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PREFERENTIAL ADSORPTION OF MEAT PROTEINS DURING EMULSIFICATION.

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The aim of this work was to investigate if there exists a competition between various kinds of proteins in the formation of protein films as suggested by Schut (1), i.e. a preference of some protein(s) over others to be adsorbed at the fat-water interface. Another object, closely related to the first, was whether a protein film once formed can be replaced by another protein or not. And finally the role played by the non-soluble protein particles (meat- and connective tissue) in the formation and stability of meat emulsions was investigated.

Extraction procedures and definitions.

All extractions were so performed as to imitate as closely as possible the normal practice in meat emulsion production, viz. the minced meat was chopped with 60 % of either 0.02 M KCl or 0.35 M NaCl solution in a normal bowl chopper, followed by rapid dilution with the same solution and immediately centrifuged.

a) Meat proteins soluble in 0.02 M KCl are designated as the Water-Soluble Proteins, WSP.
b) Meat proteins insoluble in 0.02 M KCl but soluble in 0.35 M NaCl are referred to as Salt-Soluble Proteins, SSP.
c) Mixing the residue from a) with 2 % solid NaCl, followed after two hours by dilution with 0.35 M NaCl solution and centrifuging resulted in two layers, viz. SSP and a residue.
d) Chopping with 2 % solid NaCl instead of mixing produced three distinct layers viz. SSP, a swollen granular fraction, designated as K-fraction (1) and a residue R. The insoluble fractions were refined by washing.

As a comparative protein a sodium caseinate solution was used. The experiments were performed with WSP, SSP, K, R and sodium caseinate, SC.

Emulsifying Capacity (EC) - determination.

The Swift system (2) was used with some modifications, the most important of which was that the emulsification of soybean oil was performed under vacuum. The endpoint indication was based upon electrical conductivity according to Moerman (3). Unless otherwise stated, 50 grams of 0.4 % protein solutions in 0.35 M NaCl were submitted to the test.

Results and discussion.

1. Competition between the proteins of WSP and SSP and sodium caseinate (intra-competition).

Increasing quantities of oil were pre-emulsified under vacuum in a Sorvall mixer, into 200 gram lots of the three solutions, which all contained 2 % NaCl and 1 % protein, except SSP which contained 0.6 % protein. The term "pre-emulsified" is used to indicate that the amounts of oil chosen, were smaller than those needed to turn the emulsion in the EC-test. The emulsions were then centrifuged for 20 minutes at 48 200 x g, resulting in three layers, i.e. oil, emulsion and water. The water layers were analysed for remaining protein, diluted with 0.35 M NaCl solution to the standard 0.4 % protein concentration and submitted to the EC-test. The resulting EC-values are plotted in figure 1a against the protein consumed by the pre-emulsification, as a percentage of the initial protein. The amounts of pre-emulsified oil are indicated by different symbols.
Since the water layers of the SSP-emulsions with more than 100 grams of oil contained less than 0.4 % protein, the EC-tests were also performed with 0.3 % protein in this case. Where this concentration was too high for the last two points, these were found by extrapolation in accordance with the linear relationship between EC and SSP concentration in the corresponding water layers.
The distinct steps of the EC-curve of SSP after pre-emulsification indicate that SSP consists of several proteins with different EC. Apparently the proteins with the highest EC are preferentially adsorbed at the fat-water interface.

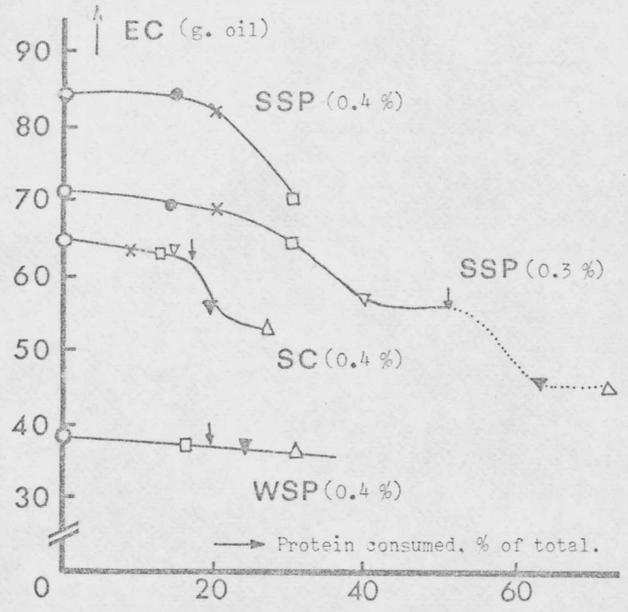


fig. 1a Change in emulsifying capacity with protein consumed by pre-emulsification of various amounts of oil.

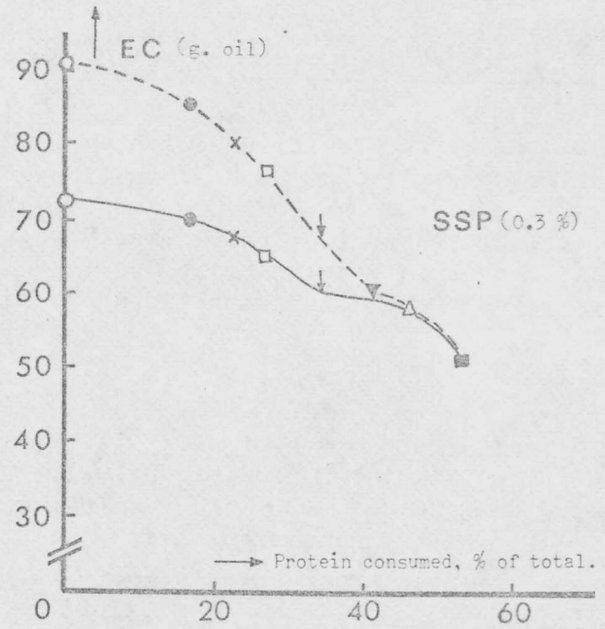
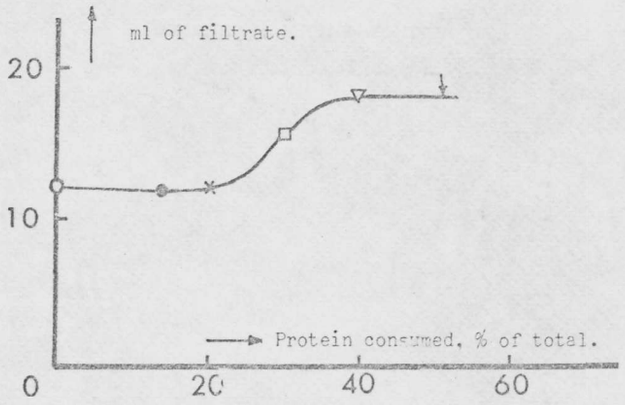


fig. 2 EC-values as in fig. 1a before (——) and after (– – –) treatment with Mg^{++} and $P_2O_7^{----}$.



Symbols of fig. 1 and 2.

200 grams of the solutions were pre-emulsified with:

○	none
●	25
×	50
□	100
▽	150
↓	200
▼	300
△	400
■	800

grams of oil.

fig. 1b Amounts of filtrate after one hour from heat-gelled SSP water layers (0.3 % protein), obtained after pre-emulsification of various amounts of oil.

The EC-curve of sodium caseinate shows a similar form. In the WSP-extract apparently there are no proteins which are preferentially adsorbed.
Since it is well known that SSP-extracts set to a gel on heating it was interesting to investigate how the different water layers after pre-emulsification would behave in this respect. Therefore equal amounts of these water layers were heated in a waterbath at 70°C for 2½ minutes, cooled down to 20°C and filtered. The amounts of filtrate obtained after one hour are plotted in figure 1b against the amounts of protein consumed by pre-emulsification. This figure suggests that those proteins with the highest EC also have the greatest ability for gel formation on heating. To investigate which proteins are preferentially adsorbed, the SSP-extract was dialysed to an ionic strength of 0.25 M and the resulting precipitate was identified as actomyosin; the supernatant was further dialysed to 0.04 M and the resulting precipitate appeared to be myosin. The original SSP-extract and the isolated actomyosin solution in 0.35 M NaCl were submitted to the EC-test before and after dissociation by means of Mg^{++} and $P_2O_7^{----}$ according to Gränicher and Portzehl (4). Table I shows the EC-values, measured at 0.4 % protein concentration. In addition, the experiments dealt with in fig. 1a were repeated for SSP, but this time the water layers also were submitted to the EC-test after dissociation. Fig. 2 shows the results. The data suggest that myosin has the highest EC, followed by actomyosin.

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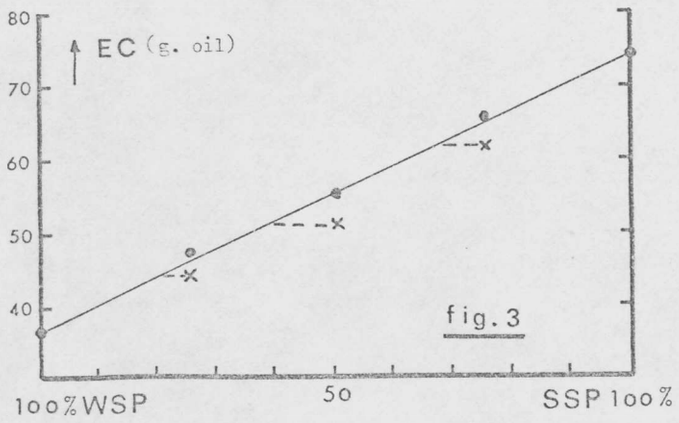
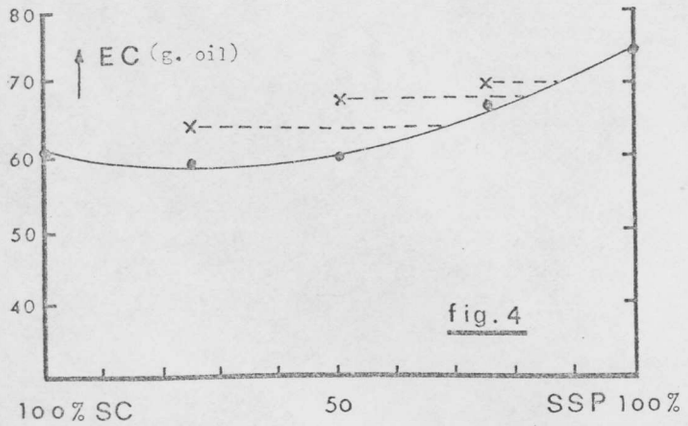
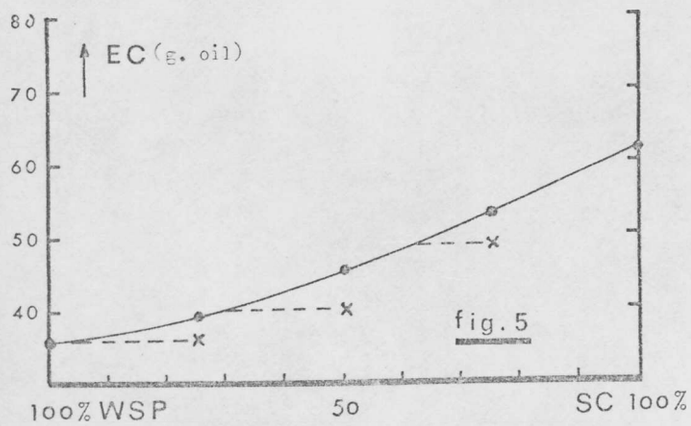
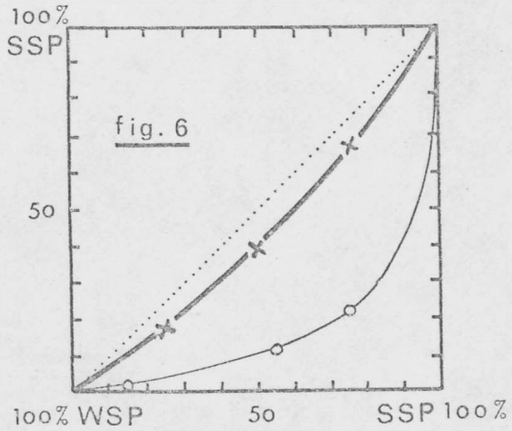
2. Competition between the soluble meat protein fractions WSP and SSP and sodium caseinate (inter-competition).

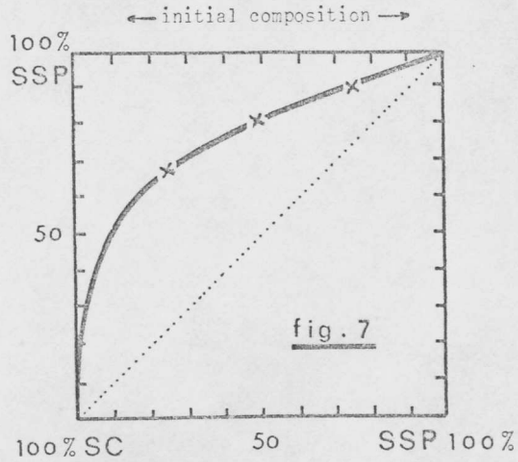
Into 200 gram lots of standardised WSP (1.0 %) and SSP (0.6 %) solutions, 100 grams of oil were pre-emulsified. The emulsions were then centrifuged and the water layers diluted to 0.4 % protein by means of 0.35 M NaCl solution. Of these diluted water layers a number of mixtures was made, ranging from 100 % WSP and 0 % SSP to 0 % WSP and 100 % SSP. The curve in fig. 3 shows the EC-values of these mixtures plotted against the WSP-SSP composition. After that mixtures of the original WSP and SSP extracts of known composition were pre-emulsified with











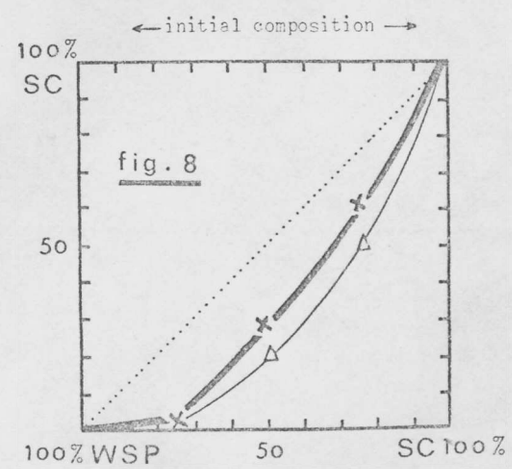


Fig. 3-5 EC-values of mixtures of pre-emulsified protein solutions (solid lines) and of pre-emulsified mixtures of protein solutions, marked X. The original compositions, corresponding to X, actually changed by the emulsification process to those, indicated by the intersection of the solid lines with the dashed ones.

Fig. 6-8 The initial composition of the protein solution mixtures before emulsification is shown on the abscissae, the changed composition after emulsification is shown on the ordinates. Pre-emulsified amounts of oil were 100 (×), 150 (Δ) or 200 grams (o).

the same quantity of oil, i.e. 100 grams. After centrifuging here also the water layers were diluted with 0.35 M NaCl solution to 0.4 % protein. The resulting EC-values are presented in the same fig. 3 and marked X. Although the original extracts were different in protein (1.0 and 0.6 %), the figures on the abscissa represent the true protein composition. So, the figure shows the change in WSP-SSP composition, brought about by pre-emulsification of oil.
The figures 4 and 5 represent similar experiments with mixtures of SSP and sodium caseinate SC (SC in 1.0 % solution) and of WSP and SC. Figures 6, 7 and 8 give the results in an other way, i.e. the abscissae representing the original SSP-WSP, SSP-SC, and WSP-SC composition respectively and the ordinates the corresponding compositions after pre-emulsification of oil. From these curves a preference of SSP and sodium caseinate over WSP and a preference of sodium caseinate over SSP as regards the film formation can be deduced.
From the curves in figures 6 and 8 it can be seen that the change in composition of the mixtures increases with increasing quantities of pre-emulsified oil.

When increasing quantities of oil were emulsified into the meat protein fractions WSP and SSP, the protein content of the continuous phase decreased more and more and did not increase again when the emulsion collapsed. After centrifuging the consumed protein was found as a thin film of insoluble material between the separated oil and the water layer. This suggests, that if meat protein films are disturbed during the emulsification process, where oil droplets are reduced to smaller ones, these protein films are not able to emulsify oil again and the more preferred protein of the continuous phase can then be expected to take over the film formation. Unlike the case of WSP and SSP, sodium caseinate dissolved again almost completely when the emulsion collapsed with no significant change in its EC.

3. The role of the K-fraction and the residue R.

To study the role of K and R, increasing quantities of oil were pre-emulsified into 200 gram lots of 1.0 % K and R suspensions, containing 2 % NaCl. The water layers, obtained by centrifuging, could not be submitted to the EC-test, as described for the soluble proteins, since they hardly contained any protein. Therefore the protein content of the emulsion layers was determined after centrifuging and plotted in fig. 9 as a percentage of the total initial protein against the amounts of pre-emulsified oil. These curves indicate that both K and R are adsorbed at the fat-water interface, the K-fraction, however, in a considerably higher amount than the residue R.

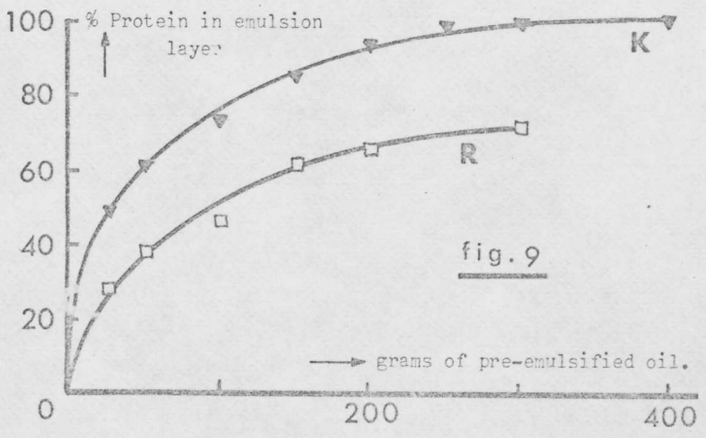


fig. 9

To investigate how K and R behave in combination with soluble proteins, 100 grams of oil were pre-emulsified into 200 gram lots of mixtures of either WSP, SSP or sodium caseinate with K and R respectively (0.6 % protein for SSP, 1.0 % for the others). The water layers, centrifuged from the emulsions, were diluted with 0.35 M NaCl solution to 0.2 % protein and the EC-test was carried out. On the right hand side of fig. 10 the EC-values are presented as a function of the mixture composition, whereas the left hand side of the diagram gives the EC-data of the non pre-emulsified protein fractions, also in a 0.2 % concentration. It can be seen that the EC-values of the water layers remained exactly the same, either with or without K and R. Since the soluble protein in the water layers originated from the WSP, SSP or sodium caseinate, the diagram suggests that the actual emulsification is performed by the soluble proteins and that K and R are additionally adsorbed. This adsorption will probably contribute to the stability of the emulsion, since it results in a higher viscosity of the system.

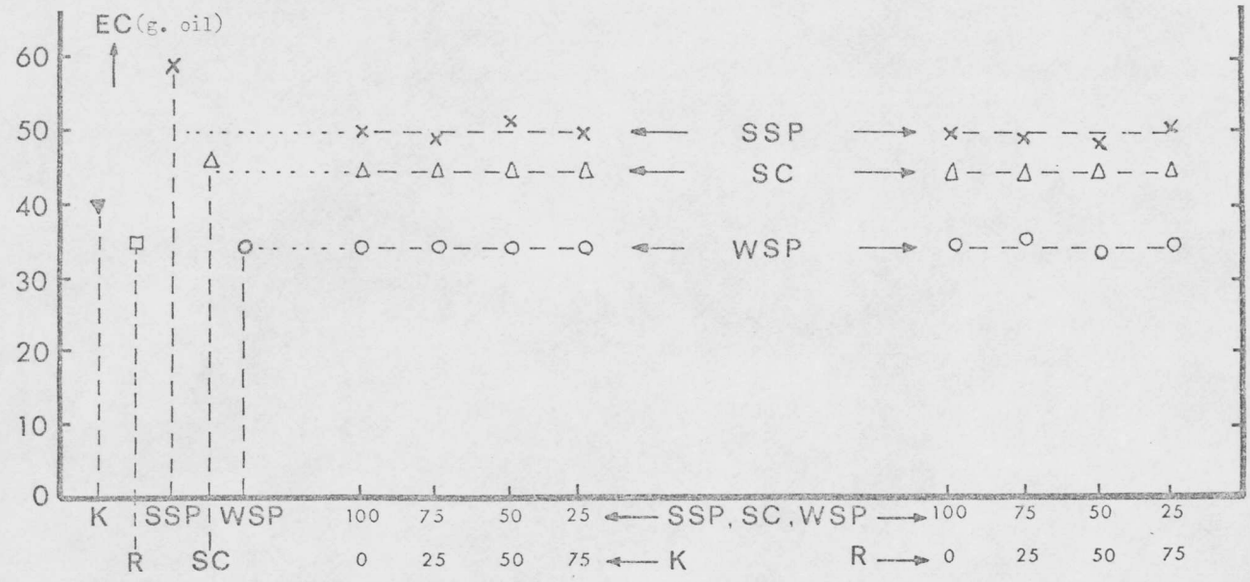


fig. 10 EC-values of the pre-emulsified mixtures of the soluble proteins WSP, SSP, and SC with K and R (right), compared with the EC-values of the non pre-emulsified pure samples (left). All EC-values for 0.2 % protein solutions.

By determining the protein content of the various layers (the precipitates on the bottom included), obtained after centrifuging the emulsions dealt with on the right hand side of fig. 10, the origin of the protein in the emulsion layers was estimated. Fig. 11 shows how much soluble protein SP, so either WSP or SSP, and how much total protein ends up in the emulsion layer. It can be seen that the percentages of WSP and SSP that are found in the emulsion layer are fairly constant. The same holds for the fractions of K and R that are adsorbed in combination with SSP, but in combination with WSP a great affinity of K and R for the emulsion layer is observed. From fig. 11 it can also be seen that a high percentage of the adsorbed protein in the case of 100 % SSP is in fact insoluble. This is due to the mechanical treatment in the Sorvall blender. This was especially the case in SSP-extracts from fresh meat.

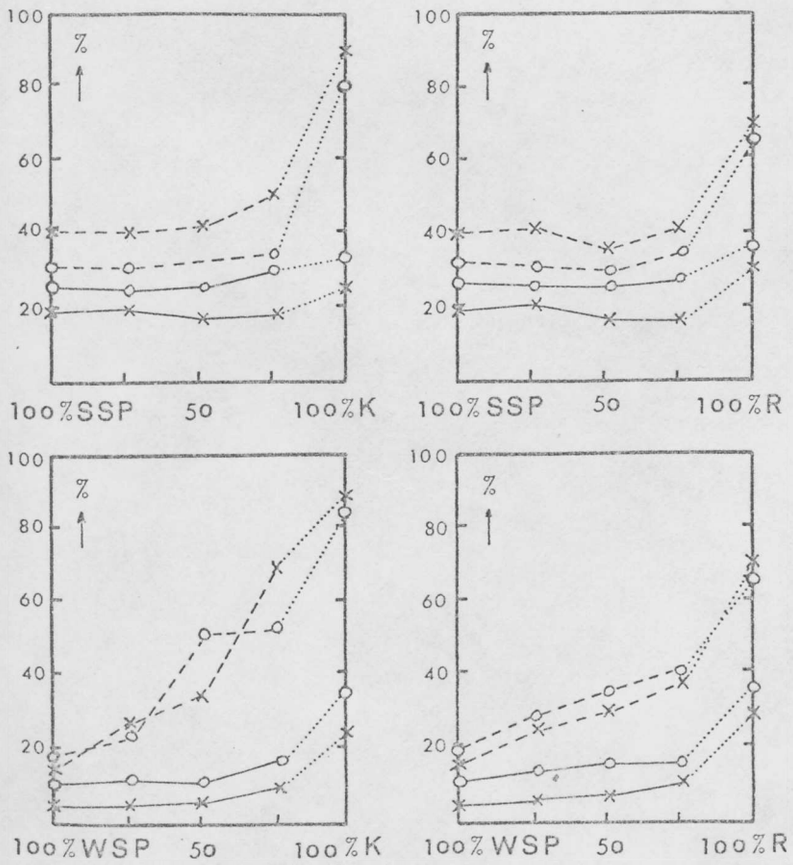


fig. 11

Soluble protein SP (——) and total protein (----) in the emulsion layer as a percentage of total protein content in the emulsion. Experiments with fresh (×) and frozen meat (○).

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To examine the possibility that K and R consist at least partly of myofibrillar protein and thus must contain actomyosin, EC-values of K and R were determined before and after treatment with Mg^{++} and $P_2O_7^{----}$. As table I shows, the treatment caused a great increase in EC and since no effect was observed on the other fractions of the myofibrillar protein, it seems likely indeed, that an appreciable part of "aggregated" actomyosin is present in K and R. The dissociation treatment of K and R not only caused an increase in EC, but also in the solubility, whereas a severe mechanical treatment of the soluble actomyosin from SSP (1 minute high speed in a Sorvall blender) caused flocculation of the protein and a considerable fall in EC. Here also subsequent dissociation of the precipitate by means of Mg^{++} and $P_2O_7^{----}$ restored the solubility and the EC (table I). It is generally accepted that comminuting meat with salt and water favours the extraction and solubility of some proteins, but the data of table I suggest that an exaggerated mechanical treatment may be less favourable for other proteins.
The increases in the EC-values after dissociation of both K and R are of the same order since R was found to contain approximately 40 % connective tissue. In spite of this resemblance in the EC-values, the difference between K and R cannot be explained only by differences in particle size, since the two fractions appear as distinct layers after centrifuging of the meat-salt-water mixtures. The K-fraction possibly consists of myofibrillar protein freed from connective tissue, in contrast to the residue R, where it may be still enclosed in the connective tissue.

Meat sample		fraction	before	after
I	fresh	WSP	38	34
		SSP	92	100
		SSP, minus actomyosin and myosin	72	73
		Myosin	114	
II	fresh	SSP	99	123
	frozen	SSP	88	109
		SSP, minus actomyosin	96	97
		Actomyosin from SSP	75	100
		Idem, after mechanical treatment	40	99
		K	49	80
		R	42	64
		Sodium caseinate	65	70
		0.35 M NaCl	26	

Table I EC-values in grams of oil, measured at 0.4 % protein solutions in 0.35 M NaCl, before and after treatment with Mg^{++} and $P_2O_7^{----}$.

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