# Using NIR Hyperspectral Imaging For The Differentiation Of Pathogenic Bacteria

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Rapid methods for detection and differentiation of pathogenic bacteria is becoming increasingly important in the food industry as conventional microbiological and immunological methods are time-consuming and labour intensive [1, 2]. Culturing and colony counting could take several days to detect and identify bacteria [3]. This may lead to a delay in product distribution or may result in contaminated fresh produce or ready-to-eat products being distributed before microbiological tests have been completed.

This study aims to use wavelengths in the NIR region to differentiate between Gram positive and Gram negative bacteria, pathogenic and non-pathogenic bacteria and similar species of the same genera. The bacteria will include, *Bacillus cereus, Escherichia coli, Salmonella enteritidis*, *Staphylococcus aureus* and *S. epidermidis*.

## MATERIALS AND METHODS

Four commonly encountered foodborne pathogens (*Salmonella enteritidis*, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*), as well as non-pathogenic *Staphylococcus epidermidis* were studied. All bacteria were imaged on Luria Bertani (LB) agar in glass petri dishes after 20 h incubation at 37 °C. Images were acquired with a SisuChema short wave infrared (SWIR) camera, in the range 1000 to 2500 nm.

Standard normal variate (SNV) correction and the Savitzky-Golay technique (2nd derivative, 3rd order polynomial; 25-point smoothing) was applied to wavelengths 1103 to 2471 nm. Three mosaics were created, each containing a different combination of bacteria. Group 1 included all bacteria which appeared white/cream in colour on the agar (*B. cereus, E. coli* and *S. enteritidis*). Group 2 included all Gram positive bacteria (*B. cereus, S. aureus* and *S. epidermidis*) and group 3 included the two *Staphylococcus* species of the same genera (*S. aureus* and *S. epidermidis*). PLS-DA models were calculated for each group. The calibration models included the first set of 5 Petri dishes and the models were independently validated on the duplicate plates (test sets).

## RESULTS AND DISCUSSION

Chemical differences were evident, along PC1 (59 % SS) *B. cereus* (Gram positive) could be distinguished from *E. coli* and *S. enteritidis* (Gram negative), and *E. coli* from *S. enteritidis* in the direction of PC2 (6.85 % SS) (Fig. 1). For group 2, pathogenic *B. cereus* and *S. aureus* were separated from non-pathogenic *S. epidermidis* along PC1 (37.5 % SS). The loading line plots and mean spectra of all three groups showed that the main chemical contributors permitting distinctions between bacteria were variances in amino acids, carbohydrate and teichoic acid content. Teichoic acid of certain bacteria consists of glycerol units while others contain ribitol units[4]. The peak at 1405 nm (O-H stretch, ROH) was only present in loading lines where Gram positive bacteria were present, leading to the assumption that this peak represented teichoic acid. Partial least squares discriminant analysis (PLS-DA) models were used to confirm the PCA data.

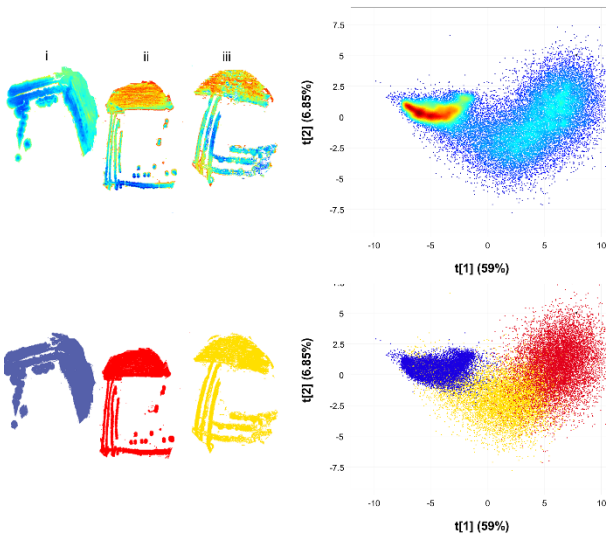


**Fig. 1 a** PC1 score image of (i) *B. cereus,* (ii) *E. coli* and (iii) *S. enteritidis*; **b** score plot of PC1 vs. PC2 for the mosaic of *B. cereus, E. coli* and *S. enteritidis*; **c** score image showing bacteria coloured according to class membership, *i.e. B. cereus* (blue)*, E. coli* (red) and *S. enteritidis* (yellow). **d** Projection of class membership onto score plot permitting easier visualisation of clusters.

Certain PLS-DA classification results were satisfactory, where the model for groups 1 and 2 was calculated with four components, while group 3 was calculated with three. Coefficients of determination ($R^2$) for groups 1, 2 and 3 were 0.41, 0.74 and 0.89, respectively. For group 1, 78.2 % of *B. cereus* pixels and 53.9 % of *S. enteritidis* were correctly predicted, while only 2.34 % of pixels belonging to *E. coli* were predicted correctly. Group 2 had a higher percentage of correctly predicted pixels than group 1, where 93.0 % of *B. cereus,* 86.7 % of *S. aureus* and 82.0 % of *S. epidermidis* were correctly predicted. When predicting the *Staphylococcus* species, 99.96 % and 91.0 % of *S. aureus* and *S. epidermidis* pixels were predicted correctly. The best predictions were made for the identification of *B. cereus* and the two *Staphylococcus* species, where results ranged from 82.0-99.96% correctly predicted pixels.

## CONCLUSION

NIR-HSI and MIA techniques was successfully used to separate Gram positive and Gram negative bacteria, differentiate between bacteria which appear similar in colour and distinguish between pathogenic and non-pathogenic bacteria. It was also possible to recognise variation among Gram positive bacteria and distinguish between similar species of the same genera on solid growth media.

Differences in teichoic acid, protein structures and lipid content were found to be the possible sources of variation in the analysed images. To test the reliability of the PLS-DA models for discrimination between bacteria, a model containing more replicates of the same bacteria should be used in a training set. Colonies of the same species can vary significantly, therefore more replicates would contribute variation, possibly making improving classification accuracy. Although chemical variances can provide a more comprehensive representation of bacterial content, this study did not include verification (with NIR spectroscopy) of the pure chemical components (teichoic acid) mentioned.

## REFERENCES

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