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Study on factors interfering in sarcomere length measurements.

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Introduction

Sarcomere length measurements have been widely used as an index of meat toughness, as far as it is related to muscle contraction. However does not seem that other factors, then temperature, have been studied in details for their influence in post mortem muscle contraction. In the following, observations are presented about the influence of muscle, time of preservation, and temperature, on sarcomere length measurements using two different methods.

Materials and Methods

The study was carried out in Longissimus dorsi and Trapezius muscles of 18 Friesian beef animals. Muscle samples were taken one hour post-mortem and preserved for six days in $+1^{o}C$ and others in $+14^{o}C$.
Sarcomere length measurements in muscle homogenates took place at 5,30,100 and 150 houres post-mortem, from both temperature levels of sample preservation. For the preparation of homogenates samples wheihting 200 ± 5mg were put in 20ml of 1% formalin and macerated in a Turrax apparatus. For improved visualisation of sarcomeres a few drops of 1% solution of methylene blue were put in the homogenates. After sedimentation a Pasteur pipette was used for recovering one drop of the sediment which was placed on a microscope slide for observation. An occular micrometer was used, after appropriate calibration for measurements of sarcomere lengths. In each preparation the length of ten sarcomeres was measured in 20 different muscle fiber fragments. After the same preservation periods,pieces of tissue were taken for histologic examination. For this the samples were routinely prepared by fixing in 10% formalin, embbeding in parrafin wax and staining in HXE. Sarcomere length measurements took place as it is described for homogenates.

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For each group of measurements, which refered to certain time after slaughtering for samples preserved in certain temperature, mean values, standard deviations and standard errors were calculated. For all the groups of measurements factorial analysis of variance was performed for two muscles, two temperatures and four preservation periods. The statistical significance of the differences, for the mean values for different preservation periods for all the combinations of muscles and temperatures, was tested by means of one way analysis of variance and the Duncan - Kramer test or the Student's t test analysis.

Results

In Tables I and II the mean values of sarcomere length measurements, using the two different methods of measuring, are given seperately for the two muscles. Also, the statistical significance of the differences

TABLE I

Means ± standard error of the mean (in μ) of sarcomere length measurements in Longissimus dorsi muscle homogenates and histologic sections

Preservation Temperature	+1°C					+14°C				
Repetition of measurements (houres post mortem)	1	5	30	100	150	1	5	30	100	150
Muscle homogenates	—	1.58 ±0.04	1.47 ±0.04	1.44 ±0.04	1.48 ±0.05	—	1.66 ±0.04	1.71 ±0.03	1.72 ±0.02	1.80 ±0.03
Histologic sections	1.69 ±0.02	1.92 ±0.06	1.56 ±0.03	1.57 ±0.05	1.54 ±0.05	1.69 +0.03	2.11 +0.04	1.80 +0.05	1.89 +0.04	1.92 +0.03
Statistical significance of differences (Student's t test)	—	$p<0.001$	N.S.	N.S.	N.S.	—	$p<0.001$	N.S.	$p<0.001$	N.S.

between the two methods of mesuring sarcomere length, for each group of measurements made in the same muscle after the same time and temperature preservation, are shown.

TABLE II

Means ± standard error of the mean (in μ) of sarcomere length measurements in Trapezius muscle homogenates and histologic sections

Preservation Temperature	±1°C					±14°C				
Repetition of measurements (houres post mortem)	1	5	30	100	150	1	5	30	100	150
Muscle homogenates	—	1.71 ±0.04	1.48 ±0.03	1.54 ±0.05	1.60 ±0.07	—	1.67 ±0.02	1.70 ±0.04	1.78 ±0.04	1.71 ±0.04
Histologic sections	1.65 ±0.03	1.95 ±0.05	1.76 ±0.03	1.73 ±0.04	1.59 ±0.05	1.65 ±0.03	2.16 ±0.04	1.85 ±0.05	1.94 ±0.04	1.97 ±0.03
Statistical significance of differences (Student's t test)	—	$p<0.05$	$p<0.01$	$p>0.05$	N.S.	—	$p<0.001$	N.S.	$p<0.02$	$p<0.001$

TABLE III

Table of factorial analysis of variance for the changes in the values of sarcomere length measurements in homogenates for two muscles, two preservation temperatures and four repetitions of measurements

Source of Variation	Degrees of Freedom	Mean Squares	F Value	Statistical Significance
Blocks	17	0.15211	7.167	N.S.
Muscles	1	0.14987	7.062	$p<0.01$
Temperatures	1	2.42550	114.288	$p<0.01$

Time	*3*	*0.06091*	*2.870*	*N.S.*
Muscles X Temperatures	*1*	*0.17552*	*8.270*	*p 0.01*
Temperatures X Time	*3*	*0.21882*	*10.311*	*p 0.01*
Muscles X Time	*3*	*0.02851*	*1.343*	*N.S.*
Muscles X T° X Time	*3*	*0.02484*	*1.170*	*N.S.*
Error	255	0.02122		
Total	287			

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Table of factorial analysis of variance for the changes in the values of sarcomere length measurements in histologic sections for two preservation temperatures and four repetitions of measurements

Source of Variation	Degrees of Freedom	Mean Squares	F Value	Statistical Significance
Blocks	12	0.04747	1.621	N.S.
Muscles	1	0.27769	7.378	$p < 0.01$
Temperatures	1	3.68889	98.022	$p < 0.01$
Time	3	0.82038	21.799	$p < 0.01$
Muscle X Temperatures	1	0.07846	2.085	N.S.
Temperatures X Time	3	0.12741	3.313	$p < 0.05$
Muscles X Time	3	0.08415	2.236	N.S.
Muscles X T° X Time	3	0.03455	0.918	N.S.
Error	180	0.03763		
Total	207			

In Tables III and IV appear the factorial analysis of variance for two muscles, two preservation temperatures and four repetitions, for the two methods. The values according to experimental factors as well as the significance of differences are shown in Table V.

TABLE V

Sarcomere length mean values (in μ) according to experimental factors

Sources of Variation	Homogenates	Histologic Sections
Muscles		
Longissimus dorsi	$1.61^{a,k}$	1.79^{k}
Trapezius	$1.65^{a,k}$	1.84^{k}
Temperatures		
+1°C	$1.54^{c,k}$	$1.69^{b,k}$
+14°C	$1.72^{c,k}$	$1.95^{b,k}$
Preservation time (houres post mortem)		
1	-	1.67^{k}
5	$1.65^{d,e,k}$	2.00^{k}
30	$1.59^{d,f,k}$	$1.73^{e,f,g,k}$
100	$1.62^{d,e,f,k}$	$1.77^{e,k}$
150	$1.65^{d,e,i}$	$1.75^{e,f,i}$

NOTE

-Vertical pairs of values, when marked with a,b,c, are statistically significantly different respectively at $p<0.05$, $p<0.01$ and $p<0.01$ level.
-Vertical groups of values marked with d,e,f,g,h,are not statistically significantly different (level at least $p<0.05$) respectively from the first, second, third, fourth highest value of the group.
-Horizontal pairs of values, when marked with i,j,k, are statistically significantly different respectively at $p<0.05$, $p<0.01$ and $p<0.001$ level.

Discussion

Two are the most important differences in the structure of the L.Dorsi and Trapezius muscles which were taken in acount in selecting them for the present study. First,beef animal Trapezius muscle is a red muscle and L.Dorsi is a white one. Evidence of the histochemical profile of both of these muscles has been presented elswhere. (Rantsios, 1981; Rantsios and Papavasileiou, 1981). Second,there is a marked difference in the amount of content of connective tissue between the two muscles. According to Bendall (1967), the content in collagen is 8.6% and elastin 0.65% for beef animal Trapezius muscle, while the same parameters in L.Dorsi are 2.3% and 0.07 respectevily. Data presented so far are not conclusive about the role of the muscle histochemical profile in the formulation of the values of sarcomere length measurements. For example the relationship between beef muscle redness and cold shortening was questioned earlier (Bendall, 1973). In a more recent study (Rantsios, 1981) values for sarcomere length measurements did not appear to be statistically significantly different between red, intermediate and white fibers in the same muscle. One therefore could consider the implication of connective tissue in post mortem muscle contractions. Connective tissue implication in meat toughness, because of muscle contraction, have been already indicated by some workers (Bouton et al, 1975; Rowe, 1974).

Considering the significance of differences which are shown in tables I and II, it could be suggested that the tendency for significantly higher sarcomere length measurement values in histologic sections against muscle homogenates is stronger in Trapezius muscle rather then is L.Dorsi.The same is more clear in table V where the values are shown according to experimental factors. Again values for the Trapezius muscle are significantly higher. Also, it is of some interest to be noted that,even in cases in which statistically signifficant deferences do not exist,the values for Trapezius muscle are always higher; (compare tables I and II). In addition (again table V) measurements in histologic sections are significantly higher from measurements in homogenates for both muscles. Further to that, it should be mentioned that the values from the two ways of measuring sarcomere length have a statistically significant corelation coefficient ($r=0.7564$; $p<0.001$).