Xanthophyll and Carotene Loss during Pilot and Industrial Scale Alfalfa Processing

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Xanthophyll losses, from 28 to 73%, and carotene losses, from none to 33%, occurred during pilot and industrial scale alfalfa dehydration. These losses were correlated with the moisture content of the meal. Twenty samples of fresh alfalfa were analyzed and the effect of dehydration on the three major xanthophylls was determined. Lutein was more stable than either neoxanthin or violaxanthin under normal drying conditions.

The importance of alfalfa meal as a source of xanthophyll for poultry pigmentation makes it imperative to know the processing conditions which will provide a meal of high xanthophyll content. Both xanthophyll and beta-carotene are subject to oxidation losses during storage (Griffith and Thompson, 1945; Silker *et al.*, 1944; Thompson *et al.*, 1960; Thompson and Maclay, 1952; Wilder and Bethke, 1941) and stereoisomerization during dehydration and heating (Bickoff *et al.*, 1954; Thompson *et al.*, 1950) resulting in loss of provitamin A activity of the beta-carotene and pigmenting potency of the xanthophylls. A preliminary pilot scale alfalfa dehydration study at this laboratory revealed that as much as 50% of the xanthophyll of alfalfa meal was destroyed during dehydration (Livingston *et al.*, 1966). This loss could be correlated with both the outlet temperature of dehydration and moisture of the meal. In contrast, the total carotene was relatively unaffected during dehydration, although the beta-carotene isomers increased with higher dehydration temperatures and lower meal moisture levels.

The present study was conducted on both a pilot and industrial scale to delineate further the effects of dehydration variables on total xanthophyll and carotene. Effects on the three principal xanthophylls of alfalfa, lutein, violaxanthin, and neoxanthin, and on three lutein isomers were also studied by thin-layer chromatographic analysis of fresh alfalfa samples and their corresponding dehydrated meals.

MATERIALS AND METHODS

Two full-scale industrial plants were employed, one utilizing an Arnold alfalfa dehydrator, and the other a Stern-Rogers dehydrator. A pilot scale Arnold dehydrator was also employed at one of the plant sites. The sites were sufficiently close so that it was possible on a given day to dehydrate alfalfa from the same field in all three dehydrators. The output temperature of the dehydrators was regulated by controlling gas flow to the burner; in addition, it was possible at the Stern-Rogers dehydrator to regulate the speed of flow of alfalfa through the drum so that the moisture content of the meal varied while the output temperature remained constant, or conversely, the output temperature was varied while the moisture of the meal remained constant.

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Fresh alfalfa was collected from the dehydrator elevator by collecting three grab samples at 1-minute intervals and combining them for freezing, grinding, and analysis. Throughput time was then ascertained by applying a high-temperature-resistant aluminum paint to the fresh alfalfa and using its throughput time as a guide. A resultant dehydrated alfalfa sample was obtained by collecting and combining a series of small samples as the dried material left the dehydrator.

The fresh alfalfa was placed in plastic bags, sealed, and quickly frozen between layers of dry ice. The frozen material was returned to the laboratory and immediately freeze-dried. Following freeze-drying, the plant material was ground through a No. 40 screen and analyzed for xanthophyll and carotene by the procedure of Kohler *et al.* (1967). This consists of treating the extract with alkali prior to chromatography and eluting the xanthophyll fraction from the chromatographic column with a mixture of hexane–acetone–methanol (80:10:10). Portions of extract were also concentrated and the xanthophylls isolated by the thin-layer chromatography (TLC) procedure of Nelson and Livingston (1967). Lutein, violaxanthin, and neoxanthin, as well as three isomers of lutein, were quantitatively determined by this TLC procedure.

Moisture of the dehydrated alfalfa meal was determined by drying in a forced draft oven at 110° C. for 24 hours. Total xanthophyll, carotene, the three principal xanthophylls, and the three lutein isomers were determined in the same manner as that described above for the fresh freeze-dried alfalfa samples.

Identification of Lutein Isomers. Solutions of pure lutein, neoxanthin, and violaxanthin in hexane were treated with iodine in the sunlight by the procedure of Zechmeister and Tuzson (1938). The isomerized xanthophyll solutions were then streaked on TLC plates and the isomers of lutein separated in the same manner as that used for the alfalfa extracts. Although neither neoxanthin or violaxanthin gave isomers which were separated from the parent compound by the TLC procedure employed, cis isomers of violaxanthin were apparently formed as indicated by a shift of the spectrum to a shorter wavelength, and a shift of the spectrum of neoxanthin to the longer wavelength suggested the formation of a trans isomer of neoxanthin (Curl, 1965). Table I presents the spectral absorption maxima of the isomers prepared from pure crystalline lutein and the apparent lutein isomers isolated from the alfalfa samples by TLC. Aliquots of the isomers

of authentic lutein were also compared on TLC with the apparent lutein isomers prepared from the alfalfa samples and their respective R_f values were identical. The spectral and TLC comparisons showed that the compounds from the crude alfalfa extracts were indeed isomers of lutein.

RESULTS AND DISCUSSION

In the previous study, little carotene loss was detected during the dehydration operation; this study indicates a loss of from none to 33% of the initial carotene depending on the dehydration conditions. The apparent difference may be accounted for in the method of analysis of the fresh alfalfa for carotene and xanthophyll. In the previous procedure, a few minutes elapsed during the interval of chopping and blending, allowing the very active carotenoid enzymes time to destroy both carotene and xanthophyll. This results in low apparent carotene and xanthophyll values for fresh alfalfa, making the losses during dehydration appear lower than they actually are. In the present procedure, the plant material was quickly frozen and freeze-dried prior to analysis. A trial comparison of the two methods of analysis on two samples of fresh alfalfa demonstrated an apparent loss of 15% of carotene and 10 to 17% of xanthophyll during the chop and solvent blend procedure (Table II). Accordingly, the fresh alfalfa samples were analyzed by the freeze-dried method during the present study.

Although it was not possible to separate the two process variables, output temperature and moisture of meal, in the pilot and industrial Arnold dryers, because of the design of the control system, direct correlation between xanthophyll content and moisture of meal was achieved in the operation of the Stern-Rogers dehydrator (Table III). In trial 1, the outlet temperature of all three dehydrators was varied through a similar range; however, the retention time in the two Arnold dehydrators remained constant, while that of Stern-Rogers was decreased at the higher outlet temperatures resulting in an almost constant moisture content of the alfalfa meals produced at the four outlet temperatures. At the highest outlet temperature (330° F.) there was a slight decrease in meal moisture resulting in a slight decrease in xanthophyll content; however, at the three lower outlet temperatures the moisture of the meals was almost constant, and as a result, despite differences in dryer temperatures, the loss of xanthophyll was held nearly constant. In trial 2, the outlet temperature of the Stern-Rogers dehydrator was held constant while the moisture of the meal was varied by changing the retention time (Table III). The results show an inverse correlation between moisture of meal and xanthophyll loss during dehydration. Figure 1 demonstrates that adequate moisture of meal is a critical factor in minimizing xanthophyll loss.

Lutein, the principal xanthophyll of alfalfa, proved to be the most stable of the three major xanthophylls during de-

Table I. Comparison of Isomerization Products of Pure Crystalline Lutein and Xanthophylls Purified by TLC from Alfalfa Meal

Sample	Spectral Absorption Maxima, Mμ, Ethanol–Hexane (1 to 1)
All-*trans*-lutein	475, 446, 419
Alfalfa lutein	475, 446, 419
Lutein isomer I	467, 441, 330
Alfalfa lutein isomer I	466, 440, 330
Lutein isomer II	469, 441, 329
Alfalfa lutein isomer II	470, 443, 329
Lutein isomer III	471, 443, 420, 331
Alfalfa lutein isomer III	471, 443, 420, 331

Table II. Comparison of Methods of Analysis of Fresh Alfalfa

	Freeze-Dried, Mg./Kg.[a]		Solvent Blend, Mg./Kg.[a]	
Sample	Carotene	Xanthophyll	Carotene	Xanthophyll
1	339	717	295	652
2	232	528	198	450

[a] Dry basis, average of duplicate analyses.

Table III. Loss of Xanthophyll and Carotene during Dehydration

Dryer	Outlet Temperature of Dryer, °F.	Moisture of Meal, %	Carotene, Mg./Kg.[a]			Xanthophyll, Mg./Kg.[a]		
			Fresh	Meal	Loss, %	Fresh	Meal	Loss, %
Industrial	300	9.2	378	344	9	831	508	39
Arnold	310	7.8	353	267	24	736	368	50
(trial 1)	330	2.3	353	290	18	798	286	64
Pilot Arnold	270	2.8	329	290	12	784	363	53
(trial 1)	300	1.6	334	290	13	750	295	61
	330	1.5	348	281	19	821	220	73
Stern-Rogers	240	8.3	411	387	6	876	552	37
(trial 1)	270	9.5	407	353	13	905	542	40
	300	9.9	411	363	12	861	552	36
	330	5.9	363	353	3	822	450	45
(trial 2)	250	12.2	349	353	0	784	568	28
	250	7.1	411	339	17	861	484	44
	250	2.5	339	290	14	808	329	59
	275	7.1	445	377	15	972	556	43
	275	3.1	445	300	33	1005	397	60
	275	1.5	363	286	21	847	310	63

[a] Dry basis, average of duplicate analyses.

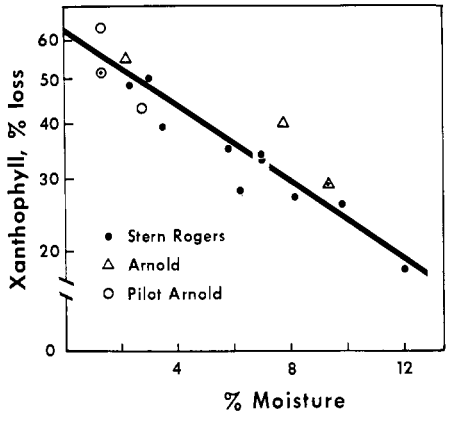

Figure 1. Correlation of xanthophyll loss at three dehydrators with moisture of meal

hydration (Table IV), undergoing losses from 21 to 74%, depending on the processing conditions. Although detectable levels of the isomers of lutein were found in the fresh alfalfa at the dehydrator, these isomers were probably due to light and enzyme activity, since 15 to 30 minutes elapsed from the time the alfalfa was cut until it reached the dehydrator. Two of the three isomers of lutein actually increased during dehydration. This might be expected, since isomerization of xanthophylls takes place during heating. However, this increase in the isomers of lutein accounts for only a small part of the loss of all-*trans*-lutein under the more severe dehydration conditions. Under these severe dehydrating conditions, the zone termed lutein isomer III was actually made up of at least three minor bands, suggesting the formation of additional isomers as well as possible oxidation products of lutein.

Neoxanthin, which is a trihydroxy monoepoxide xanthophyll (Curl, 1965; Donohue *et al.*, 1966), was considerably more stable than violaxanthin, a dihydroxy diepoxide, except at the highest outlet temperature and lowest meal moistures in the Arnold dryers. This is probably due to the labile nature of the epoxide groups. Neoxanthin losses ranged widely (15 to 94%) while the violaxanthin losses were consistently high (65 to 87%).

During the course of this investigation, 20 samples of fresh alfalfa were analyzed by TLC for the three principal

Tab.e IV. Loss of Xanthophylls during Dehydration

Dryer	Outlet Temperature of Dryer, °F.	Moisture of Meal, %	Neoxanthin, Mg./Kg.[a]			Lutein Isomer I, Mg./Kg.[a]			Violaxanthin, Mg./Kg.[a]		
			Fresh	Meal	Loss, %	Fresh	Meal	Loss, %	Fresh	Meal	Loss, %
Industrial	300	9.2	92	53	42	19	29	+50	116	15	87
Arnold	310	7.8	92	39	57	24	48	+100	150	29	81
(trial 1)	330	2.3	82	10	88	24	19	20	121	29	76
Pilot	270	2.8	102	34	67	15	24	+60	140	29	79
Arnold	300	1.6	92	24	74	15	19	+27	136	24	82
(trial 1)	330	1.5	121	7	94	53	10	81	150	24	85
Stern-	240	8.3	97	48	50	19	34	+79	169	29	83
Rogers	270	9.5	116	48	58	19	34	+79	213	34	84
(trial 1)	300	9.9	102	48	53	19	34	+79	169	34	80
	330	5.9	106	34	68	15	24	+60	140	29	79
Stern-	250	12.2	97	82	15	15	48	+220	160	56	65
Rogers	250	7.1	102	63	38	19	48	+153	179	29	84
(trial 2)	250	2.5	92	34	63	15	48	+220	179	22	87
	275	7.1	97	71	27	19	48	+153	242	38	84
	275	3.1	111	44	60	19	48	+153	252	34	87
	275	1.5	92	24	74	24	44	+83	198	26	87

Dryer	Outlet Temperature of Dryer, °F.	Moisture of Meal, %	Lutein Isomer II, Mg./Kg.[a]			Lutein, Mg./Kg.[a]			Lutein Isomer III, Mg./Kg.[a]		
			Fresh	Meal	Loss, %	Fresh	Meal	Loss, %	Fresh	Meal	Loss, %
Industrial	300	9.2	44	29	34	532	344	35	15	29	+93
Arnold	310	7.8	34	9	74	421	225	47	9	19	+110
(trial 1)	330	2.3	44	0	100	508	160	69	15	71	+374
Pilot	270	2.8	30	15	50	484	247	49	9	19	+110
Arnold	300	1.6	39	5	87	465	194	59	15	34	+130
(trial 1)	330	1.5	34	5	85	450	117	74	15	82	+440
Stern-	240	8.3	34	9	74	552	421	24	9	15	+67
Rogers	270	9.5	39	9	77	514	402	22	9	15	+67
(trial 1)	300	9.9	34	15	56	528	397	25	9	24	+150
	330	5.9	34	9	73	518	315	39	9	38	+300
Stern-	250	12.2	15	24	+60	484	349	28	15	24	+67
Rogers	250	7.1	24	15	38	518	305	41	19	19	...
(trial 2)	250	2.5	34	9	74	479	208	57	9	19	+110
	275	7.1	48	19	60	562	358	36	9	24	+167
	275	3.1	39	15	62	576	242	58	9	19	+110
	275	1.5	57	9	84	455	189	59	19	34	+79

[a] Dry basis, average of duplicate analyses.

Table V. Percentage of Principal Xanthophylls in 20 Fresh and Dehydrated Alfalfa Samples

Neoxanthin		Lutein Isomer I		Violaxanthin		Lutein Isomer II		Lutein		Lutein Isomer III	
Fresh[a]	Meal[a]	Fresh[a]	Meal[a]	Fresh[a]	Meal[a]	Fresh[a]	Meal[a]	Fresh[a]	Meal[a]	Fresh[a]	Meal[a]
13	9	2	6	18	8	4	4	62	68	1	5
12	7	2	8	17	7	5	2	62	65	2	11
15	5	3	5	19	10	4	3	57	39	2	38
11	16	3	6	18	11	4	3	62	61	2	3
11	11	2	6	14	3	5	6	66	68	2	6
11	4	3	6	14	10	6	...	65	55	2	24
11	9	2	6	19	4	4	2	63	76	1	3
13	9	2	6	24	6	2	2	58	74	1	3
12	9	2	6	20	6	4	3	61	72	1	4
13	8	2	6	17	7	4	2	63	69	1	8
11	14	2	9	20	8	2	4	60	61	2	4
12	13	2	10	21	6	3	3	60	63	2	4
12	10	2	14	22	6	4	3	59	60	1	6
11	8	3	13	23	7	7	3	54	57	2	11
11	11	2	12	25	8	4	4	57	60	1	5
10	13	2	9	24	7	5	3	57	63	1	4
13	16	3	1	22	9	4	6	57	51	2	6
12	8	3	9	21	5	5	3	58	70	1	5
15	10	3	9	20	10	4	3	55	61	2	5
15	12	4	8	19	8	5	3	56	65	2	3
Mean 12.2	10.1	2.5	7.8	19.9	7.3	4.3	3.3	59.6	62.9	1.5	7.9

[a] Average of duplicate analyses.

xanthophylls. The percentage each of these comprised of the total xanthophyll content is presented in Table V. Since neither zeaxanthin nor cryptoxanthin comprised over 2 to 5% of the total xanthophylls, and they were not readily separated (zeaxanthin from lutein, and cryptoxanthin from carotene), they were not determined in this study. In an earlier study in this laboratory, Bickoff *et al.* (1954), using a single sample of fresh alfalfa and a single sample of meal, found the relative proportions of the xanthophylls to be: in fresh alfalfa, lutein, 40%; neoxanthin, 19%; and violaxanthin, 34%; in the meal, lutein, 46%; neoxanthin, 14%; and violaxanthin, 16%. In the present study, the average values in the fresh alfalfa were: lutein, 60%; neoxanthin, 12%; violaxanthin, 20%; in the meal, lutein, 63%; neoxanthin, 10%; and violaxanthin, 8%.

Because of the acid-labile nature of violaxanthin and neoxanthin, one would expect these xanthophylls to be less effective pigmenters than lutein. Accordingly, total xanthophyll assay of the fresh alfalfa and of mildly treated meals, where neoxanthin and violaxanthin make up a larger percentage of the total, might overestimate the broiler or egg pigmenting potency of that material. The analytical method used, however, underestimates neoxanthin and, to some extent, violaxanthin. Furthermore, much of the violaxanthin is lost even under the mildest dehydration conditions. The total xanthophyll analysis of the meal, therefore, reflects chiefly its lutein content and may be used as a guide to pigmentation potency.

The quantity of lutein loss is dependent upon the moisture content of the meal and is sensitive to changes in processing conditions. This study indicates that an alfalfa dehydrator could, by maintaining a meal moisture level of 7 to 9%, produce a product containing up to 77% more lutein, which would possess correspondingly greater poultry pigmenting potency.

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