NIR imaging of agar on glass and plastic petri dishes: effects of sample presentation

Ana Herrero-Langreo[1,2], Gauri Ravindra Chemburkar[1], Amalia G.M. Scanell[2,3,4], Aoife Gowen[1,2] *
[1] UCD School of Biosystems and Food Engineering, University College of Dublin (UCD), Belfield, Dublin 4 Ireland *ana.herrero-langreo@ucd.ie
[2] UCD Institute of Food and Health
[3] UCD Center for Food Safety
4 UCD School of Agriculture and Food Science

I. INTRODUCTION

Polystyrene plastic petri dishes are one of the most common sample presentations in microbiological studies. They have the advantage of being cheap, disposable, transparent to the eye and easy to handle as well as being easily available and well standardized. Nevertheless, cultures on agar on plastic petri dishes present a series of challenges for the acquisition of NIR hyperspectral images: the absorption band of polystyrene can interfere with the spectral profile of the sample, while the water content in agar can mask a large portion of the spectra of the constituents of interest. In turn, glass petri dishes are preferred for NIR acquisition, but they are heavier and inconvenient for handling in the preparation of microbiological cultures. Their use is time consuming and involves more safety hazards, as they are prone to breakage and require additional protocols for safe disposal of the samples and clean-up of the dishes.

The objective of this work is to study the spectral features of Luria Bertani agar spectra at different sample presentation schemes. This study was carried out as part of the protocol development for a larger experimental set up. The spectral characterization of sample presentations that it illustrates, and the effect of spectral pre-treatments on the spectral features of the different sample presentations can be a useful resource for other works dealing with samples presented on agar petri dishes.

II. MATERIALS AND METHODS

Two different volumes (15 and 20 ml) of Luria Bertani agar poured on plastic and glass petri dishes were analysed. Agar volumes were measured with a graduated cylinder to assure consistency in the agar thickness. Samples were imaged with a push-broom hyperspectral camera at a range between 900 and 1700 nm, with a spectral resolution of 7 nm (Hyperspectral pushbroom system (DV Optics, Padova, Italy). Illumination was provided by a Tungsten Halogen white light lamp source attached to a fibre optic line light positioned at an angle of 45º to the moving table. Two replicates were taken for each sample presentation set up. All dishes were imaged both uncovered and covered with a glass lid, as covering the dishes is usually required to avoid contamination in microbiology studies. White porcelain was used as a background to place the samples on. Samples were always located in the same position, allowing to use a single mask for the segmentation between the petri dish and the background for all samples. Exploratory analysis of the spectral features of each type of sample presentation setup was carried out through visual assessment of mean and average spectra for each sample and Principal Component Analysis (PCA). Several pre-treatments were tested in their ability to attenuate the multiplicative effects produced by different agar volumes and the differences between sample presentations.

III. RESULTS AND DISCUSSION

PCA of reflectance spectra of samples presented on uncovered plastic petri dishes (two replicates for each agar volume) showed 99.3% of variance in the first two Principal Components (PC). The first PC appears as a multiplicative effect (flat loading) affecting the border of the petri dishes. This could be interpreted as the effect of multiple scattering produced by the edge of the petri dish. For easier characterization of the difference samples on further analysis, a circular mask in the centre of the petri dish (50% of the whole petri dish area) was used to define the region of interest for each sample. Second PC enhances the difference between different volumes of agar and shows a similar profile to the average agar samples on uncovered plastic petri dishes.

The frequency of reflectance values equal to 0 throughout the spectra, calculated for each sample, indicates the region from 1349 nm to 1643 nm contain no spectral information, with 38% to 100% of the spectra showing 0 reflectance in this range, likely due to water absorption.

Average and standard deviation of the spectra including two replicates of each type of sample

presentation was used to compare spectral profiles and the effects of different pre-treatments (Fig.1A). For empty plates, the variance of reflectance spectra on plastic petri dishes was much greater than for glass. This is reflected in all agar samples, that consistently present wider variance in plastic than in glass. Smoothing with Savisky-Golay and a window of 5; and SNV pre-treating the spectra effectively reduced the variance between plastic and glass petri dishes with the same type of covering - Glass lid or uncovered - (Fig.1.B). Similar results were obtained for smoothed and SNV absorbance. Petri dishes with a glass cover presented a spectral profile with lower corrected absorbance in the range from 1104 nm to 1156 nm and a lower absorbance with different spectral features for both types of presentations on the range from 1160 nm to 1188 nm.

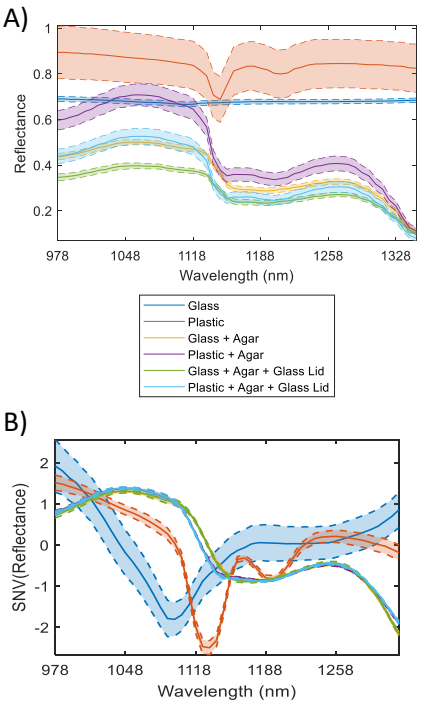

Fig. 1. Average and standard deviation of the spectra for each type of sample setup. A) Acquired reflectance spectra; B) Smoothed SNV pre-treated reflectance

Within samples of same petri dish material and lid, multiplicative differences were observed both between replicates and between different agar volumes. SNV pre-treatment both in reflectance and absorbance was able to attenuate these differences (Fig.2).

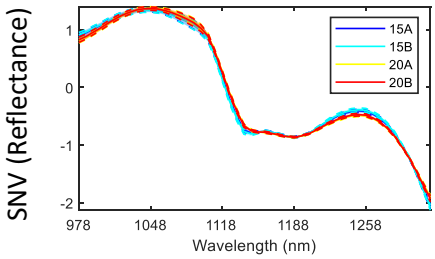

Fig. 2. Average and standard deviation of reflectance spectra for uncovered plastic petri dishes samples, two replicates, labelled as A and B and two volumes of Agar 15 and 20 ml. A) Acquired reflectance spectra; B) Smoothed and SNV pre-treated reflectance spectra

External Parameter Orthogonalization (Roger et al., 2003) was tested to project agar samples on plastic petri dishes orthogonally to the plastic spectra from the empty plastic petri dish. This operation, rather than correcting the small spectral features of plastic peaks present in the agar on plastic samples, produced further artefact peaks in the spectra. Orthogonalization to a spectral base extracted from samples with different volumes of agar on plastic dishes (2 replicates of 15 and 20 ml agar on plastic petri dishes), removed all spectral features of agar. This approach could be tested on further experiments dealing with microbial growth on agar petri dishes.

IV. CONCLUSIONS

The application of spectral pre-treatments, such SNV, for removing multiplicative differences between replicates and differences in agar thickness, is recommended for experiments based on these types of sample presentation. For all agar samples, the spectral range between 1349 nm to 1643 nm was completely absorbed by the agar. A different imaging or illumination configuration might improve the signal acquisition in this range. Samples on borosilicate glass, presented a greater absorbance while samples on polystyrene plastic showed greater multiplicative spectral variance. Characteristic spectral peaks of plastic could be recognized on the spectral profile of samples on plastic plates. As no pre-treatments effectively removed this effect without introducing further artefacts in the spectra, glass petri dishes are recommended if this interference needs to be avoided.

Bibliography

Roger JM, Chauchard F, Bellon-Maurel V. EPO-PLS external parameter orthogonalisation of PLS application to temperature-independent measurement of sugar content of intact fruits. Chemom Intell Lab Syst. 2003;66(2):191–204.

Acknowledgments: Authors would like to thank Multi-scale Hyperspectral Imaging for Enhanced Understanding and Control of Food Microbiology (HyperMicroMacro) project, Funded by Science Fundation Ireland.