4-21 ESTIMATION OF Z-LINE DESTRUCTION DURING MUSCLE RESERVATION

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Introduction

Measurements of Z-line destruction have been widely used as an indicator of the advancement of meat maturation. This can be demonstrated by muscle fiber fragmentation imposed by mechanical action. The phenomenon and its measurement are particularly important as an index of meat toughness, due to Z-line destruction. In the following, the results of using various methods for measuring muscle fiber fragmentation and the influence upon it, of muscle, time and temperature of preservation are presented

Materials and Methods

The study was carried out in Longissimus dorsi and Trapezius muscles of eighteen Friesian beef animals. Muscle samples were taken one hour post-mortem and preserved for six days in +1°C and others in +14°C. Measurements of Z-line destruction in muscle homogenates took place at 5, 30, 100 and 150 hours post-mortem, from both temperature levels of sample preservation. A technique, described originally by Love (1960) and used in fish muscles, was used for the nephelometric estimation of muscle fiber fragmentation index. Accordingly, for securing standard length of intact muscle fibers, which were to be homogenised, a specially modified knive, with two parallel blades, three mm apart, was used. Samples were taken from places with no macroscopically visual connective tissue elements, cutting perpendicularly to muscle fibers direction. For the preparation of homogenates samples weighing 200±5mg were put in 20ml of 1% formalin and macerated in a Turrax homogeniser. Absorbance was measured at 430nμ after agitation with a Vibro-Fix of IKA-Werk apparatus.

A Pasteur pipette was used, after sedimentation of the homogenate, for recovering one drop of the sediment. This was placed on a microscope slide for observation. Lenght of muscle fiber fragments was measured, by using an occular micrometer and appropriate calibration against a microscopic slide, bearing divisions at 10μm intervals level. In each preparation the length of 20 muscle fiber fragments was measured.

Other samples, taken again as it is described above, were homogenised in incubation medium for the demonstration of succinic dehydrogenase (SDH). This medium consisted of:

Phosphate buffer, pH 7.4	17ml
Sodium succinate sol., 0.2 M	1ml
Tetranitrotetrazolium salt, 0.5mg/ml	2ml

An incubation time of 15 min., at +37°C, was following. After that, muscle fiber fragments length was measured, in microscopic preparations, using the method described above. Measurements took place in 20 muscle fiber fragments, for each of the histochemical muscle fiber types, I (red), II (white) and intermediate.

Pieces of muscle tissue were, also, taken at the same afforementioned periods of preservation, for histologic examination. These samples were routinely prepared by fixing in 10% formalin, embedding in paraffin wax and staining in HXE. Destruction of Z-line in muscle fibers was subjectively evaluated in the histologic sections and the intensity of destruction was graded, using a five point scale.

For each group of measurements, which referred to certain time from slaughtering, for samples of the two different muscles 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 muscle fiber fragmentation indexes, using the four different methods of measuring, are given separately for the two muscles. There are no statistically significant differences in fiber fragment length between measurements according to muscle fiber type. Therefore, mean values, in each case, from the measurements according to the three different histochemical muscle fiber types, were produced. These means are the ones presented in Tables I and II in the line "muscle homogenates (histochemical)". In Tables III, IV, V and VI appear the factorial ana-

lysis of variance for two muscles, two preservation temperatures, five repetitions of measurements for the histologic and four repetitions for the other methods. The values, according to experimental factors as well as the significance of differences, are shown in Table VII.

TABLE I

Mean ± standard error of the mean of muscle fiber fragmentation indexes in Longissimus dorsi muscle

Preservation Temperature	+1°C					+14°C				
Repetition of measurements (hours post mortem)	1	5	30	100	150	1	5	30	100	150
Nephelometric determination	-	0.79 ±0.05	0.86 ±0.05	1.08 ±0.05	1.07 ±0.05	-	0.78 ±0.06	0.86 ±0.06	1.10 ±0.06	1.11 ±0.04
Muscle homogenates	-	606.61 ±63.81	801.53 ±51.93	620.02 ±65.73	454.82 ±36.77	-	558.17 ±63.85	726.57 ±49.89	361.73 ±36.90	287.30 ±25.67
Muscle homogenates (histochemical)	-	248.77 ±12.93	308.27 ±25.25	314.51 ±11.78	206.99 ±9.47	-	227.74 ±22.46	287.53 ±18.63	227.12 ±13.70	227.73 ±18.18
Histologic sections	1.50 ±0.22	2.20 ±0.30	1.61 ±0.24	3.00 ±0.27	2.93 ±0.40	1.50 ±0.22	2.00 ±0.38	2.16 ±0.24	3.16 ±0.32	3.77 ±0.30

TABLE II

Mean ± standard error of the mean of muscle fiber fragmentation indexes in Trapezius muscle

Preservation temperature	+1°C					+14°C				
Repetition of measurements (hours post mortem)	1	5	30	100	150	1	5	30	100	150
Nephelometric determination	-	0.70 ±0.06	0.79 ±0.05	0.91 ±0.05	0.99 ±0.05	-	0.73 ±0.04	0.81 ±0.07	1.09 ±0.06	1.09 ±0.05
Muscle homogenates	-	706.88 ±59.61	592.49 ±45.42	487.06 ±42.90	403.39 ±31.71	-	598.66 ±42.59	602.96 ±33.62	402.79 ±39.42	334.18 ±22.62
Muscle homogenates (histochemical)	-	257.17 ±12.92	262.34 ±11.68	235.52 ±23.87	224.01 ±10.40	-	253.88 ±13.41	279.57 ±11.78	239.14 ±8.67	209.20 ±7.06
Histologic sections	1.30 ±0.13	2.42 ±0.32	2.61 ±0.29	2.87 ±0.39	2.16 ±0.37	1.30 ±0.13	2.00 ±0.58	1.78 ±0.29	2.91 ±0.31	3.53 ±0.29

TABLE III

Table of factorial analysis of variance for the changes in the values of nephelometric determination of muscle fiber fragmentation

Source of variation	Degrees of freedom	Mean squares	F value	Statistical significance
Blocks	17	0.22136	6.179	N.S.
Muscles	1	0.31435	8.775	p<0.01
Temperatures	1	0.17875	4.990	p<0.05
Time	3	1.79613	50.140	p<0.01
Muscles X Temperatures	1	0.09882	2.758	N.S.
Temperatures X Time	3	0.03505	0.978	N.S.
Muscles X Time	3	0.00363	0.101	N.S.
Muscles X T° X Time	3	0.01883	0.525	N.S.
Error	255	0.03582		
Total	287			

TABLE IV

Table of factorial analysis of variance for the changes in the values of muscle fiber fragments measurements in muscle homogenates

Source of variation	Degrees of freedom	Mean squares	F value	Statistical significance
Blocks	17	248358.98101	3.019	N.S.
Muscles	1	4484.12391	0.054	N.S.
Temperatures	1	380050.31320	4.620	p<0.05
Time	3	1709897.38722	20.788	p<0.01
Muscles X Temperatures	1	307940.47818	3.743	N.S.
Temperatures X Time	3	146660.98619	1.783	N.S.
Muscles X Time	3	371759.47218	4.519	p<0.01
Muscles X T° X Time	3	8586.60807	0.104	N.S.
Error	255	85250.11063		
Total	207			

TABLE V

Table of factorial analysis of variance for the changes in the values (mean of the measurements according to the three different histochemical muscle fiber type) of muscle fiber fragments measurements in muscle homogenates, incubated for the demonstration of succinic dehydrogenase

Source of variation	Degrees of freedom	Mean squares	F value	Statistical significance
Blocks	17	45382.75269	10.060	N.S.
Muscles	1	1557.70362	0.345	N.S.
Temperatures	1	32.38112	0.007	N.S.
Time	3	52586.87974	11.657	p<0.01
Muscles X Temperatures	1	218.87550	0.048	N.S.
Temperatures X Time	3	245.40861	0.054	N.S.
Muscle X Time	3	4263.46521	0.945	N.S.
Muscles X T° X Time	3	5168.51128	0.945	N.S.
Error	255	4510.97522	1.145	N.S.
Total	287			

TABLE VI

Table of factorial analysis of variance for the changes in the values of muscle fiber fragmentation evaluations in histologic sections

Source of variation	Degrees of freedom	Mean squares	F value	Statistical significance
Blocks	15	1.62472	2.058	N.S.
Muscles	1	0.09687	0.122	N.S.
Temperatures	1	1.62562	2.059	N.S.
Time	3	21.42282	27.140	p<0.05
Muscles X Temperatures	1	2.03775	2.581	N.S.
Temperatures X Time	3	4.97760	6.306	p<0.05
Muscle X Time	3	1.31362	1.664	N.S.
Muscles X T° X Time	3	1.80375	2.285	N.S.
Error	225	0.78934		
Total	255			

TABLE VII

Muscle fiber fragmentation indexes mean values according to experimental factors

Source of variation	Nephelometric determination	Muscle homogenates	Muscle homogenates (histochemical)	Histologic sections
Muscles				
Longissimus dorsi	0.960[a]	551.73	249.93	2.49
Trapezius	0.894[a]	514.66	245.05	2.44
Temperatures				
+ 1°C	0.919	584.74[c]	244.56	3.43
+14°C	0.950	482.96[c]	247.04	3.55
Preservation time (hours post mortem)				
3	-	-	-	1.40
5	0.75	617.60[d]	247.83[d,f]	2.15[g]
30	0.83	681.40[e]	284.07[e]	2.04[f]
100	1.05[d]	467.90	241.06[e]	2.98[e]
150	1.07[e]	369.92	217.30[f]	2.79[d]

Note

- Vertical pairs of values, when marked with a, b, c, are statistically significantly different respectively at $p<0.05$, $p<0.01$ and $p<0.001$ 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 1st, 2nd, 3rd, 4th highest value of the group.

Discussion

The study of F values appearing in Tables III-VI shows that all measurements depend primarily on the time and secondly on the temperature of preservation. Dependence of muscle fiber gragmentation indexes on time and temperature is a demonstration of faster Z-line degradation in +14°C then in +1°C This, of course, is not surprising and is in agreement with other observations (Henderson et al, 1970 Parish et al, 1973a; Parish et al, 1973b; Dutson and Yates, 1978).

Muscle is influencing nephelometric determinations of muscle fiber fragmentation index (Table III). Trapezius and L. dorsi muscles are different in two respects. 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 elsewhere (Rantsios, 1981; Rantsios and Papavassiliou, 1981). Second in connective tissue content. Connective tissue content in Trapezius muscle is more than three times higher than in L. Dorsi (Bendall, 1973). As it is already mentioned there were no statistically significant differences in muscle fiber fragments length between the three muscle fiber histochemical types. Therefore, differences between muscles due to different histochemical muscle profile do not appear in muscle fiber fragmentation index. The probability then of differences being due to muscle connective tissue rises. This is supported by the fact that mean values for measurements in Trapezius muscles are lower than in L. Dorsi, although they are not always of a statistically significant difference (Table VII) . As a possible explanation is suggested the mechanical prevention of muscle fiber fragmentation, during homogenisation, by connective tissue.

The microscopic measurement of muscle fiber fragments is an absolute index of muscle fiber fragmentation. Nephelometric estimations of the same index show statistically significant correlation coefficient r values with values taken with direct measurements ($r=-0.7451$, $p<0.001$). It could, therefore, substitute the direct measurements being in addition less laborious, easily performed and referred to a larger number of muscle fibers. This method, the way was used in the present study is different from the ones used by other workers (Davey and Gilbert, 1969; Olson et al, 1976; Olson and Parish, 1977; Olson et al, 1977). It is almost identical with the one used in fish muscles (Love, 1960). Two problems are associated with the practical application of the method. They are the standardisation of homogenisation and the quantity of the sample, so that the same homogenisation force to be applied to the same sample size, in order to detect the differences between samples. It has been suggested (Davey and Gilbert, 1969) the homogenate to be reduced to standard protein content, before measuring, by sequential washings, filtrations and dilutions. This is, however, complicated and in addition starting from samples with various muscle fiber lengths produces different muscle fiber fragments lengths after standard mechanical action. In addition the influence of connective tissue cannot be avoided. With the technique suggested originally by Love (1960) and applied almost identical-

ly in the present work these problems are minimized. Standard sample size with standard length of original muscle fibers is secured. It is, therefore, suggested as a method for practical application for estimating muscle fiber fragmentation index in meat. The use of histologic sections for evaluating muscle fiber fragmentation cannot be used for practical application, because, apart from being absolutely subjective, it is time-consuming, in preparing sections, which, in addition, should be perfectly parallel to the direction of muscle fibers. Nevertheless, a statistically significant correlation coeficient r value ($r=0.4657$) at the level of $p<0.05$, with direct microscopic measurements in homogenates allows for using the technique when preservation of preparations is of some value.

Mean values, for each case appearing in Tables I and II, for muscle homogenates (histochemical) were compared, by applying Student's t test, in pairs with mean values for direct microscopic measurements in homogenates, regardless of the histochemical muscle fiber type. Statistically significant differences within each muscle were found in all cases except one. However, shorter muscle fiber fragments, in homogenates in SDH incubation medium, do not imply necessarily originally shorter length. Sodium succinate in the incubation medium, for the SDH demonstration, is suggested as a source of energy for muscle fibers contraction. This is in contradiction with the lack of statistically significant differences between muscle fibers of different histochemical type treated identically. Logically, red (I) fibers should have been contracted more than white (II) ones, because of better oxygen utilisation. It is, therefore, concluded that maximum ability of contraction, under the experimental conditions examined, for red muscle fibers does not exceed the one of white fibers, as it is manifested by oxygen utilization and contraction stimulation in $+37^{o}C$, which is the incubation temperature for SDH demonstration.

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