ASTOGRAPHY OF BEEF MUSCLE

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Elastography, a technique that uses ultrasonic pulses to track the internal displacements of small tissue elements in response to an ernally applied stress, has been applied to beef muscle. Beef Longissimus (one day postmortem) and Semimembranosus (five days mortem) muscles were obtained from A maturity beef carcasses. Samples were vacuum-packaged and frozen to -20°C. For stography measurements, muscles were equilibrated to a constant temperature (30°C±.5) in a water tank. Custom transmitters and ivers were used in conjunction with a 2.25 MHz medical transducer. The transducer was driven by a 286 PC, and the radio-frequency es digitized at 50 MHz and 8 bits. The pre- and post-compression echo trains (A-lines) were subjected to crosscorrelation analysis. ual interpretation of beef elastograms demonstrate circular areas of relatively inelastic tissues and smaller, banding areas of elastic tissues the cross-section of beef Longissimus muscle. The dark inelastic areas from the elastograms may be related to myofibrillar areas from the muscle sections; the light, elastic band areas from the elastograms may be related to perimysial connective tissue or intramuscular fat. ty septa and a calcified abscess could be easily identified on the elastogram. These preliminary results demonstrate that elastography may e potential as a nonintrusive method of visualizing tissue components of beef muscle.

TRODUCTION

Structural components of muscle have been related to quality and palatability attributes of meat. For example, the total amount of llagen and the solubility of collagen, which is a quantitative evaluation of heat-labile cross-linking within collagen structure, has been lated to beef tenderness (GOLL et al., 1964; HILL, 1966; HERRING et al., 1967; BAILEY, 1972; SHIMOKOMAKI et al., 1972; ROSS et al., 1973). Additionally, the contractual state of the myofibrillar component of muscle (LOCKER and HAGYARD, 1963; MARSH and LEET, 1966) and the degradative state of myofibrillar proteins postmortem (OLSON et al., 1977; DUTSON, 1983; GOLL et , 1983; KOOHMARAIE et al., 1984; KOOHMARAIE, 1988) have been associated with tenderness differences in meat. A third muscle mponent, intramuscular fat, has been correlated to meat tenderness and palatability (SAVELL and CROSS, 1988). These three mponents of muscle are generally assessed when palatability or quality differences are examined in meat systems. The fact that each mponent originates within the structure of muscle indicates that a method to assess muscle structure could eventually provide information r developing value determinations for meat quality or palatability. To date, collagen content and solubility, myofibrillar contractile state, d protein degradation are assessed chemically; and intramuscular fat content is determined either visually (marbling scores) or chemically. A nonintrusive, rapid method of assessing muscle and/or meat structure could eventually provide needed information into quality determinations for meat systems.

We have recently described a method for making quantitative cross-sectional images of tissue elasticity (OPHIR et al., 1991). The technique, called elastography, uses ultrasonic pulses to track the internal displacements of small tissue elements in response to an externally applied stress. These displacements are converted into local strain values along the axis of compression by comparing pre- and post-compression signals from within the tissue. The strain values may be converted into calibrated Young's modulus values with additional knowledge of the applied stress and its distribution along the compression axis. The resultant image is termed an elastogram. For practical reasons, elastograms actually display the inverse Young's modulus values, such that lighter regions in the image correspond to softer, more elastic structures.

We have shown (OPHIR et al., 1991) that elastograms have several interesting properties: 1. The signal-to-noise ratio in elastograms of homogeneously elastic regions is on the order of 5. As a comparison, the signal to noise ratio in standard sonograms cannot exceed 1.91 (WAGNER et al., 1983). As a result, elastograms exhibit less artifactual speckle than do sonograms; 2. Large regions of interest in elastograms with contrast ratio of 2 (6dB) or more are easily visualized; 3. The resolution of elastography appears to be on the order of the underlying sonographic resolution; 4. It is possible, in general, to obtain good quality elastographic data in acoustically shadowed regions which allow only poor sonographic visualization, as long as a signal is present; and 5. Elastograms can convey absolute elastic modulus information. In principle, some of these attributes may make elastography a suitable modality for evaluation of bovine muscle structure and composition.

In this paper we describe some preliminary investigations into the elastographic appearance of bovine muscle in vitro. We have found that elastography may be capable of depicting muscle structure at the muscle bundle level, and of demonstrating differences in the elastic moduli of muscle bundles, connective tissue and fatty septations.

MATERIALS AND METHODS

Elastography

The basic elastography method has been described previously (OPHIR et al., 1991). We present here a brief discussion of the principles of the method. When an elastic medium, such as tissue, is compressed by a constant stress applied from one direction, all points in the medium experience a resulting level of strain along the axis of compression. In the simplest form of a one-dimensional case, all strains are equal and constant as long as the elastic moduli of the local tissue elements are equal. If one or more of the tissue elements has a different elastic modulus than the others, the level of strain in that element will be higher or lower; a harder tissue element will experience less strain than a softer one.

In order to convert local strains to local elastic modulus values, it is necessary to know the local stress. In a general three-dimensional case, the applied axial stress diminishes with depth. The diminution of the stress along the axis of compression depends on the size and shape of the compressor, on the depth of interest, and on the boundary conditions. It can be shown (OPHIR et al., 1991) that for a given target depth, the stress distribution along the axis of compression is a monotonic function of the parameter (z/a), where a is the radius of a circular compressor, and z is the depth, viz.

$$\sigma(z)=\sigma(0)D(z/a) \qquad \text{(Eq. 1)}$$

where $\sigma(0)$ is applied stress and $D(z/a)$ is a theoretical stress decay function derived from the work of SAADSA (1974). This expression also holds well for finite tissue depths under certain conditions (PONNEKANTI et al., 1992). The applied stress $\sigma(0)$ is measured separately as discussed below.

The Young's modulus of a tissue element at depth z is given as

$$E(z) = \frac{\sigma(z)}{s(z)} \qquad \text{(Eq. 2)}$$

where s(z) is the local strain. The strain is estimated from ultrasonic measurements using standard medical ultrasound diagnostic equipment. This is accomplished by acquiring a set of digitized radio frequency echo sequences (A-lines) from the tissue region of interest; compressing the tissue with the ultrasonic transducer along the ultrasonic radiation axis by a small amount; and acquiring a second, post-compression set of A- lines from the same region of interest. Parts of congruent A-lines are then subdivided into small temporal windows and compared by using crosscorrelation techniques, from which the arrival times of the echoes can be estimated. The strain in a given window is calculated as

$$S_{window} = \frac{(t_{1b} - t_{1a}) - (t_{2b} - t_{2a})}{t_{1b} - t_{1a}} \qquad \text{(Eq. 3)}$$

ere t_{1a} = arrival time of the pre-compression echo signal from the proximal edge of the window; t_{1b} = arrival time of the pre-compression
o signal from the distal edge of the window; t_{2a} = arrival time of the post-compression echo signal from the proximal edge of the
dow; and t_{2b} = arrival time of the post-compression echo signal from the distal edge of the window. The window is translated along the
poral axis of the A-line, and the calculation is repeated. The strain values so obtained constitute the s(z) function.

The applied stress $\sigma(0)$ is normally calculated from the strain measured in a compressible standoff layer of known Young's modulus,
ch is interposed between the compressing transducer and the tissue. In the work reported here, the applied stress was not measured, and
s the elastograms which were produced were uncalibrated.

mple Preparation

Three different samples were prepared for this study. Sample one was a 80 x 50 x 50 mm section of the Longissimus dorsi muscle
m an A maturity beef carcass at one day postmortem. The sample was vacuum-packaged in surlyn film (4 mm thick bottom film and 3
n thick top film; oxygen transmission rate of 3,000 to 4,000 cc/m^2) on a Biovac top-forming, heat sealing, vacuum-packaging machine
merican National Can Corp., Neenah, WI) to remove excess oxygen from the package and to avoid the development of excess purge or
ntamination. The plane of interest was carefully marked so that the plane was perpendicular to the fiber orientation of the muscle. The
mple was stored at -20°C until elastography measurements were obtained. Sample two was a 150 x 130 x 100 mm section from a Low
lect (USDA, 1989) beef top round (*Semimembranosus* muscle excised at five days postmortem) prepared similarly to sample one. The
ne of interest again was carefully marked. Sample three was a 60 x 60 x 100 mm section from a High Select beef top round muscle which
s embedded in a 100 x 100 x 140 mm block of 1.5% Agar gel.

lastography Experiments

Elastography requires good acoustic coupling between the transducer and the target, therefore tests were performed in a 120 gal
mperature regulated water tank. A 2.25 MHz 13 mm diameter transducer focused at 7 to 19 cm was used. The system was controlled by a
ompaq 286 computer via an IEEE 488 bus. A stepper motor controller enabled transducer movements in steps of 2.5 microns. A
ansmitter was used to shock excite the transducer. The received signal from the target was amplified by an input protected TGC
ntrollable amplifier, and fed into an 8-bit digitizer operating at 25 MHz.

An annulus was attached to the transducer so as to form a compressor with a diameter of 44 mm. This was done in order to reduce the
ue of depth dependent stress decay (OPHIR et al., 1991). A block of reticulated polyester foam with a porosity of 30 pores per inch with
mensions of 140 x 140 x 50 mm was used as an acoustic standoff (OPHIR et al., 1991), such that the focal area of the transducer was
rmed inside the meat sample.

Measurements

Samples one and two were transferred to a refrigerator (60°C) for approximately 24 h and then transferred to a water tank maintained at
0°C ±.5 for approximately 8 hours before the test. The samples were placed on a platform and immobilized with lead blocks. The acoustic
andoff was placed on the sample and the transducer was positioned such that good contact was made with it, and the first A-line was
btained. The transducer then was slowly moved down by 1.0 mm so as to compress the sample, and a second A-line was obtained. The
rocess then was repeated at 1 mm increments along the plane of interest to obtain forty A-line pairs for sample one and sixty A-line pairs for
ample two. The gel block containing sample three was placed on a platform and immobilized using lead blocks and sixty A-line pairs were
btained in a way similar to the one described above for samples one and two.

To obtain the strain profile along the compression axes, the A-line pairs were subjected to crosscorrelation computations as described above. The strain data was then used to generate a two-dimensional uncalibrated elastogram.

RESULTS AND DISCUSSION

Figure la shows the sonogram obtained from sample one, while Fig. lb shows the uncalibrated elastogram obtained from the same image plane. The plane of interest was taken so that the muscle was imaged across the fibers. The elastographic appearance of this cross section is suggestive of enclosed, dark (hard) areas, surrounded by light (soft) bands. The size of these hard areas is on the order of 5 to 10 mm. This structure is similar to the myofibrillar bundle cross-sectional structure which is surrounded by bands of perimysium. A textbook appearance of this structure is given in Fig lc (JUDGE et al., 1989).

Figure 2a shows the sonogram obtained from sample two, and Fig 2b shows the uncalibrated elastogram obtained from the same plane. A photograph of the muscle cut at the scan plane is shown in Fig 2c. The diagonal fatty septations (< 1 mm thickness) present in the muscle can be identified in the elastogram as lighter (softer) bands; they are not demonstrated on the sonogram. In previous work (Cross et al., 1991), we have shown that bacon fat bands appeared softer than adjacent muscle structures. Muscle bundles are surrounded by connective tissue defined as perimysium. Connective tissue and intramuscular fat have been shown to develop from fibroblasts. As intramuscular adipocytes develop, these cells mature and fill with lipid while surrounded by connective tissue. An electromicrograph of cooked beef top round (Fig. 3) illustrates how intramuscular adipose cells are embedded in connective tissue which surrounds muscle bundles. Since connective tissue and fat tend to be soft compared to muscle tissue, these results indicated that the soft areas in the elastogram are most likely connective tissue and/or fat tissue, with the surrounding relatively inelastic areas being comprised mainly of muscle components. Note that at the bottom left quadrant of Fig. 2c, the textural definition diminishes and intramuscular fat is not visible. Similarly, the elastogram of Fig. 2b has a noticeable lack of white banding in this area indicating a lack of soft, elastic tissues. Figure 4 shows results from the third sample. Figure 4a shows the cut surface of the muscle (embedded in gelatin), which exhibits the presence of a partially calcified collapsed abscess in what appears to be an old muscle site injury or intrusion. Figure 4b shows the sonographic appearance of the muscle. The abscess is clearly visible, but high level echoes obscure its structure. Figure 4c shows the resulting elastogram, where a pocket-like abscess is seen, which contains a harder core surrounded by a soft perimeter.

The appearance of sonograms and elastograms of tissue are expected to be fundamentally different. The reason is that while sonograms display the acoustic backscatter energy from tissue components, elastograms display its static elastic modulus. In general, these two parameters are not expected to be correlated with each other. It is therefore not surprising that certain structures which may not be seen on the sonogram would be clearly seen on the elastogram, and vice versa. The use of elastography may convey new information about the tissue structural components which was heretofore unavailable.

CONCLUSIONS

This preliminary work shows that muscle structures not seen or not well identified with standard sonography may be identified with elastography, and that elastography has potential as a noninvasive method of evaluating structural characteristics of beef muscle. Additional research is needed to more specifically define the role of elastography in characterizing quality attributes of beef muscle. Research is currently being conducted in our laboratories to relate the connective tissue, muscle fiber and intramuscular fat components of muscle to information derived from elastograms. Additionally, the correlation between sensory properties and elastography images is being evaluated. These studies should further define the efficacy of elastography as a non-invasive method of quantitating beef muscle quality and structure.

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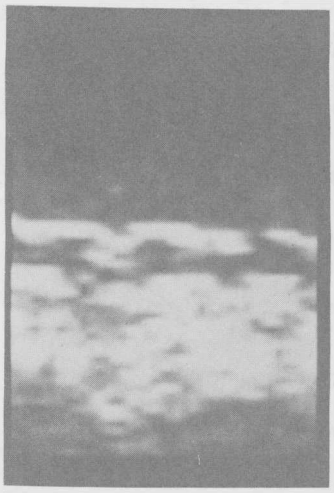
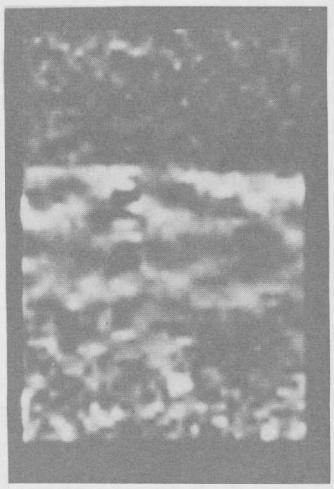
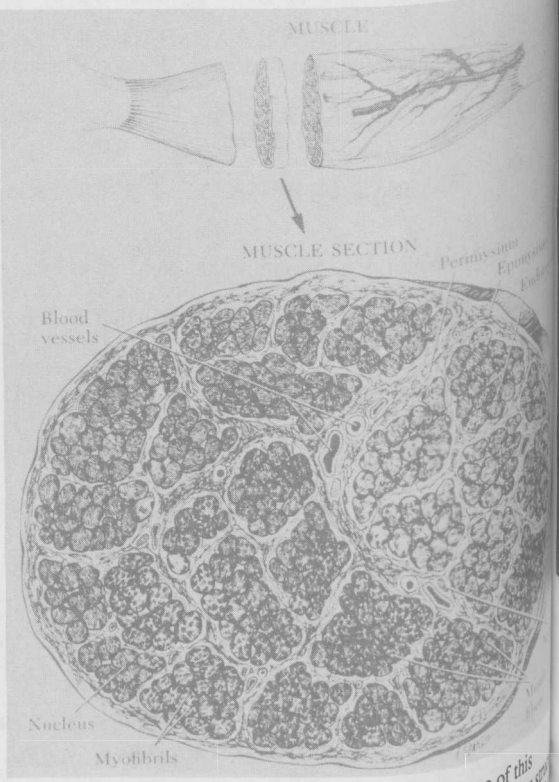
Figure 1. Images from sample one taken from the same scanning plane across the muscle fibers.





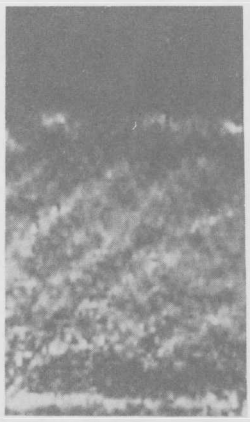


a. Sonogram

b. Elastogram. Observe dark (hard) areas surrounded by light (soft) bands.

c. A textbook appearance of this structure. Observe a similar to that seen in b. (Adapted from et al., 1989)

Figure 2. Images from sample two.

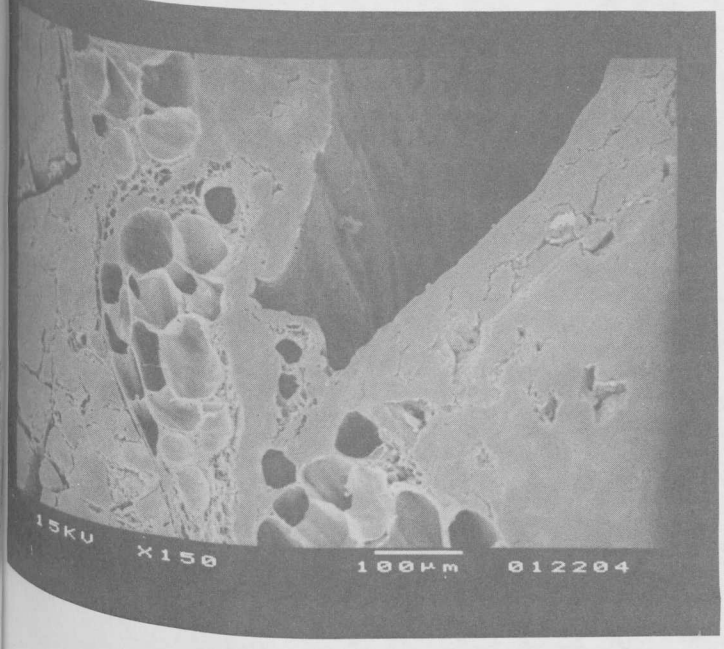


a. Sonogram

b. Elastogram. Observe diagonal altering soft and hard bands.

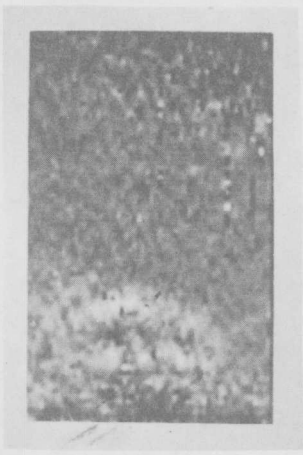
c. Photograph of the sample cut at the image plane. Observe the presence diagonal fatty septations.

igure 3. An electromicrograph of cooked beef top round, demonstrating intramuscular adipose cells embedded in connective tissue which rrounds muscle fiber bundles.

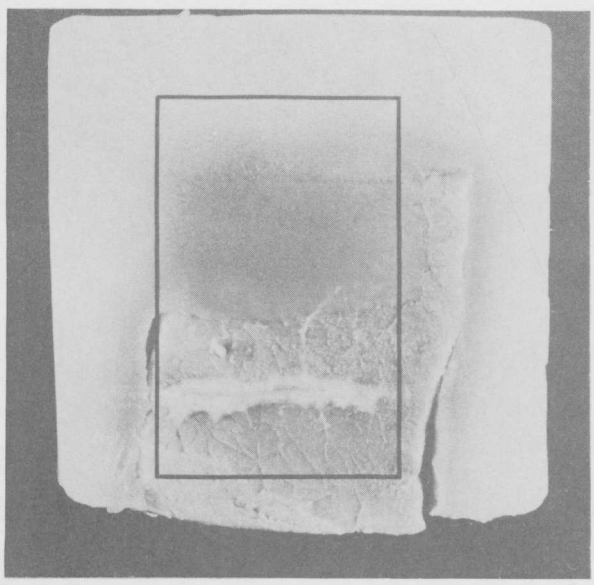


igure 4. Images from sample three.

a. Sonogram. Note the presence of a highly echogenic region near the bottom of the image.



b. Elastogram. Note the pocketlike structure near the bottom of the image.



c. Photograph of the sample cut at the image plane. Note the presence of a partially calcified abscess due to an old muscle injury or intrusion.