

Chemical Changes in Proteins Produced by Thermal Processing

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Various processing procedures are applied to food systems for preservation, quality improvement, and consumer convenience. These procedures generally provide food that is satisfying, nutritious, economical, safe, and convenient. Thermal processing is used by much of the food processing industry to impart these attributes to its products, and many different procedures including cooking, blanching, pasteurization, and sterilization (1) are employed. Thermal processing has both beneficial and detrimental effects on food systems; therefore, the food scientist attempts to optimize the beneficial effects and to minimize the negative effects of heat processing (1, 2).

Heat is also one of the primary factors in the enhancement of palatability of most foods, with many of the beneficial palatability changes related to food proteins. These changes involve flavor and structural components; as most consumers will agree, properly cooked food products are generally more palatable than those in the uncooked stage or those which have been overcooked. Other factors which must be considered, in addition to those of preservation and palatability, are the alterations in nutritional properties of proteins caused by heating. Endogenous enzymes found in many food systems can produce either beneficial or deleterious effects on components of a particular food system or on the type of products generated by a particular enzyme. Heating of foods generally denatures these enzymes and prevents their action upon food components.

Effects of Thermal Processing on Proteins

Maillard Reaction

One of the predominant reactions which occurs upon heating of protein foods is the Maillard reaction (non-enzymatic browning) which occurs between free amine and carbonyl groups within food systems. Although most free amines and carbonyl groups have the capacity to participate in Maillard-type reactions, the major food substances participating in these reactions are the carbonyl groups of reducing sugars and free amino groups of proteins and other components (3). Although the Maillard reaction can occur at ambient and lower temperatures, increased temperatures have a marked effect upon its activation. Recent reviews fully de-

scribe characteristics and properties of the Maillard reaction (3-5); therefore, only the salient features of this reaction will be presented here.

Figure 1 shows the first stages of the Maillard reaction, which involves the production of a Schiff base with the carbonyl and amine groups. The Schiff base produced by the reaction sequence in Figure 1 normally undergoes an Amadori rearrangement to produce a ketoseamine. Both the Schiff base and the Amadori compound then undergo further reactions to produce a wide variety of reaction products which eventually produce the melanoidins responsible for the brown color. A group of these reaction products is presented in Figure 2. The Amadori compound and its products have the capability of participating in other Schiff base and aldol condensation reactions, producing a myriad of products which are difficult to characterize in food systems (3, 5). Much is known about the chemistry of the Maillard reaction, however, its complexity in food systems is such that only a fraction of the total number of reactions is understood. In fact, many of the reaction products resulting from Amadori compounds can cause cross-linking between molecules, resulting in the formation of numerous polymers (5).

Although there are many beneficial effects of the Maillard reaction in foods, such as improved flavor (3, 5), the reaction can also produce detrimental effects in foods, primarily from a nutritional standpoint; however, if the Maillard reaction in foods is extremely severe, detrimental flavors will also ensue. One of the possible adverse effects of the Maillard reaction is the reduction of protein nutritional quality through losses in availability of specific amino acids, particularly lysine, or through development of toxins or mutagens (8).

Satterlee and Chang (8) have described the nutritional changes induced by the Maillard reaction as follows: (1) decrease in the bioavailability of lysine, as well as several other essential amino acids; (2) decrease in protein digestibility; and (3) possible formation of growth inhibitory and/or toxic compounds. Figure 3 appears to corroborate these statements by demonstrating a reduction in the growth rate of rats fed a browned versus control diet (5). On the other hand, according to Hegarty (10), if dietary intake of protein is high and if sufficient quantities of the essential amino acids are consumed, the reduction in protein quality due to Maillard-type

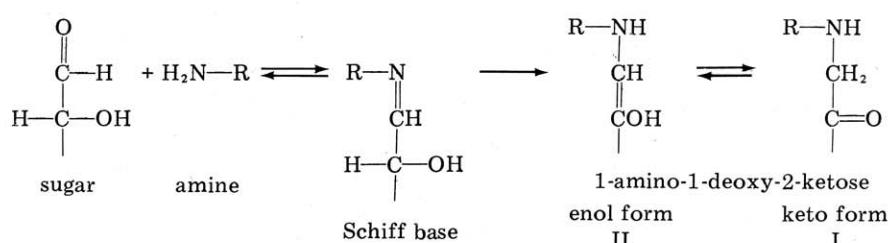


Figure 1. Proposed type reactions involved in initiation of nonenzymatic browning. Adapted from Paul (6) with permission (©John Wiley & Sons, Inc.).

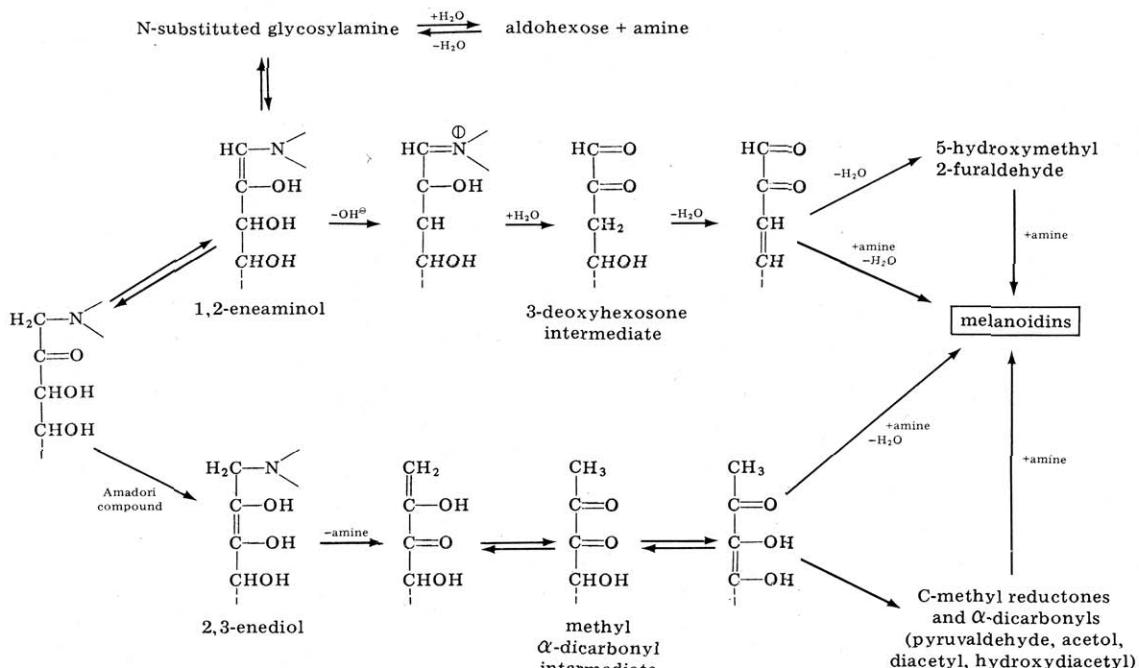


Figure 2. Sugar-amine (Maillard) browning reactions: two pathways to melanoidins and by-products. Adapted from Hodge and Osman (7) with permission (© Marcel Dekker, Inc.).

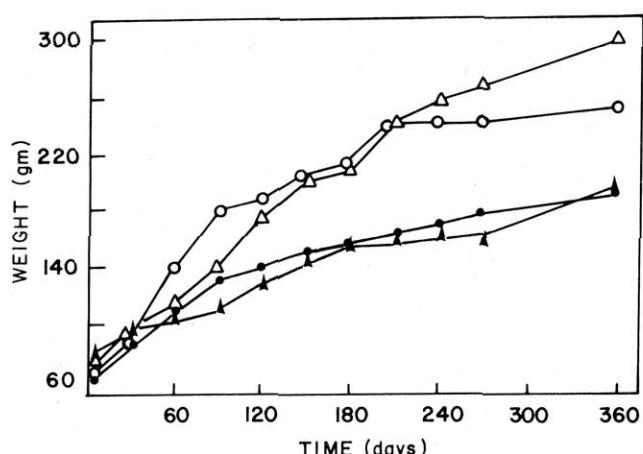


Figure 3. Growth curve for rats on browned and control diets. Key: browned, male ▲; browned, female ●; control, male, Δ; and control, female, O. (Average of five or six rats in each group; the weight gains shown here are for the rats which were fed for 12 months. Adapted from Kimiagar et al. (9) with permission (© J. Agric. Food Chem.).

reactions may be of academic interest only. It must be noted, however, that people on restricted diets (i.e., the elderly and people from lower income groups) may be vulnerable to decreased protein nutrition in view of the limited variety of foods they are likely to consume.

Heat Denaturation of Proteins

Thermal denaturation of protein occurs when hydrogen and other noncovalent bonds, such as ionic and van der Waals bonds, within the protein are disrupted by heat (11). Thus, the secondary and higher native-state structures of proteins are interrupted, changing the protein from its native state to an altered configuration, or denatured state. A diagram (Fig. 4) presented by Ledward (12) describes the transition between the native and denatured state (predenaturational state), whereby proteins are able to interact with other components within the system. From the intermediate state of denatura-

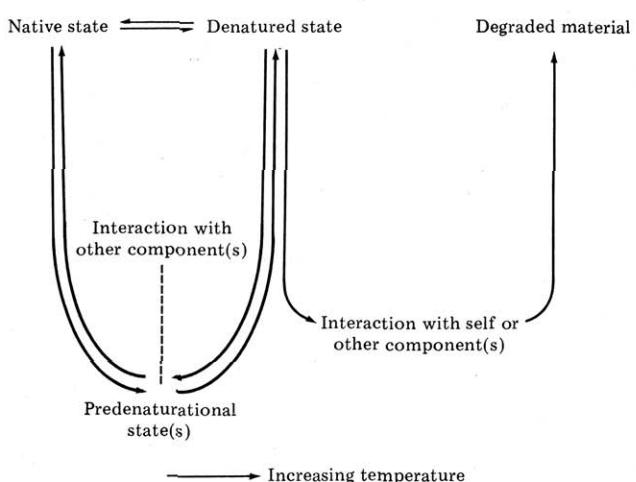


Figure 4. The transition between the native and denatured (pre-denaturational) state. Adapted from Ledward (12) with permission (© Applied Science Publishers Ltd.).

tion, and depending upon the amount of thermal perturbation of the noncovalent linkages within the protein, proteins can either return to a native state or continue to the denatured state. From the denatured state, provided sufficient thermal energy is supplied, proteins can continue to be degraded or react with other components within the system (Fig. 5), e.g., the Maillard reaction. Figure 5 demonstrates the changes in proteins from dissociation to degradation caused by heating at different temperatures, and the associated changes in properties of the protein.

The thermal stability of food proteins, or the amount of heat denaturation, is markedly affected by the protein's environment, such as the amount of water associated with the protein

| Property | Heating temperature (°C) | | | | |
|------------------------------------|--------------------------|--------------|------------------------|-----|-----|
| | 80 | 100 | 120 | 140 | 160 |
| Subunit | dissociation-unfolding | | degradation | | |
| | decrease-precipitation | | increase in solubility | | |
| Viscosity | increase-decrease | | decrease | | |
| Hydration | increase | decrease | | | |
| Gelation (following heating) | regular | hard-fragile | soft elastic | sol | |

Figure 5. The changes in proteins from dissociation to degradation due to heating at different temperatures and associated changes in the properties of the proteins. Adapted from Kinsella (13) with permission (©J. Amer. Oil Chem. Soc.).

(14). Tropocollagen molecules in solution have a denaturation temperature of about 37°C; whereas those same molecules, when organized into collagen fibers of connective tissue, have a denaturation temperature of about 60°C (12). Inorganic salts, ionic strength, pH, type of solvent, and other factors also have an effect on the thermal stability of proteins (15). Since the noncovalent bonds within each individual protein are different, each protein reacts differently to thermal energy, and each protein is affected differently by the same type of components in the food system (12, 16).

Aggregation, Precipitation, Gelation, and Degradation

In food systems containing several different proteins, thermal processing causes co-aggregation among proteins and protein-protein interactions. These interactions could be very important in determining the properties of heated food. Although the exact mechanism for aggregation of proteins during thermal processing is not clear, some type of covalent bonding must be involved because of the stability of most protein aggregates (12, 14).

Depending upon the food system utilized and the components within that food system, thermal processing of proteins can proceed beyond the aggregation stage and cause precipitation and/or gelation. Normally, when protein precipitation occurs, there is a decrease in water-holding capacity and a reduction in the functional properties of proteins in most food systems. However, when gelation occurs, there may be an increase in water-holding capacity of the food system by entrapment of water within the gel matrix (12). When an increasing amount of thermal energy is applied to protein systems, degradation occurs, including destruction of amino acids, hydrolysis of peptide bonds, and other reactions.

According to Neucere and Cherry (14), excessive heat treatment causes proteins to undergo many complex reactions which decrease their digestability. As shown in Table 1, cooking in boiling water, by both conventional and microwave means, decreases the amount of amino acids in peas (17). Microwave cooking was found to be slightly more destructive to amino acids than conventional cooking but produced slightly greater retention of thiamin and riboflavin (14). This indicates that more research is needed on the type and amount of energy absorbed relative to nutrient content of thermally processed foods (14).

Other Thermally Induced Protein Reactions

In addition to the Maillard reaction, thermal processing causes other reactions to occur in food proteins due to the unfolding of the proteins during denaturation and the modification of protein side chains. These reactions include: disulfide bond formation and hydrogen sulfide liberation; isopeptide formation; racemization of amino acids; and alteration of enzyme activities with the food system.

Disulfide Bonds and Hydrogen Sulfide. During heat de-

naturation of bovine serum albumin (BSA), lateral association of protein molecules occurs through a chain reaction of sulfhydryl groups with disulfide groups (18). Kolthoff and Tan (19) found that heat denaturation of BSA is not reversible in the absence of a sulfhydryl binding agent but that heat denaturation is reversible when the sulfhydryl groups are protected.

One of the milk proteins (β -lactoglobulin) is thermally very unstable, and the stability of milk proteins is affected by this thermal instability of β -lactoglobulin (20). However, when β -lactoglobulin is heated with κ -casein, a disulfide linkage is formed, which reduces the denaturation and aggregation of β -lactoglobulin and, hence, milk proteins (21). The functionality of milk powders in bread and bakery products has been increased by warming the milk before powder production to promote the disulfide linkage between β -lactoglobulin and κ -casein (11).

The formation of disulfide bonds in meat proteins occurs between 70 and 120°C, with a concomitant decrease in the number of sulfhydryl groups. The formation of disulfide bonds in meat proteins is not likely the cause of coagulation of the proteins, since coagulation begins to occur at 45°C; however, the formation of disulfide bonds may be related to other textural changes occurring in meats between 70 and 120°C (22, 23). When portions of meat are heated for long periods of time at temperatures substantially above 80°C, the sulfhydryl and disulfide groups in the meat proteins are almost completely lost. This occurs by oxidation to cysteic acid, or by the splitting off of hydrogen sulfide. The majority of the hydrogen sulfide originates from sulfhydryl groups on the proteins and increases rapidly with increasing temperature (22).

Isopeptide Formation. The formation of isopeptides by cross-linking of peptide chains is increased at higher temperatures (8, 14), producing some of the following products: lysinoalanine; lanthionine; and ornithinoalanine. These products have been demonstrated to reduce the ability of proteolytic enzymes to cleave proteins (8) and to reduce the

Table 1. Effect of Cooking on Amino Acid Content of Colossus Peas.^a

| Amino acid | Raw | Concentration—g/100 g dry wt | |
|---------------|------|------------------------------|-------------|
| | | Conventional | Microwave |
| Lysine | 1.82 | 1.72 (5.5) | 1.68 (7.7) |
| Histidine | 0.86 | 0.80 (7.0) | 0.75 (12.8) |
| Arginine | 1.41 | 1.35 (4.2) | 1.28 (9.2) |
| Asparagine | 2.94 | 1.76 (6.1) | 1.67 (9.2) |
| Glutamine | 4.46 | 4.20 (5.8) | 4.05 (9.2) |
| Threonine | 1.04 | 0.98 (5.8) | 0.94 (9.6) |
| Serine | 1.41 | 1.35 (4.2) | 1.28 (9.2) |
| Proline | 1.09 | 1.05 (3.7) | 1.01 (7.3) |
| Glycine | 0.93 | 0.89 (4.2) | 0.83 (10.7) |
| Alanine | 1.09 | 1.04 (4.6) | 1.00 (8.2) |
| Cystine | 0.11 | 0.10 (9.1) | 0.09 (18.2) |
| Valine | 1.31 | 1.26 (3.8) | 1.20 (8.4) |
| Methionine | 0.23 | 0.22 (4.3) | 0.20 (13.0) |
| Isoleucine | 1.16 | 1.09 (6.0) | 1.07 (7.8) |
| Leucine | 2.13 | 1.01 (5.6) | 1.98 (7.0) |
| Tyrosine | 0.74 | 0.70 (5.4) | 0.68 (8.1) |
| Phenylalanine | 1.53 | 1.47 (3.9) | 1.40 (8.5) |

^a Values in parenthesis are percent losses. Adapted from Chung et al. (17) with permission (©Journal of Food Science).

Table 2. D-Aspartic Acid (D-Asp) Content of Commercial Foods and Food Ingredients

| Product | D-Asp/L-Asp | D-Asp ^a /L-Asp |
|-----------------------------|-------------|---------------------------|
| Texturized soy protein | 0.095 | 0.09 |
| Baby formula (soy-based) | 0.108 | 0.10 |
| Simulated bacon (soy-based) | 0.143 | 0.13 |
| Corn chips | 0.164 | 0.14 |
| Dairy (casein-based) | 0.208 | 0.17 |

^a Relative ratios of total aspartic acid and its D- and L-enantiomers (D-Asp, L-Asp). Adapted from Satterlee and Chang (8) with permission (© American Chemical Society).

absorption of some other amino acids (14). Isopeptides are, therefore, detrimental to nutritional quality, but they may have a beneficial effect on the stability of the protein matrix of some food systems.

Racemization of Amino Acid Residues. An excellent review has been published recently describing the effects of amino acid racemization on the nutritional quality of proteins (8). According to Satterlee and Chang (8), a portion of the amino acid residues found in proteins which have been treated with heat and/or alkali is racemized. This racemization occurs in many common foods, and reduces the nutritional value of food proteins by decreasing their digestion by proteases. Both the absorption of D-amino acid residues and their subsequent conversion to the L-enantiomer are slow. One note of caution, as pointed out by Satterlee and Chang (8), is that some of the observed racemization of amino acids may occur during protein hydrolysis in preparation for conducting amino acid analysis (24).

Table 2 (8) presents the amount of D-aspartic acid present in commercial foods and food ingredients. For those foods presented, the D-enantiomer comprises between 9 and 17% of the total aspartic acid in those foods.

Enzymatic Reactions. All raw materials destined to become foods, or parts of food systems, contain endogenous enzymes which can have either beneficial or deleterious effects on the resulting food. Many food systems also contain added, or exogenous, enzymes from various sources which, when properly controlled, have tremendous beneficial effects upon the food system. Many of the enzymes present in foods are proteolytic in nature and, by their action upon food proteins, can affect the resultant food product, again, either beneficially or detrimentally. Enzymes in food systems can be activated by exposing the food to temperatures near physiological temperatures, and enzymes can be destroyed by increasing the temperature of the food system to a point where the enzyme is irreversibly denatured (14). Although many components within the food system, in addition to temperature, have an effect on an enzyme's activity (such as pH, ion concentration, and inhibitors), temperature control can be used, alone or in combination with these other components, to control more efficiently the proteolysis of food proteins.

An example of an exogenous proteolytic enzyme used in a food system is the addition of rennet to milk, where its action on the protein casein aids in the curd formation of the cheese-making process. The proper temperature is very important during the curd setting period and affects the rate of formation, firmness, elasticity, and other properties of the curd (25). The proteolytic activity of rennet in the resulting cheese whey (a by-product of curd formation) can be detrimental, since dried whey proteins are sometimes used subsequently in other milk products which contain casein. Calf rennet is more susceptible to heat denaturation than fungal rennets, and, therefore, whey which contains calf rennet is more useful for incorporation into casein-containing dairy products (26).

The proteolytic enzymes contained in muscle that degrade muscle proteins and increase tenderness (27) are an example

of an endogenous enzyme system having an effect on a food system. Activation of these natural proteolytic enzymes can be accomplished by high temperature conditioning of muscle during the early postmortem period, resulting in an improvement in the tenderness of the meat (28, 29). However, when the meat is cooked at high temperatures, these enzymes are rapidly denatured, so very little tenderization occurs during or after the cooking process.

Effects of Thermal Processing on Proteins of Specific Food Systems

Due to the large number of food systems which employ thermal processing in some form before consumption and the effects of heat on proteins in many of these systems, a comprehensive review is not possible within the scope of this paper. However, a few examples of the effects of heat on proteins in the major food systems and the resultant improvement or decrease in quality should suffice to illustrate the importance of thermal processing control on food proteins and ultimate food quality. These examples will also illustrate some of the reactions previously discussed that occur in food proteins due to thermal processing. The reader is directed to reviews by Høyem and Kvåle (30), Priestly (31), Fox and Condon (32), and Cherry (33) for more complete discussions of this topic.

Muscle Foods

Since the proteins within skeletal muscle of red meats, fish, and poultry are quite similar, and, since thermal processing affects these proteins in a similar manner, the interaction of heat and proteins from all muscle foods will be discussed together.

Denaturation of myosin (the primary myofibrillar protein in muscle) and sarcoplasmic proteins occurs to a large extent between 40 and 70°C, as shown in Figure 6. Associated with the denaturation of muscle proteins is a hardening of the tissues and a release of juice (22). Figure 7 demonstrates the effect of heating on meat tenderness (shear force value). This figure illustrates the toughening of meat (increase in shear force value) between 40 and 50°C, which corresponds to the temperature of the most marked protein denaturation (Figure 6). Little toughening occurs between temperatures of 50 and 65°C, but a further increase in toughness occurs between 65 and 75°C. This second phase is closely associated with collagen

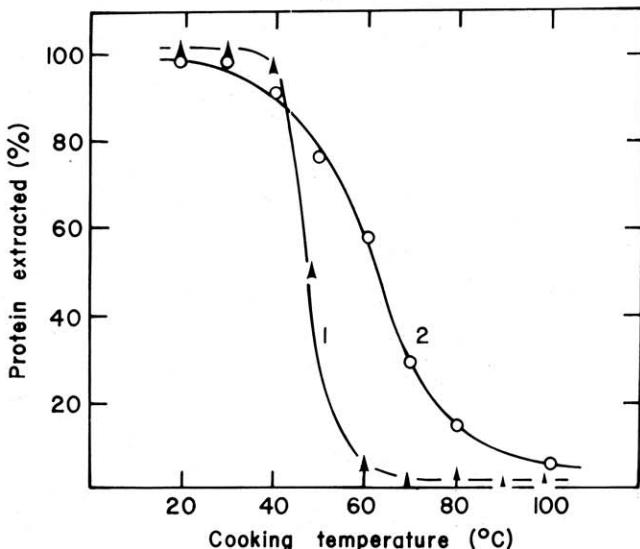


Figure 6. Myosin and sarcoplasmic protein denaturation and collagen shrinkage. Curve 1, percentage denaturation of myosin in the myofibril. Curve 2, percentage denaturation of the sarcoplasmic proteins in the muscle. Oblong horizontal area, the temperature zone of collagen shrinkage. Adapted from Davey and Gilbert (34) with permission (© Blackwell Scientific Publications Ltd.).

shrinkage (34, 35). The toughening of the second phase (between 65 and 75°C) may also be affected by loss of juice and the formation of new cross-linkages in the coagulated myofibrillar proteins (22). The decrease in shear force (improved tenderness) which occurs above 80°C may be associated with the degradation of cross-links within the myofibrillar proteins as evidenced by the production of hydrogen sulfide from disulfide groups in this temperature range (22).

Egg Proteins

Eggs have been used in many food products because of the functional properties of both egg white and yolk proteins (36–39). Some of the more familiar food products which, in order to make use of these functional properties, incorporate egg proteins are: cakes, meringues, souffles, custards, etc.

The beneficial functional properties of egg proteins are due to their denaturation and coagulation at specific temperatures and the formation of a stable matrix upon coagulation (40). A majority of egg proteins coagulate between 60 and 75°C (36–39); however, the coagulation temperature is affected by pH and salt concentration, as would be expected since salt and pH alter the hydrogen bonding characteristics of proteins.

Hydrogen sulfide, produced from both sulfhydryl and disulfide groups within the proteins (40), begins to be released when egg products are heated to temperatures exceeding 60°C. The green color formed on the surface of the yolk of hard-cooked eggs is a result of hydrogen sulfide liberation from albumin and its interaction with iron from the yolk (40). This green discoloration can be reduced by controlling the temperature of heating and subsequent cooling.

Fruits and Vegetables

Enzymes comprise one of the protein components of fruits and vegetables: if the activity of these enzymes is not limited, a reduction in the quality of the food can ensue. Phenolase is one such enzyme and is responsible for enzymatic browning of fruit and vegetable products (41). Peroxidase, another enzyme present in fruits and vegetables, can lead to reduction in nutrition, color, and flavor of the fruit and vegetable food systems (41). Phenolase is relatively unstable to heat and can be inactivated by a mild heat treatment, whereas peroxidase is much more resistant to heat and needs higher temperatures for its destruction (41, 42). These two examples illustrate the effects of thermal processing on enzymatic proteins within

foods and how specific control of these enzymes through selective denaturation and coagulation may improve the quality of the food product.

Cereal Grains

During the mixing of bread doughs, thiol disulfide interchange reactions are important in the development of desired elasticity of the protein matrix of the doughs (11). In addition, activities of the amylase enzymes are extremely important for the proper degradation of starch within the dough matrix and for the production of maltose required for the action of the yeast (41, 43, 44). Temperature control is essential to proper dough development: in order to ensure proper activity of amylase enzymes, and proper development of bond interactions between the elastic gluten proteins, temperatures must be altered depending upon the relative content of enzymes and gluten in the dough (43).

During baking of the bread, as temperature rises, yeast activity increases until inactivation occurs near 54°C. At about 65°C, starch granules begin to gelatinize, allowing the amylases to attack the starch material and produce the proper crumb consistency. Amylase enzymes are inactivated at approximately 75°C, at which time the gluten network also begins to denature and coagulate. Coagulation continues up to the point at which baking is complete, at a temperature of 95–100°C (43, 44).

The loaf surface will reach temperatures of 150–160°C and the color and flavor of the crust are partially a result of the occurrence of Maillard-type reactions at the bread surface (43, 44). The length of time the crust is maintained at these high temperatures determines the extent of Maillard reaction and the thickness of the crust. Maillard reactions are also important in some special bread products, such as German pumpernickel bread, where the extremely long baking time at intermediate temperatures produces appreciable quantities of sugars as a consequence of amylase activity. The reaction of these sugars with amino groups in Maillard-type reactions produces a very hard bread with special flavors (43). As a result of the large amount of Maillard-type reactions, the protein value of this bread may be reduced.

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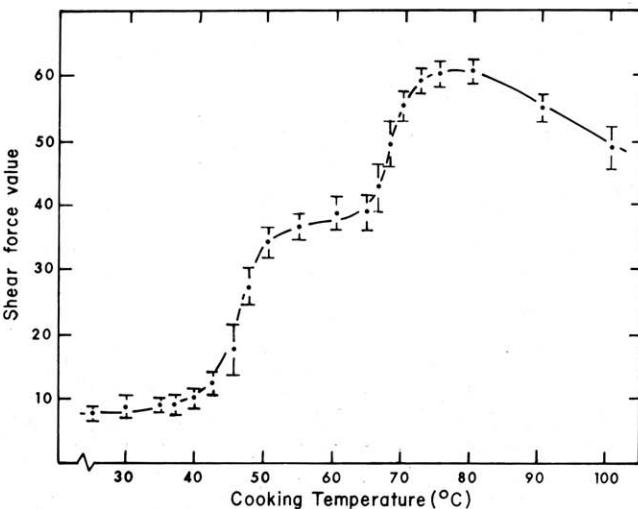


Figure 7. The two-phase effect of cooking temperature on shear-force values. Standard deviations are given by vertical lines. Each point is the mean of 8–16 determinations from the muscle of four bulls. Adapted from Davey and Gilbert (34) with permission (©Blackwell Scientific Publications Ltd.).

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