

Environmental Scanning Electron Microscopy of Raw and Heated Veal Semimembranosus Muscle

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ABSTRACT

The environmental scanning electron microscopy (ESEM) is a new development in the field of electron microscopy. In this study ESEM has been used to study the structure of veal semimembranosus muscle. Four treatments, raw (control), conventional heating, domestic and industrial microwave heating, were observed using ESEM. The temperature used in conventional heating was 163 °C. Frequency applied for microwave heating was 2450 MHz with two wattage levels of 700 (domestic microwave) and 12000 (industrial microwave). All samples were heated to 70 °C internal temperature. Occasional cracks across the individual muscle fiber and shrinkage were present in all images of the structure for heated muscle. Erosion at the edges of muscle fibers was clear and increased with continued heating. A gap between perimysium of each muscle bundle was effected with the domestic microwave samples in cross section. This phenomenon was more apparent in industrial microwave heating and the depth of gap between perimysial collagenous fiber is more than that in domestic microwave heating. More damage was observed in the connective tissue network for conventional heating as compared with microwave heating. Denaturation and distortion of connective tissue caused more damage during longer time of conventional heating. Surface damage in structure of semimembranosus was not observed in either conventional heating or the low powered microwave heating. Rapid increase of heat and penetration of the microwaves, at the 12000 wattage level, caused granulation and separation of some parts of the muscle fibers.

Keywords: Environmental scanning electron microscopy, Veal, Semimembranosus Muscle, Microwave heating, Conventional heating.

INTRODUCTION

Environmental Scanning Electron Microscopy (ESEM) has been used for the examination of living and fresh botanical samples (Danilatos, 1981). Fungal mycelium and cross sections of stems from different plant sources have also been represented. Danilatos and Postle (1982) have studied common application of biological specimens. Many researchers have reported the use of ESEM in hydrated biological samples (Wallace *et al.* 1992; Klose *et al.*, 1992). Only a small amount of research has been

undertaken comparing unprocessed ESEM specimens and samples prepared by conventional methods (Danilatos, 1981; O'Brien *et al.* 1992). Schaller and Powrie (1972) applied Conventional Scanning Electron Microscopy in the study of muscle in beef structure. Much research has been done on the study of wool fiber. Investigations in many parts of this process can be largely enhanced using ESEM. Early results in this area of research have been briefly reported elsewhere by Danilatos and Brooks (1985).

Preliminary conclusions on a variety of novel applications made by ESEM, have

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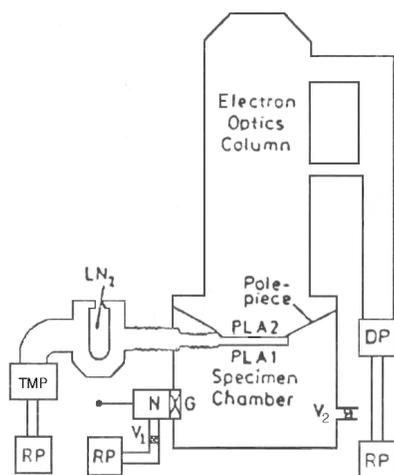


Figure 1: Diagrammatic representation of a two-stage differential pumping system for an ESEM. PLA1 = first pressure limiting aperture, PLA2 = second pressure limiting aperture, RP = rotary pump, DP = diffusion pump, TMP = turbomolecular pump, LN2 = liquid nitrogen trap (cryopump), N = airlock (specimen exchange chamber), V₁, V₂, G = valves. Source: Danilatos (1991).

been noted by Bolon *et al.* (1989). Application of ESEM has been demonstrated by Baumgarten (1990) and Peters (1990). In this study ESEM has been applied to study the microstructure of veal semimembranosus muscle, using raw muscle as a control and comparing it with different heat treatments including conventional and microwave heating. The preference of ESEM usage technique has been indicated properly in the explanation of its mechanism.

ESEM has been known as one of the most interesting new developments in the field of electron microscopy. The environmental scanning electron microscopy (ESEM) is capable of examining specimens in a gaseous environment, saturated with water vapour while higher resolution micrographs have been illustrated in the presence of gas (Danilatos, 1989). The environmental scanning microscopy has been described as a scanning electron microscopy technique to retain a minimum water vapor pressure of at least 609 pa in the chamber specimen (Dani-

latos, 1991). ESEM creates the possibility of testing practically any sample which is wet (Danilatos, 1991). The difference between ESEM and conventional SEM is in its capacity to examine materials consisting of liquids and oils in their natural state without any initial preparation for the samples (Uwins, 1994). A diagram of an ESEM is shown in Fig 1.

The two main parts of the instrument include an electron gun chamber or electron optics column and specimen chamber. The gun chamber is located at the top part of instrument and provides a flow of electrons through heating a tungsten filament, lanthanum hexaboride filament or applying a field emission source. The specimen chamber is capable of working at a very low vacuum unlike any other type of ESEM which requires high vacuum. It is based on an integration of efficient differential pumping with a secondary electron detector designed to operate at low vacuum. The specimen will not dry and therefore ESEM is used to observe specimen in the fresh state (Uwins, 1994). The high-vacuum gun chamber and the low-vacuum sample chamber are separated by two pressure limiting apertures (PLA). The “gaseous” SE detector (Danilatos, 1981) collects the low energy SE (and ions) with biased wires or plates. In the electroscan ESEM an environmental secondary electron (SE) detector is integrated with the lower PLA immediately above the sample in order to maximize collection efficiency and minimize working distance. Some important advantages have been shown for the gas around the samples in the specimen chamber. Accumulation of charge on insulating samples can be recognised as a basic advantage for this technique. This phenomenon occurs by ionization of the gases inside the specimen chamber and nowadays much work has been done on it (Pfefferkorn *et al.*, 1972; Parsons *et al.*, 1974; Moncrieff *et al.*, 1978; Crawford, 1979). Danilatos (1983, 1986, 1990) discovered that the gas itself can be employed as a detector in the microscope system which is another advantage for ESEM.

MATERIALS AND METHODS

Semimembranosus (SM) muscle was removed from veal at room temperature. Thirty samples were used for each treatment. Oven model Glendale fan forced was used for conventional heating (roasting) of muscle. An oven temperature of 163°C with an internal meat temperature of 70°C was used for conventional heating. Domestic and industrial microwave heating was applied with a frequency of 2450 MHz. The wattages for domestic and industrial microwave were 700 and 12000 W respectively. In all heat treatments the internal temperature was regulated to 70 °C. For the study of microstructure of muscle, small samples with dimensions of 2×3×3 mm were taken from SM muscle. ESEM model E-3 was used.

RESULTS AND DISCUSSION

Structure of veal SM muscle was studied using Environmental Scanning Electron Microscopy. Micrograph shows, in raw veal, individual muscle fibers are located parallel to each other (Fig 2a and 2b). The diameter of muscle fibers is low in veal which probably causes a less shrinkage during preparation. The diameter of muscle fibers is shown in the micrographs.

Images for conventional method (roasting) are illustrated. Conventional heating causes more disruption and breakdown in the muscle fibers due to subtle tissue. Occasional cracks across fibers as mentioned by Paul (1963) were seen in our samples of cooked fibers, although some tissue appears to be stretched across the crack indicating incomplete separation. Thin muscle fiber in veal muscle structure, makes it more sensitive to heat and large parts of muscle fiber separate during heating (Fig 2c). This is also shown in cross section images in Fig 2d. The damage is as a result of disruption of connective tissue during the thermal process. A number of these muscle fibers have been located in different bundles. Microwave heating was applied for study of veal SM muscle.

Shrinkage of individual muscle fiber is apparent in Fig 2e in cross section view of domestic microwave heated veal (700 W), and also division of perimysial from connective tissue is clear in this sample.

Bundles of muscle fibers are located inside the perimysial collagenous tissue. A gap also appeared after heat treatment (Fig 2f). This is probably caused by stretching force resulting from the domestic microwave heating. During industrial microwave heating, the depth of gap between perimysial connective tissue is more than that in domestic microwave heating. This phenomenon is increased in industrial heating and the depth of gap in perimysial area is much clearer.

Industrial heating altered general configuration of muscle fiber in parts of the structure. This is clear in high magnification of veal structure in Fig 3a. Bending of individual muscle fiber and also separation and granulation of some parts of muscle structure, affected by high electromagnetic field, is clearly shown in this micrograph.

The erosion at the edges of muscle fibers was reported by Doty and Pierce (1961) to increase with continued heating. Such erosion can be seen in Fig 3b. In another image of this treatment (Fig 3c), collagen network which surrounds muscle fiber was observed. Connective tissue is visualized in microwaves micrographs at both levels which seems that connective tissue has suffered less damage as compared with conventional heating. Hui (1992) also agreed with this result and believed that since the rise of temperature in microwave heating occurs very rapidly, there is not adequate time for connective tissue to be broken. He also indicated that the rapid rise of temperature will prohibit the aging of individual muscle fibers. In conventional heating images, connective tissue was not observed.

As shows in Fig 3d, transverse fracture of myofibrils was seen in cooked fibers and this is in agreement with Paul (1963) who reported the same result in his study. This phenomenon is increased in industrial heating and the depth of gap in perimysial connective tissue is more clear. Perimysial



Figure 2. Environmental scanning electron microscopy of veal semimembranosus (SM) muscle. (a) Low thickness of raw muscle fibers appeared. Mf: muscle fiber. (b) Another presentation of raw veal. Arrows pointing to the muscle fibers. (c) Conventional heating of veal. Fragmentation of muscle fibers has been shown by arrows, Mf: muscle fiber. (d) Conventional heating of veal in cross section. Separation of some parts of muscle fiber has been illustrated. (e) Domestic microwave heating of veal. Shrinkage of individual. (f) Another micrograph of domestic microwave heating of veal. Endomysial (e) and perimysial (p) connective tissues have been presented in transverse view of muscle structure.

connective tissue is also apparent inside the gap. The myofiber surfaces do not appear to be normal presenting little evidence of tissue damage in microwave heating particularly in 12000 wattage levels.

Further application of the ESEM illustrated that domestic microwave caused less physical damage to the connective tissue and myofiber elements as compared to the conventional technique. Generally it is difficult to say that microwave heating of veal SM

Figure 3. Environmental scanning electron microscopy of veal semimembranosus (SM) muscle. (a) Industrial microwave heating of veal in longitudinal visualization. Bending of individual muscle fiber and also separation and granulation of some parts of muscle structure affected by high electromagnetic field, have been visualized. (b) Another illustration of industrial microwave heating of veal. Erosion at the edge of muscle fiber was apparent. (c) Industrial microwave heating of veal. Visualization of connective tissue network surrounding muscle fiber is apparent. Ct: connective tissue. (d) Transverse view of industrial microwave heating of veal. Existence of a gap is apparent. Connective tissue inside the gap is visualised and pointed by arrows.

muscle causes more damage to the structure of both the connective tissue and the myofibrillar elements than conventional method.

In veal SM muscle, more damage was observed in the connective tissue network for conventional heating as compared with microwave heating. Surface damage in structure of semimembranosus was not observed either in conventional heating or the low powered microwave heating. This was similar to the results of Schaller and Powrie (1972) who applied conventional SEM in the study of beef structure. Rapid increase of heat and penetration of the microwave field in 12000 wattages level causes granulation and separation of some parts of the muscle fibers. The granular materials were seen in the images, thought to be formed by a mix-

ture of heat-denatured collagen and coagulated sarcoplasmic protein which possibly accumulated in the spaces between the muscle fibers. Occasional cracks across the individual muscle fiber and shrinkage were presented in all images of the structure for heated SM muscle. This agreed with the results of Jones *et al.* (1977) who investigated the effect of conventional heating on bovine semitendinosus muscle. Their results indicated that more damage was sustained by the muscle fiber at higher temperatures. Similar results were obtained by Hearne *et al.* (1978) in a structure study of conventionally heated bovine semitendinosus muscle by SEM. The differences in the above research obtained, were due to various ranges of heating applied, while in this study



conventional heating was adjusted at 163°C and an internal muscle temperature of 70°C. However structural damage was revealed and when we compare the result of conventional heating with microwave heating, it seems less damage were observed with conventional heating. A gap between perimysium of each muscle bundle was apparent with the domestic microwave sample. This phenomenon was even more apparent with industrial microwave heated samples.

As a result of these investigations, it is possible to identify and characterize the fine structure of veal semimembranosus muscle. Moreover to study and compare the effect of various heat treatments such as conventional and microwaves at two wattage levels (700 and 12000 W) on structure of this muscle. Results also show that Microwave heating causes more structural damage, at both levels, as compared with conventional heating. Distribution of heat in microwave application is responsible for the surface damage to muscle fiber and separation of some parts as well as denaturation of collagen. Further research is required to study the fine structure of semimembranosus or other muscles from various animals.

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REFERENCES

1. Baumgarten, N. 1990. Introduction to the Environmental Scanning Electron Microscope. Scanning 90 Abstract. FACMS Inc. pp. 64-66.
2. Bolon, R. B., Roberstson, C. D., and Lifshin, E. 1989. The Environmental SEM: A New Way to Look at Insulators. In: "Microbeam Analysis" (Ed): Russel P. E. San Francisco Press, San Francisco pp. 449-452.
3. Crawford, C. K. 1979. Charge Neutralization Using Very Low Energy Ions. *Scanning Electron Microsc.*, **II**, 31-46.
4. Danilatos, G. D. 1981. The Examination of Fresh or Living Plant Material in an Environmental Scanning Electron Microscope. *J. Microsc.*, **121**: 235-238.
5. Danilatos G. D., and Postle, R. 1982. -The Environmental Scanning Electron Microscope and its Application. *Scanning Electron Microsc.* I, 1-16.
6. Danilatos, G. D. 1983. A Gaseous Detector Device for an Environmental SEM. *Micron Microsc. Acta.*, **14**: 307-319.
7. Danilatos, G. D., and Brooks, J. H. 1985. Environmental SEM in Wool Research. Present State of the Art Proc. 7th Int. Wool Text Res. Conf (Ed. by M. Sakamoto) I, 263-272. Nichiei Kosen co., Ltd.
8. Danilatos, G. D. 1986. Cathodoluminescence and Gaseous Scintillation in the Environmental SEM. *Scanning.*, **8**: 279-284.
9. Danilatos, G. D. 1989. Environmental SEM; A New Instrument, A New Dimension. Proc. EMAG-MICRO 89, London. Inst Phys. Conf. Ser., **98**: 455-458.
10. Danilatos, G. D. 1990. Theory of the Gaseous Detector Device in the ESEM *Adv. Electronics Electron Phys.*, **78**: 1-102.
11. Danilatos, G. D. 1991. Review, and Outline of Environmental SEM at Present. *Journal of Microscopy.*, **162**: 391-402.
12. Doty, D. M., and Pierce, J. C. 1961. Beef Muscle Characteristics as Related to Carcass Grade, Carcass Weight, and Degree of Aging *U.S.D.A. Agr. Marketing Serv. Techn. Bul.* No. 1231.
13. Hearne, L E. 1976. Tenderness and Structural Changes in Beef Semitendinosus muscles heated at Two Rates to Four End Point Temperatures. PhD. thesis, The University of Tennessee.
14. Hsieh, Y. P. C., Cornforth, D. P., Pearson A. M., and Hooper, G. R. 1980. Ultrastructural Changes in Pre- and Post-rigor Muscle Caused by Conventional and Microwave Cookery. *Meat Sci.*, **4**: 299-311.
15. Hui, Y. K 1992. *Encyclopedia of Food science and Technology* Volume 3, J. Wiley, Interscience Publication, United States, pp 1772-1776.
16. Jones, S. B., Carrol. R.J., and Cavanaugh, J. R. 1977. Structural Changes in Heated Bovine Muscle: a Scanning Electron

- Microscope Study. *J. Food Science.*, **42**:125-131.
17. Klose, M. J., Webb, R. I., and Teakle, D. S. 1992. Studies on the Virus Pollen Association of Tobacco Streak Virus Using ESEM and MDD Techniques. *J. Comput. Ass. Microsc.*, **4**:213-220.
 18. Moncrieff, D. A., Robinson, V. N. E., and Harris, L. B. (1987). Charge Neutralization of Insulating Surfaces in the SEM by Gas Ionization. *J. Phys.*, **11**:2315-2325.
 19. O'Brien, G. P., Webb, R.K., Uwins, P. J. R., Desmarchelier, P. M., and Imrie, B. 1992. Suitability of the Environmental Scanning Electron Microscope for Studies of Bacteria on Mungbean seeds. *J. Comput. Ass. Microsc.*, **4**: 225-229.
 20. Parsons, D. F., Matriccardi, V. R., Moretz, R. C., and Turner, J. N. 1974. Electron Microscopy and Diffraction of Wet Unstained and Unfixed Biological Objects *Adv. Biol. Mcd Phy.*, **15**: 161-271.
 21. Paul, P. C. 1963. Influence of Methods of Cooking on Meat Tenderness. In: "Proceedings Meat Tenderness Symposium". Campbell Soup Company, Camden, New Jersey, PP. 225-242.
 22. Peters, K.R. 1990. Introduction to the Technique of Environmental Scanning Electron Microscopy. Scanning 90 Abstract, FACMS Inc. pp. 71-72
 23. Pfefferkron, G. F., Gruter, H., and Pfatusch, M. 1972. Observation on the Prevention of Specimen charging. *Scanning Electron Microsc.*, 147-152.
 24. Schaller, O.R., and Powrie, W. D. 1972, Scanning Electron Microscopy of Heated Beef Chicken and Rainbow Trout Muscles. *Can Inst. Food Sci. Technol. J.*, **5**:184-190.
 25. Uwins, P. J. P. 1994. Environmental Scanning Electron Microscopy. *Materials Forum.*, **18**: 51-75.
 26. Wallace, H.M., Uwins, P. J. R., and McChonchie, C. A. 1992. Investigation of Pollen-Stigma Interaction in *Macadamia* and *Grevillea* using ESEM. *J. Comput Ass. Microsc.*, **4**: 231-234.

بررسی ساختمان میکروسکوپی عضله خام و حرارت دیده سمیممبرانوسوس گوساله با میکروسکوپ الکترونی Environmental

چکیده

میکروسکوپ الکترونی Environmental پیشرفتی جدید در زمینه تحقیقات انجام گرفته با میکروسکوپ الکترونی است. در این تحقیق میکروسکوپ الکترونی ذکر شده برای مطالعه ساختمان عضله ای موسوم به Semimembranosus در گوساله مورد استفاده قرار گرفته است. چهار تیمار شامل عضله حرارت داده نشده (شاهد)، عضله حرارت داده شده با روش سنتی (Conventional)، حرارت داده شده با روش مایکروویو معمولی و مایکروویو صنعتی بوسیله این میکروسکوپ الکترونی مورد مشاهده قرار گرفت. دمای بکاررفته در روش حرارتی سنتی 163°C بود. فرکانس بکار رفته برای روش حرارتی مایکروویو 2450 مگاهرتز با دو میزان توان مصرفی 700 وات (مایکروویو معمولی) و 12000 وات (مایکروویو صنعتی) بود. دمای داخلی تمام نمونه ها به 70°C رسانده شد. در تصاویر بدست آمده شکاف هایی در میان فیبر عضلانی منفرد ملاحظه شد و جمع شدگی (چروکیدگی) در کلیه تصاویر نمونه های حرارت دیده مشهود بود. حالت ساییدگی در لبه فیبرهای عضلانی مشخص بود و با ادامه حرارت دادن افزایش یافت. با بکاربردن دمای



مایکروویو معمولی، یک شکاف مابین غلاف perimysium هر دسته باندهای عضلانی نمونه ها در برش عرضی پدیدار گشت. این پدیده در روش حرارتی مایکروویو بیشتر آشکار بود و عمق شکاف مابین فیبر کلاژنی perimysium در این روش از روش حرارتی مایکروویو معمولی بیشتر بود. صدمه بافتی بیشتری در شبکه بافت پیوندی حرارت دیده با روش حرارتی سنتی در مقایسه با روش حرارتی مایکروویو مشاهده گردید. از هم گسستگی و از دست دادن شکل طبیعی بافت پیوندی صدمه بافتی بیشتری را در حین زمان طولانی تر روش حرارتی سنتی باعث گردید. صدمه سطحی ساختمان عضله Semimembranosus در روش حرارتی سنتی و روش حرارتی مایکروویو با توان کمتر، مشاهده نشده بالا رفتن سریع حرارت درون بافت در اثر نفوذ مایکروویو، باتوان ۱۲۰۰۰ وات موجب گرانبه شدن و جدا شدن بعضی قسمت های فیبر عضلانی گردید.