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The added water and heat separate the caffeine from its natural complexes and aid its transport through the cell wall to the surface of the beans. Solvents are then used to remove the caffeine from the wet beans.

Up to the 1980s man-made organic solvents were commonly used. The caffeine is removed either by direct contact of solvent with the beans or by contact with a secondary water system that has previously removed the caffeine from the beans (12). In either case additional steaming or stripping is used to remove solvent from the beans. The beans are dried to their original moisture content of about 10–12% prior to roasting.

In the 1980s decaffeination processes were commercialized making use of solvents that occur in nature or can be made from substances that occur in nature. The use of these processes are the basis of positioning a coffee product as naturally decaffeinated.

Carbon dioxide under supercritical conditions is a very specific solvent for caffeine. This is based on a 1970 patent of Studiengesellschaft Kohler of Mulheim, FRG. Subsequent patents have been issued disclosing and claiming the use of this technology in a commercial process (17). Fats and oils, including oil from roasted coffee, are disclosed and claimed for a decaffeination process (19). Edible esters, including ethyl acetate, which is present in coffee, are also disclosed and claimed for a decaffeination process. A process using direct water contact with green beans is also in use. This contact removes caffeine as well as some noncaffeine solids. The noncaffeine solids, containing flavor precursors, are reabsorbed on green beans prior to drying and roasting.

In all of the above decaffeination processes prewetting of the green beans is necessary and drying afterward is needed prior to roasting. These steps, in addition to caffeine removal cause changes in the beans that affect roast flavor development.

The degree of decaffeination as claimed on the product is based on the caffeine content of the starting material and the time–temperature process conditions used by the manufacturer to achieve a desired end point.

Roasted decaffeinated coffee is vacuum packed as ground or whole beans for consumer use. The roasted decaffeinated coffee can also be made into a soluble coffee by methods previously described. Soluble coffee can also be made by extracting nondecaffeinated roasted coffee and then removing caffeine from the extract of the roasted coffee containing caffeine, by using many of the solvents described previously.

Roasted and ground decaffeinated coffee is vacuum packed or made into instant coffee by methods previously described. Decaffeinated coffee represented about 23% of cups of coffee consumed in 1989 in the United States (20).

BIBLIOGRAPHY

This article was adapted and updated from the article "Coffee" by A. Stefanucci, W. P. Clinton, and M. Hamell in M. Grayson, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 6, 3rd ed., John Wiley & Sons, Inc., New York, 1979, pp. 511–522.

1. W. A. Ukers, *All About Coffee*, 2nd ed., Tea & Coffee Trade Journal, New York, 1935, pp. 1–3.

2. Foreign Agricultural Service, *USDA World Coffee*, July, 1989.
3. A. Stefanucci and K. Sloman, *Internal Report*, Technical Center, General Foods Corp.
4. A. G. W. Bradbury and co-workers, "Polysaccharides in Green Coffee," paper presented at the Association Scientifique Internationale du Cafe, 12th colloquium, Montreux, Switzerland, 1987, p. 266.
5. H. Thaler and R. Gaigal, *Zeitschrift fuer Lebensmittel-Untersuchung und-Forschung* **120**, 449 (1963).
6. J. R. Feldman and co-workers, *Journal of Agriculture and Food Chemistry* **17**(4), 733 (1969).
7. L. Gariboldi and co-workers, *Journal of the Science of Food and Agriculture* **15**, 619 (1964).
8. I. Flament, "Research on the Aroma of Coffee," paper presented at the A.S.I.C. 12th colloquium, 1987, p. 146.
9. W. Clinton, "Evaluation of Stored Coffee Products," paper presented at the A.S.I.C. 9th colloquium, London, 1980, p. 273.
10. U.S. Pat. 4,169,164 (Sept. 25, 1979), M. Hubbard (to Hills Bros. Coffee Co.).
11. U.S. Pat. 4,737,376 (Apr. 12, 1988), L. Brandlein and co-workers (to General Foods Corp.).
12. U.S. Pat. 2,309,092 (Jan. 26, 1943), N. E. Berry and R. H. Walters (to General Foods Corp.).
13. R. J. Clarke and R. Macrae, eds., *Coffee*, Vol. 5, *Related Beverages*, Elsevier Applied Science, Publishers, Ltd., Barking, UK.
14. H. Foote, M. Sivetz, *Coffee Processing Technology*, Vols. 1, 2, Avi Publishing Co., Westport, Conn., 1963.
15. U.S. Pat. 3,549,380 (Dec. 22, 1970), J. M. Patel and co-workers (to Procter & Gamble).
16. U.S. Pat. 3,438,784 (Apr. 15, 1969), W. P. Clinton and co-workers (to General Foods Corp.).
17. U.S. Patent 4,820,537 (Apr. 8, 1989) S. Katz (to General Foods Corp.).
18. U.S. Pat. 897,763 (Sept. 1, 1908), J. F. Meyer and co-workers (to Kaffee-Handels-Aktien-Gesellschaft).
19. U.S. Pat. 4,465,699 (Aug. 14, 1984) F. A. Pagliaro and co-workers (to Nestlé, SA).
20. International Coffee Organization, *Coffee Drinking Study*, U.S.A., Winter, 1989.

General References

Reference 2 is a good general reference.

- R. J. Clarke and R. Macrae, eds., *Coffee*, Vols. 1–6, Elsevier Applied Science Publishers, Ltd., Barking, UK, 1985–1988.
- M. Sivetz, *Coffee Processing Technology*, Vol. 2, AVI Publishing Co., Westport, Conn., 1963.

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COLE CROPS. See VEGETABLE PRODUCTION.

COLLAGENS

Collagens are a family of proteins that are widely distributed in vertebrates and invertebrates and are the most abundant proteins in animals (1). Macromolecular assemblies of collagen support and hold the body together. The

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protein of bone is collagen; tendon, which connects muscle to bone, is collagen; and deep layers of skin contain collagen. In food, collagen contributes to the texture of meat and meat products. It is used as stabilizer in frozen dairy products, as gelatin in desserts, and as casings for sausages. Collagen is also the principal component of leather and is used as glues and as binders for emulsions in photographic films.

STRUCTURE

Collagen molecules are unique in their composition. Like other proteins, they are high-molecular weight polymers composed of amino acids joined by peptide bonds. Collagen has a special amino acid sequence, a repeating Glycine-X-Y where the X residue is frequently proline and the Y residue is frequently hydroxyproline (2,3). This repeating sequence forms a left-handed helical conformation (4) of the collagen chain. Three chains of collagen monomer combine to form the collagen molecule. Collagen molecules contain a fibrous domain that is made up of three left-handed helices fitted together to form a right-handed triple helix. The length of the triple-helical portion of the molecule varies with the type of collagen and is from 100 to 450 nm in length (3). Collagen molecules also contain globular *N*- and *C*-terminal domains. The exact composition and structure of the *N*- and *C*-terminal globular domains are collagen-type dependent (3) and are referred to as the telopeptides.

The three chains may or may not be identical. If they are different, the chains are coded on separate genes (3). Collagens undergo extensive posttranslational modification such as hydroxylation of proline residues to make hydroxyproline (necessary for collagen stability at body temperature) in the fibrous domain and the formation of hydroxylysine in both the fibrous and the telopeptide domains (3). Hydroxyproline and hydroxylysine are amino acids almost unique to collagens. Hydroxyproline and proline restrict the chain to the left-handed collagen helix (4), and hydroxylysine forms part of the covalent intermolecular and intramolecular cross-links that stabilize fibrils (macromolecular forms of collagen formation (3)).

CLASSIFICATION

Collagens are classified into three groups depending on the macromolecular form and type of cross-links present. The banded (or striated) fibrous group (group I) consists of Types I, II, and III (3). Recently, Types V and XI have been included based on their cross-links (3) and their genomic structure (5), which is the pattern of the exons (coding DNA sequences) and introns (noncoding DNA sequences) in the triple-helix coding region of the particular collagen gene. Type I collagen predominates in vertebrates (6) and is found in bone, skin, tendon, dentine, and muscle (3). Type II is found in cartilage, disk, and vitreous humor (3). Type III is usually associated with Type I in skin (7), muscle (3), and supporting connective tissue in internal organ systems (7). Type V collagen is found in tissues associated with Type I collagen, and Type XI collagen is found in tissues associated with Type II collagen (5).

The nonfibrous collagen (group II) consists of Type IV collagen that in the collagenous component of nonfibrous sheets, called the basement membrane, that underlie the epithelial and endothelial cells surrounding muscle and nerve and that provide the filtration properties of glomeruli (3). Type IV collagen has its triple-helical domain interrupted by nonglycine residues; it assembles as a tetramer and the macromolecular assembly is described as a chicken-wire structure rather than a fibril (3). The nonglycine portions, which interrupt the triple-helical domains, are theorized to add to the flexibility of the basement membrane structure, and the tetramer is stabilized by disulfide and lysino-alanine cross-links in the *C*-terminal end and by disulfide cross-links in the globular *N*-terminal end.

The microfibrillar collagens (group III) are Types VI, VII, IX, and X. Their macromolecular assembly is in the form of fine fibers, and they do not have the striated or banded appearance typical of group I fibers (3). Type VI collagen has been reported to link cell surfaces with the extracellular matrix and to form an interconnecting mesh work between collagen and elastin. Type VII collagen is found as anchoring fibrils for skin basement membrane and was first isolated from the placenta. These two collagens (Types VI and VII) are matrix-associated collagens. Types IX and X are cell-associated collagens and are found in cartilaginous tissue. Type IX is known to be the core protein for glycosaminoglycans.

Different criteria have been used to classify the collagens for groups II and III (6). Group II (the nonfibrous) collagens do not laterally aggregate as do the group I collagens and, therefore, Types VI, VII, and VIII are classified (6) as group II collagens along with Type IV. Type VIII collagen is found in bovine aorta and possibly as part of the basement membrane of the eye (2). Group III collagens have chain molecular weights of <95,000 and only Types IX and X are included in this group (6).

There are other types of collagen that have not been classified into groups. Type XII collagen is found in the bovine periodontal ligament as well as in chicken tendon (8), and a recent report (9) describes the characterization of a portion of another collagen type, Type XIII, which was isolated from the basement membrane of the bovine anterior lens capsule. Collagens have also been isolated from nonvertebrates such as annelids and insects (3).

Collagen genes for the individual chains of Types I (7) and IV (10), and the pro $\alpha 1$ (II) (7), pro $\alpha 1$ (III), pro $\alpha 2$ (V), and pro $\alpha 1$ (XI) (5) chains have been isolated, characterized, and, in some cases, cloned. The transcription of the collagen gene (DNA to RNA) is regulated by a particularly complex system that is probably cell specific (11).

BIOSYNTHESIS

Collagens are made on ribosomes and are then modified by cotranslational and posttranslational processing. Each collagen gene product undergoes slightly different processing steps depending on the type of collagen. The processing steps outlined here are for the $\alpha 1$ (I) chain, which eventually combines with itself and one $\alpha 2$ (I) chain to form Type I collagen, the most abundant type of collagen (Fig. 1). Each named chain is both chemically and geneti-

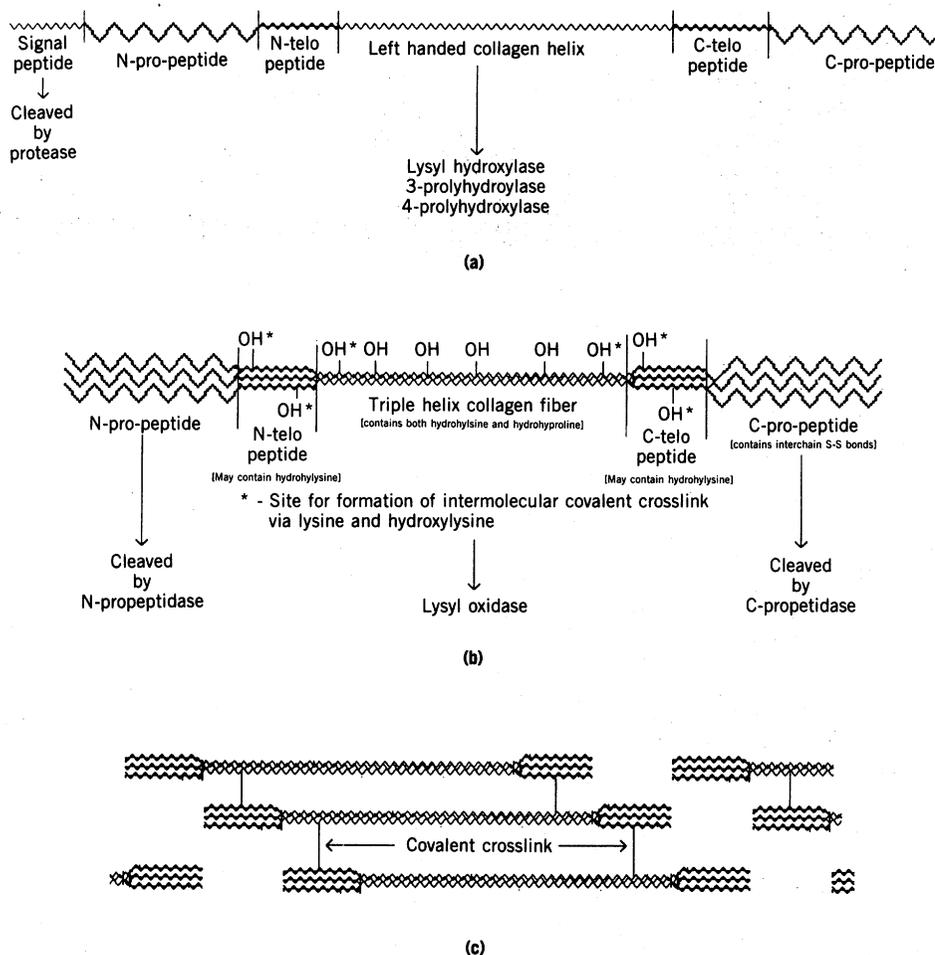


Figure 1. Post-translational assembly of type I collagen: (a) pre pro collagen chain; (b) pro collagen molecule; and (c) collagen fibril. Courtesy of Elizabeth D. Strange.

cally distinct even though there is considerable homology among the different chains even between species.

Collagens are translated from the mRNA (which also must be processed to remove the introns or noncoding sequences) starting with the *N*-terminal end. The first series of amino acids is called the signal peptide and allows the collagen to penetrate the membranes of the rough endoplasmic reticulum (where the protein is manufactured from the RNA). After the collagen has been secreted into the lumen of the rough endoplasmic reticulum, the signal peptide is cleaved by a protease. The next series of amino acids to be translated forms the aminopropeptide, which has several functions. It is involved in fibrillogenesis, the building of the ultimate structure of collagen in bone or connective tissue (6), the control of formation of the collagen fiber, and the feedback control of collagen synthesis. Along with the *C*-terminal propeptide, it also prevents the formation of collagen fibrils (macromolecular assemblages of collagen molecules) within the cell (3).

After the aminopropeptide is translated, the *N*-telopeptide domain of the collagen chain is made. This portion of the procollagen molecule is the first section made which will not eventually be cleaved in subsequent processing and is a site for the eventual formation of the covalent cross-links that stabilize the collagen molecule. Other cross-link sites are in the helical and the *C*-telopeptide domains (3), which, respectively, are the next sections of the procollagen molecule to be synthesized.

As the collagen molecule is being translated on the ribosomes of the endoplasmic reticulum, a series of three enzymes are hydroxylating the prolines and lysines as they are added to the growing collagen chain. In addition, galactose is added to hydroxylysine while the polypeptide chains are assembled on the ribosomes and the galactose can be glucosylated. The *C*-terminal propeptide chain also contains an asparagine residue that is glycosylated with a high mannose oligosaccharide.

Although the procollagen chains form on the ribosomes and the cotranslational modifications of the amino acids take place from the *N*-terminal to the *C*-terminal, the eventual assembly of the triple-helical collagen molecule takes place from the *C*-terminal end to the *N*-terminal end after the collagen has been released from the ribosome. Cross-links between the collagen chains are formed. In the *C*-terminal propeptide, intrachain and interchain disulfide linkages are formed. The interchain disulfide linkages are between the two $\alpha 1(I)$ chains and between one of the $\alpha 1(I)$ chains and the $\alpha 2(I)$ chain.

After the triple helix has been assembled, it goes to the Golgi apparatus and is then secreted. The final assembly of the collagen outside the cell requires the proteolytic cleavage of both the *N*-terminal and *C*-terminal propeptides. The rate at which propeptides are cleaved varies with the type of collagen even within the same structural groupings.

Group I collagens assemble into a quarter-stagger pat-

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tern (7) with the *N*-telopeptide of one triple-helical collagen overlapping the *C*-telopeptide of another triple-helical collagen molecule. The procollagen *N*-domain helps align the triple-helical molecules during fibril formation. During extracellular processing these procollagen peptides are cleaved (3). The spaces that have less proteinaceous material lead to the characteristic classic banding pattern with a polarized repeat (D) of 65–70 nm seen in electron microscopy of negatively stained group I collagens (7) and found by x-ray diffraction.

CROSS-LINKS

At least two types of covalent cross-links are formed extracellularly between the telopeptide regions of one collagen molecule and the triple helix of another collagen molecule in the assembled fibril. Lysine and hydroxylysine are enzymatically oxidized. The oxidized lysines (oxidized lysine is an aldehyde instead of an amine) or hydroxylysines react spontaneously with each other or with unoxidized lysines and hydroxylysines. The reaction between two oxidized lysines (to form allysine aldol) is largely intramolecular and connects the *N*-telopeptide regions of the monomers. The reaction between the oxidized lysine and hydroxylysine (to form dehydrohydroxylysinonorleucine) is largely intermolecular and connects the *N*-telopeptide of one chain of one molecule with the helical domain of another molecule and connects the *C*-telopeptide with another site in the helical domain of another collagen molecule. These linkages are acid labile, easily reduced, and form the majority of cross-links in fetal collagen. These covalent linkages change as the animal matures to form more stable types of cross-links. Maturation affects the solubility and denaturation behavior of collagen. The alteration of the cross-links as collagen ages is extremely important in the texture of meat products. It makes the texture of meat from relatively young animals more desirable than the meat from older animals. Meat preparation steps that involve an acid treatment, for example, marinating, take advantage of the acid lability of the immature collagen cross-links. The cross-links stabilize the collagen molecule and give the strength and resistance to deformation that is characteristic of connective tissue.

MEAT

Figure 2 shows the architectural structure of muscle connective tissue (3). Connective tissue is mostly aggregated collagen with a small amount of other molecular species such as complex carbohydrates, proteins, and fats. The tendon (Type I collagen) (3) attaches the muscle to the bone so that work generated by muscle fibers can be translated into movement. Epimysial (Type I and III collagens) connective tissue surrounds the muscle; muscle fiber bundles are, in turn, surrounded by perimysial (Type I and III collagens) connective tissue; and the muscle fibers are surrounded by endomysial (Type I, III, IV, and V collagens) connective tissues. Both the myofibrillar and connective tissues contribute to the texture of meat; the connective

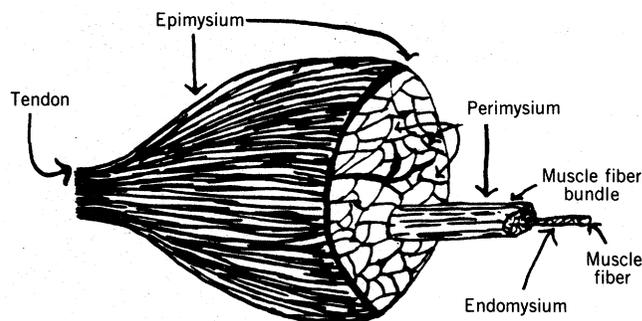


Figure 2. Muscle connective tissue. Courtesy of Elizabeth D. Strange.

tissue contribution is more subtle than the myofibrillar contribution.

Studies have concentrated on the role that the intramuscular connective tissue (ie, perimysium and endomysium) plays in the texture of muscle. Correlations of the total amount of collagen with relative tenderness of muscle have been demonstrated (12). The solubility of collagen also correlates with relative tenderness (13), and solubility is affected by collagen type and by age of the animal (14).

Excessive collagen (or connective tissue) can have an adverse effect on the acceptability of restructured meat products that are made by reducing the size of the muscle pieces and then reforming them into a steaklike product. In this kind of product, the myofibrillar structures are from a variety of muscles and the larger macromolecular connective tissue aggregates (tendon and epimysium) are not trimmed from the starting material. Different connective tissue structures have differing effects on hedonic perception of texture (15). Tendon is most objectionable, epimysium less so. Added perimysium and endomysium have little effect on the overall acceptability of the restructured product.

Collagen is also present in emulsion products such as frankfurters and high-collagen content by-products are sometimes added for economic reasons. In emulsion products, the finely chopped fat is stabilized by a coating of solubilized protein. Actomyosin is a hydrophilic protein that favors an oil-in-water emulsion, whereas collagen is a more hydrophobic protein that favors a water-in-oil emulsion. The addition of the starting materials that have a high collagen content destabilizes emulsions and causes fat pockets and water losses when products are heated to temperatures that cause gelation (unraveling of the triple helix and melting) of the collagen as well as undesirable texture changes. Successful meat emulsion products that contain high-collagen content by-products can be made if the amount of by-product added is carefully controlled (3).

Another aspect of the addition of high-collagen content by-products is the dilution of the protein quality of the product. Collagen is an incomplete protein. It lacks the essential amino acid tryptophan and is low in all the other essential amino acids. However, collagen is an excellent source of proline, which can become the limiting amino acid during times when collagen is being rapidly laid down by the body, such as during wound healing (3).

CASINGS

Denatured collagen is used to make two different types of food products: casings for sausages and gelatin. Casing collagen is usually made from the hides of mature cattle. The collagen is treated with alkali and decalcified, which opens up the fiber bundles, destroys some of the hydrogen bonding, deamidates the amide amino acids, destroys cross-links, and lowers the isoelectric point (pI) of the collagen. The prepared collagen is ground, acid swollen, extruded, and dried; it is then used as sausage casing. The extent of the alterations is critical to the success of the product (3).

GELATIN

Gelatin is made from collagen that has been solubilized with minimal cleavage of the peptide bonds. Class A gelatin is made from the hide of young animals and is acid extracted. It has pI of 6–9 and carries a net positive charge in all food systems. Class B gelatin is made from bone and hide of more mature animals and is alkali extracted. It is fully deamidated, has a pI of 5, and carries a net positive charge in acidic food systems and a net negative charge in neutral foods. Class A gelatin has a higher proportion of Type III collagen and class B gelatin contains some Type II collagen. The molecular weight distribution of the proteins of class A gelatin is lower than that of class B gelatin. Gelatin gels by the partial reformation of the triple-helical forms of the native collagen. The collagen in gelatin gels is not in exact register (triple helices are formed but they will be a variety of lengths) or as extensively aggregated as native collagen. This results in a lower melt temperature, from 60°C for native collagen to 40°C for gelatin (3).

Gelatin acts as a stabilizer for ice cream and frozen desserts. It prevents the formation of large ice crystals by increasing the viscosity of the ice cream mix. This imparts a desired smooth texture and firm body to the frozen product (16).

Some basic aspects of collagen must be elucidated. The chemical nature of the mature cross-links and the mechanisms for control of the biosynthesis of collagen are active areas of research. The solutions to these and other questions on collagen will have an impact on both the medical and food fields.

BIBLIOGRAPHY

1. H. J. Swatland, *Structure and Development of Meat Animals*, Prentice-Hall, Inc., Englewood Cliffs, N.J., 1984, p. 43.
2. D. A. D. Parry, "The Molecular and Fibrillar Structure of Collagen and Its Relationship to the Mechanical Properties of Connective Tissue," *Biophysical Chemistry* **29**, 195–209 (1988).
3. A. M. Pearson, T. R. Dutson, and A. J. Bailey, eds., *Advances in Meat Research, Vol. 4. Collagen as a Food*, AVI Publishing Co., Westport, Conn., 1987.
4. G. N. Ramachandran, "Stereochemistry of Collagen," *International Journal of Peptide and Protein Research* **31**, 1–16 (1988).
5. M. Bernard, H. Yoshioka, E. Rodriguez, M. van der Rest, T. Kimura, Y. Ninomiya, B. R. Olsen, and F. Ramirez, "Cloning and Sequencing of Pro- α 1(XI) Collagen DNA Demonstrates That Type XI Belongs to the Fibrillar Class of Collagens and Reveals That the Expression of the Gene Is Not Restricted to Cartilagenous Tissue," *Journal of Biological Chemistry* **263**, 17159–17166 (1988).
6. E. J. Miller and S. Gay, "The Collagens: An Overview and Update," in L. W. Cunningham, ed., *Methods in Enzymology*, Vol. 144. *Structural and Contractile Proteins, Part D, Extracellular Matrix*, Academic Press, Inc., Orlando, Fla., 1987.
7. K. L. Piez and A. H. Reddi, eds., *Extracellular Matrix Biochemistry*, Elsevier Science Publishing Co., Inc., New York, 1984, Chaps. 1–3.
8. B. Dublet, E. Dixon, E. de Miguel, and M. van der Rest, "Bovine Type XII Collagen: Amino Acid Sequence of a 10 kDa Pepsin Fragment from Peridontal Ligament Reveals a High Degree of Homology with the Chicken α 1(XII) Sequence," *Federation of European Biochemical Societies Letters* **233**, 177–180 (1988).
9. R. Dixit, M. W. Harrison, and S. N. Dixit, "Characterization of 26k Globular Domain of a New Basement Membrane Collagen," *Connective Tissue Research* **17**, 71–82 (1988).
10. S. L. Hostikka and K. Tryggvason, "The Complete Primary Structure of the α 2 Chain of Human Type IV Collagen and Comparison with the α 1(IV) Chain," *Journal of Biological Chemistry* **263**, 19488–19493 (1988).
11. P. Bornstein and J. McKay, "The First Intron of the α 1(I) Collagen Gene Contains Several Transcriptional Regulatory Elements," *Journal of Biological Chemistry* **263**, 1603–1606 (1988).
12. E. Dransfield, "Intramuscular Composition and Texture of Beef Muscles," *Journal of the Science of Food and Agriculture* **28**, 833–842 (1977).
13. N. D. Light, A. E. Champion, C. Voyle, and A. J. Bailey, "The Role of Epimysial, Perimysial and Endomysial Collagen in Determining Texture in Six Bovine Muscles," *Meat Science* **13**, 137–149 (1985).
14. D. E. Goll, R. W. Bray, and W. G. Hoekstra, "Age-Associated Changes in Muscle Composition. The Isolation and Properties of a Collagenous Residue from Bovine Muscle," *Journal of Food Science* **28**, 503–509 (1963).
15. E. D. Strange and R. C. Whiting, "Effects of Added Connective Tissues on the Sensory and Mechanical Properties of Restructured Beef Steaks," *Meat Science*, **27**, 61–74 (1990).
16. W. S. Arbuckle, *Ice Cream*, 2nd ed., AVI Publishing Co., Westport, Conn., 1972, pp. 98–99.

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COLLOID MILLS

A colloid mill is a device used in the preparation of emulsions and dispersions. The name implies that it can generate colloidal size droplets for the disperse phase, but in reality the colloid mill produces emulsions with droplets in the size range of 1–5 micrometers. It is sometimes used for dispersing solid particulates throughout the continuous phase, but it does not grind particles, rather it deagglomerates and disperses the solids (1).