Food Control 60 (2016) 361–364

Contents lists available at ScienceDirect

Food Control

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

Short communication

Fourier Transform Infrared Spectroscopy enables rapid differentiation of fresh and frozen/thawed chicken

Tom Grunert [a, *], Roger Stephan [b], Monika Ehling-Schulz [a], Sophia Johler [b]

[a] Functional Microbiology, Institute of Microbiology, University of Veterinary Medicine Vienna, Veterinaerplatz 1, 1210 Vienna, Austria
[b] Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Winterthurerstrasse 272, 8057 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 20 May 2015
Received in revised form
10 August 2015
Accepted 12 August 2015
Available online 15 August 2015

Keywords:
FTIR
Chicken meat
Freezing
Refrigeration
Mislabeling

ABSTRACT

Freezing and thawing affect the sensory profile and the quality of chicken meat, resulting in lower marketability. Retailers are faced with the risk of mislabeling, as fresh and frozen/thawed chicken meat are visually indistinguishable and as there is currently no fast, reproducible, and inexpensive technique for the differentiation of fresh and frozen/thawed chicken implemented in practice. Fourier Transform Infrared (FTIR) spectroscopy represents a new promising technique that determines the overall chemical composition of a sample, thus creating a metabolic spectral fingerprint that can be analyzed by various pattern recognition algorithms. In this study, we aimed to assess the performance of FTIR spectroscopy when applied to the differentiation of fresh and frozen/thawed chicken meat. To this end, we compared the FTIR spectra of chicken stored at 4 °C to those of chicken that was frozen and stored at −20 °C for 2, 5, 15, 30, 60, 70, and 85 days. Hierarchical cluster analysis of FTIR spectra allowed to distinguish fresh samples from samples that have been frozen for longer periods. Samples of frozen storage of 15, 30, 75 and 85 days could be clearly identified as such. Further, the potential of combining FTIR spectroscopy with artificial neuronal network (ANN) analysis to enable identification of even shortly frozen products was determined. Twenty out of 21 samples were correctly classified in either fresh or frozen/thawed chicken meat based on the internal validation including frozen/thawed chicken meat samples derived from day 2 and 5. In conclusion, we provide proof of principle that FTIR spectroscopy enables rapid and reliable discrimination of fresh from frozen/thawed chicken meat. Due to its high−throughput capacity, it could represent a promising tool in routine inspections differentiating fresh from previously frozen meat products such as beef, pork, lamb and turkey.

1. Introduction

Chicken is a highly perishable meat product. Freezing and frozen storage of chicken and other meat products is a common practice used in the striving global meat export industry, which is currently worth more than 13 billion USD (Leygonie, Britz, & Hoffman, 2012). According to the Food and Agriculture Organization of the United Nations, freezing of poultry can extend the practical storage life to up to two years (Cano-Muñoz, 1991). However, freezing and thawing negatively affects the sensory profile and therefore the quality of meat through formation of ice crystals, oxidation of lipids and degradation of proteins, reduced tenderness, and reduced water holding capacity (Ali et al., 2015; Leygonie et al., 2012). The impaired quality of frozen/thawed chicken and the resulting impact on marketability is also reflected in lower pricing. In December 2014, the EU implemented a new regulation specifying that frozen/thawed products have to be labeled “defrosted”, as safety, taste and the physical quality of food items − in particular meat and fish − could be affected (EU Regulation 1169/2011). However, fresh and frozen/thawed chicken meat are virtually indistinguishable. In addition, most of the currently available testing methods for differentiation of fresh and frozen/thawed chicken are laborious, time consuming, and cost intensive (Bae et al., 2014). These include enzymatic methods, DNA based techniques, spectroscopic methods using light in the ultraviolet, visible (UV−VIS) and near-infrared (NIR) electromagnetic spectrum, bio imaging and sensory methods (Ballin, 2010; Ballin & Lametsch, 2008; Jung et al., 2011). Consequently, retailers often rely on information provided by subcontractors, which bears the risk of mislabeling and its negative impact on the consumers' repurchase behavior. Therefore, a novel

* Corresponding author.
E-mail address: tom.grunert@vetmeduni.ac.at (T. Grunert).

http://dx.doi.org/10.1016/j.foodcont.2015.08.016
0956-7135/

high-throughput tool suited for reliable differentiation of fresh and frozen/thawed chicken is urgently needed.

Fourier Transform Infrared Spectroscopy (FTIR) is a new promising tool that measures the overall chemical composition of a sample, thus creating a metabolic spectral fingerprint that can be analyzed by various pattern recognition algorithms (Wenning & Scherer, 2013). It is successfully employed as an analytical tool in a wide range of fields and industries including microbiological and medical diagnostics, as well as food science and technology (Naumann, 2008; Rodriguez-Saona & Allendorf, 2011). For instance, FTIR spectroscopy was used to analyze minced meat subjected to two weeks of frozen storage (Al Jowder, Kemsley, & Wilson, 1997). However, chicken is commonly sold as intact muscle meat, which exhibits vastly different degradation processes than minced meat. We therefore investigated the potential of FTIR spectroscopy for discrimination of fresh and frozen chicken muscle meat after short time storage for two days as well as long time storage for up to three months. This approach could be useful for the evaluation of previously frozen meat products by retailers as well as governmental control agencies.

2. Materials and methods

2.1. Chicken meat samples

An overview of all chicken meat samples used is provided in Table 1. A total of 16 samples of 50 g of chicken breast muscle were purchased from a local poultry meat producer (producer A). The fresh meat was immediately packed in plastic bags in line with the industry standard and vacuum-sealed. The cooled samples (4 °C) were transported to the laboratory. The samples were randomly assigned to two groups: fresh samples that were only refrigerated (R; n = 6), and frozen/thawed samples (FT; n = 10). Refrigerated samples were kept at 4 °C and were prepared for FTIR spectroscopy immediately (T_0) and after 2 and 5 days. The frozen/thawed samples were stored at −20 °C for 2, 5, 15, 30, 60, and 70 days and were gently thawed at 4 °C for 6–7 h prior to sample preparation for FTIR spectroscopy. Two samples from a different poultry meat producer (producer B) were used for validation and were frozen for 85 days prior to sample preparation for FTIR spectroscopy.

2.2. Sample preparation and FTIR spectroscopy

We transferred 46.9 ± 1.09 (mean ± SD) grams of the samples to 50 mL polypropylene centrifuge tubes. Upon centrifugation at 15,000 × *g* for 15 min at 4 °C, the aqueous phase ("press-juice") was removed and triplicates of 1:10 and 1:15 dilutions in 0.9% NaCl were prepared for two samples of each group and time point. A volume of 30 µl of the press-juice dilutions was spotted on a zinc selenite (ZnSe) optical plate and dried at 40 °C for 30 min. Infrared spectroscopy absorption spectra were recorded using a HTS-XT microplate adapter coupled to a Tensor 27 FTIR spectrometer (Bruker Optics GmbH, Ettlingen, Germany). Spectral acquisition was performed in transmission mode in the spectral range of 4000 to 500 cm^{-1} using the following parameters: 6 cm^{-1} spectral resolution, zero-filling factor 4, Blackmann-Harris 3-term apodization and 32 interferogramms were averaged with background subtraction for each spectrum (Grunert et al., 2014).

2.3. Spectral processing and chemometrics

Spectral evaluation and processing were performed using the software OPUS 7.2 (Bruker Optics GmbH). Second derivatives of the original spectra with a 9-point Savitzky–Golay filter were calculated to increase spectral resolution and to minimize problems with baseline shifts. Subsequent vector normalization was performed for the whole spectral range to adjust biomass variations among different sample preparations (Grunert et al., 2014). Subtractive spectral analysis was used to define spectral regions of critical relevance to the discrimination of the different experimental groups. An average spectrum was calculated from the recorded, second derivative and vector normalized IR spectra of the R and FT group separately and differential spectra analysis was performed. The average spectrum of R samples was subsequently subtracted from the average spectrum of FT samples. Chemometric analysis was performed on preprocessed data employing unsupervised hierarchical cluster analysis (HCA). Unsupervised methods are not based on prior knowledge and allow reduction of data complexity, while maintaining most of the original variance (Wenning & Scherer, 2013). The spectral window 1660–1628 cm^{-1}, was selected for HCA, offering the maximum discriminatory power.

To develop and to validate the potential of an artificial neuronal network (ANN) for the discrimination between fresh and frozen/thawed chicken meat, the software NeuroDeveloper (version 2.5b; SynthonGmbH, Heidelberg, Germany) was used (Udelhoven, Novozhilov, & Schmitt, 2003). Preprocessed spectra used for HCA (n = 108) were subdivided into two groups: (1) 86 spectra served as a reference data set (n = eight to ten spectra/group) and (2) 21 spectra (n = two to three spectra/group) were used for internal validation. One spectrum was excluded based on an outlier analysis using the software Unscrambler X (CAMO Software, Oslo, Norway). The reference data set used to calibrate the model was randomly divided into a training set (n = six to eight spectra/group) and a prevalidation set (n = two spectra/group). To reduce the dimensionality in the spectral data set, the most discriminative wavenumbers were identified prior to the training process using the multivariate COVAR algorithm of the NeuroDeveloper software (based on a covariance analysis combined with the sequential forward selection search strategy).

3. Results and discussion

We evaluated the performance of FTIR spectroscopy as a tool for the differentiation of fresh (refrigerated only) and frozen/thawed

Table 1
Random assignment of chicken meat samples into two groups: fresh samples that were refrigerated only (R, n = 6); and frozen/thawed samples (FT, n = 12). Two samples per group and time point were analyzed in triplicates of 1:10 and 1:15 dilutions to generate a total of 108 FTIR spectra.

Group	Sample ID	Storage duration	Storage conditions	Number of samples	Sample source
R	R_0	0 days	4 °C	2	Producer A
	R_2	2 days	4 °C	2	Producer A
	R_5	5 days	4 °C	2	Producer A
FT	FT_2	2 days	−20 °C	2	Producer A
	FT_5	5 days	−20 °C	2	Producer A
	FT_{15d}	15 days	−20 °C	2	Producer A
	FT_{30d}	30 days	−20 °C	2	Producer A
	FT_{70d}	70 days	−20 °C	2	Producer A
	FT_{85d}	85 days	−20 °C	2	Producer B