Dihydroquercetin as an Antioxidant

E. F. KURTH [1] and FRANK L. CHAN [2]

DIHYDROQUERCETIN has been found in Douglas fir heartwood (4), and as much as 20% of this material has been obtained from the cork portion of Douglas fir bark, *Pseudotsuga taxifolia* (Poir.) Britt. (7). It also has been isolated in this laboratory from the bark of Jeffrey pine, *Pinus jeffreyi* Grev. and Balf. It differs chemically from the naturally occurring yellow flavonol, quercetin, by having two more hydrogen atoms in the molecule. The conversion of dihydroquercetin to quercetin in the laboratory is effected with hot dilute mineral acids or with bisulfite solutions.

Dihydroquercetin is white in color and crystallizes from hot aqueous solutions with water of crystallization that is lost upon heating at approximately 125°C. The solubility has been found to be 9.3% in boiling water and 0.51% in water at 15°C. The melting point of dihydroquercetin had been reported previously to be approximately 242°C. Recent work with large quantities of dihydroquercetin has shown that this melting point is too low. When the crude dihydroquercetin is recrystallized from boiling 0.5% sulfuric acid solution and then from boiling water containing a little decolorizing charcoal, the melting point is 252 to 253°C. as determined on a Fischer-Johns melting point block.

Douglas fir is the major lumber, plywood, and pole and piling species in this country. The potential annual supply of Douglas fir bark is estimated to be in excess of two million tons. Recent analyses in this laboratory of Douglas fir bark taken from sawlogs are given in Table I. The analytical data show that a ton of such bark may contain from 80 to 152 pounds of dihydroquercetin.

TABLE I

Analyses of Douglas Fir Bark from Sawlogs
(Percentage of oven-dry weight of bark)

Source of bark	Tannin	Waxes	Dihydroquercetin
	%	%	%
Rockport, California	8.8	6.5	6.0
Taos, New Mexico	13.6	9.2	7.6
Fall Creek, Oregon	7.3	10.2	4.0
Molalla, Oregon	8.1	8.0	5.5

Preparation of Dihydroquercetin

Dihydroquercetin may be obtained along with a small amount of reddish-brown tannin by re-extracting benzene extracted (wax-free) bark with ethyl ether or isopropyl ether. Also it may be obtained by re-extracting the solid or concentrated hot-water extracts from Douglas fir bark with either of these ethers. A crude reddish-brown crystalline product was usually obtained when the hot-water extract from the cork fraction of the bark was evaporated to dryness or when powdered hot-water extracts from the bark were re-extracted with acetone or other ketones.

The dihydroquercetin used in this work was prepared from the hot-water extracts from Douglas fir bark by re-extraction with ethyl ether. Distillation of the ethyl ether left a light brown powder, which was further purified by recrystallization once from a hot 0.5% solution of sulfuric acid and then from boiling water that contained a little decolorizing charcoal. When purified in this manner, the product was obtained as white crystals having a melting point of 252 to 253°C. If the recrystallization from dilute sulfuric acid was omitted, the product usually melted at 241 to 242°C.

Crude dihydroquercetin also was purified by recrystallization from a hot sodium bisulfite solution. On cooling, the bisulfite addition compound separated as white crystals, which decomposed on heating at about 225°C. The free flavanone and some quercetin were recovered from the bisulfite compound upon acidification with dilute sulfuric acid. When recrystallized from hot water, the product had a melting point of 246 to 247°C.

Antioxidant Properties of Dihydroquercetin

The antioxidant activity of dihydroquercetin and certain of its esters was determined on lard, cottonseed oil, and butter oil by methods similar to those used by other investigators (2, 6, 11). Weighed amounts of the lard or oils were placed in open glass containers and exposed to the atmosphere at 60°C., 97.7°C., and at room temperature. A constant-temperature bath was used for the tests made at 60°C. and 97.7°C. When lard samples were tested for stability at 97.7°C., a rapid stream of air was allowed to bubble through the samples. The procedure and apparatus recommended by the American Oil Chemists' Society Committee on Analysis of Commercial Fats and Oils were used (1, 3, 10, 13). Changes in peroxide values were determined by the Henderson and Young modification (5) of the Wheeler method (14) as well as by the method recommended by the American Oil Chemists' Society Committee on Analysis of Commercial Fats and Oils (10). Each of these procedures gave the same peroxide value when applied to a given oil. Free fat acid tests were made in accordance with the method of The Association of Official Agricultural Chemists (9). Under the experimental conditions employed in these tests no appreciable change in free fat acid was noticed.

Lard samples containing no antioxidant were obtained from local markets. Cottonseed oil, U. S. P., was procured from the School of Pharmacy, Oregon State College. This oil had been in storage for some time and had a golden color. Butter oil was prepared for this work by G. A. Richardson, Dairy Chemistry Laboratory, Oregon State College. No color was added to this oil, and its melting point was approximately 32 to 33°C.

The antioxidant was added by mixing one ml. of an alcoholic solution of the antioxidant to 100 grams of the lard or oil. For the tests at 60°C., 10-gram samples of the control and the test specimens were placed in 25 mm. test tubes and then stored in the constant temperature bath whereas the samples tested at room temperature were placed in 250-ml. Erlenmeyer flasks. In this way, for each temperature condition, the ratio of surface exposed to the air to the weight of the sample was constant. Samples were tested for per-

[1] Oregon Forest Products Laboratory and Department of Chemistry, Oregon State College, Corvallis, Ore.
[2] Oregon Forest Products Laboratory, Corvallis, Ore.

oxide values, expressed as milliequivalents per kg. of fat, at recorded intervals.

Lard. The effects of 0.015, 0.03, 0.06, and 0.1% of dihydroquercetin on the stability of lard are shown in Figure 1. Mixtures containing more than 0.03% dihydroquercetin were cloudy at 60°C. The results indicate that the resistance of lard to oxidation increased as the proportion of dihydroquercetin was increased.

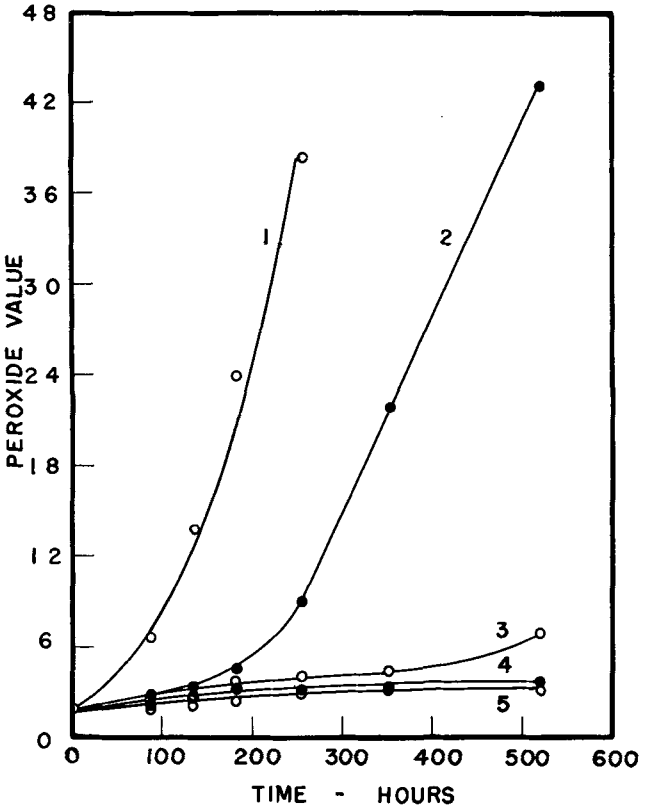

FIG. 1. Peroxide values of lard with different amounts of dihydroquercetin at 60°C.
1. 0.0% dihydroquercetin as control.
2. 0.015% dihydroquercetin.
3. 0.030% dihydroquercetin.
4. 0.060% dihydroquercetin.
5. 0.100% dihydroquercetin.

This same lard sample was subjecetd to the fat stability test by the active oxygen method recommended by the Committee on Analysis of Commercial Fats and Oils (10). The results shown in Table II are in agreement with the results shown in Figure 1.

A study of the comparative antioxidant effects of dihydroquercetin and quercetin on lard was made, and the results are given in Figure 2. The peroxide values indicate that quercetin has a greater antioxidant activity than dihydroquercetin. Quercetin however has the disadvantage of giving a yellow tint to lard.

TABLE II

Effect of Dihydroquercetin on the Stability of Lard at 97.7°C.

Mixture	Stability (A.O.M.)
	Hrs.
Control	5
Lard + 0.015% dihydroquercetin	16
Lard + 0.03%	43
Lard + 0.06%	79

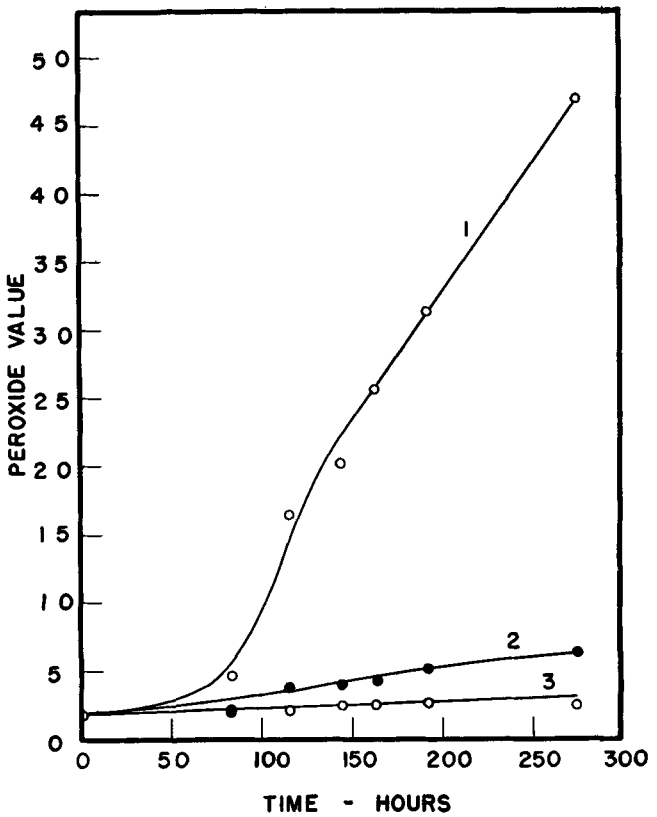

FIG. 2. Peroxide values of lard with dihydroquercetin and quercetin at 60°C.
1. 0.0% antioxidant as control.
2. 0.030% dihydroquercetin.
3. 0.030% quercetin.

The effect of adding citric acid and dihydroquercetin to lard is shown in Figure 3. In this experiment the peroxide values of lard a) without an antioxidant, b) with 0.015% dihydroquercetin and 0.015% of citric acid, and c) with 0.03% of dihydroquercetin were determined. The experimental results indicate that citric acid enhances the antioxidant activity of dihydroquercetin in lard.

The lard sample used for the experiment on the synergistic action of citric acid at 60°C. was also tested by the active oxygen method (10). The results are shown in Table III.

TABLE III

Stability of Lard with Citric Acid and Dihydroquercetin

Mixture	Stability (A.O.M.)
	Hrs.
Control	4.5
Lard + 0.015% dihydroquercetin	18.0
Lard + 0.015% each of dihydroquercetin and citric acid	34.0
Lard + 0.03% dihydroquercetin	41.0
Lard + 0.03% each of dihydroquercetin and citric acid	57.0

It has been reported that tests by the active oxygen method show no constant relationship between the stability of lard and storage at room temperature (12). The lard stability tests with different quantities of dihydroquercetin therefore were repeated at room temperature (20 to 30°C.). In the latter tests 10.0 ± 0.01 grams of the lard mixtures were placed in 250-ml. Erlenmeyer flasks and stored away from dust in a cabinet. The ratio of surface to weight was 5.0 sq. cm. per gram. Peroxide values were deter-

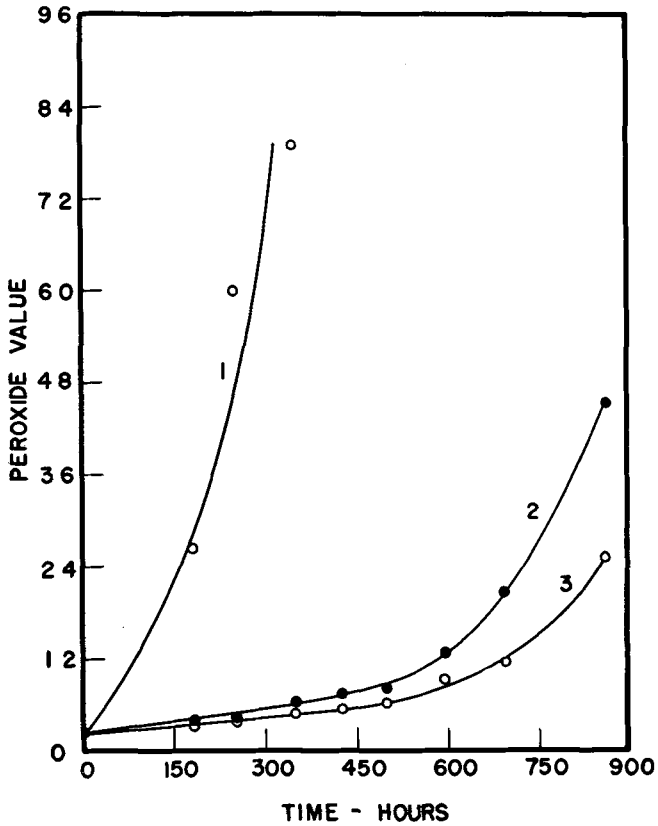

FIG. 3. Peroxide values of citric acid and dihydroquercetin in lard at 60°C.
1. 0.0% antioxidant as control.
2. 0.030% dihydroquercetin.
3. 0.015% citric acid and 0.015% dihydroquercetin.

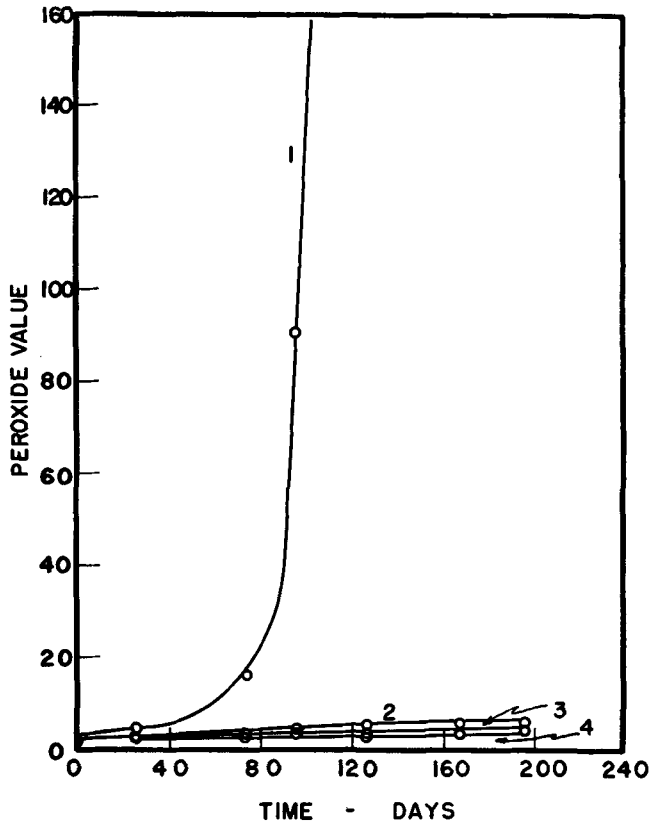

FIG. 4. Peroxide values of lard at room temperature.
1. 0.0% dihydroquercetin as control.
2. 0.030% dihydroquercetin.
3. 0.060% dihydroquercetin.
4. 0.100% dihydroquercetin.

mined over a period of 196 days, and the results are shown in Figure 4.

Butter Oil and Cottonseed Oil. The initial peroxide value of the sample of cottonseed oil on hand was greater than expected. However the effect of dihydroquercetin on the stability of this oil was found similar to that for lard as is shown in Figure 5. The

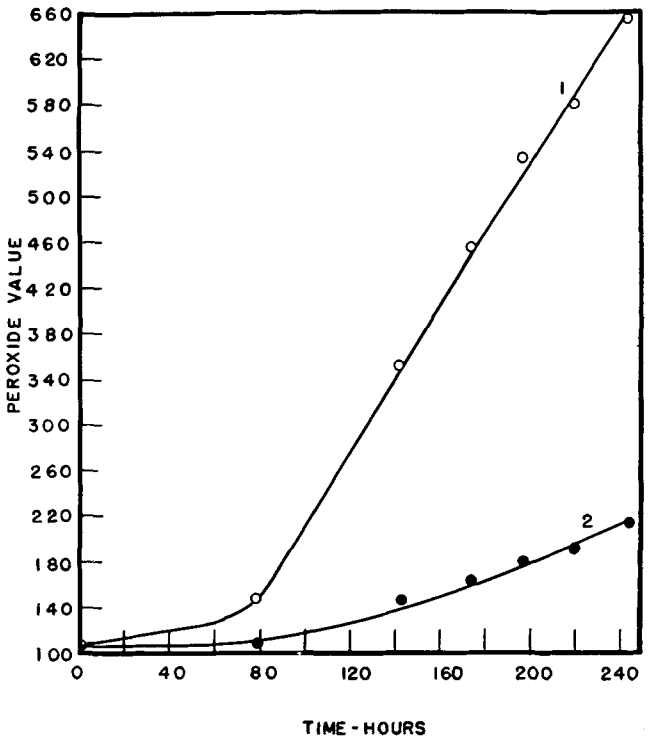

FIG. 5. Peroxide values of dihydroquercetin in cottonseed oil at 60°C.
1. 0.0% dihydroquercetin as control.
2. 0.100% dihydroquercetin.

sample of butter oil contained a considerable amount of natural antioxidant. Several 10 ± 0.01-gram samples, in the presence of and in the absence of dihydroquercetin and citric acid, were tested. The results are shown in Figure 6.

At the start of this experiment the control as well as the butter oil containing dihydroquercetin and citric acid had a golden appearance. At the end of 350 hours of incubation at 60°C. the golden color of the control samples faded considerably, and at the end of 568 hours these samples were colorless. On the other hand, the sample in the presence of dihydroquercetin and citric acid retained its original golden color, as viewed with the naked eye. The change of color of butter oil is an indication of the end of its induction period.

Esters of Dihydroquercetin

The acetyl, propionyl, and benzoyl esters of dihydroquercetin were prepared and tested for antioxidant activity. The pentaacetate was prepared with acetic anhydride and pyridine and had a melting point of 86 to 88°C. When the dihydroquercetin was similarly treated with propionic anhydride and pyridine, the pentapropionate was obtained with a melting point of 46 to 48°C. The pentabenzoate was prepared with benzoyl chloride and pyridine and had a melting point of 108 to 110°C. It was found that none of

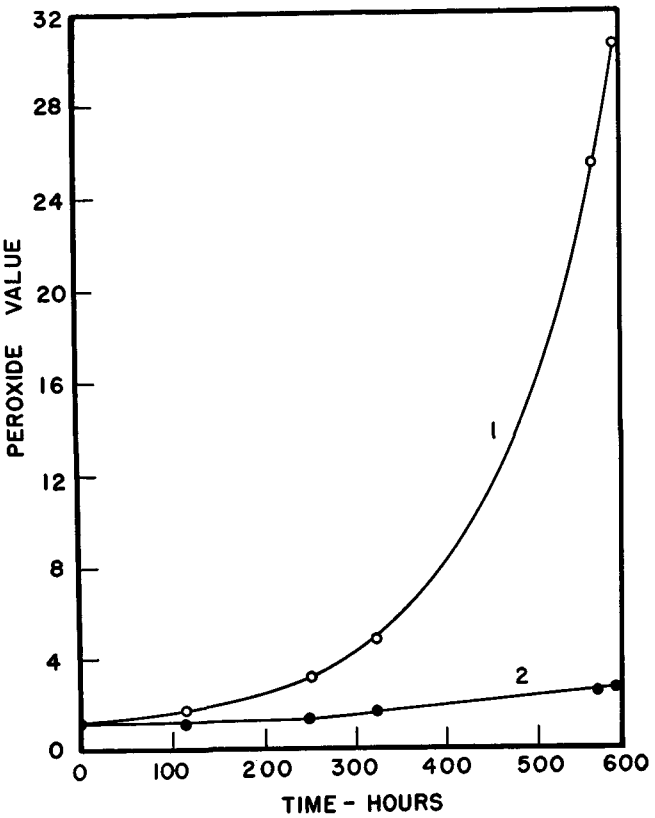

FIG. 6. Effect of dihydroquercetin and citric acid on the stability of butter oil at 60°C.
1. 0.0% antioxidant as control.
2. 0.015% dihydroquercetin and 0.015% citric acid.

these derivatives had any antioxidant activity. This confirms previous findings described in the literature that the antioxidant activity is associated with hydroxyl groups (8).

Discussion

The results of the tests on the antioxidant activity of dihydroquercetin to fats indicates that this compound is effective in the prevention of rancidity. Adequate stability of fats is accomplished with 0.03% dihydroquercetin. When used in conjunction with citric acid, an effective stability is achieved with 0.015% dihydroquercetin. Some of the findings of this laboratory were confirmed by K. F. Mattil, Research Laboratories, Swift and Company, Chicago, Illinois. He found by the active oxygen stability method that the presence of 0.05% dihydroquercetin increased the stability of lard from 5 hours to 69 hours, and he commented that this compound appears to be similar to propyl gallate in its antioxidant activity.

Dihydroquercetin is a white, crystalline, tasteless compound, which imparts no taste or color to lard and colorless oils. It has a high melting point, 252 to 253°C., and is stable at relatively high temperatures. It is easily converted into quercetin in the laboratory. A comparison of quercetin and dihydroquercetin in antioxidant activity indicated that when equal amounts (0.03%) of these substances were added to lard, dihydroquercetin was slightly less effective than quercetin. However the latter imparts a yellow color to lard.

Summary

Dihydroquercetin, a white crystalline pentahydroxyflavanone, occurring in large quantities in Douglas fir and Jeffrey pine barks, was found to be an effective antioxidant for lard, cottonseed oil, and butter oil. This compound imparts no taste and color to fats and oils and, like quercetin, appears to be nontoxic. It was found that 0.03% quercetin was slightly more effective than 0.03% dihydroquercetin as an inhibitor of rancidity in lard. The presence of a small amount of citric acid increased markedly the antioxidant effect of dihydroquercetin on lard. The pentaacetate, pentapropionate, and the pentabenzoate esters of dihydroquercetin showed no antioxidant activity.

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Viscosity of Cottonseed Meal Dispersions

JOSEPH T. HOGAN and JETT C. ARTHUR JR., Southern Regional Research Laboratory,[1] New Orleans 19, Louisiana

THE apparent viscosities of cottonseed meal dispersions, i.e., properties of the dispersions which offer definite resistance to change in form or flow, are important in adapting cottonseed meal for utilization as plywood glues or special sizes. One application of these data is in developing a cottonseed meal glue with a long "working life," i.e., a glue having a low viscosity for several hours, to facilitate the application of the glue to the laminate. In previous publications these properties have been reported for cottonseed meal glues and proteins (1-3, 5). The purpose of the present report is to present data on the viscosity properties of usable cottonseed meal dispersions *per se* and to compare these properties with those of cottonseed protein dispersions and cottonseed meal glues.

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.