DESTRUCTION OF LYSINE BY NONREDUCING SUGARS

by

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Raffinose, the nonreducing sugar of cottonseed, is shown to decrease the number of free epsilon amino groups in cottonseed meal or flour proteins, and evidence is presented that the initial reaction is the aminolysis of the 1-2 glycosidic linkage between glucose and fructose residues of raffinose by the epsilon amino group of lysine in the meal or flour protein. The practical significance resides in the fact that the nutritional quality of vegetable proteins is correlated directly with the number of epsilon amino groups of lysine in the proteins that are free to react with 2,4-dinitrofluorobenzene (2,3,4,5,7,10) and that the number of such groups is decreased when the sources of proteins are subjected to processing (1,4,5,6,8,9,10,12).

Experimental Results and Discussion:

Effect on Optical Rotation:

To 50 ml aliquots of an aqueous sucrose solution (ca. 0.26 g/ml) were added 1 or 3 ml of 2-amino-2-methyl propanol, or 1 or 3 ml of ethanolamine, and the resultant solutions were treated as indicated in Table I, and the optical rotation then determined [20°, D line of sodium].

Included in Table I are the anticipated rotations based on the algebraic sums of the observed rotations of the sugar solutions plus the contribution to the rotation made by the amino acid. The data are reported as degrees rotation for a 25 cm light path.

Table I. Effect of Amines and Amino Acids on Optical Rotation of Nonreducing Sugars

Treatment	Sugar								
	Sucrose			Raffinose			Trehalose		
	Calcd. Rotation [Rotation of Sugar + Soln of amine or amino acid]	Observed Rotation	Initial % Reduction	Calcd. Rotation [Rotation of Sugar + Soln of amine or amino acid]	Observed Rotation	% Reduction	Calcd. Rotation [Rotation of Sugar + Soln of amine or amino acid]	Observed Rotation	Reduction
1 ml 2-amino-2-methyl propanol[1]	26.9	22.0	18	12.3	9.2	25	-	-	-
3 ml 2-amino-2-methyl propanol[1]	25.9	21.5	17	11.8	6.5	45	-	-	-
1 ml 2-amino-2-methyl propanol[2]	26.9	22.0	18	-			-	-	-
3 ml 2-amino-2-methyl propanol[2]	25.9	21.8	16	-			-	-	-
1 ml ethanol amine[1]	25.9	21.7	19	12.3	8.0	35	2.16	21.8	0
3 ml ethanol amine[1]	25.9	21.7	16	11.8	5.5	53	-		
1 g lysine[1]	32.5	26.3	19.1	17.9	12.7	29	27.4	26.2	4
1 g lysine[2]	32.5	26.0	20.0	-	-		-	-	-
1 g arginine[1]	39.5	28.3	7.2	15.5	14.0	9.7	-	-	-
1 g alanine[1]	29.5	28.8	2.4	-	-	-	-	-	-

1/ Stored at room temperature overnight
2/ Heated in steam bath for 2 hrs.

Table I also contains the data obtained with raffinose and trehalose when 2.5 g of raffinose and 3.5 g of trehalose per 50 ml of water were used.

It was established through replicate determinations that the differences between the calculated and the observed rotations are highly significant.

Heating the mixtures did not increase the extent of the reaction between the sugars and amines or amino acids, and it is presumed, therefore that the reactions went substantially to completion on storage of the solutions overnight at room temperature.

Two significant results are revealed by the data in Table I: a) while ethanolamine and lysine each markedly reduced the optical rotation of the

sucrose and raffinose solutions, they had only minor effects on the trehalose solutions; b) lysine markedly reduced the optical rotation of the sucrose solution, but alanine had relatively little effect. The reduction in optical rotation induced by arginine was intermediate between those observed for lysine and alanine. These data suggest that the alpha amino group of amino acids is not involved in the reactions that reduce the optical rotations and that it is the 1-2 glycosidic linkage and not the 1-1 glycosidic linkage that is attacked.

The suggestion that the glycosidic linkage is attacked is supported by fact that the Benedict and picramic acid tests for reducing sugars become positive when lysine or ethanolamine are added to solutions of sucrose or raffinose, while the tests remain negative when alanine is added.

Effect of Sucrose on Amino Acid Analyses:

The reproduction of a thin-layer chromatogram obtained on the chromatography of mixtures of lysine and sucrose and of alanine and sucrose is shown in Figure 1. The mixture of lysine and sucrose gave only one spot

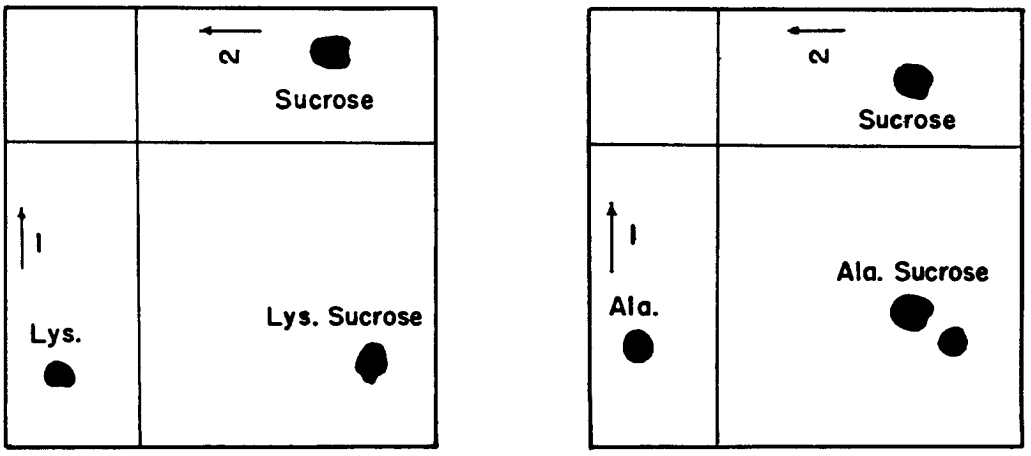

Fig. 1. Thin-layer chromatographs of mixtures of lysine and sucrose and of alanine and sucrose. Solvent No. 1, propanol: 37% ammonium hydroxide solution 70:30; solvent No. 2, methyl ethyl ketone:acetic acid; water, 60:20:20. Adsorbent, silica gel containing 2% sodium acetate. Developers, ninhydrin and aniline diphenylamine.

[Rf 0.10-0.15]. This may be compared with the Rf values for sucrose and lysine of 0.25 and 0.08 respectively. Alanine and sucrose, on the other hand, gave two spots with Rf values corresponding to those observed for alanine and sucrose respectively.

The silica gel from the positions of lysine-sucrose, from alanine in the mixtures of alanine and sucrose, and from reference alanine and reference lysine in the undeveloped chromatographs was removed and the gel from each position was transferred to a 10-ml volumetric flask. Half a milliliter of ninhydrin solution was added, and the volume was brought up to the mark with methanol. The solutions were then filtered and the optical densities (at 570 millimicrons) for triplicate determinations are reported in Table II.

TABLE II. Effect of Sucrose on the Amino Acid Analysis

Materials	O. D. 570 mμ				% Reduction in Lysine Peak (A.A.A.)
	I	II	III	Average	
Reference Lysine	0.330	0.371	0.331	0.344	
Lysine Sucrose Spot	0.235	0.263	0.241	0.246	27.49
Reference Alanine	0.220	0.223	0.230	0.224	
Alanine Spot from Alanine Sucrose	0.221	0.217	0.211	0.216	

Notes:
1. Original solutions contain: Amino acid 1.00 g.; sucrose 13.00 g.; water, 50 ml.
2. Reaction at room temperature overnight.
3. Amino acid; used as hydrochlorides and calculated amounts of sodium bicarbonate was added to neutralize HCl.
4. 20 μl of solutions used in T.L.C.; and 1 μ mol of lysine used in the amino acid analyzer.

There was a marked reduction in the ninhydrin-positive material produced by the reaction between lysine and sucrose. The partition of squares in an analysis of variance of the data involving alanine indicates no difference between test and control. Alanine was not affected.

An aliquote of the sucrose-lysine solution was subjected to analysis by the Moore, Speckman, and Stein ion-exchange procedure (11) - a Phoenix Analyzer Model K-8000[1/] was used. The area under the lysine peak for the mixture was ca. 28% less than that for the reference lysine. Moreover, lysine was the only ninhydrin-positive constituent eluted from the column. The products formed on the reaction between sucrose and lysine are either ninhydrin negative or are not eluted from the column.

Solutions containing 3 g of sucrose or 3 g of raffinose and 100 mg of lysine in 20 ml of water were autoclaved at 15 pounds per square inch for 4 hours, and then subjected to analyses by the Moore, et al. procedure. Thirty four percent of lysine in the sucrose and 24% of the lysine in the raffinose solution were lost. Lysine was the only ninhydrin-positive constituent eluted from the columns.

Changes in the Infrared Spectrum:

An aliquot of the lysine-sucrose solution was evaporated to dryness by lyophylization and the infrared spectrum (KBr disc) was determined for residue. This is compared in Figure 2 with that for sucrose. One is impressed with the lack of fine detail in the spectrum for the reaction product, and the relatively smooth curve taken to imply the sample is a mixture of substances.

Effect of Raffinose on Lysine Availability in Cottonseed Proteins:

The cottonseed protein (N, 13.8%; moisture, 9.8%; total sugars, 0.02%; crude fiber, 0.02%; lipids, 0.04%; epsilon free amino lysine 4.08 g/16 g N) was prepared from alkaline extraction of defatted glandless cottonseed flour. Raffinose was added to aliquots of the protein preparation to yield mixtures having ratios of raffinose to protein of 0.01, 0.05, 0.10, 0.50, and 1.0. Available lysine was then determined in 30 minutes by the procedure described

1/ Use of a company or product name by the Department does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

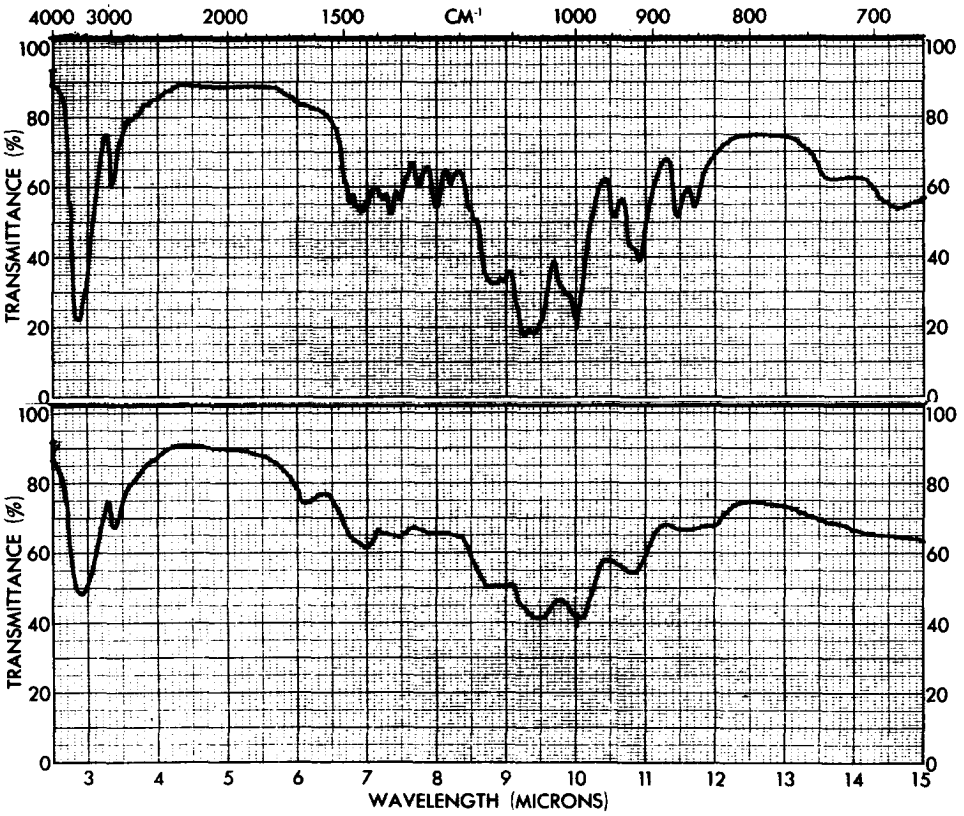

Fig. 2. Infrared spectra. Upper spectrum, sucrose; lower spectrum, sucrose-lysine reaction product.

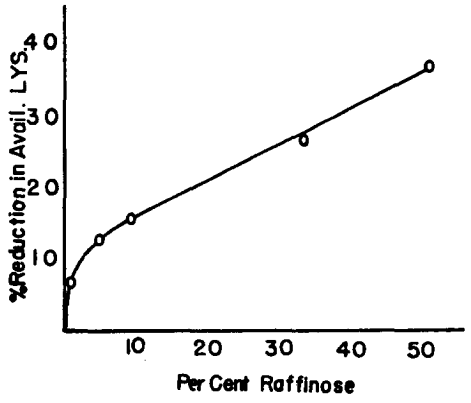

Figure 3. Effect of raffinose on lysine availability of cottonseed protein concentrate.

by Rao, et al. (12). The percent reduction in available lysine is plotted against the ratio of raffinose to protein (expressed as percent) in Figure 3.

Summary and Conclusions:

The data are interpreted to mean that the 1-2 glycosidic linkage in sucrose and in raffinose undergoes aminolysis in the presence of epsilon amino groups of lysine and lysine is lost. Reaction products appear which give positive tests for reducing sugars. The alpha amino group of amino acids are not involved, but a group liberated on the aminolysis of the 1-2 glycosidic linkage may react with the alpha amino groups. The data suggest that the reduction in availability of lysine in oilseed meals, such as cottonseed meal, peanut meal or soybean meal, is a consequence of reactions between the epsilon amino groups of lysine in the proteins and the 1-2 glycosidic linkage in nonreducing sugar constituents of the seed.

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