SPECIAL GUEST EDITOR SECTION

The Impact of Processing on the Nutritional Quality of Food Proteins

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During many food processing regimens, food proteins may undergo a variety of chemical modifications. Despite the enormous consumption of processed foods worldwide, much remains to be learned about the exact nature of these modifications. This is partly due to the complex nature of the changes involved, and partly to the problems of analysis imposed by the intractable nature of the food matrix. Such difficulties are compounded by the paucity of chemically based analytical tests that accurately measure amino acid availability in biologically relevant terms. In this review, we explore the known changes that proteins and amino acids undergo during food processing and the consequences of these changes on the physical and nutritional qualities of the food. We also examine the impact of these protein derivatizations for the analysis of food proteins and amino acids, and highlight areas that require future research.

This contribution explores the effect of food processing on amino acids and proteins, in particular, the chemical modifications induced and the implications of these modifications for amino acid analysis and bioavailability. Other aspects of amino acid analysis are covered elsewhere in this special section. The topic of bioavailability, including digestibility, absorption, and metabolism of processed food amino acids and protein, is also discussed in accompanying papers. Here, we focus on the chemistry of protein modifications and how this chemistry might impact on these various measures of protein quality.

The consequences of food processing on the quality of food proteins and their analysis are somewhat understudied, considering the vast amounts of processed protein consumed in Western diets. Processed protein can be introduced into the human diet directly from the processing of the food to be consumed, or indirectly via food that is fed to animals in the intensive livestock industry. There is a need to understand how modification of proteins impacts on both intensive livestock performance and human nutrition. Several seminal reviews have been published on the effect of processing on food proteins (1–3), but they do not cover the more recent literature. This contribution builds on and updates these reviews, with a particular focus on how the chemical modifications introduced during food processing impact on the measurement of amino acid composition and bioavailability.

For the purposes of this paper, we will treat food storage as part of food processing because, in many cases, the distinction between processing and storage is an arbitrary one. Precise details of food processing conditions are often not in the public domain, so we have restricted this review to the research literature and focused on general processing regimens that are relevant to a wide range of specific contexts.

Food Proteins and Analysis

The complexity of biological systems renders the analysis of their components inherently problematic in any research endeavor. In food science, these difficulties are compounded by the addition of chemically reactive ingredients and the frequent use of heat, which result in an extremely heterogeneous mixture in an often intractable matrix. Analysis of food proteins requires that the protein is isolated in a form that is free from substances that will interfere with the analytical test, using an extraction method that minimizes analytical artifacts. There is an inevitable compromise between these 2 factors, which is often tacitly made rather than explicitly stated (4).

A more detailed understanding of the chemistry involved —both during food processing itself and during extraction and subsequent analysis—is required for researchers to have confidence that the measurements they make are, in fact, relevant to the food in the form in which it is consumed. For example, it was recognized as early as 1946 that standard amino acid analysis could be used to ascertain the exact composition of food proteins which, in turn, could provide information as to the nutritional quality of the proteins (5, 6). The technique relies on breakdown of the food protein to its constituent amino acids followed by liquid chromatography (LC) analysis, which is accurate and reproducible. However, the hydrolysis step is very harsh, typically subjecting the proteins to refluxing hydrochloric acid over 24 h (7, 8). Improvements to these methods have been made (9) but,

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Table 1. Factors affecting protein quality and adequate protein supply[a]

Factor	Examples
Protein related	Amino acid composition, primary through to quaternary structure, stability, interactions, digestibility. Molecular form of the amino acid, whether protein-bound, contained in a small peptide, or free
Sociological	Economic, hygiene, and sanitation
Dietary	Total protein, total calorific intake, dietary fiber, frequency of feeding
Consumer-related, physiological	Species, age, gender, reproductive status (pregnancy and lactation), general health, and pathological states (trauma, stress, neoplasia)
Processing history	Alkali, chemical, heat, or pressure treatment, etc., storage, contamination by bacteria, presence of antinutritional factors

[a] Adapted from ref. 10. Note that, in a diet where amino acids are present in excess, the modifications due to processing have a much lower nutritional impact.

clearly, any acid hydrolysis step means that modifications to the food protein that are acid-labile will not be detected. Similarly, some modifications that are observed may have taken place during the hydrolysis step, rather than during the food processing itself. Both possibilities need to be carefully considered when interpreting the results. From the earliest studies, some foods showed a lower nutritive value than had been predicted from their amino acid composition (6), reflecting the fact that modified amino acids that were nutritionally unavailable had been hydrolyzed to liberate the free amino acid during the analysis.

Food Protein Quality

In foods, many factors can affect protein quality, in addition to amino acid composition and inherent digestibility. There are intrinsic factors, such as the source of the protein and whether the protein itself has antinutritional properties, the processing and storage history of the food, as well as factors extrinsic to the protein, such as the health status of the individual consuming the food (10). While this review focuses on the processing history of the protein, it is worth noting that all results need interpretation in the context of these wider issues (Table 1). Thus, the impact of food processing on the diet of an overfed, sedentary member of Western society may well be much less significant than it would be for an infant fed solely on formula milk or a hospitalized patient in a hypercatabolic state.

The specific protein and amino acid requirements for various consumers are well documented (11) and may vary widely. The nutritional value of a protein depends on both the specific distribution of amino acids and whether these amino acids are bioavailable (5). When amino acids are chemically modified, they may still be nutritionally adequate, but several factors may impair their use by the body. Proteolytic enzymes in the gut may be unable to digest proteins if the protein is not recognized by the enzyme-active site. If the protein is hydrolyzed successfully, the modified amino acid or peptide may be unable to pass through the gut wall. If the modified amino acid is absorbed through the gut, the body may be unable to convert it back to the parent amino acid or any other useful metabolite, in which case it will simply be excreted in the urine (1). A full understanding of how processing impacts on bioavailability, therefore, requires a detailed knowledge of the physiology of digestion, as well as the more obscure metabolic pathways and the chemistry and biochemistry of amino acid modification. Various methods are available to establish which of these factors may be important in determining the bioavailability of specifically modified amino acids and proteins, and these are covered elsewhere in this section.

Food Proteins and Processing

It is often mistakenly assumed that a food that has been constructed from raw ingredients meeting all dietary protein requirements will remain an adequate nutritional source until the time of consumption. Processing conditions encompass a wide range of chemical and physical environments, including variations in pressures, water content, and temperatures. Mixtures of food proteins combine with other food components, such as sugars, oxidizing agents, acids, alkali, and enzymes (1, 3). Thus, it is no surprise to a chemist that many processing regimens radically alter the structure of food proteins.

Especially in the Western diet, the processing of food has become routine. The benefits of food processing are diverse and range from preservation and sterilization to improved palatability or added convenience (12). In protein-rich diets, the perceived benefits of processing are often unrelated to nutritional concerns, but aimed at improving the important functional properties that food proteins impart on our food (13). Functional properties were first defined by Kinsella (14) as "those physical and chemical characteristics, which determine how the food performs during preparation, processing and storage." For example, egg proteins in cakes and desserts give these products desirable functional and, hence, sensory properties. Other examples of the functional roles of food protein are given in Table 2. The change in functionality of proteins can be attributed to the changes in

Table 2. Functional roles of food proteins that may be modified during processing[a]

Function	Proposed protein characteristics that play an important role	Food examples	Proteins involved
Solubility	Hydrophilicity	Beverages	Whey proteins
Viscosity	Water binding, hydrodynamic size	Soups, gravies, salad dressings, desserts	Gelatin
Water binding	Hydrogen bonding, ionic hydration	Meat sausages, cakes, breads	Muscle, egg, and whey proteins
Gelation	Water entrapment and immobilization, network formation	Meats, gels, cakes, bakery products, cheese	Muscle, egg, and milk proteins, gelatin
Cohesion-adhesion	Hydrophobic, ionic, and hydrogen bonding	Meats, sausages, pasta, baked goods	Muscle, egg, and whey proteins
Elasticity	Hydrophobic bonding, disulfide cross-links	Meats, baked goods	Muscle and cereal proteins
Emulsification	Adsorption and film formation at interfaces	Sausages, bologna, soup, cakes, dressings	Muscle, egg, and milk proteins
Foaming	Interfacial adsorption and film formation	Whipping toppings, ice cream, cakes, desserts	Egg and milk proteins
Fat and flavor binding	Hydrophobic bonding, entrapment	Low-fat bakery products, doughnuts	Milk, egg, and cereal proteins

[a] Adapted from ref. 19.

their physical state, hydrolysis of the protein into smaller peptides, and modifications of their amino acid residues. However, food science has yet to gain a detailed understanding of how specific changes to protein structure account for particular changes in functionality, except in a handful of well documented cases (15–18).

The drive toward food protein processing has, thus, been driven by a desire to improve consumer appeal, often with scant concern for the nutritional impacts of the processing regimen. This is despite concerns about the potential for reduced bioavailability of nutrients and the production of antinutritional or toxic products through the reaction of the food proteins with other food components. Excepting the destruction of particular vitamins, the reactions that proteins undergo are the major chemical reactions that occur during food processing (20). It is, thus, of great importance to understand the range of modifications open to proteins and amino acids under in vitro conditions associated with processing, how these influence bioavailability and nutritional quality, and how these changes impact upon measurements of amino acid composition and bioavailability.

Postharvest Changes

When food proteins are harvested and stored prior to processing, the proteins are subjected to a wide range of conditions that would not be encountered in vivo. The resulting modifications can be divided into 2 areas, postharvest modifications prior to processing and modifications due to the processing conditions. The postharvest modifications prior to processing include those that result from the changing concentrations of many metabolites when metabolism is shut down. New compounds may be introduced, and a wide range of chemistry becomes available to the protein that was inaccessible in vivo (4). For example, proteolytic enzymes, which in living tissues are carefully compartmentalized to avoid damage to tissues, may be liberated, resulting in proteolysis and the generation of new peptides. Such changes may alter the properties of the raw material and the available chemistry during processing, and they may be of particular importance in certain food types, as discussed below.

Processing-Induced Modifications

Processing of food generally includes the mixing of protein with different food components. These food components include fats and their oxidation products, vitamins (particularly B_6 and C), polyphenols, sugars, and other food additives, all of which are known to react with food proteins under appropriate conditions (21; Figure 1).

Processing conditions can include physical treatments, such as milling or heat; biological treatments, such as fermentation or enzymatic reactions; and chemical treatments, such as the use of an oxidizing agent or strong alkali (1, 22). Modern food processing may also include the use of microwave radiation to heat the food (23–25) or γ-radiation to sterilize the food (26). Recent literature has also described the use of high voltage electric pulses as a sterilization method that has consequential changes for enzyme activity and mechanical properties of protein-based foods (27). As each new processing regimen is introduced, a raft of new chemistry opens up to the food proteins, producing novel protein modifications that often remain uncharacterized. All of these treatments have profound consequences for the structure and reactivity of the protein.

Commonly, processing regimens result in the denaturing of the protein, which forms a random coil with many possible

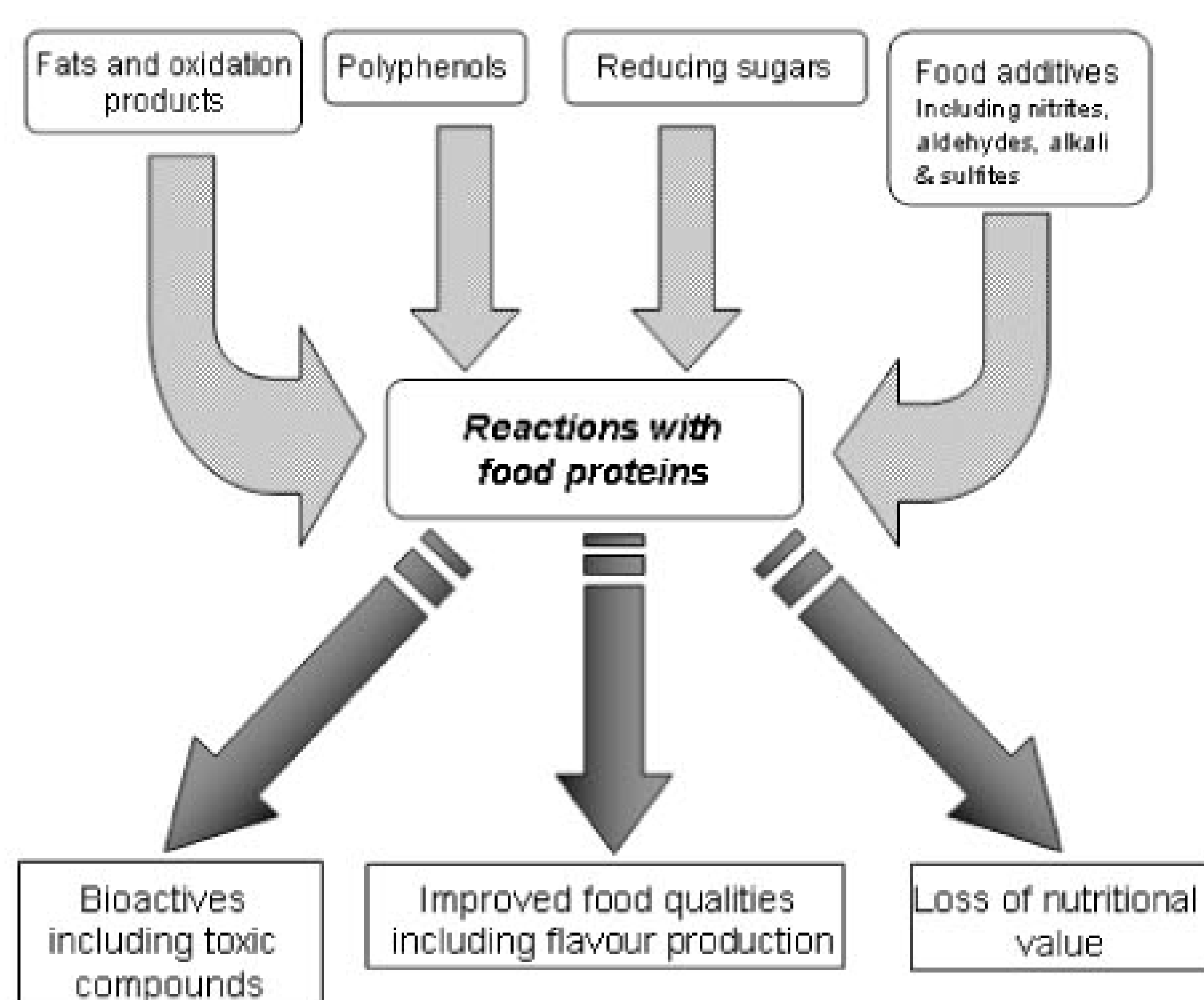

Figure 1. Major reactions of food proteins during food processing. Adapted from Hurrell and Carpenter (ref. 20).

conformations. In addition to the direct consequences of unraveling of the protein on its functionality, the change in conformation exposes reactive groups to chemistry not available when the protein remains in its native fold (28). These amino acid side chains can react with each other as well as with other food components. Many chemical reactions during food processing, notably the formation of color and flavor compounds, are highly desirable (4). Indeed, such modifications, often involving proteins, are the very reason that the food processing is performed. A detailed discussion of the chemistry of flavor and color production is beyond the scope of this review. However, many of these changes also have nutritional consequences.

In the following discussion, we will first consider general modifications to proteins that involve several amino acids, and then focus on the chemistry of individual amino acids that commonly undergo chemical change. Particular attention is focused on those amino acids that are nutritionally essential and vulnerable to chemical modification. In proteins, the primary amino group of lysine residues is the most reactive group under most circumstances (29), and often lysine is the first-limiting amino acid nutritionally. Cereals are the major source of protein for the majority of the earth's population (30). Like most foods of plant origin, cereals as sole food sources are of limited nutritional value because they are deficient in some essential amino acids, particularly lysine (28). Loss of nutritional value is also associated with reactions of the essential amino acids methionine, cysteine, and tryptophan and, to a lesser extent, arginine, histidine, phenylalanine, and tyrosine (1).

General Protein and Amino Acid Modifications Due To Processing

There are numerous protein modifications that affect a range of amino acid residues: proteolysis, protein crosslinking, amino acid racemization, protein-polyphenol interactions, and reactions induced by heat and high pressure. Each of these will be considered, in turn, in this section. However, it should be noted that it is a combination of all of these, and other factors, that ultimately determines the impact of processing on the nutritional quality of a particular food protein. While focusing on individual factors provides a simplifying view of the chemistry involved, each discussion must be interpreted in the framework of the overall impact of the entire processing regimen on the food in question, because synergies are often displayed between the many various factors. In this context, case studies examining the nutritional consequences of processing on particular food systems are extremely valuable. For example, Rérat et al. (31) examined the nutritional and metabolic consequences of Maillard treatment of milk, Mbithi-Mwikya et al. (32) described the impact of a variety of processing methods on the nutritional quality of kidney beans used for infant foods, and Grewal and Hira (33) explored the effects of processing and cooking on the overall amino acid composition of wheat.

In addition to the factors considered in the following sections, all processing modifications of protein have the potential to lead to production of bioactive compounds and/or loss of nutritional value. The production of new bioactive compounds can be beneficial if the compounds created are health-giving, or detrimental if the products are antinutritional or toxic. For example, the Maillard reaction, (discussed below) may produce antioxidant compounds (34, 35) that are generally thought to be beneficial but may also result in highly carcinogenic substances (36). As well as direct nutritional concerns, the potential for the products of the reaction of food components to interfere with accurate amino acid analysis, directly or indirectly, should not be overlooked.

(**a**) *Proteolysis*.—Proteolysis may be a result of endogenous enzymes within the raw materials being used during food processing, or may be due to commercial enzymes added as a food processing aid. In either instance, proteolytic activity changes the functional properties of the food, especially the texture. For example, unwanted proteolytic activity in wheat, such as that resulting from insect damage (37, 38), can lead to a dramatic drop in quality in baked produce (39). On the other hand, the use of protein hydrolysates in hypoallergenic infant formulas improves product quality (40). Proteolytic activity also opens up the potential for additional chemistry during food processing. In particular, the amino terminus of each peptide generated is vulnerable, like lysine, to Maillard reactions (*see* below; 41). This can be good for the color and flavor of the product, but may be nutritionally detrimental.

Meats, for example, are generally eaten cooked, not raw, and, thus, the changes experienced postharvest, but precooking, are difficult to relate directly to product quality. However, the characteristic flavor of cooked meat is thought to derive from thermally induced reactions occurring during heating, principally the Maillard reaction and the degradation of lipids. Research in this area has focused on product quality aspects, such as flavor and texture of the meat, with little attention to the nutritional consequences (42).

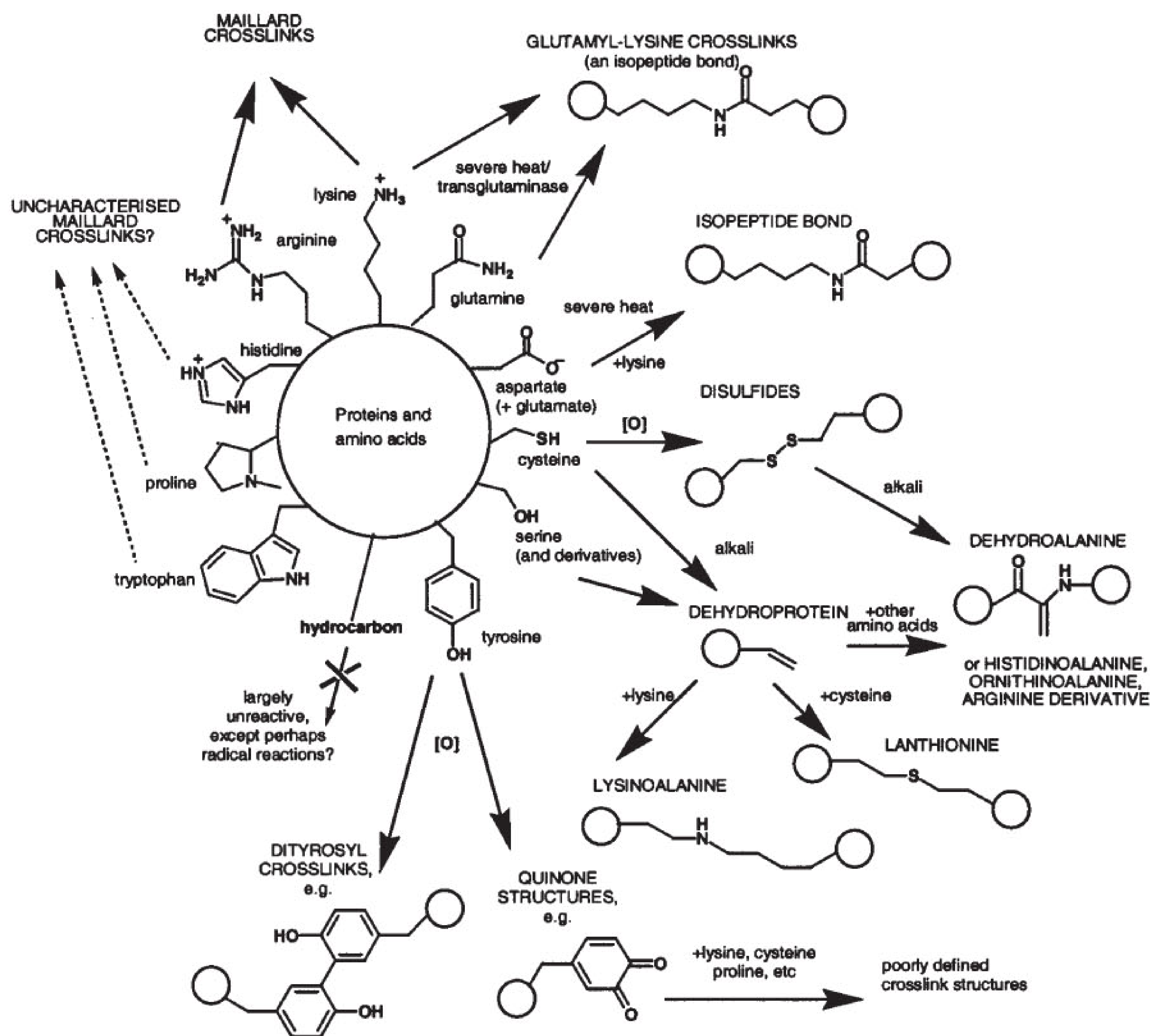

Figure 2. A summary of crosslinking reactions that may occur during food processing. Adapted from ref. 44.

(**b**) *Protein crosslinking*.—If food processing involves high temperatures, extremes in pH (particularly alkaline), and exposure to oxidizing conditions and uncontrolled enzyme chemistry, protein crosslinks often result, producing substantial changes in the structure of proteins and, therefore, the functional (43, 44) and nutritional (45–47) properties of the final product. Crosslinking has been shown to lower the digestibility of food because the crosslinked, aggregated protein is less accessible to digestive enzymes (43). Additionally, the chemical modification that constitutes the crosslink may render the residue no longer bioavailable and remaining amino acid residues more slowly digested (31). A summary of known protein crosslinking in foods is given in Figure 2, in which the information is organized according to the amino acids that react to form the crosslink. Not all amino acids participate in protein crosslinking, no matter how extreme the processing regimen. Those that react do so with differing degrees of reactivity under various conditions.

Disulfide bonds are the most common and well characterized types of covalent crosslink in food protein systems. They are formed by the oxidative coupling of 2 cysteine residues that are adjacent within a food protein matrix. The oxidation in food processing can be caused by a range of oxidant systems, including oxygen in the presence of catalytic metal ions, photooxidation due to pigments sensitive to light, enzymes such as polyphenol oxidases, or oxidizing lipids (1). Sterilization of packaging by hydrogen peroxide also has the potential to cause oxidation of proteins (1). The ability of proteins to form intermolecular disulfide bonds during heat treatment is considered to be vital for the gelling of some food proteins, including milk proteins, surimi, soybeans, eggs, meat, and some vegetable proteins (48). Gels are formed through the crosslinking of protein molecules, generating a 3-dimensional, solid-like network that provides food with desirable texture (49). Disulfide bonds are important in the formation of dough (50) and meat (43). The formation of disulfide bonds does not generally result in a change in nutritional quality, unless the disulfide bond itself is subject to further oxidation.

Under alkaline conditions, or when heated, proteins can undergo crosslink formation by other mechanisms (22). This is often due to the reaction of lysine residues with

Table 3. Percentage of D-amino acids for 4 alkali-treated proteins[a]

Amino acid	Casein	Wheat gluten	Fish	Soybean
D-Ala	15.2	18.6	19.3	15.8
D-Asp	29.2	25.6	25.0	30.8
D-Cys	—	32.0	22.8	21.0
D-Glu	19.7	32.3	18.9	21.1
D-Ileu	3.3	4.0	3.6	3.9
D-Leu	7.4	7.2	6.8	6.3
D-Lys	8.1	9.4	11.5	11.3
D-Met	24.7	33.1	29.2	24.3
D-Phe	24.4	24.4	28.0	25.5
D-Ser	41.0	42.2	42.1	44.2
D-Thr	29.3	30.0	32.8	27.8
D-Tyr	15.0	19.5	16.3	13.7
D-Val	2.6	4.0	3.1	2.5

[a] Adapted from ref. 47. Proteins were treated with 0.1M NaOH at 75°C for 3 h.

dehydroalanine, the product of a β-elimination from cysteine, serine, threonine, or phosphoserine, to give lysinoalanine (*see* below; 2, 30). Such chemistry is likely to reduce the nutritional quality of the protein concerned. In contrast, severe heating may also lead to isopeptide bond formation, due to the formation of peptide bonds between the ε-amino group of lysine and the amide groups in asparagine or glutamine residues (2). The latter isopeptides are reported to be between 80–100% biologically available (1) and would, thus, appear to protect lysine residues from other, deteriorative reactions.

Unsaturated lipids can undergo oxidation in the presence of heat, light, or catalysts to form hydroperoxides, aldehydes (including malondialdehyde), ketones, and carboxylic and polymerization products (2). The first 3 of these can react with proteins and modify some amino acids. Hydroperoxidases can oxidize the essential amino acids methionine, cysteine, and tryptophan, whereas the aldehydes and ketones are most reactive with the amino group of lysine residues (21). Quinones can also react with methionine, cysteine, tryptophan, and lysine (21). Quinones are produced from polyphenols that have been oxidized under alkaline conditions or in the presence of the enzyme polyphenoloxidase (28). Oxidizing conditions have also been shown to promote the formation of various dityrosine crosslinks (51) that, for example, have been shown to be important in wheat-based produce (52). In general, this category of crosslinks, produced under oxidizing conditions, is likely to lower the nutritional quality of food proteins.

Although generally considered detrimental to nutritional quality and bioavailability, recent work has suggested that manipulation of the number and nature of protein crosslinks during food processing offers a means by which the food industry can manipulate the functional properties of food without damaging the nutritional quality (44). In particular, the enzyme transglutaminase (TGA) may afford some protection against lysine loss by protecting the amino acid within a pseudopeptide bond that remains bioavailable (53). The reaction of TGA in food systems has great potential to improve the firmness, elasticity, viscosity, heat stability, and water-holding capacity of processed foods (54). TGA has been used to produce favorable changes in functional properties of many foods, including those derived from fish (55), meat (56–58), and dairy products (59). It has also been reported to reduce the allergenicity of wheat flour, offering a potential solution to the celiac intolerance to wheat proteins (60).

(**c**) *Amino acid racemization*.—Racemization of food proteins by alkali treatments was first identified early in the 20th century (61–63). Since then, racemization of amino acids has been reported in a wide variety of foods (47, 64, 65), including alcoholic beverages, baked products, bean products, coffee, corn meal products, dairy products, eggs, food colorants, fruits and vegetables, honey, meat and meat products, fermented cheese, sauces, (mustard, pepper, soy), soups, spices and flavor enhancers, vinegar, and yeast extract. The extent of racemization that can be induced in 4 common food proteins is shown in Table 3.

Amino acids residues can undergo racemization in a range of conditions commonly encountered in food processing, particularly at nonneutral pH (66) and upon heating (67, 68). Racemization may be more likely to occur during peptide cleavage (69) and, therefore, may be more prevalent in foods undergoing proteolysis (*see* above).

Proteins containing D-amino acids have a lower nutritional quality than their L-counterparts due to the combined effects of the inability of proteolytic enzymes to cleave peptide bonds containing a D-amino acid and the subsequent poor uptake and utilization of some essential amino acids. The relative growth response of mice fed D-amino acids is shown in Table 4.

Peptide bonds that contain a D-amino acid, for example L-D, D-D, or D-L, are not fully accessible to normal proteolytic enzymes (70, 71). Absorption of D-amino acids in the intestine (72) and kidney (73) has been reported to be significantly slower than for L-amino acids. If absorbed, 2 metabolic pathways for the utilization of the D-amino acid are potentially available: epimerases or racemases may catalyze the epimerization of the D-amino acid into the corresponding L-amino acid, or a racemic mixture; or amino acid oxidases may catalyze an oxidative deamination, followed by a selective reamination to form the L-amino acid (74). Most mammals have the required D-amino acid oxidases present in the liver and kidney, which catalyze the oxidative deamination of D-amino acids to the corresponding α-keto acid (75).

Because humans are unable to utilize D-lysine, D-leucine, D-threonine, D-trytophan, D-isoleucine, or D-valine (65, 70), it has been proposed that biochemical pathways do not exist to interconvert all D-amino acids for humans (47), which suggests that amino acid racemization has potential nutritional consequences in the human diet. Furthermore, a diet including the simultaneous feeding of several different D-amino acids has been reported to reduce the interconversion of D-amino acids due

Table 4. Relative growth response to D-amino acids in mice fed all amino acid diets[a]

Amino acid	Relative potency (compared to L-form), %
D-Methionine	79.5
D-Phenylalanine	51.6
D-Tryptophan	24.7
D-Leucine	12.4
D-Histidine	8.5
D-Valine	5.1
D-Threonine	3.1
D-Isoleucine	1.2
D-Lysine	–10.3[b]

[a] Adapted from ref. 47.

[b] Average of 2 separate experiments. Negative sign indicates a loss of weight relative to the control diet lacking L-lysine.

to the overloading of the amino acid oxidase system (76). Therefore, a diet high in nonessential D-amino acids has the potential to inhibit the bioavailability of essential D-amino acids (66). The nutritional value of both racemized and native proteins may be adversely affected by racemized amino acid residues that may occupy the active sites of the digestive proteinases, thus reducing access to the active site of the native proteins (47).

A number of factors are known to affect the rate of racemisation of amino acid residues in proteins, including the reaction conditions and protein sequence. It is not yet possible to predict the extent of racemization due to particular food processing conditions, so amino acid analysis must be performed in order to establish the degree of racemization in any given case. Under most (achiral) circumstances, the physical and chemical properties of enantiomers are indistinguishable and, thus, racemization of amino acids is often overlooked.

Ideally, an analysis would measure all the D- and L-amino acids in a single experimental procedure, but quantitation of some 40 amino acids in one assay provides a challenging analytical problem. Currently, quantitation of the enantiomeric composition of amino acid residues in food protein is a 2-step process. First, the hydrolysis of the protein is performed, followed by separation and identification of the residues. One widely used approach is the separation of derivatized amino acids on chiral LC columns and/or analysis by gas chromatography (GC) and coupled GC/mass spectrometry (GC/MS). There are numerous literature reports detailing these approaches (65, 77–79). The search for a robust, fast, economical, facile, and automated chiral amino acid analyzer for all food proteins continues. However, methodology has improved markedly in the past decade, with the range of specialized analysis methods increasing (80–83).

(d) *Oxidative reactions*.—As discussed above, derivatives of unsaturated lipids generated during oxidative reactions can modify several amino acid residues, including methionine, cysteine, tryptophan, and lysine (22). Many of these reactions result in protein crosslinks (*see* above), but others do not. In general, this chemistry, which often results from free radical chain-reaction processes of an ill-defined nature, is not well characterized. However, most oxidative reactions in food lower the nutritional quality of the protein. Many of these reactions are exacerbated by heat and pressure, and they are discussed further in the next section.

(e) *Reactions induced by heat and high pressure*.—Heat is used for a variety of reasons during food processing, including removal of water, fats, and volatiles from raw materials; controlling enzyme activity; inactivation of particular food components and microorganisms; pasteurization and sterilization; and development of desirable sensory properties. Heating is, thus, the most common food processing operation and the one that has the greatest role in protein denaturation and the derivatization chemistry of food proteins. The mechanism of denaturation in food proteins, and the extent to which this governs functionality, has been reviewed elsewhere (84, 85). This is a complicated field, and one that provides some apparent paradoxes. For example, a denatured protein is more likely to react with sugars than a protein in its native state, as discussed above. However, ingredients such as sugars have been shown to decrease the extent of denaturation (84).

Over recent years, the use of extrusion as a method of food processing has become widespread (86–88). During extrusion, food proteins are subjected to high temperatures and pressures (10 000–20 000 kPa) and mechanical shearing. Such shearing forces can lead to breakage of the peptide chain, with consequences similar to those seen for proteolysis (*see* above). The increased denaturation can lead to an increase in digestibility (89, 90) but may also increase the prevalence of Maillard chemistry, with a consequent reduction in nutritionally available lysine (*see* below; 89). However, such influences are critically dependent on the water content of the extruded food and the specific details of the processing regimen. In some studies, the nutritional impacts of extrusion do not appear to be markedly different from other food processing regimens that attain similar temperatures and water content, such as baking (91, 92).

The use of high-pressure conditions in food processing allows higher temperatures to be reached than would be the case at atmospheric pressure. Thus, the specific effects of pressure on food proteins are often hard to disentangle from the consequences of these higher temperatures. The effect of pressure per se on proteins, particularly dairy proteins (93), has been reviewed. Not surprisingly, pressure tends to induce aggregation in proteins (94), but the extent to which this impacts on nutritional quality is unclear.

Of all the possible reactions that proteins may undergo during food processing and storage, the reactions between reducing sugars and proteins are thought to be the most prevalent (2) and are the major cause of the degradation of the nutritional quality of protein in food (2). These so-called Maillard reactions are discussed below.

Table 5. Nutritional impacts of processing of proteins and amino acids

Processing conditions	Phenomenon	Nutritional effects
Heat treatment	Protein denaturation	Improvement in intrinsic digestibility
		Different residues exposed
	Heat-sensitive amino acids	Destruction (ref. 95)
	Intramolecular reaction	Crosslink formation
	Reaction with sugars	Maillard reaction
		Destruction of lysine
	Racemization	Bioavailability
High pressure	Protein denaturation	Improvement in intrinsic digestibility
		Different residues exposed
pH change	Solubilization	Improvement of solubility
	Acid/alkaline hydrolysis	Unspecific peptide bond breakage
	pH-sensitive amino acids	Destruction
		Crosslink formation
		Racemization
Protein fractionation	Membrane technology	Protein/peptide enrichment
	pH modification	Change in amino acid composition
Enzymatic reaction	Proteases	Oxidation of amino acids through lipid or polyphenol oxidation
	Oxygenases	
Modifications to improve food properties	Structural modifications	Reductive alkylation, acylation
	Enzymatic modifications	Proteolysis, crosslinkages, loss of amino acids, less allergenic, amino acid fortification
	Maillard reation	
	Proteolytic enzymes	
	Covalent fixation of amino acids	
Milling (friction and shear forces)	Protein denaturation and aggregation	Different residues exposed
Pressure	Protein denaturation	Different residues exposed (ref. 93)
Fermentation	Racemization	Bioavailability

[a] Adapted from ref. 1.

A summary of the nutritional effects, both positive and negative, of general processing conditions on amino acids and proteins is given in Table 5.

Effect of Processing on Individual Amino Acid Residues

Lysine

The literature on the chemical modification of amino acids during food processing is dominated by reactions of lysine. The reasons for this are 2-fold. First, lysine is an essential amino acid, frequently nutritionally limiting in staple crops such as cereals (30, 35). Compounding this, lysine is also the most chemically reactive of the amino acids, with its ε-amino group particularly vulnerable to damage. Lysine is known to undergo numerous changes during food processing; a summary of the types of reaction is shown in Table 6.

Derivatives of lysine have a spectrum of bioavailability. In some instances, modified lysine is fully bioavailable and, in others, it is not available at all. The biological availability of a range of modified lysine derivatives varies, as is shown in Table 7 (1).

Thus, assessing the availability of lysine in food systems is complicated, because many different species must be considered. Different combinations of these lysine derivatives are detected by different analytical methods, and working out the interrelationship between different measures of lysine and their bioavailability is fraught with difficulty. Table 8 highlights some of the difficulties for a selection of foods.

As mentioned above, severe heating of proteins in the absence of fats and carbohydrates can lead to the reaction of lysine residues with amide side chains, such as asparagine or glutamine, to form internal peptide linkages called isopeptides (101). ε-(γ-Glutamyl)-lysine isopeptide crosslinks

Table 6. Reaction of lysine in food systems—a summary

Reaction
Thermal decomposition (ref. 95)
Maillard reaction
Formation of lysinoalanine
Formation of isopeptides
Reaction with oxidized polyphenols
Acylation

are also introduced by endogeneous or exogeneous transglutaminase. In vivo experiments have shown that a synthetic isopeptide, free ε-(γ-glutamyl)-lysine, is between 80–100% biologically available to rats (97). The isopeptide ε-(γ-glutamyl)-lysine is reported to be as bioavailable as the free synthetic form, due to the proteolytic enzymes in the intestine. However, free synthetic ε-(β-aspartyl)-lysine has been reported to not be biologically available (97). Thus, not all isopeptide bonds afford nutritionally available lysine.

(**a**) *The Maillard reaction.*—The major cause of the degradation of lysine in food proteins is the Maillard reaction (1, 2), an extremely complex network of reactions first described by L.-C. Maillard in 1912 (106). The Maillard reaction is largely responsible for color and flavor development in many foods, and it also causes changes in texture (4).

The details of Maillard chemistry have been reviewed elsewhere (4). Briefly, the first step of the reaction occurs when a free amino group, such as in lysine residues in proteins, reacts with a carbonyl group, such as those found in reducing sugars or fat breakdown products, to give a Schiff's base. This undergoes a rearrangement to form a reasonably stable adduct, first reported by Amadori in 1931 (107), and now known as the Amadori product. A wide range of reactions can occur, depending on conditions such as the pH, water content, temperature, and other molecules present in the particular food system in question. These reactions can include rearrangements, fissions, cyclizations, dehydrations, retroaldolizations, isomerizations, and further condensations, ultimately leading to the formation of melanoidins—brown nitrogenous polymers and copolymers (108). Thus, the eventual fate of lysine residues in food proteins is hard to predict or measure (4).

There are many difficulties inherent in the study of the Maillard reaction, due to both the complex nature of the reaction and the vast variety of potential products, which can vary from those of a low molecular weight, to large, macromolecular and crosslinked structures (109). Hence, while research into the Maillard reaction has been undertaken for many years, it is only more recently that the structures of some Maillard reaction products have been elucidated (109). Even the major reaction products can be very difficult to isolate, because isolation methods may alter the product or advance the reaction along a different path (108, 110, 111).]

The role of the Maillard reaction on the nutritional quality of foods has been extensively reviewed (35, 112). Recent work in this area has focused on the importance of fat and its oxidative breakdown products, detailed identification of the plethora of products produced in this complex system, and the impacts of these in foods on the body (4, 109, 113).

Although the scope for changing Maillard reaction conditions in the context of a specific food process is limited, food processors have invested considerable effort in investigating the effect of process variables on the chemistry and its consequences in terms of product quality (109). The temperature–time combination is an important parameter. For example, a long, gentle heating, rather than a short, high temperature burst, may improve the nutritional value of the final product. Water activity and pH also alter Maillard reaction pathways and may strongly influence the product profile, a fact that may prove useful for food processors trying to preserve the bioavailability of lysine residues (108, 114).

As a general rule, model studies of the Maillard reaction have focused on buffered systems in the pH range 4–7.5 (108), but unbuffered systems have also been studied. The buffers themselves have been found to influence the reaction pathway (115–118), so model studies should be interpreted with caution. Extrapolation to real food systems is inherently problematic although, in some cases, the reaction products may show striking similarities under different regimens (119). Pressure has also been found to affect Maillard chemistry in model systems, and in a pH-dependent way (108). Substances that may well be present during Maillard chemistry, such as metals (120), atmospheric

Table 7. Biological availability of the modified lysine derivatives[a]

Lysine derivatives	Biological availability, %
Alkyl derivatives	
Schiff's bases of aliphatic aldehydes	100
Schiff's bases of reducing sugars	100
Schiff's bases of aromatic aldehydes	0
Amadori compounds	0
"Advanced" Maillard compounds	0
Lysinoalanine	0
Oxidized polyphenols	0
Acylated derivatives	
ε-(γ-Glutamyl)-lysine (free)	80–100
ε-(β-Aspartyl)-lysine (free and bound)	0
ε-(Amino acyl)-lysine (free)	70–100
ε-Formyl-lysine and ε-acetyl-lysine	60–80
ε-Propionyl-lysine (and long-chain fatty acyl-)	0

[a] Adapted from ref. 1; using data from refs. 96–104.

Table 8. Lysine contents; calculated values for inactivated, available lysine; and the percentage of lysine losses in several common foods[a]

Foods	Total lysine[b]	Inactivated lysine[b]	Available lysine[b]	Destroyed, %	Inactivated, %
Breads	2.4 ± 1.4	0.3 ± 0.2	2.1	20	10
Processed breakfast cereals	2.9 ± 1.1	0.6 ± 0.8	2.3	9	19
Pasta	2.6 ± 0.6	0.2 ± 0.2	2.4	0	8
Biscuits	1.4 ± 0.4	0.5 ± 0.3	0.9	46	19
Condensed milk	8.3 ± 0.3	1.2 ± 0.3	7.1	6	14
Chocolate	7.0 ± 0.8	2.0 ± 0.7	5.0	13	25

[a] Adapted from ref. 105.
[b] All values in % of the protein = in g/16 g nitrogen.

oxygen (120, 121), and Maillard inhibitors such as sulfite (122) may all result in dramatic changes in the observed product ratios, and hence, the nutritional quality of the final food as eaten.

In addition to the loss of individual amino acids after heating, a decrease in digestibility of protein can also occur. This can be due to the decreased digestibility of protein aggregates formed during the Maillard reaction, because the digestive enzymes may fail to recognize their substrate or cannot reach the point of action due to solubility or denatuaration limitations. The variety of Maillard reaction products also includes those with the potential to inhibit proteolytic and glycolytic digestive enzymes (2, 35). In low-water-content foods, such as dried fruit, the extent of Maillard chemistry that takes place during storage can be quite large. Amadori compounds can be specifically detected in dried fruit, such as apricots and raisins (123). There is mounting evidence that advanced glycation endproducts consumed in the diet of diabetics pose an added risk factor for those with renal impairment (124). Whether these factors influence the nondiabetic population is not clear.

(**b**) *Impact of lysine damage on lysine measurement*.—Quantitation of lysine in foods can be achieved either by the measurement of total lysine or reactive lysine. Total lysine is determined by amino acid analysis after acid hydrolysis treatment. It is known that the total lysine measured by this method can be significantly different compared to the levels of nutritionally available lysine (125). A number of chemical methods have been developed for the estimation of reactive lysine, that is, lysine residues with an unreacted ε-amino group (125–127). It is presumed that quantitation of this reactive lysine gives a good estimation of the nutritionally available lysine (125).

The main priority with all amino acid testing for nutritional purposes is to measure only those amino acids that are relevant to human or animal nutrition. Figure 3 illustrates the initial lysine content of food or feed as being comprised of both lysine that is available for utilization by the animal for growth and metabolism and unavailable lysine that the animal cannot utilize and subsequently excretes, if it is not metabolized by the gut flora. This lysine may be unavailable because the protein containing it is not digestible by the animal concerned, or because it has been chemically altered so it is no longer metabolized (2). An ideal nutritional test will only measure the available lysine and not any unavailable lysine.

In general, in vivo testing gives a more accurate indication of the true lysine content of a food but tends to be expensive and time-consuming, whereas in vitro testing is quicker and cheaper, but less accurate. Many varied techniques exist within each of these categories, each with advantages and disadvantages, depending on the circumstances of their use. These are discussed in detail elsewhere in this section. For the purposes of this contribution, it is important to note that each of the many different testing methods will detect a different combination of the many possible lysine derivatives found in processed food. In addition to the specific chemistry that may have taken place, the solubility of the proteins, moisture content, and potential interference by other food components must all be taken into account when an assay method is chosen, particularly if large amounts of proteolysis have occurred during the course of processing (*see* above). Some systems that are often tested for lysine loss include infants' foods and formula, milk and dairy products, and many animal

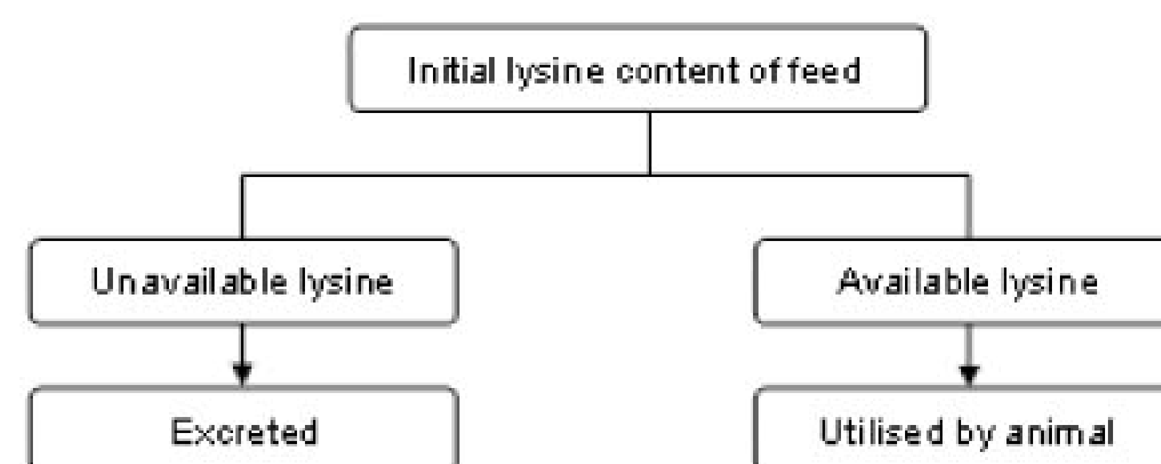

Figure 3. The difference between total, unavailable, and available lysine.

Table 9. A comparison of methods that have been used to determine available lysine levels in foods

Test	Strengths	Weaknesses	References
Feed trials/growth assays	Relatively accurate, relevant to in vivo condition	Expensive, time-consuming, cannot determine dietary needs of nongrowing animals; assumption that animal model relevant to human case	(155)
Plasma amino acid assays	Relatively quick, relatively convenient; can be used to determine limiting amino acids in diet	Dependent on physiological status of bird, catabolic or anabolic; assumption that animal model relevant to human case	(156)
Excreta digestibility assays	Technically simple; can be used on nongrowing animals; surgery/death of animal not required; digested but unmetabolized residues generally not taken into account	Interference from microbe action in hindgut, expensive, time-consuming; assumption that animal model relevant to human case	(156)
Ileal digestibility assays	No interference from microbe action in the hindgut; digested but unmetabolized residues not taken into account; adapted for use with Maillard reacted proteins	Surgery/death of animal required; surgery/cannula can interfere with normal physiology of animal; expensive, time-consuming; assumption that animal model relevant to human case	(142, 161–163)
Microbiological assays	Relatively cheap and efficient compared to other bioassays	Many microbes can utilize modified residues, e.g., early Maillard reaction product ε-fructosyl-lysine, giving results that may be inaccurate for human nutrition	(164, 165)
Amino acid analysis	Accurate, reliable, can be used to estimate total lysine, i.e., both reacted and unreacted lysine content; microwave heat reduces hydrolysis time	Expensive, time-consuming; modified lysine residues that are not digestible but are acid-labile; method could overestimate nutritionally available lysine; can underestimate total lysine if total destruction of lysine residue has occurred	(8, 9, 20, 30, 166)
Enzymatic assays using pepsin with pancreatin or pronase	Lysine release results correlate well with a rat assay	Hydrolysis is never complete and only free lysine is measured; unmeasured reactive lysine may still be present in small peptides	(164, 167)
Enzyme-based biosensors	Fast and accurate	Limited to food systems such as milk; will not detect mild modification that may still be nutritionally available	(168)
FDNB[a] method	Fast and cheaper than in vivo tests	Unsuitable for samples containing high levels of polysaccharides; does not measure free or *N*-terminal lysine; free arginine will react; time-consuming, long acid hydrolysis step; reagent not water-soluble; slightly overestimates blocked lysine	(125, 164, 169, 170)
FDNB difference method	Faster and cheaper than in vivo tests; yields more information than simple FDNB method	Long acid hydrolysis step; reagent not water-soluble	(20)
TNBS[b] method	Reagent water-soluble, acid hydrolysis step is shorter than in the FDNB method; only peptides, not amino acids, are required for reaction; free arginine does not give a colored product	Product more susceptible to interference from carbohydrates than the FDNB method	(20, 126, 171)

Table 9. (*continued*)

Test	Strengths	Weaknesses	References
MIU[c] (guanidination)	Gives results similar to those obtained by animal tests	Time-consuming method taking 2–4 days to complete; variable absolute values are obtained	(20, 142, 162–165)
Furosine	Quantitation of early Maillard reacted lysine can be made; useful in milk systems	Only relevant when lysine is blocked as Maillard derivatives; late Maillard reaction products are assumed not to degrade to give lysine	(21)
Sodium borohydride	Used in conjunction with amino acid analysis; reacts with deoxyketosyllysine to give a product that does not produce lysine on acid hydrolysis	Sodium borohydride also reduces the biologically available Schiff's base form of lysine	(20, 172)
Dye-binding lysine	Does not erroneously detect late Maillard products as nutritionally available	Azo dyes react with deoxyketosyl lysine, skewing results	(20, 173, 174)
Ninhydrin	A one-step protein extraction/lysine content assay; very small quantities of samples are required	Sample homogeneity can be an issue	(175–178)
OPA[d]	Fluorescent or visible light quantitation can be used; very sensitive if fluorescence used, cheap and quick	Fluorescence can be quenched by peptide bonds; terminal amino group can react	(154)
Fluorescamine	Very fast reaction, sensitive	Does not appear to have been used extensively for lysine analysis	(179)

[a] FDNB = 1-Fluoro-2,4-dinitrobenzene.
[b] TNBS = Trinitrobenzene sulfonic acid.
[c] MIU = *o*-Methylisourea.
[d] OPA = *o*-Phthaldialdehyde.

feedstuffs, especially those used by the intensive livestock industries, including poultry, swine, and fish (128–155).

It is commonly assumed that the best way to work out the efficacy of a food or feed as a nutritional source is, ultimately, to feed it to an animal. However, performance in animal feeding trials may not always be related to the variable in question. Determination of bioavailability ["being in a form appropriate for digestion, absorption and utilization" (156)] is rarely straightforward, and measurement may be confounded by animal variation (157), diet variation and feed intake, antinutritional factors in the diet, interactions between amino acids, and the typically curvilinear response graph for the amino acid under investigation (156). For example, if the Maillard reaction has occurred to a greater degree in a particular feedstuff, this may result in greater (or lesser) palatability, the formation of growth depressants and anti-nutritional compounds, or even the suppression of microbial activity (158, 159).

A summary of methods used to monitor nutritionally available lysine post-processing, with the strengths and limitations of each, is given in Table 9. The large number of methods reflects the lack of a general analytical test that is appropriate for all purposes. With all methods, sample preparation must also be taken into account. Poor sample preparation will affect the reliability of almost all methods. A good review of the necessary steps of sample preparation has been given by Finley (160).

A summary of the interrelationships between the different types of in vitro lysine testing methods is given in Figure 4.

Given the number of methods available for measuring the lysine content, choosing the best method for a particular situation can be a challenging task. In Figure 5, data are presented from a study by Hurrell and Carpenter (20), in which various methods were compared for their ability to give absolute values for lysine contents in an unheated albumen/glucose sample. Compared with growth assays, all tests slightly underestimated lysine contents, and both the TNBS and the *o*-methylisourea (MIU) methods did so significantly. However, later research has not confirmed that MIU underestimates lysine content (P.J. Moughan personal communication, 2003).

In Figure 6, the results of further studies by Hurrell and Carpenter (20) are shown, in which the ability of various methods to estimate lysine loss through processing is examined by comparing the results found from each test type with that given by a growth assay. From these results, it can be

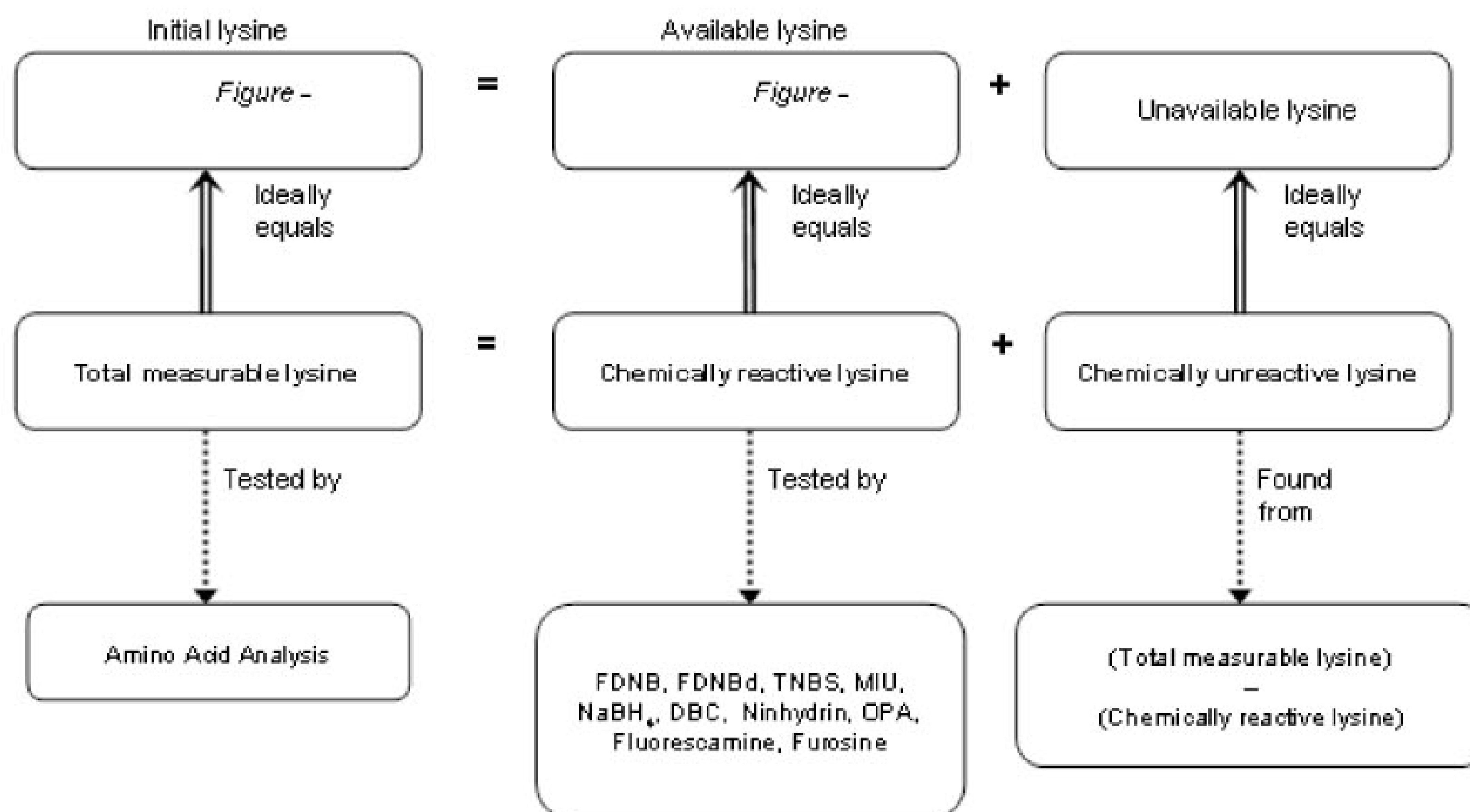

Figure 4. An overview of chemical analyeis for reactive lysine. The major assumption made is that chemically reactive lysine is equivalent to available lysine (there are exceptions, e.g., glutamyl-lysine is not reactive, but remains bioavailable). FDNBd = 1-fluoro-2,4-dinitrobenzene, difference method; DBC = dye binding capacity.

seen that, as damage to the protein increases from condition a to c, so does the difference between the results found from growth assays and those from most test types. A small increase in variability would be expected as a result of the available lysine found from the growth assay, decreasing from 78% in condition a to 27% in condition c. However, this does not account for the large variations shown by a number of the tests for condition c. This highlights the difficulty of testing for lysine after Maillard reaction has occurred.

The FDNB (1,fluoro-2,4-dinitrobenzene) method gave the best overall comparison with the growth assays under the conditions used. The sodium borohydride method also gave reasonably close approximations, but it does tend to underestimate available lysine, which can be explained by the propensity of this method to give a negative result for the biologically available Schiff's base. The dye-binding lysine (DBL) method closely approximated growth under these conditions, as did the MIU method. In contrast, amino acid analysis (total lysine) and the FDNBd method overestimated available lysine under all conditions. The TNBS method, which was only used in condition c, also strongly overestimated available lysine. The conclusion from Hurrell and Carpenter (20) was that the direct FDNB, $NaBH_4$, and MIU methods were the most suitable of the methods tested for measuring lysine after early Maillard reactions.

(c) *Lysinoalanine*.—Alkali and heat treatment of food proteins results in the formation of crosslinked amino acids, such as lysinoalanine. This derivative of lysine is not usually bioavailable (Table 7) and may represent serious nutritional damage to the protein concerned. A study by Maga (180) discovered lysinoalanine in a variety of foods (Table 10).

The presence of lysinoalanine in proteins has been shown to decrease digestibility and nutritional quality in rodents and primates, but to enhance nutritional quality in ruminants (45). Lysinoalanine in milk and milk products has been implicated in kidney damage in rats (181). Its formation is hindered under oxidizing conditions, due to preferential formation of oxidized forms of cysteine, such as cysteic acid (182).

Beyond Lysine

Although the best documented, lysine is not the only amino acid residue that reacts during the processing of food proteins. As noted in the introduction, loss of nutritional value is also

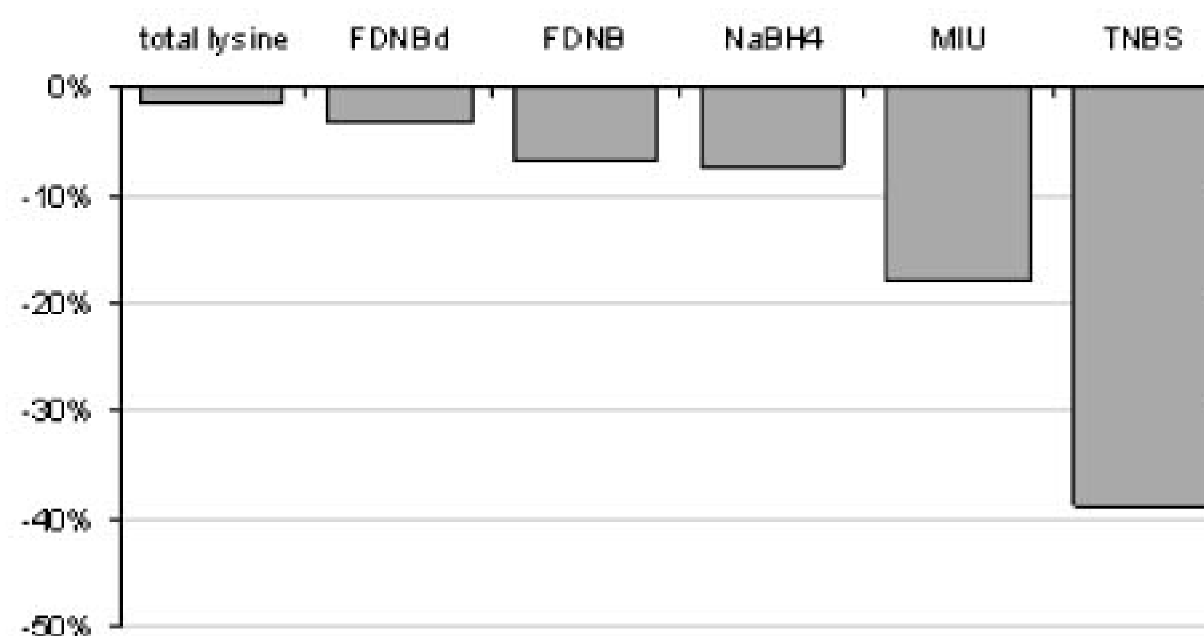

Figure 5. Comparison of the ability of various methods to give an absolute value for the amount of lysine in an unheated albumen control sample. Data are presented as percentage differences from the values found from rat and/or chick growth assays (adapted from data presented in ref. 20).

associated with reactions of the essential amino acids methionine, cysteine, and tryptophan and, to a lesser extent, arginine, histidine, phenylalanine, and tyrosine (1). Figure 7 shows a selection of the better characterized amino acid derivatives that have been reported to occur during food processing. Some of these reactions are detailed in the following sections.

Arginine

Arginine is a "conditionally essential" amino acid, which means it is normally synthesized in vivo but, under some specific physiological conditions it is not synthesized at a sufficient rate to meet the body's needs. It is a reactive amino acid through its guanidine group, which is known to take part in the Maillard reaction and to undergo a variety of modifications at alkaline pH, resulting in covalent modifications, including crosslinks (*see* above). One of the earliest qualitative tests for arginine was based on an observation by Sakaguchi in 1925 that certain guanidine derivatives gave a color change on reaction with α-napthol at high pH (183, 184). The popularity of this method, with various modifications, continued for some time. Today, the more common method for determining arginine concentration is via automated amino acid analysis, but this involves acid hydrolysis, that may lead to artefactual results, in an analogous fashion to the determination of lysine (*see* above; 185).

A more convenient method for testing arginine content, which is 1000 times more sensitive than the Sakaguchi method, was developed by Smith and MacQuarrie in 1978 (185). This method uses 9,10-phenanthrenequinone, a diketo compound that forms a stable fluorescent adduct upon reaction with the guanidine group of arginine. Unlike the Sakaguchi method, the protein does not need to be hydrolyzed prior to analysis, making it a more suitable method for quantitating arginine residues that may have been derivatized during the Maillard reaction.

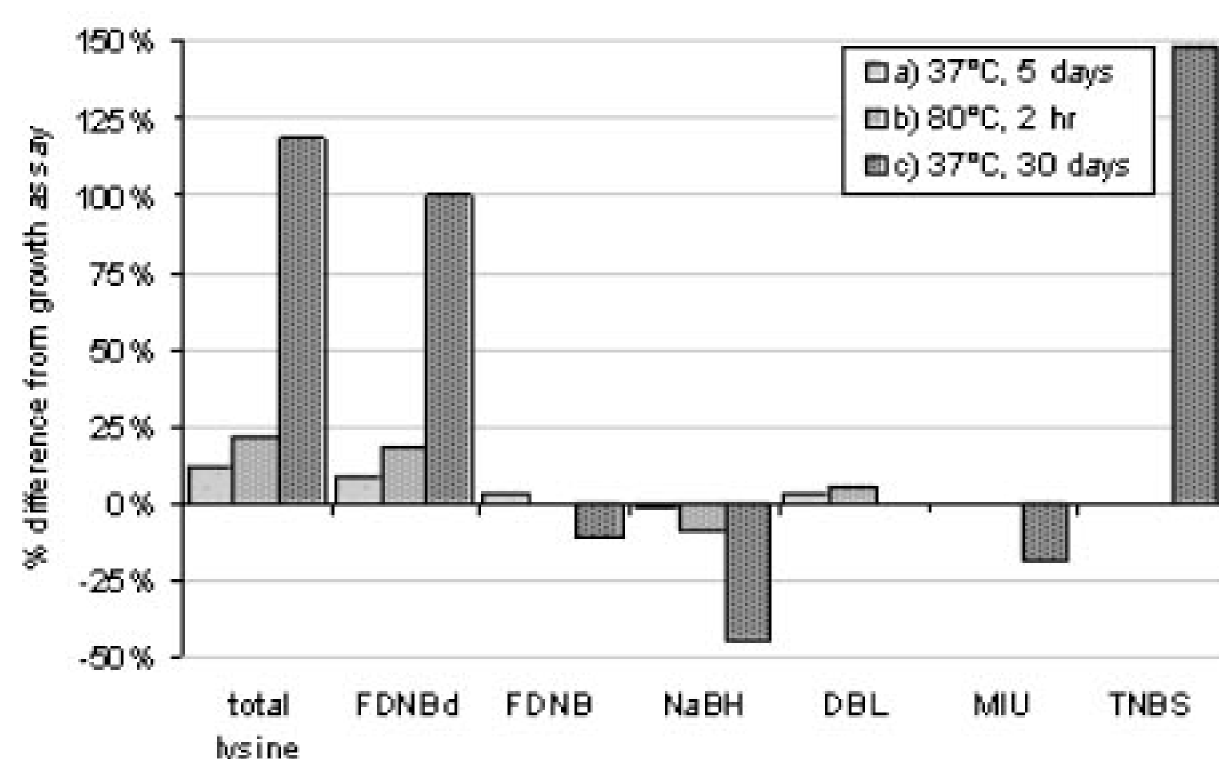

Figure 6. Comparison between different techniques for measuring lysine in albumen–glucose mixtures, for samples heated under different regimens. Data are presented as percentage differences from values found from rat and/or chick growth assays. Growth assays showed 78% lysine remaining for condition a; 69% for condition b; and 27% for condition c. Adapted from data in ref. 20. (No data available for DBL, condition c; MIU, TNBS conditions a and b.)

Table 10. Lysinoalanine (LAL) content of common food and food ingredients[a]

Product	Type	LAL, μg/g protein
Cereal	Corn	Not detected
	Puffed rice	1000
	Rice	Not detected
	Toasted oat	160
	Wheat	Not detected
Chicken thigh	Raw	Not detected
	Retorted	100
	Oven baked	110
	Charcoal broiled	150
	Retorted in gravy	170
	Cooked in microwave	200
Egg white	Fresh	Not detected
	Boiled 3 min	140
	Boiled 10 min	270
	Boiled 30 min	0–370
	Pan-fried 10 min at 150°C	350
	Pan-fried 30 min at 150°C	1100
Frankfurter	As purchased	Not detected
	Boiled	50
	Fried	50
	Charcoal-broiled	150
	Oven-baked	170
Milk	Infant formula	0–640
	Evaporated	200–860
	Skim evaporated	520
	Condensed (as manufactured)	360–540
	Dry	Not detected

[a] Adapted from ref. 180.

Tryptophan

Tryptophan is an essential amino acid with a wide range of biological functions. Although lysine and methionine are the most limiting amino acids in many food proteins, tryptophan has been reported to be the second limiting amino acid in maize. Friedman and Cuq (186) produced a seminal review on the chemistry, analysis, nutritional value, and toxicology of tryptophan in food.

Tryptophan can undergo a great diversity of modifications in food processing systems, including racemization; carboline formation; and reaction with oxygen and other food-oxidizing lipids, vitamins, reducing sugars, carbonyl compounds, nitrites, halogens, and sulfites (187, 188). These

modifications have multifarious consequences, including the production of antinutritional and toxic compounds. The reactivity of tryptophan is mainly due to the reactivity of the aromatic electron-rich indole ring. Tryptophan is susceptible to a number of modifications, including oxidative cleavage and substitution, and reaction with aldehydes. In food systems, the hydrophobic tryptophan residues are often buried within the interior of the protein and, therefore, are only readily accessible to react with other food ingredients if the protein is denatured. Detailed analysis of tryptophan in food systems has been hampered by the lack of a reliable analytical technique (189).

While free tryptophan undergoes thermal degradation in the presence of oxygen to form kynurenine, protein-bound tryptophan is less sensitive to such oxidation. In food systems under moderate processing conditions, protein-bound tryptophan does not appear to suffer significant oxidative loss. Tryptophan has been shown to be susceptible to Maillard chemistry, forming carbolines under appropriate conditions (190). It is also reported to react during protein-oxidized lipid interactions (2). For example, the changes in available tryptophan during industrial production of wheat, rye, barley, and oat flakes have been investigated. Tryptophan levels were found to be lowered significantly after hydrothermal treatment and during the flaking process. The decrease in tryptophan levels has been found to correlate with

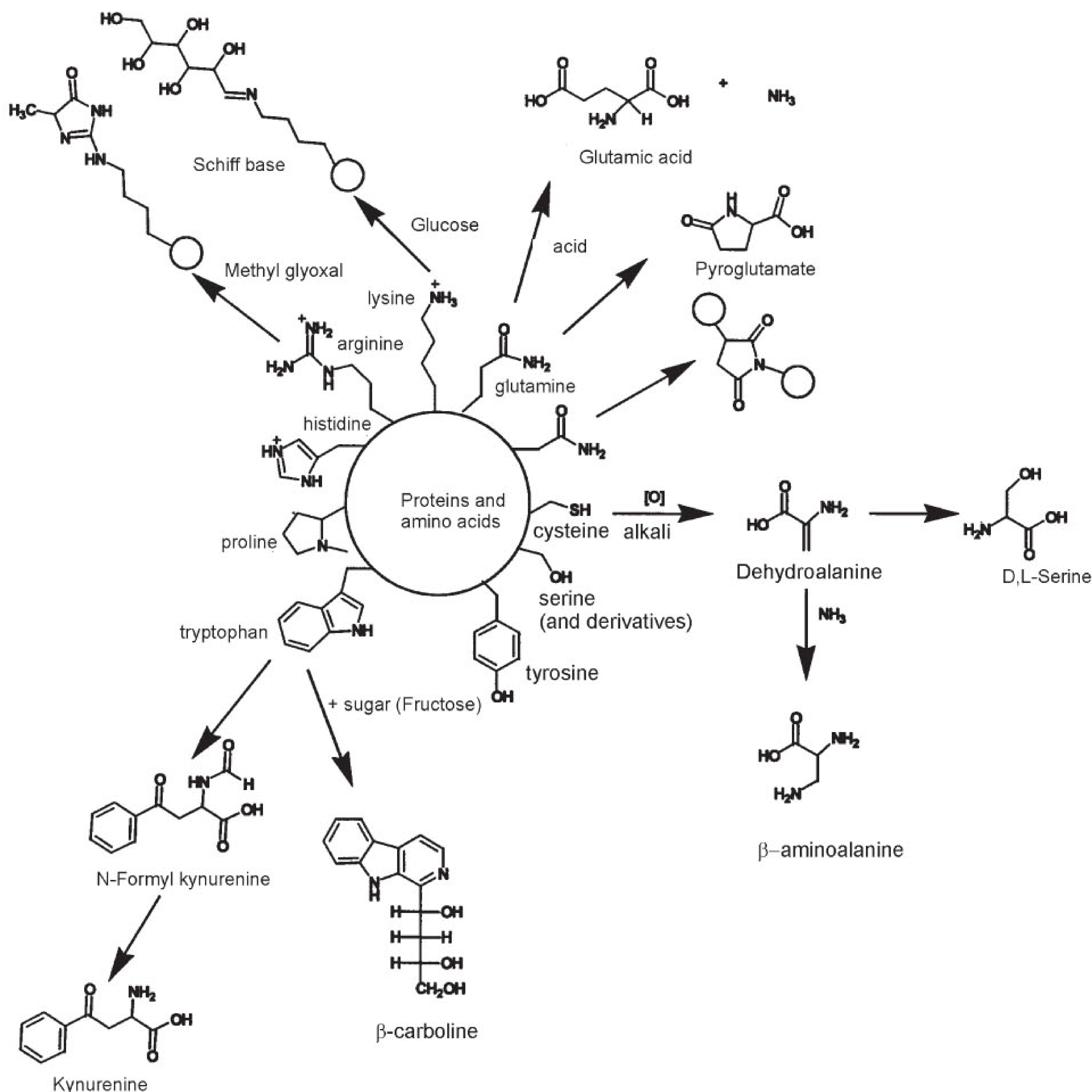

Figure 7. A selection of the better characterized amino acid derivatives that may form during food processing. (Details are discussed in the text.)

the levels of lipid oxidation products, thus implying the loss of tryptophan is due to reaction with these lipid oxidation products (191).

Analysis of protein-bound tryptophan, like many other amino acid residues, is usually measured by LC after hydrolysis. However, for tryptophan analysis, analytical difficulties are encountered because the parent amino acid is destroyed by acid. Therefore, alkaline hydrolysis followed by LC is the most widely used method for analysis of tryptophan in foods. This method, therefore, cannot detect alkali-labile derivatives (187). Rats, chicks, and pigs are reported to be suitable animal models for estimating tryptophan bioavailability in food (187, 192). However, the bioavailability of modified tryptophan has been under debate for some time (189). There exists contradictory evidence as to the effect of heat on the bioavailability of both free and protein-bound tryptophan. There remains a pressing research need to better understand the chemistry of tryptophan during food processing, particularly to examine the toxicology of the products formed in a variety of processing conditions.

Conclusions

The literature in this field has been dominated by the chemistry of lysine during food processing, but despite years of research, there is still much to learn and much improvement to be made to our methodology for measuring and monitoring the damage to this important amino acid during food processing.

With few exceptions, the reactions and bioavailability of other protein and nonprotein (193) amino acids have largely been overlooked. Many amino acid residues have low chemical reactivity and, therefore, are not thought to undergo major modifications in food processing. This lower chemical reactivity means modifications are likely to occur at a slower rate or only under extreme conditions. Additionally, the modification of nonessential dietary amino acids has not been a priority for those interested in the effects of food processing on nutrition.

However, given the paucity of information in this area and the potential for a wide range of food chemistry to take place during food processing, more research is required on the chemistry of modification of all amino acid residues. Particular attention should be paid to the generation of toxic compounds, to preempt high profile public concerns such as those surrounding recent reports of acrylamide forming during the Maillard reaction of asparagine residues under high temperature food processing conditions (193–195).

Acknowledgments

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