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Non-destructive evaluation of ATP content and plate count on pork meat surface by fluorescence spectroscopy

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ABSTRACT

The potential of fluorescence spectroscopy was investigated for the non-destructive evaluation of ATP content and plate count on pork meat surface stored aerobically at 15 °C during three days. Excitation (Ex) Emission (Em) Matrix of fluorescence intensity was obtained and fluorescence from tryptophan (Ex = 295 nm and Em = 335 nm) and NADPH (Ex = 335 nm and Em = 450 nm) was detected. Because tryptophan and NADPH fluorescence changed along with the growth of microorganisms, microbial spoilage on meat could be detected from fluorescence. By applying PLSR (Partial Least Squares Regression) analysis, ATP content and plate count were predicted with good determination coefficient (0.94–0.97 in calibration and 0.84–0.88 in validation).

1. Introduction

Muscle foods, which include both meat and poultry, are an integral part of the human diet and have been so for several thousand years. Meat demand and consumption is very high especially in developed countries. In 2009, the major livestock numbers were 1.4 billion (cattle), 941 million (pig) and 19 billion (poultry) in the world (FAO, 2011). Meanwhile, in Japan, the livestock numbers were 4.4 million (cattle), 9.9 million (pig) and 285 million (poultry). The livestock production of Japan was 2637 billion yen. Consequently in 2007, supply of meat per capita was 49 kg while supply of rice as a staple food of Japan was 57 kg (Statistics Bureau, MIC Japan, 2009). Therefore, quality characteristics and freshness of meat can be public concerns. Over the past two decades, microbiological food safety has received greater attention from regulatory authorities, researchers, public health officials, and consumers (Eifert, Arritt, & Kang, 2006).

In general, the muscle tissues of healthy animals and birds, before slaughter, can be considered sterile. In contrast, surfaces of the animal exposed to the environment such as hide, pelt, the mouth and the gastrointestinal tract may be heavily contaminated. These parts of the animal are major sources of meat and poultry carcass contamination. Moreover, not only the carcass itself but also processing environment can be the source of microbial contamination (Koutsoumanis, Geornaras, & Sofos, 2006). Food borne bacteria may contaminate the meat products during processing, e.g. during slicing (Jessen & Lammert, 2003). Therefore, hygienic practices, sanitation procedures, product handling and processing procedures, and conditions of storage and distribution are the most important factors that determine the microbiological quality of the final meat and poultry products (Koutsoumanis et al., 2006). In Japan, there are 17 large scale poultry processing plants which processes more than 30,000 t of poultry per year (MAFF, 2011). Hence, sanitation monitoring of a processing plant or a processing line is directly related to food safety.

At a meat processing plant, rapid and accurate detection system for microbial spoilage of meats is demanded. Sanitation monitoring at a meat processing plant is carried out in several ways: visual inspection, swabbing for microbiological analysis or ATP bioluminescence. However, the visual inspection is not always accurate. Meanwhile, ATP is present in all living organisms and is also present in a variety of foodstuffs in the form of non-microbial ATP. The presence of ATP in both food debris and viable microorganisms allows the dual detection of these sources of contamination using the technique of ATP bioluminescence (Hawronskyj & Holah, 1997). Thus, the total ATP estimate is employed for routine cleanness assessment as swabbing method. However, the swabbing method requires a skilled worker, a lot of time, and processing line has to be stopped. Additionally, because swabbing is a spot detection method, it cannot evaluate the whole processing line. Considering these conditions, optical sensing, i.e., spectroscopy can be a solution to these problems, as it is a non-destructive method. The sanitation monitoring by spectroscopic method can be real-time and easier than that by microbial analysis or ATP bioluminescence.

Actually, spectroscopic methods have gained importance in the evaluation of food quality attributes during the last decades (Nádai,

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1983; Nádai & Mihályi-Kengyel, 1984). The fact that NIR spectra reflect several parameters of the material suits the method for evaluating complex quality (Williams & Norris, 2001). VIS/NIR spectroscopy technology has been used in pork to determine the following factors; intramuscular fat (Hoving-Bolink et al., 2005; Savenije, Geesink, van der Palen, & Hemke, 2006), fatty acid composition (Fernandez-Cabanas, Garrido-Varo, Gracia-Olmo, De Pedro, & Dardenne, 2007; Gonzalez-Martin, Gonzalez-Perez, Alvarez-Gracia, & Gonzalez-Cabrera, 2005; Gonzalez-Martin, Gonzalez-Perez, Hernandez-Menderz, & Alvarez-Gracia, 2003), color (Cozzolino, Barlocco, Vadell, Ballesteros, & Gallieta, 2003), water-holding capacity (Brondum et al., 2000), presence of RN^- genetic allele (Josell, Martinsson, Borggaard, Anderson, & Tornberg, 2000), and classification of pork into Duroc and Iberian by neural network (del Moral et al., 2009). UV–vis spectroscopy has also been applied to monitoring the cleanness (Oshita et al., 2011; Oto et al., 2012).

Fluorescence is the light emission subsequent to absorption of ultraviolet or visible light of a fluorescent molecule or substructure, called a fluorophore. Fluorescence spectroscopy has just, recently, become quite popular as a tool in biological science related to food technology (Karoui & Blecker, 2011). Studies on applications of fluorescence from meat have primarily been focused on measurements of collagen in connective and adipose tissues. Moreover fluorescence from protein and some oxidation compounds have also been reported. In 1986, intrinsic fluorescence (autofluorescence) for analysis of meat was first proposed in a patent (Jensen, Reenberg, & Munck, 1986) suggesting a method for quality control of meat and fish products. The method was based on excitation at 340 nm and the fact that bone, cartilage, connective tissues, and meat possess different fluorescent properties. Since 1987 Swatland has written a series of papers on different aspects of the autofluorescence of meat. His work was focused on measuring collagen and elastin fluorescence from the connective tissues in meat using excitation at 365 nm. The obtained autofluorescence signals of various meat samples were correlated to several sensory-related quality parameters such as gristle content in beef (Swatland, 1987), skin content and processing characteristics of poultry meat slurry (Swatland & Barbut, 1991) and turkey meat (Swatland, 1995; Swatland & Barbut, 1995), and palatability (Swatland, Nielsen, & Andersen, 1995), chewiness (Swatland, Gullett, Hore, & Buttenham, 1995), and toughness (Swatland & Findlay, 1997) of beef. Subsequently, the bilinear methods PCA and PLS were applied in evaluation of autofluorescence emission spectra of meat obtained from selected excitation wavelengths in the UV region between 300 and 400 nm. Fluorescence emission spectra assigned to various types of collagen in meat products were found to correlate with tensile properties (Egelandsdal, Kvaal, & Isaksson, 1996), tenderness (Egelandsdal, Wold, Sponnich, Neegard, & Hildrum, 2002), and water-holding capacity (Brondum et al., 2000), and recommended for quantification of connective tissue and collagen (Egelandsdal, Dingstad, Togersen, Lundby, & Langsrud, 2005; Wold, Lundby, & Egelandsdal, 1999). Fluorescence emission spectra assigned to fluorescent oxidation products have been found to correlate with lipid oxidation (Olsen et al., 2005; Wold & Mielnik, 2000) and rancidity (Wold, Mielnik, Pettersen, Aaby, & Baardseth, 2002) of meat. Moreover, tryptophan fluorescence (excitation at 290 nm and emission at 305–400 nm) has been correlated to the texture of meat emulsions and sausages (Allais, Viaud, Pierre, & Dufour, 2004). Also autofluorescence images have been used for quantification of the intramuscular fat content and connective tissue in beef (Wold, Kvaal, & Egelandsdal, 1999) as well as for mapping of the lipid oxidation in chicken meat (Wold & Kvaal, 2000). As an example of a more peculiar meat application, the presence of autofluorescence of dietary porphyrins has been suggested for detection of fecal contamination in meat (Ashby et al., 2003). Recently, this technique has also been used for identification of bacteria (Ammor, Yaakoubi, Chevallier, & Dufour, 2004; Leblanc & Dufour, 2002) and for early detection of bacterial spoilage of chicken breast filets (Sahar, Boubellouta, & Dufour, 2011) and minced beef (Aït-Kaddour, Boubellouta, & Chevallier, 2011). Although these studies show that microbial load such as total viable count or *Pseudomonas* can be detected by fluorescence spectroscopy, ATP content as parameter for sanitation monitoring hasn't been examined.

From these current conditions, this study aimed to develop a non-destructive and real-time evaluation of ATP content and plate count on pork meat surface by fluorescence spectroscopy that could be used for sanitation monitoring.

2. Materials and methods

2.1. Meat sample

The lean part of pork loin sliced with 5 mm thickness was obtained from a retailer. The time between the day of slaughter and that of purchase at the retailer was seven days in the Experiment 1 and six days in the Experiments 2 and 3. The sliced samples were cut into pieces of about 4.5×4.5 cm and placed individually in sterilized Petri dishes with lids, but the lids did not prevent oxygen supply. The samples were stored in a storage chamber at a constant temperature of 15 °C. The storage temperature was selected as the highest temperature in a working room of a processing plant, where the temperature is controlled from 10 to 15 °C in consideration of the worker's health according to our conversation with the slaughterhouse management.

During the storage, fluorescence intensity, ATP content, and plate count of meat samples were measured. In the Experiment 1, 15 samples were prepared and three samples were used for each measurement at 0, 24, 36, 48, and 72 h of storage. The samples were used only once because of swabbing process described later. In the Experiment 2, five samples were used for each of six measurements at 0, 12, 24, 36, 48, and 60 h of storage. In the Experiment 3, three samples were used for each of four measurements at 0, 20, 40, and 60 h of storage. The storage times of measurements were different in the experiments to ensure variability in the experimental data for an appropriate estimation.

2.2. Fluorescence intensity

Fluorescence spectrophotometer (F7000, HITACHI, Tokyo, Japan) was used to record the fluorescence intensity from the surface of a pork meat sample. The area of the measurement portion was about 3×10 mm. In order to overcome the small detecting area, a sample was rotated 90° after a measurement and four points on the surface of each sample were measured. The meat sample was covered by quartz glass with 0.5 mm thickness due to the rotation process. A wavelength range of both excitation and emission were from 200 to 900 nm with the resolution of 5 nm. The measurement was conducted in the constant temperature room at 20 °C.

2.3. Sampling protocol and microbial analysis

2.3.1. Sampling protocol

Sampling of materials on the pork meat surface (4×4 cm) covering the area for fluorescence intensity measurement was carried out using the swab technique. To ensure adequate sampling, the sample was swabbed in a horizontal pattern and again in a vertical pattern, being rotated between the index finger and the thumb in a back and forth motion, according to Bautista, Sprung, Barbut, and Griffiths (1997). The quartz glass which covered the meat sample was also swabbed to sample the materials on pork meat surface. The swab sample was then examined for plate count and ATP determination.