blood produces complexes that contain 11.67% N and 6.39% Fe. Almost 97% of the input iron ion is found associated with the complexes.

These results indicate that all of the coagulant–protein complexes studied form under conditions that require only a small excess of coagulant and that the complexes contain low percentages of heavy metals or nutritionally beneficial (sodium polyphosphate) or nontoxic (sodium lignosulfonate) protein coagulants. Thus, several of the coagulants tested may be feasible for industrial use in the "cold" processing of animal blood. Pilot projects designed to test utilization of the coagulants on an industrial scale, followed by feed testing of these complexes blended as a part of animal diets, are necessary. Results of these tests should enable industrial packers to evaluate the digestibility, amino acid availability, safety, and economic advantages or disadvantages of cold blood processing relative to conventional methods.

ACKNOWLEDGMENT

We are thankful to Delbert Doty for his insight and suggestions concerning this research. We also thank Tom Dieter (Emge Packing Co.) for supplying industrial whole blood samples and William Detroit (American Can Co.) for supplying sodium lignosulfonate samples and data on their content. We thank Belinda Woods for her assistance in the preparation of the manuscript.

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Received for review August 31, 1981. Revised manuscript received March 19, 1982. Accepted April 26, 1982. The manuscript was written during a summer professional development leave from Murray State University. This research was supported by a grant from the Fats and Proteins Research Foundation, Inc., Des Plaines, IL, and by the Murray State University Committee on Institutional Studies and Research, CISR 784. The manuscript was taken from data contained in the Master's Thesis of T.M.J., submitted to the Department of Chemistry, Murray State University, Murray, KY.

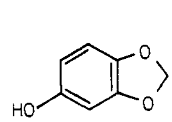
Antioxidant Properties of Synthetic 5-Hydroxy-1,3-benzodioxole Derivatives

Edward R. Cole, George Crank,* and Hai Minh

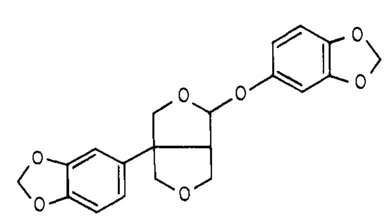
The sesamol analogues 2,2-disubstituted-5-hydroxy-1,3-benzodioxoles all show greater antioxidant effects than the parent sesamol. Cycloalkyl-substituted compounds are the most efficient, but alkyl derivatives are also good antioxidants with optimum activity at C_7. Protection is shown to a variety of lipid substrates, with best effects observed for lard. The most efficient analogues are comparable in activity to butylated hydroxyanisole and propyl gallate.

In spite of their proven utility, remarkably few antioxidants have been approved for use in foods. Stringent toxicity testing has limited the compounds permitted and has eliminated several formerly important commercial antioxidants such as nordihydroguairaretic acid (NDGA). There is a continuing need for new agents which will be highly effective without undesirable side effects.

Sesamol (5-hydroxy-1,3-benzodioxole), which occurs naturally in sesame oil (Budowski and Markley, 1951) both free and in bound form as sesamolin (Beroza, 1954, 1955),



sesamol



sesamolin

is responsible for the crude oil's considerable resistance to oxidation. The oil's resistance to autoxidation has been accurately correlated with the sesamol content (Budowski, 1950; Budowski et al., 1950). This property of the oil has been overshadowed by its insecticidal synergistic effects.

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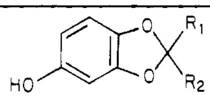
Table I. 2,2-Disubstituted-5-hydroxy-1,3-benzodioxoles



compd no.:	1	2	3	4	5	6	7	3	9
R_1	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	$-C_6H_5$	$(CH_2)_4$	$(CH_2)_5$
R_2	$-C_2H_5$	$-C_4H_9$	$-C_7H_{15}$	$-C_9H_{19}$	$-C_{11}H_{23}$	$-C_6H_5$	$-C_6H_5$		

standards: **10** = sesamol
11 = butylated hydroxyanisole
12 = propyl gallate

Recognition of the effect, attributed to sesamolin (Beroza, 1954, 1955), stimulated an intensive synthetic program leading to the development of several important commercial synergists such as sesamex (Beroza, 1956). Thus although sesamol had been shown to be an effective antioxidant for various fats and oils (Olcott and Mattill, 1941; Budowski, 1950; Fukuzumi and Ikeda, 1969, 1970), there have been few serious attempts to exploit the potential of molecular modification.

The introduction of the *tert*-butyl group in the benzene ring at the 4 position (Thompson and Symons, 1956) gave only marginally better activity than sesamol as an antioxidant for lard.

In the present work attention has been given to modifications giving analogues that are more soluble in lipids and of lesser volatility, achieved by the introduction of lipophilic side chains at position 2 on the dioxole ring. (Cole et al., 1980 a,b). It has also been established that benzodioxoles doubly substituted at position 2 are more resistant to oxidative degradation. The presence of even one hydrogen atom at this position renders the system liable to dioxole ring opening (Cole et al., 1980c). Most other synthetic work on benzodioxoles, directed toward compounds with synergistic activity, has involved introduction of substituents in the benzene ring.

EXPERIMENTAL SECTION

Materials. 2,2-Disubstituted-5-hydroxy-1,3-benzodioxoles were synthesized as previously described (Cole et al., 1980a,b) and were shown to be of analytical purity. Commercial antioxidants sesamol (Aldrich), propyl gallate (Koch-Light), and BHA (May and Baker) were recrystallized until samples of high purity were obtained. Methyl linoleate, prepared by alkaline hydrolysis of safflower oil and esterification of the freed acids with anhydrous methanol containing a little sulfuric acid, was obtained pure by preferential precipitation of saturated esters as urea adducts and fractional distillation of the residual esters. The fraction boiling at 162–163 °C/0.3 mm Hg was shown by GLC analysis to be >99% pure. It was stored under nitrogen at −5 °C until used. Squalene (Sigma) and commercial safflower oil of food grade were purified by passage through a column of alumina grade I activity under nitrogen and were stored under nitrogen at −5 °C until used. Food-grade lard, in petroleum ether (bp 40–60 °C), was similarly purified by filtration through alumina. The solvent was removed on a rotary evaporator, and the product was dried at 70 °C/1 mm Hg for 24 h and was stored under nitrogen at −5 °C.

Antioxidants were dissolved in substrates to give solutions equimolar with 1% BHA and were diluted to a concentration equivalent to 0.01% BHA as required. All such operations were carried out under nitrogen as far as possible. All glassware used was first washed with 50% nitric acid, rinsed thoroughly in demineralized water, and dried in a dust-free oven.

Test Procedures. (*a*) *Weight Gain Method* (*Olcott and Einset, 1958*). Test samples in triplicate (1 g) in 40-mL beakers (4-cm o.d.) were placed in a convection oven at 60 ± 1 °C. At daily intervals beakers were cooled in a desiccator and accurately weighed. The procedure was continued until a significant weight gain was noted.

(*b*) *Oxygen Absorption Method.* Identical Pyrex flasks (5 mL) under an oxygen atmosphere immersed in a water bath at 60 ± 1 °C were connected to manometers. One flask contained the test sample (1 g) stirred with a small magnetic stirrer; the other provided a blank to allow for slight variations in atmospheric temperature and pressure. Volume changes were measured under atmospheric pressure, and the test was continued until a significant uptake of oxygen was observed.

RESULTS AND DISCUSSION

Preparation of 5-hydroxy-2-substituted-1,3-benzodioxoles have been discussed previously (Cole et al., 1980a,b). Compounds chosen for antioxidant testing are listed in Table I.

Since variation in the performance of most antioxidants is noted for different substrates, it is necessary to use a variety of substrates to properly evaluate new materials. Squalene is a popular medium for testing antioxidants due to its low k_3 value (Bateman, 1954), and it is an example of a system containing isolated double bonds. Methyl linoleate is a typical example of a more activated system; moreover, linoleic acid is the most common polyunsaturated fatty acid encountered in foods. Two representative food fatty products were also included, safflower oil, a popular cooking and general-purpose polyunsaturated oil, and lard, which has often been used in past antioxidant assessments.

Considerable attention was given to the choice of methods of testing the compounds. The many methods for testing antioxidants described in the literature are of varying reliability and applicability. The active oxygen method (AOM) is probably the most widely used technique but can give misleading results due to the thermal instability of the initially formed hydroperoxides.

Probably the most reliable and accurate method of monitoring oxidation is measurement of oxygen uptake (Eckey, 1946; Pohle et al., 1962; Quencer et al., 1964), and this was adopted as the reference method in the present work. However, due to the limited number of samples which can be handled at any one time by this process, an additional method capable of dealing more rapidly with a larger number of samples was also used. The weight gain test, based on measuring increases in weight due to oxygen adsorption, does not require special apparatus and has been used to examine the stability of various products (Olcott and Einset, 1958; Ikeda and Fukuzumi, 1977; Ikeda et al., 1976).

All the tests were carried out at 60 °C. Oxygen adsorption tests were carried out 3 times on each sample, while the weight gain tests were run 3 times on triplicate samples, with close agreement between runs. Tests on

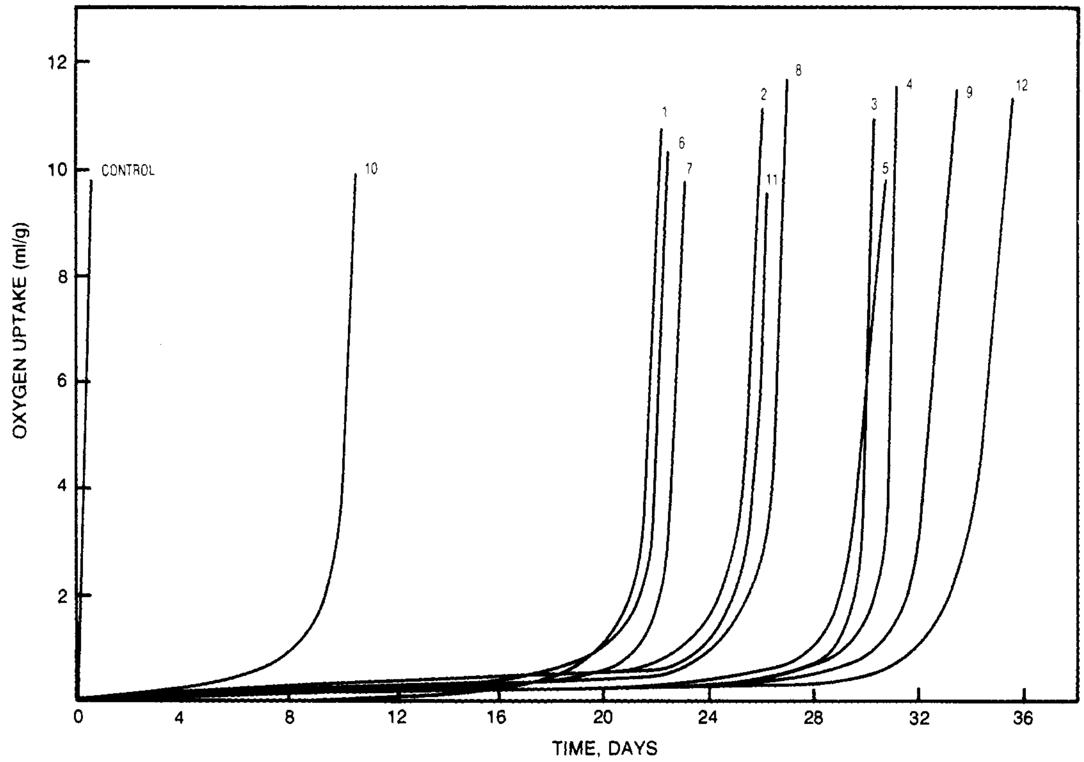


Figure 1. Effect of antioxidants on squalene by using the oxygen absorption method. Antioxidant concentrations were molecularly equivalent to 0.01% BHA. The temperature was 60 ± 1 °C. Reference numbers of antioxidants are shown in Table I. **10**, **11**, and **12** are sesamol, BHA, and PG.

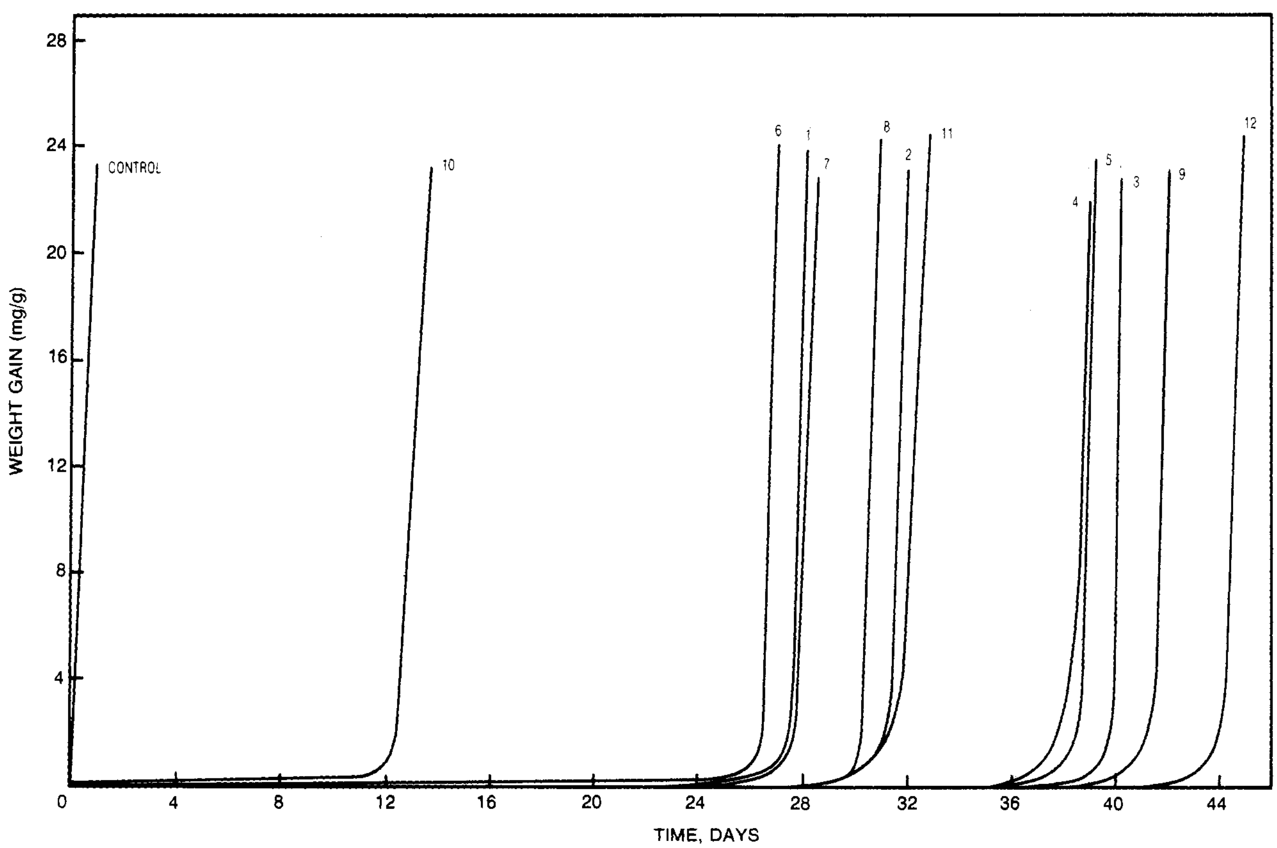


Figure 2. Effect of antioxidants on squalene by using the weighing method, run no. 1. Antioxidant concentrations were molecularly equivalent to 0.1% BHA. The temperature was 60 ± 1 °C. Reference numbers of antioxidants are shown in Table I. **10**, **11**, and **12** are sesamol, BHA, and PG.

commercial antioxidants butylated hydroxyanisole (**11**) and propyl gallate PG (**12**) were also carried out for reference.

Results for the oxygen absorption tests in squalene (Figure 1) show that all the synthetic dioxoles were much more efficient than sesamol (**10**). Although PG gave best results in this test, several of the the benzodioxoles were superior to BHA. Compound **9** was the best of the series of synthetic materials. These results were confirmed by

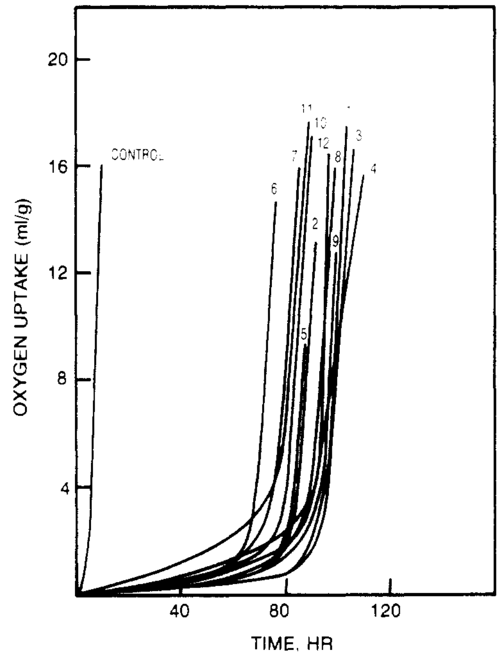


Figure 3. Effect of antioxidants on methyl linoleate by using the oxygen absorption method. Concentrations of antioxidants were molecularly equivalent to 0.01% BHA. The temperature was 60 ± 1 °C. Reference numbers of antioxidants are shown in Table I. **10**, **11**, and **12** are sesamol, BHA, and PG.

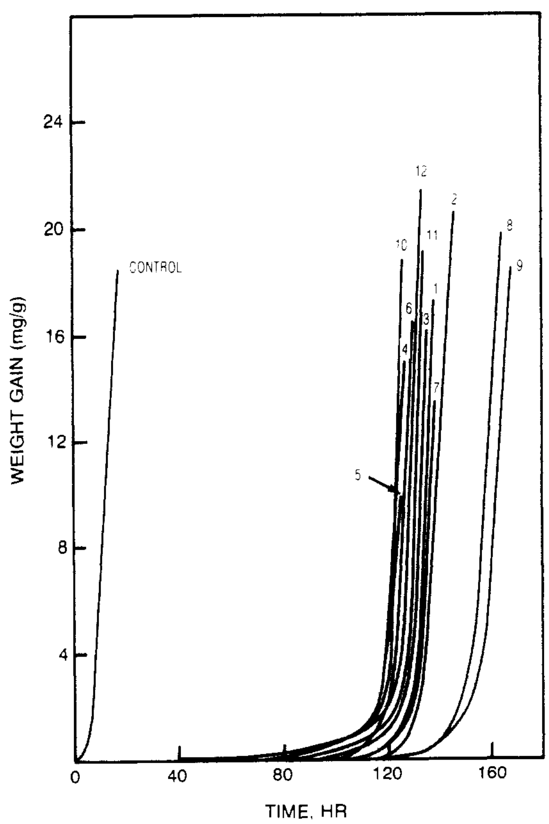


Figure 4. Effect of antioxidants on methyl linoleate by using the weighing method, run no. 1. Concentrations of antioxidants were molecularly equivalent to 0.01% BHA. The temperature was 60 ± 1 °C. Reference numbers of antioxidants are shown in Table I. **10**, **11**, and **12** are sesamol, BHA, and PG.

the weight gain test in squalene (Figure 2) where the same general order is observed.

Among the benzodioxoles substituted with alkyl groups there appears to be a general trend of increasing efficiency with an increase in the length of the chain up to seven carbon atoms (**3**) with a slight diminution for larger chains. The aryl derivatives (**6** and **7**) appear to be less effective than the alkyl compounds.

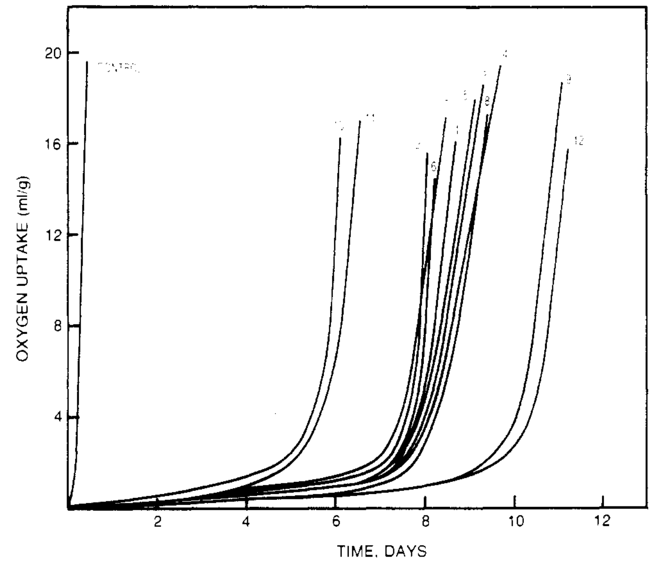


Figure 5. Effect of antioxidants on safflower oil by using the oxygen absorption method. Concentrations of antioxidants were molecularly equivalent to 0.01% BHA. The temperature was 60 ± 1 °C. Reference numbers of antioxidants are shown in Table I. **10**, **11**, and **12** are sesamol, BHA, and PG.

Table II. Effect of Concentration on Antioxidant Efficiency of Compound 9

	inducation period, days	
concn, %	squalene	safflower oil
0.005	20	7
0.01	33	14
0.02	55	21
0.05	130	32
0.10	210	50

Tests in the substrate methyl linoleate are illustrated in Figures 3 and 4 for the two methods. In general the benzodioxoles offer less protection to this substrate. Although no clear trends emerge from these tests the synthetic benzodioxoles appear to be better than sesamol and some of them are superior to BHA and PG. Compound **9** again emerges as the most efficient of the series.

Results in safflower oil are shown in Figures 5 and 6, where it can be seen that the benzodioxoles offer more protection to this medium than to methyl linoleate. However, the same general pattern of activity may be discerned, with compound **9** the best of the dioxoles with efficiency practically identical with that of PG.

The benzodioxoles offer outstanding protection to lard (Figure 7) where they are clearly better than either BHA or PG. The best compounds, including **9**, prevented the onset of autoxidation for more than 100 days at 60 °C. The time taken in the oxygen absorption method precluded completion of tests on the number of samples necessary to confirm these results.

Compound **9**, spiro[1,3-benzodioxole-2,1′-cyclohexan]-5-ol, was also tested for the effect of concentration on its antioxidant efficiency in squalene and safflower oil (Table II). It is to be noted that no prooxidant effect was detected up to 0.1% concentration whereas for BHA the maximum antioxidant power has been determined to be 0.02% with falloff in efficiency at higher concentrations (Kraybill et al., 1949).

The sesamol analogues examined in this work were clearly efficient antioxidants and show a number of in-

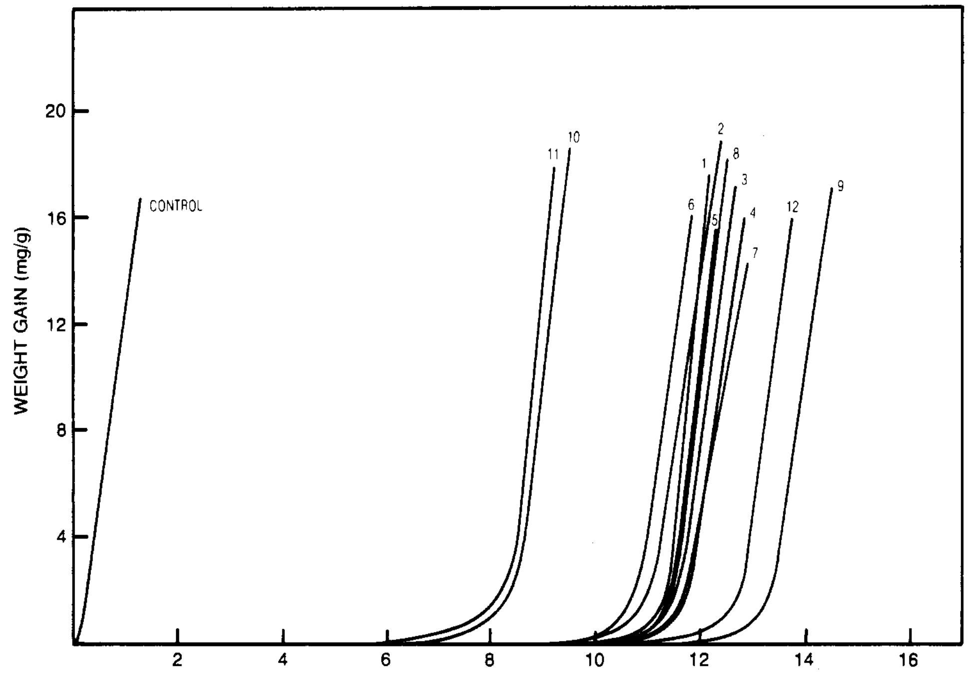


Figure 6. Effect of antioxidants on safflower oil by using the weighing method, run no. 1. Antioxidant concentrations were molecularly equivalent to 0.01% BHA. The temperature was 60 ± 1 °C. Reference numbers of antioxidants are shown in Table I. **10**, **11**, and **12** are sesamol, BHA, and PG.

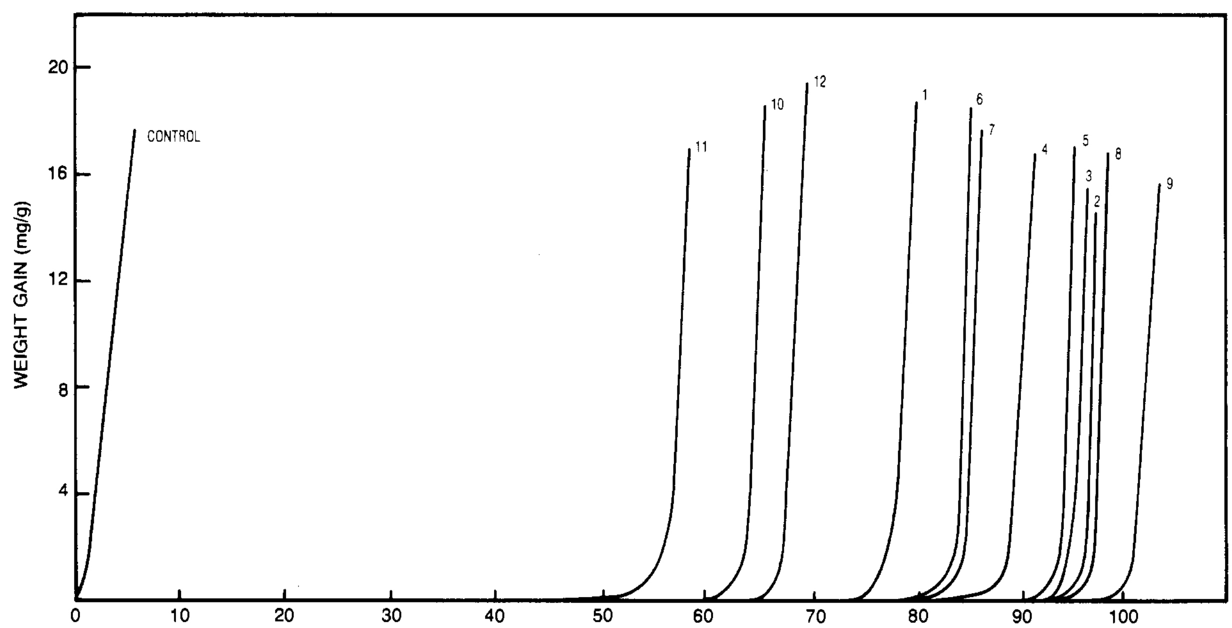


Figure 7. Effect of antioxidants on lard by using the weighing method, run no. 1. Antioxidant concentrations were molecularly equivalent to 0.01% BHA. The temperature was 60 ± 1 °C. Reference numbers of antioxidants are shown in Table I. **10**, **11**, and **12** are sesamol, BHA, and PG.

teresting trends. They are all better than the parent substance sesamol, with the efficiency increasing with increasing chain length to seven carbon atoms. Derivatives with aromatic substituents are slightly less efficient. Best results were given by derivatives with cycloalkyl substitution, in particular the cyclohexyl derivative. This material was better than BHA or PG in lard, equivalent to PG in methyl linoleate and safflower oil, and only marginally less effective in squalene.

These preliminary results suggest a possible role of synthetic hydroxybenzodioxoles as food antioxidants (Cole et al., 1981), provided that they satisfy toxicity testing. Further assessment of these materials is under way.

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Received for review July 27, 1981. Revised manuscript received March 8, 1982. Accepted March 26, 1982.

Effect of Fiber in Corn Tortillas and Cooked Beans on Iron Availability

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Availability of iron from a diet of corn tortillas and cooked beans with different levels of fiber was determined by hemoglobin repletion. Neutral detergent fiber (NDF) in corn tortillas and cooked beans and soluble, ionizable, and total iron the the test foods were determined. Weanling male rats were fed an iron-free diet until anemic and then divided into groups. The rats were fed diets with 6.5, 10, and 15% NDF and 25, 30, and 35 ppm of total iron. Iron availability was significantly reduced by 15% NDF. The amount of iron in the diet affected iron repletion. A significant correlation between soluble iron at pH 1.35 in vitro and percent efficiency of hemoglobin repletion was obtained. Iron from corn tortillas and cooked beans is approximately 50% less available than ferrous sulfate.

Iron deficiency is a commonly recognized nutritional deficiency in developing as well as in affluent societies (Goodhart and Shils, 1980). Reinhold et al. (1975) suggested that fiber largely determines the availability of bivalent metals in wheat bread. Solomons et al. (1979) proposed that the high consumption of fiber from corn tortilla by rural populations in Guatemala may affect zinc absorption. Incorporation of corn into the diet has been reported to decrease iron absorption. Miller (1978) found that incorporation of iron in hemoglobin was reduced when a corn diet was fed at 2-day intervals with a casein diet. In another study Layrisse et al. (1968) reported a decrease in iron absorption from veal after corn was introduced into the diet of experimental subjects.

Fiber is thought to interfere with iron absorption because of its binding capacity (Reinhold et al., 1975). Ismail-Beigi et al. (1977) reported wheat bran and hemicellulose exhibited high iron binding capacity. More recently Reinhold et al. (1981) found the neutral detergent fiber (NDF) fraction from corn tortilla bound as much as 0.3 mg of iron/g of NDF at pH 6.45. Since corn tortillas along with cooked beans are the main staple of the Mexican diet, the purpose of this study was to investigate the effect of fiber from corn tortillas and cooked beans on iron availability.

MATERIALS AND METHODS

Experimental Animals and Diets. Weanling male 28-day-old Long Evans rats were obtained from Charles River Breeding Laboratories (North Wilmington, MA). The rats were housed individually in stainless steel cages with wire mesh floors. Lighting was automatically controlled to provide 12 h of darkness. Feed (in aluminum cups) and deionized water (in rubber-stoppered glass bottles) were supplied ad libitum. Final weight gain as well as total feed consumption were recorded.

The compositions of the experimental diets are shown in Table I. The diets were formulated by using corn tortillas and cooked beans to provide 66.49 ± 1.98% of the total calories. The experimental diets had three levels of NDF (6.5, 10, and 15%) and three levels of iron (25, 30, and 35 ppm). Corn tortillas and cooked beans were substituted in the diets at the expense of glucose and casein. The amount of cooked beans in each of the experimental diets was held constant to 25.2% while the level of corn tortilla plus cellufil was increased to achieve different NDF levels. The three different iron levels were obtained by supplementing the endogenous iron level of the corn tortilla and the cooked beans with ferrous sulfate heptahydrate to the desired level. At the lowest level of iron, 25 ppm, no ferrous sulfate addition was necessary. Three control diets containing 0, 25, and 35 ppm of iron as ferrous sulfate heptahydrate were used in the study.

The commercially made corn tortillas and the raw beans (*Phaseolus vulgaris*) were obtained from Guadalajara, Mexico. The corn tortillas were sun-dried and ground to pass a 18-mesh screen. The powder was stored at −10 °F until blended into the diet. The beans were soaked overnight in tap water at room temperature (3:1 ratio) and cooked until soft in an open vessel at boiling temperature. The cooked beans and the broth were freeze-dried and ground to pass a 20-mesh screen (Oregon Freeze Dried Foods, Inc., Albany, OR). The remaining ingredients used in the diet were all purchased from United States Biochemical Corp. (Cleveland, OH). After the diets were completely mixed, the material was placed in plastic bags and held at −10 °F until fed.

Iron Availability. Iron availability was assessed by the depletion–repletion method (Fritz and Pla, 1972). The rats were fed an iron-free diet for 51 days. At the end of this period the hemoglobin concentration in whole blood was

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