Effect of pH and temperature on the extractability of myofibrillar proteins

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S u m m a r y

Myofibrils were prepared /normal preparate/ from porcine longissimus dorsi muscle, a part of which was contracted. Normal and contracted myofibrils were incubated for 4 hrs in a media of 0,16 M KCl,pH varied between 5,5-6,5, temperature varied between 4-37 C°. After incubation, myofibrils were extracted at 4 C° in a solution adjusted to pH 6,1 containig 0,6 M KCl with or without 1 mM $Na_4P_2O_7$. Effects of pH, temperature and contraction of myofibril preparate were evaluated in relation to extractability of proteins in the presence of $Na_4P_2O_7$. Low pH /5,5/ reduced the extractability of normal preparate with 0,6 M KCl in case of 4° and 25 C° incubation. There was only a slight difference in extractability between 4 and 25 C°incubation temperature. 37 C° incubation temperature strongly reduced the extractability in the whole pH range as compared to lower temperatures. 1 mM $Na_4P_2O_7$ combined with 0,6 M KCl increased the extractability especially when incubation had been conducted at a lower pH. Thus, extractability in the presence of $Na_4P_2O_7$ only slightly depended on the pH of incubation media. The amount of extractable protein from contracted myofibrils was generally lower than that from normal either with 0,6 M KCl or with additional $Na_4P_2O_7$. There was a reduction in 0,6 M KCl - extractability parallel with the pH of incubation media, whereas temperature of incubation did not seem to influence the extractability significantly. Contrary to the normal preparate, promoting effect of $Na_4P_2O_7$ was much more obvious after incubation at high pH level. These results contribute to our knowledge of salt-soluble proteins and their interaction with pyrophosphate.

Die Wirkung des pH-Wertes und der Temperatur auf die Extrahierbarkeit der myofibrillären Proteine

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ZUSAMMENFASSUNG

Aus "Musculus longissimus dorsi" von Schwein prerigoren Zustandes haben Verfasser Myofibrillenpräparat /Normalpräparat/ hergestellt, ein Teil dessen kontrahiert wurde. Die Myofibrillenpräparate normalen und kontrahierten Zustandes wurden in 0,16 M KCl bei pH-Werten von 5,5 - 6,5 und bei Temperaturen von 4 - 37 C° 4 Stunden lang inkubiert. Demnach wurden die Präparate in einer auf einen pH-Wert von 6,1 eingestellten 0,6 M KCl Lösung, bzw. in einer 1 mM $Na_4P_2O_7$ enthaltenden 0,6 M KCl Lösung bei einer Temperatur von 4 C° extrahiert. Der niedrige pH-Wert /5,5/ hat die aus dem Normalpräparat mit 0,6 M KCl extrahierbare Proteinmenge - bei 4 C° und 25 C° inkubiert - verringert. Im Falle der Inkubation bei 4 C° und 25 C° hat sich in der extrahierbaren Proteinmenge nur ein geringer Unterschied gezeigt. Eine Inkubationstemperatur von 37 C° hat die Extrahierbarkeit innerhalb des ganzen pH-Bereiches im Vergleich zu der niedrigen Temperatur stark vermindert. Das zusammen mit 0,6 M KCl angewendete 1 mM $Na_4P_2O_7$ hat die Extrahierbarkeit erhöht, besonders im Falle, wenn die Inkubation bei einem niedrigen pH-Wert erfolgte. So hing die Extrahierbarkeit in der Anwesenheit von $Na_4P_2O_7$ nur in geringem Masse von dem pH-Wert des Inkubationsmediums ab. Die Menge des aus dem kontrahierten Präparat sowohl mit 0,6 M KCl, als auch mit $Na_4P_2O_7$ - Komplettierung extrahierbaren Proteins war im allgemeinen kleiner, als im Falle des Normalpräparates. Die Menge des mit 0,6 M KCl extrahierten Proteins hat sich parallel mit dem pH-Wert des Inkubationsmediums vermindert, aber die Temperatur hat die Extrahierbarkeit kaum beeinflusst. Im Gegensatz zu dem Normalpräparat war die das Inlösunggehen befördernde Wirkung des $Na_4P_2O_7$ weitaus ausgedrückter in dem Falle, als die Extraktion durch eine bei hohem pH-Wert durchgeführte Inkubation überholt wurde.

Diese Ergebnisse ergänzen unsere Kenntnisse im Zusammenhang mit den salzlöslichen Proteinen sowie mit deren Wechselwirkung mit Pyrophosphat.

4.3

L'influence de la valeur pH et de la température à l'extractibilité des protéines

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Conclusion

On a produit des préparations myofibrillaires /préparations normales/ à la base du longissimus dorsi de porc avant rigor, dont l'une part a été contractée. Avec les préparations myofibrillaires normales et contractées on a fait l'incubation de 4 heures dans 0,16 M de KCl, pH 5,5-6,5 et à la température de 4 - 37 C°. Ensuite les préparations ont été extraites à la température de 4 C° dans 0,6 M de solution de KCl, avec pH 6,1 et dans 0,6 M de solution de KCl contenant 1 mM de $Na_4P_2O_7$.

Le pH bas /5,5/ a réduit la quantité de protéine qui était extractible de la préparation normale par l'intermédiaire de 0,6 M de KCl sous l'incubation à la température de 4 C° et 25 C°. En cas de l'incubation faite à 4 C° et à 25 C° la quantité de protéine ne montrait qu'un peu de différence.

La température d'incubation de 37 C° a réduit fortement l'extractibilité dans le domaine entier de pH. L'extractibilité a été augmentée en cas où 0,6 M de KCl était mis en application avec 1 mM $Na_4P_2O_7$, surtout quand on a fait l'incubation à pH bas. Par conséquant l'extractibilité ne dépendait que dans une faible mesure du pH du médium d'incubation.

La quantité de protéine extractible de la préparation contractée, extraite tant par 0,6 M de KCl, que par l'addition $Na_4P_2O_7$ était en général moindre à la préparation normale. La quantité de protéine étant extraite par 0,6 M de KCl diminuait en parallele au pH du médium d'incubation, par contre la température n'influencait guere l'extractibilité. Au contraire de la préparation normale, l'effect du $Na_4P_2O_7$ favorisant la dissolution était plus expressif, quand l'extraction avait été anticipée par une incubation à pH élevé. Ces résultats complètent nos connaissances étant en connexion avec les protéines solubles dans la saumure et leur action réciproque avec le pyrophosphate.

Влияние pH и температуры на экстрагируемость миофибриллярных белков.

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Из свиной мышцы longissimus dorsi в состоянии pre rigor пригтовили нормальный препарат, часть которого контрагировали(усадили). Нормальные и контрагированные миофибриллярные препараты инкубировали в течение 4 часов при температуре 4-37°C в растворе 0,I6M KCI при pH= 5,5-6,5. Затем препарат экстрагировали при температуре 4°C в 0,6M растворе KCI с pH= 6,I, т.е. в 0,6M растворе KCI, содержащем I мМ $Na_4P_2O_7$.
Во время инкубации при температуре 4°C и 25°C и низком pH/5,5/ снизилось количество белка, которое можно экстрагировать из нормального препарата 0,6M-ным раствором KCI. Инкубация при 4°C и 25°C показала небольшую разницу в количестве экстрагируемого белка. Инкубационная температура 37°C по сравнению с низкой температурой снизила экстрагируемость в полном интервале pH.
Применяя IмМ $Na_4P_2O_7$ с 0,6M раствором KCI значительно повысилась экстрагируемость особенно в том случае, когда инкубация проводилась при низком pH. Таким образом, в присутствии $Na_4P_2O_7$ экстрагируемость лишь в небольшой степени зависит от pH среды инкубации. Количество белка, извлечённое из контрагированного препарата с помощью 0,6M раствора KCI и из этого же раствора с добавкой $Na_4P_2O_7$, было обычно меньше, чем в случае нормального препарата. Количество белка, экстрагированное с помощью 0,6M раствора KCI, снижалось параллельно с pH инкубационной среды, температура же почти не влияла на экстрагируемость. В противоположность нормальному препарату, эффект $Na_4P_2O_7$ ускоряющий растворение, был более лее выражен, если перед экстрагированием провели инкубацию при высоком pH.
Эти результаты являются дополнением к данным, связанным с растворимыми в солях белками и их взаимосвязью с пирофосфатами.

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Introduction

Detection of structural changes of meat proteins under post mortem conditions is of great importance in relation the meat processing. The inferior quality of PSE meat initiated several workers to investigate the changes of protein structure by extraction. As they established, a considerable reduction in the extractable myofibrillar protein with high ionic strength was found as a result of denaturation caused by the combined effect of the high temperature and low pH early post mortem /Bendall & Wismer-Pedersen,1962, Sayre & Briskey,1963, Penny, 1967 a, b, 1969, Sung et al. 1976/. The importance of extractable proteins with high ionic strength in the water binding and emulsifying capacity was confirmed by Sayre et al. 1964, Penny, 1969, Saffle, 1968/.
Van Oord & Wesdorp /1978/ investigated the influence of the conditions of extraction procedure ie. pH, concentration of salt and pyrophosphate, pm age of meat and discussed the significance of solubility as an index of meat quality.
Surveying the reports on salt soluble proteins, there are no uniform methods applied for extraction /composition and concentration of solution, post mortem age of meat etc./. Such properties of myifibrils like shortening /roughly characterized by sarcomere length/ or tension at rigor also may contribute to the final quality of muscle but there are only a few data about their significance /Cook, 1967/. Detailed investigations are needed for better understanding the relationship between salt-extracted protein and the structural status of myofibrils .

In our work the effect of pH and temperature on isolated myofibrils was studied in terms of the amount of extracted proteins.

Materials & Methods

Normal preparate

Porcine longissimus dorsi muscle /pH 6,7/ was removed at post mortem 10 min, then cooled and ground. Ground muscle batches were homogenized for 3x20 sec with 5 volumes of ice-cold 0,16 M KCl by Ultra-Turrax homogeniser and centrifuged for 10 mins at 1000g.Myofibrils were passed through a muslin layer to remove connective fibers, then suspended again in 0,16 M KCl solution and centrifuged. Washing was repeated 3 times. Protein content of myofibril-suspension was determined by Gornall's Biuret method then diluted to 20mg/ml with 0,16 M KCl. Biuret method was standardized by bovine serum albumin /Serva/. During the procedure the pH of the suspension was held at 6,7 and temperature below 4°C. pH was measured by Radiometer pH meter with glass electrode.

Contracted preparate

Normal preparate was divided to two parts, one of them was centrifuged and suspended in a solution consisting of $4x10^{-3}$M $MgCl_2$, $1x10^{-4}$M $CaCl_2$, $4x10^{-2}$M Tris/HCl buffer pH 7,2 and KCl. The final ionic strength was 0,14 μ. Having suspended the myofibrils $4x10^{-3}$M ATP /ATP-Na salt, Reanal/ was added carefully mixed for 5 minutes at 20°C. After centrifuging contracted myofibrils were washed 3 times with 0,16 M KCl. After protein determination the suspension was diluted to 20 mg/ml with 0,16 M KCl.

Incubation conditions

9-9 aliquotes of 150 ml volumes from both normal and contracted preparates were poured

into plastic tubes and 3-3 of them were adjusted to pH 5,5; 5,9; and 6,5 with HCl. One sample of each pH level was incubated at 4°,25° and 37°C respectively for 4 hours and periodically mixed. After incubation tubes were held at a temperature above 4°C, were cooled to 4°C, then parallel samples were taken from each tubes for extraction with 0,6 M KCl and also parallel samples for extraction with 0,6 M KCl+1 mM $Na_4P_2O_7$.

Extraction procedure

Ionic strength of samples from treated myofibrils was adjusted to 0,6 M KCl and the pH of all samples was adjusted with HCl or KOH to 6,2. The media of another group of samples were adjusted in the same way and $Na_4P_2O_7$ was added. The final concentration of $Na_2P_2O_7$ in the mixture was 1 mM. Mixture was shaken frequently and left overnight at 4°C.
The undissolved protein was removed by centrifuging at 10.000g for 10 minutes. The protein content of the supernatant was determined by Biuret method. Soluble protein in the supernatant was expressed as the percentage of the total protein content of the suspension before extraction.

Results

In figure 1. the KCl-extractability of normal and contracted preparates are shown with and without pyrophosphate.
The percentage of extractable protein obtained from normal and contracted preparates previously exposed to different pH and temperature effect were compared. Significance of these factors/preparate, pH, temperature/ were evaluated by analysis of variance for KCl-extractability and also for KCl-pyrophosphate-extractability.
Figure 2. shows the statistically significant interactions between preparate-temperature, preparate-pH and pH-temperature.

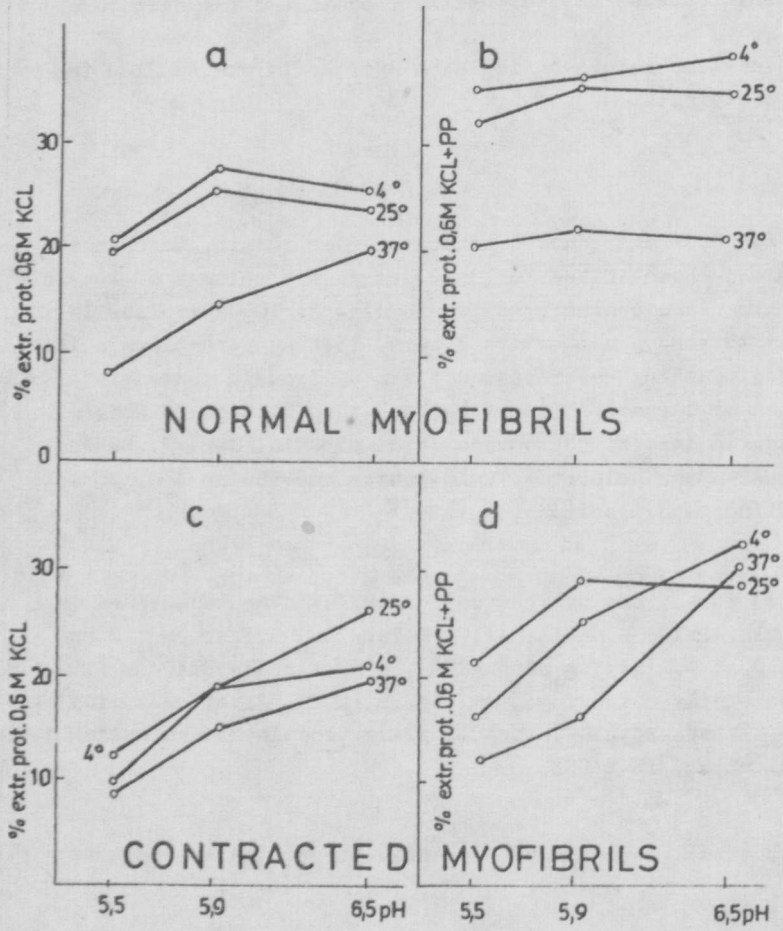
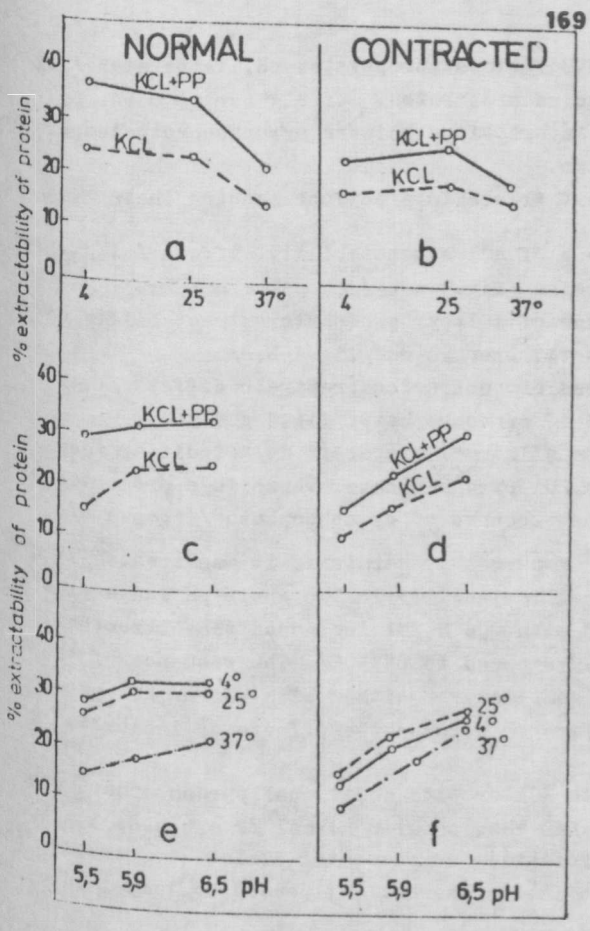


figure 1. The percentage of extractable protein from normal myofibrils in a medium of 0,6M KCl /a/, 0,6M KCl + 1 mM $Na_4P_2O_7$/b/ and from contracted myofibrils in a medium of 0,6M KCl /c/ 0,6M KCl + 1 mM $Na_4P_2O_7$ /d/, at pH 6,2



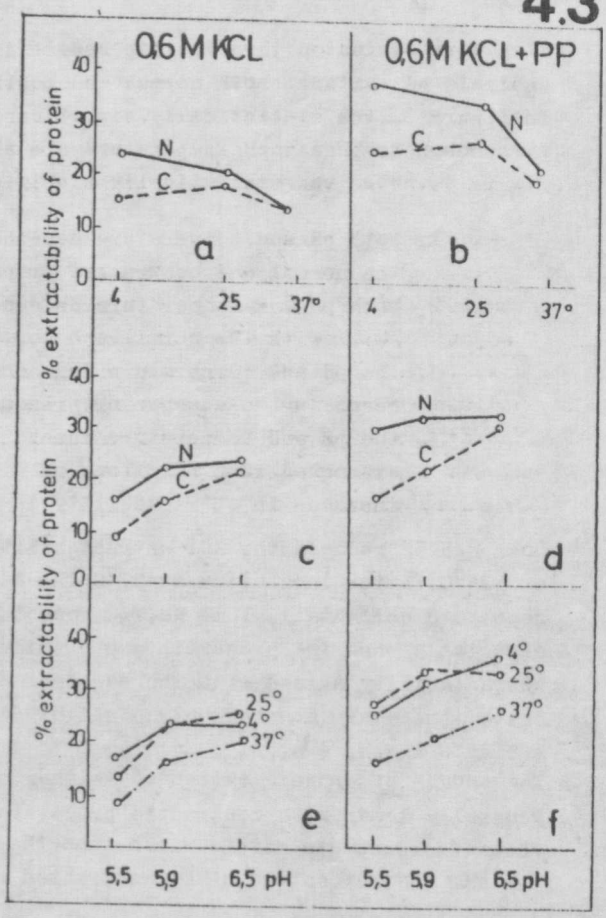


Figure 2.
Significant interactions between preparate-temperature /a,b/, preparate-pH /c,d/ and pH-temperature /e,f/. Normal/N/ and contracted /C/ myofibrils were extracted by 0,6 M KCl/a,c,e/ and 0,6 M KCl + 1 mM $Na_4P_2O_7$ / b,d,f/.

Figure 3.
Significant interaction between pyrophosphate-temperature /a,b/, pyrophosphate-pH /c,d/ and pH-temperature /e,f/. Normal/a,c,e/ myofibrils and contracted /b,d,f/ myofibrils were contracted with 0,6 M KCl and 0,6 M KCl + 1 mM $Na_4P_2O_7$/KCl+PP/.

Also were evaluated the significance of the 3 factors /preparate, pH, temperature/ by analysis of variance both normal and contracted preparates.
In figure 3. the statistically significant interactions between pyrophosphate-temperature, pyrophosphate-pH and pH-temperature are shown.
Figure 4. shows the statistically significant triple interactions meaning that:

1, both pH and temperature dependence of KCl-extractability differs /+++/ with the normal and contracted preparates /fig.4 a c /
2, both pH and temperature dependence of KCl+pyrophosphate-extractability differs /+++/ with the normal and contracted preparates /fig.4 b d/
3, the pH and temperature dependences of contracted preparate differs /+++/ according to absence or presence of pyrophosphate /fig.4 c d/
4, the pH and temperature dependence of normal preparate do not differ when extracted with KCl alone or with KCl+pyrophosphate. There is a proportional increase in extractability in the presence of pyrophosphate /fig.4 a b/

Low pH/5,5/ reduced the KCl-extractability of normal preparate if it was treated at 4°C and 25°C. After incubation at 37°C the KCl-extractability in the whole pH range /1 a,4 a/ decreased definitely. 1 mM $Na_4P_2O_7$ combined with 0,6 M KCl increased the extractability, especially when the preparate was previously exposed to pH/5,5/. The sequence of the extractability agreed with the sequence of temperature, either with KCl or KCl+pyrophosphate, there are however only a slight difference between the effects of incubation at 4°C and 25°C/1 a,b; 4 a,b/.
The amount of protein extracted either with KCl or with additional pyrophosphate was generally lower with contracted preparate than that of with normal /2 a,b,c,d; 3 a,b,c,d/. The difference was more obvious when the myofibrils were treated at low temperature and low pH. The percentage of KCl-extracted myofibrils was not influenced by temperature, but

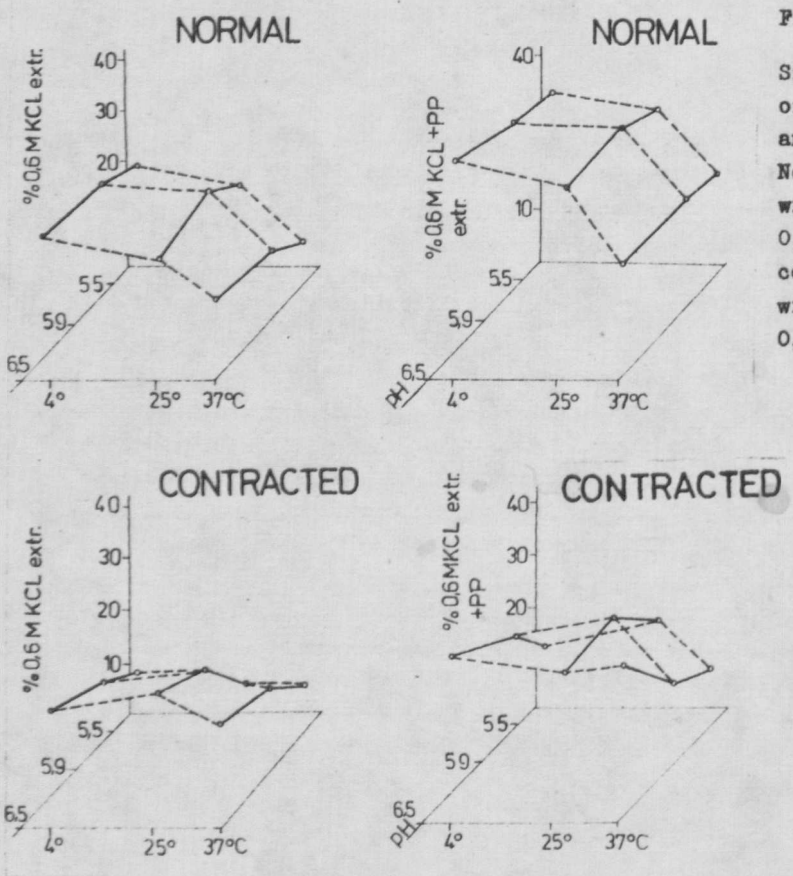


Figure 4.

Significant triple interactions of preparate-pH-temperature and pyrophosphate-pH-temperature. Normal preparates extracted with 0,6 M KCl /a/, and 0,6 M KCl+ 1 mM $Na_4P_2O_7$ /b/, contracted preparate extracted with 0,6 M KCl /c/ and 0,6 M KCl+ 1 mM $Na_4P_2O_7$ /d/

was rather reduced by pH /1 c, 4 c/. Comparing the KCl-extractability obtained with normal and contracted preparate it can be observed, that their 37°C isotherms are almost identical /1 a,c,4 a,c/. As compared to 0,6 M KCl, addition of 1 mM $Na_4P_2O_7$ caused a moderately enhanced solubilisation with contracted myofibrils after incubation at 5,9-6,5 and 4-25°C temperature intervals, moreover after treatment at 37°C and 6,5 pH KCl-pyrophosphate extractability reached much higher level than that of with normal myofibrils treated under the same condition. There was no direct relationship between the extractability and the temperature of incubation, a maximum can be observed at 25°C. KCl-solubilisation shows its maximum at 6,5 pH and 25°C, while KCl-pyrophosphate extractability attained the highest level at pH interval of 5,5-5,9 and at 25°C.

Discussion

At the time of death and rigor the tension, the rate of ATP-ase and glycolysis can vary muscle to muscle and animal to animal. In the period elapsed from death to rigor a wide variation of pH, temperature and tension can develop. As it was used by several authors /Scopes 1964, Penny,1967/b, Sung et al.1976/ myofibrils were exposed to variation of pH and temperature that might occur in this period.

Apart from the excess of water and the extremely contracted myofibrils brought about in the medium by MgATP, myofibril suspension can be considered as a useful model for revealing the meat properties.

It has been observed, that solubility showed maximum of 40%. This would be attributed to the contraction effect of endogen ATP which was present at the beginning of preparation process. Penny,/1967/ also mentioned this effect.

The results confirmed the well known effect of pyrophosphate which increased the extractability both with normal and contracted myofibrils.

This character of pyrophosphate is explained by its dissociating effect upon actomyosin /Bendall, 1954, Yasui 1964/. However, it is questionable whether the denatured myofibrils can be dissociated by pyrophosphate. In this respect Izumi, et al. 1977 suggested an irreversibly fix bond between actin and myosin / in the denatured PSE meat which was not able to relax by MgATP/. Similar result was published by Sung et al./1976/. It has been found, that the KCl- and KCl+pyrophosphate extractability depend upon the pH and temperature. This relationship is nevertheless not proportional to tempera ure. This result could be interpreted by the finding of Penny /1967 a,b/, who suggested that myosin which did not bind to actin was readily denatured, whereas when bound to actin, myosin in the myofibril was more resistant and denatured only after long exposure to denaturing effects. Sung et al./1976/ studied the extractable protein with Hasselbach-Schneider solution as depended on the pH_{ult} and also analysed the proportion of actin/myosin. They also found that myosin lost its extractability parallel with the reduction of pH. This result is in good agreement with findings of Penny/1967 a,b/. We presume that a higher proportion of myosin was bound to actin in the contracted preparate. This view is confirmed by the fact, that a lower amount of extractable protein at lower temperatures /4° and 25°C/ was obtained with 0,6 M KCl alone while applying pyrophosphate in a concentration of 1 mM, a high percentage of extractable protein found even after a treatment of 37°C provided that incubation was performed at pH 6,5.

In this case, the solubility proved to be much higher than that of with normal.

Results suggested contracted preparate exhibit a non linear relationship between extractability and the temperature. This relationship could be related to the interactions of actomyosin complex with Me^{2+} ions. Such interactions which influence the protein structure are also affected by the temperature and pH /Szent-Györgyi 1949, Berman & Swift 1961, Yasui et al. 1964/.

Our results suggested, that sensitivity of myofibrillar proteins to low pH or elevated temperature altered according to their molecular structure in the myofibrils. Further studies are required on the extractability including analysis of extractable fractions and on the relation between extractability and swelling properties of myofibrils.

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