Talanta 153 (2016) 111–119

Contents lists available at ScienceDirect

Talanta

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

The potential of spectral and hyperspectral-imaging techniques for bacterial detection in food: A case study on lactic acid bacteria

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ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form
20 February 2016
Accepted 25 February 2016
Available online 27 February 2016

Keywords:
FT-NIR spectroscopy
FT-IR microscopy
Hyperspectral imaging
Principal component analysis (PCA)
Lactic acid bacteria (LAB)
Cooked ham

ABSTRACT

Official methods for the detection of bacteria are based on culture techniques. These methods have limitations such as time consumption, cost, detection limits and the impossibility to analyse a large number of samples. For these reasons, the development of rapid, low-cost and non-destructive analytical methods is a task of growing interest.

In the present study, the capability of spectral and hyperspectral techniques to detect bacterial surface contamination was investigated preliminarily on gel cultures, and subsequently on sliced cooked ham. In more detail, two species of lactic acid bacteria (LAB) were considered, namely *Lactobacillus curvatus* and *Lactobacillus sakei*, both of which are responsible for common alterations in sliced cooked ham.

Three techniques were investigated, with different equipment, respectively: a macroscopic hyperspectral scanner operating in the NIR (10,470–5880 cm^{-1}) region, a FT-NIR spectrophotometer equipped with a transmission arm as the sampling tool, working in the 12,500–5800 cm^{-1} region, and a FT-MIR microscopy operating in the 4000–675 cm^{-1} region.

Multivariate exploratory data analysis, in particular principal component analysis (PCA), was applied in order to extract useful information from original data and from hyperspectrograms. The results obtained demonstrate that the spectroscopic and imaging techniques investigated can represent an effective and sensitive tool to detect surface bacterial contamination in samples and, in particular, to recognise species to which bacteria belong.

1. Introduction

Food labelling regulations highlight the importance of providing the consumer with exact information about ingredients and additives present in food [1,2]. Such a key concern is reported also in the recent UE regulation, which applies from 2014 [3]. In particular, commission regulations also lay down microbiological criteria for foodstuffs, maximum limits for bacterial contamination, toxins and biogenic amines [4]. A direct consequence of this is the requirement for new analytical methodologies aimed at simplifying the use and improving the efficiency of existing control tools.

The shelf-life of cooked and sliced meat products, like cooked ham, is limited mainly because of microbiological safety and spoilage issues. This is because manipulations like slicing and packaging unavoidably reintroduce bacterial contaminants after the cooking process, and because the product has a near-neutral pH (around 6) and water activity higher than 0.945 [5,6].

Spoilage of packaged sliced ham is mostly accompanied by souring, slimy meat juice exudates and swelling of the pack due to gas production and is usually caused by lactic acid bacteria (LAB), together with *Pseudomonas*. Spoilage of cooked meat products results in sensory quality defects such as sour off-flavour, discolouration, gas production, and ropy slime formation.

Official methods for the detection of these bacterial species (ISO 15214:1998, ISO 13720:2010) are based on culture techniques. These methods have limitations such as time consumption, cost, detection limits and the impossibility to analyse a large number of samples. For these reasons, the development of rapid, low-cost and non-destructive analytical methods is a task of growing interest.

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http://dx.doi.org/10.1016/j.talanta.2016.02.059
0039-9140/

Methods based on classical spectroscopy and on the analysis of conventional images (RGB) have been studied extensively for several years [7]. Although applied to numerous food issues, these conventional approaches have a limited capacity for obtaining information from food samples, discarding either the spatial information – in the former approach – or the spectral one – in the latter [8]. In recent years, hyperspectral imaging (HSI) has emerged as a very efficient solution for the quality and safety control of food products, being able to thoroughly characterise individual components within complex matrices, allowing both chemical identification and localisation [9–13]. In fact, if the conventional image analysis gives an answer to the question 'where' and conventional spectroscopy may give answers to the two questions 'what' and 'how much', HSI gives an answer to the combined question 'where and how much of what', thus providing a comprehensive characterisation, particularly useful for identification and control purposes. In addition, collecting spectral profiles at multiple points in a given area increases the representativeness of the information obtained.

HSI can be used either on a macroscopic or a microscopic scale and in different spectral ranges, depending on the level of investigation and the spatial resolution required, the chemical information of interest to the particular issue and the type of implementation needed. The mid and near infrared regions are among the most useful from which to derive chemical information and to provide informative spectral fingerprints of the samples studied. In the case of food samples, for which colour is a basic quality characteristic to be assessed, also the visible region is often considerably informative.

HSI methods usually provide a considerable amount of data, structured in three dimensions (two spatial and one spectral) and are often highly complex, especially when working in the near infrared region, which is characterised by spectral overtone bands that often overlap considerably. In addition, the effective application of HSI techniques in routine food controls is currently limited by a series of problems, such as the presence of unwanted variations in the signals, due to factors beyond our control.

In order to minimise these problems and to extract information relevant for analytical purposes, it is essential to apply suitable signal processing and multivariate pattern recognition techniques [14].

In the last decades, great attention has been devoted to the application of FT-IR spectroscopy in the field of microbial safety, thanks both to the ability of the technique to resolve complex mixtures in terms of composition and to the technological progress of modern instruments [15,16]. In particular, the coupling of microscopic devices and MIR spectroscopy offered significant advantages for the determination of compounds present at low concentration within heterogeneous matrices. Potential of FTIR techniques for identification of contaminant bacteria in food has been systematically investigated since the nineties of the 20th century, coupled with chemometric techniques, and proved to be able to perform not only species but also strain differentiations [17,18]. Recent studies efficiently coupled FTIR spectroscopy and microscopy for the characterisation of bacteria in fruit juices, dairy products and meat [19,20]. It is worth remarking that most of the approaches described in the literature include a separation step, aimed at extracting the bacterial cells from the complex food matrices before analysis. This compromises the advantages of low invasiveness towards sample and the performances of the method in terms of analysis time and cost.

In the present study, the capability of spectral and hyperspectral techniques to detect bacterial surface contamination was investigated preliminarily on gel cultures and, subsequently, on sliced cooked ham. In more detail, two species of LAB were considered, namely *Lactobacillus curvatus* and *Lactobacillus sakei*, both of which are responsible for common alterations in sliced cooked ham.

LAB have been successfully studied by FTIR methods in the NIR and in the MIR spectral regions.

In particular, FTIR spectroscopy in the MIR range allowed the discrimination of the pure bacteria analysed in transmission mode [21]. In the present study, the potential of three techniques were investigated, with different equipment, respectively: a macroscopic hyperspectral scanner operating in the NIR (10,470–5880 cm^{-1}) region, a FT-NIR spectrophotometer equipped with transmission arm as sampling tool, working in the 12,500–5800 cm^{-1} region, and a FT-MIR microscopy operating in the 4000–675 cm^{-1} region.

Multivariate exploratory data analysis was applied in order to extract useful information from the data in order to verify the capability to distinguish the bacterial strains both in the culture and in the food matrix.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The study was carried out using *L. curvatus* VZ22 and *L. sakei* VZ35 strains, isolated from Varzi PDO salami, and belonging to the bacterial collection of the Institute of Sciences of Food Production of the National Research Council of Italy (CNR-ISPA). The strains were identified by partial 16S rRNA sequencing according to McCabe and co-workers [22].

Before each experiment, the cultures were incubated overnight at 37 °C, in MRS broth (Scharlau Microbiology, Barcelona, Spain). *Lactobacillus* strains were streaked out in triplicate on MRS agar (Scharlau Microbiology), incubated at 37 °C for 72 h under anaerobic conditions (Anaerocult A, Merck Millipore, Darmstadt, Germany) and submitted to spectral and hyperspectral analysis.

Visual appearance of Petri dishes is shown in Fig. S1 (Supplementary Material), with agar gel only (Fig. S1a) and with LAB cultures (Fig. S1b and c).

A commercial sliced cooked ham (about 2 mm thickness), purchased from an Italian supermarket, was used for studying superficial contamination by LAB. The total load of bacteria of the slices before inoculum was $< 10^3$ cfu/g. A weighted amount (10 g) of sample was serially diluted in one-quarter-strength Ringer's solution and plated in Aerobic Count Plates (AC) Petrifilm (3M Canada, London, Ontario, Canada). Subsequently, 1 mL of overnight cultures of *Lactobacillus* strains (about 10^8 cfu/mL) was centrifuged and washed twice with 1 mL of phosphate buffered saline (PBS), and then re-suspended in 1 mL of Ringer solution. Different defined spots within ham surface were artificially contaminated by deposition of 100 μL LAB suspensions (about 10^7 cfu/spot). After contamination, the suspension was allowed to dry at room temperature and samples were immediately submitted to spectroscopic analyses.

2.2. Analytical techniques

2.2.1. FT-NIR spectroscopy

FT-NIR measurements were performed using a Bruker Optic MPA FT-NIR spectrophotometer equipped with transmission arm as sampling tool, working in the 12,500–5800 cm^{-1} region. The spectra were acquired in transmittance mode at 4 cm^{-1} resolution as the average of 32 scans. A rotating sample holder was used during spectra acquisition. This sampling procedure was necessary since the FT-NIR spectrophotometer used was not equipped with sampling tools suitable for punctual analysis. On the one hand, the rotation allows to eliminate the variation due to the intrinsic