MICROSCOPICAL OBSERVATIONS DURING HEATING OF MEAT PROTEIN FRACTIONS AND EMULSIONS STABILIZED BY THESE

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Water-soluble (WSP), salt-soluble (SSP) and K-fraction (swollen actomyosin) proteins were extracted from beef muscle tissue. In a number of experiments oil was emulsified in each of these fractions and mixtures. Both the separate protein fractions and the emulsions were examined during heating up to 95°C, applying a combined interferential/phase contrast (Interphako) microscopical technique. The microscope was equiped with a precise object-heating device.

As contrasted with the WSP-fraction, in the SSP and K-fraction sudden changes in the microscopic protein structures were observed at 55 - 60°C. At these temperatures the structures became far more contrasted, probably as a result of denaturation. During heating the protein material, initially present as loose, flocculent "clouds", aggregated in dense zones as well as in a kind of network structures.

About the same process was observed in the emulsions. Before heating it was striking to find all the very small oil globules (< 1 µm)intimately settled in the protein floccules. The larger oil globules appeared to be rather immobilized between the (network of) protein floccules. Especially during heating this became apparent.

In the K-fraction and SSP-stabilized emulsions no coalescence of oil globules was observed at all during heating. On the contrary the WSP-stabilized emulsions showed a rather high proportion of the larger oil droplets to coalesce during heating from about 32°C. The smaller oil globules appeared to be stable.

MIKROSKOPISCHE BEOBACHTUNGEN VON FLEISCHEIWEISSFRAKTIONEN UND DEREN STABILISIERTEN EMULSIONEN WÄHREND DER ERHITZUNG

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Aus Rindermuskelgewebe wurde die wasserlösliche (WSP) und salzlösliche (SSP) Eiweissfraktion, sowie das gequollene Aktomyosin (K-Fraktion) isoliert. In einer Versuchsreihe wurde in diesen Eiweisslösungen, bzw. in dieser Eiweissdispersion und deren Gemische Öl einemulgiert. Die separaten Eiweissfraktionen, deren Mischungen und Emulsionen, wurden dann während der Erhitzung bis auf 95°C mikroskopisch beobachtet, und zwar mittels einer kombinierten Interferenz/Phasekontrast (Interphako) Technik. Das Mikroskop war dazu mit einem genauen Objekterhitzungsgerät versehen.

Im Gegensatz zu der WSP-Fraktion, fanden bei der SSP- und K-Fraktion im Temperaturbereich von 55 - 60°C ziemlich plötzlich Veränderungen der Eiweisstruktur statt. Die Eiweisstruktur wurde bedeutend kontrastreicher, möglicherweise einer Denaturierung zufolge. Diese Fraktionen die in unerhitztem Zustand eine lockere und wolkige Beschaffenheit zeigten, aggregierten bei Erhitzung zu dicken, kompakten Zonen und einer Art Netzwerkstruktur.

Ein etwa ähnlicher Vorgang wurde bei Erhitzung der Emulsionen beobachtet. In unerhitztem Zustand waren die kleinen Ölteilchen (< 1 µm) ohne Ausnahme in den Eiweissflocken eingebettet und integriert. Es zeigte sich dass die groberen Öltropfen mehr zwischen den Eiweissflocken immobilisiert waren. Insbesondere während der Erhitzung wurde die Aufnahme ins Eiweissnetzwerk deutlich erkennbar.

In der von der K-Fraktion bzw. SSP-Fraktion stabilisierten Emulsionen wurde keine Koaleszenz von Öltropfen während der Erhitzung beobachtet.

In der von WSP stabilisierten Emulsionen dagegen fand Koaleszenz der Öltropfen häufig statt ab einer Temperatur von 32°C. Nur die ganz kleinen Öltropfen blieben stabil.

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OBSERVATIONS MICROSCOPIQUES, PENDANT LE CHAUFFAGE, DES FRACTIONS PROTEIQUES DE LA VIANDE ET DE LEURS EMULSIONS STABILISEES

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Des protéines solubles dans l'eau (WSP) et dans le sel (SSP), ainsi que de l'actomyosine gonflée (fraction K) furent extraites du tissu musculaire du maigre de boeuf. Dans une série d'expériences de l'huile fut émulsifiée dans chacune de ces fractions protéiques resp. dans cette dispersion protéique et ses mélanges. Puis les fractions protéiques différentes ainsi que les émulsions furent examinées pendant le chauffage jusqu'à 95°C en utilisant une technique microscopique combinée d'interférence et de contraste de phase (Interphaco). Le microscope était équipé d'un dispositif de précision pour l'échauffement d'objet.
Par opposition de la fraction WSP, dans les fractions SSP et K il se produisait tout à coup des changements dans les structures protéiques à une température de 55 à 60°C. Les structures protéiques furent considérablement plus contrastées, probablement à cause de la dénaturation. Pendant le chauffage la matière protéique, à l'origine présente sous la forme de flocons séparés, s'agrégeait en zones compactes et en une structure qui ressemble à un réseau.
Environ le même processus fut observé dans les émulsions, où il se trouvait, avant le chauffage, les petites sphères d'huile (< 1 µm) parfaitement intégrées aux flocons de protéine, tandis que les plus grandes sphères d'huile prouvaient être immobilisées plutôt entre les flocons de protéine. Surtout pendant le chauffage ceci s'accentua.
Dans les émulsions stabilisées par les fractions protéiques K et SSP la coalescense ne fut observée pas du tout pendant le chauffage; par contre, les émulsions stabilisées par la fraction WSP montraient un assez grand nombre de sphères d'huile qui se réunissaient pendant le chauffage en partant d'environ 32°C. Les plus petites sphères d'huile prouvaient être stables.

Наблюдения методом микроскопии, проведенные во время нагрева фракций белка, выделенного из мясной ткани, и эмульсий, ими стабилизированных.

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Из мышечной ткани коровы были выделены белки - водорастворимый (ВРБ), солерастворимый (СРБ) и К-фракция (набухший актомиозин). В ряде экспериментов осуществлялось эмульгирование масла в каждой из этих фракций и смесей. И те и другие, т.е. разделенные фракции белка и эмульсии, были исследованы во время нагрева их до 95 °С. Наблюдения производились комбинированным интерференционным фазово-контрастным методом микроскопии (Интерфако). Микроскоп оборудован агрегатом точного нагрева объекта.

В противоположность фракциям ВРБ, неожиданные изменения в микроструктуре белка в СРБ- и К-фракциях наблюдались при температуре 55-60 °С. При этих температурах структуры контрастировали значительно сильнее, вероятно, в результате денатурирования. Во время нагрева белковый материал, первоначально представленный в виде рыхлых, хлопьевидных "облачков", агрегировался в плотные зоны, а также в структуры, напоминающие сетчатые.

Более или менее подобные процессы наблюдались и в эмульсиях. Перед нагревом эмульсии встряхнули с целью выявления мельчайших масляных шариков (<1 µ), равномерно осевших в хлопьях белка. Более крупные масляные шарики казались до некоторой степени связанными среди (сетки) хлопьев белка. Особенно во время нагрева это стало более очевидным.

В К-фракции и стабилизированных СРБ эмульсиях никакой коалесценции масляных шариков во время нагрева вообще обнаружено не было. В противоположность этому, в стабилизированных ВРБ эмульсиях наблюдалось довольно высокое содержание более крупных масляных капелек, коалесцирующихся во время нагрева, при температуре где-то от 32 °С. Более мелкие масляные шарики производили впечатление устойчивых.

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INTRODUCTION

It is generally accepted nowadays that the myofibrillar meat proteins play the main role in the stabilisation of many processed meat products like e.g. finely comminuted sausages. Difference of opinion, however, exists on whether the solubilized fraction of these proteins or the swollen, insoluble fibre fragments are most important in the water- and fatbinding mechanisms (1.2). Preceding research at our laboratory (3.4) indicated the presence of an intermediate myofibrillar protein fraction in lean meat/salt/water slurries prepared in a bowl chopper. This fraction was called K-fraction, consisted of swollen actomyosin and accounted for about 30 - 40% of total meat protein. When fat was chopped into the lean meat slurry the K-fraction gradually swelled with chopping time and took up considerable amounts of water. A mutual influence of fat- and water binding and a role for the K-fraction protein in this was suggested (5).
The influence of heat on the different isolated meat protein fractions has been studied by many investigators characterizing e.g. denaturation, solubility, gelling properties and enzymatic activities (6). Samejima et al (7) and Kotter et al (2) accompanied their studies with some electron-microscopical observations, but generally microscopy was applied very scarcely in these studies. Especially the influence of finely comminuted fat on the different characteristics during heating meat protein fractions seems worth studying. Recently we studied the different protein fractions, including the K-fraction, in a model meat emulsion system (8). Interferential microscopy appeared a way of visualising the swollen protein material, also in the presence of fat. In this study the protein fractions and emulsions were observed directly during heating applying the same microscopical technique.

MATERIALS AND METHODS

Meat protein extraction

Postrigor lean beef was chopped during 1 minute with 0.6 parts 0.02 M KCl-solution in a 30 ltrs Kilia bowl chopper (temp. 3°C). The slurry was gently diluted with 0.4 parts of 0.02 M KCl and centrifuged at 3,000 g for 20 min. The supernatant (WSP-fraction) contained 3.09% protein. The residue was washed twice with 0.02 M KCl-solution to remove the rest of the WSP.
The washed residue was given to the bowl chopper and chopped 0.5 min. with 4% dry NaCl and then for 2.5 min. with 0.6 parts ice/water. The slurry (3°C) was diluted with 1.4 parts 2.5% NaCl-solution and centrifuged for 20 min. at 30,000 g. Three layers were separated:

SSP (salt-soluble protein):		1.72% protein
K-fraction	:	3.49% protein
Residue	:	6.35% protein

The different fractions were purified as follows:
The WSP-fraction was centrifuged at 30,000 g and a clear WSP supernatant was obtained (2.89% protein). Before use the WSP-fractions were adjusted to 2.5% NaCl.
The SSP-fraction was diluted with an equal amount of 2.5% NaCl-solution and centrifuged for 30 min. at 40,000 g. A clear SSP-supernatant was obtained containing the real salt-soluble proteins (0.50% protein).
The K-fraction was washed twice with 2.5% NaCl-solution and the final K-fraction sediment contained 6.48% protein. Before use this fraction was diluted to 3.25% protein.
For simultaneous extraction of WSP, SSP and K-fraction the lean beef was chopped during 0.5 min. with 4% dry NaCl and then for 3.5 min. with 0.6 parts water/ice (temp. 3°C).
The slurry was diluted with 1.4 parts 2.5% NaCl-solution and centrifuged at 30,000 g for 20 min. The viscous upper layer was separated from the residue. This fraction was called (WSP + SSP + K)-fraction and amounted 5.7% protein.

The emulsification of oil (5% corn oil) was done in a Starmix blendor during 45 seconds.
The final temperature was 14°C. Before microscopical examination of the different protein fractions without oil they were blended in the same way in the Starmix.
For gelling experiments the fractions were diluted with 2.5% NaCl-solution and given to glass tubes (Ø 1.5 cm). Different concentrations were heated at temperatures between 30°C and 80°C, and gelling was controlled by gently turning the tubes upside down.

Microscopy

For microscopical examination of the different fractions and emulsions a Zeiss Peraval Interphako microscope was used applying the Interphako technique (combined interference-phase-contrast). The usual magnification was 400x and diapositives in color were made. In addition some black and white microphotographs were made for documentation in this paper although contrast decreased in this way. The swollen protein material showed good contrast to the fat and water in using the second order blue interference band (664 nm).
The fractions and emulsions were heated during microscopical observation using a precise Zeiss object heating/cooling assembly equiped with a direct object temperature indicator (Digitherm P14). Drying out of the samples during heating was reduced by covering the object glass with a glass box in which added water was evaporated.

RESULTS

WSP-fraction

The WSP fraction, centrifuged at 30,000 g was a clear red fluid showing no microscopically visible material at all on 400x magnification. The WSP-fraction, centrifuged at only 3,000 g, showed some structured clumps (2 - 5 µm) of protein material, possibly consisting of some organella from the sarcoplasm. During heating, from 65°C in the clear WSP-fraction numerous pinpoints (< 1 µm) could be observed microscopically, however no coherent structure between these pinpoints was visible, neither on heating up to 95°C.
When oil was emulsified in the WSP-fraction the resulting emulsion showed quick flocculation and creaming of oil globules. Microscopically clumps of larger and small oil globules were visible. During heating the larger globules showed deformation and often coalescence. The emulsion destabilized completely at temperatures > 70°C and at 90°C only part of the smaller oil globules (< 1 µm) was present in emulsified condition. At 65°C no formation of pinpoints in the external phase was observed, possibly as a result of previous adsorption of the protein in the oil-water interphase.
Heating the WSP-fraction in glass tubes resulted in gelation from about 65°C at protein concentrations down to about 2%. The resulting gels were very brittle.

K-fraction

As reported before (8) at lower temperatures the K-fraction microscopically is composed of cloudy, flocculent material. When oil or fat was emulsified into the K-fraction it was striking to find all the very small oil globules intimately settled in the protein floccules. The larger oil globules appeared to be rather immobilized within large fields of loosely aggregated protein floccules. Especially in the emulsions some channels or holes of clear fluid material were visible. In these channels during heating streaming of the fluid with individual protein floccules could be observed. At about 48 - 50°C this streaming often ceased, indicating a kind of gelation of this phase. Heating up to 55°C caused no change in the microscopic picture of the protein material, neither in the pure K-fraction nor in the emulsion. Between 55°C and 60°C, more precisely at about 59°C, the protein material suddenly became far more contrasted and seemed to shrink to a kind of protein network trapping small and large holes of fluid or gel. In these initially clear holes also some "new" protein became visible. The same was true for the protein material in the emulsions. (see microphotographs).
During heating up to 95°C no deformation or coalescence of oil/fat globules could be observed.
Heating the K-fraction in glass tubes microscopically showed gelation to occur at 50°C. Dilutions down to about 0.5 % protein still showed gelification at 60°C.

Microphotograph

A. K-fraction 20°C magn. 400x, B. K-fraction 80°C magn. 400x,
C. K-fraction/oil emulsion 20°C magn. 400x, D. K-fraction/oil emulsion 80°C magn. 400x.

SSP-fraction

The undiluted SSP-fraction (1.72% protein), microscopically appeared to contain a considerable amount of the same "cloudy" material as the K-fraction. However, the material seemed to be somewhat finer and less contrasted. In the presence and absence of oil about the same observations were made during heating as with the K-fraction. However, at temperatures above 60°C the network seemed to be somewhat more open.
The clear SSP-fraction, diluted and centrifuged, contained no visible material. However, when the fraction was treated mechanically in the Starmix (as usual) membraneous and irregular structures became microscopically visible. Centrifuging at 30,000 g yielded a significant sediment of this material. On heating the SSP-fraction from 48 - 50°C some faint changes started while at 59°C a very open kind of network structure became visible. This structure, however, was far less contrasted as the swollen material in the K-fraction. Moreover this visible SSP-material originated the clear fluid phase as opposed to the swollen K-fraction material.
When oil was introduced the resulting emulsion showed hardly any deformation and no coalescence.
The clear SSP-fraction showed no gelation on heating it up to 75°C in a glass tube. The original SSP-fraction formed a gel at 50°C in concentrations down to about 0.7% protein.

(WSP + SSP + K)-fraction

This fraction showed the same pictures as the K-fraction, both in the presence and absence of fat/oil. Because of the highly viscous character of this fraction some muscle fibre fragments and collagen fibres were present. The muscle fibre fragments clearly showed shrinkage on heating as did the collagen fibres around 60°C. At higher temperatures the collagen fibres seemed to desintegrate, and a kind of an gellified aureole developed in the fibre region.
Gelation of the fraction occured at 50°C but not lower than in 1.5% protein dilutions.

DISCUSSION

Many investigators have reported the most drastic changes on heating isolated myofibrillar proteins to occur between 30°C and 50°C, and to reach almost completion at 60°C (6). In accordance with this the gelation of our SSP- and K-fraction already took place in this temperature range, which is considerably lower than the 59°C at which microscopical changes in the swollen, undissolved protein were observed. This change could be specific for a protein interactive force in the swollen protein.
Up to now the genesis of the swollen actomyosin fraction is not completely understood. Since hardly any publication on meat protein extraction has mentioned it, we believe the occurence of the K-fraction to be specific for our extraction procedures that are close to practice. It could be a certain fraction of the native actomyosin complex, freed from the sarcolemn. However the possibility that it consist of a partly denaturated actomyosin cannot be excluded. For the interaction between finely dispersed fat and the actomyosin fraction, possibly resulting in an increased swelling, we could not get a point of contact in this microscopical study.
The observed change at 59°C in the swollen protein, giving rise to a kind of network formation, might give the already developed gel a better elasticity and firmness. Due to the strong shrinkage of a.o. the fibre fragments there is no doubt that during heating of meat emulsions some water will become free. The same could be true for an amount of fat that had not been emulsified or stabilized within the original fatty tissue. Observing the network microscopically one could imagine that this myofibrillar protein network immobilizes this water and fat. It is possible that the very finely dispersed fat-globules in close contact with the protein floccules will prevent the swollen protein to shrink too strongly and thereby give rise to the development of a more open, elastic gel, able to hold more water.
The gelling properties of our WSP-fraction are in agreement with the results of Hamm & Grabowska (9). The microscopical absence of any coherent structure in the heated globular WSP-fraction could explain the very brittle character of the WSP-gel.

Our results once again show the myofibrillar proteins to be far better emulsifiers/stabilizers than the globular sarcoplasmic proteins. In earlier experiments (3) we found a meat emulsion prepared with WSP-free meat to be more heat stable than the original meat emulsion. The same tendency was found by Macfarlane et al (10) for the binding capacity of a mixture of myosin and sarcoplasmic proteins in the presence of salt.

Of course this microscopic study is mainly a phenomenologic one and it is therefore difficult to draw straight conclusions. Results from other experimental techniques are needed in addition. However, these direct observations during heating possibly can contribute to a better understanding of stabilization mechanisms in processed meat products.

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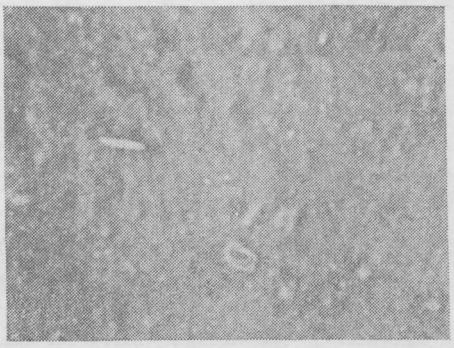
A.



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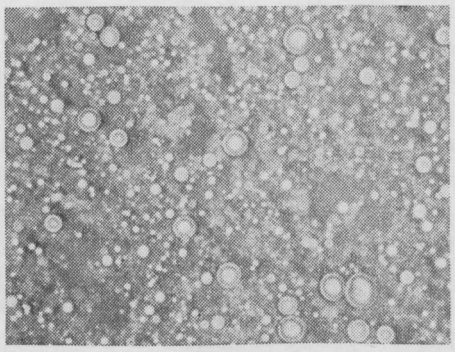

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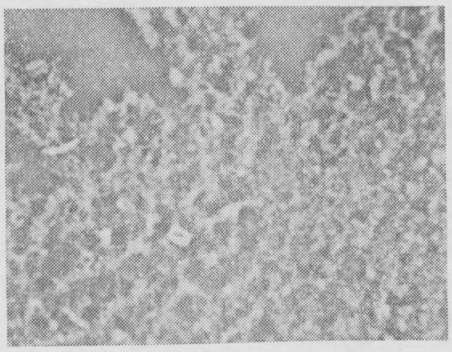

D.