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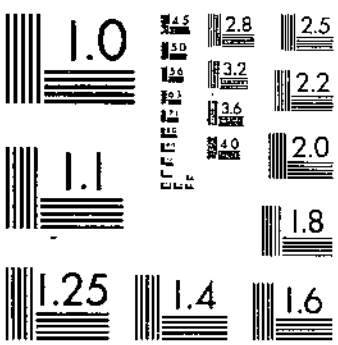

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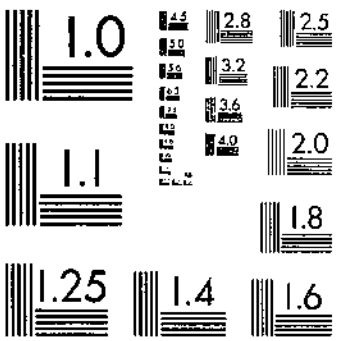

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Xanthophyll, Carotene, and α-Tocopherol Stability in Alfalfa as Affected by Pilot- and Industrial-Scale Dehydration

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Xanthophyll, Carotene, and α-Tocopherol Stability in Alfalfa as Affected by Pilot- and Industrial-Scale Dehydration

By A. L. Livingston, R. E. Knowles, and G. O. Kohler[1]

INTRODUCTION

Dehydrated alfalfa meal is a valuable source of vitamins and nutrients for addition to poultry and livestock mixed feeds. Three of these nutrients, xanthophyll, carotene, and α-tocopherol are susceptible to oxidation (*4, 5, 12, 16, 20, 21*)[2] and isomerization (*1, 2, 4, 19*) losses during drying and storage of the plant material. Recent studies at this laboratory have indicated that up to 70 percent of the total xanthophyll of fresh alfalfa may be lost during dehydration (*9, 10*). The ease of isomerization and oxidation of the xanthophyll is due principally to the large number of conjugated double bonds in the molecule (*22*). Since the xanthophyll of dehydrated alfalfa is an effective source of pigment for broiler skin and eggs (*7*), it is important to know the dehydration conditions that will retain a high proportion of xanthophyll in the dehydrated meal. Moreover, the conditions that serve to produce high-xanthophyll meal will produce a meal high in carotene (provitamin A), α-tocopherol (vitamin E), and other nutrients.

Lutein, violaxanthin, and neoxanthin make up about 90 percent of the total xanthophylls of fresh alfalfa (*1, 10*). In addition to these three, cryptoxanthin and zeaxanthin make up 6 to 8 percent, while minor components such as zeinoxanthin make up the remaining 2 to 4 percent (*11*). Lutein, which comprises 60 to 70 percent of the total xanthophyll, is an effective pigmenter, while violaxanthin and neoxanthin have little if any pigmenting activity for broiler skin

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[2] Italic numbers in parentheses refer to literature cited, p. 18.

(*8*). Therefore, retention of lutein during dehydration is of utmost importance in the production of high-quality poultry feeds. In addition to oxidative and enzymatic losses, isomerization of xanthophyll and carotene readily takes place during heating (*9*, *19*, *22*). Thus, a previous study at this laboratory demonstrated that while fresh alfalfa produced only 5 major xanthophyll bands during chromatography, dehydrated meal produced at least 40 xanthophyll bands (*1*).

The present study was carried out to ascertain the retention of xanthophyll, carotene, and α-tocopherol during pilot- and industrial-scale alfalfa dehydration and subsequent meal storage.

PROCEDURE

Dehydration of Alfalfa

Initial studies used a pilot Arnold dryer (model 5D45–12) with a rated capacity of 1,000 pounds of water per hour. The flame to the burner was regulated manually, controlling both the input and output temperature. In a second study, a fan was incorporated into the system to provide a negative air pressure and regulate the throughput time of the alfalfa chops. The flame to the burner was automatically regulated by the output temperature of the dryer.

Following initial evaluation on a pilot scale, studies were extended to include an industrial-scale Arnold dryer at Dixon, Calif., and a Stearns-Roger dryer at Clarksburg, Calif. The industrial dryers were in sufficiently close proximity that fresh alfalfa chops from the same field could be dehydrated in both dryers simultaneously.

The throughput time of the alfalfa chops was determined by the addition of heat-resistant aluminum paint to the fresh plant material as it moved up the elevator, and ascertaining the time required for the paint-treated alfalfa to appear among the dehydrated chops.

Chemical Analyses

Total xanthophyll and carotene were determined by the improved (TX) procedure of Kohler and others (*6*). The individual xanthophylls were determined by the thin-layer chromatographic (TLC) procedure of Nelson and Livingston (*14*). α-Tocopherol and related isoprenoid compounds were determined by the method of Livingston and others (*12*).

Meal moisture determinations were made by drying the samples for 24 hours at 105° C. in a forced-draft oven. The fresh plant material was prepared for analysis by rapid freezing between layers of dry ice, followed by drying in a Virtis freeze dryer. Both the freeze-dried material and the dehydrated chops were ground so as to pass through a 40-mesh screen before analysis.

RESULTS AND DISCUSSION

Stability of Xanthophylls and Carotene During Dehydration

Pilot-scale dehydration

Study 1.—The first study employed the pilot Arnold dehydrator. The input temperature was regulated manually by controlling the flame to the burner. The feed rate of the fresh chops entering the dehydrator, as well as the input temperature, determined the outlet or rear-end temperature. The average retention time of the alfalfa chops in the drum was 15 minutes in this initial study.

A direct correlation of xanthophyll retention, dehydrated meal moisture, and, inversely, the outlet temperature of the dehydrator, was obtained as presented in table 1. At the three high levels of meal moisture, 90 percent or more of the xanthophyll was retained, but xanthophyll losses were very large at the three lowest moisture levels. The carotene, however, was relatively stable during dehydration.

Although xanthophyll was the most stable in the higher moisture meals during dehydration, that in the 3 to 4 percent moisture meals was more stable during storage. Accordingly, during dehydration plus storage the total xanthophyll and carotene retention of the medium-moisture meals was greater than that of the high-moisture meals. This emphasizes the necessity of treating the dehydrated meal with

TABLE 1.—*Stability of xanthophyll and carotene during pilot alfalfa dehydration and storage* [1]

Dryer temperature		Moisture of meal	Xanthophyll			Carotene		
			Initial content in fresh alfalfa	Retained in meal		Initial content in fresh alfalfa	Retained in meal	
At inlet	At outlet			After dehydration	After dehydration and storage		After dehydration	After dehydration and storage
° F.	*° F.*	*Pct.*	*Mg./kg.*	*Pct.*	*Pct.*	*Mg./kg.*	*Pct.*	*Pct.*
1, 600	220	11. 0	498	93	29	225	100	28
1, 400	220	9. 0	573	90	31	267	100	35
1, 200	220	8. 6	456	92	28	205	100	35
1, 600	270	4. 0	538	68	34	238	100	49
1, 400	270	3. 2	626	57	34	275	100	58
1, 200	270	1. 6	575	65	32	258	100	50
1, 600	320	1. 2	616	41	26	282	100	52
1, 400	320	0. 7	570	50	26	269	100	49
1, 200	320	0. 3	616	42	24	284	88	43

[1] Stored 12 weeks at 90° F.

an antioxidant such as ethoxyquin (*17*, *18*), or storage of the meal under an inert atmosphere (*3*), or storage in the cold (*5*) in order to preserve the xanthophyll and carotene of the higher moisture meals.

Study 2.—In order to achieve efficient regulation of the flow of meal through the drum and to maintain a higher moisture meal more easily, a variable-speed fan was installed on the outlet side of the cyclone. In this way a negative air pressure was established at the outlet of the drum. Gas flow to the burner was made automatic depending on the quantity of fresh plant material entering the drum, thus maintaining a fixed outlet temperature. The results are presented in table 2.

At the lowest outlet temperature of 235° F. there was a direct correlation of xanthophyll retention with meal moisture, fan speed and, inversely, with retention time. This same correlation was present at the 255° F. outlet temperature, except at this outlet temperature all of the meals contained less than 3 percent moisture. The relationship of the outlet temperature to meal moisture and hence to xanthophyll retention may be illustrated at the constant fan speed of 1,800 r.p.m. and constant retention time of 7 to 11 minutes. The maximum xanthophyll retention, 66 percent, was obtained at the middle outlet temperature of 245° F. when the meal moisture level, 5.2 percent, was highest. The sample obtained at the 255° F. outlet temperature had the lowest meal moisture and lowest xanthophyll retention.

Carotene was again found to be

TABLE 2.—*Effect of outlet temperature, retention time, and meal moisture on stability of xanthophyll and carotene during pilot alfalfa dehydration*

Dryer temperature at outlet	Fan speed	Time in dryer	Moisture of meal	Retained in meal	
				Carotene	Xanthophyll
° F.	*R.p.m.*	*Min.*	*Pct.*	*Pct.*	*Pct.*
235	2, 200	4–8	8. 4	92	86
235	2, 000	6–10	4. 2	87	69
235	1, 800	7–11	3. 9	88	60
245	2, 200	4–8	9. 6	95	76
245	1, 800	7–11	5. 2	99	66
245	1, 400	10–14	6. 4	93	57
255	2, 000	6–10	2. 8	90	59
255	1, 800	7–11	2. 9	92	50
255	1, 600	9–13	2. 7	87	48
255	1, 400	10–14	2. 2	93	48

more stable than xanthophyll during dehydration. Even at the low moisture levels in the meals obtained at the 255° F. outlet temperature, there were only small losses of carotene.

Industrial-scale dehydration

An industrial-scale dehydration study was conducted, using an Arnold dryer at Dixon, Calif., and a Stearns-Roger dryer at Clarksburg, Calif.

In the Arnold dryer the fan speed was varied to change retention time and outlet temperature, resulting in different meal moisture levels.

During trial 1 at the Stearns-Roger site, a variable-speed fan was adjusted to regulate the flow of alfalfa chops through the drum, maintaining a nearly constant meal moisture level while varying the outlet temperature. In trial 2 the burner or inlet temperature was varied along with the draft, so that a constant outlet temperature was maintained while varying the meal moisture. Total carotene and xanthophyll as well as the three principal xanthophylls—lutein, violaxanthin and neoxanthin—were determined. The results are presented in table 3.

In this study the dryers were frequently operated at less than optimum conditions, therefore, the most severe carotene losses might not occur under normal operating conditions. No clear correlation of carotene loss to any of the variable conditions was demonstrated, except that in each trial the highest carotene retention was found in the highest moisture meal.

Xanthophyll was much less stable than carotene during dehydration and was directly dependent on dehydrated meal moisture. Dehydrator outlet temperature changes for the range studied had little effect on xanthophyll retention when meal moisture was maintained constant. The highest moisture meal (12.2 percent), prepared in the Stearns-Roger dryer (trial 2), retained 72 percent of the initial xanthophyll. The lowest moisture meal (1.5 percent) retained only 37 percent of its initial xanthophyll.

Lutein was the most stable of the three principal xanthophylls. In only a single sample (Arnold dryer 330° F.) was there less than 50-percent retention. Neoxanthin was less stable than lutein but considerably better preserved during dehydration than violaxanthin. The retention of the three xanthophylls as well as total xanthophyll could be correlated with meal moisture (*fig.* 1). Neoxanthin loss was most rapid in lower moisture meals, while violaxanthin loss was severe in both high- and low-moisture meals. Loss of violaxanthin during dehydration is not as critical as the loss of lutein, since violaxanthin is not effective as a pigmenter for either broiler skin (*8*) or hens eggs (*13*).

Isomerization of lutein

Isomerization of carotenoids causes the formation of *cis*-isomers,

TABLE 3.—*Stability of xanthophyll and carotene during industrial-scale alfalfa dehydration*

Dryer temperature		Time in dryer	Moisture of meal	Content in fresh alfalfa					Retained in dehydrated meal				
At inlet	At outlet			Carotene	Total xanthophyll	Lutein	Neoxanthin	Violaxanthin	Carotene	Total xanthophyll	Lutein	Neoxanthin	Violaxanthin
° *F.*	° *F.*	*Min.*	*Pct.*	*Mg./kg.*	*Mg./kg.*	*Mg./kg.*	*Mg./kg.*	*Mg./kg.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
						Industrial Arnold Trial 1							
1,600	300	3–5	9.2	378	831	610	92	116	91	61	71	58	24
1,600	310	4–6	7.8	353	736	488	92	150	76	50	62	43	19
1,600	330	5–7	2.3	353	798	591	82	121	82	36	42	12	13
						Stearns-Roger Trial 1							
850	240	13–17	8.3	411	876	614	97	169	94	63	62	50	17
900	270	8–10	9.5	407	905	581	116	213	87	60	79	42	16
900	300	4–6	9.9	411	861	590	102	169	88	64	80	47	20
900	330	5–7	5.9	363	822	576	106	140	97	55	67	32	21
						Stearns-Roger Trial 2							
420	250	3–5	12.2	349	784	529	97	160	100	72	84	85	35
820	250	4–6	7.1	411	861	580	102	179	83	56	67	62	16
1,000	250	4–6	2.5	339	808	537	92	179	86	41	53	37	13
900	275	3–5	7.1	445	972	638	97	242	85	57	70	63	16
920	275	2–4	2.1	445	1,005	643	111	252	67	40	50	40	13
950	275	2–4	1.5	363	847	555	92	198	79	37	50	26	13

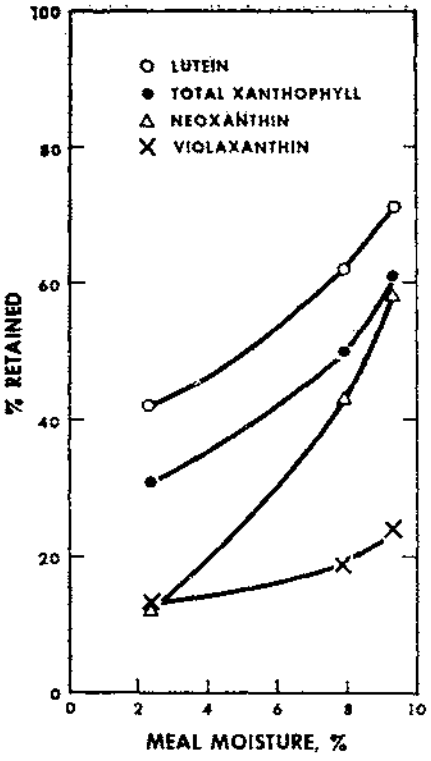

FIGURE 1.—Correlation of xanthophyll retention and alfalfa meal moisture level during alfalfa dehydration.

with shifts in the spectra to lower wavelengths and consequent loss of absorption at the all-*trans* peaks. Accordingly, the formation of *cis*-isomers of lutein during dehydration may actually contribute a very significant part of the total measured lutein loss during dehydration.

Dehydrated meal samples prepared in all of the dryers were analyzed for total lutein and two lutein isomers, employing a TLC procedure (*14*) for the analysis (table 4).

It is readily apparent that dehydrated meal moisture is inversely correlated with both the percentage of lutein isomers present and the loss of lutein during dehydration. In meals with lower moisture levels, the total lutein absorbance at 475 $m\mu$ represents a greater percentage of *cis*-isomers of lutein. In all samples the increase in the total quantity of lutein isomers during dehydration ranged from 50 to 150 percent more than that present in the freeze-dried material.

In all three dryers substantial losses of lutein, from 29 to 61 percent, occurred during alfalfa dehydration. The higher losses for each dehydrator were in the lower moisture meals.

Stability of Individual Xanthophylls During Storage

A substantial part of the dehydrated alfalfa meal commercially produced is stored for several months before being fed. Stability of the individual xanthophylls over this extended period is essential.

Meal samples prepared in the pilot Arnold dryer were stored up to 10 weeks in open shell vials at 90° F. The samples were analyzed initially and at 4 and 10 weeks for all-*trans* lutein, violaxanthin, neoxanthin, 2 lutein *cis*-isomers, and deoxylutein. The results are presented in table 5.

During storage at high temperature, losses would be expected from

TABLE 4.—*Isomerization of lutein during alfalfa dehydration*

Dryer temperature at outlet	Moisture of meal	Fresh alfalfa		Dehydrated meal	
		Total lutein content	Content of isomers [1]	Total lutein content	Content of isomers [1]
°F.	Pct.	Mg./kg.	Pct.	Mg./kg.	Pct.
			Industrial Arnold		
300	9.2	610	13	431	20
310	7.8	488	14	301	25
330	2.3	591	14	250	36
			Pilot Arnold		
270	2.8	538	10	305	19
300	1.6	534	13	252	23
330	1.5	552	18	214	45
			Stearns-Roger		
275	7.1	638	12	449	20
275	3.1	643	10	324	25
275	1.5	555	18	276	32

[1] Percent isomers calculated as percent of total lutein absorbance at 475 mμ.

TABLE 5.—*Stability of individual xanthophylls during storage* [1] *of*

Dryer temperature		Moisture of meal	Neoxanthin			All-*trans* lutein			Isomer-I (lutein)		
			Content in fresh alfalfa	Retained in meal		Content in fresh alfalfa	Retained in meal		Content in fresh alfalfa	Retained in meal	
At inlet	At outlet			Stored 4 weeks	Stored 10 weeks		Stored 4 weeks	Stored 10 weeks		Stored 4 weeks	Stored 10 weeks
°F.	°F.	Pct.	Mg./kg.	Pct.	Pct.	Mg./kg.	Pct.	Pct.	Mg./kg.	Pct.	Pct.
1,600	220	11.0	70	50	19	297	50	35	37	50	30
1,400	220	9.0	68	53	19	352	53	40	35	53	34
1,200	220	8.6	31	50	50	275	67	41	24	67	48
1,600	270	4.0	44	70	40	242	80	65	22	71	54
1,400	270	3.2	48	75	55	245	82	68	31	72	36
1,200	270	1.6	22	94	80	216	99	69	18	100	100+
1,600	320	1.2	22	90	35	117	74	62	18	75	55
1,400	320	0.7	18	65	60	148	66	62	18	54	70
1,200	320	0.3	22	70	45	145	67	63	15	60	60

[1] Stored at 90° F.

isomerization and oxidation of the all-*trans* xanthophylls. In turn, *cis*-isomers would be formed from the all-*trans* xanthophylls, and simultaneously there would be losses of the *cis*-derivatives due to oxidation. In the 3.2 percent (or less) moisture meals, lutein isomer-II increased during storage. Only in the 1.6 percent moisture meal did lutein isomer-I increase. The all-*trans* lutein was least stable in the high-moisture (8.6 to 11.0 percent) meals, and most stable in the medium-moisture (3.2 to 4.0 percent) meals. These meals had been prepared at an outlet temperature of 270° F. and inlet temperatures of 1,400 and 1,600° F. Due to the higher initial lutein content of the high-moisture meals the content of lutein following storage was about the same in the medium- and high-moisture meals.

Lutein was better retained during storage than either neoxanthin or violaxanthin as shown in figure 2. Losses of neoxanthin and violaxanthin are due to oxidation and isomerization of 5,6-epoxy xanthophylls to their 5,8-epoxy derivatives, which absorb at shorter wavelengths in the visible spectra. This may account for the more rapid loss of violaxanthin, a 5,6,5′,6′-diepoxy xanthophyll, compared to neoxanthin, a 5,6-monoepoxy or lutein, a nonepoxy xanthophyll.

Loss of total xanthophyll was greater in the high-moisture (9 percent) alfalfa meal than in the medium-moisture (4 percent) or low-moisture (1.2 percent) meal during storage (fig. 3). This indicates that the more labile xanthophylls are lost more rapidly during dehydration to low moisture levels and those

dehydrated alfalfa, effect of dehydration conditions

Isomer-II (lutein)			Deoxylutein			Violaxanthin		
Content in fresh alfalfa	Retained in meal		Content in fresh alfalfa	Retained in meal		Content in fresh alfalfa	Retained in meal	
	Stored 4 weeks	Stored 10 weeks		Stored 4 weeks	Stored 10 weeks		Stored 4 weeks	Stored 10 weeks
Mg./kg.	*Pct.*	*Pct.*	*Mg./kg.*	*Pct.*	*Pct.*	*Mg./kg.*	*Pct.*	*Pct.*
18	50	38	29	47	40	29	47	40
15	55	52	31	58	32	40	30	25
13	34	49	18	64	62	57	25	13
13	67	45	22	70	54	59	25	21
7	80	100+	22	80	60	33	54	40
4	100+	100+	15	100+	70	20	80	67
11	72	60	59	63	48	31	72	55
9	52	100+	66	51	52	37	55	52
4	52	100+	42	53	55	22	70	70

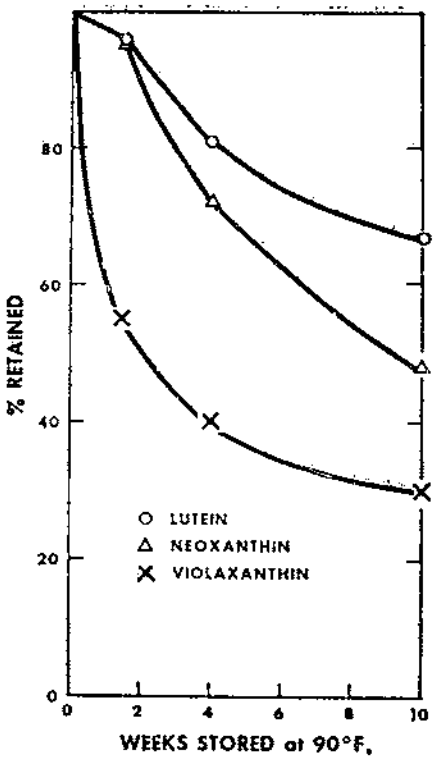

FIGURE 2.—Retention of lutein, neoxanthin, and violaxanthin during storage of dehydrated alfalfa meal.

remaining are more stable during storage.

The overall loss of total xanthophyll through the dehydrator and during a 10-week storage period was greater for the very low moisture meal than for either the high- or medium-moisture meals (fig. 4). Thus although actual storage loss of xanthophyll was greater for the high-moisture meals, this loss did not offset the very large loss of xanthophyll during dehydration to a low-moisture meal. It would therefore be most desirable for a dehydrator to control meal moisture carefully in order to preserve xanthophyll during dehydration. Measures such as inert-gas storage and antioxidant treatment should then be employed to enhance xanthophyll retention during storage.

Stability of α-Tocopherol and Related Isoprenoids During Dehydration and Storage

Vitamin E (α-tocopherol) is becoming increasingly important in poultry and animal nutrition. Fresh alfalfa and also dehydrated alfalfa meal are excellent sources of this vitamin. Stability of α-tocopherol and related compounds that possess antioxidant activity is an important factor in determining the value of the dehydrated meal in poultry and animal rations.

Fresh alfalfa was dehydrated in both the industrial Arnold and Stearns-Roger dryers as described above in the xanthophyll studies. Certain of the meals were stored for 12 weeks at 90° F. in open shell vials. The samples were analyzed for both α-tocopherol and related reducing isoprenoids.

The results (table 6) show that α-tocopherol is better retained during dehydration of alfalfa to the higher moisture levels. In each series of samples the lowest-moisture meal lost more α-tocopherol during dehy-

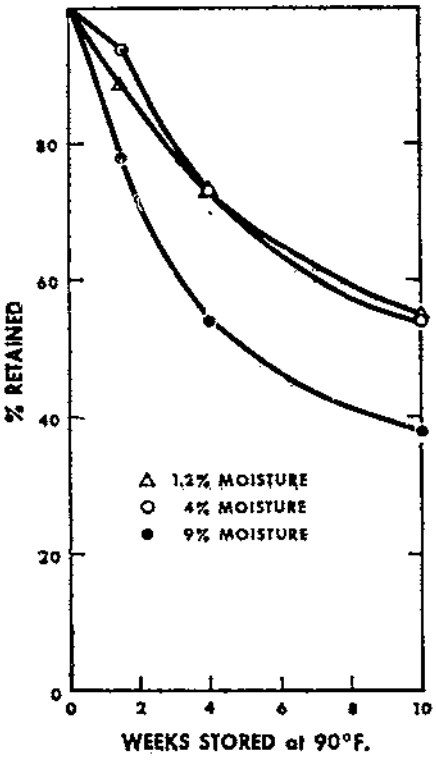

FIGURE 3.—Effect of alfalfa meal moisture level on total xanthophyll retention during storage.

dration than the higher moisture meals. The retention of α-tocopherol was much greater than the retention of xanthophyll for comparable moisture samples. In three of the samples only 5 percent of the α-tocopherol was lost during dehydration.

Retention of the related isoprenoid compounds, which include solanachromene, was negatively correlated with meal moisture. The 1.5 percent moisture meal prepared in the Stearns-Roger had no apparent isoprenoid loss during dehydration. This may be due to cyclization of plastoquinone to form solanachromene during dehydration (*15*). Likewise the isoprenoid content of two of the four stored samples actually increased during storage. However, the α-tocopherol was rapidly lost during storage; 69 to 80 percent of the α-tocopherol originally present in the fresh plant material was lost during dehydration and storage.

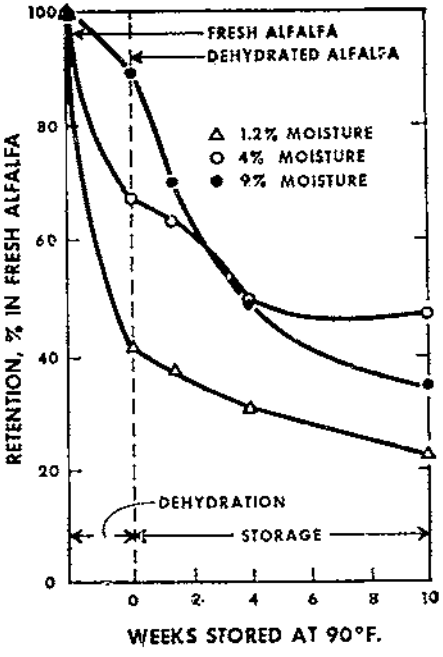

FIGURE 4.—Effect of meal moisture on retention of total xanthophyll during alfalfa dehydration and storage.

TABLE 6.—*Stability of α-tocopherol and related compounds during alfalfa dehydration and storage*

Dryer temperature		Time in dryer	Moisture of meal	Fresh alfalfa		Retained in meal							
						After dehydration				After dehydration and 12 weeks' storage			
						α-Tocopherol		Related compounds		α-Tocopherol		Related compounds	
At inlet	At outlet			Content of α-tocopherol	Content of related compounds	Amount	Percent	Amount	Percent	Amount	Percent	Amount	Percent
° F.	° F.	Min.	Pct.	Mg./100 g.	Mg./100 g.	Mg./100 g.	Pct.	Mg./100 g.	Pct.	Mg./100 g.	Pct.	Mg./100 g.	Pct.
						Arnold Dehydrator							
1, 600	300	3–5	9. 2	18. 1	9. 3	17. 2	95	10. 9	100+	4. 9	27	12. 9	100+
1, 600	330	5–7	2. 3	22. 8	13. 9	18. 1	79	13. 6	98	---	--	----	-----
						Stearns-Roger Dehydrator							
420	250	3–5	12. 2	18. 8	10. 1	13. 7	73	5. 0	50	3. 7	20	8. 7	86
820	250	4–6	7. 1	17. 5	11. 5	12. 9	74	3. 8	33	3. 7	21	9. 1	79
1, 000	250	4–6	2. 5	20. 6	9. 4	13. 8	67	7. 9	84	6. 3	31	12. 1	100+
900	275	3–5	7. 1	19. 6	14. 7	18. 7	95	9. 2	65	---	--	----	-----
920	275	2–4	3. 1	19. 0	13. 9	18. 1	95	11. 0	79	---	--	----	-----
900	275	2–4	1. 5	21. 9	9. 1	17. 8	81	9. 1	100	---	--	----	-----
1, 000	250	4–6	7. 1	17. 5	11. 5	12. 9	74	3. 8	33	[1] 9. 0	52	[1] 21. 2	100+

[1] Added 0.15% ethoxyquin.

SUMMARY

The effects of dehydration conditions on the stability of xanthophyll, carotene, and α-tocopherol in alfalfa meal were studied, by using industrial-scale Arnold and Stearns-Roger dryers and a pilot-scale modified Arnold dryer. The conclusions follow:

1. Xanthophyll losses during dehydration ranged from 28 to 73 percent, depending on meal moisture, outlet temperature of dehydrator, and dehydrator retention time, in both industrial-scale dehydrators and the pilot-scale model.

2. The xanthophyll in alfalfa meals dehydrated to medium-moisture (3 to 4 percent) levels was better retained during storage than that in high-moisture (8 to 12 percent) meals.

3. Lutein was more stable during dehydration than either neoxanthin or violaxanthin.

4. Isomerization during dehydration and storage accounted for considerable loss of all-*trans* lutein.

5. Carotene and α-tocopherol were more stable than xanthophyll during dehydration.

6. α-Tocopherol was better retained during dehydration in the alfalfa meals dried to the higher moisture levels.

7. α-Tocopherol was rapidly lost during storage.

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