EFFECT OF TIME-TEMPERATURE COMBINATIONS ON WARNER-BRATZLER SHEAR PARAMETERS IN BEEF MUSCLES.

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INTRODUCTION.

Estimates of the relative contribution of the two main structural components of tenderness -the myofibrillar and the connective tissue strength- have been the subject of many investigations. Instron adhesion measurements have been used for determination of connective tissue strength (Bouton and Harris 1972 a; 1972 c) and Warner-Bratzler (WB) shear force value (Bouton and Harris 1972 b; Bouton et al. 1973) or Instron tensile strength measurements (Bouton and Harris 1972b; 1972c; Penfield et al. 1976) have been used as estimates of myofibrillar strength. Rhodes et al. (1972) studied force-deformation curves from roasted meat samples by using Volodkevich jaws and suggested that some of the parameters measured were related to the mechanical properties of different elements of the meat structure. Studies on the force-deformation curves obtained by using the WB shear device have shown that treatments influencing predominantly the muscle fibers such as aging, cooking and myofibrillar contraction affect mainly the initial force values, whereas differences between the initial yield and peak force values reflect changes due to connective tissue differences (Bouton et al. 1975). In studies on texture of beef semitendinosus by using WB deformation curves, consistent differences in yield position were found between the yield taken as the myofibrillar strength (termed WB M-force) resp. the connective tissue strength (termed WB C-force) (Møller 1980). Increasing the end point temperature from 60°C to 80°C was found to increase the WB M-force and to decrease the WB C-force. Also, the WB M- and C-force appeared to be more significant estimators of sensoric evaluations of tenderness and collagen solubility than the usual measured WB peak force value.

The purpose of the present investigation was to make further studies on the WB parameters as influenced by time at different final temperatures in beef semitendinosus and biceps femoris. Rectangular muscle strips were cooked by water bath method and beside the WB parameters, solubility of collagen, cooking loss and sensoric evaluations of tenderness were measured.

MATERIALS AND METHODS

Six semitendinosus (ST) and biceps femoris (BF) muscles from 2-4 years old cows were obtained from a local slaughter-house. After 2 days aging at 6°C, the muscles were excised and further stored for six days at 4°C. Muscle pH values ranged from 5.3-5.5 . Then the muscles were wrapped in polyethylene bags and frozen at -18°C for no longer than ten weeks. The meat was allowed to thaw slightly to permit the removal of 24 subsamples, 2 x 2 x 5 cm, cut parallel to the muscle fibers. Subsamples of ST muscles were subjectively classified as R (Red), M (intermediary) and W (White) according to sample location. The subsamples were weighed and placed in 100 ml glass tubes, randomly assigned to the various groups of heat treatment and stored in the refrigerator at 2-3°C 16 hr. before heating.

Different heat treatment were assigned by using three different endpoint temperatures and three different times at each final temperature as shown in table 1. A 0,9% NaCl solution was added to the tubes just covering the meat blocks. For temperature control a thermocouple was inserted lengthwise into the center of the sample. The glass tubes containing the meat sample were placed in a water bath (Heto type 02 PG623) at 35°C, and allowed to equilibrate at that temperature. The water bath was then programmed to produce the assigned temperature gradient. After removal of samples from the water bath, the meat blocks were rinsed by tap water and immediately placed in the refrigerator at 2-3°C for no longer than 24 hr., the blocks were reweighed and cooking losses calculated.

Samples of rectangular cross section, 1,0 x 1,0 cm, and 4 cm along the fiber axis were cut from the cooked meat. These samples were sheared at right angles to the fiber axis using a WB shear blade with a triangular slot angular cutting edge (Instron Cat, No. T 372-66).An Instron Universal Testing Maschine table model 1140 was used to measure the WB shear forces with the following operating parameters: Crosshead speed 50 mm pr. minute and chart speed 100 mm pr. minute. The distance travelled by the shear blade was standardized to 2,5 cm in all measurements which corresponds to a 5 cm distance on the recorder chart. The final position of the cross head was standardized to .7 cm from the point where the blunt WB shear blade had been cutting through the meat sample. From the WB deformation curves three parameters were measured. (a) WB M-force or initial yield taken as a measurement of the myofibrillar component of tenderness. This parameter was measured on the shear deformation curve as the peak occuring approximately 2,5 cm from the starting point of the recorder. (b) WB C-force taken as a measurement of the connective tissue component of tenderness. This parameter or final yield was measured on the shear deformation curve as the peak occuring approximately 3,5 cm from the starting point of the recorder, which almost corresponds to the position of the cross head, where the shear blade had cut through the meat sample. (c) WB peak force taken as the maximum force recorded i.e., the usual measurement when using the WB shear device. Typical shear deformation curves from ST muscles are earlier shown (Møller 1980).

The extractable collagen was measured as earlier described (Møller 1980) on a 6 g sample of the minced meat after heat treatment equivalent to the treatment given to the meat blocks, but only in the experimental groups referring to treatment A. A five member taste panel of laboratory staff was used. The panel members were asked to rate ease of first biting at right angle to the fiber axis and juiciness on a 9 point scale. The end points of the two scales were defined as extremely tender/juicy (=5) and extremely tough/dry (=1). The panel members were also asked to count the number of chewing movements necessary before swallowing. The meat was presented after equilibrating to room temperature as approximately 1 cm^3 cubes, i.e. the cubes remaining after the WB measurements. The results including the total material were analyzed by the following design (a), while differences between sections of the ST muscle were investigated by design (b).

(a)

Effect.	Collagen analyses d.f.	Other parameters d.f.
Muscle (M)	1	1
Animal (A)	5	5
Final Temperature (FT)	-	2
Time (T)	2	2
Interactions		
T x FT	-	4
M x FT	-	2
M x T	2	2
Model	10	18
Error	25	89
Total	35	107

(b)

Effect	d.f.
Section of muscle	2
Animal	5
Final temperature (FT)	2
Time (T)	2
Interaction FT x T	4
Model	15
Error	146
Total	161

Least significant differences were obtained using the error term from the analysis of variance. Level of significance is denoted by *** (P< .001), ** (P< .01),* (P< .05) and NS (not significant). All calculations were carried out using the Statistical Analysis System (Barr et al. 1976).

RESULTS.

Means and F-values from the analysis of variance for WB parameters, sensoric evaluations, cooking loss and collagen solubility are presented in table 2. As the interactions muscle x temperature and muscle x time were nonsignificant except for cooking loss, the two muscles are treated together in the statistical analysis.

WB C-force and WB peak force differed with respect to muscles (P < .001) while no difference was found in WB M-force between the two muscles. This indicate that the myofibrillar shear strength measured after heat treatment seems identical in ST and BF muscles whereas the connective tissue strength is significant higher in the BF muscle (P < .05). Supporting that observation BF muscles contained higher amount of total collagen and required more chewing movements. The ease of biting, juiciness and percent cooking loss did not differ between muscles.

The variation of final temperature revealed significant effects on WB M-force and WB C-force (P < .001) but final temperature had no influence on the WB peak force values. The WB M-force increased only between 60°C and 70°C (P < .05) while WB C-force decreased both between 60°-70°C and 70°-80°C. The nonsensitivity of the WB peak force value in measuring heat induced changes due to final temperature was clearly demonstrated from these results. The sensoric evaluations including ease of biting (P < .001), no. of chewings (P < .05) and juiciness (P < .001) were all affected by final temperature. The amount of cooking loss increased both between 60-70°C and 70-80°C (P < .05).

Among the WB parameters only WB C-force was found to be affected by prolonged cooking time (P < .05). As seen from table 1 a 30 min. period of prolonged cooking decreased the WB C-force. A 30 min. additional cooking time provided still lower WB C-force but the difference between 30 min. and 60 min. cooking time was not significant. Prolonged cooking time had no influence on sensoric evaluations of ease of biting and no. of chewings. On the other hand the percentage of cooking loss and solubility of collagen increased due to prolonged cooking time (P < .001).

The significant interactions between final temperature and time as found for WB C-force and WB peak force was due to a much larger effect of prolonged cooking time from 0 to 30 min. at 60°C than at 70° or 80°C. The percentage of cooking loss and juiciness also showed time x temperature interactions caused by a lesser difference between 30 and 60 min. cooking time at 70°C and 80°C final temperature, than at 60°C final temperature.

Means and F-values from the analysis of variance for WB M-force and WB C-force in the different locations in ST muscles are presented in table 3. As shown, the WB M-force did not differ according to intramuscular location. On the other hand, the WB C-force seemed to be significant higher in the red part of the ST muscle. In accordance to this observation measurement of total collagen in ST muscles from four additional animals showed, that the red section contained significant higher amount of total collagen as compared to the white section (P < .01). Main effects as shown by the F-values in table 3 were in accordance to the total material as earlier described.

Simple correlation coefficients between the various tenderness parameters measured in the experiment are presented in table 4 for BF and ST muscles. The WB parameters M-force and C-force were correlated to the WB peak shear force in both muscles ($P < .001$). The correlation between WB M-force and WB C-force was also highly significant in BF ($P < .001$) whereas no relationship between these parameters appeared in the ST muscles. This apparent deviation is not quite understood. The panel score for ease of biting was higher correlated to the WB M-force and the WB peak force value than to the WB C-force. The panel evaluation for the number of chewing movements was higher correlated to all WB parameters in BF muscles than in ST muscles. The correlation coefficients between WB M-force and percent cooking loss were highly significant ($r = 0.6-0.7$, $P < .001$).

DISCUSSION.

Several years ago it was proposed that meat tenderness could be resolved into at least two different components: "Background toughness", which is the toughness due to presence of connective tissue and other stromal proteins, and "Actomyosin toughness", which is the toughness that can be attributed to the myofibrillar proteins (Locker 1960, Marsh and Leet 1966). This simple division of tenderness into two components has been extremely useful in interpretations of various treatments and in devising new experiments to investigate the molecular basis for variations in meat tenderness.

The present investigation focused on the separate effects of heat induced changes on myofibrillar and connective tissue strength in beef BF and ST muscles. Using the WB shear device it was found that the yield taken as a measurement of myofibrillar toughness (WB M-force) and the yield taken as a measurement of connective tissue toughness (WB C-force) occurred at fairly well defined positions on the force-deformation curves. The results showed with those of an earlier study (Møller 1980) that heat induced changes in meat due to increased final temperature were significantly related to changes in WB M-force and WB C-force whereas the WB peak force values showed lower relationship. Obviously, some information is lost when only peak force value is taken as a measurement of tenderness.

Earlier workers (Laakonen et al. 1970, Penfield and Meyer 1975, Hearne et al. 1978) have reported a greater decrease in WB peak shear value from 50-60°C but no significant changes in shear values with heating from 60 to 70°C in beef semitendinosus. Dubé et al. (1972) found that WB shear values increased with increasing cooking temperature above 60°C for bovine meat samples. In contrast, Paul et al. (1973) found that end point temperature did not influence shear values of bovine ST cores heated from 58 - 82°C. This disaggreement from earlier reports is probably caused by the insensibility of the WB peak shear values in reflecting heat induced changes in a given sample of meat due to the antagonistic changes in the mechanical properties of the myofibrillar and connective tissue proteins.

Bouton et al. 1975, found no significant reduction in WB initial yield force or WB peak force values as measured in ST muscles obtained from 2-4 yr old steers by using prolonged cooking time at 60°C from 1-24 hr. . In the present study only prolonged cooking time up to 60 min. was used, and among the WB parameters only WB C-force was found to be affected. However, the latter effect was most pronounced at 60°C, which is in the region of the shrinkage temperature of collagen. The prolonged cooking period at 60°C resulted in higher amount of soluble collagen but also in a significant increase in cooking loss. The cooking tenderizing of beef muscles as represented in this study was found optimal by using either a 30 min. cooking time at 60°C or 70°C as final temperature without holding period when also the effect of heat treatment on cooking loss is considered.

Comparisons between BF and ST muscles showed that the main difference in tenderness was caused by a higher connective tissue component of toughness in the BF muscles whereas the myofibrillar component was similar in the two muscles.

The WB M-force and the WB C-force were generally higher correlated to sensoric evaluations of tenderness as compared to the WB peak shear values. Therefore, it is concluded that the possibility for interpretation of the effect of different heat treatment on meat tenderness may be increased by measuring the individual response of the myofibrillar and connective tissue components as suggested in this study.

Table 1. Experimental design. Each group contains three samples from each muscle and animal.

Time at final temperature	Final temperature[1)] 60°C	70°C	80°C
0 min.	A1	B1	C1
30 min.	A2	B2	C2
60 min.	A3	B3	C3

[1)] The temperature was increased from 35° to the final temperature by 0.6°C/min. in all groups.

Table 2. Means, F-values from analysis of variance and significant differences among means for Warner-Bratzler (WB) parameters, sensoric evaluations, cooking loss and collagen solubility.

Source of variation	WB parameters, kg			Sensoric evaluations			Collagen		
	M-force	C-force	Peak force	Ease of biting 1)	No. of chewings	Juiciness 1)	% Total 2)	% Soluble 3)	% Cooking loss
Muscle (M)									
Biceps femoris	4.33^{a}	5.62^{a}	5.95^{a}	3.17^{a}	19.4^{a}	3.02^{a}	1.42^{a}	9.12^{a}	36.8^{a}
Semitendinosus	4.37^{a}	3.83^{b}	4.79^{b}	3.12^{a}	17.7^{b}	3.01^{a}	$.91^{b}$	8.97^{a}	36.3^{a}
F-values	$.07^{NS}$	93.69 ***	37.29***	$.39^{NS}$	9.30 **	$.01^{NS}$	85.29 ***	$.41^{NS}$	1.88^{NS}
Final Temperature (FT)									
60°C	3.44^{a}	5.22^{a}	5.36^{a}	3.69^{a}	17.5^{a}	3.85^{a}	1.16	9.05	27.4^{a}
70°C	4.60^{b}	4.71^{b}	5.39^{a}	3.01^{b}	19.6^{b}	2.88^{b}	-	-	38.3^{b}
80°C	5.01^{b}	4.24^{a}	5.35^{a}	2.74^{c}	18.5^{ab}	2.32^{c}	-	-	43.9^{c}
F-values	31.30 ***	9.33 ***	$.01^{NS}$	42.89 ***	4.42 *	177.74 ***	-		594.55***
Time at final temp. (T)									
0 min.	4.19^{a}	5.09^{a}	5.62^{a}	3.23^{a}	18.9^{a}	3.26^{a}	1.14^{a}	7.91^{a}	31.9^{a}
30 min.	4.38^{a}	4.59^{b}	5.23^{a}	3.09^{a}	18.4^{a}	3.01^{b}	1.19^{a}	9.16^{b}	37.8^{b}
60 min.	4.48^{a}	4.48^{b}	5.26^{a}	3.11^{a}	18.3^{a}	2.77^{c}	1.15^{a}	10.08^{c}	39.9^{c}
F-values	1.04^{NS}	4.15 *	1.71^{NS}	1.03^{NS}	$.36^{NS}$	18.19 ***	$.29^{NS}$	24.55***	145.95***
Animal									
F-values	17.75***	12.00***	12.74 ***	19.84 ***	13.53***	2.75*	7.39***	2.53	17.04 ***
Interactions									
T x FT	$.68^{NS}$	4.97**	5.39 ***	1.99^{NS}	$.43^{NS}$	8.11 ***	-	-	2.97 *
T x M	$.43^{NS}$	1.25^{NS}	$.30^{NS}$	1.17^{NS}	$.34^{NS}$	1.50^{NS}	$.08^{NS}$	2.59^{NS}	$.37^{NS}$
FT x M	$.88^{NS}$	1.05^{NS}	$.69^{NS}$	2.67^{NS}	2.09^{NS}	$.34^{NS}$	-	-	4.49 *

Means with same subscript are not significant different at the 5% level

1) 5 extremely tender/juicy, 1=extremely tough/dry. 2) % of total mass 3) % of total collagen

Table 3. Means, F-values from analysis of variance and significant differences among means for Warner-Bratzler (WB) parameters in different sections of M. Semitendinosus.

	WB M-force	WB C-force
Red section	4.51^{a}	4.29^{a}
Intermediary section	4.36^{a}	3.90^{b}
White section	4.25^{a}	3.13^{c}
F-values for:		
Section	1.87^{NS}	22.8***
Final temperature	45.7 ***	19.9***
Time	$.88^{NS}$	14.6***
Animal	9.85 ***	14.4***
Final temperature x time	2.29^{NS}	6.3***

Means with same subscribt are not significant different at the 5% level.

Table 4. Correlations between Warner-Bratzler (WB) parameters, sensory evaluation and cooking loss in M. Biceps Femoris [1)] and M. Semitendinosus [1)] (No. of samples 54)

	1	2	3	4	5	6	7
1. WB M-force		.51***	.71***	-.89***	.72***	-.66***	.58***
2. WB C-force	.07		.95***	-.45***	.55***	-.02	-.14
3. WB peak force	.55***	.82***		-.61***	.62***	-.21	.03
4. Ease of biting	.74***	-.20	-.53***		-.73***	.78***	-.66***
5. No. of chewings	.26	.31*	.28*	-.45***		-.41**	.26
6. Juiciness	-.63***	.42**	-.03	.52***	-.01		-.87***
7. % cooking loss	.70***	-.46***	-.02	-.46***	.04	-.87***	

[1)] correlations above the diagonal represents Biceps Femoris and correlations below the diagonal represents Semitendinosus.

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