EFFECT OF ALKALINE PROCESSING OF CORN ON ITS AMINO ACIDS

J. SANDERSON, J. S. WALL, G. L. DONALDSON, and J. F. CAVINS, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture,[1] Peoria, IL 61604

Cereal Chem. 55(2): 204-213

ABSTRACT

Alkaline treatment of corn to prepare tortilla flour (masa) or hominy results in losses of the amino acids arginine and cystine. Laboratory-prepared masa and hominy and commercial masa and tortillas contained the amino acid lysinoalanine. The masa, tortillas, and hominy also contained lanthionine and ornithine, amino acids derived from alkaline breakdown of cystine and arginine. A new chromatographic procedure separates tryptophan, lysinoalanine, ornithine, and lysine on a single column of the amino acid analyzer. The analyzer permits study of major alkaline degradation products of amino acids of food proteins in conjunction with a column that separates lanthionine.

American Indians long used cooking corn in alkaline solutions as a means of rendering the grain more suitable for food products (1). In Mexico and Central America, corn is heated in lime water, which causes it to soften and swell; the washed product is ground to masa that is suitable for preparing tortillas. In an earlier era in the United States, hominy was prepared by cooking corn in water containing wood ashes. Today, not only are tortillas and related products prepared in the home in many areas but also commercial products, dried masa flour, tortillas, corn chips, and canned hominy are available in the food market to maintain traditional demands and to supply the growing desire for tasty, novel food products (2).

De Groot and Slump (3) showed that the alkaline processing of several food proteins decreases the quality of the protein and causes loss of amino acids and formation of an amino acid, lysinoalanine (N^{ϵ}-[DL-2-amino-2-carboxyethyl]-L-lysine). Lysinoalanine is formed by condensation of lysine with dehydroalanine, an alkaline degradation product of cystine and serine (4). Woodard and Short (5) observed a toxic reaction on kidneys of rats that were fed semipurified diets containing alkali-treated soybean protein; they considered the lysinoalanine to be the causative agent. While de Groot *et al.* (6) found that 0.2% free lysinoalanine in the diets induced renal abnormalities in rats, 0.2% lysinoalanine present in alkaline-treated soy protein did not. They concluded that protein-bound lysinoalanine is poorly digested and absorbed. Furthermore, they report no renal defects in mice, dogs, or monkeys that were fed diets adequate in protein and containing free or protein-bound lysinoalanine. Thus, according to Gould and MacGregor (7), who recently reviewed this topic, many questions still exist concerning the toxicity of lysinoalanine in proteins to various species.

Bressani *et al.* (8) have used masa preparations in nutritional studies with children and have not reported any toxic symptoms in diets supplemented to compensate for the amino acid deficiencies of corn protein. In another study, however, Bressani and Scrimshaw (9) found significant changes in some amino acids during conversion of corn to tortillas.

[1]Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

0009-0352/78/000025$03.00/0

We investigated in detail the changes in amino acid content of corn during cooking in alkali to form masa and hominy and analyzed the amino acids in commercial tortillas and hominy preparations. In addition to demonstrating lysinoalanine formation as Sternberg *et al.* (10) recently reported, we found that lanthionine and ornithine, amino acids not common to proteins, formed during alkaline cooking of corn.

MATERIALS AND METHODS

Preparation of Masa

Tortilla flour, or masa, was prepared from DeKalb SX-70 hybrid corn that had been dried after harvest to 15% moisture at moderate temperatures (50° C) to ensure high retention of biologically available lysine. The procedure for masa preparation was based on that of Bressani and Scrimshaw (9). Generally, 250 g of corn was heated in 480 ml of water containing 4.0 g of calcium hydroxide at 95° C for 90 min, with occasional stirring. After cooling to room temperature, the cooking solution was discarded and the swollen grain washed several times with 500 ml of water. The wet corn was ground in a coffee mill, and the resulting masa was dried by lyophilization. It was reground to 40 mesh in a Wiley mill.

High-lysine *opaque-2* corn was also converted into masa and changes in its amino acid composition determined, since its higher level of lysine and other essential amino acids (compared with that of normal corn) might cause it to be more sensitive to alkaline treatment. The *opaque-2* corn was obtained from PAG Hybrid Seed Co., Aurora, IL. Processing of this corn into masa was similar to that for the normal corn.

Preparation of Hominy

Preparation of hominy in the laboratory followed the procedure of the Continental Can Company (11), scaled down to laboratory proportions. To investigate the effects of bisulfite bleaching of the hominy on lysinoalanine formation, three batches were prepared. Initially, 200 g of DeKalb hybrid corn in 1,000-ml Erlenmeyer flasks were heated and stirred in 400 ml of water containing 5.4 g of sodium hydroxide at 100° C for 50 min. After cooling to room temperature, contents of the flasks were washed several times with distilled water and hulls and tip caps were removed manually. The samples were next boiled in water for 15 min. After cooling, the corn was again rinsed with cold water and the process was repeated. The hominy was then allowed to soak in water overnight.

For the bleaching step, 0, 0.9, or 3.6 g of sodium metabisulfite was added to one of the three hominy preparations that was then boiled in 300 ml of water for 30 min. The preparation was cooled to room temperature and the cooking solution discarded. The corn was then boiled in 300 ml of water and rinsed to remove the bisulfite. Finally, the wet hominy was lyophilized to dryness and ground to 40 mesh in a Wiley mill.

Commercial Products

In addition, commercially available products of alkaline-treated corn were analyzed for amino acids. Masa harina (Quaker Oats Co.), frozen tortillas, and canned yellow and white hominy were obtained from food market shelves. The

masa and tortillas were reground to 40 mesh. The hominy kernels were lyophilized and ground.

Analytic Procedures

Nitrogen was determined by a semimicro Kjeldahl procedure and protein calculated by multiplying per cent of nitrogen by 6.25.

Samples containing 1 mg of nitrogen were hydrolyzed for amino acid analysis by refluxing in constant-boiling HCl (2 ml/1-mg sample) for 24 hr. Five milligrams of nitrogen samples of meals were hydrolyzed to permit quantitative determination of lysinoalanine, lanthionine, and ornithine, which occur in small amounts. After removal of acid under vacuum and addition of internal standards, both concentrations of hydrolysates were diluted to 10 ml with pH 2.2 citrate buffer.

Most amino acids were quantitatively determined in duplicate hydrolysates with a Beckman Model 121 Automatic Amino Acid Analyzer using a standard two-column procedure (12). The results were integrated on an Infotronics Model CRS-210 Integrator and calculated by computer according to a program similar to that of Cavins and Friedman (13).

Amino acids that are not generally present in proteins, including *l*-cysteic acid and methionine sulfone (Pierce Chemical Co.), *l*- and *meso*-lanthionine (Calbiochem), and ornithine monohydrochloride (Mann Research), were incorporated into a standard mixture (Pierce Chemical Co.) to calibrate the chromatographic analyses. Purified lysinoalanine used as a standard was a gift from Dr. James Woodard, University of Florida, Gainesville. Norleucine and α-amino-β-guanidinopropionic acid (AGPA) were added as internal standards to the amino acid mixture and to hydrolysates.

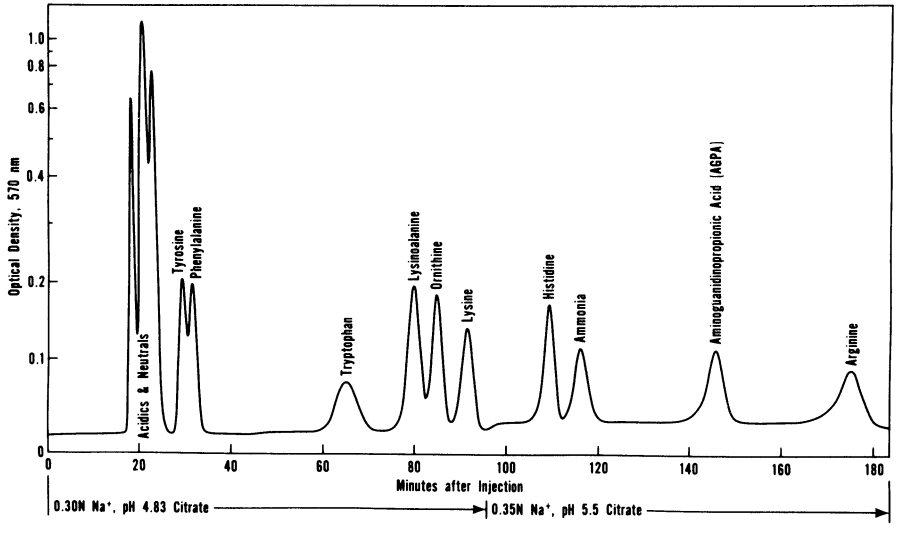

Fig. 1. Separation of mixture of basic amino acids (0.25 μM each) on 20 × 0.9 cm column of Durrum DC-1A ion exchange resin, at 55°C, 70 ml/hr buffer flow rate.

Cystine and cysteine were determined as cysteic acid produced by performic acid oxidation of samples of the corn and alkali-treated products by the method of Moore (14). Methionine was also determined in hydrolysates of the oxidized samples as methionine sulfone (14).

In initial experiments, lysinoalanine was separated from any remaining tryptophan in hydrolysates and determined on a modified short ion exchange column in the amino acid analyzer according to the method of Hugli and Moore (15). To separate and determine ornithine and lysine, the basic column for separation of amino acids in physiologic fluids described by the manufacturer was used at first. It was then demonstrated that these four amino acids could be conveniently separated on a single 20-cm column of Durrum DC-1A resin operated at 55° C and 70 ml per hour flow rate. Use of Pierce pH 5.28, 0.35*N* sodium citrate buffer diluted to 0.30*N* and adjusted to pH 4.83 for the first 70 min of operation eluted tryptophan, lysinoalanine, ornithine, and lysine, in that order. Just after lysine, a shift to pH 5.5 buffer eluted the remaining basic amino acids (Fig. 1). Total running time of the column is 180 min.

The *l* and *meso* diastereomers of lanthionine were resolved between proline and glycine on the standard 56-cm column used for separating acidic and neutral amino acids by lowering the pH of the first buffer from 3.25 to 3.05 as shown in Fig. 2. The procedure is similar to that of Inglis and Nicholls (16). Both peaks were combined in the calculations as lanthionine.

RESULTS

Accurate determination of lysinoalanine in small amounts in acid protein hydrolysates requires that it be separated from any traces of residual tryptophan, because tryptophan and lysinoalanine elute together on the standard basic column. Also, ornithine and lysine elute together on this standard short column

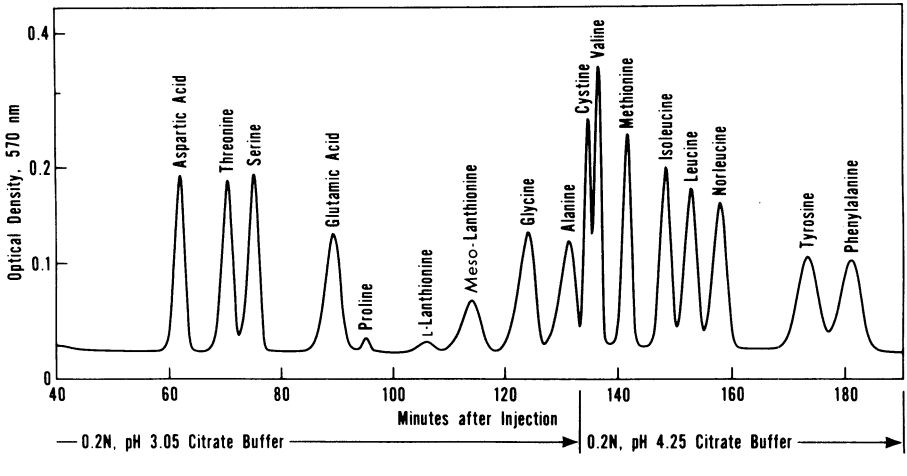

Fig. 2. Separation of mixture of acidic and neutral amino acids (0.25 μM each) on 56×0.9 cm column of Beckman AA-15 ion exchange resin, at 55°C, 70 ml/hr buffer flow rate.

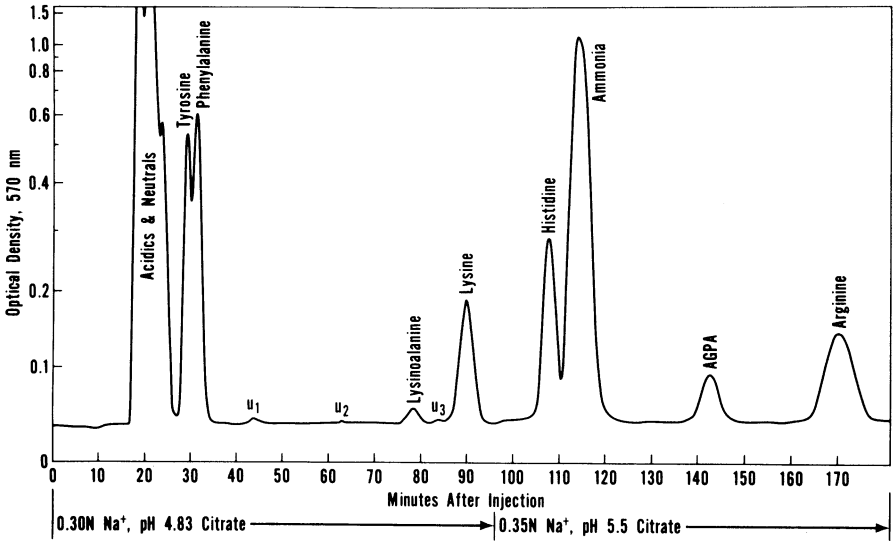

Fig. 3. Separation of basic amino acids in hydrolysate of laboratory-prepared unbleached hominy sample. Column same as in Fig. 1.

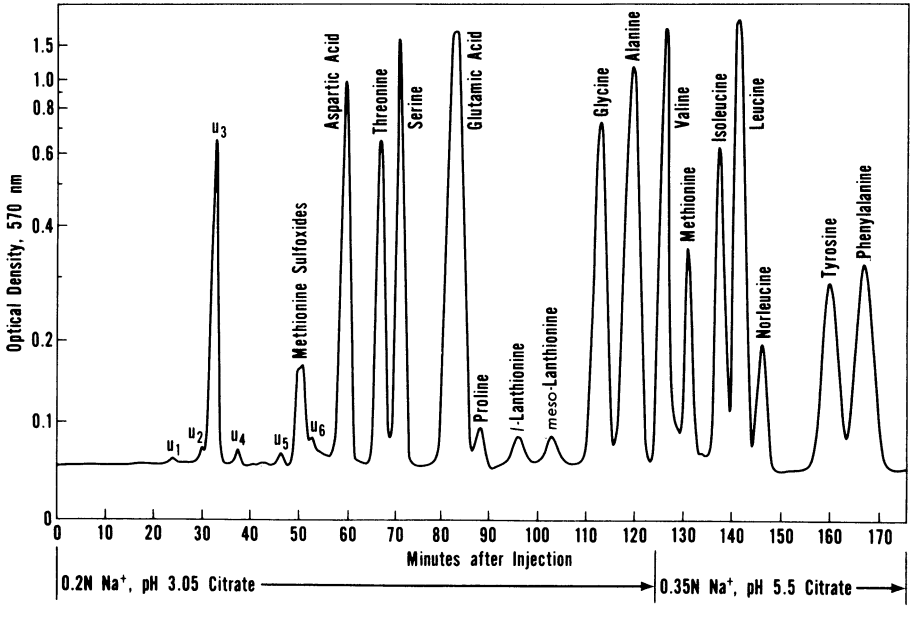

Fig. 4. Separation of acidic and neutral amino acids in hydrolysate of laboratory-prepared unbleached hominy sample. Column same as in Fig. 2.

and must be resolved if either of these amino acids is to be measured accurately. Since lanthionine is not well resolved from other amino acids on the standard acidic and neutral column, recourse to a different elution system is essential to demonstrate formation of this amino acid in alkaline-treated proteins. The chromatographic systems illustrated in Fig. 1 and 2 resolve most of the major unique amino acids found in alkaline-treated proteins. Figures 3 and 4 illustrate typical chromatograms of fivefold concentrated hydrolysates of hominy by this chromatographic procedure.

Complete amino acid analyses of the original corns and various masa and tortilla products are summarized in Table I. Analyses of both commercial and laboratory-prepared hominy are given in Table II. Included in the data are contents of major amino acids that arise as artifacts of alkaline processing.

Small losses in the amino acids arginine and cystine occurred during the laboratory processing of normal dent corn to masa (Table I). Arginine and cystine are known to be unstable to alkali. The arginine breaks down to urea and

TABLE I
Amino Acid Compositions of Corn and Alkaline Processed Corn Products

Amino Acids	Normal Corn	Normal Corn Masa	High-Lysine Corn	High-Lysine Corn Masa	Commercial Masa	Tortilla
			Protein (%)			
	7.9	8.5	10.7	10.7	8.1	9.5
			% of Protein			
Lysine	3.0	2.7	5.1	5.2	3.2	2.9
Histidine	2.9	2.8	3.9	3.8	3.5	3.5
Ammonia	2.8	2.4	2.3	2.4	2.9	3.1
Arginine	5.4	4.6	8.3	7.9	6.3	5.5
Aspartic acid	7.2	6.9	8.4	8.4	6.0	5.8
Threonine	3.8	3.8	3.6	3.6	3.5	3.4
Serine	5.0	5.0	4.4	4.5	4.6	4.7
Glutamic acid	18.8	19.5	15.4	15.7	17.8	18.9
Proline	9.2	10.7	7.0	7.6	9.1	8.7
Glycine	4.0	4.3	4.7	4.6	3.9	3.5
Alanine	7.7	8.1	6.1	6.1	7.2	7.6
1/2 Cystine[a]	2.0	1.7	2.5	2.2	2.3	1.9
Valine	4.8	5.3	5.1	5.0	5.0	4.9
Methionine[a]	2.8	2.9	1.9	1.9	1.4	2.3
Isoleucine	3.7	3.8	3.4	3.3	3.4	3.5
Leucine	12.6	13.4	8.3	8.3	11.3	12.1
Tyrosine	4.5	4.6	3.8	3.7	4.2	4.4
Phenylalanine	5.0	5.2	4.3	4.2	4.5	4.7
Lysinoalanine[b]	...[c]	0.059	ND[d]	0.049	0.020	0.081
Lanthionine[b]	...[c]	0.208	ND[d]	0.056	...[c]	0.049
Ornithine[b]	0.034	0.025	ND[d]	0.038	0.025	0.021

[a]Determined by performic acid oxidation.
[b]Determined on fivefold concentrated hydrolysates.
[c]... = None observed.
[d]ND = not determined.

ornithine, while the cystine undergoes loss of H_2S to yield dehydroalanine and other products. Similar losses in these amino acids were observed in the production of masa from the high-lysine corn (Table I).

The commercial masa had higher lysine, arginine, and cystine than did either the normal control corn or the masa derived from it (Table I). Evidently, the corn that was used to prepare the commercial masa harina was distinctly different from the normal control corn, and conclusions cannot be made concerning changes in this product during processing.

Although efforts were made to simulate the commercial production of hominy in the laboratory, problems were encountered in heating and stirring the samples evenly. Also, the excessive rinsing and agitation of the kernels caused the loss of germ in some cases, resulting in slight variation of protein content and some amino acid contents between preparations (Table II). Substantial losses of cystine occurred in laboratory-prepared hominy compared with commercial hominy in which only small losses were observed (Table II). The difference may indicate that the laboratory process was more rigorous than was the commercial

TABLE II
Amino Acid Compositions of Commercial and Laboratory-Prepared Hominy

Amino Acids	Normal Corn	Commercial Hominy		Laboratory-Prepared Yellow Hominy		
		Yellow	White	Bisulfite 0 g	Bisulfite 0.9 g	Bisulfite 3.6 g
				Protein (%)		
	7.9	8.2	10.0	6.8	7.9	6.5
				% of Protein		
Lysine	3.0	2.7	2.4	2.5	3.4	2.6
Histidine	2.9	3.4	3.4	3.5	3.3	3.1
Ammonia	2.8	2.0	2.1	2.8	2.0	2.6
Arginine	5.4	5.0	4.8	4.2	6.2	4.5
Aspartic acid	6.9	6.4	5.9	5.6	6.5	6.2
Threonine	3.8	3.8	3.8	3.4	3.7	3.6
Serine	5.0	5.0	5.2	4.4	4.8	4.8
Glutamic acid	18.8	21.3	22.3	21.4	19.3	21.2
Proline	9.2	8.1	8.5	11.7	10.2	11.2
Glycine	4.0	4.0	3.8	3.7	4.4	3.8
Alanine	7.7	8.4	8.5	7.9	7.9	7.9
1/2 Cystine[a]	2.0	1.6	1.9	0.75	0.88	0.82
Valine	4.8	5.7	5.6	5.4	6.0	5.5
Methionine[a]	2.8	3.0	2.6	2.7	2.7	2.6
Isoleucine	3.7	4.0	4.1	3.8	3.9	3.8
Leucine	12.6	14.7	15.4	14.0	12.4	13.6
Tyrosine	4.5	5.0	5.1	4.5	4.5	4.7
Phenylalanine	5.0	5.7	5.7	4.8	5.2	5.2
Lysinoalanine[b]	...[c]	...[c]	...[c]	0.27	0.18	0.18
Lanthionine[b]	...[c]	0.75	0.66	1.2	1.5	1.6
Ornithine[b]	0.034	0.033	0.034	...[c]	...[c]	...[c]

[a]Determined by performic acid oxidation.
[b]Determined on fivefold concentrated hydrolysates.
[c]... = None observed.

one. Losses in lysine and arginine were also evident in the samples with no bisulfite and addition of 3.6 g of bisulfite.

Lysinoalanine was found in masa that was prepared from normal and high-lysine corn at levels of 0.059 and 0.049 g/100 g of protein (Table I). No lysinoalanine was found in the original corn. In the commercial masa, only 0.020 g/100 g of protein were found, indicating that this product is not subjected to as vigorous conditions of alkaline cooking as is the laboratory-prepared masa, which was prepared by traditional methods. The tortillas contained 0.081 g of lysinoalanine per 100 g of protein. These values are higher than are those that Sternberg *et al.* (10) reported for tortillas.

Varying amounts of lysinoalanine were found in the laboratory-prepared hominy, whereas no lysinoalanine was observed in either commercial hominy. Lysinoalanine content of the bisulfite-treated hominies was markedly decreased compared with that of the untreated sample (Table II). Finley *et al.* (17) and Feairheller *et al.* (18) have shown that addition of sulfide ions or mercaptoamino acids to alkali-treated proteins to reduce cystine to cysteine inhibits lysinoalanine formation by favoring formation of lanthionine from any dehydroalanine that is produced. In the commercial hominy that we analyzed, the analogous inhibition of lysinoalanine formation by bisulfite treatment presumably may be enough to reduce formation of this amino acid. In the laboratory-prepared hominy, however, the lysinoalanine levels were three to four times the levels that Sternberg *et al.* (10) reported in commercial hominy. Variations in the extent of alkaline treatment may be the major factor in amount of lysinoalanine formation.

Lanthionine is also an alkaline degradation product of cystine; it results from the condensation of dehydroalanine with cysteine (19). Lanthionine was present in most of the alkaline-treated food products but absent in the untreated corn (Table I). Masa that was prepared from normal corn contained 0.208 g/100 g of protein. No lanthionine was found in commercial masa. High levels of lanthionine were found in both commercial and laboratory-prepared hominy (Table II). The amounts found in the laboratory-prepared samples were generally twice as high as those found in the commercial hominy samples, but this increase in lanthionine is to be expected in view of the greater loss of cystine in the laboratory samples. Also, more lanthionine was observed in the two bisulfite-treated samples than in the untreated sample, which seems to provide additional support for the findings that Finley *et al.* (17) reported.

Ornithine was found in whole corn as well as in the alkaline-processed corn products. Christianson *et al.* (20) reported ornithine in corn as the free amino acid. In the process of washing the alkali-treated corn, however, much of the free ornithine is lost and the remainder is mostly protein-bound. The level of ornithine in the alkaline-treated masa is low, and its origin from arginine is possible in view of the lower arginine levels in the treated grain. No measurable ornithine was found in the laboratory-prepared hominy samples.

Other novel amino acids that are possibly formed by alkaline treatment of proteins, such as ornithinoalanine (which Finley and Friedman [21] reported nearly to coelute with lysinoalanine) and β-aminoalanine (which Nashef *et al.* [19] reported to elute between histidine and ammonia on their chromatographic system), were not detected. No standards were available to determine accurately the location of ornithinoalanine and β-aminoalanine in our chromatographic

system. Various small unknown peaks, however, were observed on the chromatograms of the alkali-treated products (Figs. 3 and 4). Peak u_2 in Fig. 3 elutes at a position close to that of tryptophan (Fig. 1), while peak u_3 is probably a trace amount of ornithine. No peak was observed at the latter position in most of the laboratory-prepared hominy samples. The two diastereomers of lanthionine eluted between proline and glycine (Fig. 4) in the alkali-treated products. Several unknown peaks eluted ahead of aspartic acid, but these are probably various breakdown products of the sulfur amino acids or carbohydrate material in the corn. Peak u_3 (Fig. 4) is most likely levulinic acid, which is formed from breakdown of carbohydrates in all acid hydrolysates of corn or corn products.

DISCUSSION

The initial breakdown of cystine in the glutelin fraction of corn protein during alkaline processing may be beneficial in disrupting the matrix of protein that tightly binds protein and starch and makes it difficult to grind the horny endosperm of corn. The disulfide bonds of cystine link the matrix protein. Bressani and Scrimshaw (9), however, report that zein and glutelin in masa are less soluble in the alcoholic and alkaline solvents used respectively to extract those proteins. In addition to denaturation due to heating, new cross-linkages through lanthionine or lysinoalanine may further insolubilize those proteins. These same workers note, however, that *in vitro* digestion of alkaline-treated corn proteins by enzymes proceeds more rapidly than with protein in normal corn meals. The improved digestibility by enzymes may be due to better accessibility to the corn proteins caused by starch gelatinization and changes in the protein matrix. The loss of cystine in masa and hominy would be important in diets that combine these products with beans, since legumes are generally deficient in sulfur amino acids.

The formation of small quantities of lysinoalanine in masa raises a question as to any physiologic effects of this compound in populations consuming considerable tortillas and related products. More careful investigation of this problem is necessary. Lanthionine has long been known to be present in alkaline-treated protein products. No work on physiologic effects of lanthionine has been reported thus far. Studies should be made on lanthionine metabolism in animals. Since ornithine is a regular component of animal body fluids, its presence in normal corn and alkaline-treated grain products has not elicited concern. Ornithine is a natural intermediate in arginine biosynthesis and is involved in the urea synthesis cycle in humans.

Literature Cited

1. KATZ, S. H., HEDIGER, M. L., and VALLEROY, L. A. Traditional maize processing techniques in the new world. Science 184: 765 (1974).
2. INGLETT, G. E. Food uses of corn around the world. In INGLETT, G. E. (ed.). Corn, culture, processing, products. p. 138. Avi Publishing Co.: Westport, Conn. (1970).
3. DE GROOT, A. P., and SLUMP, P. Effects of severe alkali treatment of proteins on amino acid composition and nutritive value. J. Nutr. 98: 45 (1969).
4. BOHAK, Z. N^{ϵ}-(DL-2-amino-2-carboxyethyl)-L-lysine, a new amino acid formed on alkaline treatment of proteins. J. Biol. Chem. 239: 2878 (1964).
5. WOODARD, J. C., and SHORT, D. D. Toxicity of alkali-treated soy protein in rats. J. Nutr. 103: 569 (1973).

6. DE GROOT, A. P., SLUMP, P., FERON, V. J., and VAN BEEK, L. Effects of alkali-treated proteins: Feeding studies with free and protein-bound lysinoalanine in rats and other animals. J. Nutr. 106: 1527 (1976).
7. GOULD, D. H., and MacGREGOR, J. T. Biological effects of alkali-treated protein and lysinoalanine: An overview. Adv. Exp. Med. Biol. 86B: 29 (1977).
8. BRESSANI, R., WILSON, D., CHUNG, M., BEHAR, M., and SCRIMSHAW, N. S. Supplementation of cereal proteins with amino acids. V. Effect of supplementing lime-treated corn with different levels of lysine, tryptophan and isoleucine on the nitrogen retention of children. J. Nutr. 80: 80 (1963).
9. BRESSANI, R., and SCRIMSHAW, N. S. Effect of lime treatment on *in vitro* availability of essential amino acids and solubility of protein fractions in corn. J. Agric. Food Chem. 6: 774 (1958).
10. STERNBERG, M., KIM, C. Y., and SCHWENDE, F. J. Lysinoalanine: Presence in foods and food ingredients. Science 196: 992 (1975).
11. CONTINENTAL CAN CO., INC. The canning of hominy. Canning memorandum. 8th rev. Continental Can Company, Inc.: Chicago.
12. BENSON, J. V., and PATTERSON, J. A. An accelerated automatic chromatographic analysis of amino acids on a spherical resin. Anal. Chem. 37: 1108 (1965).
13. CAVINS, J. F., and FRIEDMAN, M. Automatic integration and computation of amino acid analysis. Cereal Chem. 45: 172 (1968).
14. MOORE, S. On the determination of cystine as cysteic acid. J. Biol. Chem. 238: 235 (1963).
15. HUGLI, T. E., and MOORE, S. Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolyzates. J. Biol. Chem. 247: 282 (1972).
16. INGLIS, A. S., and NICHOLLS, P. W. Determination of lanthionine in wool hydrolyzates. Anal. Biochem. 24: 209 (1968).
17. FINLEY, J. W., SNOW, J. T., JOHNSTON, P. H., and FRIEDMAN, M. Inhibition of lysinoalanine formation during alkali treatment of proteins. J. Agric. Food Chem. 25: 1421 (1977).
18. FEAIRHELLER, S. H., TAYLOR, M. M., and BAILEY, D. G. ^{35}S-Sulfide incorporation during alkaline treatment of keratin and its relation to lanthionine formation. Adv. Exp. Med. Biol. 86B: 177 (1977).
19. NASHEF, A. S., OSUGA, D. T., LEE, H. S., AHMED, A. L., WHITAKER, J. R., and FEENEY, R. E. Effects of alkali on proteins. Disulfides and their products. J. Agric. Food Chem. 25: 245 (1977).
20. CHRISTIANSON, D. D., WALL, J. S., and CAVINS, J. F. Location of nonprotein nitrogenous substances in corn grain. J. Agric. Food Chem. 13: 272 (1965).
21. FINLEY, J. W., and FRIEDMAN, M. New amino acid derivatives formed by alkaline treatment of proteins. Adv. Exp. Med. Biol. 86B: 123 (1977).

[Received March 7, 1977. Accepted October 4, 1977]