Chapter 19

Microstructure of Muscle Foods

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19.1 Introduction on Food Microstructure

The chemical components of a food are organized into a characteristic microscopic pattern that constitutes its microstructure. The food’s microstructure is currently being presented as a new challenge in multidisciplinary studies of foods and a way of contributing to our understanding of scientific and technological progress.

In foods from animal and plant tissues, macromolecules such as proteins, lipids, and polysaccharides and their interactions with other chemical components, including water, perform important functional roles. These chemical components constitute the fundamental structural components from which the characteristic microstructures of each food are built. For instance, within muscle foods such as Spanish “serrano” dry-cured ham, the organization of the muscle fibers, with their
myofibrils and free fat infiltration among the fibers, constitutes the microstructural basis for the characteristic texture and sensory quality that consumers appreciate.

The different stages of the processing and storage of foods cause modifications that are manifested by macroscopic changes that impart the characteristic texture, color, flavor, etc., to the finished food. The microstructure is directly affected by the chemical, biochemical, and physical changes undergone by the foods during processing, and the final properties of the elaborated foods, desirable or not, depend on their microstructure. For example, there are foods where a total microstructural change is desired and all the original plant or animal tissue organization disappears (products such as patés or vegetable purees). In other cases, a predefined microstructure is imitated to obtain processed foods such as surimi, artificially reproducing a particular tissue organization. However, the original submicroscopic structure in processed foods, where the appearance of a “fresh food” has to be retained, needs to be preserved as much as possible during the various treatments (fresh-cut fruits and vegetables, refrigerated fresh meat packed in inert atmospheres, marinated fish, and so on). It is also possible to seek an optimum microstructure, which is defined both by the arrangement of the chemical components and by the size and distribution of the gaps or air bubbles, in bubble foods such as certain emulsions and foams or in bakery products, for example. Additionally, and always depending on the temperature, the physical state of the chemical compounds also affects the final structure. For instance, the solidification of the ingredients and the even distribution of the air bubbles in ice creams are key aspects of their microstructure. The presence of microscopic foreign bodies can occasionally lead to the detection of unsuitable practices or even fraud.

However, the microstructure of the food plays an important role in many processes where transport takes place within the food: (i) saline agents and other preservatives are carried through the intercellular spaces and through the cell walls and membranes when pickling vegetables, curing hams, and salting and smoking fish, (ii) the heat transmission in thermal processes such as sterilization, blanching, freezing, etc., (iii) water/solutes move intra- and intercellularly during dehydration by hot air or by osmosis, and (iv) matter is transferred intra- and intercellularly in solid/liquid extraction of oils and fats from food tissues, etc. The establishment of mathematical transport models, which provides sufficiently accurate predictions, requires a real understanding of the mechanisms that govern them. These mechanisms are influenced by the chemical and biochemical components and their structural distribution within the food. Consequently, a better understanding of the microstructure of foods should, in turn, allow for better modeling of the processes.

Understanding the organization of chemical components is key to describing, predicting, or controlling the changes undergone by foodstuffs during processing. Conceptually, this adds a new viewpoint to the classic chemical or physical aspect of foods; microstructure is thus seen as a fundamental aspect of the combined, multidisciplinary study of food.

Nowadays, different microscopy techniques, particularly electron microscopy (scanning or SEM, transmission or TEM), offer powerful tools for clarifying the microstructure of foods and establishing its interrelationships with their physical, chemical, and biochemical behaviors. Coupled with image analysis, it also allows the quantification of the morphological changes that take place during food processing. Additionally, current X-ray detection systems (EDX, WDX) make it possible to microanalyze foods at submicroscopic levels. Above all, broad horizons have opened up in the field of food microstructure and its multidisciplinary study. It could be interesting to take research carried out in this field as a complement for solving problems and developing new products in the food industry. Furthermore, this could also be a very useful approach defining the properties of functional foods and the importance of their components for the health of the consumers.
As the aim of this chapter is to give a brief overview of these issues, certain interesting aspects concerning the microstructure of different foods of animal origin with, for example, native, modified or altered, destroyed, restructured, or imitated tissue structures will be discussed.

19.2 Main Microscopy Strategies for Studying the Structure of Muscle Foods

The invention of the light microscope (LM) at the end of the sixteenth century provided food scientists with the first tool that allowed them to observe samples magnified up to 20 or 30 times their original size. LM uses visible light as its illumination source and was used to study food microstructure despite its low resolution, which is limited due to the wavelength of the light (Figure 19.1a). Nowadays, modern LMs have a resolution (200–500 nm) that is about $10^3$ times that of the human eye and they produce an image that is magnified 4–1500 times (4–1500×) (Figure 19.2). The LM is a versatile and useful tool that works in different applications such as bright field, which is the most common application for muscle foods; phase contrast or differential interference contrast (Nomarski), where the contrast of unstained tissues is enhanced; polarizing microscopy, where a plane-polarized light is used to illuminate the sample (useful with birefringent structures); or fluorescence microscopy, which is the basis of the modern technique of confocal laser scanning microscopy (CLSM). There are different ways of preparing samples for LM observation (Figure 19.3); the only essential requirement is that the sample be translucent, in other words, sufficiently thin to allow light to pass through it when mounted in a suitable medium. Consequently, depending on the physical characteristics of the sample, different methods are available [1]. Powdered foods (normally under 200 µm) are fine enough to be observed directly, mounted in an inert oil or an appropriate dye solution. Fluid foods such as emulsions and sauces are spread

Figure 19.1 (a) Visible light, high wavelength, only interferes with big objects. (b) Accelerated electrons, short wavelength, only interfere with very small objects.
on the slide as translucent films. Lastly, solid foods are prepared in thin sections. The sections can be obtained after embedding the food in paraffin or resins, but the simplest and quickest way is to use a cryotome, placed in a freezer, to obtain cryosections of samples after having frozen their constituent water along with their other chemical constituents. In this method, the solid water acts as the embedding medium. The use of a hot stage coupled to the microscope helps to reproduce food processes that include a heating step.

In recent years, an important advance in the field of food structure has been the introduction of CLSM. In this technique, as the muscle food materials of structural interest are not autofluorescent, the sample should be stained with fluorescent dyes before examination under the microscope. Optical sectioning is one of the major advantages of this technique, since physical slicing is not necessary. This leads to the possibility of generating three-dimensional (3D) images of biological cells and tissues [2]. In CLSM, the illumination of the sample is restricted to a

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**Figure 19.2 Diagram of a light microscope (LM).**
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To prepare for slicing:
- Cold knife
- Embedding with paraffin or resin
- Freezing (CO₂, liquid N₂)

Specimen portions (slice, powder, pellet, ...)

To prepare for slicing:
- Semithin sections (0.1–2 µm)

To prepare for slicing:
- Mounting in glass slides
- Staining specimen

LM observation

Figure 19.3 Preparation of samples for LM observation.
Figure 19.4  Diagram of a confocal laser scanning microscope (CLSM).

single point (or an array of points), which is scanned to produce a complete image (Figure 19.4). Another major advantage of CLSM is the exclusion of out-of-focus blur, since the fluorescence emissions from the illuminated regions of the sample above and below the focal plane are not allowed to reach the photomultiplier and form an image.

The development of electron microscopy (EM) has allowed food structure to be studied in greater detail. The electron microscope takes advantage of the much shorter wavelength of the accelerated electron compared to light (Figure 19.1b). This makes a 1000-fold increase in magnification possible, together with a parallel increase in resolution. There are two microscopes that use electron beams as source of illumination: transmission electron microscope (TEM) and scanning electron microscope (SEM) (Figure 19.5). In both methods, the samples first need to be prepared. Both the working conditions (the electron microscope works under high vacuum) and the nature of the sample itself (in the case of muscle foods, a biological tissue) make long and occasionally tedious sample preparation protocols essential.

The TEM projects accelerated electrons through a very thin slice of specimen [3]; the transmitted electrons produce a 2D image on a phosphorescent screen (Figure 19.6). TEM gives an image with better resolutions (0.2–1 nm) and higher magnifications (200–300,000×) than LM. The steps in the preparation of the sample (Figure 19.7) are primary fixation, washing, secondary fixation, dehydration, infiltration with resin, embedding, cutting ultrathin sections (100–500 Å) in an ultramicrotome, and staining with heavy metals. The resin permeates the sample, replacing all the water within the structural components and making the sample firm enough for sections
Figure 19.5  Two basic electron microscopes. TEM: image is formed from the electrons transmitted by the electron beam. SEM: image is formed from the signal (secondary electrons, etc.) emitted by the sample scanned by the electron beam.

Figure 19.6  Diagram of a transmission electron microscope (TEM).
Figure 19.7 Preparation of samples for TEM observation.
to be cut. This technique is very useful for observing the characteristic banding of animal muscle fibers and the ultrastructure of the cells, differentiating the cell organelles.

The SEM uses a spot of electrons that scans the surface of the specimen to generate secondary electrons, which are the primary signal emitted by the specimen and form a 3D image (Figure 19.8). SEM gives resolutions (3–4 nm) and magnifications (20–100,000×) that are intermediate between those of LM and TEM. The SEM method observes the surface of the sample, so there is no need to section it. Surface and internal structures can be observed, depending on the preparation techniques used, and these are much easier than the TEM. In essence, there are two ways of preparing samples for SEM: chemical fixing and physical fixing (Figure 19.9). In the former, the sample preparation steps are chemical fixation, dehydration in a series of ethanol dilutions of increasing concentration, change to a transition fluid (acetone), critical point drying, mounting, and coating with a conducting metal or carbon. When physical fixation is used, the sample is frozen in liquid nitrogen and then freeze-dried before being mounted, coated, and observed. In recent years, considerable progress has been made in the field of SEM through vitrification techniques. In cryo-SEM, the sample is frozen in slush nitrogen and quickly transferred to a cold stage fit on a microscope where the frozen sample is coated and observed (Figure 19.10); in this way, the sample can be observed with all its constituent water.
Figure 19.9 Preparation of samples for SEM observation.
Figure 19.10 Preparation of samples for cryo-SEM observation.

Besides the secondary electrons, other emanations or signals such as X-rays and backscattered electrons may be generated as a result of the electron beam striking the specimen [3]; the different signals can be captured by the appropriate detector in each case. In this way, ions or molecules can be identified and quantified in situ using specific detectors coupled to the electron microscope.
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19.3 The Muscle Structure of Meat

The muscle structure of meat is similar in all species. Although meat includes a number of different tissues, the majority is skeletal muscle tissue. The muscle mass is elongated and covered by a connective tissue termed the epimysium which binds both individual fiber bundles and groups of muscle bundles together [5]. It tapers into a tendon that connects the muscle to the skeletal structure. The perimysium forms partitions within the muscle at irregular intervals to form both primary and secondary muscle bundles. Within the perimysium, the sarcolemma or cell membrane retains the sarcoplasmic fluids, which bathe each muscle fiber. The muscle fiber is surrounded by a connective tissue called the endomysium, and inside the cells there are numerous myofibrils (Figure 19.12).

The contractile unit of the myofibril is known as sarcomere. The dark band is called the A-band, the light band is called the I-band, and the dark line bisecting the light band is called the Z-disk. The sarcomere is the distance from one Z-disk to the next (1.5–2.5 µm in length, depending on the state of contraction) and is made up of two sets of interdigitating filaments: thin and thick. The thin filaments are predominantly composed of actin molecules, wrapped around one another, and of tropomyosin and troponin, connected to the Z-disk; the thick filaments are composed of densely packed myosin proteins, running from the center of the sarcomere toward the Z-disk (Figure 19.12). The myofibrils are linked to the sarcolemma by filamentous structures called costameres; the protein constituents of the costameres (desmin, actin, vinculin, talin, etc.) extend into the muscle cell where they encircle the myofibrils at the Z-disk and run from myofibril to myofibril and from myofibril to sarcolemma [6].

Figure 19.11 Different signals emitted by the sample when scanned by the electron beam.
Figure 19.12  Schematic representation of the skeletal muscle. (a) A transverse section of the muscle bundles and the connective tissues: epimysium, perimysium, and endomysium. (b) Longitudinal muscle fiber composed of myofibrils, which are composed of groups of myofilaments and surrounded by the sarcolemma. (c) Muscle proteins organized into ultrastructural features or sarcomeres. Costameres are protein filaments anchored in the Z-disk and run from myofibril to myofibril and from myofibril to sarcolemma. (From Lluch et al., Chemical and Functional Properties of Food Proteins, Technomic, Lancaster, PA, 2001. With permission.)
19.4 Influence of Postmortem Processing on Meat Microstructure

Different microscopy techniques have been used to characterize meats and to analyze the changes in muscle tissue that take place under different postmortem storage or cooking conditions. Some authors [7,8] have observed the microstructure of rabbit semimembranosus muscles by LM. They found that the muscle tissue of the dead animal presented a type of structure in which the myofibrils were perfectly packed and the intercellular connections of the connective tissue remained unchanged; as the postmortem progressed, the structure of the muscle fibers and endomysial connective tissue gradually broke down. Transverse sections of rabbit semimembranosus muscle showed the typical muscle tissue structure (Figure 19.13a); at 24 h postmortem, gaps caused by the destruction of the perimysium were observed in the muscle bundles in some zones and the fibers inside each cell bundle appeared fairly separate due to degradation of the endomysial connective tissue (Figure 19.13b).

SEM was employed by Palka [9] to study the influence of postmortem aging of bovine semitendinosus muscle. The structure of the intramuscular connective tissue and myofibrillar structure of the meat after five days of aging was regular. In 12-day-old samples, the fibrous and myofibrillar structures were less distinct, damage appeared in the endomysium tubes, and the fibers of the perimysium were swollen.

CLSM was used by Straadt et al. [10] to study aging-induced changes in microstructure and water distribution in fresh pork. At day 1 (24 h postmortem) a few muscle fibers appeared to be swollen.

Larrea et al. [11,12] have used several techniques to observe muscle in pork meat. No structural differences were observed between the biceps femoris and semimembranosus muscle tissues. A cross section of the semimembranosus muscle from a sample of pork meat observed by SEM shows the perimysial connective tissue that separates the bundles of fibers (Figure 19.14a). The cells are surrounded by endomysial connective tissue, a reticular structure primarily composed of collagen, which keeps the muscle fibers firmly attached to one another. This technique provides a clearer picture of the myofibrils inside the cells, where they are strongly attached to one another and to the sarcolemma (Figure 19.14b).

![Image](a)
![Image](b)

**Figure 19.13** Transverse sections of rabbit meat at 1 h postmortem (a) and 24 h postmortem (b) observed by light microscopy (LM); e: endomysial connective tissue; p: perimysial connective tissue. (From Sotelo, I., Pérez-Munuera, I., Quiles, A., Hernando, I., Larrea, V., and Lluch, M.A., *Meat Sci.*, 66, 823, 2004. With permission.)
When ultra-thin sections of pork muscle tissue are studied by TEM, it is possible to observe ultrastructural details that pass unnoticed with other methods. Figure 19.14c shows the inside of a muscle fiber with perfectly bundled myofibrils. In this figure, several mitochondria can be observed between the myofibrils. Figure 19.14d shows a more detailed view of a number of myofibrils; it is even possible to distinguish their component sarcomere filaments.

The microstructure of broiler chicken muscles was studied by Wattanachant et al. [13] using SEM. The thickness of the perimysium directly contributes to the texture of raw chicken muscles, whereas the cross-linked collagen content does not.

The amount of fat in different meats and muscles depends on a wide range of factors. Fat is located in the adipocytes, in the adipose tissue. Figures 19.15a and 19.15b show the intramuscular fat of pork meat observed by SEM and cryo-SEM [11]. As observed by SEM, it is made up of spherical adipocytes closely arranged and surrounded by a membrane, with perimysial connective tissue fibers among them.

Nishimura et al. [14] used SEM to investigate changes in the structures and mechanical properties of intramuscular connective tissue during the fattening of steers. They observed that the...
development of adipose tissue in longissimus muscle appears to disorganize the structure of the intramuscular connective tissue and contributes to tenderization of highly marbled beef during the late fattening period.

19.5 Influence of Processing on Meat Microstructure

The effect of cooking on different types of meat has been studied by several authors [9]. Roasting to an internal temperature of 50°C slightly affected the structure of bovine meat. During roasting to an internal temperature of 60–90°C, significant changes occurred both in the myofibrils and in the intramuscular connective tissue. The degree of postmortem aging of meat had a significant effect on the thermal stability of tissue structures. The changes in myofibril structure during roasting were considerably smaller in meat aged for 5 days than in that aged for 12 days postmortem.

Using SEM, Wattanachant et al. [13] have studied the effect on broiler muscle fibers after cooking (80°C/10 min). The broiler muscle fibers shrank more in a parallel than in a transverse direction to the fiber axis and expanded transversally after cooking, resulting in increasing muscle tenderness.

Sorheim et al. [15] have studied the effects of carbon dioxide on the microstructure of cooked beef by LM. Beef meat, previously stored in an environment containing 20–100% CO₂, had higher cooking losses compared to meat stored in 100% N₂ and under vacuum. Decreases in the pH of raw meat and the formation of small pores and microstructural changes upon heating due to CO₂ exposure were likely to cause the increased cooking losses. During cooking, structural changes in the meat protein matrix showed an increase in water loss due to pores and gaps in the matrix created by heat denaturation of the myofibrillar proteins and collagen.

Ueno et al. [16] have studied the effect of high-pressure treatments on intramuscular connective tissue from beef using SEM; it seems that high pressure may have a different effect from aging on the intramuscular connective tissue membrane structure. Structural changes in the endomysium and perimysium occurred and disruption of the honeycomb-like structure of the endomysium was observed.
19.6 Microstructure of Model Systems Based in Meat Components

In recent years, a huge number of works have focused on the effects of using different ingredients in meat product processing, employing model systems. These lines of research help to elucidate the interactions between the ingredients and the main meat components.

Iwasaki et al. [17] have studied the effect of hydrostatic pressure pretreatment on thermal gelation of chicken myofibrils using SEM and TEM. The structure of the myofibrils observed by SEM was disrupted at 200 MPa and the myofilaments were dispersed. The myofibril debris was observed at 300 MPa and the aggregated structures were observed in the debris periodically. The dispersed myofilaments became short at 300 MPa. The structures and characteristics of pressure/heat-induced gels of chicken myofibrils were investigated by TEM. The M-line and the Z-line in the chicken myofibril in the 0.2 M NaCl treatment were seen to be disrupted, and both the thin and the thick filaments were dissociated by the pressure treatment. The microstructure of pressure/heat-induced chicken myofibrillar gel was composed of a 3D matrix of fine strands.

Chen et al. [18] investigated the microstructure of salt-soluble meat protein (SSMP) and flaxseed gum (FG) mixtures by using SEM. The addition of FG changes the microstructure of SSMP gels. SEM observations showed that an interaction between FG and SSMP might occur. The results of adding a destabilizer to SSMP gels indicated that electrostatic forces seemed to be the primary force involved in the formation and stability of a protein-polysaccharide gel. The structure of SSMP gels showed a granular aggregated structure of large open spaces within the matrix, which seemed to show less linkage and be somewhat discontinuous between protein strands. The structure of SSMP–FG gels showed a well-structured matrix with a highly interconnected network of strands that may cause more resistance to applied stress and impart great water-holding capacity. These microstructural changes helped to explain functionality differences among the gels. A fine, uniform structure with numerous small pores would probably result in a higher absorption capacity and better retention of fat and water compared to a coarse structure with large pores.

References