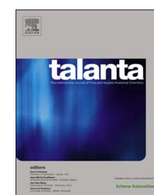
Talanta 161 (2016) 606–614

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta



Exploiting multispectral imaging for non-invasive contamination assessment and mapping of meat samples

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ARTICLE INFO

Article history:
Received 9 June 2016
Received in revised form
1 September 2016
Accepted 5 September 2016
Available online 6 September 2016

Keywords:
Surface chemistry
Multi-spectral sensor
Spoilage of meat
Non-invasive
Machine learning

ABSTRACT

Recently, imaging and machine vision are gaining attention to food stakeholders since these are considered to be the emerging tools for food safety and quality assessment throughout the whole food chain. Herein, multispectral imaging, a surface chemistry sensor type, has been evaluated in terms of monitoring aerobically packaged beef filet spoilage at different storage temperatures (2, 8, and 15 °C) and storage time. Spectral data acquired from the surface of meat samples (with/without background flora; +BF/–BF respectively) along with microbiological analysis. Qualitative analysis was employed for the discrimination of meat samples in two microbiological quality classes based on the values of total viable counts (TVC < $2\log_{10}$ CFU/g and TVC > $2\log_{10}$ CFU/g). Furthermore, a Support Vector Regression model was developed to provide quantitative estimations of microbial counts during storage. Results exhibit good performance with overall correct classification rate for the two quality classes ranging from 89.2% to 80.8% for model validation. The calculated regression results to an R-square of 0.98.

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1. Introduction

Spoilage of foods determined as the result of the biochemical activity of microbial populations [1,2] is driven mainly by two categories of factors: (i) intrinsic (e.g. water activity, acidity, redox potential, available nutrients and natural antimicrobial substances) and (ii) extrinsic (storage conditions of temperature, humidity, atmosphere composition and packaging) [2,3]. This inherent complexity of determining the food deterioration is also apparent in food quality assessment where the acquisition of reliable information through non-destructive methods is a major challenge of the food industry [4–6]. To address this, development and application of effective quality and safety assurance systems based on controlling, monitoring, and recording the critical parameters throughout the food chain need to be employed by the food industry. This goal cannot be reached using conventional microbiology or molecular based techniques [7] since both are time-consuming, destructive, limited to the number of samples to be analyzed and require highly trained personnel [2,8]. So, it is obvious that rapid non-destructive techniques for automatic monitoring of food processes in all stages of the food chain is a must. The potential of using non-destructive methods (e.g. vibrational spectroscopy and surface chemistry sensors) to overcome the limitations of conventional food microbiology gains more and more attention [9–13].

Multi- and hyper-spectral imaging emerge as highly promising techniques since they give insight to the chemical composition of the surface of the samples. Up to date, all the available methods for contamination assessment based on multi-/hyper-spectral imaging data follow the same, more or less, roadmap for analysis. Specifically, they make use of the average intensities of the sample at specific wavelengths along with the corresponding standard deviations. Then those average values are used as inputs for a classification and/or prediction system, in order to train a representative model for predicting the total viable counts (TVC) of the sample [11–17]. Popular methods for prediction/classification used are partial least square regression (PLSR) [18], support vector machine regression (SVR) [18], artificial neural networks [18], and principal components analysis (PCA) [18]. Although the aforementioned general approach exhibits good results in terms of prediction, the use of average values as predictor factors cannot be considered as a reliable/robust indicator. This argument is supported by the fact that in the corresponding studies, the samples are all under the same conditions, e.g. same temperature. Thus, the prediction outcome for samples originated from different conditions is poor.

Herein, a novel methodology is presented that exploits the multi-spectral technology. Instead of using the mean reflectance values of the samples, (i.e. averaging the values of contaminated

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http://dx.doi.org/10.1016/j.talanta.2016.09.019
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and non-contaminated sites present on a sample) the spectra related to each contamination-wise condition are identified. Next, the pixel-wise spectra are classified and categorized according to those "signature" spectra, previously identified. To do so, unsupervised clustering techniques are employed and applied in two cascade steps. At the first step, the data are reduced by the application of a pre-clustering approach. Then, the least number of spectra, which constitutes the condition related characteristic spectra, are automatically determined, i.e. each spectrum reflects to either contaminated or non-contaminated sites. The characteristic spectra are used as prediction factors since they are considered much more robust than the corresponding average values. The justification for this is that they express the central spectra that characterize the contamination state (either contaminated or not) of sites on the surface of the samples. The evaluation performed, exhibits that the proposed framework leads to reliable results when compared to the conventional microbiology technique's measured contamination abundancy. More importantly, the performance is consistent and robust when different storage conditions are applied on the samples (temperatures at 2 °C, 8 °C, & 15 °C).

2. Materials and methods

2.1. Materials

For this study, a block of de-boned beef was obtained from a local processing plant, transported under refrigeration to the laboratory within 1 h and divided to two separate block. The first meat-block was treated according to [19]. Specifically, it was sprayed with 100% alcohol and ignited with a gas burner so as to reduce/eliminate the initial microbial load. Then, it was placed onto a laminar cabinet, where the external burnt surface tissue was removed aseptically and the sterile tissue below was excised and cut into beef fillets of 20–30 g. Samples were placed into sterile Petri dishes in order to avoid environmental contamination. Those samples are considered to be without Background Flora (−BF). As far as the second meat-block concerns, the one with the Background Flora (+BF), it was also cut into smaller fillets of about 20–30 g, placed into Petri dishes and stored under the same storage conditions. In conclusion, two groups of samples have been created, one that has no background flora (no contamination was evident, e.g. no visible colonies on TGY medium was present) and one that has background flora (i.e. number of bacteria colonies progressively increased during storage). In the case of the samples with background flora, the bacteria present are mixed populations of mesophilic and psychrophilic bacteria (i.e. the indigenous flora). This microbial association comprise of different microorganisms, as consequence of the effect of applied temperatures [2].

2.2. Experimental design for samples collection

Meat samples (−BF and +BF) were stored in 2, 8, and 15 °C using high-precision (±0.5 °C) incubation chambers (MIR-153, Sanyo Electric Co., Osaka, Japan). At appropriate time intervals, [0, 6, 10, 20, 26, 32, 38, 44, 47, 50, 53 and 60 h] for 15 °C, [20, 26, 38, 44, 50, 56, 62, 68, 74, 80, 83, 86, 88, 94, 97, 100 and 103 h] for 8 °C, and [26, 32, 50, 62, 74, 80, 86, 95, 98, 101, 104, 107, 110, 118, 124, 128, 132, 136, 140, 151, 157,171, 195 and 222 h] for 2 °C, samples were analyzed microbiologically while images from the multispectral imaging instrument were acquired at the same time points. In total 164 beef fillets samples (74 −BF and 90 +BF) were obtained and represent time-series and coupled storage experiments. Hereafter, the datasets with no background flora (−BF) will be referred as SD2, SD8, SD15 (the number indicates the storage temperature) and with background flora (+BF) as D2, D8, and D15.

2.3. Microbiological analysis

Samples (1 g) from meat were weighed aseptically, added to sterile quarter-strength Ringer solution (9 ml), and homogenized in a stomacher (Lab Blender 400, Seward Medical, London, UK) for 60 s at room temperature. For the enumeration of Total Viable Count (TVC), duplicate 0.1-ml samples of the appropriate decimal dilutions in quarter-strength Ringer solution were spread onto Tryptic Glucose Yeast Agar (Biolife) by incubating aerobically at 30 °C for 48–72 h.

3. Methods

3.1. Multispectral image acquisition and image analysis

The proposed workflow is described in detail below while its flow diagram is shown in Fig. 1. For multispectral image acquisition the VideoMeterLab imaging system was used [20] (Fig. 1a–c). VideoMeterLab captures surface reflection images of samples at 18 different wavelengths ranging from 405 to 970 nm, creating a data hypercube for each sample. The acquisition system, records the surface reflections with a standard monochrome charged coupled device chip (CCD). The sample is placed inside a sphere, called Ulbricht sphere, which has a matte white coating so as to ensure a diffused and spatially homogenous illumination of the sample. At the rim of the sphere, light emitting diodes (LEDs) with narrow-band spectral radiation distribution are positioned side by side. During data acquisition, the diodes are strobing successively, resulting in a monochrome image with 32-bit floating point precision for each wavelength. Finally, a data cube of spatial and spectral data for each sample of size $m \times n \times 18$ (where $m \times n$ is the image size in pixels) is acquired.

The acquired images are then processed in order to exclude any background/environment areas (such as Perti dish, fat, and connective tissue areas), non-relevant to the region of interest, i.e. meat sample. This is done by an automated and unbiased image processing pipeline that has been developed within our group previously [21]. In brief, it is based on unsupervised machine learning methods (e.g. Gaussian Mixture Models [22]) and a novel unsupervised scheme of spectral band selection for segmentation process optimization. The aforementioned image analysis application (Fig. 1d) results to region of interest extraction (ROI), pixel-wise spectral information (i.e. spectrum of each sample pixel at the 18 image acquisition wavelengths) and other collectible statistics of the sample (e.g. spectral average intensity, standard deviation etc.).

3.2. Data analysis

At this point the region of interest and the individual pixel-wise spectra along the wavelengths (Fig. 1e) are extracted. Each spectrum is a vector of the reflectance values over the 18 wavelengths, i.e. an 18 dimensional vector. Typically, for each sample image, we usually get more than 300,000 spectra (size of the sample area in pixels). This constitutes an enormous data size. In order to reduce the complexity and the size of the data, a pre-clustering step is applied, based on the well-known k-means algorithm [18]. Here the number of clusters is set to 20 so as not to oppose a strict limitation and lose informative clusters (Fig. 1f). The choice of 20 clusters is justified by the fact that we do not expect to have more than 20 different areas on a sample in terms of surface coloration. Collaterally, 100 instead of 20 clusters were used, leading to the same results in terms of the "natural" clusters determination. At

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