6 - 47 THE INFLUENCE OF DIFFERENT NON-MEAT PROTEINS ON THE HEAT GELLING PROPERTIES OF VARIOUS MEAT PROTEIN FRACTIONS.

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INTRODUCTION

The basic process to manufacture finely comminuted meat products is in fact the preparation of a meat batter. This includes actually two steps, namely first chopping of lean meat with salt, phosphate and ice and subsequently the more fatty ingredients.The homogeneous mass that is obtained, should have no visible fat, except show fat, and all the water and fat being bound. The stability of such meat products during and after processing, as well as its texture mainly depend upon the presence and performance of certain meat proteins. The same is true for non comminuted or coarsely comminuted meat products like hams and burgers in which the meat proteins are responsable for waterbinding and meatbinding.

In meat, the proteins can be divided into the following groups:

- sarcoplasmic proteins (25-30 %)
- myofibrillar proteins (45-55 %)
- connective tissue proteins (15-30 %)

It is generally accepted that the myofibrillar fraction constitutes the most important contribution to the stability of the above described meat products (1, 2, 3, 4)

Part of the myofibrillar proteins can be solubilized with salt, the so-called SSP (salt soluble protein), and when set free from the tissue, these proteins can emulsify fat or bind water by forming a gel on heating. An other part of myofibrillar proteins swell in the presence of salt, thus binding water in the raw product, not only in extracted condition, but also within intact tissue cells.

The sarcoplasmic proteins, called WSP (water soluble protein), are also capable to emulsify fat and to

gellify. However, in meat homogenates WSP is believed not to contribute to the WHC (5). In addition to this, SSP is found to be absorbed preferentially, over WSP in the fat/water interphase during emulsification (6).
Since the emulsifying, swelling and gelling is so important for stability, waterbinding, texture and consistency of meat products during processing and in the end product, it is obvious that during chopping of lean meat with ice and salt, or during massaging and tumbling of hams, conditions should be optimal for the solubilization, extraction and swelling of the different protein fractions.
To improve product stability and consistency different non-meat proteins can be added, although their performance may vary considerably, e.g. depending on product formulation, procedures, lean meat quality and of course the specific functionality of such non-meat proteins. Caseinates have a high capacity to emulsify fat and this will safe the meat proteins for water binding in the meat emulsion. Whey proteins may bind water by heat gelling and also soy proteins have similar properties. The performance of non-meat proteins in meat products will not only depend on their specific characteristics but also on the interactions with the solubilized and swollen meat proteins, which would determine the over all functionality. Such phenomena are noticed e.g. with caseinate which, although not capable of gelling, improves the consistency and water binding of meat products, whereas whey proteins often lead to lower water binding and less consistency despite their heat gelling properties (7, 8).
This study aims to examine the effect of some non-meat proteins on the gelling properties of the different meat protein fractions and to elucidate the mechanism that determines the practical performance of such non-meat proteins in meat products.

E X P E R I M E N T A L

EXTRACTION AND SEPARATION OF WSP-, SSP-, K-, AND R-FRACTIONS OF BOVINE M.SEMIMEMBRANOSIS.

Extraction

After trimming off any visible fat and tendons, the lean meat is ground through a 8 mm plate, made into a homogeneous blend and then packed in polythene bags and frozen at -20 °C. For the further experiments a portion of the frozen meat is thawed during 15 hrs to reach a temperature of -5 °C, then transfered to the high speed bowl chopper (Kilia, 25 l), to prepare a lean batter with salt and water.
Lean batter formula: meat 62.73% , salt 2.13% , water 35.14%.
Meat is first chopped with salt (1 min, high speed) then the water is added (3 min, high speed), resulting in a lean batter with the temperature between -3 and 0 °C.

Separation

In order to facilitate the centrifugal seperation of the meat protein fractions, the above lean batter is diluted with 2 volumes of a 2.5 % W/w salt solution. Centrifugation at 27,000 x g for 30 minutes (Sorvall superspeed RCB-2) leads to separation of three phases:

- supernatant, clean purpered - WSP and SSP fractions;
- intermediate, pastry grey - K-fraction (swollen, non dissolved myofibrillar proteins) (9);
- sediment - Residue or Stroma (non swollen, insoluble myofibrillar and connective tissue proteins);

WSP and SSP fractions can be separated by centrifuging (15 min, 27,000 x g) the supernatant after cold dialysis against 0.02 m KCl during 24 hrs. WSP will remain soluble while the SSP fraction precipitates at the lower salt concentration, due to the dialysis. The WSP concentration in the supernatant is very low because of the dilution of the lean batter and in order to test the gelling properties, its concentration is increased by partial freeze-drying (Christ α 1-5 Retsch). Each different protein fraction is stored under cooling and samples are analyzed on solids and protein content (table 1).

GELLING CONDITIONS OF MEAT PROTEIN FRACTIONS

Standardized solutions containing 1.0, and 2.0 % protein in 2.5 % salt solutions are prepared for each protein fraction using a Hamilton Beach mixer (908s), (5 sec speed I + 5 sec speed II). Test tubes containing 15 ml of said solutions are placed in a 75 °C waterbath and every two minutes a tube is taken out to observe the gel setting and macrostructure.
The following indices are used to describe the observed situation:

gelling	gel structure
---	no gelling	liquid
+--	some floccules	poorable
++-	almost homogeneous gel	gel remains fixed on turning the tube upside down
+++	complete gelation	gel remains fixed on shaking the tube upside down

GEL STRENGTH OF MEAT PROTEIN FRACTIONS IN COMBINATION WITH DIFFERENT NON-MEAT PROTEINS

Solutions

Standardized solutions of the WSP, SSP and K-fractions are prepared, containing 3 % of the respective proteins and 3 % salt, to which an additional 1 % non-meat protein is added.
The different protein products in this study are:

- sodium caseinate, (Na-cas), roller dried, DMV - Holland;
- calcium caseinate, (Ca-cas), roller dried, DMV - Holland;

- Whey Protein Isolate, (WPI), spray dried, DMV-Holland;
- Soy Protein Isolate, (SPI), spray dried, Ralston Purina Company-St.Louis.

These proteins are added to the meat protein solution, using a Sorvall omnimixer (17106) (10 seconds, speed 4). Of these solutions 40 ml is transferred into glass jars (50 ml, ø 4 cm) and then sealed with a lid. After 15 minutes heating in a 75 °C waterbath, the jars are cooled for 1 hr in ice. The gel strength of the samples is measured with the penetrometer (PNR 6), using a 23,3 gram conus and falling time 0.1 second.

Emulsions

Part of the 3% protein, 3% salt solutions, including also the residue, is used to make emulsions with soy bean oil, incorporating also the mentioned non-meat proteins. The emulsion is formulated on the basis of 70% meat protein solution, 30% soy bean oil and additional 1.0% of the non-meat proteins. The emulsions are again prepared in the Sorvall omnimixer, then heated in glass jars at 75 °C in a waterbath (15 minutes) and subsequently cooled in ice. Penetrometer readings using 62.5 gram conus and 1.0 second falling time are collected for each product and registered as reciproke penetrations.

RESULTS AND DISCUSSION

Basic consideration in these experiments was, although splitting up the meat emulsion system and extracting and separating the proteins, to simulate as much as possible the normal meat processing conditions, like chopping (extraction) procedures and apparatus, salt concentrations, ratio meat / non-meat proteins etc. In various countries an average formulation for a finely comminuted meat product can be for instance: 40% lean meat, 20% water, 35% fat, 1.5% salt, 0.3% phosphate, 2.0% non-meat proteins, 1.2% others.
The ingredients are comminuted in a bowl-chopper and the chopping time in total is about 5 minutes of which 2 - 3 min. are used for preparing the lean batter. As from the formulation can be seen the salt centrations on the lean meat and on the total amount of water are resp. 3.8 and 3.0 % and the meat / added water ratio is 2/1.

After several preliminary trials we came to the meat / salt / water ratio as mentioned in the experimental part. The salt in water concentration during extraction and thus of the dilution brine, had to be 2.5 % instead of 3.0 %, in order to be able to separate the K-fraction from the WSP/SSP. At 3.0% salt in water the K-fraction was too voluminous to distinguish a clear borderline with the SSP/WSP layer. The meat used for the experiments and the obtained fractions after chopping, diluting, centrifuging and dialysis showed the analysis as given in table I. The observed gelling behaviour of the individual meat protein fractions in test tubes is presented in graphs (fig.1).

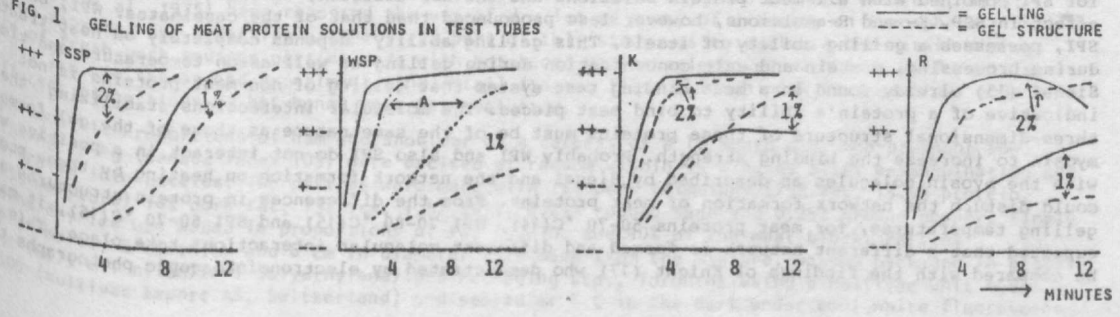
Table I:

	% dry matter	% protein*
Meat	25.10	20.25
WSP	1.35	1.04
SSP	7.78	7.09
K	6.14	4.02
R	15.10	12.90

* protein= N x 6.25

At this stage of the study WSP was not freeze-dried yet so we were bound to a max. of 1% protein solution. It appeared that the WSP fraction had the best gelling ability (after 5 minutes homogeneous gelation), however the gel structure was poor compared with this gelling ability. The gel structure improved a little in the absence of salt (see lines a) but at the same time the gelling ability decreased somewhat. The SSP fraction also gellified in a 1% protein concentration, but here it took 10 minutes. 1% protein was insufficient to get a homogeneous gel out of the K-fraction and the residue. At a protein concentration of 2% there was not much difference in the gelling of SSP, K and the residue. With the residue syneresis of the gel occurred after 8 minutes, probably due to the shrinkage of connective tissue in the meat-fibres. The K-rraction showed a very "short" and brittle gel structure. Contrary to the WSP fraction, we found in additional trials both improved gelling ability and better gel structure for the SSP fraction at higher salt concentrations.
In order to be able to measure the strength of the meat protein gels as well as of the combinations with non-meat proteins, these probes had to be gellified in wide glass jars and any separation of water had to be avoided to allow factual comparisons. In this procedure only a 2% WSP, 2.5% salt solution showed no separation on heating.

FIG. 1 GELLING OF MEAT PROTEIN SOLUTIONS IN TEST TUBES



Experiments in test tubes and glass jars showed that for the other fractions a 3% meat protein, 3% salt solution is required for gel strength measurements. The necessity of a higher salt concentration can be explained by the previously mentioned better gel structure and gelling ability of the SSP-fraction at higher ionic strength. As the K-fraction is also constituted of myofibrillar proteins similar effects could be expected. The influence of the protein concentration can be understood from fig.1.

In fig. 2 and 3 the results of the measured reciproke penetrations of the different gels and gellified emulsions are presented. It was not possible to produce gels of dispersions of the residue without water separation, whereas it was possible for emulsions made with the residue. Fig. 2 shows clearly that the SSP-fraction yields by far the highest gel strength, followed by the K-fraction and the WSP-fraction, this despite the best gelling ability of WSP.

FIG. 2 GEL STRENGTH OF MEAT PROTEIN FRACTIONS COMBINED WITH 1% OF NON-MEAT PROTEINS

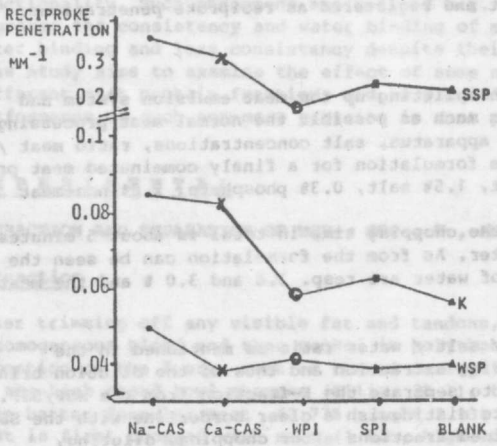
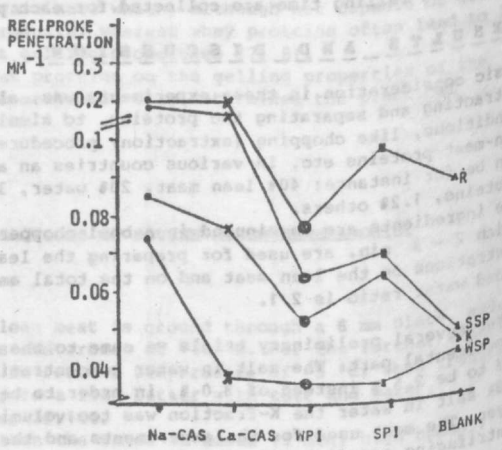


FIG. 3 GEL STRENGTH OF MEAT PROTEIN EMULSIONS COMBINED WITH 1% OF NON-MEAT PROTEINS



Although we may not predict from fig.2 behaviour of these fractions in meat products, it confirms the findings of meat scientists who claim a more important contribution of SSP than WSP to the waterbinding and consistency of meat products. Besides the results of Mc Farlane (10) showing WSP decreasing the binding of myosin, Grabowska (11) found an improved gel strength of fish muscle homogenates after WSP had been extracted. The Japanese fish paste industry in fact applies this technique by washing fish meat (extracting WSP) to obtain the desired high gel strength (12). Recent unpublished experiments in our laboratory (8) confirm the findings of Grabowska for beef muscle.

The above mentioned findings, however are mainly noticed in low fat systems and it is questionable whether this can be transferred to meat emulsions. In fig.3 it can be seen that the differences in gel strength completely disappear when emulsions are made with the meat protein fractions, but without non-meat proteins. Only the residue shows a higher value.

From fig.2 as well as from fig.3 it can be observed that, except for Ca-cas in the WSP fraction, caseinates significantly improve the gel strength of the individual fractions. Apparently Ca-cas, having a more conglomerated structure than Na-cas, disturbes the gelling of the globular sarcoplasmic proteins whereas the unfolded, thread-like Na-cas with high viscosity supports the WSP-gelling. The disturbance by Ca-cas seems not to take place with the myofibrillar proteins, thus resulting in an increased gel strength. So the practical experience of improved consistency of meat products by adding caseinates is also noticeable in the gelling of the individual fractions. A remarkable observation during the gelling in various test tube and glass jar trials was that caseinates retarded the SSP and K gelling but also that retarded gelling resulted in an improved gel strength and gel structure. Here we find a similarity with the pure WSP and SSP gelling: SSP shows a retarded gelling as compared to WSP, however resulting in an increased gel strength. WPI, in combination with different meat protein fractions, reduced the gel strength or had no effect both in solutions and emulsions. The same is true for SPI combined with all meat protein solutions and the WSP emulsion, whereas the SPI has a positive effect in SSP, K- and R-emulsions, however less pronounced than that of the caseinates. WPI, but also SPI, possesses a gelling ability of itself. This gelling ability depends completely on heat treatment during processing, protein and salt concentration during gelling as well as on temperature and time. Siegel (13) already found in a meat binding test system that gelling of non-meat proteins is not indicative of a protein's ability to bind meat pieces. The molecular interactions stabilizing the three-dimensional structure of these proteins must be of the same nature as those of the gel formed by myosin to increase the binding strength. Probably WPI and also SPI do not interact in a positive way with the myosin molecules as described by Siegel and the network formation on heating by these proteins could disturb the network formation of meat proteins. From the differences in protein structures and gelling temperatures, for meat proteins 50-70 °C(14), WPI 70-80 °C(15) and SPI 60-70 °C(16), it may be expected that a different network is formed and different molecular interactions take place. This may be compared with the findings of Knight (17) who demonstrated by electronmicroscopic photographs that

the gel structure of sarcoplasmic proteins is completely different from a myosin gel and after mixing of these proteins no homogeneous gel is obtained. These photographs visualize the more loose structure of a WSP-SSP gel which seems to be in accordance with other studies (6, 9, 11, 12). Caseinates would not hinder the formation of meat protein gels, since these random-coil structured proteins do not gellify on heating. Although caseinates by themselves are not able to bind meat pieces together (13), it has become clear through these experiments that they do increase the gel strength.
The above conclusions may have certain implications for the formulation and processing of both meat emulsion systems and reformed products like hams, even though waterbinding capacity and binding strength between meat pieces were not measured in this study. In meat products the type of interactions between heat gelling meat proteins and added non-meat proteins appears to be more important than the gelling ability of non-meat proteins as such. This could be an explanation of the superior effect of caseinates, noticed in practice, on the waterbinding and consistency of meat products in comparison with globular non-meat proteins.

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