Amino Acid Stability during Alfalfa Dehydration

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The stability of 18 amino acids during alfalfa dehydration on a pilot and industrial scale was determined. Retention of lysine, methionine, cystine, arginine, histidine, and aspartic acid was correlated with meal moisture and dehydrator outlet temperatures. Lysine and methionine were the most labile, undergoing maximum losses of 47 and 30%, respectively. The amino acid contents of 13 commercial alfalfa meals were determined. The results of analysis on commercial blends of 15, 17, 20, and 22% protein alfalfa meals are presented.

Dehydrated alfalfa meals contain relatively high amounts of protein and are used in most rations of poultry and livestock. The relative amounts of the essential amino acids are particularly important in poultry and swine nutrition. Losses of amino acids as well as other vitamins and nutrients have been found to occur during processing of human and animal foods.

Studies at this laboratory have demonstrated that substantial losses of xanthophyll and carotene (Livingston *et al.*, 1966, 1968b) or α-tocopherol (Livingston *et al.*, 1968a) may occur during alfalfa dehydration, depending to a great extent on conditions.

Lyman *et al.* (1953) found that excessive heating during processing of soybean oil meal damaged the digestibility of the protein. Lysine was found to be the most labile of the essential amino acids. Halevy and Guggenheim (1953) autoclaved wheat gluten–glucose mixtures and found that the *in vitro* digestibility of the essential amino acids was reduced by the autoclaving. In a study on the chemistry of the spontaneous heating of stored alfalfa pellets, Ruliffson *et al.* (1956) concluded that the heating was initiated and sustained by sugar–protein interactions of the Maillard type, resulting in reduction of the free amino acid content. Beauchene and Mitchell (1957) found no difference in the total nitrogen content of alfalfa meal dehydrated at 50° C from that which was dehydrated commercially. However, they did find that the low temperature meal contained more α-amino nitrogen and less protein nitrogen than did meal prepared at high temperature.

In light of these earlier studies concerning the effects of treatment on plant protein and amino acids, the present study was undertaken to ascertain the stability of the amino acids of alfalfa during pilot and industrial scale alfalfa dehydration.

EXPERIMENTAL

Pilot-Scale Dehydration—Study 1. Freshly harvested alfalfa was dehydrated in a pilot Arnold (Model SD45-12) dehydrator. In Study 1 the flame to the burner or the inlet temperature was regulated manually. The outlet temperature was regulated by a combination of the inlet temperature and the feed rate of the fresh alfalfa entering the drum.

Pilot and Industrial-Scale Dehydration—Study 2. Following the preliminary study, a second investigation of dehydration effects was made employing the pilot Arnold dryer, an industrial-scale Arnold (Model SD8-24) and a Stearns Roger dehydrator. The operations of the dehydrators and the collection of samples has been previously described (Livingston *et al.*, 1966, 1968b).

The samples of fresh alfalfa were quickly frozen between layers of Dry Ice and kept frozen until dried in a vacuum freeze-dryer (Repp sublimator Model 15 FFD, Repp Industries, Inc., Gardiner, N.Y.). (Freeze-dried controls were not prepared in Study 1.) The freeze-dried alfalfa, the dehydrated alfalfa chops, and all dehydrated meals were ground so as to pass through a 40-mesh screen. Moisture in the ground meal was determined by drying at 105° C for 24 hr in a forced draft oven.

Study 3. Commercial samples of alfalfa leaf meals, blended leaf and whole meal, whole meal, and stem meal were obtained from major alfalfa producers for amino acid analysis.

Study 4. Thirty-nine lots of four commercial grades of dehydrated alfalfa from all major production areas of the country were combined to give four composite commercial blends for analyses. These were prepared by the Midwest Research Institute for the American Dehydrators Association analytical program.

The amino acids were determined by the modified ion-exchange chromatography procedure of Kohler and Palter (1967) on a modified Phoenix amino acid analyzer (Model K-8000). Tryptophan was determined by the procedure of Knox *et al.* (1970).

RESULTS AND DISCUSSION

It was apparent in Study 1, which employed the pilot Arnold dryer, that a correlation existed between the lysine content of the meal and both the outlet temperature of the

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Table I. Retention of Amino Acids During Pilot-Scale Alfalfa Dehydration
(g of amino acid/16 g nitrogen) (dehydrated meal)

Inlet temp., °F	1200			1400			1600		
Outlet temp., °F	220	270	320	220	270	320	220	270	320
Meal moisture, %	8.6	1.6	0.3	9.0	4.0	0.7	11.0	3.2	1.2
Amino acid									
Lysine	5.70	5.52	4.29	5.35	5.10	5.25	5.53	5.05	3.79
Histidine	2.19	2.12	2.23	2.20	2.25	2.32	2.25	2.29	2.17
Ammonia	2.60	1.83	1.85	2.04	1.93	2.16	1.70	2.13	2.04
Arginine	4.69	3.51	2.19	4.87	4.71	5.20	4.58	4.62	4.36
Aspartic acid	10.34	10.61	10.04	10.84	10.80	10.41	11.05	11.14	10.20
Threonine	4.34	4.63	4.66	4.65	4.49	4.92	4.67	4.48	4.40
Serine	4.42	4.84	4.62	4.78	4.70	4.90	4.82	4.70	4.56
Glutamic acid	10.34	10.96	10.45	10.81	10.45	11.39	10.76	10.50	10.22
Proline	4.59	4.53	4.40	4.54	4.48	4.95	4.58	4.34	4.29
Glycine	5.11	5.21	5.16	5.10	5.05	5.61	5.09	5.10	5.14
Alanine	5.48	5.69	5.61	5.81	5.67	6.09	5.78	5.58	5.53
Valine	6.08	6.14	6.14	6.25	6.09	6.59	6.16	5.35	5.97
Isoleucine	4.86	4.97	5.01	5.11	4.95	5.45	4.75	4.54	4.85
Leucine	7.58	7.87	7.91	7.84	7.77	8.65	7.84	7.59	7.57
Tyrosine	3.35	3.13	3.10	3.37	3.16	2.59	2.89	3.03	3.27
Phenylalanine	4.77	5.43	5.26	5.29	5.12	4.37	5.03	5.09	6.08
Methionine	1.72	1.74	1.66	1.56	1.50	1.63	1.61	1.71	1.59
Cystine	1.24	1.43	1.22	1.01	1.03	1.22	1.24	1.24	1.10
% Nitrogen recovery	80.7	80.4	75.4	86.3	80.5	87.5	84.0	71.8	79.1

Table II. Retention of Amino Acids During Alfalfa Dehydration
(g of amino acid/16 g nitrogen) (dehydrated meal)

Dehydrator	Pilot Arnold						Stearns–Roger						Industrial Arnold				Lyophilized controls (average of three samples)
Outlet temp., °F	270		300		330		270		300		330		300		330		
Meal moisture, %	2.8		1.8		1.5		9.5		9.9		5.9		9.2		2.3		
Amino acid		% Ret		% Ret		% Ret		% Ret		% Ret		% Ret		% Ret		% Ret	
Lysine	4.78	78	4.36	71	3.41	56	5.25	86	5.38	88	4.60	76	4.88	80	3.25	53	6.11
Histidine	2.05	89	2.07	90	1.95	85	2.19	95	2.15	93	2.14	93	2.20	96	1.98	86	2.31
Ammonia	2.17	93	1.76	77	1.96	86	2.06	91	2.37	104	2.23	98	2.59	114	2.08	92	2.27
Arginine	4.68	95	4.50	90	4.52	90	4.82	96	4.74	94	4.47	89	4.72	94	4.02	80	5.04
Aspartic acid	11.42	100	10.48	92	9.86	86	11.19	98	11.07	97	10.51	92	11.31	99	10.92	96	11.45
Threonine	4.46	96	4.33	93	4.34	93	4.37	94	4.43	95	4.47	96	4.37	94	4.51	97	4.66
Serine	4.66	98	4.46	94	4.32	91	4.52	92	4.89	101	4.50	92	4.53	93	4.53	93	4.79
Glutamic acid	10.27	99	9.67	94	9.78	95	9.93	96	10.03	98	9.98	97	10.21	99	10.79	105	10.31
Proline	4.23	91	4.20	90	4.17	90	4.22	91	4.94	107	4.26	92	4.35	93	4.73	101	4.65
Glycine	5.29	102	5.04	97	5.21	100	5.07	98	5.07	98	5.23	101	5.15	99	5.73	109	5.22
Alanine	5.69	99	5.31	92	5.45	95	5.40	94	5.47	96	5.64	98	5.52	96	5.87	103	5.76
Valine	6.05	99	5.82	95	5.88	95	5.83	95	6.40	104	5.77	94	6.05	99	6.62	107	6.11
Isoleucine	5.09	100	4.84	96	4.92	97	4.81	95	4.88	97	5.00	99	4.95	98	5.34	105	5.05
Leucine	7.91	103	7.59	99	7.63	99	7.27	91	7.76	97	7.81	98	7.81	100	8.33	106	7.66
Tyrosine	3.33	98	3.37	99	3.19	94	3.42	100	3.50	102	3.40	100	3.38	100	3.44	102	3.39
Phenylalanine	5.30	101	5.96	114	5.12	98	5.21	100	5.22	100	5.35	103	5.25	100	5.46	104	5.24
Methionine	1.66	92	1.56	87	1.43	80	1.74	97	1.74	97	1.66	93	1.27	71	1.25	70	1.79
Cystine	1.08	84	1.08	84	1.02	80	1.18	92	1.19	94	1.05	82	1.13	89	0.92	72	1.27
% Nitrogen recovery	82.9		77.9		77.2		82.0		83.4		81.7		85.1		81.4		87.1

drum and meal moisture (Table I.) However there did not seem to be a strong correlation between the inlet temperature and lysine retention.

The second dehydration study, which employed both the pilot Arnold and the industrial-scale dryers, demonstrated more definite correlations among the factors of meal moisture, outlet temperature of the dehydrator, and amino acid retention during dehydration. Six amino acids appeared to be substantially lower in the dehydrated alfalfa meal than in the lyophilized control (Table II). All of these except aspartic and methionine were significantly lower at P 0.05 level as measured by the Student's t test. Of the 17 amino acids analyzed, the essential amino acid, lysine, had the lowest retention in all three dehydrators and was the most affected by dryer outlet temperature and meal moisture. At the lower moisture level in the pilot and industrial Arnold dehydrators, almost half of the lysine was lost during dehydration. This laboratory has previously demonstrated that as much as 60% of the xanthophyll may be lost during alfalfa dehydration to similar moisture levels (Livingston *et al.*, 1968b). This possible large loss of both lysine and xanthophyll during dehydration makes it essential that the dehydration conditions be carefully regulated.

The retention of the labile amino acids decreased very

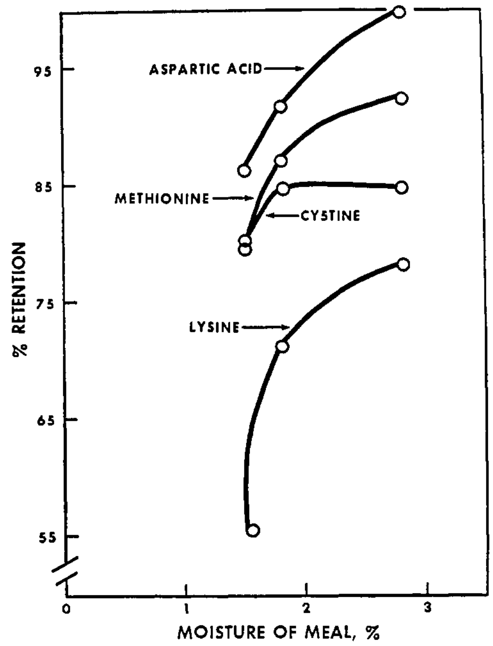

Figure 1. Stability of amino acids in alfalfa meals prepared to low moisture content

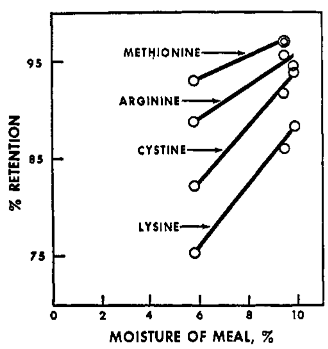

Figure 2. Stability of amino acids in alfalfa meals prepared to medium and high moisture contents

rapidly at the lower moisture levels in the pilot Arnold dryer (Figure 1). A decrease of 1% in the meal moisture resulted in a 20% decrease in lysine retention. At higher moisture levels a 13% decrease in retention was found between meals differing 4% in moisture levels (Figure 2). The relationship between moisture of meal and retention was more linear in the industrial dryers than in the pilot model, apparently due to the higher moisture levels of the meals prepared in the industrial-scale dryers.

Table III shows a comparison of amino acid retention among the three dehydrators. This table gives the dehydrator × amino acid interaction means that were found significant in the analysis of variance shown in Table IV. Lysine and methionine showed the greatest retention differences among the three dehydrators. The amino acid retention on temperature regression coefficients shows that the lysine rate of loss increases most with increased temperature. A major part of the lysine losses undoubtedly results from reaction of the ε amino group with reducing carbohydrates followed by a series of rearrangements and dismutations that lead to brown-colored polymers and evolution of carbon dioxide gas. These reactions referred to collectively as non-enzymatic browning or the Maillard reaction are known to reduce nutritive value and biological availability of amino acids.

Table III. Least Square Means and Standard Errors for Amino Acid Retentions: Dehydrator × Amino Acid and Amino Acid × Temperature Interaction

	Mean	Standard error	5% h.s.d.
Pilot Arnold			
Lysine	66.96	1.71	8.12
Histidine	87.75		
Arginine	91.35		
Aspartic acid	91.79		
Methionine	85.58		
Cystine	82.42		
Stearns–Roger			
Lysine	82.31	1.71	8.12
Histidine	93.54		
Arginine	92.56		
Aspartic acid	95.29		
Methionine	95.42		
Cystine	88.71		
Industrial Arnold			
Lysine	76.63	2.58	12.26
Histidine	94.75		
Arginine	92.25		
Aspartic acid	98.63		
Methionine	70.88		
Cystine	86.88		
Amino acid		**Amino acid retention on temperature regression coefficients**[a]	
Lysine		−0.483	0.266
Histidine		−0.144	
Arginine		−0.222	
Aspartic acid		−0.144	
Methionine		−0.100	
Cystine		−0.267	

[a] Shown as a significant amino acid × temperature interaction in Table IV. Standard error of regression coefficient = 0.056.

Table IV. Least Squares Analysis of Variance for Amino Acid Retention

	DF	Mean squares	F calcd	F 0.05 required
Dehydrator	2	219.34	25.68	3.88
Amino acid	5	325.44	38.10	3.11
Dehydrator × amino acid	10	63.14	7.39	2.76
Dehydrator × temperature	2	90.13	10.55	3.88
Amino acid × temperature	5	52.41	6.14	3.11
Dehydrator × amino acid × temperature	10	22.19	2.60	2.76
Temperature	1	833.68	97.60	4.75
Residual	12	8.54		

The reactions leading to loss of methionine, cystine, histidine, arginine, and serine are not known. It seems quite likely that oxidation might be involved, especially in the case of cystine, which is readily oxidized to cysteic acid.

The two aliphatic basic amino acids, arginine and histidine, were also subject to losses during dehydration, depending on the final meal moisture. Although only small losses of aspartic acid occurred, even at low meal moisture levels, the retention of this dicarboxylic acid could be correlated with moisture. Excessive heat, such as autoclaving above 120° C, has previously been found to have a destructive effect on the proteins of soybeans (Evans and McGinnis, 1946). The addition of methionine, lysine, or cystine improved the nutritive value of the overheated soybean proteins (McGinnis and Evans, 1947). Undoubtedly, the overheating of alfalfa and the loss of essential amino acids during drying results in loss of nutritive value of the alfalfa amino acids, particularly

Table V. Variation of Amino Acids in Commercial Samples of Dehydrated Alfalfa Meals

(g of amino acid/16 g nitrogen) (dehydrated meal)

Amino acid	Leaf meal				Blends of whole and leaf alfalfa meals		Whole alfalfa meal				Pelleted and reground		Stem meal
Lysine	4.40	4.51	4.41	3.76	4.53	4.83	4.80	4.80	3.63	4.01	3.71	3.27	5.15
Histidine	2.05	2.16	2.16	2.03	2.05	2.19	2.17	2.06	1.79	1.95	2.10	1.74	2.06
Ammonia	1.62	1.78	1.74	1.74	1.74	1.84	1.78	1.75	1.93	2.02	2.57	2.45	2.01
Arginine	4.68	4.95	5.03	4.53	4.62	5.64	4.90	4.53	3.81	4.29	4.28	4.26	4.21
Aspartic acid	8.58	9.17	9.15	9.01	9.06	9.81	10.30	9.37	9.37	11.02	11.61	7.20	10.64
Threonine	4.26	4.57	4.58	4.14	4.49	4.74	4.62	4.32	3.83	4.22	4.15	2.88	4.32
Serine	3.98	4.24	3.51	3.64	4.24	4.51	4.51	4.35	3.76	4.16	4.38	3.64	4.43
Glutamic acid	9.60	10.58	10.52	9.54	10.53	11.03	10.32	10.28	7.86	9.45	9.39	11.94	9.51
Proline	3.95	4.24	4.28	4.05	4.25	4.50	4.42	4.14	3.67	4.33	4.70	4.96	4.11
Glycine	4.77	5.15	5.20	4.87	5.13	5.41	5.07	5.09	4.38	4.77	4.80	3.70	4.52
Alanine	5.51	5.85	5.84	5.34	5.85	6.10	5.86	5.72	4.85	5.32	5.16	4.10	5.43
Valine	5.70	6.06	6.17	5.70	6.07	6.39	6.24	6.02	5.48	5.75	5.65	4.24	5.48
Isoleucine	4.76	5.05	4.98	4.78	5.03	3.99	4.99	4.89	4.33	4.68	4.59	3.48	4.55
Leucine	7.61	8.09	8.15	7.63	8.07	8.45	8.06	7.86	6.81	7.48	7.01	6.11	7.08
Tyrosine	3.17	3.26	3.68	3.20	2.99	3.09	3.10	2.80	2.35	2.83	3.18	2.62	2.22
Phenylalanine	5.10	5.24	5.50	5.11	5.53	6.06	5.32	5.09	4.63	5.02	4.10	3.84	4.84
Methionine	1.73	1.74	1.79	1.77	1.74	1.83	1.59	1.59	1.74	1.62	1.83	1.69	1.55
Cystine	1.11	0.91	1.05	0.98	1.00	1.06	1.00	1.02	1.06	1.18	0.96	1.06	1.04
% Nitrogen recovery	75.2	80.0	80.4	74.8	78.5	84.1	81.5	78.2	69.6	77.6	79.9	70.3	77.2

Table VI. Amino Acid Analysis on Blended Lots of Commercial Pelleted Dehydrated Alfalfa

(g amino acid/16 g nitrogen)

Protein grade %[a]	15	17	20	22
Amino acid				
Alanine	5.21	5.28	5.44	5.95
Arginine	3.85	4.14	4.74	4.51
Aspartic acid	10.65	10.20	9.93	10.68
Glutamic acid	9.21	9.43	9.85	10.83
Glycine	4.73	4.86	4.91	5.37
Histidine	1.97	1.92	2.02	2.10
Isoleucine	4.47	4.63	4.76	5.16
Leucine	6.93	7.25	7.45	8.19
Lysine	3.92	4.03	4.23	4.57
Methionine	1.48	1.56	1.59	1.67
Phenylalanine	4.35	5.05	5.06	5.60
Proline	4.15	4.35	4.33	4.67
Serine	4.07	4.20	4.26	4.58
Threonine	3.95	4.15	4.28	4.68
Tryptophan	1.70	1.80	1.86	1.93
Tyrosine	2.74	3.00	3.10	3.05
Valine	5.47	5.76	5.78	6.29
Cystine	1.10	1.01	1.11	1.06
% Nitrogen recovery	81.5	78.7	80.5	84.6

[a] Nitrogen × 6.25; 8% moisture basis.

lysine, which has previously been found to be the most sensitive to heat (Kuiken, 1952). Accordingly, the dehydrator operator must carefully process alfalfa in order to preserve best nutritive qualities such as amino acids, xanthophylls, and α-tocopherol.

The correlation in amino acid content with increasing protein levels in the commercial blended meals is apparent from the data in Table VI. These meals were especially prepared by the American Dehydrator's Association from 39 lots of four grades of dehydrated alfalfa from all major production areas of the United States to represent typical lots of commercially available meals.

Table V presents the variations of amino acids in commercial samples of dehydrated alfalfa meals that have undergone leaf–stem separation to give leaf–poultry and cattle–stem fractions. The lysine level of the protein in the stem meal was significantly higher than that in the leaf or whole meals. A possible explanation is that leaves dry faster in the dehydrator than stems because of the thickness of the particles. Thus, the leaves are dried to a lower moisture content and are subjected to higher temperatures (*e.g.*, dry bulb temperatures) for a longer period of time in the dryer. The stems, with a higher moisture level at the discharge end of the dryer, remain at essentially the wet bulb temperature longer and would be subjected to less loss of lysine or other labile components. From this information, a feed formulator might prepare feeds which might contain sufficient amino acids to meet the requirements of a particular poultry or livestock feed.

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