Solubility of Rabbit Muscle Proteins after Various Time-Temperature Treatments

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Samples of rabbit longissimus dorsi were heated up to 10 hours at 40° to 80° C. Proteins of the muscle were separated by solubility into sarcoplasmic, myofibrillar, denatured, and stroma fractions. The sarcoplasmic fraction was further separated into nonprotein and protein nitrogen. With increasing temperature, or increasing time at a given temperature, the nitrogen-containing compounds in the sarcoplasmic and myofibrillar fractions decreased, while the denatured proteins increased, and the stroma fraction remained relatively constant. Increase in the sarcoplasmic total and nonprotein nitrogen, and in the myofibrillar nitrogen after 10 hours in the 45° to 60° C. range suggests the possibility of proteolytic activity. Shear data on samples heated for 60 minutes showed relationship only to the amount of denatured protein. Data are given on the composition of a number of muscles of the rabbit.

HEAT causes major alterations in proteins. Studies on these changes have usually been made on isolated proteins. Little information could be found on the chemistry of the changes which occur in the structural proteins of voluntary muscle, when heated in situ, although Engelhardt (*1*) has pointed out that proteins are much more resistant to heat in the muscle structure than when isolated. Histological studies (*8*, *9*) have shown that major alterations occur in the structural organization of the contractile and connective tissue proteins, and in the staining properties of the collagenous fibers, when the muscle is heated. A complementary study was initiated to explore the chemical changes involved. The first phase was to separate the total protein sarcoplasmic, myofibrillar, denatured and stroma, and ascertain how these fractions are altered by heat treatment.

Materials and Methods

The longissimus dorsi (LD) muscles of year-old female rabbits were utilized as experimental material. The animals were killed and dressed and the carcasses stored at 5° C. for 24 to 48 hours before sampling. Previous studies (*7*) had shown that 24 hours' cold storage was ample time for completion of the major portion of the cycle of chemical changes which occur after slaughter.

The LD muscles were excised and strips 2 mm. in width and thickness, and 15 mm. in length, cut parallel to the direction of the muscle fibers. Any obvious fat deposits or connective tissue were removed. Strips weighing approximately 2 grams were packaged watertight in aluminum foil, and immersed in a constant-temperature water bath. Temperatures used included 40°, 45°, 50°, 55°, 60°, 65°, 70°, 75°, and 80° C. Determinations were made on raw samples, on samples as soon at the tissue reached the temperature of the water bath (0 time), and after various intervals of heating up to 10 hours. The time required for the samples to reach the temperature of the water bath averaged 3 minutes. The samples were then ground and extracted. Matching samples subjected to the same treatment were fixed in neutral formalin for histological examination. The histological results will be reported in another paper.

The extraction scheme was based on separation into sarcoplasmic, myofibrillar, and stroma fractions for raw muscle. These names are used for both raw and heated samples, although it is recognized that the heat treatments used would cause shifts in the proteins extracted by each solution. Also, an NaOH extract was added.

The samples were disintegrated with a Virtis high-speed mixer, in 50 ml. of KCl–borate, ionic strength (I) = 0.05, pH = 7.5 (*4*), then centrifuged at 1200 × G for 10 minutes. The supernatant was decanted, and the residue was extracted again with 35 ml. of KCl–borate. The supernatants from the two extractions were combined and made to volume, and aliquots taken for determination of total nitrogen, and for separation into nonprotein and protein nitrogen by precipitation of the protein in 5% trichloroacetic acid. The total nitrogen of this extract is designated as sarcoplasmic protein.

The residue from the sarcoplasmic protein extraction was treated with KI–borate buffer, I = 0.6, pH = 7.5, to remove the myofibrillar proteins. Two extractions were made, using 50 and 35 ml. of the buffer solution, with centrifugation at 1200 × G for 10 minutes. KI rather than KCl was used (*10*) to minimize gelation. Disintegration of the original sample, and extraction and centrifugation in KCl and in KI solutions were carried out at 0° to 2° C., to minimize denaturation of the proteins during these procedures.

The residue from KI-borate extraction was further extracted with 50, 20, and 20 ml. of 0.1N NaOH at room temperature, to remove the heat-denatured protein. The first mixture was held for 45 minutes before centrifugation, the second for 30 minutes. The samples were stirred gently during these holding periods. The residue from these extractions was designated as the stroma fraction.

The four solutions (total sarcoplasmic, sarcoplasmic NPN, myofibrillar, and denatured) and the two residues (TCA-precipitated sarcoplasmic, stroma) were analyzed for nitrogen content by the micro-Kjeldahl procedure. pH measurements were made on raw and heated muscle samples. In addition, samples of the raw LD and of several other muscles of the carcass were analyzed for total solids, fat by ether extraction, total nitrogen, and hydroxyproline content. Hydroxyproline measurements are considered useful in estimating the amount of connective tissue, since collagen is the only protein known to contain a large amount. Elastin contains a much smaller amount, but histological examination suggests that the rabbit LD contains only a very small amount of elastin (*8*). The hydroxyproline analyses were made according to Method II of Woessner (*13*), using a hydrolysis time of 15 hours at 124° C.

One section, 4 to 5 cm. in length, of the whole LD was heated for 60 minutes after attaining the temperature of the water bath, then cooled and tested for force required to shear, following the technique outlined for series II (*7*).

Results and Discussion

Data on the weight and composition of nine of the muscles of the rabbit carcass are given in Table I. The per

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cent dry matter and per cent nitrogen were very uniform, but ether extract and hydroxyproline content were much more variable. The high coefficient of variation for hydroxyproline in the triceps brachii is largely a sampling problem, as this muscle is divided into several segments. This makes it difficult to avoid the connective tissue insertions, the inclusion of which increases the hydroxyproline content appreciably. Analysis of variance on the total nitrogen and ether extract from raw LD of 10 animals (20 muscles, right and left sides) showed highly significant variation among animals for ether extract on both the wet and the dry basis, and for nitrogen on the wet basis, but not on the dry, fat-free basis. None showed a significant variation between the right and left sides of the same animal.

One LD muscle was sampled in 18 places for hydroxyproline content. The values obtained ranged from 322 to 474 μg. per gram of wet muscle. The anterior portion tended to have higher amounts of hydroxyproline than the posterior, but the values did not form a uniform progression. The differences between adjacent samples ranged from 2 to 73 μg. per gram of muscle. These data illustrate the sampling problem within a single muscle.

The values for sarcoplasmic, myofibrillar, denatured, and stroma nitrogen are shown in Table II, while Table III gives the data on nonprotein and protein nitrogen of the sarcoplasm. Coefficients of variation are included for the raw samples, to show the amount of variation among animals.

In general, with increasing temperature or increasing time at a given temperature, the amount of nitrogen-containing compounds extracted in either of the salt solutions decreased, the amount soluble in 0.1*N* NaOH increased, and the amount of stroma remained relatively constant. Also, in the soluble fractions, the change was more rapid as the temperature increased. However, there were several interesting exceptions to these patterns.

In the time interval from 2 to 10 hours, the amount of nitrogen in the sarcoplasm fraction increased at 50°, 55°, and 60° C., the myofibrillar portion increased at 45°, 50°, and 55° C., and the heat-denatured protein decreased at 50°, 55°, and 60° C. Since these temperatures are known to increase enzymic activity, the changes in solubility could be the result of proteolytic enzymes naturally present in the muscle tissue. The parallel significant increase in the nonprotein nitrogen of the sarcoplasmic fraction at these temperatures tends to support this possibility.

The increase in nitrogen in the stromal residue at the longer times at 75° and 80° C. parallels the increase noted in beef (*6*). This is thought to be due to insolubilization of some of the protein constituents by the extreme heat treatment.

The nonprotein nitrogen content of the sarcoplasmic fraction was uniform, with the exception of the samples held for 10 hours at 50°, 55°, or 60° C. After heating to 60° C. or higher the sarcoplasmic fraction consisted primarily of nonprotein nitrogen, but even after 10 hours at 80° C. there was still a small amount of protein nitrogen in this fraction, suggesting that a small part of the total enzyme system of the muscle may be resistant to heat. However, the centrifugal force employed did not

Table I. Average Weights and Composition of Muscles from Year-Old Female Rabbits (5 Animals)

Muscle	Raw Weight, G.	% Dry Matter Av.	% Dry Matter C.V.[a]	% Ether Extract, Dry Basis Av.	% Ether Extract, Dry Basis C.V.	% Nitrogen, Dry, Fat-Free Av.	% Nitrogen, Dry, Fat-Free C.V.	% Hydroxyproline, Dry, Fat-Free Av.	% Hydroxyproline, Dry, Fat-Free C.V.
Triceps brachii	18.7	24.62	2.76	4.43	18.74	14.50	2.41	0.40	42.42
Psoas major	22.4	24.83	1.93	4.17	21.82	14.13	5.94	0.12	16.24
Biceps femoris	50.2	26.45	0.95	6.89	16.40	14.79	2.37	0.29	12.71
Semimembranosus	14.5	26.48	0.53	8.28	28.50	14.65	1.98	0.35	18.97
Adductor longus	20.4	25.85	0.97	4.55	32.97	14.78	2.03	0.16	16.13
Adductor magnus	26.8	28.50	1.61	14.61	7.39	14.61	2.33	0.23	11.89
Rectus femoris	16.3	27.48	1.67	12.83	30.09	14.89	2.96	0.52	11.99
Vastus lateralis	17.6	25.65	1.17	3.93	34.10	14.89	2.15	0.31	12.94
Longissimus dorsi	128.5	27.52	2.25	6.19	37.80	14.84	1.89	0.14	17.99

[a] Coefficient of variation.

Table II. Average Percentage of Total Nitrogen Soluble in Extracting Solutions and in Stroma (Dry, Fat-Free Basis), with Coefficient of Variation for the Raw Samples

Heating Temp., ° C.	Raw	0° (to Temp.)	Heating Time 10 min.	30 min.	1 hr.	2 hr.	4 hr.	7 hr.	10 hr.
			Sarcoplasm, KCl–Borate, I = 0.05						
40	38.3	38.2	38.7	37.9	37.3	35.2			30.7
45	42.4	40.2	37.2	34.7	34.2	30.6			26.6
50	38.4	33.3	28.3	27.9	24.9	21.0			24.2
55	37.1	29.5	25.3	20.8	19.2	18.2			24.2
60	41.6	26.1	18.6	15.2	15.9	15.4			22.9
65	36.9	17.1	15.0	14.1	14.4	14.2	14.9	14.9	16.0
70	40.4	14.3	14.5	14.1	13.4	14.0	14.0	13.4	14.0
75	38.3	13.8	12.6	12.2	11.9	11.7	11.7	9.8	10.1
80	41.1	13.0	12.9	11.7	12.6	11.9	13.0	12.2	11.1
C.V.	11.2								
			Myofibrillar, KI–Borate, I = 0.6						
40	54.3	55.3	52.4	51.8	48.7	42.8			31.3
45	53.8	50.6	43.4	33.4	28.0	20.8			29.2
50	48.2	37.4	21.6	21.3	20.4	20.5			27.4
55	54.0	25.6	22.4	22.0	21.6	23.4			34.7
60	48.0	19.9	18.8	17.8	19.2	20.2			19.6
65	53.0	21.6	20.5	21.7	23.4	21.3	17.9	16.5	17.3
70	48.7	18.0	16.0	13.6	13.0	10.7	11.2	11.2	12.3
75	52.8	12.4	9.9	9.2	9.2	9.3	8.2	9.1	8.1
80	47.4	10.4	10.0	8.9	9.0	5.3	4.4	5.0	5.3
C.V.	10.1								
			Denatured, 0.1*N* NaOH						
40	7.1	6.0	8.6	9.5	15.0	22.5			37.6
45	5.2	8.1	20.2	30.1	37.9	43.7			41.9
50	11.1	28.2	46.8	48.7	53.5	54.3			46.4
55	8.7	44.5	50.6	57.1	59.8	59.1			42.4
60	10.2	55.5	63.4	66.2	65.9	62.9			55.2
65	9.2	62.2	66.0	64.6	62.1	64.2	64.2	64.5	67.2
70	11.2	69.1	67.5	68.6	68.7	75.4	70.4	69.5	73.7
75	9.3	70.7	69.2	78.6	70.0	73.4	75.8	73.4	74.8
80	11.0	77.7	76.9	77.3	76.2	78.9	79.2	80.3	77.0
C.V.	34.9								
			Stroma, Insoluble in Any of Solutions Used						
40	0.92	0.60	0.82	0.74	0.84	0.64			0.76
45	0.84	0.81	0.76	0.90	0.85	0.78			0.86
50	0.87	0.74	0.78	0.92	0.70	1.05			0.75
55	0.78	0.70	0.64	0.88	0.72	0.66			0.53
60	0.83	0.61	0.55	0.63	0.49	0.62			0.34
65	0.88	0.76	0.73	0.73	0.57	0.30	0.36	0.48	0.48
70	0.64	0.39	0.61	0.58	0.75	0.67	0.25	0.53	0.64
75	0.76	0.52	0.67	1.00	0.64	0.55	0.96	1.31	1.58
80	0.76	0.54	0.76	0.80	1.64	1.47	1.36	1.14	3.75
C.V.	22.50								

Table III. Average Nonprotein and Protein Nitrogen in KCl–Borate Extract, as Per Cent of Total Nitrogen (Dry, Fat-Free), with Coefficient of Variation for Raw Samples

Heating Temp., ° C.	Raw	0° (to Temp.)	Heating Time 10 min.	30 min.	1 hr.	2 hr.	4 hr.	7 hr.	10 hr.
			Sarcoplasm Nonprotein Nitrogen						
40	12.7	11.4	12.3	11.9	12.5	12.4			13.3
45	11.2	11.0	11.5	10.8	12.9	13.0			12.4
50	10.7	11.3	11.3	11.4	13.2	12.0			13.6
55	11.0	11.5	11.6	13.7	11.5	13.0			18.7
60	12.6	12.4	12.9	12.4	13.5	13.8			18.8
65	13.2	13.5	12.3	12.3	12.8	15.2	14.9	15.5	15.7
70	13.4	13.0	12.8	12.8	13.0	12.3	13.1	12.9	14.3
75	12.0	12.6	12.4	12.1	11.7	10.7	10.1	9.1	9.3
80	12.6	11.8	12.0	11.9	11.6	12.5	11.9	11.7	11.7
C.V.	9.6								
			Sarcoplasm Protein Nitrogen (TCA Precipitate)						
40	26.0	26.4	26.3	24.2	24.8	22.9			17.0
45	29.8	27.6	23.6	22.3	21.0	18.0			12.1
50	27.3	23.0	17.7	14.8	13.0	10.2			6.2
55	25.6	17.6	12.0	8.7	5.9	4.1			4.4
60	29.5	12.8	5.7	2.4	2.6	2.8			3.6
65	24.5	4.6	1.4	1.4	1.2	1.3	1.1	0.8	0.8
70	27.5	1.5	0.8	0.8	0.6	0.8	0.6	0.6	0.6
75	26.1	0.7	0.4	0.4	0.7	0.6	0.3	0.3	0.6
80	28.9	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3
C.V.	14.1								

Table IV. Average pH of Rabbit LD after Various Time-Temperature Treatments, and Average Shear Force Values after Heating for 60 Minutes

Heating Temp., ° C.	Raw	0° (to Temp.)	Heating Time 10 min.	30 min.	60 min.	2 hr.	4 hr.	7 hr.	10 hr.	Warner-Bratzler Shear, Lb.
40	5.68	5.73	5.70	5.72	5.70	5.76			5.75	2.96
45	5.66	5.68	5.74	5.76	5.80	5.82			5.88	4.55
50	5.64	5.74	5.83	5.78	5.78	5.84			5.93	12.94
55	5.69	5.82	5.92	5.90	5.95	5.96			5.96	11.25
60	5.79	5.94	5.93	5.97	5.95	6.04			6.05	7.93
65	5.72	5.88	5.94	5.94	5.95	5.90	5.95	5.93	5.92	3.79
70	5.69	5.91	5.94	5.94	5.94	6.00	6.07	6.06	6.08	8.15
75	5.77	6.01	6.00	6.04	6.00	6.05	6.03	6.20	6.14	16.08
80	5.74	5.90	5.92	5.94	5.94	5.99	6.03	6.04	6.10	16.19

always give clear-cut separations of supernatant and residue, so small variations may be artifacts. Also, after 10 hours at 80° C., about 5% of the total protein was still soluble in the KI–borate solution. Hydroxyproline analyses would be necessary to determine whether or not this was solubilized collagen. A limited number of trials on hydroxyproline analysis of the various extracts have indicated that some modifications in the methods will be necessary before accurate results can be obtained. This will be followed up in further studies.

The small amount of protein soluble in 0.1*N* NaOH in the raw samples may represent protein denatured by the grinding and extraction procedures and/or alkali-soluble collagen. If this is denatured protein, the amount is small in relation to that produced by heating. Again, further work on the hydroxyproline analysis on this extract should show whether or not collagen is involved.

The pH of the muscle tissue increased with heating, more rapidly and to a higher level as the temperature increased (Table IV). The maximum change at 40° C. was <0.1. From 45° to 65°, the maximum change was between 0.2 and 0.3, while at 70° to 80° C. the change was about 0.4 pH unit. A number of other workers have reported increase of pH on heating meat: Kauffman *et al.* (*3*); Hamm and Deatherage (*2*); Paul (*7*). Hamm and Deatherage (*2*) attribute the shift in pH to loss of free acidic groups by formation of new stable cross linkages.

The average shear values for LD heated 60 minutes are also given in Table IV. These results resemble in part those obtained by Machlik and Draudt (*5*) on beef, and Tuomy *et al.* on beef (*12*) and pork (*11*). Machlik and Draudt (*5*) discuss the roles of the collagen shrinkage reaction, heat denaturation of the myofibrillar proteins, and the collagen-gelatin transformation in influencing the force required to shear. They consider the decrease in shear between 50° and 65° C. to be due to collagen shrinkage, and the increase between 65° and 75° C. as possibly due to changes in the myofibrillar proteins.

The shear data showed no relation to the hydroxyproline content of the raw muscle, or to the per cent nitrogen extracted by the various solutions at 60 minutes' heating time, with the exception of the 0.1*N* NaOH. The correlation coefficient between shear and the alkali extract was 0.63, high enough to be highly significant statistically, but too low to have any major predictive value.

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