## 2. Die spezifische Wirkung von Pyrophosphat auf die Löslichkeit von Fleischproteinen

A.H.A. VAN DEN OORD und J.J. WESDORP

Unilever Research Duiven, Zevenaar, Niederlande

Proben von magerem Schweinefleisch (Biceps femoris) wurden mit einer fünffachen Volumenmenge an Lake, die 1-6% Natriumchlorid in Gegenwart von 0,01-1% Pyrophosphat enthielt, im pH-Bereich 5,50 bis 7,00 extrahiert. Die beachtliche positive Wirkung des Pyrophosphats auf die Extrahierbarkeit von Fleischproteinen nahm ständig zu mit wachsender Ionenstärke und steigendem pH-Wert. Pyrophosphat beeinflusst die Ionenstärke an sich, obwohl geringfügig, während dieser Einfluss nicht mit dem Effekt auf die Proteinextrahierbarkeit korreliert ist. Letzterer Effekt ist als eine spezifische Wirkung zu betrachten, welche vermutlich durch das $P_2O_7^{4-}$ Ion verursacht wird. Darüber hinaus gibt es Andeutungen dafür, dass das Triphosphat seinen spezifischen Effekt erst nach der Hydrolyse zu Pyrophosphat aufweist.

## 2. The specific effect of pyrophosphate on protein solubility of meat

A.H.A. VAN DEN OORD and J.J. WESDORP

Unilever Research Duiven, Zevenaar, The Netherlands

Lean pork (Biceps femoris) was extracted with 5 volumes of brine, containing 1-6% sodium chloride in combination with 0,01-1% pyrophosphate, over the pH range 5.50-7.00. The marked positive effect of pyrophosphate on the extractability of meat proteins generally became stronger with increasing ionic strength and increasing pH. Pyrophosphate does influence ionic strength itself, but only to a limited extent, while this influence is not correlated with its effect on protein extractability. This effect is concluded to be a specific one and is suggested to be caused by the $P_2O_7^{4-}$ ion. It is further suggested that triphosphate exerts a specific effect only after hydrolysis into pyrophosphate.

# D 13:2

## 2. L'effet spécifique du pyrophosphate sur la solubilité des protéines de viande

A.H.A. VAN DEN OORD et J.J. WESDORP

Unilever Research Duiven, Zevenaar, Pays-Bas

On a extrait du porc maigre (Biceps femoris) en utilisant 5 volumes de l'eau salée (contenant 1-6% de chlorure de sodium et 0,01-1% de pyrophosphate) près duquel le pH final se situait entre 5,50 et 7,00. L'effet positif très clair du pyrophosphate sur l'extractabilité de protéines de viande s'augmentait généralement à une force ionique et un pH croissants. Le pyrophosphate influe, sans doute, sur la force ionique même, mais seulement à un degré limité; en outre, il n'y a pas de corrélation entre cette influence et son effet sur l'extractabilité des protéines. Cet effet est considéré comme spécifique et les résultats obtenus font suggérer qu'il est dû à l'action de l'ion $P_2O_7^{4-}$. Il est aussi suggéré que le triphosphate a seulement un effet spécifique après hydrolyse en pyrophosphate.

## 2. Специфический эффект пирофосфата на растворимость мясных протеинов

А.Х.А. ВАН ДЕН ООРД И И.Я. ВЕСДОРП

Юнилевер Рисёрч Дейвен, Зевенаар, Голланд

Постная свинина (Biceps femoris) проэкстрагирована 5 объемами раствора, содержащего I-6% поваренной соли и 0,0I-I% пирофосфата, в пределах pH от 5,5 до 7,0. Выраженный положительный эффект пирофосфата на экстрагируемость мясных протеинов усилился при возрастающей ионной силе и повышении pH. Пирофосфат оказывает влияние на саму ионную силу, но только до определенной степени, в то время как взаимосвязь влияния на ионную силу с эффектом данного вещества на экстрагируемость протеинов отсутствует. Сделан вывод о специфичности этого эффекта.

По результатам исследования предполагается, что активная форма пирофосфата - ион $P_2O_7^{4-}$.

Далее высказывается мнение, что трифосфат имеет лишь специфический эффект после его гидролиза в пирофосфат.

## 2. The specific effect of pyrophosphate on protein solubility of meat

A.H.A.van den OORD and J.J.WESDORP

Unilever Research Duiven, Zevenaar, The Netherlands

Pyrophosphate has a marked positive effect on the solubilization of proteins from lean beef and pork; this effect can not be explained by its effect on ionic strength or pH (1). The specific effect of pyrophosphate was exerted only when a sufficiently high ionic strength had been attained with sodium chloride and when pH was 6 or higher (1). At low ionic strengths of sodium chloride or at pH-values below 6.00, hardly any effect of pyrophosphate is observed, as is shown in Figures 1 and 2. For the extraction of the proteins from lean pork (silverside) the meat was dispersed in five volumes of brine. Details of the method have been described elsewhere (1).

Ionic strength and pH, which both promote the effect of pyrophosphate, also have an effect on the dissociation of this phosphate. The dissociation of pyrophosphate in dependence of ionic strength is given by the relation (2):

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

in which $z_a$ is the charge of the acid under consideration, $\mu$ is the ionic strength and A is a constant, being 0.50 at 20°C. In fact this formula 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. Moreover, the effects of dispersed meat and a possible absorption of ions to proteins on the ionic strength have not been accounted for.

The effect of high salt concentrations, or high ionic strengths, on the dissociation of polyphosphate is an increase of dissociation (pK' values become lower than pK values). The four pK values for pyrophosphate (3) together with their corrected pK' values at some ionic strengths, are:

CRC D167

| | | $\mu$=0 | $\mu$=0.1 | $\mu$=0.5 | $\mu$=1.0 |
|---|---|---|---|---|---|
| $H_4P_2O_7 \rightleftharpoons H^+ + H_3P_2O_7^-$ | $pK'_1$ | 0.85 | 0.75 | 0.69 | 0.66 |
| $H_3P_2O_7^- \rightleftharpoons H^+ + H_2P_2O_7^{2-}$ | $pK'_2$ | 1.49 | 1.17 | 1.00 | 0.91 |
| $H_2P_2O_7^{2-} \rightleftharpoons H^+ + HP_2O_7^{3-}$ | $pK'_3$ | 5.77 | 5.24 | 4.95 | 4.82 |
| $HP_2O_7^{3-} \rightleftharpoons H^+ + P_2O_7^{4-}$ | $pK'_4$ | 8.22 | 7.49 | 7.07 | 6.89 |

Although these values are only valid by approximation in the meat-brine mixtures studied, the practical result of this is that under influence of sodium chloride polyphosphates will yield a fair amount of $P_2O_7^{4-}$ ions in meat at pH values as low as 5.75 to 6.0. In solutions of pyrophosphate this only occurs at pH 7 or higher.

With increasing pH, the availability of $P_2O_7^{4-}$ ions is increased too. With 4% sodium chloride present, the effect of pyrophosphate already has a maximum at around pH 6.00. At higher pH values the solubility decreases, presumably because of increased diphosphatase activity (4).

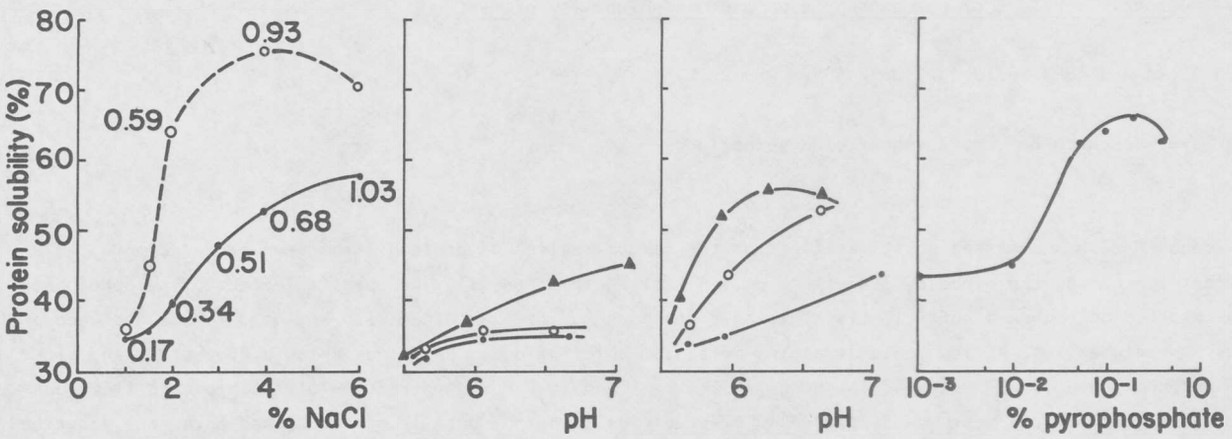
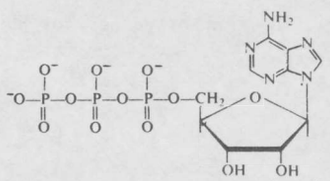



Fig.1. Protein extractability from pork silverside (% of total protein) at pH 6.00 in dependence of % NaCl with 1% pyrophosphate (---) and as such (——). Concentrations of the salts are given on total water in the meat-brine mixtures. The figures at the measuring points indicate ionic strengths (1). For details of the extraction procedure see (1).

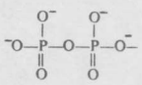
Fig.2. Protein solubility at low concentrations of NaCl (● 1%, o 1.5%, ▲ 2%) over the pH range 5.50 to 7.00.
The left figure is for NaCl as such, the right figure is for NaCl + 0.5% pyrophosphate.
The extractability is expressed as percent of total protein.
Salt and pyrophosphate concentrations are given on total water.

Fig.3. Relation of solubility of protein from pork silverside in a brine containing 2% NaCl and various low concentrations of pyrophosphate.
Solubility is expressed as percent of total protein.
For details of the extraction method see (1).

Pyrophosphate resembles ATP in its effect on muscle, as was shown by Weber (5). Comparing the formulas of ATP and pyrophosphate, it is obvious that the electronic structure of the two phosphates poly-ion moieties are very much the same:



adenosine triphosphate (ATP)



pyrophosphate

Using triphosphate instead of pyrophosphate gave similar results of protein extractability as obtained with the latter phosphate. This is also reported in literature (6,7,8). However, it has been well established that meat is capable of hydrolysing triphosphate into pyrophosphate fairly quickly (9,10). Furthermore, Yasier et al.(11) have established that triphosphate can cause changes in size and shape of myosin B only after being hydrolysed by B-tri-polyphosphatase.

The observed effect of pyrophosphate resembles that of ATP in one more respect. ATP exerts its biological function at a concentration of $5.10^{-3}$M, or 0.25% (3). We found that pyrophosphate affects protein extractability of meat proteins already at a level as low as 0.02% (Figure 3).

From the evidence described, we suggest that pyrophosphate is the only polyphosphate effective in solubilization of meat proteins and that the effect is most probably exerted via its $P_2O_7^{4-}$ poly-ion.

References

1. A.H.A.van den Oord and J.J.Wesdorp, Solubility of meat proteins (part 1). These proceedings.
2. Biochemists' Handbook, Ed. C.Long, E. & F.N.Spon Ltd., London 1961, p.23.
3. Handbook of Chemistry and Physics, Ed. R.C.Weast, The Chemical Rubber Co., Cleveland, 46th Edition, 1965/1966, p.D 79.
4. R.Hamm and R.Neraal, Z.Lebensm.Unters.-Forsch. 163, 213 (1977).
5. H.H.Weber, Proc.Roy.Soc. 139B, 512 (1952).
6. R.Hamm, Kolloidchemie des Fleisches, P.Parey, Berlin, 1972.
7. L.Kotter, Zur Wirkung kondensierter Phosphate und anderer Salze auf tierisches Eiweiss, Verlag M.& H.Schaper Hannover, 1960.
8. T.Yasini, M.Sakanishi and Y.Hashimoto, Agric.Fd.Chem. 12, 392 (1964).
9. A.H.Sutton, J.Fd.Technol. 8, 185 (1973).
10. R.Neraal and R.Hamm, Proceedings of the 19th European Meeting of Meat Research workers, Paris 1973, p.1419.
11. T.Yasui, T.Fukarawa, K.Takahashi, M.Sakanishi and Y.Hashimoto, Agric.Fd.Chem. 12, 399 (1964).