ALFALFA CAROTENE

Quinoline Derivatives as Antioxidants for Carotene

E. M. BICKOFF, A. L. LIVINGSTON, JACK GUGGOLZ, and C. RAY THOMPSON

Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Albany 6, Calif.

Because carotene stabilization in alfalfa meal is an important problem and because 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline had proved to be an effective antioxidant, a study was made of the ability of certain related chemicals to inhibit oxidation of carotene. The compounds tested were chosen to permit observation of variation in activity with systematic variation in structure. Several were effective in both dehydrated alfalfa meals and mineral oil solutions.

THE ANTIOXIDANT, 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline, exerts a protective effect on carotene in alfalfa meal (*4*, *5*). Recent tests with rats and poultry have revealed that its toxicity is low, and treated meal (containing not more than 0.015% of antioxidant) for use in poultry feeds is now shipped in interstate commerce. Results of these tests, which were conducted by the Agricultural Experiment Stations of Colorado and Illinois, Poultry Producers of Central California, Monsanto Chemical Co., and Western Utilization Research Branch of the U. S. Department of Agriculture, have been summarized in an unpublished report (*7*). A study has now been made of closely related compounds which might have a similar or more effective protective action. The compounds tested were chosen to permit observation of variation in activity with systematic variation in structure.

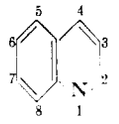
Stability of Carotene

In Oil Solution The effectiveness of the antioxidants in preserving carotene in oil solution was determined first. A solution of 1.2 mg. of purified crystalline carotene in 1.0 ml. of medicinal grade mineral oil was used as the substrate. The stability test consisted of a determination of the time required for breakdown of 20% of the carotene in the oil solution stored as a thin layer at 75° C. under specified conditions (*8*). To facilitate the comparative evaluations, the antioxidants were incorporated on an equivalent molecular basis. Thus in all cases the test solution contained the added antioxidant in the proportion of 1 molecule of antioxidant to 2 molecules of carotene. Chromatographic adsorption on magnesia was employed to remove the colored oxidation products of carotene which developed during the storage period of the test (*6*).

In Alfalfa Meal The effect of the antioxidants was also tested on carotene in alfalfa meal. A solution of the compound (0.125% on a dry meal basis) in ethylene glycol monoethyl ether was sprayed on a sample of meal while it was tumbled at 12 r.p.m. in a rotary mixer. The initial carotene content was about 200 p.p.m., depending on age of the meal. Residual carotene was measured after 2 weeks at 65° C. and after 18 months at 25° C. (*4*). Beauchene *et al.* showed that 6-ethoxy - 2,2,4 - trimethyl - 1,2 - dihydroquinoline did not interfere with carotene assay (*1*).

Stability of Derivatives

In Mineral Oil Solution The antioxidant effectiveness of quinoline and a number of its derivatives is reported in Table I. The quinoline nucleus is as follows:



Column 3 shows the comparative antoxidant effects of the various compounds tested for carotene in mineral oil solution. The stability value of the control—i.e., the time in hours required for 20% loss of carotene in the absence of antioxidants—was 1 hour.

Although neither quinoline itself nor several of its substituted derivatives (compounds 1 to 12) possessed antioxidant activity, the tetrahydroquinoline was moderately active, whereas the decahydroquinoline was inactive (compounds 13 and 26). The presence of three methyl groups on the ring, as in 2,2,-4-trimethyl-1,2-dihydroquinoline (compound 24), did not enhance the activity over that of the tetrahydroquinoline. The presence of a fourth methyl group on the 6-position of the ring gave a compound having threefold more antioxidant activity (compound 33). When this fourth methyl group was present at the 8-position on the ring, the activity was reduced to one fifth that of the 6,2,2,4-tetramethyl derivative (compound 18). A compound containing 2 molecules of 8,2,2,4-tetramethyl-1,2-dihydroquinoline linked through the 6-position (compound 29) had considerably more activity than the unlinked compound. The presence of either a phenyl or indanyl group on the 6-position of 2,2,4 - trimethyl - 1,2 - dihydroquinoline gave a fivefold increase in antioxidant activity, while a chlorine atom on position 7 reduced the activity to half that of the parent compound (compounds 19, 30, and 32).

The most effective antioxidant studied was 6-hydroxy-2,2,4-trimethyl-1,2-dihydroquinoline (compound 40). The following alkoxy derivatives (in descending order of effectiveness) were also potent antioxidants: 6-*n*-propoxy-, 6-ethoxy-, 6-methoxy-, and 6-isopropoxy-2,2,4 - trimethyl - 1,2 - dihydroquinoline (compounds 36 to 39). Somewhat less effective was 6-*n*-butoxy-2,2,4-trimethyl-1,2-dihydroquinoline (compound 35). The presence of two or more alkoxy groups on the ring (compounds 16, 21),

Table I. Efficiency of Antioxidants for Stabilizing Carotene in Mineral Oil Solution and in Alfalfa Meal

Compound No.	Name of Compound	Effect on Carotene Stability: Mineral oil[a], hours	Carotene Remaining in Alfalfa Meal[b], %: 2 weeks, 65° C.	18 mo., 25° C.
Control	None	1	22	13
1	Quinoline	1	24	..
2	8-Aminoquinoline	1	20	..
3	4,8-Dimethyl-2-hydroxyquinoline	1	22	15
4	8-Hydroxyquinoline	1	22	8
5	5,7-Dichloro-8-hydroxyquinoline	1	4	2
6	Isoquinoline	1	24	..
7	6-Methoxyquinoline	1	27	16
8	6-Phenylquinoline	1	27	14
9	6-Ethoxy-2,4-dimethylquinoline	1	27	15
10	2,6-Dimethylquinoline	1	28	16
11	2-Hydroxy-4-methylquinoline	1	28	..
12	3-Aminoquinoline	1	28	..
13	Decahydroquinoline	2	28	16
14	6-Phenyl-2,3-diisobutyl-2-methyl-1,2-dihydroquinoline	3	30	23
15	5,8-Dimethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	4	39	44
16	6,7-Dimethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	5	48	29
17	8-Ethyl-2,2,4-trimethyl-1,2-dihydroquinoline	8	42	41
18	8,2,2,4-Tetramethyl-1,2-dihydroquinoline	9	53	41
19	7-Chloro-2,2,4-trimethyl-1,2-dihydroquinoline	10	50	..
20	8-Methoxy-2,2,4-trimethyl-1,2-dihydroquinoline	10	40	37
21	6,7,8-Trimethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	11	43	44
22	6-Ethoxy-2-ethyl-2,3,4-trimethyl-1,2-dihydroquinoline	11	41	33
23	6-Butyl-2,2,4-trimethyl-1,2-dihydroquinoline	12	30	23
24	2,2,4-Trimethyl-1,2-dihydroquinoline	16	43	47
25	6-(2-Benzothiazoyl)-2,2,4-trimethyl-1,2-dihydroquinoline	17	32	25
26	1,2,3,4-Tetrahydroquinoline	18	39	41
27	6-Ethoxy-2,4-diethyl-2-methyl-1,2-dihydroquinoline	23	53	53
28	8-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	22	38	40
29	6,6′-Bis(8,2,2,4-tetramethyl-1,2-dihydroquinoline)	40	34	28
30	6-Phenyl-2,2,4-trimethyl-1,2-dihydroquinoline	40	48	45
31	6-Isopropyl-2,2,4-trimethyl-1,2-dihydroquinoline	47	53	46
32	6-(1-Indanyl)-2,2,4-trimethyl-1,2-dihydroquinoline	48	45	42
33	6,2,2,4-Tetramethyl-1,2-dihydroquinoline	48	53	50
34	Acid-rearranged 2,2,4-trimethyl-1,2-dihydroquinoline	50	27	15
35	6-*n*-Butoxy-2,2,4-trimethyl-1,2-dihydroquinoline	68	59	58
36	6-Isopropoxy-2,2,4-trimethyl-1,2-dihydroquinoline	88	58	61
37	6-Methoxy-2,2,4-trimethyl-1,2-dihydroquinoline	110	57	59
38	6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	112	59	60
39	6-*n*-Propoxy-2,2,4-trimethyl-1,2-dihydroquinoline	135	56	57
40	6-Hydroxy-2,2,4-trimethyl-1,2-dihydroquinoline	155	38	20

[a] Time in hours for 20% loss of carotene at 75° C.
[b] % of initial carotene retained after 2 weeks' storage at 65° C. and after 18 months' storage at 25° C.

or the presence of the alkoxy group on the 8 rather than the 6-position, resulted in the loss of most of the antioxidant effectiveness (compounds 20, 37; 28, 38). Compounds containing alkyl groups in the 6-position were less than half as effective as the corresponding alkoxy derivatives (compounds 23, 35; 31, 36; 33, 37), while the compounds containing the alkyl groups in the 8-position were even less effective (compounds 18 and 33). The presence of methyl groups at the 2,2,4-positions on the ring was important for antioxidant effect. Compounds containing higher alkyl groups in one or more of these positions had much less antioxidant activity (compounds 14, 30; 27, 38).

In Alfalfa Meal

Column 4 of Table I shows the comparative antioxidant effects of the various compounds tested for carotene in alfalfa meal in the accelerated storage test. After 2 weeks' storage at 65° C. the control sample of meal retained only 22% of its original carotene. The 6-hydroxy - 2,2,4 - trimethyl - 1,2 - dihydroquinoline, which was the most effective antioxidant in mineral oil, gave about a twofold increase in stability over the control in alfalfa meal (compound 40). The most effective antioxidants in alfalfa meal were the 6-alkoxy derivatives. The five such derivatives tested were all about equally effective, producing a threefold increase in carotene stability (compounds 35 to 39). As in the oil medium, the presence of the alkoxy group on the 8-position of the ring resulted in considerable loss in antioxidant activity (compounds 20, 37; 28, 38). Likewise, the di- and trialkoxy derivatives were not as effective antioxidants as the monoalkoxy derivatives (compounds 16 and 21). The monoalkoxy derivatives were also much more effective than the corresponding alkyl derivatives (compounds 23, 35; 33, 37; 31, 36). Although the acid-rearranged 2,2,4-trimethyl-1,2-dihydroquinoline was an effective antioxidant in the mineral oil media, it had very little activity in the alfalfa meal (compound 34).

One derivative, 5,7-dichloro-8-dihydroxyquinoline, proved to have a pro-oxidant effect on carotene in alfalfa meal (compound 5).

As the accelerated storage tests at 65° C. may not give results comparable to those obtained in commercial storage, a comparison was made between results obtained at 65° and 25° C. Column 5 of Table I shows the comparative antioxidant effects of the various compounds tested for carotene in alfalfa meal after 18 months' storage at 25° C. In most cases, the relative order of effectiveness of the compounds at 25° C. was comparable to the results obtained at 65° C.

Discussion

Considerable agreement was found between the results in alfalfa meal and in oil solution. Thus, the 6-alkoxy-2,2,4-trimethyl-1,2-dihydroquinoline derivatives, which were among the most effective derivatives for carotene in mineral oil solution, were also the most effective for carotene in alfalfa meal. This is in contrast to earlier results with pyro-

gallol and other phenolic antioxidants, which were effective for carotene in oil solution but relatively ineffective in alfalfa meal (*2, 3*). A notable exception is 6-hydroxy-2,2,4-trimethyl-1,2-dihydroquinoline, which, although the most effective compound tested in mineral oil solution, was only moderately effective in the meal (compound 40). As has been pointed out (*2, 3*), if a compound is to be capable of preventing carotene loss in a complex substance such as alfalfa meal, it must not only be an effective antioxidant, but must also satisfy a number of other conditions, including solubility in that phase which it is designed to protect. The oily nature of the alkoxy derivatives seems to facilitate their passage through the meal to the site of the carotene. The hydroxy derivative, on the other hand, is a crystalline solid.

The sensitive positions on the quinoline ring were shown to be the 2-, 4-, and 6-positions. Furthermore, the specificity at these positions was very pronounced. Thus, alkyl substitution of all three positions enhanced antioxidant activity, and alkoxy substitution on the 6-position was even more effective than alkyl substitution.

Acknowledgment

The authors are indebted to the Monsanto Chemical Co. for many of the quinoline derivatives used in this study. The technical assistance of I. V. Ford is gratefully acknowledged.

Literature Cited

(1) Beauchene, R. E., Mitchell, H. L., Parrish, D. B., and Silker, R. E., J. AGR. FOOD CHEM., **1**, 461 (1953).
(2) Bickoff, E. M., *J. Am. Oil Chemists' Soc.*, **28**, 65 (1951).
(3) Bickoff, E. M., Coppinger, G. M., Livingston, A. L., and Campbell, T. W., *Ibid.*, **29**, 51 (1952).
(4) Thompson, C. R., *Ind. Eng. Chem.*, **42**, 922 (1950).
(5) Thompson, C. R., U. S. Patent 2,562,970 (Aug. 7, 1951).
(6) Thompson, C. R., and Bickoff, E. M., *J. Assoc. Offic. Agr. Chemists*, **34**, 219 (1951).
(7) Western Utilization Research Branch, U. S. Dept. Agriculture, unpublished report, January 1954.
(8) Williams, K. T., Bickoff, E., and Lowrimore, B., *Oil & Soap*, **21**, 161 (1944).

Received for review August 16, 1954. Accepted October 23, 1954. Presented before the Division of Agricultural and Food Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, *New York, N. Y.*

GARVAN MEDAL ADDRESS

PROTEINS IN FLOUR

Review of the Physical Characteristics of Gluten and Reactive Groups Involved in Change in Oxidation

BETTY SULLIVAN
Russell-Miller Milling Co., Minneapolis, Minn.

The present state of our knowledge of the proteins of flour is reviewed. The reactive groups of gluten involved in the oxidation and reduction of flour are, so far as is known, the sulfur-containing amino acids, cysteine, cystine, and methionine. Maturing agents exert their beneficial effect by action on free and combined mercapto groups of the protein, but the mechanism of their action is still unsolved.

WHEAT FLOURS vary widely in their baking characteristics and in their response to oxidation. These variations are due, in large measure, to the amount and the physical properties of the proteins of the flour.

Osborne (*27*), in his classical work on the wheat proteins, separated the proteins of flour into five main fractions based on solubility: gliadin, a prolamine soluble in 70% ethyl alcohol; glutenin, soluble in dilute acid and dilute alkali; a neutral salt-soluble globulin; a water-soluble, heat-coagulable albumin; and an ill-defined "proteose." The classification of these proteins on the basis of solubility behavior has not proved very satisfactory. Gortner (*18, 19*), Blish (*8–10*), McCalla (*23–25*), Rich (*33*), and others have shown that, in the separation of wheat flour glutens, one is not dealing with true solubility effects, but rather with a complex mixture of components which possess different degrees of peptizability with varied ionic environment. The globulin fraction of the wheat flour proteins has since been shown to be made up of three individual components (*12, 30*) and the albumin fraction to consist of at least six individual components similar in molecular weight but differing in electrophoretic patterns (*29*).

Gluten

We are concerned in practice with a complex mixture of gliadin and glutenin, together with small amounts of lipides and starch, commonly known as gluten. Neither gliadin nor glutenin is a homogeneous protein and the so-called glutenin fraction, particularly, cannot be dispersed in any solvent sufficiently well to permit the use of the ultracentrifuge, electrophoresis, or usual physical techniques. It is consequently ill characterized.

Beccari (*2*) in 1745 first reported the separation of gluten from the starch of flour. Gluten is conveniently prepared by adding 60 to 65% water to a hard wheat flour, mixing and allowing the dough to rest about 30 minutes, then washing out the bulk of the starch and other more soluble constituents under a steady stream of water. An elastic, rubberlike material holding, roughly, two thirds of its weight of water is obtained.

Gluten has the approximate composition shown in Table I, naturally varying