GERMAN

Titel: Veränderungen in den myofibrillaren Proteinen und ihr Verhältnis zu der Erweichung von skeletaren Rindermuskeln.

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Chemische und physikalische Veränderungen in den myofibrillaren Proteinen der Rindermuskeln longissimus, semitendinosus und psoas major wurden festgestellt während postmortaler Lagerung bei 2°C und 25°C. Der Myofibrilfragmentationsindex, festgestellt durch Messung der Absorption einer myofibrilen Suspension, vergrösserte sich während der postmortalen Lagerung, und Fragmentation fand schneller statt bei 25°C als bei 2°C Lagerung in den Muskeln longissimus und semitendinosus,änderte sich aber nur wenig in dem Muskel psoas major. Veränderungen von Zärte von Steaks von den Muskeln longissimus, semitendinosus und psoas major (gemessen nach dem Warner-Bratzler Verfahren) stimmten während postmortaler Lagerung mit der Veränderung in dem myofibrillaren Fragmentationsindex überein. Weiterer Beweis für dieses Verhältnis war der Korrelationskoeffizient zwischen myofibrilem Fragmentationsindex und SensorizÄrte von 0,9 und 0,7 im Falle von Steaks von Kälbern, bzw. A- und C-Alter bei Rümpfen. Sodiumdodecylsulfate (SDS) und Polyacrylamidegelerte von Myofibrien zeigten, dass während der postmortalen Lagerung eine sehr kurz bemessene spezifische Proteolyse in den myofibrillaren Proteinen der Muskeln longissimus und semitendinosus stattfand, aber nicht in dem Muskel psoas major. Ein Polypeptid mit einem molekularen Gewicht von ungefähr 30.000 Daltonen erschien und eine untergeordnete Einheit von Troponin, Troponin R, verschwand während postmortaler Lagerung. Ein von Kalzium aktiviertes proteolytisches Enzym wurde aus den Muskeln longissimus, semitendinosus und psoas major postmortal isoliert. Die Aktivität dieses Enzyms war hoch in longissimus und semitendinosus, die Aktivität in psoas major war aber nur halb so gross wie in longissimus und semitendinosus. Inkubation dieses Enzyms mit Myofibrilen zur Zeit des Todes produzierten eine Degradation der myofibrilen Proteine ähnlich der Degradation in Myofibrilen der Muskeln longissimus und semitendinosus, postmortal gelagert.

FRENCH

Le titre: Des changements en myofibrillar proteines et leur relation a la tendresse du muscle squéletique bovine.

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Les changements chimiques et physiques en myofibrillar proteines des muscles bovines longissimus, semitendinosus et psoas major étaient determinés pendant le magasinage après la mort à 2° C et 25° C. L'indice fragmentation myofibril determiné par la mésure de l'absorption de la suspension myofibril agrandissait pendant la magasinage après la mort, et la fragmentation se présentait plus vite à 25° C qu'à 2° C enmagasinage, en muscles longissimus , semitendinosus mais changeait peu en psoas major. Le changement en la tendresse (mesuré par la toute force Werner-Bratzler) les tranches des muscles longissimus semitendinosus et psoas major pendant la magasinage après la mort s'est accordé avec le changement dans l'indice myofibrillar de la fragmentation. La plus grande evidence de cette rélation étaient les correlation coefficients entre l'indice myofibril fragmentation et la tendresse sensoriale de 0.9, 0.7 et 0.7 pour les tranches au veau, les carcasses A-maturité et C-maturité respectivement. Sodium dodecyl sulfate (SDS) polyacrylamide gels de myofibryls montraient que pendant le magasinage après la mort un proteolysis très limité et tres specifique se presentait dans les proteines myofibrillars des muscles longissimus et semitendinosus mais pas du muscle psoas major. Un polypeptide avec le pesant des molécules d'approximatif 30,000 doltons se présentait dans les proteines et sub-unité de troponin, troponin T a disparu pendant le magasinage après la mort. Un calcium enzyme fait-actif proteolytic s'était isolé d'après la mort des muscles longissimus, semitendinosus et psoas major. L'activité de cet enzyme était grande en longissimus et semitendinosus. L'incubation de cet enzyme avec "a la mort" myofibrils a produit la degradation des proteins myofibrillar comme à ceux qui se trouvent en myofibrils de "apres la mort" dans les muscles longissimus et semitendinosus.

Title: Changes in myofibrillar proteins and their relationship to bovine skeletal muscle tenderization.

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Chemical and physical changes in myofibrillar proteins of bovine longissimus, semitendinosus and psoas major muscles were determined during postmortem storage at 2° C and 25° C. Myofibril fragmentation index, determined by measuring absorbance of a myofibril suspension, increased during postmortem storage, and fragmentation occurred more rapidly at 25° C than at 2° C storage, in longissimus and semitendinosus muscles, but changed only slightly in psoas major muscle. Change in tenderness (measured by Warner-Bratzler shear force) of steaks from longissimus, semitendinosus, and psoas major muscles during postmortem storage coincided with the change in the myofibrillar fragmentation index. Further evidence of this relationship was the correlation coefficients between myofibril fragmentation index and sensory tenderness of 0.9, 0.7 and 0.7 for steaks from veal, A-maturity and C-maturity carcasses, respectively. Sodium dodecyl sulfate (SDS) polyacrylamide gels of myofibrils showed that during postmortem storage a very limited and specific proteolysis occurred in the myofibrillar proteins from longissimus and semitendinosus muscles, but not from the psoas major muscle. A polypeptide having a molecular weight of approximately 30,000 daltons appeared and a subunit of troponin, troponin T, disappeared during postmortem storage. A calcium activated proteolytic enzyme was isolated from postmortem longissimus, semitendinosus and psoas major muscles. Activity of this enzyme was high in longissimus and semitendinosus, but activity in the psoas major was only half as much as in the longissimus and semitendinosus. Incubation of this enzyme with at-death myofibrils produced degradation of myofibrillar proteins similar to that found in myofibrils from postmortem stored longissimus and semitendinosus muscles.

Заглавие: Изменения в миофибрилловых белках и их отношение к мускулатурному смягчению бычачьего каркаса.

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Химические и физические изменения в миофибрилловых белках бычьих longissimus, semitendinosus и psoas major мускулов были определены посмертно (postmortem) в хранении при 2°C и 25° C. Индекс миофибрилловой фрагментации, определённый измерением абсорбции миофибрилловой суспензии, увеличился в течение postmortem хранения, и фрагментация в хранении произошла быстрее при 25°C чем при 2° C в мускулах longissimus и semitendinosus , но в мускуле psoas major перемена была незначительна. Перемена в мягкости (измерена по Варнер-Брецлер срезывающему усилию) мяса из longissimus , semitendinosus и psoas major мускулов во время хранения postmortem , совпала с переменой в индексе миофибрилловой фрагментации. Дальнейшим доказательством этой зависимости явилась корреляция коэфициентов между индексом миофибрилловой фрагментации и сенсорной мягкости 0,9; 0,7; и 0,7 телятины в указаном порядке туш зрелости А и зрелости С. Гели миофибриллов натрия додекила сульфата (SDS) полиакриламида доказывают, что в продолжение хранения postmortem происходит весьма ограниченный и специфический протеолиз в миофибрилловых белках мускулов longissimus и semitendinosus , но не мускула psoas major . Появился полипептид молекулярного веса приблизительно 30,000 далтонов, а еденица прилежащей части тропонина, тропонин Т, исчезла во время хранения postmortem . Протеолитический энзим, активированый кальцием, был выделен из postmortem мускулов longissimus , semitendinosus и psoas major . Активность этого энзима была высока в longissimus и semitendinosus но активность в psoas major была в половину меньше чем в longissimus и semitendinosus . Инкубация этого энзима при отмирании миофибриллов произвела деградацию миофибрилловых белков, сходную с находящейся в миофибриллах мускулов postmortem хранения longissimus и semitendinosus .

Postmortem changes in myofibrillar proteins and their relationship to bovine skeletal muscle tenderization

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Tenderness is one of the most important palatability characteristics of meat. It also is a very complex characteristic because many variables affect tenderness. Although some of these variables are understood, the factors responsible for the large increase in tenderness during postmortem storage are still enigmatic. Myofibril structure, especially Z-disk structure, is altered during postmortem storage, but the cause of this structural alteration or how strongly this event is related to tenderness is still unknown. Although it is clear that postmortem muscle tenderization is influenced primarily by changes in myofibrillar proteins and not by changes in sarcoplasmic or stroma proteins, the nature of the myofibrillar protein changes that directly influence meat tenderness and what causes these changes have not been elucidated.

The purpose of this paper is to describe some of our recent evidence showing that Z-disk degradation in postmortem myofibrils is one of the most important factors influencing tenderization in beef, and indicating that postmortem Z-disk degradation is caused by a calcium activated proteolytic enzyme endogenous to muscle.

Materials and Methods

Muscle samples and steaks were obtained from bovine animals weighing 400-500 Kg, about 18 months of age, and on similar feeding regimes at the Iowa State University Nutrition Farm. At-death samples were obtained from longissimus, semitendinosus and psoas major muscles within one hour after exsanguination, and subsequent samples were removed from the companion side of the carcass at 1, 2, 3, 6, 7, 10 or 13 days postmortem (not all postmortem times were used in each experiment).

Wholesale short loins from thirty-five A-maturity carcasses and twelve C-maturity carcasses were obtained from a commercial packing company. Wholesale short loins of six veal were obtained from animals originating from the Iowa State University Dairy Farm. Samples and steaks were removed after 1 and 7 days of postmortem storage at 2° C.

Myofibrils were isolated from longissimus, psoas major and semitendinosus muscle by six washings and centrifugations at 2° C in 100 mM KCl, 20 mM K phosphate (pH 7.0), 1 mM EDTA, and 1 mM sodium azide. Myofibril fragmentation was determined by measuring absorbance of a myofibril suspension containing 0.5 mg/ml of myofibril protein at 540 nm. Myofibrils were examined with a Zeiss Photomicroscope equipped with phase and polarized light optics.

Troponin was prepared from 100 gm of at-death bovine longissimus according to the procedure described by Arakawa et al. (1970a, b, c) and was further purified by DEAE-cellulose column chromatography (van Eerd and Kawasaki, 1973).

Crude CAF was prepared from 100 gm of muscle immediately after death and after 1, 3 and 6 days postmortem muscle according to the procedure of Busch et al. (1972) and purified porcine CAF was prepared according to the procedure of Dayton et al. (1974a, b).

Myofibrils and purified troponin were run on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis according to the method of Weber and Osborn (1969).

Warner-Bratzler (W-B) shear and sensory panel evaluations were determined on steaks oven-broiled to 65° C internal temperature. All steaks for W-B shear were allowed to cool to room temperature (about 25° C) before three 1.27 cm diameter cores were removed and each sheared twice. Sensory panel members (10) received warm samples to evaluate tenderness, flavor and juiciness according to an eight point hedonic scale, with a score of 8 being the most desirable.

Data was analyzed according to methods described by Snedecor and Cochran (1967) and Steel and Torrie (1960).

Results and Discussion

Initially phase-contrast and polarized light microscopy were used to determine myofibril fragmentation of longissimus, semitendinosus and psoas major muscles during postmortem storage. It was found that the increase in myofibril fragmentation of longissimus and semitendinosus muscles confirmed earlier observations of this phenomenon by Davey and Gilbert (1967, 1969), Fukazawa et al. (1969), Hay et al. (1973), Henderson et al. (1970), Parrish et al. (1973) and Sayre (1970). Only a slight increase in fragmentation, however, was observed in myofibrils from the psoas major muscle during postmortem storage.

Although microscopy is valuable in visualizing the structural changes in the myofibril, it does not provide a good objective, representative method of determining fragmentation. To overcome this problem, a method of measuring the absorbance of a myofibril suspension was developed, and a myofibril fragmentation index was calculated to give a relative value of the degree of disintegration of myofibrils. Fragmentation of myofibrils from longissimus and semitendinosus increased substantially during postmortem storage at 2° C, but fragmentation of myofibrils from psoas major increased only slightly. Table 1. Measurement of myofibril fragmentation by absorbance confirms measurements of fragmentation by microscopy and absorbance measurements can be used to quantitate amount of myofibril fragmentation. Myofibrils from longissimus and semitendinosus muscles stored 1, 2 and 3 days postmortem at 25° C were much more highly fragmentated than myofibrils from psoas major muscles stored under similar conditions.

Table 1. Effect of postmortem storage (2° C) on myofibril fragmentation index (FI) and Warner-Bratzler (W-B) shear-force of longissimus (L), semitendinosus (ST) and psoas major (PM) muscles[1]

	Days of postmortem storage		
	1	3	6
FI[2]			
L	49.6±1.3	69.8±1.1	76.3±0.9
ST	48.8±0.8	68.2±1.1	77.6±1.0
PM	47.1±0.9	49.3±1.1	54.7±1.0
W-B[3]			
L	2.60±0.20	2.23±0.17	2.13±0.12
ST	3.27±0.11	2.72±0.09	2.64±0.11
PM	2.16±0.12	1.94±0.11	1.86±0.17

[1] Means ± standard errors of five carcasses. Means not underscored by the same line are significantly different ($P<0.05$).

[2] Absorbance per 0.5 mg myofibril protein X 200.

[3] kg of shear-force per cm.

W-B shear force values showed that tenderness increased significantly during postmortem at 2° C for longissimus and semitendinosus muscles, but not for psoas major muscles. Table 1. Storage at 25° C reduced shear force in all three muscles, but little change was found for psoas major muscles. Hence, the association between W-B shear force and myofibril fragmentation seems strong.

These initial observations on myofibril fragmentation and W-B shear force during postmortem storage suggested that a relationship existed between myofibrillar proteins and changes during postmortem storage. To determine the extent of this relationship, myofibrils prepared from bovine longissimus muscle (at-death and after 1, 3, 6 and 10 days of postmortem storage at 2° C), were electrophoresed on SDS 7½ and 10% polyacrylamide gels. Between 0 and 10 days postmortem storage, no major changes (appearance or disappearance of protein bands) occurred in the bands above actin (myosin and α-actinin); however, obvious changes in protein bands occurred below actin. The most noticeable change was a gradual decrease and disappearance of the troponin-T protein band and a gradual appearance of a protein band in the 30,000-dalton molecular weight region. Nearly identical changes were observed in the myofibrillar proteins of the semitendinosus. Both SDS 7½% and 10% polyacrylamide gels, however, showed only very slight changes in myofibrillar proteins of psoas major muscle during postmortem storage. The troponin T band, which appeared very faintly in these gels, did not completely disappear during 0 to 10 days of postmortem storage. Likewise only a very faint 30,000-dalton band appeared at 10 days postmortem. These slight changes in the myofibrillar proteins of psoas major muscle during postmortem storage are in sharp contrast to the obvious changes in the troponin-T and the 30,000-dalton bands in myofibrils from longissimus and semitendinosus muscles during postmortem storage. Moreover, these changes in SDS-polyacrylamide gels parallel similar changes during postmortem storage in myofibril fragmentation (as observed with the light microscope), myofibril fragmentation index and W-B shear-force.

Higher postmortem storage temperature (25° C) of carcasses accelerated the changes in myofibrillar proteins of longissimus and semitendinosus muscle, and did not cause additional degradative changes. On the other hand, no major differences were noted between the gels of myofibrils either among days of postmortem storage or between storage temperatures for psoas major muscle.

A calcium-activated-factor (CAF), endogenous to muscle tissue, has been shown to selectively remove Z-disks and degrade the myofibrillar proteins, troponin, tropomyosin and component C (Dayton et al., 1974a, b). CAF was prepared from bovine longissimus, psoas major and semitendinosus muscles immediately after death and after 1, 3 and 6 days of postmortem storage at 2° C, and its proteolytic activity was measured on casein. CAF activity of the three muscles was maximal at-death and its activity decreased between 0 to 60 days postmortem. Nearly identical at-death and postmortem CAF activity was noted for longissimus and semitendinosus muscles with the greatest decrease in CAF activity in these two muscles occurring between 0 and 1 day postmortem. CAF activity from at-death psoas major muscle was less than half the at-death CAF activity of the longissimus and semitendinosus muscles and it diminished to almost zero at 1 day postmortem.

To determine the effect of CAF on myofibrils, purified CAF from porcine muscle was incubated with myofibrils from at-death bovine longissimus, semitendinosus and psoas major muscles. Phase microscopy of myofibrils incubated in the absence of CAF showed no structural damage, whereas myofibrils incubated in the presence of CAF had no Z-disks. To further demonstrate the role of CAF, at-death myofibrils from the longissimus, semitendinosus and psoas major muscles were incubated in the absence and presence of CAF and electrophoresed on SDS 7½% and 10% polyacrylamide gels. Those gels of at-death myofibrils treated with CAF showed the absence of α-actinin, troponin-T and troponin-I bands and the presence of a 30,000-dalton band. These results clearly show that CAF mimics almost exactly the affect of postmortem storage on myofibrillar proteins.

To determine the origin of the 30,000-dalton band, purified troponin prepared from at-death bovine longissimus muscle was incubated with purified CAF from porcine muscle for 0, 5, 10, 20 and 40 min. As incubation time increased from 5 to 40 min, the troponin-T band decreased in intensity and almost disappeared at 40 min incubation. The troponin-I band decreased slightly as incubation time with CAF increased, but the troponin-C band appeared to be unaffected by CAF treatment. The 30,000-dalton band increased in intensity up to 10 min incubation and then decreased in intensity as incubation time increased beyond 10 min because it apparently is further degraded by CAF into smaller molecular weight proteins. Troponin, with and without CAF treatment was also electrophoresed along with myofibrils from at-death bovine longissimus muscle and at 10 days of postmortem storage on SDS-10% polyacrylamide gels. The gel of at-death myofibrils (control) showed the presence of troponin-T and the absence of the 30,000-dalton band. When troponin (with no CAF treatment) was electrophoresed along with the at-death myofibrils, troponin subunit bands showed increased intensity but the 30,000-dalton band was not present. When CAF-treated troponin was electrophoresed with the at-death myofibrils, however, the troponin subunit bands showed greater intensity and the 30,000-dalton band appeared. Likewise gels of myofibrils from muscle stored at 10 days postmortem showed the absence of the troponin-T band and the presence of the 30,000-dalton band. When troponin (with no CAF treatment) was electrophoresed with myofibrils from postmortem muscle, the troponin-T band appeared in the same location as that shown in the gel of at-death myofibrils. The troponin-I and C bands were of greater intensity and the 30,000-dalton band was of similar intensity. When CAF-treated troponin was electrophoresed with myofibrils from postmortem muscle the 30,000-dalton band increased in intensity. These results clearly show that the band in at-death myofibrils that disappears from the gel of myofibrils after postmortem muscle storage is troponin-T and the 30,000-dalton band that appears in the gel of myofibrils from postmortem muscle originates from the degradation of troponin-T.

Because myofibrillar proteins, especially troponin-T, are degraded and associated with myofibrillar fragmentation, a study was carried out to determine the relationship of myofibril fragmentation to W-B shear force and sensory panel evaluation of loin steaks from veal, A-maturity and C-maturity postmortem aged for 1 and 7 days at 2° C. Significant correlation coefficients (Table 2) between myofibril fragmentation and W-B shear force ranged from -0.65 to -0.75 and between myofibril fragmentation index and sensory tenderness ranged from 0.65 to 0.75 in the A- and C-maturity groups. These results closely agree with the results of Moller et al. (1973) who found when using a method of measuring myofibril fragmentation similar to that used in this investigation, a correlation coefficient of -0.78 between myofibril fragmentation and W-B shear force of longissimus muscles from A-maturity bovine carcasses at 7 days postmortem. Correlation coefficients between fragmentation index and tenderness measurement were even higher for veal. Hence, myofibril fragmentation index is one of the most highly correlated measures between raw muscle attributes and cooked meat tenderness. It accounts for about fifty percent of the tenderness of beef steaks from A- and C-maturity carcasses broiled to 65° C internally.

Table 2. Effect of postmortem storage (2° C) on correlation coefficients among myofibril fragmentation index (FI), Warner-Bratzler (W-B) shear-force and sensory panel tenderness (TEND), flavor (FLA) and juiciness (JU) of longissimus muscles from veal, A-maturity and C-maturity carcasses

	Veal		A-maturity		C-maturity	
	Days of postmortem storage					
	1	7	1	7	1	7
FI vs W-B	-0.95**	-0.97**	-0.65**	-0.75**	-0.68*	-0.72*
FI vs TEND	0.88*	0.95**	0.67**	0.73**	0.68*	0.65*

Table 2. Continued.

*Significant at the 5% level.
**Significant at the 1% level.

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