Carotenoid Storage Stability in Drum Dried PRO-XAN

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The storage stability of the carotenoids in PRO-XAN, a low fiber high protein product from alfalfa, was studied and found to be comparable to that reported for carotenoids in alfalfa meal. The moisture level to which PRO-XAN was drum dried did not appear to affect the subsequent storage stability of the carotenoids. The necessity of reducing carotenoid loss due to oxidation during storage was clearly demonstrated. Ethoxyquin, commonly used commercially to protect carotenoids in dehydrated alfalfa meal, and added vegetable oil were effective in reducing these losses in the product. The water soluble constituents were also instrumental in retarding these losses and, in addition, their effect was additive with ethoxyquin.

Alfalfa has traditionally been a prime source of nutrients for poultry. Dehydrated alfalfa meal (dehy) is one of the major sources of xanthophylls used in poultry rations and is still sold with a guaranteed carotene content. Since these carotenoids rapidly deteriorate during unprotected storage, the dehydration industry uses the following means to improve their storage life: inert gas storage (Graham, 1944; Hoffman *et al.*, 1945) and treatment with an antioxidant (ethoxyquin, Thompson, 1951), alone or in combination with oil (Bickoff *et al.*, 1955; Livingston *et al.*, 1955; Mitchell *et al.*, 1954; Thompson *et al.*, 1960; Van Atta *et al.*, 1953). These means are effective in preventing oxidative losses.

High fiber and low energy ingredients are not desirable in modern high energy poultry rations, and alfalfa meal's xanthophyll content is one of the main reasons that it is still included in such rations. PRO-XAN (the name given to alfalfa protein xanthophyll concentrate) is a product of "wet fractionation" of alfalfa (Kohler *et al.*, 1968; Spencer *et al.*, 1970) and is ideally suited for use in high energy rations. A dark green free flowing powder, PRO-XAN contains most of the same nutrients as dehydrated alfalfa meal but in different proportions. For example, it contains two to three times as much xanthophyll, carotene, and protein as dehy, yet is virtually devoid of fiber. Studies have shown that xanthophyll extracts produce greater pigmentation in broilers than the meals from which they are prepared (Kuzmicky *et al.*, 1968; Livingston *et al.*, 1969). Preliminary feeding trials with PRO-XAN indicate that a similar improvement in xanthophyll availability will also be exhibited by this product (Kuzmicky, 1970).

Because of these factors, it is important to the poultry industry to know the storage stability of PRO-XAN. This paper reports the effects of a number of variables, including temperature, drying conditions, the water solubles, ethoxyquin, and added oil on carotenoid stability during long term storage of this product.

EXPERIMENTAL

Preparation of Concentrates. Wet protein-xanthophyll concentrate was prepared in the WRRL pilot plant as previously described (Spencer *et al.*, 1970). One batch of concentrate was water washed several times to remove the water soluble constituents. Several levels of the water solubles, 12½, 25, and 37½% on a dry weight basis, were added back to the washed concentrate prior to final drying. Samples of these preparations as well as samples from the two different batches of unwashed concentrate were dried on a 12 by 18 in. chromeplated double drum drier (Spencer *et al.*, 1970). The moisture content of the dried product was controlled by varying either the retention time on the drum or the drum temperature. Drum temperatures ranged from 280° to 320° F. In addition, comparable samples of the washed and the unwashed concentrates were freeze-dried in a RePP Sublimator (Model 15 FFD). Prior to analysis and storage, the PRO-XAN preparations were kept in sealed bottles at −10° F.

Preparation of Samples for Storage. The dried preparations were ground through a 20 mesh screen in a Wiley Mill. Ethoxyquin, 0.0125 to 0.125% by weight, and Wesson Oil, 5 to 20% by weight, either alone or in combination, were added to the dried samples following the procedure of Livingston *et al.* (1955). Five percent oil was readily absorbed by PRO-XAN without altering its friability. Moisture levels were determined by drying for 2 hr at 110° C in a forced-draft oven.

Storage Tests. One gram samples of the various treated and untreated PRO-XAN preparations were stored in the dark, either in open test tubes or in sealed vials at 36° or 100° F. Samples stored at 100° F were analyzed after 4, 8, and 12 weeks, and at 36° F, after 13, 26, and 39 weeks.

Carotenoid Analyses. Before and during storage, duplicate samples of each preparation were analyzed for total carotene and xanthophyll by the method of Kohler *et al.* (1967). The percent transmittance of the carotenoid solutions was determined on an Evelyn Photoelectric Colorimeter using its No. 440 filter for carotene and No. 470 filter for xanthophyll. The transmittance readings were converted into absorbance using a calibration curve which had been prepared by comparing identical solutions on the Evelyn colorimeter and on a Cary Model 15 spectrophotometer.

RESULTS AND DISCUSSION

Storing the concentrates in open containers permitted their moisture contents to equilibrate with the moisture in the air. Moisture levels, determined on selected samples after 4 weeks at 100° F, indicated that the equilibrium moisture for PRO-XAN was about 6 to 8%. The moisture equilibration which occurred during this study probably improved the storage stability of both high and low moisture samples, since Bailey *et al.* (1949) have shown that a moisture content ranging from 5 to 8% is optimum for carotene retention during the storage of open alfalfa meal samples. Nevertheless, the storage differences found should reflect the effects of drying conditions on the composition and, therefore, the stability of the concentrates.

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Freeze-drying is generally considered to be one of the mildest methods of drying available, and the one which has the least effect on the composition of the dried product. Freeze-dried samples, therefore, provide a convenient means of measuring the effects of drying on carotenoid stability during subsequent storage. It has been reported that ethoxyquin does not improve the excellent storage stability of freeze-dried alfalfa meal (Knowles *et al.*, 1968). However, ethoxyquin markedly improved carotenoid stability in the freeze-dried concentrate (Figure 1). An explanation for this difference between alfalfa meal and PRO-XAN may lie in the manner in which the concentrate is prepared. When alfalfa juice is heat coagulated, the concentrate separates from a second product, the brown juice (Spencer *et al.*, 1970). This brown juice, when concentrated, is called Alfalfa Solubles and contains the water soluble constituents, including the water soluble antioxidants, that were in the alfalfa juice. Although PRO-XAN is drained to remove the major portion of the brown juice, part of the water solubles, about 25% on a dry weight basis, are occluded in the concentrate. Consequently, PRO-XAN would contain less of the natural antioxidants to protect its carotenoids than would whole alfalfa. The ethoxyquin would replace these missing natural antioxidants, thereby increasing the stability of the freeze-dried concentrate.

Knowles *et al.* (1968) compared carotenoid stability in freeze-dried and dehydrated alfalfa meals during storage at 90° F, and found freeze-dried meals to be considerably more stable than dehydrated meals. They attributed this greater stability to the presence of natural antioxidants. Two types of natural antioxidants are present in alfalfa: those which are fat-soluble (Thompson *et al.*, 1960; Polesello and Vistarini, 1966), such as the tocopherols; and those which are water-soluble (Grossman *et al.*, 1969). The fat-soluble antioxidants are heat-labile (Livingston *et al.*, 1968), whereas the water-soluble ones are not affected by heat (Ben Aziz *et al.*, 1968). Drum drying, like dehydration, apparently destroys some of the naturally occurring fat soluble antioxidants since drum-dried PRO-XAN was less stable than the comparable freeze-dried samples (Figure 2a). Similarly, the drum dried preparation, washed to remove the water solubles, was less stable than the comparable freeze-dried material (Figure 2b). Another possible explanation for these observations would be

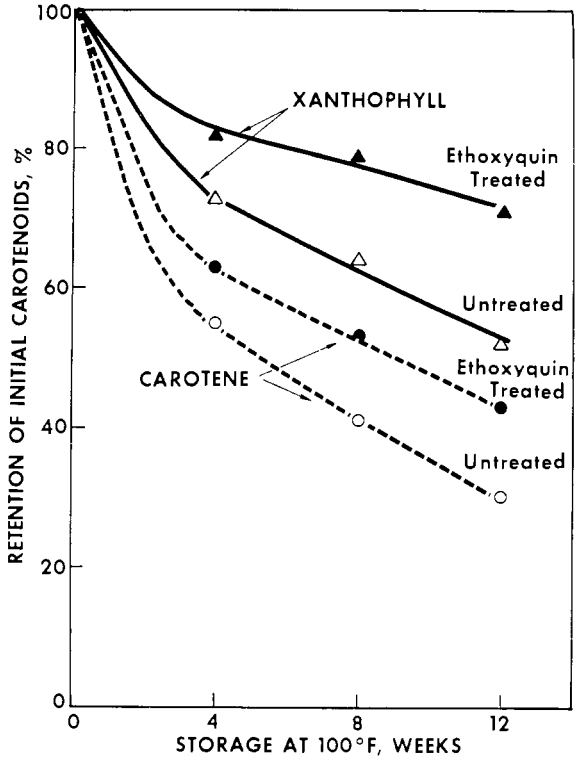

Figure 1. Effect of ethoxyquin (0.125%) on carotenoid stability during open storage of freeze-dried PRO-XAN

Carotene ○ ●
Xanthophyll △ ▲

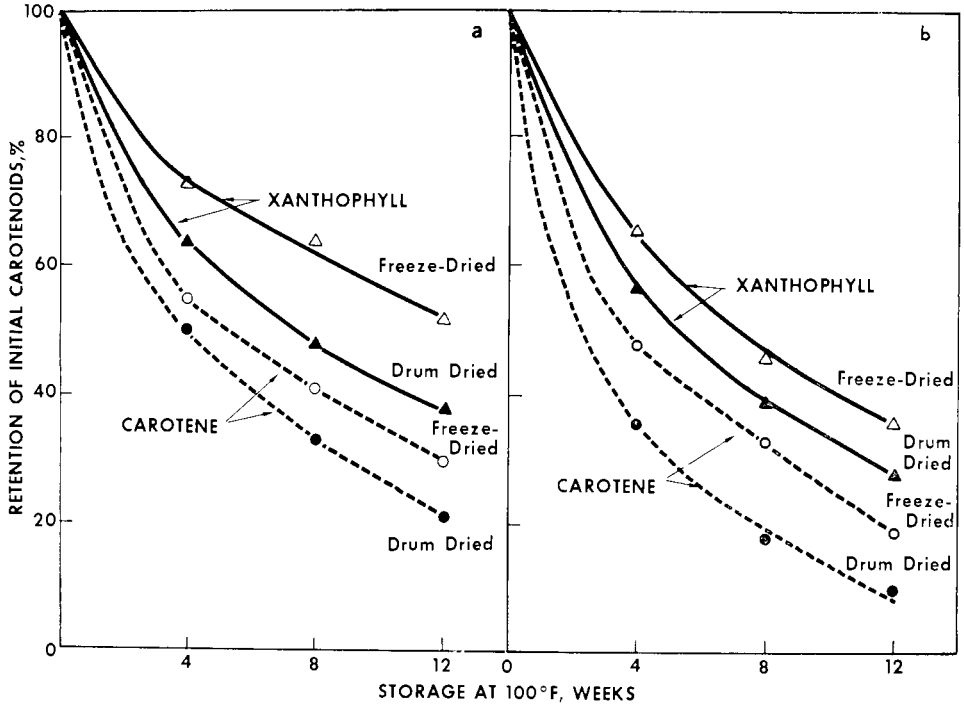

Figure 2. Effects of freeze-drying and drum drying on carotenoid stability in PRO-XAN (a) and washed concentrate (b) during open storage

Carotene ○ ●
Xanthophyll △ ▲

Table I. Effect of Water Solubles on Carotenoid Stability in Drum Dried PRO-XAN[a] Stored at 100° F

% Solubles Added	Initial, mg/lb		% Carotene Retained				% Xanthophyll Retained			
			4 Weeks		12 Weeks		4 Weeks		12 Weeks	
	Carotene	Xanthophyll	Untreated	Treated[b]	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	455	646	36	52	10	25	57	76	28	51
12½	403	581	42	61	15	34	59	79	32	59
25[c]	350	499	49	71	21	46	62	74	38	60
37½	292	399	55	71	25	52	60	72	38	56

[a] Samples dried to 11–13% moisture, stored in open containers. [b] Samples treated with 0.125% ethoxyquin. [c] Equivalent to normally prepared PRO-XAN.

Table II. Carotenoid Stability in PRO-XAN Drum Dried to Different Moisture Contents and Stored at 100° F[a]

Sample	% Moisture	Initial, Mg/lb		% Carotene Retained				% Xanthophyll Retained			
				4 Weeks		12 Weeks		4 Weeks		12 Weeks	
		Carotene	Xanthophyll	Untreated	Treated[b]	Untreated	Treated	Untreated	Treated	Untreated	Treated
PRO-XAN[c] Batch 1	7	339	489	56	75	25	56	69	79	42	68
	9	327	473	59	73	27	52	70	79	46	69
	11	330	482	57	67	28	47	69	78	47	68
	13	330	491	57	67	29	47	69	77	48	69
PRO-XAN[c] Batch 2	8	209	286	42	74	12	46	60	83	27	56
	9	208	275	40	73	13	44	56	82	28	59
	11	215	294	42	72	13	45	56	79	28	58

[a] Stored in open containers. [b] Samples treated with 0.125% ethoxyquin. [c] Samples prepared from unwashed concentrate containing about 25% water solubles.

Table III. Effect of Ethoxyquin Concentration on Carotenoid Stability in Drum Dried PRO-XAN[a] Stored at 100° F

% Ethoxyquin Added	% Carotene Retained		% Xanthophyll Retained	
	4 Weeks	12 Weeks	4 Weeks	12 Weeks
0	42	12	60	27
0.0125	52	19	70	36
0.025	59	25	73	42
0.050	68	34	78	48
0.075	71	39	80	53
0.125	74	46	83	56

[a] Samples prepared from unwashed concentrate containing about 25% water solubles. Initial mg/lb carotene = 208; xanthophyll = 277, stored in open containers.

the formation of "pro-oxidants" (*e.g.*, peroxides) during drying as a result of oxidation of fatty acids. These peroxides have been shown to accelerate carotene loss (Budowski and Bondi, 1960).

In order to study the effectiveness of the water soluble antioxidants further, a series of drum-dried samples containing increasing levels of water solubles was stored at 100° F (Table I). The solubles had a greater stabilizing effect on carotene than on xanthophyll. Ethoxyquin greatly increased carotenoid stability, even in the samples which contained 37½% solubles. Some water solubles were necessary for ethoxyquin to provide maximum protection against oxidative losses. This latter effect showed a leveling at 12½% in the case of xanthophyll, but had not reached a plateau at the highest level in the case of carotene. Ethoxyquin can effectively replace the natural antioxidants, as shown by the comparable stabilities of ethoxyquin-treated washed drum dried and unwashed freeze-dried concentrates (51 and 52% xanthophyll retention after 12 weeks, respectively). The effectiveness of ethoxyquin under these circumstances provides further evidence that the natural antioxidants are partly destroyed during drum drying.

It has been shown that alfalfa meals dehydrated to high moisture levels (8 to 12%) are more stable than those dried to low moisture levels (<4%) (Knowles *et al.*, 1968; Livingston *et al.*, 1970). Within each of the above moisture ranges, the moisture content had relatively little effect on carotenoid storage stability. Similarly, in PRO-XAN drum dried to 7 to 13% moisture, the initial product moisture level had little effect on the subsequent stability (Table II). More important were variations between batches, which were prob-

Table IV. Effect of Oil and Ethoxyquin on Carotenoid Stability in Drum Dried PRO-XAN[a] Stored at 100° F[b]

% Oil Added	% Carotene Retained				% Xanthophyll Retained			
	4 Weeks		12 Weeks		4 Weeks		12 Weeks	
	Untreated	Treated[c]	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	40	73	13	44	56	82	28	59
5	53	85	21	46	60	84	29	57
10	64	91	28	59	59	88	27	53
15	68	84[d]	32	55[d]	57	75[d]	25	43[d]
15	68	94	32	60	57	87	25	51
20	70	97	28	59	58	88	23	49

[a] Initial mg/lb carotene = 212; xanthophyll = 287. [b] Stored in open containers. [c] Samples treated with 0.125% ethoxyquin except where noted. [d] Sample treated with 0.0125% ethoxyquin.

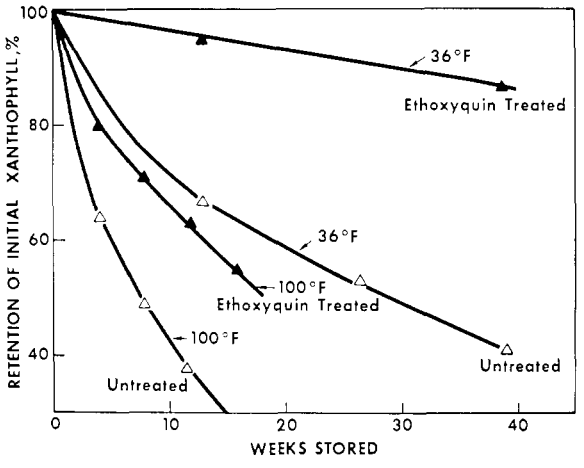

Figure 3. Effect of temperature and ethoxyquin on xanthophyll stability in drum dried PRO-XAN during open storage

Untreated △
Ethoxyquin treated ▲

ably due to differences in the amount of natural antioxidants removed during processing. Ethoxyquin treatment afforded a similar degree of protection, regardless of the initial carotenoid content. After 12 weeks at 100° F, xanthophyll retention averaged 46% for Batch 1 and 28% for Batch 2 (Table II). This increased to 68 and 58%, respectively, when the PRO-XAN preparations were treated with ethoxyquin prior to storage. This level of xanthophyll retention is similar to that reported for dehydrated alfalfa meal stored at a lower temperature (90° F) (Knowles *et al.*, 1968).

In the laboratory, a level of 0.125% is commonly added to show ethoxyquin's antioxidant properties (Bickoff *et al.*, 1954; Knowles *et al.*, 1968). This high level has been shown to exert a somewhat greater stabilizing effect on carotenoids in alfalfa than lower concentrations. Since the above results with ethoxyquin were all at the 0.125% level, the effect of increasing levels of ethoxyquin on carotenoid stability in PRO-XAN was studied. As shown in Table III, as ethoxyquin concentration was increased ten-fold, carotenoid stability increased. The increase was greater for carotene than xanthophyll, which is similar to the results reported for alfalfa meal (Livingston *et al.*, 1955).

Vegetable oil, added either alone or in combination with ethoxyquin to alfalfa meal and mixed feeds, is very effective in reducing carotene ioss (Bickoff *et al.*, 1955; Thompson *et al.*, 1960) and, to a lesser extent, xanthophyll loss (Livingston *et al.*, 1955) during storage. Adding increasing amounts of vegetable oil to PRO-XAN progressively improved carotene stability (Table IV). Ethoxyquin afforded greater protection than oil to the carotene, especially as the length of storage increased, and was also very effective in improving xanthophyll stability. Both oil and ethoxyquin were necessary for maximum stabilization of carotene (Table IV). Oil, 15%, plus as little as 0.0125% ethoxyquin, more than quadrupled carotene retention after 12 weeks storage. Increasing the ethoxyquin level to 0.125% caused little additional increase in carotene retention. Combinations of oil and ethoxyquin were only slightly more effective than ethoxyquin alone in improving xanthophyll stability. Bickoff *et al.* (1955) and Livingston *et al.* (1955) reported that this synergistic effect between oil and antioxidants was probably due to the oil acting as a mutual solvent for the carotenoids and antioxidants (both natural and added), thereby enhancing carotenoid retention.

PRO-XAN stored in sealed containers with no access to air showed virtually no carotenoid loss after 6 months at 100° F, whereas comparable samples in open storage had lost nearly half of their xanthophyll after 2 months. Inert gas storage has become the method of choice for preserving carotenoids in dehydrated forages prior to marketing. A marked improvement in xanthophyll stability can also be obtained by cold storage. PRO-XAN was considerably more stable at 36° F than at 100° F (Figure 3). Moreover, ethoxyquin-treated PRO-XAN retained most of its xanthophyll even after 6 months when stored at the lower temperature. Inert gas storage superseded refrigerated storage because of its lower cost. With a low bulk, high density product, such as PRO-XAN, it may be feasible to reconsider the use of cold storage for xanthophyll retention under conditions where inert gas storage is not practical.

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