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# ON-LINE PROBE PREDICTION OF BEEF TOUGHNESS, CORRELATING SENSORY EVALUATION WITH FLUORESCENCE DETECTION OF CONNECTIVE TISSUE AND DYNAMIC ANALYSIS OF OVERALL TOUGHNESS

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## ABSTRACT

*The main muscles of commercially competitive cuts of beef ($n=16$) from a variety of sources were probed to detect ultraviolet (UV) fluorescence of connective tissue, together with a dynamic analysis of electromechanical signals for overall toughness. The main muscles were cut into 1.2-cm cubes after being frozen. Muscle cubes were cooked for 20 minutes to an internal temperature of 70°C and evaluated by a trained panel. Dynamic analysis showed that tough regions of meat cuts had a relatively high frequency of narrow fluorescence peaks subtending a small area under the fluorescence signal. Thus, for probe measurements made perpendicularly across muscles, the area under the fluorescence signal was correlated ($p<0.01$) positively with tenderness ($r=0.57$), and negatively with chewiness ($r=-0.61$) and residual tissue ($r=-0.58$). Thus, despite variation in post-mortem treatment and cooking, connective tissue toughness may contribute to the overall toughness of commercially competitive cuts of beef, and sensory responses may be partly predicted from rapid, relatively non-destructive measurements on the raw meat.* © *1997 Elsevier Science Ltd*

*Keywords: Beef; on-line evaluation; tenderness; connective tissue; fluorescence.*

## INTRODUCTION

Tenderness is one of the main factors influencing consumer acceptability of cooked beef (Miller *et al.*, 1995) and prediction of toughness from on-line carcass measurements could be of great value in the beef industry. This was first attempted with the Armour Tenderometer (Hansen, 1972), using an electromechanical signal from a battery of relatively short penetration needles. More recent methods use a single, long needle coupled with either a force transducer (Gordon, 1994), or a depth detector (Swatland, 1994*a*). Thus, when the probe tip encounters tough meat, it is detected either by an increase in resistance or by a deceleration. However, beef toughness has several different causes. Some causes originate on the farm, like connective tissue (Loyd and Hiner, 1959; Smith and Judge, 1991) or the effects of growth rate (Van Eenaeme *et al.*, 1994), while others are modified by events after slaughter, such as cold-shortening (Marsh and Carse, 1974), aging (Etherington, 1984; Eilers *et al.*, 1996), or pH (Bouton *et al.*, 1973).

Research was undertaken to determine if on-line measurements of heterogeneous, commercially competitive beef cuts could be used to predict sensory responses. Connective tissue was detected by UV fiber-optic fluorescence, augmented by dynamic analysis of the electromechanical signal for overall toughness. Although fluorescence prediction of sensory toughness has been reported for Canada grade A beef (Swatland *et al.*, 1995*a*) and Danish dairy beef (Swatland *et al.*, 1995*b*), the samples in these trials were treated uniformly post-mortem whereas those of the present study were not. Post-mortem pH has a strong effect on connective tissue fluorescence (Swatland, 1995*a*) so that the fluorescence method was pushed to its limit by not controlling post-mortem sample treatment, thus introducing pH as a confounding variable, as might occur in a realistic commercial application of the technology.

## MATERIALS AND METHODS

### Samples

Sixteen beef cuts of interest to the research sponsor were obtained from Canada grade A beef carcasses with marbling scores of A and AAA, from United States

Department of Agriculture (USDA) Choice and Select carcasses, and from Australian and New Zealand boxed beef. Specifications for the Canadian and USDA grades are given by Swatland (1994*b*). Different samples were treated in different ways, as specified by the sponsor, including post-mortem aging from two to seven weeks, with vacuum packed primals being held at temperatures from 4 to 8°C. Proprietary results are not given, and this report is restricted to general scientific observations on how on-line and sensory data may be related in commercially competitive meat cuts. The muscles tested were *longissimus lumborum* ($n = 14$), *psoas major* ($n = 1$) and *biceps femoris* ($n = 1$).

## On-line measurements

The connective tissues of meat are arranged in a series of concentric patterns. Epimysium is located around whole muscles, perimysium surrounds bundles of muscle fibers, and endomysium surrounds individual muscle fibers. Thus, most of the peaks detected with a fluorescence probe are perimysial, because the epimysium is only on the muscle surface and layers of endomysium are microscopic, below resolution with a 1-mm diameter optical fiber. However, because of this concentric pattern, it is important to control the direction of measurement (Swatland *et al.*, 1996). Thus, hand-held probe measurements were made in two directions: coaxially, along the longitudinal axes of the main muscle in each cut and, perpendicularly, across the muscle axes. In the first orientation, the probe window tended to pass layers of perimysium in a tangential direction. In the second orientation, the probe window tended to cut through layers of perimysium obtusely. Fluorescence measurements of connective tissue were made on initial penetration of the meat (way-in). Dynamic analysis of the electromechanical signal relating to overall toughness was undertaken using both way-in, and way-out signals made as the probe was withdrawn. Samples were measured at, and after equilibration to, 10°C.

The probe was a hand-held MQM fat-depth probe (kindly donated by the Danish Meat Research Institute, Roskilde), modified to measure connective tissue instead of fat to muscle boundaries. The modifications are described in detail elsewhere (Swatland, 1991, 1992), and may be summarised as follows. The MQM probe was dismantled and an optical fiber (type HFBR, Hewlett-Packard, Palo Alto, California) was inserted through the shaft to form a window behind the cutting head. The optical surface in contact with the meat was approximately 1 $mm^2$ but the optical fiber was cut at an angle so that the interface optics were asymmetrical. Light from a 100 W short-arc mercury source (Osram HBO 100, Berlin, Germany) was directed through a heat absorbing filter (type KG1; Zeiss, Oberkochen, Germany), a red-attenuation filter (Zeiss BG38) and a dichroic mirror (Zeiss FT395). Light peaking at 365 nm was directed into the proximal end of the optical fiber with a microscope objective (Zeiss Neofluar ×6.3, NA 0.2). Fluorescence from connective tissues in contact with the distal end of the optical fiber was measured through the dichroic mirror at the proximal end of the fiber using a side-window photomultiplier with S-20 characteristics (Hamamatsu HTV-R928, Hamamatsu City, Japan) and 5 kHz damping (essentially a raw signal). Thus, the dichroic mirror was used as a chromatic beam splitter to separate the outgoing excitation ($\lambda < 395$ nm) from the incoming fluorescence emission ($\lambda > 395$ nm). The electromechanical vector describing the depth of the optical window in the meat was obtained from a precision potentiometer geared to the plate of the MQM probe remaining on the meat surface.

The main controller for the probe was a Hewlett-Packard workstation (360 CMA) programmed in BASIC, adapting regression analysis statistics from Steel and Torrie (1980). In searching for relationships between fluorescence signals and sensory responses, both data sets were reduced to their main components. For fluorescence, the following were used: peaks $cm^{-1}$, peaks $cm^{-1}$ above background rejection (minimum ×1.1), area under signal $cm^{-1}$, mean peak height, mean peak height above background rejection, mean half-peak width, mean half-peak width above background rejection, depth vector disorder (n/total), mean length of disorder, and the heights of the three largest peaks in each transect. For sensory evaluation, the following were used, tenderness, chewiness, and residual tissue. For stepwise multiple regression analysis, the level for accepting or rejecting independent variables was $F = 2$.

Fluorescence signals may have contained some noise, inextricably mixed with the signal from small endomysial connective tissues. Imposed on this were larger signal peaks derived from larger elements of connective tissue and arteries (elastin has stronger UV fluorescence than collagen; Swatland, 1995*b*). The separation of large peaks from the overall population was made at the minimum signal in each transect ×1.1. The objective was to determine if sources of intense fluorescence were specially important.

The maximum depth of penetration available with the probe was used for most samples, although some trans-

**TABLE 1.** Attributes and their definitions for line scale evaluation

| Attribute (low → high scale) | Definition |
|---|---|
| 1. Tenderness (tough → tender) | Force to chew a 1.2-cm cube of meat, measured after 3 chews |
| 2. Chewiness (not chewy → very chewy) | Energy (time and force) required to prepare the meat sample for swallowing |
| 3. Residual tissue (very little → very much) | Quantity of meat particles remaining in the mouth after swallowing main bolus |

ects were terminated prematurely before the probe tip passed completely through small samples. However, data were corrected for the length of the transect so this would have had minimal effect on the results.

### OCA CT-probe

The Ontario Cattlemen's Association (OCA, 130 Malcolm Road, Guelph, Ontario N1K 1B1) in cooperation with Sciencetech (45 Meg Drive, London, Ontario N6E 2V2) manufactures a connective tissue probe called the OCA CT-Probe. Independent measurements of the 16 beef cuts were made coaxially using the OCA CT-Probe (data courtesy of Steven Nadeau) and a brief summary of their analysis is given in this report.

### Sensory panel training

In 24 one-hour training sessions, 12 panelists were trained to evaluate three attributes of tenderness → toughness (Table 1) using the Compusense five computerized sensory analysis system (Compusense, 150 Research Lane, Guelph, Ontario N1G 4T2). This displays a 10-cm line on-screen with the panelist response logged with a light pen. Examples of the range of each attribute were experienced. Discussions were held to ensure that panelists understood the attribute definitions. A pre-trial test session was held using spare samples from the experiment. Evaluations were conducted in individual, computerized booths under red lighting. Panelists placed a meat cube between their back molars with the fiber direction perpendicular to the teeth.

### Protocol

After on-line measurements, the meat cuts were evenly frozen to −18°C in blast freezer, then cut into slices 1.2 cm thick with a band saw. The slices were subdivided into 1.2-cm cubes, vacuum packaged, and sealed.

A completely randomized design was used for sensory testing. At each session, each panelist evaluated four samples giving a total of three replications of each sample per session. Altogether, 16 sessions were required to complete the sensory testing. Presentation of samples to panelists was randomized within each session.

Each day of testing, bags of samples were removed from the freezer and sealed in a large vacuum package bag in a refrigerator at 4°C for 5 h. Meat cubes thawed completely without any noticeable fluid exudate. The bags were immersed in a water bath at 72.5°C and heated for 20 min to an internal end point temperature of 70°C. Temperature was monitored with nickel-chromium thermocouples inserted into the center of the cube package. Upon removal from the water bath, samples were cooled to room temperature (21.5°C). The cubes were placed into plastic cups which were closed with a lid and labelled with a three digit code before presentation to panelists for evaluation.

## RESULTS AND DISCUSSION

### Sensory data

Overall means for tenderness, chewiness and residual tissue were, respectively, $5.96 \pm 0.90$, $4.52 \pm 0.73$, and $3.94 \pm 0.69$. As expected, these traits were strongly ($p < 0.0005$) interrelated: tenderness with chewiness ($r = -0.97$) and residual tissue ($r = -0.97$), and chewiness with residual tissue ($r = 0.94$).

### Fluorescence analysis of connective tissue

Way-in fluorescence probe measurements for the least and most tender cuts are shown in Fig. 1 where fluorescence intensity was normalized (maximum value = 1) because intensity measurements were arbitrary (photomultiplier cathode current, mA). Fluorescence measurements are difficult to quantify radiometrically, particularly those made via fiber-optics. Differences in the distance of probe penetration were corrected by expressing data on a per centimetre basis. Subjectively, the tender cut appeared to have lower fluorescence than the tough cut (i.e., most of the squares are below the line in Fig. 1).

The analysis of way-in fluorescence probe signals made along the longitudinal axes of muscles is shown in Table 2 (fluorescence intensity is not normalised as in Fig. 1). Panelists correlated with the frequency of all fluorescence peaks combined, but there was no advantage in isolating a subset of intense peaks related to elements of connective tissue with strong fluorescence. Panelists did not correlate with the fluorescence intensity of peaks, but with the width of all peaks. However, the nature of the correlation was paradoxical: samples with wider peaks were more tender, less chewy, and had less residual tissue than those with narrow peaks. No correlation of the area under the fluorescence signal with sensory responses was detected.

Measurements made in the direction across, rather than along the muscles may give different results because

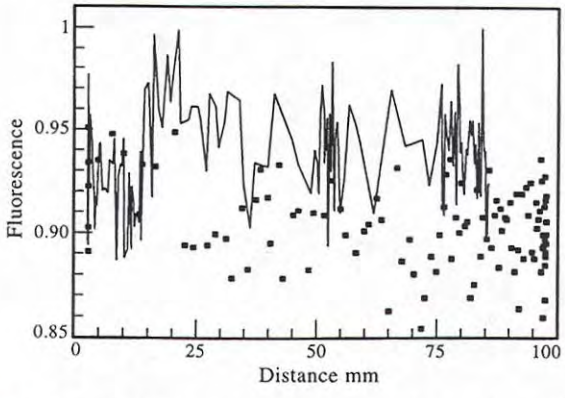


**FIG. 1.** Way-in fluorescence probe signals for the least (line) and most tender (squares) beef cuts with the probe measuring perpendicularly across muscles.

**TABLE 2.** Features of way-in fluorescence probe signals made along the longitudinal axes of muscles and their simple correlations (r) with sensory attributes

| Feature | Mean | SD | r | | |
|---|---|---|---|---|---|
| | | | Tenderness | Chewiness | Residual tissue |
| Frequency $cm^{-1}$, all peaks | 4.21 | 0.64 | −0.42 | 0.45[a] | 0.43[a] |
| Frequency $cm^{-1}$, intense peaks | 0.79 | 0.85 | −0.06 | 0.05 | 0.04 |
| Intensity, all peaks | 6.73 | 1.22 | 0.07 | −0.12 | −0.9 |
| Intensity, intense peaks | 8.81 | 2.46 | 0.01 | 0.01 | 0.08 |
| Width, all peaks, mm | 0.37 | 0.11 | 0.43[a] | −0.57[b] | −0.40 |
| Width, intense peaks, mm | 0.06 | 0.12 | −0.03 | 0.00 | −0.03 |
| Area under signal $mm^{-1}$ | 873 | 192 | −0.06 | 0.05 | 0.04 |

[a] $p < 0.05$, [b] $p < 0.025$

of the geometry of tissue contact. Thus, going down the length of a muscle, tangential contact with connective tissues is more likely than with perpendicular penetration across layers of perimysium around bundles of muscle fibers. Also, for similar reasons, there may have been differences in the degree of tissue deformation (Swatland, 1995*b*). Measurements made with the probe perpendicularly to the longitudinal axes of muscles gave a similar pattern (Table 3) to those made coaxially (Table 2) but, except for one feature, relationships were weaker. The variance in measuring the area under the fluorescence signal (adjusted for distance) was far lower when measurements were made perpendicularly to muscles than when they were made coaxially, probably because of fewer tangential contacts, and relatively strong correlations with sensory responses were detected. Thus, with multiple regression, the area under the signal and the mean width of all peaks were correlated with tenderness ($R = 0.77$, $p < 0.01$), chewiness ($R = 0.74$, $p < 0.01$) and residual tissue ($R = 0.71$, $p < 0.01$). Stepwise regression of coaxial and perpendicular data combined was viewed cautiously (because of relatively small number of samples), but the strongest prediction model of tenderness ($R = 0.81$, $p < 0.01$) used signal area from perpendicular probing, combined with mean peak width from both perpendicular and coaxial probing (in order of decreasing importance).

In summary, the most robust fluorescence predictor of sensory tenderness was a perpendicular probe measurement of overall fluorescence (area under the fluorescence signal $cm^{-1}$). Small improvements were gained by further analysis of the signal, but there was no indication of any further advantage from separating major fluorescence peaks from the overall signal. Results were paradoxical, however, because wide peaks of connective tissue fluorescence were associated with tender meat.

## Dynamic analysis of overall toughness

Depth measurements were made at a constant rate (68 Hz) which enabled the depth vector to be reassembled as a histogram, counting the number of data in each millimetre of depth (Figs 2 and 1). Positions at which the probe decelerated as it encountered strong resistance were detected by the number of data that accumulated without any advance in depth. Thus, the higher the histogram column, the longer the probe decelerated at the depth indicated for that column. The actual numbers are unimportant (because they were determined by the data collection rate) and they were normalized (adjusted so that the height of the tallest histogram cell = 1). This enabled the depth vector to be compared with the fluorescence signal in the same graphics frame (Fig. 2, B), allowing subjective examination of the microstructural resistance at a particular depth in the meat with fluorescence at the same depth. When the probe tip encountered resistance from numerous seams of connective tissue at a certain depth (for example, from

**TABLE 3.** Features of way-in fluorescence probe signals made perpendicularly to the longitudinal axes of muscles and their simple correlations (r) with sensory attributes

| Feature | Mean | SD | r | | |
|---|---|---|---|---|---|
| | | | Tenderness | Chewiness | Residual tissue |
| Frequency $cm^{-1}$, all peaks | 3.37 | 0.92 | −0.20 | 0.23 | 0.13 |
| Frequency $cm^{-1}$, intense peaks | 0.49 | 0.57 | −0.33 | 0.40 | 0.25 |
| Intensity, all peaks | 6.16 | 0.92 | −0.25 | 0.28 | 0.13 |
| Intensity, intense peaks | 7.50 | 4.65 | −0.05 | 0.09 | 0.02 |
| Width, all peaks, mm | 0.51 | 0.24 | 0.39 | −0.30 | −0.29 |
| Width, intense peaks, mm | 0.24 | 0.61 | 0.34 | −0.33 | −0.24 |
| Area under signal $mm^{-1}$ | 880 | 5 | 0.57[a] | −0.61[a] | −0.58[a] |

[a] $p < 0.01$

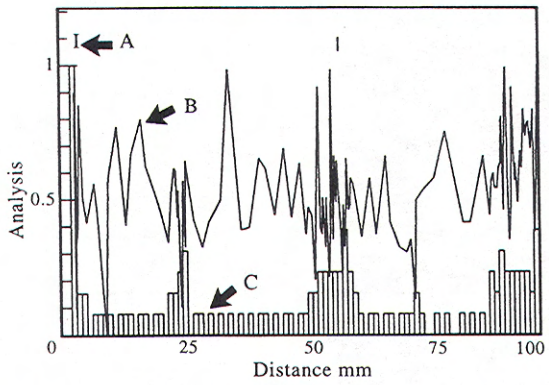


**FIG. 2.** Example of dynamic analysis of the way-in signals from the toughest sample with the probe measuring coaxially along muscle length. The analysis shows three aspects: depths at which the probe tip recoiled (A), the fluorescence signal (B), and velocity histograms (C).

50 to 60 mm in Fig. 2), data accumulated in the histogram cells for that depth (as the probe decelerated) close to where the window detected numerous seams of fluorescent connective tissue. At the top of the graphics frame, above the normalized data (Fig. 2, A) are markers whose x-axis coordinates show the depths at which the probe actually stopped or went in a reverse direction.

Not all the samples had logical relationships between internal connective tissue structure and probe penetration dynamics (as in Fig. 2), but this was expected because connective tissue is only one of several possible causes of toughness. However, the comparison of fluorescence signals with dynamic analysis explained the paradoxical effect noted earlier (tender meat having wide fluorescence peaks). In tough parts of samples (for example, from 23 to 25 mm, 50 to 60 mm, and 85 to 95 mm in Fig. 2), the fluorescence peaks tended to be frequent and narrow, subtending a relatively low area under the signal. Tender samples which lacked these zones of toughness tended to have less frequent, wide peaks subtending a high area under the signal. Thus, the relationships revealed in Fig. 2 provided the key to

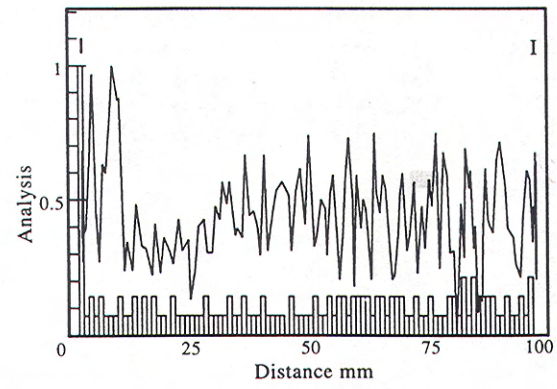


**FIG. 3.** Example of dynamic analysis of the way-out signals from the most tender sample with the probe measuring coaxially along muscle length.

understanding the otherwise paradoxical relationship between the width of fluorescence peaks and sensory evaluation, which also had been detected in an earlier study on beef from immature cattle (Swatland *et al.*, 1995*a*). In mature beef, however, the effect acts the other way (wide peaks are associated with tough beef; Swatland *et al.*, 1995*b*) and, with equal numbers of youthful and mature beef, could be lost.

When the probe tip encountered resistance, the momentary recoil often generated a disordered sequence of numbers in the otherwise smoothly increasing way-in depth vector. For the calculation of parameters to describe the extent and magnitude of this disorder, the depth after a disorder was used as a reference from which to determine the length of the disorder. For example, consider an imaginary sequence ($n=6$) of integer depth measurements, 1, 2, 3, 3, 4, 5 mm. If the measurements were taken at a constant rate, it is evident that the probe paused for 1 measuring cycle at 3 mm. This would have been logged as a disordered incidence of 1/6. Another imaginary sequence ($n=7$) where the probe bounced at 3 mm, such as 1, 2, 3, 2, 3, 4, 5 mm would have been logged as a disordered incidence of 2/7. Real numbers, the full result of analogue to digital conversion without rounding, were used for actual calculations. Similar effects occurred as the probe was withdrawn, when the rear barb of the arrow-head tip of the MQM probe snagged on seams of connective tissue.

Dynamic analysis of way-in signals was not particularly useful for the particular set of meat cuts examined, although the technique appeared to be working because the mean distance of disorder was correlated with chewiness ($r=0.45$, $p<0.05$). For the way-out signals of probe measurements made coaxially from muscles, however, there were relatively strong simple correlations ($p<0.005$) of the incidence of disorder with tenderness ($r=0.72$), chewiness ($r=-0.72$) and residual tissue ($r=0.72$). When the meat was tough, as in Fig. 2, the probe delayed in regions where there were numerous, narrow fluorescence peaks, before moving to the next region. For tender meat (Fig. 3), the probe moved rapidly and smoothly through the meat so that velocity irregularities associated with starting and stopping the probe were the numerator to a smaller denominator (less total measurements because probe did not often decelerate). With stepwise multiple regression, way-out disorder in depth was the primary predictor of tenderness ($R=0.87$, $p<0.01$), chewiness ($R=0.90$, $p<0.01$) and residual tissue ($R=0.87$, $p<0.01$). Probe measurements made perpendicularly to muscles had a similar pattern of relationships to coaxial measurements, but correlations with tenderness ($R=0.62$, $p<0.05$), chewiness ($R=51$, NS), and residual tissue ($R=0.60$, $p<0.01$) were weaker.

In summary, a strong correlation of toughness with dynamic analysis was detected ($r=0.72$, $p<0.005$), but the relationship was complex and may not have been robust. The main value of dynamic analysis was that it

explained the relationships detected between fluorescence and sensory responses.

### Oca CT-probe

It was not expected that the OCA CT-Probe would give exactly the same results as the modified Danish MQM probe because of optical differences in window geometry affecting the resolution of fluorescent structures. For the OCA CT-Probe, the best predictor was the frequency of fluorescent peaks, which was correlated with tenderness ($r = -0.48$, $p < 0.05$), with chewiness ($r = 0.44$, $p < 0.05$) and with residual tissue ($r = 0.57$, $p < 0.025$). The two parameters selected by stepwise regression to predict sensory tenderness from OCA CT-Probe data were the incidence of major peaks $cm^{-1}$ and the mean width of major peaks ($R = 0.61$, $p < 0.05$).

These results emphasize an important feature of CT-Probe usage: that the information contained in fluorescence signals is relative rather than absolute, and users must be prepared to optimize measuring procedures and signal analysis on-site, using representative samples of the general population to be graded. Relationships found between fluorescence and sensory evaluation in certain types of meat cannot automatically be assumed to apply to all situations. Far from making sensory evaluation redundant, this increases the need for sensory evaluation to verify and calibrate on-line measuring methods. There is much to commend combining both methods, because only sensory evaluation can explain consumer responses, while only on-line measurement can be applied to vast numbers of carcasses or meat cuts in a commercial enterprise.

In summary, independent verification using measurements made by a different operator with a different probe supported the validity of relationships between connective tissue fluorescence and sensory evaluation of tenderness, but confirmed the necessity for sample specific, on-site calibration.

## CONCLUSIONS

1. Connective tissues made an appreciable contribution to the sensory toughness of commercially competitive cuts of beef from different sources, despite differences in the post-mortem treatment of samples and complete cooking of small cubes of meat for 20 min to an internal temperature of 70°C
2. Sensory evaluation of cooked beef toughness could be partly predicted by rapid, relatively non-destructive measurements of connective tissue fluorescence made on the raw meat.
3. Dynamic analysis was useful in helping to explain correlations of fluorescence with sensory evaluation.
4. Sensory evaluation was essential for the verification and calibration of on-line measurements.

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