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REVIEWS

Lysinoalanine in Foods

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The formation of lysinoalanine has been primarily associated with the alkaline treatment or heat processing of proteins. The chemistry, food occurrences, nutritional significance, and methods available for the analysis of this phenomenon, relative to the literature, are discussed.

Lysinoalanine (LAL) [N^{ϵ}-(DL-2-amino-2-carboxyethyl)-L-lysine] represents a unique amino acid that apparently has been present in our food supply for centuries, especially in products that undergo alkaline processing and/or heating of a protein-containing food. In addition, Gross et al. (1975) report that LAL is a natural constituent of cinnamycin and duramycin, which are two peptides present in certain species of *Streptomyces*.

However, it was not until the mid 1960s that LAL was actually identified (Patchornik and Sokolovsky, 1964b; Bohak, 1964). Since that time numerous studies have been reported in an attempt to more completely understand its formation mechanism(s), ways of minimizing its formation, and levels present in foods and, most importantly, to identify its specific nutritional and potential toxicological significance relative to food processing and preparation. Several reviews have appeared that discuss certain aspects of the above (Sternberg and Kim, 1977; de Groot et al., 1977; Struthers, 1981), but no review has attempted to provide an inclusive overview of the entire subject.

Thus, the primary objective of this review is to more extensively discuss the literature to date in an effort to summarize our knowledge of LAL in food and to point out areas that need further investigation or clarification.

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Chemistry. On the basis of manuscript receipt date, Patchornik and Sokolovsky (1964b) were the first to actually identify LAL. When they treated S-dinitrophenylated reduced ribonuclease with alkali, followed by acid hydrolysis, a new amino acid, which they assigned the trival name lysalanine, was identified. At that time they proposed that LAL was formed by the reaction of ϵ-amino groups of lysine residues with dehydroalanine residues.

However, Bohak (1964) is usually given credit for first identifying LAL. He reported that during the alkali treatment of ribonuclease lysine was lost with a corresponding new amino acid (LAL) formed. He assigned the trival name lysinoalanine to the compound and demonstrated that it was formed in alkali-treated lysozyme, papain, chymotrypsinogen, bovine plasma alubumin, and phosvitin.

Ziegler (1964) also found LAL to be present in alkali-treated wool. Ziegler et al. (1967) later identified ornithinoalanine as well as LAL in an alkali-treated silk protein fraction.

The roles of cysteine and serine in the formation of dehydroalanine was reported at approximately the same time (Patchornik and Sokolovsky, 1964a; Sokolovsky et al., 1964). The overall reaction was reported to occur in three stages: first, a conversion of cysteine to its *S*-dinitrophenyl derivative or to a tertiary sulfonium salt; second, dehydroalanine formation by a β-elimination reaction under basic conditions; third, either hydrolytic or

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oxidative scission of the dehydroalanine derivative followed by oxidation. Later, Mellet (1968) also demonstrated that seryl residues could be involved in the formation of α-aminoacrylic acid residues, which in turn could combine with lysine to form LAL. Recently, Finley et al. (1982) demonstrated that the degree of cysteine oxidation apparently is a key factor in LAL formation. They found that partial oxidation of cysteine to the disulfide or monoxide forms resulted in significant LAL formations, whereas when oxidation resulted in sulfinic or cysteic acids, no LAL formation was noted.

In certain food systems, the presence of phosphoserine can also be an important contributor to LAL formation via the same pathway as described above, except the β-elimination of phosphorus occurs (de Groot and Slump, 1969; Provansal et al., 1975; Sternberg et al., 1975b; Aymard et al., 1978; Sen et al., 1977; Sternberg and Kim, 1977; Lorient, 1979; de Koning and van Rooijen, 1982). For example, in the case of casein, Manson and Carolan (1980) found significantly higher levels of LAL associated with α_{S0}-casein as compared to α_{S1}-casein. Earlier work by Manson et al. (1977) showed that structurally these two caseins differed only in that α_{S0}-casein had a phosphoseryl residue immediately adjacent to a lysyl residue, which apparently makes interaction easier.

The reaction of reduced bovine serum albumin and reduced whole wheat gluten with acrylontrile resulted in the formation of LAL and N^{ϵ},N^{ϵ}-bis(β-carboxyethyl)-L-lysine (Cavins and Friedman, 1967), thus indicating the possibility that other lysine derivatives of unknown properties can also form, in addition to other amino acid derivatives (Finley and Friedman, 1977). Friedman (1977) has proposed that a total of 14 major cross-linked amino acids may exist, which because of isomeric forms, can result in at least 53 derivatives. Thus, the chemist has a major task ahead of him if he wishes to isolate and identify all such compounds formed in a food system. The names and proposed structures for the 14 major compounds are summarized in Figure 1.

Since LAL contains two asymmetric centers, Tas and Kleipool (1976) postulated that two enantiomeric pairs of diastereoisomers could exist. Thus, they synthesized and isolated LD-, DL-, LL-, and DD-LAL. All compounds were noted to decompose upon heating at approximately 192 °C for the LD and DL forms and 174 °C for the LL- and DD-forms.

The possibility of amino acid racemization has also been extensively investigated. Early work by Levene and Bass (1929) indicated that free amino acids were quite stable to racemization in alkali; however, studies involving proteins have shown that amino acid racemization does occur with alkali treatment (Dakin and Dudley, 1913; Dakin and Dale, 1919; Pickering and Li, 1964; Pollock and Frommhagen, 1968). Recently, Masters and Friedman (1979, 1980) subjected various common 1% protein sources to 0.1 N NaOH at 65 °C for 3 h and measured the amount of racemization for seven amino acids. Interestingly, D-lysine/L-lysine ratios were not measured. In any event, they found that soy protein was the most easily altered, followed by casein, wheat gluten, and lactalbumin. it is thought that the naturally occuring L form can be converted to the D form by abstraction of the proton from an amino acid or amino acid residue, resulting in a negatively charged planar carbanion. Then a proton can be added back to either side of this intermediate, thus ether regenerating the L form or producing the D enantiomer.

In a later study, Freidman and Masters (1982) reported on the extent of amino acid residue racemization in casein

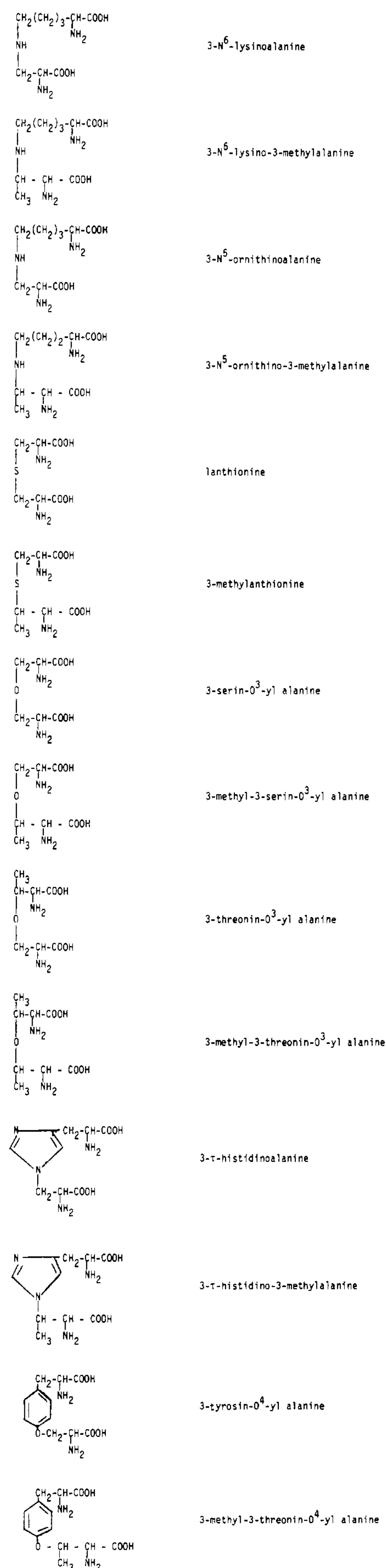


Figure 1. Names and proposed structures of cross-linked amino acids.

that had been exposed to 0.1 N NaOH at 65 °C for up to 24 h. From this study the authors concluded that racemization and LAL formation are independent since, when using acetylated casein, racemization continued whereas no LAL formation was detected.

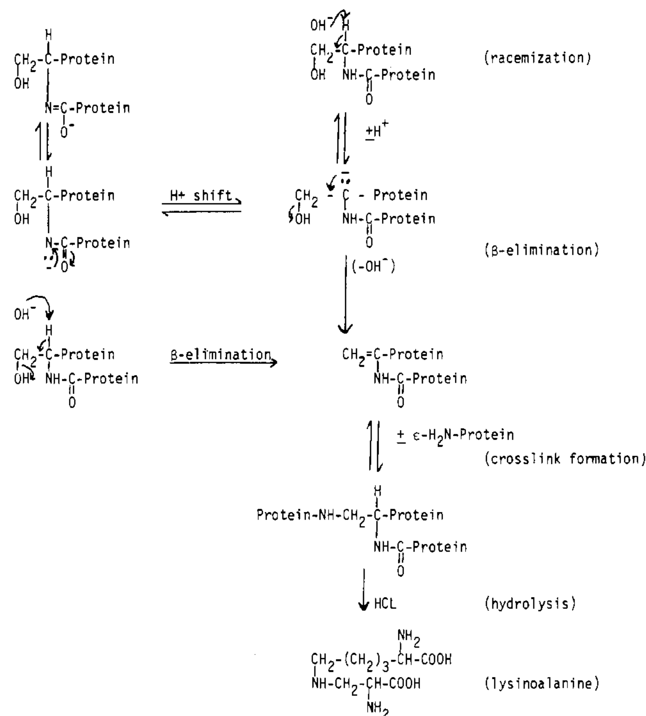
Recently, Liardon and Hurrell (1983) looked at the degree of amino acid racemization in heated and alkali-treated proteins. Significant racemization of aspartic acid, cysteine and serine resulted in bovine plasma albumin and solvent-extracted chicken muscle that had been heated up to 27 h at 121 °C. Whole milk powder that was stored for 9 weeks at 60 or 70 °C or heated in sealed glass bottles for 1 h at 120 °C showed no racemization. However, racemization of aspartic and glutamic acids did result when whole milk powder was heated for 20 min at 230 °C. Also, extensive racemization of all amino acids was noted for casein that had undergone alkaline treatment (1 h at 80 °C in 0.2 or 1 M NaOH).

The extent of amino acid racemization in conventionally processed foods was recently evaluated by Bunjapami et al. (1982). Products evaluated included raw and cooked beef, chicken, soy flour, peanuts, corn, and wheat flour. No major differences were observed, but in all products aspartic acid appeared to be the amino acid most susceptible to racemization. The authors also clearly demonstrated that the isolation process can contribute to the formation of D-amino acids, and thus, the absolute degree of racemization is difficult to quantitate.

Earlier, de Groot and Slump (1969) had subjected various protein sources to severe alkali treatment (pH 12.2 at 40 °C for 4 h) and reported on the formation of LAL in all products tested except coconut meal. Alkali-treated protein sources that did have measurable LAL levels included soy meal, casein, animal protein concentrate, peanut meal, sesame, and brewer's yeast protein. No discussion was provided as to why alkali-treated coconut meal was found not to contain LAL while the other sources did contain LAL. Perhaps another look should be given to coconut meal to verify this observation.

The severe alkaline treatment of other protein sources such as lysozyme, myoglobin, ribonuclease, and insulin (Asquith et al., 1969), cartilage (Whiting, 1971), and keratin (Asquith and Carthew, 1972) has also resulted in LAL formation. Perhaps at this point it should be noted that treatment conditions as severe as those used in the above studies seldom occur in normal food processing operations.

Historically, the formation of LAL was thought to occur by one of three methods: (1) hydrolysis or nucleophilic substitution involving the displacement of sulfur from the amino acid by a hydroxide ion; (2) α-elimination initially involving the abstraction of a proton on a carbon α to a sulfur, followed by heterolytic cleavage of the sulfur–sulfur bond, thus resulting in a mercaptide anion and a thioaldehyde or thioketone, which in turn would decompose into an aldehyde or ketone and hydrogen sulfide; (3) β-elimination involving the abstraction of the acidic proton from a carbon β to a sulfur atom, which was followed by the rearrangement of the formed carbonion into an olefin or unstable persulfide, which upon further decomposition resulted in a mercaptide anion and basic sulfur. The work of Nashef et al. (1977) would indicate that the last proposed mechanism is most likely through the action of the intermediate dehydroalanine. In addition, the reader is referred to Figure 2, which summarizes the proposed mechanism by Masters and Friedman (1980) whereby racemization and LAL formation can occur through a common carbanion intermediate.



From: Masters and Friedman (1980)

Figure 2. Proposed pathway for racemization and lysinoalanine formation.

In working with the formation of LAL in alkali-treated wool, Touloupis and Vassiliadis (1977) found that the type of phosphate used for pH adjustment could influence the levels of LAL formed. Sodium phosphate was found to be the most active compound in promoting LAL formation evaluated. Since numerous types of phosphates are utilized in certain foods, this area deserves further research consideration.

Thus, from this brief discussion it should be apparent that the chemistry associated with LAL formation is rather complex, even when attempts are made to evaluate its formation using model systems. Therefore, its mechanism of formation in foods or food components can be expected to take a good deal of time and effort to resolve.

Food Occurrences. Due to the fact that alkaline exposure to proteins has been shown to result in significant LAL formation (de Groot and Slump, 1969; Provansal et al., 1975; Chu et al., 1976; de Groot et al., 1977; Karayiannis et al., 1979a; Hasegawa and Okamoto, 1980; Hasegawa et al., 1981; Dworschak et al., 1981; Hayashi and Kameda, 1980a,c; Meyer et al., 1981), one would suspect that foods that are traditionally exposed to alkaline conditions during processing or preparation would probably contain LAL. This in fact is the case. For example, the traditional production of tortillas is such that one part of corn is placed in two parts of water containing 1% lime, and the mixture is heated to approximately 80 °C for 20–45 min (Cravioto et al., 1945; Bressani and Scrimshaw, 1958; Bressani et al., 1958; Katz et al., 1974).

Chu et al. (1976) evaluated LAL formation in whole white corn as influenced by type of alkaline treatment. Compounds evaluated included lime, calcium hydroxide, sodium hydroxide, and potassium hydroxide. The corn was heated at 77 °C for 15 or 30 min in the presence of up to 0.273 M base and held overnight. It should be noted that in the original article molar concentrations of up to 4.1 were reportedly used. This value is an error. They reported that LAL formation was alkaline concentration dependent. Also, sodium hydroxide produced the highest

amounts of LAL (1033–1330 ppm), followed by potassium hydroxide (724 ppm), then lime (133 ppm), and finally calcium hydroxide (103 ppm). It is also apparent that the use of sodium or potassium hydroxide resulted in significant amounts of LAL as compared to the use of lime or calcium hydroxide. However, for some unexplained reason, heating time was not beyond 30 min, and to more closely approximate preparation procedures of tortillas, longer times should have been evaluated. Also, lime purity could have an influence that perhaps should be evaluated.

Other major uses of alkali are in the isolation of proteins and to improve their solubility and functionality. For example, raw yeast protein sources usually have the problem of associated high levels of nucleic acids, which have to be decreased to acceptable levels before human consumption. Traditionally, the easiest way to accomplish this as well as improve protein extraction from the yeast cell is through an alkaline extraction step (Hedenskog and Ebbinghaus, 1972; Hedenskog and Mogren, 1973; Lindblom, 1974; Viikari and Linko, 1977; Vananuvat and Kinsella, 1975a,b; Cunningham et al., 1975; Zee and Simard, 1975; Shetty and Kinsella, 1980; Achor et al., 1981). Shetty and Kinsella (1980) concluded that with yeast protein, LAL formation was greater (3.59 g/16 g of nitrogen) using high-alkali, low-temperature conditions (pH 12.5 at 65 °C) than at low-alkali, high-temperature (0.49 g/16 g of nitrogen) conditions (pH 10.5 at 85 °C). The first set of conditions produced approximately 7 times more LAL than the second. Achor et al. (1981) also noted that increased yeast cell concentration, time, temperature, and pH all promoted formation of greater quantities of LAL.

Alkaline extraction has also been utilized to make fish protein concentrate more soluble (Carpenter et al., 1952; Tannenbaum et al., 1970; Hermansson et al., 1971; Spinelli et al., 1971), but I am not aware of any specific studies relative to LAL formation and content in this type of product. However, Raymond (1980) did report that canned sardines contained 120 μg of LAL/g of sample while canned tuna was found to have no measurable level of LAL. However, it was not reported if the sardines were packed plain or with condiments, which could perhaps account for the difference found in the two fish-based products. Also, can sizes were not reported, and if of different sizes, the degree of heating during canning could be a factor.

Spun soy protein, which usually is manufactures from an alkali-extracted soy isolate (Thulin and Kuramoto, 1967; Rosenfield and Hartman, 1974) also is exposed to an additional alkali step in its manufacture (Hamdy, 1974). Recently, Katsuta et al. (1982) evaluated the influence of soy protein concentration on LAL formation during the spinning of soy. Over a protein concentration range of approximately 10–20%, LAL levels increased from 0.1 to 1.1 mg/g of protein respectively, and thus they concluded that in this system LAL concentration is protein concentration dependent. However, Freidman (1982) has reported that LAL formation is not soy protein concentration dependent. It should be noted, however, that in the first study 1% NaOH was used for 10 min at protein concentrations of 10–20%, whereas in the latter study low protein levels were used (0.5–5%) with 0.1 NN NaOH for 3 h. Thus, both conclusions may be valid for the systems evaluated. Katsuta et al. (1982) also found that as would be expected, LAL content increased with increasing time and temperature of alkali treatment. After 2 h at 20 °C LAL concentration gradually increased to 0.5 mg/g of protein, while at 80 °C the corresponding value was 2.0 mg. Increasing sodium hydroxide concentration from 0.2% to 1.0% also demonstrated a near linear increase from 0.13 to 0.29 mg of LAL/g of protein.

Hayakawa and Katsuta (1981) also investigated the variables that influenced spun soy fiber functionality and LAL formation. Products made with 18–25% protein and 1.0–2.0% sodium hydroxide were optimum but also had undisclosed levels of LAL. The addition of L-cysteine hydrochloride at levels up to 10% reduced LAL amounts by one-third to one-half without compromising product functionality.

The alkaline extraction of residual meat from beef (Jelen and Edwardson, 1979) and poultry (Lawrence and Jelen, 1982) bones has been proposed. In the case of poultry bones, Lawrence and Jelen (1982) used alkaline conditions of pH 9.2, 10.0, 10.7, and 11.5 for 1, 4, and 16 h at 22, 35, and 50 °C for meat extraction. No LAL was detected for any variables treated for 1 h, but at 50 °C and a 16-h incubation LAL started to appear at pH 9.2 (100 ppm) and increased to a high of 4450 ppm when the pH was at 11.5. After 4 h, LAL was 175 ppm at 50 °C and a pH of 10.7, and when the pH was 11.5, LAL was detected at 100 ppm at 22 °C, while at 50 °C the level had increased to 690 ppm. However, since optimum extraction conditions were found to be pH 10.5 for 30 min at room temperature, the authors concluded that their process would not produce LAL.

Alkaline extraction procedures have also been proposed for the production of protein concentrates from wheat-processing byproducts (Saunders et al., 1975; Edwards et al., 1980), but LAL levels in these products have not been reported. Alkaline processing of sunflower protein (Provansal et al., 1975) and peanut protein (Bensabat et al., 1958) have been shown to result in LAL formation.

Other miscellaneous alkaline processing conditions that are available to the food industry include the lye peeling of various fruits and vegetables (Hart et al., 1970, 1974; Graham et al., 1969), the processing of quick cooking beans (Rockland and Metzler, 1967), and the proposed detoxification of peanut meal containing aflatoxin (Sreenivasamurthy et al., 1967). However, none of these procedures have taken into account potential LAL and thus should be investigated further.

Numerous investigators have utilized various model protein systems to demonstrate LAL formation, with soy and milk protein being the most often evaluated. Along these lines de Groot and Slump (1969) presented data indicating that certain proteins are perhaps more susceptible to LAL formation than others. For example, alkali-treated soybean meal and soy isolate were found to contain 0.57 and 0.80 g of LAL/16 g of N, respectively, whereas alkali-treated casein contained 1.15 g of LAL/16 g of N. de Rham et al. (1977) also found higher LAL in an alkali-treated milk-based product (whey protein) as compared to soy protein that has been treated in a similar manner (90 s at pH 12.5 and 65 °C). The respective values were 0.5 and 0.2 g of LAL/100 g of protein.

Dworschak et al. (1981) recently reported that milk, soy, and egg proteins are more susceptible to LAL formation than wheat protein. It should be noted that their procedure called for heating a 2.5% protein solution at 100 °C 1 h at pH 13.2. Wheat gluten had an LAL level of 0.25 mol/100 g of protein, while egg and soy proteins had approximately 0.75 and casein had a value of 1.25. They concluded that the quantity of LAL formed was proportional to the lysine molar ratio multiplied with the sum of cystine and serine molar ratios.

Karayiannis et al. (1979a) also demonstrated that under most conditions of alkaline pH, time, and temperature that milk proteins produced more LAL than soy protein. They

concluded that under moderate alkaline conditions cystine was the primary precursor of LAL, but as the alkalinity increased serine amounts became an important precursor. Thus, if one were to look at the amino acid composition of various proteins, one may be able to predict how prone that protein may be to LAL formation. Interesting data were also presented by Karayiannis et al. (1979a) demonstrating that if alkali conditions are severe enough and the time of exposure long, the LAL level can decrease. For example, at 90 °C and pH 12 the protein sources evaluated (lactalbumin, casein, soy) reached maximum LAL levels (70, 100, and 135 μmol/g of dry protein, respectively) after 2 h but then decreased during the following 4 h to values of 25, 50, and 110. However, when the reaction was performed at 25 instead of 90 °C, there was a gradual rise in LAL over the 6-h period with all three protein sources having between 20 and 40 μmol of LAL/g of dry protein. It should be noted that even under severe conditions of temperature and pH, the LAL values reported by Karayiannis et al. (1979a,b) appear to be low relative to other published data. The major reason for this discrepancy is the fact that they conducted their research using steam and not a direct aqueous method as is more commonly done. In the steaming method moisture conditions are not optimum for LAL formation. Hasegawa and Okamoto (1980) also looked at longtime heating at 80 °C using 0.1 N NaOH on LAL formation in soy protein but found little difference between 1 and 5 h. This is not surprising in view of data generated by de Rham et al. (1977) as discussed below.

Recently, Weder and Sohns (1983) evaluated the role of dry heating on protein composition and LAL formation. Lysozyme (LSH) and ribonuclease (RNase) were heated at either 160 or 180 °C for up to 24 h. At 160 °C it took 4 h of heating to obtain measurable levels of LAL (0.10 and 0.21 mol of LAL/mol of unheated protein) for LSH and RNase, respectively. At 180 °C LAL appeared after 4 h of heating (0.10 mol) in LSH and after 2 h (0.22 mol) for RNase. Independent of heating temperature, approximately 0.22 mol of LAL was present in LSH after 24 h of heating, while with RNase approximately 0.44 mol was present after 24 h. Thus, it would appear that these proteins are relatively stable to LAL formation when dry heated.

de Rham et al. (1977) used a pH of 12.5 in their studies but used shorter heating times and temperatures. For example, after soy protein was heated at pH 12.5 for 1.5 min at 55 °C, 1000 ppm of LAL was found, whereas, when the temperature was 65 °C, 1800 ppm of LAL resulted. In the case of whey protein, 4500 and 5400 ppm of LAL were found at 55 and 65 °C, respectively.

In looking at specific proteins, Hasegawa et al. (1981) concluded that in the case of the protein α-lactalbumin, the amount of LAL formed was related to the number of lysine residues with a cystine disulfide bond in the adjacent position rather than the total amounts of each amino acid. With α- and β-caseins they concluded that the total number of phosphoserine residues, regardless of their proximity to lysine residues, was the primary factor controlling final levels of LAL in these proteins. Other researchers have shown that milk protein and various milk protein fractions can form LAL under relatively mild processing conditions (Lorient, 1979; Manson and Carolan, 1980; Friedman et al., 1981).

Recently, Sovoie and Parent (1983) fractionated rapeseed protein into the soluble, 12S, and 2S fractions and soy protein into soluble, 11S, 7S, and 2S fractions. A 5% aqueous concentration of each fraction was treated with 0.2 N sodium hydroxide and incubated at 60 °C for up to 8 h. Changes in amino acid composition and LAL were then measured. In general, they found that rapeseed protein was more susceptible to LAL formation than soy protein. For both products there was no fraction where the LAL level was vastly different from other fractions; however, no statistical data were presented. In the case of soy protein LAL primarily resulted due to losses in lysine and cystine, while with rapeseed protein the amino acids lysine, cystine, and threonine were primarily involved.

At this point perhaps the controversial role of pH in LAL formation should be further discussed. Some authors believe that alkaline conditions are required for the formation of LAL (de Groot et al., 1976, 1977; Karayiannis et al., 1979a; Friedman et al., 1981), while others (Sternberg et al., 1975a,b; Sternberg and Kim, 1977; Raymond, 1980; Aymard et al., 1978) have found measurable LAL levels in foods or food ingredients that apparently have not been exposed to alkaline conditions. For example, Sternberg and Kim (1977) have reported that LAL formation in casein can occur even at a pH as low as 5. However, it is generally agreed that alkaline processing will significantly increase LAL levels.

Thus, it would appear that another factor to consider is the degree of heat processing a product receives. For example, Asquith and Otterburn (1977) demonstrated that the formation of LAL during the dry heating of proteins is temperature dependent.

For the information of the reader, common foods and food ingredients that have been reported to contain LAL are summarized in Table I. As can be seen, a wide variety of foods have measurable levels. It is also interesting to observe the foods that had no detectable levels of LAL and try to postulate as to why.

It is also suggested that other foods be evaluated and that special attention be given to investigate methods of preparation. For example, from Table I it would appear that with chicken, microwave cooking resulted in higher level of LAL than other forms of preparation. Would this also be true for other foods, and if so, why?

Inhibition of LAL Formation. From the brief discussion above it should be fairly apparent that a wide variety of foods are prone to LAL formation, and because of certain nutritional and toxicological concerns, whether justified or not, which will be discussed in detail in later sections of this review, one has a choice of eliminating such foods from the diet, which because of the diversity of food items becomes almost next to impractical, or to find means to minimize or eliminate the formation of LAL in foods of concern. Much research has been performed in this latter area and is reviewed below.

Several different techniques have been investigated as a means of reducing LAL formation. For example, Dworschak et al. (1981) reported that depending upon the cystine content of the protein being evaluated, the addition of ascorbic acid could either impede LAL formation or enhance it. This could be of unique significance in foods that are fortified with ascorbic acid. They found that if the protein was low in cysteine, ascorbic acid inhibited LAL formation, but if the protein was high in cystine, ascorbic acid increased LAL formation. This was due to the fact that ascorbic acid–alkali-treated sulfide groups in protein are transformed into sulfhydrols, which take part in β-elimination more actively than disulfide groups. However, it should be noted that this ascorbic acid effect was observed in model systems that contained equal weight amounts of ascorbic acid and protein. Further model

Table I. LAL Content of Common Foods and Food Ingredients

product[c]	LAL, μg/g of protein
frankfurter, as purchased[a,b]	not detected
frankfurter, boiled[a]	50
frankfurter, fried[a]	50
frankfurter, charcoal broiled[a]	150
frankfurter, oven baked[a]	170
chicken thigh, raw[a]	not detected
chicken thigh, retorted[a]	100
chicken thigh, oven baked[a]	110
chicken thigh, charcoal broiled[a]	150
chicken thigh, retorted in gravy[a]	170
chicken thigh, cooked in microwave oven[a]	200
sirloin steak, frying pan scrapings[a]	130
egg white, fresh[a]	not detected
egg white, boiled 3 min[a]	140
egg white, boiled 10 min[a]	270
egg white, boiled 30 min[a,b]	0–370
egg yolk, boiled 30 min[b]	not detected
egg white, pan fried 10 min at 150 °C[a]	350
egg white, pan fried 30 min at 150 °C[a]	1100
egg, spray dried whole[b]	not detected
egg, strained egg yolks (infant)[b]	1540
egg white solids, dried (different manufacturers)[a]	160–1820
corn chips[a]	390
potato chips[b]	not detected
pretzels[a,b]	220–500
hominy[a,b]	0–560
tortillas[a]	200
taco shells[a]	170
masa harina[a]	480
milk, infant formula[a,b]	0–640
milk, evaporated[a,b]	200–860
milk, skim, evaporated[a]	520
milk, condensed (different manufacturers)	360–540
milk, dry[b]	not detected
casein, from nonpasteurized whole milk[b]	not detected
casein, commercial[b]	70
sodium caseinate (different manufacturers)[a,b]	310–6900
calcium caseinate[a]	370–1000
acid casein[a]	70–190
Parmesan cheese[b]	not detected
cheddar cheese[b]	not detected
imitation cheese[b]	1070
spaghetti[b]	not detected
egg noodles[b]	not detected
white bread[b]	not detected
ginger cookies[b]	not detected
soy sauce[b]	not detected
instant chocolate mix[b]	not detected
gelatin[b]	not detected
peanut butter[b]	not detected
Tomatoes, canned[b]	not detected
pears, canned[b]	not detected
corn cereal[b]	not detected
wheat cereal[b]	not detected
rice cereal[b]	not detected
toasted oat cereal[b]	160
puffed rice cereal[b]	1000
hydrolyzed vegetable protein (different manufacturers)[a]	40–500
whipping agent (different manufacturers)[a]	6500–50 000
soy protein isolate (different manufacturers)[a]	0–370
yeast extract[a]	120
bologna[b]	not detected
thuringer[b]	not detected
bratwurst[b]	not detected
beef jerky[b]	not detected
Vienna sausage[b]	not detected
canned tuna[b]	not detected
canned sardines[b]	270
infant strained beef[b]	160
tomato paste[b]	not detected
catsup[b]	not detected

[a] Sternberg et al. (1975b). [b] Raymond (1980). [c] Note: Where both references are cited for a product, the lower value is from Raymond (1980) and the higher is that of Sternberg et al. (1975b).

system studies using lower ascorbic acid levels, which would be more realistic for most foods, is suggested.

Dworschak et al. (1981) also demonstrated that the addition of 15 mg of glucose/50 mg of protein reduced LAL formation by 50%. However, the addition of this amount of glucose under alkaline conditions resulted in significant amounts of Maillard-type browning in the systems, which from a practical and perhaps toxicological standpoint is not desirable (Finot et al., 1977). Likewise, Murase (1980) reported that glucose limited LAL formation, the extent of which was directly dependent upon the ratio of glucose to protein.

From a purely experimental standpoint, Dworschak et al. (1981) reported that through the use of certain heavy metals, such as copper sulfate, LAL production was dramatically reduced. In their experiment 1 mg of copper ion reduced LAL formation by 50%. However, the toxic effects of copper sulfate would limit its use in foods. Also, hydrogen peroxide was shown to be effective, but the level required to be significant from a practical standpoint would almost certainly eliminate its use. Also, from an experimental standpoint, Meyer et al. (1981) have shown that if casein is enzymatically dephosphorylated, it is less subject to LAL formation.

Several researchers have shown that the addition of various sulfur compounds in the form of bisulfite or bisulfide can reduce LAL formation (Finley and Kohler, 1979; Friedman, 1978). Compounds of this type are thought to function by at least two different mechanisms. First, by direct competition they can trap dehydroalanine residues. Second, the additives can cleave protein disulfide bonds, forming free SH groups, which in turn can combine with dehydroalanine residues (Friedman, 1978). However, it should be noted that the addition of these compounds can dramatically alter protein structure, thus raising questions as to the nutritional quality of the resulting protein.

Various groups have demonstrated that the addition of mercaptoamino acids can also be effective in lowering LAL formation (Finley et al., 1977, 1978; Sternberg and Kim, 1977). Specific compounds that have been reported to be effective include cysteine, reduced glutathione, and propionylmercaproglycine. However, levels of up to 3% addition are required.

Protein acylation has also been proposed as a means of minimizing LAL formation (Freidman, 1978). Acetic and succinic anhydrides were utilized to chemically modify samples of wheat gluten, soy protein, and lactalbumin. When 10% acetylated gluten or 1% succinylated gluten were exposed to alkaline conditions, no measurable levels of LAL were detected. In the case of soy protein acylation was not as effective as with wheat gluten. However again it should be noted that the procedure results in the chemical modification of protein, which could have nutritional and/or toxicological consequences.

However, Finley and Kohler (1979) have shown that where possible, minor changes in manufacturing technique can influence LAL formation. They reported that apparently oxygen is required for the formation of high levels of LAL since bubbling air through a reaction mixture enhanced LAL levels, while bubbling nitrogen or not mixing the experimental solution resulted in lower LAL levels. The use of nitrogen to minimize LAL formation has also been proposed by Steinig and Montag (1982). This concept should perhaps be further investigated using other model systems.

Thus, it would appear that in the laboratory chemical techniques have been demonstrated that can significantly

lower LAL levels in alkaline-treated proteins. However, most procedures result in significant alteration to protein structure or result in the inclusion of potentially harmful or unproven compounds. Therefore, it would appear that any practical application from any of these techniques is in the distant future. However, the use of less severe processing techniques relative to time and temperature, allowing for adequate processing from a health standpoint, perhaps would be a means to at least lower existing LAL levels in some foods.

Methods of Analysis. This portion of the review will be relatively short for two primary reasons. First, not a wide variety of diverse methods have been developed specifically for the analysis of LAL, and second, a relatively recent review has appeared in this area (Haagsma and Slump, 1978).

Basically, all analyses start with a fairly standard form of protein acid hydrolysis. From there traditional forms of chromatographic separation and identification techniques are applied. By far the most common method has been via separation using ion-exchange chromatography followed by colorimetric detection with ninhydrin similar to the procedures as described by Robson et al. (1967), Slump (1977), and Raymond (1980). This latter method, after initial digestion, takes approximately 40 min to perform.

The combination of high-voltage electrophoresis and paper chromatography has been utilized (Asquith et al., 1969), but operation time was approximately 20 h, thus limiting the usefulness of this technique.

Forms of thin-layer chromatography (TLC) have also been used (Woods and Wang, 1967; Sternberg et al., 1975a), and although the lower level of sensitivity is rather low (3 ng), it requires a long separation time (Haagsma and Gortemaker, 1979), and when compared to ion-exchange techniques, large standard deviations (Haagsma and Slump, 1978) make the widespread use of the technique somewhat limited. It should be noted that separation time has now been shortened to approximately 4 h (Aymard et al., 1978). Also, with some products, problems with interference due to galactosamine can make the TLC method rather limiting. However, galactosamine interference can also be a problem with the more traditional ion-exchange procedure (Raymond, 1980).

Gas-liquid chromatography has been also utilized (Sakamoto et al., 1975, 1977; Hasegawa and Okamoto, 1980; Hasegawa and Iwate, 1982). All employ the use of trifluoroacetylbutyl esters and a means to volatilize the hydrolysate mixture before analysis.

Recently the technique of high-pressure liquid chromatography has been shown to function in the detection of LAL (Wood-Rethwill and Warthesen, 1980). Basically, the dansyl derivative of LAL is formed and measured. The technique has a reported coefficient of variation of 5.4% and is as rapid as the common ion-exchange technique.

It should be noted that all of the above techniques begin with a composite hydrolysate, which in turn may result in incomplete separations and/or erroneous identification of LAL or closely related species. For these reasons it is imperative that a LAL standard be utilized to verify identification. If not readily available, the synthetic procedure as outlined by Finley and Snow (1977) is suggested.

Biological Effects. The fact that the processing of proteins in general may in some cases have a net negative effect on their biologial value has been demonstrated by numerous early researchers as summarized by Greaves et al. (1938). Alkali-treated casein or fish also was reported to have toxic effects on rats (Ramasarma et al., 1949) and chickens (Carpenter and Duckworth, 1950).

Concern was expressed but no data were provided to the use of alkali-treated barley for animal feeding (Davidson et al., 1982). They did find that when ground barley was treated with 35 g of NaOH/kg, 1.16 g of LAL was produced/kg of barley.

Hayashi and Kameda (1980b) have proposed that in the case of alkali-treated proteins at least part of their decreased biological value can result due to the fact that racemized amino acid residues cannot be used as efficiently as substrate binding sites for proteases, thereby resulting in decreased proteolysis.

However, it was not until several reports that began to appear in the mid to late 1960s that the potential detrimental role of LAL on health was considered. One of the key publications in this area was that of Newberne and Young (1966), who demonstrated that diets containing α protein, which is a soy protein modified by alkaline processing to provide some unique functional properties for industrial uses, resulted in rats that had obvious microscopic lesions in the inner cortex of the kidney along with noncalcified, mucoid structures in the urinary bladder.

This observation was followed by a series of studies reported by Woodward and his group (Woodward and Alvarez, 1967; Woodward, 1969, 1971a,b; Reyniers et al., 1974; Woodward and Short, 1973, 1977) that confirmed the formation of renal lesions in rats fed alkali-treated soy protein diets that were characterized by nephrocalcinosis and cytomegalic changes in the straight portion of the proximal tubule. They also reported that the female rat was more prone to this former problem than males. It should be noted that the original report by Newberne and Young (1966) concerning the presence of mucoid structures in the bladder has not been confirmed by Woodward's group and apparently does not result from LAL toxicity.

de Groot and Slump (1969) also observed nephrocalcinosis in female rats when they were fed alkali-treated soy, milk, and peanut proteins but reported that the problem could be prevented with additional dietary calcium. However, the Woodward group believe that this observation is due to the feeding of semipurified diets and is not caused by LAL.

When alkali-treated spun soy isolate was evaluated in rat diets at levels up to 20% for 90 days, no nephrocytomegalia was observed, but nephrocalcinosis did appear in female rats (van Beek et al., 1974), which they demonstrated was due to the high dietary level of available phosphorus naturally inherent in the diet.

A major study by de Groot et al. (1976) provided more information to the puzzle by demonstrating that nephrocytomegalia apparently only occurred in rats when free LAL was included in the diet. In this case levels of 100 ppm of free LAL induced significant renal changes. When LAL was fed in the bound form associated with protein, no renal changes were noted. However, if bound LAL was subjected to acid hydrolysis and then fed, nephrotoxic properties again appeared. This then should lead to the following questions: How much free LAL is there in certain foods or is it all present in the bound form; also, if an alkali-treated protein source is used as an ingredient in a food system that is exposed to acidic conditions during further processing or storage, does free LAL form? From a purely inquisitive standpoint these two questions should be answered. However, one would have to devise an analytical procedure that would specifically measure free LAL, if it does indeed exist to any degree in the free form.

However, the above proposed analysis may be only academic since in the above study de Groot et al. (1976)

reported that when up to 1000 ppm of synthesized LAL was fed to mice, hamsters, rabbits, quail, dogs, or monkeys, no renal changes were noted, thus suggesting a species specificity. This concept was endorsed by O'Donovan (1976) and observed by Leegwater (1978) and Finot et al. (1977).

Apparently, some researchers feel that different strains of rats react differently to alkali-treated soy protein. For example, Struthers et al. (1977) reported that the Sprague-Dawley strain was more susceptible to renal cell cytomegaly than the Wistar strain, whereas the latter strain was more prone to renal calcification. It is interesting to note that a later report by Struthers et al. (1978a) demonstrated that LAL-produced nephrocytomegaly in rats apparently is at least partially reversible if LAL is removed from the diet. Rats were fed for 8 weeks a diet containing 3000 ppm of protein-bound LAL and then fed a normal diet for another 8 weeks. This resulted in a 60% decrease in the extent of cytomegalia. However, Karayiannis et al. (1979b) reported no quantitative or qualitative difference between the renal responses of the same two rat strains fed up to 2630 ppm of LAL bound in alkali-treated soy protein. Therefore, they concluded that perhaps certain lines within a particular strain of rats may also vary in their sensitivity of LAL. They also concluded that bound LAL did not induce nephrocytomegaly in mice, whereas apparently free LAL does (Feron et al., 1978; Sternberg and Kim, 1979). Thus, from these reports it would appear that renal cytomegaly induced by free LAL is not exclusive to rats but can occur in other species as well. Robbins et al. (1980) also noted nephrocytomegaly in rats fed free LAL, but its presence or absence in chicks fed the same diet was not noted.

In addition, Tas and Kleipool (1976) demonstrated that the stereoisomeric form of LAL present is a significant factor in the degree of renal cytomegaly observed. They concluded that LD-LAL is approximately 10–30 more active than LL- and DD-LAL, respectively, while the DL form is less active than the LD form and the DD form is less active than DL-LAL. In a preliminary rat feeding study, in which no details are given, Tas and Kleipool (1976) reported that 100–300 ppm of the LD-LAL isomer induced nephrocytomegalic changes, while no such changes were noted with LL-LAL. Thus, not only is the level of free LAL apparently important but also the isomeric form that is predominant appears to be important.

Feron et al. (1978) reported that perhaps another factor to consider is the length of time rats are on diets containing LAL. With an alkali-treated casein diet they found no significant signs of cytomegaly after feeding for up to 26 weeks; however, after 52 weeks cytomegaly was observed.

Gould and MacGregor (1977) have also suggested that other nutritional factors beside the level and type of LAL present may significantly influence whether nephrocytomegaly is or is not observed. These include the presence or absence of LAL-containing oligopeptides, methionine level, overall diethro amino acid source, carbohydrate source, and salt concentration. Thus, one can appreciate the amount of work and number of experiments that would be required to verify the significance of these variables.

They also suggested that the biological activity of LAL bound to protein may vary significantly among protein sources dependent upon the nature of the LAL cross-link, which, in turn, may be influenced by the concentration of protein undergoing reaction. In addition, they noted that the role of intestional flora type and amount on bound and/or free LAL may be also critical.

A key question relative to the potential harmful effect of LAL in either free or bound form that has received some research attention is the question of the metabolic fate of these compounds. de Groot and Slump (1969) and de Groot et al. (1976) reported that when rats were fed various alkali-treated proteins, 38–65% of the ingested LAL was recovered in the feces, with up to 6%, dependent upon protein source, being found in the urine. The major question is what happened to the rest? Finot et al. (1977) concluded that a high proportion of free LAL can be oxidized by gut bacteria to carbon dioxide.

Other data generated by Finot et al. (1977) would indicate that LAL can be actively metabolized via numerous pathways. For example, radioactively labeled LAL, when given intravenously, appeared in the lumen of the cecum and intestines of rats. In addition, they detected more than 10 LAL metabolites in the urine with at least 5 of these compounds appearing as acetylated LAL derivatives. They found similar metabolites in the urine of mice and hamsters.

An in vitro study by Engelsma et al. (1979) on LL-LAL and LD-LAL utilizing L-amino acid oxidase at pH 7.6 demonstrated that the primary digestion product of LD-LAL was the lactam 3-(2-piperidonyl)alanine, which upon hydrolysis yielded 2-amino-4-azanonanedioic acid.

Struthers et al. (1980) have also reported on the metabolic fate of LAL in rats. A control group of male rats were fed a LAL-free diet for 4 weeks and an experimental group fed a diet containing 3000 ppm of LAL. Both groups were dosed by stomach tube with radioactively labeled LAL. Total radioactive label excreted in 72 h in the feces and urine was approximately 58% for the controls and 63% for the experimentals. Another 5% of the label was detected in expired carbon dioxide in each group. Apparently a small amount (0.5%) of [^{14}C]LAL did accumulate in the kidneys of rats not previously fed LAL. In both groups lysine was found to be the major accumulated metabolite, thus indicating at least some ability of the rat to metabolize LAL. Specific organ data were given but no organ except the kidney appeared to be active in accumulation. For some unexplained reason, female rats were not included in this study.

Earlier, Struthers et al. (1978b) reported on the teratological effects of feeding female rats up to 3000 ppm of LAL. They found no significant differences in birth weight, mortality, live births per litter, or number of pups per litter as influenced by LAL level in the diet. However, it is not entirely clear as to how long the rats were fed the LAL-containing diets. In one experiment it was stated that the experimental diets were initiated at pregnancy. Up to three liters were evaluated. It was noted by the authors that the pups resulting from dams on 2000 and 3000 ppm of LAL had significantly decreased weight gains, probably due to reduced milk production in these groups. Also, no LAL was found in any of the milks.

Recently Hayashi (1982) has provided some in vivo insight as to the possible toxicological mode of LAL. Its role as a metal chelator was demonstrated by using yeast alcohol dehydrogenase and carboxypeptidases A and B.

The toxicity of LAL to humans is a difficult factor to resolve; however, since in other species free LAL has been shown to be significantly more toxic than its protein-bound form, the ability of the human gut to proteolytically release LAL is still unanswered (Vachon et al., 1982).

The question of the nutritional bioavailability of LAL has been investigated. For example, Robbins et al. (1980) reported that LAL is 37% available as a lysine source to chicks and is not available at all in the rat, although

Struthers et al. (1980) reported that labeled LAL was partially available to the rat. With mice, Friedman et al. (1982) found LAL to be 3.8% as effective as lysine. Karayiannis et al. (1979c) reported that LAL could be used as the lysine source for certain lysine-requiring microorganisms, namely, *Erwinia chrysanthemi* and *Escherichia coli*. Sternberg and Kim (1979) also reported that the bacteria *Tetrahymena pyriformis* and certain mutant strains of *E. coli*, *Bacillus subtilis*, and *Aspergillus niger* can utilize LAL instead of lysine. Thus, it would appear that LAL metabolism and utilization is very species dependent, which in turn would dictate further in vivo studies.

There is no question that bound LAL and perhaps free LAL exist in our food supply. However, it has not been unequivocally determined relative to humans if the levels present in a normal diet can significantly influence human health. It should also be noted that this review dealt specifically with LAL and the human nutritional and health significance of other protein-based cross-linked compounds have received only minor investigation.

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Rice Product Volatiles: A Review

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The flavor composition of rice and its products has been extensively investigated during the last two decades. This review summarizes the compounds that have been identified in the volatiles of wild rice, scented rice, raw rice, cooked rice, rice bran, Soong-Neung, and rice cake.

In many parts of the world rice is the major component of the diet. To most Americans rice is a rather bland-tasting food, but in many cultures minor chemical changes can make rice and its products unacceptable for human consumption. As a result, flavor chemists for the last two decades have been attempting to better understand rice flavor chemistry. A brief summary of this work was published earlier (Maga, 1978a), but significant advances have been made since then, and since most of the research in this area has appeared in foreign journals that may not be readily available to the American researcher, this current review was compiled. Specific topics covered in this review include the volatiles identified in wild rice, scented rice, raw rice, cooked rice, rice bran, Soong-Neung, and rice cake.

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Wild Rice. North American wild rice (*Zizania aquatica*) is unique in that the grain is harvested in a moist state and permitted to undergo a biochemical and micro-

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