variable quantities. The presence of the above three constituents indicates the occurrence of a small quantity of hemicellulosic material in the original flour. The relatively greater quantity of uronic acid may stem in part from pectic substances present in the flour or alternatively it may come from a rather acidic hemicellulose fraction. Attempts to characterize the starch of the rye-wheat Triticale have not yet been made but are projected. An interesting possibility arises in that an interaction of the rye and wheat genomes could possibly be present in a chemically detectable form.

Total nitrogen of the ether-extracted flour was determined and is expressed as crude protein. The values obtained were in close agreement with those which were obtained in some previous investigations (*13*). More detailed studies are underway on the flour proteins, and the results will be reported at a later date. The hexaploid Triticale generally contained more 80% alcohol-soluble nitrogenous substances than did the hard red spring wheat varieties and the synthesized bread wheat (6A49) (Table I). Some 6A-Triticale varieties (6A190, 6A20.1, 6A20.2, 6A67.1) and two of the tetraploid wheats (4B113 and 4B276) contained considerably more soluble nitrogen than the other species of these two classes. The two octaploid Triticale were also relatively high in soluble nitrogen as were the two durum varieties and the rye variety. Both the rye and durum wheat genomes may contribute toward this character although in some specific varieties (some of the 6A67 selections) a suppression seems to be evident. Paper chromatographic examination of the soluble nitrogenous fractions indicated the presence of at least 14 amino acids in varying relative quantities. This aspect will be further investigated using gas chromatographic techniques in conjunction with other available procedures.

Except for a few isolated exceptions, the hexaploid and octaploid Triticale were relatively low in fat and compared in this respect to rye. The durum varieties contained about twice as much fat. Whether the relatively higher fat content of a few Triticale is an expression of the durum wheat genome cannot be ascertained at this time, but it is an attractive possibility. A more detailed comparative study of the nature of the lipids is projected.

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FOOD ADDITIVES MEASUREMENT

Determination of Antioxidants in Certain Food Products and Packaging Materials by Gas Chromatography

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A method is described for measuring p.p.m. quantities of BHA and BHT in food products. The method includes a rapid extraction step and analysis with GLC utilizing 3,5-di-*tert*-butyl-4-hydroxyanisole (di-BHA) as an internal standard. The gas chromatographic column, containing a mixture of Silicone gum SE-30 and Tween 80, can be operated at a relatively low temperature (150° C.) with complete separation of the BHA and BHT from interfering compounds. The usefulness of the method is illustrated with data on ready-to-eat cereals, potato products, and waxed paper.

BUTYLATED HYDROXYANISOLE (BHA) and butylated hydroxytoluene (BHT) are added to many food products as freshness preservers. They may be added directly to the food or indirectly through the medium of packaging materials. Since these compounds act as free radical acceptors, small quantities of BHA and BHT very effectively protect foods from autoxidation. Present regulations allow 10 p.p.m. of the combination of BHA and BHT in potato granules and 50 p.p.m. in dry breakfast cereals. Therefore, a method for the determination of BHA and BHT in food products must be sensitive to at least the part per million level. In addition to sensitivity, a method suitable for quality control use must be rapid, accurate, and inexpensive.

Colorimetric procedures have been proposed by Anglin *et al.* (*2*); Filipic and Ogg (*6*); Lazlo and Dugan (*9*); and Sloman *et al.* (*12*). Hall and Clark (*7*) used infrared spectrophotometry to study complex mixtures of food antioxidants, and ultraviolet spectrophotometry was used by Phillips and Hinkel (*11*); Hansen *et al.* (*8*); and Berger *et al.* (*3*). Mitchell (*10*) suggested a paper chromatographic method for the analysis of mixtures of known antioxidant compounds, and Anderson and Nelson (*1*) and Buttery and Stuckey (*4*) have used gas liquid chromatography techniques. Sloman *et al.* (*12*) pointed out that most of these methods, except those based on GLC, work best at concentrations above 10 p.p.m.

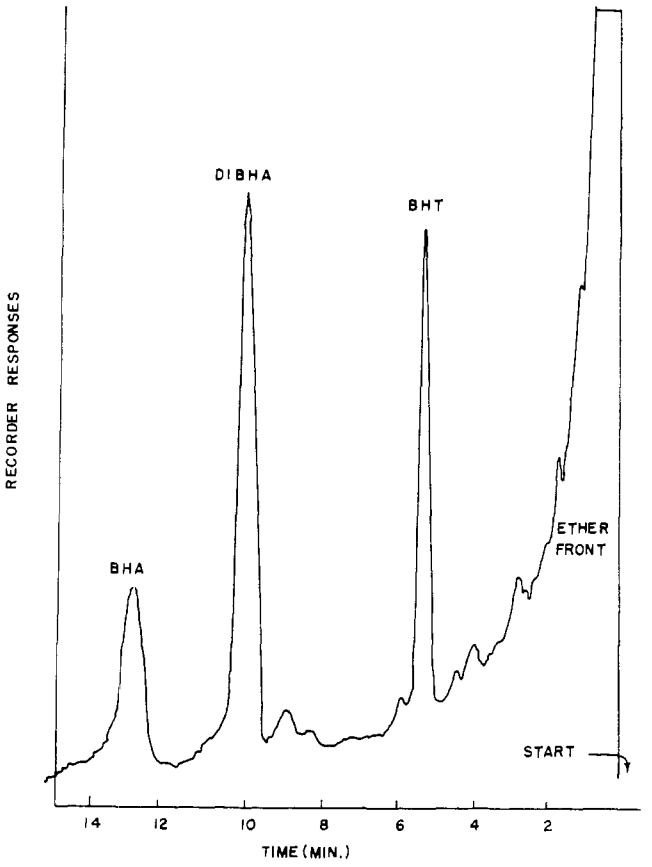

Figure 1. Typical gas chromatogram of an extract from wheat cereal containing 7.3 p.p.m. BHA and 6.1 p.p.m. BHT

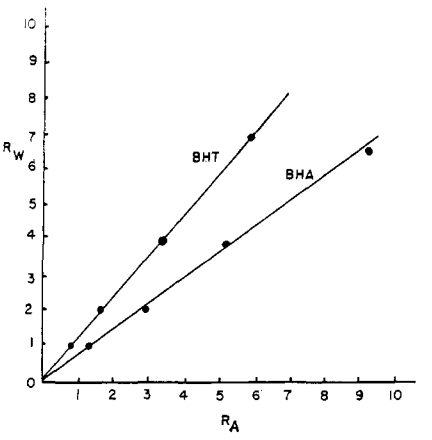

Figure 2. Relationship of peak area response (R_A) to weight ratio (R_W)

They pointed out that the method of Buttery and Stuckey, although sensitive, requires a long extraction time and a careful concentration step. Anderson and Nelson (*1*) suggested that extracts of cereal products contained materials which interfere with spectrophotometric or GLC methods and that column chromatography and steam distillation were not suitable cleanup procedures. Their procedure utilized a gas chromatograph which allowed programming of the column to a very high temperature to separate and isolate BHA and BHT from cereal extracts.

By combining a rapid extraction step with an analysis by GLC on a selective column at a relatively low column temperature, the authors determined p.p.m. quantities of BHA and BHT in dry breakfast cereals, potato granules, potato slices, vegetable oils, waxed paper, and other package-liner material. The method presented is sensitive, accurate, rapid, and inexpensive.

Experimental

Apparatus. A Model 609, F & M gas chromatograph with a flame ionization detector was used. A 10-foot aluminum column packed with SE-30 silicone gum and Tween 80 coated on Chromosorb W–type ABS, 70–80 mesh (Analabs, Hamden 18, Conn.) was used. No special treatment of the chromosorb was necessary. One per cent of Tween 80 and 2% of silicone gum were dissolved in chloroform and applied to the chromosorb.

Instrument Parameters. Column temperature, 150° C.; injector temperature, 250° C.; detector temperature, 250° C.; helium flow rate, 51 ml. per minute; hydrogen flow rate, 50 ml. per minute; air flow rate, 550 ml. per minute; attenuation range, 10 × 4; sample size, approximately 2 µl.

Reagents. 2,6-Di-*tert*-butyl-*p*-cresol (BHT) and 3-*tert*-butyl-4-hydroxyanisole (BHA) were obtained from Eastman Chemical Products, Inc., Kingsport, Tenn. The 3,5-di-*tert*-butyl-4-hydroxyanisole (di-BHA) was obtained from Universal Oil Products Co., Des Plaines, Ill.

Procedure

A 10.0-gram sample of product, reduced to a small particle size, is poured into a glass column made from 1-cm. glass tubing. The column should be approximately 40 cm. long and tapered at one end to allow plugging with glass wool. Diethyl ether is percolated through the column until approximately 20 ml. are eluted from the column. Ten milliliters of an ether solution containing 20 µg. of di-BHA per ml. of solution are added to the 20 ml. of eluate. The resulting solution is swirled to promote mixing, and the ether is evaporated with a gentle stream of nitrogen by immersing the flask in a 35° to 40° C. water bath. The solution should be concentrated to approximately 0.5 ml. By means of a microliter syringe, approximately 2 µl. of solution are injected into the gas chromatograph for analysis. Figure 1 shows a typical chromatogram of a wheat cereal extract containing BHA, BHT, and the internal standard (di-BHA). The chromatogram takes approximately 12 minutes for completion. The area of the peaks is measured by multiplying the peak height times the width at half the height. The ratio of the area of the di-BHA peak to the area of the BHA or BHT peak is then calculated.

Modified procedures are necessary for preparing packaging material samples for analysis. For waxed paper, 1 gram (cut up with a paper punch) is weighed into a round-bottomed flask, and 40 ml. of Skellysolve B and 10 ml. of a Skellysolve B solution containing 120 µg. of di-BHA per ml. of solution are added. The mixture is shaken for 15 minutes on a mechanical shaker; the supernatant liquid is decanted and concentrated to approximately 2 ml. as described above. Fifty milliliters of absolute methanol is added to the concentrate, and the mixture is shaken several times. The precipitated wax is filtered off, and the filtrate is concentrated to approximately 5 ml. Two to 3 µl. of the concentrated methanol solution are used for the analysis as described above.

Samples of breakfast cereal containing 5, 20, and 50 p.p.m. of BHA and BHT were prepared by slurrying the cereal in a solution of the known amount of BHA and BHT in diethyl ether.

Table I. Recoveries of BHA and BHT from Cereals and Potato Granules

BHA, P.P.M.			BHT, P.P.M.		
Added	Found	% Recovered	Added	Found	% Recovered
		Wheat Flakes			
6.3	5.7	90.5	6.3	6.0	95.2
20.0	21.0	105.0	20.0	19.0	95.0
50.0	49.0	98.0	50.0	48.6	97.2
		Corn Flakes			
...	...	...	13.0	13.0	100.0
18.7	18.4	98.4	18.7	19.1	102.1
32.0	31.0	97.0	32.0	32.0	100.0
		Potato Granules			
...	...	...	10.0	9.8	98.0

Table II. Recovery of BHT from Waxed Paper

BHT in Wax,[a] %	BHT Added to Extract (Wax Basis),[b] %	BHT Recovered (Wax Basis), %
	Waxed Glassine without BHT	
0.0	0.20	0.22
0.0	0.60	0.60
0.0	1.00	1.03
	Waxed Glassine with BHT	
0.20	0.0	0.15
0.60	0.0	0.55
1.20	0.0	1.25

[a] Calculated by manufacturer.
[b] Extract of waxed paper as described in procedure.

Samples were shaken while the solvent was evaporated under vacuum. The residue of antioxidant on the inside of the flask was rinsed out with diethyl ether, the rinsings were concentrated, and the concentrate was dripped on the flakes. The potato granule samples were prepared in the same general manner.

Samples of commercial waxed glassine paper containing 0.2, 0.6, and 1.2% of BHT in the wax were obtained for this study. For recovery studies, known amounts of BHT were added to extracts of BHT-free waxed paper, and these extracts were analyzed by the stated procedure.

Calculations

Equal quantities of BHA, BHT, and di-BHA will not excite equal responses from the hydrogen flame detector cell; therefore, the response of the detector for BHA and BHT must be measured relative to the response of the detector to di-BHA. Figure 2 shows the linear relationship of the area response to weight for BHA and BHT relative to di-BHA. Solutions containing ratios of 1.0, 2.0, 4.0, and 6.5 parts of BHA and BHT on a weight basis were analyzed by GLC. The results are plotted in Figure 2. This figure shows that the response of the detector to the di-BHA is greater than the response to BHA but is less than the response of the detector to BHT.

To calculate p.p.m. of antioxidant, determine the ratio of the area of the di-BHA peak to that of the antioxidant peak (R_A), convert the area ratio (R_A) to the weight ratio (R_W) by using Figure 2; then, p.p.m. antioxidant $= \frac{200}{10R_W} = \frac{20}{R_W}$, where 200 = μg. of di-BHA added to eluate; 10 = grams of sample; and $R_W = \frac{\text{weight of di-BHA added to eluate}}{\text{weight of antioxidant in extract}}$.

For routine use where the sample size and amount of internal standard added are constant, the graph in Figure 2 can be calibrated directly in p.p.m. antioxidant.

Table III. Comparison of Results from Different Methods

	Recovery, P.P.M.					
	Sample 1		Sample 2		Sample 3	
	BHA	BHT	BHA	BHT	BHA	BHT
Anderson and Nelson (*1*)	6	6	20	20	...	32
Proposed Method	5.3	6.1	19.4	21.4	...	31.0

Results and Discussion

Extraction Procedure. Table I lists the recovery data for wheat cereal, corn cereal, and potato granules. The average recovery is 98.2%, and a typical extraction of cereal takes 10 to 15 minutes, while a sample of potato granules requires 20 minutes before 20 ml. of eluate are collected.

Table II shows the recovery of BHT from waxed paper and gives the results of analyses on commercial products containing different levels of BHT. That no loss of BHT occurs during the preparation of the sample is shown by the good recoveries when BHT is added to the extracts of waxed paper. The recoveries from commercially prepared papers are also good.

Chromatography. The silicone gum–Tween 80 column used in this analysis is especially suited to give a rapid analysis with a very satisfactory separation of BHA and BHT and di-BHA. This column very nicely separates these three components from the myriad of organic compounds present in extracts from breakfast cereals or potato granules as shown in Figure 1. The relative retention times for the compounds are BHT, 0.55; di-BHA, 1.00; and BHA, 1.28.

Because of the selectivity of this column, commercial fats and oils may be directly analyzed for their BHA and BHT content. Operation of this column at 150° C. allows a chromatogram to be completed in approximately 12 minutes. When operated at this temperature, the column is very stable and may last for years if operated continuously. A silicone gum–Tween 80 column has been in use in the authors' laboratory intermittently for 6 months, and no degradation or loss of resolving power has been observed.

The calibration curve illustrated in Figure 2 varies slightly from time to time and must be restandardized at least once a week by plotting at least two points on the curve. This observation was reported earlier by Coffman and Schwecke (*5*), who did work on vanilla flavors, and by Wesselman (*13*) in his work on the quantitative determination of ethanol.

Several samples analyzed by the procedure of Anderson and Nelson (*1*) were also evaluated by the method proposed in this paper. The results are presented in Table III and show good agreement between methods. The proposed method does not require temperature programming, yields peaks completely resolved from the solvent front, and uses small samples which allow better column efficiency and longer column life.

This method has been used successfully in the authors' laboratory for more than a year. Although GLC equipment, in general, is expensive, this procedure requires a gas chromatograph of simple design. When GLC

equipment is used as standard equipment in quality control laboratories, the initial cost is not such an important factor, especially if the equipment is of rugged design so that it will give many years of service.

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CURED MEAT PIGMENTS

Studies of the Photooxidation of Nitrosomyoglobin

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The effects of ascorbic acid, isoascorbic acid, nicotinamide, niacin, orthophosphate, and reduced diphosphopyridine nucleotide on the formation and light stability of denatured globin nitric oxide hemochromes and nitrosomyoglobin were studied. Cured meat pigments of hams were more stable to light irradiation in the presence of nicotinamide and sodium ascorbate than were those treated with ascorbate alone or those treated with a mixture of ascorbate and niacin. The color of ham slices treated with orthophosphate buffer at pH 6.8 was more desirable during storage under light than that of slices from hams treated with pH 6.2 orthophosphate buffer. Nitrosomyoglobin was formed more readily from metmyoglobin in the presence of nicotinamide than in its absence. It was more stable to light irradiation at pH 6.8 than at pH 6.2.

THE HEME PIGMENT responsible for the desirable color of freshly cured meat and its instability to light irradiation has been the object of considerable study. Many adjuncts are currently being used by industry to improve color stability of cured meat products during storage, but the practical solution of the problem has not been forthcoming.

Past work concerning use of various additives on the stability of nitrosomyoglobin can be divided into three general areas of study: the effect of ascorbates and other reducing agents (*1, 3, 12, 13, 18, 24, 25*); the influence of phosphates or other materials which affect hydrogen ion concentration (*2, 4, 10, 17, 20, 21*); and the reactivity of pyridine compounds and meat pigments to form hemachromes (*5, 6, 22*).

The objective of studies reported herein was to determine the influence of nitrogen-containing compounds such as niacin, nicotinamide, and reduced diphosphopyridine nucleotide; ascorbates; and pH on the stability of cured meat pigments.

Methods and Materials

Meat Cuts. Seventy-six hams and 12 picnics were studied in these experiments. These consisted of 32 hams purchased from a commercial source and 44 hams and 12 picnics obtained from animals slaughtered at the University of Missouri.

Curing Procedure. All hams were pumped with a 70° pickle consisting of 0.825% sodium nitrite, 0.54% sodium nitrate, 15.0% sugar, and 83.6% salt and certain other chemical additives. Sodium ascorbate was used at the level of 0.03*M* and the nitrogen ring compounds at 0.01*M*. Sodium orthophosphate (0.05*M*) was used to adjust the brine pH. Control samples included hams pumped with brine containing all constituents except the nitrogen ring compounds or samples pumped with brine containing all constituents except the nitrogen ring compounds and ascorbate. The ascorbates and nitrogen ring compounds were added to the commercial curing pickle just prior to pumping of the hams and picnics. Hams were artery pumped and the picnics stitch pumped to 10% of their trimmed weight, and one-third ounce per pound dry cover cure containing the same proportion of curing ingredients as above was applied. The meat samples were cured at 38° ± 2° F. for 6 days.

Cooking and Smoking Procedures. Following curing for 6 days, the samples were washed with running tap water for 30 minutes to remove excess cover cure. They were then placed in stockenettes and dried for 12 hours at 120° F. in an electric smoke house. Smoke was applied after drying, and the temperature raised 10° F. each hour until it reached 150° F. The cuts were processed to an internal temperature of 142° F. After smoking and cooking, the cuts were cooled to room temperature and stored at 38° ± 2° F. until used for display and analysis purposes.

Preparation of Samples. Five center slices were removed from each of the hams and picnics. Two slices were wrapped individually with a laminated