Annu. Rev. Nutr. 1992. 12:119–37

DIETARY IMPACT OF FOOD PROCESSING[1]

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KEY WORDS: food browning, food processing, browning prevention, food safety, nutrition

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INTRODUCTION

A variety of processing methods are used to make foods edible, to permit storage, to alter texture and flavor, to sterilize and pasteurize food, and to destroy toxic microorganisms. These methods include baking, cooking, freezing, frying, and roasting. Many such efforts have both beneficial and harmful

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effects. It is a paradox of nature that the processing of foods and feeds can improve nutrition, quality, safety, and taste, and yet occasionally can lead to the formation of antinutritional and toxic compounds. These multifaceted consequences of food processing arise from molecular interactions among nutrients and with other food ingredients. Billions of new compounds can, in principle, be formed from such interactions among the approximately 60 known nutrients.

Beneficial and adverse effects of food processing are of increasing importance to food science, nutrition, and human health. A better understanding of the molecular changes during food processing and the resulting nutritional and safety consequences is needed to optimize beneficial effects such as bioavailability, food quality, and food safety, and to minimize the formation and facilitate the inactivation of deleterious compounds. Such an understanding will encompass multidisciplinary studies of the chemistry, biochemistry, nutrition, and toxicology of food ingredients. This limited review uses examples largely based on our own studies to illustrate general concepts. It describes the nutritional impact of two major food processing conditions: pH and heat. The references cited offer the reader an entry into the comprehensive, but widely scattered, relevant literature (1–107).

EFFECT OF pH

Racemization of Amino Acids

Since the early part of this century, alkali and heat treatments have been known to racemize amino acids (9). As a result of food processing using these treatments, D-amino acids are continuously consumed by animals and man. Because all of the amino acid residues in a protein undergo racemization simultaneously, but at differing rates, assessment of the extent of racemization in a food protein requires quantitative measurement of at least 36 optical isomers, 18 L and 18 D. Analytically, this is a difficult problem not yet solved (48, 54, 55, 74, 75, 77).

Racemization of an amino acid proceeds by removal of a proton from the α-carbon atom to form a carbanion intermediate. The trigonal carbon atom of the carbanion, having lost the original asymmetry of the α-carbon, recombines with a proton from the environment to regenerate a tetrahedral structure. The reaction is written as

$$\text{L-amino acid} \underset{k'_{rac}}{\overset{k_{rac}}{\rightleftharpoons}} \text{D-amino acid} \qquad 1.$$

where k_{rac} and k'_{rac} are the first-order rate constants for the forward and reverse racemization of the stereoisomers.

The product is racemic if recombination can take place equally well on either side of the carbanion, giving an equimolar mixture of L- and D-isomers. Recombination may be biased if the molecule has more than one asymmetric center, resulting in an equilibrium mixture slightly different from a 1:1 enantiomeric ratio.

Because the structural and electronic factors that facilitate the formation and stabilization of the carbanion intermediate are unique for each amino acid, it follows that the reaction rate for the isomerization of each amino acid is also unique. Thus, the inductive strengths of the R-substituents have been invoked to explain differing racemization rates in the various amino acids. Plotting racemization for individual amino acids in casein and soybean proteins against the inductive parameters clearly demonstrates strong correlations (74, 75, 77).

Two pathways are available for the biological utilization of D-amino acids: (*a*) racemases or epimerases may convert D-amino acids directly to L-isomers or to (DL) mixtures; or (*b*) D-amino-acid oxidases may catalyze oxidative deamination of the α-amino group to form α-keto acids, which can then be specifically reaminated to the L-form. Although both pathways may operate in microorganisms, only the second has been demonstrated in mammals.

The amounts and specificities of D-amino acid oxidase are known to vary in different animal species. In some, the oxidase system may be rate limiting in the utilization of a D-amino acid as a source of the L-isomer. In this case, the kinetics of transamination of D-enantiomers would be too slow to support optimal growth. In addition, growth depression could result from nutritionally antagonistic or toxic manifestations of D-enantiomers exerting a metabolic burden on the organism.

The nutritional utilization of different D-amino acids varies widely, both in animals and humans (3, 42–44, 47, 48, 50, 54, 55, 77). In addition, some D-amino acids may be deleterious. For example, although D-phenylalanine is nutritionally available as a source of L-phenylalanine, our studies have shown that high concentrations of D-tyrosine inhibit the growth of mice (43). The antimetabolic effect of D-tyrosine can be minimized by increasing the L-phenylalanine content (protein bound, or free) of the diet. Similarly, L-cysteine has a sparing effect on L-methionine when fed to mice (44); however, D-cysteine does not. The wide variation in the utilization of D-amino acids is exemplified by the fact that D-lysine is not utilized as a source of the L-isomer for growth. The utilization of methionine is dose dependent, reaching 76% of the value obtained with L-methionine. Both D-serine and the mixture of L-L and L-D isomers of lysinoalanine induce histological changes in the rat kidneys. D-tyrosine, D-serine, and lysinoalanine are produced in significant amounts under the influence of even short periods of alkaline treatment.

Unresolved is whether the biological effects of D-amino acids vary depending on whether they are consumed in the free state or as part of a food protein.

Indications are that L-D, D-L, and D-D peptide bonds in food proteins may not hydrolyze as readily as naturally occurring L-L peptide bonds (50, 58, 82). Possible metabolic interaction, antagonism, or synergism among D-amino acids in vivo also merits further study. The described results with mice complement related studies with other species and contribute to the understanding of nutritional and toxicological consequences of ingesting D-amino acids. Such an understanding will make it possible to devise food processing conditions to minimize or prevent the formation of undesirable D-amino acids in food proteins and to prepare better and safer foods.

Lysinoalanine Cross-links

Lysinoalanine [$HOOCCH(NH_2)CH_2CH_2CH_2CH_2NHCH_2CH(NH_2)COOH$] (LAL), is an unnatural amino acid that has been identified in hydrolyzates of processed food proteins, in particular those subjected to alkali (16, 25, 53, 58, 75).

Detailed studies revealed that base-catalyzed synthesis of lysinoalanine proceeds by the addition of the ϵ-NH_2 group of lysine to the double bond of a dehydroalanine residue. This residue is derived from cysteine and/or serine. From a nutritional standpoint, lysinoalanine formation results in a decrease of the essential amino lysine, and the semiessential amino acid cystine, as well as in a decrease in digestibility of the modified protein (51, 53, 58).

In rats, studies have found histological changes in the kidneys related to dietary exposure to this substance, either isolated or as part of intact proteins (104). The lesions are located in the epithelial cells of the straight portion of the proximal renal tubules and are characterized by enlargement of the nucleus and cytoplasm, increased nucleoprotein content, and disturbances in DNA synthesis and mitosis.

Because of these observations, concern has arisen about the safety of foods that contain LAL and related dehydroalanine-derived amino acids known to produce similar lesions. However, since the mechanism by which these compounds damage the rat kidney is unknown, it is difficult to assess the risk to human health caused by their presence in the diet.

LAL has two asymmetric carbon atoms, making possible four separate diastereoisomeric forms: LL, LD, DL, and DD. Its structure suggests that it should have excellent chelating potential for metal ions, a property that may be relevant to its toxic action. Accordingly, we have examined LAL for its affinity towards a series of metal ions, of which copper (II) was chelated the most strongly (40b, 57, 87). On this basis, we have suggested a possible mechanism for kidney damage in the rat involving LAL's interaction with copper within the epithelial cells of the proximal tubules.

Of the four isomers of LAL, LL and LD are derived from L-lysine and the other two from D-lysine. Since L-lysine is the natural amino acid present in

proteins, most of the LAL formed during food processing can be expected to be a mixture of LL and LD. However, since exposure of food proteins to heat and alkali may racemize a small fraction of L-lysine to the D-isomer, treated food proteins may also contain small amounts of DL- and DD-LAL.

As described in detail elsewhere (57, 87), it is possible to predict the equilibria in vivo between histidine, the major low-molecular weight copper carrier in plasma, and competing chelating agents such as LAL (Equation 2).

$$CuHis_2 + H_2LAL \rightleftharpoons CuLAL + 2HHis \qquad 2.$$

A mathematical analysis of the equilibrium shown in Equation 2 was used to calculate LAL plasma levels needed to displace histidine as the major copper carrier in vivo. The calculated values are 27 μM for LD-LAL, 100 μM for LL-LAL, and 49 μM for the mixture of the two.

The above considerations suggest that LD-LAL would be a better competitor for copper (II) in vivo than the LL-isomer, i.e. it should take about one fourth as much LD-LAL as LL-LAL to displace the same amount of histidine from copper-histidine. This difference could explain the greater observed toxicity of the LD-LAL. The apparent direct relationship between the observed affinities of the two LAL isomers for copper (II) ions in vitro and their relative toxic manifestation in the rat kidney is consistent with our hypothesis that LAL exerts its biological effect through chelation of copper in body fluids and tissues. Limited studies on the binding of LL- and LD-lysinoalanines to cobalt (II), zinc (II), and other metal ions imply that lysinoalanine could also influence cobalt utilization in vivo. Animal studies are needed to confirm the predicted role of lysinoalanine in metal ion transport, utilization, and histopathology (13a, 19, 21, 81, 89a).

EFFECT OF HEAT

Maillard Browning

Maillard-type reactions of primary amino groups with reducing sugars, and other nonenzymatic browning reactions with nonreducing carbohydrates, cause deterioration of food during storage and commercial or domestic food processing. The loss in nutritional quality, and potentially in safety, is attributed to some or all of the following factors: (*a*) destruction of essential amino acids, (*b*) decrease in digestibility, and (*c*) production of antinutritional and toxic compounds (1a, 1b, 8, 13a, 14, 17, 18, 22–29, 34, 35, 38–40, 52a, 60, 61, 70–73, 76, 78–82, 88, 90, 98, 102, 106, 107).

Although extensive efforts have been made to elucidate the chemistry of both desirable and undesirable compositional changes during browning, parallel studies on the nutritional and toxicological consequences of browning are

limited. This is understandable since, in principle, each combination of a specific amino acid or protein with a particular carbohydrate needs to be investigated to understand the scope of the problem. Reported studies in this area include (*a*) influence of damage to essential amino acids, especially lysine, on nutritional quality; (*b*) effects of fortifying browning products with essential amino acids on recovery of nutritional quality; (*c*) nutritional damage as a function of processing conditions; (*d*) biological utilization of characterized browning compounds, such as ϵ-*N*-deoxy-fructosyl-L-lysine; and (*e*) formation of mutagenic and clastogenic products.

A number of investigators have examined the effects of the Maillard browning reaction on digestibility and nutritional quality (14, 17, 18, 61–64, 73, 82, 83, 85). Our studies (61) show that loss of nutritional quality of heat-treated casein is related to decreased nitrogen digestibility rather than to simple destruction of essential amino acids. The influence of glucose and starch was minimal compared to observed effects of heat on casein alone under the conditions used. Glucose and perhaps starch augment protein degradation and loss of nutritional quality under moderate, dry-heat conditions. Further studies are needed to explain the molecular basis for the extent and nature of the heat-induced destruction of essential amino acids and the formation of undigestible browned and cross-linked products. These changes impair intestinal absorption and nutritional quality in general. Toxic compounds formed under these conditions might also modulate nutritional quality. Thus, such studies should differentiate antinutritional and toxicological interrelationships and develop means for preventing or minimizing the formation of deleterious compounds in foods.

Ascorbate Browning

When a nutritionally complete, low-protein basal diet containing 10% casein was supplemented with 20% protein from unheated casein, wheat gluten, or soybean, test mice exhibited a significantly increased weight gain (52, 52a, 107). In contrast, weight gain was markedly reduced when the supplement was soy protein or gluten heated at 200° or 215°C for 72 min in the dry state (simulated crust baking). Baked casein was nonnutritive. Adding carbohydrates to gluten during heating prevented subsequent growth inhibition. After heating with sodium ascorbate (but not L-ascorbic acid), soy protein (at 200°C) and gluten (at 215°C) completely prevented growth when added to the basal diet. Growth inhibition was also aggravated by a heated casein-ascorbate mixture, but less than with the other proteins. The extent of nutritive damage increased sharply with heating temperature in the range 180 to 215°C, and with sodium ascorbate concentration in the range 1 to 20%.

In a related study, Oste & Friedman (85) showed that sodium ascorbate heated with amino acids, especially tryptophan, results in the formation of antinutritional compounds.

The reduced weight gain of mice fed a nutritionally adequate diet supplemented with these materials suggests that heating induces the formation of nutritionally antagonistic or toxic compounds that interfere with essential metabolic pathways such as digestion, transport, absorption, and utilization of nutrients. Further studies of the chemical basis of these effects may be more conveniently performed with tryptophan or other amino acid/ascorbate mixtures than with the more complex protein/ascorbate blends, since the heat-induced products may be easier to isolate and characterize.

Our heating experiments used proportionately much more sodium ascorbate than is used in thermal food processing to improve bread-dough characteristics such as loaf volume and bread texture, and to inhibit nitrosamine formation in bacon. However, since our findings do not rule out possible cumulative biological effects, additional studies are needed to determine whether consumption of low levels of the heat-derived compounds can be a human health hazard.

Our results suggest that deleterious material formed during heating of gluten or soy protein, and to a lesser extent casein, may represent the degradation of protein to nitrogenous materials without nutritional value. At the nominal protein level fed, such materials would represent a severe metabolic burden (toxic effect) to the animal, which must then eliminate them. The protective effect of carbohydrates in diminishing the formation of toxic gluten is interpreted as a thermochemical volatilization of deleterious products, while sodium ascorbate appears to reduce vaporization.

These considerations suggest the need (*a*) to characterize the compound(s) in heated protein- and amino acid-sodium ascorbate mixtures that may be responsible for the observed growth inhibition; (*b*) to determine the safety of the pure compounds in laboratory animals and measure their prevalence in commercial foods in order to define possible human risk; (*c*) to carry out studies with related food ingredients such as sodium citrate, sodium gluconate, and sodium glutamate in order to define the mechanism of this type of growth inhibition; and (*d*) to devise processing conditions to prevent the formation of the growth inhibitors in food (10, 13, 69).

Food Allergenicity

As noted earlier, carbohydrates interact with proteins to form Maillard browning products. Oste and colleagues (83, 84) studied the effects of these transformations on the antigenicity of the Kunitz soybean trypsin inhibitor (KTI) with two monoclonal antibodies. They report that solid mixtures of KTI and carbohydrates were heated in an oven at 120°C, dialyzed, freeze-dried, and analyzed by enzyme-linked immunosorbent assay (ELISA). Glucose, lactose, and maltose decreased the antigenicity of KTI to levels 60–80% lower than those observed in a control sample heated without carbohydrate. Starch was less effective than the three reducing sugars. The decrease was

rapid, occurring within 10 min when glucose was heated with KTI, with retention of 60% of the chemically available lysine. Longer heating times increased browning and reduced the level of available lysine in KTI, without further reducing antigenicity. The results suggest that relatively mild conditions of heating food proteins with carbohydrates can reduce the antigenicity of the protein and possibly modify sites known to elicit allergenic responses.

The nature and extent of browning reactions, as well as the magnitude of antigenicity changes, are probably highly dependent on the experimental conditions. In addition, the relative importance of the Maillard reaction and the reactions of nonreducing carbohydrates merit further study. Nevertheless, the results of our studies lead us to hypothesize that the early stages of the Maillard reaction can significantly affect protein antigenicity. Note that these reactions can also introduce new antigenic determinants into a food protein (6, 11, 12).

The Schiff's base formed in the first step of the Maillard reaction is biologically available (14, 17). Therefore, it does not adversely affect protein nutrition. Many foods present a favorable medium for the Maillard reaction, including allergenic foods such as milk (67b). These results suggest that the antigenic sites of food proteins responsible for adverse allergenic responses, by eliciting production of IgE and possibly other isotypes that trigger allergic reactions, could be selectively altered by modification with reducing carbohydrates under mild conditions. Chemical and structural modification during food processing could at least partly account for the observation that cow's milk is less antigenic in vivo after heat treatment and for apparent differences in the allergenicities between liquid and powdered soybean infant formulas. Conditions might be developed to exploit such beneficial effects of nonenzymatic browning and related food-processing-induced changes.

Inactivation of Inhibitors of Digestive Enzymes

Adverse effects of ingestion of raw soybean meal have been attributed to the presence of soybean inhibitors of chymotrypsin and trypsin and to other factors. To improve nutritional quality and safety of soy products, inhibitors are partly inactivated by heat treatment during food processing. The approach used is exemplified by the following summary of a study by Friedman et al (37).

The content and heat stability of protease inhibitors of a standard cultivar (Williams 82) and an isoline (L81-4590) lacking the Kunitz trypsin inhibitor (KTI) were measured by using enzyme inhibition and enzyme-linked immunosorbent assays (ELISA). Steam heating of the isoline flour (121°C, 20 min) resulted in a near-zero level of trypsin inhibitory activity, while 20% remained in the Williams 82 sample. The raw soy flour prepared from the isoline was nutritionally superior to the raw flour prepared from the standard

variety, as measured by protein efficiency ratio (PER) and pancreatic weights. The increased PER was likely due to the lower level of trypsin inhibitor activity in the isoline. Steam heating the flours for up to 30 min at 121°C progressively increased the PER for both strains. Less heat was needed to inactivate the inhibitors in the isoline than in the standard cultivar.

Related studies showed that treating raw soy flour with cysteine, *N*-acetyl-L-cysteine, or reduced glutathione introduces new half-cystine residues into native proteins, with a corresponding improvement of nutritional quality and safety (46). The proteins are modified through formation of mixed disulfide bonds among added thiols, protease inhibitors, and structural protein molecules. This leads to decreased inhibitory activity and increased protein digestibility and nutritive value (40a). The SH-containing amino acids also facilitate heat inactivation of hemagglutinins (lectins) in lima bean flour. Exposure of raw soy flour to sodium sulfite was also nutritionally beneficial (40a, 40b, 45, 46).

Naturally occurring enzyme inhibitors, such as Bowman-Birk inhibitor (BBI) in which every sixth amino acid residues is cystine, also have beneficial effects such as prevention of development of colon cancer in mice (97). Although the molecular basis for such beneficial effects needs to be ascertained, one possibility is that the inhibitors or inhibitor-protease complexes act as free radical traps, whereby the free electrons on damaging oxygen radicals are transferred or dissipated to the sulfur atoms of the sulfur-rich inhibitors or complexes. [For reviews of free radical chemistry of proteins, see Friedman (20) and Swallow (96).] These considerations suggest the need for further studies to enrich our knowledge about possible beneficial effects of plant protease inhibitors in relation to sulfur amino acids (60).

BIOAVAILABILITY OF AMINO ACIDS

Lysine and Derivatives

Wheat gluten, the major protein in many baking formulations, is considered a poor-quality protein, primarily because it has insufficient amounts of two essential amino acids: lysine, the first-limiting amino acid, and threonine, the second-limiting one. To compensate for the poor quality of most cereal proteins such as gluten, the minimum recommended daily allowance (RDA) for these proteins has been set at 65 g, compared to 45 g for good-quality proteins such as casein (63).

As noted by Ziderman & Friedman (106), during baking, the mixture of protein, carbohydrate, and water plus additives in dough is exposed to two distinct transformations. Desiccation of the surface on exposure to temperatures reaching 215°C produces the crust. The crust encloses part of the dough in steam phase at approximately 100°C, resulting in the formation of the crumb.

Because lysine's ϵ-amino group interacts with food constituents to make it nutritionally less available (94), the baking process further reduces the dietary availability and utilization of lysine, especially in the crust, which makes up about 40% of the bread by weight. Many such interactions have been described including (*a*) the reaction of the amino group with carbonyl groups of sugars and fatty acids to form Maillard browning products; (*b*) the formation of cross-linked amino acids such as lanthionine, lysinoalanine, and glutamyllysine; (*c*) the interaction with tannins and quinones; and (*d*) steric blocking of the action of digestive enzymes by newly introduced cross-links, as well as native ones such as disulfide bonds. Because these reactions of lysine with other dietary components may lead to protein damage and to the formation of physiologically active compounds, an important objective of food science and nutrition is to overcome these effects (1b, 21–36, 38, 39, 99, 102, 103).

In principle, it is possible to enhance the nutritional quality of bread by amino acid fortification (1b). A major problem encountered when free lysine is used to fortify foods is that the added amino acid can itself participate in browning and other side reactions. Because ϵ-acyl-lysine derivatives are less susceptible to Maillard reactions than is free lysine (14, 17) our objective was to compare lysine and glutamyllysine as nutritional sources of lysine for mice fed bread crust, crumb, and whole bread co-baked with these amino acids. Since the ϵ-NH_2 group of N$^{\epsilon}$(γ-L-glutamyl)-L-lysine (glutamyllysine) is blocked in the form of an isopeptide bond with the γ-COOH group of glutamic acid, expectations were that this peptide should also undergo less damage than lysine during baking.

To assess whether the glutamyllysine can serve as a nutritional source of lysine, we compared the growth of mice fed (*a*) an amino acid diet in which lysine was replaced by four dietary levels of glutamyllysine; (*b*) wheat gluten diets fortified with lysine; (*c*) a wheat bread–based diet (10% protein) supplemented before feeding with lysine or glutamyllysine, not co-baked; and (*d*) bread diets baked with these levels of lysine or glutamyllysine (40). For the amino acid diet, the relative growth response to glutamyllysine was about half that of lysine. The effect of added lysine on the nutritional improvement of wheat gluten depended on both lysine and gluten concentrations in the diet. With 10 and 15% gluten, 0.37% lysine hydrochloride produced markedly increased weight gain. Further increase in lysine hydrochloride to 0.75% proved somewhat detrimental to weight gain. Lysine hydrochloride addition improved growth at 20 to 25% gluten in the diet and did not prove detrimental at 0.75%. For whole bread, glutamyllysine served nearly as well as lysine to improve weight gain. The nutritive value of bread crust, fortified or not, was markedly less than that of crumb or whole bread. Other data showed that lysine or glutamyllysine at the highest level of fortification, 0.3%, improved the protein quality (PER) of crumb over that of either crust or whole bread, indicating a possible greater availability of the second-limiting amino acid,

threonine, in crumb. These data and additional metabolic studies with [U-^{14}C]glutamyllysine suggest that glutamyllysine, cobaked or not, is metabolized in the kidneys and utilized in vivo as a source of lysine; it and related peptides merit further study as sources of lysine in low-lysine foods (105).

Amino acids are used both metabolically, as building blocks for protein biosynthesis, and catabolically, as energy sources. Catabolism for most amino acids proceeds through transamination pathways; the exceptions are lysine and threonine. These nutritionally limiting amino acids are catabolized by nonaminotransferase-specific enzymes: threonine dehydratase acts on threonine and lysine ketoglutarate reductase on lysine. The concentrations of these enzymes in the liver of rats are subject to adaptive responses that control the utilization of these two amino acids (63). Although both enzymes are induced by feeding diets high in protein, rats differ in the mechanism of the adaptive response to high-protein diets and to diets whose threonine or lysine content is less than needed for growth. Thus, reductase falls to very low levels in the liver of rats fed wheat gluten. This appears to be an adaptive response conserving body lysine. At the same time, catabolism of body proteins increases, producing endogenous lysine needed for survival. These considerations imply that as the level of wheat gluten in the diet decreases, lysine is no longer the limiting amino acid. Total protein or some other amino acid then becomes limiting.

In contrast to the apparent mechanism of lysine catabolism, threonine dehydratase does not appear to be substrate induced (63). Therefore, when lysine is the limiting amino acid, the catabolic enzyme falls to low levels and lysine is apparently conserved at the expense of body proteins. Loss of tissue proteins is much less when a diet low in threonine is fed, since the level of threonine dehydratase does not seem to be significantly affected by the protein or threonine content of the diet. Additional studies are needed to establish whether the catabolic enzyme patterns in mice parallel those of rats.

Our results also show that mice provide a good animal model to study protein quality of native, fortified, and processed wheat proteins. Mouse bioassays have a major advantage in applications to label foods for protein nutritional quality. They require about one fifth of the test material needed for rats and can be completed in 14 instead of 28 days (68, 92). They are especially useful to evaluate nutritional and safety impacts of new food ingredients formed during processing (2, 13, 14, 41–44, 47, 50, 51, 59, 61, 92, 92a, 92b) and of new plants and plant parts (35a, 36), when amount of material available for bioassays is limited.

Methionine and Derivatives

The low content of the essential amino acid L-methionine limits the nutritive value of many food proteins of plant origin. These include soybeans and other legumes. The problem is further compounded for two reasons. First, during

food processing and storage L-methionine and other amino acids are chemically modified, further reducing nutritional quality. In the case of methionine, such modifications include oxidation to methionine sulfoxide and methionine sulfone, racemization to D-methionine, and degradation to compounds with undesirable flavors. Second, protein-bound methionine in some plant foods is poorly utilized, presumably because of poor digestibility (1a, 7, 45, 47, 95, 99).

A related aspect is the widespread use of L-methionine to fortify low-methionine foods in order to improve nutritional quality. Because of the reported antinutritional or toxic manifestations of high levels of free methionine in the diet, a need exists to find out whether methionine analogs and derivatives lack the apparent toxicity of L-methionine and whether they can be used as methionine substitutes in the diet.

As part of a program to evaluate the nutritional and toxicological potential of novel amino acids formed during food processing, we compared weight gain in mice fed amino acid diets containing graded levels of L-methionine and 16 methionine derivatives, isomeric dipeptides, and analogs (47). Because the mice received no other source of sulfur amino acids, the results reflect the ability of each of the compounds to meet the animals' entire metabolic demand for dietary sulfur amino acids, relative to that for L-methionine.

Linear response was closely approximated for concentrations below those yielding maximum growth. Derivatization of L-methionine generally lowered potency, calculated as the ratio of the slopes of the two dose-response curves. However, the three isomeric dipeptides (L-L, L-D-, and D-L-methionylmethionine), *N*-acetyl- and *N*-formyl-L-methionine, L-methionine sulfoxide, and D-methionine were well utilized. The double derivative, *N*-acetyl-L-methionine sulfoxide, reduced potency below 60%. D-methionine sulfoxide, *N*-acetyl-D-methionine, and D-methionyl-D-methionine possessed potencies between 4 and 40%. The calcium salts of L- and D-α-hydroxy analogs of methionine had potencies of 55.4 and 85.7%, respectively. Several of the analogs were less growth-inhibiting or toxic at high concentrations in the diet than was L-methionine. These results imply that some methionine dipeptides or analogs may be better candidates for foritfying foods than L-methionine.

The data for mice demonstrate that (*a*) the assay is highly reproducible, exhibits excellent dose-response characteristics, and yields useful estimates of relative potency for the 16 methionine analogs; and (*b*) somewhat rigorous control of concentration may be required for dietary supplementation with L-methionine in order to achieve maximum nutritional benefit while preventing toxicity problems. This constraint may be alleviated or avoided by using one or more analogs as alternatives. Whether these compounds will also alleviate the reported adverse flavor aspects of sulfur amino acid sup-

plementation associated with methionine when it is added to foods awaits further study.

Tryptophan and Derivatives

The essential amino acid tryptophan contributes to normal growth and protein synthesis and participates in numerous biochemical processes. Since tryptophan is a nutritionally second-limiting amino acid in maize, and since cereals and processed foods are increasingly used to meet human dietary needs, it is of paramount importance to develop an understanding of thermally induced changes in tryptophan in order to improve the quality and safety of our food supply (38).

The stability of free or protein-bound tryptophan during processing and storage depends on temperature and the presence of oxygen or other oxidizing agents, especially lipid peroxides, and radiation. In the absence of oxidizing agents, tryptophan is a stable amino acid, even in strongly basic or acidic conditions. Free or bound tryptophan is relatively stable during heat treatments such as industrial or home cooking in the presence of air or steam sterilization. Only severe treatments cause a significant degradation of this amino acid. In the presence of carbonyl compounds and/or at high temperatures, however, carboline formation occurs. Both carbolines and tryptophan-derived nitroso compounds are potential carcinogens. Tryptophan losses during food processing cannot always be monitored because of the lack of reliable analytical methods.

The losses in tryptophan bioavailability during heat treatment such as home cooking or industrial sterilization appear less important than other detrimental effects, particularly on lysine or methionine. Some of the reported variabilities in the utilization of D-tryptophan could be due to the fact that the value (potency), of D-tryptophan as a nutritional source of L-tryptophan is strongly dose dependent (38).

Possible consequences for nutrition, food safety, and human health of halogenated tryptophans (38), light-induced tryptophan adducts (93a); tryptophan-derived carbolines (38), and tryptophan-induced eosinophilia myalgia (96a) are active areas of current research.

PREVENTION OF ADVERSE EFFECTS

Sulfur-containing amino acids such as cysteine, *N*-acetylcysteine, and the tripeptide glutathione play key roles in the biotransformation of xenobiotics by actively participating in their detoxification. These antioxidant and antitoxic effects are due to a multiplicity of mechanisms including their ability to act as (*a*) reducing agents, (*b*) scavengers of reactive oxygen (free-radical species), (*c*) strong nucleophiles that can trap electrophilic compounds and in-

termediates, (*d*) precursors for intracellular reduced glutathione, and (*e*) inducers of cellular detoxification.

For these reasons, fruitful results were expected from evaluation of the effectiveness of sulfur amino acids and sulfur-rich proteins to (*a*) prevent the formation of toxic browning products by trapping intermediates, and (*b*) reduce the toxicity of browning products in animals by preventing activation of such compounds to biologically active forms (10, 35, 46, 56, 79, 80).

These expectations were realized, as evidenced by the following observations on the prevention of both enzymatic and nonenzymatic browning by sulfur amino acids (20, 35, 46, 56, 79, 80, 103). To demonstrate whether SH-containing sulfur amino acids minimize nonenzymatic browning, β-alanine, *N*-α-acetyl-L-lysine, glycyl-glycine, and a mixture of amino acids were each heated with glucose in the absence and presence of the following potential inhibitors: *N*-acetyl-L-cysteine, L-cysteine, reduced glutathione, sodium bisulfite, and urea. Inhibition was measured as a function of temperature, time of heating, and concentration of reactants. The results suggest that it should be possible to devise conditions to inhibit browning in amino acid–carbohydrate solutions used for parenteral nutrition (59, 72, 73, 89, 91, 93).

Reflectance measurements were used to compare the relative effectiveness of a series of compounds in inhibition browning in freshly prepared and commercial fruit juices including apple, grape, grapefruit, orange, and pineapple juices (79). For comparison, related studies were also carried out with several protein-containing foods such as casein, barley flour, soy flour, and nonfat dry milk, and the commercial infant formula "Isomil." The results revealed that under certain conditions, SH-containing *N*-acetyl-L-cysteine and the tripeptide reduced glutathione may be as effective as sodium sulfite in preventing both enzymatic and nonenzymatic browning.

In a related study designed to develop sulfite alternatives (15), Russet Burbank potatoes, Washington golden delicious apples, and Washington red delicious apples were subjected to enzymatic browning in air and evacuated plastic pouches in the absence and presence of browning inhibitors (80). Studies on the effects of concentration of inhibitors, storage conditions, and pH revealed that *N*-acetyl-L-cysteine and reduced glutathione were nearly as effective as sodium sulfite in preventing browning of both apples and potatoes (66, 86, 101).

CONCLUSIONS

This overview shows that pH, heat, and oxygen have both beneficial and adverse effects on many nutrients. To maximize beneficial effects, future studies should emphasize the prevention of browning and the consequent

antinutritional and toxicological manifestations of browning products in whole foods. Many of the safety concerns cited, especially those of genotoxic potential, are based on in vitro data that may not always be relevant to in vivo effects following the consumption of whole food products containing the browning-derived constituents. The presence of other dietary constituents in the food and the process of digestion and metabolism can be expected to decrease or increase the adverse manifestations of browning products. Most urgent is the need to develop food processing conditions to prevent the formation of carcinogenic heterocyclic amines (38, 65, 101). For nutrition and food safety, possible consequences of chelation of nutritionally essential trace materials to processing-induced food ingredients (17, 21, 40b, 64, 67, 81, 87, 89a), beneficial effects of processing on food allergy and the immune system (4, 5, 11, 12, 49, 62, 67b, 83, 84), and differentiating adverse and beneficial effects of heat and oxygen on lipids (67c, 69a, 86a) and vitamins (1, 6a, 52, 52a, 59, 64, 67a, 69, 75a, 78, 85, 88, 90, 93) also merit study.

ACKNOWLEDGMENTS

I thank my colleagues whose names appear on the cited references for excellent scientific collaboration.

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