the metabolic profiles of the natural spoiled pork chops and those contaminated with *S. typhimurium*. The analysis identified 17 metabolites of significant interest (including various types of amino acid and fatty acid) in the discrimination of pork contaminated with the pathogenic microorganism.

Introduction

Food spoilage is the result of the activities of microorganisms on the food matrix resulting in the decomposition of carbohydrates and proteins,[1,2] and the synthesis and subsequent release of malodorous substances and metabolites from the breakdown of meat products or microorganisms. The quality of food is usually defined with respect to organoleptic changes that make the meat unacceptable to the consumer and these include the development of off-odours, colour changes or slime formation;[3] this deterioration eventually makes the food unsuitable to eat and this occurs at around 10^7 colony forming units (cfu) per square centimetre.[4] It is particularly hazardous when the food is contaminated by pathogenic microorganisms, for example *Salmonella* bacteria, as these can attack the stomach and intestine and in severe cases result in blood poisoning. Various tools have been developed to monitor the hygiene status of foods, from general flavours and aroma analysis to some very specific tool including PCR assays and immunosensors among others for detecting specific hazardous pathogen(s); for a review see Ellis and Goodacre.[2] In recent years, the approach of spectral "fingerprinting" of the metabolic profile of a biological system has attracted significant attention, especially in the field of metabolomics. This is because of the rapidity of the sampling which leads to immediate action being taken if a contamination is detected. In addition, methods that measure the phenotype of the cell are advantageous as this shows how the organism is responding to an environment rather than a somewhat static readout such as PCR. The metabolic profile is a 'holistic' method to determine metabolites from a diverse range of metabolic pathways, so to define system-wide differences in metabolism. By utilizing powerful analytical tools such as gas chromatography-mass spectrometry, a wide range of metabolites can be detected and identified and, at least, semi-quantified. This provides abundant information to further the understanding of the biological process under investigation and paves the way for developing more specific and sensitive tools for pathogen detection.[5–8]

Given the advantages of metabolic profiling approaches, we believe that such approach can be adopted as a valuable tool to improve the traceability of the contaminations of microorganism. There are a few reports in the literatures investigating rapid natural meat spoilage detection by proton transfer reaction mass spectrometry[9] and FT-IR spectroscopy[10,11] yet these studies were based on a single isolated experiment on naturally spoiled meat. In this study we explore the use of a metabolic profiling approach to characterise the microbial contamination of pork and, in addition, discriminate samples colonised with natural spoilage microorganisms from those contaminated

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by *Salmonella typhimurium*. Two objectives were investigated: (1) the temporal progression of the spoilage process and (2) to determine metabolic differences associated with contamination by *Salmonella* or natural spoilage microorganisms. Two independent experiments were conducted; the first experiment monitored the progression of the spoilage process during the incubation period following contamination by *S. typhimurium*. Metabolic profiles were generated at multiple time points ($n = 11$) across a 72 hour period for each experimental condition (natural spoiled and *Salmonella* contaminated). The second experiment provided robust statistical testing and identified specific metabolites to differentiate between the experimental conditions. It is worth noting that even *S. typhimurium* is not a commonly observed microorganism found in food 'spoilage'; however, when this food 'poisoning' organism is found, it usually leads to very serious consequences.[12,13]

Experimental

Culture and chemicals

S. typhimurium strain 4/74 was kindly provided by Professor Tim Brocklehurst (The Institute of Food Research, Norwich, UK). The strain was sub-cultured on Lab M LAB028 blood agar plates (Lab M Ltd., Lancashire, UK). A single colony was inoculated into nutrient broth (250 mL) and incubated at 37 °C for 16 h, which resulted in a culture of $\sim 5 \times 10^7$ cfu mL^{-1}. An aliquot (5 mL) of the culture was harvested by centrifugation at 4810*g* for 10 min. The supernatant was removed and the pellet was re-suspended in 50 mL of sterile saline solution and centrifuged at 4810*g* for 10 min, the supernatant was removed and this process was repeated two further times. The pellet was re-suspended in 50 mL of sterile saline solution and used for the artificial contamination of the pork.

Sample collection

A total of 33 boneless pork chops (weight 200–300 g) were used in the initial experiment. They were purchased from a local supermarket. Each pork chop was sampled to provide a unified surface area of 12–14 cm^2 followed by a process of butterflying into two pieces with equal surface area. In butterflying the meat is cut laterally and as spoilage organisms are not intramuscular this provides a virtually sterilized surface for the study. For each piece of pork, a digital photo was taken alongside with a ruler. The number of pixels on the pork surface was counted and divided by the number of pixels within one cm^2 area (estimated from the ruler in the same photo), thus providing an estimation of the surface area of the pork. Each piece of pork was placed in a large glass Petri dish lined with sterilized filter paper (Whatman grade 40 cat. no. 90-7501-06) to which 2 mL of sterilized saline solution (0.9% NaCl, w/v) was added to act as a moisture source and prevent the surface of the meat from drying out. The matched pieces of meat from the same pork chop were used as either a control or an artificial contamination surface. To the control sample, an aliquot (1 mL) of sterilized saline solution was added and the sample was incubated to allow the natural spoilage to progress. To the other piece, 1 mL of saline suspension of *S. typhimurium* (*vide supra*) was added and spread by using a sterile plastic loop. The Petri dishes were covered and incubated at 25 °C for various time points. A total of 11 time points were monitored: 0, 12, 24, 28, 32, 36, 40, 44, 48, 60 and 72 h during the incubation. At each time point, the biomass from the surface of 3 pork chops, *i.e.* 6 pieces were harvested using sterile swabs. Two swabs were used for each piece of pork and both were transferred directly into 1 mL of ice-cold methanol stored on dry ice (−48 °C). The suspension was extracted with three freeze–thaw cycles (frozen in liquid nitrogen and allowed to thaw on dry ice). The suspensions were then centrifuged at 16 060*g*, at −9 °C for 5 min. The supernatants were immediately lyophilised and subjected to GC-MS analysis (see below). A second experiment was performed approximately 6 months after the first experiment, the sample collection procedures were the same with the exception that only 4 time points were monitored: 0, 24, 48 and 72 h after the contamination. Samples from 6 pork chops were collected for each time point with 50% of the samples being applied as the controls and the other 50% applied as contaminated with *Salmonella*. Total viable counts (TVCs) were also performed in this experiment. This was performed by harvesting the microbial load from a 1 cm^2 area (randomly selected) of each pork piece using a sterile swab and transferred it into 1 mL of sterilized saline solution. The TVC data were hereby obtained using the plate count method.[3]

GC/MS analysis

An aliquot of 1000 μL of each metabolite extract was spiked with 100 μL of internal standard solution (0.19 mg mL^{-1} succinic d_4 acid, 0.27 mg mL^{-1} malonic d_2 acid and 0.22 mg mL^{-1} glycine d_5 in HPLC grade water) and then lyophilised in a HETO VR MAXI vacuum centrifuge attached to a HETO CT/DW cooling trap (Thermo Life Sciences, Basingstoke, UK). Samples were subsequently derivatized in two stages. An aliquot (50 μL) of 20 mg mL^{-1} *O*-methylhydroxylamine solution in pyridine was added and heated at 60 °C for 45 min followed by adding an aliquot (50 μL) of MSTFA (*N*-acetyl-*N*-(trimethylsilyl)-trifluoroacetamide) and then heating at 60 °C for 45 min. A retention index solution was added for chromatographic alignment (20 μL, 0.6 mg mL^{-1} $C_{10}/C_{12}/C_{15}/C_{19}/C_{22}$ *n*-alkanes).

The samples were analyzed in a random order by employing a GC/TOF-MS (Agilent 6890 GC coupled to a LECO Pegasus III TOF mass spectrometer) using a previously described method for yeast footprint samples.[14] Raw data were processed using LECO ChromaTof v2.12 and its associated chromatographic deconvolution algorithm, with the baseline set at 1.0, data point averaging of 3 and average peak width of 2.5. A reference database was prepared, incorporating the mass spectrum and retention index of all metabolite peaks detected in a random selection of samples (one sample per contamination or time-point class) so to allow detection of all metabolites present, whether or not expected from the study of bibliographic data. Each metabolite peak in the reference database was searched for in each sample and if matched (retention index deviation $< \pm 10$; mass spectral match > 750) the peak area was reported and the response ratio relative to the internal standard (peak area-metabolite/peak area-succinic d_4 acid internal standard) calculated. These data (matrix of N samples × P metabolite peaks) representing normalised peak lists were exported in ASCII format for further analysis. Metabolites were definitively

identified[15] by matching the mass spectrum and retention index of detected peaks to those present in the mass spectral library constructed at the University of Manchester.[16] A match is defined as a match factor greater than 750 and a retention index ±10.

Data analysis

We employed a three-way model named PARAllel FACtor analysis 2 (PARAFAC2)[17,18] to model the data generated by the two experiments. PARAFAC2 is an extension of the well known PARAFAC model,[19–21] a generalization of principal component analysis[22] to the situation where a series of matrices to be analysed (*e.g.* a three-way matrix). In PARAFAC, it models a 3 dimensional matrix $\underline{\boldsymbol{X}}$ with a dimension of ($I \times J \times K$) as a summation over R outer products of triads of vectors where R is the number of PARAFAC components. For the k^{th} ($k = 1, 2,\ldots, K$) "slab" (*e.g.*, all the sample at one certain time point) $\boldsymbol{X}_k$ in $\underline{\boldsymbol{X}}$, PARAFAC decompose it into a product of two loadings matrices as shown in eqn (1):

$$\boldsymbol{X}_k = \boldsymbol{F}\boldsymbol{D}_k\boldsymbol{A}^{\mathrm{T}} + \boldsymbol{\varepsilon}_k \quad (1)$$

where $\boldsymbol{F}$ is the loadings matrix of the row units (*e.g.*, samples), $\boldsymbol{A}$ is the loadings matrix of the column units (*e.g.*, the peak areas of the metabolites), D_k is the weight scalar for the k^{th} slab and $\boldsymbol{\varepsilon}_k$ is the residue matrix of the k^{th} slab. In PARAFAC related literatures, row units is normally named mode 1, column units is named mode 2 and a diagonal matrix $\boldsymbol{D}$, which contains the weights of all the slabs on its diagonal, is named mode 3, while $\boldsymbol{F}$, $\boldsymbol{D}$ and $\boldsymbol{A}$ are called the loadings of mode 1, 3 and 2 respectively. PARAFAC fits the three-way matrix X by minimizing each of the ε_k and this is typically achieved by using an alternative least square optimization.

However, PARAFAC requires that the size of each slab has to be exactly the same which sometimes cannot be met, even with a balanced experiment design (*e.g.*, a few samples may have failed to be derivatized and cannot generate good GC/MS data). PARAFAC2 was developed to cope with such problem and allows one dimension (normally rows, containing the samples) to be unequal between different slabs. The PARAFAC2 model is given by

$$\boldsymbol{X}_k = \boldsymbol{F}_k\boldsymbol{D}_k\boldsymbol{A}^{\mathrm{T}} + \boldsymbol{\varepsilon}_k \quad (2)$$

Instead of giving a global loadings matrix for mode 1, PARAFAC2 gives K loadings matrices, one for each slab, and each matrix may have different rows which matches the original size of $\underline{\boldsymbol{X}}$. By using PARAFAC2, a minor sample lose can be afforded without the need to "trim" the whole 3-D data matrix to make each slab equal.

In this study, two samples were lost during the first experiment and therefore a flexible PARAFAC2 was needed. We re-arranged the data matrix into a three-way matrix as illustrated in Fig. 1, then employed a PARAFAC2 model to fit the data. Using this model, the relative distribution of the samples at each time point can be revealed at loadings plots of the mode 1, the loadings of mode 2 can help identifying potential interesting variables (*i.e.*, metabolites), while the loadings of mode 3 (*i.e.*, the weight of each slab) reveal the global change of metabolic profiles over time. The number of PARAFAC2 components was 2 as once 3 or more components were used, the results appeared to be very unstable (*i.e.*, different runs generated different results), indicating too many components were introduced causing distortions. Once the model was built, the separation between two types of samples, *viz. S. typhimurium* contaminated and natural spoiled samples, at each time point was measured using a Hotelling T^2 statistic[23] using the model 1 loadings. Two PARAFAC2 models were built, one for each experiment, with the same parameter setting and the results were compared.

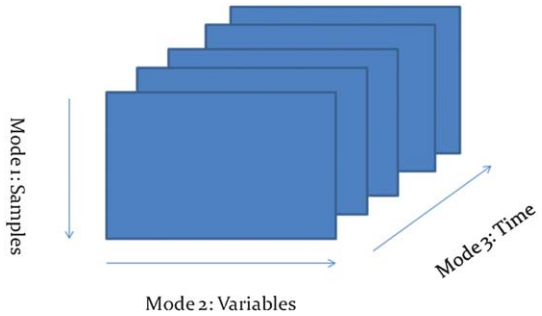

Fig. 1 Illustration of PARAFAC2 model. Mode 1 details information about the samples that have been analysed (*e.g.* naturally spoiled and contaminated with a food poisoning pathogen), Mode 2 details the variables (in this case identified metabolites), and Mode 3 the time course of the experiment (*e.g.* 11 time points from 0–72 h in the first experiment).

Although mode 2 of the PARAFAC2 model can help identifying potentially interesting metabolites as stated above, it provides no statistical assessment of the metabolites. Hence we also employed univariate statistics test to identify statistically significant metabolites from the two experiments. Since the two experiments have different sample compositions, different statistical tests have to be used. For the first experiment, we monitored a fairly large number of time points (11 in total) while the number of samples at each time point is rather limited (6 in total, 3 for each type). In this dataset, the peak areas of each metabolite of the same type of samples at the same time point were averaged and resulted in 11 pairs of averaged peak areas indicating the difference of the abundance of a certain metabolite at each time point. A paired two-sample Wilcoxon signed rank test[24] was then employed to identify the metabolites which showed significant difference between two types of samples. Since the number of levels (time points) is larger than the number of samples at each level in this experiment, it is difficult to identify the metabolites which showed significant difference between different levels based on the data obtained from this experiment. For the second experiment, a reduced number of time points (4 in total) was monitored while the number of samples at each time point was doubled which makes it more suitable for statistics test. We employed a non-parametric two-way ANOVA test (Friedman's test)[25] to identify metabolites which showed significant differences between different types of samples or different time points or both. Since the statistics tests were used for multiple hypothesis testing (*i.e.* simultaneously testing a family of variables/metabolites), the significant threshold of p-values was adjusted by using false discovery rate (FDR)[26] to control the increased chance of type I error (false positive).

Results and discussion

In both experiments, a distinct odour can be smelt after 24 h of incubation and such odour intensified rapidly afterwards. After 48 h of incubation the odour developed into a very unpleasant putrid smell, indicating the onset of proteolysis. Transparent to light green slimes could be seen on the surface of the pork from 36–48 h onwards. Few visual differences were observed between naturally spoiled pork and those contaminated by *Salmonella* after 48 hours. The total viable counts at 0, 24, 48 and 72 h in the second experiment are provided in Table 1. The TVCs increase as spoilage progresses to a level of approximately 10^8 to 10^9 cm^{-2} after 72 h of incubation. The increase in TVC was different for the two types of spoilage with the naturally spoiled meat showing higher TVCs at each time point.

A total number of 126 peaks were detected from the first experiment while 116 peaks were detected from the second experiment from the GC-MS analysis. In multivariate analysis, principal component analysis (PCA) is the most commonly used tool to gain an intuitive view of the multivariate data. However, when there are 2 or more underlying influential factors PCA is not always the best method to reveal the influence of these factors. In this study there are two factors of interest, these are the progression of spoilage and the type of samples (natural *vs.* *Salmonella* spoilage). As suggested by Cattell,[27] when classical factor analysis models like PCA are applied to such dataset, the latent factors obtained will be "neither clear species differentiator nor optimal individual differentiators". A three-way model is most suited to the data generated in this study such that the influential parameters *i.e.*, time, experimental conditions (natural spoilage and *Salmonella* contamination) and progression of spoilage can be investigated individually. The influence of time, *i.e.* the progression of spoilage, can be explicitly modelled by adding a third dimension into the model (mode 3) whilst separating it from the influence of the experimental condition *i.e.* natural spoilage and *salmonella* contamination which are modelled in mode 1 (Fig. 1). The plots of the loadings of mode 1 which models the relative position of the samples at each time point from the PARAFAC2 model based on the data from the first experiment are shown in Fig. 2. For brevity, only 4 time points: 0, 28, 32 and 72 h of the mode 1 loadings were shown in Fig. 2(a) and this illustrates that the separation of the sample types (*S. typhimurium* contaminated and natural spoiled) becomes greater as the spoilage progresses. This trend is easier to see by plotting the Hotelling's T^2 statistics which measures the separation between two types of samples against time as shown in Fig. 2(b). The Hotelling's T^2 statistics allows a *p*-value to be calculated. If one considers $p < 0.05$ as "significant", it appears that in this experiment between 32 and 36 h (and later time points) after the contamination, the sample types (contaminated *vs.* naturally spoiled) can be "significantly" separated. Note that at each time point the TVCs (Table 1) are equivalent so this is not

Table 1 Average total viable counts per cm^2 from the 4 time points monitored[a]

	0 h	24 h	48 h	72 h
N[b]	0–500	1.9×10^7 (1.8×10^6)	3.9×10^8 (5.1×10^7)	1.5×10^9 (9.5×10^7)
C[c]	4.8×10^5 (5.7×10^4)	1.4×10^7 (5.1×10^6)	2.3×10^8 (9.5×10^6)	5.3×10^8 (1.3×10^7)

[a] The standard deviations of the TVCs are given in the parentheses. [b] N: naturally spoiled samples. [c] C: *S. typhimurium* contaminated samples.

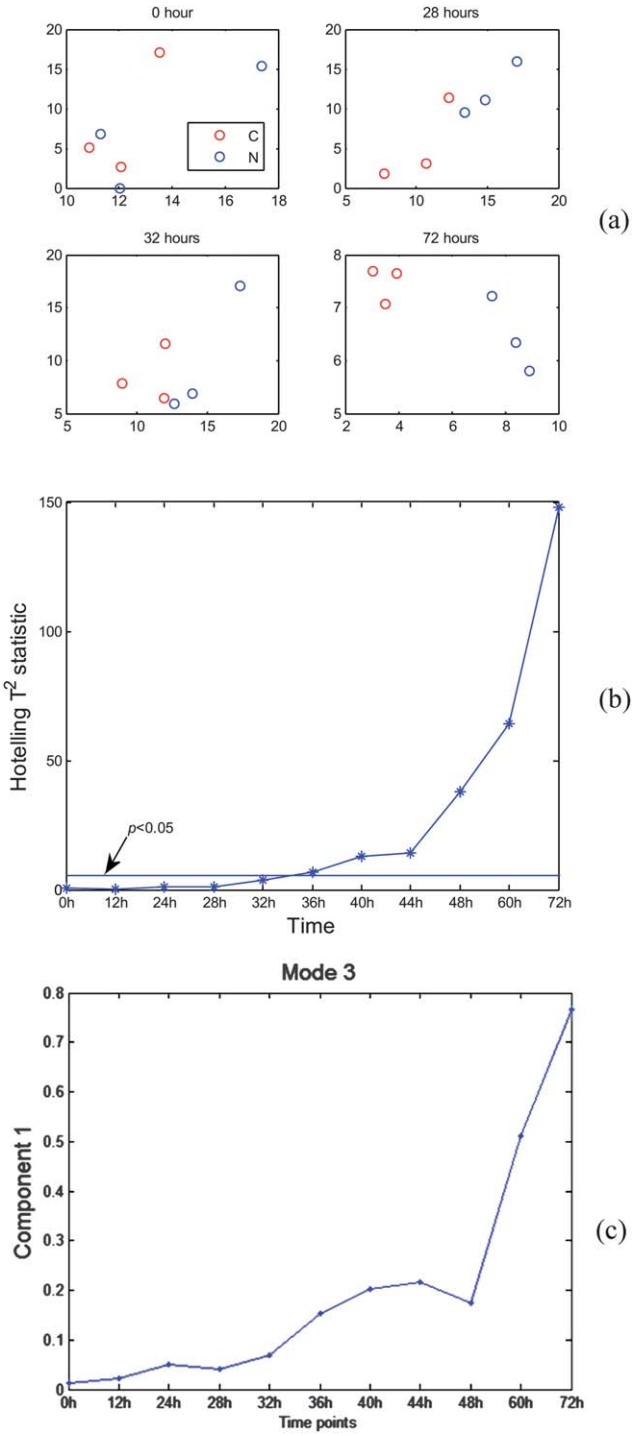

Fig. 2 (a) PARAFAC2 mode 1 loadings plot from the first experiment: C = *Salmonella* contaminated samples; N = natural spoiled samples; (b) Hotelling's T^2 statistics *versus* time, the value corresponds to $p = 0.05$ was marked by a horizontal line and (c) the first component in the PARAFAC2 mode 3 loadings plotted against time.

a bacterial load influence but a metabolic difference, *i.e.* different types of samples appeared to have different metabolic profiles. Due to the restricted sample size, the statistical implication of the *p*-value derived from the Hotelling's T^2 statistics is limited and can only be used for reference purpose, therefore in the second experiment we increased the number of samples at each sampling point. The mode 3 loadings plot (Fig. 2(c)) showed an approximately monotonically increasing trend which suggests that the abundance of the majority of the metabolites was increasing as the spoilage progressed, and thus can be used as indicator for the progression of the spoilage. The increase in the general abundance of metabolites is most likely caused by the increase in the abundance of the microorganisms on the surface of the meat as shown in the TVC data (Table 1).

The mode 1 loadings plot from the PARAFAC2 model based on the second experiment is shown in Fig. 3(a) which showed a similar trend as observed in the first experiment (Fig. 2). The inclusion of multiple replicates increases the statistical power of the Hotelling's T^2 statistics, and allows a clear discrimination of the early sample points (24 h) after the contamination ($p < 0.05$) as shown in Fig. 3(b).

By applying a paired *t*-test on the data from the first experiment, 16 peaks were identified as significant when the false discovery rate (FDR) was set to 0.05; 12 of these peaks were chemically identified by the matching of mass spectra to those in metabolomics-specific mass spectral libraries. The two-way ANOVA test with the FDR set to 0.05 on the data from the second experiment suggested that nearly half of the metabolite features (55 out of 116 detected peaks) showed significant differences between different time points. The majority of peaks showed a monotonically increasing trend which matched the trend shown in the mode 3 loadings plot from the PARAFAC2 model based on the data from the first experiment (Fig. 2 (c)). In addition, 16 peaks were identified as significant and 11 of them were definitively identified through mass spectra matching.[15,16] Among these 11 metabolites with chemical identifications, 6 of them were also detected and identified as significant in the first experiment. In addition, there is one compound (inosine) which was identified as significant in the first experiment but not significant in the second experiment. It seems that this compound was not detected in 33 of 48 samples analysed in the second experiment. This is most likely a result of the metabolite concentration being lower than the analytical limit of detection. The 17 significant (identified as significant in either of the 2 experiments) and identifiable metabolites are detailed in Table 2 with an indication as to if they are increased in natural spoilage relative to *S. typhimurium* contaminated meat, or *vice versa*; the time trajectories for these metabolites are shown in the ESI†. In addition to these a further 9 metabolite features (peaks) were significant but remain unidentified. Ignoring the differences between natural *vs.* contaminated meat, many of the metabolites identified are due to proteolysis occurring after 24 h. This is associated with the post-glucose utilization of amino acids by pseudomonads and is coincidental with organoleptic changes indicating the production of malodorous protein breakdown products.[2] Several amino acids (valine, tryptophan, aspartic acid, lysine and glycine), amino acid derivatives (hydrocinnamic acid from microbial action in the phenylalanine pathway) and creatinine (a breakdown product from general enzymatic proteolysis) all increase (see ESI†) after 24 h. Methylmalonic acid is a product from the metabolism of fat and protein and also increases after 24 h. In addition, several fatty acids increased during the spoilage process and these included dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecenoic acid and ethanolamine (which produces an ammoniacal odour). The production of fatty acids is likely to result from the breakdown of meat and incorporation into the lipid membrane of the bacteria that are growing on the meat surface.

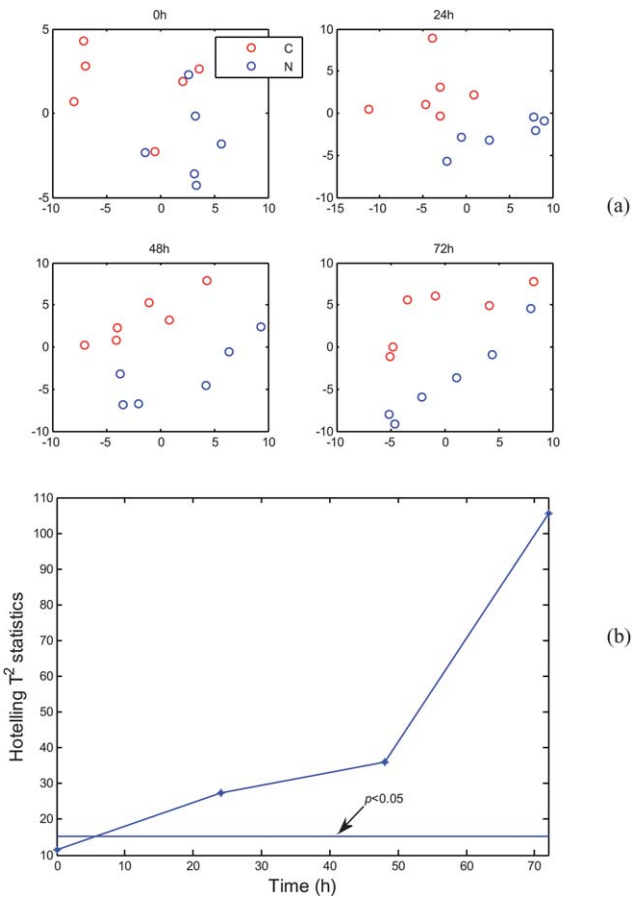

Fig. 3 (a) PARAFAC2 mode 1 loadings plot from the second experiment: C = *Salmonella* contaminated samples; N = natural spoiled samples; (b) Hotelling's T^2 statistics *versus* time.

With respect to differences between *S. typhimurium* contamination and natural spoilage it is clear that several amino acids are increased in *Salmonella* contamination relative to natural spoilage and these include aspartic acid, lysine and glycine, whilst lysine, valine and tryptophan are increased during natural spoilage. It is known that bacteria utilise amino acids preferentially and in a species specific manner[28] and this information could be used to differentiate this food poisoning bacterium from background harmless microflora. In addition, the same is also the case for fatty acids content in bacteria and hexadecanoic acid could be a marker for *S. typhimurium* contamination. However, from this study there is no direct evidence of this and future studies will be targeted at the amino acid utilisation pattern from *S. typhimurium* strain 4/74, and also undertake a fatty acid profile.

Table 2 The significant metabolites identified in the discrimination of the natural spoiled samples and those contaminated with *S. typhimurium*

Name	*p*-value in exp. 1	*p*-value in exp. 2	More abundant in	HMDB i.d.
Valine	0.0119	0.0219	N[c]	HMDB00883
Methylmalonic acid	0.0163	N/A[b]	N	HMDB00202
Phosphate	0.0204	0.0091	N	—
Ethyl pipecolinate	0.0165	N/A	N	—
Hydrocinnamic acid	0.0153	N/A	C[d]	HMDB00764
Creatinine	0.0073	0.00035	C	HMDB00562
Dodecanoic acid[a]	0.0086	N/A	N	HMDB00638
Tetradecanoic acid[a]	0.0197	0.0034	N	HMDB00806
Hexadecanoic acid[a]	0.0169	0.0099	C	HMDB00220
Octadecenoic acid[a]	0.0199	0.012	N	HMDB00207
Tryptophan	0.0239	N/A	N	HMDB00929
Inosine	0.0188	0.162	N	HMDB00195
Ethanolamine	N/A	0.0209	C	HMDB00149
Aspartic acid	N/A	0.0007	C	HMDB00191
Lysine	N/A	0.0077	N	HMDB00182
Myo-inositol	N/A	0.0199	N	HMDB00211
Glycine	N/A	0.0226	C	HMDB00123

[a] Multiple possible isomers exist, only one typical HMDB i.d. was given. [b] N/A: not detected. [c] N: naturally spoiled samples. [d] C: *S. typhimurium* contaminated samples.

Conclusion

In this study we have conducted two independent experiments which were separated by a period of 6 months to explore the possibility of applying metabolic profiling to characterise the hygiene status of pork chops which undergo a spoilage process. The results from the PARAFAC2 analysis on the two experiments appeared to be highly consistent. The mode 3 loadings from the PARAFAC2 analysis as well as the non-parametric two-way ANOVA test (Friedman test) suggested that the intensities of the majorities of the metabolites generally increased over time and reflects the fact that as the spoilage progresses, the microbial load increases and hence the metabolites from the cells become more abundant in the extraction solvent. More interestingly, the mode 1 loadings from the PARAFAC2 suggested that the *salmonella* contaminated samples have metabolic profiles which are *different* from those acquired from naturally spoiled meat. Such differences can be observed as early as 24 h after contamination at room temperature and the findings were consistently observed in the two independent experiments. By employing statistical tests, 17 metabolites were discovered which showed significant differences between two types of samples and 6 of these were identified as significant in both experiments. These included valine, creatinine, tetradecanoic acid, hexadecanoic acid, and octadecenoic acid. In further studies we shall employ a targeted approach to quantify the changes of the 6 metabolites highlighted in this investigation and also test their validity as biomarkers against the contamination of other closely related strains (*e.g. Escherichia coli*). Our investigation demonstrates the use of metabolic profiling as a tool to discriminate between natural spoilage and pathogenic microorganisms without the need for time-consuming and laborious methods traditionally employed in microbiology to detect pathogenic microorganisms. The outlined method provides valuable information for tracing the source of accidental or intentional contamination of food products by pathogenic and natural spoilage microorganisms. Although this study focused on *S. typhimurium* detection, our metabolic profiling technique could readily be applied to detect other commonly observed foodborne pathogenic bacteria that are involved in food poisoning such as *Campylobacter jejuni*, *E. coli*, *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes*.

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