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EFFECTS OF HEAT ON MEAT PROTEINS– IMPLICATIONS ON STRUCTURE AND QUALITY OF MEAT PRODUCTS

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Abstract

Globular and fibrous proteins are compared with regard to structural behaviour on heating, where the former expands and the latter contracts. The meat protein composition and structure is briefly described. The behaviour of the different meat proteins on heating is discussed. Most of the sarcoplasmic proteins aggregate between 40 and 60°C, but for some of them the coagulation can extend up to 90°C. For myofibrillar proteins in solution unfolding starts at 30-32°C, followed by protein-protein association at 36-40°C and subsequent gelation at 45-50°C (conc.> 0.5 % by weight). At temperatures between 53 and 63°C the collagen denaturation occurs, followed by collagen fiber shrinkage. If the collagen fibers are not stabilised by heat–resistant intermolecular bonds, it dissolves and forms gelatin on further heating. The structural changes on cooking in whole meat and comminuted meat products, and the alterations in water-holding and texture of the meat product that it leads to are then discussed.

Introduction

Cooking of meat products is essential to achieve a palatable and safe product. The meat proteins, approximately 20 % of a muscle's weight, represent the main constituents that make up the structure of the meat product. They undergo substantial structural changes on heating and therefore the quality of the meat product, which is mainly governed by the meat structure, also changes drastically after cooking. This review will try to cover the aspects of structural changes on cooking for meat proteins *per se*, in whole meat and comminuted meat products, and the quality alterations of the meat product that it leads to. Earlier reviews in this area are among others Hamm, 1977, Offer, 1984 and Asghar et al., 1985.

Proteins in general

A protein is built up from a long polymer chain of amino acids, a polypeptide chain. The variable side chains give each protein chain its distinctive character. There are three general categories of side chains: nonpolar, polar but uncharged and charged polar (Dickerson and Geis, 1969).

The build-up of the polypeptide chain of the proteins is called the *primary structure* (Figure 1). This polypeptide chain tends to form specific conformations in solutions, the so called localised *secondary structures,* i.e. α-helix, β-pleated sheet or random coil (Figure 1). *Tertiary and quaternary structures* of proteins are the denomination of the three-dimensional structure and the association of protein entities in solution, respectively (Figure 1).

The stabilisation of these structures of a given protein system is dependent mostly on non-covalent forces, such as hydrogen bonding, van der Waal's forces, electrostatic and hydrophobic interactions. The formation of *globular* proteins is a typical example of how hydrophobic interactions stabilises this type of tertiary structure. There is a driving force for the nonpolar, hydrophobic side chains of a protein to be removed from an aqueous to a nonpolar environment. The protein gains an extra 4 kcal of free energy stabilisation for every

nonpolar side chain group buried in the interior of the globular protein, mainly as an entropy effect (Kauzmann, 1959). Among the meat proteins myoglobin, giving meat its colour, is a typical example of a globular protein (Figure 2). Another structural form of proteins, which is highly prevalent among meat proteins, is the *fibrous* form of proteins. Actin, myosin and collagen in meat are typical fibrous proteins. These proteins are built up from three main structures, namely the α-helix, the antiparallel β-pleated sheet and the triple helix. Myosin is α helical, whereas collagen uses the triple helix (Figure 2). To stabilise these structures hydrogen bonding is frequent (Dickerson and Geis, 1969).

What happen to these type of protein structures on heating? At increased temperatures the hydrophobic side chains, for entropy reasons, can more favourably stay in the aqueous environment and in the case of the compact globular proteins leads to an expansion and partial unfolding. Furthermore, a free energy gain can be achieved by the association of two partially unfolded proteins, thereby shielding the hydrophobic side chains from the aqueous environment.

If the degree of association of protein entities is too large, leading to less colloidal stability of the system, the *solubility* of the proteins is lowered, and a precipitate is formed. If, however, the three-dimensional association of the proteins occurs in such a way that the attractive and the repulsive forces are so well balanced that a three-dimensional network is formed, a gel has set. This gel binds the water in the former solution, mainly by capillary forces, being solid-like in its mechanical behaviour. The gels can in turn vary from being transparent, containing a network of strands of small cross-sections to opaque gels containing much coarser aggregated structures (Hermansson, 1986). For one of the latest review on thermally-induced gelling of globular proteins see Clark, 1998.

For the fibrous proteins the large amount of hydrogen bonds and electrostatic interactions that keep the stretched molecules in register in the large building blocks, where the fibrous proteins take part, are broken on heating. This results in the molecules having a greater freedom to form any random configuration, as driven by entropy. Since the proteins are relatively stretched in the fibrous form fibrous proteins contract on cooking in contrast to globular proteins which expand.

Meat proteins, composition and structure

The muscle consists of 75 % water, 20 % protein, 3 % fat and 2 % soluble non-protein substances. Out of the latter 2 %, metals and vitamins constitute 3%, non-protein nitrogen 45 %, carbohydrates 34 % and inorganic compounds 18 %. The proteins can be divided into three groups; myofibrillar, sarcoplasmic and stromal proteins. The myofibrillar proteins constitute between 50 to 55 % of the total protein content, while the sarcoplasmic proteins account for approximately 30 to 34 %. The remaining 10 to 15 % of the proteins is the stromal fraction, frequently referred to as the connective tissue proteins.

The myofibrillar proteins are further divided into three subclasses: the myofilamentous fibrous proteins myosin and actin building up the myofibrillar structure, the regulatory proteins including the tropomyosin-troponin complex, α- and β-actinin, M-protein and C-protein and ultimately the scaffold proteins, such as titin, nebulin, desmin, vimentin and synemin, supporting the whole myofibrillar structure. Titin is a massive protein with a molecular weight of around 1 million Dalton.

The sarcoplasmic proteins are the soluble proteins of the sarcoplasm, to which belong most of the enzymes of the glycolytic pathway, creatine kinase and myoglobin. About 100 different proteins are known to be present in the sarcoplasmic fraction and they are globular proteins of relatively low molecular weight ranging from 17000 (Myoglobin) to 92500 (Phophorylase b).

The structures built up by the connective tissue proteins starts with an external covering sheet of connective tissue, the epimysium, around the whole muscle. This layer of connective tissue binds the individual bundles of muscle fibres into place and also binds groups of muscles together. The muscle fiber, which is the muscle cell, can vary in diameter from 10 to 100 μm and have a length of up to 30 cm. The cell has a membrane, called the sarcolemma, and is also surrounded by another type of connective tissue called the endomysium. The fibers are collected into fiber bundles, where the third type of connective tissue (perimysium) envelopes the fiber bundle (Ashgar and Pearson, 1980).

The connective tissue proteins collagen, reticulin and elastin are all fibrous proteins. Collagen, a glycoprotein, is the main structural component of the connective tissues (55-95 % of the dry matter content) and is composed of tropocollagen monomers about 2800Å long and 14-15Å in diameter with a molecular weight of 300,000. These tropocollagen molecules aggregate to form either extended fibers in the epimysium and perimysium or as a structural matrix in the endomysium. Collagen exists in several different genetic forms (I-V), which are present in muscle. Bailey et al., 1979 used immmunoflourescence to show that type I is present in the epimysium, types I and III are present in the perimysium and types III, IV and V collagen are present in the endomysium.

The muscle fibers constitute 75-92% of the total muscle volume and it holds long, thread-like structures, the myofibrils, wherein the sarcomere, the smallest contractile unit, is lined up.

The structural build-up of the sarcomere can be overviewed in Figure 4. The diameter of the myofibrils is about 1 µm and the length of a sarcomere is about 2.2 µm in a resting muscle.

According to Figure 3 the sarcomere is built from two ”building blocks”, that consist of a thick filament, extending over the A-band and a thin filament, extending from the Z-line towards the A-band in the H-zone. The thick filaments are composed of myosin. There are 200-400 molecules of myosin in each thick filament, with each being 1.5µm long and 130 Å in diameter(Knight and Trinick, 1987). By tryptic digestion, the myosin filament can be split into a heavy head, called heavy meromyosin (HMM), and a tail called light meromyosin (LMM). The water-insoluble LMM fraction has a molecular weight of 150,000 and is composed of either a double or triple-stranded α-helical structure. The head region consists of two globular units, each about 70 Å in diameter with 45 % α-helical content (Knight and Trinick, 1987).

The second major myofibrillar protein is actin. The fibrous actin (F-actin) is formed from longitudinal polymerization of globular actin (G-form, M_W 47,000 Da). In solution at low ionic strength actin exists in the monomeric globular form. When the ionic strength is raised, the monomers are polymerized into the fibrous structure, consisting of a double, twisted helix with a diameter of 70 Å (Figure 4, Ashgar and Pearson, 1980).

Behaviour of meat proteins in heating

Conformational changes of the proteins occurring on heating are usually called denaturation. The cooking temperature, where conformation changes occur is commonly called denaturation temperature and has been mostly investigated using differential scanning calorimetry (DSC). The unfolding of the proteins (the loss of helical structure) can also be followed by optical rotary dispersion (ORD) and circular dichroism (CD). Another away to follow the unfolding of the proteins is to measure the surface hydrophobicity of the proteins, using a flourescent probe 8-anilino-1-naphtalene sulfonate (ANS). The next step in the structural changes to occur on heating are the protein-protein interactions, resulting in the aggregation of proteins. These processes are mainly studied by turbidity measurements and loss in protein solubility. The gel forming ability and the type of gels formed by the proteins are usually studied, using some sort of mechanical and micro-structural measurements.

Sarcoplasmic proteins

According to the review by Hamm, 1977 most of the researchers have found that most sarcoplasmic proteins (i.e. those muscle proteins soluble in water or at low ionic strength) coagulate or aggregate between 40 and 60°C. Davey and Gilbert, 1974 found for the bull neck muscle that the heat coagulation of the sarcoplasmic proteins could extend up to 90°C. They were also the first to suggest that the sarcoplasmic proteins might have a role in the consistency of cooked meat in such a way that the heat-induced aggregated sarcoplasmic proteins can form a gel in between the structural meat elements and thereby link them together. In our investigations on tenderness of meat cooked to different temperatures (Tornberg et al., 1997) our measurements suggest that might well be the case. The mechanism by which this will operate will further be elaborated on later in this review.

Of special interest among the sarcoplasmic proteins is the myoglobin, as it is the carrier of the colour of meat. Myoglobin aggregates at about 65°C in meat, whereas in pure solution it denatures at a higher temperature (Draudt, 1969).

Another interesting aspect of the sarcoplasmic proteins is the tenderizing effect these enzymes can have, using low temperature long time heating (heating rate of 0.1°C/min) on beef muscles. Laakkonnen et al., 1970 have shown that collagenase could remain active in the meat at cooking temperatures < 60 °C, whereas at faster heating and reaching a higher end temperature of 70-80°C they were inactivated. They also showed that a heating time of at least six hours was needed to achieve a substantial lowering of the shear force, i.e. a tenderizing effect. Over the same time most of the water losses between 25 and 30 % (w/w) had occurred.

Myofibrillar proteins

Changes in secondary and tertiary structure

With regard to changes in secondary structure on heating of myosin Morita and Yasui, 1991 have for example measured the change in helix content (circular dichroism) and surface hydrophobicity (ANS) of LMM, i.e. the tail portion of the myosin molecule, at pH 6 and 0.6 M KCL. The helix content of LMM began to decrease at about 30°C and attained a minimum at 70°C. Simultaneously, heating to 65°C progressively increased the surface hydrophobicity, whereas at higher temperatures it decreased again. The decrease in hydrophobicity observed at the higher temperatures suggests that part of the hydrophobic residues take part in protein-protein interactions leading to a network formation of aggregates, a gel.

The advantage of the DSC method is that it can be used in complex mixtures and at high concentrations of proteins, which are the situation occurring in meat. A typical curve (Figure 4) from thermal transitions found in a muscle is composed of three major transition zones A, B and C. The first transition displays its maximum between 54 and 58°C and has been attributed to myosin (Martens and Vold, 1976, Wright et al., 1977). The second transition, which occurs between 65 and 67°C, was assigned to collagen (Martens and Vold, 1976, Stabursvik and Martens, 1980) and to sarcoplasmic proteins (Wright et al., 1977). The third transition has been assigned to actin and is found between 80 and 83°C (Wright et al., 1977). For the second transition it has also been shown that both isolated actomyosin and myosin and its sub-units undergo transitions in the same temperature range (Wright and Wilding, 1984). Recently, the thermal denaturation of titin from pork and beef has been investigated , using DSC. Denaturation was characterised by a single DSC peak at 78.4 and 75.6°C for beef and pork titin, respectively (Pospiech et al., 2002).

Ziegler and Acton, 1984 reviewed and summarised conformational changes of natural actomyosin. In the 30-35°C range, native tropomyosin is dissociated from the F-actin backbone, while at 38°C the F-actin superhelix dissociates into single chains. The myosin light chain sub units dissociate from the heavy chains at about 40°C, followed by conformational changes in the head and the hinge regions of the myosin molecules. Dissociation of the actin-myosin complex occurs at 45-50°C and light meromyosin (LMM) undergoes a helix-to-coil transformation at 50-55°C. Above 70°C major conformational changes in globular actin takes place. However, the exact temperatures for these changes is influenced by such factors as ionic strength, pH, heating rate, muscle source and type.

Changes in quaternary structure

Xiong and Brekke, 1990 have studied salt-soluble proteins (SSP, the myofibrillar proteins) from pre- and postrigor chicken on heating at 0.6 M NaCl, pH 6, using ANS, turbidity and rigidity measurements for unfolding(hydrophobicity), protein-protein association and gelation, respectively (Figure 5 a-d). Despite some differences between breast and leg muscles and between pre- and postrigor SSP samples, a general trend was seen in all samples that protein- protein association beginning at 36-40 °C was preceded by protein unfolding at 30-32°C. Gelation at 45-50°C was initiated after interactions of proteins had taken place. They further stated from these investigations that the hydrophobicity was not dependent on the type of muscle (white, breast and red, leg) nor on muscle rigor state, but rather the protein-protein association varied due to these variables, thereby forming different type of gels. According to Figure 5b the optical density of the SSP solution increased drastically above 40°C and after 45°C the increase was less. There are no differences between pre- and post-rigor meat, which could be due to the fact that actomyosin complex dissociate at 40-45°C. However, breast SSP showed a great difference from leg SSP in aggregation behaviour. But when gels are formed the penetration force is the highest for the breast post-rigor SSP (Figure 5c). Comparing the water-holding of these different gels, as visualised in Figure 5d, the best is achieved by breast, pre-rigor.

High penetration force for gels usually reflect highly aggregated junction points in the gel, as Hermansson, 1982 has shown for blood plasma gels, and good water holding gels suggest a fine-stranded gel with a higher amount of junction points per volume unit. This suggests that breast prerigor SSP form more the last type of gel, as revealed by figure 8b,d, having a lower degree of aggregation and better water-holding, whereas the leg SSP forms the former type of gel, having a high degree of aggregation and a higher penetration force.

As shown above the denaturation of myofibrillar proteins in solution usually results in gel formation, because especially myosin is unique in the sense that it form gels at very low concentration of 0.5 % by weight (Hermansson and Langton, 1988). For comparison sarcoplasmic proteins need about 3 % by weight to gel (Hamm and Grabowska, 1978). When purified myosin is heated, the firmness of the gel reaches its maximum at 45°C at pH 5.5 or at 60°C at pH 6 (Sharp and Offer, 1992). If actin is present in the solution a firmer gel is obtained (Yasui et al., 1980). Ionic strength and pH are important factors since they determine if the myosin exists in monomeric form or as filaments. At ionic strengths >0.3 and at neutral pH, the myosin molecules are dispersed as monomers, forming a coarse network with large pores. At lower ionic strength the myosin molecules are assembled in filaments, resembling the natural thick filaments in the muscle. During heating a firmer gel is formed, especially if the filaments are very long. Such a gel consists of a finer and more uniform network, with smaller pores (Sharp and Offer, 1992).

The gel formation of myosin occurs in two steps, in two separate temperature regions on heating. The first part of the reaction occurs between 30-50°C and the second step at temperatures above 50°C.The first step involves aggregation of the globular head of myosin. Sharp and Offer, 1992 have studied heating of purified myosin at different constant temperature for 30 minutes in an electron microscope. They found that after heating at 30°C in 30 minutes the appearance of the myosin molecule had not changed. After heating at 35°C, the presence of native myosin molecules with two heads was still dominating, but other types of new structure had been formed, such as two myosin molecules that had aggregated by dimerisation of their heads. After heating at 40°C there were no native myosin molecules left and the only monomers present had coalesced heads. Heating to 50°C resulted in further aggregation. At this temperature it was hard to distinguish between the individual tails. Heating at 50-60°C lead to the formation of large globular aggregates. No tails where seen after these temperatures. The second stage involved structural changes in the helix-structure of myosin leading to network formation, where hydrophobic groups interact with each other.

Connective tissue proteins

At temperatures between 53 and 63 °C the collagen denaturation occurs according to DSC measurements (Martens et al., 1982), which probably involves first the breakage of hydrogen bonds loosing up the fibrillar structure and then the contraction of the collagen molecule. If unrestrained, collagen fibers shrink to one-quarter of its resting length on heating to temperatures between 60 and 70°C. If the collagen fibres then are not stabilised by heat-resistant intermolecular bonds, it dissolves and forms gelatine on further heating. The presence of heat-stable bonds means that intermolecular linkages are retained at these temperatures and a proportion of the fibre matrix does not dissolve (Light et al., 1985). In young animals the epimysium contains primarily of thermally labile cross-links, the perimysium a mixture of thermally labile and stable cross-links and the endomysium of thermally stable cross-links (Sims and Bailey, 1981). As the animal age increases the thermally labile increasingly converts into thermally stable cross-links (Shimokomaki et al., 1972). Higher levels of heat-stable cross-links lead to the development of greater tension in the connective tissue during cooking (Sims and Bailey, 1981).

Wu et al., 1985 have followed the structural alterations, using scanning electron microscopy, of the epimysium, perimysium and endomysium from Bovine *sternomandi bularis* caused be heating to 60 and 80°C for 1 hour. The epimysium did not show large alterations after cooking, whereas the perimysium and endomysium became granular at 60°C and start to gelatinise at 80°C. There are also differences in solubilisation for the different type of collagens, where type I is more easily solubilised on heating than type III (Burson and Hunt, 1986).

Structure and qulity of meat products on cooking

Different quality aspects of meat peoducts

Important quality aspects of meat can embrace quality factors, such as fat- and water-holding, appearance, and the eating quality of meat products. The concept of the eating quality implies texture/tenderness, juiciness, flavour and aroma. In this review we will confine ourselves to quality aspects such as water-holding and texture of meat products

Besides whole meat, meat products, such as hamburgers and emulsion sausages, will be referred to in this review. The procedure of manufacturing hamburger patties normally includes mincing, blending and forming, after which the patties are either frozen or fried and then frozen. Frankfurters and similar sausages are made by chopping meat, with the addition of water and NaCl, in a bowl chopper into a fine meat homogenate, in which pork fat and is further dispersed and emulsified. Heat treatment of the stuffed sausage batter is performed in a smoke-chamber. The microstructure of the different meat products, such as a transverse cut of whole meat (A), a beef burger (B) and an emulsion sausage (C) can be compared in Figure 6. A hamburger is composed of more or less intact meat fibers and fiber bundles up to 50 - 70 % but randomly distributed compared to the well-defined anisotropic structure of the whole muscle. In the emulsion sausages, however, meat protein network formation constitutes the major part of the structure, as shown by Andersson et al. (2000).

Structural changes of meat products during cooking

Whole meat

The structural events during cooking of the different proteins in their structural environment of the meat have been investigated by among others by Cheng and Parrish, 1976; Jones et al., 1977 and Bendall and Restall, 1983. During heating, the different meat proteins denature as described above and they cause meat structural changes, such as the destruction of cell membranes (Rowe, 1989), transversal and longitudinal shrinkage of meat fibers, the aggregation and gel formation of sarcoplasmic proteins and the shrinkage and the solubilisation of the connective tissue.

Transverse shrinkage to the fibre axis occurs mainly at 40-60°C, which widens the gap already present at rigor between the fibers and their surrounding endomysium. There is, however, a controversy regarding these observations. Davey & Gilbert (1974) for example found no change in the cross-sectional area on cooking of the neck muscle, whereas Bendall & Restall (1983) found that the transverse shrinkage of both fibres and fibre bundles of M. psoas major starts at about 40°C. There is also a disagreement between the results presented in the literature with regard to the temperature, where the longitudinal shrinkage of the fibre starts. Offer, 1984 and Bendall & Restall, 1983 have observed that fibres do not shorten until 60°C, whereas Hostetler & Landman, 1968 have reported that both sarcomere and fibre shortening usually begin at temperatures of 40-50°C. At 60-70°C the connective tissue network and the muscle fibres co-operatively shrink longitudinally, the extent of shrinkage increasing with temperature. This is mainly based on the fact that the intra-muscular collagen (mainly perimysium) shrinks longitudinally at 64°C (Mohr & Bendall, 1969).

We have in one of our investigations (Tornberg et al., 1997) followed the structural changes in the different protein systems of the meat during cooking. Firstly, we separated the water phase from whole meat and studied the aggregation of the sarcoplasmic proteins, recording the change in absorbance in a spectrometer (Figure 7 a). From this figure it can be deduced that the increase in absorbance starts at about 40°C and is more or less terminated at 60°C. This is in accordance with the behaviour of the sarcoplasmic proteins on heating as discussed earlier. Secondly, the shrinkage during cooking of the separated connective tissue per se and fibre shrinkage, both transversally and longitudinally, have been followed under the light microscope during cooking. The micrographs taken continuously during heating were quantified with an image analysing system (Figure 7 b-d).

The transverse shrinkage of the fibre starts at 35-40°C and then increases almost linearly as a function of temperature. The total shrinkage at 80°C can, though, according to Figure 7 b, vary from 3 up to 14 % area. The shrinkage of the connective tissue per se starts at 60°C, where after at around 65°C it contracts more intensively. However, the amount of shrinkage varies substantially from about 7 % area up to 19 % area. The

discrepancy in results could, among other things, be due to large biological variation within a muscle and between different muscles. These observations are similar to those observed by Bendall and Restall, 1983.

Comminuted meat products

Comminution in combination with salt addition and subsequent heating, which are processes used in the making of hamburgers and emulsion sausages, drastically alter the structure of the meat system. On cooking hamburgers the higher occurrence of whole fibers and pieces of fibers causes more shrinkage as compared to emulsion sausages. In the latter product, higher amounts of myofibrillar proteins are extracted, which on heating create a dense protein network, a gel, that holds water efficiently by capillary forces.

The quality of that protein network is influenced by a number of factors interacting in a complicated way as visualised in Figure 8. The amount of myofibrillar proteins which are extracted into the water-phase during comminution and blending is generally considered to be the most important factor for the quality of the meat network. Moreover, the type of gel matrix formed is related to the disperse or aggregated state of the protein prior to gelation. The complex meat system consists of, not only dissolved proteins, but also insoluble components like meat fibers, connective tissue and fat. The amount and state of these components have a large impact on the gelation properties. The properties of the gel are also influenced by the heating process.

Water-holding of meat products on cooking

Whole meat

Considering the structural basis of water-holding in whole meat, the work of Offer *et al*. cannot be overlooked (Offer, 1984, Offer and Knight, 1988 and Offer et al., 1989). It is important to point out that the structural origin of water-holding in whole meat and highly comminuted products is different. In the former it is the shrinkage and swelling of myofibrils that is the crucial factor (Offer & Knight, 1988), whereas the ability of meat proteins to form different types of gel comes more into play in comminuted meat products (Hermansson, 1986).

Water loss from whole raw meat can be obtained by evaporation from the surface and as exudate, when a muscle is cut. This exudate, a solution of sarcoplasmic proteins, is drained from the cut surface of the meat by gravity, if the viscosity of the water is low enough and the capillary forces do not retain it. Offer *et al.* (1989) have confirmed that this drip loss arises predominantly from the longitudinal channels through the meat between the fiber bundles. The main question then arisis, in order to be able to control and understand changes in water-holding, is how water is accumulated and lost in those channels.

Most of the water in the living muscle is held within the myofibrils (≈ 80 %), in the spaces between the thick and thin filaments (Offer *et al.,* 1989). Any large changes in the distribution of water within the meat structure, by necessity, originate from changes in this spacing. Lateral shrinkage of the filament lattice is brought about by a pH-fall closer to the isoelectric point, rigor contraction and myosin denaturation (Offer and Knight, 1988). There will only be changes in the water distribution, if the myofibrils change in volume. The fact that fiber and fiber bundles shrink when their constituent myofibrils shrink, has been shown by Offer and Cousins (1992), thereby giving rise to the two extracellular compartments around fibers and fiber bundles.

Cooking induces structural changes, which decrease the water-holding capacity of the meat. The review by Offer (1984) summarises the structural changes occurring on cooking as follows: When the transverse shrinkage to the fibre axis occurs mainly at 40-60°C this widens the gap already present at rigor between the fibers and their surrounding endomysium. At 60-70°C the connective tissue network and the muscle fibres co-operatively shrink longitudinally, the extent of shrinkage increasing with temperature. This longitudinal shrinkage causes the great water loss that is obtained on cooking. It is then presumed that water is expelled by the pressure exerted by the shrinking connective tissue on the aqueous solution in the extracellular void.

A convenient way of studying water holding in meat is to investigate the multi-exponential decay of the relaxation time, T_2, of water protons in the muscle, using the ^{1}H-pulse-NMR (Nuclear Magnetic Resonance). Two dominating, discernible relaxation processes have mostly been observed, where the major fraction (≈ 80 %) of the muscle water has a T_2 (called T_{23}) between 35-50 ms, while the rest of the water relaxes in the range of 100-150 ms (called T_{22}). The percentage of water relaxing with the shortest relaxation time can be considered as mainly held by the myofibrils, since a very high fraction of the water is occupied by the myofibrils. Tornberg and Larsson (1986) have further shown, by comparing the percentage

of water having a T_2 = 100-150 ms with the percentage of area around the fiber bundles (evaluated by light microscopy), that the latter could be predicted with an 80 % probability using the pulse-NMR method.

Studies of the water distribution on cooking of meat, using ^{1}H-pulse-NMR, have been done by Fjelkner-Modig and Tornberg, 1986; Tornberg and Larsson, 1986 and Borisova and Oreshkin, 1992, Tornberg et al., 1993; Micklander et al. 2002 and Bertram et al., 2004. It was noted both by Fjelkner-Modig & Tornberg, 1986, Tornberg and Larsson, 1986 and Tornberg et al., 1993 that the T_2 for the major fraction of the water (T23) in both beef and pork was lowered on cooking from around 40 ms to around 30 ms. This suggests a more aggregated, dense protein structure in the myofibrils on heating, which is in accordance with the denaturation behaviour of the proteins as discussed earlier.. For the cooked meat samples, the percentage water of T_{22} and extracellular space around fiber bundles, as evaluated by light microscopy, was compared at the different cooking temperatures (Figure 9). The amount of water around fiber bundles increases up to 50°C, in comparison with the raw meat, which seems to be in accordance with the transverse shrinkage of fibers and fiber bundles. Above 50°C this widened gap diminishes, again up to 70°C, probably mainly due to the shrinkage of the connective tissue. The increase in extracellular space from 70 to 90°C is more difficult to comprehend but, according to the light micrographs a swelling of the perimysium seems to occur at these cooking temperatures.

Comminuted meat products

On comminution of meat and salt addition, leading to the extraction of meat proteins, which occurs when producing hamburger patties and/or emulsion sausages more of the structure consists of a protein gel network after heat treatment. This is especially the case for the highly comminuted emulsion sausages(4 % salt on meat basis), which is reflected in the water-holding of this meat product, being superior to both hamburgers (0.4 % salt) and whole meat, reflected in Figure 15. In this figure the cooking loss of the whole meat, the hamburger and the emulsion sausage of beef *M. biceps femoris* can be compared at different cooking temperatures (The heating gradient was 1.5°C/minute up to 60°C, thereafter 0.7°C/ minute was used until the final temperature was reached in the centre of the meat sample). The temperature dependence on cooking loss for the whole meat shown in this Figure, where the highest increase in water loss is achieved in the temperature region of 60 to 80 °C, is similar to other investigators (Davey and Gilbert, 1974; Bouton et al., 1976; Bendall and Restall, 1983; Honikel, 1987). It is further interesting to note that although the hamburgers have been comminuted, the cooking losses are almost as large as for the whole meat. This is probably due to the more prevalent shrinkage of whole fibers and pieces of fibers, causing larger water losses in the hamburgers as compared to emulsion sausages. Because in the latter product, the higher amounts of myofibrillar proteins extracted create a dense protein network that holds more water (in combination with 4 % added potato starch, which is usually added in Swedish emulsion sausages).

In one of our latest study on beefburgers patties was fried on a double-sided pan fryer to a mid-temperature of 72°C, having different pan temperatures (100, 150 and 175°C). Kovascne et al., 2004 found that the water losses, sometimes reaching up to 60 % of the initial water content, never went below 80 % drip loss. This means that the pressure driven water loss, due to meat protein contraction, is a substantially more important mechanism governing the water loss on frying of beefburgers, than the evaporation losses occurring at the surface crust formed at the higher frying temperatures of 150 and 175 °C.

Textural properties of meat products on cooking

Whole meat

Meat texture can be evaluated by both sensory and instrumental methods. Numerous studies have been conducted in the area of meat cookery, but especially attempts to relate sensory evaluated tenderness with mechanical and structural changes in the meat during cooking are scarce (Martens et al., 1982, Josell and Tornberg, 1994 and Tornberg et al., 1997). Textural properties of meat with emphasis on the instrumental methods have been reviewed by amongst others Purslow (1991), Lepetit and Culioli (1994) and Harris and Shorthose (1988). In this review only the cooking temperature dependence of texture properties of meat will be dealt with.

During the mastication of meat, deformation and fracture of the samples takes place. The mechanical forces acting on meat can include shear, compressive and tensile forces and they should be defined in the mechanical test in use. As meat is a composite, it is important to study in which structural elements failure takes place, and where cracks propagate, to be able to understand its mechanical properties. Tensile tests on cooked strips of both beef (Bouton & Harris, 1972; Munro, 1983; Purslow, 1985) and pork (Josell and Tornberg, 1994; Mutungi et al., 1995; Christensen et al., 2000) have been used.

For the assessment of the texture of whole meat, the empirical method of the Warner-Bratzler shear device is the most widely used (Bratzler, 1932). The most commonly used configuration is the one in which the shearing plane is perpendicular to the muscle fibres. Tensile, shear and compression forces operate in this type of test. The W-B-technique is, however, the instrumental technique that usually yields the best correlation with sensory panel scores for meat toughness.

When relating the sensory properties of the meat with the mechanical, structural traits Josell and Tornberg, 1994 and Tornberg et al., 1997 used in the former case *M. longissimus* from pork and in the latter case whole and minced meat from beef (M. *biceps femoris*). From the former investigation the sensory evaluated tenderness of pork loin increases substantially from 50 to 65°C, where after it decreases again to 80°C (Figure 11). In this study also the temperature dependence of longitudinal and transversal fracture stress and strain were recorded, using tensile measurements(Figure 12 a and b). As shown by Purslow (1985) for beef the longitudinal rupture of the fibers require higher stresses than transversal rupture, which was also the case for pork. The increase in tenderness in the temperature interval 50-65°C was observed as decrease in the transversal fracture stress and in the transversal and longitudinal fracture strain (Figure 12 a, b). The elastic modulus of the meat registered both at small and large deformations increased with cooking temperature from 50 up to 80°C (Figure 12 d).

Bouton and Harris, 1981 showed, using W-B shear peak values, that there is a decrease between 50 and 65°C, which they attributed to a weakening of the collagenous connective tissue, due to denaturation. This phenomenon could also be the reason for the fracture stress to diminish from 50 to 65°C in our investigation. However, we are more inclined to suggest another hypothesis to explain the observed phenomena. In the tensile measurements the strain at fracture tells us how much the meat yields on tearing and this yielding would increase between 50 and 65°C, if the weakening of the perimysium connective tissue would be the dominating mechanism in the tensile measurements. However, the opposite is observed, i.e. from 50 to 65°C a decrease in fracture strain from 70 to 30 % is achieved (Figure 12 b). Concomitantly, the elastic modulus of the whole meat system increases (Figure 12 c, d), which means that the meat converts from a viscoelastic to a more or less elastic material.

We suggest that the reason for the toughness observed for the raw meat up to about 50°C is based on the fact that the applied stress during mastication is reduced by viscous flow in the fluid-filled channels in between fibres and fibre bundles. This situation is, however, improved from 50 to 65°C by the formation of a gel of aggregated sarcoplasmic proteins gluing the fibres and fibre bundles together. The viscous flow becomes then lower as the elasticity of the meat increases in that temperature region. That means there is a higher probability of the applied stress being transferred within the material to the crack without any viscous dissipation of energy and there propagating it. The piece of meat is then more easily fractured in the mouth and mastication is facilitated and tenderness improved. Above 65°C elasticity acts adversely and impairs the tenderness. For a fully brittle fracture in a linearly elastic material (which we suppose cooked meat above 65°C behaves like) higher elastic modulus gives rise to larger tensile stresses to extend a crack (Jowitt, 1979), i.e. a tougher meat. We suggests that the contraction of the connective tissue, mainly occurring after 65°C as seen in Figure 10, gives rise to an increase in the elasticity of the meat by forming a much denser material in the temperature region of 65 to 80°C and thereby a tougher meat.

Comparison between whole and minced meat

These findings are further substantiated, when comparing the rheological properties of whole and minced meat (M.*biceps femoris*, beef) in relation to sensory and structural characteristics (Tornberg et al., 1997). The sensory evaluation (Figure 13) showed that, for the whole meat, the toughness decreased drastically from 55 to 60°C, thereafter increased again up to 80°C. For the minced meat, however, the hardness increased over the whole temperature range and was significantly lower than the toughness of the whole meat at cooking

temperatures below 60°C. The rheological properties of the whole meat, cut transversally to the fibre direction, and the minced meat were also recorded, using a low deformation dynamic shear test, and the results can be seen in Figure 14. The storage modulus increases steeply with the temperature from 50 to 65°C both for the whole and the minced meat, where after it levels off to values for the modulus of 80 kPa and 70 kPa for the whole and minced meat, respectively. The phase angle, which is the quotient between the loss and the storage modulus, decreases already from 35-40°C down to a low plateau round 65-70°C. These results tell us that the spacial arrangement of the fibers is of utmost importance for the textural behaviour of the meat. In the whole meat the crack that fractures the meat has to pass over the fluid filled channels at temperatures below 55 °C (for the case of biceps femoris) and therefore the fracture energy will dissipate as viscous flow and is therefore anticipated as tough. For the minced meat, however, the structure is no longer anisotropic and the more or less disintegrated muscle fiber and bundles are now randomly distributed in the batter and the crack then preferentially goes through the extracted myofibrillar mass that holds the fiber together in the batter. The fracture stress to propagate a crack in that material is much less than the stress needed to pass through a meat fibre. As the temperature increases from 45 to 65°C in the beef burger batter the extracted proteins form a gel and after 65°C the contraction of the connective tissue and the substantial loss of water contribute to a denser product. All these structural changes give rise to an increased elasticity of the minced meat (Figure 14), and also an enhanced sensorial perceived toughness (Figure 13).

If this increased toughness of the minced meat on heating was beneficial for the overall impression of it was not investigated in that study. For an emulsion sausage, however, where different textures were produced by using varying recipes, salt concentrations and pH (Andersson et al., 1997), the overall acceptability of the emulsion sausage was mostly governed by the properties of the protein network. This was best characterised by the fracture strain in the tensile test used. When comparing the stress-strain curves obtained for aggregated and non-aggregated sausages, the latter were characterised by having larger fracture strains and they were also rated the highest overall acceptability. Even the elastic modulus (G') was significantly higher for non-aggregated sausage (31.5 kPa) compared to the aggregated (24.9 kPa). Evidently, a dense, elastic meat protein gel is sensorially preferred to a more brittle, grainy protein gel in an emulsion sausage.

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Figures

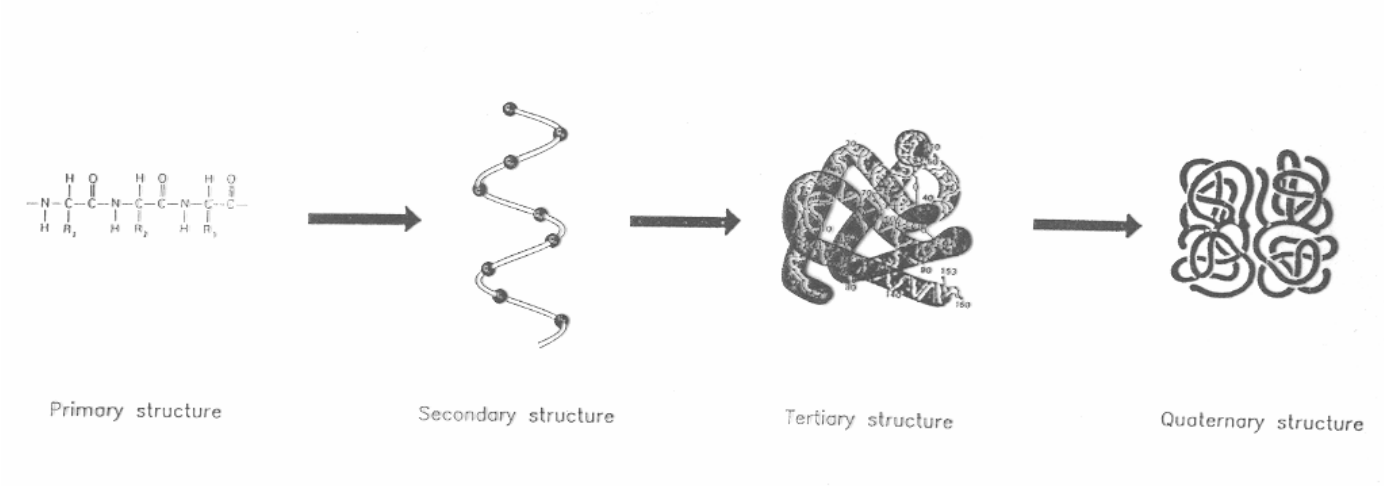
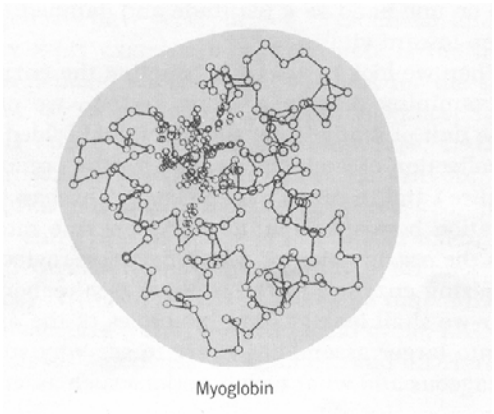


Figure 1: The different type of structure of proteins in solution. From Tornberg et al., 1990.



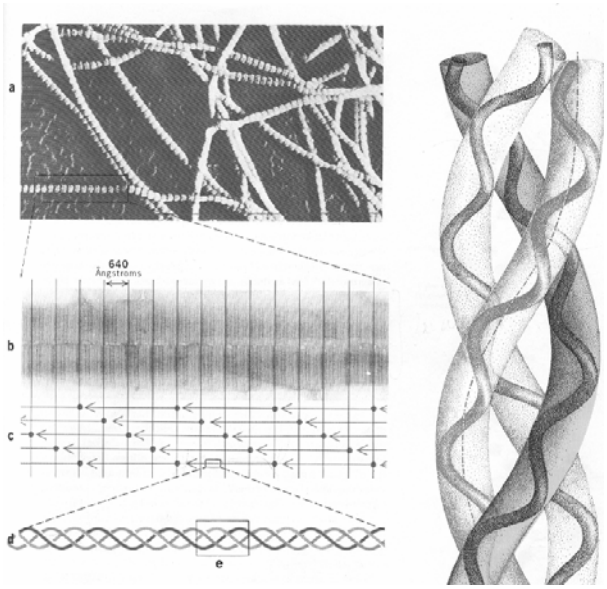


Figure 2: Example of meat proteins being: 1) globular: Myoglobin; 2) fibrous: The build-up of a collagen fibril (a) from the tropocollagen molecule(e). From Dickerson & Geis, 1969.

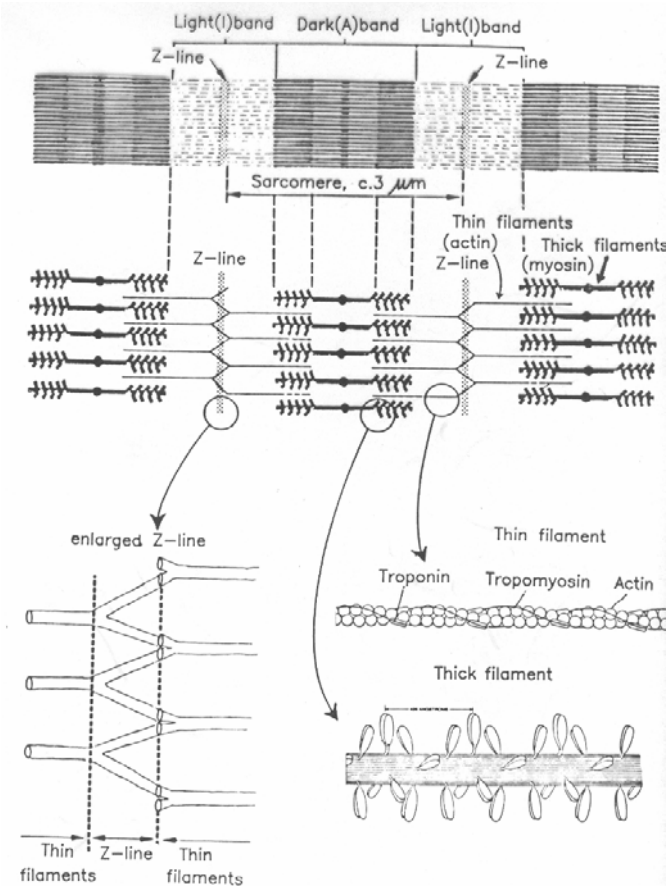


Figure 3: The structural build-up of the sarcomere, the thin and thick filaments. From Tornberg et al., 1990

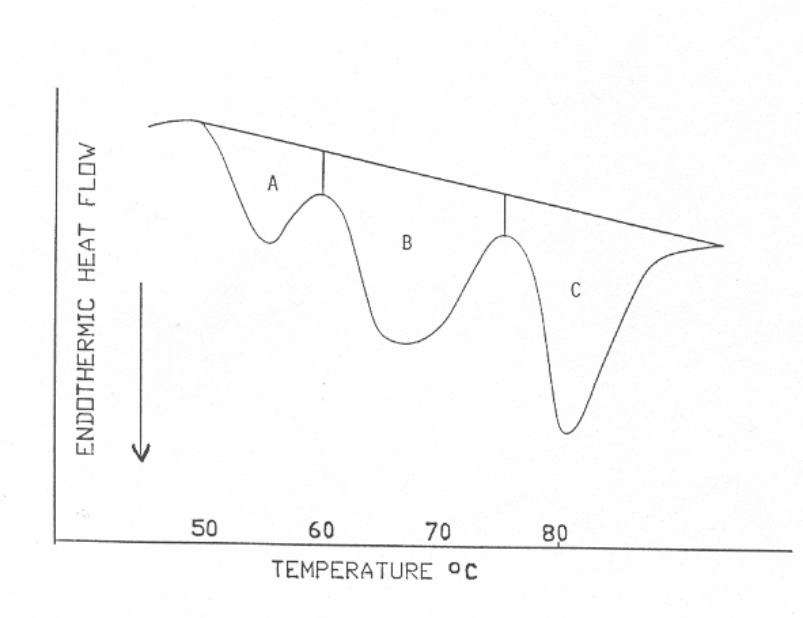


Figure 4: A typical thermal curve of muscle is composed of three major zones: A: Myosin subunits; B: Sarcoplasmic proteins and collagen, and C: Actin. From Findlay et al., 1986.

a) b)

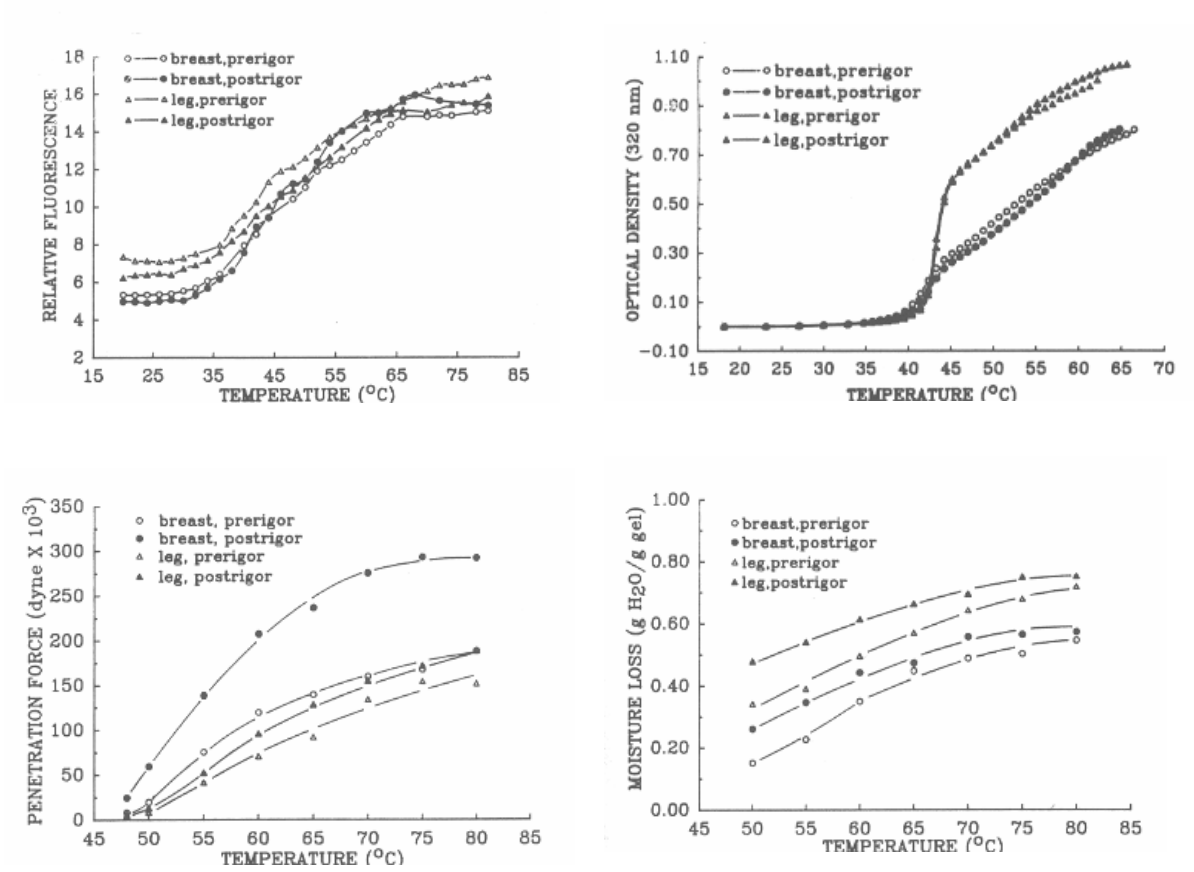


Figure 5: Salt-soluble proteins(SSP) prepared from pre- and postrigor chicken muscles as a function temperature. SSP were suspended in 0.6 M NaCl, pH 6.0. Heating rate = 1°C/min. a) ANS-protein flourescence intensity. b) Protein-protein interactions measured as turbidity. c) Gel strength as penetration force. d) Water loss. From Xiong and Brekke, 1990.

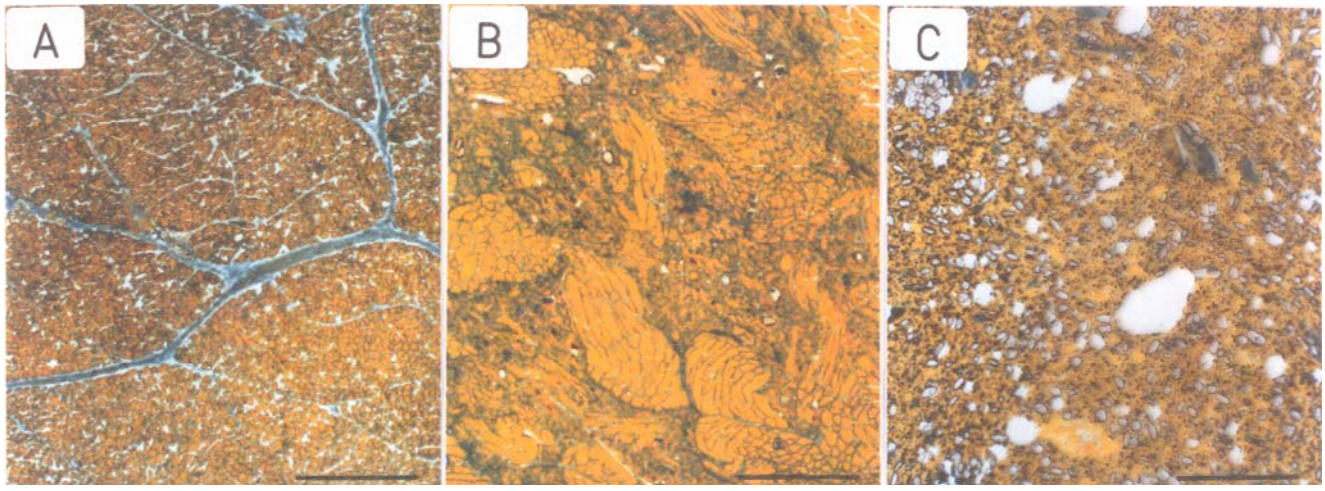


Figure 6: Microstructure of three meat products, using light microscopy. A: A transverse cut of a cutlet. B: Beef burger. C: Emulsion sausage. The bar represents 1 mm.

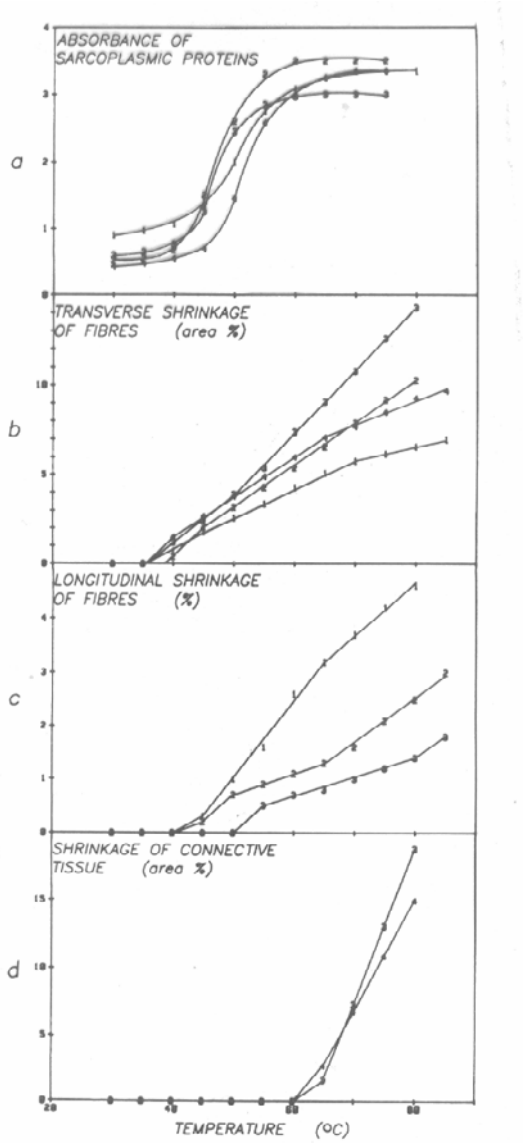


Figure 7: Effect of cooking temperature on the absorbance of the sarcoplasmic fraction (a), the transverse (b) and longitudinal (c) shrinkage of fibres and the shrinkage of connective tissue (d) prepared from four different animals, using beef *M. biceps femoris*. From Tornberg et al., 1997.

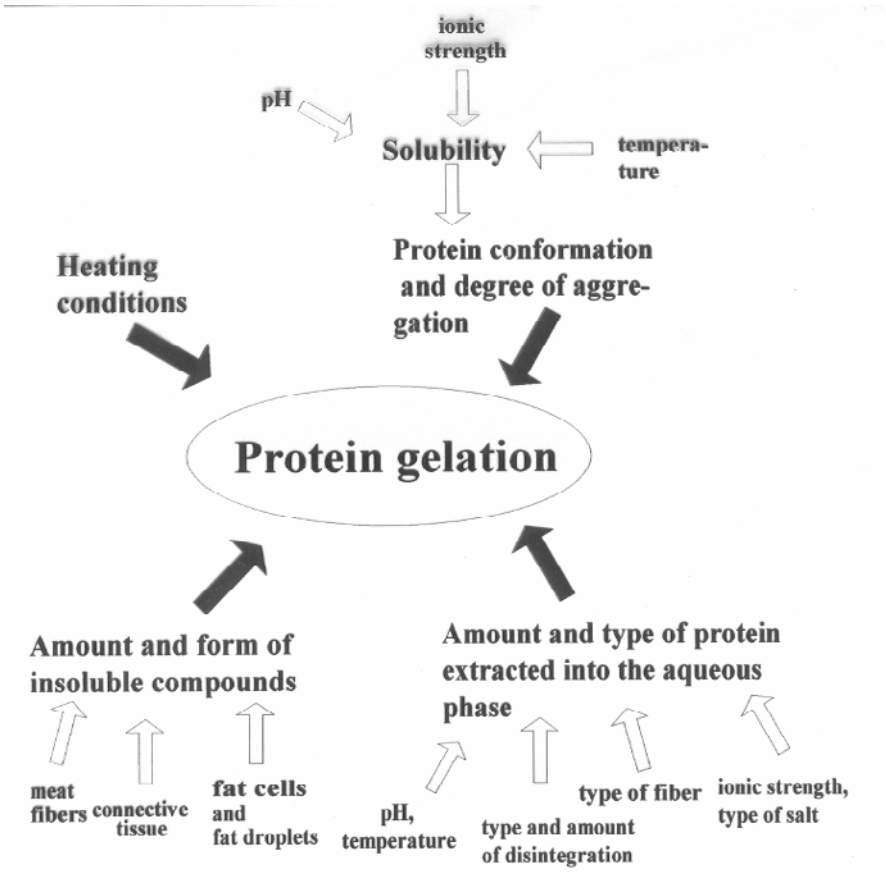


Figure 8: Factors affecting protein gelation, when cooking a meat batter. From Andersson et al., 1997.

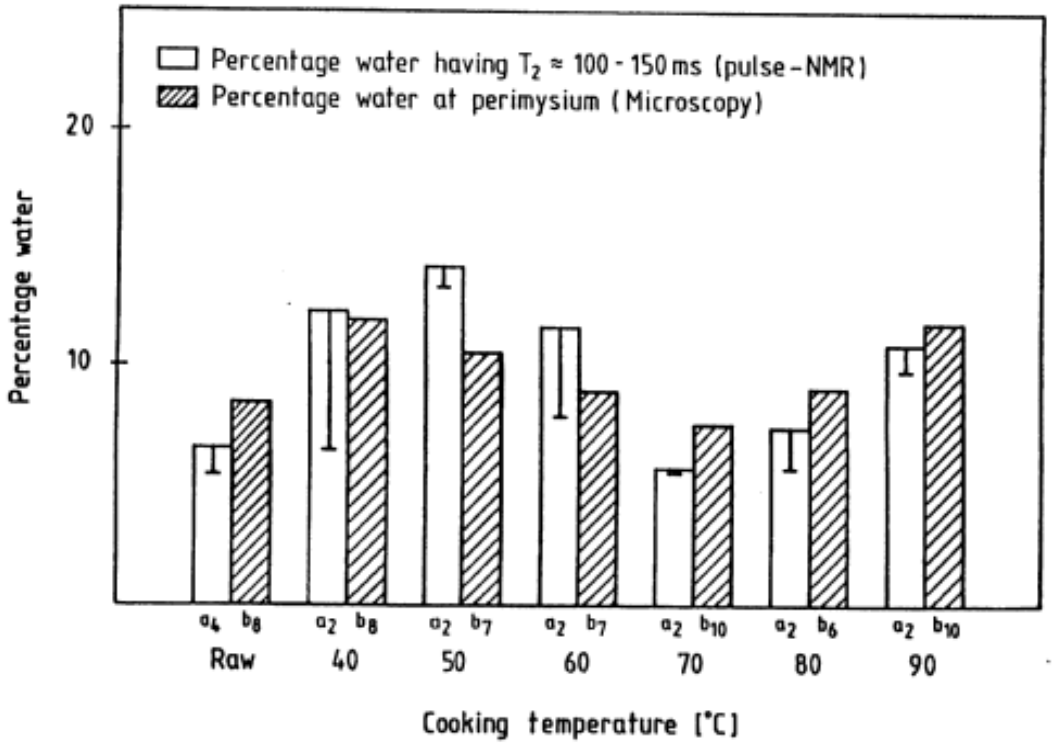
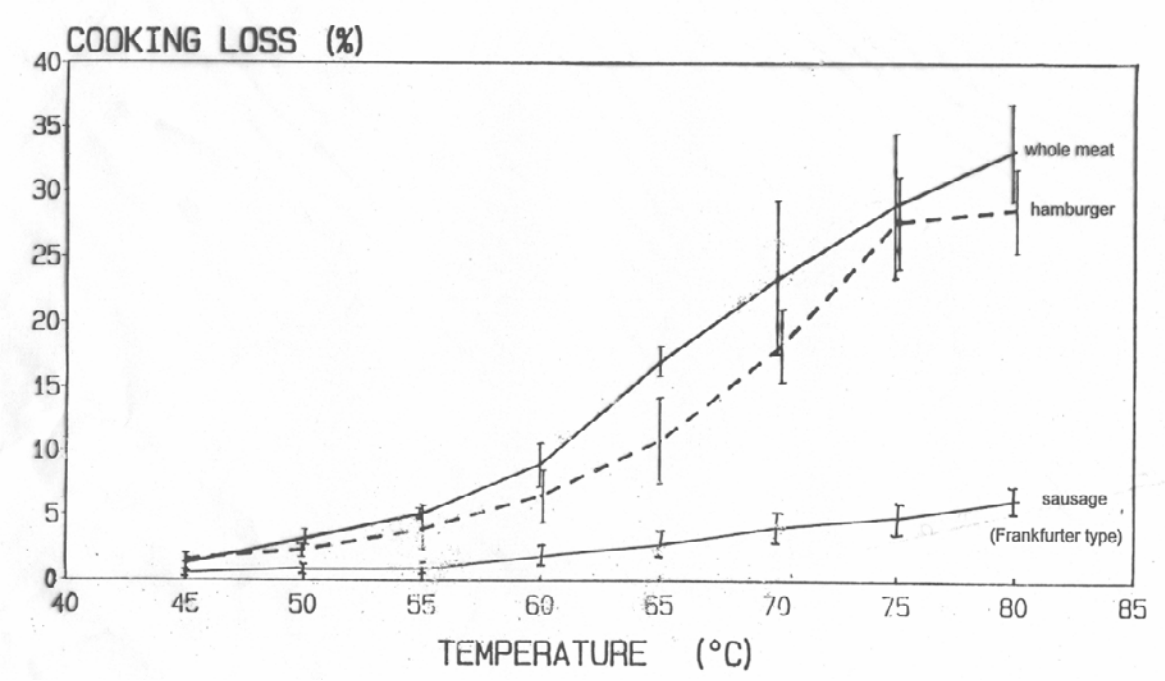


Figure 9: Percentage water having a T_{22} of 100-150 ms, using proton pulse NMR, and percentage water around fiber bundles (at perimysium), as determined with light microscopy, as a function of



cooking temperature (a_n: n = number of samples; b_n: n = number of photos).

Figure10: Cooking losses (%) as a function of cooking temperature for whole meat, hamburger and an emulsion sausage. The meat raw material used in all cases was *M. biceps femoris.*

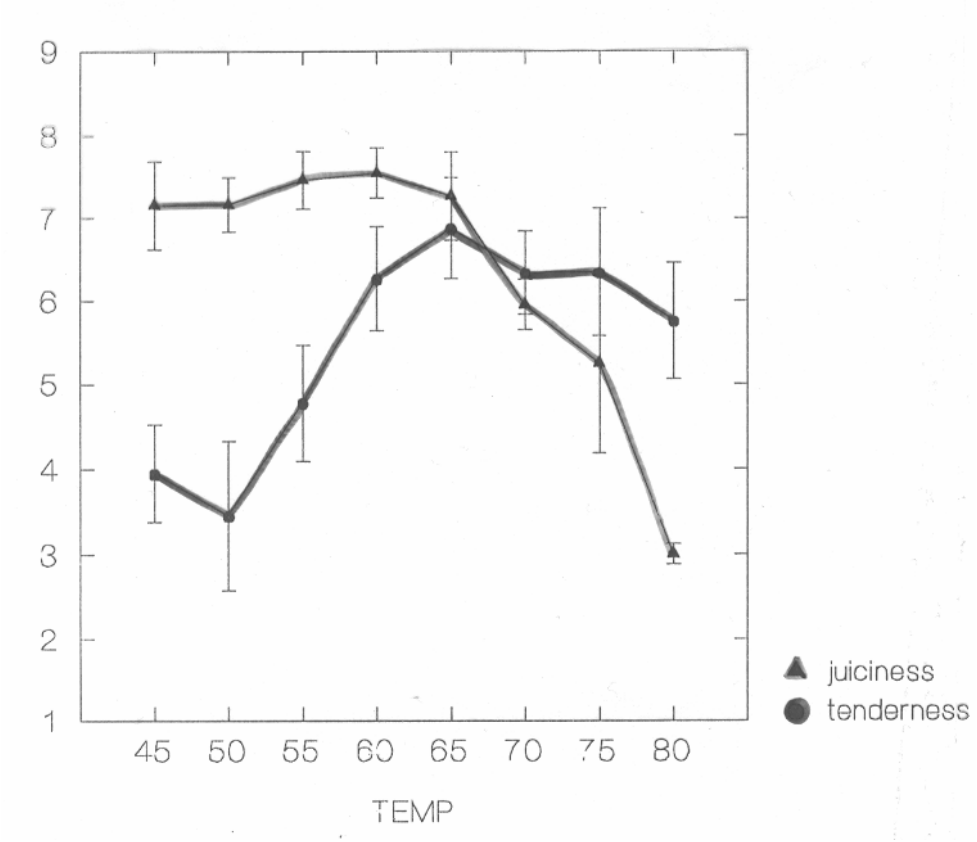


Figure 11: Sensory evaluated tenderness and juiciness for pork *M. longissimus dorsi* versus cooking temperature. From Josell and Tornberg, 1994.

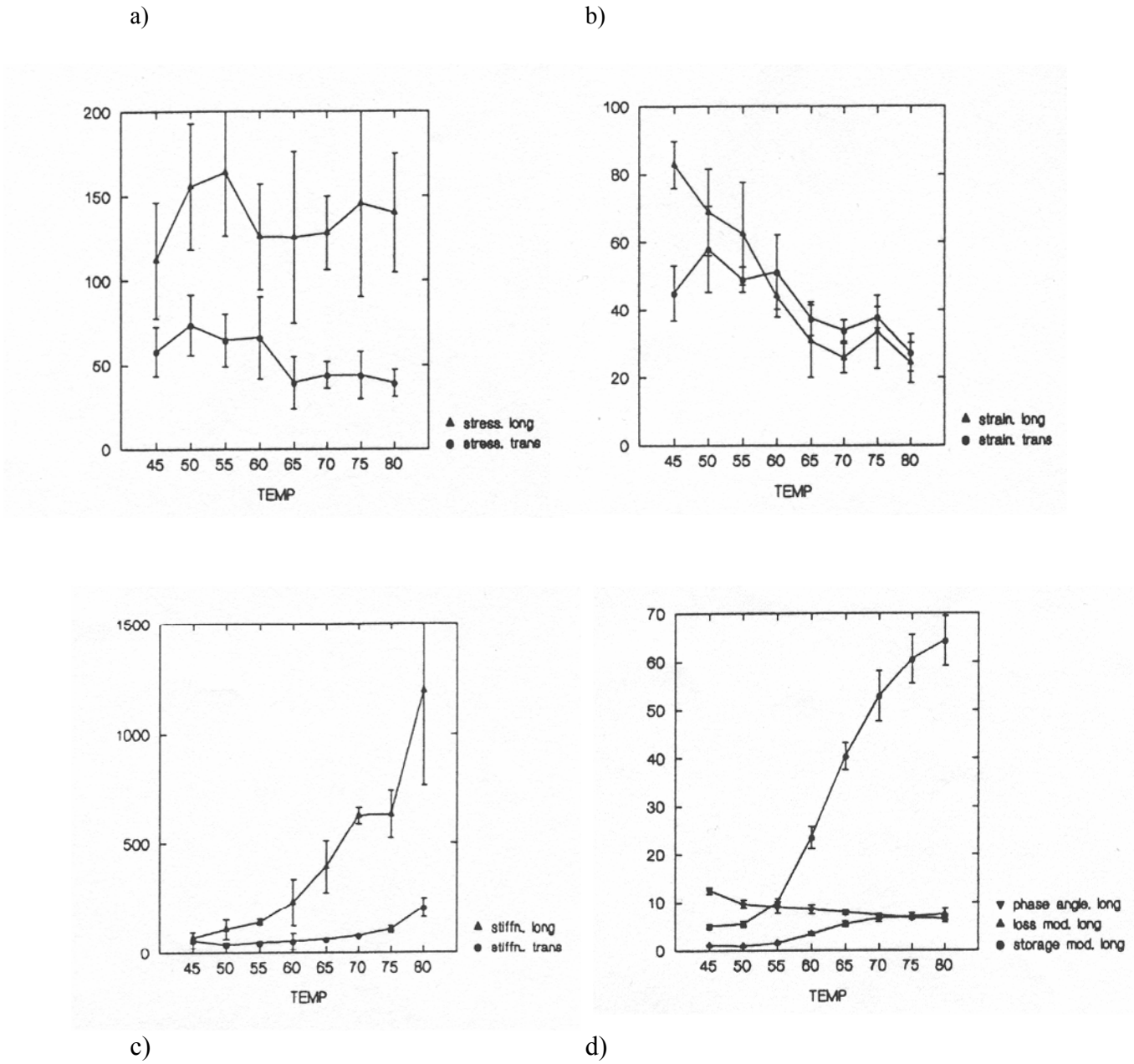


Figure 12: Different mechanical properties for pork *M. longissimus dorsi* versus cooking temperature. a) Longitudinal and transversal fracture stress (kPa). b) Longitudinal and transversal fracture strain (%). c) Longitudinal and transversal initial stiffness (kPa). d) Longitudinal storage and loss moduli (kPa). From Josell and Tornberg, 1994.

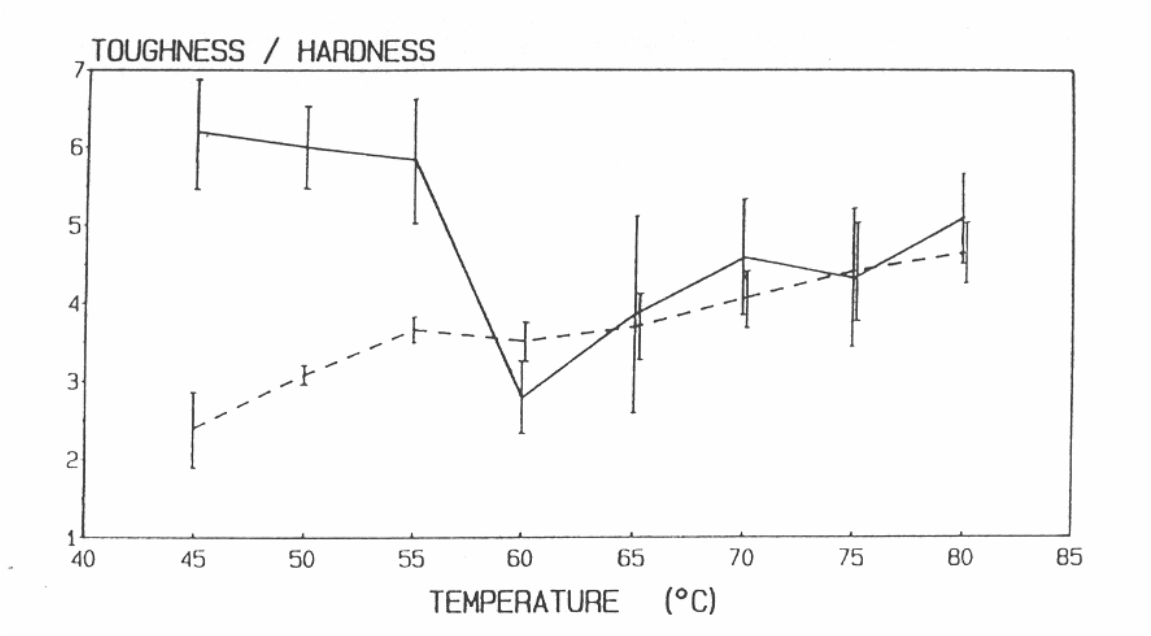


Figure 13: Toughness of whole meat (—) and hardness of minced patties (---), made out of beef *M. biceps femoris,* as a function of cooking temperature. The bars give the standard deviation. From Tornberg et al., 1997.

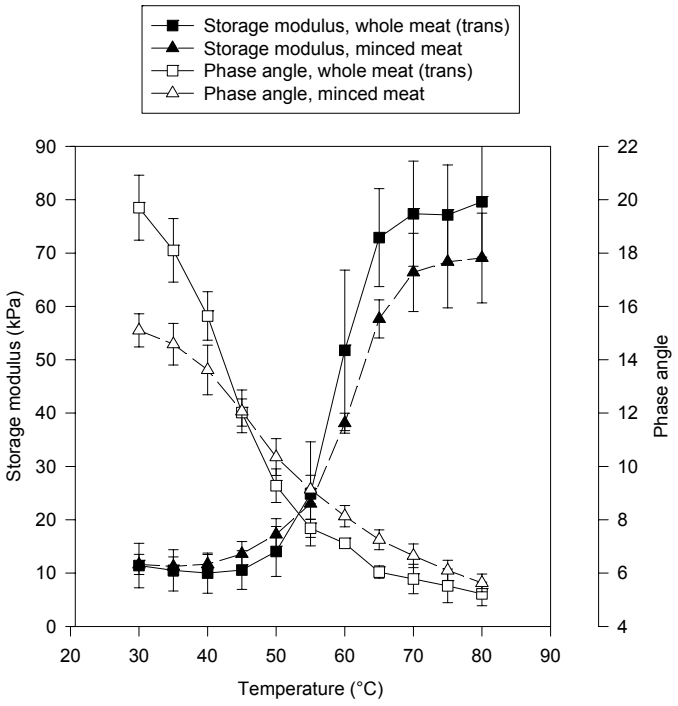


Figure 14: Storage modulus (kPa) and phase angle (degrees) for whole meat (■, □) and for minced meat (σ , Δ), respectively, made out of beef *M. biceps femoris,* as a function of cooking temperature. The bars give standard deviation. From Tornberg et al., 1997.