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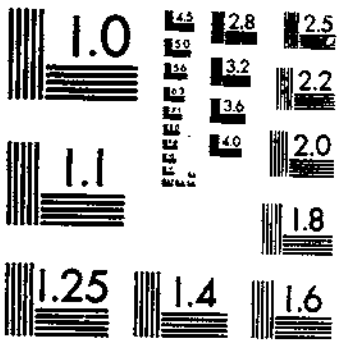

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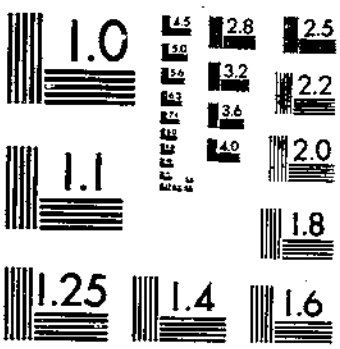

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CAROTENE STABILITY IN ALFALFA
As Affected by Laboratory- and Industrial-Scale Processing

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[1] Mention of specific products does not imply recommendation by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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Carotene Stability in Alfalfa as Affected by Laboratory- and Industrial-Scale Processing

By C. R. Thompson, E. M. Brickoff, G. R. Van Atta, G. O. Kohler, J. Guggolz, and A. L. Livingston, *Western Utilization Research and Development Division, Agricultural Research Service* [1]

NEED FOR THIS STUDY

Dehydrated forages, principally alfalfa, are used widely as "vitamin concentrates" for addition to mixed poultry and livestock feeds. These products supply provitamin A (carotene), vitamin E (tocopherols), xanthophylls (poultry pigmenting factors), vitamin K, vitamin C, and B-vitamins. Of special interest are carotene, vitamin E, and xanthophylls, for these nutrients are unstable during dehydration and subsequent storage. From the feed manufacturer's viewpoint, they are expensive ingredients possibly because they have not been synthesized in quantity. Many factors may influence the retention of these valuable nutrients, such as the starting plant material, conditions of dehydration, addition of stabilizers, and conditions of storage.

Although little data are available on the carotene content of any particular variety of alfalfa or grass, it is generally recognized that the leaves of legumes are 3 to 10 times higher in valuable nutrients than the stems. Thus, as the plant matures and the ratio of leaf to stem decreases, the vitamin and protein content per unit weight also decreases.

Dehydration may have a number of effects on nutrients. Thompson and others (*15*) [2] and Brickoff and Thompson (*2*) observed that dehydration of alfalfa caused moderate isomerization of the carotene but that about 90 percent of the total provitamin was retained. Xanthophylls are isomerized in a similar way (*1*), except that many more separate compounds appear after dehydration because the fresh plant material contains five major plus seven minor xanthophylls when harvested.

Several chemical stabilizers have been proposed for preserving fat-soluble vitamins in dehydrated forages. Kephart in 1949 (*8*) proposed the use of N,N'-diphenyl-p-phenylenediamine (DPPD) and two diphenylamine derivatives. DPPD was used in dehydrated alfalfa and mixed feeds for several years until adverse toxicity reactions were observed with rats and its use was disallowed by the Federal Food and Drug Administration. Thompson (*13, 14*) showed that several phenylenediamines and dihydroquinolines, particularly

[1] Western Regional Research Laboratory, Albany, Calif.

[2] Italic numbers in parentheses refer to literature cited, p. 13.

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1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (Ethoxyquin), preserved carotene in alfalfa meal. Ethoxyquin is presently allowed at the rate of 0.3 pound per ton in dehydrated forages.

Mitchell, Beauchene, and Silker (*11*) synthesized several phenylenediamines which were active in preserving carotene in forages and made a significant contribution by defining how oils and heating can aid antioxidants in stabilizing fat-soluble vitamins. Bickoff and others (*3*) showed that several different oils gave increased carotene stability in alfalfa meal, and later Livingston and others (*10*) showed that animal fats and antioxidants were effective in preserving xanthophyll concentrates in mixed feeds.

A number of ways of storing dehydrated forages for preserving vitamins have been proposed. The first method that was applied commercially was cold storage. The effect of temperature on the stability of carotene and vitamin E in dehydrated forages was described by Kohler and others (*9*). Losses occurred even at low temperatures though at a greatly reduced rate. Because of this factor and the high expense of installation and operation of cold storage, this method of storing dehydrated forages was abandoned when a cheaper, more effective method became available. By this new method forage was stored under inert gas as patented by Graham (*4*). Hoffman and others (*7*) published data which confirmed Graham's work and showed the effect of different levels of oxygen on carotene retention. Inert gas storage as proposed by Graham is now used widely. It is estimated that about 40 percent of the dehydrated forage produced is presently being stored in this way.

Hart and Halverson (*5*, *6*) tried sealing alfalfa meal in gas-tight plastic-lined bags and showed that this treatment plus adjustment of moisture content to about 10 to 12 percent gave good carotene retention. However, this method never proved to be practical, because the bags weren't sturdy enough and were too expensive, and producing a gas-tight closure was difficult.

The studies presented here were carried out at the Western Regional Research Laboratory, Albany, Calif., to define more clearly a number of factors that influence the carotene stability in dehydrated forages. Kinds of dehydration equipment; drying conditions, both laboratory and industrial; variety of alfalfa; and effect of antioxidants and oils were all studied. Most of the analyses show how carotene is affected but, because both xanthophylls and tocopherols occur abundantly in dehydrated forages and have similar stabilities, the results can be extended to predict how these nutrients are affected as well.

CAROTENE STABILITY DURING COMMERCIAL DEHYDRATION

Stability in Alfalfa Meal From Different Dehydrators

Samples of freshly dehydrated alfalfa meal were obtained from as many U.S. dehydration firms as possible. Samples were received within 1 week of production. They were stored in closed containers at −20° F. until storage stability tests were begun. Moisture con-

tents of 7 to 10 percent were representative of trade samples. This amount depends upon the relative humidity of the atmosphere in which they were kept during shipment. Information was obtained as to the source and variety of alfalfa, the kind of dryer, and the inlet-outlet temperature (table 1). Carotene, tocopherol, and "antioxidant activity" were determined. The carotene was assayed by chromatography (*2*) and tocopherols by the method of Parker and McFarlane (*12*).

"Antioxidant activity" is a term used to express the amount of total stabilizing effect for carotene contained in a particular alfalfa meal. This effect is caused partly by tocopherols but also by other unidentified antioxidants. It is a value obtained by extracting the dried alfalfa meal with petroleum ether. This crude fraction containing all the antioxidant is then serially diluted and added to a standard amount of carotene dissolved in white mineral oil. Alpha tocopherol is likewise added in serial dilutions to carotene in mineral oil and all samples are stored at 167° F. The amount of tocopherol required to stabilize 50 percent of the carotene for 24 hours is taken as a standard value. The amount of extract required to give an equal effect is likewise determined. Thus it is possible to measure the antioxidant activity against alpha tocopherol and arrive at this value.

The results showed a considerable variation in initial carotene. However, these values were typical of market samples produced during 1952. Total antioxidant activity was four to seven times the tocopherol value, showing that alfalfa contains appreciable antioxidant-active material which will preserve carotene in mineral oil but which remains to be identified.

Carotene retention in the intact meal at 149° F., a measure of storage stability, showed that a two-stage dryer produced the most unstable meal. All other dryers gave meals of comparable stability. The cause for instability in samples from the two-stage dryer was unknown. Additional tests were conducted to find out whether something was indeed reducing the carotene stability.

Stability at Different Stages in Two-Stage Dehydration

In a small followup study of the two-stage drying equipment, alfalfa was sampled before drying and throughout the drying process. The dehydrator was peculiar in having a predryer which removed the first part of the moisture. It consisted of a revolving tube approximately 6 feet in diameter and 60 feet in length. Conveyor flights picked up the green material and showered it down through the hot gases from an oil-fired furnace. After the predryer, the partially dried material was finished in a single-pass conventional drum dryer fired by a separate furnace. In the study the initial sample was oven dried, a comparable sample was air dried, two samples were taken after the predryer and finished in two ways, and two final samples were taken at the end of the drum dryer. All samples were stored for 1 week at 149° F. As shown in table 2, the samples taken after predrying were less stable than the oven- or air-dried samples. Further attempts to find the cause of this reduction in stability were fruitless.

TABLE 1.—*Total antioxidant content and carotene stability in commercial alfalfa meals*

Source	Variety	Dryer	Dryer temperature		Carotene	Tocopherol	Antioxidant activity [1]	Carotene retained 1 week—149° F.
			Inlet	Outlet				
			°F.	°F.	P.p.m.	P.p.m.	P.p.m.	Percent
California	Chilean	Home built drum	1, 200	200	229	174	694	39
Do	California common	Arnold	1, 650	275	241	200	879	41
Do	do	do	1, 375	265	199	175	912	37
Do	do	Two-stage	1, 800	130	185	181	939	28
Do	do	Tunnel	350	125	260	246	1, 057	46
Do	do	Arnold	1, 800	200	189	140	1, 207	48
Do	Chilean	do	1, 600	275	214	144	808	42
Do	California common	Tunnel	350	125	192	193	913	49
Nebraska		Arnold 3 stage	1, 600	240	158	143	927	37
Kansas	Kansas common	Arnold	1, 700	215	138	118	896	49
Do	do	do	1, 700	210	144	141	921	46
Colorado	Buffalo	do	1, 700	180	209	182	1, 000	37
Ohio					243	270	1, 175	46
South Dakota	Cossack	Arnold	1, 000–1, 600	200	235	182	927	46
Minnesota					233	172	939	45
Texas		Arnold			143	100	1, 004	44
New York	Grimm		270–280	120	207	199	917	40
Missouri					125	107	787	41
California	(Leaf meal)	Laboratory dried			423	266	1, 727	46
Do		Sun cured			20	22	233	37

[1] Stabilizing effect of petroleum ether extract.

TABLE 2.—*Stability of carotene in alfalfa during two-stage dehydration*

Sample	Initial carotene	Carotene retained 1 week—149° F.
	P.p.m. (m.f.b.) [1]	*Percent*
Green:		
Oven dried (266° F.—10 minutes)	211	45
Air dried (room temp.—24 hours)	152	33
After predryer:		
Finished (194° F.—5 minutes)	209	24
Finished (77° F.—24 hours)	213	23
Fully dehydrated	195	23
	190	27

[1] M.f.b.=moisture free basis.

CAROTENE STABILITY AS AFFECTED BY BLANCHING AND HEATING

Effect of Blanching

To find out whether blanching would reduce the stability of carotene during storage, three lots of freshly cut alfalfa were each subdivided into four aliquot samples. One lot was oven dried at 266° F. for 50 minutes; the others were steam blanched in an autoclave at 15 pounds per square inch for 3 minutes. One blanched lot was vacuum dried for 16 hours at 149°; the other was oven dried as above. As shown in table 3, carotene retentions were similar in all samples after 1 week at 149°. Thus, blanching did not alter the storage stability of carotene, nor did it appear to have an immediate effect upon the carotene itself.

TABLE 3.—*Effect on carotene stability of blanching fresh alfalfa before drying*

Minutes of blanching	Subsequent drying		Initial carotene	Carotene retained 1 week—149° F.
	Time	Temperature		
	Minutes	*° F.*	*P.p.m.*	*Percent*
0 (control)	50	[1] 266	[2] 281±12	36±0.9
3	50	[1] 266	307±17	33±2
	Hours			
3	16	[3] 149	305±10	34±0.4

[1] Oven dried.
[2] Mean and standard error.
[3] Vacuum dried.

Effect of Heating

To test the carotene stability of alfalfa heated at 212° F., fresh-frozen alfalfa was chopped to short lengths and held at 212° in a circulating air oven for increasing periods up to 7½ hours. The samples were dry after three quarters of an hour and heating beyond this time represented excessive exposure to this temperature. The carotene was destroyed progressively during the heating, but the storage stability of the pigment remaining after heating for periods up to 2 hours was equal to that in material barely dried (table 4). After 2 hours of heating, the storage stability of carotene declined. These results indicate that prolonged heating not only destroys carotene but also reduces the stability of the remaining pigment.

To test the carotene stability of alfalfa heated to high temperatures, dried whole alfalfa was placed in a circulating air oven and held for periods up to 4 minutes at temperatures above 400° F. At this temperature scorching was evident after 1 minute. Carotene was destroyed rapidly and the stability of the remaining pigment was low (table 5). Thus, if excessive scorching occurs in dehydration, carotene stability is reduced.

TABLE 4.—*Effect of heating chopped alfalfa*[1] *on carotene loss during subsequent storage of meal*

Hours in oven at 212° F.	Initial carotene	Carotene retained 1 week—149° F.
	P.p.m.	*Percent*
¼	229	37
1	228	37
2	197	37
3	176	28
4	149	21
5½	112	18
7½	86	15

[1] Fresh-frozen alfalfa from Ryer Island, California.

TABLE 5.—*Effect of overheating dried whole alfalfa on carotene loss during subsequent storage of the meal*

Minutes in oven	Temperature	Initial carotene	Carotene retained 1 week—149° F.
	°F.	*P.p.m.*	*Percent*
0 (control)		468	42
1	478	295	18
3	464	38	13
4	455	44	13

Effect of Overheating and Underdrying at Dehydrator

A full-scale industrial trial was conducted at Dixon, Calif., to observe the effect on carotene stability in alfalfa meal of drying at different temperatures and also of underdrying and overdrying. The equipment used was a Heil-Arnold dryer[4] with automatic controls.

In the first trials drying was conducted at inlet temperatures ranging from 800° to above 1,500° F. In the second series, the rate of feed was varied and drying was conducted at 1,200°, 1,400°, and 1,500° to 1,600°, so that underdried, normal, and overdried materials were obtained at each temperature. Normal material is considered to be a product that contains from 8 to 9 percent moisture after grinding, underdried material contains from 10 to 12 percent, and overdried material contains less than 6 percent moisture. The storage tests showed that the inlet temperatures had little effect on carotene stability (table 6). Also, drying to 10 to 11 percent moisture gave the same stability as overdried, or even slightly scorched material. Thus, this equipment could be operated to give a rather wide range of moisture contents and the products would still have comparable stabilities. Why scorching in the commercial equipment gave no reduction in carotene stability is unknown, but perhaps the short time of heating caused the difference.

TABLE 6.—*Effect of underdrying and overdrying on carotene stability in a commercial dehydrator*

Inlet temperature, °F.	Dehydrator control gage	Moisture	Initial carotene[1]	Carotene retained 1 week—149° F.
		Percent	*P.p.m.*	*Percent*
800	Normal	10.9	308	29
1,200	Normal	9.8	260	28
1,400	Normal	7.6	282	27
1,530	Normal	7.0	255	29
1,540	Normal	7.6	211	31
1,500	Normal	6.3	301	26
1,200	Slightly wet	10.9	260	31
	Normal	10.2	237	29
	ditto	9.0	188	29
	ditto	8.3	181	37
	Slightly scorched	7.9	212	32
1,400	Slightly wet	10.9	190	24
	ditto	11.9	127	29
	Normal	5.9	292	33
	ditto	8.8	302	29
	ditto	9.0	159	29
	Slightly scorched	8.7	172	28
	Scorched	5.7	197	38
1,500	Wet	21.3	194	28
1,600	Normal	6.1	256	30
1,600	Slightly scorched	4.7	193	37

[1] Corrected for moisture.

[4] Mention in this publication of commercially manufactured equipment does not imply endorsement by the U.S. Department of Agriculture over similar equipment not mentioned.

Stability in Different Varieties of Alfalfa After Drying

A limited test was conducted to check the carotene stability of pure varieties of alfalfa. Samples were made available by the University of California at Davis. These varieties were grown in experimental plots and harvested at about the bud stage of maturity, and the materials were oven dried. All samples were more stable than the materials tested previously (table 7), possibly because the plants were less mature. Much more study would be necessary to prove that carotene stability is greater in one variety than in another.

TABLE 7.—*Carotene stability of pure varieties of alfalfa, harvested at equal maturity* [1]

Variety	Initial carotene content	Carotene retained after storage at 149° F. for—	
		1 week	2 weeks
	P.p.m.	*Percent*	*Percent*
Iran 28–4–23	306	53	31
Dwarf resistant 28–12–5	281	47	25
Wilt resistant 27–6–45	282	49	27
Hairy Peruvian	273	48	25
Wilt resistant 28–7–38	292	50	26
Turkistan	282	50	28
Grimm	266	52	29
Wilt resistant 28–7–39	301	49	27
Iran 28–3–19	312	48	23
Dwarf resistant 28–12–45	270	46	27
Dwarf resistant 28–11–5	288	47	25
Iran	247	47	22
Chilean	288	44	22
California common	236	53	27
Hardistan 270	270	47	23
Pilca Butta	254	49	27
India	214	47	23
Atlantic	235	50	25
Buffalo	248	49	25

[1] Dried in laboratory oven.

LABORATORY APPLICATIONS OF ANTIOXIDANTS FOR CAROTENE

Ethoxyquin and DPPD

In previous laboratory studies several chemical antioxidants (*13*, *14*) improved the stability of carotene in dehydrated alfalfa. To compare the stabilizing effect of Ethoxyquin and DPPD, a laboratory-scale experiment was performed in which increasing levels of these two antioxidants were tested. In each test 4 ml. of a Cellosolve (ethylene glycol monomethyl ether) solution of the antioxidant concerned was sprayed into 200 grams of alfalfa meal which was

being tumbled in a rotary mixer. Ethoxyquin was much more active per unit weight (table 8). Because the two series of results are not in the same range, direct comparison is impossible. However, extrapolation of the stability curves indicates that Ethoxyquin is about 10 times as active as DPPD in this trial.

TABLE 8.—*Effect of increasing levels of Ethoxyquin and DPPD on carotene stability*

Treatment, percent	Carotene retained 16 weeks—77° F.
	Percent
Control (untreated)	27
Solvent (no antioxidant)	26
Ethoxyquin:	
0.015	64
0.030	75
0.045	71
0.060	81
DPPD:	
0.015	32
0.030	37
0.045	40
0.062	46
0.125	58

Effect of Increasing Levels of Animal Tallow and Ethoxyquin on Carotene Stability

In another laboratory trial, five levels of animal tallow—from 1 to 5 percent—were added to dehydrated alfalfa with three levels of Ethoxyquin—0.005, 0.010, and 0.015 percent. The tallow and antioxidant were added as a solution to 25-gram samples of meal in evaporating dishes. Petroleum ether was added until a heavy slurry was obtained. The slurry was stirred before a fan to distribute the antioxidant and tallow and to remove the solvent. Two-gram samples were stored in shell vials at 77° F. for 1 year. The results showed that the untreated meal retained only 16 percent of the original carotene (fig. 1). As the level of oil and/or antioxidant was increased, better retention of carotene resulted.

INDUSTRIAL-SCALE APPLICATIONS OF ANTIOXIDANTS FOR CAROTENE

Ethoxyquin, DPPD, and DBH to Dehydrated Alfalfa

Despite many laboratory studies no published data were available on the effect of several chemical antioxidants for carotene in actual full-scale application. Accordingly, three compounds—Ethoxyquin,

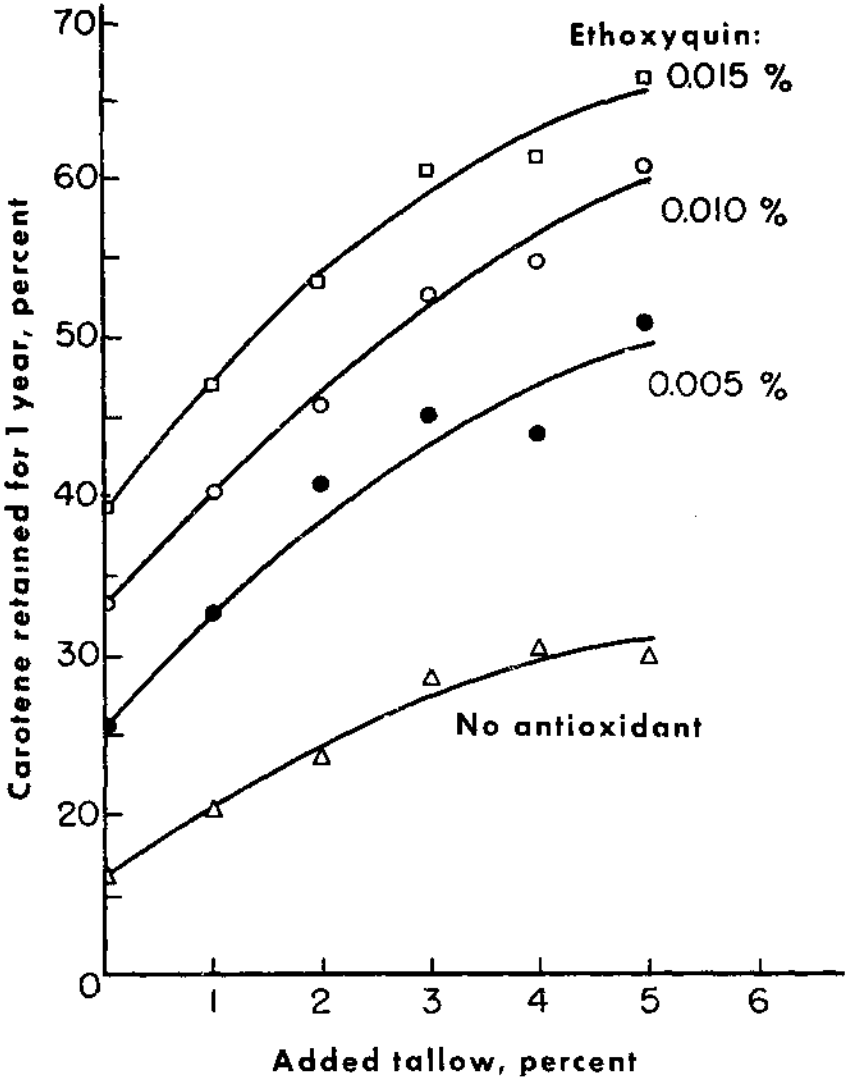

FIGURE 1.—Effect of increasing levels of animal tallow and of Ethoxyquin on carotene stability in alfalfa.

DPPD, and 2,5-ditert-butyl-hydroquinone (DBH) [5]—were tested experimentally on an industrial scale at the two-stage dehydrator referred to previously. For application the chemicals were dissolved in a carrier—rice oil or propylene glycol—in the proportions shown in table 9 and were added to dehydrated chopped "hay" after drying but before hammer milling. The carrier solution was metered by a

[5] According to the U.S. Food and Drug Administration, data on DBH are presently insufficient for determining its tolerance.

variable speed drive pump into an air stream which carried the dried hay from the dehydration plant to the hammer mill. No spray nozzle was used, but the high-velocity air stream broke up the oil solution into small droplets as it issued from a ¼-inch copper discharge tube. No trouble with buildup of oil solution or blinding of screens was encountered. Dust nuisance was reduced to about one-fourth that prevailing earlier.

TABLE 9.—*Effect of antioxidants on carotene retention in dehydrated chopped alfalfa "hay"; an industrial-scale application*

Antioxidant, percent	Carrier, percent	Carotene retained 24 weeks—77° F.
		Percent
Control (untreated)		23
Ethoxyquin: [1]	Rice oil:	
0.015	1.0	66
0.015	0.4	65
0.046	0.5	78
DPPD, 0.014 [2]	0.6	33
DBH, 0.06 [3]	Propylene glycol:	
	0.5	54

[1] 1,2-Dihydro-6-ethoxy-2,2,4-trimethylquinoline.
[2] N,N′-diphenyl-p-phenylenediamine.
[3] 2,5-Ditert-butyl-hydroquinone.

Ethoxyquin was much more effective than DPPD or DBH (table 9). Little if any difference was observed between the samples that had 0.4 and 1.0 percent of rice oil plus Ethoxyquin. Propylene glycol was used with DBH because of its poor solubility in vegetable oils. The temperature of 77° F. which was maintained during the 24-week storage period is considerably above that of average, temperate-climate, warehousing conditions. Thus, in actual practice the loss of carotene would be less than this amount.

Ethoxyquin and BHT as Antioxidants

Further industrial-scale trials of antioxidants were made at a dehydration plant in Firebaugh, Calif. Large samples of butylated-hydroxy-toluene (BHT) in both the crystal, feed-grade form and a stabilized powder were made available for experimental use. They were applied at different levels and the effects compared with these of Ethoxyquin.

The procedure was as follows: Antioxidant application was begun about 30 minutes before sampling to allow the plant to come to equilibrium. Then a control sample of untreated meal was taken from the main stream just after the hammer mill. Antioxidant, either dissolved in animal tallow or as a dry powder, was added immediately after as the meal entered a high-speed mixer. A second sample was

taken at the discharge of the mixer, timed so as to obtain the same material as previously. The treated meal was then pelleted. A final sample was taken of the finished, cooled pellets. This, too, was timed to obtain the same material as far as possible. Four to six replicates were taken, each set being taken at half hour intervals. Thus, an untreated sample, a treated sample, and a treated, pelleted sample were obtained in quick succession, after which a half hour was allowed to elapse before another set of replicates was taken. Several hours or days were allowed to elapse between runs with different antioxidants to allow the residual compound to be "washed-out" from the equipment.

Samples were analyzed for carotene and then stored at 77° F. for 24 weeks and reanalyzed. BHT at 0.025 or 0.05 percent in a solution of tallow had a small stabilizing effect (table 10). Dry, powdered BHT had a small favorable effect also. Ethoxyquin, as observed previously, gave a marked reduction in carotene loss. Pelleting seemed to cause no decrease or increase.

TABLE 10.—*Effect of antioxidants and pelleting on carotene stability after 24 weeks' storage, an industrial-scale test*

Replicates, number	Carotene remaining in—		
	Control	Meal	Pellets
	Percent [1]	*Percent*	*Percent*
		0.025% BHT+tallow	
4	37	41	40
4	35	39	37
4	38	40	40
		0.05% BHT+tallow	
6	39	42	43
		0.035% BHT (dry powder)	
6	41	44	46
		0.015% Ethoxyquin+tallow	
5	40	63	62
5	40	75	76

[1] Storage temperature 77° F.; average standard deviation of all values = ±2.4 percent.

SUMMARY

Studies were made at the Western Regional Research Laboratory, Albany, Calif., to define more clearly a number of factors that influence the stability of carotene in dehydrated forages, with the following results:

1. When dehydrated alfalfa from several processing plants was studied, it was found that all plants except one produced meal of comparable carotene stability. The cause of the carotene instability in the product of this single plant is unknown, but the effect is known to have been caused by the predrying treatment.
2. Blanching alfalfa before drying had little effect on subsequent carotene stability.
3. Continued oven heating of dehydrated alfalfa meal after drying destroyed much of the carotene and reduced the stability of the carotene that remained.
4. Underdrying or overdrying alfalfa in a commercial dehydrator gave meal that was comparable in carotene stability and content to material produced under more optimum drying conditions.
5. Increasing levels of animal tallow alone or with Ethoxyquin increased carotene retention in dehydrated alfalfa.
6. Carotene stability in several varieties of alfalfa was similar.
7. Industrial-scale application of antioxidants to alfalfa meal showed that Ethoxyquin was much superior to DPPD, DBH, or BHT. The superiority of Ethoxyquin over DPPD as an antioxidant was confirmed by laboratory studies.

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