THE USE OF ANTIOXIDANTS FOR STABILIZATION OF CAROTENE

BY

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INTRODUCTION

Much time has been spent in an attempt to stabilize carotene against atmospheric oxidation. Oxidized carotene cannot be converted by the animal body into vitamin A. Dehydrated alfalfa meal is a rich source of carotene and is the chief source of vitamin A for many mixed feeds. Hence the question of stabilizing carotene in dehydrated alfalfa meal is a problem of great economic importance.

Early attempts to stabilize the carotene of dehydrated alfalfa meal involved (a) storage in refrigerated warehouses (b) high moisture storage in air tight cans or bags and (c) storage in an inert gas. Other studies have been concerned with the use of antioxidants for inhibiting carotene oxidation. Silker, Schrenk, and King (7) inhibited the destruction of carotene in alfalfa by the addition of diphenylamine, thiourea, hydroquinone, and sodium cyanide. Bickoff, Williams, and Sparks (1) found that nordihydroguaiaretic acid improved the stability of carotene which had been added to edible oils. Kephart (4) sprayed alfalfa meal with certain aryl amines, including N,N'-diphenyl-p-phenylenediamine, and obtained a marked increase in carotene stability. Thompson (9) evaluated a series of compounds as antioxidants for carotene in dehydrated alfalfa. He reported that alkyl substitution in the benzene ring of hydroquinone greatly increased the antioxidant effect as compared to hydroquinone itself. Thus, he found the 2,5-ditertiarybutyl derivative of hydroquinone to be a very good antioxidant.

However, Bickoff (2) found that hydroquinone was a better stabilizer for beta-carotene dissolved in mineral oil than was its methyl derivative, toluhydroquinone, and that the 2,5-ditertiarybutyl derivative was actually a pro-oxidant. More recently, Bickoff, Coppinger, Livingston, and Campbell (3) reported certain pyrogallol derivatives to be effective antioxidants for carotene dissolved in oils, but ineffective for the stabilization of the carotene in dehydrated alfalfa meal. Thus, the activity of a given antioxidant is influenced by the system into which it is incorporated. The ease of penetration of the antioxidant into the site of the carotene in the meal may be involved. The work herein reported was an attempt to clarify some of the problems involved in the use of antioxidants for the stabilization of the carotene of dehydrated alfalfa meal.

EXPERIMENTAL

Effect of Solvents

In studying the effect of antioxidants applied to alfalfa meal, Kephart (4) dissolved the chemicals in cottonseed or soybean oil and sprayed the resulting solution on the meal. Thompson (9) used Cellosolve as the solvent. However, he reported that the stabilizing properties of an antioxidant may be influenced by the solvent.

In the work presented herein the substances to be tested were applied to the meal in three different forms to determine the effect of solvent. Each antioxidant was used at the rate of 0.45 g per one-half pound of meal (4#/ton). The first form

consisted of the antioxidant dissolved in 20 ml of acetone. The second form consisted of the antioxidant and 1.8 g of Wesson oil in 20 ml of acetone. The third form consisted of an emulsion of the antioxidant and 1.8 g of Wesson oil in 40 ml of a 4 per cent solution of Triton X-100 in water. These materials were sprayed on the meal by means of a DeVilbiss No. 15 nasal atomizer connected to a source of air pressure by means of a rubber tube. The spray was applied to the meal while the latter was being tumbled in a mixer rotating at 37 r.p.m. The mixing chamber was a cylindrical metal can 10 inches in diameter and 12 inches high. The lid of the can contained a small hole through which the sprayer nozzle was inserted during the spraying operation. Mixing was continued for about ten minutes after the spraying operation was completed to insure uniformity of sample. The treated meal then was placed on papers to allow the solvent to evaporate. Samples of the treated meals were put in eight-ounce screw cap bottles, with an inch of headspace remaining to insure the presence of oxygen. The samples were stored in a dark room maintained at 25°C. The carotene content was determined at the time of storage and at monthly intervals thereafter by the method of Silker, Schrenk, and King (8). The results are shown in Table 1.

In general, the solvent had no marked effect on the antioxidant activity of a given compound. One compound, hydroquinone, was slightly more effective in the water-oil emulsion than when applied in an acetone solution with or without oil. Two compounds, monomethylaniline and propyl gallate, were more effective when applied as an oil-acetone solution. 2,5-Ditertiarybutyl-

Table 1. Effect of the solvent on the stabilizing properties of five compounds when sprayed on dehydrated alfalfa meal.

Compound	Initial carotene	Carotene destruction: Months at 25° C.					
		1	2	3	4	6	8
	mg/100g	Percent					
None							
Unsprayed meal	22.5	13	27	42	57	69	75
Oil in acetone	22.6	18	35	47	58	68	74
N,N'-Diphenyl-p-phenylenediamine							
Acetone	27.7	9	27	32	35	35	46
Oil & acetone	25.8	3	17	22	24	39	43
Emulsion	25.0	3	19	24	38	37	42
2,5-Ditertiarybutylhydroquinone							
Acetone	22.2	12	25	35	36	42	45
Oil & acetone	22.9	12	25	32	34	42	46
Emulsion	22.5	12	24	32	34	42	48
Monomethylaniline							
Acetone	22.4	16	25	35	50	62	69
Oil & acetone	21.6	13	19	28	41	48	61
Emulsion	22.9	14	20	30	42	54	62
Hydroquinone							
Acetone	22.3	17	33	48	53	64	70
Oil & acetone	23.1	22	36	48	52	64	72
Emulsion	22.3	15	27	40	44	57	66
Propyl gallate							
Acetone	22.8	3	36	50	60	68	74
Oil & acetone	21.2	14	25	38	50	63	69
Emulsion	22.8	15	27	44	54	65	71

hydroquinone showed almost an equal amount of stabilizing activity regardless of the form in which it was applied. The data concerning the form in which N,N'-diphenyl-p-phenylenediamine was applied is not reliable. From a comparison of the initial carotene values of the various samples, it will be seen that all samples treated with this compound were appreciably higher. Since all samples in this experiment were prepared from the same lot of meal, it is obvious that the compound interfered with the determination of the carotene. This analytical difficulty will be considered later.

The application of oil only in acetone proved slightly adverse to carotene stability. However, in previous experiments in this laboratory, oil has sometimes improved carotene stability. Thus, perhaps the quality of Wesson oil which was used varied because of differences in processing, age of the oil, or content of natural antioxidants.

Comparison of the Efficiency of Antioxidants on Carotene Concentrates and on Alfalfa Meal

To study further the possibility that penetration of the antioxidant to the site of the carotene in the meal may be a factor, a series of compounds was applied to both solid carotene concentrates and to dehydrated alfalfa meal. Also, since the antioxidant probably would be applied commercially to the hot meal as it came from the dehydrator, the effect of a heat treatment was studied.

Solid carotene concentrates were prepared by the procedure of Mitchell, Schrenk, and King (5). This procedure consists of

extracting alfalfa meal with Skellysolve B, removing the chlorophylls and xanthophylls by adsorption on tricalcium phosphate, and concentrating the resulting solution on a steam plate. Carotene content of the solution was determined by the method of Silker et al. An aliquot containing approximately 75 mg of carotene was placed in a 600 ml beaker and most of the Skellysolve B removed by evaporation on a steam plate. Acetone was added to bring the total volume up to about 15 ml, and 0.15 g of the chemical to be tested was added. Seventy five grams of Cerelose were added, and the contents of the beaker were thoroughly mixed with a stirring rod. The mixture was spread on a sheet of paper and placed in a dark cabinet to allow the solvent to evaporate. The concentrate was thoroughly blended with a spatula, and a portion was placed in a 4-ounce screw-cap bottle with an inch of head space remaining. Carotene determinations were made at the time of storage and at weekly intervals by the method of Silker et al. The results are shown in Table 2.

The dehydrated alfalfa meal samples were prepared by the spraying procedure previously described. However, the compound to be tested as an antioxidant was applied in only one form. This consisted of a solution of the compound and Wesson oil in acetone. The rate of application was the same as previously described. Two portions of each treated meal were placed in 4-ounce screw-cap bottles. One bottle of each treated meal was given a heat treatment. This consisted of heating the bottle of meal for 1 hour at 100° C. The samples were stored in a dark room maintained at 25° C.

Table 2. Comparison of the efficiency of antioxidants when incorporated in Cerelose concentrates.

Compound	Initial carotene	Carotene destruction — Weeks at 25° C.						
		2	4	6	8	12	17	25
	mg/100g	Percent						
None	104	98	100					
2,5-Ditertiarybutylhydroquinone	112	2	4	9	17	27	34	44
Toluhydroquinone	112	5	13	19	25	34	42	55
Hydroquinone	112	8	16	31	55	79	87	94
Monomethyl ether of hydroquinone	109	89	97	99	100			
Dimethyl ether of hydroquinone	116	99	100					
Dibenzyl ether of hydroquinone	110	99	100					
1,5-Dihydroxynaphthalene	109	32	46	56	65	75	83	97
2,5-Ditertiarybutyl-p-cresol	115	20	6	40	45	72	93	98
Propyl gallate	111	44	58	66	73	81	86	94
4-(alpha-methylbenzyl)-2-phenylphenol	112	98	100					
Alpha-methylbenzylphenol mixture	109	98	100					
4-Tert-butyl-2-(alpha-methylbenzyl)-phenol	114	97	100					
p-alpha-Cumylphenol	107	99	100					
Monomethylaniline	103	94	98	99	100			
2-Mercapto-4,6,6-trimethylthiazine	117	4	15	97	100			
N,N'-Di-beta-naphthyl-p-phenylenediamine	116	76	88	92	95	96	100	
Trimethyldihydroquinoline polymer	111	86	92	93	94	95	96	100
Tetramethylthiuram disulfide	115	21	39	50	61	71	76	84
2-Aminopyridine	97	75	84	89	92	96	100	
N-Nitrosodiphenylamine	103	99	100					

Carotene determinations were made at the time of storage and at monthly intervals thereafter. The results are shown in Table 3.

Carotene stability varied markedly, depending on the compound used as an antioxidant. In the series of hydroquinone compounds, there seemed to be a relationship between the structure of the compound and its antioxidant properties. This was true with both the concentrates and the meal samples. Hydroquinone itself had some stabilizing activity. Its methyl derivative, toluhydroquinone, was considerably more effective, while its 2,5-ditertiarybutyl derivative was the best antioxidant tested, not only of the hydroquinones but of all of the 20 compounds. It seems, therefore, that alkylation of the benzene ring of hydroquinone increases the effectiveness both for the concentrates and for the alfalfa meal.

The monomethyl ether of hydroquinone showed less stabilizing action than hydroquinone and the di-ethers such as dimethyl and dibenzyl ethers showed no stabilizing characteristics. Thus, blocking of the hydroxyl groups through ether linkages was detrimental to carotene stability.

Of the other hydroxy compounds studies, 1,5-dihydroxynaphthalene possessed the greatest stabilizing ability, both on the concentrates and on alfalfa meal. During the storage of the concentrate treated with this compound a deep brown color developed. Possibly it was a condensation product of the antioxidant with Cerelose. It seemed to offer no analytical trouble, however. Propyl gallate was more effective with the concentrates than it was with the alfalfa meal. Bickoff et al. (3) also reported this to be true. From the data

Table 3. Effect of heat on the activity of antioxidants when sprayed on dehydrated alfalfa meal.

Compound	Initial carotene	Carotene destruction: Months at 25° C.				
		1	2	3	5	6
	mg/100g	Percent				
None						
Unsprayed and unheated meal	26.1	20	43	56	69	74
Wesson oil, unheated	26.8	21	42	55	68	72
Wesson oil, heated	26.6	17	33	49	61	65
2,5-Ditertiarybutylhydroquinone						
Unheated	26.7	17	24	31	40	45
Heated	26.5	8	15	17	24	32
Toluhydroquinone						
Unheated	26.0	19	28	38	44	52
Heated	25.6	11	21	31	38	45
Hydroquinone						
Unheated	26.7	22	36	50	56	62
Heated	26.4	16	28	42	53	58
Monomethyl ether of hydroquinone						
Unheated	26.1	19	39	56	64	72
Heated	26.1	13	30	47	62	65
Dimethyl ether of hydroquinone						
Unheated	26.8	24	41	59	67	73
Heated	25.8	12	28	50	61	69
Dibenzyl ether of hydroquinone						
Unheated	26.6	25	47	59	69	73
Heated	26.2	21	37	52	62	69

Table 3 (cont.)

Compound	Initial carotene	Carotene destruction: Months at 25° C.				
		1	2	3	5	6
	mg/100g	Percent				
1,5-Dihydroxynaphthalene						
Unheated	27.1	20	34	41	49	56
Heated	26.3	13	25	36	46	53
2,5-Ditertiarybutyl-p-cresol						
Unheated	26.9	20	33	43	51	55
Heated	26.4	14	25	36	43	47
Propyl gallate						
Unheated	26.5	23	27	47	60	66
Heated	26.3	14	27	43	57	62
4-(Alpha-methylbenzyl)-2-phenylphenol						
Unheated	26.6	18	35	56	66	70
Heated	26.4	12	27	52	61	66
Alpha-methylbenzylphenol mixture						
Unheated	26.7	20	40	59	65	72
Heated	26.2	12	31	51	61	66
4-Tert-butyl-2-(alpha-methylbenzyl)-phenol						
Unheated	26.3	21	42	60	69	75
Heated	26.2	13	32	51	62	69
p-alpha-Cumylphenol						
Unheated	26.7	24	46	60	70	75
Heated	26.8	16	39	53	65	72

Table 3 (concl.)

Compound	Initial carotene	Carotene destruction: Months at 25° C. 1	2	3	5	6
	mg/100g	Percent				
Monomethylaniline						
Unheated	26.5	16	23	38	49	53
Heated	26.4	13	14	28	40	44
2-Mercapto-4,6,6-trimethylthiazine						
Unheated	25.5	15	29	39	51	57
Heated	25.7	14	25	32	44	51
N,N'-di-beta-naphthyl-p-phenylenediamine						
Unheated	25.7	14	37	51	62	66
Heated	25.3	6	21	40	51	56
Trimethyldihydroquinoline polymer						
Unheated	26.9	17	32	44	58	64
Heated	26.8	10	21	34	52	61
Tetramethylthiuram disulfide						
Unheated	25.8	18	38	56	65	71
Heated	25.6	14	27	43	56	61
2-Aminopyridine						
Unheated	26.2	28	49	59	70	74
Heated	25.6	15	38	53	65	70
N-Nitrosodiphenylamine						
Unheated	25.2	79	89	90	100	100
Heated	22.0	33	51	56	63	68

it would appear that 2,5-ditertiarybutyl-p-cresol improved the stability of both the concentrates and alfalfa meal, but analytical difficulties prevented a reliable evaluation of the compound. During the chromatographic operation a yellow band moved ahead of the carotene. Frequently this substance was not entirely eliminated before the carotene was eluted, thus leading to high carotene values. Alpha-methylbenzylphenol mixture, 4-tertiarybutyl-2-(alpha-methylbenzyl)-phenol, 4-(alpha-methylbenzyl)-2-phenylphenol, and p-alpha-cumylphenol showed no stabilizing activity with either the concentrates or the alfalfa meal.

Of the nitrogen containing compounds tested, the most effective were monomethylaniline on the meal and tetramethylthiuram disulfide on the concentrates. However, monomethylaniline imparted but little stability to the concentrates, and tetramethylthiuram disulfide but little stability to the meal. Thus, the antioxidant properties of a compound may vary depending on the carotene-containing system into which the compound is incorporated.

Three of the other nitrogen containing compounds, 2-mercapto-4,6,6-trimethylthiazine, N,N'-di-beta-napthyl-p-phenylenediamine, and trimethyl-dihydroquinoline polymer were moderately active on both the meal and the concentrates. 2-Mercapto 4,6,6-trimethylthiazine was very effective on the concentrates during the first month of the storage period, but quickly lost its antioxidant properties thereafter. In contrast, the concentrate containing the trimethyldihydroquinoline polymer lost the greater part of its carotene content during the first two weeks of the storage period, but the loss of the remaining carotene was spread over

a 15 week period. 2-Aminopyridine reduced carotene loss in the concentrates but not in the meal. N-Nitrosodiphenylamine imparted no stability to the concentrates, but behaved as a pro-oxidant with the unheated sample of meal.

Heating the meals after application of the chemicals improved carotene stability to some extent in all cases. These data indicate that if penetration is a factor, the application of heat did not greatly improve it. It is interesting to note that heat markedly improved carotene retention when N-nitrosodiphenylamine was used, the effect of the heat partially counteracting the pro-oxidant activity observed in the unheated sample.

A possible explanation for the effect of heat may be that with additional heating the carotene is isomerized to a greater extent. The isomerized forms might be oxidized at a slower rate than beta-carotene. A study of the rates of oxidation of the carotene isomers would be of value in this respect.

Still another possible explanation may be that the oil contained some peroxides, and that these were destroyed by the heat. Such an explanation would be consistent with the results of Riemenschneider (6), who found that the peroxide number of lard was reduced by heating, and that the stability of the lard was greatly improved.

Analytical Difficulties

During chromatography of the extract of the meal treated with N,N'-diphenyl-p-phenylenediamine, it was observed that the eluate remained colored for a longer period of time than did the

eluate from untreated samples. Normally the eluate becomes colorless after about 175 ml of eluting agent (4 per cent acetone in Skellysolve B) have passed through the column. However, with the N,N'-diphenyl-p-phenylenediamine treated sample, the eluate was not entirely colorless even after 250 ml had passed through the column. This resulted in high carotene values, which can be seen by comparing the initial carotene values of the samples as shown in Table 1. In subsequent analyses this difficulty was observed consistently in connection with the N,N'-diphenyl-p-phenylenediamine sample. Experiments were conducted to study this phonomenon.

Alfalfa meal was sprayed with an acetone solution of N,N'-diphenyl-p-phenylenediamine as described previously. Duplicate determinations were made on this sample and on the untreated meal by the method of Silker et al. After the extracts were placed on the column, 235 ml of the eluting agent were used to wash each column. These eluates were made to a volume of 250 ml and were designated as the "initial eluates." Each column then was washed with an additional 35 ml of the eluting agent. The duplicates of the latter were combined and the volume was reduced to 25 ml under reduced pressure. Absorption curves were obtained both from the initial eluates and from the concentrated tailings. These curves are shown in Fig. 1.

It will be observed that the optical density of the initial eluate of the treated meal was greater at any given wavelength than the optical density of the initial eluate of the untreated

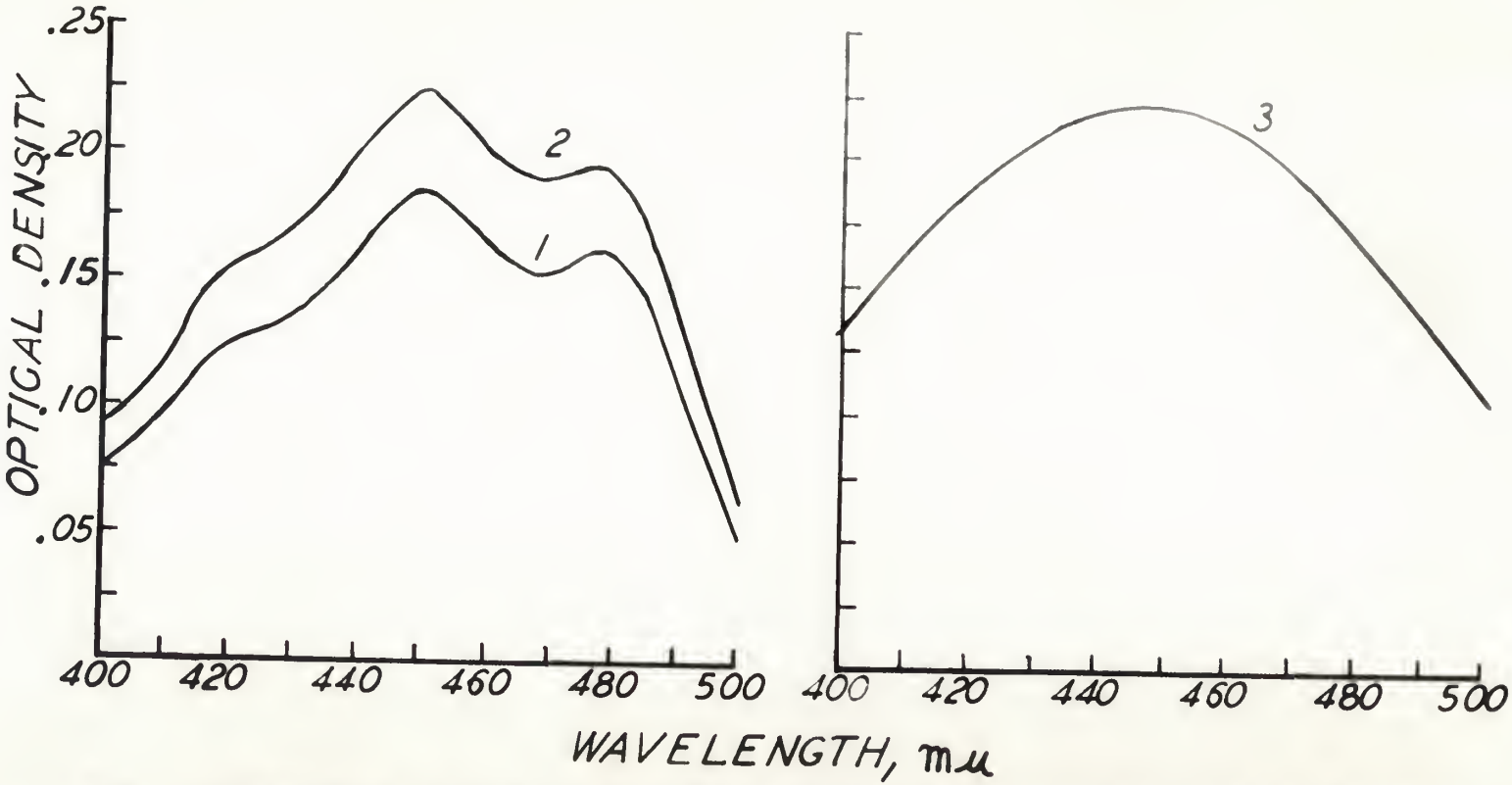

Fig. 1. Absorption spectra obtained from eluates of untreated meal and meal treated with N,N'-diphenyl-p-phenylenediamine; 1, initial eluate from untreated meal; 2, initial eluate from treated meal; 3, tailings from treated meal.

meal. However, the general shapes of the two curves are similar. This indicates that the use of the antioxidant resulted in extraneous color but not to a sufficient degree to alter the general shape of the curve. The tailings of the untreated sample did not contain any color, and hence an absorption curve was not obtained. The tailings of the treated meal was yellow, but its absorption curve differed markedly from that of the initial eluate of the untreated meal. Hence, the pigment in the tailings was not carotene. It will be noted that the absorption curve has only one maximum, which coincides with one of the peaks of carotene.

The source of the extraneous color was realized when a small amount of the N,N'-diphenyl-p-phenylenediamine was dissolved in a solution of 4 per cent acetone in Skellysolve B and a small amount of the active component of the adsorbent, magnesium oxide, was added to the solution. A yellow color developed. It was found that the Skellysolve B could be left out of the reaction mixture and the color still would be produced. However, acetone was necessary for the reaction to occur. Possibly the substance producing the color is a condensation product of the acetone and the diamine, the condensation taking place under the influence of the magnesium oxide.

An attempt was made to estimate the amount of contamination from this source which would result during an actual carotene determination. Eighty milligrams of the N,N'-diphenyl-p-phenylenediamine were dissolved in 40 ml of acetone and this solution was diluted to 1 liter with Skellysolve B. A 25 ml aliquot of this

solution was chromatographed on a magnesium oxide column. The amount of the compound (2 mg) thus was equivalent to the amount which was present on a one gram sample of the treated meal analyzed previously. The chromatogram was developed with 210 ml of 4 per cent acetone in Skellysolve B. This eluate was made to a volume of 250 ml and designated as the "initial eluate." The optical density of this solution measured at 4360 A^{o} corresponded to 6.8 mg of carotene per 100 g of alfalfa meal. Tailings were obtained by further developing the chromatogram with 35 ml of the eluting agent. The tailings from two such chromatograms were combined and concentrated to 25 ml as described above. Absorption curves were obtained both from the initial eluate and from the tailings. These curves are shown in Fig. 2.

The absorption spectrum of the initial eluate was similar to that of the tailings, differing only in height. Hence, the constituents of the two eluates appear to be identical. Also, a comparison of the absorption spectrum of the tailings of this experiment (Fig. 2) and the tailings of the treated meal (Fig. 1) reveals their great similarity. This is additional evidence that the material is not a carotenoid and is not derived by interaction of the antioxidant with some constituent of the meal, since neither carotenoid nor alfalfa meal was present at any time during the course of this experiment.

It was noticed that color appeared in the eluate very quickly during the development of the chromatogram of the 25 ml aliquot of N,N'-diphenyl-p-phenylenediamine solution. Thus, it appears

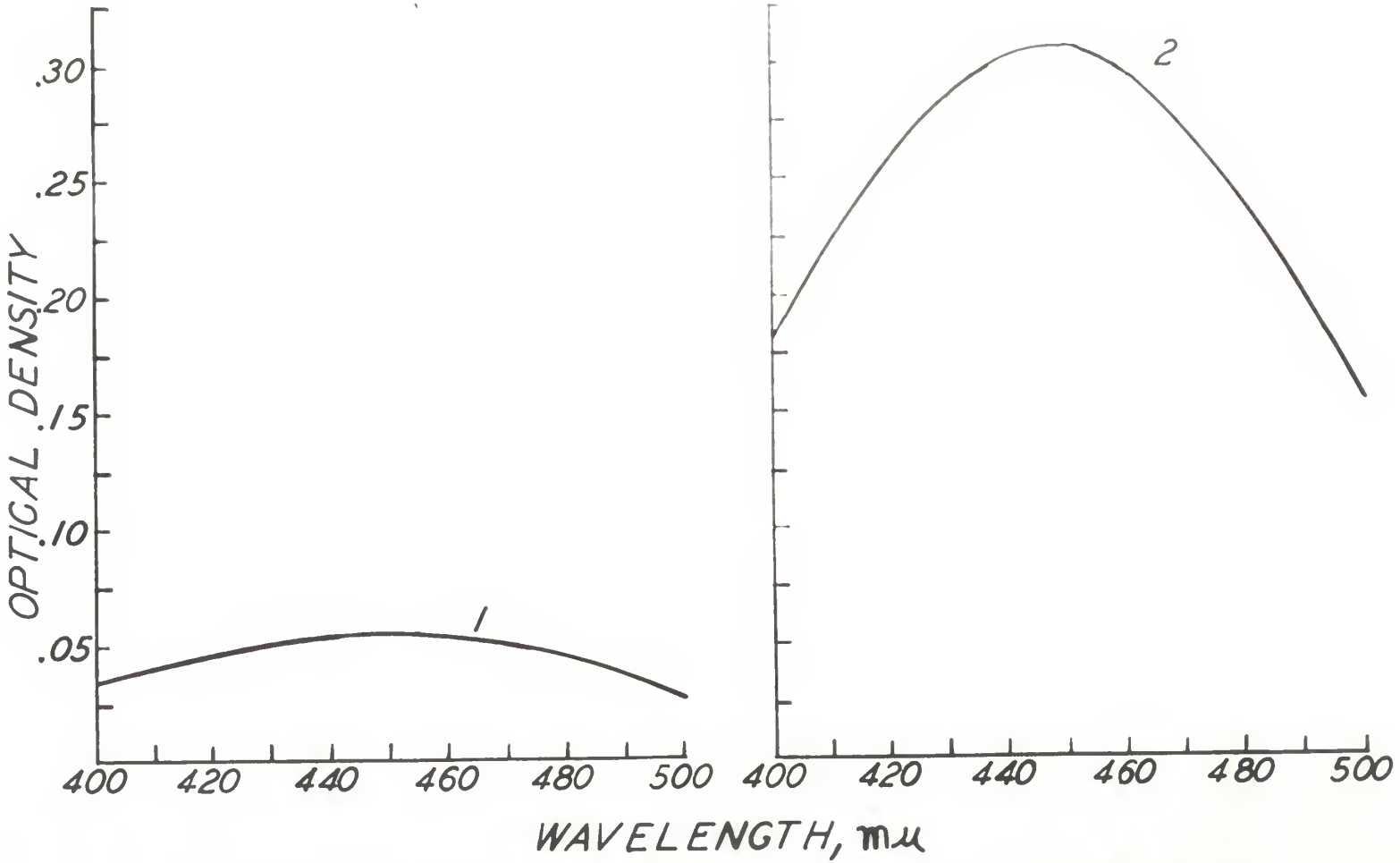

Fig. 2. Absorption spectra obtained from eluates of the solution of N,N'-diphenyl-p-phenylenediamine; 1, initial eluate; 2, tailings.

that the substance elutes continuously, and it would be impossible to elute carotene during an analysis of meal treated with this compound without also eluting some of it. In view of these observations, it would seem improbable that this compound can be evaluated as a carotene antioxidant by methods employing magnesium oxide as an adsorbent. A correction factor would be of questionable value, since its magnitude would depend on the concentration of the antioxidant on the meal, the dimensions of the adsorption column, and the amount of eluting agent used.

Some of the other compounds which were investigated may have interfered with carotene determination also. As previously mentioned, 2,5-ditertiarybutyl-p-cresol produced a yellow band which could not be separated entirely from the carotene during elution. N,N'-di-beta-naphthyl-p-phenylenediamine developed an orange band on the column above the carotene band. Although the carotene appeared to be eluted completely before the orange band began to elute, there is always the possibility that closely adjacent bands will not be separated sharply. With most of the antioxidant-treated meal samples there was a tendency for the eluate to remain colored for a longer period of time than with the untreated samples. 2,5-Ditertiarybutylhydroquinone especially seemed to "tail", but evidently did not contribute sufficient extraneous color to cause the initial carotene values of the treated and untreated samples to differ significantly. Thus, caution should be used in evaluating all antioxidants as stabilizers for the carotene in alfalfa meal. Perhaps careful bioassays will be needed, in conjunction with

chemical determinations, for the final evaluation of those compounds which appear promising as carotene antioxidants.

SUMMARY

Five antioxidants were sprayed on dehydrated alfalfa meal, each in an acetone solution, an acetone-oil solution, and a water-oil emulsion. The solvent system had little effect on the carotene stability obtained with a given antioxidant.

Twenty compounds were tested as antioxidants when incorporated into concentrates of carotene on Cerelose and when sprayed on dehydrated alfalfa meal. 2,5-Ditertiarybutylhydroquinone, toluhydroquinone, and 1,5-dihydroxynaphthalene were effective on both the concentrates and meal. Monomethylaniline was quite effective on the meal but imparted little stability to the concentrates. Tetramethylthiuram disulfide showed appreciable antioxidant activity on the concentrates but was of little value when applied to the meal. Of the other compounds tested, none showed strong antioxidant activity on either the concentrates or meal.

A portion of each of the meals treated with one of the twenty compounds was subjected to a temperature of 100° C. for one hour. The heat treatment improved carotene stability slightly in every case, as compared to the non-heated portion of meal.

In the series of hydroquinone compounds tested, alkylation of the benzene ring improved carotene stability both with the concentrates and alfalfa meal. Blocking the hydroxyl groups of

hydroquinone through ether linkages proved detrimental to the stabilizing activity of the compound. The structures necessary for nitrogen-containing compounds to function as carotene antioxidants could not be ascertained from the compounds tested.

N,N'-Diphenyl-p-phenylenediamine produced an extraneous color during development of the chromatogram which interfered with carotene determination. 2,5-Ditertiarybutyl-p-cresol developed a yellow band on the column below the carotene which could not be separated entirely from the carotene. Other compounds had a tendency to cause "tailing." Thus, the present method of determining carotene may not permit an accurate evaluatation of the stabilizing action of a compound on carotene.

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Much time has been spent in an attempt to stabilize carotene against atmospheric oxidation. Oxidized carotene cannot be converted by the animal body into vitamin A. Dehydrated alfalfa meal is a rich source of carotene and is the chief source of vitamin A for many mixed feeds. Hence the question of stabilizing carotene in dehydrated alfalfa meal is a problem of great economic importance. This investigation is concerned with the use of certain chemicals as antioxidants for carotene.

Five antioxidants were sprayed on dehydrated alfalfa meal, each in an acetone solution, an acetone-oil solution, and a water-oil emulsion. The treated samples were stored in a dark room maintained at 25° C. Carotene content was determined at the time of storage and at regular intervals thereafter. The solvent system had little effect on the carotene stability obtained with a given antioxidant.

Twenty compounds were tested as antioxidants when incorporated into concentrates of carotene on Cerelose and when sprayed in an acetone-oil solution on dehydrated alfalfa meal. The compounds tested included hydroquinone and its derivatives, other aryl hydroxyl compounds, and nitrogen-containing compounds. The treated samples were stored at 25° C. and carotene content determined at regular intervals as above. 2,5-Ditertiarybutylhydroquinone, toluhydroquinone, and 1,5-dihydroxynaphthalene were effective on both the concentrates and meal. Monomethylaniline was quite effective on the meal but imparted little stability to the concentrates. Tetramethylthiuram disulfide

showed appreciable antioxidant activity on the concentrates but was of little value when applied to the meal. Of the other compounds tested, none showed strong antioxidant activity on either the concentrates or meal.

In the series of hydroquinone compounds tested, alkylation of the benzene ring improved carotene stability both with the concentrates and alfalfa meal. Blocking the hydroxyl groups of hydroquinone through ether linkages proved detrimental to the stabilizing activity of the compound. The structures necessary for nitrogen-containing compounds to function as carotene antioxidants could not be ascertained from the compounds tested.

The effect of a heat treatment on the meal samples treated with each of the twenty compounds was also studied. The heat treatment consisted of subjecting a portion of the treated meals to a temperature of 100° C. for one hour. In every case the heated sample showed greater stability than the treated sample not receiving the heat treatment.

It was found that N,N'-diphenyl-p-phenylenediamine produced an extraneous color during development of the chromatogram which interfered with carotene determination. It seems that the color is due to the condensation of the antioxidant with acetone when in the presence of magnesium oxide, the active constituent of the absorbent. 2,5-Ditertiarybutyl-p-cresol developed a yellow band on the column below the carotene which could not be separated entirely from the carotene. Other compounds had a tendency to cause "tailing." Thus, the present method of determining carotene may not permit an accurate evaluation of the stabilizing action of a compound on carotene.