• *Technical*

A Direct Spectrophotometric Determination of Butylated Hydroxyanisole in Lard and in Hardened Lard

P. V. HANSEN,[1] F. L. KAUFFMAN, and L. H. WIEDERMANN, Research Laboratories, Swift and Company, Chicago, Illinois

THE USES and advantages of antioxidants in edible fats and oils have been adequately described in the literature (5, 6, 10, 11); however the development of methods for the determination of these antioxidants has lagged considerably behind the application of these stabilizers in fat products. With a few exceptions (1, 7, 10, 13) all of the published methods for the estimation of edible antioxidants in fats and oils depend upon the formation of a color complex which can be measured in the region of the visible spectrum (1, 2, 3, 9, 12). These colorimetric methods are suited for systems containing one or more antioxidants when they are present in appreciable quantities.

Two areas of particular deficiency encountered in the above methods are that they do not allow rapid and convenient procedures nor are they effective for small quantities of antioxidant(s), *i.e.*, a few parts per million (p.p.m.). Since butylated hydroxyanisole [BHA; 2-(and 3-)*tert*-butyl-4-hydroxyanisole] and butylated hydroxytoluene (BHT; 3,5-di-*tert*-butyl-4-hydroxytoluene) are of particular interest in fat technology, it is necessary to have available a convenient method for their quantitative determination over a practical range of concentrations having 100 p.p.m. as its higher limit.[2]

Hall and Clark (7) have reported the detection of BHA and other antioxidants by infrared analysis, and this approach may offer definite possibilities for the development of a quantitative method of analysis. Whetsel *et al.* (13) have developed a direct spectrophotometric method for the determination of BHA and propyl gallate in commercial antioxidant preparations. Their method takes advantage of the *iso* absorptive points of the absorption curves of the two substances; however its practical limitations in the low range of antioxidant concentrations have not been explored, nor does it provide either the presence or separation of oleaginous materials. In a recent publication (10) a method for analyzing butylated hydroxytoluene in lard and shortenings was described. This method utilizes chromatographic separation for the isolation of the antioxidant in the eluate, which is then analyzed for BHT by using its ultraviolet absorption curve between 240 and 320 $m\mu$. This is a satisfactory method for the analysis of BHT (BHA being noninterfering); however its applicability over an extended range of antioxidant concentrations has not been reported.

The present paper describes a method for determining butylated hydroxyanisole in lard and in hardened (iodine value less than 10) lard by means of a direct spectrophotometric technique and allows the estimation of the antioxidant when it is present in amounts as low as a few parts per million. This method is based on the strong absorbability of BHA at 290 $m\mu$ because of the hydroxyl group in the aromatic ring. The quantity of antioxidant present is determined from a standard curve after its extraction from the fat sample with methanol. It should be noted that such other antioxidants as BHT and tocopherols interfere with this determination, and this method cannot be successfully applied to BHA in their presence.

Experimental

Materials. Food-grade butylated hydroxyanisole was used and was obtained from Eastman Chemical Products Inc., Kingsport, Tenn. The fats used in the sample preparations were a) deodorized, prime steamed lard (66.5 iodine value) and b) deodorized, commercially hardened lard (6.5 iodine value). The solvents used, chloroform and methanol, were ACS grade. Spectrophotometric analyses were made with a Beckman DU spectropohotometer, using matched 1-cm. silica cells and a hydrogen lamp.

Method. Weigh 25.0 g. of hardened lard into a 250-ml. glass-stoppered flask, melt on a steam bath, and add 10 ml. of chloroform and 100 ml. of methanol. Heat to just below the boiling point of the solvent. Shake vigorously for 1 min. while the contents of the flask are still molten. Then chill in an ice bath to precipitate most of the dissolved fat, filter through No. 1 Whatman filter paper, and set aside the methanol layer. Remelt the remaining fat, add 5 ml. of chloroform and 50 ml. of methanol, and extract as before. Repeat this procedure a third time. Evaporate the combined extracts on a steam bath to a volume of approximately 10 ml., chill thoroughly in an ice bath, and filter through No. 1 Whatman filter paper into a 25-ml. volumetric flask. Wash filter with previously chilled methanol and, after attaining room temperature, dilute the contents of the flask to volume with methanol.

The extraction of BHA from lard was performed in much the same manner as described above for hardened lard. In place of a glass-stoppered flask the extraction was carried out in a separatory funnel at a temperature just below the boiling point of the solvent mixture. After cooling to room temperature, the methanol layer is decanted and filtered through No. 1 Whatman filter paper. This procedure is repeated three times, and the combined extracts are handled as described above.

Spectrophotometric readings were made by filling a 1-cm. silica cell with the solution and measuring its optical density at 270, 290, and 310 $m\mu$ with the

[1] Present address: Dana College, Blair, Nebr.
[2] The maximum level (0.01%) allowed by the Meat Inspection Bureau.

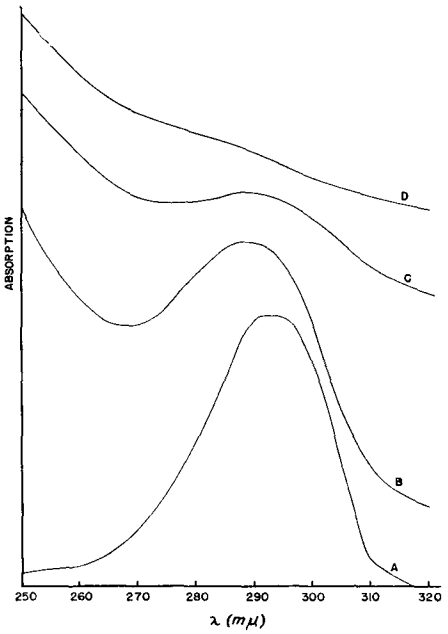

FIG. 1. Ultraviolet absorption curves of methanol solutions (curves are displaced on the vertical axis): A, containing 50 p.p.m. BHA; B, from the extraction of hardened lard containing 50 p.p.m. BHA; C, like B, containing 10 p.p.m. BHA; D, like B, containing no added BHA.

instrument adjusted to read 0.00 optical density for the solvent.

The corrected optical density (to account for dissolved fat) is calculated from the formula:

$$d_{1(290)} = d_{290} - \left(\frac{d_{270} - d_{310}}{2} \right)$$

where $d_{1(290)}$ is corrected optical density at 290 $m\mu$
d_{290} is actual optical density at 290 $m\mu$
d_{270} is actual optical density at 270 $m\mu$
d_{310} is actual optical density at 310 $m\mu$

The parts per million of BHA in the sample are read from a standard curve made by plotting corrected optical density [$d_{1(290)}$] *versus* p.p.m. BHA of known samples.

Results and Discussion

The effect of dissolved fat on the ultraviolet curve for an alcoholic solution of BHA is shown in Figure 1, as are the effects of fat absorption with changing BHA concentrations. The significant changes observed are the rounding-off of the characteristic peak for BHA at 290 $m\mu$. These changes can however be compensated for and will not adversely affect the determination of BHA in these solutions.

To correct for dissolved fat a straight line is drawn through the points at 270 and 310 $m\mu$ of an ultraviolet absorption curve for the solution made between 250 and 320 $m\mu$, as shown in Figure 2. These points were chosen because the straight line, ab, is then tangent to the characteristic minimum and the nearly flat portion of the curve.[3] Alcoholic solutions containing dissolved fat with varying quantities of BHA produce curves of generally the same form with the peak of the curve at approximately 290 $m\mu$ being shifted upward with an increase in the BHA content. The effect of small amounts of dissolved fat is to shift the whole curve upward. This does not affect the distance from the straight line, ab, to the peak or point of the curve at 290 $m\mu$, line cd, but only increases the distance from the abscissa to this peak. This then gives a basis for the measurement of the BHA concentration in a solution containing dissolved fat. Rather than plot each set of points and measure the distance cd, it was found satisfactory to subtract the mean of the readings at 270 and 310 $m\mu$, point *d*, from the reading at 290 $m\mu$, point c, thus giving the optical density resulting from only the BHA in the solution under investigation.

A plot of the corrected optical density values from the extraction of hardened lard samples against the known BHA content of these samples gives a linear relationship (curve A in Figure 3). The small correction because of the slight absorption at 290 $m\mu$ of solutions from the extraction of samples containing no BHA is inherent in the $d_{1(290)}$ *vs.* $m\mu$ plot in Figure 3.

The validity of this curve was further checked by the analysis of samples containing known levels of BHA, particularly in the lower range of concentrations. These data are contained in Table I and generally show an accuracy of plus-or-minus 2 p.p.m. for BHA concentrations below 50 p.p.m. This allows analysis of samples containing approximately 2 p.p.m. of BHA.

Curve B in Figure 3 also shows a straight line rela-

TABLE I

Recovery of BHA from Known Samples of Hardened Lard

BHA added (p.p.m.)	BHA recovered (p.p.m.)						
	Trial					Avg.	Deviation
	I	II	III	IV	V		
3	3.5	3.2	2.85	2.7	3.15	3.07	±0.4
6	5.75	5.9	5.8	6.0	5.8	5.85	±0.2
9	9.1	8.7	9.2	9.2	9.25	9.09	±0.3
25	24.2	....	....	....	....	....	....
50	51.0	....	....	....	....	....	....
100	95.3	....	....	....	....	....	....

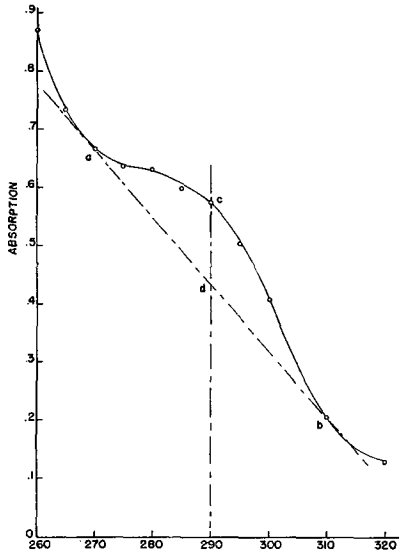

FIG. 2. Spectrophotometric curve of alcoholic solution from the extraction of hardened lard containing 10 p.p.m. BHA. Distance cd is a direct measure of absorption because of BHA and is independent of any dissolved fat.

[3] The minimum at 270 $m\mu$ is observed for curves of solutions containing low BHA concentrations, and while this point shifts slightly at higher BHA levels (to 265 $m\mu$ at 100 p.p.m.), the use of its optical density at 270 $m\mu$ was found to give consistent results throughout the tested range of concentration.

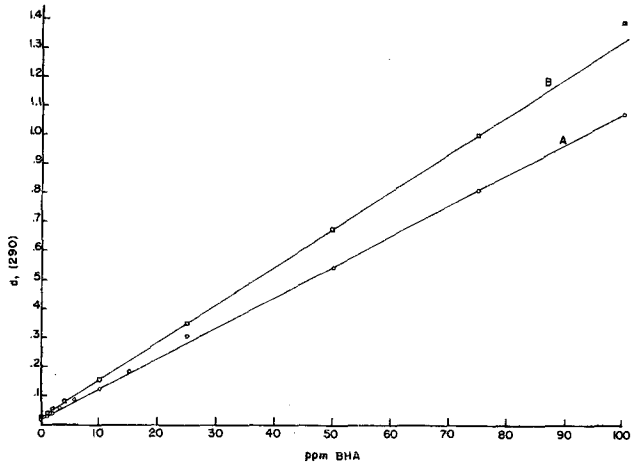

FIG. 3. Corrected optical density *versus* p.p.m. BHA in known samples: A, curve for hardened lard samples; B, curve for lard samples.

tionship when this method is applied to the determination of BHA in lard samples. With fats much softer than lard the accuracy of this method falls off rapidly on account of their increased solubility in the solvent. When measures are taken further to decrease their solubility, the solubility of BHA is also reduced but not to a reproducible level. It is therefore a necessary restriction of this method that the fat samples under investigation not be softer than lard or rather that they do not contain more lower-melting, more-soluble triglycerides.

This method was used to determine how effectively BHA is removed from lard or hardened lard during deodorization. In the deodorization process steam is bubbled through the fat, which has been heated to a temperature of 220°C. at 1-mm. pressure for 4 hr. Under these conditions the steam distillable BHA should be completely removed from the fat. Thirty p.p.m. of BHA were added to hardened lard both before and after deodorization, and samples of each were then analyzed by the present method. Table II compares these results with a control as well as the analysis of these samples by the 2,2′-bipyridine-ferric chloride method. The colorimetric procedure for analysis gives low results, and while it does indicate the complete removal of BHA during deodorization, the results cannot be considered reliable for low concentrations of BHA. The use of borax-2,6-dichloroquinonechlorimide (9) is under optimum conditions capable of the qualitative detection of BHA. However this method is of little practical value to the processor who has to restabilize a deodorized stabilized lard (11).

TABLE II

BHA Content of Hardened Lard Before and After Deodorization

	BHA recovered (p.p.m.)	
	Present method	Colorimetric method (1, 9)
1. Control (no BHA added)	0	0
2. 30 p.p.m. BHA added to control	29.3	20
3. Sample 2 after deodorization	0	0
4. 30 p.p.m. added to Sample 3	29.6	20

It was observed that, on re-examination of the hardened lard-BHA test samples after 40 and 100 days of storage at room temperature, there was a decrease in the antioxidant level. After 40 days there were 68 p.p.m. and after 100 days 57 p.p.m. for the 100-p.p.m. BHA sample. This decrease was found to be proportional at all BHA concentrations at each time interval, thus exhibiting a continued linear relationship. We can attribute this to the oxidation of BHA in its role as an antioxidant with the loss of its characteristic phenol-grouping. This loss through oxidation is not thoroughly understood, but it is thought that the isomeric phenoxy radicals will rearrange to give a semiquinone structure (8) or, as in the case of the oxidation of butylated hydroxytoluene (4), undergo some degree of dimerization. Either path would result in the observed decrease of BHA.

Summary

A direct spectrophotometric method for the determination of butylated hydroxyanisole has been presented. This procedure allows good precision in the analysis of lard and hardened lard containing from 0 to 100 p.p.m. of BHA and is more reliable than existing colorimetric techniques, especially in the lower range of antioxidant concentrations.

REFERENCES

1. Anglin, Constance, Mahon, J. H., and Chapman, R. A., J. Agr. & Food Chem., *4*, 1018–1023 (1956).
2. Austin, J. J., "Quantitative Analysis of Butylated Hydroxytoluene (BHT) in Fats and Oils," private communication.
3. Austin, J. J., "The Qualitative and Quantitative Analysis of BHA Using 2,6-Dichloroquinonechlorimide," private communication.
4. Cook, C. D., Nash, N. G., and Flanagan, H. R., J. Am. Chem. Soc., *77*, 1783 (1955).
5. Dugan, L. R. Jr., Marx, Lotte, Weir, C. E., and Kraybill, H. R., American Meat Institute Foundation, Bull. 18 (June, 1954).
6. Gearhart, W. M., and Stuckey, B. N., J. Am. Oil Chemists' Soc., *32*, 287–290 (1955).
7. Hall, L. A., and Clark, G. L., Food Technology, *10*, 384 (1956).
8. Koch, R. B., Bakers Digest, April 1956.
9. Mahon, J. H., and Chapman, R. A., Anal. Chem., *23*, *8*, 1116–1120 (1951).
10. Phillips, M. A., and Hinkel, R. D., J. Agr. Food Chem., *5*, 379–384 (1957).
11. Sims, Rex J., and Hilfman, Lee, J. Am. Oil Chemists' Soc., *33*, 381–383 (1956).
12. Stroupe, H. F., and Austin, J. J., "The Analysis of BHT, BHA, and 50-50 Mixtures of the Two in Paraffin Wax and Waxed Paper," private communication.
13. Whetsel, K. B., Robertson, W. E., and Johnson, F. E., J. Am. Oil Chemists' Soc., *32*, 493 (1955).

[Received January 9, 1959]

Analysis of Surfactant Mixtures. I.

J. D. KNIGHT and R. HOUSE, California Research Corporation, Richmond, California

MOST SCHEMES for characterizing or analyzing surfactants rely on specific reactions of the hydrophilic part of the molecule (*e.g.*, sulfate, sulfonate, or polyethoxyethanol groups). Much additional useful information can be obtained by also identifying the hydrophobic raw material used in the manufacture of the surfactant. Identification of the hydrophobic portion of the molecule is very difficult on the intact surfactant and can be accomplished much more easily if it can be decomposed into a