Table V. Nitrogen Recovery by Grasses Following Top Dressing with Uran Solution or Pelleted Ammonium Nitrate, Leon Fine Sand, pH 5.8

Treatment and Initial Rains	Nitrogen Recovered,[a] Net Lb.			
	Coastal Bermudagrass		Pensacola Bahiagrass	
	Uran	NH_4NO_3	Uran	NH_4NO_3
Area *A*				
54.0 lb. N applied 4/13, 0.93 inch rain on 4/18, cut 5/27	12.9	27.2[b]	7.1	11.5[c]
Refertilized 45.3 lb. N on 6/17, 3.50 inches rain 6/18, cut 7/20	16.8	18.0	18.9	24.4[b]
Area *B* (Adjacent to *A*)				
45.1 lb. N applied 4/22, 0.10 inch rain immediately, 1.03 inches rain 4/26, cut 5/27	16.8[b]	10.5	8.7	8.7
Refertilized 44.7 lb. N on 6/24, 1.83 inches rain 6/27, cut 7/20	17.3	17.2	17.1	18.4

[a] Av. of six replicates.
[b] Statistically significant difference at 0.01.
[c] Statistically significant difference at 0.05.

subject to greater volatile loss of nitrogen than another material under a given set of conditions does not necessarily imply lower efficiency for crop production. This type of loss is only one of several factors to be considered. However, it does strongly suggest that agricultural practices leading to measurable volatile losses of ammonia, such as surface application of urea to light soils and turf, or of ammoniates to calcareous or top-limed soils, should be taken into consideration and cultural practices adjusted within reason to reduce such losses and thus increase the efficiency of the material in question.

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FOOD ANTIOXIDANTS

Determination of Butylated Hydroxyanisole and Butylated Hydroxytoluene in Potato Granules by Gas-Liquid Chromatography

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A rapid and specific method for the quantitative analysis of low levels of butylated hydroxyanisole and butylated hydroxytoluene in dehydrated potato granules using gas-liquid chromatography with flame ionization detection is described. The method is shown to give analyses with an average error of less than 11% from the true concentration for both antioxidants within the range 0.5 to 10 p.p.m. of antioxidant in the granules.

BHA (2- and 3-*tert*-butyl-4-hydroxyanisole) and BHT (3,5-di*tert*-butyl-4-hydroxytoluene) are used at low levels (<10 p.p.m. of both) in commercial dehydrated potato granules to protect the natural fat of the potato from oxidative deterioration. Many naturally occurring phenolic materials in potatoes interfere with analysis of these antioxidants by colorimetric methods unless a tedious steam distillation procedure (*2*) is used. Even with steam distillation, the authors have found the colorimetric method to be questionable below 10 p.p.m. for BHT.

A gas-liquid chromatographic method (GLC) for BHT in paperboard has been reported (*3*). Thermal conductivity detection was satisfactory for the levels used in paperboard. With the low levels used in potato granules, however, it is impossible to obtain accurate analysis with thermal conductivity detection unless an excessively large sample is taken. The low levels are, however, well within the sensitivity of the flame ionization detector.

Experimental

Materials. Dehydrated Potato Granule Samples. Idaho Russet Burbank potato granules prepared by the conventional add-back process were received directly from the manufacturer—dried to 5.7% moisture—and stored under a nitrogen atmosphere (<0.2% O_2) at 75° F. until the present work, 1 year later. The only additive was sodium bisulfite at a level of 300 p.p.m. of SO_2. Weighed amounts of antioxidants were mixed carefully with granules in the laboratory; first by grinding with mortar and pestle and later with a rotating mixer to give levels used in the analyses—i.e., 0.50 p.p.m., 2.50 p.p.m., 5.00 p.p.m., and 10.00 p.p.m. of each antioxidant. This was done within a day prior to analysis.

Reagents. Petroleum ether, b.p. 40° to 60° C.

BHA (Tenox BHA). Twice recrystallized from petroleum ether, m.p. 59° to 60° C.

BHT (Koppers Food Grade). Purity, 98.7%; m.p. 69° to 70° C.

Standard solution. It contained 0.100 mg. of BHA per ml. and 0.100 mg. of BHT per ml., in petroleum ether.

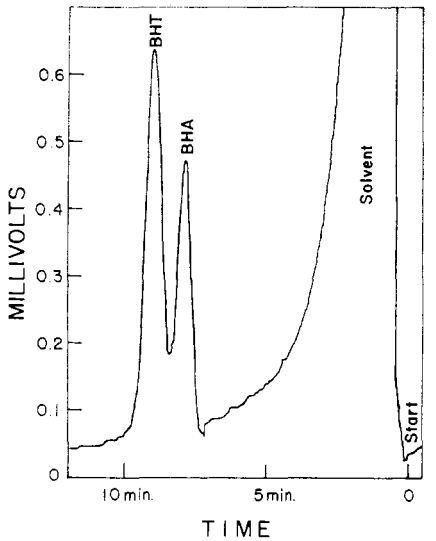

Figure 1. GLC curve from the injection of 30 μl. of the standard solution containing 0.100 mg. of BHA per ml. and 0.100 mg. of BHT per ml. in petroleum ether

Table I. Analyses of Samples of Potato Granules Containing Various Amounts of Antioxidants

Sample	Found, P.P.M. BHA	Found, P.P.M. BHT
Added 5.00 P.P.M. of BHA and 5.00 P.P.M. of BHT[a]		
A	4.5	4.2
B	4.7	5.0
C	4.4	4.2
D	4.2	4.2
E	4.3	4.1
F	4.2	4.2
G	4.7	4.0
H	5.0	4.3
Av.	4.5	4.3
Av. dev.	±0.2	±0.2
Av. % error from added amount	−9.5%	−14.5%
Max. % error from added amount	−16%	−20%
Added 0.50 P.P.M. of BHA and 0.50 P.P.M. of BHT[a]		
I	0.44	0.44
J	0.47	0.45
K	0.46	0.46
L	0.42	0.46
Av.	0.45	0.45
Av. dev.	0.02	0.01
Av. % error from added amount	−10.5%	−9.5%
Max. % error from added amount	−16%	−12%
Added 2.50 P.P.M. of BHA and 2.50 P.P.M. of BHT[a]		
M	2.3	2.4
N	2.2	2.1
Added 10.00 P.P.M. of BHA and 10.00 P.P.M. of BHT[a]		
O	8.9	8.9
P	9.1	9.6

[a] By careful addition and mixing.

Apparatus. Gas-liquid chromatography. The unit was laboratory constructed. The column was heated by a cylindrical aluminum oven.

The flame ionization detector was constructed by using the design of Thompson (*4*). A close-fitting aluminum foil cap placed over the top of the detector improved the stability of the baseline.

The electrometer was also constructed according to the design of Thompson (*4*) except that a 10-turn, 5000-ohm helipot was used as the variable zero control. A 0 to 1-mv. range, 1-second response time recorder was used with a chart speed of 24 inches per hour.

The column used for the antioxidant analysis was a stainless steel tube 5 feet long by 0.25 inch in O.D., 0.21 inch in I.D., packed with 40- to 60-mesh diatomaceous earth firebrick coated with 20% of Apiezon L, high vacuum grease. It was found essential to age the column for 1 week at 220° C. under operating conditions for satisfactory resolution of BHA.

The conditions used for analysis were: column temperature, 220° C.; carrier gas, nitrogen at a flow rate of 80 ml. per minute, inlet pressure, 10 p.s.i.; hydrogen flow rate to the detector, 60 ml. per minute; air flow rate to the detector, 600 ml. per minute. The detector and injector were at the same temperature as the column.

The sensitivity of the amplifier was set to give the size of peaks shown in Figure 1 for 30 μl. of the standard containing 0.100 mg. per ml. of each antioxidant.

Glass Apparatus. The glassware was standard except for two distillation columns used in concentration. These were a 500-mm. over-all length, 12 mm. in O.D. column (24/40 joints), packed with 1/8-inch Nichrome helices for 340 mm. of its length, and a 250-mm. over-all length, 9-mm. in O.D. column (14/20 joints), packed with Nichrome helices for 120 mm. of its length.

Procedure. Weigh a 100.0-gram potato granule sample into a 500-ml. flat-bottomed flask. Add 200 ml. of petroleum ether, stopper, shake, and allow to stand overnight at room temperature (75° F.) in the dark. Filter the resulting mixture through a sintered glass funnel into a 1-liter filtering flask, washing the residue and 500-ml. flask with three 50-ml. lots of petroleum ether. Transfer the filtrate and washings to a 500-ml. round-bottomed flask and concentrate to about 20 ml. by heating on a steam bath and using a 500-mm. helix-packed distillation column. Wash the column back into the flask with a small quantity of petroleum ether (10 ml.).

Transfer the concentrate and washings to a 50-ml. conical flask and concentrate further to about 3 ml. on the steam bath, using a 250-mm. helix-packed column. Wash the column back into the flask with a small quantity of petroleum there (1 ml.). Transfer the concentrate and washings to a 5.0-ml. volumetric flask and make up to the mark with petroleum ether. Inject 30 μl. of the standard solution (0.100 mg. per ml. each of BHA and BHT) into the GLC apparatus under the condition stated above. Immediately after emergence of the BHA and BHT peaks of the standard, and giving time for the base line to level out, inject 30 μl. of the unknown concentrate.

The peaks emerge as in Figure 1, the BHA peak coming at 7 minutes, 45 seconds, and the BHT peak at 9 minutes, 0 second. There will be some variation in retention times from column to column and with the age of column. It is best to determine the exact retention times from the standard, which is injected immediately prior to the unknown. Determine the area of each peak by multiplying peak height by peak width at 1/2 peak height. Use a French curve to determine the base line. (Note: In this work for the 0.5 p.p.m. level, the peak height was used instead of the peak area.) Calculate the parts per million of BHA and BHT in the granule sample from the formula.

$$\text{P.p.m.} = \frac{\text{area of unknown peak} \times 5.0}{\text{area of standard peak}}$$

The gas-chromatography analysis is repeated and the two results are averaged.

Results and Discussion

Sensitivity and Accuracy. Table I lists results found for analyses carried out on granule samples containing the levels 0.50, 2.50, 5.00, and 10.00 p.p.m. of each antioxidant. With results for the 5.00-p.p.m. level, the mean for the eight analyses was −0.5 p.p.m. different from the added amount for BHA with an average deviation from the mean of ±0.2 p.p.m., and −0.7 p.p.m. different from the added amount for BHT with an average deviation from the mean of ±0.2 p.p.m. For the levels 0.50 to 10.00 p.p.m., the average per cent difference from the added amount for 16 analyses was −10.0% for BHA and −10.9% for BHT. The maximum difference found from the added amount was −16% for BHA and −20% for BHT.

Analytical results are fairly consistently low with an average deviation from the mean of ±4%. The low figures are apparently due to incomplete extraction of the antioxidant from the granule sample. It would seem feasible to apply a correction factor to the result by multiplying by 1.1. This would give an analysis closer to the actual. It is desirable, of course, that each analyst determine his own particular correction factor.

Discussion of Method. The accuracy and sensitivity of the method are within the present needs of the industry. The main error seems to result from incomplete extraction of antioxidant from the granule sample. Petroleum ether is a poor solvent for elution from adsorbant material. However, it has other advantages over stronger solvents, which make it desirable for the present use. Chlorinated solvents give rise to corrosive vapors in the flame ionization detector and thus are undesirable for routine use in detectors constructed from metal. Diethyl ether may contain peroxides which could react with small concentrations of antioxidants. Alcohol solvents cause undesirable, excessive tailing with the Apiezon L firebrick column and extract considerable quantities of potato sugars, etc.

The GLC conditions were chosen to give a rapid analysis with a satisfactory separation of BHA and BHT. The Apiezon L column has several advantages over other columns for the present analysis. It is stable and normally has a long life. At the high temperature required, Apiezon L gives a minimum of "bleeding," an essential feature with the sensitivity capabilities of the flame ionization detector. Polar columns give better separation of BHA and BHT, but they cause excessive base line "noise" when used near or above 200° C. at the sensitivity required for the present analyses.

It is important that the Apiezon L column be well "aged"; otherwise it is not satisfactory for BHA. BHA seems to be decomposed to some extent on the column and its peak is smaller than the BHT peak. The extent of decomposition seems also to vary with age of the column. This is no problem if the standard sample is injected immediately prior to the unknown. The decomposition probably occurs on the active centers of the firebrick and it may be advantageous to use one of the several known methods (*1*) of deactivating these centers.

Although the Apiezon L column causes little bleeding, the potato lipide which is injected into the GLC apparatus along with antioxidants is thermally unstable and produces some base line noise, which limits the sensitivity at which the detector can be used.

Because potato lipide is injected into the GLC apparatus along with antioxidants, it would be desirable to use an injection system which can be periodically cleaned with solvent to remove accumulated lipide, which otherwise would eventually result in deterioration of the column. The quantity of lipide injected with each analysis is, however, small (1 mg.). The columns used without periodic cleaning of the injection system had a reasonably long life. It is estimated that columns used under such conditions would last at least 1 month with daily routine use.

Possible Simplification of the Procedure. About the same accuracy can be obtained by a single concentration step to about 5 ml. with the 500-mm. helix-packed column. This concentrate is then transferred directly to a 10-ml. volumetric flask, and 30-μl. samples are injected into the GLC apparatus. Depending upon accuracy required and level of antioxidant used, the volume of concentrate may be changed to simplify the concentration step. Another simplification is to use peak heights instead of peak areas. This seems to be just as accurate, if the standard is injected immediately before the unknown.

A method used by one of the authors (B. N. Stuckey) is to add 2 ml. of iso-octane to the extract and carry out concentration at room temperature using a rotating, high vacuum type evaporator. A convenient volume of iso-octane remains after the relatively rapid removal of the more volatile petroleum ether. The concentrate is transferred to a 10.0-ml. volumetric flask with iso-octane washing and is made up to the mark with iso-octane.

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FEED ADDITIVES

Metal Binding Properties of Bacitracin

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BACITRACIN, a mixture of polypeptide antibiotics, was first isolated by Johnson, Anker, and Meleney (*9*) in 1945 from cultures of *B. licheniformis*. Since its discovery, bacitracin has not enjoyed as widespread pharmaceutical use as other well known antibiotics, but it has been effectively employed in formulated feedstuffs as a growth promoter (*11, 18, 19*).

Two groups of workers, Craig and associates in this country and Newton and Abraham in England, have shown the polypeptide mixture to consist of a main component, bacitracin A, and lesser amounts of bacitracin B, D, E, and F (*2, 13, 16*). Independently, these same two groups (*17*) arrived at the structural formula of bacitracin A (I).

One of the major problems associated with the use of bacitracin in animal feed materials is gradual loss of activity on standing (*3*). More stable forms, such as zinc bacitracin (*6*) and bacitracin methylene disalicylate (*15*), have been prepared, and recently manganese bacitracin has been added to the list of metal bacitracins (*5*). Numerous observations have been published concerning the binding of other antimicrobial com-