LWT - Food Science and Technology 68 (2016) 14–20

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Fiber optics fluorescence fingerprint measurement for aerobic plate count prediction on sliced beef surface

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ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form
12 November 2015
Accepted 30 November 2015
Available online 8 December 2015

Keywords:
Excitation emission matrix
Partial least squares regression
Multivariate analysis
Meat

ABSTRACT

The ability to predict the aerobic plate count (APC) on a meat surface by rapid, nondestructive, and at-line measurement using fluorescence fingerprint (FF) spectroscopy coupled with fiber optics is reported here. The FF of beef slices stored for 3 days aerobically at 15 °C was measured using fiber optics at excitation wavelengths ranging from 200 nm to 500 nm and emission wavelengths ranging from 200 nm to 900 nm, which was followed by an APC of the same samples. The APC was successfully predicted by partial least squares regression (PLSR) of the measured FF dataset with a coefficient of determination of 0.831. From the variable importance in projection (VIP) score and the sign of the regression coefficient obtained by PLSR, it was indicated that tryptophan, NAD(P)H, Vitamin A, porphyrins, and flavins changed over time and were correlated with predicted APC. Rapid, nondestructive, and at-line analysis of the APC using FF spectroscopy coupled with fiber optics was shown to be applicable.

1. Introduction

Modern food chain distribution requires less time to deliver fresh foods to consumers. Nevertheless, care should be taken to avoid contamination with microorganisms which could result in severe food-borne outbreaks (Sodha et al., 2011; Walsh, 2013). In particular, microorganisms in meat pose the most severe threat to human health. Pathogenic bacteria such as *Salmonella* spp, *Escherichia coli*, and *Campylobacter* spp are well known microorganisms of meat that could be a source of food-borne outbreaks (Kinross et al., 2014; Koutsoumanis, Geornaras, & Sofos, 2006; Matulkova et al., 2013; Schneider et al., 2011). Therefore, a rapid, nondestructive, and quantitative method of microbiological analysis is essential for meat-production monitoring. However, conventional methods for monitoring microorganisms such as aerobic plate counts (APCs) by the culture method, are time-consuming and laborious.

Spectroscopy is a rapid and nondestructive technique which can be used to solve this problem. Among the several spectroscopic techniques, fluorescence spectroscopy has better sensitivity and selectivity than absorption spectroscopy (Karoui & Blecker, 2010). Fluorescence fingerprint (FF) is a set of fluorescence spectra acquired at consecutive excitation wavelengths. FFs have been used as a nondestructive technique for both qualitative and quantitative food assessment for mycotoxins (Fujita, Tsuta, Kokawa, & Sugiyama, 2010), gluten and starch (Kokawa et al., 2012), and buckwheat content (Shibata et al., 2011).

Several studies on employing fluorescence spectroscopy to determine meat quality have been reported. These studies cover the prediction of beef toughness, texture, and tenderness (Allais, Viaud, Pierre, & Dufour, 2004; Egelandsdal, Wold, Sponnich, Neegård, & Hildrum, 2002; Swatland & Findlay, 1997), turkey meat paleness (Swatland & Findlay, 1997), rancidity (Wold, Mielnik, Pettersen, Aaby, & Baardseth, 2002), and lipid oxidation (Gatellier et al., 2007; Veberg et al., 2006). Among them, a few studies on fluorescence spectroscopy in relation to microorganisms in food, such as the alteration of raw-milk cheese by *Pseudomonas* spp. (Leriche et al., 2004), microbial spoilage (Aït-Kaddour, Boubellouta, & Chevallier, 2011), and lactic acid bacteria from sausages (Ammor, Yaakoubi, Chevallier, & Dufour, 2004), have been reported.

There are two major drawbacks to the above studies related to

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http://dx.doi.org/10.1016/j.lwt.2015.11.065
0023-6438/

fluorescence spectroscopy. Firstly, in many studies only a single set of excitation and emission wavelengths was used, leading to a loss of information when using fluorescence spectroscopy to determine the quality of food systems. Therefore, it would be interesting to measure fluorescence at different excitation wavelengths and emission wavelengths to simultaneously collect information on several constituents. Secondly, many measurements were conducted inside the chamber of a fluorescence spectrophotometer, which is not suitable for at-line analysis. FF spectroscopy with fiber optics is a potential technique for overcoming these problems. FF spectroscopy coupled with multivariate analysis appears to be applicable to the nondestructive determination of the APC on the surface of lean beef (Yoshimura et al., 2013). However, in their study, the sample holder, which was made from quartz glass, was in contact with the surface of the lean beef. Sampling that requires contact with samples is undesirable for at-line monitoring because contaminated devices could be a source of cross-contamination (Davies & Board, 1998). However, contactless applications of FF that can be measured outside the fluorescence spectrophotometer has not yet been reported. A breakthrough in solving this sampling problem could be achieved by coupling FF measurement with fiber optics. Fiber optics is an outgrowth of the communication industry and allows the transmission of light over long distances with high efficiency (Daneshvar et al., 1999).

Therefore, the objective of this study was to develop an estimation method for the APC on the surface of beef by FF measurement through fiber optics and multivariate analysis. Thanks to fiber optics, FF spectroscopy was successfully applied to sliced beef without any contact and was conducted outside the fluorescence spectrophotometer.

2. Material and methods

2.1. Beef preparation

Beef slices were purchased from a local meat retailer and transported to the laboratory of the National Food Research Institute, National Agriculture and Food Research Organization (NARO) in Ibaraki, Japan. Beef samples were cut at the store on the starting day of storage, to a cut size of 50 × 50 mm per slice and a thickness of 8 ± 1 mm. The samples were then stored in a refrigerator at 15 °C during experiment periods (72 h). During storage, the samples were placed in sterilized plastic Petri dishes with lids. In the beginning of the experiment, a total of 35 beef slices on petri dishes were prepared, 24 beef slices were measured and the rest were spares. The spare samples were prepared in order to avoid samples with rough surface. Thus, the average distance between fiber optics and meat surface were approximately equal for all samples. Fig. 1 shows a flowchart of the experiments. Two independent experiments were conducted with same procedure on this study.

2.2. FF measurements

FF measurements were performed on beef slices using a fluorescence spectrophotometer (F-7000, Hitachi High-Technology Corp., Tokyo, Japan) with fiber optics (5J0-0114-F-7000, Hitachi High-Technology Corp., Tokyo, Japan). The fluorescence spectrophotometer is equipped with a 150 W Xenon arc lamp as the light source and two grating monochromators coupled with a slit as the excitation and emission wavelength selectors. The scan speed was set to 60000 nm/min. Consequently, the measurement time was 2 min per point. The spectrophotometer was calibrated in advance with Rhodamine B solution. As shown in Fig. 2, the system consisted of a spectrophotometer, fiber optics, and an XY stage controller. The excitation and emission wavelength ranges for FF measurement were 200–500 nm and 200–900 nm, respectively, both with wavelength intervals of 5 nm. A 500 × 500 × 500 mm box was placed outside the fluorescence spectrophotometer and used as a darkroom for the measurement. The box walls were coated with a black cloth to prevent exposure to the outside light. Excitation light was transferred through the fiber optics to the darkroom where the sample was placed. The emission signal from the sample surface was captured by the same fiber optics then sent to the detector of the fluorescence spectrophotometer. The fiber optics used for excitation were placed perpendicular to the light source, while the fiber optics used for emission were perpendicular to the detector of the fluorescence spectrophotometer. A Y-shaped joint was used to bundle both the excitation and emission fiber optics in a single casing. The random arrangement of excitation and emission fiber optics at the end of the probe was oriented toward the sample at distance of 3–5 mm.

A Petri dish containing the beef sample was placed on an XY stage controller inside the darkroom under the fiber-optic probe. The XY stage controller was used to move the sample to measure the FF signal from nine points on the sample surface. The movement of the XY stage controller was controlled using a program developed by system development software (Labview 8.6, National Instruments Inc., USA).

The FF measurement and microbial counts were performed after 12, 24, 36, 48, 60, and 72 h of storage. Two slices were measured for each storage time.

2.3. Microbiological analysis

The nine points used in the FF measurement on beef sample surfaces were wiped with a sterile swab after the measurement. An area of 1 × 1 cm^2 was swabbed with one cotton swab kit (GSI Creos Corp., Japan). To ensure valid sampling, the area was swabbed in a horizontal pattern and again in a vertical pattern. This allowed the entire surface to be swabbed evenly (Bautista, Sprung, Barbut, & Griffiths, 1997; Oto et al., 2012). After swabbing, the cotton swabs were placed into its container that contained 10 ml of 0.1% peptone to be used for plate counts.

Serial decimal dilutions were prepared with Phosphate Buffer Saline (PBS), and the diluted samples were pour plated (1 ml) in duplicate on standard agar (Nissui, Japan). Samples with a high range of bacterial load were plated using an Eddy Jet Spiral Plater (IUL, Japan). The APC was determined by counting colonies after 48 h of incubation at 35 °C. A total of 216 (nine points/sample × six storage time × four samples/storage time) APC were determined in the two experiments.

2.4. Fluorescence fingerprint data analysis

2.4.1. FF data extraction

The extraction of FF data and preprocessing were performed in accordance with a previous study (Fujita et al., 2010). The software for the control of F-7000 (FL Solutions, Hitachi High-Technology Corp., Tokyo, Japan) was used to acquire the data. The original FF data consisted of 8601 recorded intensities for one-point measurement. The FF data were preprocessed and then unfolded from 3D to 2D (Guimet, 2004; Obeidat, Glasser, Landau, Anderson, & Rayson, 2007) with Microsoft Office Excel 2007 (Microsoft, USA) and Matlab 2007b (MathWorks, Inc., USA). The preprocessing procedure included (a) the removal of data with emission wavelengths shorter than the excitation wavelength, because fluorescence data always comes from emission wavelengths longer than the excitation wavelengths. (b) The removal of the scattered light and second, third, and fourth-order light. These were generated by light scattering from the surface of the diffraction grating, and were