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Combined use of variable pressure scanning electron microscopy and confocal laser scanning microscopy best reveal microstructure of comminuted meat gels

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ABSTRACT

Observing the distribution of protein and fat phases in comminuted meat products can be helpful to understanding the mechanisms of texture development and fat/water binding. In this study variable pressure scanning electron microscopy (VP-SEM) was compared to conventional scanning electron microscopy (SEM), and contrasted with confocal laser scanning microcopy (CLSM), as tools to characterize gel morphology of cooked meat batters or non-fat pastes. Gel morphology was varied by inclusion of whey protein isolate (WPI) that gels only at high temperature, in partial substitution of myofibrillar protein (MFP). CLSM (magnification:10–1000×) revealed that, when no WPI was added, a homogeneous gel structure was produced enmeshing small, well-distributed fat particles. Substituting 30 g/100 g MFP by WPI produced a coarse gel structure with clear microphase separation of fat. VP-SEM (magnification:1000–2000×) enabled visualization of small pore structure of gel matrix whereas SEM obscured details of this, as well as of the relationship between fat globules and gel matrix that were visible by VP-SEM. Since meat gels properties can be affected by multiple morphological features, visible only at different levels of magnification, the relationships between microstructure and important properties of meat gels can be most advantageously observed when both VP-SEM and CLSM are used in tandem.

1. Introduction

Comminuted products from mammalian and avian meats, such as hot dogs and bologna, are prepared by chopping meat with the addition of salt, ice water, fatty tissues and seasonings to a fine homogenate, which forms a stable gel matrix upon heating. The microstructure of this matrix has been widely investigated by microscopy as the basis of texture fat/water holding properties of meat products (Barbut, 1997; Barbut, Gordon, & Smith, 1996; Borchert, Greaser, Bard, Cassens, & Briskey, 1967; Froning, Aanersen, & Mebus, 1970; Gordon & Barbut, 1992; Hansen, 1960; Hermansson, 1988; Jones & Mandigo, 1982; Lee, 1985; Lee, Carroll, & Abdollahi, 1981; Schmidt, Acton, & Ray, 1985; Theno & Schmidt, 1978; Wu, Xiong, Chen, Tang, & Zhou, 2009; Youssef & Barbut, 2009). Computer-aided image analysis has been used to more quantitatively assess changes in structure or fat particle distribution as affected by formulation or processing (Koolmees, 1989; Lee, 1985; Stevenson, Liu, & Lanier, 2012).

The microscopy techniques used in these studies often require sample preparation steps that can lead to artifacts and thus perhaps false interpretation of photomicrographic results. When using light microscopy (LM) for examination of gels at lower magnification (10–1000×), the sample preparation methods are not only time consuming due to the staining process but often result in poor resolution such that it can be difficult to distinguish lipid droplets from protein components, especially near a cut surface (Gordon, 1993). Confocal laser scanning microscopy (CLSM) is a technique employing a pair of pinhole apertures to acquire in-focus images from a process of optical sectioning where a specimen can be imaged in successive volumes with the optical (z) axis of the microscope incorporating a series of sections (Inoue, 1995). This technique should eliminate these issues in the LM magnification range, and moreover allows non-invasive, optical sectioning, essentially free from out-of-focus blur. These sections can then be reconstructed to yield a three dimensional image (Shotton, 1989).

Conventional scanning electron microscopy (SEM) is a technique for producing high-resolution images of a sample by scanning it with a focused beam of high-energy electrons (Oatley, Nixon, & Pease, 1965). It has been widely used to study the microstructure of meat products and also has several disadvantages

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(Gordon, 1993). Extensive sample preparation is required before analysis due to the high water and/or fat content of comminuted meat gels. Dehydration from either physical or chemical fixation is required to prevent water vaporizing within the microscope chamber. Fat should be fixed or extracted before the sample is viewed under the electron beam. These preparations may change the original gel microstructure and cause artifacts, thus making image interpretation very difficult (Gordon, 1993; Hassan, Frank, & Elsoda, 2003; Stevenson et al., 2012). Another type of electron microscope, similar to SEM, is named as variable pressure SEM (VP-SEM) where high-energy electron chamber is maintained at high vacuum while the sample chamber vacuum level can be adjusted (Stokes, 2008). Use of this technique should minimize shrinkage or deformation of hydrated specimens because direct microscopy of wet, fat-containing and non-conductive samples in their natural state can be carried out with no special sample preparation required (Yamada & Kuboki, 1993). This micrographic technique, and a similar approach of cryo-SEM (Gordon & Barbut, 1990a), has already been successfully utilized to characterize the microstructure of non-fat-containing meat gels (Kubota, Tamura, Matsui, Morioka, & Itoh, 2006; Kubota, Tamura, Morioka, & Itoh, 2003). However, VP-SEM has not been used to examine high fat-containing gels of traditional comminuted meat products.

We investigated the utility of VP-SEM and CLSM as techniques to characterize the structure of fat-containing and non-fat comminuted meat gels, as modified by partial substitution of whey protein isolate (WPI) for myofibrillar protein (MFP). VP-SEM was directly compared to conventional SEM in this regard, and CLSM results could be compared to previous published results to evaluate its possible added advantages.

2. Materials and methods

2.1. Materials

Boneless skinless chicken breast meat was purchased from a local chicken processor (Pilgrim's Pride, Sanford NC), packaged in ~3.5 kg portions in freezer bags and frozen at −10 °C until use (<5 months). Frozen chicken was thawed at 4 °C for at least 12 h before the trimming of visible fat and connective tissue. Pork fat, in the form of lard, was obtained from a local grocery store (International Foods, Raleigh NC), packaged in 9.08 kg portions in plastic containers, and kept at 4 °C until use (<2 months).

2.2. Preparation of meat batters and pastes

Finely comminuted fat-containing batters (HF) were prepared by chopping thawed chicken (51.17 g/100 g), water (24.83 g/100 g), fat (lard; 20 g/100 g), salt (2 g/100 g), sugar (1.7 g/100 g), and tripolyphosphate (0.3 g/100 g) in a Stephan UMC-5 vertical-cutter/mixer (Stephan Machinery Corp., Columbus, OH) under vacuum for 6 min at 25,000 rpm. This formulation closely simulated that of a leading brand of frankfurter. Lean, non-fat meat pastes (NF) were prepared in the same way except that fat was deducted from the formula and the ratio of the remaining ingredients was kept constant. Final chopping temperature did not exceed 10 °C (Acton, Ziegler, & Burge, 1983; Gordon & Barbut, 1989; Brown & Toledo, 1975; Schut, 1976). Meat batters (pastes) were vacuum-packaged in Cryovac B-series bags (Cryovac, Duncan, SC, U.S.A.) with a Multivac 8941 (Multivac, Allgau, Germany) to remove air pockets. A corner was clipped and the bag placed into a manual sausage stuffer for extruding into cellulose casings (inner diameter 1.9 cm; tied off to lengths of 17.8 cm). These were then vacuum packed inside water impermeable plastic bags for subsequent heat processing in a water bath.

To vary the gelling quality of the protein fraction of this formulation, the content of myofibrillar protein (MFP) of these HF batters or NF pastes was adjusted downward by addition of 30 g/100 g whey protein isolate (WPI) in substitution of MF protein (chicken breast meat), holding moisture content constant (designated hereafter as '30 g/100 g WPI' treatment, vs. 0 g/100 g WPI treatment) (Liu, 2012).

2.3. Heat processing

Meat batters/pastes were cooked in a programmable water bath (Neslab Instruments Inc., Portsmouth, NH) from 5 to 70 °C at the rate of 0.5 °C/min. Actual product temperature was confirmed with a thermocouple inserted into the geometrical center of one tube gel. Samples were removed from the water bath at the endpoint temperature and cooled in an ice-water bath for 20 min.

2.4. Confocal laser scanning microscopy (CLSM)

The method of Liu (2012) was employed. Meat gels, held in sealed bags at 4 °C overnight, were sliced into sections approximately 5 mm × 5 mm × 1 mm thick using a razor blade. A 0.2 g/100 g solution of Nile Blue A Sulfate (MP Biomedicals, LLC; Solon, OH) fluorescent dye in de-ionized water was filtered twice using Whatman No. 3 (Maidstone, Kent UK) filter paper, and 20 μL of the solution was pipetted onto the cut surface of each gel slice. The dye was allowed to absorb into the gel at room temperature for 10 min. Gel samples were then turned over (dyed gel surface against the coverslip) onto a single-welled slide with a #1.5 coverslip adhered to the bottom of the slide via silicone grease. Samples were imaged on a Zeiss LSM 710 Confocal laser scanning microscope (CLSM) attached to a Zeiss Axio Observer Z1 inverted microscope using 10× 0.45 NA (Numerical Aperture) dry, 20x 0.8 NA dry and 40× 1.2 NA water immersion objectives. A 488 nm argon laser (to excite Nile Blue in the fat phase) and a 633 nm helium neon laser (to excite Nile Blue in the protein phase) were used sequentially to image the samples. Emission spectra were collected from 500 to 650 nm for the fat phase and 650–800 nm for the protein phase, and the resulting images were overlaid. The pinhole was optimized to the size of 1 airy unit (diameter of the airy disk produced by the objective) to reduce unwanted light dispersion that might blur the image, and the zoom was maintained at 1.0000 across all images, regardless of z-stack position. For each gel treatment, 2 samples were prepared; 3 images were taken per sample at the 10× objective, resulting in a total of 6 images per treatment.

2.5. Variable pressure scanning electron microscopy (VP-SEM)

Cylindrical meat gels (ϕ: 2 cm) were held at 4 °C until cut into blocks of 2 cm thick using a razor blade. To avoid the error from the surface smashing due to melted fat, the blocks were lightly frozen for 20 s on a metal stabber surrounding by liquid nitrogen. The internal structures of the gels were exposed by cutting the gels in half. The samples were mounted on a copper specimen holding and the exposed surface was observed under a SEM (S-3400, Hitachi) with a VP-mode (sample stage, <−5 °C; pressure: 30 Pa; accelerating voltage, 20 kV). For each gel treatment, 2 samples were prepared; 3 images were taken per sample at each magnification: 250×; 500×; 1000×, resulting in a total of 18 images per treatment.

2.6. Conventional scanning electron microscopy (SEM)

SEM-observation of comminuted meat gels was conducted according to a modification of the method of Stevenson et al. (2012). They had noted that chemical fixation during sample preparation