Liquid-Liquid Extraction of BHT from Vegetable Oils

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ABSTRACT

Partition behavior of the antioxidants BHT and BHA has been investigated between *n*-heptane and the polar phases; DMSO, DMF, acetonitrile and 80% methanol-water v/v. The distribution of four vegetable oils in these solvent pairs was also examined, as well as the influence of the oil on the partition of the antioxidants. Consideration has been given to requirements for quantitative extraction in the more promising cases.

INTRODUCTION

The separation of the antioxidant BHT (4-methyl, 2, 6 di-tertiary butyl phenol) from vegetable oils and other products has presented considerable difficulty due to the lipophilic character and relative instability of the compound. Quantitative determinations have been reported for steam distillations (1) and for a direct gas chromatographic method in which the oil was removed on a stainless steel or glass wool precolumn (2). Chromatographic identification of antioxidants, however, generally requires a prior separation, and this would undoubtedly be true for any technique in which the sample was not a pure oil but a mixture containing other volatile components, e.g., cosmetic formulations. Several liquid-liquid extraction systems effect complete separation of the other common antioxidants but are all much less satisfactory for BHT. A very limited amount of quantitative data has been reported, however.

An investigation has therefore been undertaken of the distribution of BHT between *n*-heptane and the polar solvents dimethylsulfoxide (DMSO), N, N-dimethyl formamide (DMF), acetonitrile and 80% methanol-water v/v. The distribution of BHA (4-methoxy 2- and 3-tertiary butyl phenol), commonly used in conjunction with BHT, has been included for a comparison.

EXPERIMENTAL PROCEDURES

Reagents

ACS reagent grade solvents were used with the exception of *n*-heptane and dimethyl sulfoxide which were spectrograde. BHT (4-methyl, 2, 6 di-tertiary butyl phenol) was obtained from Eastman Organic Chemicals. The two isomers of BHA (4-methoxy, 3-tertiary butyl phenol and 4-methoxy, 2-tertiary butyl phenol) were Food Chemicals Codex reference standards.

Procedure

Equilibration of 25 ml of *n*-heptane saturated with the appropriate polar solvent and 25 ml of the polar solvent saturated with *n*-heptane was carried out in 125 ml separatory funnels. After equilibration the concentration of antioxidant in each phase was determined spectrophotometrically with a Beckman DK-2A spectrophotometer. Absorbance values at the UV maxima were used, except in the case of the more dilute *n*-heptane solutions of 2- and 3-BHA which were determined colorimetrically by the ferric chloride-bipyridine reaction (3) and the dichloroquinone chloroimide reaction (4), respectively. The distribution data were checked by performing the equilibration with the antioxidant originally present in each of the two phases. The concentration range for the antioxidant solutions was 0.05-0.15 mg/ml.

RESULTS AND DISCUSSION

The distribution of the antioxidants has been calculated in terms of the experimentally determined partition ratio *Kp*—the ratio of the analytical concentration of antioxidant in the polar phase to that in the *n*-heptane phase. The partition ratios for BHT and the isomers of BHA in *n*-heptane and four polar solvents are shown in Table I. The *Kp* value of propyl gallate and nordihydroguaiaretic acid (NDGA), other common antioxidants, was too large in each case to be calculated by this method, due to their virtual insolubility in *n*-heptane.

While qualitative extractions of BHT from aliphatic hydrocarbons have been reported with aqueous alcohol (5,6), it is unlikely on the basis of this data that a quantitative method could be feasible.

The number of equilibrations (n) between equal volumes of solvent, which would be required for 99% of the BHT initially present to be extracted into the polar solvent, may be obtained by placing equal to 0.01 the cross current expression: ${}_nq = (1 + Kp)^{-n}$, where ${}_nq$ is, in this case, the solute fraction remaining in the heptane phase after n equilibrations. Rounded to the next highest integer, the values of n are 4, 6 and 10 for DMF, DMSO and CH_3CN, respectively.

The amount of material, other than antioxidant, extracted by these solvent pairs from four refined vegetable oils is shown in Table II as the percentage of a 5 g sample of oil present in the polar phase after equilibration between 25 ml each of *n*-heptane and polar solvent, previously saturated with respect to each other. The actual percentages vary somewhat from lot to lot of oil, but several lots of peanut oil have shown the same relationship (factor of five) between DMSO and CH_3CN.

It has been observed (7) that DMF extracts more "background" material from petroleum waxes in heptane than either CH_3CN or DMSO. Similarly it was found (5) that CH_3CN was more effective than 80% MeOH for the extraction of BHT from petroleum ether solutions, but the alcoholic solvent was used because the extraneous material

TABLE I

Antioxidant Partition Ratios, Polar Solvent to *n*-Heptane

Polar solvent	BHT	3BHA	2BHA
DMF	2.65	36	40
DMSO	1.40	130	170
Acetonitrile	0.65	19	22
$MeOH/H_2O$ 80:20 v/v	0.02	10	15

TABLE II

Extraction of Vegetable Oil by Polar Solvents from *n*-Heptane Solution

Solvent	Peanut, %	Corn, %	Soy, %	Cottonseed, %
DMF	3.01	3.75	4.55	3.37
DMSO	0.08	0.04	0.09	0.13
Acetonitrile	0.41	0.53	0.65	0.73
$MeOH/H_2O$ 80:20 v/v	0.04	0.04	0.03	0.02

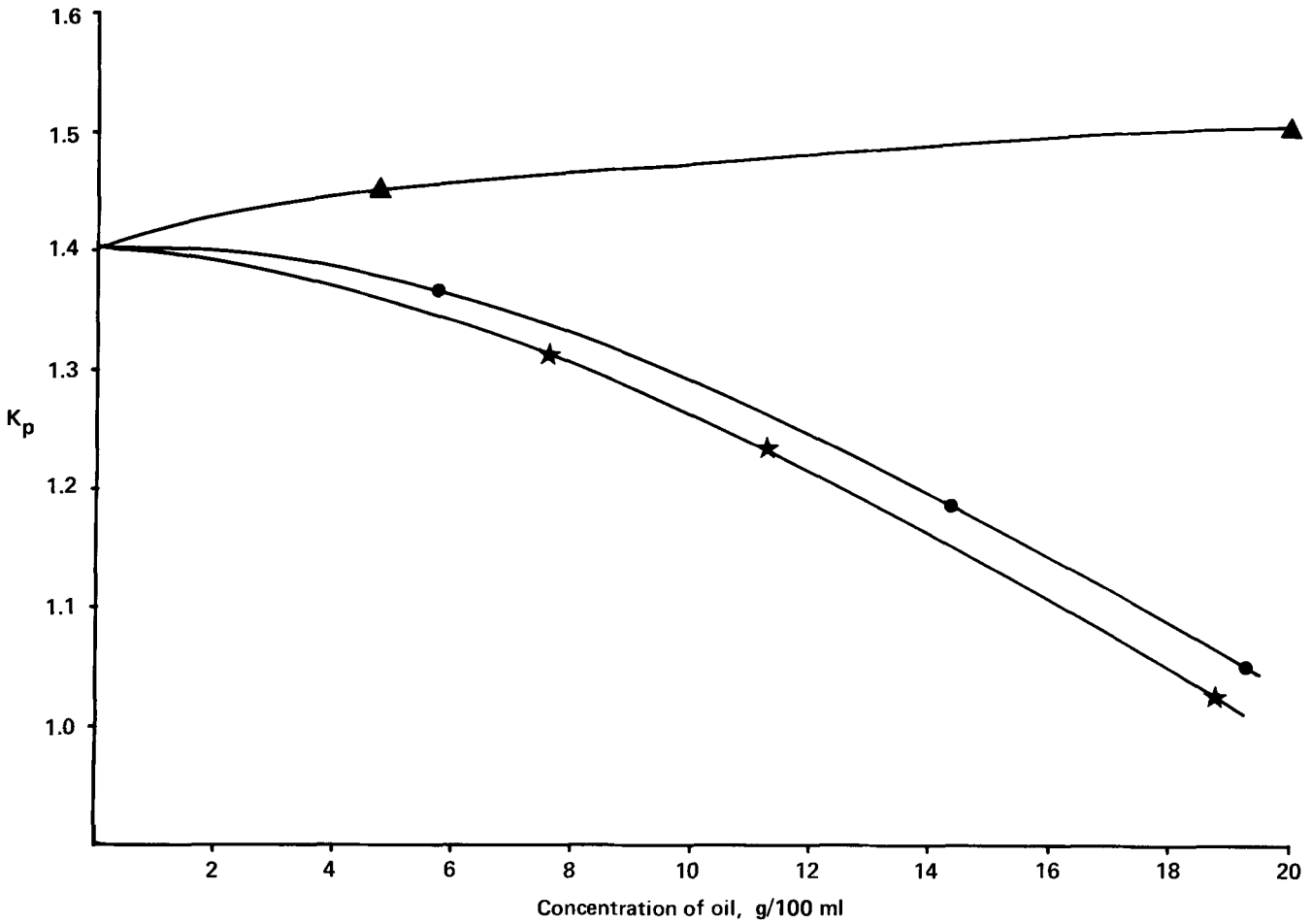

FIG. 1. Dependence of partition ratio on concentration of oil in *n*-heptane. ▲ = Mineral oil; ● = peanut oil; and ★ = corn oil.

extracted by the CH_3CN interfered with the qualitative analysis. The amount of oil components extracted by DMF is so great that it would very likely interfere appreciably with any subsequent chromatography. The extraneous material extracted by CH_3CN is also excessive, considering the large volume of solvent or number of equilibrations that would be necessary for quantitative extraction given the low value of the partition ratio.

The influence of the presence of vegetable oil on the distribution of BHT between DMSO and *n*-heptane is indicated in Figure 1. A similar reduction in *Kp* was found for the CH_3CN-*n* heptane solvent pair. This is the behavior to be expected on making the nonpolar phase more polar with triglycerides. The converse effect has been observed in the distribution of methyl palmitate between hexane and DMSO on adding sucrose to the DMSO phase (8). The slight increase in *Kp* caused by the presence of mineral oil is probably due to the reduction of the DMSO concentration in the heptane. The mutual solubilities of the pure solvents are assumed to be ca. 1.5% by weight in each case, as reported for the DMSO-*n* hexane system (9).

On the basis of the results given in Tables I and II it would appear that DMSO/heptane is the most efficient of the solvent pairs examined.

DMSO has received considerable attention in the field of separations over the past 10 years due to its highly selective solvency properties and relatively high dielectric constant. It has been used extensively as an extractant, particularly for glycosides, hemicelluloses, lignin, etc. Its use in liquid-liquid systems has generally been in conjunction with a third solvent, usually water. This has also been true for ion-exchange applications. Very few quantitative data have been reported for anhydrous systems (10,11). Will (12) determined *Kp* values for five fatty acids (C_{10}-C_{18}) in the solvent pair DMSO/petroleum ether. Renon and Prausnitz examined the partition of 5-nonanone between DMSO and *n*-hexane (9). Haenni et al. (7) determined the *Kp* of five chlorinated pesticides in the solvent pair DMSO/*n*-heptane.

Two quantitative extraction methods employing acetonitrile and petroleum ether have been reported. In one of these the low value of *Kp* is overcome by the use of a very large volume of acetonitrile and a clean-up of extraneous material on an alumina column (13). In the other method eight extractions are performed (14), but even allowing a *Kp* of 0.65 only 80% of the BHT would theoretically be extracted on the basis of the volumes used.

In general, a disadvantage to the use of DMSO as a liquid-liquid extraction solvent is that because of the high boiling point the extracted materials must be re-extracted by shaking with water and a low boiling solvent before the solution can be concentrated. In the case of BHT, however, it is necessary that the concentration step be carried out in as inert and low boiling a solvent as possible. We have found that when a solution of BHT in spectrograde methanol is concentrated from 25 ml to 3 or 4 ml by heating on a steam bath in a gentle stream of air, then returned to 25 ml, the absorbance at the wavelength maximum (275 nm) is reduced 24%. BHT solutions in *n*-heptane, acetonitrile and petroleum ether treated under identical conditions showed absorbance reductions of 10%, 5% and 4%, respectively. Campbell and Coppinger (15) followed the reaction of BHT with *t*-butyl peroxide through the decrease in absorbance at 275 nm.

BHT readily undergoes auto-oxidation in alcoholic solutions in the presence of hydroxide ion (16). Matsuura et al. (17) found that a variety of products are produced by refluxing a solution of BHT in pure methanol in a stream of oxygen. The presence of a photosensitizer was required for

the recovery of macroscopic amounts of material, but our data indicate that this is not necessary for significant oxidation of dilute solutions (ca. 8 mg/100 ml). It may also be mentioned that BHT has been added to various eluents to prevent oxidation of plant lipids during column or thin layer chromatography and then removed simply by evaporation (18). Consideration should be given to this effect in any quantitative extraction of BHT. Several published procedures (6,13,19) call for evaporation of the extracting solvent at elevated temperatures or in a stream of air, or both.

Procedure

For vegetable oils containing 0.005-0.01% BHT and BHA we have employed the following procedure: A 4 g sample dissolved in 20 ml of *n*-heptane is extracted four times with 25 ml portions of DMSO. This will provide an extraction of over 95% calculated on the basis of the values in Fig. 1. To the combined DMSO extracts are added 100 ml of water and 100 ml of saturated aqueous sodium chloride. This solution is extracted twice with 75 ml portions of petroleum ether. The combined petroleum ether extract is filtered and evaporated to 2-3 ml at room temperature and transfered to a 5 ml volumetric flask. BHT is readily extracted from DMSO/H_2O solutions, but BHA requires a concentration of ca. 2 M sodium chloride for complete salting-out under the conditions given here.

This solution is suitable for analysis by thin layer chromatography using any of several systems described for antioxidant mixtures. However, for specific identification of BHT, elution with *n*-hexane on silica gel followed by spraying with Folin-Ciocalteau reagent (Rf ca. 0.5) is preferable to the general procedures due to the nonpolar character of the compound. Similarly, high speed liquid-solid chromatography on a 1 m column packed with Corasil II and eluted with *n*-heptane provides a quantifiable peak for vegetable oil extracts obtained by this procedure.

Using the latter technique with peanut oil we have obtained an average BHT recovery of 97% in the 0.01-0.02% range. A small amount of extraneous material, having a retention time similar to that of BHT, is extracted from the blank so that we have been limited to a recovery of 90 ± 10% at the 0.005% level.

REFERENCES

1. Anglin, C., J.H. Mahon and R.A. Chapman, J. Agr. Food Chem. 4:1018 (1956).
2. Hartman, K.T., and L.C. Rose, JAOCS 47:7 (1970).
3. Mahon, J.H., and R.A. Chapman, Anal. Chem. 23:1116 (1951).
4. Mahon, J.H., and R.A. Chapman, Ibid. 1120 (1951).
5. Heindrick, P., and H.W. Conroy, J. Ass. Off. Agr. Chem. 45:244 (1962).
6. Schneider, W., Kosmetic 43:559 (1970).
7. Haenni, E.O., J.W. Howard and F.L. Joe, J. Ass. Off. Agr. Chem. 45:67 (1962).
8. Ranny, M., Fette, Seifen, Anstrichm. 69:70 (1967).
9. Renon, H., and J.M. Prausnitz, Ind. Eng. Chem. Process Des. Devel. 7:220 (1968).
10. Waksmundski, A., K. Stelmach and T. Wolski, Przemysi Chem. 43:194, 445 (1964); CA 61:3735; CA 62:7166.
11. Phipps, A.M., Anal. Chem. 40:1769 (1968).
12. Will, F., Ibid. 33:647 (1961).
13. Schwien, W.G., B.J. Miller and H.W. Conroy, J. Ass. Off. Agr. Chem. 49:809 (1966).
14. Sahasrabudhe, M.R., Ibid. 47:888 (1964).
15. Campbell, T.W., and G.M. Coppinger, J. Amer. Chem. Soc. 74:1469 (1952).
16. Brieskorn, C., and K. Ullmann; Chem. Ber. 100:618 (1967).
17. Matsuura, T., K. Omura and R. Nakashima, Bull. Chem. Soc., Japan 38:1358 (1965).
18. Wren, J.J., and D. Szczepanowska, J. Chromatogr. 14:405 (1965).
19. Scheidt, S.A., and H.W. Conroy, J. Ass. Off. Agr. Chem. 49:807 (1966).

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