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AN ANALYSIS OF MICROSTRUCTURAL FACTORS WHICH INFLUENCE THE USE OF MUSCLE AS A FOOD

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Abstract

Study of structure of muscle provides information on the location and arrangement of various components and the changes which may be inflicted upon them. The structural features of muscle have been described in detail down to the molecular level, but regarding its use as a food, special interest centers on the connective tissue component and on myofibrillar proteins. Muscle comprises about 1/3 of the live weight of the animal and is not static, but rather is subject to major changes in properties associated with growth, repair and senescence. It is apparent that the nervous and hormonal systems exert a strong influence on the properties of muscle and great potential exists for regulation of muscle via control of these systems. The microstructure of emulsions has been described with interest concentrated on the protein membrane surrounding the lipid globules, but as of yet, utilization of this information by the industry has not occurred. The challenge for the future is to utilize the enormous amount of structural information known about muscle to improve its use as a food.

Introduction

Knowledge of structure is an obvious prerequisite to successful study of or research on muscle. It follows then that almost any organized consideration of muscle has at least one presentation dealing with general structural factors. This discussion of meat microstructure is no exception. The exception, however, is that we do not intend to repeat what can be found in detail in numerous textbooks and countless reviews. Our objective is to analyze what is known and discuss how the information might be used to improve the properties of fresh meat and its use as a processed product.

Study of structure results in knowledge about (1) location and arrangement of various components and (2) changes in the location and arrangement as a result of some procedure. While such descriptive information is relatively easy to obtain, the key is to devise a means of utilizing it to improve something. Lewis (1981) has discussed this and pointed out that morphological results should have application and lead to control of manufacturing conditions.

Structural Features

Even though skeletal muscle by sheer volume alone is the overwhelming source of meat, the other types (cardiac and smooth) may be present in or incorporated into meat. Cardiac muscle, for example, may influence color because of its relatively high content of cytochrome c (Lozano and Cassens, 1984). Structural features of the three types of muscle together with information about protein composition, role of membrane components, neuromuscular function, contraction, etc., are readily available (Bourne, 1972; Cassens, 1984; Weiss and Greep, 1977). For our purpose here, however, only two figures will be used. Figure 1 is a diagrammatic cross-section of skeletal muscle. Most apparent is the highly organized connective tissue. An individual muscle is encased within a layer of connective tissue known as the epimysium. The muscle is divided into segments or groups of muscle fibers known as bundles by a layer of organization of connective tissue termed the perimysium. And, finally, each individual muscle fiber is surrounded by a thin...
layer of connective tissue known as the endomysium. The connective tissue serves as a scaffold to hold the muscle in place and as a pathway for the circulatory and nervous systems to distribute themselves throughout the muscle, eventually making contact with each individual cell. While the connective tissue does a marvelous job of organizing the muscle and keeping components in place, it also presents a formidable barrier to breaking up the muscle or to selectively extracting specific components from it. Note should be made that intramuscular fat cells are also embedded in connective tissue (i.e., perimysial planes), and lipid also occurs intracellularly as a component of membranes or as droplets of free lipid.

Figure 2 presents in diagrammatic form the spectrum of structure from whole muscle to the ultrastructural level. The "bellied" shape of the whole muscle is the simplest example and is termed fusiform. The muscle is composed of individual muscle fibers which vary in size and appear striated when viewed with the light microscope. They are multinucleate with the nuclei being placed in a subsarcolemmal or peripheral location. Each muscle fiber is fitted with a motor end plate giving it communication with the central nervous system which signals the fiber to contract. The sarcoplasm or non-structured portion of the cell is composed primarily of soluble proteins (myoglobin and enzymes functioning in metabolism) and contains organelles such as mitochondria, and inclusions such as glycogen. The major components of the fibers are the subunits termed myofibrils. These are in register giving rise to the striated appearance and are, in fact, the actual contractile units of muscle. The myofibrils are in turn composed of smaller subunits shown as thick and thin filaments. The substructure of the filaments is known — they are built up of protein molecules which have been relatively well characterized. In brief, an enormous amount of information is now known about the structure of the cell. We are thus aware of the structure to the molecular level. Therefore, new information appears slowly and most often is a refinement which allows further conclusion regarding the mechanisms of function.

Biological Properties

When muscle is considered as meat, the role of the live animal is all too often overlooked. It is well to recall that simple management practices (genetics, nutrition) can influence the amount and properties of muscle produced by the animal. From a biological viewpoint, the function of skeletal muscle is contraction which translates into movement of the animal and the associated advantages. Muscle comprises about 1/3 of the live weight of a typical meat animal and, therefore, from the simple aspect of mass alone, is an important metabolic component. Finally, muscle gives shape to the animal. It is suggested that the serious student of muscle structure must devote some time to gross anatomy (see, for example, Getty, 1975; Swatland, 1984) not only because of the above points but also because different muscles are used for very different functions and, therefore, have very different properties. For example, Kaufman and Safanie (1967) determined looseness of the fascicular organization and found that it paralleled fat content of the muscle. In high fat content muscles, the fascicular organization was distinct and orderly with highly separated large fasciculi.

Active Processes

Muscle is not a static entity. It grows and matures and finally enters a stage of senescence. If damaged or injured, it may undergo some degree of repair. This also is another area in which an enormous amount of information is available, and the meat technologist should have an awareness of the processes in order to better understand the tissue (meat) with which he or she works.

Embryonic development of muscle occurs when mononucleated myoblasts fuse to form multinucleated myotubes. These myotubes accumulate muscle-cell-specific proteins, show the characteristic cross-banded pattern, are innervated and have contractile activity. At a given time, the number of myotubes is stabilized, they begin to grow, and the centrally placed nuclei migrate to the periphery of the cell. Information indicates that the number of muscle fibers is essentially constant and that growth of the muscle occurs from then on by hypertrophy rather than by hyperplasia. Depending on the species and the muscle, the fiber also begins to differentiate and display the characteristics of fiber type during fetal development. During early development of the animal, the fiber type proportions change until an adult condition is approximated (Suzuki and Cassens 1980, 1983). Aside from an increase in mass of muscle by hypertrophy, the other general change as the animal reaches adulthood is compositional in that the amount of fat increases. See Swartland (1984) for a complete account of muscle fiber differentiation and growth and development of the animal.

Special mention is made of the concept of fiber type and the reader is referred to several early reviews on the topic (Needham, 1926; Denny-Brown, 1929; Cassens and Cooper, 1971). The important point is that muscle fibers are not homogeneous but differ greatly in properties. In the most general sense, these are red and white and the proportions present in a given muscle are responsible not only for the gross appearance (i.e., red or white) of the muscle but also for its function. In mammalian muscle, both types are twitch while the white are physiologically faster than the red. The red are designed for more aerobic type metabolism having more myoglobin, more lipid, and less glycogen than the white.

Aging is a progressive development in the muscle of living animals which is recognized first as a loss of strength and endurance. In humans and various laboratory animals, senile muscular atrophy is well recognized and the degenerative changes accompanying it have been described.
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Figure 1: A diagrammatic cross-section of muscle which illustrates the arrangement of connective tissue (taken from Ham, 1965).

Figure 2: Diagrammatic representation of the levels of organization in muscle. (a) the entire muscle, (b) muscle fibers showing different sizes and innervation, (c) single fiber showing myofibrils and location of nuclei and mitochondria, (d) striation pattern of a myofibril, (e) a single sarcomere, and (f) the arrangement of thick and thin myofilaments (taken from Huxley, 1958).

It is recognized that the nervous and hormonal systems exert a strong regulatory influence on muscle and must be intimately involved in the growth and senescence processes just discussed. It is further recognized that muscle has the ability to change or to adapt to its environment or to the demands placed on it by actually changing its properties.

The work of Buller et al. (1960) proved that the properties of muscle are under neural control. Their classical cross-reinnervation studies demonstrated that when nerves to red and white muscles are surgically severed and reimplanted on the muscle of opposite properties, the properties of the muscle change to those of the nerve. Other recent observations have given evidence for the strong role that the thyroid hormones have in influencing the expressed properties of skeletal muscle. Ianuzzo, et al. (1977) reported that thyroid hormone caused a slow-twitch muscle to become more fast-twitch.

Cassens et al. (1983) have discussed how the above information can be used to regulate properties of muscle to improve its utilization as either fresh or processed meat. The concept of using regulation in the live animal to produce muscle with specific properties is an area deserving increased research effort.

Post-mortem Factors

The conversion of muscle to meat occurs post-mortem and the changes associated with rigor mortis have been described (Bendall, 1973). From a structural viewpoint, most emphasis has been placed on describing changes in morphology associated with rigor mortis. For example, Greaser et al. (1969), published detailed work on subcellular fractions from normal and pale, soft exudative porcine muscle prepared at various times post-mortem. Myofibrils from the PSE samples at 24 hr post-mortem had more granular appearing filaments and wider Z lines than normal muscle. Several other structural differences were observed.

A significant finding arose from the observation that the Z-line was degraded in aging of some muscle; the responsible enzyme known as CAF was isolated (Dayton et al., 1976).

At present, considerable emphasis is being focused on the so-called cytoskeleton of muscle. It is thought that the protein connectin, in some way, attaches A filaments of adjacent sarcomeres while the protein desmin plays a role in transverse linking at the level of the Z-line. The pertinent information as it affects properties of
meat is reviewed by Locker (1982) in a complete account of his theory of tenderness based on gap filaments.

It seems obvious, although there is no relevant hard evidence to cite, that during post-mortem change, permeability characteristics of the cells are drastically altered. This could be an important aspect for better understanding of conversion of muscle to meat and one where careful morphological investigation could produce needed and useful information.

**Fresh Meat**

Early morphological investigations of fresh meat were limited largely to measurements of fiber dimensions. This work was, for the most part, correlated with growth of the animal and little attention was paid to association with meat. However, as pointed out by Stanley and Swatland (1976), variations in microstructure of muscle can cause rather large changes in rheological properties. The goal of the morphologist working on fresh meat should be to identify such. The observation by Locker (1960) that contraction state of muscle affected tenderness ushered in a new era. Numerous investigations followed in which sarcomere length was shown to be associated with tenderness of the meat. The objective of our present work is to describe in a more refined way this association especially as related to aging (see previous section).

Major reviews (Voyle, 1979, 1981; Howgate, 1979) have been written which relate structural characteristics of muscle to its properties as food. Other than the already described structural changes occurring post-mortem and the association of contraction state with tenderness, it should be mentioned that surface texture is a reflection of the bundle arrangement. Color is due in part to the proportion of fiber types.

**Processing**

Another area of morphological study has been the description of the effect of various processing procedures on meat. Birkner and Auerbach (1960) reported that during heating, collagenous fibers swelled, shrank, and then disintegrated, and that fat translocated. Classical work on the morphology of muscle during freezing has been published by Rapatz and Luyet (1959). They described several ice patterns which occur in frozen muscle and cited evidence that the cell favors development of longitudinal spears rather than lateral growth of ice.

Morphological study of processed meats has concentrated mainly on emulsion type products and two early papers are mentioned as being significant. Hansen (1960) demonstrated that a protein membrane surrounded the lipid globules in meat emulsions and Borchert et al. (1967) used electron microscopy to confirm the Hansen finding and also to reveal the extremely small lipid droplets present in meat emulsions (down to 0.1 μm diameter). Since then, numerous papers have appeared which have attempted to relate this emulsion structure to properties of the meat. Very recently, Kempton and Trupp (1983) have used a very refined image analysis system to study morphology in wiener batters. They found no relationship of any feature of microstructure to firmness of the product.

Two areas for study deserve comment. The first is compartmentalization. Structural (and histochemical) studies reveal where components (such as fat) are concentrated and located. The investigator should be constantly aware of this situation as it may influence the procedure for preparation of a meat product and also how it might influence properties and storage life of the finished product. The second area of interest is interfaces. (Cassens et al., 1979) in which polar and non-polar groups are aligned. These are present in muscle (where lipid of a fat cell contacts the surrounding sarcoplasmic protein, for example) or may be found in processed meats (where lipid globules contact surrounding protein membrane), and they may influence chemical reactions.

**Specific Identifications**

One final aspect of morphology is identification. The trained person may distinguish the different types of muscle in a product purely on the basis of structural characteristics. Similarly, components such as connective tissue, organs, glands or foreign bodies can be identified. Staining techniques such as for connective tissue or the mucopolysaccharides of salivary glands can be used to increase sensitivity and give very specific identification. Such identification procedures have the greatest application in regulation and do lend themselves to semi-quantitation. European scientists have been especially active in this area of morphology (Prandl, 1961).

The rapidly emerging immunological methods offer great potential for specific identification. Species identification by immunology has been available for some years and enzyme-linked-immunosorbent-assay (ELISA) procedures now offer precision and automation. When antibodies are coupled to markers, they can be used microscopically to relate presence to specific location. For example, antibody to myosin heavy chains from red and white muscle may be used to distinguish fiber types (Carpenter et al., 1984).

**Conclusions**

An enormous amount of detail is known about the structure of muscle. Major morphological accomplishments have been made regarding muscle as a food. These are (1) relating state of contraction to tenderness, (2) characterizing aging associated morphological changes with properties of the meat, and (3) description of the structure of meat "emulsions". Significant work is ongoing in the area of relating degradation of muscle proteins to properties of meat. The challenge to significant progress in the future is substantial but the rewards are likewise major. The era of description without utilization of the information is drawing to a close. The most likely opportunities lie in...
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regulating biological processes to produce custom made meat, in devising morphological control procedures for manufacturing processes and in utilizing the now available immunological procedures.

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References


Discussion with Reviewers

J. G. Sebranek: When one considers various equipment designs for meat comminution, claims are often made of improvement in particle definition, better visual differentiation between fat and lean, etc., in one system vs. another. Is a microstructural difference likely to result from use of different types of equipment for relatively coarse products like salami or summer sausage?

Authors: We cannot visualize that there would be any microstructural difference in such large chunks unless a "cleaner" cut were actually made at the surface by sharper cutting edges.

J. G. Sebranek: How important is the microstructural arrangement at the interfaces of sectioned and formed or restructured products for particle adhesion or binding strength?

Authors: Theno et al. (J. Food Sci. 1978, 43, 392-400) showed that in the emulsion-like area which binds pieces of meat together in these products, optimal binding was associated with a high degree of junction alignment. It should be noted that these interfaces in sectioned formed products are not the same as the interface between polar and non-polar groups as described in the body of our text.

D. W. Stanley: Have the authors any thoughts on the relatively recent findings that attribute a major role in meat quality to the state of water, and has consideration been given to a possible role for a muscle cell cytoskeleton as a microstructural factor?

Authors: Because of the large amount of water present in meat and because of its effect on physical properties due to hydration, etc., we are not surprised that it plays a major role in determining meat quality. Work on the so-called cytoskeleton is progressing rapidly now and as more is learned about the basic properties, then undoubtedly, further explanation will be forthcoming regarding the cause of various meat quality attributes. Reference is made to the discussion of gap filaments by Locker in this Food Microstructure volume.

C. A. Voyle: Is it possible to monitor the effect of biological processes in producing "custom made meat" by analysis of biopsy samples? What parameters, in addition to fiber size, should be determined? Is there a preferred sampling site which would provide an index of the whole?

D. F. Lewis: Do you envisage meat manufacturers using biopsy techniques on every cut of meat they process?

Authors: The biopsy technique can be used to follow changes occurring in muscle of live animals over time but it is not without its problems. The surgical procedure may influence the animal and thereby subsequent results. Sampling site is a major problem. No one muscle reflects the properties of the total muscle mass of the animal. Also a given muscle may not be large enough to allow serial biopsies required to plot a change. Fiber typing, probably with the APHase method, is in our opinion the best way to determine changes which occur in the muscle.

H. J. Swatland: It has recently been suggested that the epimysium and perimysium are more or less mechanically independent of the endomysium. (Moore, M. J., (1983). Muscle and Nerve 6:416-422). What are the authors' opinions on this idea?

Authors: We have no argument with this idea. We would point out a reference by Ramsey and Street (1940). J. Cell. Comp. Physiol. 15:11-34) wherein single muscle fibers, the resting length tension relationship is essentially identical whether the fiber is left intact or injured in such a way as to disrupt the contractile proteins but not the sarcolemma and remnants of remaining connective tissue. This would be proof that resting tension of the muscle fibers is not due to the contractile component but rather to the sarcolemma and remnants of remaining connective tissue.

H. J. Swatland: Much of the early literature on the effect of cooking on meat structure was obtained by the examination of paraffin embedded sections which sometimes introduced drastic changes to the microstructure, such as the shrinking of fibers during hot-wax embedding. Are the classical descriptions of Birkner and Auerbach (1960) compatible with results obtained by the more recent methods of EM fixation and frozen sectioning?

Authors: The early work on changes in fiber size due to heating, must be viewed with some question for the reason you suggest. The observations on changes in connective tissue are in our opinion still correct. Careful use of frozen sections reveals much more information reflective of the in vivo state and electron microscopy, obviously, gives much more structural detail.

D. F. Lewis: You indicate that there is a vast amount of knowledge available on the structure of muscle and imply that the responsibility for applying this knowledge lies with the meat technologist. To what extent do you think the microscopist should act as a bridge between the animal scientist and the meat technologist?
Can you suggest how a meat technologist might modify his processes to cope with:

i) Meat with a high red fibre content?

ii) Meat with a high white fibre content?

iii) Meat from an animal in which a state of muscular senile atrophy has developed?

iv) Meat from an animal which has recovered from muscle injuries?

Authors: The microscopist can be most effective if he is aware of the influence the live animal exerts on the sample of muscle to be examined and if he maintains an awareness of the biochemistry of muscle. We believe progress is much easier with an integrated approach.

D. F. Lewis: Attempts to prove a concrete link between fibre type and meat behaviour on processing have been largely inconclusive. How would you suggest that such a link could be established?

Authors: Sair et al. (J. Food Sci. 1972, 37, 659-663) demonstrated that muscle from stress-susceptible pigs have large numbers of intermediate and white fibers compared to normal animals. It is well recognized that the PSE (pale, soft, exudative) muscle from the stress-susceptible animals is undesirable for manufacture into hams or processed meats.

D. F. Lewis: What effect does growth by hypertrophy rather than hyperplasia have on meat performance? Is it alterable?

Authors: See answer above -- muscle from stress-susceptible animals generally has hypertrophied fibers.

D. F. Lewis: Does the fact that nuclei migrate from the centre to the edge of the cell during embryonic development of muscle hold any practical significance to the behavior of meat on processing?

Authors: None that we know of at this time.

D. F. Lewis: Could you give practical details of your myosin antibody technique including availability of materials and applicability to processed meats?

Authors: The method is published in complete detail in the Proceedings of the 30th European Meeting of Meat Research Workers, Bristol, U.K. (see Carpenter et al., 1984). With proper specimen fixation and appropriate antibody labelling, we see no reason why myosin heavy chain could not be immunohistochemically localized at the light or electron microscope level in a processed meat product.

D. F. Lewis: It is possible that changes in the permeability of the cell membrane will affect distribution of salts in meat. Can you suggest ways in which the membrane permeability changes may be controlled? Which do you think has the greater effect of salt distribution -- the state of the connective tissue or the cell membranes?

Authors: We cannot suggest means to control membrane permeability. Several years ago we noticed during work on staining of myoglobin in skeletal muscle that if fixation did not occur immediately post-mortem, the differential localization of myoglobin in red and white fibers was lost. We interpreted this as meaning that cell permeability changed rapidly allowing the soluble myoglobin to diffuse. If this is in fact the case, then the connective tissue may present a more effective barrier to diffusion of salt than does the cell membrane.

Editor: Following references were suggested by reviewers as relevant to the subject of this paper:


