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# Microstructure of Fermented Sausage

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*ABSTRACT*

*A protein matrix is necessary for the desired texture of fermented sausages suitable for slicing. The formation of this network is predominantly induced by myosin and actin proteins. A change in the structure of native muscle proteins results from different technological processes such as chopping, salting, and fermentation. During chopping with simultaneous release of meat proteins, the salt brings about a change in the original structure of proteins by swelling and partial solution of myofibrils. The dissolved proteins are transformed into a thin fluid colloidal transition state, the so-called 'sol-state' with unstable coagulation bonds. During sausage ripening, as a result of denaturation by lactic-acid and due to gradual loss of water (drying), the unstable bonds are replaced by condensation bonds, and thus the sol-state is converted into the 'gel-state'. Both gel formation (condensation structure) and water evaporation (syneresis) result in the development of a matrix in fermented sausage and, consequently, in the texture of the sliceable product.*

## INTRODUCTION

Depending on their texture and the degree of mincing, fermented sausages can be differentiated into those suitable for spreading and those suitable for slicing. Those suitable for slicing are storable at temperatures

exceeding 10°C. During storage their texture gradually changes. The sausages become firmer as a result of ripening and drying (water evaporation). In the present view, this firmness is brought about by protein solutions which moisten the fat and muscle particles overall and form a gel. According to Kotter & Prändl (1958) a thin coating 'adhesive substance' of meat proteins—either in a liquid state as a gel solution or in a slightly firmer state as a 'loose' gel—covers the surfaces of all particles and binds them. During ripening of fermented sausage, the gradual drying leads to a gel solidification at the edge of the particles (Kotter *et al.*, 1962).

What has been known hitherto about the basic structure of fermented sausage is somewhat vague and therefore we studied the fine structure and the development of the matrix of fermented sausage using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The results are reported here and the contribution of this matrix to the texture of the fermented sausage is discussed.

## MATERIALS AND METHODS

Finely ground fermented sausage (Cervelat-type) was used for this study, and the ripening process was followed for 28 days.

### Scanning electron microscopy

The SEM methods applied have been described in detail by Katsaras and Stenzel (1983) as well as by Katsaras *et al.* (1989).

### Transmission electron microscopy

From each sausage investigated five 1 × 1 mm blocks were obtained. These originated from both ends and the middle of the product. From the latter region three specimens were taken, from the surface, the centre and from an intermediate position. The blocks were fixed in 4% glutaraldehyde, followed by immersion in 1% osmium tetroxide. The blocks were embedded in Epon and stained *en bloc* or in sections with uranyl acetate. The TEM methods have been described in detail by Katsaras *et al.* (1986).

### Extraction of meat protein

Extracts of meat proteins from bovine muscle were obtained using the method of Grabowska and Hamm (1978), which was slightly modified.

We removed the *Musculus sternomandibularis* from beef carcasses immediately after slaughter (hot-boning), and the muscle was stored at room temperature for 5 h in order to prevent 'cold-shortening' and subsequently at 4°C for 16 h. Thereafter the muscle tissue was trimmed of external fat and connective tissue and crushed in a meat grinder (2 mm disc). Fifty grams of the ground muscle tissue were placed in 200 ml of water containing 2% NaCl and homogenized with a Moulinette. The homogenate was stirred at 4°C for 6 h, and subsequently centrifuged at 18 000 rpm for 30 min with a refrigerated Beckman centrifuge. The supernatant contained both water- and salt-soluble proteins.

## RESULTS AND DISCUSSION

During the manufacture of fermented sausage the meat is first mechanically chopped. During this process, the muscle cells are cut open and their integrity is more or less destroyed. By 'opening of the sarcolemma' the myofibrils, which constitute 83% of the cell content (Offer, 1984), are exposed. The added NaCl penetrates the myofibrils. In the sarcomeres the chloride ions are mainly absorbed by the actomyosin filaments and by the Z- and M-bands, which leads to an increase of the electrostatic charge of the proteins (Hamm, 1960). Consequently, on the one hand the repulsive forces between actin and myosin filaments are intensified while on the other hand the disintegration of the proteins—with loosening of their transverse filament binding—accelerates. The network of the Z- and M-bands becomes unstable and the cohesion of the bands diminishes. A consequence of this process is an enlargement of the interfilament spacing (lattice spacing) (Offer & Trinick, 1983). Due to the hygroscopic property of NaCl, these lattice spacings are capable of binding a greater amount of water. Furthermore, water molecules penetrate between the protein molecules of the filaments and cause the swelling of the myofibrils (Fig. 1). The addition of sodium chloride results in an increase in the water-holding capacity of swelling of the tissue (Voyle *et al.*, 1984). NaCl is thought to attack first the filament-stabilizing transverse bonds of the M-line which then either disappear (Fig. 2) or at least become less distinct (Fig. 3). This halolytic process progressively splits the myosin filaments into intrafilamentous fragments of fine felt-like structures and/or into fine granular or floccular structures. A consequence of this is the loosening or partial disintegration (Fig. 3) of the H-zone which consists exclusively of myosin filaments. The Z-disc is slightly loosened and shows two parallel lines (Fig. 4). However, it remains the most stable portion of the sarcomere. Weakened Z- and M-lines allow an

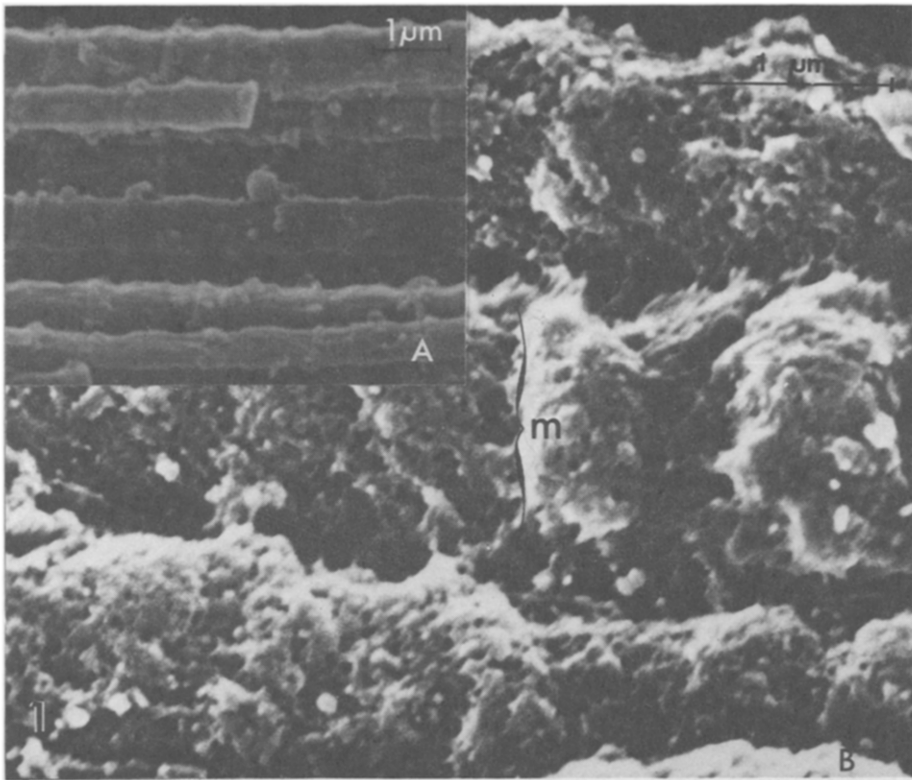


Fig. 1. (A) Untreated myofibrils (magnification × 2400). (B) Swollen myofibrils (m) in the sausage matrix (magnification × 9400) (SEM).

enlargement of the interfilamentous space, which goes along with a swelling of the myofibrils. An appropriate NaCl concentration and a fine chopping of the muscle meat may have such a pronounced effect on the swelling that the filament bonds, i.e. the Z- and M-lines as well as the acto-myosin transverse bonds, may partially or even totally disintegrate. In this case, not only the filaments or filamentous fragments, respectively, separate but beyond that, their protein molecules go into solution and form a filigree network (Fig. 5). By the extraction of the myosin proteins of the A-bands and especially of the H-zone, clefted and more or less dis-integrated filaments are formed which probably are relics of the actin filaments. These changes allow the Z-lines to come closer together with the sarcomeres becoming shorter and greatly deformed. The band pat-tern, typical for sarcomeres has now clearly changed (Fig. 6). Hence, in-tensive chopping and a higher concentration of NaCl result in structural changes of the meat proteins with the loss of the typical band pattern of the myofibrils in the treated muscle tissue.

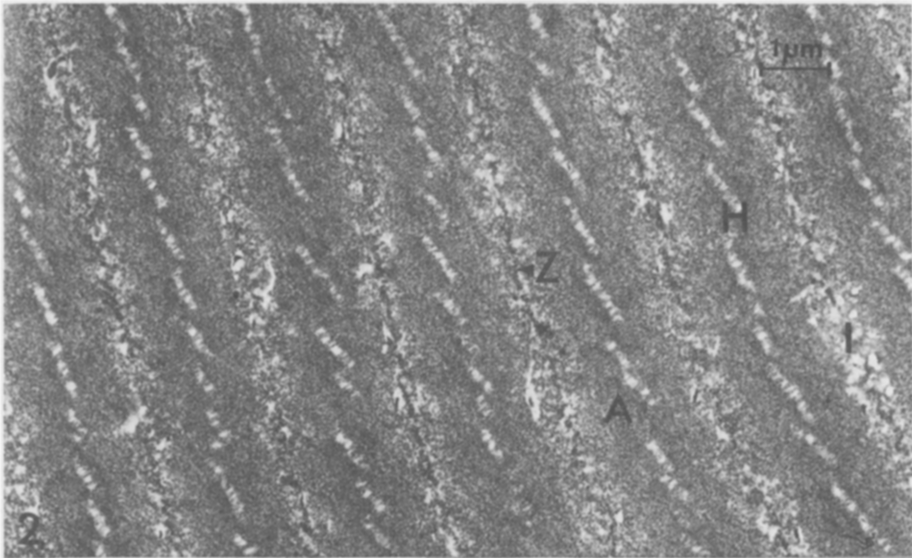


**Fig. 2.** Sodium chloride is thought to attack first the structure of the M-line which then disappears (magnification × 5000) (TEM).

**Fig. 3.** As for Fig. 2, except M-line has become less distinct (magnification × 20 000) (TEM).

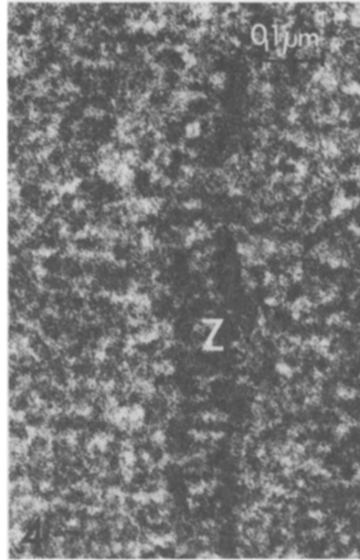


**Fig. 4.** The Z-disc is slightly loosened and shows two parallel lines (magnification ×40000) (TEM).

The changes, especially on the surfaces of the myofibrils include the formation of swollen but also of dissolved proteins and hence of protein-rich solutions. The swelling of the myofibrils coincides with the dissolving process of the proteins. Salt- and water-soluble proteins enter the spaces between the particles or moisten film-like the surfaces of the meat and

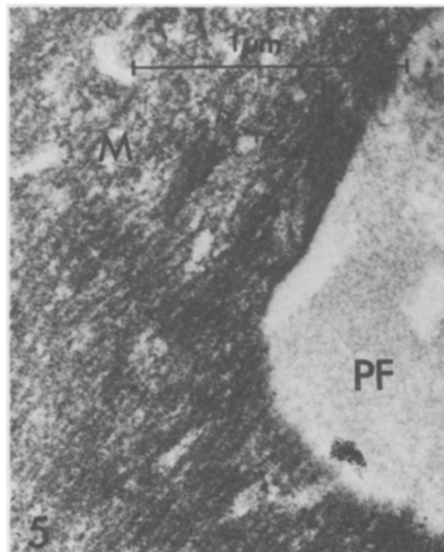


**Fig. 5.** Swollen myofibrils (M); del. protein felt (PF) (magnification × 20000) (TEM).

**Fig 6.** Shortened and deformed sarcomere (magnification × 20000) (TEM).

fat particles. Here, they build a fine-meshed, three-dimensional network during the ripening of the sausage. This structural network is the stabilizing factor of the sausage batter (Fig. 7). At the periphery of the myofibrils the swollen proteins can be considered an 'adhesive substance' which holds the meat and the fat particles of the sausage batter together (Fig. 8).

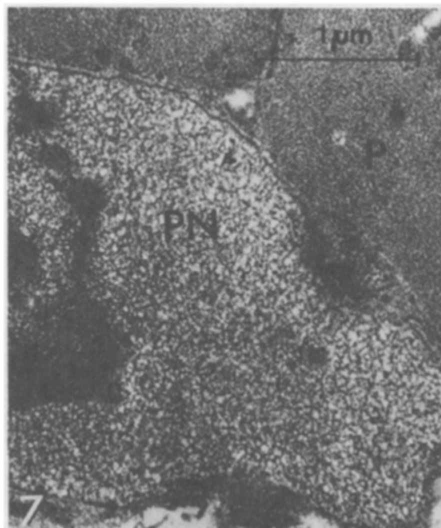


**Fig. 7.** Swollen protein (P); p-network (PN) (magnification × 12500) (TEM).

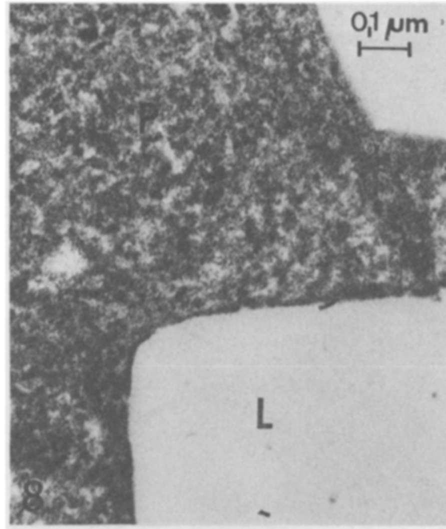


**Fig. 8.** Swollen protein (P); which holds the lipid (L) particles together (magnification × 40 000) (TEM).

The fibrous connective tissue layers among the sausage batter show morphological changes which largely depend on the type of collagen as well as on the number of intra- and intermolecular crosslinks. The perimysium, for example, is still arranged in a nearly glomerular network, but the collagenous fibrils already expose a clear decrease of

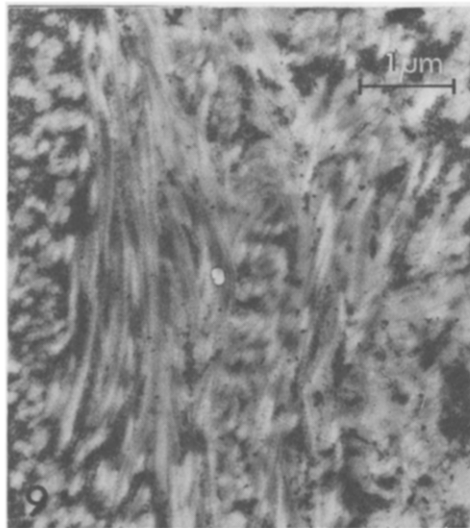


**Fig. 9.** Collagen fibrils (perimysium) between meat particles (magnification × 12 500) (TEM).

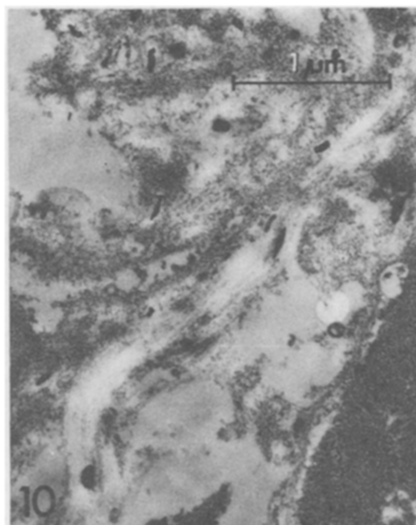


**Fig. 10.** Collagen fibrils (endomysium) show a fine network (magnification × 10 000) (TEM).

osmiophility and periodicity (Fig. 9), which suggests a partial disintegration of these fibrils. Simultaneously, numerous electron-dense, dark, indistinctly-shaped, localized granula can be observed not only at the periphery but also between the lighter collagenous fibrils. These granula are probably derived from the transformed (assimilated) myofibrillar proteins and the intercellular matrix.

In contrast to the perimysium, the endomysial system, largely consisting of reticular argyrophylic microfibrils, is obviously less lactic acid-resistant and therefore swells. The carbohydrate coat possibly is digested by the lactic acid bacteria and the acid itself produces the splitting of the reticular fibrils. With onset of denaturation these fibrils may lose their typical morphological appearance and change into a fine felt-like network (Fig. 10).

*What are the factors that make matrix-formation possible?*

The dissolved proteins, especially of myosin molecules, react at a low ion concentration (0·1–0·3M KCl at pH 6·0–8·0) and spontaneously form aggregations. Some of these aggregations are long and spindle-shaped and are called synthetic filaments (Huxley, 1963). Other proteins form floccular coagulations, however, and do not show signs of gel development. The dimensions and formation of the so-called synthetic filaments depend on such factors as pH value and NaCl concentration (Pollard, 1975; Knight & Trinick, 1984). These filaments can therefore be

distinguished by size and arrangement from native filaments. The synthetic filaments which were observed in the negatively stained specimens often aggregate at almost right angles to each other and show bonds extending from the end of one filament to the middle of the other filament. These Y-shaped bonds and the parallel arrangements of the filaments result in 'ladder-formations' (Hermansson & Langton, 1988) which finally build up a loose reticular structure. These synthetic filaments are thus the 'building components' of a three-dimensional network. Types of reactive aggregates are bound to each other by hydrogen bonds and electrostatic charges to form a continuous reversible 'coagulation structure' (Sokolow & Tschechowskaja, 1976*a*,*b*). This structure is therefore regarded as interesting as it results from an aggregation of mainly dissolved myosin molecules. The bonds of the coagulation structure are initially unstable and therefore not yet capable of giving the sausage components the necessary firmness. Within this three-dimensional protein network a large amount of 'free' water is adsorbed. The water has two functions: (a) as a hydration layer it separates the coherent protein aggregates; (b) on the other hand, via the hydrogen bonds it constitutes a link between the protein threads. If there is a sufficient amount of water available then the protein remains in a colloidal 'sol-state'. The firmness of the coagulation bonds is still rather unstable and in addition it is weakened by the intermediate layer of water molecules. Consequently, the coagulation structure in this state is still capable of flowing and is flexible. It is this flexibility that allows this transitional structure to follow all changes in shape caused by chopping and filling the sausage batter into casings. In these phases of sausage processing some coagulation bindings are torn and easily rebuilt elsewhere. These processes of structural destruction and reconstruction partially run simultaneously, with the exception that reconstruction takes less time. Therefore, the meat mixture shows a relatively good coagulation structure (Kiessling, 1982).

A further, final firmness of the coagulation structure, however, can only be achieved by releasing the immobilized water molecules that occupy the spaces between the protein aggregates. Dehydration is thus an essential prerequisite for the firmness of the sausage batter. For a continuous dehydration it is necessary that at the onset of ripening the conditions in the climatic chamber are properly adjusted and controlled, so that the degree of moisture differs only slightly (3 to 5%) between sausage and air of the climatic chamber. In this moisture differential the sausage continuously loses considerable amounts of water.

During the process of sausage ripening, after a certain period of adaptation, bacterial populations increase in number. The growth of lactic

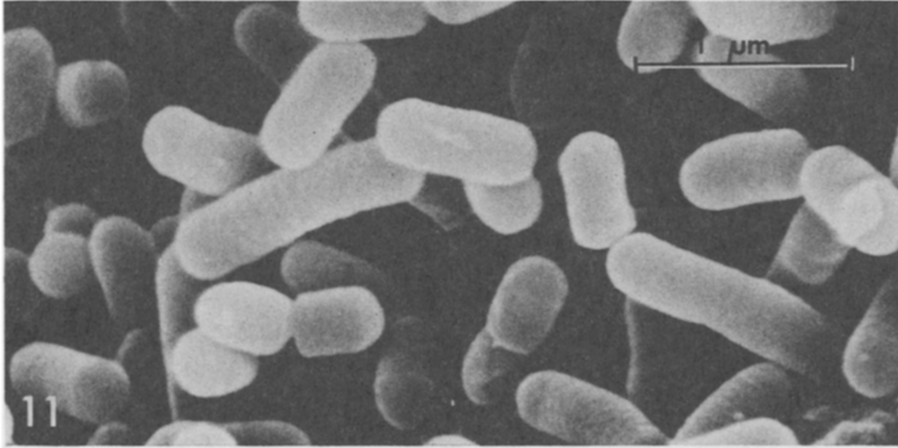


**Fig. 11.** Lactobacilli in a small group within the fermented sausage mass (magnification × 7800) (SEM).

acid bacteria in 'nests' is dominant (Fig. 11), because these bacteria enjoy the advantage of selection in the milieu of fermented sausages (Katsaras & Leistner, 1988). The growth of lactic acid bacteria results in a breakdown of sugar and thus in the formation of lactic acid.

The continuous release of lactic acid brings the meat proteins gradually close to their isoelectric state. This involves a decrease in number of the negatively charged groups and a simultaneous reduction of the capacity to bind water in the muscle protein. As soon as the sausage has reached the isoelectric level of about pH 5·3, the negative and positive charges compensate each other and bound water is released, i.e. the water binding capacity in the fermented sausage has reached its minimum (Hamm, 1972). Consequently, the amount of immobilized water between the coherent protein threads is reduced and its function as 'spacer' becomes increasingly ineffective so that protein aggregates and also meat particles can gradually approach each other. Thereby, the appearance of the spindle-shaped protein aggregates is subjected to a series of changes and, in addition, the intermolecular interactions successively create new bonds, until an extended, dense, three-dimensional network of continuous protein threads is built (Fig. 12). This process, which is accompanied by water loss and shrinkage of the sausage, is called syneresis (Kiessling, 1982). The water release from the interior of the sausage takes place partially by diffusion, due to the moisture difference between sausage and ripening chamber, and partially the water is released via slits and gaps. The formation of slits and gaps results from syneresis, halolytic and enzymatic (calcium-activated factors = CAF) processes of

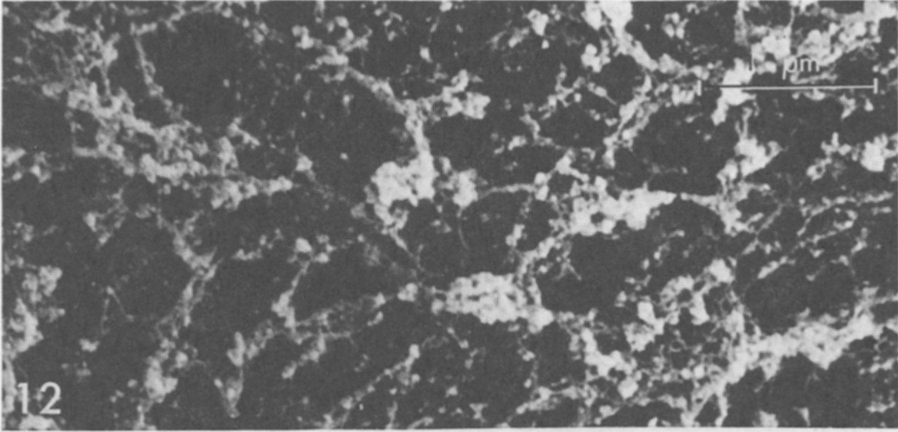


**Fig. 12.** Released muscle proteins form a network (magnification × 7800) (SEM).

decomposition between myofibrils and greater meat particles, i.e. between the peri- and endomysium, as well as in the muscle cells (Fig. 13). Moreover, the influence of acid leads to more intensive and more stable aggregations between the proteins. These relatively firm bonds, which in contrast to other coagulation bonds are not rebuilt when destroyed, strengthen the developing protein network and thus contribute to the structural firmness. Therefore, it can be concluded that during the period of sausage ripening the initially unstable coagulation bonds are gradually converted into firmer condensation bonds by the gradual drying of the sausage (loss of water) and the denaturation by acid (Sokolow & Tschechowskaja, 1976*a*,*b*; Raeuber & Kiessling, 1982), i.e. the viscous

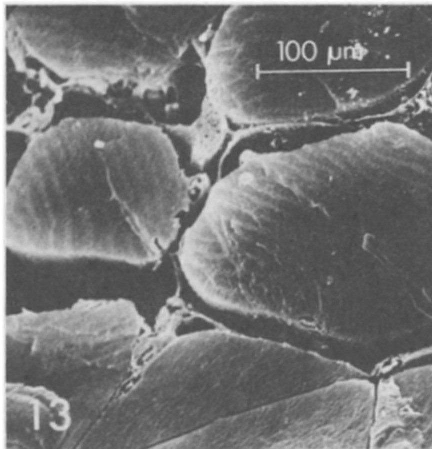


**Fig. 13.** Endomysial slits between muscle cells (magnification × 200) (SEM).

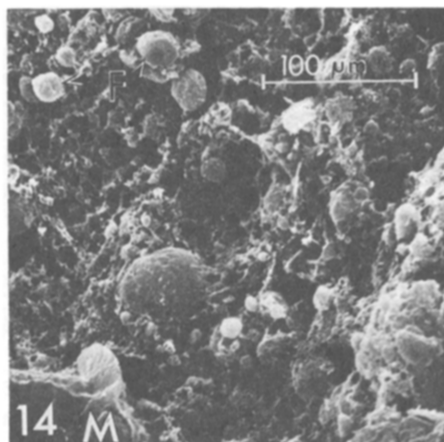


**Fig. 14.** Protein network stabilizing the fat (F) and meat (M) particles in the matrix (magnification × 200) (SEM).

protein system is transformed from the 'sol-state' into the colloidal viscous 'gel-state'. The condensation structure constitutes an irreversible network, which due to its ramification binds the meat and fat particles at their surfaces with the effect that the sausage batter becomes firm for slicing (Fig. 14).

The modified connective tissue fibrils form microfibril webs either among themselves or among the newly developed structures (Oelker, 1986). From this it can be concluded that the connective tissue proteins also (although to a minor degree) belong to the structure-forming substances. All these processes that occur during sausage ripening—gel formation with contribution of myofibrillar and connective tissue proteins, acid denaturation of the transformed proteins, and the water loss—result in the development of a matrix in fermented sausage. The matrix is the necessary requirement for desired texture in the product.

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