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Evaluation of structural changes in raw and heated meat batters prepared with different lipids using Raman spectroscopy

Jun-Hua Shao, Yu-Feng Zou, Xing-Lian Xu, Ju-Qing Wu, Guang-Hong Zhou *

Key Lab of Meat Processing and Quality Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing, 210095, PR China

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ABSTRACT

Structural changes, textural properties, water- and fat-binding properties and their relationships in meat batters prepared with different lipids and with heating were studied by Raman spectroscopy. Results revealed that the meat batters prepared with soybean oil (SO) showed the lowest fluid losses, greatest ($P<0.05$) hardness, springiness, cohesiveness, chewiness, resilience values compared with batters prepared with pork fat (PF) or butter (DB). There was a significant decrease ($P<0.05$) in α-helix content accompanied by a significant increase ($P<0.05$) in β-sheet structure after heating in PF and SO samples, but no significant ($P>0.05$) difference was found in DB batters. A positive significant ($P<0.05$) correlation between β-sheet structure and textural properties was found in heated samples. Further, there was a significant positive ($P<0.05$) correlation between α-helical structure and total expressible fluid and a significant negative ($P<0.05$) correlation between β-sheet structure and total expressible fluid. Results suggest that different lipid additions and thermal treatments induced different changes in meat proteins structural, expressible fluid, and textural properties of meat batters.

1. Introduction

Raman spectra provide information on the micro-environment and chemistry of protein side chains as well as on the conformation of the protein polypeptide backbone (Herrero, 2008a; Tuma, 2005). Changes in Raman bands can give information on modifications in secondary structures of proteins (amide conformation region, C-C stretching vibration) and also in local environments (tryptophan residues, tyrosyl-doublet, aliphatic amino acid bands) (Herrero, 2008a; Li-Chan, 1996). In processed meat food systems, identification of these structural changes in proteins could provide information leading to an understanding of the mechanisms involved in meat emulsions, thereby allowing the possibility to improve the conditions of handling, processing and storage of these foods (Herrero, 2008b).

Addition of fat to meat products plays an important role in the rheological and structural properties as well as providing a unique taste profile (Cáceres et al., 2008; Hughes, Cofrades et al., 1997; Jiménez-Colmenero, 2007). The structure and physico-chemical properties of proteins and lipids influence the formation and stability of emulsions (i.e., forming an interfacial protein film around the fat globules in finely comminuted meat products.) and thus the texture of many food products (Hansen, 1960; Howell, Herman, & Li-Chan, 2001). Fats interact with other ingredients to develop texture, mouthfeel and assist in the overall sensation of lubricity of foods (Giese, 1996). Using Fourier transform Raman spectroscopy, Howell et al. (2001) studied systems containing lysozyme (25% in D_2O), corn oil, and their emulsions (10% w/w oil/D_2O solution) and their findings suggested that the presence of lipids can alter the molecular structure of proteins and result in changes in exposure of hydrophobic groups, secondary structures, and conformation of disulfide groups. Lee, Lefévre, Subirade, and Paquin (2007) reported that high-pressure homogenization led to a decrease in the content of α-helix and an increase of β-sheeting, indicating the formation of fewer interactions with the lipid phase and more interaction between adsorbed proteins respectively. The reasons of these results may be that the α-helix structure exposed to the hydrophobic region plays an important role in the adsorption and interaction with oil phase of emulsions and intermolecular β-sheet formation after adsorption reflects the formation of strong interactions and aggregations between neighboring adsorbed proteins to form gel-like interfacial layers. Herrero, Carmona et al. (2011) showed that the extent of lipid-chain disorder, or lipid–protein interactions in emulsions, depended on the formulation of the stabilizing system. It was observed that the protein secondary structures of the emulsifying systems studied changed to a more orderly protein backbone when formed with olive oil-in-water emulsion.

However, the structural changes occurring in meat proteins produced by the specific interactions of different types of lipid and meat protein during heat-induced processing are not clearly understood. In general, most published studies concern with the surface behavior of proteins, and less attention has been paid to changes in the lipid phase, and interactions between protein and lipid molecules (Herrero et al., 2011). Moreover, these studies have been performed in model

* Corresponding author. Tel./fax: +86 25 84395939.
E-mail address: ghzhou@njau.edu.cn (G.-H. Zhou).

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emulsions which contain only one or a few individual proteins and therefore do not represent meat products. Also, to our knowledge, there have been no Raman spectroscopic studies performed on meat batters that have been prepared with different types of lipids added.

A better understanding of the structural changes of meat proteins and lipids occurring in meat products induced by addition of different lipids and thermal treatments could be helpful to elucidate their role in the protein matrix structure and for the development of new healthy meat products. The goal of this work was to use Raman spectroscopy to determine the structures of components of meat batters manufactured in situ with different types of lipids. From this we wished to establish causal relationships between the structural and physical properties of emulsions. Further, we investigated the relationship between the changes in protein structures and changes in water holding capacity and textural properties of the meat batters. The ultimate aim was to gain a thorough understanding of the behavior of these emulsions with different fat ingredients.

2. Materials and methods

2.1. Materials

Fresh pork center loin muscle (70.3% moisture, 22.2% protein, and 6.2% fat) and pork fat (89.9% fat, 8.1% moisture, 1.7% protein; AOAC 2000) were purchased from a local market (24 to 48 h postmortem, pH5.6 to 5.9). All visible connective tissue and fat were trimmed from the meat. The meat and fat were separately mixed and passed through a grinder (MM-12, Guangdong, China) using a 0.6 cm plate. Lots of approximately 500 g were vacuum-packed in a polyethylene bags and stored at −18 °C until use. Soybean oil (Arowana, 100% fat) and butter (Suki, 82% fat) were obtained from a local supermarket. Others ingredients and additives used were sodium chloride, sodium tripolyphosphate (STP).

2.2. Preparation of meat batters

Individual meat batters were prepared using one of three different types of fat, each having a target final protein level of 12.5% and fat level of 18%. The fats used were pork fat (PF), soybean oil (SO) and dairy butter (DB). The formulations are shown in Table 1.

The meat and fat were thawed (5 °C overnight). The preparation procedure according to Youssef and Barbut (2010) was as follows: the lean meat was chopped in a bowl chopper (Stephan UMC-5C, Germany), at the low speed setting for 30 s, followed by addition of 2% salt and 0.3% sodium tri-polyphosphate, while chopping at the high speed setting for 1 min. This was followed by a 1.5 min break (allowing time for protein extraction). Next, lipids and 1/3 ice water were added and the batter chopped at the high speed setting for 1 min, followed by addition of ice water and chopping for another 3 min at the high-speed setting. Final batter temperature did not exceed 12 °C. Each batter was vacuum-packed to remove trapped air. Ten 35 g samples were stuffed into 50 mL polypropylene tubes, which were hermetically sealed. The plastic containers were centrifuged (Model 225, Fischer Scientific, Pittsburgh, Pa., U.S.A.) at 500 *g* (4 °C) setting for 3 min to remove any remaining air bubbles. Each of the meat batter formulations in the plastic containers was subjected to two different treatments. Half of the containers with each meat batter formulation were chilled at 4 °C and analyzed as fresh (un-heated) batters. The rest of the containers in each case were heated in a water bath at 70 °C for 30 min. Both unheated and heated samples were stored in a chiller at 4 °C until analyses.

Table 1
Formulations of meat batters prepared with different lipids.

Samples	Meat (g)	Lipids (g)	Ice water (g)	Total (g)
PF	270	100	118.75	500
SO	270	90	128.75	500
DB	270	110	108.75	500

All formulated with 2.0% sodium chloride and 0.25% sodium tri-polyphosphate (STP).

2.3. Chemical analysis

Moisture and ash contents of meat and meat batters were determined (AOAC, 2000) in triplicate. Fat content was determined by a Soxhlet solvent extraction system (Soxtec® Avanti 2050 Auto System, Foss Tecator AB, Höganäs, Sweden) and protein was determined by an automatic Kjeldahl nitrogen analyzer (Kjeltec® 2300 Analyzer Unit, Foss Tecator AB, Höganäs, Sweden).

2.4. Water- and fat-binding properties

Water- and fat-binding properties were determined (six replicates) by measuring water and fat loss according to Cofrades, Serrano, Ayo, Carballo, and Jiménez-Colmenero (2008) with minor adjustments. The samples were heated at 70 °C for 30 min, and then the containers were opened and left to stand upside down (for 1 h) to release the separated fat and water onto a plate. Total loss (TL) was expressed as % of initial sample weight. Water loss (WL) was determined as % weight loss after heating the total released fluid (TL) for 16 h on a stove at 101 °C. Fat loss (FL) was calculated as the difference between TL and WL. WL and FL were expressed as % of total loss. Determinations were carried out six times.

2.5. Texture profile analysis

For heated samples, texture profile analysis (TPA) was performed using a texture analyzer (Texture Technologies Corp., Model TA.XT2, Scarsdale, N.Y., U.S.A.). Samples were tested at room temperature. TPA parameters were determined using 6 cooked cores (each diameter 16 mm, height 10 mm) per treatment. Cores were compressed twice to 75% of their original height by a texture analyzer using a 25 kg load cell at a crosshead speed of 1.5 mm/s. Attributes were calculated as follows: hardness (Hd), peak force (N) required for first compression; cohesiveness (Ch), ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp), distance (mm) of sample recovery after the first compression; and chewiness (Cw), Hd × Ch × Sp (N mm). Measurement of samples was carried out at room temperature.

2.6. Raman spectroscopic analysis

Raman experiments were conducted with a Jobin Yvon Labram HR800 spectrometer (Horiba/Jobin. Yvon, Longjumeau, France). A microscope equipped with a 50× lens was used to focus the excitation laser beam (514.5 nm excitation line of a Spectra Physics Ar-laser) on the sample and collection of the Raman signal in the backscattered direction. The laser power at the sample surface was controlled at about 100 mW. The laser spot diameter reaching the sample was about 1 μm. For measurement, the sample was spread on a glass slide. The spectra were recorded in the range of 400–3600 cm^{-1}. Each spectrum was obtained under the following conditions: 3 scans, 30 s exposure time, 2 cm^{-1} resolution, a sampling speed of 120 cm^{-1}/min with data collected every 1 cm^{-1}. The time required for the acquisition of 1 spectrum was about 2 min. Spectra were smoothed, baselines corrected and normalized against the phenylalanine band at 1003 cm^{-1} using Labspec version 3.01c (Horiba/Jobin. Yvon, Longjumeau, France).

The Phe ν-ring band located near 1003 cm^{-1} was used as internal standard to normalize the spectra, as it has been reported to be insensitive to the micro-environment (Herrero, 2008a; Herrero, 2008b; Li-Chan, 1996; Li-Chan, Nakai, & Hirotsuka, 1994; Xu, Han et al., 2011). Assignment of the visible bands to vibrational modes of peptide