12 Meat

12.1 Foreword

Much evidence from many civilizations has verified that the meat of wild and domesticated animals has played a significant role in human nutrition since ancient times. In addition to the skeletal muscle of warm-blooded animals, which in a strict sense is "meat", other parts are also used: fat tissue, some internal organs and blood. Definitions of the term "meat" can vary greatly, corresponding to the intended purpose. From the aspect of food legislation for instance, the term meat includes all the parts of warm-blooded animals, in fresh or processed form, which are suitable for human consumption. In the colloquial language the term meat means skeletal muscle tissue containing more-or-less adhering fat. Some data concerning meat production and consumption are compiled in Tables 12.1–12.3.

Table 12.1. World meat production in 2006 (1000 t)[a]

Continent	*(Beef/Veal)* Cattle meat	Buffalo	*(Mutton/Lamb)* Sheep meat	Goat
World	61,033	3183	8633	4945
Africa	4565	270	1167	932
America, Central-	1992	—	49	43
America, North-	13,301	—	101	21
America, South- and Caribbean	15,672	—	293	138
Asia	13,664	2911	4653	3706
Europe	11,033	1	1294	128
Oceania	2797	—	1126	20

Continent	Pork	Horse	Chicken meat	Duck meat
World	105,604	772	73,057	3846
Africa	838	11	3472	57
America, Central-	1225	84	3103	20
America, North-	11,448	44	16,941	93
America, South- and Caribbean	6011	191	16,567	37
Asia	62,013	333	24,226	3226
Europe	24,767	169	10,908	423
Oceania	526	22	944	10

Continent	Meat, grand total
World	272,884
Africa	12,528
America, Central-	6542
America, North-	45,574
America, South- and Caribbean	39,628
Asia	118,103
Europe	51,204
Oceania	5846

Table 12.1. Continuation 1

Country	Beef/Veal	Country	Buffalo	Country	*(Mutton/Lamb)* Sheep meat
USA	11,910	India	1488	China	2540
Brazil	7774	Pakistan	571	Australia	626
China	7173	China	351	New Zealand	500
Argentina	2980	Egypt	270	Iran, Islamic Rep of	389
Australia	2077	Nepal	142	United Kingdom	331
Russian Fed.	1755	Philippines	70	Turkey	272
Mexico	1602	Thailand	63	India	239
France	1473	Indonesia	40	Spain	227
Canada	1391	Myanmar	24	Syrian Arab Republic	200
India	1334	Iran	14	Algeria	185
Germany	1167	∑(%)[b]	95	Pakistan	172
Italy	1109			Sudan	148
South Africa	804			Russian Federation	136
Colombia	792			South Africa	117
UK	762			Morocco	112
New Zealand	700			Kazakhstan	106
Spain	671			Nigeria	103
Ukraine	592			France	99
∑ (%)[b]	75			∑ (%)[b]	75

Country	Goat	Country	Pork (swine)	Country	Horse
China	2161	China	52,927	China	200
India	475	USA	9550	Mexico	79
Pakistan	392	Germany	4500	Russian Fed.	56
Sudan	186	Spain	3230	Kazakhstan	56
Nigeria	147	Brazil	3140	Argentina	56
Bangladesh	137	Vietnam	2446	Italy	41
Iran	105	Poland	2092	Mongolia	41
Greece	57	France	2011	USA	26
Indonesia	53	Canada	1898	Australia	21
Mali	51	Denmark	1749	Brazil	21
∑ (%)[b]	75	∑ (%)[b]	75	∑ (%)[b]	75

[a] Slaughtering in each region is independent of the origin of the animals.
[b] World production = 100%

12.2 Structure of Muscle Tissue

12.2.1 Skeletal Muscle

Skeletal muscle (Fig. 12.1) consists of long, thin, parallel cells arranged into fiber bundles. Each of these muscle fibers exists as a separate entity surrounded by connective tissue, the endomysium. Numbers of these primary muscle fibers are held together in a bundle which is surrounded by a larger sheet of thin connective tissue, the perimysium. Many such primary bundles are then held together and wrapped by an outer, large, thick layer of connective tissue called the epimysium. Figure 12.2 shows a cross-section of rabbit *Psoas major* muscle in which the endomysium and perimysium are readily recognized.

Table 12.1. Continuation 2

Country	Chicken meat	Country	Duck meat	Country	Meat, grand total
USA	15,945	China	2673	China	81,733
China	10,701	France	233	USA	41,081
Brazil	8507	Malaysia	105	Brazil	19,783
Mexico	2411	Viet Nam	86	Germany	6868
India	2000	USA	86	India	6103
Russian Fed.	1534	Thailand	85	Mexico	5331
Japan	1337	Korea, Rep.	67	Spain	5293
Indonesia	1333	India	65	France	5206
UK	1331	Myanmar	60	Russian Fed.	5153
Argentina	1156	UK	42	Argentina	4537
Iran	1153			Canada	4493
Thailand	1100	∑(%)[b]	91	Australia	3941
Spain	1048			Italy	3915
Canada	997			Poland	3490
South Africa	971			UK	3389
Poland	960			Viet Nam	3151
Turkey	937				
Malaysia	914			∑ (%)[b]	75
France	819				
∑ (%)[b]	75				

[a] Data refer to slaughtered animals irrespective of the possibility of being imported as live animals.
[b] World production = 100%

Table 12.2. Annual meat consumption in FR Germany (kg/person)

Year	Beef/Veal	Pork	Poultry	Total
1960	18.7	29.3	4.2	59.0
1964/65	19.2	33.9	6.0	66.5
1970	22.9	36.1	7.4	72.0
1972/73	20.5	42.0	9.0	79.0
1974/75	21.0	44.6	8.8	82.5
1976/77	21.6	45.5	9.1	84.9
1993	19.7	56.1	12.4	95.2
1995	16.6	54.9	13.4	92.0
1997	14.5	53.8	14.7	89.9
2003	12.8	55.1	18.2	90.7

Table 12.3. Meat consumption in selected countries (kg/person/year)

Country/ region	Year	Beef/ Veal	Pork	Poultry	Total
EEC (European Economic Community)	1960	19.9	19.2	5.2	52.2
	1970	25.2	23.7	8.9	65.7
EU-15	1995	20.1	41.0	20.0	93.2
	1997	19.0	40.8	20.7	92.3
	2003	20.0	43.4	23.4	96.6
France	1960	29.2	19.8	8.6	74.9
	1970	35.9	24.8	11.3	89.3
	1995	28.1	35.9	22.6	107.9
	1997	26.7	35.4	23.9	106.5
	2003	27.1	36.4	24.5	109.6
Italy	1960	12.9	7.2	3.6	30.0
	1970	18.9	9.1	9.2	43.5
	1995	25.9	33.1	18.4	88.7
	1997	24.2	34.4	18.6	88.2
	2003	25.1	39.4	18.0	91.5
UK	1995	17.5	23.1	25.8	133.2
	1997	16.6	23.3	26.6	128.1
	2003	20.0	22.1	28.5	83.0
USA	2004	30.0	22.3	15.3	100.4

The membrane surrounding each individual muscle fiber is called the sarcolemma (thickness ca. 75 nm). It consists of three layers: the endomysium, a middle amorphous layer and an inner plasma membrane. Muscle fibers are polynuclear cells. The nuclei are surrounded by the sarcoplasm and by other cellular elements (mitochondria, sarcoplasmic reticulum, lyso-

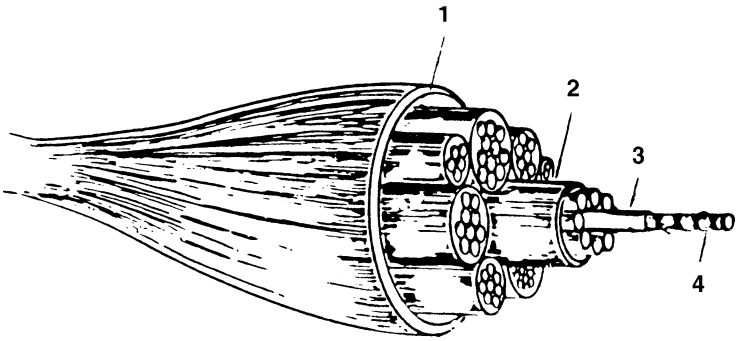


Fig. 12.1. The structural element of skeletal muscle (according to *Gault*, 1992). *1* epimysium, *2* perimysium, *3* endomysium, *4* muscle fiber

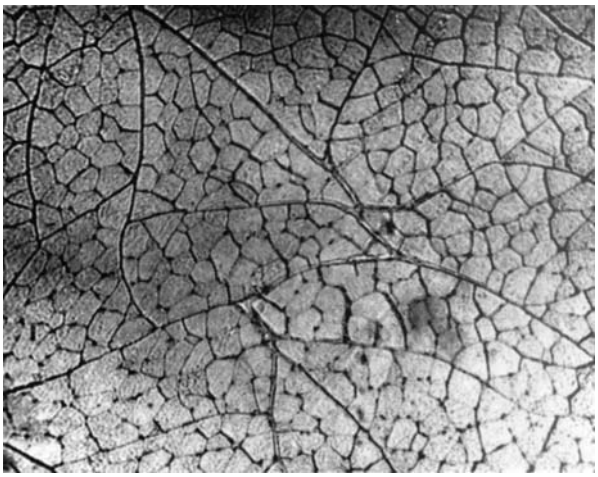


Fig. 12.2. A cross-section of *M. psoas* rabbit muscle. (From *Schultz, Anglemier*, 1964)

somes). Under aerobic conditions, the bulk of the cellular energy is produced in the form of ATP in the mitochondria. The lysosomes are the source of the endopeptidases which participate in the aging of meat (cf. 12.4.3). The muscle fibers or muscle cells have a diameter of 0.01 to 0.1 mm and attain a length of 150 mm and more.

The main components of muscle cells are the myofibrils, each of which has a diameter of 1–2 μm. Up to 1000 myofibrils arranged parallel to each other stretch through the muscle cell in the direction of the fiber.

White muscle (birds, poultry), which has a high ratio of myofibrils to sarcoplasm, contracts rapidly but tires quickly. It can be distinguished from red muscle, which is poor in myofibrils but rich in sarcoplasm. Red muscles are used for slow, long-lasting contractions and do not tire quickly. Figure 12.3 shows a cross-section of a muscle fiber with numerous myofibrils. A greatly magnified, oblique view of a fiber of this type is presented in Fig. 12.4 and Fig. 12.5 shows separated myofibrils.

The organization of the muscle contractile apparatus is revealed in a longitudinal section of the muscle fiber. The characteristic crossbondings ("striations"; Fig. 12.6) of skeletal muscle are due to the regularly overlapping anisotropic A bands, which double refract polarized light, and the isotropic I bands. The dark bands, the Z line, are in the middle of the light I bands and perpendicular to the axis of the fiber. The dark A bands are crossed in the middle with light H bands, while the dark M line is situated in the middle of the H bands (Fig. 12.7). A single contractile unit of a myofibril, called the sarcomere, stretches from one Z line to the next and consists of thick and thin filaments. Figure 12.8a shows

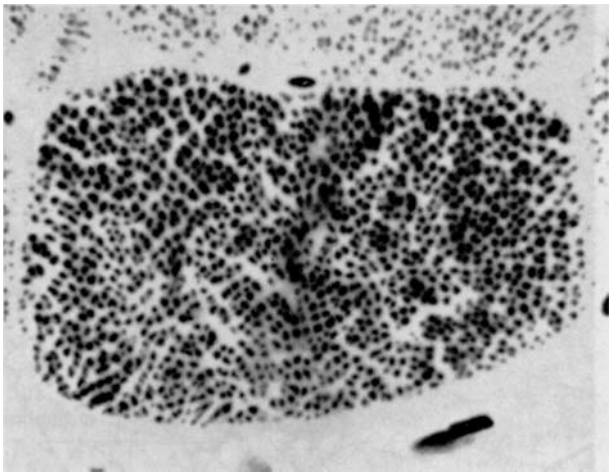


Fig. 12.3. A cross-section of a muscle fiber. (from *Schultz, Anglemier*, 1964)

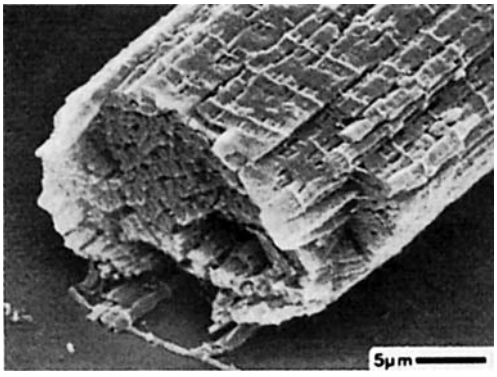


Fig. 12.4. An oblique view of a fractured muscle fiber; scanning electron microscopy at −180 °C (*Sargent*, 1988)

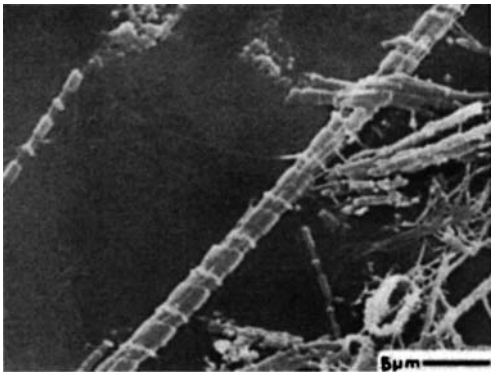


Fig. 12.5. Separated myofibrils; scanning electron microscopy at −180 °C (*Sargent*, 1988)

a schematic representation of the structure of a sarcomere derived from Fig. 12.6 and 12.7. The thick filaments are formed from the protein myosin. They stretch through the entire A band and are fixed in a hexagonal arrangement by the bulge at the center (M line) (Fig. 12.8 a, IV). Thin filaments consist mainly of actin. They original from the Z line, pass across the I band and between the thick filaments, and penetrate the A bands (Figs. 12.7 and 12.8). During muscle contraction, the mechanism of which is explained in section 12.3.2.1.5, the thick filaments penetrate into the H zones and the Z lines move closer to each other. Thus, the width of the I band gradually decreases and finally disappears. Figure 12.8b schematically presents these changes which take place during muscle contraction.

12.2.2 Heart Muscle

The structure of heart muscle is similar to striated skeletal muscle but has significantly more mitochondria and sarcoplasm.

12.2.3 Smooth Muscle

The smooth muscle cells are distinguished by their centrally located cell nuclei and optically uniform myofibrils which do not have crossstri-

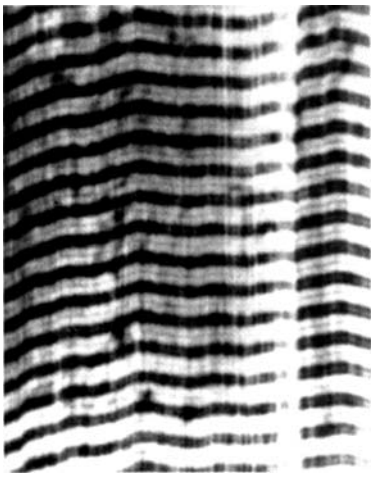


Fig. 12.6. A longitudinal section of two adjacent muscle fibers. (from *Schultz* and *Anglemier*, 1964)

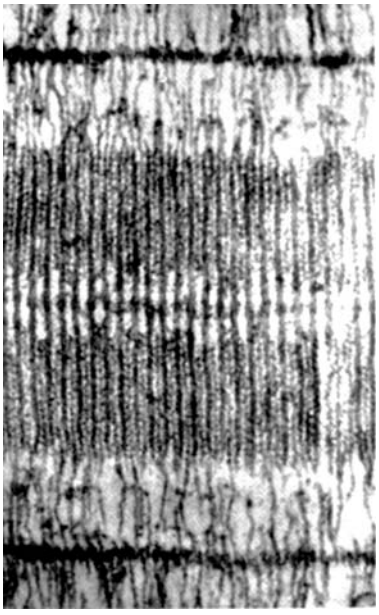


Fig. 12.7. A longitudinal section of a sarcomere. (from *Schultz, Anglemier*, 1964)

ations. Smooth muscles occur in mucous linings, the spleen, lymphatic glands, epidermis and intestinal tract. Smooth muscle fibers are useful in the examination of meat products; preferentially for the detection of pharynx (esophagus), stomach or calf pluck (heart, liver and lungs).

12.3 Muscle Tissue: Composition and Function

12.3.1 Overview

Muscles freed from adhering fat contain on the average 76% moisture, 21.5% N-substances, 1.5% lipids and 1% minerals. In addition, variable amounts of carbohydrates (0.05–0.2%) are present. Table 12.4 provides data on the average composition of some cuts of beef, pork and chicken.

12.3.2 Proteins

Muscle proteins can be divided into three large groups (cf. Table 12.5):

- Proteins of the contractile apparatus, extractable with concentrated salt solutions (actomyosin, together with tropomyosin and troponin).
- Proteins soluble in water or dilute salt solutions (myoglobin and enzymes).
- Insoluble proteins (connective tissue and membrane proteins).

12.3.2.1 Proteins of the Contractile Apparatus and Their Functions

About 20 different myofibrillar proteins are known. Myosin, actin and titin quantitatively predominate, acounting for 65–70% of the total protein. The remaining proteins are the tropomyosins and troponins, which are important for contraction, and various cytoskeletal proteins, which are involved in the stabilization of the sarcomere.

12.3.2.1.1 Myosin

Myosin molecules form the thick filaments and make up about 50% of the total proteins present in the contractile apparatus. Myosin can be isolated from muscle tissue with a high ionic strength buffer, for example, 0.3 mol/l KC1/0.15 mol/l phosphate buffer, pH 6.5. The molecular weight of myosin is approx. 500 kdal. Myosin consists

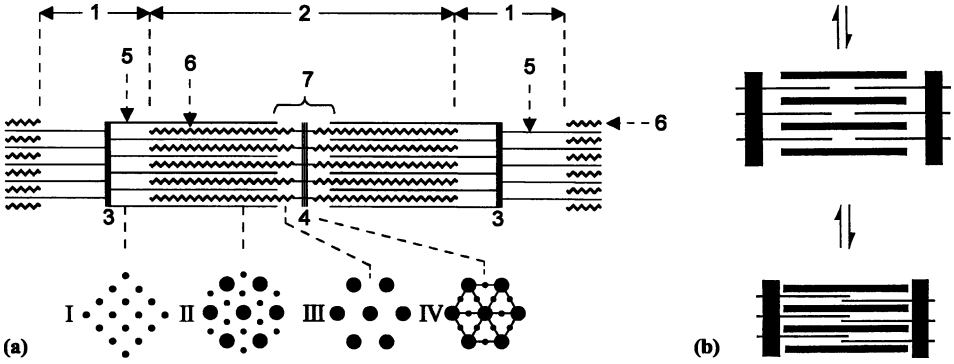


Fig. 12.8. Schematic representation of a sarcomere in the relaxed (**a**) and contracted (**b**) state (according to *Gault*, 1992). *1* I band, *2* A band, *3* Z line, *4* M line, *5* thin filament, *6* thick filament, *7* H zone. Cross section: I thin filaments near the Z line, II overlapping thick and thin filaments, III thick filaments, IV M line

Table 12.4. Average composition of meat (%)

Meat	Cut	Moisture	Protein	Fat	Ash
Pork	Boston butt (*M. subscapularis*)	74.9	19.5	4.7	1.1
	Loin (*M. psoas maior*)	75.3	21.1	2.4	1.2
	Cutlets, chops[a]	54.5	15.2	29.4	0.8
	Ham	75	20.2	3.6	1.1
	Side cuts	60.3	17.8	21.1	0.85
Beef	Shank	76.4	21.8	0.7	1.2
	Sirloin steak[a]	74.6	22.0	2.2	1.2
Chicken[b]	Hind leg (thigh + drum stick)	73.3	20.0	5.5	1.2
	Breast	74.4	23.3	1.2	1.1

[a] With adhering adipose tissue.
[b] Without skin.

of two large (M_r ca. 200,000) and four small (M_r ca. 20,000) subunits and is a very long molecule (measurements 140×2 nm). The two large subunits form a long, double-stranded α-helical rod with a double head of globular protein, both heads being joined at the same end of the coil (head dimensions, 5×20 nm) (Fig. 12.9a). The myosin ATPase activity is localized in the heads and is required for the interaction of the heads with actin, the protein constituent of the thin filaments. Myosin is cleaved by trypsin into two fragments: light (LMM, M_r 150,000) and heavy meromyosin (HMM, M_r 340,000). The HMM fraction contains the globularheaded region and has the ATPase activity and the ability to react with actin. Further proteolysis of HMM yields two subfragments S1 and S2, which correspond to the actual head and neck. The four smaller subunits mentioned above are found in the head region.

Up to 400 myosin molecules are arranged in the thick filaments ($l \sim 1500$ nm, $d \sim 12$ nm). By bringing the tails together, a major cord is formed and on its surface the heads are spirally located. The distance between two adjacent heads on such a spiral is 14.3 nm, and that between the two repeating heads in the same row or line is 42.9 nm. Their association is reversible under certain conditions. Myosin is stabilized by titin during muscle contraction (cf. Fig. 12.9b).

12.3.2.1.2 Titin

Apart from actin and myosin, titin is the third filament in the sarcomere (Fig. 12.9b). It connects the myosin filaments with the Z line and forms an "elastic" region with actin. Therefore, titin is the "backbone" of the sarcomere. As a result of its size ($M_r = 3 \times 10^6$), it moves very slowly

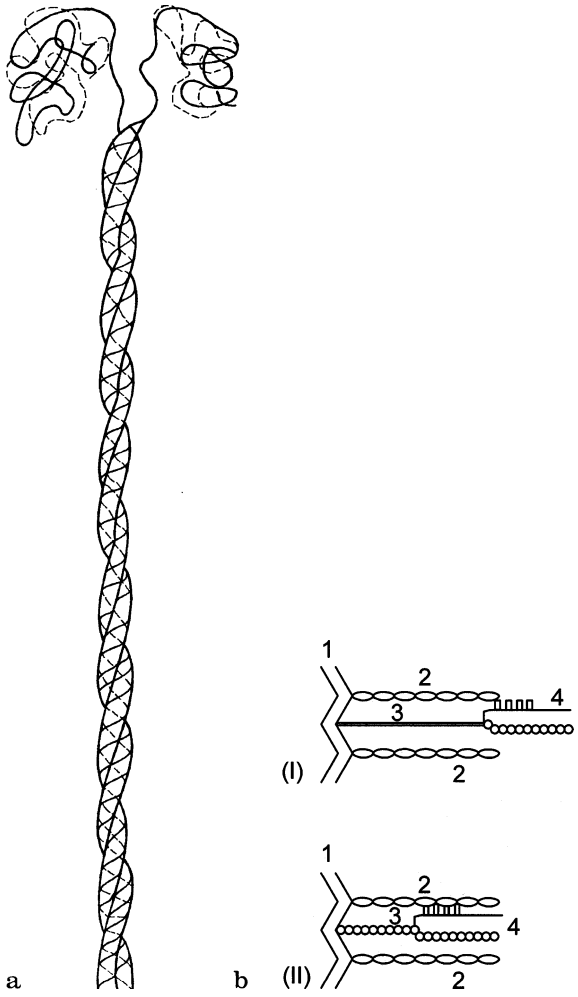


Fig. 12.9. Schematic representations of (**a**) a myosin molecule (according to *Lehninger*, 1975), (**b**) arrangement of the Z line *(1)*, actin *(2)*, titin *(3)* and myosin *(4)* in the sarcomere; **(I)** stretched muscle, **(II)** contracted muscle

in an electric field and, consequently, was not noticed for a long time in electrophoretic separations of muscle proteins. Titin is the largest known protein until now. Its sequence consists of 26,926 amino acid residues. In fact, 90% of the molecule consists of globular domains, a large part of which bind to other proteins, especially to myosin.

12.3.2.1.3 Actin

Actin is the main constituent of the thin filament. It makes up ca. 22% of the total protein of the contractile apparatus. It is substantially less soluble than myosin, probably because it is fixed to substances in the Z line. Actin can be isolated, for example, by extraction of pulverized, acetone-dried muscle tissue with an aqueous ATP solution.

The monomer G-actin (globular actin) consists of 375 amino acids, has a molecular weight of 42,000 and is able to bind ATP and a doubly charged cation. G-actin exists only at low ionic strengths. The addition of singly and doubly charged cations starts the polymerization to F-actin (fibrillar actin) with the cleavage of ATP to ADP, which remains in the bound state.

F-actin in the thin filaments ($1 \sim 1000$ nm, $d \sim 8$ nm) is in the form of a double-stranded helix in which the G-actin beads are stabilized by two

Table 12.5. Muscle proteins

Proteins	Percentage[a]	
Myofibrillar proteins	60.5	
Myosin (H-, L-meromyosin, various associated components)		29
Actin		13
Titin		3.7
Tropomyosins		3.2
Troponins C, I, T		3.2
α, β-, γ-Actinins		2.6
Myomesin, N-line proteins etc.		3.7
Desmin etc.		2.1
Sarcoplasma proteins	29	
Glyceraldehydephosphate dehydrogenase		6.5
Aldolase		3.3
Creatine kinase		2.7
Other glycolytic enzymes		12.0
Myoglobin		1.1
Hemoglobin, other extracellular proteins		3.3
Connective tissue proteins		
Proteins from organelles	10.5	
Collagen		5.2
Elastin		0.3
Mitochondrial proteins (including cytochrome c and insoluble enzymes)		5.0

[a] Average percentage of the total protein of a typical mammalian muscle after rigor mortis and before other post mortem changes.

tropomyosin fibrils (cf. 12.3.2.1.3), as shown in Fig. 12.10. Altogether, it is a four-stranded filament. Six F-actin strands surround a thick filament; consequently, each F-actin strand adheres to the heads of three thick filaments (Fig. 12.8a, II).

12.3.2.1.4 Tropomyosin and Troponin

Tropomyosin (ca. 5% of the contractile proteins) is a highly elongated molecule (2×45 nm) with a molecular weight of about 68,000, and is assumed to be a double-stranded α-helix. Although each chain contains the same number of amino acids, their sequences differ in 39 positions. Tropomyosin contains no di-sulfide bridges and no proline. Indeed, 100% of tropomyosin is an α-helix. The monomer readily forms polymeric fibrils which are bound to F-actin on the thin filament.

Troponin (ca. 5% of the contractile proteins) sits on the actin filaments (cf. Fig. 12.10) and controls the contact between the filaments of myosin and actin during muscle contraction by means of a $Ca^{2\oplus}$ concentration-dependent change in conformation (cf. 12.3.2.1.5). It is a complex of three components, T, I, and C. Troponin T consists of a peptide chain with 259 amino acid residues and binds to tropomyosin. Troponin I (179 amino acid residues) binds to actin and inhibits various enzyme activities (ATPase). Troponin C (158 amino acid residues) binds $Ca^{2\oplus}$ ions reversibly through a change in conformation.

12.3.2.1.5 Other Myofibrillar Proteins

Apart from the main components of sarcomeres, myosin and actin, a series of cytoskeletal proteins exist that are responsible for the stabilization of the structure of the sarcomeres. The most important component is *connectin* (ca. 10% of the contractile proteins), an insoluble protein (M_r ca. 2,000,000) capable of forming fine filaments (g-filaments, $d = 2$ nm) which start at the Z line and proceed between the thick filaments of neighboring sarcomeres. These g-filaments greatly contribute to the elasticity and firmness of meat because they can be stretched from 1.1 µm to a length of 3.0 µm. Another protein of the cell skeleton is *myomesin* (subunit: $M_r = 165{,}000$). As the main component of the M line, myomesin is involved in fixing the thick filaments of the A band and in connecting neighboring myofibrils. Since myomesin strongly binds to myosin, it is possibly involved in the packing and cohesion of the myosin molecules in the thick filaments as well.

Among other proteins, α-*actinin* ($M_r = 200{,}000$), *desmin* ($M_r = 55{,}000$), *vimentin* ($M_r = 58{,}000$) and *synemin* ($M_r = 23{,}000$) are localized in the Z lines. Desmin appears to connect neighboring myofibrils.

A *N line protein* ($M_r = 60{,}000$) has been isolated from the N lines which run parallel to the Z lines on both sides and through the I bands.

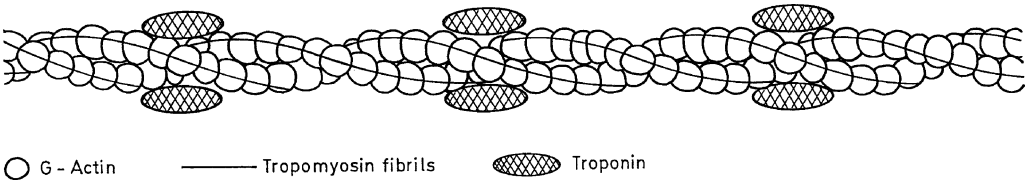


Fig. 12.10. A schematic representation of a thin filament. (according to *Karlsson*, 1977)

12.3.2.1.6 Contraction and Relaxation

Muscle stimulation by a nerve impulse triggers depolarization of the outer membrane of the muscle cell and thus release of Ca^{2+} ions from the sarcoplasmic reticulum. The Ca^{2+} concentration in the sarcoplasm of the resting muscle increases quickly from 10^{-7} to 10^{-5}mole/l. The binding of this Ca^{2+} to the troponin complex causes a conformational change in this protein. As a consequence, displacement of the tropomyosin fibrils occurs along the F-actin filament. Thus, the sterically hindered sites on the actin units are exposed for interaction with the myosin heads. The energy required for the shifting of the unbound myosin heads is obtained from the hydrolysis of ATP. The hydrolysis products of ATP, ADP and inorganic phosphate (P_i), remain on the myosin heads, which then bind to the actin monomers (Fig. 12.11a). Consequently, the myosin heads, now bound to actin, are forced to undergo a conformational change, which forces the thin filament to move relative to the thick filament (Fig. 12.11b).

The thin filaments and the heads of the thick filaments reverses half way between the Z lines. Therefore, the two thin filaments which interact with one thick filament are drawn toward each other, resulting in a shortening of the distance between the Z lines.

When the myosin heads release ADP and P_I and become detached from the thin filaments (Fig. 12.11c), the heads are ready to take up a fresh charge of ATP (Fig. 12.11d). If the Ca^{2+} concentration in the sarcoplasm remains high, the ATP will again hydrolyze and the interaction of the myosin heads with the thin filament is repeated (Fig. 12.11a). However, if the Ca^{2+} concentration drops in the meantime, no ATP hydrolysis occurs, tropomyosin again blocks the access of myosin heads to the actin binding sites

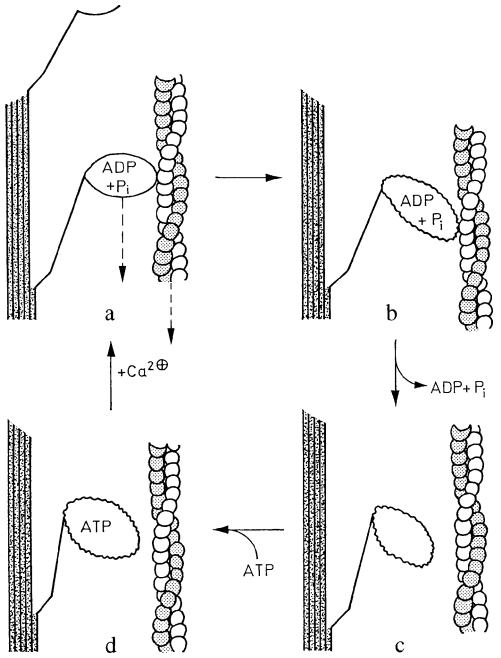


Fig. 12.11. Molecular processes involved in muscle contraction (see text; according to *Karlsson*, 1977)

and the muscle returns to its resting state. The decrease in Ca^{2+} concentration when muscle excitation has ceased, as well as the increase in Ca^{2+} during stimulation, i. e. the flow of calcium ions, is controlled by the sarcoplasmic reticulum. The Ca^{2+} concentration is low in the sarcoplasm of the resting muscle, while it is high within the sarcoplasmic reticulum. When the ATP level is low, detachment of the myosin and actin filaments does not occur. The muscle remains in a stiff, contracted state called rigor mortis (cf. 12.4). Hence, relaxation of muscle depends on the presence of regenerated ATP.

12.3.2.1.7 Actomyosin

Solutions of F-actin and myosin at high ionic strength ($\mu = 0.6$) *in vitro* form a complex called actomyosin. The formation of the complex is reflected by an increase in viscosity and occurs in a definite molar ratio: 1 molecule of myosin per 2 molecules of G-actin, the basic unit of the double-helical F-actin strand. It appears that a spike-like structure is formed, which consists of myosin molecules embedded in a "backbone" made of the F-actin double helix. Addition of ATP to actomyosin causes a sudden drop in viscosity due to dissociation of the complex. When this addition of ATP is followed by addition of Ca^{2+}, the myosin ATPase is activated, ATP is hydrolyzed and the actomyosin complex again restored after the ATP concentration decreases.
Upon spinning of an actomyosin solution into water, fibers are obtained which, analogous to muscle fibers, contract in the presence of ATP. Glycerol extraction of muscle fibers removes all the soluble components and abolishes the semipermeability of the membrane. Such a model muscle system shows all the reactions of *in vivo* muscle contraction after the readdition of ATP and Ca^{2+}. This and similar model studies demonstrate that the muscle contraction mechanism is understood in principle, although some molecular details are still not clarified.

12.3.2.2 Soluble Proteins

Soluble proteins make up 25–30% of the total protein in muscle tissue. They consist of ca. 50 components, mostly enzymes and myoglobin (cf. Table 12.5). The high viscosity of the sarcoplasm is derived from a high concentration of solubilized proteins, which can amount to 20–30%. The glycolytic enzymes are bound to the myofibrillar proteins *in vivo*.

12.3.2.2.1 Enzymes

Sarcoplasm contains most of the enzymes needed to support the glycolytic pathway and the pentosephosphate cycle. Glyceraldehyde-3-phosphate dehydrogenase can make up more than 20% of the total soluble protein. A series of enzymes involved in ATP metabolism, e. g., creatine phosphokinase and ADP-deaminase (cf. 12.3.6 and 12.3.8) are also present.

12.3.2.2.2 Myoglobin

Muscle tissue dry matter contains an average of 1% of the purple-red pigment myoglobin. However, the amounts in white and red meat vary considerably.
Myoglobin consists of a peptide chain (globin) of molecular weight of 16.8 kdal. It has known primary and tertiary structures (Fig. 12.12). The pigment component is present in a hydrophobic pocket of globin and is bound to a histidyl (His^{93}) residue of the protein. The pigment, heme, is the same as that in hemoglobin (blood pigment), i. e. Fe^{2+}-protoporphyrin (Fig. 12.13).

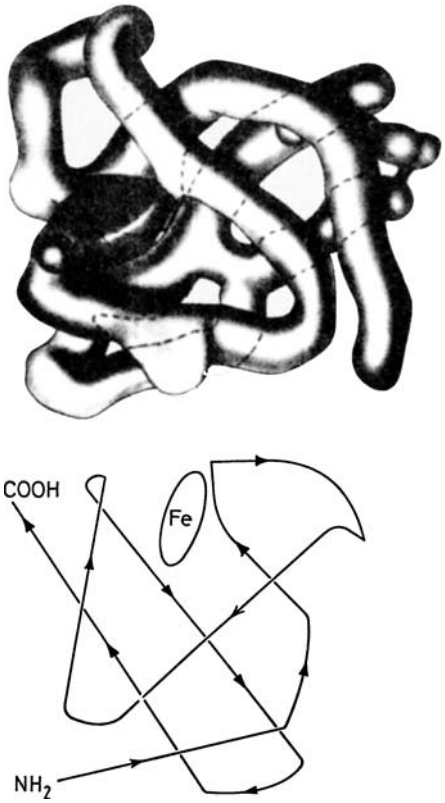


Fig. 12.12. Molecular model of myoglobin (**a**) and a schematic representation of peptide chain course (**b**). (from *Schormueller*, 1965)

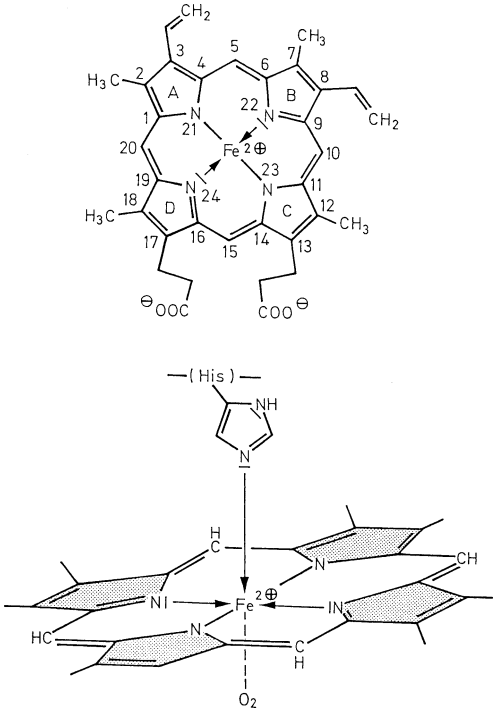


Fig. 12.13. Octahedral environment of Fe^{2+}-protoporphyrin with the imidazole ring of a globin histidine residue and oxygen (according to *Karlsson*, 1977)

Myoglobin supplies oxygen because of its ability to bind oxygen reversibly. Comparison of the oxygen binding curves for hemoglobin and myoglobin (Fig. 12.14) shows that at low p_{o_2}, such as exists in muscle, hemoglobin releases

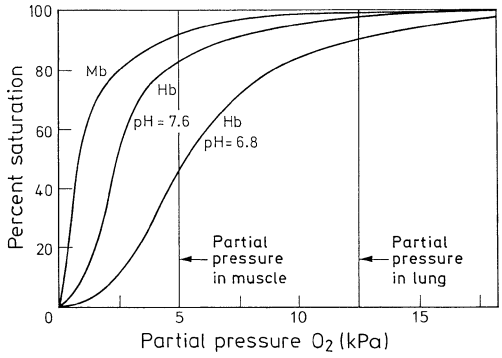


Fig. 12.14. Oxygen binding curves of myoglobin and hemoglobin

oxygen to myoglobin. The sigmoidal shape of the O_2-binding curve for hemoglobin is due to its quaternary structure. It consists of four polypeptide chains, with one pigment molecule bound to each. The binding of O_2 to the four pigment molecules occurs cooperatively because of allosteric effects. Therefore, the degree of saturation, S, is expressed by the following equation (p_{O_2} = oxygen partial pressure; k = dissociation constant for the O_2-protein complex):

$$S = \frac{k \cdot p_{O_2}^n}{1 + k \cdot p_{O_2}^n} \qquad (12.1)$$

For hemoglobin, $n \sim 2.8$ (sigmoidal saturation curve), and for myoglobin, $n = 1$ (hyperbolic saturation curve). The efficiency of O_2 transfer from hemoglobin to myoglobin is further enhanced by a decrease in pH since oxygen binding is pH-dependent (the *Bohr* effect).

While in the living animal approx. 10% of the total iron is bound to myoglobin, 95% of all the iron in well-bled beef muscle is bound to myoglobin. Unlike myoglobin, hemoglobin contributes little to the color of meat. The contribution of other pigments, such as the cytochromes, is negligible. However, attention must be paid to the fact that the visual appearance of a cut of meat is influenced not only by the light absorption of pigments, i. e. primarily myoglobin, but also by light scattering by the surface of muscle fiber. A bright red color is obtained when the coefficient of absorption is high and that of light scattering is low. Myoglobin (Mb) is purple (λ_{max} = 555 nm); oxymyoglobin (MbO_2), a covalent complex of ferrous Mb and O_2, is bright red (λ_{max} = 542 and 580 nm); and the oxidation product of Mb in the ferric state, metmyoglobin (MMb^+), is brown (λ_{max} = 505 and 635 nm). Some other ligands, such as electron pair donors (e. g. CO, NO, N_3^-, CN^-), like O_2, bind covalently, giving rise to low-spin complexes with similar absorption spectra and hence to a color similar to MbO_2. Figure 12.15 shows several absorption spectra of myoglobins.

Heme devoid of globin (free heme, Fe^{2+}-protoporphyrin) does not form the O_2-adduct, but oxidizes rapidly to hemin (Fe^{3+}-protoporphyrin). A prerequisite for reversible O_2 binding is the presence of an effective donor ligand on the iron's axial site, which is bound by formation of a quadratic-pyramidal complex. The imidazole

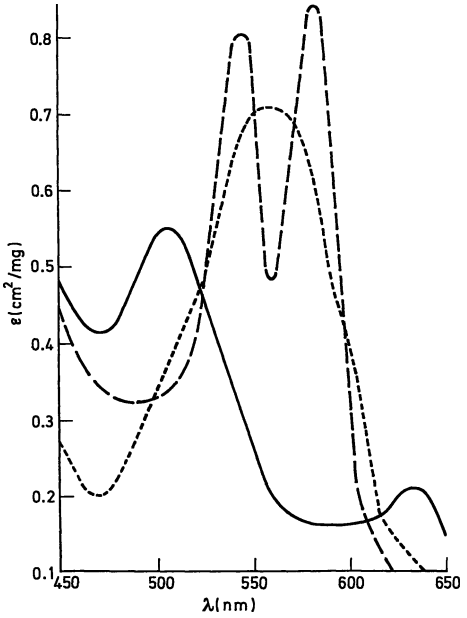
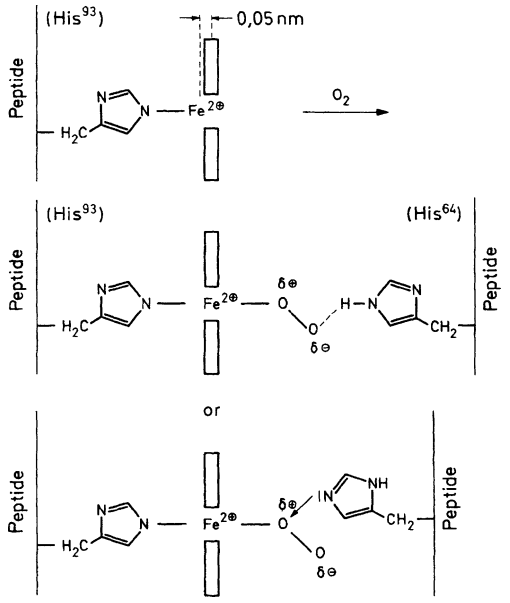


Fig. 12.15. Absorption spectra of myoglobin - - -, oxymyoglobin – – – and metmyoglobin ——. (according to *Fennema*, 1976)

side chain of His^{93} of myoglobin has this function. Upon interaction with this fifth ligand, iron is raised above the heme plane by about 0.05 nm:



(12.2)

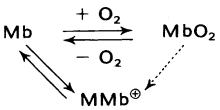
Binding of the sixth ligand moves the iron to its original position in the heme plane. Since the Fe–N bond distance (His^{93}) remains constant, dislocation of the fifth ligand occurs (His^{93}, proximal His), i. e. a conformational change of the globin takes place.

The basicity of the fifth ligand affects the binding of the sixth ligand. The imidazole ring of His^{93} is a good π-donor and, hence, stabilizes the O_2-adduct. A weaker base would enhance oxidation of the iron rather than adduct formation, while a stronger base would increase the stability of the adduct and diminish the probability of iron oxidation. From a biochemical viewpoint, the latter effect is rated as (O_2 supplier) negative; while from a food science point of view, it is desirable and positive (stable, bright red meat color).

As mentioned above, His^{93} is located in a hydrophobic pocket of the myoglobin molecule. The electron density and, therefore, the oxidation state of the iron are regulated by protonation and deprotonation of the imidazole ring. With an increase in pH, there is an increase in basicity and, hence, an increase in binding of O_2 (the *Bohr* effect; cf. Fig. 12.14). A second histidine residue of myoglobin, His^{64} (distal His), contributes to heme-O_2-complex stabilization by formation of a hydrogen bridge or ionic bond between N and O (cf. Formula 12.2).

12.3.2.2.3 Color of Meat

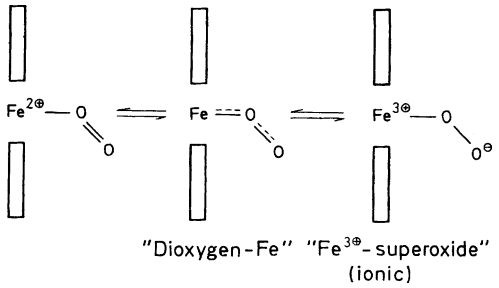
The color of fresh meat is determined by the ratios of myoglobin (Mb), oxymyoglobin (MbO_2) and metmyoglobin (MMb^+):



(12.3)

Stable MbO_2 is formed at a high partial pressure of oxygen. Fresh cuts of meat, to a depth of about 1 cm, acquire a bright cherry-red color which is considered a mark of quality. A slow and continuous oxidation to MMb^+ occurs at a low partial pressure of O_2. The change of $Fe^{2+} \rightarrow Fe^{3+}$ is reflected in the change in color from red to brown. MMb^+ does not form an O_2-adduct, since Fe^{3+} appears to be a less efficient π donor than Fe^{2+}. With better donor ligands than O_2 (CN^-,

NO, N_3^-), low-spin complexes are formed, the spectra of which are similar to those of MbO_2.
The change of $Fe^{2+} \rightarrow Fe^{3+}$ is designated as autoxidation:



(12.4)

The oxygen molecule dissociates from the heme, taking along an electron from the iron, after protonation of its outer, more negative oxygen atom to form a hydroperoxy radical, the conjugate acid of the superoxide anion (cf. 3.7.2.1.4). The proton may originate from the distal histidine residue or other globin residues or from the surrounding medium. Autoxidation is accelerated by a drop in pH. The reason is the increased dissociation of the protein-pigment complex:

$$Globin + Heme \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Myoglobin \qquad (12.5)$$

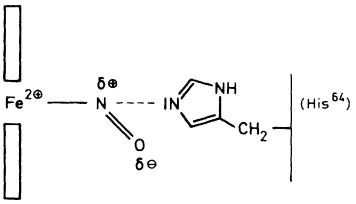
Soon after slaughter, the meat has a pH at or near 7, at which the equilibrium constant of the above reaction is $K = k_1/k_{-1} = 10^{12}-10^{15}\,mole^{-1}$. Since, during post-rigor, glycolysis decreases the pH of the meat to 5–6, myoglobin becomes increasingly susceptible to autoxidation.
The stability of MbO_2 is also highly dependent on temperature. Its half-life, τ, at pH 5 is 2.8 h at 25 °C and 5 days at 0 °C. Fresh meat has a system which can reduce MMb^+ back to Mb. Although various enzymatic and non-enzymatic reactions take part in this system, their contribution to the preservation of the color of fresh meat is unclear. One proposal suggests the participation of a NADH-cytochrome b_5-reductase. This enzyme which was detected in meat reduces $MMb^{\oplus}$ and $MHb^{\oplus}$. In addition, a series of non-specific reductases, also known as diaphorases, are supposed to play a role in this system. The slow formation of MMb^+ can be reversed at the low partial pressure of O_2 which is found inside the cut of meat or in packaged, sealed meat. Therefore, for color stabilty, packaging of meat in O_2-permeable materials is not suitable since, after a time, its reduction capacity is fully exhausted. A non-O_2-permeable material is suitable for packaging meat. All of the pigment is present as Mb and is transformed to the bright red MbO_2 only when the package is opened.
Stabilization of the color of meat is also possible under controlled atmosphere packaging. A gaseous mixture of CO and air appears to be advantageous.
Copper ions promote autoxidation of heme to a great extent, while other metal ions, such as Fe^{3+}, Zn^{2+} or Al^{3+}, are less active.

12.3.2.2.4 Curing, Reddening

Color stabilization by the addition of nitrate or nitrite (meat curing) plays an important role in meat processing. Nitrite initially oxidizes myoglobin to metmyoglobin:

$$Mb + NO_2^{\ominus} \rightarrow MMb^{\oplus} + NO \qquad (12.6)$$

The resulting NO forms bright-red, highly stable complexes with Mb and MMb^+, MbNO and MMb^+NO:



(12.7)

Reducing agents, such as ascorbate, thiols or NADH, accelerate the *reddening* by reducing nitrite to NO and MMb^+ to Mb. Nitrosylmyoglobin, MbNO, is formed, which is highly stable when O_2 is absent. However, in the presence of O_2, the NO released by dissociation of MbNO is oxidized to NO_2.
The color of cured meat is heat stable. Denatured nitrosylmyoglobin is present in heated meat, or, due to dissociation of the proteinpigment complex, heme occurs with NO ligands present in

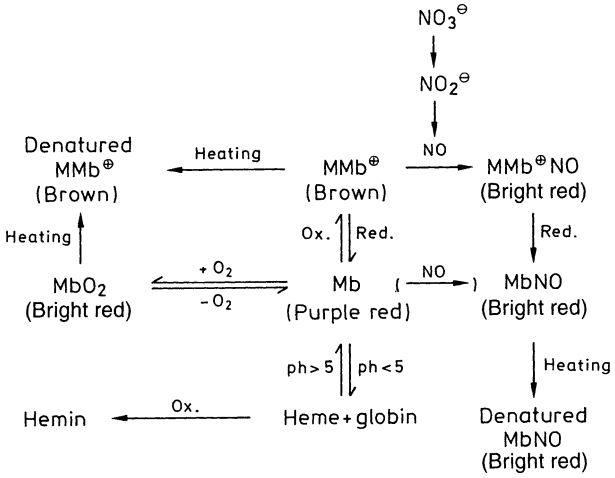
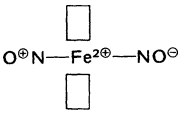


Fig. 12.16. Myoglobin reactions (Mb: myoglobin, MMb^{+}: metmyoglobin, MbO_2: oxymyoglobin, MbNO: nitrosylmyoglobin, $MMb^{+}NO$: nitrosylmetmyoglobin)

both axial binding sites:



(12.8)

MbNO antioxidatively protects the meat against lipid peroxidation. As shown in Formula 12.9, it traps fatty acid peroxyl radicals $ROO^{\bullet}$ with the formation of myoglobin and nitrite. MbNO is reformed in the presence of the above mentioned reducing agents.

$$ROO^{\bullet} + MbNO + H_2O \rightarrow ROOH + Mb + HNO_2$$
$$ROOH + 2MbNO + H_2O \rightarrow ROH + 2Mb + 2HNO_2 \quad (12.9)$$

A color change to brown is observed when noncured meat is heated. A Fe^{3+} complex is present which has its fifth and sixth coordination sites occupied by histidine residues of denatured meat proteins.
The myoglobin reactions relevant to meat color are presented schematically in Fig. 12.16.

12.3.2.3 Insoluble Proteins

The main fraction of proteins insoluble in water or salt solutions are the proteins of connective tissue. Membranes and the insoluble portion of the contractile apparatus are included in this group (cf. 12.3.2.1.4).

Connective tissue contains various types of cells. These cells synthesize many intercellular amorphous substances (carbohydrates, lipids, proteins) in which the collagen fibers are embedded.
Lipoproteins are present mostly in membranes. The lipids make up 3–4% of muscle tissue and are located in membranes. They consist of phospholipids, triacylglycerols and cholesterol. The phospholipid portion varies greatly: it makes up 50% of the plasma membrane and 90% of the mitochondrial membrane.

12.3.2.3.1 Collagen

Collagen constitutes 20–25% of the total protein in mammals. Table 12.6 shows data on its amino acid composition. The high contents of glycine and proline and the occurrence of 4-hydroxyproline and 5-hydroxylysine are characteristic. Since the occurrence of hydroxyproline is confined to connective tissue, its determination may provide quantitative data on the extent of connective tissue incorporation into a meat product.
Collagen also contains carbohydrates (glucose and galactose). These are attached to hydroxylysine residues of the peptide chain by O-glycosidic bonds. The presence of 2-O-α-D-glucosyl-O-β-D-galactosyl-hydroxylysine and of O-β-D-galactosyl-hydroxylysine has been confirmed.
Various types of collagen are known. They are characteristic of different organs and also of dif-

Table 12.6. Amino acid composition of muscle proteins (values are in g/16 g N)

Amino acid	Beef muscle total	Poultry muscle total[a]	Myosin	Actin (calf skin)	Collagen	Elastin
Aspartic acid	9.7–9.9	9.7–11.0	10.9	10.4	5.4	1.0
Threonine	4.8	3.5–4.5	4.7	6.7	2.1	1.1
Serine	4.1–4.5	–	4.1	5.6	2.9	0.9
Glutamic acid	15.8–16.2	16–18	21.9	14.2	9.7	2.4
Proline	3.0–4.1	–	2.4	4.9	13.0	11.6
Hydroxyproline					10.5	1.5
Glycine	4.6–6.1	4.6–6.7	2.8	4.8	22.5	25.5
Alanine	6.1–6.3	–	6.7	6.1	8.2	21.1
Cystine	1.3–1.5	–	1.0	1.3	0	0.3
Valine	4.8–5.5	4.7–4.9	4.7	4.7	2.9	16.5
Methionine	4.1–4.5	–	3.1	4.3	0.7	Trace
Isoleucine	5.2	4.6–5.2	5.3	7.2 }	4.8[b]	3.7
Leucine	8.1–8.7	7.3–7.8	9.9	7.9 }		8.6
Tyrosine	3.8–4.0	–	3.1	5.6	1.2	1.3
Phenylalanine	3.8–4.5	3.7–3.9	4.5	4.6	2.2	5.9
Lysine	9.2–9.4	8.3–8.8	11.9	7.3	3.9	0.5
Hydroxylysine					1.1	–
Histidine	3.7–3.9	2.2–2.3	2.2	2.8	0.7	0.1
Arginine	5.3–5.5	5.7–6.1	6.8	6.3	7.6	1.2
Tryptophan	–	–	0.8	2.0	0	–

[a] Chicken, duck, turkey: average values.
[b] Sum of isoleucine and leucine.

ferent connective tissue layers of muscular tissue (cf. 12.2.1). An overview is presented in Table 12.7. The amino acid sequence of an α^1-chain of collagen type I of mammalian skin is shown in Table 12.8. It is typical that every third residue in this sequence is glycine. Deviations from this regularity have been observed only at the ends of a chain. A frequently recurring sequence is:

Gly – Pro – Hyp – .

As a result of the specificity of the hydroxylating enzyme in vertebrates, hydroxyproline is always located, as shown in the sequence (Table 12.8) before glycine.

Collagen consists of three peptide chains which can be different or identical, depending on the type (cf. Table 12.7). The three peptide chains, each of which has a helical structure, form together a triple-stranded helix which has a structure corresponding to that of polyglycine II. A triple helix of this type is shown in Fig. 12.17.

The basic structural unit of collagen fibers is called tropocollagen. It has a molecular weight of approx. 30 kdal. With a length of approx. 280 nm and a diameter of 1.4–1.5 nm, collagen is one of the longest proteins. Tropocollagen fibers associate in a specific way to form collagen fibers, as presented schematically in Fig. 12.18. The association of adjacent rows is not in register, but is displaced by about one-fourth of the tropocollagen length (a "quarter staggered" array). This is responsible for cross-striations in the collagen fibers.

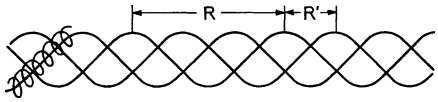


Fig. 12.17. Schematic representation of the conformation of tropocollagen (period R = 8.7 nm, pseudoperiod R′ = 2.9 nm)

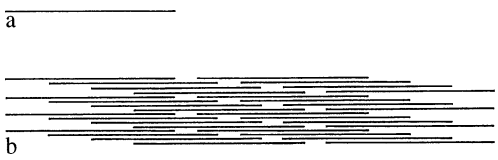


Fig. 12.18. Build up of a collagen fiber (**b**) from tropocollagen (**a**) molecules

Table 12.7. Types of collagen

Type	Peptide chains[a]	Molecular composition	Occurrence
I	α^1, α^2	$[\alpha^1(I)]_2\ \alpha^2(I)$	Skin, tendons, bones, muscle (epimysium)
II	α^1	$[\alpha^1(II)]_3$	Cartilage
III	α^1	$[\alpha^1(III)]_3$	Fetal skin, cardiovascular system, synovial membranes, inner organs, muscle (perimysium)
IV	α^1, α^2	$[\alpha^1(IV)]_3(?)$[b]	Basal membranes, capsule of lens, glomeruli
		(?)	Placental membrane, lung, muscle (endomysium)
V	αA, αB, αC (?)	$[\alpha B]_2\ \alpha$ A or $(\alpha B)_3$ + $(\alpha A)_3$	Placental membrane, cardiovascular system, lung, muscle (endomysium),
		$(\alpha C)_3$ (?)	secondary component of many tissues

[a] Since the α chains of various types of collagen differ, they are called α^1(I), α^1(II), αA etc.
[b] (?) Not completely elucidated.

Figure 12.19 shows an electron micrograph of collagen fibers of bovine muscle.
During maturation or aging, collagen fibers strengthen and are stabilized, primarily by covalent cross-linkages. Thus, cross-links confer mechanical strength to collagen fibers.
Cross-link formation involves the following reactions:

- Enzymatic oxidation of lysine and hydroxylysine to the corresponding ω-aldehydes.
- Conversion of these aldehydes to aldols and aldimines.
- Stabilization of these primary products by additional reduction or oxidation reactions.

It appears that only certain residues undergo reaction, mainly in the terminal, non-helical regions of the peptide chains.
Lysine and hydroxylysine residues within the peptide chain are oxidized by an enzyme that requires Cu^{2+} and pyridoxal phosphate for its activity and which is related to amine oxidase.

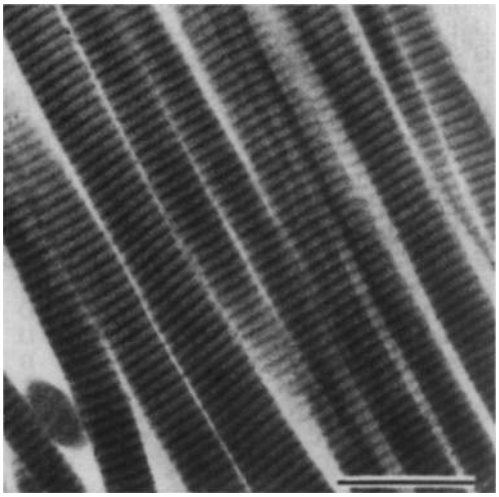
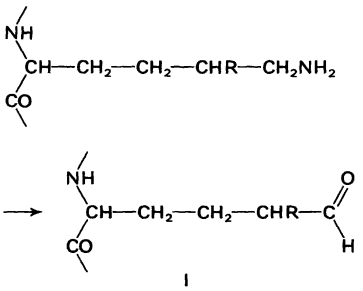


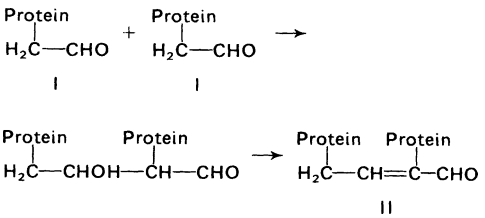
Fig. 12.19. Collagen fibers of bovine muscle (*Extensor carpi radialis*); transmission electron microscopy; sample fixed with 2% glutaraldehyde/paraformaldehyde, bar: 0.5 µm. (according to *Elkhalifa* et al., 1988)

This reaction yields an α-aminoadipic acid semialdehyde residue bound to the existing peptide chain (R = H or OH):



I (12.10)

The two aldehyde-containing chains may interact through an aldol condensation followed by elimination of water, forming a cross-link:



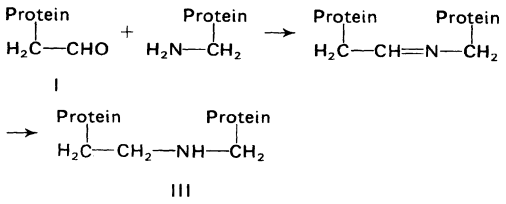
II (12.11)

Table 12.8. Sequence of mammalian skin collagen, α^1-chain[a]

														Z*	M	S	Y	G	Y	D	E	K	S	A	G	V	S	V	P
G	P	M	G	P	S	G	P	R	G	L	P*	G	P	P*	G	A	P*	G	P	Q	G	F	Q	G	P	P*	G	E	P*
G	E	P*	G	A	S	G	P	M	G	P	R	G	P	P*	G	P	P*	G	K	N	G	D	D	G	E	A	G	K	P
G	R	P*	G	Q	R	G	P	P*	G	P	Q	G	A	R	G	L	P*	G	T	A	G	L	P*	G	M	K*	G	H	R
G	F	S	G	L	D	G	A	K	G	N	T	G	P	A	G	P	K	G	E	P*	G	S	P*	G	E	N	G	A	P*
G	Q	M	G	P	R	G	L	P*	G	E	R	G	R	P*	G	P	P*	G	S	A	G	A	R	G	D	D	G	A	V
G	A	A	G	P	P*	G	P	T	G	P	T	G	P	P*	G	F	P*	G	A	A	G	A	K	G	E	A	G	P	Q
G	A	R	G	S	E	G	P	Q	G	V	R	G	E	P*	G	p	P*	G	P	A	G	A	A	G	P	A	G	N	P*
G	A	D	G	Q	P*	G	A	K	G	A	N	G	A	P*	G	I	A	G	A	P*	G	F	P*	G	A	R	G	P	S
G	P	D	G	P	S	G	A	P*	G	P	K	G	N	S	G	E	P*	G	A	P*	G	N	K	G	D	T	G	A	K
G	E	P*	G	P	A	G	V	Q	G	P	P*	G	P	A	G	E	E	G	K	R	G	A	R	G	E	P*	G	P	S
G	L	P*	G	P	P*	G	E	R	G	G	P*	G	S	R	G	F	P*	G	A	D	G	V	A	G	P	K	G	P	A
G	E	R	G	S	P*	G	P	A	G	P	K	G	S	P*	G	E	A	G	R	P*	G	E	A	G	L	P*	G	A	K
G	L	T	G	S	P*	G	S	P*	G	P	D	G	K	T	G	P	P*	G	P	A	G	Z	B	G	R	P*	G	P	A
G	P	P*	G	A	R	G	Q	A	G	V	M	G	F	P*	G	P	K	G	A	A	G	E	P*	G	K	A	G	E	R
G	V	P*	G	P	P*	G	A	V	G	P	A	G	K	D	G	E	A	G	A	Q	G	P	P*	G	P	A	G	P	A
G	E	R	G	E	Q	G	P	A	G	S	P*	G	F	Q	G	L	P*	G	P	A	G	P	P*	G	E	A	G	K	P*
G	E	Q	G	V	P*	G	D	L	G	A	P*	G	P	S	G	A	R	G	E	R	G	F	P*	G	E	R	G	V	E
G	P	P*	G	P	A	G	P	R	G	A	N	G	A	P*	G	N	D	G	A	K	G	D	A	G	A	P*	G	A	P*
G	S	Q	G	A	P*	G	L	Q	G	M	P*	G	E	R	G	A	A	G	L	P*	G	P	K	G	D	R	G	D	A
G	P	K	G	A	D	G	A	P	G	K	D	G	V	R	G	L	T	G	P	I	G	P	P*	G	P	A	G	A	P*
G	D	K	G	E	A	G	P	S	G	P	A	G	T	P	G	A	P*	G	D	R	G	E	P*	G	P	P*	G	P	A
G	F	A	G	P	P*	G	A	D	G	Q	P*	G	A	K	G	E	P*	G	D	A	G	A	K	G	D	A	G	P	P*
G	P	A	G	P	A	G	P	P*	G	P	I	G	N	V	G	A	P*	G	P	K*	G	A	R	G	S	A	G	P	P*
G	A	T	G	F	P*	G	A	A	G	R	V	G	P	P*	G	P	S	G	N	A	G	P	P*	G	P	P*	G	P	A
G	K	E	G	S	K	G	P	R	G	E	T	G	P	A	G	R	P*	G	E	V	G	P	P*	G	P	P*	G	P	A
G	E	K	G	A	P*	G	A	D	G	P	A	G	A	P*	G	T	P	G	P	Q	G	I	A	G	Q	R	G	V	V
G	L	P*	G	Q	R	G	E	R	G	F	P*	G	L	P*	G	P	S	G	E	P*	G	K	Q	G	P	S	G	A	S
G	E	R	G	P	P*	G	P	M	G	P	P*	G	L	A	G	P	P*	G	E	S	G	R	E	G	A	P*	G	A	E
G	S	P*	G	R	D	G	S	P*	G	A	K	G	D	R	G	E	T	G	P	A	G	P	P*	G	A	P*	G	A	P*
G	A	P*	G	P	V	G	P	A	G	K	S	G	D	R	G	E	T	G	P	A	G	P	I	G	P	V	G	P	A
G	A	R	G	P	A	G	P	Q	G	P	R	G	B	K*	G	Z	T	G	Z	Z	G	B	R	G	I	K*	G	H	R
G	F	S	G	L	Q	G	P	P*	G	P	P*	G	S	P*	G	E	Q	G	P	S	G	A	S	G	P	A	G	P	R
G	P	P*	G	S	A	G	S	P*	G	K	D	G	L	N	G	L	P *	G	P	I	G	P‡	P*	G	P	R	G	R	T
G	D	A	G	P	A	G	P	P*	G	P	P*	G	P	P*	G	P	P*	G	P	P	S	G	G	Y	D	L	S	F	L
P	Q	P	P	Q	Q	Z	K	A	H	D	G	G	R	Y	Y														

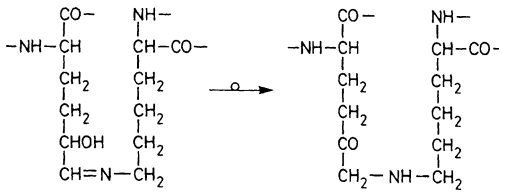
Z*: Pyrrolidone carboxylic acid, P*: 4-hydroxyproline, K*: 5-hydroxylysine, P‡: 3-hydroxyproline.
[a] The sequence is derived from very similar sequences of skin collagen of various mammals.

A polypeptide chain with an aldehyde residue (I) can interact with a lysine residue of the adjacent chain to form an aldimine, which can be further reduced to peptide-bound lysinonorleucine (III):



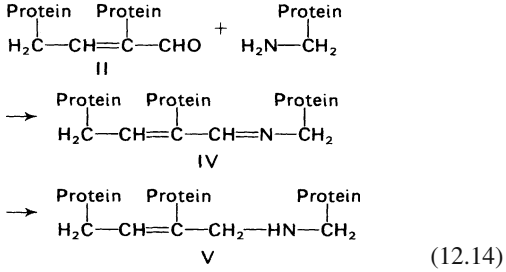
(12.12)

If hydroxylysine is involved, an aldimine formed initially can be converted to a more stable β-aminoketone by *Amadori* rearrangement (cf. 4.2.4.4.1):



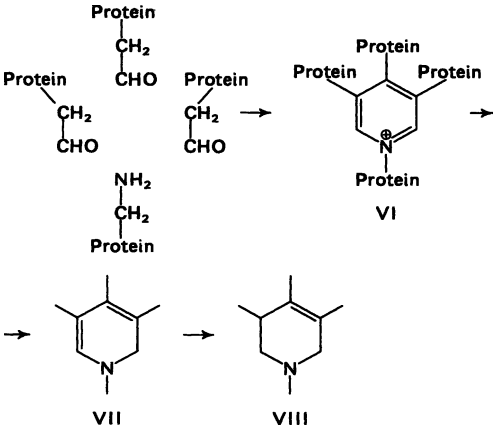
(12.13)

Likewise, aldehyde II can interact with one lysine residue through the intermediary dehydromerodesmosine (IV) to merodesmosine (V) and, thus, provide cross-links between the three adjacent polypeptide chains:



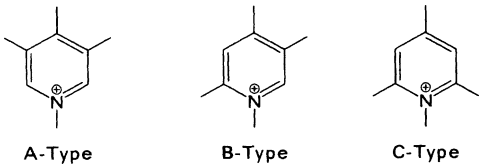
(12.14)

During the reaction of three aldehyde molecules of type I with a lysine residue (actually a total of four lysine side chains are involved), a pyridine derivative is formed which, depending on the extent of reduction, yields desmosine (VI), dihydro- (VII) and tetrahydrodesmosine (VIII):



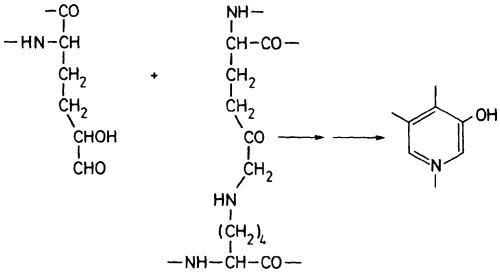
(12.15)

Depending on the kind of condensation, in addition to desmosine VI, designated as an A-type condensation product, rings with other substitution patterns are observed, i. e. B- and C-type condensation products:



(12.16)

Pyridinolines have also been detected. They are probably formed from β-aminoketones and the ω-aldehyde of a hydroxylysine residue:

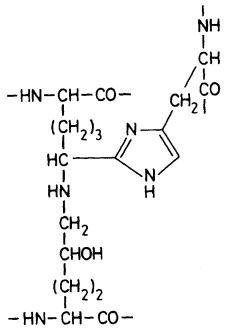


(12.17)

Studies of bovine muscle collagens have shown that the pyridinoline content increases with increasing age of the animal and, like the collagen content, negatively correlates with the tenderness. In intensively fattened cows, the pyridi-

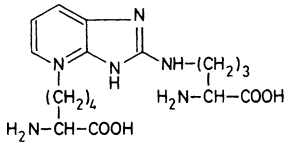
noline content was higher than in extensively fattened animals.

Histidine can also be involved in cross-linking reactions, as shown by the detection of histidino-hydroxylysino-norleucine:



(12.18)

The amino acid pentosidine was also obtained from collagen, which indicates the bonding of lysine and arginine with the participation of a pentose:



(12.19)

The outlined reactions can also occur with hydroxylysine residues present on collagen fibers. Of all the compounds mentioned, hydroxylysino-norleucine and dihydroxylysino-norleucine have been isolated from collagen in significant amounts.

In the case of type I, collagen biosynthesis (Fig. 12.20a–h) involves first the synthesis of pro-α^1- and pro-α^2-precursor chains. The N-terminus of these precursors contains up to 25% of extended α^1- and α^2-chains (a). Immediately after the chains are released from polysomes, hydroxylation of the proline and lysine residues occurs (cf. reactions under 12.20).

Realignment of the chains follows: two strands of pro-α^1 and one chain of pro-α^2 are joined to form a triple-stranded helix (b–d). The extended peptides at the N-terminus appear to play a distinct role in these reactions. Disulfide bridging occurs between the strands at this stage in order to stabilize the structure. The procollagen thus formed will cross the membrane of the cell in which it was synthesized (e). The N-terminal peptides are removed by limited proteolysis (f) and the pro-

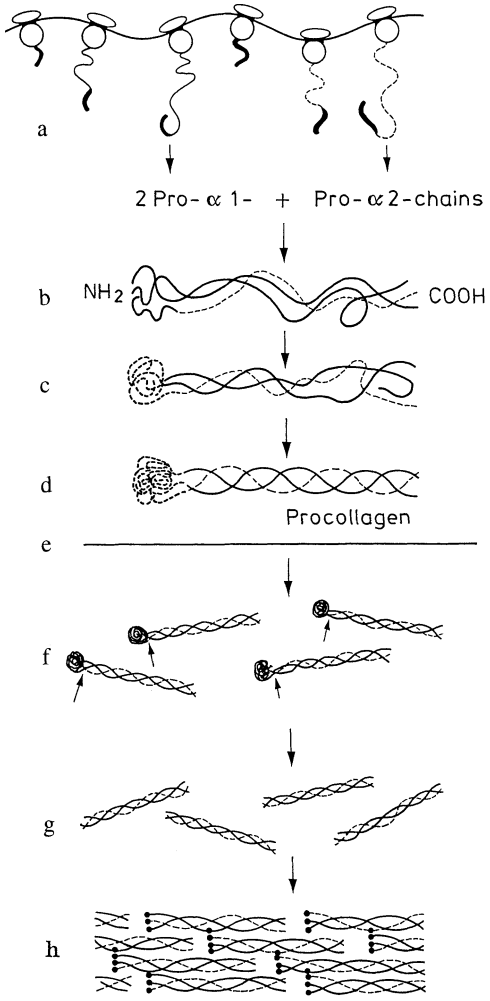
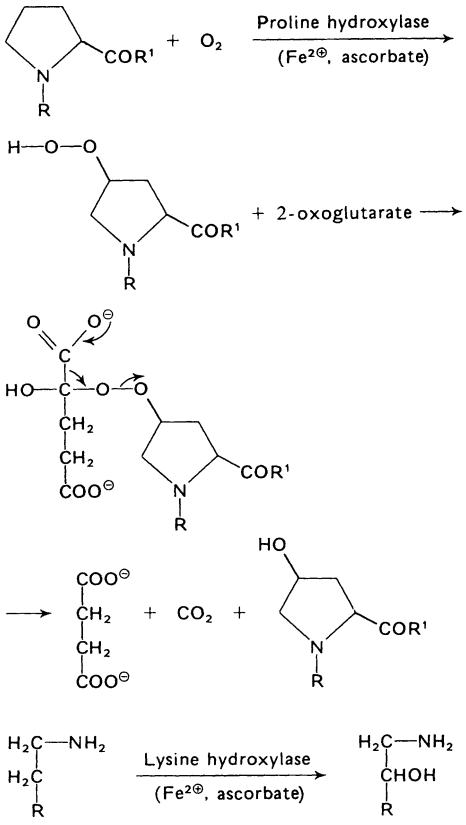


Fig. 12.20. Collagen biosynthesis (according to *Bornstein*, 1974). **a** Polysome, **b** hydroxylation, **c** chain straightening, **d** disulfide bond formation, **e** cell membrane, **f** membrane crossing, **g** a limited hydrolysis to tropocollagen, **h** collagen fiber formation, cross-linking

collagen is converted to tropocollagen (g). Finally, the tropocollagen is realigned to form collagen fibers (h). At this stage, collagen maturation, which coincides with strengthening of collagen fibers by covalent cross-linking along the peptide strands, begins. The maturation is initiated by oxidation of lysine and is followed by the reactions described above.

Collagen swells but does not solubilize. Enzymatically, it can be hydrolyzed to various extents with



(12.20)

a series of collagenases from different sources and with different specificities. A vertebrate animal collagenase, which is a metal proteinase, splits a special bond in native collagen while the collagenase from *Clostridium histolyticum*, also a metal proteinase, cleaves collagen preferentially at glycine residues, forming tripeptides:

$$-\text{Pro}-\text{X}\downarrow-\text{Gly}-\text{Pro}-\text{X}\downarrow-\text{Gly}-\text{Pro}- \qquad (12.21)$$

Collagenase enzymes which are serine proteinases are also known.

Denatured collagen, as formed post-mortem by the action of lactic acid, can also be cleaved by lysosomal enzymes, e. g., lysosomal collagenase and cysteine proteinase cathepsin B_1. Thermally denatured collagen is attacked by pepsin and trypsin.

One characteristic of the intact collagen fiber is that it shrinks when heated (cooking or roasting). The shrinkage temperature (T_s) is different for different species. For fish collagen, the T_s is 45 °C and for mammals, 60–65 °C. When native or intact collagen is heated to $T > T_s$, the triple-stranded helix is destroyed to a great extent, depending on the cross-links. The disrupted structure now exists as random coils which are soluble in water and are called gelatin. Depending on the concentration of the gelatin solution and of the temperature gradient, a transition into organized structures occurs during cooling. Figure 12.21 schematically summarizes these transitions. At low concentrations, intramolecular back-pleating occurs preferentially with single-strands. At higher concentrations and slow rates of cooling, a structure is rebuilt which resembles the original native structure. At even higher concentrations and rapid cooling, structures are obtained in which the helical segments alternate with randomly coiled portions of the strand. All these structures can immobilize a large amount of water and form gelatin gels.

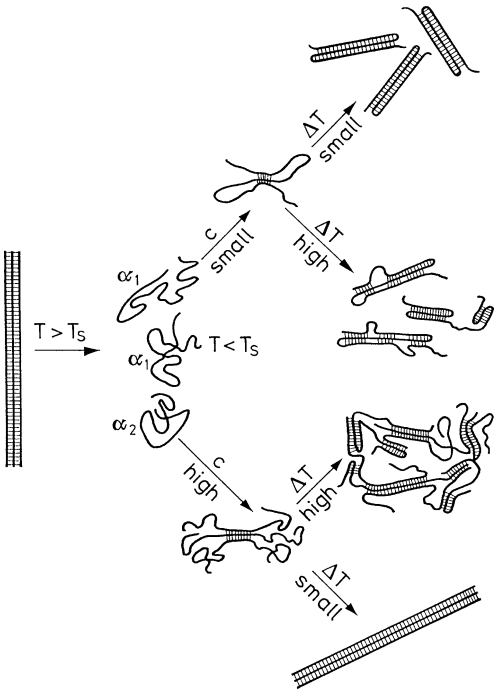


Fig. 12.21. Collagen conversion into gelatin. (according to *Traub* and *Piez*, 1971). T_s: shrinkage temperature, T: temperature, c: concentration; (see text)

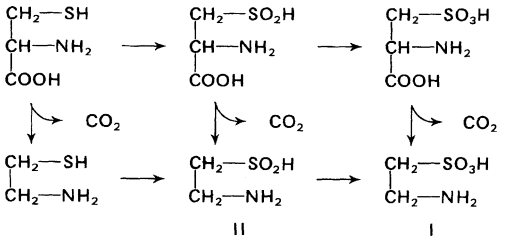
The transition of collagen to gelatin outlined above occurs during the cooking and roasting of meat. The extent of gelatinization is affected by the collagen cross-linking as determined by the age of the animal and the amount of heat applied (temperature, time, pressure).
Gelatin plays a role as a gelling agent. It is produced on a large scale from animal bones or skin by treatment with alkali or acid, followed by a water extraction step. Depending on the process, products are obtained which differ in molecular weight and, consequently, in their gelling properties. Some brands are used as food gelatins, others play an important role in industry (film emulsions, glue manufacturing).

12.3.2.3.2 Elastin

Elastin is found in lower amounts in connective tissue along with collagen. It is a nonswelling, highly stable protein (M_r 70,000) which forms elastic fibers. The protein has rubber-like properties. It can stretch and then return to its original length or shape. Large amounts of elastin are present in ligaments and the walls of blood vessels. The ligament located in the neck of grazing animals is an exceptionally rich source of this protein. Table 12.6 shows that the amino acid composition is different from that of collagen. The amount of basic and acidic amino acids is low, e. g., hydroxylysine is absent, while the content of amino acids with nonpolar side chains (Ala, Val) is greatly increased. This difference explains that, unlike collagen, elastin lacks the capability of swelling on heating in water. The elastic properties are based on very strong cross links, which involve the desmosines described in the section on collagen (cf. Formula 12.15).
Elastin is hydrolyzed by the serine proteinase elastase, which is excreted by the pancreas. This enzyme preferentially cleaves peptide bonds at sites where the carbonyl residue has a nonaromatic, nonpolar side chain.

12.3.3 Free Amino Acids

Fresh beef muscle contains 0.1–0.3% free amino acids (fresh weight basis). All amino acids are detectable in low amounts (<0.005), with alanine (0.01–0.05%) and glutamic acid (0.01–0.05%) being most predominant.
The free amino acid fraction also contains 0.02–0.1% taurine (I). As such, taurine should be regarded as a major constituent of this fraction. It is obtained biosynthetically from cysteine through cysteic acid and/or from a side pathway involving cysteamine and hypotaurine (II):



(12.22)

The biochemical role of taurine includes derivatization of bile acids (taurocholic and taurodeoxycholic acids). A neurotransmitting function has also been ascribed to this compound.

12.3.4 Peptides

The characteristic β-alanyl histidine peptides, carnosine, anserine and balenine, of muscle are described in section 1.3.4.2. Their contribution to taste is discussed in 12.9.1.

12.3.5 Amines

Methylamine in fresh beef muscle is present at 2 mg/kg, while the other volatile aliphatic amines (dimethyl-, trimethyl-, ethyl-, diethyl- and isopropylamine) are detected only in trace amounts.
Of the biogenic amines produced on the decarboxylation of amino acids (cf. 10.2.8.3), histamine, tyramine, putrescine and cadaverine have been identified in beef and pork. Since these substances are microbial metabolic products, they were the proposed indicators of microbial quality and were listed in the biogenic amine index (BAI = concentration of the four amines in mg/kg). A BAI value of <5 indicates clean meat, 5–20

acceptable (early stages of microbial infestation), 20–50 inferior quality and >50 spoiled meat. The BAI values of fermented meat products are naturally higher; a limit of 500 mg/kg was proposed for salami.

Other biogenic amines are spermidine [N-(3-aminopropyl)-1,4-butandiamine] and spermine [N, N′-bis-(3-aminopropyl)-1,4-butandiamine], which are biogenetically formed from putrescine and belong to the constituents of meat. The main compound is spermine, with a concentration in the range of 25–65 mg/kg.

12.3.6 Guanidine Compounds

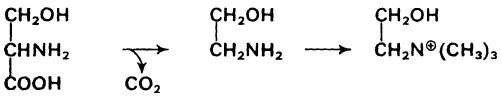
Creatine and creatinine (I and II, respectively; cf. Formula 12.23) are characteristic constituents of muscle tissue and their assay is used to detect the presence of meat extract in a food product. Creatine is present in fresh beef at 0.3–0.6% and creatinine at 0.02–0.04%.

In living muscle, 50–80% of creatine is in the phosphorylated form, creatine phosphate (III, cf. Formula 12.23), which is in equilibrium with ATP. The reaction rate is highly influenced by the enzyme creatine phosphokinase. Creatine phosphate serves as an energy reservoir (free energy of hydrolysis, $\Delta G^0 = -42.7\,kJ/mole$; of ATP: $\Delta G^0 = -29.7\,kJ/mole$). Creatine phosphate has a higher phosphoryl group transfer potential than ATP. Hence, when muscle is stimulated for a prolonged period in the absence of glycolysis or respiration, the supply of creatine phosphate will become depleted within a couple of hours by maintaining the ATP concentration. This is especially the case in post-mortem muscle, when the ATP supply has declined significantly through oxidative respiration.

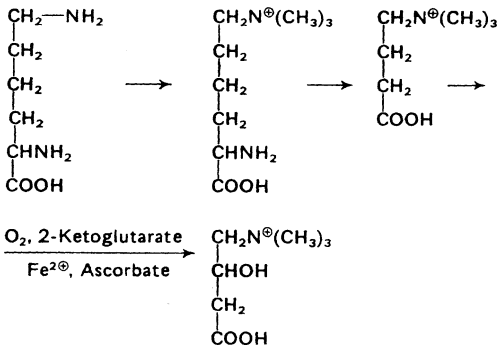
12.3.7 Quaternary Ammonium Compounds

Choline and carnitine are present in muscle tissue at 0.02–0.06% and 0.05–0.2%, respectively (on a fresh weight basis). Choline is synthesized from serine with colamine as an intermediary product (cf. Reactions 12.24) and carnitine is obtained from lysine through ε-N-trimethyllysine and butyrobetaine (cf. Reactions 12.25).

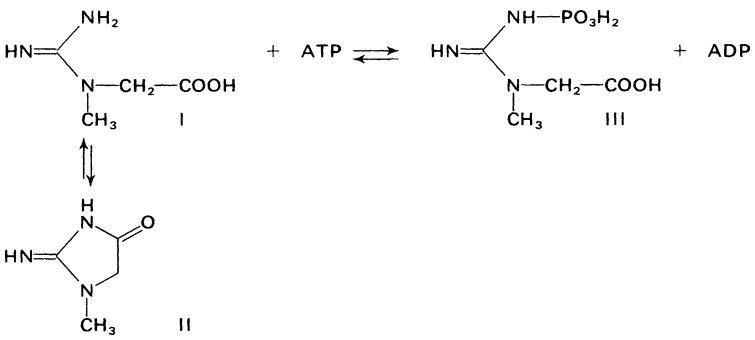
The carnitine fatty acid esters, which are in equilibrium with long chain acyl-CoA molecules in living muscle tissue, are of biochemical importance. The carnitine fatty acid ester, but not the acyl-CoA ester, can traverse the inner mitochondrial membrane. After the fatty acid is oxidized within the mitochondria, carnitine is instrumental in transporting the generated acetic acid out of the mitochondria.



(12.24)



(12.25)



(12.23)

12.3.8 Purines and Pyrimidines

The total content of purines in fresh beef muscle tissue is 0.1–0.25% (on a fresh weight basis). ATP, present predominantly in living tissue, breaks down to inosine-5′-monophosphate (IMP) in the post-mortem stages. The breakdown rate is influenced by the condition of the animal and by temperature. IMP is then slowly decomposed through successive steps to hypoxanthine, with inosine as an intermediary product:

$$\begin{aligned}
&\text{ATP} \quad \rightleftarrows \text{ADP} + \text{P}_{\text{in}} \ (\text{Myosin-ATPase})\\
&\text{2ADP} \quad \rightleftarrows \text{ATP} + \text{AMP} \ (\text{Myokinase})\\
&\text{AMP} \quad \rightarrow \text{IMP} + \text{NH}_3 \ (\text{Adenylate-deaminase})\\
&\text{IMP} \quad \rightarrow \text{Inosine} + \text{P}_{\text{in}} \ (5' - \text{Nucleotidase})\\
&\text{Inosine} \rightarrow \text{Hypoxanthine} + \text{Ribose} \ (\text{Nucleosidase})
\end{aligned} \tag{12.26}$$

Post-mortem data on the *Psoas major* rabbit muscle are given in Table 12.10. They relate to nucleotide breakdown and to other important muscle tissue constituents.

The changes in water holding capacity of meat resulting from ATP transition to IMP are dealt with in Section 12.5. Unlike purines, pyrimidine nucleotide content in muscle is very low (Table 12.9).

Table 12.9. Purines and pyrimidines in fresh-beef muscle

Compound	Content (%)
Inosine-5′-phosphate	0.02–0.2[a]
Inosine	Trace
Hypoxanthine	0.01–0.03
Adenosine-5′-phosphate	0.001–0.01
Adenosine-5′-diphosphate	<0.3[b]
Adenosine-5′-triphosphate	
Nicotinamide-adenine-dinucleotide	0.1
Guanosine-5′-phosphate	0.002
Cytidine-5′-phosphate	0.001
Uridine-5′-phosphate	0.002

[a] Until approx. 1 h post-mortem no IMP is found in muscle.

[b] There is a fairly rapid decrease in post-mortem concentration influenced by cooling and other muscle handling conditions.

Table 12.10. Post-mortem changes in the concentration of some constituents of rabbit muscle (*M. psoas*)

Compound	µmol/g Fresh tissue	
	living muscle	post-rigor muscle
Total acid-soluble phosphorus	68	68
Inorganic phosphorus	<12	>48
Adenosine triphosphate (ATP)	9	<1
Adenosine diphosphate (ADP)	1	<1
Inosine monophosphate	<1	9
Creatine phosphate	20	<1
Creatine	23	42
NAD/NADP	2	1
Glycogen	50	<10
Glucose-1-phosphate	<1	<1
Glucose-6-phosphate	5	6
Fructose-1,6-bisphosphate	<1	<1
Lactic acid	10	100

12.3.9 Organic Acids

The predominant acid in muscle tissue is the lactic acid formed by glycolysis (0.2–0.8% on a fresh meat weight basis), followed by glycolic (0.1%) and succinic acids (0.05%). Other acids of the *Krebs* cycle are present in negligible amounts.

12.3.10 Carbohydrates

The glycogen content of muscle varies greatly (0.02–1.0% on a fresh tissue weight basis) and is influenced by the age and condition of the animal prior to slaugher. The rate of the post-mortem decrease in glycogen als varies. Sugars are only 0.1–0.15% of the weight of fresh muscle, of which 0.1% is shared by glucose-6-phosphate and other phosphorylated sugars. The free sugars present are glucose (0.009–0.09%), fructose and ribose.

12.3.11 Vitamins

Table 12.11 provides data on water-soluble vitamins in beef muscle.

Table 12.11. Vitamins in beef muscle

Compound	mg/kg Fresh tissue
Thiamine	0.6–1.6
Riboflavin	1–34
Nicotinamide	40–120
Pyridoxine, pyridoxal, pyridoxamine	1–4
Pantothenic acid	4–10
Folic acid	0.03
Biotin	0.05
Cyanocobalamine (B_{12})	0.01–0.02
α-Tocopherol	4.8
Retinol	0.2
Vitamin K	0.13

12.3.12 Minerals

Table 12.12 provides data on minerals in meat. Table 12.13 provides data on the occurrence of soluble and insoluble iron in meat of different animals. The other trace elements, which are 1 mg/kg fresh meat tissue, are not listed individually.

Table 12.12. Minerals in beef muscle

Element	% in fresh tissue	Element	%in fresh tissue
K	0.25–0.4	Zn	0.001–0.008
Na	0.07–0.2	P (as	
Mg	0.015–0.035	P_2O_5)	0.30–0.55
Ca	0.005–0.025	Cl	0.04–0.1
Fe	0.001–0.005		

12.4 Post Mortem Changes in Muscle

Immediately after death, the muscle is soft, limp, and dry and can be reversibly extended by using a low load (5–15 kPa). Cadaveric rigidity (rigor mortis) occurs after a few hours. The muscle can then be extended only by using a heavy load (>200 kPa) and becomes moist or wet. Rigor can occur in various stages of contraction or stretching. It subsides after some time and the muscle can be easily extended, but irreversibly. Meat with a more or less tender consistency is formed from the muscle. This process is caused by complicated proteolytic reactions, which are discussed in 12.4.3.

12.4.1 Rigor Mortis

Cessation of blood circulation ends the O_2 supply to muscle. Anaerobic conditions start to develop. The energy-rich phosphates, such as creatine phosphate, ATP and ADP, are degraded. The glycolysis process, which is pH and temperature dependent and which is influenced by the presence of glycogen, is the sole remaining energy source. The lactic acid formed remains in the muscle, thereby decreasing the muscle pH from 6.5 to less than 5.8.

Table 12.10 gives an example of post mortem changes in rabbit *Psoas major* as related to concentrations of some of the more important muscle tissue constituents. The data shown in Fig. 12.22 illustrate the post mortem decreases in pH, creatine phosphate and ATP in beef *Longissimus dorsi* and *Psoas major* muscles and emphasizes that the changes are dependent on the type of muscle.

Table 12.13. Occurrence of iron in meat of different animal species

Animal species	Concentration (μg/g)[a]		Distribution of soluble iron (%)[a]			
	Insoluble Fe	Soluble Fe	Ferritin	Hemoglobin	Myoglobin	Free Fe
Beef (rump steak)	5.9	20.0	1.6	6.0	89.0	3.4
Pork (loin)	3.0	3.6	8.4	22.2	64.0	5.4
Lamb (loin)	5.9	12.3	7.3	13.0	74.0	5.7
Chicken (leg)	4.7	3.4	26.4	55.7	12.1	5.8

[a] Average value of three meat samples.

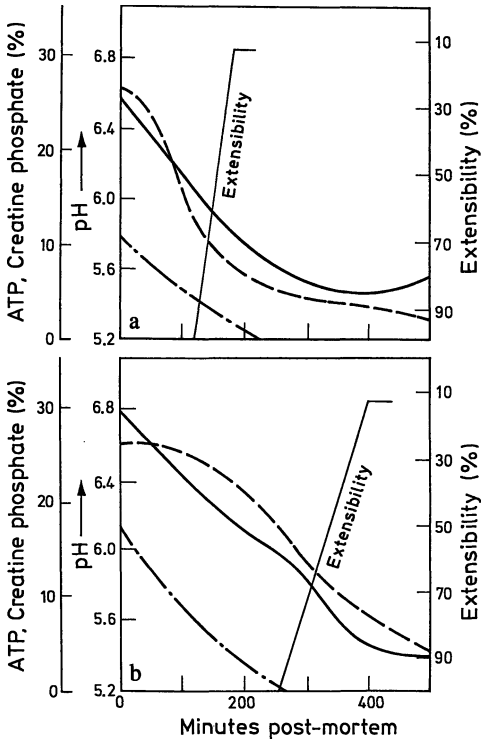


Fig. 12.22. Post-mortem changes in beef muscle. **a** *M. longissimus dorsi*; **b** *M. psoas*; ——: pH value; - - -: ATP as % of the total acid-soluble phosphate; -·-·-: creatine phosphate as % of the total acid soluble phosphate. (according to *Hamm*, 1972)

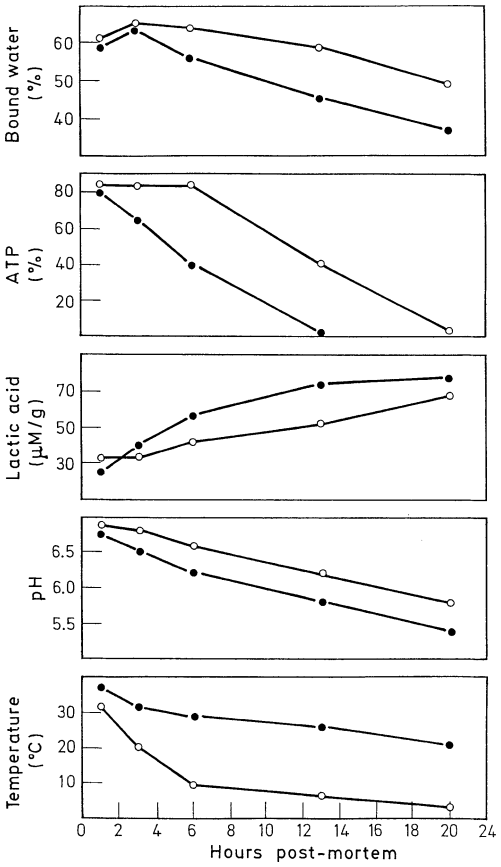


Fig. 12.23. The effect of temperature on post mortem changes in beef muscle.
M. semimembranosus ●—●: normal cooling, animal carcass kept for the first hour post-mortem at 2–4 °C then posterior hind quarters cut and kept at 14 °C for 10 h followed by 2 °C; ○—○: cooling in ice, hind quarters 11 h in crushed ice, followed by 2 °C. Temperature measurement of the meat at 4 cm depth; bound water as percent of total water; lactic acid results are on fresh weight basis and ATP expressed as percent of total nucleotides. (according to *Disney* et al., 1967)

Although muscle tissue is soft and flexible and dry on the surface immediately following death, its flexibility or extensibility is lost very rapidly. ATP breaks down (Fig. 12.22). The muscle tissue becomes stiff and rigid (death's stiffening, rigor mortis; cf. 12.3.2.1.5 and 12.3.2.1.6) and, as the rigor proceeds, the muscle tissue surface becomes wetter. The depletion of the energy reserves results in the distribution of the calcium ions, which are stored in the mitochondria and in the sarcoplasmic reticulum, throughout the entire intracellular matrix.

The onset of rigor mortis occurs in beef muscle within 10–24 h; in pork, 4–18 h; and in chicken, 2–4 h.

The rate of decrease in pH and the final pH value of meat are of significance for water holding capacity and, therefore, for meat quality. Figure 12.23 shows that a more rapid and intensive cooling of the post mortem muscle results in meat with a noticeably higher water holding capacity than that of muscle cooled slowly.

12.4.2 Defects (PSE and DFD Meat)

Rapid drops in ATP and pH (Fig. 12.24) cause pork muscle to become pale and soft and to undergo extensive drip loss because of lowered wa-

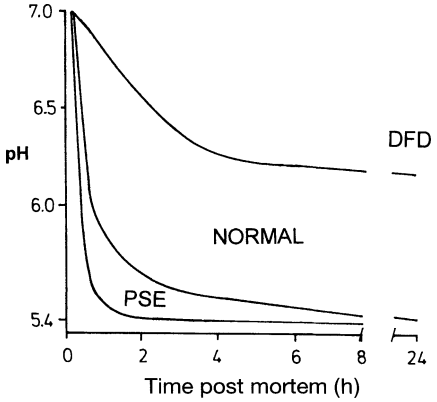


Fig. 12.24. Post mortem decrease in pH in normal meat, PSE meat and DFD meat in the case of pork (according to *Moss*, 1992)

Table 12.14. Some differences between normal and faulty meat[a]

	Quality	pH (1 h)	pH (24 h)	ATP	Glycogen	Lactate
Normal meat		6.5	5.8	2.2	6.2	4.7
PSE-Meat	Pale, exudative, loose soft texture	5.6	5.6	0.3	1.9	9.0
DFD-Meat	Dark, sticky, firm texture	6.5	6.3	1.1	1.5	4.0

[a] Pork meat: *M. longissimus dorsi*. Values are averages expressed as mg/g muscle 1 h post mortem; pH 1 h (initial) and pH 24 h (final) values post mortem.

ter holding capacity (PSE meat: pale, soft, exudative). PSE meat has a low tensile strength and loses a substantial amount of weight when hung and, when thawed, drip losses occur. Such defects are typical of hogs with an inherited sensitivity towards stress, such as fear prior to slaughter, anxiety during transport, exposure to temperature changes, etc. Immediately prior to or during slaughtering, an abnormally rapid ATP breakdown occurs and, consequently, the rate of glycolysis is accelerated. The pH value falls rapidly and the body temperature, which would normally drop (from 38 °C to 36 °C in 45 minutes post mortem), rises to 40–41 °C as a result of the intensified metabolism.

The falling pH value and the high temperature cause protein denaturation. Soluble proteins precipitate and scatter light. Consequently, the meat appears paler in spite of the unchanged myoglobin content. At the same time, cell membranes disintegrate and water loss increases. In fact, PSE pork incurs up to 15% drip loss in 3 days and normal meat only ca. 4%.

The occurrence of dark and firm pork meat (DFD meat: dark, firm and dry) is likewise characteristic of a stress-impaired hog. Since glycogen is largely used up due to stress, only a little lactic acid is formed after slaughtering and the pH hardly falls (Fig. 12.24). The microfibrils which are more swollen at higher pH values bind more water (dry texture). As a result of this effect and the higher stability of oxymyoglobin at higher pH values (cf. 12.3.2.2.3), the color appears darker than normal meat. The relatively high pH value makes DFD meat susceptible to microbial infection and, therefore, not suitable for raw meat products.

Data relating to normal and faulty cuts of meat are summarized in Table 12.14. Both defects mentioned may occur in different muscles of the same animal. The PSE effect is not significant in beef muscle tissue since energy is available from fat oxidation so glycogen breakdown can occur slowly. These meat defects may be avoided in hog muscles by careful handling of stress-sensitive animals and by rapid cooling of carcasses.

12.4.3 Aging of Meat

Rigor mortis in beef muscle tissue is usually resolved 2–3 days post mortem. By this time, the meat again becomes soft and tender (aging). Further aging of the meat to improve tenderness and to form aroma requires various amounts of time, depending on the temperature. At temperatures around 3 °C (−1 °C to +7 °C), aging of poultry requires at least 36 h, pork 60 h, veal 7 days and beef 14 days. Apart from the animal species, the age of the animal (degree of cross linkage of collagen) and the released enzymes influence the duration of aging. A slight rise in pH is observed with aging, the water holding capacity is increased somewhat and, also, fluid loss from heat-treated meat is slightly decreased.

Table 12.15. Endopeptidases involved in the aging of meat

Origin	Enzyme	M_r	pH range	Hydrolysis
Sarcoplasma	μ-Calpain[a]	110,000	6.5–7.5	Z line proteins
	m-Calpain[a]	110,000	6.5–7.5	Z line proteins
Lysosomes	Cathepsin B[a]	25,000	3.5–6.0	
	Cathepsin L[a]	28,000	3.0–6.0	Myosin, actin,
	Cathepsin D[a]	42,000	3.0–6.0	troponin, collagen

[a] Cysteine endopeptidase.
[b] Aspartic acid endopeptidase.

Maturation or aging is accompanied by morphological changes which primarily affect the cytoskeleton. Microexaminations show that the Z lines, which as cross structures (cf. 12.2.1) separate the individual sarcomeres in the muscle fibril, are broken up during aging. In addition, the fibrillar proteins titin and desmin are degraded. In comparison, the contractile proteins myosin and actin are stable. They are attacked only at temperatures above 25 °C. The connective tissue present outside the muscle cells also remains intact.

The degradation of the myofibrillar proteins is catalyzed by endopeptidases. The participation of the enzymes listed in Table 12.15 is under discussion. Special attention is directed to μ-calpain, which, like m-calpain, is activated by the Ca ions liberated during the rigor phase. Both calpains are cysteine endopeptidases, which consist of a large (80 kdal) and a small (28 kdal) subunit. The large subunit contains the active center. These two calpains can be distinguished by the Ca concentration required for their activation. μ-Calpain requires about 30 μmol/l and m-calpain 250–270 μmol/l. The activity of the calpains is regulated, among other factors, by their endogenous inhibitor calpastatin. It has been proposed that the calpains synergistically cooperate in the aging of meat with the cathepsins shown in Table 12.15. Most of the cathepsins are also cysteine endopeptidases, which are similar to papain. Their endogenously occurring inhibitors are the cystatins. On the whole, the processes involved in the aging of meat are so unclear that it has not been possible to define markers which can predict the development of tenderness in meat.

12.5 Water Holding Capacity of Meat

Muscle tissue contains 20–25% protein and approx. 74–76% water, i. e. 350–360 g water per 100 g protein. Of this total water not more than 5% is bound directly to hydrophilic groups on the proteins. The rest of the water in the muscle tissue, i. e. 95%, is held by capillary forces between the thick and thin filaments. When a larger amount of water is bound to the network, the muscle is more swollen and the meat is softer and juicier. Hence, water holding capacity, protein swelling and meat consistency are intimately interrelated. The extent of water holding by the protein gel network depends on the abundance of cross-links among the peptide chains. These links may be hydrophobic bonds, hydrogen and ionic bonds and may involve divalent metal ions. A decrease in the number of these cross-linkages results in swelling, whereas an increase in the number of cross-linkages results in shrinkage (syneresis) of the protein gel. The transversal swelling of the myofibrils caused by NaCl has been visualized by phase-contrast microscopy. On washing with 40.6–1.0 mol/L of NaCl, first the centers of the myosin polymer A bands (thick filaments) (cf. 12.3.1 and 12.3.2.1.1) are extracted, and, with increasing concentration, the entire bands are extracted. There is a 2.5 fold increase in the diameter of the myofibrils, corresponding to a 6 fold increase in volume. The cause of these changes is attributed to the depolymerization of the thick filaments to give soluble myosin molecules and the weakening of the bonding of myosin heads to actin. Furthermore, weakening of transversal structural ele-

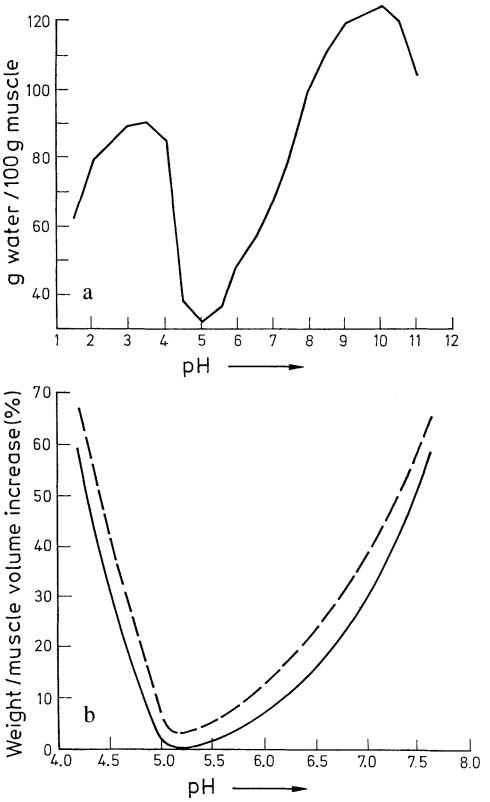


Fig. 12.25. Swelling of meat as affected by pH. **a** Beef muscle homogenate, 5 days post mortem, **b** beef muscle cut in cubes 3 mm edge length, – – – weight increase, — volume increase (according to *Hamm*, 1972)

ments (M line, Z line, cf. 12.3.2.1.4) probably occurs, which facilitates the extension of myofibrils. The water holding capacity of meat is of great practical importance for meat processing and is affected by pH and the ion environment of the proteins (cf. 1.4.3.1 and 1.4.3.3).

The total charge on the proteins and, hence, their electrostatic interactions are the highest at their isoelectric points. Therefore, meat swelling is minimal in the pH range of 5.0–5.5 (Fig. 12.25). Addition of salt to meat shifts the isoelectric point and, hence, the corresponding swelling minimum to lower pH values, due to the prefered binding of the anion. This means that, in the presence of salts, water holding is increased at all pH's higher than the isoelectric point of the unsalted meat (Fig. 12.26).

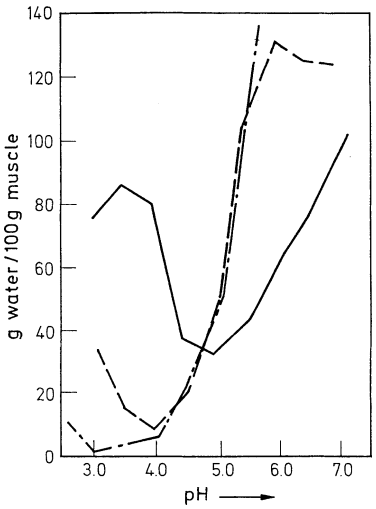


Fig. 12.26. Swelling of meat as affected by salts. Beef muscle homogenate; the ionic strength of the salt added to homogenate is $\mu = 0.20$; — control, – – – NaCl, – · – · – NaSCN (according to *Hamm*, 1972)

The water holding capacity of muscle tissue soon after slaughter is high because the muscle

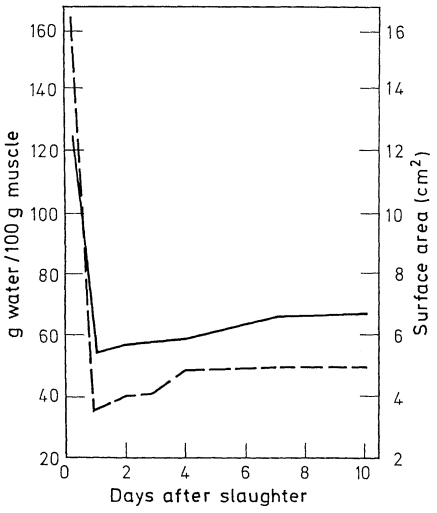


Fig. 12.27. Water holding capacity and rigidity of beef muscle. — Water holding capacity, – – – rigidity (stiffness) expressed as the surface area acquired by homogenate after being pressed between filter papers, under standardized conditions (according to *Hamm*, 1972)

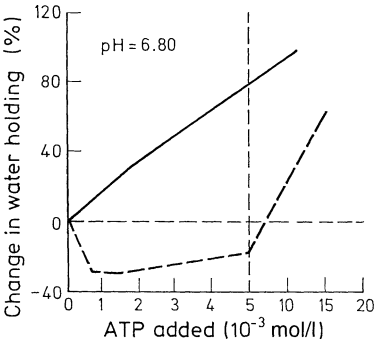


Fig. 12.28. Swelling of meat as affected by ATP addition. Beef muscle homogenate; pH 6.8; — 2 h post-mortem, – – – 4 days post-mortem (according to *Hamm*, 1972)

is still warm and due to the presence of high concentrations of ATP. After the onset of rigor mortis ATP breaks down, the rigidity of the tissue increases and the water holding capacity starts to decrease (Fig. 12.27). Addition of ATP to muscle tissue homogenates prior to the onset of rigor mortis brings about a rise in tissue swelling (Fig. 12.28). Addition of low levels of ATP (to about 1×10^{-3} molar) during post-rigor brings about tissue contraction or shrinkage, while higher levels of ATP cause tissue swelling (Fig. 12.28). This influence on swelling, however, is of short duration since, as ATP breaks down, contraction and shrinkage take place. Nevertheless, these studies amply illustrate the softening effect of ATP and, as already mentioned, the ability of ATP to dissociate actinmyosin complexes (cf. 12.3.2.1.5 and 12.3.2.1.6). Thus, because of high ATP levels and high pH, the slaughtered muscle which is still warm has a high water holding capacity, whereas post-rigor meat, with low ATP and low pH, has a low water holding capacity.

12.6 Kinds of Meat, Storage, Processing

Modern slaughterhouses are higly automated. After the delivered animals are stunned either electrically, or by using a bolt apparatus, or with CO_2, they are bled. The blood (3–4% of the live weight) can be processed into plasma (60–70%) and blood concentrate (30–40%, hemoglobin). The animal bodies are then passed to skinning machines via scalding vats and unhairing machines. Subsequently, the animals are disemboweled, the red organs and the stomach/intestine package are separated for further processing. The animals sides are passed through a shock tunnel (air temperature −4 to −10 °C, 1–2 h). They are stored in the cold until they are cut up on conveyor belts. During processing, accumulating fat is fed to the grease boiler. All discarded materials and bones are processed into meat and bone meal in carcass processing plants. The waste water is treated using specific processes.

12.6.1 Kinds of Meat, By-Products

12.6.1.1 Beef

The most important categories are:

- Young bull meat from full-grown animals (18–22 months, live weight >300 kg): fine fibered, well marbled.
- Cow meat from animals (>2 years) which have already calved: medium red to brown red, moderately fine to coarse fibered, yellow fat, marbled.
- Heifer meat from young, full-grown female animals (15 to 24 months) which have not calved: red, fine fibered, white fat.

The meat of bulls (>5 years) and oxen (2–3 years) is of little economic importance. The average amount of waste from slaughterhouse oxen is 40–55%; that from cows, 42–66%. Beef carcasses are hung for 4–8 days before being cut up for soup meat, and 10–14 days for roasts or steaks.

12.6.1.2 Veal

Meat from young cattle (ca. 4 months) with a body weight up to 150 kg when slaughtered. Color: pale red. The meat aroma is weaker than that of beef. The meat is hung for 8 days before use.

12.6.1.3 Mutton and Lamb

Depending on the age of the animals, the meat has a light, brick, or dark red color and is generally interpersed with fat tissue. The most important types are:

- Lamb from animals not older than 6 months (milk lamb) or 12 months (fattened lamb).
- Mutton from male, castrated and female animals not older than 2 years. The meat of older animals is called *sheep meat*.
- Sheep meat

The odor and taste of mutton and sheep meat are specific.

12.6.1.4 Goat Meat

Goat meat is generally from young animals (2–4 months).

12.6.1.5 Pork

The meat is from very young animals (sucking pig) or from 5–7 month old animals. It exhibits a fairly soft consistency and is fine fibered with a pale pink, pink or whitish grey color. The meat should be hung for 3–4 days before use. The meat becomes greyish-white when cooked, making it different from all other meats. Pork is interspersed and entwined with fat.

12.6.1.6 Horse Meat

The meat of a young horse is bright red, whereas that of older horses is dark or reddish-brown or, when exposed to air, darkens to a reddish-black color. The consistency of the meat is firm and compact and the muscle tissue is not marbled with fat. During cooking, the white fat (melting point 30 °C) appears as yellow droplets on the surface of the broth. The characteristic sweet flavor and taste of the meat are derived from the high glycogen content. In addition to the determination of glycogen, an immunoassay (cf. 2.6.3) and fatty acid analysis can be used to detect horse meat. Horse fat is characterized by a higher content of linolenic acid than beef or pork lard.

12.6.1.7 Poultry

The color of poultry meat differs according to age, breed and body part (breast meat is light, thighs and drumsticks are dark). Species of poultry which have dark meat (geese, ducks, pigeons) can be distinguished from those with light meat (chickens, turkeys, peacocks). The age, breed and feeding of the bird influence meat quality. Poultry fat tends to become rancid because of its high content of unsaturated fatty acids.

12.6.1.8 Game

Wild game can be divided into fur-bearing animals: deer (antelope, caribou, elk, white-tailed deer), wild boars (wild pigs) and other wild game (hare, rabbit, badger, beaver, bear); and birds or fowl (heathcock, partridge, pheasant, snipe, etc.). The meat of wild game consists of fragile fibers with a firm consistency. The meat remains red to red-brown in color. It has low amounts of connective and adipose tissues. The taste and flavor of each type of wild meat is characteristic. Aging of the meat requires a longer time than meat from domestic animals because of the thick and compact muscle tissue structure. The meat then becomes dark-brown to black-red.

12.6.1.9 Variety Meats

Meats of various animal organs are called variety meats. They include tongue, heart, liver, kidney, spleen, brains, retina, intestines, tripe (the first and second stomachs of ruminants), bladder, pork crackling (skin), cow udders, etc. Many of these variety meats, such as liver, kidney or heart, are highly-valued foods because they contain vitamins and trace elements as well as high quality protein. Liver contributes the specific aroma of liver sausage and pastes (goose liver). Liver is also consumed as such. Heart, kidney, lungs, pork or beef stomach, calf giblets and cow's udders are incorporated into sausages: spleen is also made into sausage. Tongues are cooked, pickled and smoked, used for the production of better-quality sausages, and canned or sold as fresh meat. Calf brain and sweetbreads (thymus glands) are especially valued as food for patients. The compo-

Table 12.16. Average composition of some internal organs and blood (g/100 g edible portion)

Organ	Moisture	Protein	Fat	Carbohydrate	Caloric value (kJ)
Heart					
beef	75.5	16.8	6.0	0.56	517
pork	76.8	16.9	2.6	0.4	390
Kidney					
beef	76.1	16.6	5.1	–	471
pork	76.3	16.5	3.8	0.80	435
Liver					
beef	69.9	19.7	3.1	5.90	550
pork	71.8	20.1	4.9	1.14	542
Spleen					
beef	76.7	18.5	2.9		422
pork	77.4	17.2	3.6	–	426
Tongue, beef	66.8	16.0	15.9	0.4	867
Lung, pork	79.1	13.5	6.7	–	477
Brain, veal	80.4	9.8	7.6	0.8	461
Thymus, veal	77.7	17.2	3.4	–	418
Blood					
beef	80.5	17.8	0.13	0.065	309
pork	79.2	18.5	0.11	0.06	319

sitions of some variety meats are shown in Table 12.16.

Intestines, with their high content of elastin, make excellent natural sausage casings. These and beef stomach are specialty dishes.

Pork skin is an ingredient of jellied meat and blood sausage. It is also consumed directly and is a good source of vitamin D. Cartilage and bones contain tendons and ligaments which are collagen- and elastin-type proteins. Cartilage and bones are similar in composition, with the exception of their mineral content; the former contains 1% minerals and the later averages 22% minerals, ranging from 20–70%. The fat content of bones can be as high as 30% and commonly varies between 10–25%. Spinal cord and ribs, when boiled in water, release gelatin-type substances and fat and, therefore, both are used in soup preparations (bouillon, clear broth or bouillon cubes or concentrated stock).

12.6.1.10 Blood

The blood which drains from a slaughtered animal is, on the average, about 3–4% of the live weight (oxen, cows, calves) but is particularly high for horses (9.98%) and low for hogs (3.3%). Blood has been used since ancient times for making blood and red sausages and other food products.

Blood consists of protein-rich plasma in which the cells or corpuscles are suspended. They are the red and white blood cells (erythrocytes and leucocytes, respectively) and the platelets (thrombocytes). The red blood cells do not have nuclei and are flexible round or elliptical discs with indented centers. The diameters of red blood cells vary (in μm: 4 in goat; 6 in pig; 10 in whale; and up to 50 or more in birds, amphibians, reptiles and fish). Red blood cells contain hemoglobin, the red blood pigment. White blood cells contain nuclei but no pigments, are surrounded by membranes, are 4–14 μm in diameter and are fewer in number than red blood cells. In addition to salts (potassium phosphate, sodium chloride and lesser amounts of Ca-, Mg- and Fe-salts), various proteins, such as albumins, globulins and fibrinogen, are present in blood.

The N-containing low molecular weight substances ("residual nitrogen") of blood comprise primarily urea and lesser amounts of amino acids, uric acid, creatine and creatinine. During coagulation or clotting of blood, the soluble fibrinogen in the plasma is converted to insoluble fibrin fibers which separate as a clot. Coagulation is a complex reaction catalyzed by the enzyme thrombin, the precursor of which is prothrombin. Thrombin reacts with fibrinogen to form insoluble fibrin. The mesh of long fibrin fibers traps and holds blood cells (platelets, erythrocytes and leucocytes). Hence, the clot is colored red. The remaining fluid, which contains albumins and globulins, is the serum. Blood plasma contains 0.3–0.4% fibrinogen and 6.5–8.5% albumin plus globulin in the ratio of 2.9:2.0.

The composition of blood is given in Table 12.16. Blood clotting requires the presence of Ca^{2+} ions. Hence, Ca^{2+}-binding agents, such as citrate, phosphate, oxalate and fluoride, prevent blood coagulation. In the processing of blood into food, coagulation is occasionally retarded by stirring the blood with metal rods onto which the fibrin deposits. Currently, blood clotting is inhibited by using Ca^{2+}-complexing salts. After centrifugation, blood stabilized in this way yields about 70% of plasma containing 7–8%

protein. The proteins can be processed further by spray-drying into powdered plasma. Recovery of liquid plasma is permitted only from the blood of cattle (excluding calves) and hogs. Addition of dried and liquid plasma to processed meats is legal. Citrate and/or phosphate are used as calcium-binding agents.

12.6.1.11 Glandular Products

Animal glands, such as the adrenal, pancreas, pineal, mammary, ovary, pituitary and thyroid glands, provide useful by-products for pharmaceutical use. Some of these products are adrenalin, cortisone, epinephrine, insulin, progesterone, trypsin and thyroid gland extract.

12.6.2 Storage and Preservation Processes

Meat must be appropriately treated to allow storage.

12.6.2.1 Cooling

Refrigeration (cooling or freezing the meat) is an important process for prolonged preservation of fresh meat. Carcasses in the form of sides or quarters are cooled. Cooling is performed slowly (e. g., with a blast of air at 0.5 m/s at 4 °C) or quickly (e. g., stepwise for 3 h with a 3.5 m/s blast of air at −10 °C, for 19 h with a blast of air at 1.2 m/s at 2 °C, and over 18 days with air at 4 °C). The shelf-life of meat at 0 °C is 3 to 6 weeks. Weight loss due to moisture evaporation is low at high relative humidities, and decreases as the water holding capacity increases.

If meat is cooled to cold storage temperatures (<10 °C) before rigor occurs, it shrinks and becomes tough. This is due to the fact that at low temperatures, binding of $Ca^{2\oplus}$ by the sarcoplasmic reticulum and mitochondria is reduced, the $Ca^{2\oplus}$ concentration in the intracellular space is increased, inducing contraction (cf. 12.3.2.1.5). To prevent this phenomenon, meat is kept at 15–16 °C for 16–24 h and cooled after rigor has occurred. Electrical stimulation is also possible. This process causes rigor by accelerating glycolysis and a decline in pH. The same effect is achieved by stunning the animals with CO_2.

As long as the meat is stored in the cold in large cuts, lipid oxidation is very slow. Only chopping or mincing or warming of the muscle tissue causes a high rate of peroxidation. Muscle disintegration results in a low but significant release of highly unsaturated membrane phospholipids and Fe^{2+} ions from myoglobin. This non-heme iron is an effective catalyst of lipid peroxidation. Its concentration increases during cooking, as shown for beef in Table 12.18. Even after short cold storage of heated meat and subsequent warming, a rancid off-flavor may develop (warmed over flavor, WOF) (cf. 12.6.2.6 and 12.9.4).

Curing prevents WOF. Myoglobin is stabilized by nitrite, therefore, no additional non-heme iron is formed during cooking (Table 12.18). In addition, the MbNO formed has an antioxidative effect (cf. 12.3.2.2.4). Lipid peroxidation does not occur and new aroma substances are formed that are characteristic of cured meat.

12.6.2.2 Freezing

The shelf life of meat is substantially lengthened by freezing. Freezing can be performed in a single step (direct freezing) or in a two step process (initial cooling followed by freezing) using an air

Table 12.17. Loss of quality of frozen chicken from producer to consumer[a]

Frozen food chain	Average storage temperature (°C)	Shelf-life (day)	Quality loss (% per day)	Average storage time (day)	Quality loss (%)
Producer	−23	540	0.186	40	7.5
Transport	−20	420	0.239	2	0.5
Wholesaler	−22	520	0.196	190	37.1
Transport	−16	370	0.370	1	0.4
Retailer	−20	420	0.239	30	7.2
	−14[b]	210	0.476	3	1.4
Transport	−7	60	1.67	0.17	0.3
Consumer	−12	150	0.666	14	9.3
Σ				280	63.7

[a] For definition cf. Fig. 12.29.

[b] A temperature estimate for food storage on the surface of open freezers.

Table 12.18. Oxidative fat deterioration in cooked beef

	Beef			
	Raw	Cooked	Cured[a]	Cured and cooked[a]
Non-heme iron (μg/g)	6.62	10.8	6.65	6.80
Storage at 4 °C	Malonic aldehyde	(mg/kg)[b]		
0. Day	0.58	0.56		
5. Day	1.55	0.48		
12. Day	2.78	0.47		
21. Day	2.83	0.54		

[a] Cured with 156 mg/kg nitrite.
[b] Determined with the thiobarbituric acid test.

blast freezer with an air temperature of −40 °C and an air stream velocity of 3–10 m/s. The shelf life for storage at −18 °C to −20 °C and 90% relative humidity is 9 to 15 months. The shelf life of frozen chicken, as affected by storage temperature, is presented in Fig. 12.29, while Table 12.17 shows the deterioration of frozen chicken as it is shipped from producer to consumer. The shelf life is largely determined by oxidative changes affecting the lipids, which take place more readily in poultry (ducks, geese, chickens) and pork than in beef or mutton.

The water holding capacity of frozen meat increases as the freezing temperature decreases.

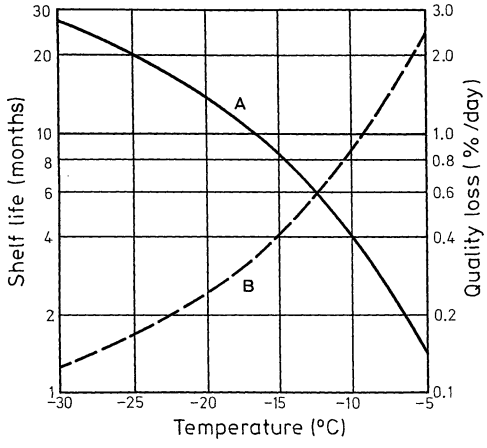


Fig. 12.29. Shelf life of frozen chicken as affected by storage temperature. — Shelf life A, – – – quality loss B = 100/A (according to *Gutschmidt*, 1974)

Water holding capacity also remains high when freezing is performed rapidly. Under these conditions the formation of large ice crystals is suppressed and damage to membranes and the irreversible change in myofibrillar proteins caused by temporary high salt concentrations are avoided.

Freezing meat immediately after slaughtering, without precooling (single-stage process), causes substantial shortening and high fluid losses on thawing, if the meat freezes completely before rigor occurs. However, this is possible only with smaller cuts. The reason for this phenomenon is an extremely fast ATP breakdown with a corresponding decrease in the water holding capacity. The sudden high rate of ATP breakdown is initiated by release of Ca^{2+} ions from the sarcoplasmic reticulum, which triggers the high activity of myosin-ATPase ("thaw rigor"). This "thaw rigor", which is associated with toughness, can be avoided if the warm meat is frozen and then minced in the frozen state after addition of NaCl. Thaw rigor may also be avoided by disintegrating warm meat in the presence of NaCl and then freezing it.

Freezing matured meat results in lower fluid losses than freezing meat in a prerigor or rigor state. However, this process is not widely used for economic reasons. Rigor can be induced before freezing by using electrical stimulation. Long storage of frozen meat results in a decrease in water holding capacity. Solubility changes and shifts in the isoelectric point of proteins of the sarcoplasm and contractile apparatus are observed.

Slow thawing of frozen meat is generally considered more favorable than rapid thawing, although some opposing data exist. Obviously, freezing, storage and thawing should be considered as related process steps, which should be coordinated.

12.6.2.3 Drying

Drying is an ancient method of meat preservation. Drying is frequently used in combination with salting, curing, and smoking. Some processes are: drying in a stream of hot air (40–60 °C), drying in vacuum under variable conditions, e. g., in hot fat, and freeze-drying, the most gentle process. The moisture content of the end product is usually 3–10%. Important quality criteria of such dried meat products are the rehydration capacity, which can be determined by water uptake under standard conditions, and the fraction of firmly-bound water. The drying process should not affect the water holding and aroma characteristics of the meat. The shelf life of dried meat products is limited by the development of off-flavors due to fat oxidation and by discoloration due to the *Maillard* reaction. Dried beef and chicken are important ingredients of many soup powders. In addition to pieces of meat, minced meat, with or without binders, and processed meats, e. g., meat balls or dumplings, are also dried for this purpose.

12.6.2.4 Salt and Pickle Curing

Salt in high concentrations inhibits the growth of microorganisms and curtails activity of meat enzymes. Hence, salt is considered as a meat preservative. Salting meat at a level up to 5% NaCl causes swelling (cf. Fig. 12.26). Higher salt concentrations (10–20%) induce shrinkage in meat and its products, causing a decrease in moisture to a level below that of untreated meat. The meat retains its natural color, usually dark red, since the myoglobin concentration increases due to the moisture loss. The color of such meat changes upon cooking to grayish-brown.

Salting by the addition of sodium nitrite and/or nitrate (curing or pickling) produces products with highly stable color (cf. 12.3.2.2.4). Since nitrite reacts faster and less is required for color stabilization, it is widely used in place of nitrate. Salt curing is done either by rubbing salt on the meat surface (dry curing or pickling), by submerging the meat in 15–20% brine (wet pickle curing), or by injection of brine in special automats.

Additives, such as sugar or spices, which favorably affect the red color and formation of meat aroma, are often added to pickling salts. The aroma of cured meat is specific and differs from that of noncured meat. Aroma formation is enhanced by the microflora (*Micrococcus* spp. and *Achromobacter* spp.) of curing brine, which are simultaneously involved in reduction of nitrate (NO_3^-) and nitrite (NO_2^-) ions and thereby contribute to the stabilization of the pinkish or red color of cured meat.

12.6.2.5 Smoking

Smoking of meat is usually associated with salting. Depending on the smoking procedure, the moisture drops 10–40%. Compounds present in smoke with bactericidal and antioxidative properties are deposited on and penetrate into the meat. Important smoke ingredients include phenols, acids, and carbonyl compounds. The concentration of polycyclic hydrocarbons in smoke depends on the type of smoke generation and can be largely suppressed by suitable process management, e. g., by external smoke generation with cleaning of the smoke via cold traps, showers, or filters. A distinction is made between hot smoking (50–85 °C) over a period ranging from less than one hour to several hours (e. g., used for cooked and boiling sausages), warm (25–50 °C) and cold smoking (12–25 °C) over a period ranging from two days to several weeks (e. g., used for raw sausage and ham). Special smoking processes include wet smoking processes, electrostatic processes, and the use of smoke condensates.

12.6.2.6 Heating

Heat treatment is an important finishing process and also serves for the production of canned meat. Typical changes involved in heat treatment are: development of grayish-brown color, protein coagulation, release of juices due to decrease in water holding capacity (Fig. 12.30), increase in

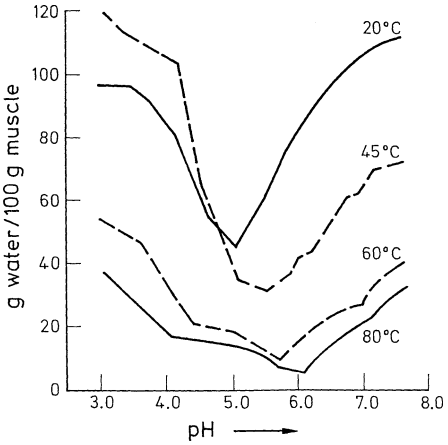


Fig. 12.30. Water holding capacity of beef muscle versus heat treatment and pH. (according to *Hamm*, 1972)

pH, development of a typical cooked or roasted meat aroma and, finally, softening induced by the shrinking and partial conversion of collagen to gelatin (cf. 12.3.2.3.1).
Refrigerated storage of heated meat and reheating may lead to WOF (cf. 12.6.2.1 and 12.9.4).

12.6.2.7 Tenderizing

Plant enzyme preparations (ficin, papain, bromelain) are used to tenderize meat. These substances are either sprayed onto the meat cuts or are distributed via the blood vessels of the animal either shortly before or after slaughtering.

12.7 Meat Products

Canned meat, ham, and sausages, and meat extracts are produced from meat.

12.7.1 Canned Meat

Examples of canned meat are beef and pork in their own juice, corned beef, luncheon meat, cooked sausages, jellied meat, and cured and pickled hams. In order to achieve sterile canned meat, the required heating time and temperature depend on the size and content of the can since heat penetration is highly variable (Table 12.19). Therefore, mathematical models have been developed which allow the temperature to be controlled in such a way that even the coldest point in the contents is heated to a temperature high enough and for long enough to kill pathogenic bacteria and microbes responsible for spoilage. Correspondingly controlled processes are also used in the production of cooked and boiling sausages.

Table 12.19. Effect of can size and product on required heating time of canned meat (time in min to reach 121 °C at the center of the can)

Canned meat	400 g	850 g	2500 g
Beef	47	57	80
Pork	58	98	120
Liver sausage	90	130	
Blood sausage	106	113	130

12.7.2 Ham, Sausages, Pastes

12.7.2.1 Ham, Bacon

12.7.2.1.1 Raw Smoked Hams

After the center ham has been cut (longitudinal or circular), *ham on the bone* is dry, then wet cured (4–7 weeks), matured (reddened) for 2–3 weeks by dry storing, followed by washing, drying and exposure to cold smoke for 4–7 weeks. In *rolled ham*, the bone is taken out, and it is subsequently processed like ham on the bone, except that the curing time is shorter. *Lightly-salted lean hams* are made from cutlet or chop meats by a mild curing process, filled into casings and warm smoked.

12.7.2.1.2 Cooked Ham

Bone-free ham is cured for 2–3 weeks, stored dry to mature, washed and warm smoked. It is subsequently cooked by gently simmering. In the cook-in process, the cured meat is first packed in foil that is resistant to boiling, then cooked

and smoked. *Praha ham* is a special cooked ham which is often baked in a bread dough.

12.7.2.1.3 Bacon

Back fat from the pig is salted, washed, dried, and cold smoked.

12.7.2.2 Sausages

Sausage manufacturing consists of grinding, mincing or chopping the muscle tissue and other organs and blending them with fat, salts, seasonings (herbs and spices) and, when necessary, with binders or extenders. The sausage mix or dough is then stuffed into cylindrical synthetic or cellulose casings or tubings of traditional sausage shape or, often, natural casings, such as hog or sheep intestines or the hog's bun (for liver sausage) are used. They are sold as raw, precooked or cooked, and/or smoked sausages. The composition of ham and sausage products is shown in Table 12.20. The different types of sausages have in common that a continuous, hydrophilic salt/protein/water matrix stabilizes a disperse phase (coarse meat/fat particles, fat globules, insoluble proteins, connective tissue, and seasoning particles). The stability of systems of this type is influenced by the pH value, ionic strength, melting range of the lipids, and by the protein content. In finely ground systems with emulsion character, the grinding temperature is also important for stability. A temperature of 14 °C is regarded as optimal, unstable products resulting at $T > 20$ °C.

In the emulsions mentioned above, a monomolecular protein film is formed around the fat globules present (Fig. 12.31). The importance of the different protein components as film formers decreases in the following order: myosin > actomyosin > sarcoplasma proteins > actin. The hydrophobic heads of the myosin molecules evidently dip into the fat globules, while the tails interact with actomyosin in the continuous phase. The monomolecular myosin layer formed in this way should have a thickness of ~130 nm. On the outside, there is probably a multimolecular actomyosin layer which binds water and contributes to the stabilization of the emulsion because of its viscous, elastic, and cohesive properties. Higher temperatures, which lead to destabilization (see above), probably cause increased protein/protein interactions in the actomyosin layer which, in turn, result in a decrease in the water binding, elasticity losses, and disturbances in the myosin film.

While the formation of myosin films on fat globules is responsible for the stabilization of raw sausages with emulsion character, protein/protein interactions and gel formation are important for the stabilization of fat and water in the system in the case of cooked and boiling sausages.

Table 12.20. Protein and fat content of ham and sausage products

Product	Moisture %	Protein %	Fat %	Caloric value (kJ) (kJ/100 g)
Salami (German style)	40	21	33	1578
Cervelat sausage	41	20	34	1598
Knackwurst	60	12	26	1166
Bratwurst (pork)	57	12	29	1277
Hunter's sausage	64	16	16	864
Gelbwurst	58	11	27	1186
Munich Weisswurst (white sausage Munich style)	62	11	25	1112
Bockwurst	59	12	25	1129
Liver sausage	52	12	29	1351
Rotwurst	56	12	29	1277
Ham, raw	43	18	33	1527
Ham, cooked	70	23	4	539
Bacon, marbled	20	9	65	2558

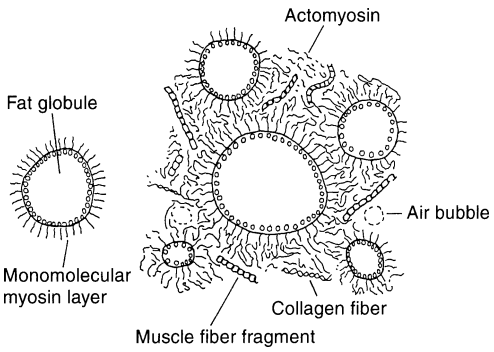


Fig. 12.31. Schematic representation of a sausage emulsion (according to *Morrissey* et al., 1987)

12.7.2.2.1 Raw Sausages

Typical products are Cervelat sausage, salami and the German Mettwurst. They are made of raw, ground skeletal muscle (pH < 5.8), fat and spices. They are cured by the addition of 2.8–3.2% of nitrite curing salt (cf. 22.2.4) or common salt and sodium nitrate (max. 300 mg/kg), max. 2% of sugar (sucrose, glucose, maltose, lactose) and other curing aids (D-gluconic acid-5-lactone, ascorbic acid etc.). Starter cultures are normally added to optimize ripening. The production of raw sausage is schematically presented in Fig. 12.32.

The grinding is preferably carried out in a cutter in which the following steps are conducted: cutting, distribution, mixing, very fine grinding, and emulsification. A cutter consists essentially of a dish rotating around a highspeed cutter head. Products of varying texture can be produced by varying the form and speed of the cutter, the size of the cutting chamber (possible insertion of stowing rings), plate speed, and cutting duration. Operation under a vacuum has advantages. Apart from batch cutters with a dish content of up to 750 l, continuously operating dish cutters are also available today.

In the case of firm types of raw sausages, frozen material is used for grinding (−20 °C) and the temperature is kept below 4 °C during the grinding process by cooling. After the mass has been stuffed, the sausage is ripened in air conditioned rooms. At first, the temperature is 20–26 °C (air humidity 90–95%) to increase lactobacilii; it is later reduced to 10–15 °C (air humidity 75%).

The specific aroma is formed in the course of ripening by microorganisms present (micrococci and lactobacilli, often added in the form of starter cultures). Reddening (cf. 12.3.2.2.4) also plays a big role. The drop in pH due to lactic acid formation (5.2–4.8) results in shrinkage of the protein gel. The sausages become stable, firm and suitable for slicing after vaporization of the water released (20–40% weight loss). Ripening takes 2–3 weeks (fast processes) or 7–8 weeks (slow processes). The raw sausage is subsequently smoked, e. g., cold smoking at 16–28 °C. The white layer on various types of salami is due to mold mycelia or, in cheaper products, a layer of lime milk dip.

12.7.2.2.2 Cooked Sausages

Cooked sausages are made from cooked starting materials. Typical products are liver sausage, blood sausage, and jellied sausage. The production of liver sausage is shown schematically in Fig. 12.33. Modern plants generally use cooking cutters in which the following steps are conducted in one machine: preliminary grinding, cooking, mixing, and cutting. In comparison with boiling sausages, cooked sausages can be cut only when cold.

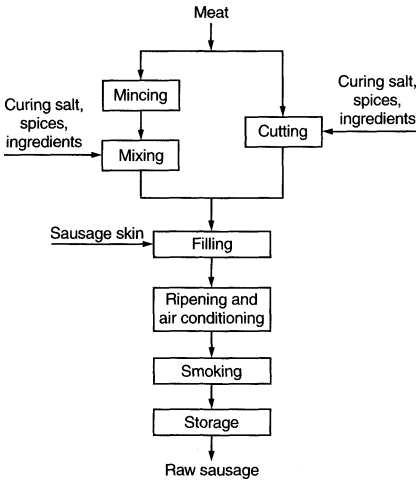


Fig. 12.32. Production of raw sausage

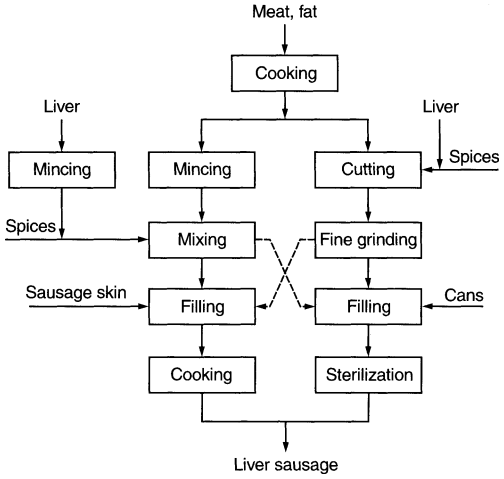


Fig. 12.33. Production of cooked sausage (liver sausage)

12.7.2.2.3 Boiling Sausages

Boiling sausages are made from raw meat (beef, pork, veal) by boiling, baking or frying. During processing in the cutter, the water holding capacity is increased by the addition of common salt and cutter aids (condensed phosphates, lactate, acetate, tartrate, and citrate). The swelling resulting from added salts is caused by an increase in the pH of the meat slurry and by the complexing of divalent cations which, in free form, suppress swelling.

The temperature during grinding/chopping has to be kept low (addition of ice or ice-cold water) since higher temperatures decrease the water holding capacity. Water retention increases as the fat component of the meat slurry is increased as long as the fat:protein ratio does not exceed 2.8 to 1. As a consequence the salt concentration is increased. After chopping and stuffing, the sausages are hot smoked and scalded at 72–78 °C. At this temperature, coagulation of protein gel, which holds the water, forms the broken texture so typical of these sausages.

Typical products are bockwurst, wieners, white and hunter's sausage and mortadella. Figure 12.34 schematically shows the production of boiling sausages.

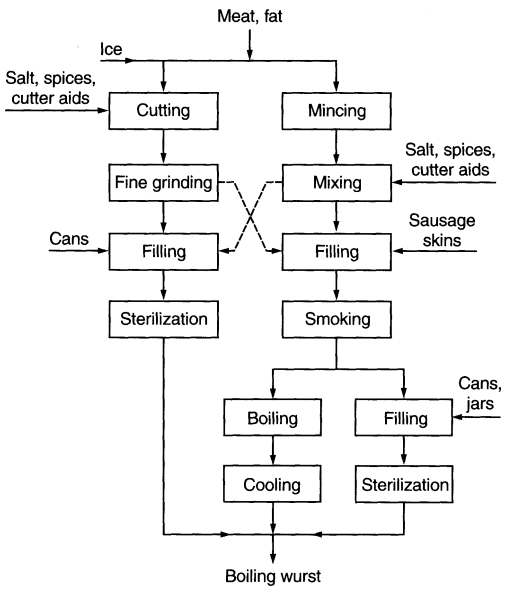


Fig. 12.34. Production of boiling sausage ("Brühwurst")

12.7.2.3 Meat Paste (Pâté)

12.7.2.3.1 Pastes

Meat pastes are delicately cooked meat products made primarily from meat and fat of calves and hogs and, often, from poultry (e. g. goose liver paste) or wild animal meat (hare, deer or boar). Unlike sausages, pastes contain quality meat and are free of slaughter scrapings or other inferior by-products. A portion of meat or the whole meat used is present as finely comminuted spreadable paste.

12.7.2.3.2 Pains

Pains usually consist of larger pieces of meat (especially game and poultry), which are processed into a cooked sausage-like mass with fat, truffle, and various spices.

12.7.3 Meat Extracts and Related Products

12.7.3.1 Beef Extract

Meat extract is a concentrate of water-soluble beef ingredients devoid of fat and proteins. Its preparation dates back to *Liebig's* work in Munich in 1847. Comminuted beef is countercurrently extracted with water at 90 °C. After removal of fat by separators and subsequent filtration, the extract containing 1.5–5% solids is concentrated to 45–65% solids in a multiple stage vacuum evaporator which operates in a decreasing temperature gradient (a range of 92 to 46 °C). The final evaporation to 80–83% solids is then carried out under atmospheric pressure at a temperature of 65 °C or higher or under vacuum on a belt dryer.

In the same way, the cooking water recovered during the production of corned beef can be processed into meat extract. Only this latter source of meat extract is of economic significance. The yield is 4% of fresh meat weight.

Table 12.21. Chemical composition of beef extract

	%
Organic matter	56–64
Amino acids, peptides	15–20
Other N-compounds	10–15
Total creatinine	5.4–8.2
Ammonia	0.2–0.4
Urea	0.1–0.3
N-free compounds	10–15
Total lipids	>1.5
Pigments	10–20
Minerals	18–24
Sodium chloride	2.5–5
Moisture	15–23
pH-value of a 10% aqueous solution	approx. 5.5

The composition of the extract is given in Table 12.21. For addition to soup powders and sauce powders, the thick pasty meat extract is blended with a carrier substance and vacuumor spray-dried.

12.7.3.2 Whale Meat Extract

This product is obtained from meat of various whales (blue, finback, sei, humpback and sperm) in a process similar to that used for beef extract.

12.7.3.3 Poultry Meat Extract

Chicken extract is obtained by evaporation of chicken broth or by extraction of chicken halves with water at 80 °C, followed by a concentration step under vacuum to an end-product of 70–80% solids.

12.7.3.4 Yeast Extract

Yeast cells (*Saccharomyces* and *Torula* spp.) are forced to undergo shrinking of protoplasm by addition of salt, which causes loss of cell water and solutes (plasmolysis), or the cells are steamed or subjected to autolysis. Cells treated in this way are extracted with water and the extract is concentrated to yield a brown paste. Yeast extract is rich in the B-vitamins. The concentrations of thiamine and thiamine diphosphate are above their taste threshold values and may contribute to the product's unpleasant flavor. On the other hand, the spicy flavor of the paste is essentially due to 5′-nucleotides freed during hydrolysis and to amino acids, particularly glutamic acid.

12.7.3.5 Hydrolyzed Vegetable Proteins

The production of this protein hydrolysate is schematically presented in Fig. 12.35. According to the given formulation, the different plant protein-containing raw materials, such as wheat and rice gluten and roughly ground soybeans, palm kernels or peanuts, are automatically delivered from raw material silos, weighed, and fed to a hydrolysis boiler (double-walled,

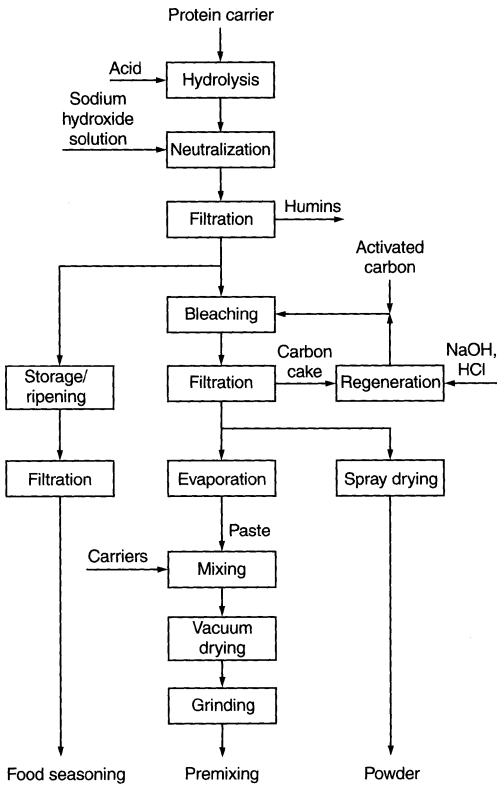


Fig. 12.35. Production of hydrolyzed vegetable protein

pressure-stable stirred tank). Hydrolysis proceeds at temperatures above 100 °C and the appropriate pressure with hydrochloric acid or sulfuric acid (salt-free seasoning).

The hydrolysate is subsequently neutralized to pH 5.8 with sodium or calcium carbonate or with sodium hydroxide solution. In this process, the pH range of 2.5–4 must be passed through as quickly as possible to repress the formation of pyrrolidone carboxylic acid from glutamic acid.

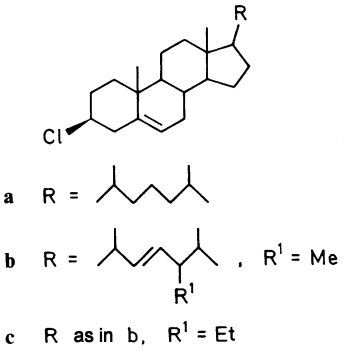
The hydroysate is filtered and the filtrate (seasoning) stored. The filtration residue is washed with water and refiltered, if necessary. The diluted filtrate is evaporated and added to the seasoning obtained in the first step.

The seasoning is subsequently stored; it is filtered several times before filling. Apart from liquid food seasoning, seasoning in paste and powder form and mixtures for use in dry soups and sauces are produced. These products are partly bleached with activated carbon and the taste is neutralized.

The compound 3-hydroxy-4,5-dimethyl-2(5H)-furanone (HD2F, cf. 5.3.1.3) is responsible for the intensive, typical seasoning aroma. The products have a meat- or bouillon-like odor and taste.

It was found in 1978 that genotoxic compounds are formed in hydrochloric acid hydrolysates of protein-containing raw materials. Thus, 3-chloropropane-1,2-diol, 2-chloropropane-1,3-diol, 1,3-dichloropropane-2-ol, 1,2-dichloropropane-3-ol, and 3-chloropropane-1-ol have been identified as secondary products of lipids in amounts of 0.1 to >100 ppm in commercial protein hydrolysates and products derived from them. In feeding experiments on rats, these dichloro compounds were found to be carcinogenic. The testing of the monochloro compounds is still in progress. The chlorinated glycerols, which are partly also present as fatty acid esters, have half life periods of several hundred days in the hydrolysates. The N-(2,3-dihydroxypropyl) derivatives of the amino acids serine and threonine as well as 3-aminopropane-1,2-diol have been detected as aminolysis products.

Chlorinated steriods, e. g., 3-chloro-5-cholestene (Formula 12.27a), 3-chloro-24-methyl-5,22-cholestadiene (Formula 12.27b) and 3-chloro-24-ethyl-5,22-cholestadiene (Formula 12.27c), have been identified in the insoluble residue of the corresponding products.



(12.27)

Moreover, there have been indications of the presence of chlorinated *Maillard* compounds in hydrochloric acid hydrolysates, e. g., 5-(chloromethyl)furfural.

To avoid or minimize the unwanted compounds mentioned above, the production process has been or is being modified, e. g., in the form of an additional alkali treatment of the hydrochloric acid hydrolysate. Thus, concentrations of <1 ppm of 3-chloro-1,2-propanediol were found in the majority of samples tested in 1990, which is clearly less than it was in previous years.

12.8 Dry Soups and Dry Sauces

Meat extract, hydrolysates of vegetable proteins, and yeast autolysate are used to a large extent in the production of dry soups amd dry sauces. For this reason, these substances will be described here. The industrial production of these products for use in home and canteen kitchens has become increasingly important in the past 20 years. In particular, a special pretreatment of the raw materials made possible the development of products which, after quick rehydration, give ready-to-consume complete meals (dry stews), snacks between meals (dry soups, instant soups), or sauces.

12.8.1 Main Components

Not only meat extracts, protein hydrolysates, and yeast autolysates, but also glutamate, ribonucleotides (inosinate/guanylate), and reaction

aromas are used as the taste-bearing substance (cf. 12.9.3). These substances are dried with and without a carrier (belt vacuum drying, spray drying). Flour (wheat, rice, corn), legume flour (peas, lentils, beans), and starches (potato, rice and corn) serve as binding agent. Apart from native flour or starch, swelling flour or instant starch that is pregelatinized by drum drying or boil extrusion is used. In fact, especially good swelling and dispersing properties are achieved by agglomeration. Legumes are precooked in pressure vessels for up to several hours before drying. The rehydration time can be reduced to 4–5 minutes by freeze drying. Standard products are normally air dried on belt dryers. Pasta is subjected to a precooking process by means of steam and/or water or used in a fat dried form, like in the Far East.

Rice is added in a pre-cooked, freeze-dried form or as reformed rice (dried rice flour extrudate). After the appropriate pretreatment (e. g., blanching), vegetables and mushrooms are dried (drum, spray, and freeze drying). Products with instant character are obtained by centrifugal fluidized bed drying. In this process, which is used on a large scale for carrots and rice, the products in a perforated and basket-shaped rotating cylinder are dried with hot air of ca. 130 °C with simultaneous puffing. The fats used are mainly beef fine tallow, hardened plant fats, chicken fat, and milk fat. These fats are often applied in powder form (cf. 14.4.7). The meat additives are primarily beef and chicken which are air dried or freeze dried. To perfect the taste, salt and spices are used as ground natural spices or in the form of spice extracts.

To improve the technological properties, dry soups and sauces contain a series of other ingredients, e. g., milk products, egg products, sugar, and maltodextrin, acids, soybean protein, sugar coloring, and antioxidants.

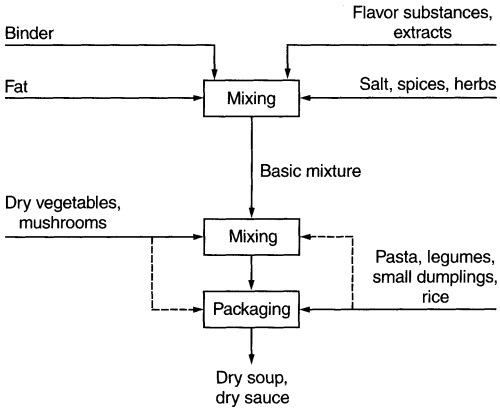


Fig. 12.36. Production of dry soups and sauces

12.8.2 Production

The production of dry soups and sauces essentially involves mixing the preproduced raw materials. The process steps are shown in Fig. 12.36. Weighing of individual components from the raw material silos and their pneumatic dosing into the mixer are conducted automatically. In soup mixtures that contain breakable components, such as pasta and dry vegetables, a basic mixture of the powdery components (binder, fat powder, extract powder etc.) is first produced in high-speed mixers. The breakable components are gently mixed in a second slow mixing step. The mixtures are agglomerated for special uses (instant soups and sauces); they generally have no coarse components. This is usually conducted in batchwise or continuously operated fluid bed spray granulators. In continuous agglomeration plants (Fig. 12.37), extract substances and fat are dosed in separated systems. Alternatively, finished soup/sauce mixtures are agglomerated by back wetting with steam or water and dried

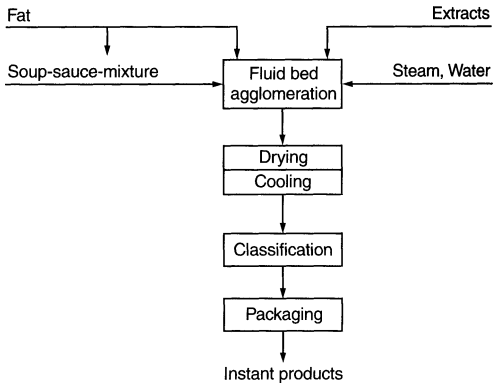


Fig. 12.37. Production of instant products by agglomeration

via a separate fluid bed. The packaging materials used protect the dry mixture from light, air, and moisture.

12.9 Meat Aroma

Raw meat has only a weak aroma. Numerous intensive aroma variations arise from heating, the character of the aroma being dependent on the type of meat and the method of preparation (stewing, cooking, pressure cooking, roasting or broiling-barbecuing). The preparation effects are based on reaction temperatures and reactant concentrations. Thus, a carefully dried, cold aqueous meat extract provides a roasted meat aroma when heated, while an extract heated directly, without drying, provides a bouillon aroma.

12.9.1 Taste compounds

Meat aroma consists of: (a) nonvolatile taste substances, (b) taste enhancers and (c) aroma constituents. The latter compounds or their precursors originate essentially from the water-soluble fraction. The constituents listed in Table 12.22 have been identified as the taste substances of beef broth and roasted meat juice. Solutions of these substances in the given concentrations (Table 12.22) give the typical taste profiles, which are composed of sweet, sour, salty, and glutamate-like (umami) notes. The meat note is produced by odorants.

Table 12.22. Taste compounds in beef broth and pot roast gravy

	Concentration (mmol /l)	
Compound/Ion	Broth[a]	Roast gravy[b]
Aspartic acid	0.05	0.18
Alanine	–[c]	9.41
Glutamic acid	0.3	1.71
Cysteine	–[c]	0.48
5′-AMP	0.14	0.64
5′-IMP	0.4	7.82
Hypoxanthine	–[c]	3.62
Carnosine	6.2	23.4
Anserine	0.7	–[c]
Lactic acid	25.6	155
Succininc acid	–[c]	2.16
Carnitine	2.0	–[c]
Pyroglutamic acid	2.6	–[c]
Creatinine	–[c]	43.3
Creatine	–[c]	20.3
Sodium	2.3	35.6
Potassium	31.3	170
Magnesium	3.0	12.1
Calcium	1.0	–[c]
Chloride	3.1	18.9
Phosphate	10.1	49.4

[a] Ground meat (500 g) suspended in 1 l of water and boiled for 2 h, followed by fat separation and filtration.
[b] Meat (2 kg) fried for 20 min and braised for 4 h after the addition of 1 l of water. The meat juice or gravy is poured off.
[c] Does not contribute to taste in the sample.

12.9.2 Odorants

Dilution analyses were used to elucidate the potent odorants (Table 12.23) of boiled beef and pork and of the meat and skin of fried chicken. Omission experiments (cf. 5.2.7) show that octanal, nonanal, (E,E)-2,4-decadienal, methanethiol, methional, 2-furfurylthiol, 2-methyl-3-furanthiol, 3-mercapto-2-pentanone and HD3F are the key aroma substances of boiled beef. These compounds are also present in boiled pork and chicken, but species-specific differences in concentration exist. The meaty/caramel-like note typical of beef is produced by 2-furfurylthiol,2-methyl-3-furanthiol and HD3F, which occur in relatively high concentrations in this meat. In comparison, the lower concentration of HD3F in pork is due to the considerably lower contents of the precursors glucose 6-phosphate and fructose 6-phosphate.

The aroma of boiled pork is not as intensive as that of beef and the fatty note is more pronounced. The concentrations of the fatty smelling carbonyl compounds, e. g., hexanal, octanal and nonanal, are lower in pork, but in proportion to the concentrations of 2-furfurylthiol, 2-methyl-3-furanthiol and HD3F, they are higher than in beef. This difference appears to favor the intensity of the fatty note in the odor profile of pork. In chicken, the fatty notes become even more noticeable due to

Table 12.23. Concentrations of odorants in boiled beef and pork and in fried chicken

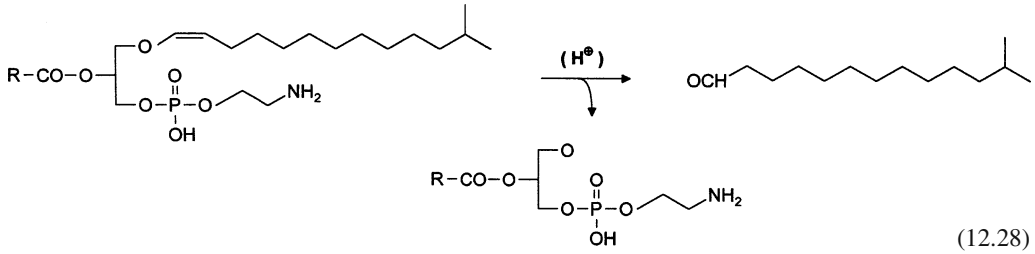
	Concentration (μg/kg)			
			Chicken[b]	
	Beef[a]	Pork[a]	Meat	Skin
Acetaldehyde	1817	3953	3815	3287
Methylpropanal	117	90	83	538
2-Methylbutanal	n.a.	n.a.	8	455
3-Methylbutanal	26	27	17	668
Hexanal	345	173	283	893
Octanal	382	154	190	535
1-Octen-3-one	9.4	4.8	7.2	10.8
Nonanal	1262	643	534	832
(Z)-2-Nonenal	6.2	1.4	5.5	10.5
(E)-2-Nonenal	32	15	23	147
(E,E)-2,4-Decadienal	27	7.4	11	711
12-Methyltridecanal	961	n.a.	n.a.	n.a.
Hydrogen sulfide	n.a.	n.a.	290	n.a.
Methanethiol	311	278	202	164
Dimethylsulfide	105	n.a.	n.a.	n.a.
Methional	36	11	53	97
2-Furfurylthiol	29	9.5	0.1	1.9
2-Methyl-3-furanthiol	24	9.1	0.4	4.1
3-Mercapto-2-pentanone	69	66	29	27
4-Hydroxy-2,5-dimethyl-3 (2H)-furanone(HD3F)	9075	2170	50	395
2-Acetyl-2-thiazoline	1.4	1.6	2.6	5.8
2-Acetyl-1-pyrroline	1.1	n.a.	0.2	2.9
2-Propionyl-1-pyrroline	n.a.	n.a.	n.a.	0.8
2-Ethyl-3,5-dimethylpyrazine	n.a.	n.a.	n.a.	4.3
2,3-Diethyl-5-methylpyrazine	n.a.	n.a.	n.a.	2.5
p-Cresol	5.9	n.a.	3.4	1.1
Guaiacol	4.3	n.a.	4.3	<1
Butyric acid	7024	17,200	8119	4817

[a] Beef (8.8% fat) and pork (1.7% fat) were boiled for 45 min at a pressure of 80 kPa at 116 °C.
[b] Chicken was fried for 1 h in coconut fat at 180 °C, breast meat (1% fat) and skin (46% fat) were analyzed separately.
n.a: not analyzed

the low formation of the two sulfur-containing odorants and HD3F.

The aroma of fried chicken is primarily caused by the *Strecker* aldehydes methyl propanal, 2-and 3-methyl butanal as well as the roast aroma substances 2-acetyl-2- thiazoline, 2-acetyl-1-pyrroline and the two alkyl pyrazines. The thiazoline and the pyrroline are also formed in lower concentrations during the boiling of meat. 2-Acetyl- 2-thiazoline is the most important roast aroma substance in meat fried for only a few minutes. It decreases on longer heating (cf. 5.3.1.5), while the stable alkylpyrazines increase further.

If beef is heated for a longer period of time, 12-methyltridecanal (MT) appears as an important odorant. Especially in a pot roast, this substance is one of the indispensable aroma substances, which develops its full effect on retronasal detection and increases mouth feeling. The precursors of MT are plasmalogens which occur in the membrane lipids of muscle and slowly hydrolyze on heating (cf. Formula 12.28).



(12.28)

Larger amounts of MT are released only on hydrolysis of the lipids in the meat of ruminants, but not from the lipids of prok and poultry meat, as shown in Table 12.24. In fact, there are indications that microoganisms present in the stomach of ruminants produce MT which is then incorproated into plasmalogens.

The MT concentration in the phospholipids of bovine muscle increases with increasing age. The studies conducted until now indicate a linear relationship, which could be of interest for the determination of the age of beef.

Important aroma substances of raw and cooked mutton are listed in Table 12.25. A special feature is the two branched fatty acids, which are already present in the raw meat and produce the "mutton" odor. (E)-2-Nonenal and the other odor substances from lipid peroxidation are also present in not inconsiderable concentrations in the raw meat. Only HD3F is formed during cooking.

Table 12.24. Release of 12-methyltridecanal (MT) on hydrolysis of lipids from different animal species

Animal species	Lipid (g/kg)	MT (µg/g lipid)
Beef[a]	14–22	55–149
Beef[b]	n.a.	44–63
Veal[a]	12	19
Red deer[a]	25	5
Springbok[a]	14	16
Pork[a]	15–19	1.3–2.7
Pork[b]	n.a.	1.6
Chicken[b]	n.a.	0.3
Turkey[b]	n.a.	1.6

The samples were refluxed: [a] with HCl (1 h), [b] at pH 5.7 (4 h).
n.a.: not analyzed

Table 12.25. Comparison of the aroma substances of raw (I) and cooked, lean mutton (II)

Compound	Amount (µg/kg)	
	I	II
4-Ethyloctanoic acid	255	217
4-Methyloctanoic acid	278	502
(E)-2-Nonenal	27	21
(E,E)-2,4-Decadienal	2.9	4.6
(E,E)-2,4-Nonadienal	1.4	3.8
(Z)-1,5-Octadien-3-one	0.8	2.1
4-Hydroxy-2,5-dimethyl -3(2H)-furanone (HD3F)	<50	9162

12.9.3 Process Flavors

Aromas obtained by heating aroma precursors are used in the aromatization of foods. An important aim of process flavors is the production of odor qualities similar to those of meat. This is achieved especially on heating cysteine with ribose, as shown in Table 12.26. Glucose is less effective and rhamnose promotes the formation of HD3F.

For economic reasons, attempts are made to replace individual precursors with inexpensive materials, e. g., a relatively inexpensive protein hy-

Table 12.26. Formation of relevant aroma substances on heating cysteine with ribose, glucose or rhamnose[a]

Compound	Amount (µg/kg)		
	Ribose	Glucose	Rhamnose
2-Furfurylthiol	12.1	2.8	0.8
2-Methyl-3-furanthiol	19.8	1.9	0.8
3-Mercapto-2-pentanone	59.9	13.9	7.3
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HD3F)	18.5	79.4	19,800

[a] Mixtures of cysteine (3.3 mmol) and the monosaccharide (10 mmol) dissolved in phosphate buffer (100 ml; 0.5 mol/l; pH 5.0) were heated to 145 °C in 20 min.

drolysate is used as the amino acid source and other important precursors of meat aromas like thiamine and monosaccharide phosphates are applied in the form of yeast autolysates.
Fats or oils are added to produce the carbonyl compounds which contribute to the animal species specific note of meat aroma.

12.9.4 Aroma Defects

If cooked meat is stored for a short time, e. g., 48 h at ca. 4 °C, an aroma defect develops, which becomes unpleasantly noticeable especially after heating and is characterized by the terms metallic, green, musty and pungent. This aroma defect, also called warmed over flavor (WOF), is caused by lipid peroxidation (cf. 12.6.2.1). The indicator of this aroma defect is hexanal, which increases as shown in Table 12.27.
Other changes which contribute to the aroma defect are the increase in metallic/musty smelling epoxydecenal, which, like hexanal, is formed in the peroxidiation of linoleic acid (cf. 3.7.2.1.9), and the decrease in HD3F. The latter is probably due to the reaction of its enolic OH group with peroxy radicals.
The WOF appears in chicken much faster because its linoleic acid content is about 10 times higher than that in beef. Apart from the changes in concentration of the odorants listed in Table 12.27, the degradation of 2,4-decadienal, which is typical of an advanced lipid peroxidation (cf. 3.7.2.1.9), has an additional negative effect on the aroma.
The WOF is inhibited by additives which bind Fe ions, e. g., polyphosphates, phytin, and EDTA. In comparison, antioxidants are almost ineffective. Therefore, it is assumed that a site specific mechanism is involved in the formation of WOF. The Fe ions liberated in the cooking process are bound by the phospholipids via the negatively charged phosphate residues and, consequently, adjoin the unsaturated acyl residues of these lipids. Radicals from the *Fenton* reaction of Fe ions with hydroperoxides (cf. 3.7.2.1.8) attack only the unsaturated acyl residues, starting their peroxidation. This hypothesis can also explain the observation that multivalent ions ($Ca^{3\oplus}$, $Al^{3\oplus}$) inhibit WOF as they probably displace the Fe ions from the phospholipids.

Table 12.27. Changes in the concentrations of important aroma substances on cold storage and reheating of roasted beef

Compound	Concentration (μg/kg)	
	I[a]	II[b]
Hexanal	269	2329
trans-4,5-Epoxy-(E)-2-decenal	1.5	10.7
4-Hydroxy-2,5-dimethyl-3(2H) -furanone (HD3F)	1108	665

[a] I: hamburgers were fried for 7 min.
[b] II: as in I, then storage at 4 °C for 48 h and heated at 70 °C for 45 min until a core temperature of 60–65 °C is reached.

12.10 Meat Analysis

12.10.1 Meat

The determination of the kind of animal, the origin of meat, differentiation of fresh meat from that kept frozen and then thawed, and the control of veterinary medicines is of interest. The latter include antibiotics (penicillin, streptomycin, tetracyclines, etc.) used to treat dairy cattle infected with mastitis, and other chemicals, including diethyl stilbestrol, used for cattle to increase the efficiency of conversion of feed into meat.

12.10.1.1 Animal Origin

The animal origin of the meat can be determined by immunochemical and/or electrophoretic methods of analysis as well as by PCR. The PCR method is described in 2.6.4.2.2. Electrophoretic protein analysis will be discussed here. The sexual origin of a meat sample can also be of interest, as discussed here for beef.

12.10.1.1.1 Electrophoresis

To determine the animal or plant origin of the food, electrophoretic procedures have often

proved to be valuable when the electropherograms of the protein extracts reveal protein zones or bands specific for the protein source. Thus, in meat analysis, such a method allows for the differentiation between more than 40 animal species, e. g., beef, pork, horse, buffalo, sheep, game, and poultry (cf. Fig. 12.38).

To carry out an analyis, the sarcoplasm proteins are extracted with water. The electrophoretic separation is predominantly conducted on polyacrylamide gels, previously also on starch and agarose gels. The application of a pH gradient (isoelectric focusing) provides excellent protein patterns. The first assignment is achieved directly after the electrophoretic separation on the basis of two red myoglobin zones (Fig. 12.38a). The ratio of the intensities of these zones, which represent met- and oxymyoglobin, changes with the storage time of the meat or extract and is not important for evaluation. The myoglobin and hemoglobin zones can be intensified by treatment with o-dianisidine/H_2O_2 (Fig. 12.38b) and subsequent staining with coomassie brilliant blue makes all the proteins visible (12.38 c).

Some animal species can be recognized via the myoglobin bands (e. g., beef, buffalo, pork, horse, red and grey kangaroo) and others are assigned to groups. The identification is achieved with electropherograms stained with coomassie blue (Fig. 12.39). A differentiation within the families Cervidae (deer) and Bovidae (horned animals) is difficult, with the exception of the subfamily Bovinae (cattle), e. g., between roe deer, fallow buck, elk, reindeer, kudu, springbok,

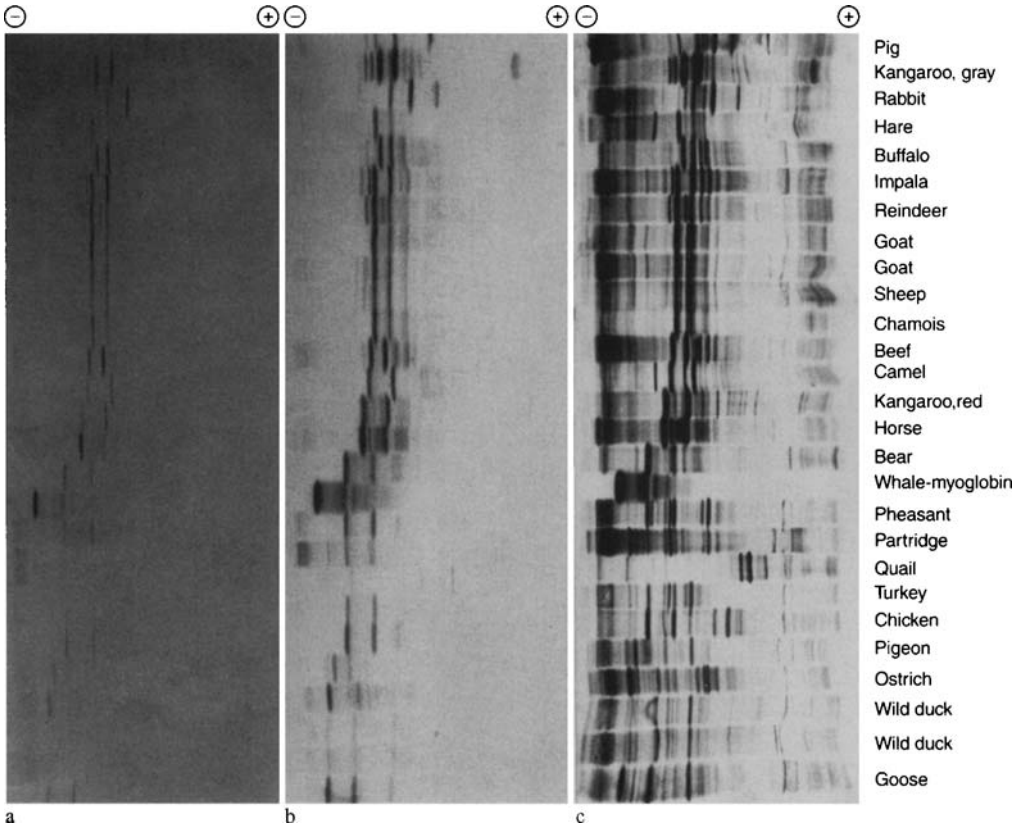


Fig. 12.38. Separation of sarcoplasm proteins of various warm blooded animals (mammals and fowl) by isoelectric focusing on polyacrylamide gels (PAGIF, PAGplate, pH range 3.5–9.5). (according to *Kaiser*, 1988). **a** Myoglobin (and hemoglobin) zones without staining; **b** Myoglobin and hemoglobin zones after treatment with o-dianisidine/H_2O_2; **c** Protein zones after staining with coomassie brilliant blue

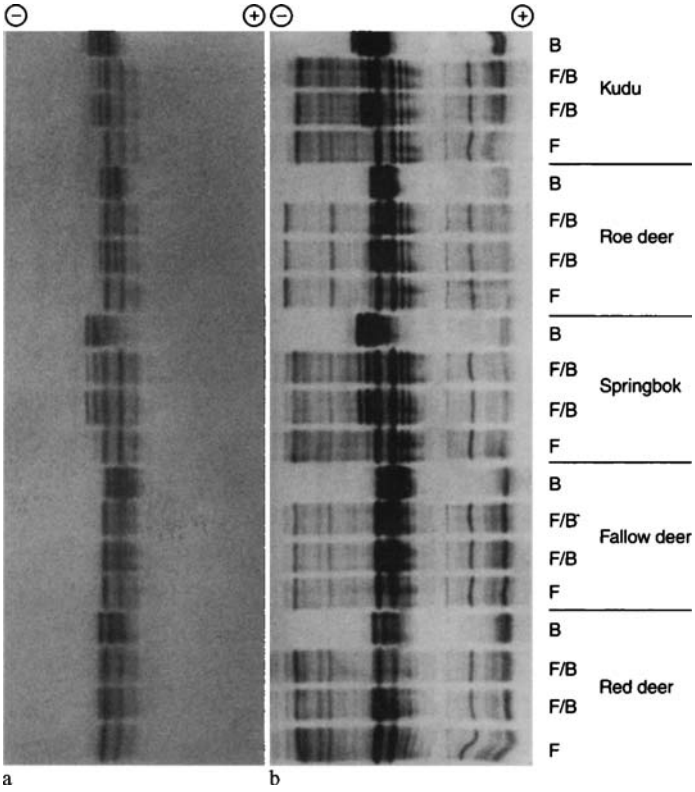


Fig. 12.39. Animal species with the same myoglobin patterns. Separation by isoelectric focusing of water soluble muscle proteins [F], blood [B], and mixtures of both [F/B] (cf. Fig. 12.38, according to *Kaiser*, 1988). **a** Myoglobin and hemoglobin zones after treatment with o-dianisidine/H_2O_2; **b** Protein zones after staining with coomassie brilliant blue

impala, sheep, goat, and chamois. Here, the hemoglobins can be used if the meat contains sufficient blood components, as is usually the case with game, or if blood is separately available (Fig. 12.39a).

The analyses mentioned above are largely limited to raw meat because protein denaturation occurs in heat treated meat. Denauration increases with temperature and time and makes the immunochemical and electrophoretic identification more and more difficult. Since DNA is more thermostable than proteins, the PCR is a promising alternative in these cases (cf. 2.6.4.2.2).

From the intensities of the indicator zones in an electropherogram, it is possible to estimate the proportion of one kind of meat in a meat mix. This is illustrated in Fig. 12.40 using a mixture of ground beef and pork.

12.10.1.1.2 Sexual Origin of Beef

The sexual origin of beef can be determined by an analysis of the steroid hormones. Since the concentrations of individual compounds vary too greatly, the ratio of progesterone/pregnenolone obtained from GC/MS is used. This value is on average 0.5 for oxen and bulls and 7.9 for heifers.

12.10.1.2 Differentiation of Fresh and Frozen Meat

The isoenzyme patterns of cell organelles, for instance mitochondria and microsomes, differ often from those of cytoplasm. When the organelle membranes are damaged by a physical or chem-

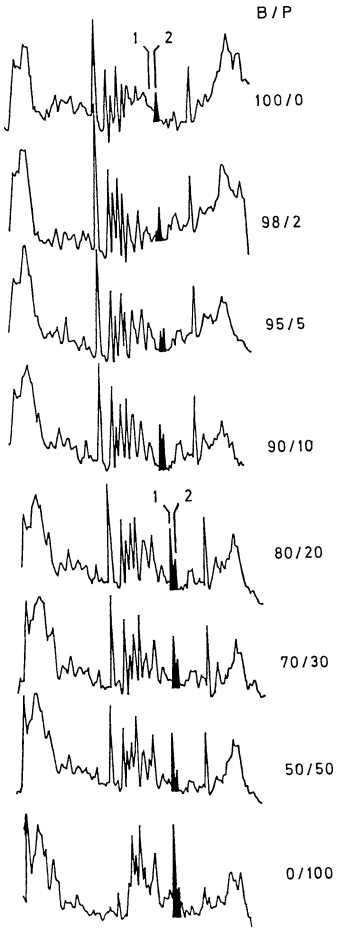


Fig. 12.40. Blended beef and pork meat: Densitograms of sarcoplasm proteins separated by PAGIF and PAG-plate; pH range 3.5–9.5. B/P (beef/pork) blend ratios in weight %. (according to *Kaiser*, 1980b)

ical process, isoenzyme blending will occur in the cytoplasm.

Such membrane damage has been observed by freezing and thawing of tissue, for example, of muscle tissue, in which the isoenzymes of glutamate oxalacetate transaminase (GOT) bound to mitochondrial membranes are partially released and found in the sarcoplasm. The pressed sap collected from fresh unfroze meat has only sarcoplasm enzymes, while the frozen and thawed meat has, in addition, the isoenzymes derived from mitochondria. The GOT isoenzymes can be separated by electrophoresis (Fig. 12.41). This procedure is also applicable to fish.

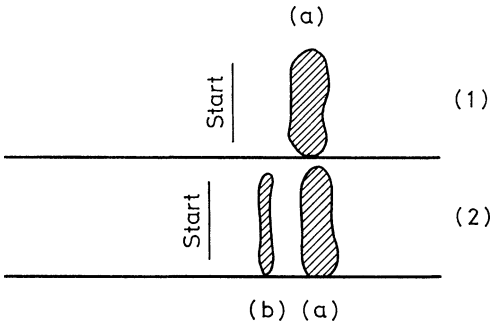


Fig. 12.41. Differentiation of fresh liver (1) from frozen and thawed liver (2) by electrophoretic separation of glutamate-oxalacetate transaminases (a) GOT sarcoplasm, (b) GOT mitochondria (according to *Hamm* and *Mašić*, 1975)

The enzyme β-hydroxyacyl-CoA-dehydrogenase (HADH, EC 1.1.1.35) is also suitable for the detection of frozen meat or fish. In the oxidation of fatty acids, HADH catalyzes the reaction shown in Formula 12.29. This enzyme is bound to the inner membrane of mitochondria and is liberated in the freeze/thaw process. Its activity can then be measured in the issuing sap with acetoacetyl CoA or with the artificial substrate N-acetylacetoacetylcysteamine.

$$\text{3-Oxoacyl-CoA} + \text{NADH} + \text{H}^{\oplus} \rightarrow \text{(S)-3-Hydroxyacyl-CoA} + \text{NAD}^{\oplus} \qquad (12.29)$$

12.10.1.3 Pigments

Pigment analysis is carried out for the evaluation of meat freshness. The individual pigments, such as myoglobin (purple-red), oxymyoglobin (red) and metmyoglobin (brown), are determined.

12.10.1.4 Treatment with Proteinase Preparations

Proteinases injected intramuscularly or through blood vessels degrade the structural proteins and, hence, proteolytic enzymes can be used to soften or tenderize meat. The enzymes are of plant or microbial origin and are used in the meat and poultry industries, while some are also used in the

household as meat tenderizers. Analytical determination of proteinases is relatively difficult.
A possible assay may be based on disc gel electrophoresis of meat extracts, prepared in the presence of urea and SDS. The band intensities of the lower molecular weight collagen fragments increase in proteinase-treated meat.

12.10.1.5 Anabolic Steroids

Anabolic compounds present in animal feed as an additive increase muscle tissue growth. Owing to a potential health hazard, some of these compounds are banned in many countries. Their detection can be achieved by the mouse uterus test or by a radioimmunoassay. Special receptor proteins which have the property of binding strongly to estrogens are isolated from rabbit or cattle uterus. The hormone-receptor complex is in equilibrium with its components:

$$\text{Receptor} + \text{estrogen} \rightleftharpoons \text{Receptor-estrogen complex} \qquad (12.30)$$

The nonlabelled estrogens bound to receptor in the test sample will be competitively displaced by the addition of 17-β-estradiol labelled with tritium for radiochemical assay.
To reach equilibrium, a suitable amount of receptor protein and a constant amount of labelled estradiol are incubated together with the test sample. The amount of the radioactive ^{3}H-estradiol receptor complex will decrease in the presence of competitive estrogens from the meat extract. The binding affinity of the estrogen receptor depends on the type of estrogen present (Fig. 12.42). Hence, detection limits differ and range from 0.3 to 50 ppb (mg per metric ton).
Anabolic compounds can be further separated by gas–liquid chromatography after derivatization of the polar functional groups, and identified by mass spectrometry. This method allows the determination of weak or nonestrogenic components too, but in the past it suffered from high losses in sample preparation and could not compete with radioimmunoassay in sensitivity. In the meantime disadvantages of the method have been eliminated.

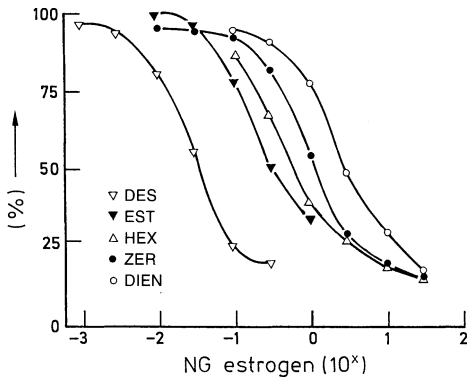


Fig. 12.42. Relative binding affinity of estrogen compounds to estrogen receptor. 50% binding achieved by: 0.034 ng diethylstilbestrol (DES), 0.33 ng 17-β-estradiol (EST), 0.6 ng hexestrol (HEX), 1.2 ng zeranol (ZER), 2.9 ng dienestrol (DIEN). (according to *Ingerowski* and *Stan*, 1978)

12.10.1.6 Antibiotics

Antibiotics are used as part of therapy to treat animal diseases and, sometimes in low concentrations, as constitutents of animal feed to increase feed utilization and to accelerate animal growth.
Detection of antibiotics is usually achieved by the inhibition of the growth of bacteria ("inhibitor test"). *Bacillus subtilis*, strain BGA, is one of the recommended test organisms.
Chemical methods must be used in order to identify and quantify the antibiotics and other veterinary medical residues. The principal method is chromatographic separation coupled with mass spectrometry. The tetracyclines, which are common antibiotics, can be determined relatively easily by fluorometric measurement of adequately prepared and purified meat extracts.

12.10.2 Processed Meats

Besides the estimation of the animal species and the control of additives, the analysis of processed meats is associated with verifying composition. Here the emphasis is on the content of extraneous added water, carbohydrate-containing thickeners and binders, nonmeat protein additives and fat. In addition, the determination of nitrites, nitrates, nitrosamines and, for enhancing the

pinkish-red color of processed meat, L-ascorbic acid is of importance in pickle-cured meat products. Other analytical problems involve the detection of condensed phosphates, citric acid and glucono-δ-lactone, as well as the detection of polycyclic aromatic compounds in smoked meats, of mycotoxins in products with desirable or undesirable growth of molds and of chlorine compounds in seasonings.

12.10.2.1 Main Ingredients

The first insight into the composition of processed meat, i. e. whether it contains an excess of fat or carbohydrate, which would lower the protein content and thus lower the value of the processed meat, is obtained by proximate analysis of the product's main ingredients: moisture, raw protein, fat and ash content. If their sum is less than $100 \pm 0.5\%$ of the sample weight, then the presence of carbohydrate binders should be verified. A positive finding should be further investigated since incorporation of liver into processed meat may provide glycogen. Hence, thorough carbohydrate analysis is required.

12.10.2.2 Added Water

Moisture content is related to protein content and is relatively constant. *Feder's* method of analysis of water added to chopped or ground meat or to emulsion-type sausages is based on these findings. The method uses the empirical equation:

$$\text{Water added}\ (\%) = \text{Moisture}\ (\%) - \text{Protein}\ (\%) \times \text{F} \qquad (12.31)$$

F for beef and pork $= 4.0$;

F for poultry leg $= 3.9$ and breast $= 3.6$

This indirect method for assessing the amount of added water has been repeatedly criticized. In spite of this, no better method has yet been developed. Moreover, the calculated water content is never used alone to evaluate a meat product. Other significant data, such as muscle protein content and the proportion of fat to protein, are also included.

12.10.2.3 Lean Meat Free of Connective Tissue

A measure of meat quality is expressed as the amount of lean meat free of connective tissue, which corresponds to meat proteins devoid of connective tissue protein (MPDCP). To obtain this value, the meat sample is analyzed for connective tissue proteins (CP), added extraneous protein (EP) and nonprotein-nitrogen (NPN), e. g., glutamate, purine and pyrimidine derivatives, urea. These values are then deducted from the value for total protein (TP):

$$\text{MPDPC} = \text{TP} - (\text{CP} + \text{EP} + \text{NPN}) \qquad (12.32)$$

Another method still being tested is based on drastic treatment (heating to 130 °C at pH 9) of a meat sample. Under these conditions, extraneous proteins, collagen and blood plasma proteins solubilize, while the residual protein is calculated as MPDCP using a constant factor.

12.10.2.3.1 Connective Tissue Protein

The amino acid 4-hydroxyproline is a marker compound for connective tissue. It occurs only in connective tissue protein. The amount of 4-hydroxyproline is determined in the acidhydrolysate of the sample or the isolated protein using an amino acid analyzer, or color-imetrically using a specific color reaction. The latter, accepted widely in practice, is a direct photometric procedure based on the oxidation of hydroxyproline in alkaline solution by H_2O_2 or N-chloro-p-toluenesulfonamide (chloramine-T). The oxidation yields a pyrrole derivative which is then condensed with p-dimethylaminobenzaldehyde to form a red pigment. The connective tissue content of meat is calculated by multiplying the hydroxyproline value by a factor of 8, which corresponds to an average of 12.4% hydroxyproline content of connective tissue.

12.10.2.3.2 Added Protein

In order to extend or improve the water holding capacity of processed meat, the product may

contain milk, egg or soy proteins. These proteins can be detected immunochemically, e. g., with the ELISA technique (cf. 2.6.3), with high sensitivity. The PCR technique (cf. 2.6.4.2) is even more sensitive and suitable for heated meat preparations. The addition of other proteins is usually limited by law so that a quantitative evaluation is required, which is fairly difficult.

12.10.2.4 Nitrosamines

Not only does the question of the content of nitrite or nitrate in pickle-cured meat arise, but also whether nitrosamines are formed and to what extent they occur in meat (cf. 9.8).

In general, nitrosamines arise only in very low concentrations. Since some of these compounds are a great health hazard, they should be detectable below 0.1 ppm in food for human consumption. The same procedures are available for identifying volatile nitrosamines which have been described earlier for the analysis of aroma constituents (cf. 5.2). However, precautions should be taken during the isolation step. Isolation of nitrosamines should not proceed at low pH since an acid medium in the presence of residual meat nitrites promotes further *de novo* synthesis of nitrosamines. Since the isolated fraction of neutral volatile compounds, which also includes nitrosamines, is highly complex in composition, reliable nitrosamines identification by gas chromatographic retention data is not possible. Additional mass spectrometric data are needed to verify the chemical structure.

12.11 References

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