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ALFALFA CAROTENE

Stability of Carotene in Dehydrated Alfalfa Meal With Effect of Antioxidants, Oil, and Heat

H. L. MITCHELL, R. E. BEAUCHENE, and RALPH E. SILKER

Kansas Agricultural Experiment Station, Manhattan, Kan.

A study of the ability of certain chemicals to inhibit the oxidation of carotene in alfalfa meal during storage showed appreciable antioxidant activity in compounds related to aniline. The most promising was *N,N'*-diphenylhexamethylenediamine. Carotene retention during storage was influenced by the amount of oil which was used in applying the antioxidants to the meal. Applications of Wesson oil at the rate of 80 pounds per ton of meal were more effective than 16 pounds per ton in reducing oxidation. Heating the samples at 100° C. for an hour after spraying the meal with the oil resulted in a further increase in carotene retention.

Stabilization of the carotene of dehydrated alfalfa meal by means of antioxidants has received much study in recent years. Kephart (*4*) proposed the use of *N,N'*-diphenyl-*p*-phenylenediamine, and this chemical has been approved by the U. S. Food and Drug Administration for use on alfalfa meal.

Table I. Antioxidant Activity of Aniline and Certain Derivatives

(0.2% antioxidant and Wesson oil at a rate of 16 pounds per ton)

Chemical	% Carotene Loss in 5 Months at 25° C.
None	60
Aniline	48
Monomethylaniline	40
Dimethylaniline	53
N,N'-Diphenylhexamethylenediamine	36
2,5-Di-*tert*-butylhydroquinone	35

The most extensive investigation has been reported by Thompson (7), who concluded that 2,5-di-*tert*-butylhydroquinone and 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline were the most effective of the 54 chemicals tested.

Kephart (*4*) recommended that the antioxidant be applied by dissolving the chemical in a vegetable oil and spraying the solution on the meal. The oil is desirable also for controlling dustiness of the product, and is used for this purpose at rates of 7.5 to 25 pounds per ton of meal (*8*).

The data presented below show that certain derivatives of aniline possess considerable antioxidant potency, and that the effectiveness of a chemical can be enhanced by heavy oiling of the meal and by the application of heat.

Aniline Derivatives as Antioxidants

Alfalfa meal containing 25 mg. of carotene per 100 grams was sprayed in a rotary mixer with Wesson oil at a rate of 16 pounds per ton and with the various antioxidants at a level of 0.2% (*2*). The meals were stored in 4-ounce bottles at 25° C. and were analyzed for carotene at intervals by the method of Silker, Schrenk, and King (*6*). The aniline, monomethylaniline, and dimethylaniline used in the experiment were purchased from various chemical supply houses. The *N,N'*-diphenylhexamethylenediamine was synthesized by reducing adipanilide with lithium aluminum hydride (*1*). The product had a melting point of 74° C.

The data are presented in Table I. The value of 2,5-di-*tert*-butylhydroquinone is included for comparison, because it was one of the better antioxidants studied by Thompson. All of the compounds had some antioxidant activity. Substitution in the amino group of aniline resulted in increased antioxidant potency, monomethylaniline having greater activity than either aniline or dimethylaniline. The activity of diphenylhexamethylenediamine was equal to that of 2,5-di-*tert*-butylhydroquinone. Thus, it may be possible to synthesize other derivatives which will have enhanced antioxidant activity, will possess the proper physical properties to facilitate application to the meal, and will have low toxicity.

Effects of Heat and Oil Level

Often it is difficult to duplicate industrial conditions on a laboratory scale. Oil frequently is applied commercially

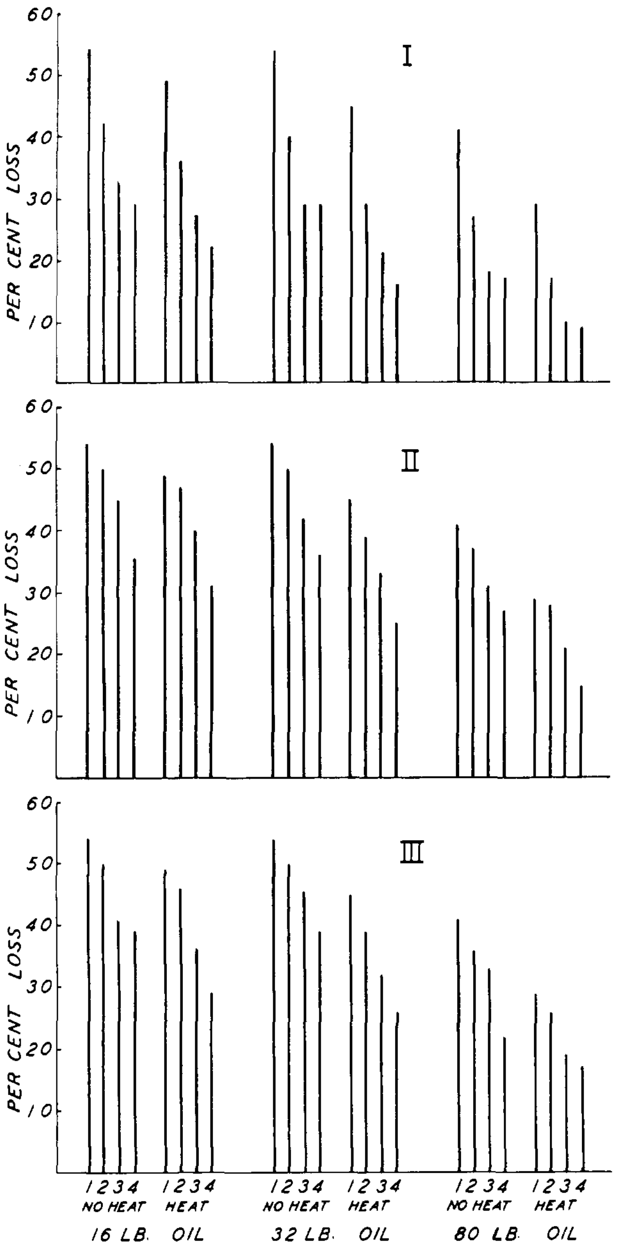

Figure 1. Effect of oil, antioxidant, and heat on loss of carotene in dehydrated alfalfa meal stored at 25° C. for 3 months

I. 6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline
II. N,N'-Diphenylhexamethylenediamine
III. 2,5-Di-*tert*-butylhydroquinone
1, 2, 3, and 4 are 0, 0.01, 0.05, and 0.1% antioxidant, respectively.

before passage of the meal through the hammer mill, and a certain amount of heat is developed at this stage. To study in the laboratory the effect of heat on antioxidant activity, alfalfa meal was sprayed with Wesson oil at a rate of 16 pounds per ton and with the antioxidants at a level of 0.2%. Two portions of each sample were placed in 4-ounce bottles. One portion was tightly stoppered and was heated in an electric oven at 100° C. for an hour, after which the samples were cooled quickly to room temperature. Moisture determinations on the heated and unheated portions indicated that there was no loss of moisture during heating. Table II shows the per cent of carotene which was lost in the heated and unheated portions during 6 months of storage at 25° C.

Preliminary work had shown that the heat treatment would improve carotene retention. This was investigated further by heating the Wesson oil at 100° C. for an hour and using this oil for treating the meal. The treated samples then were stored without further heating. The stability data of these samples are also shown in Table II.

The application of heat to the sprayed samples reduced carotene destruction appreciably with all of the antioxidants except monomethylaniline. Heating the oil before applying it to the meal did not increase stability over that obtained when no heat was applied. Hence, the effect of heat appears not to have been due to changes in the oil itself. It seems likely that the effect of heat was to improve penetration of the oil and antioxidant through the nonlipide portion of the meal.

If penetration of the antioxidant is a factor affecting the degree of stabilization, increasing the amount of oil might be expected to improve the activity of the antioxidant. To study this, alfalfa meal was treated with 0, 0.01, 0.05, and 0.1% of the antioxidant and with Wesson oil at rates of 16, 32, and 80 pounds per ton. The antioxidants used were the three which exhibited the greatest activity in earlier tests. Half of each sample was heated for an hour at 100° C. The samples were stored in 4-ounce bottles at 25° C. for 3 months. Stability data are shown in Figure 1.

Application of oil alone had an appreciable effect on carotene stability in certain instances. The untreated meal lost 49% of its carotene in 3 months (not shown in Figure 1), as compared to losses of 54 and 41% for the meals sprayed with 16 and 80 pounds of oil per ton. Oil at a rate of 32 pounds per ton likewise caused a slight increase in carotene loss. Thus, the lower levels of this particular lot of oil increased carotene destruction slightly, but this effect was overcome at the higher level. These data are in agreement with a previous report from this laboratory on the effect of various levels of cottonseed oil (*5*). Van Atta *et al.* (*8*) also reported that high levels of palm oil improved carotene stability under accelerated storage conditions, but apparently they found no deleterious effect at low levels. Although low levels of oil had a slight adverse effect in the experiments being reported, the effect is not always encountered in this laboratory. Presumably, variations between lots of oil or meal are responsible for these deviations.

Application of heat to the meals sprayed with oil resulted in less carotene loss in all instances when compared with the unheated oiled samples. With 16 and 32 pounds of oil, heating merely overcame the slightly deleterious effect of the oil alone. However, heating the meal after spraying with 80 pounds of oil greatly improved carotene retention, the combination of oil and heat reducing the loss to 29%.

Some reduction in carotene loss was obtained in all cases when 0.01% antioxidant was added along with 16 pounds of oil, although the effect at this level of antioxidant was not appreciable unless heat was applied also. Even when heated, the most stable sample at this oil level and antioxidant concentration (substituted dihydroquinoline) still lost 36% of its carotene. Marked reduction in carotene loss was obtained when a combination of 80 pounds of oil, 0.1% antioxidant, and heat was used. Under these conditions the dihydroquinoline-treated meal lost only 9% of its carotene, while losses in the presence of the substituted hydroquinone and the substituted hexamethylenediamine were 17 and 15%, respectively.

Antioxidants are being used commercially at a level of 0.015% (*3*). The authors believe that perhaps the level should be increased if no heat is applied in the process, for at a level of 0.05% and with 16 pounds of oil, the dihydroquinoline reduced carotene loss from 54 to 33%, while 0.01% of the antioxidant reduced it only to 42%. Heat and the high oil level in conjunction with 0.01% of the antioxidant reduced the loss to 17%. This degree of stabilization should be adequate to meet the needs of the dehydrated alfalfa industry. It is probable that the stabilizing effect of 0.01% antioxidant would have been greater under commercial conditions, particularly if applied before grinding, since

Table II. Effect of Heat on Carotene Loss in Alfalfa Meal Sprayed with 0.2% Antioxidant and Wesson Oil at a Rate of 16 Pounds per Ton

(Storage at 25° C. for 6 months)

Antioxidant	Loss, % Unheated	Oil applied, then heated	Oil heated, then applied
None (oil only)	79	76	78
Monomethylaniline	61	57	59
Diphenylhexamethylenediamine	45	35	42
2,5-Di-*tert*-butylhydroquinone	44	25	44
6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline	40	30	39

some heating of the meal will occur during processing. Greater amounts of oil than now are used should be beneficial, although it appears from these data that 32 pounds per ton is not appreciably better than 16 pounds.

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SUGAR CANE PROCESSING

Clarification of New Varieties of Cane On a Pilot Plant Scale

W. F. GUILBEAU, JOHN G. LIPPS, Jr., and L. F. MARTIN

Southern Regional Research Laboratory, New Orleans, La.

This research was undertaken to provide data on the suitability of new sugar-cane varieties for sugar production before they are grown on a commercial scale. Equipment and procedures were developed for grinding and processing individual 2-ton samples of cane on a pilot plant scale. The study of clarification of seven new varieties and a widely grown commercial standard on this scale provided information on the relative quantities of precipitate formed, filterability of the precipitates, and clarities of the juices, all of which are important factors in determining the efficiency of sugar recovery from the cane. Significant differences in the performance of these new varieties were established, and were considered together with the results of agronomic field testing in deciding to release two of them for commercial use. Further field testing of one of the less promising new varieties was discontinued when it was established that juice from this cane would be extremely difficult to clarify.

Almost every major sugar-producing area has come to rely upon the breeding of new varieties of sugar cane to maintain or improve the productivity of the crop. Breeding programs were initiated to combat diseases and have been continued for improving agronomic qualities, increasing the yields of sugar per acre, and maintaining disease resistance. In Louisiana, the new seedling varieties are evaluated agronomically during 6 to 8 years of multiplication before being cultivated commercially. Field tests establish the yields of sugar per acre, suitability for mechanical harvesting, early maturity, adaptability to climate and soils, and resistance to attack by diseases and insects.

Introduction of one new variety per year on the average results in complete change in predominant varieties in the crop about every 10 years. The continually changing composition of the crop alters the processing qualities of the juices obtained. New varieties that are satisfactory in field performance may yield juices that cannot be clarified readily to yield maximum recoveries of sugar. Work was initiated in 1949 to develop methods of evaluating the processing qualities of new varieties prior to commercial planting. Clarification characteristics of seven varieties of sugar cane have been determined on a pilot plant scale during four successive grinding seasons.

New varieties of sugar cane are available 2 or 3 years before they are eligible for commercial adoption, in quantities sufficient for experimental grinding and continuous processing on a pilot plant scale simulating factory conditions. The experimental Audubon Sugar Factory of Louisiana State University is equipped with a complete milling train 2 feet wide capable of grinding single bundles of approximately 2 tons of cane continuously through knives, crusher, and three three-roller mills in about an hour. Equipment for liming and clarification on a smaller scale was installed to obtain data on continuous clarification of the juice from this amount of cane in experiments of 4 to 6 hours' duration. Duplicate tests on both first-year plant cane and the stubble cane produced by a