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Heterospectral two-dimensional correlation analysis with near-infrared hyperspectral imaging for monitoring oxidative damage of pork myofibrils during frozen storage

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ABSTRACT

Near-infrared (NIR) spectra contain abundant data, heterospectral two-dimensional correlation (H2D-CS) analysis offers a good way to interpret these data. For the first time, H2D-CS was used to correlate the NIR hyperspectral imaging (HSI) data with mid-infrared spectra and to identify feature-related wavebands for developing models for monitoring the oxidative damage of pork myofibrils during frozen storage. The HSI images were acquired at frozen state without thawing and the oxidative damage of myofibrils was assessed by carbonyl content. Results showed that the simplified PLSR model based on H2D-CS identified feature wavebands obtained determination coefficient in prediction (R_P^2) of 0.896 and root mean square error in prediction (RMSEP) of 0.177 nmol/mg protein, which was better than the partial least square regression (PLSR) model based on full wavebands (R_P^2 = 0.856, RMSEP = 0.209 nmol/mg protein). Therefore, H2D-CS was effective in selecting feature-related wavebands of NIR HSI.

1. Introduction

Similar to drying (Delgado & Sun, 2002; Ma, Sun, Qu, & Pu, 2017; Pu & Sun, 2016, 2017; Qu, Sun, Cheng, & Pu, 2017; Sun, 1999; Yang, Sun, & Cheng, 2017) and cooling (McDonald, Sun, & Kenny, 2001; Sun, 1997; Sun & Zheng, 2006; Wang & Sun, 2001, 2004), freezing is one of the most frequently used techniques to preserve foods (Cheng, Sun, & Pu, 2016; Cheng, Sun, Zhu, & Zhang, 2017; Kiani, Sun, Delgado, & Zhang, 2012; Kiani, Zhang, Delgado, & Sun, 2011; Ma, Pu, Sun, Gao, Qu, & Ma, 2015; Pu, Sun, Ma, & Cheng, 2015; Xie, Sun, Zhu, & Pu, 2016). Therefore, fresh meat is often frozen in the meat industry for processing and long-term preservation because freezing inhibits microbial spoilage and retards autolytic reactions. During frozen storage, although microbial spoilage and autolytic reactions can be inhibited, a number of chemical reactions are still active, which can lead to undesirable quality modifications (Baron, Kjærsgård, Jessen, & Jacobsen, 2007; Ngapo, Babare, Reynolds, & Mawson, 1999). In recent years, the oxidation of muscle myofibrils induced by reactive oxygen (ROS) or the interaction with lipid oxidation products is one of the most innovative topics within the food science field and has been acknowledged as a major factor for quality deterioration in frozen stored meats (Estévez, 2011; Utrera & Estévez, 2013). The oxidation of specific amino acid side chains and/or the peptide backbone can lead to irreversible chemical modifications of the proteins including fragmentation, aggregation, loss of solubility and functionality, and decreased susceptibility to proteolysis (Estévez, 2011). Previous studies have related the oxidation of muscle proteins to impaired functionality, altered sensory traits, and loss of nutritional value (Estévez, Ventanas, Heinonen, & Puolanne, 2011; Xiong, 2000), therefore, it is of significance to monitor the oxidation degree of meat products in frozen storage in the meat industry.

As carbonylation is one of the most remarkable modifications in oxidized muscle proteins, the quantification of the total amount of protein carbonyls is the most frequently used method to assess the oxidation degree of proteins (Estévez, 2011). Conventionally, the carbonyl content of protein is quantified by chemical analysis method using dinitrophenylhydrazine (DNPH). However, this method is

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laborious, time-consuming, and cannot meet the demands of online detection. In recent years, by combining spectroscopy and computer vision (Du & Sun, 2005; Jackman, Sun, & Allen, 2009, 2011; Sun, 2004; Sun & Brosnan, 2003; Xu, Riccioli, & Sun, 2017; Xu & Sun, 2017) into one single system, near-infrared hyperspectral imaging (NIR HSI) as a rapid and nondestructive detection method has found wide range applications (Cheng & Sun, 2015a, 2017; Cheng, Sun, Pu, Wang, & Chen, 2015; Cheng, Sun, Pu, & Zhu, 2015; Cheng et al., 2016; Dai, Cheng, Sun, Zhu, & Pu, 2016; ElMasry, Sun, & Allen, 2013; Li, Sun, Pu, & Jayas, 2017; Ma, Sun, & Pu, 2016; Pu, Kamruzzaman, & Sun, 2015; Pu, Liu, Wang, & Sun, 2016; Pu, Xie, Sun, Kamruzzaman, & Ma, 2015; Xiong et al., 2015; Xu, Riccioli, & Sun, 2016). For meat quality inspection, HSI has been investigated to evaluate chemical composition (Barbin, ElMasry, Sun, & Allen, 2013), freshness (Cheng, Sun, & Cheng, 2016; Cheng, Sun, Pu, & Liu, 2016; Dissing et al., 2013; Tao & Peng, 2015), and sensory characteristics (Barbin, ElMasry, Sun, & Allen, 2012) of fresh meat. In particular, Xie, Sun, Xu, and Zhu (2015) and Cheng, Sun, Pu, and Wei (2018) demonstrated that NIR HSI could be used to inspect the cooking loss, drip loss, Warner–Bratzler shear force (WBSF), and myofibrils cold structural deformation degrees of frozen pork meat obtained from different freezing rates at the frozen state without thawing. However, the feasibility of NIR HSI to detect the quality of frozen stored meat, such as the oxidation degree of myofibrils has not been investigated.

Since meat is a complex food system composed of various proteins, lipids, and carbohydrates. The NIR spectrum of meat is often complex and overlapped, making the extraction of weak feature information difficult. As a result, quantitative analysis by NIR has relied heavily on the application of chemometric analysis to relate the subtle spectral changes to the variations in concentrations of certain component in the analyte (Liu et al., 2011; Shao & He, 2009; Shen et al., 2012; Wu, Nie, He, & Bao, 2012), resulting in the need for large number of experiments to identify the feature wavebands and construct a robust calibration model. Feature wavebands selection is usually a time-consuming process and the results are often empirically and varied with the chemometric methods used. Thus, a better understanding of the NIR spectra and more precise band assignments is beneficial for the establishment of robust NIR quantitative models.

Heterospectral two-dimensional correlation (H2D-CS) analysis, which can be used to augment the interpretation of peaks, which are known in one dimension but elusive in the other dimensions, offers a good way to interpret the NIR spectra because it enables one to correlate the NIR spectra to well-defined bands in other types of spectra obtained under a same or similar perturbation (Muik, Lendl, Molina-Diaz, Valcarcel, & Ayora-Cañada, 2007). In previous studies, this technique has been successfully used for spectral interpretation and band assignments of proteins (Jung, Czarnik-Matusewicz, & Ozaki, 2000), carbohydrates (Cocciardi, Ismail, Wang, & Sedman, 2006), and polymers (Amari & Ozaki, 2002; Yu, Tang, Xu, & Shen, 2011). As the carbonyl groups of proteins have distinctive absorption peaks in the mid-infrared (mid-IR) spectra (Brauner, Flach, & Mendelsohn, 2005), the aim of this study was thus to correlate the NIR spectra of frozen meat with the mid-IR spectra by the H2D-CS technique to identify the carbonyl-related wavebands of the NIR spectra. This is the first time to monitor the oxidative degree of frozen pork myofibrils during frozen storage by the NIR HSI technique, and elucidate the feature related wavebands by the H2D-CS technique combined with other type of spectra to make a better interpretation of the NIR spectra used. The performance of the identified feature wavebands was investigated for predicting the carbonyl contents of the frozen meat and compared with the commonly used successive projections algorithm (SPA) and regression coefficient (RC) methods, and the partial least square regression (PLSR) model based on full wavebands. The results of this study were expected to provide a method for interpreting the NIR HSI spectrum of frozen pork meat and offer a new method for monitoring the myofibrils carbonyl content in a rapid and accurate way.

2. Materials and methods

2.1. Freezing meat preparation

Fresh pork *longissimus dorsi* muscles were obtained from a local Lotus supermarket (Guangzhou, China) and transported to the laboratory in 30 min. Upon arrival, the connective tissue and fat portion on the muscles were removed, and the muscles were chopped into 100 samples with similar size of 10.0 cm × 5.0 cm × 4.0 cm (length × width × thickness). The samples were then frozen at −60 °C in an air-blast freezer (CTE-SE7510-05F, China-Scicooling Co., Beijing, China). T-type thermocouples were used to record the core temperature of the samples during freezing. When the core temperature of the meat samples reached −40 °C, the freezing operation was terminated and the samples were moved into a refrigerator for storage. The freezing rate of the frozen pork meat samples was about 2.8 cm/h according to the definition by the International Institute of Refrigeration (IIR) (Xie et al., 2015). Among the 100 frozen pork meat samples, 50 samples were stored at −18 °C a refrigerator (BL/BD-719H, Haier Ltd., Qingdao, China) and the other 50 samples were stored at −40 °C another refrigerator (DW-40L 188, Haier Ltd., Qingdao, China). At 0, 7, 16, 24, 38 week intervals, 10 samples stored at −18 °C and 10 samples stored at −40 °C were taken out. The frozen samples were firstly scanned by the HSI system and then subjected to chemical analysis as described below.

2.2. HSI image acquisition and preprocessing

Hyperspectral images of the frozen meat samples were collected using a pushbroom hyperspectral system. The system was mainly composed of six components: an illumination source including two 150 W halogen lamps (3900-ER, Illumination Technologies Inc., New York, USA), a line-scan spectrograph (Specim V25E, Spectral Imaging Ltd., Oulu, Finland) that decomposes light into spectral range of 916–2534 nm with a spectral increment of 6.32 nm, a high definition CCD camera (XC403, Xenics Infrared Solutions, Leuven, Belgium) with focal length of 22 mm and frame rate of 23 frames per second, a camera lens (OLES30, Xenics Infrared Solutions, Leuven, Belgium), a translation stage controlled by a stepper motor (IRCP0076-1COMB, Isuzu Optics Corp., Taiwan, China), and a computer system installed with image acquisition software (Spectral Image software, Isuzu Optics Corp., Taiwan, China). Before scanning, the temperature of frozen meat samples from both the −18 °C and −40 °C groups were equilibrated to −18 °C and moved to a portable refrigerator (KM-25YS, Jinhua Minke Trading Ltd., Jinhua, China) at −18 °C located near the HSI system. The samples were then placed to the conveyer belt for image acquisition. After image acquisition, the samples were thawed at 4 °C in a domestic refrigerator (BC/BD-241SE, Haier Ltd., Qingdao, China) for chemical analysis of carbonyl content.

The spectral data collected from the HSI system was digital numbers corresponding to the signal intensity. In order to minimize the interference from uneven light intensity, the spectral images (R) were firstly calibrated into the reflectance mode (R_c) using the standard white (W) and black (B) images:

$$R_c = (R-B)/(W-B) \times 100\% \quad (1)$$

where the white image was obtained by a standard Teflon white tile (~100% reflectance), and the black image was acquired by covering the camera lens with its opaque cap (~0% reflectance). Then, in order to extract the typical spectra of frozen meat, the background was firstly removed based on a masking operation. In this study, the mask was created by subtracting the image at band 95 (with low reflectance value) from the image at 30 (with high reflectance value), where a threshold value of 0.04 was used. After that, to remove the effects of the frost on the sample surface, a preliminary small region of interest (ROI) without frost was firstly identified and all the pixels without