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Lysinoalanine: Production, Significance and Control in Preparation and Use of Soya and Other Food Proteins[1]

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ABSTRACT

Formation of lysinoalanine (LAL) in proteins in response to alkali treatment is a well-known phenomenon. The quantity of LAL formed depends on temperature, the time of exposure to alkali, the type of protein, the concentration of protein and alkali in some instances, and probably the type of cation in the alkaline solution. Higher temperatures, longer exposure times, and higher pH's generally result in more LAL formation. The addition of mercaptoethanol or cysteine to an alkaline protein solution decreases LAL formation markedly; lanthionine is apparently a major product formed when cysteine is added to an alkaline protein solution. Some LAL is likely to be formed in any protein-containing product that is subjected to alkaline treatment, and has been shown to be formed in some protein products under extreme heat conditions. Proper control of temperature and pH in processing can reduce or eliminate LAL formation. LAL has not been shown to present a toxicological hazard to any species other than the rat. Its presence in large quantities in any protein indicates destruction of cysteine and lysine; the nutritional inferiority of severely alkali- or heat-treated proteins due to LAL formation, amino acid degradation and isomerization, Maillard product formation, and so on is well documented. The small quantities of dietary LAL in food products currently on the market seem to represent no health hazard; the reduced nutritional quality of protein products that contain relatively high levels of LAL should be considered when these products are major sources of dietary protein.

Lysinoalanine (LAL) is formed by the dehydration of either cystine or serine to dehydroalanine, followed by reaction of the epsilon-amino group of lysine with the double bond of dehydroalanine (1). Its formation in proteins exposed to alkali treatment is a well-known phenomenon (2, 3). The quantity of LAL formed is dependent on several factors, including temperature, time of exposure to alkali, type of protein, concentration of alkali and type of cation in the solution.

[1] All values of LAL have been converted to ppm for purposes of comparison in this review. The alkali concentrations in another paper were likewise converted, from moles/kg to molarity (14). Some of the LAL values were estimated from graphs and are only approximate (7).

EFFECTS OF TIME AND TEMPERATURE

DeRham (4) followed cysteine/cystine (cys) destruction by alkali at pH 12.5 at periods of 25–80 min at several temperatures. At 25 C little cys loss was observed. At 75 C, 40% of the cys in soya protein was destroyed within 45 sec, and up to 65% within 15 min. Losses of cys in whey protein were 15 and 50% under the same conditions. In soya protein exposed to pH 12.5 for 1.5 min, 1000 ppm LAL was formed at 55 C, and 1800 ppm at 65 C. Whey protein (which has a much higher cys content than soy) was subjected to similar treatment, and 4500 and 5400 ppm LAL were formed at 55 C and 65 C respectively.

Using 0.1 N NaOH at 80 C, Hasegawa and Okamoto (5) reported no apparent major difference in the quantity of LAL formed at 1 and 5 hr in a soybean protein preparation, but found 34% more LAL after a 16 hr alkali treatment than after 1 or 5 hr. When 0.2 N NaOH was used, a 15% increase in LAL was found in 5 hr compared to that found in 1 hr. The lack of increase from 1 to 5 hr in .1 N NaOH, and the small magnitude of the increase in 0.2 N NaOH is not surprising. DeRham's results indicate that the majority of easily formed LAL would be present long before the 1 hr measurement was made.

Provansal et al. (6) observed a different effect of extended alkali exposure on LAL concentration. Using 0.2 N NaOH at 80 C, and a sunflower protein isolate solution, 11,275 ppm LAL was measured after 1 hr. After 5 hr, LAL was present at 8200 ppm, and after 16 hr, at only 6150 ppm. The loss of LAL with time in strong alkaline solutions has also been observed in different proteins by other authors (7,8,9).

EFFECT OF pH AND ALKALI CONCENTRATION

As would be expected, LAL formation is usually increased by increasing pH or increasing alkali concentration. Provansal et al. (6) extracted sunflower protein with 0.05 M NaOH for 30 min, and found 4264 ppm LAL in the protein

isolate. Further alkali exposure for 1 hr at 55C resulted in 6150 ppm LAL at 0.05 M NaOH, 7175 ppm LAL at 0.1 M NaOH, and 6150 ppm at 0.2 M NaOH. Hayashi and Kameda (7) treated soya protein isolate (4 hr, 40 C) at pH's ranging from 7-12. They observed no LAL formation at pH 7 or 8. At pH 9, approximately 960 ppm LAL was formed, and at pH 10, approximately 9600 ppm. Only 8000 ppm LAL was found at pH 12. Casein treated at 60 C for 1 hr at pH 10 contained 625 ppm LAL; at pH 11, 2200 ppm, and at pH 12, 11,875 ppm LAL (9). Karayiannis et al. (8) autoclaved fifteen solutions of casein and lactalbumin for 15 or 120 min at 120 C. This method of heating produced only small amounts of LAL and little or no increase in LAL with autoclaving times, but LAL was found at approximately 260 ppm in casein and 130 ppm in lactalbumin at pH 8. This increased slightly at pH 10 to around 400 ppm and 150 ppm in casein and lactalbumin respectively, and to 670 and 300 ppm at pH 12. Sternberg et al. (10) have reported LAL formation at neutral and even acidic pH values under severe heating conditions in some foods. Their results, showing formation at acid pH's, have not been duplicated by other investigators, but several other studies do report the presence of LAL in commercial food products that have not undergone any alkali treatment (11,12,13).

EFFECT OF SULFHYDRYL REAGENTS

The presence of cysteine, n-acetyl cysteine, α-mercaptopropionylglycine, reduced glutathione (14) or mercaptoethanol (5) in alkaline solutions of treated proteins markedly inhibited LAL formation. Other sulfhydryl reagents were less effective.

EFFECT OF VARIOUS CATIONS AND ANIONS

Chu et al. (15) compared lime, $Ca(OH)_2$, NaOH, and KOH on LAL formation in whole white corn heated at 62.4 C for 15 or 30 min and allowed to stand overnight. Corn treated with 0.273 M lime for 15 or 30 min contained less than 140 ppm LAL, and $Ca(OH)_2$ treatment (30 min 0.273 M) produced only 103 ppm LAL. Treatment with NaOH, on the other hand, produced much higher LAL levels (1033-1339 ppm). No 30 min treatment using KOH was reported, but a 15 min treatment produced 724 ppm LAL, more than 5 times as much as was formed using $Ca(OH)_2$ under similar conditions. These results with whole corn are quite different from results obtained with isolated proteins at similar alkali concentrations.

Creamer and Matheson (9) compared the effects of several multivalent cations on a sample of rennet casein that had been dissolved in NaOH at pH 10 and had an initial LAL concentration of 1010 ppm. They added various metal chlorides to this solution at 0.0125 M concentrations. All of the metal chlorides used enhanced LAL formation. $MgCl_2$ produced the least increase in LAL (1320 ppm) and $AlCl_3$ the greatest (8060 ppm), compared with 500-1200 ppm when no metal chloride was added. The 0.0125 M $CaCl_2$ produced almost the same LAL concentration as did 0.225 M NaCl. This contrasts with results obtained by Chu et al. (15) on whole corn, and may be due to the difference in availability of the proteins to the cations. Exposure of RNAse-A to pH 8-10 in 0.1 M borate buffer resulted in no LAL production, although measurable LAL was formed in this protein at pH 10 in NaOH (7). Apparently, exposure of whole grain to alkali is less detrimental in terms of LAL formation than exposure of isolated protein, and the type of alkali is a significant factor.

TYPE OF PROTEIN

It is evident from the data presented above that the quantity of LAL formed under a given set of conditions depends on the particular protein. Hayashi and Kameda (7) compared the quantity of LAL formed in several proteins incubated in 0.2 M NaOH for 4 hr at 40C. Results show that despite the presence of appropriate precursors, the quantity of LAL formed was dependent on the specific protein. Their evidence, and that of other authors (1,2,4,8,11) indicates that LAL formation is usually an intramolecular, rather than intermolecular, reaction, although there is one report of LAL formation in alkaline solution containing polylysine and either polyserine or glutathione (8).

The presence of LAL in commercial protein food products varies with type, lot and source. Creamer and Matheson (9) reported 0-6800 ppm LAL in various caseins and sodium and calcium caseinates from different manufacturers. Sanderson et al. (16) detected no LAL in commercial hominy, 810 ppm in protein of tortillas, and 200 ppm in commercial masa. A sample of masa analyzed in our laboratory did not contain LAL. Several publications (10,11,12, 17) contain extensive lists of products that have been examined for the presence of LAL; these will not be reviewed here.

It is evident from data reviewed here that LAL can be formed in most proteins if subjected to the proper conditions. Soya protein can, if conditions are controlled properly, be extracted without detectable LAL being formed. Data from numerous investigators demonstrates several facts concerning LAL-caused nephrocytomegaly. Free LAL is 15-25 times more potent in causing nephrocytomegaly than protein-bound LAL, but is not likely to be found in foods. The minimum cytomegalic effect level of protein-bound LAL has been reported to be 1370-3000 ppm (17,18) The minimum cytomegalic effect level of free LAL, on the other hand, is around 100 ppm (19). The rat is the only species that has been shown to experience nephrocytomegaly at these levels (19). Nutritional effects due to alkali treatment of protein have been observed in several species in proteins containing noncytomegalic LAL levels (20,21). DeRham's data indicate that cysteine was destroyed during alkali treatments of proteins that were too mild for LAL to be formed (4).

LAL in products now on the market does not appear to present a toxicological problem at levels currently consumed. Reduced nutritional quality of foods that undergo severe alkaline treatment should be considered in food applications when foods that are major constituents of the diet are involved.

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Flatulence Caused by Soya and Its Control through Processing

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ABSTRACT

Elimination of flatulence is a challenging practical problem associated with the comsumption of soybeans as well as other food legumes and other selected foodstuffs. The problem is compounded by the variability in susceptibility among individuals. Research has established that the oligosaccharides—verbascose, stachyose, and raffinose—are the major cause of soybean flatulence. They escape digestion and are fermented by intestinal microflora to form excessive amounts of carbon dioxide and hydrogen. Hot water treatment, aqueous alcohol extraction, and isoelectric protein precipitation processes have been adapted to produce flatus-free products commercially. At the household level, soaking combined with germination appears to be a practical means of producing soybean sprouts having low flatus activity. Food legumes, which include some oilseeds, peas, and beans, as well as selected vegetables, contain enough of the oligosaccharides—verbascose, stachyose, raffinose—to be a major cause of flatulence in humans and animals. In the absence of alpha-galactosidases in the mammalian intestinal mucosa, these oligosaccharides escape digestion and are not absorbed. As a consequence, the active microflora in the ileum, colon, and fecal matter of the large intestine ferment them to form excessive levels of rectal gas, primarily carbon dioxide and hydrogen. In some instances, undigested starch and other carbohydrates contribute to the flatulent effect of diets. With 70% of the world's population being lactase-deficient (hypolactasia), susceptibility to flatulence would be more widespread with diets containing both food legumes and milk products.

Use of food additives, antibiotics, and phenolic compounds to inhibit flatulence is not a practical approach. However, soya processing technology used to manufacture protein concentrates and isolates can be adapted to produce flatus-free products from other food legumes. Hot water treatment, aqueous alcohol extraction, or isoelectric protein precipitation insolubilizes most of the protein and removes the oligosaccharides. Tempeh and tofu are two other soya products that exhibit little or no flatus activity. Soaking, fermentation, enzymatic hydrolysis, and germination can also be used to eliminate oligosaccharides. Tests with humans and rats indicate that a combination of such processes can be used to reduce flatus activity. The beneficial effects of germination on flatulence, often conflicting and contradictory, have been attributed to failure to control conditions that ensure removal of most of the oligosaccharides. Whether the high-molecular-weight soybean polysaccharides (dietary fiber), which normally do not cause flatulence, can be modified during germination to become substrates for flatus production by the intestinal microflora is not known. Such an effect could compensate for the loss of stachyose and raffinose.

INTRODUCTION

Food legumes, which include some oilseeds, peas and beans, are important sources of protein and calories for a large segment of the world's population. Elimination of flatulence associated with the consumption of such foods is a challenging scientific problem and is one of the research priorities recommended by the Protein Advisory Group of the United Nations, now referred to as the Protein Calorie Advisory Group (1). Requests for information attest to the continuing concern of many individuals for simple and practical solutions to the problem of gas production. Although there are many causes for the formation of gastrointestinal gas, consumption of certain foods accounts for most of the nonspecific gastrointestinal symptoms associated with flatulence. Abdominal pain, nausea, cramps, diarrhea, increased peristalsis, borborygmus and social discomfort may accompany the ejection of rectal gas. Several reports, after eliminating intestinal malabsorption problems associated with disease, indicate that intestinal microflora interact with certain carbohydrates in flatulent foods to produce gas, primarily carbon dioxide and hydrogen, with lesser amounts of methane. Raffinose, stachyose and verbascose cause flatulence in man and animals (2-4). Lactose is a major factor contributing to flatulence in a person with lactase deficiency.

In this report, the emphasis will be on soybean flatulence and on processing technology that can be used to prepare flatulence-free soya products. The role of the intestinal microflora and interactions between soybeans and other foods also will be described.

Oligosaccharides—Structure

Soybeans contain high levels of stachyose and raffinose and trace amounts of verbascose, whereas most other food legumes contain verbascose in the greatest amount. Many other foods also contain these oligosaccharides, which are related by having one or more α-D-galactopyranosyl moieties in their structure where the α-galactose units are bound to the glucose moiety of sucrose (glucose-fructose). Lactose, the disaccharide associated with flatulence in lactase-deficient individuals, is glucose-4-β-galactoside.