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Evaluation of the Resistance of Lysine Sulfite to Maillard Destruction

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Lysine sulfite (a salt consisting of 2 mol of lysine and 1 mol of sulfurous acid) was found to be very resistant to nonenzymic browning when heated in a glucose solution. As measured by loss of chemically available lysine, it was also found to be more resistant to Maillard destruction when compared with lysine or lysine plus sodium bisulfite. However, heat treatment of lysine-supplemented wheat–glucose diets resulted in losses of bioavailable lysine which were equally severe when either lysine sulfite or lysine hydrochloride was used as the lysine source. Thus, lysine sulfite, although quite resistant to color formation, is very susceptible to loss of nutritionally available lysine due to Maillard destruction.

Cereal grains are limiting in lysine (Howe et al., 1965). Because lysine is also susceptible to destruction via the Maillard reaction, heat treatment of proteins in the presence of reducing sugars often reduces the protein quality still further. Although supplementing the processed protein with lysine generally counteracts loss in quality, postprocessing supplementation is impractical. Addition of lysine prior to heat processing, however, also poses problems since dark colors and off-flavors sometimes occur. Moreover, protein quality may even be reduced in some instances upon heating. Adrian and Frangne (1969), for example, demonstrated that supplementing an unbaked cookie with lysine in the form of skim milk powder increased the PER from 1.09 to 2.10, but after baking, the PER dropped from 0.19 for the unsupplemented cookie to 0.02 for the lysine-supplemented cookie. Thus, it is critical to supplement with a form of lysine which is reasonably resistant to the Maillard reaction.

Kawashima et al. (1978) recently proposed that lysine sulfite (a salt consisting of 2 mol of lysine and 1 mol of sulfurous acid) would be a useful form of lysine for preprocessing supplementation. However, evidence presented for lysine sulfite's resistance to Maillard destruction was restricted to its resistance to color formation. This is a critical point since sulfites generally act on coloration without markedly lowering amino acid loss (Friedman and Kline, 1950).

Our objective was to further evaluate the resistance of lysine sulfite to Maillard destruction using a bioassay technique.

EXPERIMENTAL SECTION

Lysine sulfite was prepared by the method of Kawashima et al. (1978). Final lysine content was determined using a Beckman 119CL amino acid analyzer. Sulfurous acid content was determined by the conventional iodometric titration technique. The final product contained 76.1% L-lysine and 22.0% sulfurous acid.

Table I. Composition of the Purified Crystalline Amino Acid Diet

basal diet	%	amino acid mixture	%
cornstarch	to 100.00	L-Arg·HCl	1.15
amino acid mixture	19.84	L-Lys·HCl	0.50
corn oil	10.00	L-His·HCl·H_2O	0.45
cellulose	3.00	L-Tyr	0.45
mineral premix[a]	5.37	L-Phe	0.50
sodium bicarbonate	1.50	L-Thr	0.65
choline chloride	0.20	L-Trp	0.15
vitamin premix[a]	0.20	DL-Met	0.35
DL-α-tocopheryl acetate (20 mg/kg)	+	L-Cys_2	0.35
ethoxyquin (125 mg/kg)	+	L-Leu	1.00
		L-Val	0.69
		L-Ile	0.60
		Gly	0.60
		L-Pro	0.40
		L-Glu	12.00
		total	19.84

[a] Baker et al. (1979).

To test the stability of the reaction mixtures, 2.5 mmol of lysine base (United States Biochemical Corporation, Cleveland, OH), lysine hydrochloride, or lysine sulfite was dissolved in 10 mL of 0.5 M glucose with or without the addition of 1.25 mmol of sulfite as either sodium sulfite or sodium bisulfite. The solutions were heated in capped tubes at 100 °C and the resultant colors were read at 490 nm in a spectrophotometer.

To test the resistance to Maillard destruction, lysine base (5 mmol), lysine base plus sodium bisulfite (5 mmol and 2.5 mmol, respectively), or lysine sulfite (5 mmol) was dissolved in 10 mL of 0.5 M glucose. The solutions were heated at 100 °C for 40 min. after which remaining free lysine was determined on an amino acid analyzer.

New Hampshire × Columbian male chicks were fed a crystalline amino acid diet (Table I) to assess the bioavailability of lysine in lysine sulfite (experiment 1). The

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Table II. Composition of the Wheat-Glucose Basal Diet

basal diet	%
cornstarch	to 100.00
wheat (finely ground)	82.14
glucose	9.13
corn oil	2.00
mineral premix[a]	5.37
choline chloride	0.10
vitamin premix[a]	0.20
DL-Met	0.30
L-His·HCl·H_2O	0.05
L-Thr	0.30
L-Arg·HCl	0.15

[a] Baker et al. (1979).

basal diet contained 0.50% L-lysine hydrochloride which insured that addition of supplemental lysine would result in growth on the linear response surface. Bioavailable lysine was calculated using the slope-ratio technique (Baker, 1978). Dietary additives replaced an equal weight of cornstarch. For this experiment, triplicate groups of seven chicks were fed the experimental diets from day 8 to 15 posthatching.

A wheat–glucose diet (Table II) was used to assess the loss of bioavailable lysine with heat treatment. A preliminary experiment (unpublished) confirmed that lysine was first limiting in this diet and that the growth response to lysine was linear between 0 and 0.21% supplemental lysine. For each experiment, the wheat (ground whole wheat) and glucose portions of the diet were mixed with or without the addition of L-lysine or water and then heat treated. The remaining ingredients were added after heat treatment to insure that only the wheat–glucose–lysine portions of the diet were affected. A standard curve was obtained for each experiment by supplementing the basal diet with 0, 0.07, 0.14, or 0.21% L-lysine. To test the effect of heat treatment on lysine loss, lysine was added to the wheat–glucose mixture prior to heat treatment at a level equivalent to 0.14% of the complete diet. The generated standard curve together with the gain response of chicks fed the heat-treated lysine provided data from which bioavailable lysine could be calculated. This value divided by the level of lysine present before heat treatment provided the fraction of lysine lost due to Maillard destruction.

Experiment 2 was designed to assess the effect of high-temperature, high-humidity storage on lysine loss. The wheat–glucose and wheat–glucose–lysine mixtures were stored at 38 °C and 86% relative humidity for 3 weeks in an environmentally controlled chamber. Experiment 3 was designed to assess the effect of low-temperature, long-term heating on lysine loss. For this, 20% of the dry weight of the wheat–glucose and wheat–glucose–lysine mixtures was added as water and subsequently dried at 60 °C for 24 h in a forced-air drying oven. The effect of high-temperature, short-term heating on lysine loss was quantified in experiment 4. Water was added to the wheat–glucose and wheat–glucose–lysine mixtures at a level equal to 5% of the initial dry weight. The mixtures were then baked at 150 °C for 90 min.

In experiment 2, triplicate groups of seven chicks were fed the experimental diets from day 8 to day 16 posthatching in experiment 3, triplicate groups of six chicks were fed the experimental diets from day 8 to day 15 posthatching; and in experiment 4, triplicate groups of seven chicks were fed the experimental diets from day 9 to day 15 posthatching.

For all experiments, feed and water were provided ad libitum and chicks were housed in typical chick battery-brooders in an environmentally controlled room during the assay period. Analysis of variance procedures were used to assess treatment differences.

Table III. Effect of Sulfites on Nonenzymic Browing between Lysine and Glucose[a]

components	absorbance at 490 nm		
	30 min	60 min	90 min
glucose + Lys	1.710	*b*	*b*
glucose + Lys + $NaHSO_3$	0.201	0.415	1.277
glucose + Lys + Na_2SO_3	0.115	0.548	1.363
glucose + Lys·HCl	1.050	*b*	*b*
glucose + Lys·HCl + $NaHSO_3$	0.153	0.356	0.649
glucose + Lys·SO_3^{2-}	0.064	0.256	0.532

[a] Two and one-half millimoles of lysine was dissolved in 10 mL of 0.5 M glucose and heated at 100 °C with or without the addition of 1.25 mmol of sulfite. Data represent means of duplicate determinations. [b] Absorbance at 490 nm was greater than 5.

Table IV. Effect of Sulfites on Lysine Loss Due to Maillard Browning[a]

components	absorbance at 490 nm	Lys loss, %
glucose + Lys	*b*	51.6 ± 1.2
glucose + Lys + $NaHSO_3$	1.610 ± 0.020	45.8 ± 0.2
glucose + Lys·SO_3^{2-}	0.283 ± 0.014	33.4 ± 2.1

[a] Five millimoles of lysine was dissolved in 10 mL of 0.5 M glucose and heated at 100 °C for 40 min with or without the addition of 2.5 mmol of sulfite. Data represent mean ± SEM for duplicate determinations. [b] Absorbance at 490 nm was greater than 5.

Table V. Bioavailability of Lysine Sulfite (Experiment 1)[a]

supplemental lysine[b]			
source	consumed, mmol	gain,[c] g	gain/feed
	0	23	0.338
L-Lys·HCl	0.274	35	0.438
L-Lys·HCl	0.623	43	0.472
L-Lys·SO_3^{2-}	0.260	31	0.408
L-Lys·SO_3^{2-}	0.616	45	0.500
	pooled SEM	2.8	0.025

[a] Data represent means of triplicate groups of seven chicks fed the experimental diets from day 8 to day 15 posthatching. Average initial weight was 74 g. [b] The basal diet (Table I) contained 0.40% L-lysine which is approximately 40% of the chick's requirement. [c] Regression of gain (g) on lysine consumed (mmol) yielded the multiple regression equation: gain = 23.6 + 32.9 lysine hydrochloride + 33.9 lysine sulfite (R^2 = 0.97). The lysine moiety of lysine is therefore 33.9/32.9 × 100, or 103 ± 18% available.

RESULTS

The effect of sulfites on nonenzymic browning between lysine and glucose is shown in Table III. In the absence of sulfite, the reaction mixtures were essentially opaque following 60 min of heating. The addition of sodium sulfite or sodium bisulfite greatly reduced color formation. However, the reaction mixture containing lysine hydrochloride and sodium bisulfite was the most resistant. Lysine sulfite, although very resistant to browning, was slightly less effective than lysine hydrochloride plus sodium bisulfite. Lysine loss due to Maillard destruction (Table IV), although reduced in the presence of sulfite, was substantially greater than would be expected from the degree of color formation. Nonetheless, results of these in vitro assays indicated that lysine sulfite was more resistant to Maillard destruction than was lysine plus sodium bisulfite.

Results of experiment 1 (Table V) indicated that the lysine moiety of lysine sulfite was completely utilized by

Table VI. Effect of High-Temperature, High-Humidity Storage on the Loss of Bioavailable Lysine Due to Maillard Browning (Experiment 2)[a]

supplemental lysine[b]				
untreated, consumed, mmol	treated source	consumed, mmol	gain,[c] g	gain/feed
0			17	0.241
0.417			27	0.306
1.036			41	0.378
1.625			47	0.415
	L-Lys·HCl	0.991	37	0.361
	L-Lys·SO_3^{2-}	0.914	34	0.359
		pooled SEM	1.9	0.009

[a] Data represent means of triplicate groups of seven chicks fed the experimental diets from day 8 to day 16 posthatching. Average initial weight was 65 g. [b] Supplemental lysine was added either after (untreated) or before (treated) the wheat-glucose mixture was stored. Test ingredients were stored at 38 °C and 86% RH for 3 weeks in an environmentally controlled chamber. [c] Regression of gain (grams) on untreated lysine consumed (millimoles) provided the standard curve: gain = 18.5 + 18.8 lysine (r^2 = 0.97). Using this standard curve and the gain of chicks fed treated lysine, the bioavailability of lysine after treatment was 101 ± 7% for L-lysine hydrochloride, and 92 ± 6% for L-lysine sulfite.

Table VII. Effect of Low-Temperature, Long-Term Heating on the Loss of Bioavailable Lysine Due to Maillard Browning (Experiment 3)[a]

supplemental lysine[b]				
untreated, consumed, mmol	treated source	consumed, mmol	gain,[c] g	gain/feed
0			12	0.197
0.339			20	0.287
0.923			33	0.346
1.745			50	0.415
	L-Lys	0.710	23	0.311
	L-Lys·HCl	0.741	26	0.332
	L-Lys·SO_3^{2-}	0.741	25	0.320
		pooled SEM	1.6	0.016

[a] Data represent means of triplicate groups of six chicks fed the experimental diets from day 8 to day 15 posthatching. Average initial weight was 76 g. [b] Supplemental lysine was added either after (untreated) or before (treated) the wheat-glucose mixture was heat treated. Test ingredients were heated at 60 °C for 24 h in a forced-air drying oven. [c] Regression of gain (grams) on untreated lysine consumed (millimoles) provided the standard curve: gain = 12.4 + 21.7 lysine (r^2 = 0.99). Using this standard curve and the gain of chicks fed the treated lysine, the loss of bioavailable lysine was 32 ± 4.9% for L-lysine, 18 ± 5.5% for L-lysine hydrochloride, and 24 ± 3.0% for L-lysine sulfite.

the chick. Thus, on a molar basis, lysine sulfite was assumed to be equal to lysine in the subsequent experiments.

The bioavailability of supplemental lysine following 3 weeks storage at 38 °C and 86% relative humidity (Table VI) was 101 ± 7% for lysine hydrochloride and 92 ± 6% for lysine sulfite, indicating that both forms were completely resistant to Maillard destruction under these conditions. When diets containing supplemental lysine were heated at 60 °C for 24 h (Table VII), the loss of bioavailable lysine was 32 + 4.0% for L-lysine, 18 ± 5.5% for L-lysine hydrochloride, and 24 ± 3.0% for lysine sulfite. The loss of bioavailable lysine was greatest for L-lysine, but there was no significant difference ($P > 0.05$) between L-lysine hydrochloride and L-lysine sulfite. Heat treatment at 150 °C for 90 min (Table VIII) resulted in the loss of

Table VIII. Effect of High-Temperature, Short-Term Heating on the Loss of Bioavailable Lysine Due to Maillard Browning (Experiment 4)[a]

supplemental lysine[b]				
untreated, consumed, mmol	treated source	consumed, mmol	gain,[c] g	gain/feed
0			15	0.209
0.406			21	0.244
0.916			30	0.312
1.532			38	0.357
	L-Lys·HCl	0.735	21	0.258
	L-Lys·SO_3^{2-}	0.764	21	0.258
		pooled SEM	1.0	0.010

[a] Data represent means of triplicate groups of six chicks fed the experimental diets from day 9 to day 15 posthatching. Average initial weight was 98 g. [b] Supplemental lysine was added either after (untreated) or before (treated) the wheat-glucose mixture was heat treated. Test ingredients were heated at 150 °C for 90 min. [c] Regression of gain (grams) on untreated lysine consumed (millimoles) provided the standard curve: gain = 14.9 + 15.5 lysine (r^2 = 0.97). Using this standard curve and gain of chicks fed the treated lysine, the loss of bioavailable lysine was 46 ± 9.8% for L-lysine hydrochloride and 48 ± 2.3% for L-lysine sulfite.

46 ± 9.8 and 48 ± 2.3% bioavailable lysine when diets were supplemented with L-lysine hydrochloride and L-lysine sulfite, respectively.

DISCUSSION

Results of the in vitro studies indicated that lysine sulfite is very resistant to color formation. This, in itself, is of practical importance since formation of dark colors in processed foods may affect consumer acceptability. Furthermore, the sulfite moiety of lysine sulfite is much more resistant to oxidative degradation than are the other sulfite sources (Kawashima et al., 1978) and would thus be more stable when employed under long-term oxidative conditions, e.g., in amino acid solutions designed for parenteral nutrition. As measured by the loss of chemically available lysine, it appears that lysine sulfite is also more resistant to Maillard destruction. However, the differences were small and, moreover, criteria of the change in bioavailable lysine, rather than of chemically available lysine, would provide a better measure of lysine sulfite's value as a lysine supplement for processed foods.

Results of experiments 2, 3, and 4 demonstrated conclusively that lysine sulfite is equal to lysine hydrochloride in its susceptibility to Maillard destruction, despite its greater resistance to nonenzymic browning. Thus, from a nutritional standpoint, degree of color formation appears to provide little information about the loss of amino acids due to Maillard destruction. This is supported by the fact that the products formed during the very first stages of the Maillard reaction remain colorless even though the nutritional value of the amino acids are lost (Lea and Hannan, 1950; Lewis and Lea, 1950). Furthermore, our results support those of Friedman and Kline (1950) which demonstrated that sulfites act on coloration without markedly affecting amino acid loss.

Our results also nicely illustrate that degree of temperature and total quantity of heat applied have different effects on the rate of the Maillard reaction. In experiment 4, the temperature was 2.5 times higher than that used in experiment 3, while heating time was reduced to one-sixteenth of that used in experiment 3. However, twice the amount of available lysine was lost at the higher temperature. These results are similar to those of Adrian and

Favier (1961) which demonstrated a comparable amino acid loss when a lysine–glucose solution was heated for 25 h at 120 °C, or for 6 h at 130 °C.

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Determination of Choline in Soybean Meal by Liquid Chromatography with the Ion-Exchange Membrane Detector

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A liquid chromatographic method for the determination of choline in feeds is described, using the ion-exchange membrane detector. An 8-h acid hydrolysis–extraction step is required to quantitatively extract the bound choline. Chromatographic analysis is accomplished in 20 min. Results compare favorably with an NMR method and are shown to be more reproducible and faster than popular ammonium reineckate methods.

Choline occurs in the cells of all living things. It may occur free in small quantities; however, it is usually bound in phospholipids (lecithins and sphingomyelin), in acetylcholine and other esters, or in phosphorylcholine. Choline is an important nutrient and serves many functions in the body such as methyl donor for transmethylation. A choline deficiency interferes with normal bone growth and fat metabolism. This importance of choline as an essential nutrient creates a need for suitable analytical methods.

Many methods for the determination of choline (as well as choline esters) are based on the use of ammonium reineckate as a precipitant. The original gravimetric method (Kapfhammer and Bischoff, 1930) was later modified to include a colorimetric comparison for quantitation (Beattie, 1936). Probably the most widely used method today for the determination of choline in feeds is the reineckate method of Lim and Schall (1964). As popular as these methods have been, they still suffer from the drawback that other compounds such as acetylcholine, other choline esters, and other quaternary nitrogen compounds will also form reineckates and, therefore, interfere with any determination where separation of these interfering compounds is not first accomplished.

Harkiss (1972) surmounted this problem by precipitating choline reineckate, converting it back to choline chloride, chromatographing on Whatman No. 1 paper, and locating the sample with Dragendorff reagent. Unfortunately, this method has a range of only 0.3 to 2.4 μg of choline chloride.

Other methods that have been reported for the determination of choline, mostly with biological samples, include pyrolysis GC (Schmidt and Speth, 1975), high-voltage electrophoresis (Brooker and Harkiss, 1974), thin-layer chromatography using a Dragendorff reagent (McLean and Jewers, 1972), mass spectrometry (Johnston, et al., 1968), photometry following ion-pair extraction (Eksborg and Persson, 1971), and radioisotopic measurement (Wang and Haubrich, 1975).

Chastellain and Hirsbrunner (1976) recently reported the simultaneous determination of betaine and choline in feeds by ^{1}H-NMR spectrometry. While being a significant advance over the Lim and Schall method, it still suffers from the inherently poor sensitivity of NMR and the difficulty of quantitating NMR peaks in aqueous solutions.

The poor volatility of choline makes liquid chromatography the analytical method of choice. Unfortunately, the lack of useful UV-visible absorption bands has heretofore precluded the use of this method. We have recently described the separation and quantitation of some simple quaternary ammonium ions by liquid chromatography (Dorsey et al., 1978) using a detector developed in this laboratory (Gilbert and Dobbs, 1973).

This paper describes the application of this method to the determination of choline in soy meal. Extraction conditions are described and discussed in terms of recovery. The method is compared to both the Lim and Schall (1964) and Chastellain and Hirsbrunner (1976) methods. Precision, linearity, detection limits, and speed of analysis are discussed.

EXPERIMENTAL SECTION

Chromatographic System. The liquid chromatography system used and the ion-exchange membrane detector have been described previously (Dorsey et al., 1978).

Aminex A-4 resin (Bio-Rad Laboratories, Richmond, CA), 16–24 μm, an 8% DVB cross-linked strongly acidic cation-exchange resin with sulfonic acid functional groups, was equilibrated three times with the mobile phase and then slurry packed in a Chromatronix LC-9M column, 9.0 mm i.d. (LDC, Riviera Beach, FL). When the column was filled, the resin bed was compressed by pumping the mobile phase through the column at maximum flow rate for at least 1 h, and then the column was adjusted to this final bed height of 20 cm. The mobile phase was 70:30 acetone/water which was also 0.035 M ethylene diammonium chloride (en·2HCl) and was vacuum degassed.

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