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Effect of Fat on Carotene Stability in Dehydrated Alfalfa

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The influence of various fats on carotene retention in different samples of dehydrated alfalfa was investigated. Some samples of dehydrated alfalfa with added fat showed greatly improved carotene retention, while others did not. Carotene retention was not improved by antioxidants at the level normally used for the stabilization of fats; however, the use of antioxidants at higher levels may improve carotene retention. Fatty acids did not increase carotene destruction in dehydrated alfalfa, but caused color deterioration.

THE importance of dehydrated alfalfa as a source of carotene in feeds has made it imperative that studies be conducted on the stability of this provitamin. Research conduted by Guilbert (*7*), Fraps and Treichler (*6*), Fraps and Kemmerer (*5*), Taylor and Russell (*16*), Wilder and Bethke (*18*), Mills and Hart (*10*), Halverson and Hart (*8*), and many others has shown that the carotene content of dehydrated alfalfa decreased during storage. The rate of decrease was influenced mainly by oxygen and storage temperatures.

With the recent use of increasing amounts of fat in the alfalfa dehydrating industry, it became important to study the effect of fats and antioxidants on carotene stability in dehydrated alfalfa during storage. Mitchell, Beauchene, and Silker (*11*, *13*) showed that oil added to dehydrated alfalfa at the rate of 80 pounds per ton produced greater carotene stability than when added at the rate of 16 pounds per ton. They (*13*) showed that heating the meal for 1 hour at 100° C. after spraying with oil gave a further increase in carotene stability. Increasing the amount of fat from 1 to 5% in dehydrated alfalfa was found by Bickoff *et al.* (*3*) to improve carotene retention. Studies by Livingston, Bickoff, and Thompson (*9*) revealed that the addition of animal fats in combination with an antioxidant, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (Santoquin), improved the retention of carotenoids in mixed feeds more effectively than when the oil and antioxidant were added separately. Siedler, Enzer, and Schweigert (*15*) showed an improvement in carotene stability in mixed feeds by the use of stabilized fats. Thompson (*17*) reported that on increasing the amounts of antioxidants in alfalfa, carotene stability increased, but the level of antioxidant approached a limit (approximately 0.125%) above which no additional benefit was noted. It has also been reported that animal fats produced greater carotene stability in dehydrated alfalfa during storage than vegetable oils (*13*, *14*).

This study was designed to obtain more information concerning the effects of the addition of animal fats to samples of dehydrated alfalfa. The study encompassed the effects of stabilization of the animal fats with antioxidants, the effect of free fatty acids, and the effect of wide variations in melting point of the fats on the carotene stability in dehydrated alfalfa.

Methods

The melted fats at 80° C. were added dropwise to 200 grams of dehydrated alfalfa while it was vigorously stirred mechanically in a 3-necked distillation flask, placed in a heated water bath. Approximately 5 minutes were required to heat the alfalfa to a temperature of 80° C. and to add the fat. The alfalfa was then stirred at 80° C. for 10 minutes after the addition of the fat. Solvents were not used to introduce the fats, because of the possibility that they may bring the carotene and natural antioxidants into mutual solution to a greater extent than the fat alone, thereby improving carotene stability. Because heat is necessary for good fat dispersion and may improve carotene retention in normal pelleting operations (*13*), the above method of addition was used. Immediately after the mixing was completed, the samples were removed from the flask, spread out on paper to cool, and then placed in jars with screw-type lids. Control samples were treated in a similar manner without the addition of fat. The per cent retention of carotene, unless otherwise stated, was based on the carotene content of the control sample immediately after mixing. All samples were stored at 37° C.

Carotene determinations were made by using the AOAC method of analysis (*1*). Beauchene *et al.* (*2*) and Mitchell and Silker (*12*) have shown that the AOAC method of analysis gave high values for carotene in the presence of DPPD (diphenyl-*p*-phenylenediamine). In the following experiments, when DPPD was added to alfalfa at the rate of 0.0015% there was no color interference. Initially moisture determinations were made, but this was later discontinued, because of the slight variation which occurred at the beginning and after various lengths of time in storage. The alfalfa samples were obtained from commercial dehydrators located in various states. The time of the year when the samples were dehydrated was not known, but none of the samples had been pelletized or had added fat or antioxidant.

Results

The first experiment was designed to show the effects on the carotene retention in different samples of dehydrated alfalfa, of adding 3% bleachable fancy and No. 2 tallows stabilized with 0.05% of various antioxidants. Bleachable

Table I. Carotene Retention in Dehydrated Alfalfa Stored at 37° C.

Treatment	% Retention of Alfalfa Sample A, 56 days	A, 112 days	E, 56 days	E, 112 days
Control, no fat added	39.3	24.6	42.0	17.9
3% bleachable fancy	61.8	51.7	48.2	32.9
3% bleachable fancy + Tenox R[a]	71.0	52.0	55.2	28.1
3% bleachable fancy + Sustane 3[b]	67.6	65.5	52.5	28.4
3% bleachable fancy + Santoquin[c]	74.4	61.3	57.3	36.0
3% bleachable fancy + DPPD[d]	77.7	57.5	48.8	35.4
3% No. 2 tallow	57.6	42.3	41.5	20.7
3% No. 2 tallow + Tenox R	57.6	44.3	37.1	20.1
3% No. 2 tallow + Sustane 3	56.7	...	38.4	24.4
3% No. 2 tallow + Santoquin	62.5	38.0	44.6	20.7
3% No. 2 tallow + DPPD	63.4	36.3	37.1	22.2

[a] 20% butylated hydroxyanisole, 20% citric acid, 60% propylene glycol added at a level of 0.05% of fat.
[b] 20% butylated hydroxyanisole, 4% citric acid, 6% propyl gallate, 70% propylene glycol added at a level of 0.05% of fat.
[c] 100% 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline added at a level of 0.05% of fat.
[d] 100% diphenyl-*p*-phenylenediamine added at a level of 0.05% of fat.

Table II. Effect of Antioxidant Level on Carotene Retention in Dehydrated Alfalfa Stored at 37° C.

Treatment	Level of Antioxidant in Alfalfa, %	% Retention 28 days	56 days
Control, no fat added	None	47.6	16.5
2% Bleachable fancy	None	69.5	49.3
	0.005	85.8	63.8
	0.010	89.0	80.0
	0.015	89.0	82.8
2% No. 2 tallow	None	61.2	39.2
	0.005	68.0	61.3
	0.010	76.4	68.5
	0.015	85.0	77.8
2% oil mixture	None	51.6	32.5
	0.005	65.1	48.4
	0.010	69.0	57.0
	0.015	79.5	63.6

fancy and No. 2 tallows are inedible animal fats. Bleachable fancy tallow contains a maximum of 4.0% free fatty acids and has a minimum titer of 41.5° C. No. 2 tallow contains a maximum of 35.0% free fatty acids and has a minimum titer of 40.0° C.

Carotene was determined in the experiments after 28, 56, and 112 days of storage. The results obtained after these three storage times were comparable and those obtained after 56 and 112 days are shown in Table I. As noted with both bleachable fancy tallow and No. 2 tallow, greater carotene stability resulted by the addition of fat to alfalfa sample A than to alfalfa sample E. The results obtained, after the three storage times, were subjected to statistical analysis and it was found that the addition of fat significantly increased carotene stability in alfalfa sample A, but not in sample E. Although stabilization of the fat appeared to increase carotene stability in some samples, there was no significant affect of any of the antioxidants and no significant differences among them with respect to carotene stability. Mitchell and Silker have shown (*13*) that unstabilized choice white grease performed as well as stabilized grease in carotene stability tests.

Because Santoquin had previously been reported as an effective antioxidant in reducing losses of carotene in dehydrated alfalfa during storage (*17*), experiments were conducted to show the effect of various levels of this antioxidant in bleachable fancy tallow, No. 2 tallow, and an oil mixture. The oil mixture was composed of Swift's No. 805 Red Oil (commercial oleic acid; 97% free fatty acids as oleic acid) and a by-product from vegetable oil refining. This mixture contained 75% free fatty acids (as oleic acid). The results (Table II) showed that as the antioxidant level is increased, carotene retention increased. As reported in previous experiments, bleachable fancy tallow exhibits greater carotene stabilizing power than No. 2 tallow.

The greater improvement in carotene retention in the presence of bleachable fancy tallow and Santoquin than in the presence of the other two fats and antioxidant is apparently due to the improvement caused by the bleachable fancy tallow without the antioxidant in comparison with the other two fats without the antioxidant. However, by using greater quantities of Santoquin such as 0.75% in No. 2 tallow (0.015% in the alfalfa) greater carotene stability will result than with bleachable fancy tallow alone or in combination with a low level of Santoquin.

In addition to the carotene stability of samples of dehydrated alfalfa reported in Table I, additional samples were tested in the presence of 3% No. 2 tallow stabilized with 0.05% Tenox R (Table III). Apparently the loss of carotene from dehydrated alfalfa treated with No. 2 tallow is independent of the initial carotene content. Results showed that alfalfa A with an initial content of 138 γ per gram retained 66.1% of its carotene after 56 days, while alfalfa D with an initial content of 130 γ per gram retained only 42.3%. Both Mitchell and Silker (*12*) and Ogden (*14*) have shown that lots of alfalfa vary as to their carotene retention not only when collected from widely scattered areas, but also when collected from the same county. Results of experiments from this laboratory agreed with those of Mitchell and Ogden and revealed that alfalfa samples exhibiting approximately the same carotene stability with no treatment may show varying degrees of improvement when the same fat is added.

The effect of heating the alfalfa during the mixing on the carotene stability was studied. Mitchell and Silker (*13*) have reported that effects of heat on the carotene stability were variable in one experiment, but that the heat generated in pelleting was sufficient to retard carotene loss. Results of this limited experiment (Table IV) showed that heating during mixing in the presence or absence of fat had little influence on carotene stability. The heat treatment used in the present experiments was less severe than that employed by Mitchell and Silker. Mills and Hart (*10*) observed that heating dehydrated alfalfa in the absence of added fat for 60 minutes at 93° C. did not influence the carotene stability.

Mitchell *et al.* (*11*, *13*), Bickoff *et al.* (*3*), and Ogden (*14*) have shown that by increasing the level of fat added to the dehydrated alfalfa, carotene retention was increased. Experiments conducted in this laboratory with No. 2 tallow both stabilized with 0.05% Tenox R and unstabilized showed similar results (Table V). In these experiments 5% added fat was necessary before an increase in carotene stability could be detected in this sample of dehydrated alfalfa. Stabilization of the fat, again, had no influence on the carotene retention.

Bickoff *et al.* (*3*) reported that the free fatty acid content of fats applied to dehydrated alfalfa did not seem to be deleterious to the carotene within the limits of their experiment, and that a fat with a high free fatty acid content was not deleterious to the color of the meal. On the other hand, Ogden (*14*) reported that free fatty acids caused an increase in carotene destruction and color deterioration. Because of these

Table III. Carotene Retention[a] in Different Samples of Dehydrated Alfalfa Stored 56 Days at 37° C.

Alfalfa Sample	Initial Carotene Content after Adding 3% No. 2 Tallow, γ/Gram	% Retention[a]
A	138	66.1
B	205	44.5
C	154	52.0
D	130	42.3
E	179	41.3

[a] Per cent retention based on carotene values immediately after fat was added.

Table IV. Effect of Heat on the Carotene Retention in Dehydrated Alfalfa Stored 56 Days at 37° C.

Treatment	% Retention[a]
No heat[b]	57.3
3% No. 2 tallow, no heat	70.8
Heat	50.3
3% No. 2 tallow, heat	69.3

[a] Per cent retention based on unheated alfalfa at time stored.

[b] Heat refers to alfalfa being heated during mixing. Sample not heated: fat was melted and mixed into alfalfa at room temperature.

Table V. Effect of Various Levels of No. 2 Tallow on Carotene Retention in Dehydrated Alfalfa

% Fat	Antioxidant	% Retention, 56 Days' Storage
None	None	39.5
1	None	...
3	None	46.4
5	None	57.2
1	Tenox R	43.1
3	Tenox R	41.3
5	Tenox R	57.2

Table VI. Effect of Free Fatty Acids on the Carotene Retention in Alfalfa

Fat Added	% Retention	Days' Storage, 37° C.
None	46.6	56
3% bleachable fancy tallow	52.1	56
3% tallow–fatty acid mixture (1–1)	44.7	56
3% tallow fatty acids	44.7	56
None	39.5	42
3% Swift's No. 805 Red Oil	42.5	42
3% undecolorized Red Oil	48.5	42
3% oil mixture	41.9	42
None	42.9	51
5% palmitic acid	51.3	51

Table VII. Effect of Melting Point of Fats on Carotene Retention in Dehydrated Alfalfa

Fat	Melting Point, ° C.	Days' Storage, 37° C.	% Retention
None	..	51	40.5
3% raw cottonseed oil	7	51	55.7
3% partially hydrogenated cottonseed oil	38	51	66.7
3% completely hydrogenated cottonseed oil	60	51	39.5
None	..	42	38.8
2% No. 2 tallow	46	42	56.1
2% completely hydrogenated No. 2 tallow	60	42	42.2

divergent reports, an experiment was designed to determine under experimental conditions the influence of free fatty acids on carotene and on color preservation in dehydrated alfalfa. The fatty acids used in the tallow fatty acid mixture and in the tallow fatty acids (Table VI) were made from the same bleachable fancy tallow by saponification, acidification, and washing. The undecolorized red oil used in the second series was Swift's Saponification Grade Red Oil which contained 95% free fatty acids as oleic acid. The oil mixture was the same as that reported in Table II.

The free fatty acids were not detrimental to carotene stability in three different samples of alfalfa under these experimental conditions (Table VI). Color deterioration was greatly accelerated by samples containing appreciable amounts of free fatty acids. Under experimental conditions, fats containing in excess of 10% free fatty acids caused noticeable browning of the alfalfa. Apparently this is due to the conversion of chlorophyll to its degradation product, pheophytin (*4*). Experiments using pure oleic and palmitic acids produced the same browning effect.

Because of the availability on the market today of fats with widely different melting points, an experiment was conducted to determine the effects of fats hydrogenated to varying degrees on the carotene retention in dehydrated alfalfa. The results (Table VII) showed that the fats hydrogenated completely have little influence on carotene retention. On the other hand, raw or partially hydrogenated cottonseed oil and No. 2 tallow gave increased carotene stability. Although slight changes in melting points of the fat may not influence carotene retention (*14*), complete hydrogenation reduced the carotene stabilizing effects of fats. The hydrogenated fats did not mix well into the alfalfa, although the mixing temperature was above the melting point of the hydrogenated fat. This was evidenced by the presence of small clumps of alfalfa after mixing.

Discussion

The results of these studies revealed that animal fats apparently are stable to rancidity when mixed with dehydrated alfalfa. If the fats had been undergoing autoxidation, they would have accelerated carotene destruction (*14*). However, it is extremely important to use all possible means to ensure against rancidity.

Until more is known concerning the cause of the variations in the stability of carotene in different samples of dehydrated alfalfa, the magnitude of the increase in carotene stability arising from fat addition cannot be predicted. However, results of these experiments indicate that the better grades of fat are preferable.

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