

Literature Cited

- (1) Kassner, J. L., Krammer, H. P., Ozier, M. A., *Anal. Chem.* **20**, 1052 (1948).
- (2) Strauchen, A., Paper No. LE/61/44, International Superphosphate Manu-

- facturers' Association Technical Conference, September 1961.
- (3) Sulphuric Acid and The Manufacture of Phosphatic Fertilizers, pp. 56-58, The Organization for European Economic Co-operation, Paris, 1953.
- (4) Waggaman, W. H., "Phosphoric

Acid Phosphates and Phosphatic Fertilizers," 2nd. ed., pp. 372-4, Reinhold, New York, 1952.

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PROCESSING CHANGES

Xanthophyll and Carotene Stability during Alfalfa Dehydration

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The xanthophyll content of dehydrated alfalfa meal was greatly reduced as the outlet temperature was increased and the moisture content of the meal decreased. Although total carotene was relatively unaffected by temperature of dehydration or moisture of meal, the beta-carotene isomers increased with the higher outlet temperature and lower moisture levels.

PREVIOUS STUDIES have shown the xanthophyll, carotene, and fat-soluble vitamins of dehydrated alfalfa to be subject to oxidation losses during storage (6, 8, 11, 15-17). Treatment of the alfalfa meal with antioxidants and oils or storage at a low temperature or under an inert atmosphere has eliminated much of these losses (4, 5, 7, 10, 12, 13). However, both xanthophyll and beta-carotene form stereoisomers during dehydration (2, 3, 14) with a resulting loss of the provitamin A activity of the beta-carotene as well as possible loss of the pigmentation potency of the xanthophyll. Since dehydrated alfalfa is used in poultry rations primarily as a source of xanthophyll pigmenters and fat soluble vitamins (carotene, vitamin E, and vitamin K), the prevention of isomer formation is desirable. Furthermore, high temperatures during dehydration may not only enhance isomerization but could also cause oxidative and destructive losses of these labile compounds.

The present study presents data obtained with a pilot scale alfalfa dehydrator, showing the temperature effect, during dehydration, upon the carotene and xanthophyll content of the alfalfa meal. This information is correlated with the moisture content of the alfalfa meal and the extent of beta-carotene isomerization.

Materials and Methods

A pilot scale Arnold alfalfa dehydrator was employed at the site of an industrial plant so as to utilize the fresh plant

material being commercially dehydrated. The input temperature of the pilot dehydrator was regulated by controlling gas flow to the burner. The stack gas or outlet temperature was regulated by controlling the feed rate of fresh alfalfa to the dehydrator.

Samples of the fresh plant material were collected as it passed from the loading platform onto the dehydrator elevator. An average fresh sample was obtained by collecting three samples at 5-minute intervals. Then, after allowing ample retention time, two resultant dehydrated samples were collected (after grinding in a hammer mill) at 5-minute intervals.

The fresh plant material was thoroughly and rapidly chopped in a Hobart food chopper. The samples were placed in sealed plastic bags and aliquots taken for analyses and moisture determination within 10 minutes following chopping. Moisture of the fresh and dried alfalfa was determined by drying in a forced draft oven at 110° C. for 24 hours. Carotene and xanthophyll were extracted by mixing 50 grams of fresh alfalfa in a Virtis high speed electric blender for one minute with 175 ml. of acetone. The extract was filtered and the residue washed with 50 ml. of acetone and re-extracted by blending with 125 ml. of acetone. The combined extract was made to volume (500 ml.) and used for determining both the ratio of beta-carotene isomers and the total carotene and xanthophyll content. Extracts of the dehydrated alfalfa meal were prepared by soaking 2-gram portions overnight at room temperature with 30 ml. of Skellysolve B-acetone (7:3).

The method of Bickoff and Thompson (3) was used to separate and determine

the stereoisomers of beta-carotene. Total apparent carotene and xanthophyll of the fresh and dehydrated alfalfa were determined by the procedure of Kohler, Knowles, and Livingston (9), which consists of treating the extract with alkali to saponify the chlorophyll prior to chromatography and eluting the xanthophyll fraction from the chromatographic column with a mixture of Skellysolve B-acetone-methanol (85:10:5). The xanthophyll mixture eluted contains cryptoxanthin, lutein, zeaxanthin, violaxanthin, and neoxanthin, and their main isomers.

Yellow pigments remaining on the column after elution of the xanthophyll pigments were eluted with a mixture of Skellysolve B-acetone-methanol (80:10:10). This more polar fraction is referred to as "polyoxy" carotenoids and consists largely of oxidation products of naturally occurring carotenoids.

Results and Discussion

As shown in Table I, the total carotene was relatively constant during dehydration. In only one instance was there an appreciable loss (inlet temperature 1200° F., outlet temperature 320° F.), and this may have been due to the variability of the carotene content of the fresh plant material.

The carotene and xanthophyll content of the fresh alfalfa varied from day to day and also varied during any particular day. However, the ratio of the beta-carotene isomers in fresh alfalfa was constant; neo-B, 7% all trans, 84%; neo-U, 9%. The formation of cis isomers of beta-carotene during dehydration is apparently a function of the outlet temperature and the moisture

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Table I. Stability of Carotenoids during Alfalfa Dehydration

Temperature of Dryer, ° F.		Moisture of Meal, %	Carotene, Mg./Lb. ^a		beta-Carotene Isomers, %			Xanthophylls, Mg./Lb. ^a		Polyoxy Meal, Mg./Lb. ^a	Ratio Xanthophyll to Carotene	
Inlet	Outlet		Fresh	Meal	Neo-B	All trans	Neo-U	Fresh	Meal		Fresh	Meal
1200	220	8.6	92	97	27	63	10	207	190	None	2.3	2.0
	270	1.6	117	119	44	45	11	261	170	5	2.2	1.4
	320	0.3 ^b	129	113	46	36	18	280	118	12	2.2	1.0
1400	220	9.0	121	124	25	65	10	260	235	None	2.1	1.9
	270	4.0	108	110	40	50	10	244	166	5	2.3	1.5
	320 ^c	0.7 ^b	122	129	47	35	18	259	129	14	2.1	1.0
1600	220	11	102	110	27	63	10	226	211	None	2.2	1.9
	270	3.2	125	127	44	45	11	284	163	17	2.3	1.3
	320	1.2 ^b	128	128	47	33	20	280	116	24	2.2	0.9

^a Dry basis.

^b Slight charring due to lower moisture content of meal.

^c Actual inlet temperature was slightly over 1300° F.

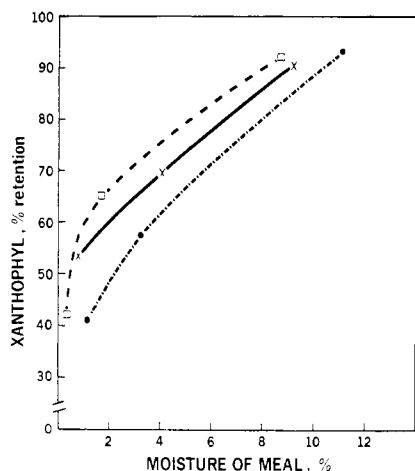


Figure 1. Correlation of xanthophyll retention at three inlet temperatures with moisture of alfalfa meal

----- 1600° F.
 ————— 1400° F.
 1200° F.

content of the meal. This is in agreement with Thompson, Bickoff, and Maclay (7-1) who found increased isomerization of the beta-carotene of dried alfalfa juice with increasing amounts of heat.

Since no polyoxy xanthophylls were present in fresh alfalfa, this formation is also a function of temperature of dehydration and moisture content of the alfalfa meal. However, only a portion of the loss in total xanthophyll is accounted for by the amount of polyoxy formed. During dehydration some of the xanthophyll has been converted to colorless products.

The xanthophyll content was greatly affected at the higher outlet temperatures and lower moisture levels (Table I). Since the temperatures measured were gas temperatures rather than product

temperatures, and since evaporation, which tends to cool the product, occurs as long as moisture is present, residual moisture appears to be important in affecting losses of xanthophylls and isomerization of beta-carotene. A plot of xanthophyll retention vs. moisture content (Figure 1) shows highly significant correlations. Further work will be necessary to delineate completely the interactions of process variables.

In the dehydrated alfalfa industry, it is a common practice to assume that the ratio of xanthophyll to carotene is constant in all meals, and to calculate the xanthophyll content of a given sample of the meal by employing a factor times the carotene content. The factor employed ranges from 1.2 to 2.0 parts of xanthophyll to 1.0 part carotene (7). As shown in Table I, the ratio of xanthophyll to carotene was quite constant in the fresh alfalfa; however, in the dehydrated alfalfa, the ratio varied from 2.0 to 0.9, depending upon the conditions of dehydration. Each sample of alfalfa meal must be analyzed for its true xanthophyll content. Alfalfa meals prepared under carefully controlled dehydration practices could contain more than twice the amount of xanthophyll of those prepared by less favorable procedures.

Acknowledgment

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Literature Cited

(1) American Dehydrators Assoc., Kansas City, Mo., private communication, 1961.
 (2) Bickoff, E. M., Livingston, A. L., Bailey, G. F., Thompson, C. R.,

J. AGR. FOOD CHEM. 2, 563 (1954).
 (3) Bickoff, E. M., Thompson, C. R., J. Assoc. Offic. Agr. Chemists 32, 775 (1949).
 (4) Bickoff, E. M., Thompson, C. R., Livingston, A. L., Van Atta, G. R., Guggolz, Jack, J. AGR. FOOD CHEM. 3, 67 (1955).
 (5) Graham, W. R., Jr., U. S. Patent 2,353,029 (July 4, 1944).
 (6) Griffith, R. B., Thompson, C. R., Botan. Gaz. 111, 165 (1949).
 (7) Hoffman, E. J., Lum, F. G., Pitman, A. L., J. Agr. Res. 71, 361 (1945).
 (8) Kohler, G. O., Beier, Elizabeth, Bolze, C. C., Poultry Sci. 34, 468 (1955).
 (9) Kohler, G. O., Knowles, R. E., Livingston, A. L., Western Regional Research Laboratory, U. S. Department of Agriculture, Albany, Calif., unpublished data, 1966.
 (10) Mitchell, H. L., Beauchene, R. E., Silker, R. E., J. AGR. FOOD CHEM. 2, 939 (1954).
 (11) Silker, R. E., Schrenk, W. G., King, H. H., Ind. Eng. Chem. 36, 831 (1944).
 (12) Taylor, M. W., Russell, W. C., J. Nutrition 16, 1 (1938).
 (13) Thompson, C. R., Ind. Eng. Chem. 42, 922 (1950).
 (14) Thompson, C. R., Bickoff, E. M., Maclay, W. D., Ibid., 43, 126 (1951).
 (15) Thompson, C. R., Bickoff, E. M., Van Atta, G. R., Kohler, G. O., Guggolz, Jack, Livingston, A. L., U. S. Dept. Agr. Tech. Bull. No. 1232, 1960.
 (16) Thompson, C. R., Maclay, W. D., Feed Age 22, October 1952.
 (17) Wilder, O. H. M., Bethke, R. M., Poultry Sci. 20, 304 (1941).

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