UTILISATION OF MEAT FRACTIONS AND PREDICTIVE MODELLING OF MEAT PRODUCT COOKING LOSSES AND TEXTURE

M.K. Knight, Leatherhead Food RA, Randalls Road, Leatherhead, Surrey, England

SUMMARY

This report deals with a new approach to meat product formulation, in which the individual effects of three major protein fractions, viz. salt-soluble protein (SSP), insoluble myofibrillar protein (IMP) and connective tissue protein (CTP), on cooking performance and texture have been elucidated. The aim of this study was to provide a greater understanding of the contributions of these individual components of meat to the quality of meat products.

The influence of each of the protein fractions and of fat on the cooking losses, texture and microstructure of a model meat emulsion has been determined. The results demonstrate both the individual and the interactive functions of the major meat fractions in meat products, and reveal ways in which meat product quality could be more closely controlled by selection of raw materials.

This approach to meat product formulations should lead to a much more precise method of predicting product performance from a knowledge of the functions of the components of meat and their interaction in the product.

INTRODUCTION

The design of new meat products and the reformulation of traditional products, to obtain a low cooking loss and particular texture, is largely an empirical process, which leads to many failures. One of the reasons for this is that there are variable amounts of the major meat proteins and fat in the meat raw materials used in products (Porteus, 1981). Consequently, it is difficult to exercise a high degree of control over product composition, and this leads to variations in cooking losses and texture.

The objectives of this study were (i) to survey the influence of the major meat protein fractions and fat on the cooking performance and texture of meat products, and (ii) to construct models from which the cooking performance and texture of meat products can be predicted from formulation. Adequate control over product composition would lead to more consistent eating quality, and result in greater consumer confidence in products made by meat manufacturers.

The approach to this study was to fractionate meat into salt-soluble protein (SSP), insoluble myofibrillar protein (IMP), connective tissue protein (CTP and fat fractions by centrifugation. To investigate interactions between these fractions, the three protein fractions were recombined at various fixed ratios and fat levels, heat set and then cooled. Texture was measured by an instrumental penetration test and cooking losses were also measured. The microstructure of the model systems was viewed in the light microscope.

MATERIALS AND METHODS

Meats

Pork shoulder meat was obtained from a local abattoir 24-48 h postmortem, cut into 5 cm cubes and randomised prior to mincing through a 4 mm plate. The minced meat was vacuum packed, blast frozen and stored at -18°C until required. Samples were thawed for 24 h at +2°C prior to extraction of the protein fractions.

Pork backfat was obtained from a local abattoir 24-48 h postmortem, derinded, cut into 5 cm cubes and randomised prior to mincing through a 4 mm plate. The fat was vacuum packed, blast frozen and stored at -18°C until required. The fat was thawed for 24 h at +2°C prior to mixing with the protein fractions.

Fractionation Technique

The minced pork shoulder was chopped with buffer solution (1.12 M sodium chloride, 9.8 mM sodium tripolyphosphate, pH 8.4) at a ratio 1:2, respectively, for 1 m in a Robot Coupé mixer. The meat slurry produced was centrifuged for 30 m at 30,000 x g, after which time three protein fractions (SSP, IMP and CTP) and fat could easily be isolated. The CTP fraction was cut into 1-2 mm fragments with a scalpel prior to use.

The fractionation conditions described above were based on those used by Turner, Jones and Macfarlane (1979), and were selected because they produce large amounts of SSP fraction.

Analysis of Protein Fractions

Collagen contents were estimated from the hydroxyproline content (collagen = hydroxyproline x 8; Ranken, 1984), which was determined by the high-performance liquid chromatography (HPLC) method described by White, Hart and Fry (1986). Protein, fat, moisture and chloride contents were determined by the methods described in the Leatherhead Food RA Analytical Methods Manual (1987). A nitrogen-to-protein conversion factor of 6.25 was used. The pH value of each protein fraction was determined after the addition of two parts distilled water to each fraction and stirring at room temperature for 30 m. The pH value was measured with an Orion 91-03 microelectrode fitted to an Orion 601-1 pH/mV meter.

Cooking Losses

Appropriate amounts of the three protein fractions and fat were weighed into a cuvette (6 x 5 x 5 cm). The fractions were mixed by hand with a glass rod until dispersed and then mixed for 1.5 m using an Ultra Turrax mixer. The fractions were again mixed with a glass rod followed by a further 1 m mixing in the Ultra Turrax mixer. The surfaces of the mixtures were smoothed with a spatula.

Each cuvette was placed into an 80°C water bath for 30 m, removed and the cooking loss drained from the cuvette and weighed.

Texture Measurements

The gels prepared for cooking loss determinations were cooled at room temperature for 30 min and transferred to a 2°C room for a further 30 m to equilibrate. Each gel was then placed centrally on to the platform of a Stevens CR analyser. A hemispherical probe (3.5 cm long x 1.2

cm in diameter) was fixed in a position above the gel and set at a speed of 50 cm/m, penetrating the gel a distance of 3.0 cm. The charge recorder traces plotted the force (g) required to penetrate the gel against distance (cm). The values measured from the traces were (i) the slope of the initial increase in force with distance - interpreted as a measure of firmness; (ii) the maximum load value - also interpreted as a measure of firmness; and (iii) the distance at which the gel broke (break distance) - interpreted as a measure of elasticity. All measurements were made at 2°C.

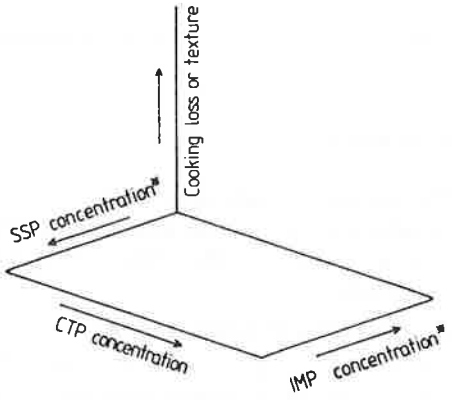
Statistical Analysis

The gels contained the following concentrations of each fraction:

SSP - 0 to 100% w/w (at increments of 25% w/w)
IMP - 0 to 100% w/w (at increments of 25% w/w)
CTP - 0 to 20% w/w (at increments of 5% w/w)
Fat - 0, 15 and 30% w/w

Graeco-Latin square arrangements were constructed such that replicate cooking loss and texture measurements were made on fractions prepared on two occasions. Results from various fixed ratios of the protein fractions were tested at each fat level. The data were analysed by factorial analysis of variance and response surface methodology.

The cooking loss and texture results are presented as response surface plots and this method of presentation is simplified in the diagram below. This method shows the effect of mixing the three protein fractions, at a fixed level of fat, on either cooking loss or texture.



*expressed as a percentage of the non-CTP component

Light Microscopy

Small amounts of the raw and cooked samples were mounted on suitable stubs and frozen in liquid nitrogen. Sections, 10-15 m thick, were cut in a cryostat at -25°C, collected on glass slides and allowed to dry. Sections were stained by the following methods and then examined in the light microscope:

(i) Toluidine blue

Sections were mounted and examined in 1% m/V toluidine blue in glycerol phenol, which stains proteins generally blue and connective tissue lilac.

(ii) Osmium and acid fuchsin

TABLE I

Proximate analyses of protein fractions

Analysis*	Protein fraction		
	SSP	IMP	CTP
Proportion % m/m	59.2 ± 0.9	36.6 ± 1.1	4.2 ± 0.2
Protein (by Kjeldahl N x 6.25) % m/m	4.5 ± 0.09	8.8 ± 0.69	11.4 ± 0.77
Collagen (hydroxyproline x 8) % m/m	0	0.76 ± 0.27	4.08 ± 0.94
Fat % m/m	0.3 ± 0.04	1.7 ± 0.09	3.0 ± 0.18
Moisture % m/m	90.7 ± 0.09	85.2 ± 0.22	81.4 ± 0.36
Chloride (expressed as sodium chloride) % m/m	4.2 ± 0.09	3.9 ± 0.08	3.7 ± 0.08
pH value	6.25 ± 0.04	6.4 ± 0.05	6.4 ± 0.04
Total**	99.7	99.6	99.5

* Mean values from duplicate determinations on protein fractions isolated on three occasions.
** Total = protein (by Kjeldahl) + fat + moisture + sodium chloride.
± = Standard deviation.

Sections were stained for 1-2 min in 0.1% m/V aqueous osmium tetroxide, washed and then stained for 10-20 m in an acid fuchsin/ponceau de-xylidene mixture (Lewis, 1978). This method stains fat brown/black and protein red.

(iii) Picro-sirius red

Sections were stained using the method of Flint and Pickering (1984). This method stains collagen red and gelatin pink. Raw and cooked collagen can be differentiated using polarised light; muscle tissue stains yellow.

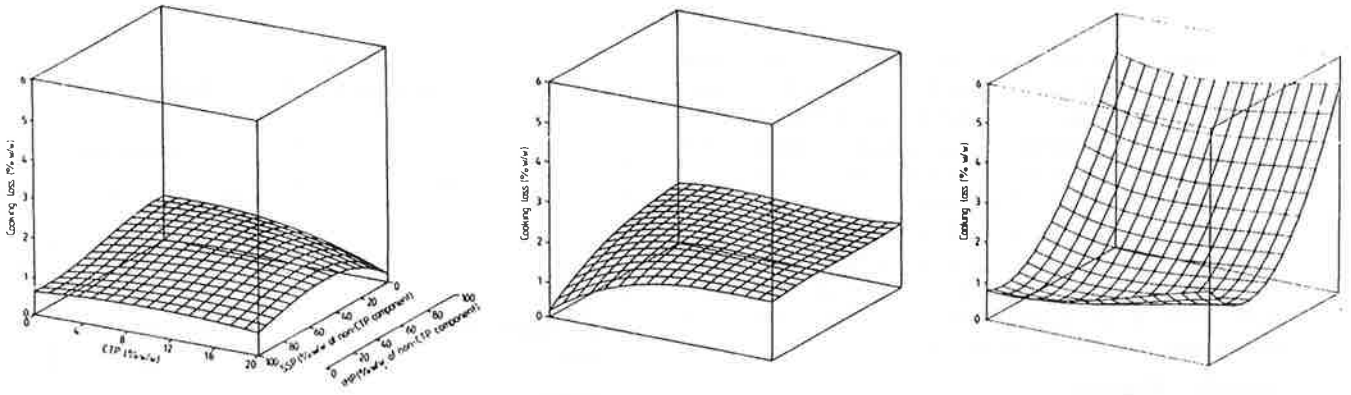
RESULTS

Characterisation of Protein Fractions

The composition of samples SSP, IMP and CTP fractions are shown in Table 1. The protein, fat, moisture and sodium chloride contents account for more than 99.5% of the composition of each fraction.

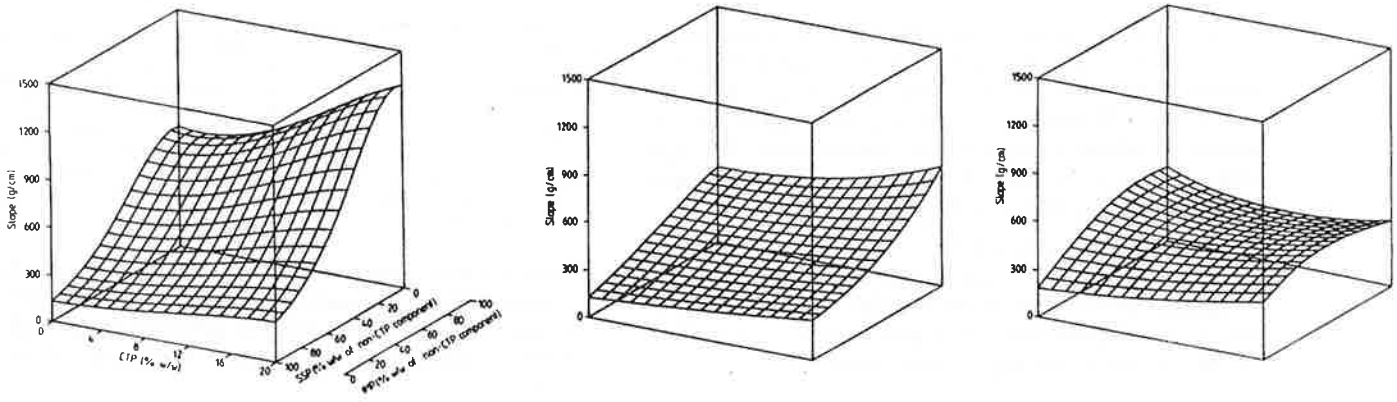
Cooking Losses

The cooking loss results for systems containing 0, 15 and 30% w/w fat are illustrated in Fig.1. The losses at 0 and 15% w/w fat were generally low (about 1-2% w/w) and are detailed in Figs. 1(a) and (b), respectively. A marked change in the behaviour of the system occurred between 15 and 30% w/w fat. Whilst at the lower fat levels cooking losses were not affected greatly by changes in the proportions of the three fractions, this was not so at 30% w/w fat. The dominant factor controlling cooking loss in systems containing 30% w/w fat is the ratio of SSP to IMP. The CTP fraction appears to play only a minor role in determining the cooking loss, which was mainly fat. The marked change in cooking loss results between 15 and 30% w/w fat may be gradual in relation to increasing fat levels or it may occur at a specific fat level.



(a) zero added fat (b) 15% w/w added fat (c) 30% w/w added fat

Fig. 1. Cooking losses



(a) zero added fat (b) 15% w/w added fat (c) 30% w/w added fat

Fig. 2. Firmness texture measurements

The lowest cooking loss from systems containing 30% w/w fat was obtained from the system containing SSP (Fig.1(c)). This fraction contained the least protein of the three fractions (4.5% protein - Table 1). The CTP and IMP fractions contained higher levels of protein (12.66 and 9.92% protein, respectively) and yet showed higher cooking losses than the SSP fraction. It appears therefore that the SSP fraction contains more functional protein, in relation to controlling cooking losses, than the IMP and CTP fractions.

Texture Measurements

Firmness (slope values)

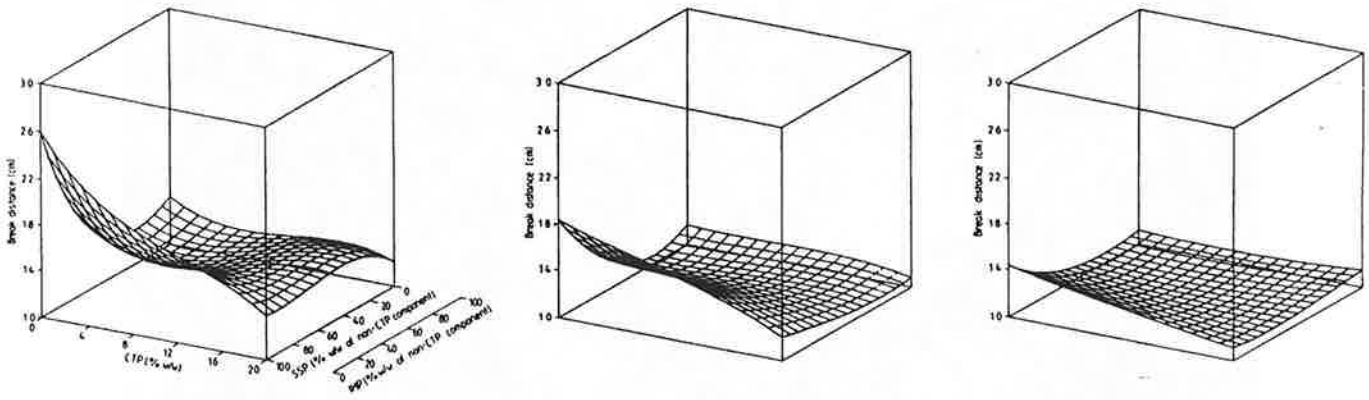
The effects of mixing various proportions of SSP, IMP, CTP and fat on the slop values obtained from the Stevens CR Analyser are shown in Fig.2. The slope values were interpreted as a measure of firmness (high values) and softness (low values). The results show that fit diminishes the effect of the IMP and CTP fractions on firmness is reduced as fat level is increased.

The soft textures (low slope values) associated with high levels of SSP are little changed by increasing the fat level. Firmer textures were produced by replacing SSP with CTP at all fat levels examined.

The maximum load values obtained from the Stevens CR Analyser showed similar trends to the slope values shown in Fig.2, and are therefore not reported, to avoid repetition.

Elasticity (break distance values)

The results in Fig. 3 show the effect of mixing various ratios of the three protein fractions and fat on the break distance values obtained from the Stevens CR Analyser. These values were taken as a measure of elasticity (high values) or crumbliness (low values). The results show a dilution of the elastic contribution of the SSP fraction as fat content is increased. At zero level of fat (Fig.3(a)) the effect of SSP fraction on elasticity is pronounced. However, this effect is progressively reduced as fat level is increased. The most elastic systems therefore contained high levels of SSP and low levels of fat, and increasing the proportion of either the IMP or the CTP fraction resulted in the formation of crumblie textures. The large effect of the CTP fraction on reducing the elasticity of the SSP fraction contrasts with its small effect on firmness at each level of added fat (e.g. Figs. 3(a) and 2(a), respectively).



(a) zero added fat (b) 15% w/w added fat (c) 30% w/w added fat

Fig. 3. Elasticity texture measurements

Microscopy

The model meat emulsions were examined before and after cooking, using different staining methods to provide information on the type of protein matrix formed on cooking and the way in which fat, when present was stabilised. The picro-sirius red stain reveals connective tissue by staining collagen red. Raw and cooked collagen can be differentiated using polarised light. Muscle tissue stains yellow and gelatine pink. Using this stain, toluidine blue, a general protein stain, and also osmium and acid fuchsin, which stains protein red and fat brown/black, the different fractions were examined.

Without fat

The IMP fraction consisted mainly of protein but also contained a small amount of associated fatty tissue and connective tissue. When cooked, this formed a firm smooth aerated matrix and this is illustrated in Plate 1. The SSP fraction was difficult to stain with picro-sirius red, giving a red/yellow colour with the cooked sample. This makes it difficult in the mixed system to distinguish between gelatin and other proteins such as SSP. The SSP fraction changed from a liquid in the raw state to a soft, elastic protein gel on cooking. A gel network was visible, as illustrated in Plat II. In mixtures of the IMP and SSP fractions the two types of structures could be detected.

The CTP fraction contained large (often several mm long) fragments of connective tissue, and cooked samples containing the CTP fraction all showed considerable pink staining, indicating a gelatin network formed from the collagen. An example of this is shown in Plate III, which represents a system containing all three fractions.

With 30% w/w fat:

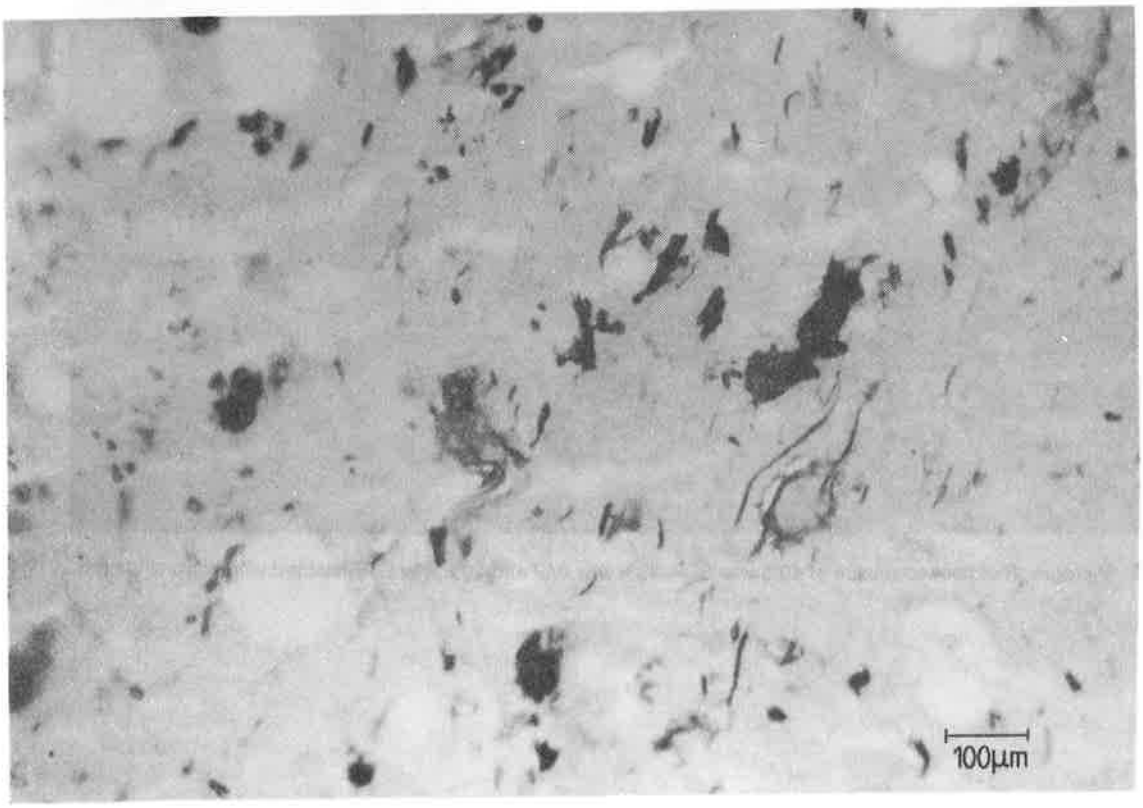


Plate 1. Micrograph of cooked IMP fraction stained with picro-sirius red.

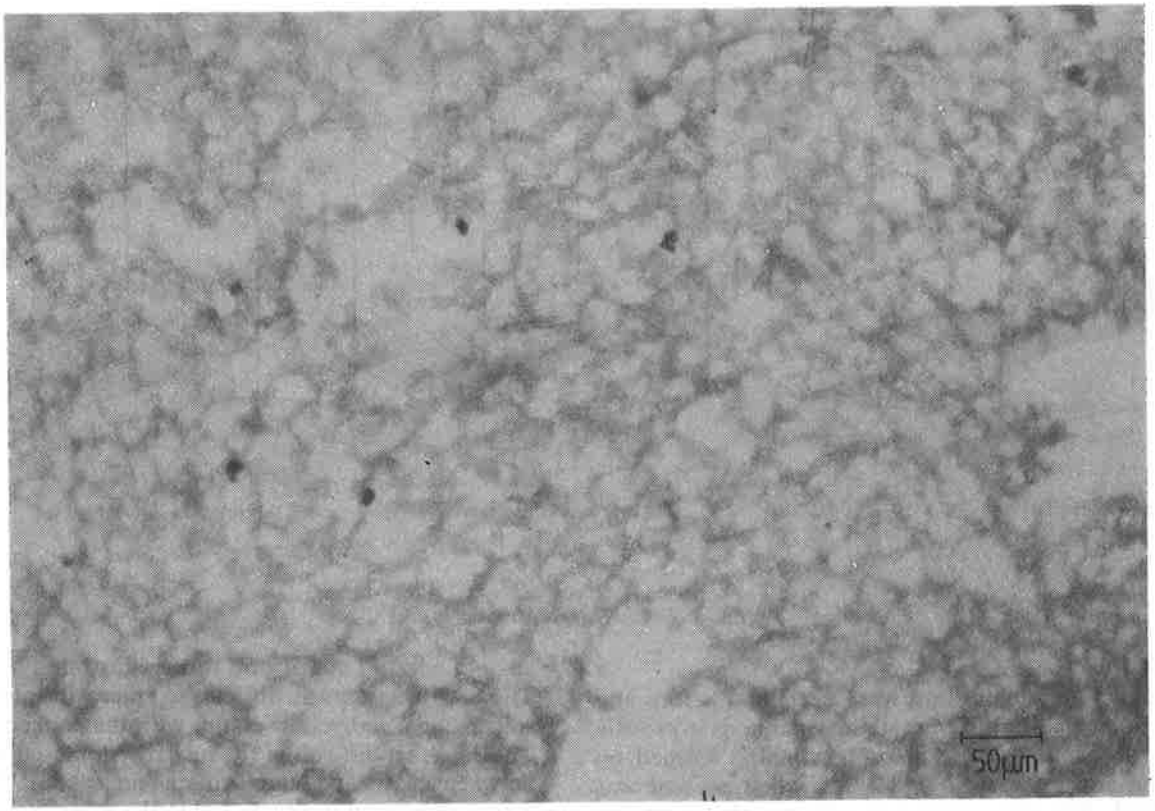


Plate II. Micrograph of cooked SSP gel network stained with toluidine blue.

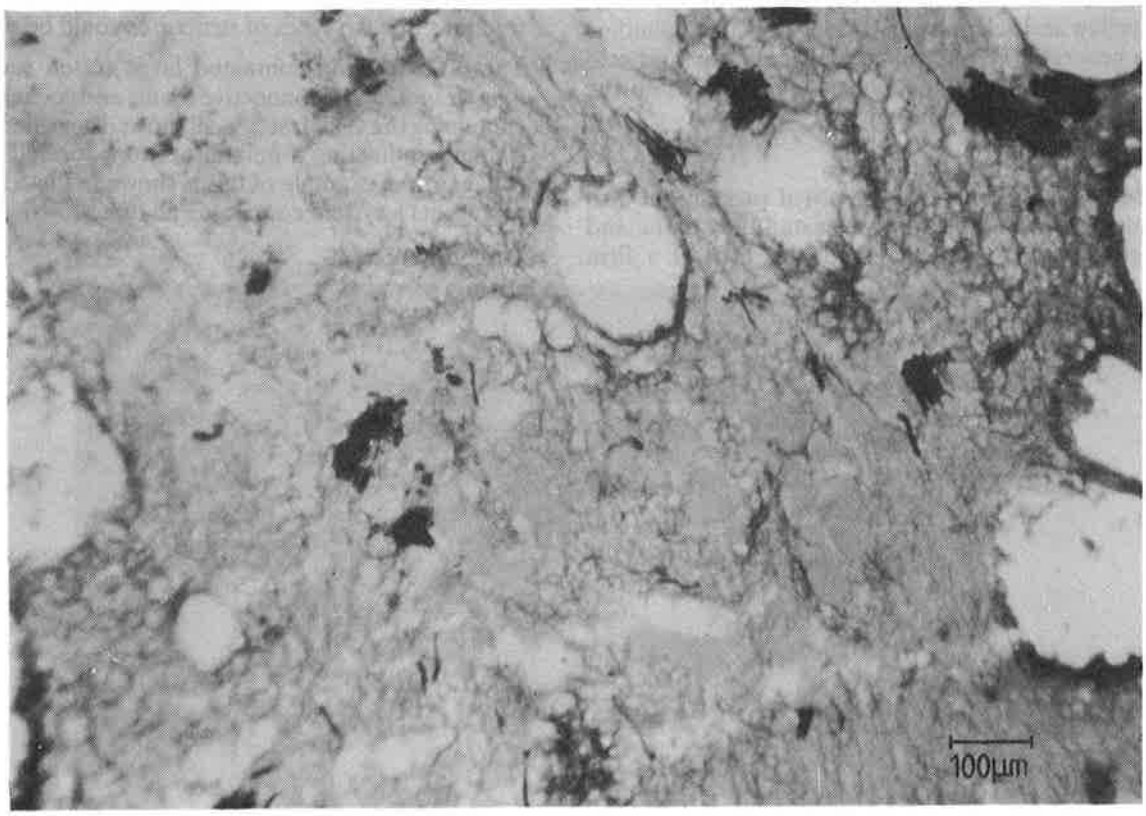


Plate III. Micrograph of cooked mixtue of 40% w/w SSP, 40% w/w IMP and 20% w/w CTP, stained with picro-sirius red.

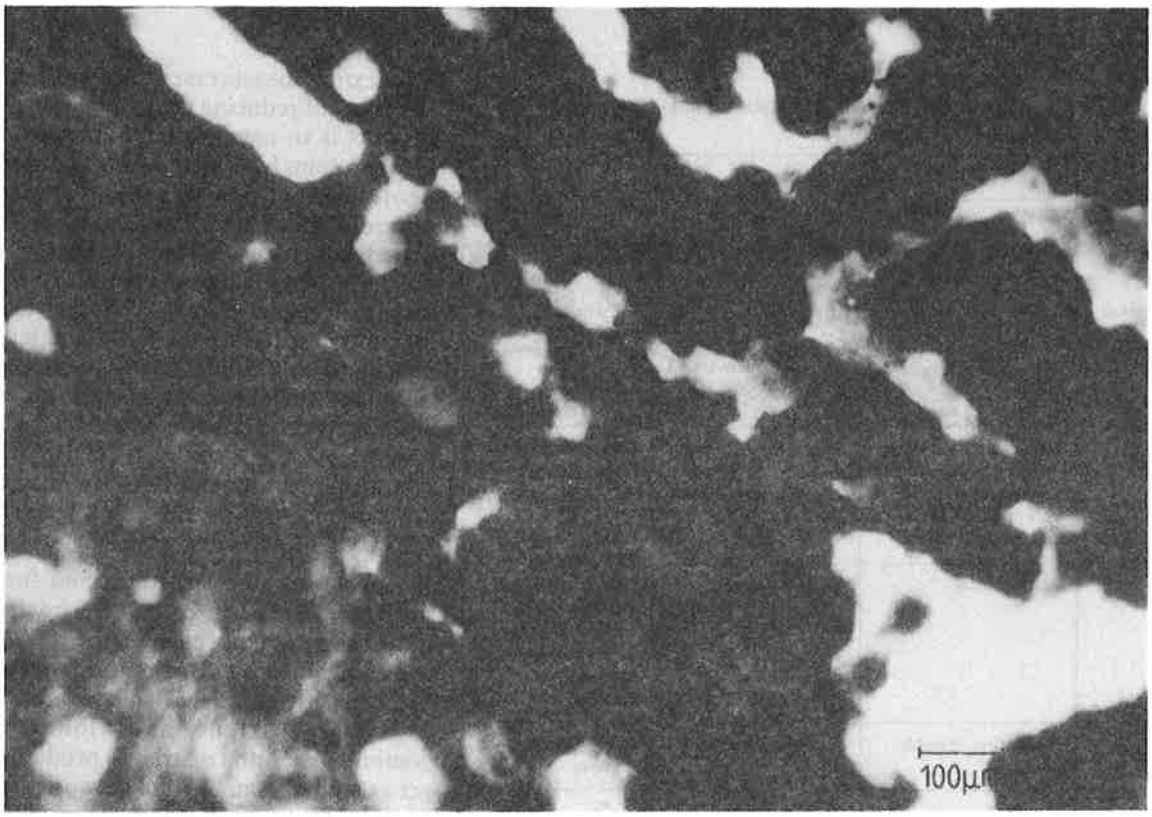


Plate IV. Micrograph of cooked mixture of 70% w/w IMP and 30% w/w fat stained with osmium and acid fuchsin.

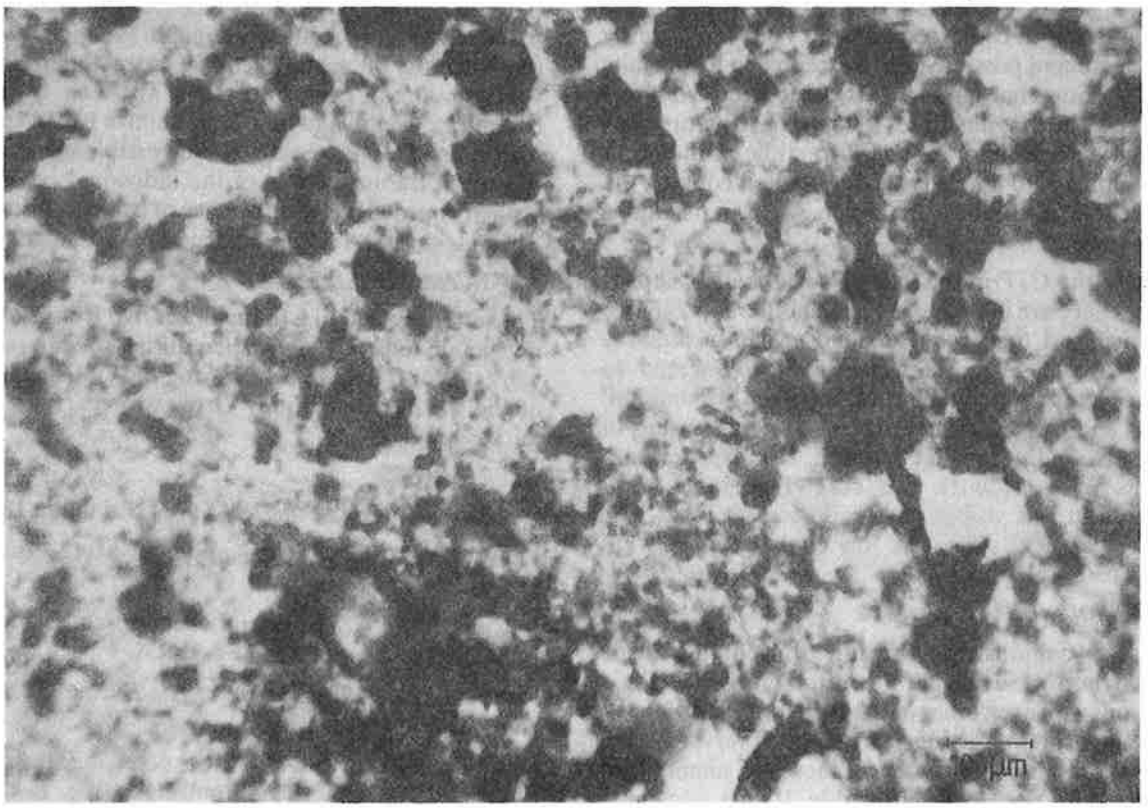
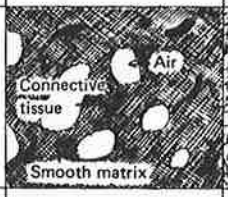
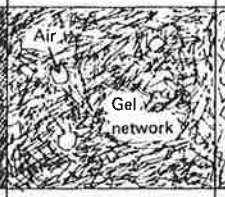
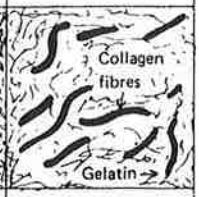


Plate V. Micrograph of cooked mixture of 70% w/w SSP and 30% w/w fat stained with osmium and acid fuchsin.

TABLE II

Functional properties of IMP, SSP and CTP fractions in meat products

Property	Fraction		
	IMP	SSP	CTP
Structure of cooked fraction (x 25)			
Influence on cooking loss	Increases fat and water losses. Contracts upon cooking, leaving channels through which fat will flow	Reduction of fat and water losses. Emulsifies and entraps free fat	Reduction of water losses. Forms good water-binding gel
Influence on adhesion	Poor adhesive	Good adhesive	Poor adhesive, but may improve adhesion indirectly by interaction with SSP gel during cooking
Influence on texture	Forms firm, crumbly textures	Forms soft, elastic textures	Forms firm textures
Partial supplements from non-meat sources	Textured soya protein, mycoprotein	Soya, caseinate, whey, alginate, gluten	Starch

When fat was mixed with the IMP fraction it was found within the protein matrix in the raw state but was not emulsified. After cooking, fat was still trapped in the matrix but areas of coalesced fat were visible, indicating fat movement and possible loss. The cooking loss results in Fig.1(c) show that high fat losses occurred from this system, and Plate IV illustrates the type of structure seen in the light microscope. The SSP fraction on the other hand did appear to emulsify the fat, and on cooking the fat was held as an emulsion in the gel. An example of this is shown in Plate V.

The role of the CTP in the structure of the emulsions is unclear. There are indications that gelatin was produced on cooking and this would enhance gelation and the trapping of fat. The CTP fraction also seemed to promote aggregation of the proteins in the IMP and SSP fractions and this was reflected by the denser areas of protein surrounding the CTP fraction. In mixtures where SSP was present with the CTP fraction, some emulsification was visible, thus reducing fat loss. The results in Fig. 1(c) show that low cooking losses were obtained from such systems.

DISCUSSION

The results demonstrate the functions of the three protein fractions in meat product matrices, their interactions with each other and their interaction with fat. Clearly, the protein fractions each possess very different functional properties, and some of these are summarised in Table II. Also listed in Table II are non-meat ingredients that are sometimes used to compensate for deficiencies of meat proteins, or alternatively to modify the properties of meat products.

The texture measurements, overall, show that the effect of reducing the fat content of a meat product is to enhance the influence that the meat proteins have on texture. The three meat protein fractions examined each exert different effects on texture, and therefore the proportion of each fraction present in the meat raw materials used in products becomes increasingly important in relation to texture as fat content is reduced. At high fat levels, the extent to which different meat proteins influence texture becomes less important, since individual effects are diminished by the presence of fat. However, in products containing large amounts of fat, cooking performance will be affected by the ratios of the protein fraction present.

In conclusion, the structural and functional properties of the three protein fractions are quite different from each other. SSP appears to be the 'function' fraction, since it both gels and emulsifies. The IMP fraction also gels but to a lesser extent, and alone forms a continuous aerated matrix. In products in may act as a filler in an SSP gel network. The role of the CTP fraction is less clear, but it does appear to contribute to gelation. Because of these differences the proportion of each fraction in the model system greatly influenced the resultant cooking loss and texture. Omission of any one of the fractions significantly changed the characteristics of the model system. It is reasonable to suppose from this that the use of meat fractions, extracted from highly variable meat raw materials, such as MRM and trimmings, could lead to more effective use of these raw materials. This technology should enable manufacturers to exploit the properties of the individual components, both to improve the performance of current products and to create new and different structures and textures.

ACKNOWLEDGEMENTS

The author would like to thank Mr. M. Robinson for his invaluable technical assistance during the course of this work, to Dr. D. Rose for carrying out statistical analysis of the data, and to Kathy Groves for carrying out the microscopy.

REFERENCES

Flint, F.O. and Pickering, K. (1984). *Analyst*, **109**:1505.

Leatherhead Food RA Analytical Methods Manual (1987).

Lewis, D.F. (1978). Manual of microscopical methods. Published by Leatherhead Food RA.

Proteus, J.D. (1981). *Can. Inst. F. Sci. Technol. J.*, **14**(3):224.

Ranken, M.D. (1984). Notes on meat products. Published by Leatherhead Food RA.

Turner, R.H., Jones, P.N. and Macfarlane, J.J. (1979). *J. Fd. Sci.* **44**:1443.

White, J.A., Hart, R.J. and Fry, J.C. (1986) *J. Autm. Chem.* **8(4)**:170.