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REVIEWS

Chemistry, Biochemistry, Nutrition, and Microbiology of Lysinoalanine, Lanthionine, and Histidinoalanine in Food and Other Proteins

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Heat and alkali treatments of foods, widely used in food processing, result in the formation of dehydro and cross-linked amino acids such as dehydroalanine, methyldehydroalanine, β-aminoalanine, lysinoalanine (LAL), ornithinoalanine, histidinoalanine (HAL), phenylethylaminoalanine, lanthionine (LAN), and methyl-lanthionine present in proteins and are frequently accompanied by concurrent racemization of L-amino acid isomers to D-analogues. The mechanism of LAL formation is a two-step process: first, hydroxide ion-catalyzed elimination of H_2S from cystine and H_2O, phosphate, and glycosidic moieties from serine residues to yield a dehydroalanine intermediate; second, reaction of the double bond of dehydroalanine with the ϵ-NH_2 group of lysine to form LAL. Analogous elimination−addition reactions are postulated to produce the other unusual amino acids. Processing conditions that favor these transformations include high pH, temperature, and exposure time. Factors that minimize LAL formation include the presence of SH-containing amino acids, sodium sulfite, ammonia, biogenic amines, ascorbic acid, citric acid, malic acid, and glucose; dephosphorylation of *O*-phosphoryl esters; and acylation of ϵ-NH_2 groups of lysine. The presence of LAL residues along a protein chain decreases digestibility and nutritional quality in rodents and primates but enhances nutritional quality in ruminants. LAL has a strong affinity for copper and other metal ions and is reported to induce enlargement of nuclei of rats and mice but not of primate kidney cells. LAL, LAN, and HAL also occur naturally in certain peptide and protein antibiotics (cinnamycin, duramycin, epidermin, nisin, and subtilin) and in body organs and tissues (aorta, bone, collagen, dentin, and eye cataracts), where their formation may be a function of the aging process. These findings are not only of theoretical interest but also have practical implications for nutrition, food safety, and health. Further research needs are suggested for each of these categories. These overlapping aspects are discussed in terms of general concepts for a better understanding of the impact of LAL and related compounds in the diet. Such an understanding can lead to improvement in food quality and safety, nutrition, microbiology, and human health.

Keywords: *Lysinoalanine; histidinoalanine; lanthionine; chemistry; nutrition; safety; microbiology*

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Table 1. LAL Contents of Foods Listed Alphabetically

food source	LAL, μg/g	reference
baby food	10−70	Kikutani et al., 1982
cereal products	200−390	Antilla et al., 1987; Hasegawa et al., 1987; Sternberg and Kim, 1977; Struthers et al., 1980
chicken meat	370	Sternberg and Kim, 1977
eggs	160−1820	Fritsch and Klostermeyer, 1981a,b; Hasegawa et al., 1987; Murase and Goto, 1977; Raymond, 1980; Sternberg et al., 1975a,b; Sternberg and Kim, 1977
gelatin	250	Fujimoto et al., 1984
infant formulas, dry	150−920	Antilla et al., 1987; Bellemonte et al., 1987
infant formulas, liquid	160−2120	Bellemonte et al., 1987; Langhendries et al., 1992; Pfaff, 1984; Pompei et al., 1987
meat products	140−540	Hasegawa et al., 1987
milk powders	150−1620	Aymard et al., 1978; Fritsch and Klostermeyer, 1981a,b; Hasegawa et al., 1987; Sternberg and Kim, 1977
noodles, Chinese	480	Hasegawa et al., 1987
skipjack, dry	410	Kikutani et al., 1984
sodium caseinate	430−6900	Aymard et al., 1977; Lee et al., 1977; Moret et al., 1994; Sternberg and Kim, 1977
soy protein isolate	370−1300	Aymard et al., 1978; Sternberg and Kim, 1975
whipping agents	6500−53150	Fritsch and Klostermeyer, 1981a,b; Sternberg and Kim, 1977

INTRODUCTION

Protein-containing foods and feeds are commonly treated with alkali in the course of preparing protein concentrates and isolates for dietary and other uses. For example, in preparing soy protein concentrates, the usual step is to extract soybeans with aqueous alkali and then precipitate the protein from the resulting solution at the isoelectric point (Liener, 1994a,b). Similar alkali treatments are used in recovering proteins from cereal grains and milling byproducts, oilseeds such as cottonseed, flaxseeds, safflower seeds, and peanuts, and dairy proteins such as sodium caseinate. Alkali procedures are also used to induce fiber-forming properties for use in textured soybean foods (meat analogue vegetable soy protein), for preparing peeled fruits and vegetables, and for destroying microorganisms (Anonymous, 1995; Betschart, 1975; Castro et al., 1995; Floros et al., 1987; Marsilio et al., 1996; McGhee et al., 1979; Moharram et al., 1981; Moss et al., 1986). Such treatments, however, may also cause side reactions. These include formation of new amino acids such as lysinoalanine (LAL), lanthionine (LAN), and histidinoalanine (HAL), which may affect the nutrition and biology of the treated proteins (Anonymous, 1989; Cuq and Cheftel, 1985; Finot, 1983; Hurrell et al., 1976; Karayiannis et al., 1980; Van Beek et al., 1974). Foods containing LAL are widely consumed (Table 1).

Although the cross-linked and dehydro amino acids are "unnatural" amino acids formed on exposure of proteins to certain processing conditions, they also appear naturally in certain antibiotic peptides and proteins and tissue proteins such as bone, dentin, and eye cataracts, where their formation may be related to the aging process.

The chemistry leading to the formation of cross-linked amino acids during alkali treatment of proteins needs to be studied and explained. The nutritional and toxicological significance of these changes needs to be defined. Strategies to minimize these reactions need to be developed. This integrated, multidisciplinary overview of the widely scattered literature emphasizes mechanistic aspects related to (a) the distribution of LAL in the food chain; (b) nutrition and safety; (c) factors that influence formation of LAL and related compounds; (d) possible approaches to minimize LAL formation; (e) the chemistry and microbiology of the unusual amino acids in natural antibiotics; (f) the in vivo formation and possible role of HAL and LAL in tissues of animals and humans; and (g) research needs designed to catalyze progress in these areas. The guiding principle is that a better understanding of the underlying chemistry and biology of these amino acids should make it possible to minimize adverse and optimize beneficial effects.

Figure 1 depicts a postulated mechanism of LAL formation, and Figure 2 gives the structures of the dehydro and cross-linked amino acids discussed in this paper.

ANALYSIS

A key element of any evaluation of a protein, especially a modified protein, is its amino acid composition. Automated procedures are now widely used to achieve this objective (Cavins and Friedman, 1968; Sarwar et al., 1983). A number of methods have been evaluated for measuring LAL and related unusual amino acids in proteins. These are briefly summarized below.

Ion-Exchange Chromatography. The following is a typical procedure (Friedman and Cuq, 1988; Friedman et al., 1984; Sanderson et al., 1978). The protein sample (5 mg) was hydrolyzed in 15 mL of 6 N HCl in a commercial hydrolysis tube. The tube was evacuated, placed in an acetone−dry ice bath, evacuated, and refilled with nitrogen before being placed in an oven at 110 °C for 24 h. The cooled hydrolysate was filtered through a sintered disk funnel, evaporated to dryness with the aid of an aspirator, and the residue was suspended in H_2O and evaporated to dryness. Amino acid analysis of the hydrolysate was carried out on an amino acid analyzer with norleucine as an internal standard. LAL is eluted just before histidine.

Modifications of this method are described by Bohak (1964), Erbersdobler et al. (1979), Haagsma and Slump (1978), Johnson (1983), Raymond (1980), Wilkinson and Heewavitherana (1997), and Zarkadas et al. (1986). Compared to ninhydrin, detection by fluorometry after postcolumn derivatization with an *o*-phthalaldehyde reagent appears to produce considerable increase in sensitivity and accuracy of the analysis (Fritsch and Klostermeyer, 1981a).

GC/MS. Four LAL isomers can be formed during exposure of food proteins to high pH. Two (LL and LD) are derived from L-lysine and the other two (DD and DL) from D-lysine. Because concurrent racemization of L- to D-lysine occurs slowly (Liardon and Friedman, 1987), only the first two isomers are usually encountered in protein hydrolysates. Liardon et al. (1991) describe a procedure to quantitate these two LAL isomers by GC/MS after protein hydrolysis and derivatization to diastereomeric *N*(*O*,*S*)-perfluoropropionyl isopropyl esters (Figure 3).

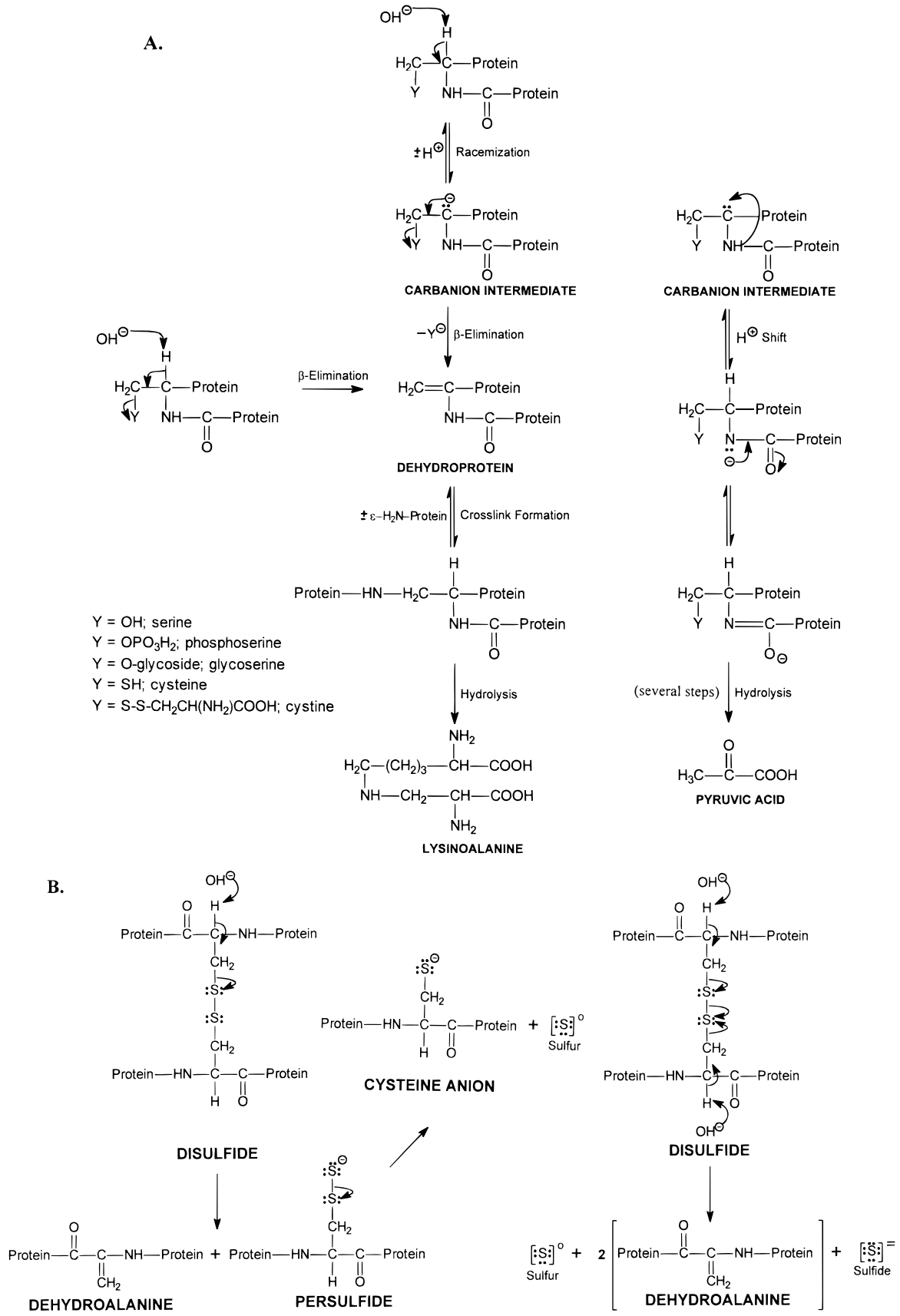
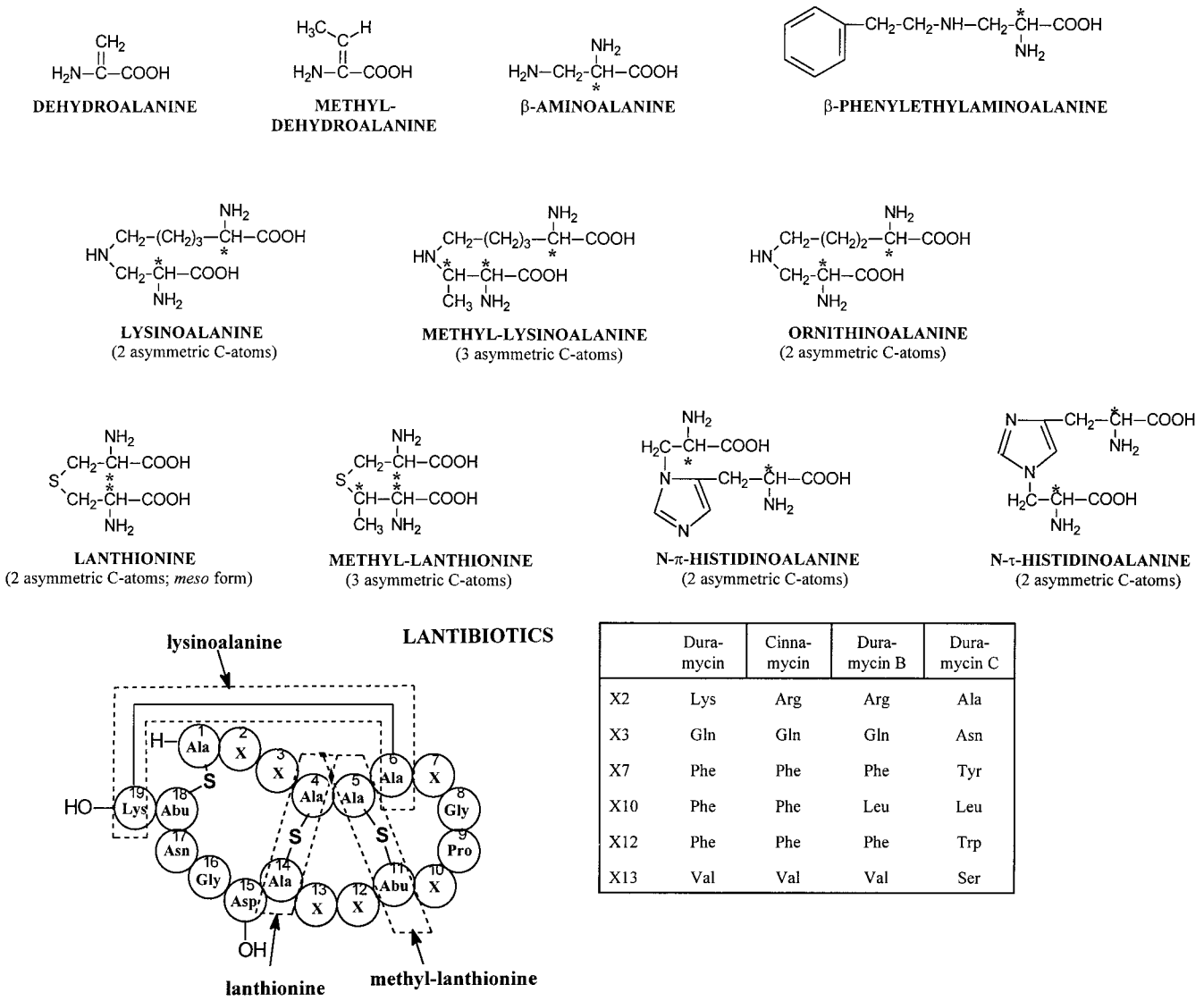


Figure 1. (A) Transformation of a reactive protein side chain to an LAL side chain via elimination and cross-link formation. An OH^- ion abstracts a proton from a C−H group of an amino acid to form a carbanion, which has lost the original asymmetry. The carbanion can then combine with a proton to regenerate the original amino acid, which is now racemic, and/or undergo an elimination reaction to form a dehydroprotein. A one-step elimination shown on the left is also possible. The dehydroprotein can hydrolyze to pyruvic acid and/or react with NH_3 to form β-aminoalanine, with the NH_2 group of phenylethylamine to form phenylethylaminoalanine, with the ϵ-NH_2 of lysine to form LAL, with the δ-NH_2 group of ornithine to form ornithinoalanine, with the NH group of histidine to form HAL, and with the SH group of cysteine to form LAN. Addition of these groups to methyldehydroalanine derived from threonine produces analogous methyl compounds. See Figure 2 for structures. (B) Mechanisms of OH^--catalyzed transformation of a protein cystine to dehydroalanine.



	Dura-mycin	Cinna-mycin	Dura-mycin B	Dura-mycin C
X2	Lys	Arg	Arg	Ala
X3	Gln	Gln	Gln	Asn
X7	Phe	Phe	Phe	Tyr
X10	Phe	Phe	Leu	Leu
X12	Phe	Phe	Phe	Trp
X13	Val	Val	Val	Ser

Figure 2. Structures of dehydro and cross-linked amino acids present in foods, body tissues, and lantibiotics. The structures of the lantibiotics were adapted from Zimmerman et al. (1993).

Related studies on the determination of LAL in processed proteins and foods by GC after derivatization to *n*-butyl esters of *N*(*O*)-trifluoroacetyl derivatives are described by Buser and Erbersdobler (1984), Hasegawa and Iwata (1982), Hasegawa et al. (1987), Sakamoto et al. (1977), and Schwass and Finley (1984).

HPLC. Badoud and Pratz (1984) developed a rapid and simple method for LAL after derivatization with dansyl chloride, separation on an HPLC column, and detection by UV. HPLC was also used by Antila et al. (1987), Moret et al. (1994), Pellegrino et al. (1996), and Wood-Rethwill and Wartesen (1980). Pellegrino et al. (1996) used fluorescence detection after derivatizing LAL with 9-fluorenylmethylchloroformate.

Other Methods. TLC methods for the quantitative analysis of LAL are described by Aymard and Cheftel (1978), Freimuth and Notzold (1980), and Sternberg et al. (1975a,b). Whiting (1971) used high-voltage electrophoresis.

Assessment. The choice of analytical method will be dictated by the availability and cost of reagents and instruments, the required sensitivity, which ranges from parts per billion (HPLC and GC) to parts per million levels (ion-exchange chromatography), and the nature of the sample. Complex foods such as dairy, soybean, and meat products contain numerous compounds that may interfere with the analysis during both hydrolysis (incomplete recovery of LAL) and quantitative determination (overlapping peaks). For example, we found that high concentrations of starch caused poor recovery of LAL during acid and alkaline hydrolysis used for proteins (Friedman et al., 1984). The cited investigators have struggled with these problems and have devised useful techniques to overcome them.

LAL CONTENT OF PROCESSED PROTEINS AND FOOD PRODUCTS

Composition and Nutrition of Dope Solutions of Proteins. The need to expose food proteins to extreme pH to enhance their utility has stimulated great interest in the resulting changes in composition and nutrition (Abe, 1986; Abe et al., 1981). According to De Rham et al. (1977), such processes, called acid spinning, make it possible to manufacture fibrous proteins resembling meat fibers. The alkaline protein solution or dope is injected through a punctured plate (the spinneret) into an acid bath, where the pH is reduced to the isoelectric point of the protein. The precipitated coagulated fibers

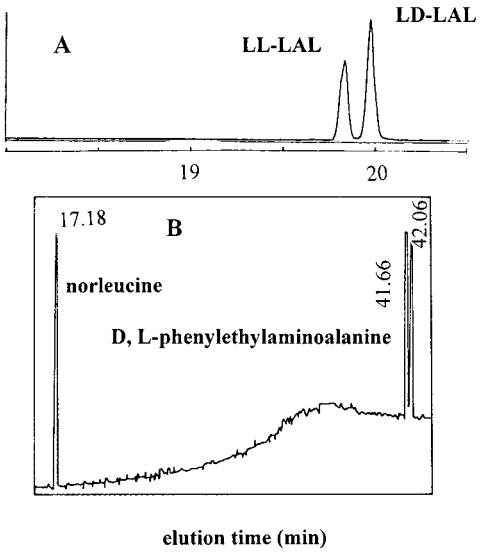


Figure 3. (A) Separation of LL-LAL and LD-LAL isomers present in a hydrolysate of alkali-treated whey proteins by GC/MS (Liardon et al., 1991). (B) Resolution of D- and L-phenylethylaminoalanine by GC (Friedman and Noma, 1986).

are then washed and used to manufacture vegetable meat analogues.

Dosako (1979) investigated the effect of aging on chemical properties of casein and soy protein dope solutions. For casein, the amount of LAL formed increased with time. Cysteine inhibited LAL formation and NaCl enhanced it. For soy proteins, the increasing formation of LAL with aging was accompanied by a decrease in cystine and an increase in cysteine residues. These changes may be responsible for the observed effects of high pH on the viscosity of the protein solutions. The LAL content of soy protein dope solutions (18−25% protein in 1−2% NaOH) decreased by up to 50% in the presence of cysteine (Hayakawa and Katsuta, 1981).

LAL (85 mg/100 g of protein) was also present in extruded fibers (pH 12, 40−50 °C) from suspensions of chicken and carp muscle proteins (Hayasho and Takahashi, 1990). The formation of heat-induced LAL cross-links contributed to the enhanced strength of collagen fibers, the main connective tissue component and most abundant animal protein (Gorham., 1992).

The combination of low-methionine soy and high-methionine cheese whey proteins resulted in enhanced nutritional value (De Rham et al., 1977). The critical pH for preparing dope solutions from the mixed proteins was higher than 11, because at that pH only a very small amount of LAL is formed. In contrast, after 2 h at pH 12.5 and 60 °C, about half of the cysteine/cystine residues had reacted to form LAL. After 90 s at pH 12.5 and 65 °C, soy proteins contained 0.2 g/100 g of LAL and whey protein, 0.5 g/100 g. These findings suggest that conditions for spinning protein fibers can be regulated to minimize adverse compositional and nutritional changes.

Cereal Proteins. Exposure of cereal protein to alkali and heat has a number of benefits. These include (a) prevention of microbial attack on moist grain, (b) enhancement of storage properties of grain (Henning and Steyn, 1984), (c) enhancement of solubility of gluten proteins (Batey and Gras, 1984), and (d) improvement in the quality of corn tortillas.

Wheat gluten contains high levels of the LAL precursor cystine and ∼2% lysine (Friedman and Finot, 1990). The factors that influence LAL formation in wheat gluten have been studied by Friedman (1977a, 1978a,b, 1979), Fujimaki et al. (1980), and Takeuchi et al. (1978). Conditions that favor LAL formation include high pH, temperature, and time of exposure. Because protein concentration did not affect LAL formation, intra- rather than intermolecular cross-links are formed in gluten proteins. Hydrolysis of gluten by Pronase followed by alkali exposure of the resulting peptides formed less LAL as compared to the undigested protein (Haraguchi et al., 1980).

The maximum amount of LAL formed when wheat gluten was treated with 10% Na_2CO_3 by weight at pH 10.5 at 100 °C for 60 min was 22 μmol/kg (Abe et al., 1981). Addition of malic acid to the Chinese wheat noodles treated with 5% Na_2CO_3 before drying resulted in the reduction of LAL content to 14.4 mg/kg. Treatment of instant noodles with malic acid completely prevented LAL formation.

The LAL content of Finnish whole grain cereal powder ranged up to 265 mg/kg, that of apple porridge powder up to 241 mg/kg, and that of rice porridge powder up to 19 mg/kg (Antila et al., 1987).

Exposure of barley to NaOH induced the formation of 1.16 g of LAL/kg of the grain. Untreated barley had no LAL (Davidson et al., 1982). Because barley contains ∼10% protein, the lysine content of which is between 2 and 3% (Friedman and Atsmon, 1988), about half of the lysine seems to have participated in LAL formation. Although LAL formation does not seem to adversely affect ruminant nutrition, the loss of lysine and cystine and the high sodium content of the treated barley could have an adverse impact.

Tortillas. Liming of corn meal during the preparation of tortillas involves exposure to $Ca(OH)_2$ and heat. Chu et al. (1976) measured the LAL and lysine content in corn flour after treatment with different alkaline solutions. No LAL was detected at concentrations below 3.1 mol of NaOH or lime/kg of corn. Significant amounts of LAL (up to 1339 μg/g of protein) were measured in corn flour exposed to high concentrations of NaOH, whereas exposure to lime or $Ca(OH)_2$ of the same concentration induced the formation of much smaller amounts of LAL (up to 140 μg/g). The LAL content of tortillas was 810 mg/kg of protein and that of commercial masa, 200 mg/kg (Sanderson et al., 1978). Alkali-processed high-lysine corn and high-lysine corn masa also contained LAN.

Young rats fed NaOH-treated zein failed to grow and showed extreme diarrhea. The effects were less severe with $Ca(OH)_2$-treated zein (Jenkins et al., 1984). The treatments induced racemization of L- to D-amino acids but no LAL formation. Evidently, alkali treatment of zein can damage its nutritional quality in the absence LAL.

We studied the formation of LAL and D-amino acids during the preparation of tortillas. The following is a brief summary of some of our findings (unpublished results). Whole dried kernels (100 g) of White Dent corn and Yellow Flint corn were each mixed with 300 mL of distilled H_2O and 1 g of $Ca(OH)_2$. The mixture was boiled for 20 min and left standing for 15 h. The kernels were then rinsed with distilled H_2O and ground in a Waring blender. Masa Harina instant tortilla mix was included as a control. Water was added to the Masa Harina to form a dough. The dough was divided into 30

Table 2. Effect of Temperature on LAL, Lysine, Arginine, Serine, and Threonine Contents of Soy Protein (Grams per 16 g of N)[a]

	untreated control	temperature (°C)						
		25	35	45	55	65	75	85
LAL	0	0.69	0.81	1.04	1.64	2.64	3.66	4.13
lysine	5.54	4.91	4.88	4.63	4.29	3.72	3.46	2.86
arginine	6.6	6.49	6.52	6.29	6.11	5.48	4.86	3.30
serine	4.78	4.47	4.48	4.07	4.08	3.32	2.69	1.73
threonine	3.40	3.39	3.35	3.25	3.00	2.83	2.24	1.84

[a] Conditions: 1% protein; pH 12.5. (Friedman et al., 1984).

g pieces, which were shaped into a ball and then pressed flat to form 10 cm diameter disks. Half of the patties were baked on a hot griddle for 1 min on each side and the other half were left raw. All of the patties were then air-dried for 24 h and ground to a fine powder.

The pH of the treated corn flour suspensions ranged from 11.8 to 12.2. The Masa Harina control sample and the tortillas prepared from Yellow Flint corn had no LAL. The LAL content of White Dent corn tortillas before baking was 0.16 g/16 g of N and after baking, 0.36 g/16 g of N. The racemization of amino acids in the tortilla samples generally was <1% with the exception of serine, aspartic acid, and tyrosine. The values (D/(D + L) × 100) of serine ranged up to 7.7%. The corresponding values for aspartic acid ranged up to 3.2% and for tyrosine up to 2.2%.

Studies were also carried out on the formation of LAL in normal and high-lysine corn proteins extracted from yellow and high-lysine corn flours according to the method of Concon (1973). The isolated proteins and commercial zein (1 g) were each suspended in 100 mL of 0.1 N NaOH and in 100 mL of saturated $Ca(OH)_2$ solutions, respectively. The samples were then heated at 65 °C in a water bath for 3 h, dialyzed against 0.01 N acetic acid, lyophilized, and analyzed for amino acid composition. Control experiments were done with H_2O as the medium. The results showed that (a) no LAL was present in the alkali-treated zein and yellow cornmeal protein isolate; (b) the alkali-treated high-lysine corn protein isolate contained 1.42 and 1.51 g of LAL/16 g of N for the NaOH and $Ca(OH)_2$ samples, respectively; (c) cystine was nearly lost as a result of both treatments; and (d) the amino acid chromatograms of all treated corn samples had one and the high-lysine sample two new unknown peaks. Zein treated at 75 °C instead of 65 °C contained 0.32 g of LAL/16 g of N. The cited findings suggest that it should be possible to devise processing conditions for tortillas to minimize LAL and D-amino acid formation.

Legume Proteins. Friedman et al. (1984) carried out a detailed study on the factors governing LAL formation in soy proteins. Exposing soy proteins to alkaline conditions (pH 8−14) for various time periods (10−480 min) and temperatures (25−95 °C at 10 °C intervals) destroyed all of the cystine and part of the arginine, lysine, serine, and threonine residues at the higher pH values and temperatures. These losses were accompanied by the appearance of LAL and unknown ninhydrin-positive compounds (Tables 2−4). LAL formation was suppressed by protein acylation and addition of SH-containing compounds, copper salts, and glucose. They also found that free and protein-bound LAL was stable to acid but not to basic conditions used for protein hydrolysis. Related studies are reported by De Rham et al. (1977), de Groot et al. (1976), and Nishino and Uchida (1995).

Table 3. Aspartic Acid (Asp) Racemization and LAL Content of Alkali-Treated Casein and Acetylated Casein[a]

protein	D/L Asp ratio	LAL (mol %)
casein, untreated	0.023	0.0
casein + alkali	0.387	2.35
acetylated casein +alkali	0.336	0.0

[a] Conditions: 1% protein; 0.1 N NaOH; 65 °C; 3 h (Friedman et al., 1981).

Table 4. Variation in LAL Content of Alkali-Treated Protein[a]

protein	LAL (g/16 g of N)	isomeric ratio [LL-LAL]/[LL-LAL + [LD-LAL]
zein	0.32	
wheat gluten	0.95	0.50
fish protein concentrate	2.75	0.40
bovine hemoglobin	3.36	0.50
casein	4.40	0.51
lactalbumin	5.38	0.51
bovine serum albumin	8.52	0.50

[a] Conditions: 0.1 N NaOH; 75 °C; 3 h (Friedman and Liardon, 1985; Liardon et al., 1991).

Conditions that promote LAL formation in sunflower proteins are described by Provansal et al. (1975). Deng et al. (1990) measured LAL levels in rapeseed protein meals and isolates and two other protein isolates. The LAL levels of <500 μg/g in mildly treated rapeseed products were similar to those present in casein, soy, and other food products. The results demonstrate the need for careful control of reaction conditions during protein extraction to minimize LAL formation.

A study of the kinetics of LAL formation in peptides derived from partial hydrolysis of field bean and casein proteins and from recombination of these peptides to plasteins showed that the LAL content decreased with the extent of hydrolysis and that LAL favored intramolecular cross-linking of the peptide chains (Notzold et al., 1984).

Milk Proteins and Dairy Products. Major classes of milk proteins contain high concentrations of LAL amino acid precursors (Erbersdobler and Holstein, 1980; Dehn-Muler et al., 1991). Thus, casein contains high concentrations of serine phosphate groups, and lactalbumin and whey proteins contain high levels of cystine residues. All milk proteins have a high lysine content, which can react with the dehydroalanine groups generated from these precursors. Because casein isolation on an industrial scale involves isoelectric precipitation followed by neutralization at alkaline pH (Jelen and Schmidt, 1976; Ward and Bastian, 1998) and because heat is widely used to pasteurize and process milk (Dehn-Muller et al., 1991), the effect of alkali and heat on the formation of LAL and other unusual amino acids has been widely studied to define its significance for food quality and nutrition. The LAL content of raw and pasteurized milk (in milligrams per kilogram of protein) ranged up to 15, that of whey protein concentrate up to 145, that of ultrahigh-temperature (UHT)-heated milk up to 400, that of autoclaved milk up to 880, that of sodium caseinate up to 1530, and that of calcium caseinate up to 1560 (Amarowicz et al., 1988, 1991; Annan and Manson, 1981; Creamer and Matheson, 1977; de Konig and van Rooijen, 1982; Hasegawa et al., 1981a,b; Friedman et al., 1981; Fritsch et al., 1983; Isohata et al., 1983; Manson and Carolan, 1980). The LAL content of 54 Finnish milk protein concentrates

and isolates ranged from 0 to 143 mg/kg of protein (Antila et al., 1987). The corresponding values for milk-based infant formulas, baby foods, and formula diets exceed 300 mg/kg in some cases (see below). Commercial milk products contain significant amounts of LAL.

Naturally ripened cheeses contained no or very low levels of LAL, whereas the LAL content of processed cheeses with added caseinates ranged from 50 to 1070 mg/kg of protein (Antila et al., 1987; Fritsch and Klostermeyer, 1981a,b). The mean value for imitation Mozzarella cheeses of 54 mg/kg ($n = 29$) was ~50 times greater than the corresponding value for the natural cheeses. The LAL content of pasteurized milk was 0.44 mg/kg of protein, that of natural Mozzarella cheeses, 0.4−4 mg/kg of protein, and that of processed and imitation Mozzarella cheeses, 15−421 mg/kg of protein. These observations are the basis for the suggestion that the LAL content appears to be a good indicator to distinguish natural from imitation Mozzarella cheeses, even those without added caseinates (Pellegrino et al., 1996).

Because peptide bonds in cheese proteins slowly hydrolyze during storage due to the action of proteolytic enzymes (Pearce et al., 1988) and because peptide bond cleavage affects both LAL formation and racemization (Hayashi and Kameda, 1980a,b; Liardon and Friedman, 1987), comparisons of LAL content should, when possible, be made with cheeses of the same age.

Infant Formulas. Table 1 shows that the LAL content of commercial powdered infant formulas ranges up to 920 ppm and that of liquid formulas up to 2120 ppm. The amino acid content of 13 liquid and powdered milk-based infant formulas produced in Italy showed a marked difference in LAL content between liquid and powdered samples (Pompeii et al., 1987). The powders contained low levels of LAL, whereas the LAL content of the liquid samples ranged up to 1032 mg/kg of protein. LAL content was a sensitive index of heat damage and correlated with other indices such as hydroxymethylfurfural content and reduction in protein quality.

Because such formulas are often the sole source of protein for infants over a significant time period, Pfaff (1984) and Pfaff and Pfaff (1984) and other authors listed in Table 1 recommended that the LAL content of infant formulas be kept <200 ppm. It is not known if growing infants and children are more sensitive to adverse nutritional effects of LAL than are adult humans, as is the case compared for growing adults rats (Possompes et al., 1989; Struthers et al., 1978). Infant formulas also contain Maillard browning products, which are also reported to induce nephrocytomegaly in rats (Erbersdobler, 1989; Sarwar, 1991). See also the section below on Primate Studies.

Egg Proteins. Alkali- and storage-induced β-elimination reactions of O-glycosidically linked carbohydrate groups of the egg white protein ovomucin and accompanying degradation of disulfide bonds are chemical events that may occur during egg white thinning (Donovan and White, 1971; Kato et al., 1979; Mine, 1997). These transformations also generated LAL (Walsh et al., 1979).

LAL was present in the egg white and yolk of hen and quail eggs sold in Japan (Murase and Goto, 1977). The values ranged up to 93 mM/kg of protein. Heating egg white in boiling water for 30 min induced LAL formation at the natural storage pH of 8 used for eggs. LAL formation increased with pH, time, and temperature of treatments and was accompanied by degradation of cystine. The LAL content of boiled eggs increased with storage time of the raw eggs (Mukai et al., 1987).

Meat Proteins. Exposure of bovine serum albumin to denaturing conditions at pH values between 9.0 and 12.5 resulted in the formation of LAN and LAL residues (Zahn and Lumper, 1968). The introduction of these cross-linked amino acids prevented the renaturation of the protein. Exposure of insulin, ribonuclease, and lysozyme to dry heat for 1−24 h at temperatures between 80 and 180 °C resulted in the formation of various amounts of LAL, *allo*-isoleucine, and ornithine. Of these, only LAL appeared to be useful as an indicator to detect amino acid damage during dry heating.

Prolongation of activity of the β-melanocyte-stimulating hormone isolated from the pituitary gland, induced by heating in 0.1 N NaOH at 100 °C for 10 min, may result from the partial conversion of arginine to ornithine and racemization of other amino acids (Geschwind and Li, 1964). The concurrent formation of LAL [the discovery of which (Bohak, 1964) was reported in the same year] probably also contributes to the enhanced activity of the hormone.

Alkaline treatment of the protein−polysaccharide complex of cartilage used to separate the protein part from the polysaccharide chain induces the formation of LAL, which was accompanied by loss of cystine, serine, and lysine residues (Whiting, 1971).

Alkaline extraction of deboning residues from poultry and red meats may be used for the recovery of additional food grade proteins (Ozimek et al., 1986). Although such treatments induce the formation of various amounts of LAL, the levels are not significant for the conditions recommended for the commercial processes (Lawrence and Jelen, 1982).

The presence of high concentrations of LAL and HAL (70 and 250 nmol/mg of protein, respectively) in lime-processed gelatin used in photography is attributed by Fuji and Kuboki (1985) to the nongelatin protein components that are coextracted with gelatin from beef bones and hides.

Some of the disulfide cross-links in the muscle protein tubulin were transformed to LAL at high pH and temperatures (Correia et al., 1993). Exposure of the anticoagulant hirudin to high pH and temperature results in the introduction of *meso*-lanthionine and LAL residues and loss of thrombin-inhibitory activity (Chang, 1991). The formation of LAN and LAL residues in insulin occurred during its isolation and purification by preparative HPLC (Schartmann et al., 1983). Inactivation of biological properties of animal proteins by heat and alkali was assessed by measuring the selective destruction of cystine and the accompanying formation of LAN and LAL residues (Chang and Knecht, 1991).

Fish Proteins. Because alkaline treatments are used in the processing of canned, dried, and frozen fish products, including the preparation of lutefisk, a traditional Scandinavian fish product, Miller et al. (1983) examined the effect of pH on LAL formation in fish muscle. Although samples heated at pH 12 and 13 contained significant amounts of LAL, the content of fish muscle treated under the milder conditions used industrially was negligible.

Kume and Takehisa (1984) detected LAL in commercial fish meals, fish solubles, and bone meal at concentrations of 6, 10, and 29 μmol/kg, respectively.

They also found that (a) the LAL content of the dry fish meals was not changed after exposure to irradiation by γ-rays; (b) dry LAL was not, whereas solutions of free and protein-bound LAL were, susceptible to degradation by γ-radiation; and (c) γ-radiation did not induce LAL formation in bovine serum albumin, lysozyme, and ovalbumin.

Wool, Feathers, and Silk. Wool, animal and human hair, feathers, silk, and skin are keratin proteins composed of amino acids linked by peptide bonds. The peptide units are cross-linked by a large number of intra- and intermolecular disulfide bonds (Feairheller et al., 1977; Friedman and Noma, 1970; Friedman and Orracah-Tetteh, 1978; Zahn and Gattner, 1997; Zahn et al., 1977). The disulfide content of wool is variable and can be altered by the nutrition of the sheep and by weathering. Extensive efforts have been made to improve the textile properties of wool by altering the structure of keratin fibers by heating, steaming, and mild alkali treatment. These efforts included an assessment of the roles of LAN and LAL formed as a result of such treatments.

Horn et al. (1941) isolated LAN from carbonate-treated wool. This was followed by the discovery that both alkali treatment and steaming of wool induce the formation of LAL (Ziegler, 1964). LAL residues in wool behaved as interchain cross-links (Kearns and MacLaren, 1979). Extensive pioneering studies on the effects of cystine−dehydroalanine−lanthionine−LAL relationships on mechanochemical, dyeing, setting, and other properties of wool fibers and fabrics provided useful models for such studies with other proteins (Asquith and Otterburn, 1977; Corfield et al., 1967; Ellison and Lundgren, 1978; MacLaren, 1987; Rivett, 1980; Ziegler, 1971).

Such studies with insoluble wool are much easier to conduct than with soluble proteins. Thus, exposure of wool to a solution of high pH is usually followed by filtering the solution and analyzing compositional changes. In contrast, with soluble proteins, the treated solution needs to be dialyzed and then freeze-dried or chromatographed to remove buffer salts and reagents before analysis.

Processed feather meal contains LAN (Han and Parson, 1991). LAL and LAN were detected in significant amounts in the α-keratin polypeptides of the outer regions of the stratum corneum derived from bovine skin (Steinert and Idler, 1979). The presence of these amino acids was attributed to the possible exposure of the keratins to soap solutions of high pH.

LAL formation in silk and wool induced by phosphate salts depended on the pH of the salt solution and the structure of the phosphate anion (Serassi-Kyriakou et al., 1978; Touloupis and Vassiliadis, 1977). Because the amount of LAL formed was greater than the cystine content of silk, serine residues probably also contributed to the formation of the dehydroalanine precursor of LAL. Silk worms synthesize an enzyme that catalyzes LAN formation in vivo (Shinbo, 1998).

Yeast Proteins. Alkaline conditions used to disrupt yeast cells to facilitate extractions of yeast proteins induced formation of significant amounts of LAL (Shetty and Kinsella, 1980). The amount (3.6 g/16 g of N) formed during the isolation of the proteins according to a high-alkali, low-temperature process (pH 12.5, 65 °C, 2 h) was 7 times greater than the value (0.49 g/16 g of N) observed when the protein was isolated according to a low-alkali, high-temperature process (pH 10.5, 85 °C, 4 h).

CHEMISTRY

Mechanisms of LAL Formation. Figure 1 depicts a postulated mechanism of LAL formation (Asquith and Otterburn, 1977; Friedman, 1973, 1977a,b, 1978a; Friedman and Noma, 1986; Lee et al., 1977; Maga, 1984; Patchornik and Sokolovsky, 1964; Whitaker and Feeney, 1977, 1983). The mechanism involves an OH^- ion-catalyzed transformation of the ϵ-NH_2 group of lysine to a LAL side chain via elimination and cross-link formation. The rate-determining second-order elimination reaction thus depends directly on the concentration of both OH^- ions and susceptible lysine side chains. This elimination reaction generates a dehydroalanine residue having a conjugated carbon−carbon double bond that then reacts with the ϵ-NH_2 of lysine to a form an LAL cross-link. This nucleophilic addition reaction is governed not only by the number of available ϵ-NH_2 groups but also by the location of the ϵ-NH_2 and reactive dehydroalanine groups in the protein chain.

After neighboring sites have reacted, additional LAL (or other) cross-links form less readily. Each protein has a limited fraction of sites for forming LAL residues (Hasegawa et al., 1981a,b). The number of such sites is dictated by the protein's size, amino acid composition, conformation, chain mobility, steric factors, and extent of ionization of reactive amino groups. These considerations suggest that LAL formation proceeds by a cascade of reactions. Thus, dehydroalanine formation is governed not only by the absolute concentration of serine and cystine residues but also by their relative susceptibilities to base-catalyzed eliminations. On the other hand, reaction of the ϵ-NH_2 groups with dehydroalanine to form LAL depends not only on steric and conformational factors but also on the pH of the medium, which governs the concentration of reactive ionized NH_2 groups. Because the pK value of the ϵ-NH_3^+ groups is near 10 for most proteins, complete ionization does not occur until pH 12. At pH 9, only ~10% of the amino groups are ionized and thus available for reaction. All of the amino groups can eventually react, however, because additional ones are formed by ionization of the protonated forms as the nonprotonated ones are used up. A mathematical analysis of the pH dependence of LAL formation is given in Friedman (1982).

Two types of LAL protein cross-links are possible: intramolecular and intermolecular. The introduction of intramolecular cross-links leaves the molecular weight of the treated protein largely unchanged, whereas the molecular weight increases proportionately with the number of intermolecular cross-links. Nutritional and biological properties of LAL-containing proteins probably strongly influence the relative numbers of the two types of cross-links.

The δ-amino group of ornithine, a degradation product of arginine, can react with dehydroalanine to form ornithinoalanine, which is sometimes seen in chromatograms of amino acid hydrolysates (Asquith and Otterburn, 1977; Miro et al., 1968; Ziegler et al., 1967). Analogous interaction of the NH_2 group of phenylethylamine, a biogenic amine present in some foods, produces phenylethylaminoalanine (Friedman and Noma, 1986).

Nutritional and other consequences of racemization (Masters and Friedman, 1979) that accompany LAL formation are examined in detail in a separate paper (Friedman, 1999).

Stereochemistry. Because nucleophilic addition may take place on either side of the carbon−carbon double bond of dehydroalanine, the resulting product is a mixture of L- and D-isomers (LL- and LD-lysinoalanines). Moreover, because some of the original L-lysine residues can undergo a hydroxide ion-catalyzed racemization to D-lysine before LAL formation, the D-lysine can, in principle, generate two additional isomers (DD- and DL-lysinoalanines) (Friedman and Liardon, 1985; Liardon et al., 1991; Tas and Kleipool, 1979). These have different chemical and biological properties (De Weck-Gaudard et al., 1988; Feron et al., 1978; Friedman and Pearce, 1989).

The following name is used for the mixture of two isomers derived from L-lysine: N^{ϵ}-[(DL)-2-amino-2-carboxymethyl]-L-lysine. D-Lysine would give rise to the following mixture of isomers: N^{ϵ}-[(DL)-2-amino-2-carboxymethyl]-D-lysine. These four LAL isomers are often abbreviated as DL, LL, DD, and LD forms. The reaction of the δ-NH_2 group of ornithine with dehydroalanine can also generate four isomers. The reaction between the ϵ-NH_2 of lysine and the double bond of methyldehydroalanine derived from threonine can give rise to 5 asymmetric centers and 32 isomers (Friedman, 1977a). Phenylethylamine generates a mixture of two isomers on reaction with dehydroalanine (Friedman and Noma, 1986; Figure 3). Reaction of the SH group of cysteine with dehydroalanine gives rise to one pair of optically active lanthionine isomers (enantiomers) and one diastereoisomeric (*meso*) form. These are correctly named as follows: (*R*)-L-lanthionine or *S*-[(2*R*)-2-amino-2-carboxymethyl]-L-cysteine; (*S*)-D-lanthionine or *S*-[(2*S*)-2-amino-2-carboxymethyl]-D-cysteine; (*S*)-L-lanthionine or *S*-[(2*S*)-2-amino-2-carboxymethyl]-L-cysteine; *meso*-lanthionine.

Factors Favoring LAL Formation. Studies on the influence of pH on LAL content of soybean proteins showed that LAL began to appear at pH 9, increased continually up to pH 12.5, and then decreased at pH 13.9 (Friedman et al., 1984). At the very high pH, LAL is both formed and degraded. Arginine, cystine, lysine, serine, and threonine were also modified during the alkaline treatment of soy proteins. At 100 °C, LAL formation in casein begins to appear at pH 5 (Sternberg and Kim, 1977).

When a 1% solution of soy protein was heated in 0.1 N NaOH (pH 12.5) at 75 °C for various time periods, LAL formation progressively increased for ~3 h. Beyond that time, formation leveled off and started to decrease. These results and those mentioned above for the pH dependence of LAL formation indicate that for each protein, conditions may exist at which LAL is formed as quickly as it is destroyed. The nature of the degradation products is not known.

Table 2 shows that exposure of a 1% solution of soy protein at pH 12.5 for 3 h at temperatures ranging from 25 to 85 °C caused a progressive disappearance of the following labile amino acids: arginine, lysine, serine, and threonine. Disappearance of arginine and lysine started at ~35 °C and of serine and threonine at ~45 °C. The treatment also induced the loss of cystine residues (not shown) and the appearance of LAL residues. LAL residues started to appear at 25 °C (0.69 g/100 g) and continuously increased up to 4.13 g/100 g at 85 °C.

Discriminating between LAL and D-Amino Acid Formation. Alkali-induced racemization of L-amino acids to D-isomers in proteins occurs concurrently with LAL formation (Figure 4). Racemization occurs by abstraction of a proton by hydroxide ion from an optically active α-carbon atom to form a carbanion, which loses its original asymmetry. The pair of unshared electrons on the carbanion can undergo two reactions: (a) recombination with a proton from the solvent to regenerate either the original amino acid side chain or its optical antipode, so that it is racemized; and/or (b) β-elimination to form a dehydroalanine derivative, which can then combine with the ϵ-amino group of a lysine side chain to form a LAL cross-link (Figure 1).

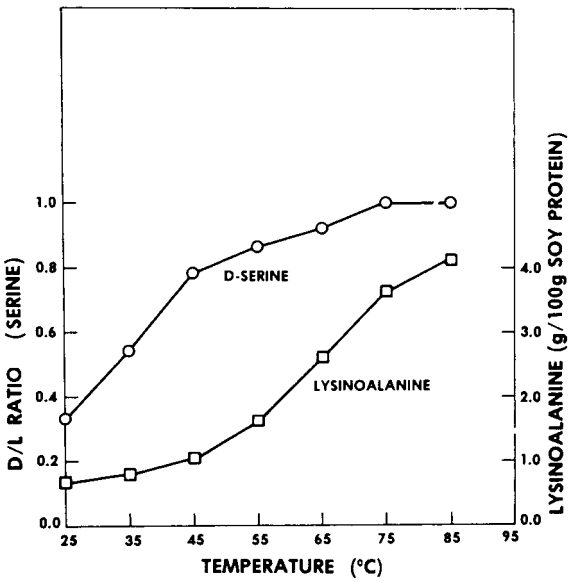


Figure 4. Effect of temperature on alkali-induced concurrent formation of D-serine and LAL in soybean proteins. Conditions: 1% protein in 0.1 N NaOH, 3 h (Friedman et al., 1984).

Because LAL formation requires the participation of the ϵ-NH_2 group of lysine, acylation of the amino group is expected to prevent LAL formation but not racemization. Table 3 shows that this is indeed the case, because alkali treatment of casein resulted in formation of both D-aspartic acid and LAL, whereas the corresponding treatment of acylated casein produced the same amount of D-aspartic acid but no LAL. This approach permits partitioning nutritional and other consequences of racemization from LAL production. Because the LAL but not the D-amino acid content of different proteins treated under the same conditions varies by a factor of ~30 (Table 4; Friedman, 1991), it is possible to produce proteins with various LAL to D-amino acid ratios, depending on whether the product is to be used in human or ruminant nutrition.

Dehydroalanine in Food Proteins. Dehydroalanine is the postulated reactive precursor for LAL (Snow et al., 1975a,b; Steenken and Zahn, 1984; Watanabe and Klostermeyer, 1977; Weder and Sohns, 1983). Friedman et al. (1977) obtained direct evidence for the reaction of dehydroalanine residues with protein functional groups. They showed that dehydroalanine esters convert lysine side chains in polylysine, casein, bovine serum albumin, lysozyme, and wool to LAL residues at pH 9−10. Related studies revealed that protein SH groups generated by reduction of disulfide bonds by tributylphosphine are alkylated at pH 7.6 to LAN residues. These observations show that LAL and LAN residues can be introduced into a protein under relatively mild conditions, without strong alkali treatment. They also imply

Table 5. Dehydroalanine Content of Alkali-Treated Casein and Acetylated Casein[a]

protein	dehydroalanine (g/l6 g of N)
casein, untreated	0
acetylated casein	0
casein, alkali-treated	0.33
acetylated casein, alkali-treated	1.39

[a] Conditions: 1% protein; pH 12.5; 70 °C; 3 h (Masri and Friedman, 1982).

that it should be possible to assess nutritional and toxicological consequences of protein-bound LAL and LAN consumption with little or no racemization.

Masri and Friedman (1982) developed a procedure for detecting dehydroalanine in alkali-treated proteins based on addition of the SH group of 2-mercaptethylpyridine to the double bond of dehydroalanine to form *S*-β-(2-pyridylethyl)cysteine (2-PEC). The cysteine derivative can be assayed by amino acid analysis (Friedman et al., 1979, 1980). The method revealed the presence of a significant amount of dehydroalanine in casein and acetylated casein (Table 5).

Because dehydroalanine residues, which are present in alkali-treated proteins and which occur naturally in peptide antibiotics (see below), can act as alkylating agents in vitro, a need exists to establish whether they can act as biological alkylating agents of essential enzymes and DNA in vivo.

PREVENTING LAL FORMATION

Until the nutritional significance and safety of LAL are established, it may be desirable to limit its formation in foods. Consideration of the mechanistic pathways for LAL formation discussed earlier suggests several possible approaches to prevent or minimize LAL formation. Their applicability, based on trapping intermediates or modifying reactants, is described below.

SH Compounds. Sulfhydryl (SH) groups in amino acids, peptides, and proteins participate in anionic and free radical reactions both in vitro and in vivo (Friedman, 1973, 1994). The chemical reactivity of negatively charged sulfur anions (RS^-) is much greater than would be expected from their basicities. Besides acting as precursors of disulfide bonds that stabilize proteins, SH groups participate directly in many and varied chemical and biochemical processes. Amino (NH_2) and SH groups may react concurrently with electrophilic compounds containing double bonds.

Our studies revealed that SH groups react more rapidly with compounds that contain double bonds than do NH_2 groups. Thus, the double bond of dehydroalanine, which is part of an activated conjugated double-bond system, reacted 34−5000 times more rapidly with the SH group of cysteine in the pH range 7−12 than with the ϵ-NH_2 group of α-*N*-acetyl-L-lysine (Snow et al., 1976). These observations provide a scientific rationale for the observed preventive effect of sulfhydryl compounds.

These model studies suggested that by adding thiols such as cysteine, it may be possible to competitively trap the dehydroalanine intermediate in alkali-treated proteins and thus prevent the formation of LAL. This expectation was realized. Structurally different SH-containing compounds partly inhibited LAL formation during alkali treatment of proteins (Finley et al., 1977, 1978; Table 6). Added nucleophiles such as thiols and

Table 6. Effect of Thiols (1 mM) on LAL Content of Alkali-Treated Soybean Proteins[a]

	additive			
	none	cysteine	*N*-acetyl-cysteine	reduced glutathione
LAL, g/100 g	4.04	1.69	1.72	1.05

[a] Conditions: 1% protein; pH 12.5; 65 °C; 3 h (Friedman et al., 1984).

sulfite ions can inhibit LAL formation by three distinct mechanisms. The first is by *direct competition*. The added nucleophile can preferentially combine with dehydroalanine residues derived from serine and cystine, thus preventing the (slower) addition of ϵ-NH_2 of lysine side chains to form LAL. The second mechanism can be described as *indirect competition*. The added nucleophile can cleave protein disulfide bonds, generating free SH-containing cysteine residues. These may also preferentially combine with dehydroalanine residues. The third possible mechanism can be described as *suppression of dehydroalanine formation*. The added nucleophile, by cleaving cystine disulfide bonds, can diminish a potential source of the dehydroalanine intermediate, because negatively charged cysteine residues (RS^-) are expected to undego elimination reactions to form dehydroalanine much less readily than the original cystine (RS−SR) precursor molecules. The extent to which these mechanisms operate individually or concurrently is undoubtedly influenced by the nature of the protein, the nature of the nucleophile, pH, etc.

Surprisingly, addition of cysteine to high-lysine corn caused an increase in the content of LAL (Warthesen and Wood-Rethwill, 1981). A possible explanation is that either cysteine underwent a β-elimination to dehydroalanine or, more likely, cysteine was first oxidized to cystine, which was then converted to dehydroalanine. This additional source of dehydroalanine then reacted with ϵ-NH_2 groups to form LAL. Also surprising was that exposure of normal and high-lysine corn to $Ca(OH)_2$ and heat induced the formation of the same low levels (200−217 ppm) of LAL in both varieties. This finding suggests that the content of serine and cystine dehydroalanine precursors, which is the same in both varieties (unlike the lysine content), governed the extent of LAL formation in corn proteins.

Adverse nutritional effects, as measured by PER, of alkali-treated (0.1 or 0.2 N NaOH at 80 °C for 1 h) casein could be nullified by adding cysteine (6.1 g/100 g of casein) (Possompes et al., 1989). This amount of cysteine completely prevented LAL formation. In contrast, supplementation of alkali-treated casein with cysteine or methionine after treatment had no effect on nutritional quality. Evidently, the low nutritional value of treated casein is due to chemical and structural modification of the protein resulting from the transformation of serine phosphate to dehdyroalanine and LAL and the formation of D-amino acids and not to a lack of sulfur amino acids.

Sodium Sulfite. Table 7 shows the LAL, LAN, and lysine contents of four proteins exposed to pH 11.6 in the absence and presence of Na_2SO_3. The amount of LAL formed under similar conditions varied greatly among the four proteins tested. The presence of Na_2SO_3 resulted in significant decreases in the LAL content. The reduction for casein was 55%, for wool 68%, for lysozyme 73%, and for bovine trypsin inhibitor 84%. Significant amounts of LAN were found only on amino

Table 7. Effect of Sodium Sulfite on LAL, Lanthionine, and Lysine Contents (Micromoles per Gram) of Proteins Treated at pH 11.6 at 60 °C for 3 h (Friedman, 1977a)

protein	LAL	lanthionine	lysine
casein, untreated	0	0	482.7
casein, pH 11.6	145.2	0	294.0
casein, pH 11.6, Na_2SO_3	65.7	0	373.0
lysozyme, untreated	0	0	397.3
lysozyme, pH 11.6	135.2	0	222.2
lysozyme, pH 11.6, Na_2SO_3	35.6	0	317.2
bovine trypsin inhibitor, untreated	0	0	319.3
bovine trypsin inhibitor, pH 11.6	235.1	0	129.2
bovine trypsin inhibitor, pH 11.6, Na_2SO_3	36.8	0	239.0
wool, untreated	0	0	222.5
wool, pH 11.6	58.7	119.5	139.0
wool, pH 11.6, Na_2SO_3	19.1	0	176.9

acid chromatograms of alkali-treated wool. Sodium sulfite completely prevented LAN formation. Our results suggest that sulfite ions may be more effective in inhibiting LAL formation than are sulfhydryl compounds such as cysteine.

Sulfite ions probably prevent LAL formation by suppressing dehydroalanine formation and/or trapping dehydroalanine. First, by cleaving protein disulfide bonds, the added sulfite ions can diminish a potential source of dehydroalanine, because cystine residues would be expected to undergo β-elimination reactions more readily than would negatively charged protein-bound cysteine ($P-S^-$) or sulfocysteine ($P-S-SO^-$) produced by sulfite ion-catalyzed cleavage of protein disulfide bonds (Friedman and Gumbmann, 1986). Second, because sulfite ions also inhibit LAL formation in casein, which has no cystine residues, sulfite ions may trap dehydroalanine residues by nucleophilic addition to their double bonds. The presence of thiols and sulfites during alkali treatment of soy proteins affects their rheological properties and spinnability (Katsuta and Hayakawa, 1984).

Dephosphorylation of Proteins. In addition to disulfide bonds of cystine residues and hydroxyl groups of serine and threonine residues, *O*-glycosyl and *O*-phosphoryl groups of glycoproteins (e.g., antifreeze protein) and phosphorylated proteins (e.g., phosvitin) also undergo hydroxide ion-induced β-elimination reactions to form dehydroalanine residues (Whitaker and Feeney, 1977). The amount of LAL produced from enzymatically dephosphorylated casein was significantly less than from native casein with intact phosphate esters (Meyer et al., 1981). This finding can be rationalized by postulating a slower elimination of the hydroxyl groups of serine residues from dephosphorylated casein than of phosphate groups from native casein.

Acetylation of Amino Groups. Because formation of LAL requires the participation of an ϵ-NH_2 group of a lysine side chain, modification of amino groups by, for example, acetylation or peptide bond formation should reduce LAL formation. This turned out to be the case; our studies revealed that acetylation (with acetic anhydride) of casein, wheat gluten, soy proteins, and wool minimized or prevented LAL formation (Friedman, 1978; Friedman et al., 1984; Friedman and Masters, 1982). The nutritional qualities (PER) of native and acetylated casein and soy proteins were the same. Prior acetylation of a protein's amino groups can reduce or prevent LAL formation.

Glucose. Under the influence of heat, the ϵ-NH_2 reacts with glucose, other carbohydrates, and quinones to form Maillard (nonenzymatic) and enzymatic browning products (Friedman, 1996a,b, 1997) resulting in loss of lysine. If such reactions occur in alkaline solution, they should reduce the formation of LAL because the blocked lysine amino groups would be unable to combine with dehydroalanine to form LAL.

LAL formation in egg white protein was partially inhibited by D-glucose (Murase, 1980). The inhibition increased with increases in the glucose-to-protein ratio. Highest inhibition was observed between pH 11 and 12. Dworschak et al. (1981) made similar observations. Our studies on the effect of glucose in soy protein suspensions exposed to 0.1 N NaOH for 1 h at 45 °C showed that the treatment did not significantly affect the amount of LAL produced (Friedman et al., 1984). When the temperature was increased to 75 °C, LAL decreased at the two concentration ratios tested. The decrease in LAL content was accompanied by significant decreases in arginine content and the appearance of several unidentified ninhydrin-positive compounds. The amounts formed of these compounds were proportional to the amount of glucose present.

Ascorbic Acid. Ascorbic acid inhibited LAL formation in several proteins heated for 1 h at pH 13.2 at 100 °C (Dworschak et al., 1981). Abe et al. (1981) also report an inhibiting effect of ascorbic acid in alkali-treated wheat proteins. The chemical basis for the inhibition is not known. Possibilities include modification of lysine ϵ-NH_2 groups, oxidative modification of SH and SS groups of cysteine/cystine, and modification of formed LAL.

Malic Acid. Addition of malic acid to both alkali-treated wheat proteins and quick-served noodles inhibited LAL formation (Abe et al., 1981). The malic acid did not alter the flavor, texture, or taste of the noodles. The mechanism of inhibition could involve alteration of hydrogen bonding and charge distribution near reactive sites involved in LAL formation.

Ammonia. Heating alkaline solutions (pH 13) of lactalbumin and phosvitin at 55 and 95 °C in the presence of ammonia enhanced β-aminoalanine formation at the expense of the formation of LAL (Mukai et al., 1986). The competitive reaction did not occur at 30 °C.

Biogenic Amines. The NH_2 group of β-phenylethylamine reacted with dehydroalanine residues of food proteins to form a mixture of two isomeric phenylethylaminoalanines, DL-3-(*N*-phenylethylamino)alanine (PEAA), analogous to nucleophilic addition reactions leading to the formation of LAL (Jones et al., 1981). Addition of phenylethylamine to cheeses, soy products, and a yeast extract followed by heating in the pH range 6−11 induced formation of various amounts of PEAA (Tucker et al., 1983). Addition of histamine and tyramine to Cheddar cheese prior to heating (120 °C, 1 h) also induced the formation of new amino acids, presumably histaminoalanine and tyraminoalanine. These have not been characterized.

The extent of formation of PEAA in casein, acetylated casein, soy protein, and acetylated soy protein is consistent with a postulated mechanism for its formation from phenylethylamine and dehydroalanine (Friedman and Noma, 1986). LAL and PEAA formation were shown to be competitive reactions. Because the same intermediate, dehydroalanine, is postulated to compete

for the ϵ-NH_2 groups of lysine and the amine, blocking of lysine NH_2 groups should minimize LAL formation. This was the case, because acetylation of the ϵ-NH_2 groups of casein and soy proteins resulted in significant decreases in LAL and corresponding increases in PEAA content compared to the native proteins.

Because (a) PEAA induced kidney lesions in rats similar to those caused by LAL (see below); (b) both LAL and PEAA inhibited metalloenzymes such as carboxypeptidase A and polyphenol oxidase (Friedman et al., 1985, 1986a,b); and (c) histamine, phenylethylamine, and tyramine in cheese, chocolate, wine, and other foods are reported to precipitate migraine headaches in dietary migraine sufferers, a need exists to better understand possible adverse and beneficial effects of removal of such biogenic amines from processed foods via dehydroalanine interactions.

Metal Salts. Metal ions can interact with the SH group of cysteine and with α-NH_2 and COOH groups of free amino acids, the NH group of histidine, and the guanidino group of arginine. Complexation with lysine may minimize or prevent LAL formation if the complex is stable in alkali. $CuSO_4$ and $FeCl_2$ caused a significant decrease in LAL content of soy proteins (Friedman et al., 1984). In contrast, aluminum, barium, calcium, lanthanum, and sodium chloride enhanced the production of LAL from 5% sodium caseinate heated at 60 °C at pH 10 for 1 h (Creamer and Matheson, 1977). These results suggest that metallic cations in foods exposed to high pH may affect LAL formation.

Nitrogen Atmosphere. Alkali treatment of food proteins under an atmosphere of nitrogen minimized LAL formation (Steinig and Montag, 1982).

Dimethyl Sulfoxide. Heating soy proteins in mixed aqueous−DMSO solutions produced less LAL than when the same experiment was carried out in aqueous solution (Friedman et al., 1984). A possible mechanism of the DMSO effect is discussed elsewhere (Friedman, 1967).

NUTRITION AND SAFETY

Digestibility and Nutritional Quality. Alkali treatment of proteins both reduces and enhances digestibility and the nutritional quality of the treated proteins (Chung et al., 1986; De Groot et al., 1976; Friedman et al., 1981; Hayashi and Kamedas, 1980b; Possompes and Berger, 1991; Slump, 1978a,b; Savoie et al., 1984, 1991; Swaisgood and Castignani, 1985; Figure 5; Tables 8 and 9). The following examples illustrate the adverse and beneficial effects of alkali treatment on protein nutrition.

Figure 5 shows that impairment of in vitro digestibility of casein is inversely proportional to the amounts of LAL and D-amino acids formed. Table 9 and Figure 6 show that alkali treatment of soy protein isolates adversely affected in vivo digestibility in rats and body weight gain in baboons. In contrast, the lower digestibility of LAL-containing soybean proteins reduced the rate of degradation of the modified proteins in the rumen of cattle by bacterial enzymes (Friedman et al., 1982a; Nishino and Uchida, 1995; Nishino et al., 1996). Such reduction is beneficial for ruminant nutrition because it improves N retention and the nutritional value of the proteins consumed by cattle and sheep.

Another beneficial effect was observed by Jyothi and Sumathi (1995), who found that exposure of the common bean (*Phaseolus vulgaris*) to $NaHCO_3$, Na_2CO_3, and

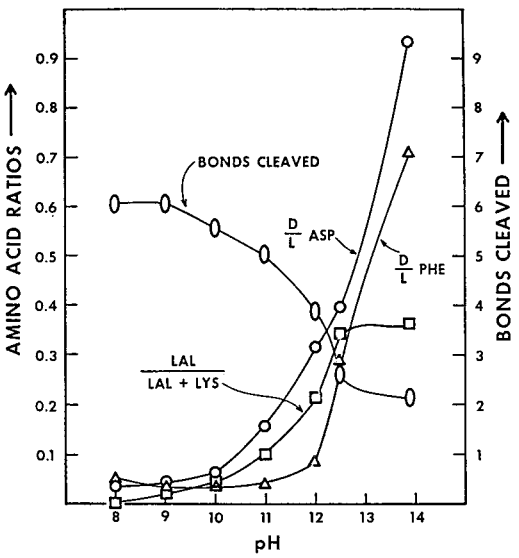


Figure 5. Inverse relationship between number of peptide bonds of caseins exposed to different pH values hydrolyzed by trypsin and their content of LAL and D-amino acids (Friedman et al., 1981).

Table 8. Effect of Alkali Treatment (0.2 N NaOH, 80 °C, 1 h) of Casein on Nutritional Parameters of Young Rats [Adapted from Possompes et al. (1989)]

	casein, untreated		casein, alkali-treated	
casein in diet (%)	10	20	10	20
food intake (g/day)	15.1	16.1	8.2	16.1
body wt gain (g)	234	277	121	207
wt gain (g/day)	4.67	7.71	0.05	5.43
N digestibility (%)	80.5	88.8	72.2	86.8
fecal N (% of dry matter)	2.45	2.96	4.02	5.55
PER (wt gain/protein intake)	3.09	2.40	0.02	1.68

NaOH enhanced digestibility and biological value (BV) of the beans. These effects result from extraction and/or inactivation of antinutritional tannins, phytates, and trypsin inhibitors and release of bound vitamins niacin and riboflavin. Thus, treatment of bean seeds with 0.1 M $NaHCO_3$ increased the digestibility coefficient from 77.3 to 93.5 and the BV from 57.9 to 66.8. The corresponding values for the casein were 96.3 and 82.3, respectively. This observation raises the question whether alkali treatment will also inactivate other antinutritional factors present in foods and feeds (Liener, 1994a,b, 1997). Steaming of feathers enhances nutritional quality and generates LAN (Han and Parsons, 1991).

Possible causes for the reduction in digestibilty−nutritional quality include destruction of proteolytic enzyme substrates such as arginine and lysine, isomerization of L-amino acids to less digestible D-forms, formation of inter- and intramolecular cross-links, which hinder access of proteolytic enzymes, inhibition by LAL of proteolytic metalloenzymes such as carboxypeptidase (Hayashi, 1982; Friedman et al., 1985, 1986a,b), and alkali-induced formation of trypsin- and chymotrypsin-inhibiting peptides (Berger and Possompes, 1987; Possompes and Berger, 1991).

Generally, the extent of nutritional damage of alkali treatment associated with loss of lysine may depend on the original lysine content of a protein. Decrease in lysine due to LAL formation in a high-lysine protein such as casein, high-lysine corn protein, or soy protein isolate may have a less adverse effect than in a low-lysine protein such as wheat gluten, for which lysine is

Table 9. Digestibilities and Assimilable (Utilizable) Nitrogen Contents of Toasted and Alkali-Treated Soy Proteins and Diets in Rats Determined by the Nitrogen Metabolism Method[a]

product	digestibility[b] (D, %)	assimilability[c] (%)	NPU[d] (%)	digestible protein content[e] (%)	assimilable protein content[f] (%)
casein	98.3 ± 0.49[g]	82.0 ± 2.85	80.6		
toasted soy	97.0 ± 0.59	64.3 ± 1.93	62.4		
alkali-treated soy	83.2 ± 0.06	34.0 ± 1.48	28.3		
toasted soy diet[h]				21.40	13.76
alkali-treated soy diet[i]				18.98	6.45

[a] J. J. Dreyer, 1998, private communication; Dreyer and van der Walt, 1985. [b] Digestibility (*D*) % = % of consumed protein nitrogen absorbed. [c] Assimilability % = % of absorbed protein nitrogen retained or assimilated by body. [d] NPU (net protein utilization) % = overall index of protein quality = (*D*) (assimilability)/100). [e] Digestible protein content = (N × 6.25 × *D*)/100. [f] Assimilable protein content = (N × 6.25 × *D*/100) (assimilability). [g] Standard error of mean. [h] N = 3.53 g/100 g of air-dried diet. [i] N = 3.65/100 g of air-dried diet.

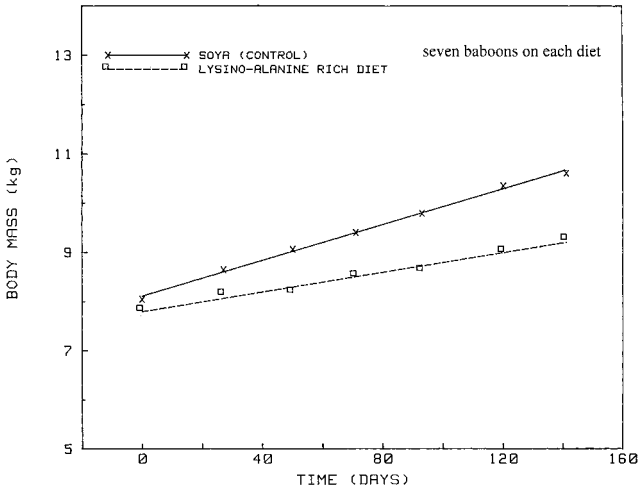


Figure 6. Mean weight gain of baboons fed a 20% toasted soy protein isolate and alkali-treated soy protein diets (see text).

a nutritionally limiting amino acid (Friedman and Finot, 1991; Friedman, 1996b).

LAL as a Source of Lysine. The microorganisms *Aspergillus niger*, *Bacillus subtilis*, *Erwinia chrysanthemi*, *Escherichia coli*, and *Tetrahymena pyriformis* can utilize LAL as a source of lysine (Karayiannis et al., 1979a; Sternberg and Kim, 1979). They appear to be able to incorporate LAL-derived lysine but not LAL into proteins. Evidently, *E. coli* and other microorganisms present in the digestive tracts of animals can transform LAL into lysine. The mechanism of such metabolic transformations must involve removal of the alanine side chain from LAL. Will the enzymes involved also transform LAL into lysine when added to processed foods?

The PER of wheat gluten in rats increased from 0.65 to 1.62 on addition of 0.3% lysine and to 1.19 with an equimolar amount of LAL (Sternberg and Kim, 1979). In contrast, although the PER of 0.62 of gluten in mice also increased (to 0.85) with lysine addition, it decreased to 0.31 with LAL. The growth-depressing effect of LAL in mice may be due to their inability to transform some of the LAL to lysine compared to rats. Robbins et al. (1980) showed that LAL is completely unavailable to the rat as a source of lysine and only partially available to the chick and that about half of the LAN was utilized as a source of cysteine by both species. Friedman and Gumbmann (1984), using an all-amino acid diet, found that the methionine-sparing effect of LAN was 27%, that of L-cysteine was 78%, and that of D-cysteine was −24% (weight loss). The contradictory results with rats need to be resolved.

The biological utilization of LAL as a source of lysine was determined in a growth assay in weanling male mice in which all lysine in a synthetic amino acid diet was replaced by a molar equivalent of LAL (Friedman et al., 1982b). The replacement produced an amount of weight gain equivalent to that expected from a diet containing 0.05% L-lysine. On a molecular equivalent basis, LAL was 3.8% as potent as lysine in supporting weight gain in mice.

Metabolism of LAL. Metabolic studies with radioactively labeled LAL and alkali-treated lactalbumin and fish and soy proteins showed that LAL is partly released by digestive enzymes and then absorbed by the intestine (Finot et al., 1977). The nonabsorbed part was partly degraded by the intestinal microflora to CO_2. The absorbed part was eliminated in the urine of rats, mice, and hamster largely as free LAL, although some of the LAL was also excreted as acetylated derivatives. In contrast to rodents, quail excreted little free LAL. In rats, those derivatives that were excreted slowly were concentrated in the cortex of the kidney, susceptible to nephrocytomegaly. Similar observations were made by Abe et al. (1981) and by Struthers et al. (1977, 1980).

The stereochemistry of synthetic LAL did not change after consumption, absorption, and excretion in the urine (De Weck-Gaudard et al., 1988). However, free LAL in urine of rats fed protein-bound LAL consisted mostly of the LL-isomer. This result could be due to the reduced ability of intestinal proteases to hydrolyze peptide bonds involving LD-LAL, resulting in a lower absorption rate of this isomer. The lower absorption is also consistent with the reported 20−100-fold greater effectiveness of free LAL to induce nephrocytomegaly as compared to bound LAL.

There seems to be a species dependence in the LAL-metabolizing activity of crude extracts of kidneys (Kawamura and Hayashi, 1987). Relative degradation rates (in nanomoles per hour per gram of wet kidney tissue) were as follows: human, 70; pig, 100; cow, 110; mouse, 145; chicken, 163; rat, 185; rabbit, 264; Japanese quail, 1551. The low degrading activity in human kidneys indicates that humans may be more sensitive to the biological effects of LAL than the other animals. Addition of the three coenzymes (acetyl-CoA; flavin mononucletide, FMN; and flavin adenine dinucleotide, FAD) and of pyridoxal 5′-phosphate to the kidney extracts stimulated LAL degradation. The addition of pyridoxal phosphate induced the highest stimulation.

Nephrocytomegaly. Feeding alkali-treated proteins to rats induces changes in kidney cells. These changes are characterized by enlargement of the nucleus and cytoplasm and disturbances in DNA synthesis and mitosis. These lesions, which have been attributed to LAL (Gould and MacGregor, 1977; Karayiannis et al., 1979a,b; Kolonkaya, 1986; Slump, 1978a,b; Woodard

Table 10. Reported Studies on Nephrocytomegaly in Rodents Induced by LAL, β-Aminoalanine, and Phenylethylaminoalanine and LAL-Containing Proteins

	reference
	Rats
lysinoalanine	De Groot et al., 1976; Feron et al., 1978; Gould and MacGregor, 1977; Jonker et al., 1993, 1996; Slump, 1978a,b; Woodard et al., 1975
β-aminoalanine	Feron et al., 1978
phenylethylamino-alanine	Jones et al., 1987
casein	De Groot et al., 1976
lactalbumin	Gould and MacGregor, 1977; Karayiannis et al., 1979a,b, 1980
soy protein	Gould and MacGregor, 1977; Karayiannis et al., 1980; Kolonkaya, 1986; Struthers et al., 1977
	Mice
lysinoalanine	Feron et al., 1978; Sternberg and Kim, 1979
soy protein	Feron et al., 1978; Sternberg and Kim, 1979

and Short, 1977; Woodard et al., 1975), are designated nephrocytomegaly (karyomegaly). The affected cells are epithelial cells of the straight portion (*pars recta*) of the proximal renal tubules (Figure 7). Enlarged nuclei tend to have more than the diploid complement of DNA, unusual chromatin patterns, and proteinaceous inclusions. Increases in total nonchromosomal protein parallel increases in nuclear volume. These events suggest disruption of normal regulatory function of the *pars recta* cells.

The renal tubular epithelial kidney cells of all animals increased in both size and DNA content. Necrosis of the cells was characterized by cytoplasmic edema and vacuolization, loss of microvilli, and increased lysosomal and cytoplasmic inclusions. β-Aminoalanine, ornithinoalanine, and β-phenylethylaminoalanine induced similar rat kidney lesions at higher doses than did LAL (Feron et al., 1978; Jones et al., 1987). The amount of LAL required for induction of cytomegaly in rats was similar to that present in some commercial foods (Table 10). The cytomegaly was partly reversed following discontinuance of the alkali-treated soy protein diets (Struthers et al., 1978).

Sternberg and Kim (1977) observed nephrocytomegaly in kidney sections of mice fed 8350 ppm of protein-bound LAL for 3 weeks, a finding similar to that observed by Feron et al. (1978). This contrasts with the effect induced in rats fed only 100 ppm of free LAL for 4 weeks.

A difficulty in formulating a simple relationship between LAL and nephrocytomegaly is that proteins of equal LAL content produce different biological responses. Thus, O'Donovan (1976) reported that feeding rats alkali-treated soy protein led to severe nephrocytomegaly, whereas a different protein with the same LAL content did not produce lesions. Alkali-treated soy protein (supplying 1400−2600 ppm of LAL) induced nephrocytomegaly, whereas 2500 ppm of LAL derived from alkali-treated lactalbumin did not (Karayannis et al., 1979b). Generally, free LAL is a much more potent inducer of kidney damage than is the same concentration of protein-bound LAL.

The divergent observations about relative potencies of various alkali-treated proteins in inducing kidney lesions could arise from dietary factors and from the combined effects of other kidney-damaging compounds present in the diet. First, adding high-quality, untreated proteins such as casein and lactalbumin to diets containing alkali-treated proteins appears to prevent the lesions (Feron et al., 1978). Diets rich in lysine and sulfur amino acids may ameliorate the LAL effect. Second, in order of cytotoxic effect, the most pronounced response is with free LAL, then low molecular weight compounds containing peptides, then LAL-containing proteins. Because the extent of peptide bond hydrolysis during alkali treatments is influenced by protein structure, different proteins having the same quantities of bound LAL may release different amounts of free LAL or LAL peptides in vivo (Liardon and Friedman, 1987). Third, because D-serine is formed concurrently with LAL (Figure 4) and because it also induced kidney lesions (Friedman, 1991; Young et al., 1994), serine may potentiate the action of LAL. Fourth, Maillard browning products and the *Aspergillus*- and *Penicillium*-derived ochratoxin A, which contaminates many foods, also induced karyomegaly (Godin et al., 1998; O'Brien and Walker, 1988; Von Wangenheim et al., 1984). Such compounds could also exert an additive and/or synergistic effect with LAL. However, Jonker et al. (1993, 1996) found that the combined exposure of LAL with other nephrotoxic compounds at nontoxic levels of the individual compounds did not enhance renal toxicity; that is, there was no apparent synergism. They did not study D-serine.

The mechanism of the observed cellular action of LAL is not well understood. On the basis of the observed inhibition of metalloenzymes by LAL and the observed high affinity of copper ions for LAL and metalloenzymes, Pearce and Friedman (1988) suggested that the damage observed in the proximal tubules probably arises from interaction of LAL with copper(II) of metallothioneins within epithelial cells. Generally, LAL may interfere with the mechanism by which the kidney conserves copper by displacing histidine as the major low molecular weight carrier of copper in vivo.

Leegwater (1978) suggested that the observed high specificity of the LAL effect for the rat kidney is probably due to the fact that nephrotoxicity in the rat is related to the high content of L-amino acid oxidase activity (which presumably catalyzes the formation of LAL metabolites responsible for toxicity) compared to other species evaluated. In addition to the rat [minimum nephrotoxic effect level (MNEL) = 100 ppm of LAL] and the mouse (MNEL = 1000 ppm of LAL), nephrocytomegaly was not observed in the kidneys of hamsters, dogs, Rhesus monkeys, rabbits, and Japanese quail fed up to 10000 ppm of LAL for 4−9 weeks (DeGroot et al., 1976; Feron et al., 1978; Jonker et al., 1993, 1996).

LAL competitively inhibited lysyl-tRNA-synthetase of prokaryotic and eukaryotic cells. It was incorporated into proteins and inhibited incorporation of lysine by a cell-free eukaryotic protein-synthesizing system (Lifsey et al., 1988). Whether these actions at the cellular level are relevant to the induction of nephrocytomegalia is not known.

Chelation of Metal Ions. Because the structure of LAL contains two amino, one imino, and two carboxyl groups, which can serve as potential metal ion chelating sites, I suggested that some of the biological properties of the molecule may be due to metal ion chelation (Friedman, 1974, 1977a,b). This prediction was later confirmed by several investigators (Hayashi, 1982; Friedman et al., 1985, 1986a,b; Furniss et al., 1985, 1989; Rehner and Walter, 1991; Sarwar et al., 1999).

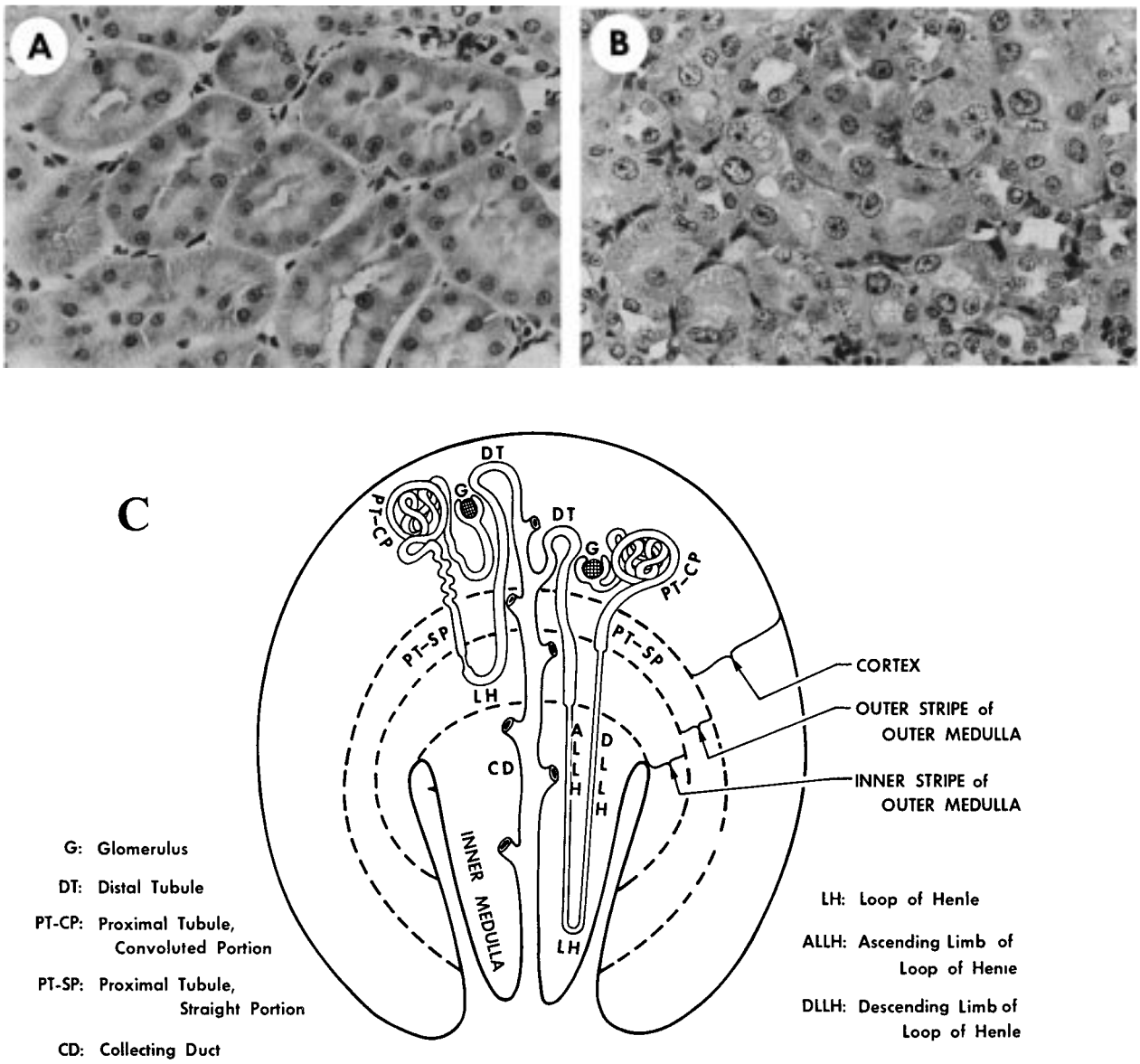


Figure 7. Nephrocytomegaly of rat kidney cells illustrated with photomicrographs of outer medullary stripe of kidneys from rats fed 20% soy protein for 8 weeks: (A) control, untreated soy protein diet (note uniformity of *pars rectae* cells); (B) alkali-treated protein (2630 ppm of dietary LAL) [note cytoplasmic and nuclear enlargement of the *pars recta* cells (Gould and MacGregor, 1977)]; (C) spatial arrangement of the tubules showing long- and short-looped nephrons (the straight portions of the affected proximal tubules, the *pars rectae*, lie in the outer stripe of the outer medulla).

In vivo studies confirmed expectations from the in vitro results. LAL and Maillard products complexed with essential trace elements and enhanced renal resorption and excretion of copper in rats. The resorption−excretion was less pronounced with iron and zinc.

To further demonstrate this possibility, we have examined LAL (a mixture of the LD- and LL-isomers as well as the individual isomers) for its affinity toward a series of metal ions, of which copper(II) was chelated the most strongly (Figure 8). On this basis, we have suggested a possible mechanism for kidney damage in the rat involving LAL interaction with copper within the epithelial cells of the kidneys.

As described in detail elsewhere (Pearce and Friedman, 1988; Friedman and Pearce, 1989), it is possible to determine p*K* values of LAL amino, imino, and carboxyl groups and the metal ion binding constants of LAL isomers and to predict the in vivo equilibria between histidine, the major low molecular weight copper carrier in plasma, and competing chelating agents such as LAL. A mathematical analysis was used to predict LAL plasma levels needed to displace histidine as the major copper carrier in vivo. The calculated values were 27 μM for LD-LAL, 100 μM for LL-LAL, and 49 μM for the mixture of the two. LD-LAL would be a better competitor for copper(II) in vivo than the LL-isomer; that is, it will 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 LD-LAL (Feron et al., 1978). The apparent direct relationship between the observed affinities of the two LAL isomers for copper(II) ions in vitro and their relative toxicities in the rat kidney are consistent with the hypothesis that LAL exerts some of its biological effects through chelation to copper and other metal ions in vivo.

Primate Studies. A key question regarding the described LAL-induced nephrocytomegaly in rodents is whether it has any significance for humans. To extend our knowledge to nonhuman primates to help assess

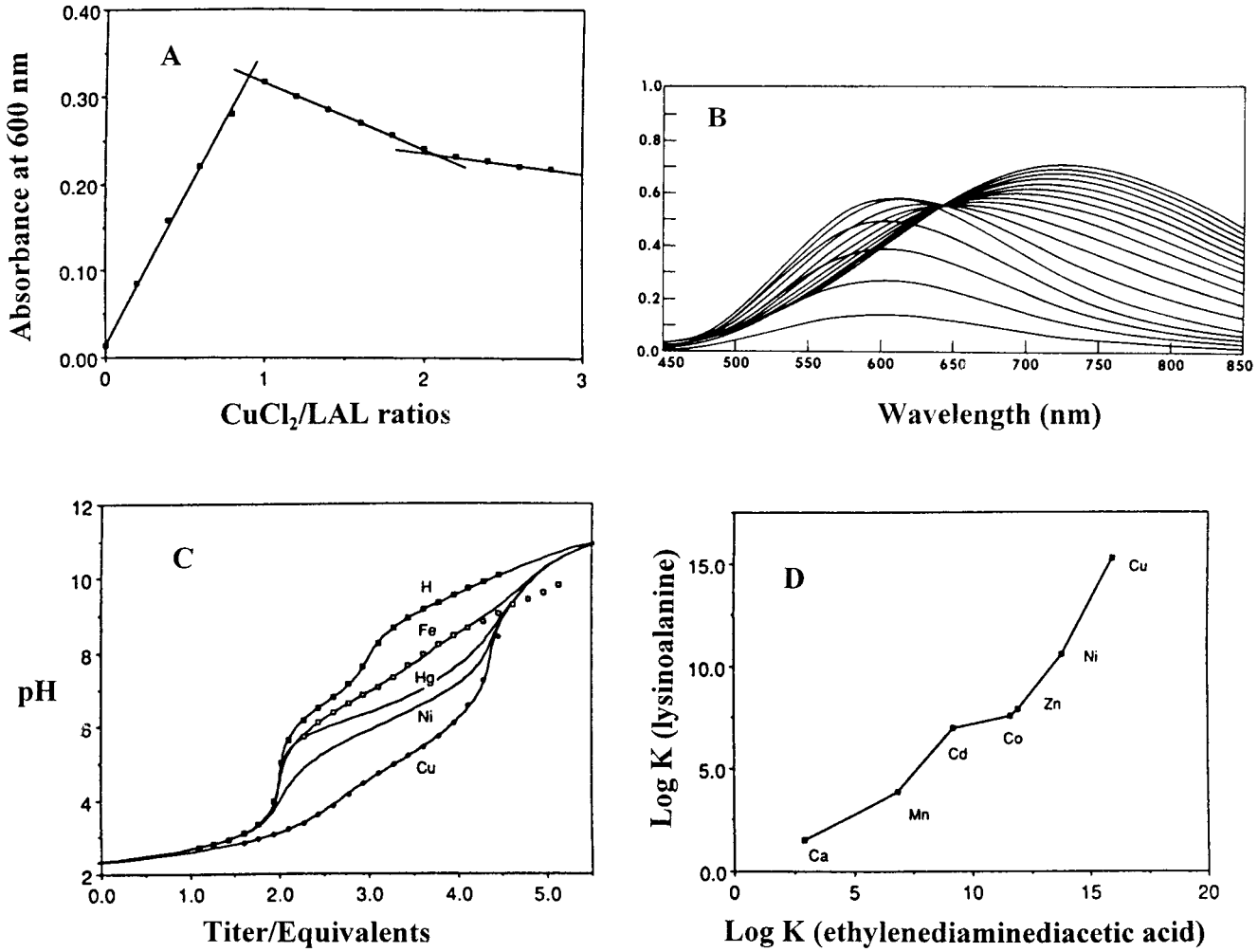


Figure 8. Affinities of metal ions for LAL: (A) spectrophotometric titration with $CuCl_2$; (B) absorption spectra of Cu(II)/LAL mixtures; (C) potentiometric titration curves for LAL in the absence and presence of various divalent metal ions; (D) relationship of stability constants of LAL and EDDA complexes of divalent metal ions (Pearce and Friedman, 1988; Friedman and Pearce, 1989).

human risk of exposure to LAL in the diet, we carried out a baboon feeding study of alkali-treated soy proteins completed ~15 years ago. We conducted this study in collaboration with the Biological Evaluation Division of the National Food Research Institute (CSIR), Pretoria, South Africa, headed by Dr. J. J. Dreyer. Previously, a short-term feeding study of alkali-treated soy protein diets to Rhesus monkeys revealed no apparent histological changes in the kidney tissue of the test animals (Feron et al., 1978). To obtain additional information on this aspect, we evaluated nutritional and histopathological consequences of feeding toasted and alkali-treated soy flours to baboons. Soy protein isolate (300 kg) (Kraft Foods) was suspended in 3000 L of 0.1 N NaOH in a large tank preheated to 60 °C. The suspension was stirred for 8 h, cooled, and neutralized with 6 N HCl to pH 4.5 (the isoelectric point). The liquid was siphoned off, and the precipitated protein was washed twice with 3000 L of H_2O, freeze-dried, and shipped to Pretoria.

The untreated commercial toasted protein isolate contained 5.74 g of lysine/16 g of N. The corresponding value for LAL was 0.37. The alkali-treated proteins contained 4.96 g of lysine/16 g of N. The corresponding value for LAL was 1.61. Figure 6 shows the growth curves for seven preadolescent male baboons each fed soy control and alkali-treated soy diets [20% protein in a standard baboon diet described in Robbins et al. (1988) and Sly et al. (1980)]. There was no difference in growth rates over the 150 day period; the slopes of the growth curves are not significantly different from each other. However, in absolute terms, weight gain of the baboons fed the treated soy diet (containing 370 mg of LAL/100 g of air-dried diet = 3700 ppm) was ~20% lower than the gain observed with the control soy diet (containing 80 mg of LAL/100 g = 80 ppm of air-dried diet). The decreased weight gains may be due to the decreased digestibility and utilization of the treated soy protein compared to the control (Table 9).

Histological evaluation of the pancreas and kidney tissues from baboons fed toasted and alkali-treated soy diets were carried out by Dr. W. L. Spangler (D.V.M.). The histological data were analyzed statistically. The effect on baboons was tested against the environmental effect, whereas that of dietary treatments was tested between-baboon within-treatment variation. Pancreatic histology was not affected by the alkali treatment.

Kidney: Nuclei per 20 mm Area. For this parameter the means of baboons within treatment were significantly different; the ranges within the three treatments were 4.13−5.67, 4.73−7.67, and 4.53−7.07, respectively. The respective significance levels were 0.03, 0.001, and 0.002. Examination of the data revealed the presence of large numbers of nuclei in some kidneys. The range

of treatment means, however, was not wide: 5.29, 6.07, and 5.80. In the nested ANOVA, the differences *between* treatments were insignificant. However, among baboons *within* treatments, the variance was highly significant, $P = 0.05$. The environmental variance component was 79%. There was no contribution to the variance by treatment differences.

These findings suggest that as far as the difference due to soy processing treatment was concerned, this was essentially a negative study. For the kidney data, although there was no treatment effect on the numbers of nuclei per 20 mm areas in the kidney, there were highly significant differences between baboons within treatments. Such differences appeared to be due to large numbers of nuclei in some sections of some of the baboons. In the tested ANOVA, the between-baboon variance component contributed 21% of the total variation. Thus, no meaningful treatment differences could be shown. Whether the differential baboon effect noted for the kidney parameter is meaningful cannot decided by these data because of the large within-treatment variations.

The histological data were also independently evaluated by our biostatistician (Dr. B. E. Mackey). He agrees that there is insufficient evidence to support an inference of differences in variances among control and alkali-treated soy diets. The significance of differences among baboons for the kidney measure is indicative of a large variance among baboons relative to the variance among tubules within kidneys. There were also no statistically significant differences in serum calcium, magnesium, zinc, alkaline phosphatase, and total protein levels.

The results of the baboon study show that although feeding alkali-treated soy protein containing a moderate amount of LAL to baboons for ~6 months adversely affected body mass, it apparently did not influence pancreatic and kidney histology. The large variation in histochemical parameters among baboons within each group tested was unexpected and should be taken into account in future studies with primates.

Short-term feeding of LAL and Maillard product-containing formulas to healthy preterm babies did not appear to induce tubular kidney damage, as determined by urinary excretion of four kidney-derived enzymes (Langhendries et al., 1992). However, the formula diets did induce a general increase in urinary microprotein levels. The authors concluded that the 10-day feeding study period may have been too short to cause significant changes in renal function.

HISTIDINOALANINE, LYSINOALANINE, AND LANTHIONINE IN AGING OF BONES AND TISSUES

Treatment of either polyhistidine or the copper complex of histidine with *N*-acetyldehydroalanine resulted in the formation of a new amino acid, which we postulated to be HAL (Finley and Friedman, 1977; Friedman, 1977a). In principle, both nitrogens of the histidine ring can react with the double bond of dehydroalanine, leading to the formation of N^{τ}- and N^{π}-(2-amino-2′-carboxymethyl)histidines, each a mixture of DL-isomers (Figure 2). Subsequent studies revealed that HAL is present in processed food proteins and is also formed in vivo, where it may be related to the aging of various tissues. Some of these studies will be briefly summarized below.

The HAL content of several proteins (bovine serum albumin, bovine tendon collagen, casein, human γ-globulin, and pepsin) heated in a pH 6.8 buffer at 110 °C for 24 h was greater than their LAL content (Fujimoto, 1984). Related studies by Henle et al. (1993, 1996) showed that heating various milk products resulted in the formation of 50−1800 mg of HAL/kg of protein. The corresponding concentrations of LAL were similar. The ratio of the N^{τ}- to the N^{π}-isomers in heated protein hydrolysates was 8:1. Efforts to demonstrate the presence of histidinomethylalanine (derived from the addition reaction of the NH group of histidine to methyldehydroalanine) and of methyl-LAL (derived from the corresponding addition of the ϵ-NH_2 group of lysine) in processed milk products were unsuccessful (Walter et al., 1994a,b).

Mechanistic considerations suggest that the ready formation of HAL at neutral pH is probably due to the fact that the p*K* value of the NH group of histidine is ~5.5, so that most of these groups are ionized at pH 7. In contrast, only a small fraction of lysine ϵ-NH_3^+ (p*K* = 10) is ionized at this pH. Only ionized protein functional groups are expected to participate with the double bond of dehydroalanine in addition reactions (Friedman and Wall, 1964; Friedman et al., 1965, 1980).

N^{τ}-Histidinoalanine was found in hydrolysates of human aorta, cataract lens proteins, collagen, connective tissues, dentine, and urine (Fujimoto, 1986; Fujimoto and Roughley, 1984; Fujimoto and Yu, 1984; Kanayama et al., 1987; Kuboki and Liu, 1998; Sugiyama et al., 1987). Its content in human tissues correlates with aging and calcification. It is striking that the HAL content of a diseased eye lens (3.35 ± 2.76 nmol/mg) is ~70 times greater than the value measured in normal eye lens (0.05 ± 0.03 nmol/mg). The content of the advanced stage cataract lens was found to be greater than in the aged human aorta. Diseased eye lenses also contained LAN (Bessems and Rennen, 1987). LAL was also discovered in calcified tissue collagen (Fujimoto et al., 1981, 1982) and bovine dentine phosphoprotein (Kuboki et al., 1984, 1991). A phosphoprotein from bovine bone with a molecular weight of 24000 contained 1.2 mol of HAL per molecule. By cross-linking tissue proteins, the formation of cross-linked amino acids probably contributes to lowering of tissue elasticity and function, increasing tissue rigidity, and hardening of the eye lens nucleus.

Diet seems to influence age-related tissue concentrations of HAL in rodents (Nagamine, 1992; Wang et al., 1995). However, the HAL content of collagen and elastin from human stomach cancer tissues was the same as in normal tissues (Kyogoku, 1988).

HAL is also present in calcium-binding phosphoproteins derived from extrapallial fluids of certain bivalve molluscs, where the N^{τ}- and N^{π}-isomers occur in a relative abundance of 3:1 (Sass and Marsh, 1983, 1984). The content of the bivalve phosphoprotein particles is an order of magnitude greater than in the vertebrate proteins (Marsh, 1986). This may be because mollusc proteins contain high levels of the dehydroalanine precursor phosphoserine as well as histidine. The phosphoprotein particles are composed of subunits cross-linked via divalent cations and HAL.

In the absence of any evidence of enzymatic catalysis of the transformations in vivo leading to the formation of HAL, LAL, and LAN cross-links, it is tempting to suggest that aging-related changes in the microenvironment near tissue proteins may favor elimination of phosphate groups from phosphoserine, generating de-

hydroalanine residues that then react with adjacent cysteine, histidine, and lysine side chains. Because not all such dehydroalanines may be appropriately situated for cross-link formation, some of them should be present in tissue proteins, as is the case for processed proteins mentioned earlier and antibiotics described below.

ANTIMICROBIAL COMPOUNDS

LAL, LAN, methyl-lanthionine, dehydroalanine, and methyldehydroalanine are formed both during food processing and naturally as a result of enzyme-catalyzed post-translational modifications during the biosynthesis of certain antimicrobial compounds. These include cinnamycin, duramycin, nisin, and subtilin (Jack and Jung, 1998; Hansen, 1994). Because some of these antibiotics are widely used in food preservation, this aspect is also relevant to an assessment of the role of cross-linked and dehydro amino acids in the diet, especially in relation to food safety. This section (a) briefly reviews the chemistry of the antimicrobial compounds, (b) discusses possible mechanisms of the antimicrobial effects, and (c) proposes new research to assess the possible value of LAL-containing peptides and proteins as antimicrobial agents for human pathogen reduction.

The following is a brief review of the literature on lantibiotics, small protein antibiotics that are produced by and act on Gram-positive bacteria (Gross, 1977; Jung, 1991; Liu and Hansen, 1993; Kaletta et al., 1991; Morris et al., 1984; Sahl et al., 1995; Zimmermann et al., 1993).

(a) The structures of these compounds contain intramolecular rings formed by LAN, methyl-lanthionine, and LAL, as well as the dehydrated amino acids 2,3-didehydroalanine (dehydroalanine) and 2,3-didehydrobutyrine (methyldehydroalanine (Figure 2).

(b) Dehydroalanine and methyldehydroalanine are formed by enzyme-catalyzed site-specific dehydration of serine and threonine residues, respectively; LAN is formed by addition of the SH group of a neighboring cysteine residue to the double bond of dehydroalanine. The corresponding addition to methyldehydroalanine produces methyl-lanthionine. LAL is generated from the nucleophilic addition of an ϵ-NH_2 group of a neighboring lysine to dehydroalanine.

(c) The cross-linked bridges may stabilize the spatial structures of the antibiotics, and the dehydroamino acids may act as alkylating agents of essential functional groups of the bacterial cells.

(d) The mechanism of antimicrobial action appears to involve (1) interference with energy transduction (depolarization) of membranes, which results in the formation of membrane channels through which low molecular weight compounds of bacterial cells may pass; (2) aggregation of the lipid components of vesicles such as lysophosphatidylethanolamine, enhancing permeability and causing hemolysis of the vesicles; and (3) alkylation of SH groups of membrane proteins and enzymes by the dehydroalanines. NMR studies (Hosoda et al., 1996) suggest that the NH group of LAL probably binds to the phosphate group of the lipid during complex formation.

(e) LAL appears to play a role in the mechanism of phosphonate ester inhibition of β-lactamases, enzymes responsible for bacterial resistance to β-lactam antibiotics (Rahil and Pratt, 1992). LAL formed from an ϵ-NH_2 group of a lysine side chain of β-lactamase and the dehydroalanine moiety generated from the elimination of an artificially introduced inhibitory phosphonyl ester group.

(f) A patented method is described by Collison et al. (1991) for inhibiting the contamination of heat-processed meat by surface application of the lantibiotics nisin and subtilin.

Because, as mentioned earlier, LAL has a strong affinity for essential metal ions such as cobalt, copper, iron, and zinc, it is quite possible that metal ion chelation by LAL-containing antibiotics such as cinnamycin and duramycin governs or contributes to the antimicrobial activity. Another possibility is that in addition to SH groups, other functional groups of bacterial cell membranes such NH_2 and NH groups associated with proteins and with DNA may also be alkylated by the dehydroalanines of the antibiotics. These considerations also suggest that free LAL and LAL-containing peptides and food proteins may also possess antimicrobial properties. These possibilities merit study.

CONCLUSIONS AND RESEARCH NEEDS

The discovery of LAL formed during the exposure of proteins to high pH in 1964 would by itself probably have been of interest largely to protein chemists. Interest grew exponentially when only three years later it was found that the introduction of LAL into food proteins under conditions widely used in the processing of foods resulted in adverse effects on nutritional quality and in kidney damage in rodents but apparently not in primates. The further discovery that LAL is also produced in vivo by microbes, animals, and humans and that these amino acids may govern antimicrobial activities and be related to the aging of tissues further broadened interest in the unusual amino acids. It is striking that just as is the case with the Maillard reaction of proteins (Friedman, 1996a), medical scientists are utilizing the extensive chemical and biological knowledge discovered by food scientists in efforts to define the role of LAL, LAN, and HAL in vivo. If these amino acids do indeed play a role in aging, the question arises whether dietary SH-containing amino acids such as cysteine, *N*-acetylcysteine, and reduced glutathione, which prevent LAL formation *in vitro*, can also prevent formation of the cross-linkekd amino acids in vivo and thus retard the aging process.

It is now generally recognized that alkali treatment can damage nutritional quality of proteins consumed by rodents and primates. In contrast, reduced digestibility enhances nuritional quality for ruminants (cattle and sheep). Possible causes for protein damage include destruction of the essential amino acids lysine and threonine and of the semiessential amino acid cystine, reduced digestibility due to formation of cross-linked and D-amino acids, loss of phosphorus from phosphoproteins such as casein, and adverse effects on mineral nutrition due to chelation of trace elements such as copper, iron, and zinc. Because impairment of digestibility could impart fiberlike properties to proteins, it would be worthwhile to ascertain whether alkali treatment would enhance the reported cholesterol lowering by plant proteins (Friedman et al., 1999).

Although the safety of LAL and related compounds for humans remains unresolved, it is reassuring that the effect on kidneys in rodents was not apparent with primates. Resolution of the safety issue will depend on a better understanding of the fundamental mechanisms of action of these unusual amino acids at the cellular level. Mechanistic studies on the formation and biologi-

cal action and fate of LAL in vivo could perhaps benefit from a proposed method for the synthesis of deuterium- and tritium-labeled free and protein-bound LAL (Friedman and Boyd, 1977). Such labeling should help ascertain the location of LAL in proteins and its metabolism in animals and humans. Although the dietary significance of LAL has been extensively studied, very little is known about the role of HAL and of the relative biological potencies of different isomers of all the cross-linked amino acids.

The amino acid penicillamine (D-dimethylcysteine), which is used to treat patients suffering from excess copper retention (Wilson's disease), does not remove copper from plasma (Friedman, 1977b). It is likely that both penicillamine and LAL interact with copper in the kidneys. If LAL is safe for human consumption, additional studies are needed to determine the possible therapeutic value of LAL and related compounds for Wilson's and related diseases.

Studies are also needed to define the dietary consequences of exposing other food ingredients to high pH. For example, in preliminary studies (M. Friedman and H. Jürgen, unpublished results) we found that the antioxidant−antimicrobial compound chlorogenic acid is degraded at high pH. To facilitate the use of such compounds in food formulations, a need exists to ascertain whether they are stable during food processing.

In conclusion, an important objective of research in food science is to more fully understand the underlying chemistry and the resulting nutritional and toxicological consequences of protein−alkali reactions to optimize processsing parameters that minimize adverse effects and maximize beneficial ones. Understanding the food-processing conditions that govern LAL formation makes it possible to minimize or maximize the LAL content of foods and feeds depending on dietary needs. It may also facilitate the development of new LAL-, lanthionine-, and dehydroamino acid-containing antimicrobial peptides effective against human pathogens and the inhibition of cross-linked amino acid formation in vivo to retard the aging process. The main objective of this overview was to further these objectives by integrating the widely scattered literature to cross-fertilize and catalyze progress that may benefit food quality, food safety, and human health.

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LITERATURE CITED

Abe, K. Lysinoalanine. *Nippon Eiyo, Shokuryo Gakkaishi* **1986**, *39*, 353−360.

Abe, K.; Ozawa, M.; Homma, S.; Fujimaki, M. Lysinoalanine formation in the processing of quick-served noodle. *Kaseigaku Zasshi* **1981**, *32* (5), 367−371.

Abe, K.; Homma, S.; Fujimaki, M.; Arai, S. Administration of LAL-containing proteins to rats and characterization of their small intestine contents. *Agric. Biol. Chem.* **1984**, *48*, 573−578.

Amarowicz, R. Lysinoalanine. *Bromatol. Chem. Toksykol.* **1991**, *24*, 89−100 (in Polish).

Amarowicz, R.; Olender, B.; Smoczynski, S. Lysinoalanine levels in powdered milk. *Rocz. Panstw. Zakl. Hig.* **1988**, *39*, 31−34 (in Polish).

Annan, W. D.; Manson, W. The production of lysinoalanine and related substances during processing of proteins. *Food Chem.* **1981**, *6*, 255−261.

Anonymous. Mechanism of toxicity of lysinoalanine. *Nutr. Rev.* **1989**, *47*, 362−364.

Anonymous. Use of alkali in manufacture of bakery products. *Ernahrung* **1995**, *19*, 80−81.

Antila, P. The formation and determination of lysinoalanine in foods containing milk proteins. *Meijeritiet. Aikak.* **1987**, *45*, 1−12.

Asquith, R. S.; Otterburn, M. S. Cystine-alkali reactions in relation to protein cross-linking. In *Protein Crosslinking: Nutritional and Medical Consequences*; Friedman, M., Ed.; Advances in Experimental Medicine and Biology 86B; Plenum: New York, 1977; pp 93−121.

Aymard, C.; Cuq, J. L.; Cheftel, J. C. Formation of lysinoalanine and lanthionine in various food proteins heated at neutral or alkaline pH. *Food Chem.* **1978**, *3*, 1−5.

Badoud, R.; Pratz, G. Simple and rapid quantitative determination of lysinoalanine and protein hydrolysate amino acids by HPLC after derivatization with dansyl chloride. *Chromatographia* **1984**, *19*, 155−164.

Batey, I. L.; Gras, P. W. Effects of using defatted gluten as a substrate for solubilization with sodium hydroxide. *J. Food Technol.* **1984**, *19*, 109−114.

Bellomonte, G.; Boniglia, C.; Carratu, B.; Filesi, C. Lysinoalanine: contents of infant formulas. *Riv. Soc. Ital. Sci. Aliment.* **1987**, *16*, 459−464.

Berger, J.; Possompes, B. Effects of severely alkali-treated casein on *in vitro* and *in vivo* digestibility and selected intestinal enzyme activities. *Nutr. Rep. Int.* **1987**, *35*, 69−78.

Bessems, G. J. H.; Rennen, H. J. J. M.; Hoenders, H. J. Lanthionine, a protein cross-link in cataractous human lenses. *Exp. Eye Res.* **1987**, *44*, 691−696.

Betschart, A. A. Factors influencing the extractability of safflower protein (*Carthamus tinctorius* L.). *J. Food Sci.* **1975**, *40*, 1010−1013.

Bohak, Z. N^{ϵ}-(DL-2-amino-2-carboxyethyl)-L-lysine, a new amino acid formed on alkaline treatment of proteins. *J. Biol. Chem.* **1964**, *239*, 2878−2882.

Buser, W.; Erbersdobler, H. F. Determination of lysinoalanine as heptafluorobutyryl isobutyl ester derivative by gas−liquid chromatography. *J. Chromatogr.* **1984**, *303*, 234−237.

Castro, A. de; Rejano, L.; Sanchez, A. H.; Montano, A. Fermentation of lye-treated carrots by *Lactobacillus plantarum*. *J. Food Sci.* **1995**, *60*, 316−319.

Cavins, J. F.; Friedman, M. New amino acids derived from reactions of ϵ-amino groups of lysine with α,β-unsaturated compounds. *Biochemistry* **1967**, *6*, 3766−3780.

Cavins, J. F.; Friedman, M. Automatic integration and computation of amino acid analyses. *Cereal Chem.* **1968**, *45*, 172−176.

Chang, J. Y. Stability of hirudin, a thrombin-specific inhibitor. The structure of alkaline-inactivated hirudin. *J. Biol. Chem.* **1991**, *266*, 10839−10843.

Chang, J. Y.; Knecht, R. Direct analysis of the disulfide content of proteins: methods for monitoring the stability and refolding process of cystine-containing proteins. *Anal. Biochem.* **1991**, *197*, 52−58.

Chu, N. T.; Pellett, P. L.; Nawar, W. W. Effect of alkali treatment on the formation of lysinoalanine in corn. *J. Agric. Food Chem.* 1**976**, *24*, 1084−1085.

Chung, S. Y.; Swaisgood, H. E.; Catignani, G. L. Effects of alkali treatment and heat treatment in the presence of fructose on digesitbility of food proteins as determined by an immobilized digestive enzyme assay (IDEA). *J. Agric. Food Chem.* **1986**, *34*, 579−584.

Collison, M. W.; Farver, T. F.; McDonald, C. A.; Herlad, P. J.; Monticello, D. J. Use of lanthionines for control of post-processing contamination in processed meat. U.S. Patent 5,015,487, 1991.

Concon, J. M. Rapid and simple method for the quantitative extraction of corn endosperm proteins. *Anal. Biochem*. **1973**, *55*, 563−572.

Corfield, M. C.; Wood, C.; Robson, A.; Williams, M. J.; Woodhouse, J. M. The formation of lysinoalanine during the treatment of wool with alkali. *Biochem. J*. **1967**, *103*, 15C−16C.

Correia, J. J.; Lipscomb, L. D.; Lobert, S. Nondisulfide cross-linking and chemical cleavage of tubulin subunits: pH and temperature dependence. *Arch. Biochem. Biophys*. **1993**, *300*, 105−114.

Creamer, L. K.; Matheson, A. R. Action of alkali on caseins. *N. Z. J. Dairy Sci. Technol*. **1977**, *12*, 253−259.

Cuq, J. L.; Cheftel, J. C. Lysinoalanine et aliments proteiques. Formation, analyse, effets biologiques. *Cah. Nutr. Diet* **1985**, *20*, 373−384.

Davidson, J.; McIntosh, A. D.; Milne, E. Note on the lysinoalanine content of alkali-treated barley. *Anim. Food Sci. Technol*. **1982**, *7*, 217−220.

De Groot, A. P.; Slump, P.; Feron, V. J.; Van Beek, L. Effects of alkali-treated proteins: feeding studies with free and protein-bound lysinoalanine in rats and other animals. *J. Nutr*. **1976**, *106*, 1527−1538.

Dehn-Muler, B.; Muller, B.; Erbersdobler, H. F. Studies on protein damage in UHT milk. *Milchwissenschaft* **1991**, *46*, 431−434.

De Koning, P. J.; van Rooijen, P. J. Aspects of formation of lysinoalanine in milk and milk products. *J. Dairy Res*. **1982**, *49*, 725−736.

Deng, Q. Y.; Barefood, R. R.; Diosady, L. L.; Rubin, L. J.; Tzeng, Y. M. Lysinoalanine concentrations in rapeseed protein meals and isolates. *Can. Inst. Food Sci. Technol. J*. **1990**, *23*, 140−142.

De Rham, O.; van de Rovaart, P.; Bujard, E.; Mottu, F.; Hidalgo, J. Fortification of soy protein with cheese whey protein and the effect of alkaline pH. *Cereal Chem*. **1977**, *54*, 238−245.

De Weck-Gaudard, D.; Liardon, R.; Finot, P. A. Stereomeric composition of urinary lysinoalanine after ingestion of free or protein-bound lysinoalanine in rats. *J. Agric. Food Chem*. **1988**, *36*, 717−721.

Donovan, J. W.; White, T. M. Alkaline hydrolysis of disulfide bonds of ovomucoid and of low molecular weight aliphatic and aromatic disulfides. *Biochemistry* **1971**, *10*, 32−38.

Dosako, S. Changes in rheological and chemical properties of soy protein dope solution through increase in aging time. *Agric. Biol. Chem*. **1979**, *43*, 803, 807, 809−814.

Dreyer, J. J.; van der Walt, W. H. *Laboratory Manual for Data Processing in Assessments of Dietary Protein Quality by an Updated Version of the Nitrogen Metabolism Methods*; National Food Research Institute (CSIR): Pretoria, South Africa.

Dworschak, E. F.; Orsi, A.; Zsigmond, A.; Trezl, L.; Rusznak, I. Factors influencing the formation of lysinoalanine in alkali-treated proteins. *Nahrung* **1981**, *25*, 44−446.

Ellison, M. S.; Lundgren, H. P. Breaking-twist angle and cross-linking in wool fibers under alkaline conditions as a function of temperature. *Text. Res. J*. **1978**, *48*, 692−697.

Erbersdobler, H. F.; Holstein, A. B. Studies on the formation of lysinoalanine in milk products. *Milchwissenschaft* **1980**, *35*, 734−737.

Erbersdobler, H. F.; Holstein, B.; Lainer, E. Simultaneous determination of lysine, lysinoalanine, furosine, and pyridosine in foods and feeds. *Z. Lebensm. Unters. Forsch*. **1979**, *168*, 6−8.

Feairheller, S. H.; Taylor, M. M.; Bailey, D. G. ^{35}S-sulfide incorporation during alkaline treatment of keratin and its relation to lanthionine formation. *Adv. Exp. Med. Biol*. **1977**, *86B*, 177−186.

Feron, V. J.; van Beek, L.; Slump, P.; Beems, R. B. Toxicological aspects of alkali-treated food proteins. In *Biological Aspects of New Protein Foods*; Alder Nissen, J., Ed.; Pergamon: Oxford, U.K., 1978; Vol. 44, pp 139−147.

Finley, J. W.; Friedman, M. New amino acid derivatives formed by alkaline treatment of proteins. In *Protein Crosslinking: Biochemical and Molecular Aspects*; Friedman, M., Ed.; Advances in Experimental Medicine and Biology 86A; Plenum: New York, 1977; pp 123−130.

Finley, J. W.; Snow, J. T.; Johnston, P. H.; Friedman, M. Inhibitory effect of mercaptoamino acids on LAL formation during alkali treatment of proteins. *Adv. Exp. Med. Biol*. **1977**, *86A*, 85−92.

Finley, J. W.; Snow, J. T.; Johnston, P. H.; Friedman, M. Inhibition of lysinoalanine formation in food proteins. *J. Food Sci*. **1978**, *43*, 619−621.

Finot, P. A. Lysinoalanine in food proteins. *Nutr. Abst. Rev. Clin. Nutr*. **1983**, *53*, 67−80.

Finot, P. A.; Bujard, E.; Arnaud, M. Metabolic transit of lysinoalanine (LAL) bound to protein and of free radioactive [^{14}C]-lysinoalanine. *Adv. Exp. Med. Biol*. **1977**, *86A*, 51−71.

Floros, J. D.; Wetzstein, H. Y.; Chinnan, M. S. Chemical (NaOH) peeling as viewed by scanning electron microscope: pimiento peppers as a case study. *J. Food Sci*. **1987**, *52*, 1312−1316.

Freimuth, U.; Notzold, H.; Krause, W. On alkali treatment of proteins. 5. Formation of lysinoalanine in β-casein and β-lactoglobulin. *Nahrung* **1980**, *24*, 351−357.

Friedman, M. Solvent effects in reactions of amino groups in amino acids, peptides, and proteins with α,β-unsaturated compounds. *J. Am. Chem. Soc*. **1967**, *89*, 4709−4713.

Friedman, M. *Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides, and Proteins*; Pergamon Press: Oxford, U.K., 1973.

Friedman, M., Ed. *Protein−Metal Interactions*; Plenum Press: New York, 1974.

Friedman, M. Cross-linked amino acids— stereochemistry and nomenclature. *Adv. Exp. Med. Biol*. **1977a**, *86B*, 1−27.

Friedman, M. Chemical basis for pharmacological and therapeutic actions of penicillamine. *Proc. R. Soc. Med*. **1977b**, *70*, 50−60.

Friedman, M. Inhibition of lysinoalanine synthesis by protein acylation. In *Nutritional Improvement of Food and Feed Proteins*; Friedman, M., Ed.; Advances in Experimental Medicine and Biology 105; Plenum: New York, 1978a; pp 613−648.

Friedman, M. Wheat gluten-alkali reactions. In *Proceedings of the 10th Conference on Wheat Utilization Research*; U.S. Department of Agriculture ARM-W-4: Washington, DC, 1978b; pp 229−253.

Friedman, M. Alkali-induced lysinoalanine formation in structurally different proteins. In *Protein Structure Related to Functionality*; Pour-El, A., Ed.; ACS Symposium Series 92; American Chemical Society: Washington, DC, 1979; pp 225−235.

Friedman, M. Lysinoalanine formation in soybean proteins: kinetics and mechanisms. *ACS Symp. Ser*. **1982**, *No. 206*, 231−273.

Friedman, M. Formation, nutritional value, and safety of D-amino acids. *Adv. Exp. Med. Biol*. **1991**, *199*, 447−480.

Friedman, M. Improvement in the safety of foods by SH-containing amino acids and peptides. *J. Agric. Food Chem*. **1994**, *42*, 3−20.

Friedman, M. Food browning and its prevention. *J. Agric. Food Chem*. **1996a**, *44*, 631−653.

Friedman, M. Nutritional value of proteins from different food sources. *J. Agric. Food Chem*. **1996b**, *44*, 6−29.

Friedman, M. Chemistry, biochemistry, and dietary role of potato polyphenols. *J. Agric. Food Chem*. **1997**, *45*, 1523−1540.

Friedman, M. Chemistry, nutrition, and microbiology of D-amino acids. *J. Agric. Food Chem*. **1999**, submitted for publication.

Friedman, M.; Atsmon, D. Comparison of grain composition and nutritional quality of wild barley (*Hordeum spontaneum*) and a standard cultivar. *J. Agric. Food Chem*. **1988**, *36*, 1167−1172.

Friedman, M.; Boyd, W. A. A nuclear magnetic double resonance study of N-β-bis-(β'-chloroethyl)-phosphonylethyl-DL-phenylalanine. *Adv. Exp. Med. Biol*. **1977**, *86A*, 727−743.

Friedman, M.; Cuq, J. L. Chemistry, analysis, nutritional value, and toxicology of tryptophan. *J. Agric. Food Chem.* **1988**, *36*, 1079−1093.

Friedman, M.; Finot, P. A. Nutritional improvement of bread with lysine and γ-glutamyl-lysine. *J. Agric. Food Chem.* **1990**, *38*, 2011−2020.

Friedman, M.; Gumbmann, M. R. The utilization and safety of isomeric sulfur-containing amino acids in mice. *J. Nutr.* **1984**, *114*, 2301−2310.

Friedman, M.; Gumbmann, M. R. Nutritional improvement of soy flour through inactivation of trypsin inhibitors by sodium sulfite. *J. Food Sci.* **1986**, *51*, 1239−1241.

Friedman, M.; Liardon, R. Racemization kinetics of amino acid residues in alkali-treated food proteins. *J. Agric. Food Chem.* **1985**, *33*, 666−672.

Friedman, M.; Masters, P. M. Kinetics of racemization of amino acid residues in casein. *J. Food Sci.* **1982**, *47*, 760−764.

Friedman, M.; Noma, A. T. Cystine content of wool. *Text. Res. J.* **1970**, *40*, 1073−1078.

Friedman, M.; Noma, A. T. Formation and analysis of phenylethylaminoalanine in food proteins. *J. Agric. Food Chem.* **1986**, *34*, 7−502.

Friedman, M.; Orraca-Tetteh, R. Hair as an index of protein malnutrition. *Adv. Exp. Med. Biol.* **1978**, *105*, 131−154.

Friedman, M.; Pearce, K. N. Copper(II) and cobalt(II) affinities of LL- and LD-lysinoalanine diastereoisomers: implications for food safety and nutrition. *J. Agric. Food Chem.* **1989**, *37*, 123−127.

Friedman, M.; Wall, J. S. Application of a Hammett-Taft relation to kinetics of alkylation of amino groups in amino acid and peptide model compounds with acrylonitrile. *J. Am. Chem. Soc.* **1964**, *86*, 3735−3741.

Friedman, M.; Cavins, J. R.; Wall, J. S. Relative nucleophilic reactivities of amino groups and mercaptide ions with α,β-unsaturated compounds. *J. Am. Chem. Soc.* **1965**, *87*, 3772−3782.

Friedman, M.; Finley, J. W.; Yeh, L. S. Reactions of proteins with dehydroalanine. *Adv. Exp. Med.* **1977**, *86B*, 299−319.

Friedman, M.; Noma, A. T.; Wagner, J. R. Ion-exchange chromatography of sulfur amino acids on a single-column amino acid analyzer. *Anal. Biochem.* **1979**, *98*, 293−304.

Friedman, M.; Zahnley, J. C.; Wagner, J. R. Estimation of the disulfide content of trypsin inhibitors as S-β-(pyridylethyl)-L-cysteine. *Anal. Biochem.* **1980**, *106*, 27−34.

Friedman, M.; Zahnley, J. C.; Masters, P. M. Relationship between *in vitro* digestibility of casein and its content of lysinoalanine and D-amino acids. *J. Food Sci.* **1981**, *46*, 127−131.

Friedman, M.; Diamond, M. J.; Broderick, G. L. Dimethylolurea as a tyrosine reagent and protein protectant against ruminal degradation. *J. Agric. Food Chem.* **1982a**, *30*, 72−77.

Friedman, M.; Gumbmann, M. R.; Savoie, L. The nutritional value of lysinoalanine as a source of lysine. *Nutr. Rep. Int.* **1982b**, *26*, 937−943.

Friedman, M.; Levin, C. E.; Noma, A. T. Factors governing lysinoalanine formation in soybean proteins. *J. Food Sci.* **1984**, *49*, 1282−1288.

Friedman, M.; Grosjean, O. K.; Zahnley, J. C. Carboxypeptidase inhibition by alkali-treated food proteins. *J. Agric. Food Chem.* **1985**, *33*, 208−213.

Friedman, M.; Grosjean, O. K.; Zahnley, J. C. Inactivation of metalloenzymes by food constitutents. *Food Chem. Toxicol.* **1986a**, *24*, 497−502.

Friedman, M.; Grosjean, O. K.; Zahnley, J. C. Inactivation of metalloenzymes by lysinoalanine, phenylethylaminoalanine, alkali-treated food proteins, and sulfur amino acids. In *Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods*; Friedman, M., Ed.; Advances in Experimental Medicine and Biology 199; Plenum: New York, 1986b; pp 531−560.

Friedman, M.; Fitch, T. E.; Levin, C. E.; Yokoyama, W. H. Tomatine and tomato reduce plasma LDL cholesterol and triglycerides in hamsters. *J. Agric. Food Chem.* **1999**, submitted for publication.

Fritsch, R. J.; Klostermeyer, H. Improved method for the determination of lysinoalanine in food. *Z. Lebensm. Unters. Forsch.* **1981a**, *172*, 101−106.

Fritsch, R. J.; Klostermeyer, H. Occurrence of lysinoalanine in milk protein-containing foods. *Z. Lebensm. Unters. Forsch.* **1981b**, *172*, 440−445.

Fritsch, R. J.; Hoffmann, H.; Klostermeyer, H. Formation of lysinoalanine during heat treatment of milk. *Z. Lebensm. Unters. Forsch.* **1983**, *176*, 341−345.

Fuji, T.; Kuboki, Y. Nongelatin components from lime-processed gelatins: presence of lysinoalanine and histidinoalanine. *J. Soc. Photogr. Sci. Jpn.* **1985**, *48*, 359−366.

Fujimaki, M.; Haraguchi, T.; Abe, K.; Homma, S.; Arai, S. Specific conditions that maximize formation of lysinolanine in wheat gluten and fish protein concentrate. *Agric. Biol. Chem.* **1980**, *44*, 1911−1916.

Fujimoto, D. Formation of histidinoalanine cross-links in heated proteins. *Experientia* **1984**, *40*, 832−833.

Fujimoto, D. Histidinoalanine in human urine. *J. Biochem.* **1986**, *100*, 809−811.

Fujimoto, D.; Rougley, P. J. Age variation in histidinoalanine cross-links in proteoglycan subunits from human articular cartilage. *Collagen Relat. Res.* **1984**, *4*, 395−398.

Fujimoto, D.; Yu, S. Y. Evaluation of histidinoalanine in calcified human aortas. *Biochem. Int.* **1984**, *8*, 553−560.

Fujimoto, D.; Hirama, M.; Iwashita, T. Occurrence of lysinoalanine in calcified tissue collagen. *Biochem. Biophys. Res. Commun.* **1981**, *103*, 1378−1383.

Fujimoto, D.; Hirama, M.; Iwashita, T. Histidinoalanine, a new cross-linking amino acid, in calcified tissue collagen. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 1102−1106.

Furniss, D. E.; Hurrell, R. F.; De Weck, D.; Finot, P. A. The effect of lysinoalanine and N-ε-fructose lysine on kidney, liver and urine trace elements in the rat. In *Trace Elements in Man and Animals*; Mills, C. F., Ed.; Commonwealth Agricultural Bureau: Slough, U.K., 1985.

Furniss, D. E.; Vuichoud, J.; Finot, P. A.; Hurrell, R. F. The effect of Maillard reaction products on zinc metabolism in the rat. *Br. J. Nutr.* **1989**, *62*, 739−749.

Geschwind, I. I.; Li, C. H. Effects of alkali-heat treatment on β-melanocyte-stimulating hormone. *Arch. Biochem. Biophys.* **1964**, *106*, 200−206.

Godin, M.; Fillastre, J. P.; Legallicier, B.; Pauti, M. D. Ochratoxin-induced nephrotoxicity in animals and humans. *Sem. Hop.* **1998**, *74*, 800−806; *Biosis* 01326498.

Gorham, S. D. Effect of chemical modifications on the susceptibility of collagen to proteolysis. II. Dehydrothermal cross-linking. *Int. J. Biol. Macromol.* **1992**, *14*, 129−138.

Gould, D. H.; MacGregor, J. T. Biological effects of alkali-treated protein and lysinoalanine: an overview. *Adv. Exp. Med. Biol.* **1977**, *86B*, 29−48.

Gross, E. α,β-Unsaturated amino acids in peptides and proteins. *Adv. Exp. Med. Biol.* **1977**, *86A*, 131−153.

Haagsma, N.; Slump, P. Evaluation of lysinoalanine determinations in food proteins. *Z. Lebensm. Unters. Forsch.* **1978**, *167*, 238−240.

Han, Y.; Parsons, C. M. Protein and amino acid quality of feather meal. *Poult. Sci.* **1991**, *70*, 812−822.

Hansen, J. N. Nisin as a model food preservative. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 69−93.

Haraguchi, T.; Abe, K.; Arai, S.; Homma, S.; Fujimaki, M. Lysinoalanine formation in wheat gluten: a conformational effect. *Agric. Biol. Chem.* **1980**, *44*, 1951−1952.

Hasegawa, K.; Iwata, S. Gas chromatography-mass spectrometry of lysinoalanine and related cross-linked amino acids in alkali-treated food proteins. *Agric. Biol. Chem.* **1982**, *46*, 2513−2520.

Hasegawa, K.; Okamoto, N.; Ozawa, H.; Kitajima, S.; Takado, Y. Limits and sites of lysinoalanine formation in lysozyme, α-lactalbumin and α_{s1}- and β-caseins by alkali treatment. *Agric. Biol. Chem.* **1981a**, *45*, 1645−1651.

Hasegawa, K.; Kitajima, S.; Takado, Y. An examination of intermolecular lysinoalanine formation using dephospho-

rylated casein and succinylated proteins. *Agric. Biol. Chem.* **1981b**, *45*, 2133−2134.

Hasegawa, K.; Mukai, K.; Gotoh, M.; Honjo, S.; Matoba, T. Determination of lysinoalanine content in commercial foods by gas chromatography-selected ion monitoring. *Agric. Biol. Chem.* **1987**, *51*, 2889−2894.

Hayakawa, I.; Katsuta, K. A study of fiber spun from soy protein. *Nippon Shokuhin Kogyo Gakkaishi* **1981**, *28* (7), 347−354.

Hayashi, R. Lysinoalanine as a metal ion chelator. An implication for toxicity. *J. Biol. Chem.* **1982**, *45*, 1430−1431.

Hayashi, R.; Kameda, I. Conditions for lysinoalanine formation during exposure of protein to alkali. *Agric. Biol. Chem.* **1980a**, *44*, 175−181.

Hayashi, R.; Kameda, I. Decreased proteolysis of alkali-treated protein: consequences of racemization in food processing. *J. Food Sci.* **1980b**, *45*, 1430−1431.

Hayasho, Y.; Takahashi, H. Fiber formation from animal protein dispersion. *Nippon Shokuhin Kogyo Gakkaishi* **1990**, *37*, 935−939.

Henle, T.; Walter, A. W.; Klostermeyer, H. Detection and identification of the cross-linking amino acids N-τ- and N-π-(2′-amino-2′-carboxyethyl)-L-histidine (histidinoalanine, HAL) in heated milk products. *Z. Lebensm. Unters. Forsch.* **1993**, *197*, 114−117.

Henle, T.; Walter, A. W.; Klostermeyer, H. Irreversible cross-linking of casein during storage of UHT-treated skim milk. *Heat Treatments and Alternative Methods*; International Dairy Federation: Brussels, Belgium, 1996; pp 290−298.

Henning, P. H.; Steyn, D. G. The response of different portions of the maize plant to NaOH treatment. *S. Afr. J. Anim. Sci.* **1984**, *14*, 142−143.

Horn, M. J.; Jones, B.; Ringel, S. J. Isolation of a new sulfur-containing amino acid (lanthionine) from sodium carbonate-treated wool *J. Biol. Chem.* **1941**, *138*, 141−149.

Hosoda, K.; Ohya, M.; Kohno, T.; Maeda, T.; Endo, S.; Wakamatsu, K. Structure determination of an immunopotentiator peptide, cinnamycin, complexed with lysophosphatidylethanolamine by ^{1}H-NMR. *J. Biochem.* **1996**, 226−230.

Hurrell, R. F.; Carpenter, K. J.; Sinclair, W. J.; Otterburn, M. S.; Asquith, R. S. Mechanisms of heat damage in proteins. 7. The significance of lysine-containing isopeptides and of lanthionine in heated proteins. *Br. J. Nutr.* **1976**, *35*, 383−395.

Isohata, T.; Abe, K.; Homma, S.; Fujimaki, M.; Arai, S. Involvement of unmasked serine residues in formation of lysinoalanine: verification by a study with dephosphorylated α_{s1}-casein and poly-L-serine. *Agric. Biol. Chem.* **1983**, *47*, 2633−2635.

Jack, R. W.; Jung, G. Natural peptides with antimicrobial activity. *Chymia* **1998**, *52*, 48−55.

Jelen, P.; Schmidt, T. Alkaline solubilization of heat-precipitated cheese whey protein. *J. Inst. Can. Sci. Technol. Aliment.* **1976**, *9*, 61−65.

Jenkins, W. L.; Tovar, L. R.; Schwass, D. E.; Liardon, R.; Carpenter, K. J. Nutritional characteristics of alkali-treated zein. *J. Agric. Food Chem.* **1984**, *32*, 1035−1041.

Johnson, D. A. A procedure for the separation and quantitation of tryptophan and amino sugars on the amino acid analyzer. *Anal. Biochem.* **1983**, *130*, 475−480.

Jones, G. P.; Rivett, D. E.; Tucker, D. J. The reaction of biogenic amines with proteins. *J. Sci. Food Agric.* **1981**, *32*, 805−812.

Jones, G. P.; Hooper, P. T.; Rivett, D. E.; Tucker, D. J.; Lambert, G.; Billett, A. Nephrotoxic activity in rats fed diets containing DL-3-(N-phenylethylamino)-alanine. *Aust. J. Biol. Sci.* **1987**, *40*, 115−123.

Jonker, D.; Woutersen, R. A.; van Bladeren, P. J.; Til, H. P.; Feron, V. J. Subacute (4-wk) oral toxicity of a combination of four nephrotoxins in rats: comparison with the toxicity of the individual compounds. *Food Chem. Toxicol.* **1993**, *31*, 125−136.

Jonker, D.; Woutersen, R. A.; Feron, V. J. Toxicity of mixtures of nephrotoxicants with similar or dissimilar modes of action. *Food Chem. Toxicol.* **1996**, *34*, 1075−1082.

Jung, G. Lantibiotics—ribosomally synthesized biologically active polypeptides containing sulfide bridges and α,β-didehydroamino acids. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1051−1068.

Jyothi, V.; Sumathi, S. Effect of alkali treatment on the nutritive value of common bean (*Phaseolus vulgaris*). *Plant Foods Hum. Nutr.* **1995**, *48*, 193−200.

Kaletta, C.; Entian, K. D.; Jung, G. Prepeptide sequence of cinnamycin: the first structural gene of a duramycin-type lantibiotic. *Eur. J. Biochem.* **1991**, *199*, 411−415.

Kanayama, T.; Miyanaga, Y.; Horiuchi, K.; Fujimoto, D. Detection of the cross-linking amino acid, histidinoalanine, in human brown cataractous lens protein. *Exp. Eye Res.* **1987**, *44*, 165−169.

Karayiannis, N. I.; Panopoulos, N. J.; Bjeldanes, L. F.; MacGregor, J. T. Lysinoalanine utilization by *Erwinia chyrsanthemi* and *Escherichia coli*. *Food Cosmet. Toxicol.* **1979a**, *17*, 319−320.

Karayiannis, N. I.; Bjeldanes, L. F.; MacGregor, J. T. Lysinoalanine formation in alkali-treated proteins and model peptides. *Food Cosmet. Toxicol.* **1979b**, *17*, 585−590.

Karayiannis, N. I.; MacGregor, J. T.; Bjeldanes, L. F. Biological effects of alkali-treated soy protein and lactalbumin in the rat and mouse. *Food Cosmet. Toxicol.* **1980**, *18*, 333−346.

Kato, A.; Ogino, K.; Kuramoto, Y.; Kobayashi, K. Degradation of the *o*-glycosidically linked carbohydrate units of ovomucin during egg white thinning. *J. Food Sci.* **1979**, *44*, 1341−1344.

Katsuta, K.; Hayakawa, I. Effects of reducing agents on rheological properties, inhibition of lysinoalanine formation, and spinnability of soy protein fibers. *Agric. Biol. Chem.* **1984**, *48*, 2927−2933.

Kawamura, Y.; Hayashi, R. Lysinoalanine-degrading enzymes of various animal kidneys. *Agric. Biol. Chem.* **1987**, *51*, 2289−2290.

Kearns, J. E.; Maclaren, J. A. Lanthionine cross-links and their effects in solubility tests on wool. *J. Text. Inst.* **1979**, *12*, 534−536

Kikutani, N.; Iguchi, M.; Tomomatsu, T.; Doguchi, M. The determination of lysinoalanine in commercial baby foods. *Ann. Rep. Tokyo Res. Lab. P. H.* **1982**, *33*, 251−254.

Kikutani, N.; Iguchi, M.; Tomomatsu, T.; Doguchi, M. Determination of lysinoalanine in high- protein foods in Japan. *Ann. Rep. Tokyo Res. Lab. P. H.* **1984**, *35*, 241−244.

Kolonkaya, D. Renal toxicity of soybean protein containing lysinoalanine. *Doga: Turk Biyol. Derg.* **1986**, 394−402 (in Turkish).

Kruse, B.; Steinhart, H. Evidence for lysinomethylalanine formation in model systems. *J. Agric. Food Chem.* **1989**, *37*, 304−307.

Kuboki, Y.; Liu, A. Two new in vitro calcification systems. *Connect. Tissue Res.* **1998**, *39*, 189−199.

Kuboki, Y.; Fujisawa, R.; Tsuzaki, M.; Liu, C. F.; Sasaki, S. Presence of lysinoalnaine and histidinoalanine in bovine dentin phosphoprotein. *Calcif. Tissue Int.* **1984**, *36*, 126−128.

Kuboki, Y.; Takita, H.; Fujisawa, R.; Yamaguchi, H.; Yamada, H.; Tazaki, M.; Ohnuma, Y.; Mizuno, M.; Tsuzaki, M. Histidinoalanine-containing phosphprotein in bone. *Calcif. Tissue Int.* **1991**, *48*, 196−203.

Kume, T.; Takehisa, M. Effect of γ-irradiation on lysinoalanine in various feedstuffs and model systems. *J. Agric. Food Chem.* **1984**, *32*, 656−658.

Kyogoku, H. Biochemical studies on the interstitial proteins of human gastric cancer tissue with special reference to cross-linking amino acids of collagen and elastin. *Hokkaido Igaku Zasshi* **1988**, *63*, 841−850.

Langhendries, J. P.; Hurrell, R. F.; Furniss, D. E.; Hirschenhuber, C.; Finot, P. A.; Bernard, A.; Battisti, O.; Bertrand, J. M.; Senterre, J. Maillard reaction products and lysinoalanine: urinary excretion and the effects on kidney function

of preterm infants fed heat-processed milk formula. *J. Pediatr. Gastroentorol. Nutr.* **1992**, *14*, 62−70.

Lawrence, R. A.; Jelen, P. Formation of lysinoalanine in alkaline extracts of chicken protein. *J. Food Prot.* **1982**, *45*, 923−924.

Lee, H. S.; Osuga, D. T.; Nashef, A. S.; Ahmed, A. I.; Whitaker, J. R.; Feeney, R. E. Effects of alkali on glycoproteins. β-Elimination and nucleophilic addition reactions of substituted threonyl residues of antifreeze glycoprotein. *J. Agric. Food Chem.* **1977**, *25*, 1153−1158.

Leegwater, D. C. The nephrotoxicity of lysinoalanine in the rat. *Food Cosmet. Toxicol.* **1978**, *16*, 405.

Leegwater, D. C.; Tas, A. C. Identification of an enzymatic oxidation product of lysinoalanine. *Lebensm. Wiss. -Technol.* **1980**, *14*, 87−91.

Liardon, R.; Friedman, M. Effect of peptide bond cleavage on the racemization of amino acid residues. *J. Agric. Food Chem.* **1987**, *35*, 661−667.

Liardon, R.; Friedman, M.; Philippossian, G. Racemization kinetics of free and protein bound lysinoalanine (LAL) in strong acid media. Isomeric composition of protein bound LAL. *J. Agric. Food Chem.* **1991**, *39*, 531−537.

Liener, I. E. Implications of antinutritional components of soybean foods. *Crit. Rev. Food Sci. Nutr.* **1994a**, *34*, 31−67.

Liener, I. E. Antinutritional factors related to proteins and amino acids. In *Foodborne Disease Handbook*; Hui, Y. H., Rorham, J. R., Murrell, K. D., Cliver, D. O., Eds.; Dekker: New York, 1994b; pp 261−309.

Liener, I. E. Plant lectins: properties, nutritional significance, and function. In *Antinutrients and Phytochemicals in Food*; Shahidi, F., Ed.; ACS Symposium Series 662; American Chemical Society: Washington, DC, 1997; pp 31−43.

Lifsey, B. J., Jr.; Farkas, W. R.; Reyniers, J. P. Interaction of lysinoalanine with the protein synthesizing apparatus. *Chem.-Biol. Interact.* **1988**, *68*, 241−257.

Liu, W.; Hansen, J. N. The antimicrobial effects of a structural variant of subtilin against outgrowing *Bacillus cereus* T spores and vegetative cells occurs by different mechanisms. *Appl. Environ. Microbiol.* **1993**, *59*, 648−651.

Maclaren, J. A. Maximum extraction of wool proteins by thiol-urea solutions. *Text. Res. J.* **1987**, *57*, 87−92.

Maga, J. A. Lysinoalanine in foods. *J. Agric. Food Chem.* **1984**, *32*, 955−964.

Manson, W.; Carolan, T. Formation of lysinoalanine from individual bovine caseins. *J. Dairy Res.* **1980**, *47*, 193−198.

Marsh, M. E. Histidinoalnine, a natural occurring cross-link derived from phsophoserine and histidine residues in mineral-binding phosphoproteins. *Biochemistry* **1986**, *25*, 2392−2396.

Marsilio, V.; Lanza, B.; De Angelis, M. D. Olive cell wall components: physical and biochemical changes during processing. *J. Sci. Food Agric.* **1996**, *70*, 35−43.

Masri, M. S.; Friedman, M. Transformation of dehydroalanine to S-β-(2-pyridylethyl)-L-cysteine side chains. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 321−325.

Masters, P.; M.; Friedman, M. Racemization of amino acids in alkali-treated proteins. *J. Agric. Food Chem.* **1979**, *23*, 507−511.

McGhee, J. E.; Bagley, E. B.; Bothast, R. J. Treating corn with aqua ammonia: effect on meal composition. *Cereal Chem.* **1979**, *56*, 128−129.

Meyer, M.; Klostermeyer, H.; Kleyn, D. H. Reduced formation of lysinoalanine in enzymatically dephosphorylated casein. *Z. Lebensm. Unters. Forsch.* **1981**, *172*, 446−448.

Miller, R.; Spinelli, J.; Babbitt, J. K. Effect of heat and alkali on lysinoalanine formation in fish muscle. *J. Food Sci.* **1983**, *48*, 296−297.

Mine, Y. Effect of dry heat and mild alkaline treatments on functional properties of egg white proteins. *J. Agric. Food Chem.* **1997**, *45*, 2924−2928.

Miro, P.; Puente, J.; Anguera, S.; Angeles-Febrer, M. Ornithinoalanine—synthesis and isolation from wool. *Invest. Inf. Text.* **1968**, *1*, 47−478.

Moharram, Y. G.; Moustafa, A. M.; Osman, H. O. A. Studies on wet dehulling of Egyptian sesame seeds by lye solutions. *Lebensm. Wiss. -Technol.* **1981**, *14*, 137−140.

Moret, S.; Cherubin, S.; Rodriguez-Estrada, M. T.; Lercker, G. Determination of lysinoalanine by high performance liquid chromatography. *J. High Resolut. Chromatogr.* **1994**, *17*, 827−830.

Morris, S.; Walsh, R. C.; Hansen, J. N. Identification and characterization of some bacterial membrane sulfhydryl groups which are targets of bacteriostatic and antibiotic action. *J. Biol. Chem.* **1984**, *259*, 13590−13594.

Moss, H. J.; Miskelly, D. M.; Moss, R. The effect of alkaline conditions on the properties of wheat flour dough and Cantonese-style noodles. *J. Cereal Sci.* **1986**, *4*, 261−268.

Mukai, K.; Shimizu, Y.; Murata, R.; Matoba, T.; Hasegawa, K. Effect of ammonia on lysinoalanine formation in proteins by alkali treatment. *Nihon Nogei Kagakkaishi* **1986**, *60*, 1009−1015.

Mukai, K.; Matoba, T.; Hasegawa, K. Influence of freshness of egg on lysinoalanine formation in boiled egg. *Kaseigaku Kenkyu* **1987**, *34* (1), 86−88.

Murase, M. Effect of glucose on lysinoalanine formation. *Nippon Nogei Kagaku Kaishi* **1980**, *54* (1), 13−19.

Murase, M.; Goto, F. Formation of lysinoalanine residues by alkaline treatment of hen's egg white. *Nippon Shokuhin Kogyo Gakkaishi* **1977**, *24*, 547−552.

Nashef, A. S.; Osuga, D. T.; Lee, H. S.; Ahmed, A. I.; Whitaker, J. R.; Feeney, R. E. Effects of alkali on proteins. Disulfides and their products. *J. Agric. Food Chem.* **1977**, *25*, 245−251.

Negamine, J. Effect of vitamin E- or C-rich diet on longevity and tissue histidinoalanine in senescence accelerated mice. *Hissu Aminosan Kenkyu* **1992**, *138*, 39−48.

Nishino, N.; Uchida, S. Formation of lysinoalanine following alkaline processing of soya bean meal in relation to the degradability of protein in the rumen. *J. Sci. Food Agric.* **1995**, *68*, 59−64.

Nishino, N.; Masaki, Y.; Uchida, S. Changes in nitrogenous compounds and rates of *in situ* N loss of soybean meal treated with NaOH or heat. *J. Agric. Food Chem.* **1996**, *44*, 2667−2671.

Notzold, H.; Winkler, H.; Wiedemann, B.; Ludwig, E. The effect of enzymatic modificaion on lysinoalanine formation in field bean protein isolate and β-casein. *Nahrung* **1984**, *28*, 299−308.

O'Brien, J.; Walker, R. Toxicological effects of dietary Maillard reaction products in the rat. *Food Chem. Toxicol.* **1988**, *26*, 775−783.

O'Donovan, C. J. Recent studies on lysinoalanine in alkali-treated proteins. *Food Cosmet. Toxicol.* **1976**, *14*, 483−489.

Ozimek, G.; Jelen, P.; Ozimek, L.; Sauer, W.; McCurdy, S. M. A comparison of mechanically separated and alkali extracted chicken protein for functional and nutritional properties. *J. Food Sci.* **1986**, *51*, 748−753.

Patchornik, A.; Sokolovsky, M. Chemical interaction between lysine and dehydroalanine in modified bovine pancreatic ribonuclease. *J. Am. Chem. Soc.* **1964**, *86*, 1860−1861.

Pearce, K. N.; Friedman, M. The binding of copper and other metal ions by lysinoalanine and related compounds and its significance for food safety. *J. Agric. Food Chem.* **1988**, *36*, 707−717.

Pearce, K. N.; Karahalios, D.; Friedman, M. Ninhydrin assay for proteolysis in ripening cheese. *J. Food Sci.* **1988**, *52*, 432−435.

Pellegrino, L.; Resmini, P.; DeNoni, I.; Masotti, F. Sensitive determination of lysinoalanine for distinguishing natural from imitation mozarella cheese. *J. Dairy Sci.* **1996**, *79*, 725−734.

Pfaff, G. Lysinoalanine contents of formula diets. *Lancet* **1984**, *1*, 448.

Pfaff, W.; Pfaff, G. Lysinoalanine content of weight reduction diets. *Apotheker J.* **1984**, *5*, 57−59.

Pintauro, S. J.; Philippossian, G.; Finot, P. A.; Lee, T.-C. Lysinoalanine: absence of mutagenic response in the *Sal-*

monella/mammalian-microsome mutagenicity assay. *Food Chem. Toxicol.* **1985**, *23*, 763−765.

Pompei, C.; Rossi, M.; Mare, F. Protein quality in commercial milk-based infant formulas. *J. Food Qual.* **1987**, *10*, 375−391.

Possompes, B.; Berger, J. Effect of severely alkali treated casein on gastrointestinal transit and selected intestinal enzyme activities. In *Nutritional and Toxicological Consequences of Food Processing*; Friedman, M., Ed.; Advances in Experimental Medicine and Biology 199; Plenum: New York, 1991; pp 517−530.

Possompes, B.; Cuq, J. L.; Guenoun, D.; Besancon, P. Use of cysteine as a reducing agent during alkali treatment of proteins. Biochemical and nutritional effects. *Food Chem.* **1983**, *11*, 15−26.

Possompes, B.; Berger, J.; Diaz, B.; Cuq, J. L. Factors affecting the evaluation of the nutritional value of severely alkali-treated casein. *Food Chem.* **1989**, *32*, 189−199.

Provansal, M. M. P.; Cuq, J. A.; Cheftel, J. C. Chemical and nutritional modifications of sunflower proteins due to alkaline processing. Fromation of amino acid cross-links and isomerization of lysine residues. *J. Agric. Food Chem.* **1975**, *23*, 938−943.

Rahil, J.; Pratt, R. F. Mechanism of inhibition of the class C β-lactamase of *Enterobacter cloacae* P99 by phsphonate monoesters. *Biochemistry* **1992**, *31*, 5869−5878.

Raymond, M. L. Studies concerning the deteremination of lysinoalanine in food proteins. *J. Food Sci.* **1980**, *45*, 56−59.

Rehner, G.; Walter, T. H. Effect of Maillard products and lysinoalanine on the bioavailability of iron, copper and zinc. *Z. Ernahrungswiss.* **1991**, *30*, 50−55.

Renner, R.; Jelen, P. Nutritional evaluation of alkali solubilized heated whey protein. *J. Inst. Can. Sci. Technol. Aliment.* **1980**, *13*, 10−13.

Rivett, D. E. The binding of polyamines in wool. *Text. Res. J.* **1980**, *50*, 440−443.

Robbins, K. R.; Baker, D. H.; Finley, J. W. Studies on the utilization of lysinoalanine and lanthionine. *J. Nutr.* **1980**, *110*, 907−915.

Robbins, D. J.; Liener, I. E.; Spangler, W. M.; du Bruyn, D. B. Effects of a raw soy protein diet on the pancreas of the *Chacma* baboon. *Nutr. Rep. Int.* **1988**, *38*, 9−16.

Sahl, H. G.; Jack, R. W.; Bierbaum, G. Biosynthesis and biological activities of lantibiotics with unique post-translational modifications. *Eur. J. Biochem.* **1995**, *230*, 827−853.

Sakamoto, M.; Nakayama, F.; Kajiyama, K. I. GC-MS of lanthionine, lysinoalanine, and S-carboxyethylcysteine. *Adv. Exp. Med. Biol.* **1977**, *86A*, 687−71.

Sanderson, J.; Wall, J. S.; Donaldson, G. L.; Cavins, J. F. Effect of alkaline processing of corn on its amino acids. *Cereal Chem.* **1978**, *55*, 204−213.

Sarwar, G. Amino acid ratings of different forms of infant formulas based on varying degrees of processing. *Adv. Exp. Med. Biol.* **1991**, *199*, 389−401.

Sarwar, G.; Christensen, D. A.; Finlayson, A. J.; Friedman, M.; Hackler, L. R.; Mackenzie, S. L.; Pellett, P. L.; Tkachuk, R. Inter- and intra-laboratory variation in amino acid analysis of food proteins. *J. Food Sci.* **1983**, *48*, 526−531.

Sarwar, G.; L'Abbe, M. R.; Trick, K.; Botting, C. G.; Ma, C. Y. Effects of feeding alkaline-treated proteins on growth and mineral status of rats. In *Impact of Food Processing on Food Safety*; Jackson, L., Morgan, J., Knize, M., Eds.; Plenum: New York, 1999; in press.

Sass, R. L.; Marsh, M. E. N^{τ}- and N^{π}-histidinoalanine: naturally occurring cross-linking amino acids in calcium-binding phosphoproteins. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 304−309.

Sass, R. L.; Marsh, M. E. Histidinoalanine: a naturally occurring cross-linking amino acid. *Methods Enzymol.* **1984**, *106*, 351−355.

Savoie, L. Effect of protein treatment on the enzymatic hydrolysis of lysinoalanine and other amino acids. In *Nutritional and Toxicological Aspects of Food Safety*; Friedman, M., Ed.; Advances in Experimental Medicine and Biology 177; Plenum: New York, 1984; pp 413−422.

Savoie, L.; Parent, G.; Galibois, I. Effects of alkali treatment on *in vitro* digestibility of proteins and the release of amino acids. *J. Sci. Food Agric.* **1991**, *56*, 363−372.

Schartmann, B.; Gattner, H. G.; Danho, W.; Zahn, H. Increased yields of insulin by recycling of non-combined insulin chains. *Z. Physiol. Chem.* **1983**, *364*, 179−186.

Schwass, D. E.; Finley, J. F. Heat and alkaline damage to proteins: racemization and lysinoalanine formation. *J. Agric. Food Chem.* **1984**, *32*, 1377−1382.

Serassi-Kyriakou, K.; Hadjichristidis, N.; Touloupis, C. Lysinoalanine formation in silk after treatment with phosphate salts. *Chim. Chron., New Ser.* **1978**, *7*, 161−164 (in French).

Shetty, J. K.; Kinsella, J. E. Lysinoalanine formation in yeast proteins isolated by alkaline methods. *J. Agric. Food Chem.* **1980**, *28*, 798−800.

Shinbo, H. An enzyme that catalyzes the synthesis of lanthionine in the silkworm, *Bombyx mori*. *Comp. Biochem. Physiol.* **1998**, *91*, 301−308.

Slump, P. Toxicological aspects of alkali treatment of food proteins. In *Biochemical Aspects of New Protein Foods*; Adler-Nissen, Ed.; Pergamon Press: Oxford, U.K., 1978a; pp 139−147.

Slump, P. Lysinoalanine in alkali-treted proteins and factors influencing its biological activity. *Ann. Nutr. Aliment.* **1978b**, *32*, 271−279.

Sly, M. R.; Du Bruyn, D. B.; de Klerk, W. A.; Robbins, D. J.; Liebenberg, N. W.; van der Walt, W. H. Evaluation using baboons, of a commercial cereal diet supplement, designed to prevent/control malnutrition. *Nutr. Rep. Int.* **1980**, *22*, 223−234.

Snow, J. T.; Finley, J. W.; Friedman, M. A kinetic study of the hydrolysis of N-acetyldehydroalanine methyl ester. *Int. J. Pept. Protein Res.* **1975a**, *7*, 461−466.

Snow, J. T.; Finley, J. W.; Friedman, M. Oxidation of sulfhydryl groups to disulfides by sulfoxides. *Biochem. Biophys. Res. Commun.* **1975b**, *64*, 441−447.

Snow, J. T.; Finley, J. W.; Friedman, M. Relative reactivities of sulfhydryl groups with *N*-acetyl dehydroalanine and *N*-acetyl dehydroalanine methyl ester. *Int. J. Pept. Protein Res.* **1976**, *7*, 460−466.

Steenken, I.; Zahn, H. Determining the dehydroalanine and amide contents of wool as a means of estimating damage caused by alkaline treatment. *Text. Res. J.* **1984**, *54*, 429−433.

Steinert, P. M.; Idler, W. E. Postsynthetic modifications of epidermal α-keratin. *Biochemistry* **1979**, *18*, 5664−5669.

Steining, J.; Montag, A. Studies on changes in lysine of food proteins. II. Formation of lysinoalanine. *Z. Lebensm. Unters. Forsch.* **1982**, *175*, 8−12.

Sternberg, M.; Kim, C. Y. Lysinoalanine formation in protein food ingredients. *Adv. Exp. Med.* **1977**, *86B*, 73−84.

Sternberg, M.; Kim, C. Y. Growth response of mice and *Tetrhymena pyriformis* to lysinoalanine- supplemented wheat gluten. *J. Agric. Food Chem.* **1979**, *27*, 1130−1132.

Sternberg, M.; Kim, C. Y.; Plunkett, R. A. Lysinoalanine determination in proteins. *J. Food Sci.* **1975a**, *40*, 1168−1170.

Sternberg, M.; Kim, C. Y.; Schwende, F. J. Lysinoalanine: presence in food and food ingredients. *Science* **1975b**, *190*, 992−994.

Struthers, B. J.; Dahlgren, R. R.; Hopkins, D. T. Biological effects of feeding graded levels of alkali treated soybean protein containing lysinoalanine in Sprague−Dawley and Wistar rats. *J. Nutr.* **1977**, *107*, 1190−1199.

Struthers, B. J.; Hopkins, D. T.; Prescher, E. E.; Dahlgren, R. R. Effect of protein-bound lysinoalanine on fetal and neonatal rats. *J. Nutr.* **1978**, *108*, 954−958.

Struthers, B. J.; Brielmayer, J. R.; Raymond, M. L.; Dahlgren, R. R.; Hopkins, D. T. Excretion and tissue distribution of radioactive lysinoalanine. *J. Nutr.* **1980**, *110*, 2065−2077.

Sugiyama, T.; Fujimoto, D.; Aai, C.; Hasegawa, M. Relationship between the age and histidinoalanine content of human aorta. *Biomed. Res.* **1987**, *8*, 349−351.

Swaisgood, H. E.; Catignani, G. L. Digestibility of modified milk proteins: nutritional implicaitons. *J. Dairy Sci.* **1985**, *68*, 2782−2790.

Takeuchi, H.; Kato, H.; Fujimaki, M. Effects of temperature and alkali on formation of lysinoalanine in wheat and soybean powder. *J. Food Hyg. Soc. Jpn.* **1978**, *19*, 44−49.

Tas, A. C.; Kleipool, R. J. C. The stereoisomers of lysinoalanine. *Lebensm. Wiss. -Technol.* **1979**, *9*, 360−363.

Touloupis, C.; Vassiliadis, A. Lysinoalanine formation in wool after treatments with some phosphate salts. *Adv. Exp. Med. Biol.* **1977**, *86B*, 187−195.

Tucker, D. J.; Jones, G. P.; Rivett, D. E. Formation of β-phenyethylaminoalanine in protein foods in the presence of added amine. *J. Sci. Food Agric.* **1983**, *34*, 1427−1433.

Van Beek, L.; Feron, V. J.; de Groot, A. P. Nutritional effects of alkali-treated soy protein in rats. *J. Nutr.* **1974**, *104*, 1630−1634.

Von Wagenheim, B.; Hanichen, T.; Erbersdobler, H. H. Histopathological investigation of the rat kidneys after feeding heat damaged proteins. *Z. Ernahrungswiss.* **1984**, *32*, 219−229.

Walsh, R. G.; Neshef, A. S.; Feeney, R. E. Intramolecular cross-linking of proteins by formation of lysinoalanine or lanthionine. Modification of disulfides in ovomucoids. *Int. J. Pept. Protein Res.* **1979**, *14*, 290−299.

Walter, A. W.; Henle, T.; Haessner, R.; Klostermeyer, H. Studies on the formation of lysinomethylalanine and histidinomethylalanine in milk products. *Z. Lebensm. Unters. Forsch.* **1994a**, *199*, 243−247.

Walter, A. W.; Henle, T.; Klostermeyer, H. Histidinoalanine, a new cross-link amino acid in milk proteins. *Milchwissenschaft* **1994b**, *45*, 284−286.

Wang, M. F.; Lee, M. I.; Wong, Y. C. Effect of nutritional conditions on the longevity and tissue histidinoalanine concentration in rats and mice. *J. Chin. Nutr. Soc.* **1995**, *20*, 229−238.

Ward, L. S.; Bastian, E. D. Isolation and identification of β-casein A^1-4P and β-casein A^2-4P in commercial caseinates. *J. Agric. Food Chem.* **1998**, *46*, 77−83.

Warthesen, J. J.; Wood-Rethwill, J. Effect of added cysteine and lysine upon lysinoalanine levels in various alkaline treated food proteins. *J. Food Saf.* **1981**, *3*, 27−37.

Watanabe, K.; Klostermeyer, H. Formation of dehydroalanine, lanthionine and lysinoalanine during heat treatment of β-lactoglobulin A. *Z. Lebensm. Unters. Forsch.* **1977**, *164*, 77−79.

Weder, J. K.; Sohns, S. Model studies on the heating of food proteins. Amino acid composition of lysozyme, ribonuclease and insulin after dry heating. *Z. Lebensm. Unters. Forsch.* **1983**, *176*, 421−425.

Whitaker, J. R.; Feeney, R. E. Behavior of O-glycosyl and O-phosphoryl proteins in alkaline solution. *Adv. Exp. Med. Biol.* **1977**, *86B*, 155−175.

Whitaker, J. R.; Feeney, R. E. Chemical and physical modification of proteins by the hydroxide ion. *Crit. Rev. Food Sci. Nutr.* **1983**, *19*, 173−212.

Whiting, A. H. Isolation of lysinoalanine from the protein-polysaccharide complex of cartilage after alkali treatment. *Biochim. Biophys. Acta* **1971**, *243*, 332−336.

Wilkinson, J.; Hewavitharana, A. K. Lysinoalanine determination in sodium caseinate using the LKB Alpha Plus Amino Acid Analyzer. *Milchwissenschaft* **1997**, *52*, 423−427.

Woodard, J. C.; Short, D. D. Renal toxicity of lysinoalanine in rats. *Food Cosmet. Toxicol.* **1977**, *15*, 117−119.

Woodard, J. C.; Short, D. D.; Alvarez, M. R.; Reyniers, J. Biologic effects of lysinoalanine. In *Protein Nutritional Quality of Foods and Feeds*; Friedman, M., Ed.; Dekker: New York, 1975; Part 1, pp 595−616.

Wood-Rethwill, J. C.; Warthesen, J. J. Lysinoalanine determination in proteins using high-pressure liquid chromatography. *J. Food Sci.* **1980**, *45*, 1637−1640.

Young, G. A.; Kendall, S.; Brownjohn, A. M. D-Amino acids in chronic renal failure and the effects of dialysis and urinary losses. *Amino Acids* **1994**, *6*, 283−293.

Zahn, H.; Gattner, H. G. Hair sulfur amino acid analysis. *EXS* (*Experientia Suppl.*) **1997**, *78*, 239−258.

Zahn, H.; Lumper, L. Formation of lanthionine and lysinoalanine cross-linkages during alkaline denaturation of bovine serum albumin. *Z. Physiol. Chem.* **1968**, *349*, 77−84.

Zahn, H.; Wortmann, F. J.; Höcker, H. Chemistry and structure of wool. *Chem. Unserer Zeit* **1997**, *31*, 280−290 (in German).

Zarkadas, C. G.; Zarkadas, G. C.; Karatzas, C. N.; Khalili, A. D.; Nguyen, Q. Rapid method for determining desmosine, isodesmosine, 5-hydroxylysine, tryptophan, lysinoalanine and the amino sugars in proteins and tissues. *J. Chromatogr.* **1986**, *378*, 67−76.

Ziegler, K. L. New cross-links in alkali-treated wool. *J. Biol. Chem.* **1964**, *239*, 2713−2714.

Ziegler, K. Degradation of wool containing lysinoalanine; synthetic peptides as model compounds. *J. Appl. Polym. Sci., Appl. Polym. Symp.* **1971**, *18*, 257−262.

Ziegler, K.; Melchert, I.; Lurken, C. Nδ-(2-amino-2-carboxyethyl)-ornithine, a new amino acid from alkali treated proteins. *Nature* **1967**, *214*, 404−405.

Zimmerman, N.; Freund, S.; Fredenhagen, A.; Jung, G. Solution structure of lantibiotics duramycin B and C. *Eur. J. Biochem.* **1993**, *216*, 419−428.

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