Effect of Added Fats and Oils on Carotene Stability in Dehydrated Alfalfa Meal during Storage

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A number of animal fats and vegetable oils, added to dehydrated alfalfa meal, were studied for their effect on carotene during storage. Animal fats in general caused a marked increase in carotene-stabilizing action over that exhibited by vegetable oils. Lower grades of animal fats may cause increased carotene degradation in some meals but this may, in part, be overcome by refining with alkali or vacuum distillation. The amount of stabilizing action exhibited by both animal fats and vegetable oils varies with different lots of alfalfa meal. The effect of animal fats on alfalfa meal in increasing carotene retention during storage may be due to a natural antioxidant in the fat in addition to their effect in bringing carotene and naturally occurring antioxidants of the meal into mutual solution. Addition of animal fats does not change β-carotene isomerization.

THE USE OF VEGETABLE OILS to reduce the dustiness of the dehydrated alfalfa meal has been increasing rapidly during the past few years. Economically this addition has been limited to 1% or less, even though this amount does not give permanently dustless meal. In some previous (unpublished) work by the writer, it was shown that although 1% oil added to alfalfa meal caused an immediate reduction in the dustiness of the meal, this effect was largely lost after 2 to 3 weeks' storage. In order to produce a permanently "dustless" meal, 2 to 3% added oil was necessary. The use of large quantities of lower cost animal fats has been retarded by the general belief in the dehydration industry that these fats would cause a more rapid loss of carotene in the meal during storage. A stabilizing effect was noted on vitamin A (*9, 10*) when stabilized animal fats were added to mixed feeds and on carotene (*7*) when expeller soybean and cottonseed meals were mixed with alfalfa.

Increased interest in the use of animal fats as an additive to alfalfa in the dehydration industry is indicated by two recent reports. Bickoff and others (*5*) report that addition of animal or vegetable fats, without added stabilizers, enhances carotene stability with about equal efficacy in alfalfa meal during storage at room temperature. Mitchell and Silker (*8*) reported that when no antioxidants were added, choice white grease gave greater carotene stability than vegetable oils.

This report on carotene stabilization in dehydrated alfalfa meal shows that the higher grades of inedible animal fats have a markedly greater stabilizing effect than vegetable oils.

Table I. Effect of Addition of 5% Fats to Alfalfa Meal[a] on Carotene Retention at 40° C.

Fat or Oil Added	% Free Fatty Acid as Oleic	Carotene Retention[b] Index
Beef tallow, dry rendered	0.7	60
Lard, dry rendered	1.1	60
Mutton tallow, dry rendered	0.4	57
Sperm oil 45° NW	1.0	55
Cocoa butter, crude	5.8	52
Soybean oil, expeller, refined	2.4	47
Corn oil, refined	0.6	46
Castor oil, refined	1.0	45
Peanut oil, crude	3.9	45
Apricot seed oil, crude	4.1	44
Cottonseed oil, refined	0.1	43
Mustard seed oil, crude	2.6	41
Coconut oil, crude	10.7	41
Sunflower seed oil, crude	1.7	41
Soybean oil, solvent, refined	1.0	40
Sesame oil, crude	9.2	37
Coffee oil, crude	10.4	36
Safflower oil, refined	1.5	35
Rice bran oil, crude	11.0	34
Olive oil, crude	36.7	33
Grape seed oil, crude	5.4	32
Perilla oil, refined	8.0	31
Linseed oil, refined	7.9	28
Walnut oil, crude	11.7	28

[a] Initial carotene content, Lot A, 104 mg./lb.

[b] % carotene retained in treated sample at time when untreated control contained only 40% of its original carotene. Untreated control required 26 days' storage to reach end point.

Methods

The melted or liquid fats and oils at 80° C. were added to 50 grams of alfalfa meal and mixed in a mortar with a pestle for 1 minute. The mixed samples were stored in open glass bottles in an incubator at 40° C. during the test period. The samples were removed from the incubator at regular intervals and each sample was thoroughly mixed before a portion was weighed for carotene determination. The samples were withdrawn from one lot of alfalfa meal (Lot A) which was stored in a freezer at −20° C. For determining carotene the chromatographic method of the Association of Official Agricultural Chemists (*1*) was used. The percentage of carotene remaining in the treated sample when 40% of the original carotene remained in the control sample was taken as an index of the effectiveness of the treatment.

Experimental

The stability of Lot A alfalfa carotene did not change during 6 months' storage in the freezer. The rate of carotene degradation at 40° C. for meal entering cold storage and after storage in the freezer was the same.

The reproducibility of results is shown by 11 experiments over a period of more than 2 years, in which 5% of a single lot

of beef tallow was added to Lot A alfalfa meal. The average index of retention effected by the tallow was 60.5, ranging from 58.8 to 62.1.

Effect of Animal and Vegetable Fats A survey was made using fats and oils from various sources at a 5% level of addition to the meal. These results (Table I) indicate that there may be a factor in animal fats, either physical or chemical, that causes a marked reduction in the rate of alfalfa carotene loss during storage which is not found in vegetable oils, with the possible exception of cocoa butter. No relationship could be found between the carotene-stabilizing action of these fats and their degree of saturation (iodine value), average molecular weight (saponification value), free fatty acid content, and the fatty acid make-up of the fat.

The difference in melting point ranges between animal fats and vegetable oils was considered. Animal fats were fractionated into several melting point ranges and these fractions used as the fat additive. The results (Table II) show that the melting point of the fat has very little if any effect on carotene in alfalfa meal during storage.

Commercial Grades of Animal Fats A number of unstabilized commercially available grades of inedible animal fats were collected and tested to determine the effect of the grade of the fat on its carotene-stabilizing ability. These data (Table III) indicate that there may be some relationship between the grade of fat and its carotene-stabilizing effect. Because the grade is largely dependent on the free fatty acid content, this factor was determined for each of these fats. These results (Table III) also show lower carotene-stabilizing action of the fat with increased percentage of free fatty acids. No relationship between the free fatty acid content and retention of carotene was found for the various vegetable oils (Table I), although a variation in acid content of any one kind of oil may well show some differences in the retention index for that oil. Although oxidative rancidity measurements were not made on these fats and oils, it is possible, perhaps probable, that peroxidants from possible fat rancidity may be exerting some effect on carotene degradation.

Table II. Effect of Melting Point Range of Animal Fats on Carotene Retention in Alfalfa Meal[a] at 40° C.

Melting Point Range, °C.	Carotene Retention Index[b] Beef fat	Pork fat	Mutton fat
<21	58.0	56.5	...
21–31	59.5	59.5	60.0
31–41	59.0	55.0	62.0
41–50	55	...	56.0
>50	...	...	54.5

[a] Initial carotene content, Lot A, 104 mg./lb.
[b] % carotene retained in treated sample at time when untreated control contained only 40% of its original carotene. Untreated control required 26 days' storage to reach end point.

Table III. Effect of 5% Added Commercial Grade Animal Fats on Carotene Retention in Alfalfa Meal[a] 40° C.

Additive	Source, Company	% Free Fatty Acids as Oleic	Rentention Index[b]
Beef tallow			
Oleo oil	B	0.9	60
Edible	A	1.4	57
Inedible			
Fancy	A	1.4	60
	F	1.5	58
	B	1.7	53
	C	2.1	58
	E	2.1	54
Prime	D	4.4	50
	C	4.5	50
Special	D	8.9	45
No. 2	D	34.6	25
	F	35.4	37
	B	36.9	36
	C	38.9	31
Oleic acid, U.S.P.			27
Linoleic acid, C.P.			24
Pork fat			
Lard	G	1.1	61
Grease			
Choice white	C	2.2	54
	E	2.4	51
White A	E	6.5	47
Poultry fat			
Chicken fat	H high grade	0.4	52
	F low grade	7.9	42
Turkey fat, skimmings	G	0.6	49

[a] Initial carotene content, Lot A, 104 mg./lb.
[b] % carotene retained in treated sample at time when untreated control contained only 40% of its original carotene. Untreated control required 26 days to reach end point.

Effect of Removing Free Fatty Acids To determine whether or not No. 2 tallow would give stabilization effects equal to the higher grade tallows if the high content of free fatty acid was reduced, a sample of this fat was dispersed in alcohol and the free fatty acids were neutralized with 0.1*N* sodium hydroxide. The alcohol solution of the acid salts was separated and the fat washed with alcohol. By this neutralization and removal of the fatty acids the carotene retention index was increased from 32 to 49 as compared to 58 to 60 for fancy inedible tallows. When No. 2 tallow was washed with alcohol without neutralization, the retention index was decreased about 10 units, showing an increase in rate of carotene degradation. When the free fatty acids were removed from No. 2 tallow by vacuum distillation at 300° C., the index was increased by 14 units. Although the neutralization and/or removal of free fatty acids does not increase the carotene-stabilization effects of No. 2 tallow to equal that of the higher grades, it is shown that the free fatty acid content of a fat plays an important role in the carotene stabilization by animal fats. Thus, it is obvious that it would be inadvisable to use the low grade fats as an additive to alfalfa meal because of their action in increasing the rate of carotene degradation. However, if the free fatty acids are removed or neutralized, the low grade fats can be used without deleterious results.

Bickoff and others (*5*) report that oils had no preservative effect on the color of the meal, although oiling appeared to produce a darker meal, and that high free fatty acid as such was not deleterious to the color of the meal. In the present work it has been found that the use of fats with a high percentage of free fatty acids causes a rapid browning of the meal. When oleic and linoleic acids alone are added to the meal, this same rapid browning action takes place, probably through action of the acids on the chlorophyll. The addition of the higher grade animal fats and vegetable oils tends to give the meal a better green color, which persists much longer than with untreated meal.

Amount of Fat Addition Mitchell and others (*6*, *8*) have shown increased carotene stability with increased amounts of some vegetable oils and choice white grease, and Bickoff and others (*5*) report similar results with animal fat and yellow grease. In an attempt to determine the maximum amount of fat that should be added to alfalfa meal, varying amounts of prime inedible beef tallow were added to Lot A meal and subjected to storage (Figure 1). Although there is not a sharp break point in the curve, it is shown that in-

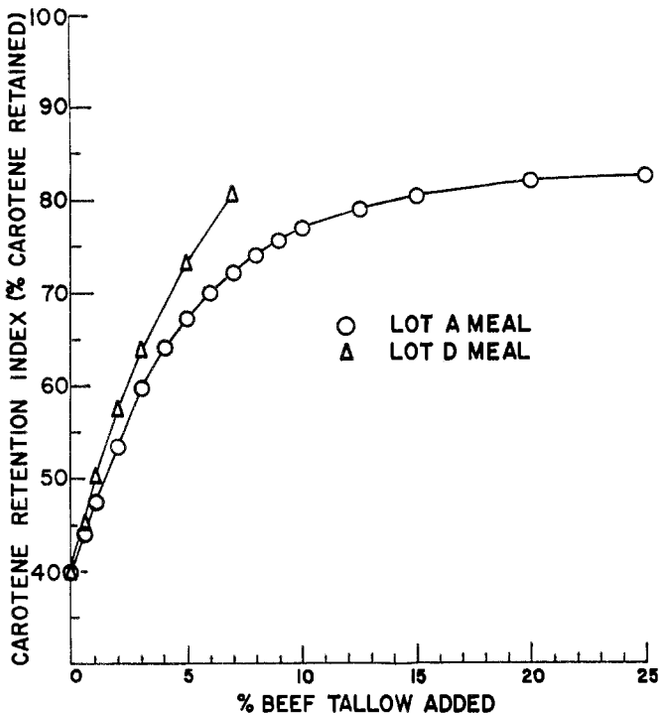

Figure 1. Effect of amount of addition of fancy tallow on carotene retention in alfalfa meal during storage at 40°C

Initial carotene content: Lot A 104 mg./lb., Lot D 110 mg./lb.

creasingly larger amounts of fat give relatively lower carotene retention effects. When another alfalfa meal was used (Lot D), the amount of retention per unit of fat added was considerably higher than for Lot A, and the curve does not appear to level off so rapidly. The amount of fat that can be added to alfalfa meal is an economic problem, in that the cost of the fat addition cannot exceed the value of the addition, considering both carotene stabilization and dustiness reduction. It seems from these data that it would not be advantageous to add more than 5% of this fat to Lot A meal, although higher percentages might be economical for Lot D meal.

Behavior of Different Lots of Alfalfa Meal

Mitchell and Silker (*8*) report that different lots of alfalfa meal vary in their rate of carotene loss during storage, as has been recognized in the dehydration industry for some time, and that this holds true when the meal is treated with vegetable oils, antioxidants, and heat. In recent experiments it has been shown that different lots of dehydrated alfalfa meal give different carotene retention indexes for addition of 5% fancy inedible beef tallow. For example, Lots A, B, and D had carotene retention indexes of 60, 64, and 73, respectively. Lot A meal was produced early in July 1952, Lot B in August 1952, and Lot D in late September 1954, all in Dawson County, Nebraska. Without treatment, Lot D meal had a much higher rate of carotene loss during storage than Lots A and B. Figure 2 shows the rate of loss of carotene for these three lots, untreated, and with addition of 5% fat. From these limited data it is not possible to state whether it would be more advantageous to add fat to meal with slow or rapid carotene loss.

Stability of Added Fat in Alfalfa Meal

Antioxidants of the type normally used in stabilizing fats and oils were added to both animal and vegetable fats without any apparent effect on carotene stability. Although animal fats seem to be stable against rancidity when mixed with alfalfa meal, it is highly desirable to protect them from oxidation, because when the fat added to alfalfa meal goes rancid during storage, the rate of carotene degradation is greatly increased. Figure 3 shows effects of fat becoming rancid during storage and of adding rancid fat to meal. Rancidity was induced in prime inedible tallow in varying degree before addition to the meal for this experiment.

Table IV. Effect of Beef Tallow on β-Carotene Isomer Ratios in Lot A Alfalfa Meal during Storage at 40° C.

Meal	Stereoisomer Composition, % Neo-β-carotene B	All trans-β-carotene	Neo-β-carotene U
Original meal before storage	30.6	55.1	14.3
Stored for 30 days			
Untreated	9.8	61.5	28.5
+5% prime beef tallow	10.9	63.0	26.1
+5% No. 2 beef tallow	8.7	63.7	27.6

Carotene Isomerization

The effect of beef tallow on the isomerization of β-carotene during storage at 40° C. was studied. The method of Bickoff and others (*3*) was used for separating the isomers, while the extracts were prepared by the method of Bickoff and Thompson (*4*). It was necessary to modify the latter procedure because the fat interfered in separation of the isomers. A large portion of the extract prepared according to Bickoff and Thompson (*4*) for dehydrated meal was first adsorbed on a 2-cm. column of activated magnesia (Micron brand 2642) and washed with 2 to 3 volumes of the solvent (Skellysolve C) to remove the fat.

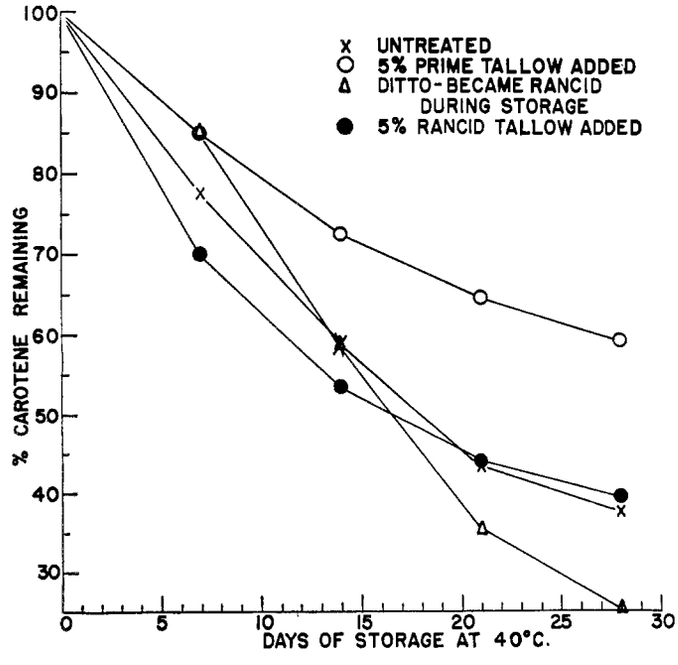

Figure 2. Rate of carotene loss for three lots of alfalfa meal

Initial carotene content: Lot A 104 mg./lb., Lot B 117 mg./lb., Lot D 110 mg./lb.

The carotenoids were eluted with a minimum amount of acetone. The acetone eluate was then treated in the same way as the acetone extract from fresh plant materials [Bickoff and Thompson (*4*)]. These data (Table IV) show that added beef tallows do not appreciably change the shift in isomer ratios other than that exhibited by untreated meal.

Discussion

The natural antioxidants in alfalfa meal, mainly tocopherol, play an important role in carotene stability of the meal. Bickoff (*2*) showed the stabilizing effects of α-tocopherol on purified β-carotene in mineral oil. Wall and Kelley (*11*) determined the tocopherol content of alfalfa leaf meal to be 118 mg. per pound. If tocopherol or other antioxidants in alfalfa meal are deposited in intimate contact with the carotene, they should be able to exert their maximum stabilizing action. However, if these constituents are deposited apart from each other, the antioxidants could not effectively stabilize the carotene. The proposal by Bickoff and others (*5*) that added fat or oil increases carotene stability by bringing carotene and the naturally occurring antioxidants of alfalfa into mutual solution, would fit the latter type of deposition or a combination of both types. Various combinations of these two types of antioxidant deposition may explain the differences in carotene stability of various lots of alfalfa meal.

Several deductions may be made from data accumulated when prime inedible tallow, No. 2 inedible tallow, and refined cottonseed oil were added to alfalfa meal Lots A and D (Table V).

The stability of carotene in alfalfa meal varies with different lots of meal, as can be seen by comparing the rate of loss of carotene in untreated meal Lots A and D. As the tocopherol contents of these two lots of meal are about equal (100 mg. per lb.), it is likely that the different rates of carotene degradation are due to the type of antioxidant-carotene deposition that exists in these meals.

Table V. Carotene Loss for Alfalfa Meal with Addition of Three Fats at 5% Level during Storage at 40° C.

	Time for Untreated Control to Retain Only 40% of Original Carotene, Days	
	26	19
	Carotene Retention Index[a]	
	Lot A[b]	Lot D[b]
5% prime beef tallow added	61	73
5% No. 2 beef tallow added	25	55
5% Cottonseed oil added	42	55

[a] % Carotene retained in treated sample when untreated control contained 40% of original carotene.

[b] Initial carotene content of alfalfa meal Lot A 104 mg./lb., Lot D 110 mg./lb.

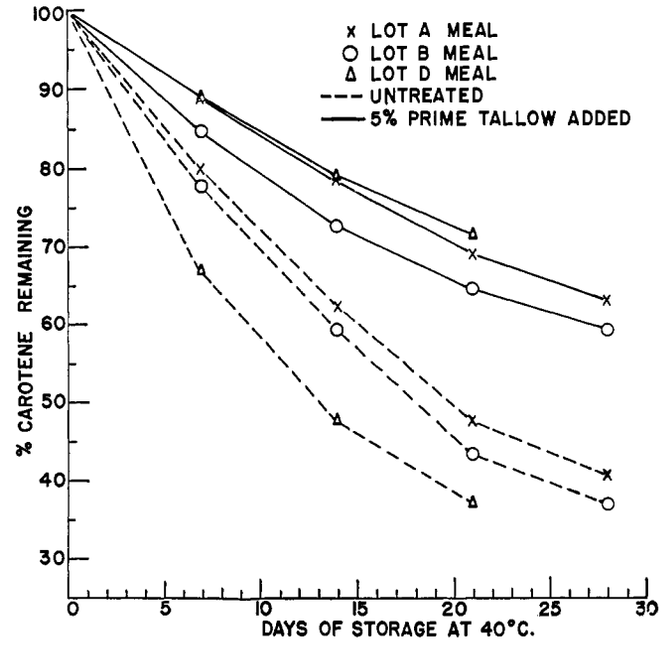

Figure 3. Effect of fat rancidity on carotene retention in alfalfa meal during storage at 40°C.

Initial carotene content of Lot A alfalfa meal: 104 mg./lb.

The ineffectiveness of cottonseed oil in increasing carotene stability in Lot A meal would indicate that this oil does not contain a carotene-stabilizing factor other than those abundantly found in alfalfa meal. Furthermore, it would appear that the natural antioxidants and carotene of Lot A meal are in intimate contact with each other, since putting them into mutual solution in the oil did not improve carotene stability.

The increased carotene stability in Lot D meal when cottonseed oil was added is probably due to a separated type of antioxidant-carotene deposition; thus, their mutual solution in the oil increases carotene stability.

Prime inedible beef tallow apparently contains a carotene-stabilization factor, as seen by the marked increase in carotene retention in Lot A meal upon addition of this fat. The increased stability noted in Lot D meal seems to be the sum of the effect of bringing the naturally occurring antioxidants and carotene into mutual solution and the effect of the carotene-stabilizing factor contained in the beef tallow.

No. 2 inedible beef tallow causes a marked decrease in carotene stability in Lot A meal, but is equal to cottonseed oil in stabilizing the carotene in Lot D meal. There are not sufficient data to explain the large difference in carotene stability of these two lots of alfalfa meal, but these apparently different effects may explain to some degree the opposite effects of free fatty acids on carotene stability as reported by Bickoff and others (*5*) and by this paper.

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