The results of these tests indicate that alfalfa samples can be stored at 0° F. prior to analysis and that the preparation for cold storage should consist of weighing, liming, and the taking of a moisture aliquot. The sample container should be sealed. Although all stored samples exhibit more variation in fluoride content than those samples analyzed soon after collection, the addition of lime to stored samples will reduce the variation. The increased variability can be obviated by carrying out replicate analyses on the stored samples. By standard statistical procedures it can be shown that two replicate analyses of stored material reduce the probable uncertainty by 30%, while a sample value based upon four replicates will have only half the probable uncertainty of a single analysis of the same material.

Acknowledgment

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ANTIOXIDANT DETERMINATION

Determination of 2,6-Di-*tert*-butyl-*p*-cresol in Edible Fats by Ultraviolet Spectrophotometry

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2,6-Di-*tert*-butyl-p-cresol is a very effective agent for inhibiting oxidation in organic substances, including edible fats. To maintain effective control over food processing operations and ensure adherence to governmental regulations, a quantitative method was developed for determining 20 to 200 p.p.m. (0.002 to 0.02%) in lards in the presence of other allowable preservatives, including butylated hydroxyanisole, nordihydroguaiaretic acid, propyl gallate, citric acid, monoisopropyl citrate, and phosphoric acid. The lard sample is dissolved in cyclohexane and percolated through a chromatographic column packed with 100-mesh silicic acid. By washing the column with successive portions of cyclohexane, 2,6-di-*tert*-butyl-p-cresol is selectively removed from the adsorbent and recovered in the column filtrates. The filtrates are subsequently analyzed by ultraviolet spectrophotometry, and the amount is calculated from absorbance measurements made at a wave length of 284 mμ. This procedure has been tested with synthetic formulations of commercial brand lards and a hydrogenated vegetable oil shortening containing this additive. Recoveries consistently better than 93% can be obtained.

A VERY EFFECTIVE OXIDATION INHIBITOR for a wide variety of organic substances, including rubber, petroleum products, and plastics, is 2,6-di-*tert*-butyl-*p*-cresol (DBPC, Koppers Co. registered trade-mark). Recently, by approval of the Meat Inspection Division, U. S. Department of Agriculture (*7*), its use has been extended to edible fats, notably lards, where prior studies have shown that the development of rancidity can be delayed effectively through the use of as little as 100 p.p.m. (0.01 weight %) of this compound.

To maintain effective production control over lard processing operations employing this antioxidant and to ensure adherence to governmental regulations, a quantitative method for the analysis of commercial products was needed. Moreover, because mixtures of preservatives are currently employed in the stabilization of lard, this method should be capable of determining 20 to 200 p.p.m. of 2,6-di-*tert*-butyl-*p*-cresol in lards in the presence of other allowable preservatives. According to present regulations, the latter could include butylated hydroxyanisole (BHA), nordihydroguaiaretic acid (NDGA), propyl gallate, citric acid, monoisopropyl citrate, and phosphoric acid.

A survey of the existing literature revealed that Chapman (*2*) and Chapman and Mahon (*3*) had developed selective solvent extraction procedures for the separation and quantitative analysis of mixtures of propyl gallate, butylated hydroxyanisole, nordihydroguaiaretic acid, and naturally occurring tocopherols in vegetable fats and oils. According to their methods, the differential solubilities of these compounds in water, ethyl alcohol, and petroleum ether were utilized to provide extracts which could be analyzed by suitable colorimetric procedures. A similar procedure was adapted by Austin to the analysis of 2,6-di-*tert*-butyl-*p*-cresol in fats and oils (*1*), and as this method appeared to be suited to the problem, it was tested in these laboratories using lards containing accurately known quantities of 2,6-di-*tert*-butyl-*p*-cresol. Unfortunately, the results obtained were variable, quantitative recoveries could not be obtained, and the elapsed time required was so long that the method seemed unsuitable for the ultimate demands to be placed on it.

Subsequent attempts were made to apply other existing procedures for the determination of 2,6-di-*tert*-butyl-*p*-cresol in various materials to its determination in lards. In general, these procedures depended on the use of visual colorimetric methods which, while adequate for the original purpose, proved to be insensitive to the small quantities

of 2,6-di-*tert*-butyl-*p*-cresol found in lards.

Because of the strong, characteristic, absorption spectra exhibited by phenolic substances in the ultraviolet region and their general utility for accurately determining low concentrations of these materials in solutions, it was decided to investigate the possible application of this technique to the problem at hand. Examination of the spectrum of 2,6-di-*tert*-butyl-*p*-cresol revealed a strong, characteristic absorption pattern in the region of 240 to 300 mμ, with specific sharp bands at 276 and 284 mμ. However, a comparison of this spectrum with those of other additives (Figure 1) showed that the presence of additives containing phenolic hydroxyl groups in benzenoid or fused ring structures could cause serious interference in the accurate, quantitative determination of this agent. It appeared therefore that the major problem of this investigation would be the separation of 2,6-di-*tert*-butyl-*p*-cresol from other substituted phenols.

Zahner and Swann (*8*) had shown that mixtures of phenol and various cresols could be separated successfully by partition chromatography, by using a column of silicic acid. Later, Sweeney and Bultman (*6*) applied a similar technique to the analysis of mixtures of substituted phenols, including cresols, xylenols, phenylphenols, naphthols, and 2,6-di-*tert*-butyl-*p*-cresol. The latter authors found that suitable separations could be effected by varying the water content of the adsorbent, and that the selective retention of isomers was dependent upon the position of the substituent relative to the phenolic hydroxyl group. Thus, ortho-substituted phenols, because of their partially hindered nature, could be separated from their meta- and para- isomers, and the relative ease of separation of mixed ortho-substituted compounds depended upon the size of the substituent group. The effect of the size and position of substituent groups on selective retention by the adsorbent was particularly evident in their experiments with mixtures containing 2,6-di-*tert*-butyl-*p*-cresol. In every case this substance could be separated successfully from the other phenols studied.

In view of this separation, it was decided to adapt the Sweeney and Bultman procedure to the separation of 2,6-di-*tert*-butyl-*p*-cresol from lard. Cyclohexane was used instead of iso-octane as the solvent, and a column of slightly smaller diameter was employed to minimize irregularity of the displacement interface as it moved through the column. The silicic acid reagent recommended by Sweeney and Bultman was used, and the initial experiments were carried out using their recommended ratio of water to silicic acid. Ultraviolet examination of the column filtrates revealed the presence of considerable amounts of interfering substances from the lard, so that quantitative determination of 2,6-di-*tert*-butyl-*p*-cresol was not possible. It was necessary, therefore, to make a more extended study of the effect of varying water content in effecting this separation. In the course of this work, other related problems were encountered, such as the effect of variations among different lots of silicic acid adsorbent, and the effect of column diameter and amount of adsorbent on the quality of separation obtained.

Apparatus

The chromatographic apparatus and auxiliary pressure filtration system are shown schematically in Figure 2. Compressed, dry nitrogen is supplied to the system through a double-stage pressure-reducing regulator and needle valve. The filtration pressure in the system is indicated by the U-tube manometer, and the overpressure safety relief device prevents the build-up of excessive pressures. Any reasonable number of chromatographic columns may be connected to the pressure manifold. In the present work, four columns were used simultaneously. The details of construction of the columns are shown in Figure 3.

Analytical spectra of the column filtrates were obtained by using a Cary Model 11 recording ultraviolet spectrophotometer and matched quartz absorption cells having a 10-cm. path length.

An auxiliary column packed with silica gel was employed to remove aromatic hydrocarbon contamination from the cyclohexane solvent. This column is made of borosilicate glass tubing 51 mm. in outside diameter and 42 inches long with an attached, 3-liter solvent reservoir at the top and a 4-mm. bore, straight-bore stopcock at the bottom.

Reagents and Materials

Cyclohexane, spectroscopic grade, suitable for ultraviolet absorption analyses. Du Pont Hytrol A solvent cyclohexane is satisfactory, if purified by percolation through the silica gel purification column described above. The purified cyclohexane is collected in 1-gallon fractions, and each fraction is examined in the ultraviolet region prior to use. The absorbance of the pure material should be less than 0.015 at 255 mμ when examined in a 1-cm. cell, with distilled water in the reference cell. Five to 6 gallons of solvent can be purified with each batch of silica gel charged to the column. The rate of filtration should not exceed 0.5 gallon per hour.

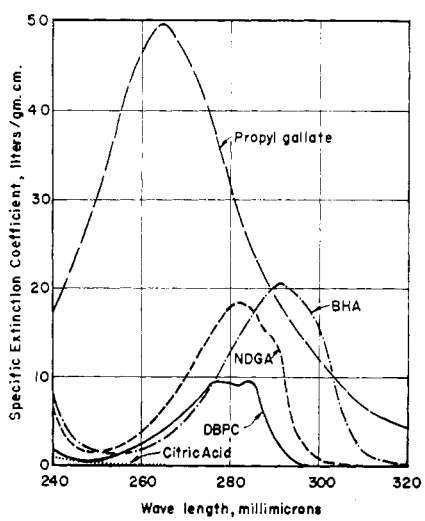
Silica gel, 28–200 mesh, Davison Chemical Corp.

Glass wool.

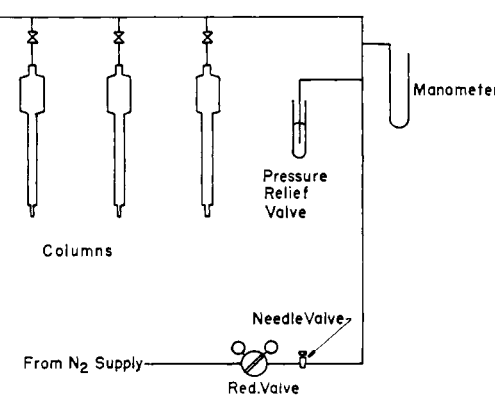
Compressed nitrogen.

Silicic acid, Mallinckrodt analytical reagent, 100-mesh powder, "suitable for chromatographic analysis by the method of Ramsey and Patterson" (*5*). The water content of each 1-pound batch of this reagent, as received, is usually around 8 to 9%, but must be determined by actual analysis. For this procedure, it must be increased to around 13%. To ensure uniform column operation over an extended period of time, it is advisable to prepare a single large batch of adsorbent from several individual 1-pound lots of the reagent.

Spread each portion into a 0.5- to 1-inch thick layer. Distribute the pre-



◀ **Figure 1. Ultraviolet absorption spectra of permissible lard additives (in cyclohexane)**



▶ **Figure 2. Diagrammatic sketch of apparatus assembly**

determined, required amount of water evenly throughout the layer with a small dropper, and then stir and mix each thoroughly. Combine all layers or portions in a suitable closed container, and subject the batch to a combined rolling and tumbling action for 16 to 24 hours to obtain thorough mixing and equilibrium. Finally, analyze a portion of the mixture to assure the proper moisture content. The water content of the silicic acid can be determined conveniently by drying at 110° C. in an oven for 1 hour and then heating at 800° C. for an additional hour in a muffle furnace.

2,6,Di-*tert*-butyl-*p*-cresol, DBPC food grade, purity 98.7% by weight (minimum), Koppers Co., Inc.

Lards, commercial brand samples.

Preparation of Apparatus

Assemble the nitrogen pressure supply system as shown in Figure 2. To pack the silicic acid chromatographic column, place approximately 30 grams of the acid in a 250-ml. beaker with 80 ml. of purified cyclohexane. Stir well to produce a slurry, cover, and allow to stand about 15 minutes. In the meantime, place a plug of glass wool in the bottom of the 25-mm. section of the column, and tamp it firmly into position. Place a container under the bottom outlet of the column, stir the silicic acid–cyclohexane slurry to redisperse the acid, and pour it quickly into the column. Use 20 ml. more cyclohexane to wash out the beaker and to wash down the solvent reservoir on the column. (No attempt need be made to transfer the silicic acid quantitatively.)

Connect the column top to the column and to the source of nitrogen pressure, and apply a pressure of about 8 pounds per square inch gage to the column. When the solvent level has drained to a point approximately 1 inch above the top of the silicic acid packing, release the pressure and carefully insert a small plug of glass wool on top of the packing. Apply nitrogen pressure again and continue the drainage of slurrying solvent until the level reaches the top of the glass wool plug. The column is now ready to receive the sample. All of the solvent drained from the column up to this point may be discarded.

Procedure

Preparation of Sample. While the chromatographic column is being prepared, melt approximately 15 to 20 grams of the lard sample on a steam bath. Weigh 12.5 grams of the melt into a 50-ml. volumetric flask, add sufficient pure cyclohexane to dissolve, and then cool to room temperature. Fill the flask to the calibration mark with cyclohexane, stopper, and shake well to ensure thorough mixing.

Chromatographic Separation. With the level of slurrying solvent just at the top of the upper glass wool plug in the chromatographic column, release the pressure, open the column, and introduce 10 ml. of the sample solution. Use a 10-ml. pipet, and insert the tip of the pipet into the column close to the top of the glass wool plug before permitting the sample to drain from the pipet.

Place a clean, dry, 50-ml. graduated cylinder under the column outlet, close the column, apply nitrogen pressure, and begin to collect the first filtrate fraction. As soon as the sample solution disappears into the packing, open the column and introduce a 10-ml. portion of cyclohexane in the same manner as the sample. When this disappears, add a second 10-ml. portion of cyclohexane, and when this has entered the packing, add sufficient cyclohexane to the column to fill the reservoir.

Collect the filtrate from the column in 50-ml. fractions, continuing filtration until at least 6 fractions have been obtained. Adjust the pressure as required throughout the filtration to maintain the filtration rate at 2 to 3 ml. per minute. Store the fractions in clean glass vessels with suitable closures. If screw-cap bottles with plastic caps are used, the caps must be provided with inner, metal-foil liners.

Spectrographic Calibration. Fill two 10-cm. absorption cells with purified cyclohexane, place them in the spectrophotometer, and standardize the instrument over the range of 235 to 320 mμ. Mark one of the pair as the sample cell and the other as the reference, and use them in this order in the subsequent analyses.

Prepare a solution containing about 0.006 gram of 2,6-di-*tert*-butyl-*p*-cresol per liter of cyclohexane (accurately determined), and replace the solvent in the sample cell with this solution. Obtain the 2,6-di-*tert*-butyl-*p*-cresol absorption curve of absorbance *vs.* wave length for the range of 235 to 320 mμ. Draw the reference base line, LL' (see Figure 4), and determine the net absorbance, A_N, between points X and Y at the 284-mμ band.

$$A_N = A_Y - A_X \qquad (1)$$

Calculate the apparent absorptivity, a, from the following Beer's law relation:

$$A_Y - A_X = A_N = abc \qquad (2)$$

where b is the optical path length in centimeters and c is the concentration of absorbing substance in grams per liter. If the same sample cell is used for the calibrating solution and filtrate fractions, as suggested, the optical path length need not be known and a new apparent absorptivity, a', can be defined such that:

$$a' = ab \qquad (3)$$

and

$$A_N = a'c \qquad (4)$$

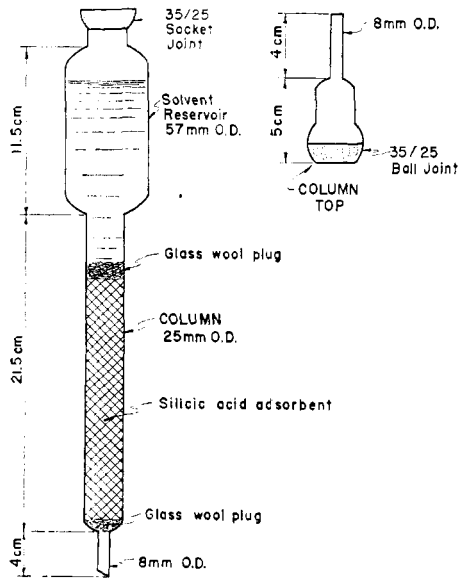


Figure 3. Chromatographic column and top

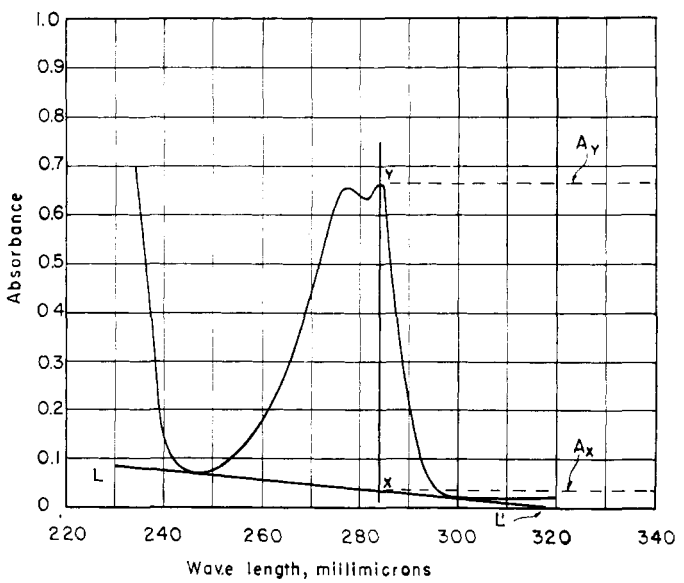


Figure 4. Ultraviolet absorption curve for DBPC

Solvent. Cyclohexane
Concentration. 0.00655 gram/liter

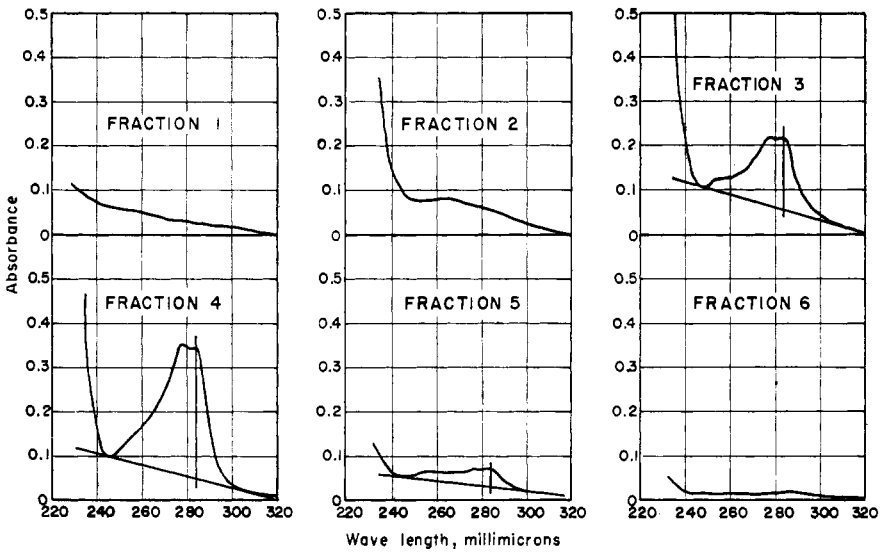


Figure 5. Ultraviolet spectra of typical filtrate fractions

Equation 4 is the working equation used in all analyses and calculations.

Analysis of Filtrate Fractions. Clean the sample cell by rinsing with several portions of cyclohexane and, finally, with a portion of the fraction to be analyzed. Fill the cell with the filtrate fraction and obtain its absorption curve over the range of 235 to 320 mμ. Draw a reference base line on each curve and measure the net absorbance, A_N, due to 2,6-di-*tert*-butyl-*p*-cresol at the 284-mμ wave length (see Figure 5). Calculate the weight of 2,6-di-*tert*-butyl-*p*-cresol in each fraction as follows:

$$\text{Grams of DBPC in fraction} = G = \frac{A_N}{a'} \times \frac{V_f}{1000} \quad (5)$$

where V_f is the volume of the filtrate fraction in milliliters. Calculate the weight per cent of 2,6-di-*tert*-butyl-*p*-cresol in the lard sample as follows:

$$\frac{\Sigma G}{2.5} \times 100 = \text{weight \% DBPC} \quad (6)$$

where ΣG is the grams of 2,6-di-*tert*-butyl-*p*-cresol in all fractions.

If the filtrate fractions are collected in constant volume quantities of 50 ml. each, the weight per cent of 2,6-di-*tert*-butyl-*p*-cresol may be calculated as follows:

$$\frac{\Sigma A_N}{a'} \times \frac{50}{1000} \times \frac{100}{2.5} = \text{weight \% DBPC} \quad (7)$$

where ΣA_N is the summation of all net absorbances.

The ultraviolet absorption spectrum of 2,6-di-*tert*-butyl-*p*-cresol (Figure 4) is characterized by two maxima bands of approximately equal intensity at 276 and 284 mμ. Either could be utilized for analysis; however, errors, if present, are generally more pronounced at the lower wave length. This is probably due to disproportionate background absorption from lard constituents eluted from the column along with 2,6-di-*tert*-butyl-*p*-cresol. If a wide deviation in results exists between calculations made at the two wave lengths, this may be regarded as an indication of faulty column operation. In such cases a decrease in filtration rate or an increase in the amount of adsorbent used may be necessary.

Results

The results of analyses of five different commercial lards are shown in Table I. Each lard was first analyzed "as received" to ensure proper conditions of column operation and to determine if any 2,6-di-*tert*-butyl-*p*-cresol had been added during processing. Accurately determined amounts of 2,6-di-*tert*-butyl-*p*-cresol were then added to each lard in various combinations with other additives, as indicated, and the analyses were carried out by the procedure described.

In all cases, recoveries fall within the range of 99 ± 3%, irrespective of the wave length of the band chosen for analysis or the composition of the additive. Additive formulation I was prepared on the basis of current permissible regulations of the Meat Inspection Division, U. S. Department of Agriculture (*7*). Formulation II was prepared to contain amounts and combinations of additives beyond permissible limits, in order to simulate a more severe test of the method than would be met in actual practice.

Discussion

Nature of Adsorbent. A characteristic feature of column chromatographic separation techniques is the fact that the efficacy of the adsorption process generally depends on physical-chemical properties of the adsorbent which are not fully understood or capable of definition. Furthermore, batches of silicic acid prepared under well-controlled conditions may be entirely different in chromatographic properties. To avoid undue difficulties related to the choice of any given brand of silicic acid adsorbent, the one selected for use in this investigation was the same as that employed by Sweeney and Bultman for similar phenolic separations (*6*).

According to the manufacturers, this reagent is selected on the basis of its suitability for chromatographic separation by the procedure described by Ramsey and Patterson (*5*). Surface area of the particles, one of the important variables which affect suitability for chromatography, is in a sense controlled by this procedure, although the manufacturers exert no specific control, as such, over this property (*4*).

In the initial experiments conducted in these laboratories, reasonably good recoveries of 2,6-di-*tert*-butyl-*p*-cresol were ob-

Table I. Analysis of Synthetic Mixtures of Commercial Lards with 2,6-Di-*tert* butyl-p-cresol and Other Additives

Lard Sample	Additive Formulation[a]	% DBPC Recovered by Analysis at 276 mμ	284 mμ
A	I	99	100
	II	98	100
B	I	96	97
	II	99	100
C	I	98	99
	II	100	102
D	I	99	99
	II	100	102
E	I	97	100
	II	97	101

[a] I. DBPC + BHA, 0.01% of each.
II. DBPC + BHA + NDGA + PG + CA + PA + IC, 0.01% of each.

Table II. Screen Analysis of Various Batches of Silicic Acid Adsorbent

Screen Fraction (U. S. Standard Screens)	Weight % of Fraction Batch 1	Batch 2	Batch 3
+ 100-mesh	3	3	2
− 100-mesh + 200-mesh	23	21	20
− 200-mesh	74	76	78

tained from cyclohexane solutions of lard when chromatographed on this adsorbent. The ratio of water to silicic acid used (1 ml. per 12 grams of acid) was the same as that employed by Sweeney and Bultman; however, the validity of this comparison is not known, inasmuch as these authors gave no indication of the total water content of their adsorbent. The present authors' experience indicated that the acid itself, before wetting, usually contains 8% of water, but occasionally as much as 12%. For the batch of silicic acid used in these first experiments, the absolute water content of the wetted adsorbent was about 15%.

As this investigation was continued and efforts were made to duplicate these experiments with different 1-pound batches of adsorbent having controlled absolute water contents, the recoveries of 2,6-di-*tert*-butyl-*p*-cresol became erratic, and variable amounts of background interference from the lard were noted in the ultraviolet spectra of the column filtrate fractions. Some erratic variation in filtration rates through the columns was also observed. These combined effects suggested variations in particle size and surface area between the various batches, but subsequent screen analyses revealed only minor differences in the fractions separated (Table II).

Because of the relatively large amount of silicic acid in the sub-200-mesh size range, it appeared that some further analysis of this fraction would be necessary to resolve the points in question, and some method of control might have to be exercised over this fraction in actual practice to obtain batches of acid having reproducible chromatographic properties. To avoid such tedious and time-consuming control, it appeared desirable to explore other means of regulating the resolution or adsorptive power of the silicic acid. Examination of the data obtained suggested that the experiments were being conducted with an adsorbent having too high a level of water content, and that, at this level, the degree of resolution or separation obtained was particularly sensitive to variations in other properties of the adsorbent.

Accordingly, a new series of tests was made using silicic acid having 13 to 13.5% water (Table III). In these tests (as before), each individual column charge of adsorbent (about 25 to 26 grams) was weighed out, and the required amount of water was added and mixed before charging according to the procedure of Sweeney and Bultman (*6*), except for the use of a dry box during the mixing. With this reduced level of water content, nearly quantitative results were obtained, although still somewhat variable. Some of this variation was believed to be due to uneven distribution of the water added during mixing and to possible erratic distortion of the particle size profile of the charge as the result of the variable grinding action exerted during mixing.

Other reduced levels of water content were also investigated for evidence of possible further improvement in operation (Table IV). In general, very satisfactory results were obtained with silicic acid having 12 to 13% water; however, no sharp line of demarcation was found to exist. With higher amounts (15 to 16%), interfering substances from the lard are eluted into the column filtrates and lead to low results. With lower amounts (9 to 10%), 2,6-di-*tert*-butyl-*p*-cresol is retained very tenaciously on the adsorbent, recoveries are usually low, and the elution times required are two- to three-fold longer than required otherwise.

After having thus established an optimum level of water content for carrying out these separations, it was decided next to try to avoid the discrepancies and disadvantages inherently associated with the Sweeney and Bultman procedure for adjusting the water content of each column charge. To do this a single large batch of silicic acid was prepared from three individual 1-pound lots of the reagent. The amount of water required was calculated and added as described above. After thorough mixing and equilibration, this material was employed throughout the remainder of the investigation.

Table III. Recovery of 2,6-Di-*tert*-butyl-p-cresol from Synthetic Formulations with Commercial Lards

(Water content of adsorbent[a] approximately 13.5%)

Lard Sample	DBPC, Weight % Added	Recovered[b]
A	0.01	94,95,97,97
B	0.002	90,105,105
	0.005	94,100
	0.01	98,100,100,101
C	0.01	99,100
D	0.002	100,100,105
	0.005	100,102
	0.01	96,99
E	0.002	85,85,100
	0.01	95,97
F	0.01	96,97
G	0.002	85,95,100,110
	0.005	90,96,96
	0.01	100,101
	0.02	96,99

[a] Water content of each column charge adjusted individually before charging.
[b] Calculated at 284-mμ wave length.

Table IV. Effect of Water Content of Silicic Acid on Recovery of 2,6-Di-*tert*-butyl-p-cresol from Synthetic Formulations with Commercial Lards

Lard Sample	Water Content of Silicic Acid, Wt. %	DBPC Recovery, %
A	16	88[a]
	13	98,100,101
	12	100
	11	94[b]
	9	..[c]
B	16	90[a]
	13	94,95,97,97
	12	98
	11	75[b]
	9	..[c]

[a] Spectral interference from other material.
[b] Incomplete elution in over 10 hours.
[c] Strongly adsorbed; no DBPC eluted in 10 hours.

Column Operation. The preliminary chromatographic separations described above were performed in columns having inside diameters of 15 to 16 mm., with 25 to 26 grams of adsorbent, and a pressure of 8 pounds per square inch gage of nitrogen for filtration. Under these conditions, the filtration rate was about 1 ml. of cyclohexane per minute, and total filtration times of 10 to 12 hours were required for an analysis. To reduce this time factor to a value suitable for routine process control, it was decided to attempt these separations in columns having internal diameters of about 22 mm. (Figure 3) and utilizing the same quantity of adsorbent and same operating pressure. With this mode of operation, the filtration rate was increased threefold and the separation of 2,6-di-*tert*-butyl-*p*-cresol from synthetic formulations with fresh lards appeared to be as good as with the smaller columns. One operator was able to operate four columns simultaneously, and complete analyses were made in 4 hours.

As this investigation was continued, several synthetic samples were formulated from lards that had acquired some degree of aging. The 2,6-di-*tert*-butyl-*p*-cresol recoveries were noticeably lower than those obtained with fresh lards, and there was a 6 to 8% deviation between results calculated at the two analytical wave lengths. Apparently some chemical changes had occurred in the lard samples which resulted in elution of other components into the filtrates with the 2,6-di-*tert*-butyl-*p*-cresol, giving rise to a considerable amount of background interference in the absorption spectra of the filtrate fractions. To correct this situation, the quantity of silicic acid charged to the 22-mm. columns was increased to 30 grams, whereupon a defi-

Table V. Effect of Quantity of Adsorbent on Recovery of 2,6-Di-*tert*-butyl-p-cresol from Synthetic Formulations with Commercial Lards Deteriorated by Storage

Lard Sample	Sample Age, Months	Quantity of Silicic Acid Used, Grams	% DBPC Recovered by Analysis at 276 mμ	% DBPC Recovered by Analysis at 284 mμ
A	3	26	90	96
			91	94
		30	97	100
			99	100
B	10	26	83	91
		30	94	96
			94	95

Table VI. Effect of Storage Time on Recovery of 2,6-Di-*tert*-butyl-p-cresol from Synthetic Formulations with Commercial Lards

Lard Sample	Weight % DBPC Added	% DBPC Recovered[a] after Storage 1 mo.	2 mo.	3 mo.	4 mo.	5 mo.
A	0.0105	100	96	93	100	94
B	0.0103	101	99	99	99	94
C	0.0106	103	105	99	102	94

[a] Calculated at 284-mμ wave length.

nite improvement in results was noted (Table V), and background spectral interference was reduced to a minimum. As this adjustment did not measurably delay the filtration and increase the time of analysis, the procedure was finally tested with 30-gram charges of adsorbent. This quantity proved adequate for all subsequent samples tested (Tables I and VI).

Effect of Lard Storage. Some governmental regulating agencies may encounter a range of application requiring analysis of commercial packaged lards of an unknown degree of shelf-aging. To determine applicability of the method under such conditions, three fresh samples of commercial brand lards were formulated with an antioxidant preparation containing 2,6-di-*tert*-butyl-*p*-cresol and stored in screwcapped glass jars at room temperature in the dark. At monthly intervals over a period of 5 months, portions of each sample were analyzed to determine the residual 2,6-di-*tert*-butyl-*p*-cresol content and the extent to which adequate separations could be effected without undue interference from degradative products of storage.

The results of such monthly analyses, listed in Table VI, indicate satisfactory results with lards as much as 3 to 4 months old. In view of the perishable nature of the product, this was considered more than satisfactory, as practical application of the method would probably never entail the analysis of lards having this degree of aging.

As a matter of interest the tests were extended for 5 months, at which time low recoveries of 2,6-di-*tert*-butyl-*p*-cresol were obtained. It is not known whether the low values are due to loss of 2,6-di-*tert*-butyl-*p*-cresol through chemical action within the lard, or to spectral background interference from chemical changes within the lard. Inspection of the ultraviolet spectra of the filtrate fractions indicated that the latter may be a contributing factor, because the 2,6-di-*tert*-butyl-*p*-cresol was displaced from the columns into an earlier filtrate fraction than ever encountered before.

Application to Other Edible Fats. Because of a general interest in the determination of 2,6-di-*tert*-butyl-*p*-cresol in vegetable fat shortenings, the procedure was also tested with a commercial, bleached product to which an accurately known quantity of 2,6-di-*tert*-butyl-*p*-cresol was added (0.01 weight %). The recovery of antioxidant, while sufficiently quantitative for most applications, was lower than that anticipated by calculation at the 284-mμ analytical wave length (Table VII, experiment 1). Furthermore, there was a deviation of 5% between the results calculated at the two analytical wave lengths, indicative of considerable background interference.

The over-all nature of the results indicated that an improved analysis should result from increasing the quantity of silicic acid adsorbent over the 30-gram quantity specified in the procedure. Consequently the experiment was repeated with a 35-gram charge of adsorbent in the chromatographic column; excellent quantitative results were obtained (Table VII, experiment 2).

The tocopherol content of the vegetable shortening used in the above formulations was not known, nor was it determined. However, because α-tocopherol, in particular, was known to be capable of serious interference in the ultraviolet determination of 2,6-di-*tert*-butyl-*p*-cresol, it was decided to add a known amount of this material to the 2,6-di-*tert*-butyl-*p*-cresol–treated shortening and to repeat the analysis. The data for experiment 3, Table VII, indicate that as much as 0.08 weight % of α-tocopherol has no effect on the analysis.

Use of the larger quantity of silicic acid adsorbent does not measurably increase the time required for the analysis.

Table VII. Analysis of Synthetic Mixtures of a Commercial Vegetable Fat Shortening with 2,6-Di-*tert*-butyl-p-cresol and α-Tocopherol

Expt. No.	Additive Formulation[a]	Silicic Acid, Grams	% DBPC Recovered by Analysis at 276 mμ	% DBPC Recovered by Analysis at 284 mμ
1	I	30	91	96
2	I	35	99	99
3	II	35	99	100

[a] I. 0.01% DBPC.
II. 0.01% DBPC + 0.08% α-tocopherol.

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