1. Die Löslichkeit von Fleischproteinen: gegenseitige Abhängigkeit von pH, Natriumchlorid, Pyrophosphat und den spezifischen Eigenschaften des Muskels

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Biceps femoris Proben vom Schwein und Rind wurden mit einer fünffachen Volumenmenge an Lake, die 1-8% Natriumchlorid in Gegenwart von 0,2-1,0% Pyrophosphat enthielt, bei einem End-pH-Wert variierend von 5,5 bis 7,0 extrahiert. Das post-mortem Alter des Muskels variierte von 0,5 Stunde bis 3 Tage für die Proben vom Schwein und von 2 Stunden bis 7 Tage für die Rindfleischproben.

Die unter verschiedenen Bedingungen bestimmte Proteinlöslichkeit war für die Proben vom Schwein und vom Rind sehr ähnlich: die Löslichkeit nahm zu mit wachsendem NaCl-Gehalt und pH-Werte, während sie ab einem Salzgehalt von 4% weitgehend unabhängig vom pH-Wert ist. Pyrophosphat in der Lake (zusammen mit $\geqslant$2% Salz; grösste Auswirkung bei 4% Salz) bewirkt einen starken Anstieg der Proteinlöslichkeit, sogar bei niedrigen pH-Werten. Der Höchstwert für die Löslichkeit wird meistens im Bereich pH 6,0 beobachtet.

Die im Bereich pH 5,5 bis 7,0 ermittelten Löslichkeitskurven für Laken mit Pyrophosphat und nach dem Rigor mortis entnommenen Fleischproben zeigen einen Höchstwert bei einem pH von etwa 6,0, im Gegensatz zu den vor der Starre entnommenen Fleischproben.

Es gibt grosse Schwankungen in der Proteinlöslichkeit des gleichen Muskels verschiedener Tiere, sowohl beim Schwein wie beim Rind, die sich nicht aus dem post-mortem Alter oder dem Alter des Tieres erklären lassen. Unterschiede in der Art des Schweinefleisches widerspiegeln sich in der Proteinlöslichkeit. Die Löslichkeitskurven ermittelt mit 2% Salz und 1% Pyrophosphat in der Lake dürften als "Qualitätsmerkmal" für Fleisch angewendet werden.

Der Einfluss von Natriumchlorid auf die Proteinlöslichkeit ist seiner Auswirkung auf die Ionenstärke zuzuschreiben. Der Effekt von Pyrophosphat ist sehr spezifisch und hängt nicht mit der Ionenstärke oder dem pH-Wert zusammen.

Die Bedeutung dieser Befunde für die Fleisch verarbeitende Industrie wird erläutert.

1. Solubility of meat proteins: interdependence of pH, sodium chloride, pyrophosphate and intrinsic properties of the muscle

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Pork and beef, Biceps femoris of both, were extracted with 5 volumes of brine, containing 1-8% sodium chloride in combination with 0.2-1.0% pyrophosphate, at a final pH value varying between 5.5 and 7.0. Post-mortem age of the muscle varied from 0.5 h to 3 days for pork or 2 h to 7 days for beef.

The protein solubility determined under various conditions is very similar for pork and beef: it increases with increasing salt level and increasing pH, while at a salt level of 4% or higher, it largely is independent of pH. Pyrophosphate in the brine (in combination with $\geqslant$2% salt; maximal effect at 4% salt) increases the protein solubility dramatically, even at the lower pH values. A maximum is usually observed at a pH of about 6.0.

The solubility patterns over the pH range 5.5 to 7.0, obtained with brines containing pyrophosphate, for post-rigor meat show a maximum at a pH of about 6.0, but not those of pre-rigor meat.

There is a large variation in protein solubility for the same muscle from various animals, both in pork and in beef, which cannot be attributed to post-mortem age or age of animal. Variation in type of pork is reflected in the protein solubility. The solubility patterns, obtained with 2% salt and 1% pyrophosphate in the brine, could be used as a "quality" index for meat.

The effect of sodium chloride on protein solubility can be attributed to its influence on the ionic strength of the brine. The effect of pyrophosphate is a specific one; it is not related to ionic strength or pH.

The relevance of the findings for actual meat processing is discussed.

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1. Solubilité de protéines de viande: corrélation entre pH, chlorure de sodium, pyrophosphate et propriétés essentielles du muscle

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On a extrait du porc et du boeuf - le Biceps femoris des deux - à l'aide de 5 volumes de l'eau salée (contenant 1-8% de chlorure de sodium et 0,2-1,0% de pyrophosphate) près duquel le pH final se situait entre 5,5 et 7,0. L'âge post-mortem du muscle était de 30 min à 3 jours pour le porc et de 2 h à 7 jours pour le boeuf.

La solubilité des protéines déterminée dans des conditions différentes est très similaire pour le porc et pour le boeuf: la solubilité s'augmente à une concentration de sel et un pH croissants. Pourtant, à une concentration de sel de 4% ou plus élevée, cette solubilité est pratiquement indépendante du pH. Le pyrophosphate dans l'eau salée (contenant également ⩾ 2% de sel, une concentration de 4% donnant un effet maximal) augmente la solubilité des protéines très considérablement, même à des valeurs pH plus basses. Le plus souvent, le maximum est trouvé à un pH de 6,0 environ.

Les solubilités dans l'intervalle pH 5,5 à 7,0 obtenues à l'aide de l'eau salée contenant du pyrophosphate, montrent un maximum à un pH de 6,0 environ pour la viande "post-rigor", mais non pas pour la viande "pré-rigor".

La solubilité des protéines montre, pour le même muscle de différents animaux, c'est-à-dire pour le porc et le boeuf, une grande variation que l'on ne peut attribuer à l'âge post-mortem ou à l'âge de l'animal. Des variations dans le type de porc sont retrouvées dans le niveau de la solubilité des protéines. Les dessins de solubilité obtenus avec 2% de sel et 1% de pyrophosphate dans l'eau salée, pourraient être utilisés comme indice de "qualité" pour la viande.

L'effet de chlorure de sodium sur la solubilité des protéines peut être attribué à son influence sur la force ionique de l'eau salée. L'effet du pyrophosphate est spécifique; il n'y a pas de corrélation avec la force ionique ou le pH.

La signification des résultats obtenus est discuté dans le cadre du traitement de viande actuel.

1. Растворимость мясных протеинов: взаимозависимость pH, хлористого натрия, пирофосфата и внутренних свойств мышца

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Свинина и говядина (мясо из бицепса бедра) проэкстрагированы 5 объемами раствора, содержащего I-8% поваренной соли и 0,2-I,0% пирофосфата. Конечные значения pH колебались в пределах 5,5-7,0. Время хранения после убоя колебалось от 0,5 ч до 3 сут для свинины и от 2 ч до 7 сут для говядины.

Растворимость протеинов свинины, определенная в различных условиях, очень близка к растворимости протеинов говядины; растворимость повышается с возрастающим содержанием соли и с повышением pH, однако при содержании соли 4% и выше она как правило не обусловливается величиной pH. Добавление пирофосфата к солевому раствору (при содержании соли ⩾ 2%; максимальный эффект при 4% соли) сильно повышает растворимость протеинов, даже при более низких значениях pH. Максимум наблюдается обычно при pH около 6,0.

Значения растворимости в интервале pH 5,5-7,0, полученные на солевых растворах, содержащих пирофосфат, в случае мяса по окончании rigor mortis имеют максимум при pH около 6,0, в противоположности мясу до сосояния rigor mortis.

Растворимость протеинов одного мышца у различных животных может колебаться в широких пределах, как в свинине, так и в говядине, чего нельзя приписать времени хранения мяса после убоя или возрасту животного. Вариация типа свинины (PSE, DFD) отражается на степени растворимости белков. Значения растворимости, полученные на растворе с 2% соли и I% пирофосфата могут служить "качественным показателем" для мяса.

Эффект NaCl на растворимость белков может быть приписан эффекту ионной силы солевого раствора. Эффект пирофосфата является специфическим; он не связан с ионной силой или pH. Обсуждено значение исследования для современного процесса переработки мяса.

1. Solubility of meat proteins : interdependence of pH, sodium chloride, pyrophosphate and intrinsic properties of the muscle

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Introduction

The importance of protein solubility for meat processing and stability of meat products, in terms of fat and jelly exudation, is stressed by several authors. Saffle (1) emphasized its importance in connection with the emulsifying capacity of meat proteins and Kotter et al. (2) pointed to the formation of a coherent gel matrix by solubilized meat proteins as a means of structuring meat products and water binding. On the other hand, Hamm (3) strongly suggests that water binding and structuring in meat products is brought about by swelling of meat particles and for swelling the protein should stay in the meat fragment rather than being solubilized.

In view of solubilization of meat proteins (1, 2) salts are required to yield a certain ionic strength, while in swelling salts are said to promote the mutual repulsion of proteins which creates the spatial condition for water binding (3). The conditions of salt type and concentration that promote either mechanism, work in the same direction for promoting the stability of meat products. However, as polyphosphates are included in the curing salt mixture, product stability is generally enhanced but the effect of polyphosphates on protein solubility and on swelling is contradictive. Solubility is increased (4) while swelling is reduced (5).

There is thus still a controversy about protein solubility and solubilized protein playing a role in the structure formation and water-binding in meat products and about which mechanism underlies these roles. This controversy may be explained by the fact that little systematic studies on protein solubility of meat have been published. Though the term soluble protein and protein solubility are often mentioned in literature on meat processing, its importance or unimportance is only substantiated by statements like "high pH promotes protein solubility" and "pre-rigor meat enhances product stability" accepting that proteins in pre-rigor meat have a high solubility. Little experimental work has been published, however, to base these statements on.

We have studied the solubility of proteins from beef and pork in detail as to the condition of pH, ionic strength, effect of pyrophosphate, post mortem age and animal-to-animal variation. The results have been discussed as to the relevance of protein solubility to meat processing, while the role of diphosphate in meat processing is also given attention.

Experimental

The meat used in the experiments was beef hind-quarter (topside, Biceps femoris) and the same muscle from pigs. The individual properties of the muscles, that is post-mortem age, age of the animal, pH and -in pigs- the type of muscle, are given in the Results section. Samples of at least 100 gram were taken for protein determination; the chilled meat was trimmed off any visible fat and minced through a plate with 2 mm holes and mixed thoroughly.

Protein solubility was determined by homogenizing 10 g of minced chilled meat with 50 g of ice-cold ($0^{o}C$) brine in a Waring Blendor for 5x15 seconds with 15 seconds' intervals. The pH-range was varied deliberately over the range 5.5 to 7.0 by adding 3 M HCl or 5 M NaOH to the meat-brine mixture before homogenization. The homogenate was equilibrated at $9^{o}C$ for 3 hours, then centrifuged at 48 000 x g for 1 hour. Protein concentration in the clear supernatant was determined by the Kjeldahl method. The amount of soluble protein is expressed relative to the total protein in meat. Soluble proteins then comprises both so-called water-soluble and salt-soluble protein (1), the level of water-soluble protein remaining constant in all extraction conditions. The protein values measured with the Kjeldahl method were corrected for the amount of low molecular nitrogeneous compounds in meat, being 5.3 and 5.5% of total nitrogen measured in beef and pork respectively .

Total protein was measured, using the Kjeldahl method, in the meat-brine homogenate obtained as described above.

The ionic strength, μ, of the meat-brine homogenates was calculated using the formula (6):

$$\mu = \tfrac{1}{2} \sum_i c_i z_i^2$$

in which c_i and z_i are concentration and charge, respectively, of the ion i. Ionic strength can only be given in large approximation: The contribution of meat to μ is neglected after the six-fold dilution with brine. Furthermore the influence of meat proteins on μ by absorbing specific ions cannot be accounted for. The contribution of pyrophosphate to μ can also be given with approximation only.

The number and type of ions set free in the dissociation of the weak pyrophosphate poly-acids, can be calculated from the dissociation constants K_1, K_2 etc. for a given pH. The dissociation constants found in literature (7) are based on concentrations at infinite dilution and are, in fact, theoretical values. In the presence of high concentrations of other ions and dealing with high concentrations of pyrophosphate itself, which is the situation in the brines used, the dissociation is influenced strongly by ionic strength. To find the real numbers and types of pyrophosphate-ions, the simplest way is to correct theoretical K values as:

$$pK' = pK + \frac{(2z_a - 1)A\sqrt{\mu}}{1+1.6\sqrt{\mu}}$$

in which z_a is the charge of the acid under consideration and A is a constant, being 0.50 at $20^{o}C$. In fact

this formula (6) holds only for ionic strengths $\mu<0.1$. As we are dealing with ionic strengths $=\mu 0.2$ to 0.7, application of the formula will yield only approximate values.

The contribution to ionic strength of pyrophosphate, being pH-dependent, is further approximated by taking only the mean value for pH range 5.75 to 6.50. The indicative ionic strengths calculated for the various brine compositions are:

% pyro-phosphate	% sodium chloride						
	1	2	3	4	6	7	8
0	0.17	0.34	0.51	0.68	1.03	1.20	1.37
0.2		0.39		0.73			
0.5		0.47		0.81			
1.0		0.59		0.93			

The concentrations of sodium chloride and pyrophosphate mentioned in this paper are expressed on total water (water from meat and added water) in the meat-brine homogenates.

Results

1. Effect of pH, salt and pyrophosphate on protein solubility

The effects of pH and concentration of sodium chloride on protein solubility of beef are illustrated in Fig. 1A. Obviously these two effects cannot be seen separately. Solubility is increased with increasing ionic strength and with increasing pH when ionic strength is low. At high ionic strength (μ= 1.37 or 8% salt) solubility becomes fairly independent of pH in the range 5.7 to 7.0. The results obtained with pork are very much the same as those obtained for beef (Fig. 1E).

Use of pyrophosphate yields a marked increase in protein solubility, both in beef and pork (Fig. 1B, C, F, G). At the 2% salt level, pyrophosphate at 0.5 to 1.0% level increases solubility from 40% to an average of 60% at pH 6.0 to 6.5. A remarkable effect is the relative large increase at lower pH-values, that is maximum solubility is attained at lower pH-values than in brines with salt alone. This phenomenon is clearly demonstrated at the 4% salt level. The effect of pyrophosphate is only slight at the 1% salt level and is maximal at the 4% salt level. The maximum is closely approached with 3% salt.

Orthophosphate exerts no such effect as pyrophosphate does (Fig. 1 D, H).

The phosphate effect on protein solubility is very much the same for beef and pork (compare Fig. 1B, C, D and Fig. 1F, G, H).

2. Differences in protein solubility in the same muscle from different animals (animal-to-animal)

Marked differences exist between the solubility of proteins from the same type of muscle from different animals, even though post-mortem age, at moment of extraction, and age and history of the animals are very much the same. For pork such differences are well known as differences in quality or type of meat, viz. PSE, DFD and "normal" type

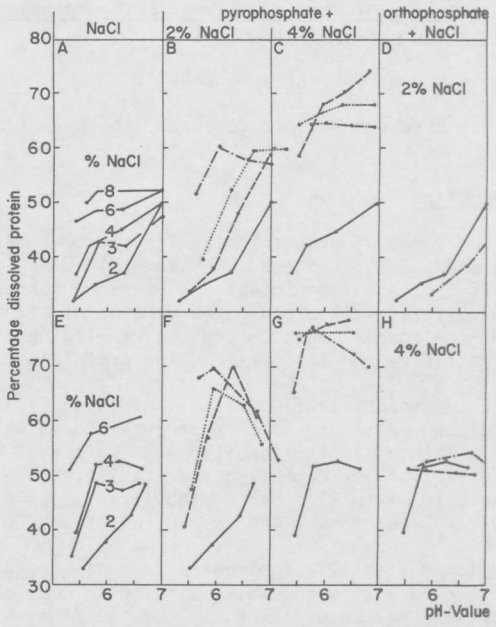

Fig. 1 Solubility of protein from beef (A-D) and pork (E-H) in the pH range 5-7. Salt varied from 2-8% on total water (A,E) and pyrophosphate was added at the 2% and 4% salt level in concentrations of 0% (—), 0.2% (--), 0.5% (...)and 1% (-·-) (B,C,F,G). The effect of orthophosphate (1% at 2% salt for beef; 0.2 and 1% at 4% salt for pork) is shown in D and H. BEEF: topside (Biceps femoris) from a 3 year old heifer (expts. were done when the muscle was 7 days post mortem and its pH was 5.38).
PORK: silverside, its pH was 6.20.

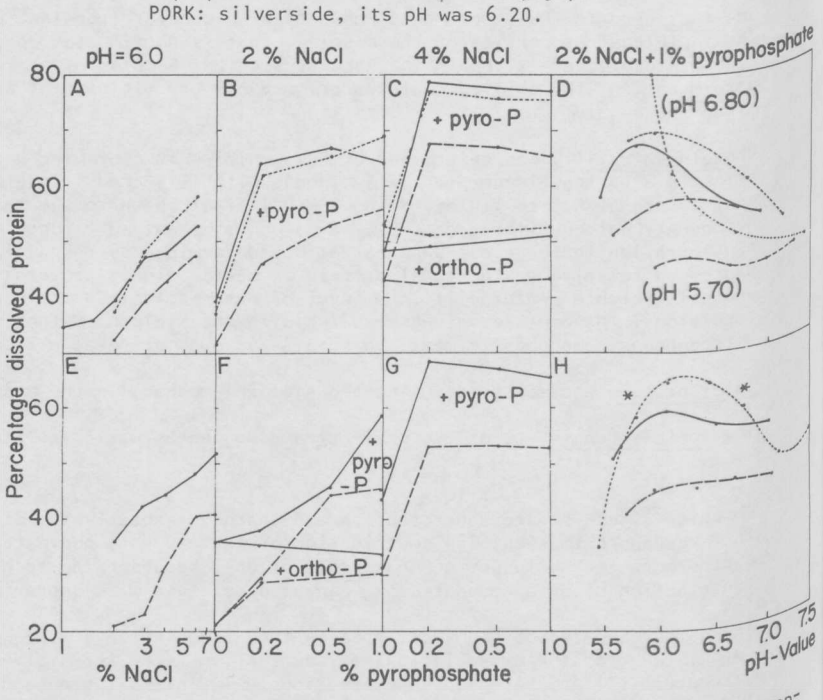

Fig. 2 Solubility of protein from pork silverside (A-D) and beef topside (E-H). Salt varied from 1-8% on total water (A,E) and pyro- and orthophosphate were added at the 2 and 4% salt level (B,C,F,G); for 2% NaCl + 1% pyrophosphate the pH was varied between 5.5 and 7.5 (D,H).
PORK (A-D): -- PSE (pH 5.42), ... DFD (pH 6.40), — normal (pH 5.96); silverside from 8 months old animals (expts. were done when the muscles were 3 days post mortem).
BEEF (E-H): topside from old dairy cow, pH 5.95 (*) and from 3 years old heifers (— pH 5.38, -- 5.48, ... pH 5.42) (expts. were done when the muscles were 4 days post mortem).

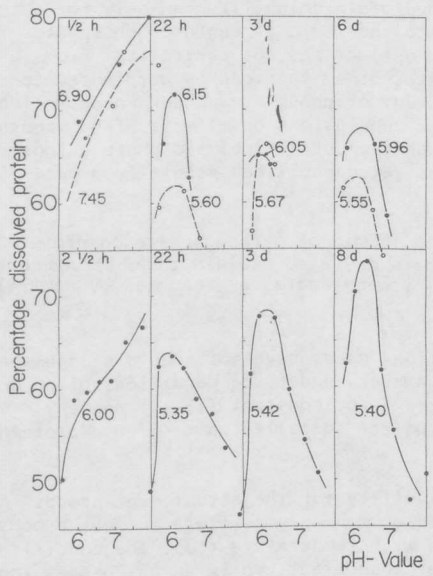

Fig. 3 Amount of soluble protein from two pork samples (½h to 6 days post mortem) and from topside beef (2½h to 8 days post mortem), with 2% salt and 1% pyrophosphate. The muscles were placed in chill immediately after they had been butchered from the carcass. The pH-values of the meat at the moment of extraction were measured at start of the various experiments in the intact muscle; the values are given at the curves in the figure.

(that is showing no pronounced PSE or DFD characteristics). Differences in protein solubility are apparent all over the pH-range studied and for all conditions of salt and pyrophosphate. The latter differences (most apparent at pH 6.0) between PSE, DFD and normal pork are summarized in Fig. 2A, B, C.

The differences over the pH-range 5.50-7.00 for the three types of ham can be best compared using the condition 2% salt and 1% pyrophosphate. This is illustrated in Fig. 2D.

The pattern of differences in solubility also exists in beef, as can be seen from Fig. 2E, F, G for the various conditions of salt and pyrophosphate at pH 6.0. Like for pork in Fig. 2D, the differences for beef, which are smaller, are shown in Fig. 2H.

3. Post-mortem changes in protein solubility

Protein solubility changes dramatically with increasing post-mortem age of the muscle, especially in the first 24 hours after slaughter. The changes are evident from the upper part of Fig. 3 which shows a maximum in the extractability curves over the pH-range 5.5 to 7.0 for two types of pork for the condition 2% sodium chloride and 1% pyrophosphate on total water. The corresponding decrease in pH post mortem of the two muscles is given at the curves. Before the onset of rigor (0.5 h post mortem), protein solubility is high and the pattern of the solubility curve over the pH-range studied is quite distinct from the patterns obtained after 22 h post mortem, when the patterns have the typical "post mortem shape" (cf. Fig. 1E-H, 2D and 2H). Minimum solubility (at pH 6.0) is attained after 3 days (pig I) or 22 h (pig II) post mortem. Pigs I and II showed no characteristics typical for PSE or DFD muscles.

With beef very similar results were obtained. The shape of the solubility pattern over the pH-range 5.5 to 7.0 obtained before onset of rigor (2.5 h post mortem) is quite different of the patterns obtained on from 22 h post mortem (lower part of Fig. 3). The level of the solubility at 2.5 h post mortem is, however, quite moderate. The pH of the beef muscle at this time, however, had fallen to 5.90 already (numbers at curves in Fig. 3), which may point to an early onset of rigor. In fact, the curve shows a transition phase from high solubility before rigor, as obtained with the pigs, to the post mortem patterns. Minimum solubility is at 22 h post mortem, after this time solubility shows a graduate increase (lower part of Fig. 3).

From the solubility curves obtained with pork and beef, it is apparent that solubility is not increasing with pH in all conditions, as generally accepted. Maximum solubility is attained around pH = 6.0.

Discussion

The results obtained clearly emphasize that protein solubility strongly depends on post mortem age of the muscle, extraction conditions and also on the individual properties of the muscle itself. The influence of post-mortem age on solubility is well-known (8), as are the effects of extraction conditions. Of these conditions, the effects of various salts, including pyrophosphate, have been extensively studied (4, 8). However, the condition of pH, and especially the interrelationships of this condition with those of salts, has not yet been studied extensively. The pH-dependence of protein solubility has been studied in some detail in connection with the influence of pressure on solubility (9). In protein-extraction studies, pH is taken as it is established by meat and the various salts used. The generally accepted rule that solubility is increased by increasing pH (10) seems to be valid only for pre-rigor meat and when only sodium chloride is used. The variation of protein solubility with pH in the range 5.5 to 7.0 is quite complicated, especially when pyrophosphate is used. With use of this phosphate, at the 2% salt level, solubility normally tends to a maximum around pH 6.0. At higher salt concentrations the solubility tends to become independent of pH on from pH 5.50.

A most prominent factor that effects protein solubility is the individual status of a muscle: there is a marked animal-to-animal variation. The variation in muscle type in pigs, resulting from distinct biochemical processes during rigor, is well-known; pronounced types are PSE and DFD muscles (8, 11). Variation in solubility from various types of meat is also well-established (12). The animal-to-animal variation in beef, or in individual status of beef muscle, is less well established in literature. There is, however, a fairly wide variation in ultimate post-mortem pH possible in beef carcasses of comparable history (13), reflecting, probably, variations in the biochemical status of the muscles. Anyhow, variation in protein solubility, which can be ascribed only to such variations, occur in beef as well as in pig muscles.

The effect of sodium chloride on protein solubility can be explained by the ionic strength provided by the salt. Our results, however, do not preclude a possibility of interaction of the chloride ions with meat proteins. Chloride ions, like other halogenide ions do have a specific effect on meat proteins (8, 14) and next to ionic strength the ratio Cl^- to meat proteins may explain the effect of sodium chloride (8).

The effect of pyrophosphate is obviously a specific one. Its effect on protein solubility can only for a small extent - not completely as held by Swift and Ellis (14) - be ascribed to the ionic strength of phosphate. The effect through pH is only of interest in conditions in which pH is not optimal and not controlled. From our results under controlled pH-conditions no direct effect of pyrophosphate, other than can be attributed to ionic strength or specificity, has been observed. On the other hand, study of phosphates under uncontrolled conditions of pH, as have been published (14, 15) may lead to erroneous conclusions on effects of polyphosphates. The specific action of pyrophosphate is not brought about by orthophosphate, but tripolyphosphate brings about very much the same effects as pyrophosphates, as is shown from as yet unpublished result from our laboratory.

By whatever mechanism, possibly by dissociation of actomyosin (16), the effect of pyrophosphate on protein solubility is marked. The increase in protein solubility by pyrophosphate may also explain the observed decrease in apparent viscosity of meat-water-salt homogenates upon addition of pyrophosphate, as reported by Hamm (17) and which contradict his theory of swelling.

The variations in protein solubility observed under the various conditions used, have not been distinguished in changes in solubility of water-soluble and salt-soluble proteins. However, under the conditions of pH and salts used, the solubility of the water-soluble proteins may be accepted to be constant (1, 17) and any variation in solubility observed thus reflect variation in solubility of the so-called salt-soluble proteins of meat.

The question arises as to the relevance of the findings on protein solubility for the actual meat processing. As concerns the conditions, the temperature of extraction (9°) is quite common a temperature in meat processing and, moreover, extractability of proteins from meat only varies little with temperature (18). The condition of amount of brine to meat used in the present experiments is much greater than practiced in actual processing. However, when ratio-to-brine was varied from 1 : 0.6 to 1 : 2, the solubility of salt-soluble proteins varied only from 34 to 40%, the amount of water-soluble protein remaining constant (17). The effects of conditions studied thus may be relevant for things happening during meat processing. In preliminary, unpublished experiments we found protein solubility at the 1 : 0.3 ratio to be higher by 5 to 10% than at the 1 : 5 ratios. The effects of conditions of sodium chloride concentrations observed are parrallel to the effects of sodium chloride on cooking yield or heat-shrinkage of meat (14, 15), in tests performed under conditions approximating those in actual processing. Likewise, the effects of pyrophosphate on protein solubility are paralleled in similar close-to-practice test on cooking yield or heat-shrinkage (15, 19), in which the specific action of pyrophosphate is also clearly demonstrated by Hellendoorn (20).

Although the conditions that increase cooking yield in actual products and those that increase protein solubility in model experiments run parallel, this offers no proof that solubilization of protein is the ruling factor or basic mechanism for meat processing. Though indications are strong, further experiments are needed to verify this assumption. At present it can be suggested that under processing conditions, meat proteins are in a potentially soluble form. Protein solubility seems to offer a fair means for quality evaluation of meats. Poor technological quality, as in PSE-hams, is reflected in poor solubility of protein. This solubility factor is already heavily encorporated in the emulsifying capacities, the quality indices as given by Saffle (1), and could as well replace them.

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